FEDERAL COURT

No. T-2030-13

BETWEEN:

NEIL ALLARD
TANYA BEEMISH
DAVID HEBERT
SHAWN DAVEY

PLAINTIFFS

AND:

HER MAJESTY THE QUEEN IN RIGHT OF CANADA

DEFENDANTS

AFFIDAVIT OF DR. CAROLINE FERRIS

I, DR. CAROLINE FERRIS, Surrey North Community Health Centre, 10697 135A St Surrey BC, MAKE OATH AND SAY AS FOLLOWS, THAT:

1. My name is Caroline Ferris and I make this affidavit of my own personal knowledge, information and belief. Where matters are stated to be on information and belief I so indicate and believe them to be true.

2. Now produced and marked as Exhibit "A" to this my Affidavit is my Rebuttal Expert Report.

3. Now produced and marked as Exhibit "B" to this my Affidavit is my signed Certificate Concerning Code of Conduct for Expert Witnesses.

4. I swear this Affidavit as an expert rebuttal witness on behalf of the Plaintiffs in this action.

SWORN BEFORE ME at the City of Abbotsford, in the Province of British Columbia, this 18th day of December, 2014

A Commissioner for Taking Affidavits in and for the Province of British Columbia

DR. CAROLINE FERRIS

This is Exhibit "A" referred to in the affidavit of Caroline Ferris sworn before me at Abbotsford, BC this 18th day of Dec. 2014

A commissioner for taking affidavits for British Columbia

(*a*) a statement of the issues addressed in the report;

This report addresses in rebuttal various issues arising in the Defendant Expert Report of Dr. Paul Daeninck of October 27, 2014.

(*b*) a description of the qualifications of the expert on the issues addressed in the report;

MD, CCFP, FCFP

Clinical Assistant Professor, UBC Department of Family Practice Site Faculty, Surrey South Fraser Family Practice Residency Program CME Lead, Surrey-North Delta Division of Family Practice Clinical cannabis experience:

- 20 years in full-service family practice including obstetrics, hospital and palliative care
- 5 years working in Mental Health and Substance Use doing primary care
- strong interest in complementary and alternate therapies;
- authorized patients in my family practice under the MMAR program since approximately 2001, recognizing cannabis as an effective herbal remedy;
- became a member of Canadian Consortium for the Investigation of Cannabinoids (CCIC) and participated in their online Cannabis Medical Education program and other CME activities;
- did MMAR consultations for a Compassion Club 2009-2010, for patients who had been screened by the Club and had all their documentation;
- medical editing of Philippe Lucas' multi-centre study of harm reduction with cannabis; and Dr Paul Hornby's published case studies on MS and chronic pain patients (anonymously);
- shifts at Medicinal Cannabis Resource Centre Inc. (MCRCI) 2011 onward (this is essentially a Chronic Pain Clinic);
- became a member of the MCRCI Advisory Board in 2011;
- became a member of the local Multidisciplinary Association of Psychedelic Studies Advisory Board in 2011; currently clinician for "MDMA for PTSD" study;
- became a member of Physicians for Medicinal Cannabis at its inception (2013);

- gave feedback to Health Canada during the public consultative process around MMAR/MMPR;
- gave feedback to College of Family Physicians of Canada regarding the policy document on cannabis, and was invited to participate in their Working Group;
- lecture Family Practice Residents on Medicinal Cannabis;
- routinely teach medical students and FP Residents how to assess a patient for cannabis recommendation, generally in the context of chronic pain, using basic principles of family practice, standard risk assessment tools and a harm reduction approach.
- Consequently, as a result of the above experiences I am familiar with the issue of "dosage" in the real world as a result of my clinical practice I am primarily a family physician and my UBC faculty appointment is a clinical one - in other words I'm a practical person not an academic. My faculty position is for Behaviour Medicine which covers interpersonal interactions, communication styles, self-awareness and resilience, as well as care of underserved populations.

(*c*) the expert's current *curriculum vitae* attached to the report as a schedule;

Attached as Schedule A

(*d*) the facts and assumptions on which the opinions in the report are based; in that regard, a letter of instructions, if any, may be attached to the report as a schedule;

I was asked to review Dr. Daeninck's report and to respond to any points contained in it that in my opinion required some rebuttal by way of clarification, or amplification or otherwise.

(*e*) a summary of the opinions expressed

I disagree with the opinion of Dr. Daeninck in a number of specific aspects as set out below in relation to each of the specific questions. In my opinion, his experiences is limited in relation to the authorization of the use of herbal cannabis to a variety of conditions and his practice appears to be primarily limited to cancer patients and to the prescription of pharmaceutical cannabinoids. In particular, I do not share his concerns about dosage as I do for other pharmaceutical drugs, given the low to virtually nonexistent lethal dose (LD50) for cannabis (marihuana)

(*f*) in the case of a report that is provided in response to another expert's report, an indication of the points of agreement and of disagreement with the other expert's opinions;

Question 1

Paragraph 16 – I disagree with schizophrenia as absolute contraindication.

Local psychiatrists often prescribe cannabis for anxiety and sleep in relation to this condition. I rely on and I am informed by local Psychiatrist Dr. Donna Dryer (ddryer@orenda.org) who advises she routinely recommends standardized high cannabidiol capsules that are at least in a ratio of CBD to THC of 2 to 1 or 3 to 1 but are usually 20 to 1 (Cannabidiol 20 mg to 1 mg of THC for patients with schizophrenia as well as for many of her patients with PTSD any psychotic disorder, Bipolar Disorder, Cancer, Anxiety, Arthritis, Tourette's, ADHD, Migraines, Parkinsons Disease, Fibromyalgia and any of the other Central Sensitization Syndrome Disorders (fibromyalgia overlapping PTSD etc) that she informs me are the physical consequences of early childhood neglect/abuse now known as Early Childhood Adversity or developmental PTSD.

In my experience patients and significantly their families have requested cannabis to assist with the above diagnosis, specifically in preference to not wishing to use prescription medications which may be addictive or have serious side effects. I have attached a few published references involving cannabis and schizophrenia as scheduled and referenced below.

Paragraph 18 - With respect to the College of Family Physicians of Canada (CFCP) document (Annex B) it is a draft or "preliminary guide" which is currently being revised. I am a member of the Committee which is working on this revision. My suggestions/recommendations have included that the indications be more inclusive as currently they do not recommend for anxiety, multiple sclerosis or osteoarthritic pain, despite ample evidence to the contrary.

Paragraphs 19, 24 – The evidence shows that patients using substances such as opioids and benzodiazapine's, whether licit or illicit, tend to reduce use of those drugs when cannabis is available and this leads to a reduction in opioid overdose rates (see Lucas papers referenced below ; Aug 2014 JAMA Article referenced below)

Paragraph 25 - I would recommend performing a urine drug screen to evaluate patients for whom substance use disorder is suspected. This eliminates any assumptions.

Question 2

Paragraph 29 – I agree in general with doses of 3-5 grams/day as being adequate for most patients. However according to the Health Canada "Information for Healthcare Professionals" (Annex A) (table 3 p. 51), the dose required for oral consumption is 2.5 times the smoked dose. Hence the dose range for those using edibles can easily be 10 -12.5 grams. One must also factor in tolerance, for which there are many factors to consider, including genetics.

Also, patients will require more product if it is of very low potency; not everyone is growing or able to access super-potent strains.

Paragraph 35 - concentrates such as hash, oil and tincture make sense from a harm reduction perspective: eliminating combustion of large amounts of plant material reduces airway irritation and other harms from smoking the dried product.

Question 3

Paragraph 37- While I acknowledge that some patients may require higher doses for edibles, I am suspicious of doses around 20 g/day and higher. I believe a small number of growers have abused their licences and have profited from selling surplus product. I would decline an application if someone asked for a dose higher than calculated "so I can pay some bills" or because a Designated Grower told them to ask for a certain amount. If, in my assessment the patient is seeking the authorization with an intention of abusing it in any way I will decline the request.

Paragraph 41 – On the "criminal coercion" issue I point to the failure of Health Canada to set up local systems for properly administering the MMAR program, leaving it open to abuse. We don't ban opiates for all patients because some people are diverting them, and therefore in my opinion we should not restrict access to or ban all home growing of cannabis because of the actions of a few, especially if this removes access for these individuals due to unaffordability. Legitimate patients with safe gardens who are growing within their dose limits should not, in my opinion, be penalized by the actions of the criminal minority resulting in restricted access to their medicine.

Paragraph 43 – Tampering – I have never personally seen this or experienced it - I would routinely get a call from Health Canada about doses authorized over 10 g, and would discuss the situation with them but I never received a call suggesting the amount had been altered or asking me to verify an amount had been altered.

(*g*) the reasons for each opinion expressed;

As a clinician I know there is no or a very low LD 50 (lethal dose ratio) for cannabis so the concern over dosage is not what it is for drugs doctors usually prescribe such as oxycontin for example for which there is also a black market demand and that can kill by overdose. What practicing doctors do is, listen to patients, try a dose, have them come back, talk to them and see what works and what doesn't within the limits of what is available and keep trying to arrive at a dosage that works for the particular patients medical issues.

There is a big problem in this area in general, exemplified Dr. Daeninks perspective. In particular, in his credentials, item 5, he states, with reference to his clinical practice, "I have used prescription cannabinoids in hundreds of patients over the past 16 years, as well as medical marijuana in approximately 20 patients under the MMPR". This is not very significant clinical cannabis experience. Many of the patients who come to see me for marijuana support have already tried "prescription cannabinoids" (e.g. nabilone or Sativex). That's why they come to try the actual plant in some form as the others have not been working satisfactorily.

The Canadian Consortium for the Investigation of Cannabinoids (CCIC), and physicians like Dr. Mark Ware, and Paul Daeninck et al are committed to "science", which is the golden calf of modern medicine. There isn't much clinical science published in this area for various reasons and the papers I have read trouble me because the researchers, not the patients, are the ones who determined the "doses" for the study. Mark Ware, who is director of CCIC, and one of those who helped develop not only the CCIC check-list that Dr. Daeninck refers to, but also the Quebec College policy on medical marijuana, and the College of Family Physicians of Canada Preliminary Guidelines paper (Annex B to Dr. Daeninck's report), is also a frequent presenter at medical education conferences. He is a medicinal cannabinoid advocate, and has limited experience with the authorization of whole plant medicine with patients.

These respected professionals are trying to legitimize cannabis as medicine, but they also seem to be paying homage to the political rhetoric that cannabis is a "dangerous drug", and their statements and the guidelines they help develop are regularly challenged by those of us doing clinical cannabis medicine.

I agree with Dr. Daeninck's concerns about high dosages under the MMAR, and I personally have noticed that the "dosage" requests under the MMPR are smaller than under the MMAR, but that is only part of the story. I think there are many reasons for higher requests under the MMAR, some of which Dr. Daeninck identifies:

- some patients diverted to the illicit market; under the MMPR there's no profit in diversion, because the licensed product is so expensive and arguably there is less diversion given the developments in the market south of the border with respect to medical and legal cannabis and the effect on the Canadian market;

- some were poor growers, or couldn't afford the hydro bills to grow properly, so asked for a higher "dosage" to get the number of plants to produce what they needed;

- most of the MMAR patients were experienced users, many having long before converted to oils or edibles (which I believe DO require more plant

product for comparable medical effects, contrary to what Dr. Daeninck says), whereas many MMPR applicants ,in my experience, are naive and early explorers, starting low. (In fact, my practice with a naive user, is START them with a medical document for 5 gm per day. I know they're not likely to use that much, but it gives them an opportunity to explore different strains and prepare different ingestible products. Why should I tie their hands with a tiny "dosage" that has nothing to do with how they are going to treat their medical problem? - these are often desperate patients, who have never before even thought of USING it, let alone SELLING it, and stand to gain nothing from having more than they need.) The important thing is to give them enough to try various things and to have them keep coming back to monitor the situation and obtain feedback from as to what works and what doesn't, and to monitor the use, just like any other drug prescribed and remembering it is not lethal, like most many of the others.

Dr. Daeninck also admits that his patient base is limited to cancer patients. Those are generally not the patients that I see who request higher doses in my clinical practice;

I think the whole issue of dose is a distraction (which Dr. Daeninck keeps referring to as if it were a complex and important question), taken way out of proportion by our profession, mainly because doctors regularly prescribe drugs that can be lethal when the dose is too high. With cannabis, if you use it for medical purposes, as far as I'm concerned, the "dose" is whatever you need (I believe this is the approach in California). Besides, there are over 400 strains. In my opinion, a medical declaration is not a prescription (a concept which is very hard to sell to doctors and their Regulatory Colleges, even though no doctor would write a prescription that said "3 gm. of any narcotic of the patient's choice").

Health Canada's Information for Health Care Professionals (Annex A to Dr. Daenink) was put together responsibly by a scientist with no agenda except to compile everything he could find in the literature that was relevant (however limited the studies). It has never been thoroughly discussed by the PMC (Practitioners for Medicinal Cannabis). The PMC now has 66 active participants, including Mark Ware, Ethan Russo, and Jeff Hergenrather of the Society of Cannabis Clinicians, from California.

Here are a few PMC comments on the CFPC (and other College) guidelines, that Dr. Daeninck includes as Annex B to his report:

- I have not seen anyone stand up and make the point that the guidelines that they have created may actually be more harmful than anything else.

- I have been a bit disheartened over the last while about policies being based on poor literature. For example, the Ontario guidelines refer to the Volkow study published in the NEJM - "Adverse effects of marijuana use". This is a terrible paper; in the words of Leon Gussow of the Poison Review - "as much a political document as a medical one".

- These new guidelines are full of mistakes. They are unscientific and do not reflect anecdotal evidence or even research articles. -Policy like this is written top-down by those with a political agenda purely. Essentially, the administration has an agenda and they force the issue in a very unscientific and non-democratic manner. Stick to the Health Canada Information for Health Care Practitioners which is well-referenced.

- What troubles me about many Regulatory College statements and "Published Guidelines" is an apparent lack of inclusion of the points of view of those practitioners whose primary "evidence" is listening to the stories of hundreds of patients who have so much to teach us about the clinical impact of cannabis on their quality of life. I'm not sure how to address this. I do respectfully suggest that our profession's veneration of "science" should not limit our commitment to listening, or our ability to see.

Dr. Daenink seems relatively open-minded however the depth and breadth of his experience is limited, as he's been treating only cancer patients, with the pharmaceutical product nabilone. By far the majority of *bona fide* medical cannabis patients are suffering with severe arthritis, MS, or HIV related illness, and most of them are using herbal cannabis in one form or another.

It's interesting what the perception of the medical community is/was re: the MMAR program. I actually heard the former FHA Addiction Medicine Physician Lead tell a group of colleagues that the MMAR program is only for patients terminally ill with cancer. I did share with the group the full range of B1 indications and typical B2 indications for the MMAR.

The professional autonomy and compassionate nature of physicians are being high-jacked by a politically driven agenda, which discourages us from considering medical cannabis as an option for fear of regulatory reprisal.

I think that medically approved patients should have the option to be allowed to grow their medicine for themselves inexpensive like other natural health care plants or other plants for food etc. and the Government should not need to regulate this anymore than they do food or natural health care plants products unless the products are being sold to the public as medicine in which case the Food and Drugs Act, Natural Health Care Products Regulations would presumably apply. This does not mean there should not be local rules involving

inspections etc. to ensure the safety and security of a premises and its inhabitants.

My general considerations, as a clinician, on these issues can be summarized as follows:

1) The very low LD50 of cannabis with comparisons to commonly used medications such as ibuprofen and opiates. For example, after reading a thorough literature review of ibuprofen with harms and risks laid out as they were for cannabis, the general public would likely elect not to use ibuprofen. Yet this relatively harmful substance is widely used and readily available over the counter. 400 Canadians die annually from GI bleeds caused by NSAID's, yet not a single Canadian has perished from using cannabis alone. (I have LD50 data for cannabis, NSAID's and opiates.)

2) The folly of basing our opinions on "scientific studies" alone while ignoring a vast body of clinical experience and patient experience. In medicine, we often use treatments that are "unproven" simply because we've always done it or it became popular on as it later turns out misguided evidence. For example, for years we recommended Vitamin E as a powerful antioxidant with general health benefits and a cardio protective effect. Then, huge studies were undertaken which revealed that people who use Vitamin E regularly had a higher all-cause mortality rate. Now we're busy telling our patients to stop taking Vitamin E.

Similarly, low-dose aspirin was recommended for years as a preventive measure for heart attacks and strokes. When the big studies were done, we noted that hemorrhagic complications (such as GI bleed and stroke) far outweighed the putative benefits of aspirin for prevention. Now, we've changed our tune and are recommending low-dose ASA only for secondary prevention for those who've already experienced a cardiac event or stroke.

3) Our duty as physicians is to "cure sometimes, relieve often and comfort always". We are gatekeepers of a system that is founded on science but operated on compassion. To give physicians another tool that we can use to relieve our patient's suffering is a gift. We shouldn't assume cannabis to be a Trojan horse, but consider it in context, using our medical skills to assess a patient and determine if cannabis could be of therapeutic benefit for them.

The principles of medicine, and of Family Practice in particular, are founded on the relationship between physician and patient. The family physician is in the best position to assess their patient, based on their longitudinal knowledge of the 'biopsychosociospiritual' context of that individual. By using our basic tools of history taking and physical examination, supplemented with other investigations as necessary, we are

able to determine whether a medical condition exists and whether a patient has had benefit from a given therapy.

When patients have a condition that hasn't responded to other therapies, and for which they have found benefit from cannabis, they need to be able to trust that their physician will treat them seriously and not dismiss them as a "stoner" seeking legitimization for recreational cannabis use. In order to assess that patient properly, a physician needs to be informed, compassionate and yes, open-minded.

I do agree with risk assessment and relative contraindications for cannabis...but I would apply the same principles as I would when considering prescription of any other substance with abuse potential.

(*h*) any literature or other materials specifically relied on in support of the opinions;

"How not to protect community health and safety: what the government's own data says about the effects of cannabis prohibition", December 2010, Stop the Violence BC Coalition

"Long term marijuana users seeking medical cannabis in California (2001-2007): demographics, social characteristics, patterns of cannabis and other drug use of 4117 applicants", Harm Reduction Journal, BioMed Central, Thomas O'Connell, November 3, 2007

"Response to CFCP Draft Guidelines for Authorizing Dried Cannabis", Caroline Ferris, MD, CCFP, FCFP

"Cannabis for therapeutic purposes: Patient characteristics, access, and reasons for use", International Journal of Drug Policy, Zach Walsh et al., August 30, 2013

"Inflammation and aging: Can endocannabinoids help?" Biomedicine & Pharmacotherapy, Yannick Marchalant, et al., February 19, 2008

"Harm reduction – the cannabis paradox" Harm Reduction Journal, Robert Melamede, September 22, 2005

"The Role of the Physician in 'Medical' Marijuana", American Society of Addiction Medicine, September 2010

"Cannabis as a substitute for alcohol and other drugs: A dispensary-based survey of substitution effect in Canadian medical cannabis patients", Informa Healthcare, Addiction Research and Theory, Philippe Lucas, et al., September 20, 2012.

"Moral regulation and the presumption of guilt in Health Canada's medical cannabis policy and practice", International Journal of Drug Policy, Philippe Lucas, et al. September 8, 2008

"Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia", Muller–Vahl. **Attached as Schedule "B"**

"Cannabinoids and Schizophrenia: Therapeutic Prospects", P.J. Robson, 2014. **Attached as Schedule "C"**

American Herbal Pharmacopoeia, *"Cannabis Inflorescence, Cannabis* spp. *Standards of Identity, Analysis, and Quality Control"*, 2014. **Attached as Schedule "D "**

(*i*) a summary of the methodology used, including any examinations, tests or other investigations on which the expert has relied, including details of the qualifications of the person who carried them out, and whether a representative of any other party was present;

Not Applicable

(*j*) any caveats or qualifications necessary to render the report complete and accurate, including those relating to any insufficiency of data or research and an indication of any matters that fall outside the expert's field of expertise;

Not Applicable

(*k*) particulars of any aspect of the expert's relationship with a party to the proceeding or the subject matter of his or her proposed evidence that might affect his or her duty to the Court.

I do not have any relationship with any party to the proceeding, and my relationship to the subject matter of my proposed evidence arises as a result of my experience as a physician and will not affect my duty to the court as an expert witness.

SCHEDULE "A"

CURRICULUM VITAE: CAROLINE FERRIS, MD, CCFP, FCFP

Education:
MD 1986, UBC
Rotating Internship, 1986-87, Saskatoon City Hospital
Internal Medicine RII year, 1988-89, UBC
Post-Grad courses in Public Health, 1990-91, UBC
Certificant of College of Family Physicians 1993
Breastfeeding Counsellor Certification, 1997, Douglas College
Fellowship College of Family Physicians 2003
Methadone Maintenance Certification, 2010

Work Experience:
Locum Tenens 1987-88, Saskatchewan
Family Practice, North Delta, 1989-2003
Family Practice Obstetrics, PAH/SMH 1989-1999
Physician, Planned Parenthood N Delta, 1989-1992
Primary Care Physician, South Fraser Mental Health, 1999-2001
Family Practice, Morgan Creek, Surrey, 2003-2010 (FHA Pilot Site for Enhanced Family Practice)
Primary Care Physician, Fraser Health MHSU 2009-2011
Staff Physician, Creekside Withdrawal Mgmt Centre 2010-present
Staff Physician, Surrey North Community Health Centre 2009-present

Hospital Privileges:
Delta Hospital, 1989-1992
Surrey Memorial Hospital, 1990-2003 and current
Peace Arch Hospital, 2003-2009

Professional Activities:
Advisory Board, Healthiest Babies Possible, 1989-1999
Member, BCMA Environmental Committee, 1990-92
Instructor, Surrey Memorial Hospital Prenatal Classes, 1990-1999
Member, South Fraser Breastfeeding Promotion Committee, 1992-1999 (including 3 yrs as Chair)
Member, Steering Committee for Advance Care

Planning, PAH, 2004-2007
Research Collaborator, Dr Jerrilyn Prior, 2004-present
Medical Director, Morgan Creek Women's Clinic, 2004-2007
Clinical Associate Professor, Faculty of Medicine, UBC, 2006-present
Examiner, Medical Council of Canada, 2009-present
Peer Tutor, Canadian College of Family Physicians, 2009-present
Member, Steering Committee Fraser Health Substance Use, 2009-2011
Organizing Committee, Berman Concurrent Disorders Conference 2010, 2011

Current Appointments: Clinical Assistant Professor, UBC Department of Family Practice
UBC Surrey South Fraser Family Practice Residency Program Site Faculty (Behaviour Medicine)

Wellness and CME Lead, Surrey/North Delta Division of Family Practice
Advisory Board, MCRCI
Advisory Board, Multidisciplinary Association of Psychedelic Studies
Member, Physicians for Medical Cannabis
Member, Canadian Consortium for Investigation of Cannabinoids
Member, Canadian Society of Addiction Medicine

Community Service: Member-At-Large, Panorama Ridge Ratepayers Ass'n, 1989-1992
PAC, Colebrook Elementary School, 1997-2002
Leader, Girl Guides of Canada, 1999-2004
Surrey North Delta Division of Family Practice, 2011-present

Contact ferris.caroline@gmail.com
604-961-6746

EXPERT REVIEWS

Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia

Expert Rev. Neurother. 8(7), xxx–xxx (2008)

Kirsten R Müller-Vahl[†] and Hinderk M Emrich

[†]Author for correspondence
Clinic of Psychiatry, Socialpsychiatry & Psychotherapy, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany
Tel.: +49 511 532 3167
Fax: +49 511 532 3187
mueller-vahl.kirsten@mh-hannover.de

Highlighting the association between schizophrenia and *Cannabis sativa* and the endogenous cannabinoid receptor system, respectively, two opposite aspects are of major relevance. On the one hand, cannabis is the most widely used illegal drug. There is substantial evidence that cannabis has to be classified as an independent risk factor for psychosis that may lead to a worse outcome of the disease. This risk seems to be increased in genetically predisposed people and may depend on the amount of cannabis used. On the other hand, during the last few years, an endogenous cannabinoid receptor system (including two known cannabinoid [CB_1 and CB_2] receptors and five endogenous ligands) has been discovered. There are several lines of evidence suggesting that, at least in a subgroup of patients, alterations in the endocannabinoid system may contribute to the pathogenesis of schizophrenia (e.g., increased density of CB_1 receptor binding and increased levels of cerebrospinal fluid endocannabinoid anandamide). Accordingly, beside the 'dopamine hypothesis' of schizophrenia a 'cannabinoid hypothesis' has been suggested. Interestingly, there is a complex interaction between the dopaminergic and the endocannabinoid receptor system. Thus, agents that interact with the cannabinoid receptor system, such as the nonpsychoactive cannabidiol, might be beneficial in the treatment of psychosis.

KEYWORDS: Δ^9-tetrahydrocannabinol • anandamide • cannabidiol • cannabinoid • cannabis sativa • CBD • psychosis • schizophrenia • THC

Schizophrenia is a common psychiatric disorder characterized by impairments in the perception or expression of reality. Since there is no laboratory test available, diagnosis is based on patients' self-reported experiences in combination with psychopathological symptoms observed by a clinician. Different systems have been used to classify schizophrenic disorders. All these categorizations describe a list of different symptoms that must be met for a certain period of time in order for someone to be diagnosed with schizophrenia [1].

Most often schizophrenic symptoms are subclassified into positive (or productive) and negative (or deficit) symptoms. Positive symptoms include delusions, auditory hallucinations and thought disorder. Negative symptoms include features such as blunted affect and emotion, poverty of speech, anhedonia and lack of motivation. In many patients, in addition, so-called disorganized symptoms may occur such as chaotic speech, thought and behavior. It is important to note, that none of these signs are diagnostic of schizophrenia, and all symptoms can occur in other medical and psychiatric conditions. Depending on the clinical symptomatology, schizophrenia is classified into the following types: paranoid, disorganized, catatonic, undifferentiated and residual type.

Schizophrenia occurs equally in males and females. However, the peak age of onset is 20–28 years for males, but 26–32 years for females. The lifetime prevalence of schizophrenia is approximately 0.5–1.0%. Schizophrenia is known to be a major cause of disability associated with social isolation and decreased life expectancy. A substantial number of patients diagnosed with schizophrenia suffer from comorbidities such as depression, anxiety disorders and substance abuse. Approximately a third of patients have a complete recovery, a third improves but do not fully recover and another third remain significantly ill. It is assumed that there is a multifactorial pathogenesis in schizophrenia including genetic and environmental factors, neurobiological alterations, as well as psychological and social processes. Pathophysiologically there is evidence for an alteration in

 10.1586/14737175.8.7.xxx ISSN 1473-7175

the dopaminergic system with increased dopaminergic activity in subcortical areas including the striatum, and decreased dopaminergic activity in cortical areas such as the prefrontal cortex. However, recent studies also suggested an involvement of the serotonergic, glutamatergic and GABAergic systems. Dopamine receptor antagonists (neuroleptics) are still the first choice treatment in schizophrenia. While positive symptoms in many cases can be controlled by these drugs, impairment in basic psychological functions such as memory, attention, executive function and problem solving is difficult to treat. Owing to the many possible combinations of symptoms, there is a controversial debate as to whether schizophrenia represents a single disorder or a number of discrete syndromes [2].

Cannabis sativa, cannabinoids & the endocannabinoid receptor system

Cannabis sativa is one the oldest and widely distributed drugs used in the form of dried buds or flowers (marijuana), resin (hashish), or various extracts collectively known as hashish oil. Cannabis plants produce a unique family of terpenophenolic compounds called cannabinoids. To date, more than 60 different cannabinoids have been detected. The two cannabinoids usually produced in greatest abundance are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the major psychoactive compound of cannabis. THC acts as a central cannabinoid CB_1 receptor agonist and, by that, mimics the endogenous cannabinoids. It was stereochemically defined and synthesized in 1964 [3].

To date, two cannabinoid receptors have been identified: CB_1 was cloned in 1990 [4] and CB_2 in 1993 [5]. However, since cannabinoid effects have been described that are not mediated by CB1 or CB2, there is increasing evidence for the existence of one or more additional, but so far unknown cannabinoid receptors (CB3) [6]. While CB_1 receptors are mainly located on neurons in the central and peripheral nervous system, CB_2 receptors predominantly occur in immune cells. Within the brain, CB_1 receptors are highly expressed in the cerebral cortex (particularly frontal areas), basal ganglia, hippocampus, anterior cingulate cortex and cerebellum. It has been demonstrated that CB_1 receptors inhibit the release of several neurotransmitters and neuromodulators including dopamine, GABA, serotonin, glutamate, noradrenaline and acetylcholine. The endogenous cannabinoid system, in addition, comprises a series of lipophilic endogenous ligands and enzymes for the biosynthesis and degradation of the endocannabinoids [7]. To date, five endocannabinoids have been identified. The two most important are anandamide (N-arachidonylethanolamide [AEA]), discovered in 1992 [8], and 2-arachidonoyl glycerol (2-AG), discovered in 1995 [9]. However, the physiological and pathophysiologically role of the endogenous cannabinoid receptor system is still widely unknown.

Cannabis has been used for medicinal purposes for approximately 4000 years. In former times it was used to treat gastrointestinal disorders, insomnia, headache, pain, emesis, epilepsy and inflammation. However, it was not reintroduced into modern medicine until approximately 20 years ago. Established therapeutic effects of THC are the treatment of refractory nausea and vomiting associated with cancer chemotherapy, and of appetite loss in cancer cachexia and AIDS wasting. Further indications are neuropathic pain in multiple sclerosis, other pain syndromes, spasticity, movement disorders such as Tourette syndrome and levodopa-induced dyskinesia, glaucoma and asthma [7].

For clinical use, in addition to THC (dronabinol, Marinol®) and CBD, the synthetic cannabinoid nabilone, the cannabis extracts Cannador® and Sativex® (containing both THC and CBD) and the selective CB_1-receptor antagonist rimonabant (SR141716A) are also available. Two further cannabinoids are under clinical investigation: dexanabinol (HU-211), an NMDA glutamate receptor antagonist and ajulemic acid (CT3), a potent analog of THC-11-oic acid.

Cannabis abuse & psychosis

The relationship between schizophrenia and the use of cannabinoids is complex and not completely understood. While it is well established that high doses of cannabis can cause a transient toxic psychosis, it is unclear whether cannabis use increases the risk of psychotic illness persisting after abstinence from the drug. However, there is substantial evidence that heavy cannabis abuse in healthy persons is a risk factor for the clinical manifestation of schizophrenia and triggers the onset of psychotic episodes in predisposed individuals and triggers relapse in patients with schizophrenia. Since the vast majority of cannabis users do not develop psychosis, it can be hypothesized that some people are genetically vulnerable to these effects. Furthermore, it has been suggested that the high prevalence of cannabis use in patients with schizophrenia could be explained as a kind of self medication, not only to reduce disease-associated symptoms such as depression, but also to diminish neuroleptic-induced side effects [10]. Alternatively to this 'self-medication hypotheses' an 'affect regulation model' has been proposed. According to this model negative emotions are associated with greater substance use problems. Thus, substance use in patients with schizophrenia could be understood as an attempt to cope with negative affects [11].

It is known that acute administration of cannabis leads to reversible, dose-dependent cognitive impairments. In the general population, it has been shown that cannabis negatively impacts cognitive functioning, although it is unclear whether cognitive deficits even persist after abstinence for a longer period. Investigating the effects of cannabis on cognitive functions in patients with first-episode psychosis, it has been demonstrated that cognitive functioning and performance is comparable or even better in those patients using cannabis compared with non-using patients [12,13]. Other studies, however, provided evidence for negative effects of THC on cognitive functioning in patients with schizophrenia [14]. In a recent

meta-analysis, including 23 studies with a total of 1807 patients with schizophrenia, it was concluded that preferential use of cannabis (but not alcohol) in persons with schizophrenia is indeed associated with higher scores for problem solving and reasoning and visual memory [15]. The authors, therefore, speculated that cannabis might have a neuroprotective effect among persons with schizophrenia [15].

More recently, several epidemiological studies have been performed investigating whether cannabis use acts as an independent risk factor in the onset of schizophrenia. In 1987, Andreasson *et al.* for the first time performed a large longitudinal study in a cohort of 45,570 Swedish conscripts and found that heavy cannabis use at age 18 years causes a sixfold increase in the risk of later schizophrenia compared with non-users [16]. In a follow-up study (surveying a period of 27 years and including 50,087 subjects) these data were corroborated suggesting a causal relationship between cannabis use and an increased risk of developing schizophrenia [17]. Arseneault *et al.* found that cannabis use in adolescence increases the likelihood for adult-onset schizophreniform disorder with a greater risk after early use (by age 15 years) compared with later cannabis use (by age 18 years) [18]. This age dependence was confirmed by a prospective population-based Dutch study [19]. These clinical data are also in line with results obtained from animal studies suggesting that chronic administration of cannabinoid agonists during the periadolescent period causes persistent behavioral alterations in adult animals [20].

In addition, from this Dutch study it is suggested that individuals with an established vulnerability to a psychotic disorder have a markedly worse outcome when using cannabis [19]. As suggested before [19], there was also evidence for a dose–response relationship between cumulative exposure to cannabis use and the psychosis outcome [23]. In a Danish study, 535 patients treated for cannabis-induced psychotic symptoms were followed for at least 3 years to establish the prognostic importance of this disorder [21]. Almost half of the patients were subsequently diagnosed with schizophrenia spectrum disorders. Age at onset of these patients was earlier compared with a comparison group with schizophrenia spectrum disorders without a history of cannabis-induced psychotic symptoms [21]. This finding was confirmed by a population-based Dutch study demonstrating a 7-year age difference between male cannabis users and non-users [22]. From a population-based sample including 2437 young people it was concluded that cannabis use increases the risk of psychotic symptoms especially in those with evidence of predisposition for psychosis [23]. Two studies from New Zealand [24,25] and one from Israel [26] further support that regular cannabis use may increase the risk of psychosis. In a recent review, Smit *et al.* concluded that the 'self-medication hypothesis' in people with schizophrenia using cannabis is not supported by the literature [27]. In addition, the authors stated that the hypothesis that other drugs than cannabis might increase the risk of becoming schizophrenic should also be dismissed owing to lack of evidence. By contrast, they summarized that cannabis has to be classified as an independent risk factor for psychosis. This risk seems to be increased in vulnerable people and may depend on the amount of cannabis used [27].

Functional interaction between the cannabinoid & the dopaminergic system

Since it has been suggested that psychosis is caused by an overactive dopaminergic system (the dopamine hypothesis of schizophrenia) [28], it has been speculated that cannabinoids might cause or exacerbate psychoses by increasing the activity of the dopaminergic system. There are several lines of evidence suggesting a complex interaction between the CB_1 receptor system and the dopaminergic system.

From preliminary clinical studies it has been suggested that second-generation antipsychotics, in particular clozapine, decrease the use of cannabis in patients with schizophrenia [29–31]. This observation raises the question as to whether the CB_1 receptor system is involved in the therapeutic effect of clozapine [32]. In rats it has been demonstrated that chronic treatment with clozapine (but not with other typical and atypical antipsychotic drugs such as haloperidol, chlorpromazine and olanzapine) results in a selective decrease of CB_1 receptor binding in the nucleus accumbens but not in other brain regions (the frontal cortex, hippocampus and striatum) [32]. This effect disappeared after withdrawal from clozapine. Inversely, a 20% decrease in striatal dopamine D_2 receptor binding could be observed immediately after smoking cannabis in a 38-year-old drug-free patient with schizophrenia when using SPECT and iodobenzamide ([^{123}I]IBZM) [33].

In addition, plasma values of the dopamine metabolite homovanillic acid (HVA) were found significantly higher in a first-admission cannabis psychosis group compared with non-cannabis using psychotic patients [34]. Since it has been demonstrated that the urine concentration of HVA is increased in cannabis-intoxicated normal volunteers, an increased dopamine turnover after cannabis use has been suggested [35]. It has been speculated that in patients with cannabis-induced psychosis a genetic vulnerability may lead to an increased dopaminergic activity [34].

There is a large number of animal studies available substantiating an interaction between the endocannabinoid and the dopaminergic system. In rats it could be demonstrated that anandamide release in the dorsal striatum was eightfold increased after administration of a D_2-like dopamine receptor agonist [36]. This response could be prevented by administration of a D_2-like receptor antagonist. Pretreatment with the cannabinoid antagonist rimonabant (SR141716A) enhanced the stimulation of motor behavior elicited by a D_2-like dopamine receptor agonist, while administration of SR141716A alone had no effect on motor activity. It can therefore be speculated that the endocannabinoid system may act as an inhibitory feedback mechanism countering dopamine stimulation of motor activity [36]. In addition, it has been demonstrated that anandamide

increases the release of dopamine both in the striatum [37] and in the mesolimbic system [38]. Treatments with the dopamine D_2 receptor antagonist haloperidol and sulpiride resulted in significantly increased cannabinoid receptor mRNA levels in the caudate-putamen. Therefore, it has been suggested that the expression of the cannabinoid receptor gene in the striatum is under the negative control of dopamine receptor-mediated events [39].

In rats it has been demonstrated that exposure to THC increases the activity and expression of tyrosine hydrolase (TH), a rate limiting enzyme in the synthesis of dopamine and other catecholamines [40]. However, postmortem analyses of patients with schizophrenia (n = 14) compared with normal controls (n = 14) failed to demonstrate changes in the levels of TH in the caudate and substantia nigra, irrespective of recent cannabis use [41]. By contrast, binding sites of the dopamine transporter (DAT), a presynaptic marker on dopaminergic neurons, were found to be decreased in the caudate in patients with schizophrenia using [^{3}H]mazindole and autoradiography [41]. Because these changes depended on recent cannabis use and were observed only in those patients who had no THC in their blood at autopsy, it has been suggested that decreased DAT binding is associated with the pathology of schizophrenia and might be reversed by THC [41].

DAT knockout (KO) mice can be used as an animal model associated with hyperdopaminergia that has been suggested to be relevant to schizophrenia. It has been demonstrated that constitutive hyperdopaminergia in DAT KO mice is associated with a significant decrease of striatal anandamide levels [42]. These results further support that hyperdopaminergia leads to alterations of the endocannabinoid system and suggest that normalization of decreased anandamide levels might constitute an alternative therapeutic strategy for disorders associated with hyperdopaminergia such as schizophrenia [42].

Schizophrenia-like clinical effects of THC

The hypothesis that the consumption of exogenous cannabinoids may contribute to the pathophysiology of psychosis is further supported by observations in healthy volunteers [14,43–45]. Administration of intravenous THC to 22 healthy individuals produced transient schizophrenia-like positive and negative symptoms such as suspiciousness, paranoid delusions, conceptual disorganization, illusions, blunted affect, psychomotor retardation and emotional withdrawal. In addition, perceptual alterations, euphoria, anxiety and deficits in working memory, recall and the executive control of attention were observed [43]. These effects of THC were found to be more robust with the inhaled and intravenous route of administration compared with oral intake and corresponded to peak drug levels [46]. In frequent cannabis users, compared with healthy controls, blunted responses to the psychotomimetic, perceptual altering, cognitive impairing and anxiogenic effects of THC were found [44]. Therefore, it has been suggested that frequent users of cannabis are either inherently blunted in their response and/or develop tolerance to these effects of cannabinoids [44]. In a parallel study, it was demonstrated that in patients with schizophrenia intravenous THC transiently exacerbated both positive and negative symptoms, as well as perceptual alternations and cognitive deficits. In addition, extrapyramidal symptoms due to neuroleptic medication increased after THC. Compared with healthy controls, patients with schizophrenia seemed to be more vulnerable to the effects of THC on learning and memory. Since THC leads to an exacerbation of symptoms in patients with schizophrenia despite ongoing treatment with dopamine D_2 receptor antagonists, it can be speculated as to whether THC-induced effects are mediated through mechanisms independent from the dopaminergic system. In another study, D'Souza *et al.* therefore investigated whether pretreatment with the dopamine D_2 receptor antagonist haloperidol alters the effects of THC in healthy subjects and frequent cannabis users, respectively [45]. While pretreatment with haloperidol had no further influence on the behavioral effects of THC, antidopaminergic medication worsened the cognitive effects of THC. Concerning the psychotomimetic effects of THC, the authors therefore suggested a crosstalk between the dopaminergic and the cannabinoid receptor system independent from dopamine D_2 receptor mechanisms [45]. However, several other mechanisms, including effects on long-term potentiation, long-term depression and inhibition of the release of different neurotransmitters such as GABA, glutamate and acetylcholine have been implicated in the amnestic effects of cannabinoids [46].

Binocular depth inversion as a model of illusionary visual perception

Based on experiments using the binocular depth inversion test (BDIT) to investigate cognitive impairment in patients with schizophrenia, Emrich *et al.* suggested that a dysfunctional endocannabinoid receptor system might underlie at least a subtype of endogenous psychoses [47]. Previous studies using the BDIT as a model of illusionary visual perception demonstrated highly impaired scores not only in patients with schizophrenia [48] but also in healthy volunteers after sleep deprivation [49] and during alcohol withdrawal [50]. People in these different states were more veridical in their judgments viewing inverted (concave) faces in the BDIT. Altered BDIT has even been demonstrated in patients in an early, prodromal state of schizophrenia, suggesting that impaired visual information processing precedes the first manifestation of acute psychosis [51]. In patients with schizophrenia impaired binocular depth inversion improved in parallel with clinically effective antipsychotic treatment [52].

In healthy volunteers, it can be assumed that cognitive factors override the binocular disparity cues of stereopsis and, thereby, correct an implausible perceptual hypothesis. Accordingly, it has been suggested that impairment of binocular depth inversion reflects a common final pathway, characterized by an impairment of adaptive systems regulating perception [48].

Investigating binocular depth inversion in THC-intoxicated normal volunteers compared with both healthy controls and patients suffering from productive psychoses, similar alterations were detected in THC-intoxicated normal volunteers and patients with schizophrenia [47]. Regular cannabis users also demonstrated reduced binocular depth inversion compared with normal controls [53]. It has therefore been speculated that comparable disturbances in the internal regulation of perceptual processes can be found in patients with schizophrenia and THC-intoxicated people [47]. By contrast, application of the nonpsychotrophic CBD did not affect BDIT in healthy male volunteers [54]. However, the combined application of both CBD and nabilone, a psychoactive synthetic 9-trans-ketocannabinoid, reduced nabilone-induced alterations in BDIT [54]. This observation further supports the hypothesis that CBD might act as an atypical antipsychotic agent that is able to abate some of the THC-induced psychotropic effects (see later) [55].

Based on a concept developed by Gray and Rawlins in 1986 [56], strategies of processing, in general, are thought to be predicated on a comparative system that adjusts incoming sensory data (bottom-up) with conceptual knowledge (top-down). In patients with schizophrenia there is evidence that these adaptive top-down mechanisms of internal correction are weakened and, thus, the generation of perceptual hypotheses is deficient to dominate bottom-up signals [57,58]. Disturbance of binocular depth inversion, as measured in acute psychosis as well as in THC-intoxicated normal volunteers, has been interpreted to represent such an impairment of the top-down processing [48,59].

Cerebrospinal fluid endocannabinoid levels in schizophrenia

An involvement of the endogenous cannabinoid receptor system in schizophrenia is further supported by findings in cerebrospinal fluid (CSF) in patients with schizophrenia. Leweke *et al.* examined CSF concentrations of different endogenous cannabinoids in 10 patients with schizophrenia compared with 11 controls [60]. While concentrations of the endocannabinoids anandamide and palmitylethanolamide (PEA) were found significantly increased in patients with schizophrenia compared with controls, levels of 2-AG were below detection in both groups [60]. The authors suggested that changes in the endocannabinoid concentrations in schizophrenia might reflect either a homeostatic adaption of the endocannabinoid system to a primary dopaminergic dysfunction or a primary hypercannabinergic state [60].

In a follow-up study, Giuffrida *et al.* found that CSF anandamide levels are eightfold higher in antipsychotic-naive first-episode paranoid patients with schizophrenia (n = 47) than in healthy controls (n = 84) [61]. In addition, an influence of antipsychotic drugs on CSF anandamide levels was obvious: while in patients with schizophrenia treated with typical antipsychotic drugs (antagonizing predominantly dopamine D_2-like receptors) CSF anandamide levels were similar to those of healthy controls; by contrast, in patients treated with atypical antipsychotics (interacting preferentially with serotonin 5-HT_{2a} receptors) levels were similar to those of drug-naive patients with schizophrenia. Furthermore, in nonmedicated acute patients with schizophrenia CSF anandamide levels were negatively correlated with psychotic symptoms. It has been hypothesized that anandamide elevation in acute paranoid schizophrenia may reflect a compensatory adaptation to the disease state that can be normalized by typical antipsychotic drugs [61].

In another study, Leweke *et al.* examined whether cannabis use alters serum and CSF anandamide levels in both first-episode antipsychotic-naive patients with schizophrenia (n = 47) and healthy controls (n = 81) [62]. In patients with schizophrenia with low-frequency cannabis use (n = 25) CSF anandamide levels were greater than tenfold higher than in high-frequency users who suffered from schizophrenia (n = 19), healthy low-frequency (n = 55) and healthy high-frequency users (n = 26). From these findings it has been concluded that frequent cannabis exposure may downregulate anandamide signaling in the CNS of patients with schizophrenia, but not of healthy individuals. It can be speculated that frequent cannabis use increases the risk for psychotic episodes only in those individuals who exhibit pre-existing pathologically hyperactive anadamide levels, as demonstrated in first episode, antipsychotic-naive patients with schizophrenia [62].

Endocannabinoid plasma levels in schizophrenia

There are only a limited number of investigations available measuring endocannabinoid levels in the plasma of patients with schizophrenia. De Marchi *et al.* found significantly increased amounts of both anandamide and the mRNA for the anandamide degrading enzyme fatty acid amide hydrolase (FAAH) in patients with schizophrenia (n = 12) compared with healthy controls (n = 20) [63]. Successful antipsychotic treatment (n = 5) led to a reduction of anandamide blood levels and of the mRNA transcripts for CB_2 receptors and FAAH. No changes, however, were detected for the CB_1 mRNA transcript. The authors hypothesized that an acute psychotic episode is associated not only with impaired endocannabinoid signaling in the CNS, but also with increased peripheral blood levels of anandamide. The simultaneously increased expression of the degrading enzyme FAAH could be explained as a compensatory attempt to normalize the circulating anandamide levels [63].

These results were, at least in part, confirmed by another study investigating endocannabinoid levels in plasma in humans [64]. Plasma anandamide levels were found to be significantly higher in first-episode neuroleptic-naive patients with schizophrenia (n = 17) compared with normal controls (n = 20). In patients with chronic schizophrenia after neuroleptic withdrawal there was a trend towards increased anandamide levels. While plasma 2-AG levels were similar in first-episode neuroleptic-naive patients with schizophrenia and normal controls, 2-AG levels were significantly lower in first-episode

neuroleptic-naive patients with schizophrenia compared with patients with chronic schizophrenia after withdrawal from neuroleptic medication. The authors speculated that an increase in plasma anandamide levels might be related to the disease independent of the state, but 2-AG might be related to disease progression [64]. By contrast, Leweke *et al.* failed to detect alterations in serum anandamide levels in first-episode antipsychotic-naive patients with schizophrenia irrespective of their cannabis use [62].

Postmortem studies of the CB_1 receptor system in schizophrenia

With regard to a cannabinoid hypothesis of schizophrenia, it is of importance that highest densities of CB_1 receptors in the brain are found in those regions that have been implicated in schizophrenia, including the prefrontal cortex, basal ganglia, hippocampus, and the anterior cingulate cortex (ACC) [65]. There are two postmortem studies available measuring CB_1 receptor binding density in the human brain in patients with schizophrenia. Using [3H]CP-55940, Dean *et al.* investigated central cannabinoid receptor binding in the dorsolateral prefrontal cortex (Brodmann's area 9), caudate-putamen and areas of the temporal lobe from patients with schizophrenia (n = 14) compared with control subjects (n = 14) [66]. In schizophrenia patients, specific binding was found to be increased in the dorsolateral prefrontal cortex. Ingestion of cannabis within 5 days to death resulted in an increase in CB_1 receptor density in the caudate-putamen in both patients with schizophrenia and normal controls.

Based on the hypothesis that the ACC plays an important role in normal cognition and the fact that cognition is grossly impaired in schizophrenia, the distribution and density of CB_1 cannabinoid receptors in the left ACC was investigated in patients with schizophrenia (n = 10) and matched control subjects (n = 9) [67]. Using the CB_1 receptor antagonist [3H]SR141716A a significant 64% increase in specific binding was found in patients with schizophrenia compared with normal controls. These changes were not related to recent cannabis use. In contrast to these findings, in a recent study Koethe *et al.* failed to demonstrate alterations of the expression of CB_1 receptors in the ACC at the protein level using immunohistochemistry in patients with schizophrenia (n = 15) compared with healthy controls (n = 15) [68]. The authors attributed the inconsistency of their results compared with prior findings to different methodological approaches and influences from medication on the expression on CB_1 receptors [68].

Since in schizophrenia abnormal glutamate, GABA and muscarinic receptor binding has been demonstrated in the posterior cingulate cortex (PCC) [69,70], and based on the known interaction of all these transmitter systems with the endocannabinoid system, Newell *et al.* investigated the binding of [^{3}H]CP-55940 to CB_1 receptors in the PCC in schizophrenia [71]. They found a significant 25% increase in CB_1 binding in the superficial (layer I and II), but not the deeper layers (layers III-VI), of the PCC of patients with schizophrenia compared with controls. Influence from recent cannabis use on these findings could be excluded.

These preliminary data obtained from postmortem analyses demonstrated for the first time alterations in CB_1 receptor binding in patients with schizophrenia. Observed changes in the endogenous cannabinoid system in the ACC, the PCC and the dorsolateral prefrontal cortex are in line with the theoretical conceptions regarding the pathology of schizophrenia.

Data from neuroimaging studies

In vivo neuroimaging using PET and SPECT to investigate different aspects of the cannabinoid CB_1 receptor system is in a very preliminary stage. Although some ligands that are suitable to measure specific binding to CB_1 receptors *in vivo* in humans are already forthcoming, there is only one single case study available investigating central cannabinoid CB_1 receptors in a patient with schizophrenia [72]. Using ^{124}I-AM281 and PET, the highest receptor binding was observed in the striatum and the pallidum, moderately high binding was seen in the frontal cortex, the temporal cortex and the cerebellum. These findings reflect results from *in vitro* studies regarding receptor distribution. It was speculated that asymmetric receptor binding in the basal ganglia (left < right) might be related to pathologic changes in schizophrenia [72].

From several MRI studies it is known that patients with schizophrenia have progressive global and regional grey and white matter brain reductions with larger decreases related to poorer outcome [73,74]. By contrast, volumetric MRI studies in (healthy) cannabis users resulted in inconsistent findings with reduced gray matter density, particularly in the right parahippocampal gyrus [75], and without detectable abnormalities [76].

In patients with recent-onset schizophrenia (n = 47), no influence of cannabis use could be detected when investigating global brain and caudate nucleus volumes [77]. However, in a recent study, it has been shown that in first-episode schizophrenia patients the decrement in brain volume is significantly more pronounced over a 5-year follow-up when patients continued using cannabis (n = 19) compared with non-users (n = 32). Using voxel-based morphometry to examine the influence of cannabis use in first-episode schizophrenia patients (15 patients with cannabis use, 24 patients without use) on gray matter volumes, a more prominent decrease in gray matter density in the right PCC could be demonstrated in patients with schizophrenia using cannabis compared with both cannabis-naive patients and healthy controls (n = 42) [78]. In another MRI study, reduced gray matter volume of the ACC was found in first-episode schizophrenia patients who used cannabis (n = 20) compared with patients with schizophrenia who did not use cannabis (n = 31) and healthy controls (n = 56) [79]. From these data, therefore, it is suggested that in patients with schizophrenia cannabis use might

amplify a pre-existent vulnerability to brain volume changes [80], in particular in regions rich in CB_1 receptors such as the PCC [78].

Cannabinoid hypothesis of schizophrenia: evidence from genetic studies

Schizophrenia has a multifactorial etiology. Based on the neurodevelopmental theory of schizophrenia it has been suggested, amongst others, that cannabis abuse may compound a pre-existing vulnerability to dopamine dysregulation owing to genetic variations or developmental damage [81]. Cannabis, therefore, may interact in a gene x environment manner.

The psychotropic effect of cannabinoids such as THC is mediated by central cannabinoid CB_1 receptors. Since it is known that CB_1 receptors are encoded by the cannabinoid receptor (*CNR1*) gene (MIM114610) [4], it is reasonable to examine whether variants within the *CNR1* gene are associated with schizophrenia to further investigate the cannabinoid hypothesis for the pathogenesis of schizophrenia. In this regard it is noteworthy that the *CNR1* gene is located at chromosome 6q14–15, which is a region of replicated linkage for schizophrenia [82]. Ujike *et al.* investigated two kinds of polymorphisms of the *CNR1* gene in patients with schizophrenia (n = 121) and age-matched controls (n = 148) in a Japanese population [83]. Interestingly, allelic and genotypic distributions of polymorphism 1359G/A at codon 453 in the coding region and AAT triplet repeats in the 3′ flanking region of the CB_1 gene in this Japanese population differed from those in a German Caucasian population [84]. While there was no association between the polymorphism 1359G/A and schizophrenia, the AAT repeat polymorphism was significantly associated with schizophrenia. Depending on the subtype of schizophrenia, an association was found for the hebephrenic type, but not for the paranoid type [83]. Based on these results it has been speculated that the endogenous cannabinoid CB_1 receptor system is overactivated in patients with hebephrenic type of schizophrenia. This hypothesis, in turn, might explain several clinical similarities between hebephrenic schizophrenia and chronic cannabis users, for example, blunted affect, diminution of ambition and motivation, and cognitive dysfunction [83]. These findings are in line with results from Martínez-Gras *et al.* who investigated alleles for the 3'-UTR CNR1 microsatellite in a Spanish population (n = 113) and 111 healthy controls, and also found an association between schizophrenia and variations within the AAT repeats polymorphism, irrespectively from substance use [85].

In another study investigating the AAT repeat polymorphism of the CB_1 gene in a French Caucasian sample including 102 patients with schizophrenia and 63 ethnic- and gender-matched controls, no significant difference was seen in the allele or genotype distribution between the whole sample of patients with schizophrenia and controls [86]. However, allelic distribution was different in non substance-abusing patients compared with substance-abusing patients (the latter being similar to the controls). These findings, however, are in contrast to data from Tsai *et al.* who failed to demonstrate any association between AAT repeats of the *CNR1* gene and schizophrenia in a Chinese population [87]. It has been speculated that this inconsistency might be due to racial differences (Chinese vs Japanese and Caucasian populations) or a different composition of the patients' groups, for example, regarding the subtype of schizophrenia (more or less patients with hebephrenic type of schizophrenia) [83]. A large study including 750 patients with schizophrenia and 688 normal controls failed to demonstrate evidence for an association between schizophrenia and cannabis use, respectively, and CNR1 genotypes [88].

Three recent studies, in addition, investigated whether there is an interaction between cannabis use, psychosis and variations within the catechol-*O*-methyltransferase (*COMT*) gene [88–90]. COMT is an enzyme that plays an important role in the degradation of dopamine, particularly in the prefrontal cortex. The functional polymorphism $Val^{158}Met$ in the *COMT* gene influences enzyme activity of COMT. Increased COMT activity may result in both reduced dopamine levels in the prefrontal cortex and increased dopamine levels in mesolimbic areas [89]. Caspi *et al.* found that carriers of the COMT valine158 allele were much more likely not only to exhibit psychotic symptoms but also to develop schizophreniform disorder if they used cannabis compared with individuals with two copies of the methionine allele [90]. This effect, however, was observed only for people using cannabis for the first time before the age of 18 years. From these data a gene x environment interaction between COMT genotype and cannabis use on risk of schizophrenia is suggested [90]. These results, at least in part, were corroborated by an experimental double-blind, placebo-controlled crossover study, investigating the effect of THC on psychosis and cognition in patients with a psychotic disorder (n = 30), relatives of patients with a psychotic disorder (n = 12) and healthy controls (n = 32) [89]. Carriers of the COMT valine158 allele were more sensitive not only to THC-induced psychosis, but also to THC-induced impairments of memory and attention. However, the interaction between THC and COMT $Vat^{158}Met$ polymorphism on psychosis was observed only in those patients with pre-existing psychosis liability [89].

In a large population of patients with schizophrenia (n = 493), Zammit *et al.* failed to demonstrate an association between $Val^{158}Met$ genotype and cannabis use [88]. These results, therefore, do not support the hypothesis that the effect of cannabis use on schizophrenia depends on variations within COMT.

Cannabidiol as an atypical antipsychotic agent

CBD is a major nonpsychotropic constituent of cannabis. There is evidence that CBD has anticonvulsive, anti-anxiety, anti-nausea, anti-rheumatoid arthritic and antipsychotic properties. In 1982, Zuardi *et al.* described that treatment with CBD can reduce anxiety provoked by THC in normal volunteers and, therefore, suggested that CBD has the opposite psychotropic

effect compared with THC [91]. In animal models predictive of antipsychotic activity, Zuardi *et al.* demonstrated that not only haloperidol but also CBD reduced the occurrence of stereotyped biting induced by apomorphine [92]. In contrast to haloperidol, CBD, however, did not induce catalepsy and, therefore, it has been suggested that CBD may have a pharmacological profile similar to that of an atypical antipsychotic agent such as clozapine [92]. Subsequently, Zuardi *et al.* tested the effect of CBD in a single case study in a 19-year-old female patient with schizophrenia and observed a significant improvement during CBD treatment (up to 1500 mg/day) [93]. However, in another case study including three patients with schizophrenia who were treatment resistant (even to clozapine) only slight or no improvement was observed after CBD treatment [94].

There is only one double-blind controlled study available investigating the effect of CBD in acute patients with schizophrenia [201]. In this clinical trial, Leweke *et al.* compared the effects of CBD and the antipsychotic drug amisulpride in 42 patients suffering from acute schizophrenia and schizophreniform psychosis, respectively [201]. They found that CBD significantly reduced acute psychotic symptoms after 2 and 4 weeks compared with baseline. In addition, the antipsychotic effect of CBD did not differ from that of amisulpride. However, CBD induced significantly less side effects compared with amisulpride. The endogenous cannabinoid system, therefore, has been suggested as a possible novel therapeutic target for the treatment of acute schizophrenia [201].

One major advantage of CBD compared with antidopaminergic drugs is that, in general, CBD is safe and well tolerated. The only side effect is sedation in very high doses [95,96]. The underlying pathophysiological mechanisms of CBD in schizophrenia remain unclear. It has been suggested that CBD acts as an antagonist at CB_1 receptors, stimulates the vanilloid receptor type 1 (VR1), inhibits the uptake and hydrolysis of anandamide, and increases the plasma level of THC by inhibiting the metabolism of THC [97,98]. This complex mode of action might explain why CBD, but not CB_1 receptor antagonists such as SR141716A (rimonabant) [99,100], may have beneficial effects in patients with schizophrenia [94]. In a recent study, hair samples of cannabis users were analyzed to examine levels of THC and CBD [101]. In addition, psychosis proneness was assessed. Compared to individuals with both THC and CBD in their hair, persons who had only THC in their hair demonstrated higher levels of unusual experiences, delusions and anhedonia. These results, therefore, further corroborate the hypothesis that CBD may have antipsychotic properties. In addition, it is suggested that use of cannabis containing both THC and CBD may be protective against THC-induced pro-psychotic symptoms [101].

Expert commentary

Even though cannabis can be misused as an illegal drug, it is reasonable to classify cannabis-derived substances as a useful medicine. It is remarkable that other substances that can be misused as well, for example, benzodiazepines, are fully accepted in modern medicine. To date, different natural and synthetic substances that interact with the endocannabinoid receptor system (either as agonists or antagonists) are available and can be dosed exactly using different routes of administration. Concerning the medicinal use

Key issues

- *Cannabis sativa* is one the oldest and widely distributed drugs worldwide. There is substantial evidence that heavy cannabis abuse in healthy persons is an independent risk factor for the clinical manifestation of schizophrenia in genetically vulnerable people.
- During the past few years, an endogenous cannabinoid receptor system that includes two known cannabinoid (CB_1 and CB_2) receptors and five endogenous ligands has been discovered.
- Δ^9-tetrahydrocannabinol (THC) is the major psychoactive compound of cannabis acting as a central cannabinoid CB_1 receptor agonist. Established therapeutic effects of THC are the treatment of refractory nausea and vomiting associated with cancer chemotherapy and of appetite loss in cancer cachexia and AIDS wasting.
- The relationship between schizophrenia and the use of cannabinoids is complex and not completely understood.
- Using the binocular depth inversion test, similar disturbances of perceptual processes in patients with schizophrenia and THC-intoxicated healthy volunteers have been observed suggesting a 'cannabinoid hypothesis' of schizophrenia.
- Highest densities of CB_1 receptors in the brain were found in those regions that have been implicated in schizophrenia, including the prefrontal cortex, basal ganglia, hippocampus and the anterior cingulate cortex. In patients with schizophrenia, increased density of CB_1 receptors were found to in the dorsolateral prefrontal cortex, and the anterior and posterior cingulate cortex.
- There are several lines of evidence suggesting a complex interaction between the CB_1 receptor system and the dopaminergic system. In this regard, it has been speculated that in patients with cannabis-induced psychosis a genetic vulnerability may lead to an increased dopaminergic activity.
- The concentration of different endocannabinoids (anandamide and palmitylethanolamide) were found to be increased in patients with schizophrenia suggesting either a homeostatic adaption of the endocannabinoid system to a primary dopaminergic dysfunction or a primary hypercannabinergic state in patients with schizophrenia.
- In a double-blind controlled trial, cannabidiol, a major nonpsychotropic constituent of cannabis, had comparable antipsychotic effects in patients suffering from acute schizophrenia compared with the atypical neuroleptic drug amisulpride.
- The endogenous cannabinoid system has been suggested as a possible novel therapeutic target for the treatment of schizophrenia.

of cannabinoids one should keep in mind that cannabinoids interact with a pre-existing physiologically endocannabinoid receptor system by stimulating or blocking central and/or peripheral cannabinoid receptors. In this regard, one might speculate that already known diseases might be caused by changes in the endocannabinoid receptor system. In schizophrenia there is substantial evidence that hyperactivity of the central cannabinoid system is involved in the pathogenesis. Based on a 'cannabinoid hypothesis' of schizophrenia, innovative therapeutic strategies could be developed.

Five-year view

For medicinal purposes cannabis has been used for approximately 4000 years. Currently, cannabinoids have been suggested to be useful drugs for several symptoms and disorders. Performing controlled clinical trials to further investigate the effect of different cannabinoids is one of the challenges for the future.

Since there is substantial evidence that *Cannabis sativa* is an independent risk factor for the clinical manifestation of schizophrenia in predisposed people, it is desirable to identify those healthy people who are on a special risk when using cannabis. Results from genetic studies probably will clarify whether the *CNR1* gene is a genetic risk factor for hebephrenic schizophrenia.

Since it has been suggested that schizophrenia represents a number of discrete syndromes rather than a single disorder, it can be speculated that a subgroup of schizophrenic syndromes may be related pathophysiologically to a functional disturbance of the endogenous cannabinoid receptor system. However, further research is needed to confirm the hypothesis of a hyperactivity of the central cannabinoid system in schizophrenia.

Neuroimaging using PET and SPECT represents a valuable tool to measure specific binding to CB_1 receptors *in vivo* in humans. During the next 5 years, new ligands will be available for clinical use to investigate CB_1 receptor binding sites in patients suffering from different neurological and psychiatric disorders such as schizophrenia to further evaluate the significance of the CB_1 receptor system in these disorders.

During the next 5 years, it is conceivable that further drugs will be available for clinical use that interact with the endocannabinoid receptor system. Not only drugs that bind selectively to only one of the cannabinoid receptors will be of interest but also drugs that inhibit the uptake or the degradation of different endocannabinoids.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this review manuscript.

References

1 Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR (Text Revision). American Psychiatric Association (2004).

2 Tandon R, Keshavan MS, Nasrallah HA. Schizophrenia, "just the facts": what we know in 2008 Part 1: overview. *Schizophr. Res.* 100(1–3), 4–19 (2008).

3 Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86(8), 1646–1647 (1964).

4 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346(6284), 561–564 (1990).

5 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365(6441), 61–65 (1993).

6 Fride E, Foox A, Rosenberg E *et al.* Milk intake and survival in newborn cannabinoid CB1 receptor knockout mice: evidence for a "CB3" receptor. *Eur. J. Pharmacol.* 461(1), 27–34 (2003).

7 Grotenhermen F. Cannabinoids. *Curr. Drug Targets CNS Neurol. Disord.* 4(5), 507–530 (2005).

8 Devane WA, Hanus L, Breuer A *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258(5090), 1946–1949 (1992).

9 Mechoulam R, Ben-Shabat S, Hanus L *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50(1), 83–90 (1995).

10 Krystal JH, D'Souza DC, Madonick S, Petrakis IL. Toward a rational pharmacotherapy of comorbid substance abuse in schizophrenic patients. *Schizophr. Res.* 35(Suppl.), S35–S49 (1999).

11 Blanchard JJ, Squires D, Henry T *et al.* Examining an affect regulation model of substance abuse in schizophrenia. The role of traits and coping. *J. Nerv. Ment. Dis.* 187(2), 72–79 (1999).

12 Stirling J, Lewis S, Hopkins R, White C. Cannabis use prior to first onset psychosis predicts spared neurocognition at 10-year follow-up. *Schizophr. Res.* 75(1), 135–137 (2005).

13 McCleery A, Addington J, Addington D. Substance misuse and cognitive functioning in early psychosis: a 2 year follow-up. *Schizophr. Res.* 88(1–3), 187–191 (2006).

14 D'Souza DC, Abi-Saab WM, Madonick S et al. Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol. Psychiatry* 57(6), 594–608 (2005).

15 Potvin S, Joyal CC, Pelletier J, Stip E. Contradictory cognitive capacities among substance-abusing patients with schizophrenia: a meta-analysis. *Schizophr. Res.* 100(1–3), 242–251 (2008).

16 Andréasson S, Allebeck P, Engström A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet* 2(8574), 1483–1486 (1987).

17 Zammit S, Allebeck P, Andreasson S, Lundberg I, Lewis G. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ* 325(7374), 1199 (2002).

18 Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ* 325(7374), 1212–1213 (2002).

19 van Os J, Bak M, Hanssen M, Bijl RV, de Graaf R, Verdoux H. Cannabis use and psychosis: a longitudinal population-based study. *Am. J. Epidemiol.* 156(4), 319–327 (2002).

20 Viveros MP, Llorente R, Moreno E, Marco EM. Behavioural and neuroendocrine effects of cannabinoids in critical developmental periods. *Behav. Pharmacol.* 16(5–6), 353–362 (2005).

21 Arendt M, Rosenberg R, Foldager L, Perto G, Munk-Jørgensen P. Cannabis-induced psychosis and subsequent schizophrenia-spectrum disorders: follow-up study of 535 incident cases. *Br. J. Psychiatry* 187(6), 510–515 (2005).

22 Veen ND, Selten JP, van der Tweel I, Feller WG, Hoek HW, Kahn RS. Cannabis use and age at onset of schizophrenia. *Am. J. Psychiatry* 161(3), 501–506 (2004).

23 Henquet C, Krabbendam L, Spauwen J *et al.* Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ* 330(7481), 11 (2005).

24 Fergusson DM, Horwood LJ, Swain-Campbell NR. Cannabis dependence and psychotic symptoms in young people. *Psychol. Med.* 33(1), 15–21 (2003).

25 Fergusson DM, Horwood LJ, Ridder EM. Tests of causal linkages between cannabis use and psychotic symptoms. *Addiction* 100(3), 354–366 (2005).

26 Weiser M, Knobler HY, Noy S, Kaplan Z. Clinical characteristics of adolescents later hospitalized for schizophrenia. *Am. J. Med. Genet.* 114(8), 949–955 (2002).

27 Smit F, Bolier L, Cuijpers P. Cannabis use and the risk of later schizophrenia: a review. *Addiction* 99(4), 425–430 (2004).

28 Meltzer HY, Stahl SM. The dopamine hypothesis of schizophrenia: a review. *Schizophr. Bull.* 2(1), 19–76 (1976).

29 Brunette MF, Drake RE, Xie H, McHugo GJ, Green AI. Clozapine use and relapses of substance use disorder among patients with co-occurring schizophrenia and substance use disorders. *Schizophr. Bull.* 32(4), 637–643 (2006).

30 Drake RE, Xie H, McHugo GJ, Green AI. The effects of clozapine on alcohol and drug use disorders among patients with schizophrenia. *Schizophr. Bull.* 26(2), 441–449 (2000).

31 San L, Arranz B, Martinez-Raga J. Antipsychotic drug treatment of schizophrenic patients with substance abuse disorders. *Eur. Addict. Res.* 13(4), 230–243 (2007).

32 Sundram S, Copolov D, Dean B. Clozapine decreases [3H] CP 55940 binding to the cannabinoid 1 receptor in the rat nucleus accumbens. *Naunyn Schmiedebergs Arch. Pharmacol.* 371(5), 428–433 (2005).

33 Voruganti LN, Slomka P, Zabel P, Mattar A, Awad AG. Cannabis induced dopamine release: an *in-vivo* SPECT study. *Psychiatry Res.* 107(3), 173–177 (2001).

34 Bowers MB, Kantrowitz JT. Elevated plasma dopamine metabolites in cannabis psychosis. *Am. J. Psychiatry* 164(10), 1615–1616 (2007).

35 Markianos M, Vakis A. Effects of acute cannabis use on urinary neurotransmitter metabolites and cyclic nucleotides in man. *Drug. Alcohol Depend.* 14(2), 175–178 (1984).

36 Giuffrida A, Parsons LH, Kerr TM, Rodríguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat. Neurosci.* 2(4), 358–363 (1999).

37 Cadogan AK, Alexander SP, Boyd EA, Kendall DA. Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J. Neurochem.* 69(3), 1131–1137 (1997).

38 Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur. J. Pharmacol.* 341(1), 39–44 (1998).

39 Mailleux P, Vanderhaeghen JJ. Dopaminergic regulation of cannabinoid receptor mRNA levels in the rat caudate-putamen: an *in situ* hybridization study. *J. Neurochem.* 61(5), 1705–1712 (1993).

40 Bonnin A, de Miguel R, Castro JG, Ramos JA, Fernandez-Ruiz JJ. Effects of perinatal exposure to delta 9-tetrahydrocannabinol on the fetal and early postnatal development of tyrosine hydroxylase-containing neurons in rat brain. *J. Mol. Neurosci.* 7(4), 291–308 (1996).

41 Dean B, Bradbury R, Copolov DL. Cannabis-sensitive dopaminergic markers in postmortem central nervous system: changes in schizophrenia. *Biol. Psychiatry* 53(7), 585–592 (2003).

42 Tzavara ET, Li DL, Moutsimilli L *et al.* Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. *Biol. Psychiatry* 59(6), 508–515 (2006).

43 D'Souza DC, Perry E, MacDougall L *et al.* The psychotomimetic effects of intravenous Δ-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29(8), 1558–1572 (2004).

44 D'Souza DC, Ranganathan M, Braley G *et al.* Blunted psychotomimetic and amnestic effects of Δ-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology* (2008) (Epub ahead of print).

45 D'Souza DC, Braley G, Blaise R *et al.* Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Δ-9-tetrahydrocannabinol in humans. *Psychopharmacology (Berl.)* (2008) (Epub ahead of print).

46 Ranganathan M, D'Souza DC. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188(4), 425–444 (2006).

47 Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. *Pharmacol. Biochem. Behav.* 56(4), 803–807 (1997).

48 Schneider U, Leweke FM, Sternemann U, Weber MM, Emrich HM. Visual 3D illusion: a systems-theoretical approach to psychosis. *Eur. Arch. Psychiatry Clin. Neurosci.* 246(5), 256–260 (1996).

49 Sternemann U, Schneider U, Leweke FM, Bevilacqua CM, Dietrich DE, Emrich HM. Pro-psychotic change of binocular depth inversion by sleep deprivation. *Nervenarzt* 68(7), 593–596 (1997).

50 Schneider U, Leweke FM, Niemcyzk W, Sternemann U, Bevilacqua M, Emrich HM. Impaired binocular depth inversion in patients with alcohol withdrawal. *J. Psychiatr. Res.* 30(6), 469–474 (1996).

51 Koethe D, Gerth CW, Neatby MA *et al.* Disturbances of visual information processing in early states of psychosis and experimental Δ-9-tetrahydrocannabinol altered states of consciousness. *Schizophr. Res.* 88(1–3), 142–150 (2006).

52 Schneider U, Borsutzky M, Seifert J *et al.* Reduced binocular depth inversion in schizophrenic patients. *Schizophr. Res.* 53(1–2), 101–108 (2002).

53 Semple DM, Ramsden F, McIntosh AM. Reduced binocular depth inversion in regular cannabis users. *Pharmacol. Biochem. Behav.* 75(4), 789–793 (2003).

54 Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM. Different effects of nabilone and cannabidiol on binocular depth inversion in Man. *Pharmacol. Biochem. Behav.* 66(1), 175–181 (2000).

55 Zuardi AW, Morais SL, Guimarães FS, Mechoulam R. Antipsychotic effect of cannabidiol. *J. Clin. Psychiatry* 56(10), 485–486 (1995).

56 Gray JA, Rawlins JNP. Comparator and buffer memory: an attempt to integrate two models of hippocamaö functions. In: *The Hippocampus.* Isaacson RL, Pribram KH (Eds), Plenum Press, NY, USA, 159–201 (1986).

57 Frith CD, Done DJ. Experiences of alien control in schizophrenia reflect a disorder in the central monitoring of action. *Psychol. Med.* 19(2), 359–363 (1989).

58 Malenka RC, Angel RW, Hampton B, Berger PA. Impaired central error-correcting behavior in schizophrenia. *Arch. Gen. Psychiatry* 39(1), 101–107 (1982).

59 Emrich HM. A three-component-system hypothesis of psychosis. Impairment of binocular depth inversion as an indicator of a functional dysequilibrium. *Br. J. Psychiatry Suppl.* 5, 37–39 (1989).

60 Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* 10(8), 1665–1669 (1999).

61 Giuffrida A, Leweke FM, Gerth CW *et al.* Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* 29(11), 2108–2114 (2004).

62 Leweke FM, Giuffrida A, Koethe D *et al.* Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr. Res.* 94(1–3), 29–36 (2007).

63 De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis.* 2, 5 (2003).

64 Yao JK, van Kammen DP, Reddy RD *et al.* Elevated endocannabinoids in plasma from patients with schizophrenia. *Biol. Psychiatry* 51, 64S-65S (2002).

65 Mailleux P, Vanderhaeghen JJ. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and *in situ* hybridization histochemistry. *Neuroscience* 48(3), 655–668 (1992).

66 Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* 103(1), 9–15 (2001).

67 Zavitsanou K, Garrick T, Huang XF. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28(2), 355–360 (2004).

68 Koethe D, Llenos IC, Dulay JR *et al.* Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J. Neural. Transm.* 114(8), 1055–1063 (2007).

69 Newell KA, Zavitsanou K, Jew SK, Huang XF. Alterations of muscarinic and GABA receptor binding in the posterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31(1), 225–233 (2007).

70 Newell KA, Zavitsanou K, Huang XF. Ionotropic glutamate receptor binding in the posterior cingulate cortex in schizophrenia patients. *Neuroreport* 16(12), 1363–1367 (2005).

71 Newell KA, Deng C, Huang XF. Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Exp. Brain Res.* 172(4), 556–560 (2006).

72 Berding G, Schneider U, Gielow P *et al.* Feasibility of central cannabinoid CB1 receptor imaging with [124I]AM281 PET demonstrated in a schizophrenic patient. *Psychiatry Res.* 147(2–3), 249–256 (2006).

73 Chua SE, Cheung C, Cheung V *et al.* Cerebral grey, white matter and CSF in never-medicated, first-episode schizophrenia. *Schizophr. Res.* 89(1–3), 12–21 (2007).

74 Cahn W, van Haren NE, Hulshoff Pol HE *et al.* Brain volume changes in the first year of illness and 5-year outcome of schizophrenia. *Br. J. Psychiatry* 189(4), 381–382 (2006).

75 Matochik JA, Eldreth DA, Cadet JL, Bolla KI. Altered brain tissue composition in heavy marijuana users. *Drug Alcohol Depend.* 77(1), 23–30 (2005).

76 Block RI, O'Leary DS, Ehrhardt JC *et al.* Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport* 11(3), 491–496 (2000).

77 Cahn W, Hulshoff Pol HE, Caspers E, van Haren NE, Schnack HG, Kahn RS. Cannabis and brain morphology in recent-onset schizophrenia. *Schizophr. Res.* 67(2–3), 305–307 (2004).

78 Bangalore SS, Prasad KM, Montrose DM, Goradia DD, Diwadkar VA, Keshavan MS. Cannabis use and brain structural alterations in first episode schizophrenia – a region of interest, voxel based morphometric study. *Schizophr. Res.* 99(1–3), 1–6 (2008).

79 Szeszko PR, Robinson DG, Sevy S *et al.* Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. *Br. J. Psychiatry* 190(3), 230–236 (2007).

80 Rais M, Cahn W, Van Haren N *et al.* Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. *Am. J. Psychiatry* 165(4), 490–496 (2008).

81 Broome MR, Woolley JB, Tabraham P *et al.* What causes the onset of psychosis? *Schizophr. Res.* 79(1), 23–34 (2005).

82 Lewis CM, Levinson DF, Wise LH *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet.* 73(1), 34–48 (2003).

83 Ujike H, Takaki M, Nakata K *et al.* CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. *Mol. Psychiatry* 7(5), 515–518 (2002).

84 Gadzicki D, Müller-Vahl K, Stuhrmann M. A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene. *Mol. Cell Probes* 13(4), 321–323 (1999).

85 Martínez-Gras I, Hoenicka J, Ponce G *et al.* (AAT)n repeat in the cannabinoid receptor gene, CNR1: association with schizophrenia in a Spanish population. *Eur. Arch. Psychiatry Clin. Neurosci.* 256(7), 437–441 (2006).

86 Leroy S, Griffon N, Bourdel MC, Olié JP, Poirier MF, Krebs MO. Schizophrenia and the cannabinoid receptor type 1 (CB1): association study using a single-base polymorphism in coding exon 1. *Am. J. Med. Genet.* 105(8), 749–752 (2001).

87 Tsai SJ, Wang YC, Hong CJ. Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. *Psychiatr. Genet.* 10(3), 149–151 (2000).

88 Zammit S, Spurlock G, Williams H *et al.* Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *Br. J. Psychiatry* 191(5), 402–407 (2007).

89 Henquet C, Rosa A, Krabbendam L *et al.* An experimental study of catechol-*O*-methyltransferase Val158Met moderation of Δ-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* 31(12), 2748–2757 (2006).

90 Caspi A, Moffitt TE, Cannon M *et al.* Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-*O*-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol. Psychiatry* 57(10), 1117–1127 (2005).

91 Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by Δ 9-THC in normal subjects. *Psychopharmacology (Berl.)* 76(3), 245–250 (1982).

92 Zuardi AW, Rodrigues JA, Cunha JM. Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology (Berl.)* 104(2), 260–264 (1991).

93 Zuardi AW, Morais SL, Guimarães FS, Mechoulam R. Antipsychotic effect of cannabidiol. *J. Clin. Psychiatry* 56(10), 485–486 (1995).

94 Zuardi AW, Hallak JE, Dursun SM *et al.* Cannabidiol monotherapy for treatment-resistant schizophrenia. *J. Psychopharmacol.* 20(5), 683–686 (2006).

95 Zuardi AW, Guimarães FS, Moreira AC. Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. *Braz. J. Med. Biol. Res.* 26(2), 213–217 (1993).

96 Consroe P, Laguna J, Allender J *et al.* Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacol. Biochem. Behav.* 40(3), 701–708 (1991).

97 Mechoulam R, Hanus L. Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. *Chem. Phys. Lipids* 121(1–2), 35–43 (2002).

98 Bisogno T, Hanus L, De Petrocellis L *et al.* Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.* 134(4), 845–852 (2001).

99 Meltzer HY, Arvanitis L, Bauer D, Rein W; Meta-Trial Study Group. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am. J. Psychiatry* 161(6), 975–984 (2004).

100 Madsen MV, Peacock L, Werge T, Andersen MB. Effects of the cannabinoid CB1 receptor agonist CP55,940 and antagonist SR141716A on d-amphetamine-induced behaviours in Cebus monkeys. *J. Psychopharmacol.* 20(5), 622–628 (2006).

101 Morgan CJ, Curran HV. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br. J. Psychiatry* 192, 306–307 (2008).

Website

201 Leweke FM, Koethe D, Gerth CW *et al.* Cannabidiol as an antipsychotic. A double-blind, controlled clinical trial on cannabidiol vs. amusilpride in acute schizophrenia. Presented at: *2005 IACM 3rd Conference on Cannabinoids in Medicine.* Leiden, The Netherlands, 9–10 September 2005. www.cannabis-med.org/english/nav/home-conference.htm.

Affiliations

- Kirsten R Müller-Vahl, MD
Clinic of Psychiatry, Socialpsychiatry & Psychotherapy, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany
Tel.: +49 511 532 3167
Fax: +49 511 532 3187
mueller-vahl.kirsten@mh-hannover.de
- Hinderk M Emrich
Clinic of Psychiatry, Socialpsychiatry & Psychotherapy, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

Current Pharmaceutical Design, 2014, *20*, 000-000

Cannabinoids and Schizophrenia: Therapeutic Prospects

P.J. Robson[1,2]*, G.W. Guy[1] and V. Di Marzo[1,3]

[1]*GW Pharmaceuticals plc, Porton Down Science Park, Salisbury SP4 0JQ, UK;* [2]*Oxford University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK;* [3]*Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli (NA), Italy*

Abstract: Approximately one third of patients diagnosed with schizophrenia do not achieve adequate symptom control with standard antipsychotic drugs (APs). Some of these may prove responsive to clozapine, but non-response to APs remains an important clinical problem and cause of increased health care costs.

In a significant proportion of patients, schizophrenia is associated with natural and iatrogenic metabolic abnormalities (obesity, dyslipidaemia, impaired glucose tolerance or type 2 diabetes mellitus), hyperadrenalism and an exaggerated HPA response to stress, and chronic systemic inflammation.

The endocannabinoid system (ECS) in the brain plays an important role in maintaining normal mental health. ECS modulates emotion, reward processing, sleep regulation, aversive memory extinction and HPA axis regulation. ECS overactivity contributes to visceral fat accumulation, insulin resistance and impaired energy expenditure.

The cannabis plant synthesises a large number of pharmacologically active compounds unique to it known as phytocannabinoids. In contrast to the euphoric and pro-psychotic effects of delta-9-tetrahydrocannabinol (THC), certain non-intoxicating phytocannabinoids have emerged in pre-clinical and clinical models as potential APs. Since the likely mechanism of action does not rely upon dopamine D_2 receptor antagonism, synergistic combinations with existing APs are plausible.

The anti-inflammatory and immunomodulatory effects of the non-intoxicating phytocannabinoid cannabidiol (CBD) are well established and are summarised below. Preliminary data reviewed in this paper suggest that CBD in combination with a CB_1 receptor neutral antagonist could not only augment the effects of standard APs but also target the metabolic, inflammatory and stress-related components of the schizophrenia phenotype.

Keywords: Phytocannabinoids, endocannabinoid system, schizophrenia, anti-psychotic, metabolic effects, chronic inflammation, stress.

"Diabetes is a disease which often shows itself in families in which insanity prevails"

Sir Henry Maudsley (1897)

INTRODUCTION

Schizophrenia typically manifests through a mixture of positive symptoms (hallucinations, delusions, thought disorder), negative symptoms (loss of motivation, social withdrawal, lack of affect, anhedonia), and cognitive deficits. In a significant proportion of patients, it is also associated with natural and iatrogenic metabolic abnormalities (obesity, dyslipidaemia, impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM)) [1,2], hyperadrenalism [3]and an exaggerated HPA response to stress [4], and chronic systemic inflammation [5].

The introduction into clinical practice of chlorpromazine in the mid-1950s revolutionised the treatment of the psychotic manifestations of schizophrenia and triggered the development of dozens of alternative 'antipsychotics' (APs). A decade or so later the synthesis of clozapine and the unanticipated discovery of its antipsychotic effects led in time to a new 'second generation' cohort of drugs that lacked the characteristic unwanted extrapyramidal effects of the 'typical' APs. The pharmacological profiles of the many 'atypical' drugs currently available vary widely, but it remains the case that all of them rely primarily for their efficacy upon their effects at the dopamine D2 receptor [6]. Approximately one third of first episode psychosis patients fail to respond adequately to a standard typical or atypical antipsychotic, and non-responders have been found to incur health costs that are twice those of responders [7]. Recent analyses suggest little if any difference in overall efficacy between the typicals and atypicals [8]. Clozapine stands alone in its ability to produce a therapeutic response in patients resistant to all other antipsychotics [9] although the pharmacological mechanisms by which it achieves this response remains unknown [6]. Unfortunately its clinical utility is limited by its propensity to cause agranulocytosis and the consequent need for haematological monitoring, along with epileptic seizures and other serious unwanted effects [10].

THE ENDOCANNABINOID SYSTEM AND PSYCHOSIS

The endocannabinoid system (ECS), first discovered in the early 1990s, consists of cannabinoid receptors, endogenous ligands ('endocannabinoids'), and proteins for endocannabinoid synthesis and degradation [11, 12]] Two G protein-coupled cannabinoid receptors have so far been identified, labeled CB_1 and CB_2. The cannabis component delta-9-tetrahydrocannabinol (THC) is a partial agonist at both, and its psychoactive effects are manifested through activation of CB_1 receptors (CB_1R), which are very abundant in the central nervous system (CNS) [11]. Furthermore, some endocannabinoids, as well as some plant cannabinoids ('phytocannabinoids'), can also interact with non-CB_1R/CB_2R targets, the most

*Address correspondence to this author at the Cannabinoid Research Institute, GW Pharmaceuticals plc, Porton Down Science Park, Salisbury SP4 0JQ; Tel:/Fax: +44 (0)1865 730969; E-mail: pjr@gwpharm.com

1381-6128/14 $58.00+.00

extensively studied of which are members of the transient receptor potential channel superfamily [13].

CB_1R are located predominantly at the presynaptic terminals of central and peripheral neurons, their main role being to mediate inhibition of neurotransmitter release. However, they are also expressed in several peripheral structures not exclusively within nervous tissue including those controlling metabolism, hormone release (e.g. cortisol and adrenaline levels), and the immune response. CB_2R are expressed mainly by immune cells, through which they modulate the release of both pro- and anti-inflammatory cytokines, and accumulating evidence suggests they also may be found in neurones. This wide distribution of the receptors accounts for the breadth of influence of the ECS on immune response, learning, food intake, energy homeostasis, pain transduction, emotion, perception, behavioural reinforcement, motor co-ordination, regulation of body temperature and wake/sleep cycle, hormonal function, bone formation and resorption, and apoptosis.

The most relevant endocannabinoids to this discussion are *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG). These are synthesized on demand in response to elevations of intracellular calcium and act in the brain as 'retrograde synaptic messengers' via presynaptic CB_1R to modulate neurotransmitter release [14]. They are then rapidly deactivated by active cellular uptake via specific transport mechanisms and the action of intracellular catalytic enzymes, notably fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL). For a fuller introduction and review see Pertwee [12]. Anandamide, but not 2-AG, also activates the transient receptor potential vanilloid type-1 (TRPV1) channels. These are abundant in sensory neurons and spinal cord but are also found in the CNS, and have been shown to be involved in the regulation of glutamate signaling as well as adipogenesis and inflammation [15].

The activity of the ECS within the CNS is essential for normal mental health. CB_1R are densely expressed in the cortex, hippocampus, amygdala, basal ganglia, and cerebellum [16]. Of relevance to schizophrenia, CB_1R modulate release of both dopamine and glutamate (as well as GABA, serotonin, glycine, acetylcholine, and noradrenaline) and in patients their expression is increased in prefrontal cortex [17] and anterior cingulate cortex [18]. CB_1R knockout mice show increased emotional reactivity, hypersensitivity to stress, reduced responsiveness to rewarding stimuli, increased aggression to intruders, enhanced development of learned helplessness, impaired extinction of aversive memories, and social withdrawal [19].

Raised levels of anandamide have been found in the cerebrospinal fluid (CSF) of untreated schizophrenia patients in comparison with controls and patients with dementia or depression [20]. Raised blood levels of anandamide have also been found in untreated schizophrenia patients, and these were reduced after clinical remission following olanzapine treatment [21]. Is this heightened ECS activity pathophysiological or an adaptive mechanism? Although a strong case can be made for the former hypothesis [22], the CB_1R antagonist rimonabant had no effect on positive or negative symptoms of schizophrenia in a placebo-controlled clinical trial [23]. The finding that CSF concentrations of anandamide were negatively correlated with psychotic symptoms [20] suggests that the ECS protects against the underlying pathophysiology, whereas the observation that remission of psychosis following atypical antipsychotic treatment was associated with normalization of increased plasma anandamide levels [21] suggests that the ECS provides an adaptive response to these symptoms. The former explanation is supported by the recent finding that the levels of anandamide in the CSF and serum of patients are increased in the initial prodromal state of schizophrenia in comparison with healthy subjects, and that patients with lower levels showed a higher risk for earlier progression to psychosis [24].

The role of the ECS in schizophrenia may be more subtle than this, however. In rats treated chronically with phencyclidine (PCP), a laboratory model of schizophrenia, THC worsened cognitive deficits whereas the CB_1R antagonist, AM251, improved both cognition and negative symptoms [25, 26]. In this model, levels of 2-AG and anandamide in the prefrontal cortex are evidently regulated in different ways, the former being elevated by PCP and the latter being reduced by THC and enhanced by AM251. This suggests that the two endocannabinoids may exert contrasting roles, with 2-AG possibly contributing to symptoms via CB_1R and anandamide counteracting them via non- CB_1R mechanisms [25, 26].

ANTIPSYCHOTIC EFFECTS OF PHYTOCANNABINOIDS

a) Cannabidiol. The most extensively investigated phytocannabinoid for potential psychiatric (and other therapeutic) applications is cannabidiol (CBD), and this has been reviewed in detail elsewhere [e.g.27, 28]. Pioneering early studies showed that CBD is able to attenuate the euphoric effects of THC [29], and that cannabis-related psychosis was less prevalent when local street cannabis contained significant amounts of CBD [30]. Black market cannabis resin characteristically containing roughly equal proportions of THC and CBD used to dominate the illicit market in the UK and many other countries, but in recent years herbal material (especially 'sinsemilla' derived from unpollinated female plants) rich in THC but containing little or no CBD has come to predominate [31]. Curran and colleagues have carried out a series of innovative studies exploring the influence of CBD on the psychoactive effects of cannabis in regular recreational consumers. CBD intake relative to that of THC was calculated either by hair analysis of THC and CBD content or by obtaining samples of the cannabis customarily consumed by each individual and subjecting this to laboratory analysis. Their studies indicate that recreational users whose habitual cannabis supply incorporates a significant CBD component differ from those smoking cannabis with negligible CBD content in the following ways: lower levels of unusual experiences (an analogue of hallucinations and delusions) and anhedonia [32]; reduced attentional bias to drug and food stimuli and lower self-rated liking of cannabis stimuli, suggesting a role for CBD in the treatment of cannabis dependence [33]; and protection against the memory impairment characteristically associated with THC consumption [34]. Protection against positive symptoms by the presence of CBD in street cannabis has been replicated in a large scale internet-based survey conducted in the Netherlands [35]. In healthy human subjects, CBD has been shown to reverse or attenuate many characteristic effects of THC such as time distortion, tachycardia, euphoria, anxiety and psychotic symptoms [28, 36] and reduced THC-induced impairment of binocular depth perception, an endophenotype of schizophrenia [37]. In a very recently published study (38), 48 healthy subjects were randomly allocated to receive either CBD (600mg) or placebo orally 3.5h ahead of an intravenous dose of THC (1.5mg). CBD significantly reduced post-THC paranoia and the likelihood of positive psychotic symptoms, and significantly improved memory.

Further light has been thrown on the oppositional effects of CBD and THC in the CNS relevant to psychosis by recent studies using functional magnetic resonance imaging (fMRI) to explore the association of behavioural responses with the activity of specific brain centres. Bhattacharyya, et al [39] showed that THC (10mg orally) induced psychotic symptoms in healthy subjects which were associated with attenuated activity in the ventral striatum. In contrast, CBD was devoid of psychotropic effects, and augmented activity in the same region. A range of behavioural tasks induced opposite effects of THC and CBD in hippocampus, amygdala, temporal cortex and occipital cortex. The same authors have demonstrated with fMRI oppositional effects of THC and CBD on prefrontal, striatal and hippocampal function during attentional salience processing [40].

CBD is active in both dopamine and glutamate laboratory models of psychosis [41, 42], and significantly inhibited ketamine-induced depersonalisation in healthy subjects [43]. Human case reports have been mixed. Significant improvement in psychotic symptoms following a large dose of CBD (up to 1.5g/24h) over four weeks of treatment was reported in a patient who had experienced serious adverse events with conventional antipsychotics [44]. In contrast, three patients with treatment resistant schizophrenia failed to respond convincingly to similar doses of CBD [45]. The most compelling human evidence to date for antipsychotic potential comes from a double-blind, four week parallel group comparison of CBD (800mg/24h) with amisulpride (800mg/24h) in 42 patients with acute schizophrenia [46]. Both treatments produced an impressive and equivalent improvement in psychotic symptoms from baseline but there were significant advantages for CBD in terms of adverse event profile. Interestingly, in contrast to the previous data with olanzapine [21], the therapeutic effect of CBD was associated with elevation, rather than reduction, of plasma anandamide levels, a finding that led the authors to suggest that the phytocannabinoid was acting via its previously reported inhibitory effect on anandamide inactivation [47].

The fact that CBD was effective in reversing social withdrawal in rats produced by the NMDA receptor-antagonist MK-801 whilst clozapine was ineffective [48] suggests that it may be active against negative symptoms. This effect of CBD was attenuated by the TRPV1 antagonist capsazepine, consistent with the previous finding of CBD stimulatory activity in this channel [47]. In the hair analysis study in cannabis smokers referred to above [32], subjects who had consumed CBD had significantly lower scores for 'introvertive anhedonia' (an analogue for negative symptoms of schizophrenia) than both the "THC only" group and control subjects who did not smoke cannabis.

The mechanism of action for the antipsychotic activity of CBD is unknown, although various theories have been proposed including augmentation of ECS through inhibition of the catalytic enzyme fatty acid amide hydrolase or the anandamide transporter [46]; via the transient receptor potential vanilloid type 1 (TRPV1) receptor [42]; through augmentation of adenosine signalling, since adenosine A_{2A} agonists have antipsychotic effects [49] and CBD inhibits adenosine uptake [50]; or activation of 5-HT_{1A} receptors [51]. The important point to note is that, whichever theory is correct, CBD does not rely on targeting the dopamine D2 receptor for this effect, so that synergy with standard APs is a plausible possibility.

b) CB1 receptor antagonists. Based on the observed psychotomimetic effects of cannabis which appear to be mediated by the effects of THC at the CB1 receptor (CB_1R), reports of increased density of CB_1R binding in key brain areas and raised CSF levels of endocannabinoids in schizophrenia patients, and evidence of a functional interaction between endocannabinoid and dopaminergic systems, a 'cannabinoid hypothesis' of schizophrenia has been put forward [52, 53]. Since this theory proposes that abnormalities in endocannabinoid signalling may be involved in the pathogenesis of schizophrenia, there has been interest in exploring the potential antipsychotic properties of CB1R antagonists. Rimonabant, a potent inverse agonist at CB1R, has been extensively investigated in a wide range of laboratory models of psychosis and has generally shown encouraging results [54]. However, in the only clinical study reported to date the results were disappointing. In a placebo-controlled, parallel group trial of six weeks treatment in schizophrenia patients, rimonabant 20mg daily did not differ from placebo on any outcome measure [23].

Another CB1R antagonist that has produced an encouraging profile in laboratory models of schizophrenia is AVE1625 [55]. The profile obtained by this programme of work suggests that whereas CB1R antagonism may not contribute significantly to the reversal of positive symptoms of schizophrenia such as hallucinations and delusions, it may well have beneficial effects on the cognitive deficits which are central to the disorder (indicated by beneficial effects on tests of episodic memory) and also ameliorate some of the unwanted effects of standard APs such as extrapyramidal motor disorders and weight gain [55]. The potential metabolic benefits of CB1R antagonism will be discussed in more detail below. Since CB1R agonists such as THC are known to produce not only positive symptoms but also inhibition of enthusiasm and interests and flattened emotion [56] there is the possibility that CB1R antagonism may also alleviate the negative symptoms of schizophrenia.

Unfortunately, prolonged treatment of obese patients with rimonabant was associated with an unacceptable prevalence of mood disorders and suicidal ideation which led to its withdrawal from US and European markets. This effect is pharmacologically not unexpected, since it has been shown that ECS deficits can result in depression and anxiety, and augmentation of CB1 signalling has antidepressant and anxiolytic potential [57]. However, the use of a neutral CB1R antagonist (as opposed to a CB1R inverse agonist such as rimonabant) may avoid this depressogenic effect [58]. Tetrahydrocannabivarin (THCV) is a natural compound present in cannabis that acts as a neutral competitive CB1R antagonist that does not reduce the constitutive effects mediated by CB1R [59]. Preliminary laboratory studies suggest that THCV has synergistic effects with a standard AP in laboratory models of psychosis and also inhibited AP-induced catalepsy and ptosis [data on file at GW Pharma].

METABOLIC ABNORMALITIES IN SCHIZOPHRENIA

Patients with schizophrenia have a pronounced excess mortality in comparison with matched controls, resulting in a 20% reduction in life expectancy [60, 61]. Two thirds of this excess are accounted for by physical illness, the remainder by accidents and suicide. CVS diseases account for a third of the physical causes of death in an equal proportion of men and women.

The association between schizophrenia and impaired glucose tolerance was first described early in the 20th Century, and many further reports followed [1]. In the 1930s, insulin coma treatment was introduced as a symptomatic treatment for psychosis and it was noticed that around 40% of patients were much less sensitive than average to the hypoglycaemic effects of insulin. When chlorpromazine was introduced in 1952, it was apparent that it had a propensity to convert impaired glucose tolerance (IGT) into full Type 2 diabetes mellitus (T2DM).

Modern studies have confirmed that the risk of IGT and metabolic dysfunction is significantly increased in schizophrenia. In a large sample (n = 689) drawn from the CATIE anti-psychotic trials in the US it was reported that 43% (males 37%, females 54%) had a fasting blood glucose greater than 5.5 mmol/litre (100 mg/dl) [2]. Even controlling for BMI, this represents an increase in risk over matched controls of 85% for males and 137% for females. It is a consistent finding in published reports that prevalence of metabolic syndrome (comprising obesity, atherogenic dyslipidaemia, hypertension, and hyperglycaemia) in schizophrenic patients lies in a range of between 37 - 43%, which is 2 - 4 times the levels encountered in matched controls. The prevalence of T2DM is between 14 - 18% in schizophrenia compared to an average of 7% in controls, with IGT occurring in up to 30% [2, 62]. Most cases with IGT go unrecognised. Intriguingly, an inverse correlation between psychosis symptomatology and insulin resistance/pancreatic β-cell function has been reported, and both improved following treatment with antipsychotics [63]. CT scanning revealed that schizophrenia patients have up to three times higher proportion of visceral fat than matched controls, alongside similar levels of total body and subcutaneous fat [64]. Collagen-induced platelet aggregation is increased [65], and was negatively correlated with psychosis ratings.

The excess risk of IGT is the result of both genetic and lifestyle factors. Family history of T2DM occurs in between 18-50% patients compared to around 5% in matched controls [60, 66, 67, 68,

69], and patients are more likely to have obese parents [70]. There is overlap in chromosomal regions for susceptibility genes for schizophrenia and T2DM, and it has been proposed that a 'common soil' effect occurs in the intra-uterine environment [71]. Poor foetal growth is linked with impaired glucose metabolism in later life, and low birth weight is also associated with neurological and psychiatric problems in adulthood, including schizophrenia. Abnormalities in genes related to energy metabolism and oxidative stress differentiated almost 90% of schizophrenia brains from controls [72].

Lifestyle risk factors include poverty, high rates of smoking, poor diet (high fat and sugar, low fibre), and lack of exercise. Risk factors amongst prior generations are now recognized to influence the phenotype also though epigenetic adaptation [73].

Oxidative stress may be important in the pathogenesis of schizophrenia [74]. Impairment of the cytoprotective enzymes superoxide dismutase and glutathione peroxidase as a result of enhanced oxidative stress leads to raised levels of malondialdehyde, the end product of lipid peroxidation. This causes increased levels of reactive oxygen species which may produce membrane defects and increased levels of catecholamines, including dopamine [74].

Metabolic syndrome is provoked by a Western diet and lifestyle, and the long-term outcome of schizophrenia is better in developing countries such as India and Nigeria (75). The disease seems to be associated with abnormalities in phospholipid metabolism, and dietary intake of omega-3 fatty acids has been found to be inversely proportional to symptom severity in schizophrenia [76]. Low cell membrane levels of the omega-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) have been found in both metabolic syndrome and schizophrenia [69]. PUFA supplementation has produced some encouraging early clinical trial results in psychosis [77], and is also known to counteract some of the parameters of the metabolic syndrome in animal models and humans [78].

Peet [66] has proposed also that brain-derived neurotrophic factor (BDNF) might provide a link between diet, T2DM, and schizophrenia. BDNF is a protein with an important role in neuronal survival, neurite outgrowth and synapse formation, and maintenance of neuronal dendrites. BDNF has an important role during brain maturation in young adults [79]. It also plays a critical role in activity-dependent neuroplasticity underlying learning and memory in the hippocampus. BDNF expression is reduced in prefrontal cortex (PFC) in schizophrenia [80], and polymorphism of the BDNF gene is associated with heightened schizophrenia susceptibility [81]. BDNF expression is reduced by high fat, high sugar diet [82]. BDNF knockout mice have reduced neuronal, somal and dendrite density in PFC [83], a profile which has been described in schizophrenia brains.

An important aggravating factor for metabolic and psychological symptoms in schizophrenia is an abnormally enhanced reaction to stress [4, 84]. This derives from both internal sources (e.g. hallucinations and persecutory beliefs) and environmental stressors such as poverty and difficult relationships. Adrenal upregulation results in mobilisation of glucose and increased hepatic glucose production. Adrenaline also inhibits pancreatic islet β-cells from releasing insulin and reduces the sensitivity of insulin receptors, thereby diminishing glucose uptake and utilisation. Sustained hyperadrenalism leads to sustained hyperglycaemia and decreased hepatic glycogen content. In schizophrenia there is raised plasma noradrenaline and a heightened response on arousal measures such as electromyographic activity, skin conductance, and resting heart rate [3].

Of even more interest in the context of stress is the role played by the hypothalamic-pituitary-adrenal axis (HPA axis). HPA axis provides the physiological response to sustained stress, and schizophrenia patients show many signs of HPA axis overactivity including raised basal cortisol levels, lack of normal cortisol inhibition during sleep, blunted response to experimental stressors, and an inverse relationship between cortisol levels and cognitive function [4, 60]. Intolerance to stress may be a schizophrenia risk factor, and this intolerance could be related to an exaggerated HPA response. Cortisol dysregulation characterises at least a sub-set of schizophrenia patients: dexamethasone non-suppression is seen in around 50% of patients regardless of whether they are experiencing primarily positive or negative symptoms, a similar proportion to that seen in patients with severe depression [4, 84]. Corticotrophin releasing hormone (CRH) levels have been found to be increased in the CSF of schizophrenia patients [85], and cortisol salivary levels correlated with symptom severity [86]. Treatment with antipsychotics tends to result in a reduction of plasma cortisol levels. In animal models, chronic stress has been found to inhibit synaptic plasticity and neurogenesis and affect dendritic morphology [87].

After an extensive review of the data, Corcoran and colleagues [4] concluded: "There is strong empirical evidence to support the notion that the biological response to stress, especially activation of the HPA axis, is capable of triggering a downstream cascade of neurochemical events that can precipitate or exacerbate psychosis." Hypothalamic glucocorticoid receptors provide a negative feedback mechanism modulating HPA response. Receptors are also prominent in medial PFC and many midbrain dopamine neurones. Chronic stress and high levels of circulating cortisol produce changes in neural organisation. In particular, negative effects on hippocampus include reduced volume, and lower levels of BDNF. Cognitive function in schizophrenia is inversely related to circulating cortisol levels.

HPA axis overactivity is associated with abdominal obesity and its metabolic consequences [88] and it is likely that this mechanism contributes significantly to the dysregulation of glucose homeostasis in schizophrenia. Levels of the satiety hormone leptin are raised in parallel with HPA upregulation [89], but cortisol diminishes leptin signalling [90]. Cortisol antagonises insulin-mediated inhibition of hepatic glucose release, glucose utilisation in muscle, and binding affinity of insulin receptors [91]. Approximately half of all patients treated long-term with glucocorticoids develop deranged glucose metabolism, which persists after withdrawal in around 50% - this is analogous to what is seen in schizophrenia [84].

The effects of antipsychotic medication on glycaemic control: After the introduction of the phenothiazines into clinical practice, the prevalence of T2DM in schizophrenic patients increased sharply: for example, up from 4.2% in 1956 to 17.2% in 1968 in one study of female inpatients [92]. Atypicals pose an even higher risk [93]. The highest risk of weight gain and metabolic disturbance is posed by clozapine and olanzapine, followed by quetiapine, zotepine, risperidone, and sertindole. Amisulpride, ziprasidone and aripiprazole carry the lowest risk, comparable to that of the typicals haloperidol, fluphenazine and pimozide [94, 95, 96].

In a recent review [97], average weight gains over an initial 10 week period of treatment were reported as follows: clozapine 4.45 kg; olanzapine 4.15 kg; sertindole 2.92 kg; risperidone 2.10 kg; ziprasidone 0.04 kg. Quetiapine was roughly equivalent to risperidone. The propensity of drugs to produce weight gain is generally proportional to the increased risk of T2DM. Although clozapine and olanzapine carry the highest risk, it seems that all atypicals are associated with some degree of metabolic impact: new onset of T2DM was reported in 6.9% patients receiving any atypical over a one-year period [98]. This consistent association has resulted in impaired glucose metabolism being regarded as a class effect for atypical antipsychotics by the FDA.

A group of 46 previously untreated schizophrenics was followed up for ten weeks after the instigation of risperidone, chlorpromazine or quetiapine [99]. Marked increases in subcutaneous and intra-abdominal fat, a tripling of circulating plasma leptin, and increases in plasma lipids and non-fasting glucose in comparison with age and gender matched healthy controls were recorded. The

authors concluded that antipsychotic treatment had interfered with the normal inhibitory control of body mass exerted by leptin. There was no correlation between anti-psychotic response and body weight gain.

The mechanism by which anti-psychotics cause weight gain and metabolic problems is still unknown, although their effects upon orexigenic and anorexigenic peptides, histamine H1, 5-HT_{2A} , 5-HT_{2C}, muscarinic M3 and adrenergic receptors has been attracting particular interest [100, 101]. There is an intriguing link with ECS: in a rat model, both acute and chronic dosing with olanzapine (and to a much lesser extent aripiprazole) was associated with significantly decreased CB1R binding in the dorsal vagal complex (DVC) [102]. Moreover, weight gain was negatively correlated to DVC CB1R binding and the authors interpreted this as evidence that brainstem cannabinoid receptors may be involved in AP related weight gain.

CHRONIC INFLAMMATION IN SCHIZOPHRENIA

Schizophrenia is typically associated with a range of physical disturbances, including abnormalities of motor function; physical anomalies including abnormal dermatoglyphics, high-steepled palate, malformed ears, epicanthus, single palmar crease, finger and toe abnormalities; increased susceptibility to infection, especially pulmonary tuberculosis; increased rates of cardiovascular and metabolic diseases, and rare genetic or idiopathic disorders such as acute intermittent porphyria and coeliac disease.

The search for a systemic explanation for the behavioural and physical characteristics has led to the hypothesis that schizophrenia might be a genetically mediated, CNS microvascular inflammatory disease [5, 103]. According to this theory, infection or other environmental stressors cause an increase in maternal pro-inflammatory cytokines which cross the placenta and foetal blood brain barrier. Foetal pro-inflammatory cytokines could also be generated by intrauterine trauma or anoxia. In a genetically primed foetus these cytokines may have several consequences: inhibition of neurotrophins such as NGF and BDNF, leading to impaired neuronal generation and connectivity and increased apoptosis; increased expression of vascular endothelial growth factor leading to enhanced angiogenesis and potential vascularisation defects; and enhanced nitric oxide production and increased oxidative stress. Mitochondrial dysfunction, either primary or secondary, has been incriminated in the schizophrenia disease process [72]. All of this would create the potential for developmental and degenerative brain changes.

In adolescence or adult life, disruption to the energy and oxygen supplies required for normal brain function could be produced periodically by inflammation in reaction to environmental stressors such as infection, hypoxia or trauma interacting with the vulnerable genotype. An important function of astrocytes is to sense neuronal activity and adjust blood flow appropriately by means of glutamate, serotonin, acetylcholine and dopamine signalling. Angiogenesis occurs in response to prolonged activation, and vice versa. Inflamed vessels would lose their coupling with astrocytes, producing disrupted regulation of cerebral blood flow (CBF), damage to the blood brain barrier (BBB), and abnormal signal processing. Raised serum levels of the brain-specific protein S100b are reported in both first episode psychosis and chronic schizophrenia suggesting the presence of abnormal BBB permeability [104].

In support of the theory [5], pioneering work from an earlier era demonstrated that genetic factors could affect susceptibility to tuberculosis [105]. Subsequently it has become clear that susceptibility and resistance to several infections are subject to genetic modulation through variations in cytokine and human leukocyte antigen (HLA) response. Other microvascular CNS diseases are associated with psychosis phenotypes, including SLE, syphilis, rheumatic fever, and Alzheimer's disease [5]. Psychotic symptoms associated with amphetamine and cocaine may result in part from induction of inflammatory genes in small vessel endothelium. CBF has been reported as abnormal in schizophrenia, with reductions in frontal regions often associated with predominant negative symptoms. Regions particularly affected include frontal cortex, cingulate cortex, thalamus, basal ganglia, parietal cortex and cerebellum. Subtle structural disruptions would be hard to identify, since the microvasculature comprises only 0.1% of the brain by volume [5].

There are numerous indications of abnormal inflammatory activity in patients with schizophrenia. In comparison with normal controls and non-schizophrenic siblings, monocytosis was reported as a correlate of the expression of the clinical phenotype [106]. Raised serum concentrations of inflammatory cytokines have been recorded in several studies, including IL-2, IL-6, IL-8, IL-10 and IFN-γ [103]. Some of these normalise in response to antipsychotic treatment, for example IFN-γ was lowered following risperidone [107] and IL-2 following haloperidol [108]. IL-6 levels were found to rise during symptomatic relapse in schizophrenia, and fall again during remission [109]. In rodents this cytokine has been shown to increase serotonin and dopamine activity in both hippocampus and prefrontal cortex [110]. It has been proposed that an imbalance in the adaptive immune system with a shift in the balance between the cellular and humoral components may correlate with the main symptoms of the disorder, and APs can correct this imbalance [111]. A meta-analysis of five randomised, placebo-controlled trials indicates that co-administration of either celecoxib or aspirin significantly augmented the efficacy of APs against both positive and negative symptoms [112]. Thus it seems that inflammation is not merely an epiphenomenon but a plausible target within schizophrenia treatment. This may extend also to the cognitive impairment component, since chronic low-grade inflammation was found to be associated with impaired executive function in a non-psychiatric sample [113].

Since it is evident that obesity and metabolic syndrome are also closely associated with subclinical chronic inflammation [114], new medicines targeting this condition could have a beneficial impact upon both the psychopathological and metabolic manifestations of schizophrenia [115].

ROLE OF THE ECS IN METABOLIC SYNDROME AND INFLAMMATION

It is now well established that ECS malfunction contributes to the accumulation of visceral adipose tissue, thereby leading to abdominal obesity and the subsequent pro-atherogenic inflammatory profile [116]. On the other hand, ECS in the hypothalamus is also activated during stress and by cortisol and counteracts HPA axis overactivity and its consequences on mood [117, 118], as well as facilitating stress-coping mechanisms [119]. In obese individuals, fasting plasma 2-AG levels correlate with the quantity of intra-abdominal adipose tissue as measured by computer tomography-scan, with triglyceride and low HDL-cholesterol levels in plasma, and with several measures of insulin resistance [120, 121]. Additionally, higher concentrations of 2-AG are found in the visceral, but not subcutaneous, adipose tissue of obese patients compared to age- and gender-matched non-obese controls [122]. A lifestyle intervention that lowered waist circumference by 8 cm in abdominally obese volunteers also reduced fasting plasma 2-AG concentrations, and the decreases in the levels of this endocannabinoid directly correlated with decreases in triglycerides and increases of HDL-cholesterol in plasma [123]. Furthermore, elevated postprandial plasma endocannabinoid levels are directly associated with BMI, IGT and liver fat [124].

In obese rodents, higher concentrations of either 2-AG or anandamide or both are found in the visceral (i.e. epididymal) adipose tissue as well as in the skeletal muscle, pancreas and liver, whereas endocannabinoid levels are reduced in the subcutaneous adipose tissue [125, 126]. Stimulation or antagonism of CB_1R respectively increases or decreases lipogenesis in adipose tissue and liver, and insulin resistance in skeletal muscle [122, 127]. This strongly sug-

gests that ECS overactivity contributes to visceral fat accumulation, liver fat, insulin resistance and impaired energy expenditure. Consistent with this conclusion, prolonged blockade of CB_1R reduces body weight, visceral fat and related metabolic dysfunction in several rodent models of congenital (i.e. through leptin deficiency) or high fat diet-induced obesity as well as in human obesity [128]. In obese patients at high risk of developing coronary heart disorders, prolonged CB_1R antagonism reduced atherogenic inflammation and/or atherosclerotic plaque formation [129]. This pharmacological profile accords with the finding of elevated, pro-inflammatory endocannabinoid tone in atherosclerotic tissues from obese humans [130].

As reviewed briefly above, HPA overactivity contributes significantly to metabolic problems in schizophrenia. ECS has a pivotal role in restoring hormonal homeostasis following exposure to stress [131]. Circulating glucocorticoids operate a negative feedback mechanism to the HPA-axis mediated by CB1R in several brain structures including hypothalamus and hippocampus. Enhancement of CB1R signalling inhibits stress-induced HPA-axis activity and CB1R antagonism has been shown to enhance the stress response [132]. CB1R knockout mice show increased HPA-axis activity and enhanced anxiety responses on exposure to stressors [133].

METABOLIC AND ANTI-INFLAMMATORY EFFECTS OF PHYTOCANNABINOIDS

It is interesting to note that the prevalence of type 2 diabetes mellitus (T2DM) is significantly lower in recreational cannabis smokers, and that this may be related to lower levels of inflammatory markers in comparison to matched, non-cannabis smoking controls [134].

The overall activity of the endocannabinoid system relevant to the schizophrenia phenotype is summarised in Figure **1**. Ideally, a pharmacological intervention should aim to re-equilibrate the ECS such that its contribution to abdominal obesity and atherogenic inflammation is toned down without impairing its homeostatic counteraction of the stress response and psychotic symptoms. This is similar to what some nutritional or lifestyle interventions which could be beneficial to patients with schizophrenia, such as dietary omega-3 PUFA supplementation or physical exercise, seem to do. The former was shown to reduce the peripheral overactivity of ECS and, concomitantly, metabolic disturbances in models of obesity, without significantly affecting endocannabinoid tone in the brain [135]. Physical exercise, on the other hand, was found in normal weight subjects to increase plasma anandamide, thus possibly contributing to raised BDNF levels [136].

Recognition of the contribution of ECS overactivity to metabolic syndrome led to the marketing, in over 66 countries though not the USA, of the CB_1R antagonist/inverse agonist, rimonabant, for weight reduction from June 2007 until October 2008. The trials consistently demonstrated significant weight loss, decrease in HbA_{1C}, improved lipidaemic profile, reduced prevalence of metabolic syndrome, and increase in adiponectin levels [137]. Unfortunately, prolonged treatment of obese patients with rimonabant was also associated with increased risk of anxiety, depression and suicidal ideation [138, 139], which led to the withdrawal of regulatory approval of rimonabant and interruption of the clinical development of several other CB_1R antagonists. One possible mechanism for these effects may relate to the modulatory role of the hypothalamic ECS during stress in an adaptive down-regulation of HPA overactivity [140]. In these circumstances, CB_1R blockade would exacerbate the increased cortisol levels observed in obese or chronically stressed individuals [141]. It is plausible that THCV, as a competitive neutral CB1R antagonist rather than an antagonist/inverse agonist, would produce the desired metabolic effects without disrupting the constitutive effects of ECS [59]. THCV at low doses induced hypophagia and weight loss in free-feeding mice without post-treatment rebound [142]. In other preliminary pharmacology studies, THCV has been shown in rodents to reduce body fat and increase energy expenditure, thermic response to food and insulin sensitivity [143; data on file at GW Pharma]. However, these observations should be treated with caution until independently replicated.

In contrast to amisulpride, CBD did not cause weight gain following 4 weeks treatment in schizophrenia patients [46]. In genetically obese mice, CBD significantly increased the HDL-cholesterol concentration [143]. When THCV and CBD were co-administered all the effects of the individual treatments were retained, suggesting

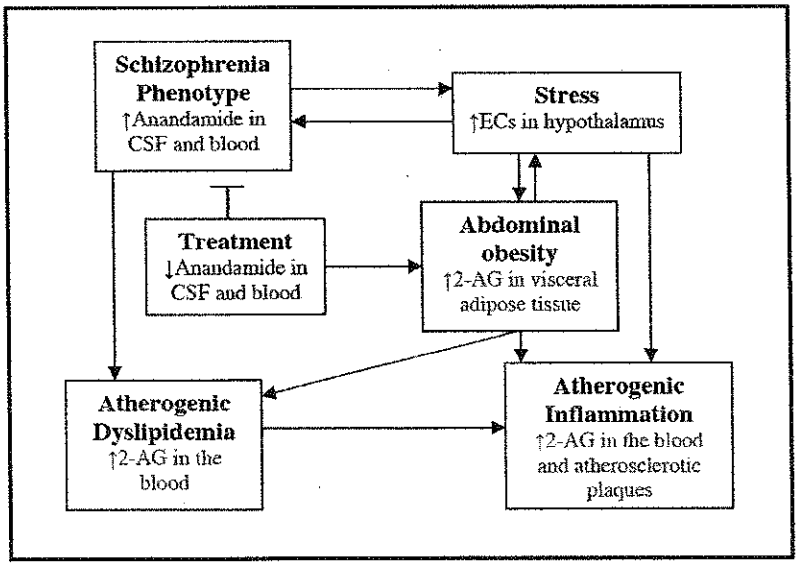


Fig. (1). The Endocannabinoid System in Schizophrenia
Adaptive Changes - blue font
Maladaptive Changes - red font

that the combination may prove effective in counteracting several components of the metabolic syndrome. It is also plausible that this mixture may have the potential to inhibit or eliminate the metabolic risks of APs but again the data upon which this speculation is based remain to be replicated.

The anti-inflammatory and immunomodulatory effects of CBD are well established. These include inhibition of T cell proliferation and macrophage migration; suppression of natural killer cells; suppression of Th1 pro-inflammatory cytokines including IFN-γ, TNF-α, IL-1, IL-2, IL-6, IL-12; stimulation of Th2 cytokines such as IL-4 and IL-10; inhibition of nitric oxide production; neutralisation of free radicals by potent anti-oxidant activity; and demonstrated efficacy in various laboratory models of inflammation [144, 45]. The mechanism of action remains speculative. The finding that CBD enhances adenosine signalling by inhibiting its uptake through an effect upon the equilibrative nucleoside transporter provides one possible explanation since adenosine agonists are known to have anti-inflammatory effects [50]. The potency of CBD in enhancing adenosine signalling also provides a possible mechanism for its antipsychotic effects described above, since adenosine dysfunction has been incriminated in both the neurobiological underpinning and symptoms of schizophrenia [146]. Since there is evidence that CB_2R antagonists may also have significant anti-inflammatory effects [147], it is possible that co-administration of THCV may have a synergistic effect. Since chronic, low-grade inflammation has been reported to be associated with impaired executive function [113], the anti-inflammatory effects of CBD may also benefit cognition. Recently, a small dose of CBD was reported to improve a sensitive marker of cognitive function (auditory-evoked mismatch negativity (MMN)) in healthy subjects [148]. Impaired MMN is a characteristic finding in schizophrenia [149].

CB_1 signalling modulates emotion, reward processing, sleep regulation, aversive memory extinction and regulation of HPA axis [116]. ECS stimulated BDNF release during neuronal insults [150]. Chronic, unpredictable stress in rodents caused dramatic down-regulation of ECS in hippocampus [151], and biosynthesis of endocannabinoids in this brain area may be inhibited by prolonged increases in circulating glucocorticoids, whereas the opposite occurs in the hypothalamus [152]. These data suggest that ECS acts as a buffer during stress, and that in conditions of chronic stress CB_1 agonism may help to normalise response by re-regulating HPA axis [117, 153]. Evidence has emerged that CBD is capable of enhancing anandamide signalling in humans [46] and may thus be beneficial in recalibrating the response to stress. The anxiolytic effect of CBD is already well established [28].

It remains to be determined which, if any, of these various potential beneficial effects of phytocannabinoids in the context of schizophrenia will prove of practical therapeutic value, and at this point they remain conjectural. However, it is intriguing to note from a very recent paper [154] that 5 and 10 year mortality risk in patients with psychotic disorders was significantly lower in those who regularly smoked cannabis compared with those who did not use the drug. The authors speculated that this might be due to either improvement in cognitive function (as has been reported as an association with cannabis smoking in schizophrenia patients in several previous studies [155] or the anti-inflammatory effects of phytocannabinoids, but improvement in metabolic parameters or reduced stress are equally plausible hypotheses.

CONCLUSION

Alongside the expected emotional, perceptual and behavioural problems, schizophrenia patients may present with metabolic abnormalities including obesity, dyslipidaemia, impaired glucose tolerance or type 2 diabetes mellitus, symptoms and signs of an abnormal stress reaction, and haematological evidence of chronic systemic inflammation. Symptomatic diversity may thus be an even bigger challenge than generally appreciated.

The ECS plays an essential role in maintaining normal mental health, is influential in many aspects of metabolic control and immune function, and modulates the response to stress and regulation of the HPA axis. Evidence summarised above suggests that a cannabinoid medicine containing a mixture of CBD and THCV may have the potential to augment existing antipsychotic drugs and reduce some of their unwanted effects, whilst at the same time targeting the metabolic problems, abnormal response to stress and chronic inflammation which form part of the schizophrenia phenotype and might be partly due to, or counteracted by, changes in ECS. Much of the data upon which this hypothesis is based requires replication and extension, but clinical trials are now justified in order to test its validity.

CONFLICT OF INTEREST

The authors are all affiliated with GW Pharmaceuticals (GWP), a public company that is researching and developing a portfolio of cannabinoid medicines. PJR and GWG also hold stock in GWP.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Kohen D. Diabetes mellitus and schizophrenia: historical perspective. B J Psych 2004; 184: s64-6

[2] McEvoy JP, Meyer JM, Goff DC, et al. Prevalence of metabolic syndrome in patients with schizophrenia: baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and comparison with national estimates from NHANES III. Schiz Res 2005; 80: 19-32

[3] Albus M, Ackenheil M, Engel RR, Muller F. Situational reactivity of autonomic functions in schizophrenic patients. Psych Res 1982; 6: 361-70.

[4] Corcoran C, Walker E, Huot R, et al. The stress cascade and schizophrenia: etiology and onset. Schizophrenia Bulletin 2003; 29: 671-92

[5] Hanson DR, Gottesman II. Theories of schizophrenia: a genetic-inflammatory-vascular synthesis. *BMC Medical Genetics* 2005, http: //www.biomedcentral.com/1471-2350/6/7

[6] Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol Psychiatry 2005; 10: 79-104.

[7] Ascher-Svanum H, Nyhuis AW, Faries DE, et al. Clinical, functional and economic ramifications of early non-response to antipsychotics in the naturalistic treatment of schizophrenia. Schiz. Bull 2008; 34: 1163-71

[8] Kendall T. The rise and fall of the atypical antipsychotics. B J Psych 2011; 199: 266-8

[9] Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. Arch Gen Psychiatry 1988; 45(9): 789-96.

[10] Lindstrom LH. The effect of long-term treatment with clozapine in schizophrenia: A retrospective study in 96 patients treated with clozapine for up to 13 years. Acta psychiatr. scand. 1988; 77: 524-29

[11] Pertwee RG. Cannabinoid receptors and their ligands. In: *Handbook of Experimental Pharmacology*; Cannabinoids. (Ed Pertwee R) 2005, 168 pp 1-51 Springer-Verlag Berlin Heidelberg

[12] Pertwee RG. Cannabinoid pharmacology: the first 66 years. B J Pharm 2006; 147: S163-71

[13] De Petrocellis and Di Marzo. Non-CB(1), Non-CB(2) Receptors for Endocannabinoids, Plant Cannabinoids, and Synthetic Cannabimimetics: Focus on G-protein-coupled Receptors and Transient Receptor Potential Channels. J Neuroimmune Pharmacol 2010; 5: 103-21

[14] Kreitzer AC. Neurotransmission: emerging roles of endocannabinoids. Curr Biol 2005; 15: 549-51

[15] Starowicz K, Nigam S, Di Marzo V. Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 2007; 114(1): 13-33.

[16] Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. In: *Handbook of Experimental Pharmacology; Cannabinoids.* (Ed Pertwee R) 2005; 168: 299-325.

[17] Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience 2001; 103: 9-15

[18] Zavitsanou K, Garrick T, Huang XF. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2004, 28: 355-60

[19] Valverde O, Karsak M, Zimmer A (2005). Analysis of the endocannabinoid system by using CB1 cannabinoid receptor knockout mice. In: *Handbook of Experimental Pharmacology; Cannabinoids.* (Ed Pertwee R) 2005; 168: 117-45.

[20] Giuffrida A, Leweke FM, Gerth CW, et al. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 2004; 29: 2108-14

[21] De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis 2003; 2: 5

[22] Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006; 58: 389-462

[23] Meltzer HY, Arvanitis L, Bauer D, Rein W. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. Am J Psych 2004; 161: 975-84

[24] Koethe D, Giuffrida A, Schreiber D, et al. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. B. J. Psych 2009; 194: 371-2

[25] Viganò D, Guidali C, Petrosino S, et al. Involvement of the endocannabinoid system in phencyclidine-induced cognitive deficits modelling schizophrenia. Int J Neuropsychopharmacol 2009; 12(5): 599-614

[26] Guidali C, Viganò D, Petrosino S, et al. Cannabinoid CB1 receptor antagonism prevents neurochemical and behavioural deficits induced by chronic phencyclidine.: Int J Neuropsychopharmacol 2011; 14: 17-28.

[27] Pertwee RG. Chapter 3: The pharmacology and therapeutic potential of cannabidiol. In: Di Marzo V. Ed Cannabinoids. Dordrecht (Netherlands): Kluwer Acad Publishers 2004; pp: 32-83

[28] Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with a wide spectrum of action. Rev Bras Psiquiat 2008; 30: 271-80

[29] Karniol IG, Carlini EA. Pharmacological interaction between cannabidiol and delta-9-tetrahydrocannabinol. Psychopharmacologia 1973; 33(1): 53-7021.

[31] Rottanberg D, Robins AH, Ben-Aire O, Teggin A, Elk R. Cannabis-associated psychosis with hypomanic feature. Lancet 1982; 11: 1364-6

[32] Potter DJ, Clark P, Brown MB. Potency of D9-THC and Other Cannabinoids in cannabis in England in 2005: Implications for psychoactivity and pharmacology. J Forensic Sci, 2008; 53; doi: 10.1111/j.1556-4029.2007.00603

[33] Morgan CJA, Curran HV. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. B J Psych 2008; 192: 306-7

[34] Morgan CJA, Freeman TP, Schafer GL, Curran HV. Cannabidiol attenuates the appetitive effects of D9-Tetrahydrocannabinol in humans smoking their chosen cannabis. Neuropsychopharmacology 2010; 35: 1879-85

[35] Morgan CJA, Schafer G, FreemanTP, Curran HV. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. Brit J Psych 2010; 197: 285-90

[36] Schubart CD, Sommer IEC, van Gastel WA, et al. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schiz Res 2011; 130: 216-21

[37] Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology (Berl) 1982; 76(3): 245-50.

[38] Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM. Different effects of nabilone and cannabidiol on binocular depth inversion in man. Pharmacol Biochem Behav 2000; 66: 175-81

[39] 38, Englund A, Morrison PD, Nottage J, et al. Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment 2012; J Psychopharmacol published online 5 October 2012 DOI: 10.1177/0269881112460109

[40] Bhattacharyya S, Morrison PD, Fusar-Poli P, et al. Opposite effects of D-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology 2010; 35(3): 764-74.

[41] Bhattacharyya S, Crippa JA, Allen P, et al. Induction of psychosis by delta-9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry 2012; 69(1): 27-36

[42] Zuardi AW, Rogdrigues JA, Cunha JM. Effects of cannabidiol in animal models predictive of antipsychotic activity. Psychopharmacology 1991; 104: 260-4

[43] Long LE, Malone DT, Taylor DA. Cannabidiol reverses MK-801-induced disruption of prepulse inhibition in mice. Neuropsychopharmacology 2006; 31: 795-803

[44] Bosi DC, Hallak JEC, Dursun SM. Deakin JFW, Zuardi AW. Effects of cannabidiol on (s)-ketamine-induced psychopathology in healthy volunteers. J Psychopharmacol 2003; 17 (Suppl): A55

[45] Zuardi AW, Morais SL, Guimaraes FS, Mechoulam R. Antipsychotic effect of cannabidiol. J Clin Psychiatry 1995; 56: 485-6

[46] Zuardi AW, Hallack JEC, Dursun SM, et al. Cannabidiol monotherapy for treatment-resistant schizophrenia. J Psychopharmacol 2006; 20: 683-6

[47] FM Leweke FM, Piomelli D, Pahlisch F, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Translational Psychiatry 2012; 2, e94; doi: 10.1038/tp.2012.15; published online 20 March 2012

[48] Bisogno T, Hanus L, De Petrocellis L, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 2001; 134(4): 845-52.

[49] Long LE, Malone DT, Taylor DA. Cannabidiol reverses MK-801-induced social withdrawal in rats. In: 16th Annual Symposium of the Cannabinoids, International Cannabinoid Research Society 2006, Abstract 31

[50] Ferré S. Adenosine-dopamine interactions in the ventral striatum: implications for the treatment of schizophrenia. Psychopharmacology 1997; 133: 107-20.

[51] Carrier EJ, Auchampach JA, Hillard J. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. PNAS 2006; 103: 7895-900.

[52] Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 2009; 156(1): 181-8

[53] Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. Pharmacol Biochem Behav 1997; 56(4): 803-7

[54] Müller-Vahl KR, Emrich HM. Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. Exert Rev Neurother 2008; 1037-48

[55] Roser P, Vollenweider FX, Kawohl W. Potential antipsychotic properties of central cannabinoid (CB1) receptor antagonists. World J Biol Psychiatry 2010; 11(2 Pt 2): 208-19

[56] Black MD, Stevens RJ, Rogacki N, et al. AVE1625, a cannabinoid CB1 receptor antagonist, as a co-treatment with antipsychotics for schizophrenia: improvement in cognitive function and reduction of antipsychotic-side effects in rodents. Psychopharmacology 2011; 215: 149-63

[57] Morrison PD, Stone JM. Synthetic delta-9-tetrahydrocannabinol elicits schizophrenia-like negative symptoms which are distinct from sedation. Human Psychopharmacol Clin Exp 2011; 26: 77-80

[58] Hill MN, Gorzalka BB. The Endocannabinoid System and the treatment of mood and anxiety disorders. CNS & Neurological Disorders - Drug Targets 2009; 8: 451-8

[59] Le Foll B, Gorelick DA, Goldberg SR. The future of endocannabinoid-oriented clinical research after CB1 antagonists. Psychopharmacology 2009; 205: 171-4

[60] Thomas A, Stevenson LA, Wease KN, et al. Evidence that the plant cannabinoid delta-9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. Br J Pharmacol 2005; 146: 917-26
[61] Ryan MCM, Thakore JH. Physical consequences of schizophrenia and its treatment: metabolic syndrome. Life Sciences 2002; 71: 239-57
[62] Thakore JH. Metabolic disturbance in first-episode schizophrenia. B J Psych 2004; 184: s76-9
[63] Expert Group. 'Schizophrenia and diabetes 2003' Expert Consensus Meeting, Dublin, 3-4 October 2003: consensus summary. B J Psych 2004; 184 (suppl. 47): s112-4
[64] Shiloah E,Witz S, AbramovitchY, et al. Effect of acute psychotic stress in nondiabetic subjects on beta-cell function and insulin sensitivity. Diabetes Care 2003; 26: 1462-7.
[65] Thakore JH, Mann JN, Vlahos I, Martin A, Reznek R. Increased visceral fat distribution in drug-naive and drug-free patients with schizophrenia. Int J Obes Relat Metab Disord 2002; 26: 137-41.
[66] Yao JK, van Kammen DP, Gurklis J, Peters JL. Platelet aggregation and dense granule secretion in schizophrenia. Psychiatry Res 1994; 54(1): 13-24.
[67] Peet M. Diet, diabetes and schizophrenia: review and hypothesis. B J Psych 2004; 184: s102-5
[68] Bushe C, Holt R. Prevalence of diabetes and impaired glucose tolerance in patients with schizophrenia. B J Psych 2004; 184; s67-71
[69] Thakore JH. Metabolic syndrome and schizophrenia. B J Psych 2005; 186: 455-6
[70] Peet M. The metabolic syndrome, omega-3-fatty acids, and inflammatory processes in relation to schizophrenia. Prosaglandins, Leukotrienes, and Essential Fatty Acids 2006; 75: 323-7
[71] Martins JM, Trinca A, Afonso A, et al. Psychoneuroendocrine characteristics of common obesity clinical subtypes. Int J Obes Rel Metab Disord 2001; 25: 24-32.
[72] Holt RIG, Peveler RC, Byrne CD. Schizophrenia, the metabolic syndrome and diabetes. Diabetic Medicine 2004; 21: 515-23
[73] Prabakaran S, Swatton JE, Ryan MM. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiat 2004; 9; 684-97
[74] Stoger R. The thrifty epigenotype: an acquired and heritable predisposition for obesity and diabetes? Bioessays 2008; 30: 156-66.
[75] Dadheech G, Mishra S, Gautam S, Sharma P. Evaluation of antioxidant deficit in schizophrenia. Indian J Psych 2008; 50: 16-20
[76] Isaac M, Chand P, Murthy P. Schizophrenia outcome measures in the wider international community. Brit J Psych 2007; 191: 71-7
[77] Mellor JE, Laugharne JDE, Peet M. Omega-3 fatty acid supplementation in schizophrenic patients. Human Psychopharm 1996; 11: 39-46.
[78] Peet M, Stokes C. Omega-3 fatty acids in the treatment of psychiatric disorders Drugs 2005; 65: 1051-9
[79] Abeywardena MY, Patten GS.Role of ω3 Longchain polyunsaturated fatty acids in reducing cardio-metabolic risk factors. Endocr Metab Immune Disord Drug Targets 2011; 11(3): 232-46
[80] Webster MJ, Weikert CS, Herman MM, Kleinman JE. BDNFmRNA expression during postnatal development, maturation and aging of the human prefrontal cortex. Dev Brain Res 2002; 139: 139-50.
[81] Weickert CS, Hyde TM, Lipska BK, Herman MM, Weinberger DR, Kleinman JE. Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. Mol Psych 2003; 8: 592-610.
[82] Szekeres G, Juhasz A, Rimanóczy A, Kériab S, Janka Z. The C270T polymorphism of the brain-derived neurotrophic factor is associated with schizophrenia. Schizophrenia Res 2003; 65: 15-8.
[83] Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brainderived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 2002; 112: 803-14.
[84] Gorski JA, Zeiler SR, Tamowski S, Jones KR. Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. J Neuroscience 2003; 23: 6856-65.
[85] Dinan TG. Stress and the genesis of diabetes mellitus in schizophrenia. B J Psych 2004; 184: s72-5
[86] Banki CM, Bissette G, Arato M, O'Connor L, Nemeroff CB. CSF corticotrophin-releasing factor-like immunoreactivity in depression and schizophrenia. Am J Psych 1987; 144: 873-7.
[87] Walder DJ, Walker EF, Lewine RJ. Cognitive functioning, cortisol release and symptom severity in patients with schizophrenia. Biological Psychiatry 2000; 48: 1121-32.
[88] Kim JJ, Yoon KS. Stress: metaplastic effects in the hippocampus. Trends Neurosci 1998; 21: 505-9
[89] Björntorp P. Do stress reactions cause abdominal obesity and comorbidities? Obes Rev 2001; 2(2): 73-86
[90] Rosmond R, Bjorntorp P. The interactions between hypothalamic-pituitary-adrenal axis activity, testosterone, insulin-like growth factor I and abdominal obesity with metabolism and blood pressure in men. Int J Obes Rel Metab Disord 1998; 22: 1184-96
[91] Zahrzewska KE, Cusin J, Sainsbury A, Rohner-Jeanrenaud F, Jeanrenaud B. Glucocorticoids are counter-regulatory hormones to leptin. Towards an understanding of leptin resistance. Diabetes 1997; 46: 717-9
[92] Meyer G, Badenhoop K. Glucocorticoidinduced insulin resistance and diabetes mellitus. Receptor-, postreceptor mechanisms, local cortisol action and new aspects of antidiabetic therapy. Medizinische Klinik 2003; 98: 266-70
[93] Thonnard-Neumann E. Phenothiazines and diabetes in hospitalized women. Am J Psychiatry 1968; 124: 978-82.
[94] Smith M, Hopkins D, Peveler RC, Holt RIG, Woodward M, Ismail K. First v second generation antipsychotics and risk of diabetes in schizophrenia: systematic review and meta-analysis. B J Psych 2008; 192: 406-11
[95] Gentile S. Long-term treatment with atypical antipsychotics and the risk of weight gain: a literature analysis. Drug Saf 2006; 29: 303-19
[96] Newcomer JW, Haupt DW. The metabolic effects of antipsychotic medications. Can J Psychiatry 2006; 51: 480-91
[97] De Nayer A, De Hert M, Scheen A, Van Gaal L, Peuskens J. Belgian consensus on metabolic problems associated with atypical antipsychotics. Encephale 2007; 33: 197-202
[98] Ucok A, Gaebel W. Side effects of atypical antipsychotics: a brief overview. World Psychiatry 2008; 7: 58-62
[99] Lambert M, Copeland L, Sampson N, Duffy SA. New-onset type-2 diabetes associated with atypical antipsychotic medications. Prog Neuropsychopharmacol Biol Psych 2006; 30: 919-23
[100] Zhang ZJ, Yao ZJ, Liu W, Fang Q, Reynolds GP. Effects of antipsychotics on fat deposition and changes in leptin and insulin levels. B J Psych 2004; 184; 58-62
[101] Roerig JL, Steffen KJ, Mitchell JE. Atypical antipsychotic-induced weight gain. CNS Drugs 2011; 25 (12): 1035-59
[102] Weston-Green K, Huang XF, Deng C. Alterations to melanocortinergic, GABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. PLoS One 2012; 7(3): e33548.
[103] Weston-Green K, Huang XF, Han M, Deng C. The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. Int J Neuropsychopharmacol 2008; 11(6): 827-35.
[104] Justo D. Smoldering systemic inflammation: a link between schizophrenia, smoking and obesity. Acta Psychiatr Scand 2006; 113: 245-6
[105] Falcone T, Carlton E, Franco K, Janigro D. Inflammation, psychosis and the brain. Psychiatric Times 2009; 26: no. 7.
[106] Kallmann FJ, Reisner D. Twin studies on the genetic variation inresistance to tuberculosis. J Hered 1943; 34: 293-301
[107] Zorrilla EP, Cannon TD, Gur RE, Kessler J. Leukocytes and organ non-specific autoantibodies in schizophrenics and their siblings: markers of vulnerability to disease? Biol Psychiatry 1996; 40: 825-33
[108] Cazzullo CL, Sacchetti E, Galluzzo A, et al. Cytokine profiles in schizophrenic patients treated with risperidone: a 3-month follow-up study. Prog Neuropsychopharmacol Biol Psychiatry 2002; 26: 33-9
[109] Zhang XY, Zhou DF, Cao LY, et al. Changes in serum interleukin-2, -6, and -8 levels before and during treatment with risperidone and haloperidol: relationship to outcome in schizophrenia. J Clin Psychiatry 2004; 65: 940-7
[110] Frommberger UH, Bauer J, Haselbauer P, Fräulin A, Riemann D, Berger M. Interleukin-6-(IL-6) plasma levels in depression and

schizophrenia: comparison between the acute state and after remission. Eur Arch Psychiatry Clin Neurosci 1997; 247: 228-33

[111] Zalcman S, Green-Johnson JM, Murray L, et al. Cytokine-specific central monoamine alterations introduced by interleukin-1, -2 and -6. Brain Res 1994; 643: 40-9

[112] Leonard BE. Is there an immunological basis for schizophrenia? Expert Rev Clin Immunol 2005; 1: 103-12

[113] Sommer IE, de Witte L, Begemann M, Kahn RS. Nonsteroidal anti-inflammatory drugs in schizophrenia: ready for practice or a good start? A Meta-Analysis. J Clin Psychiatry 2012; 73(4): 414-9

[114] Wersching H, Duing T, Lohmann H, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology 2010; 74: 1022-9

[115] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005; 115: 1111-9

[116] Fan X, Goff DC, Henderson DC. Inflammation and schizophrenia. Expert Rev. Neurotherapeutics 2007; 7(7): 789-96.

[117] Di Marzo. The endocannabinoid system in obesity and type 2 diabetes. Diabetologia, 2008; 51(8): 1356-67

[118] Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharm 2005; 16: 333-52

[119] Gorzalka BB, Hill MN, Hillard CJ. Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. Neurosci Biobehav Rev 2008; 32(6): 1152-60

[120] Lutz B, Endocannabinoid signals in the control of emotion. Curr Opin Pharmacol 2009; 9(1): 46-52

[121] Blüher M, Engeli S, Klöting N, et al. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. Diabetes 2006; 55: 3053-60.

[122] Côté M, Matias I, Lemieux I, et al. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men Int J Obes 2007; 31: 692-9

[123] Matias I, Gonthier MP, Orlando P, et al. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. J Clin Endocrinol Metab 2006; 91(8): 3171-80

[124] Di Marzo V, Côté M, Matias I, et al. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. Diabetologia 2009; 52(2): 213-7

[125] Di Marzo V, Verrijken A, Hakkarainen A, et al. Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. Eur J Endocrinol 2009; 161(5): 715-22

[126] Matias I, Petrosino S, Racioppi A, Capasso R, Izzo AA, Di Marzo V. Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: Effect of high fat diets. Mol Cell Endocrinol 2008; 286(1-2 Suppl 1): S66-78

[127] Izzo AA, Piscitelli F, Capasso R, et al. Peripheral endocannabinoid dysregulation in obesity: relation to intestinal motility and energy processing induced by food deprivation and re-feeding. Br J Pharmacol 2009; 158(2): 451-61

[128] Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 2005; 115(5): 1298-305

[129] Di Marzo, Després JP. CB1 antagonists for obesity--what lessons have we learned from rimonabant? Nat. Rev. Endocrinol 2009; 5(11): 633-8

[130] Nissen SE, Nicholls SJ, Wolski K, et al. Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA 2008; 299(13): 1547-60.

[131] Sugamura K, Sugiyama S, Nozaki T, et al. Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. Circulation 2009; 119(1): 28-36

[132] Riebe CJ, Wotjac CT. Endocannabinoids and stress. Stress 2011; 14(4): 384-97

[133] Steiner MA,Wotjak CT. Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis. Prog Brain Res 2008; 170: 397-432.

[134] Steiner MA, Wanisch K, Monory K, et al. Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice. Pharmacogenomics J 2008; 8: 196-208.

[135] Rajavashisth TB,Shaheen M, Norris KC, et al. Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. BMJ Open 2012; 2: e000494. doi: 10.1136/bmjopen-2011-000494

[136] Banni S, Di Marzo V. Effect of dietary fat on endocannabinoids and related mediators: consequences on energy homeostasis, inflammation and mood. Mol Nutr Food Res 2010; 54(1): 82-92

[137] Heyman E, Gamelin FX, Goekint M, et al. Intense exercise increases circulating endocannabinoid and BDNF levels in humans - possible implications for reward and depression. Psychoneuroendocrinology 2012; 37(6): 844-51.

[138] Christopoulou FD, Kiortsis DN. An overview of the metabolic effects of rimonabant in randomized controlled trials: potential for other cannabinoid 1 receptor blockers in obesity. J Clin Pharm Therapeut 2011; 36: 10-8

[139] Van Gaal L, Pi-Sunyer X, Després JP, McCarthy C, Scheen A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. Diabetes Care 2008; Suppl 2: S229-40

[140] Topol EJ, Bousser MG, Fox KAA, et al. Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. Lancet 2010; 376: 517-23

[141] Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. Endocrinology 2004; 145(12): 5431-8

[142] Doyon C, Denis RG, Baraboi ED, et al. Effects of rimonabant (SR141716) on fasting-induced hypothalamic-pituitary-adrenal axis and neuronal activation in lean and obese Zucker rats. Diabetes 2006; 55(12): 3403-10

[143] Riedel G, Fadda P, McKillop-Smith S, et al. Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. Br J Pharmacol 2009; 156(7): 1154-66.

[144] Cawthorne M, Stott C, Wright S, Guy G, Zaibi M, Wargent E. The metabolic effects of tetrahydrocannabivarin (THCV) and cannabidiol. In: 18th Annual Symposium of the Cannabinoids, International Cannabinoid Research Society 2008, Abstract 42

[145] Malfait AM, Gallily R, Sumariwalla PF, et al.The non-psychoactive cannabis-constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proceedings of the US National Academy of Science 2000; 97: 9561-6

[146] Croxford JL, Yamamura T. Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? J Neuroimmunology 2005; 166: 3-18

[147] Lara DR, Dall'Igna OP, Ghisolfi ES, Brunstein MG. Involvement of adenosine in the neurobiology of schizophrenia and its therapeutic implications. Progress in Neuro-Psychopharmacology & Biolog Psychiatry 2006; 30: 617-29

[148] Croci T, Landi M, Galzin AM, Marini P. Role of cannabinoid CB1 receptors and tumor necrosis factor-alpha in the gut and systemic anti-inflammatory activity of SR 141716 (rimonabant) in rodents. Br J Pharmacol 2003; 140(1): 115-22

[149] Juckel G, Roser P, Nadulski T, et al. Acute effects of Δ-9-tetrahydrocannabinol and standardised cannabis extract on the auditory evoked mismatch negativity. Schizophrenia Res 2007; 97: 109-17

[150] Javitt DC, Doneshka P, Grochowski S, Ritter W. Impaired mismatch negativity generation reflects widespread dysfunction of working memory in schizophrenia. Arch Gen Psych 1995; 52: 550-8

[151] Marsicano G, Goodenough S, Monory K, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 2003; 302: 84-8

[152] Hill MN, Patel S, Carrier EJ, et al. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 2005; 30: 508-15

[153] Hill MN, Tasker JG. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience 2012; 204: 5-16

[154] Zoppi S, Pérez Nievas BG, Madrigal JLM, et al. Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity and neuroinflammation. Neuropsychopharmacology 2011; 36: 805-18

[155] Koola MM, McMahon RP, Wehring HJ, et al. Alcohol and cannabis use and mortality in people with schizophrenia and related psychotic disorders. J Psychiatric Rese 2012; doi: 10.1016/j.jpsychires.2012.04.019

[156] Potvin S, Joyal CC, Pelletier J, Stipp E. Contradictory cognitive capacities among substance-abusing patients with schizophrenia: a meta-analysis. Schizophrenia Res 2008; 100: 242-51

Received: May 13, 2013 **Accepted: June 10, 2013**

Schedule "D"

American Herbal Pharmacopoeia®

Cannabis Inflorescence
Cannabis spp.

Standards of Identity, Analysis, and Quality Control

Revision 2014

Editors and Technical Advisors

Roy Upton RH
American Herbal Pharmacopoeia®
Scotts Valley, CA

Lyle Craker PhD
University of Massachusetts
Amherst, MA

Mahmoud ElSohly PhD
University of Mississippi
University, MS

Aviva Romm MD CPM
American Herbal Pharmacopoeia®
Lennox, MA

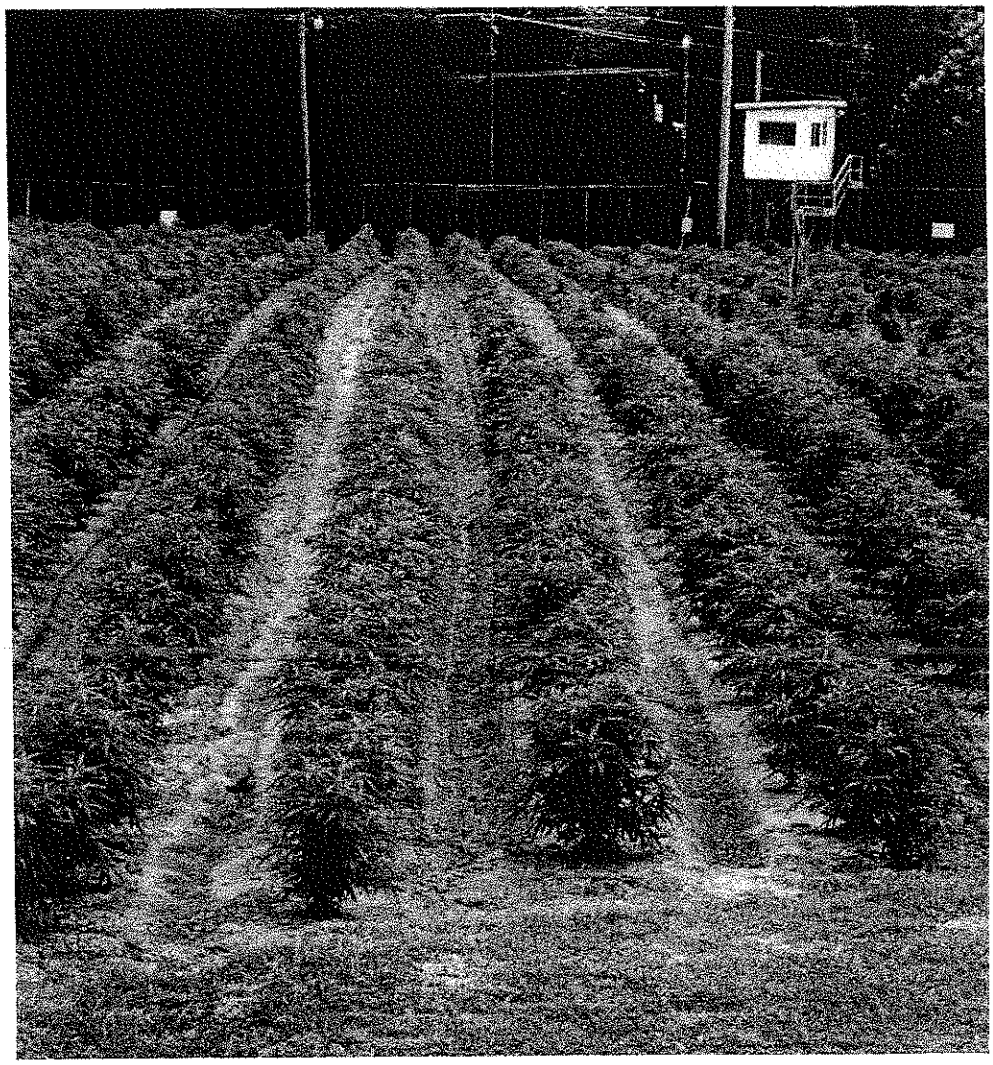
Ethan Russo MD
GW Pharmaceuticals
Salisbury, UK

Michelle Sexton ND BS
Americans for Safe Access
Washington, DC
The Center for the Study of Cannabis and Social Policy
Seattle, WA

Research Associates

Jahan Marcu PhD
Green Standard Diagnostics
Henderson, NV

Diana Swisher MA
American Herbal Pharmacopoeia®
Scotts Valley, CA



Authors

Botanical Identification

Mahmoud ElSohly PhD
Suman Chandra PhD
Hemant Lata PhD
University of Mississippi
University, MS

Macroscopic Identification

Suman Chandra PhD
Hemant Lata PhD
Mahmoud ElSohly PhD
University of Mississippi
University, MS

Microscopic Identification

Suman Chandra PhD
Hemant Lata PhD
Mahmoud ElSohly PhD
University of Mississippi
University, MS

Elizabeth Williamson PhD
University of Reading
Reading, UK

Commercial Sources and Handling

Suman Chandra PhD
Hemant Lata PhD
University of Mississippi
University, MS

Roy Upton RH
American Herbal Pharmacopoeia
Scotts Valley, CA

Daniel Harder PhD
Museum of Natural History
Santa Cruz, CA

Constituents

Mahmoud ElSohly PhD
Desmond Slade PhD
University of Mississippi
School of Pharmacy
University, MS

Analytical

Thin-Layer Chromatography (TLC)
Gas Chromatography (GC)
Mahmoud ElSohly PhD
Desmond Slade PhD
Zlatra Mehmedic PhD
Mohammed M Radwan PhD
University of Mississippi
University, MS

High Performance Liquid Chromatography (HPLC)
Kong M Li PhD
University of Sydney
Sydney, NSW

Reviewers

Wendy Appplequist PhD
Missouri Botanical Garden
St. Louis, MO

Paula Brown PhD
British Columbia Institute of Technology (BCIT)
British Columbia, Canada

Rudolf Brenneisen
University of Bern
Bern, Switzerland

Mike Corral
Wo/Men's Alliance for Medical Marijuana
Santa Cruz, CA

Staci Eisner
Cannabis Committee
American Herbal Products Association
Silver Spring, MD

Franjo Grotenhermen MD
International Association for Cannabinoid Medicines (IACM)
Ruethen, Germany

Erik W Johansen
Special Pesticide Registration Program Coordinator
Washington State Department of Agriculture
Olympia, WA

James Kababick
Flora Research Laboratories
Grants Pass, OR

ao Prof Dr Liselotte Kren
University of Vienna
Vienna, Austria

Prof Dr Reinhard Länger
AGES Pharm Med
Vienna, Austria

Etienne de Meijer
GW Pharmaceuticals
Salisbury, UK

David Potter PhD
GW Pharmaceuticals
Salisbury, UK

Eike Reich PhD
CAMAG
Muttenz, Switzerland

Jeanette Roberts PhD MPH
University of Wisconsin
Madison, WI

Steph Sherer
Americans for Safe Access (ASA)
Washington, DC

Bill Schoenbart LAc DAOM
Five Branches University
Santa Cruz, CA

Amala Soumyanath PhD
Oregon Health and Science University
Portland, OR

Elan Sudberg
Costa Mesa, CA

Elizabeth Williamson PhD
University of Reading
Reading, UK

Hugh Watson
Marijuana Agricultural Chemical Specialist
Washington State Liquor Control Board
Olympia, WA

Final Reviewers

Giovanni Appendino Laurea
Department of Pharmaceutical Sciences
University of the Eastern Piedmont
Novara, Italy

Vincenzo Di Marzo PhD
Endocannabinoid Research Group
Institute of Biomolecular Chemistry
Consiglio Nazionale delle Ricerche
Pozzuoli (NA), Italy

John McPartland DO MS
Middlebury, VT

Raphael Mechoulam PhD
Hebrew University of Jerusalem
Jerusalem, Israel

Jonathan Page PhD
National Research Council
Saskatoon, Canada

Ethan Russo MD
GW Pharmaceuticals
Salisbury, UK

Maged Sharaf PhD
American Herbal Products Association
Silver Spring, MD

Michael Steenhout
Washington State Liquor Control Board
Olympia, WA

ISBN: 1-929425-37-6 **ISSN**: 1538-0297

PO Box 66809, Scotts Valley, CA 95067 USA

The American Herbal Pharmacopoeia® is a nonprofit corporation 501(c)(3). To purchase monographs or botanical and chemical reference standards, contact the American Herbal Pharmacopoeia® • PO Box 66809 • Scotts Valley, CA 95067 • USA • (831) 461-6318 or visit the AHP website at www.herbal-ahp.org.

Medical Disclaimer

The information contained in this monograph represents a synthesis of the authoritative scientific and traditional data. All efforts have been made to ensure the accuracy of the information and findings presented. Those seeking to utilize botanicals as part of a health care program should do so under the guidance of a qualified health care professional.

Statement of Nonendorsement

Reporting on the use of proprietary products reflects studies conducted with them and is not meant to be a product endorsement.

Design & Layout

Michael Parisi
Aptos, CA

Cover Photograph

Cannabis cultivated under the Compassionate Investigational New Drug program at the University of Mississippi administered by the National Institute on Drug Abuse (NIDA). Photograph courtesy of: University of Mississippi.

Table of Contents

Legal Notification

The following Standards of Identity, Analysis, and Quality Control of *Cannabis* are intended to provide scientifically valid methods for the analysis of cannabis and its preparations that can be used to comply with state and federal regulations and policies. The analytical methods were obtained from peer reviewed literature, have been used as part of international or federal monitoring programs for cannabis, and have been verified for their scientific validity. Methods other than those presented in this monograph may be scientifically valid and provide reliable results. However, all methods must be verified as being scientifically valid prior to use for regulatory compliance.

In the United States, cannabis is a Schedule I controlled substance under federal law; therefore, any use or possession of cannabis and its preparations is illegal except pursuant to the compassionate use Investigational New Drug exemption. These standards are not intended to support, encourage, or promote the illegal cultivation, use, trade, or commerce of cannabis. Individuals, entities, and institutions intending to possess or utilize cannabis and its preparations should consult with legal counsel prior to engaging in any such activity.

The citing of any commercial names or products does not and should not be construed as constituting an endorsement by the American Herbal Pharmacopoeia. Additionally, the reliability, and therefore ability to comply with state or federal regulations, of any conclusions drawn from the analysis of a sample is dependent upon the test sample accurately representing the entire batch. Therefore, when performing all analytical tests, a formal sampling program must be employed.

Nomenclature

Botanical Nomenclature

Cannabis L. (includes *Cannabis sativa*, *C. indica*)

Botanical Family

Cannabaceae

Pharmacopoeial Nomenclature

Cannabis Inflorescentia

Pharmacopoeial Definition

Cannabis consists of the dried inflorescences and remains of subtending leaves of pistillate *Cannabis* species plants.

Common Names

Cannabis, ganja, grass, hemp, marijuana (alternatively spelled marihuana), pot, weed, sinsemilla.

Identification

Botanical Identification

Taxonomic Discussion

The taxonomic classification of *Cannabis* has been the subject of considerable debate in scientific and legal forums for decades and is driven by classical botanical taxonomy, chemotaxonomy, and molecular sequencing. Opinions regarding *Cannabis* have been split between polytypic (multiple-species) and monotypic (single-species) views of the genus. Both views usually segregate plant populations by their relative concentrations of Δ^9-tetrahydrocannabinol (Δ^9-THC; hereafter referred to as THC) and cannabidiol (CBD). For a detailed account of the taxonomic history of *Cannabis* see Hillig (2005), Russo (2004), Schultes et al. (1974), and Small and Cronquist (1976).

Following the formal description of *C. sativa* by Linnaeus in 1753, Lamarck (1785) published a description of what he considered a different species, *C. indica*, based on plant specimens collected in Asia. The *C. indica* plants were relatively shorter, had smaller leaves, narrower leaflets, smaller fruit, and, as described by Lamarck, poorer fiber quality than *C. sativa*, but greater utility as an inebriant. Since then, the name *C. indica* has been applied to variants with high levels of psychoactive THC, while the name *C. sativa* has generally been applied to plants selected for their yield of bast (phloem) fibers in the stem and relatively high CBD to THC ratio. Wild-type plants growing in southeast Europe, possibly descending from the ancestor of C. sativa, were named *C. sativa.* var. *spontanea* Vav. and *C. ruderalis* Janisch. Vavilov encountered unique, broad-leafleted plants in Afghanistan. After some equivocation, he named them *C. indica* var. *kafiristanica* (a wild-type plant) and *C. indica* var. *afghanica* (plants with traits of domestication). Numerous other botanical names have appeared in the literature (e.g., see Schultes et al. 1974 for discussion and Tropicos.org for a nearly complete list).

Schultes et al. (1974) and Anderson (1980) recognized 3 entities: *Cannabis sativa* L. (tall, branched plants, used mainly for fiber and seed and also for drugs), *C. indica* Lam. (short, densely branched plants with firm stem, broad leaflets, and high content of psychoactive THC), and *C. ruderalis* Janisch. (short, often unbranched "roadside" plants usually yielding a high CBD to THC ratio). The taxonomic treatments by Schultes and Anderson departed from the concepts of Linneaeus, Lamarck, and Janischevsky. These authors treat *C. sativa* as a source of psychoactive drugs; Lamarck's *C. indica* designates plants from India, which are relatively tall, laxly branched, with narrow leaflets; they apply *C. ruderalis* to plants from Central Asia, whose morphology departs from Janischevsky's description of European plants with moderate height, strong branching, and long, narrow leaves.

Small and Cronquist (1976) analyzed 350 world-wide accessions in a common garden experiment. These authors argued that due to the absence of reproductive barriers and the morphological discontinuities of the plant, only one polymorphic species, *C. sativa*, currently exists. They further suggested that the current gene pool of *Cannabis* was heavily influenced by human agronomic selection and proposed the recognition of subspecies *sativa* (low content of THC, grown primarily for fiber and seed use) and *indica* (high content of THC, grown primarily for intoxicant use) within the single species, *C. sativa*.

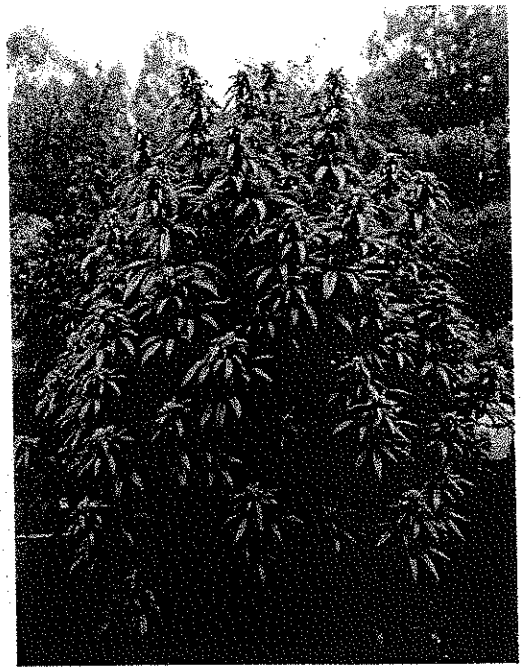
Conversly, Hillig argues that the split between *sativa* and *indica* may have pre-dated human intervention. He analyzed 157 accessions of known geographic origin in a common garden experiment, using genetic evidence (Hillig 2005), cannabinoid profiles (Hillig and Mahlberg 2004), terpenoid variation (Hillig 2004), and host-parasite data (McPartland and Hillig 2006). He recognized a *sativa* gene pool included hemp fiber and seed landraces from Europe and Central Asia and Eastern European ruderal (roadside) accessions. The *indica* gene pool comprised narrow-leaflet drug strains from Southern Asia, Africa, and South America, wide-leaflet drug strains from Afghanistan and Pakistan, Far Eastern fiber and seed landraces, and feral populations from Nepal and India. A putative third gene pool was formed by ruderal accessions from Central Asia. This classification and nomenclature was adopted and expanded by Clarke and Merlin (2013).

A vernacular taxonomy of "Sativa" and "Indica" has arisen, which conflicts with the formal botanical taxonomy of Linneaus and Lamarck, as noted by Small (2007). The 2 names have been commonly used to refer to, narrow- and wide-leafleted drug varieties, respectively (Hillig 2004). However, due to the widespread interbreeding of the species, the application of these terms to narrow and broad leafleted specimens is botanically imprecise. Recent floristic treatments of *Cannabis* recognize only one (*C. sativa*) or, rarely, 3 species, noting the confused state of taxonomic understanding: *Flora of China* and *Flora of the USSR* lists 2

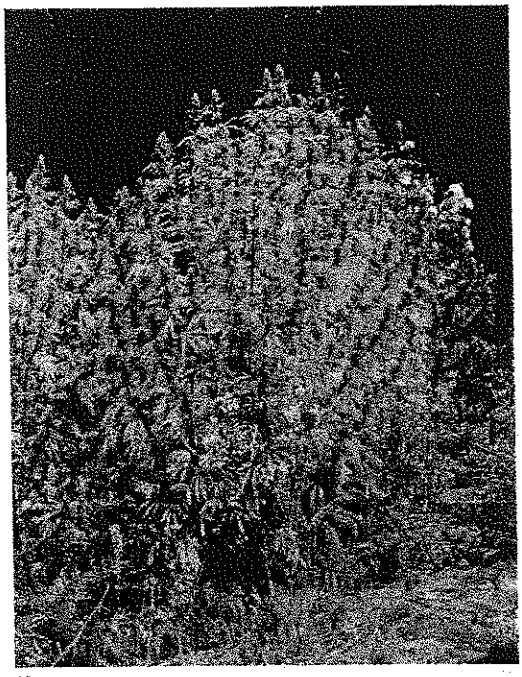
Figure 1 Morphological characteristics of *Cannabis*

A. Inflorescence of male (staminate) plant.
B. Fruiting female (pistillate).
a. Staminate flower.
b. Stamen (anther and short filament).
c. Stamen.
d. Pollen grains.
e. Pistillate flower with bract.
f. Pistillate flower without bract.
g. Pistillate flower showing ovary (longitudinal section).
h. Seed (achene) with bract.
i. Seed without bract.
j. Seed (side view).
k. Seed (cross section).
l. Seed (longitudinal section).
m. Seed without pericarp (peeled).

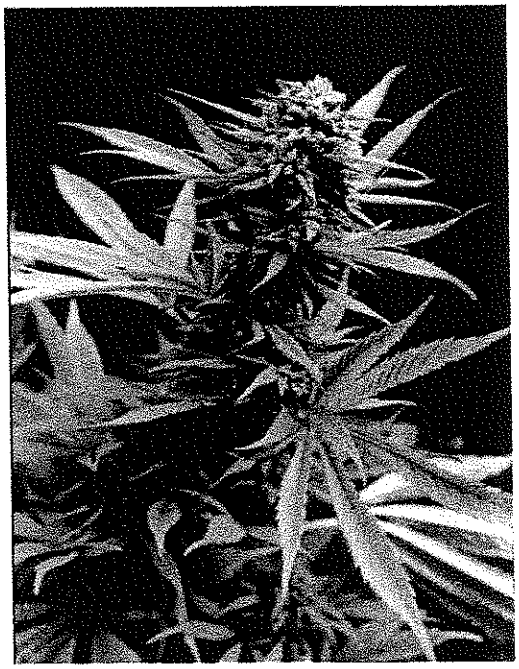
Source: Köhler, *Medizinal-Pflanzen in naturgetreuen Abbildungen und kurz erläuterndem Texte* (1887).



2a.



2b.

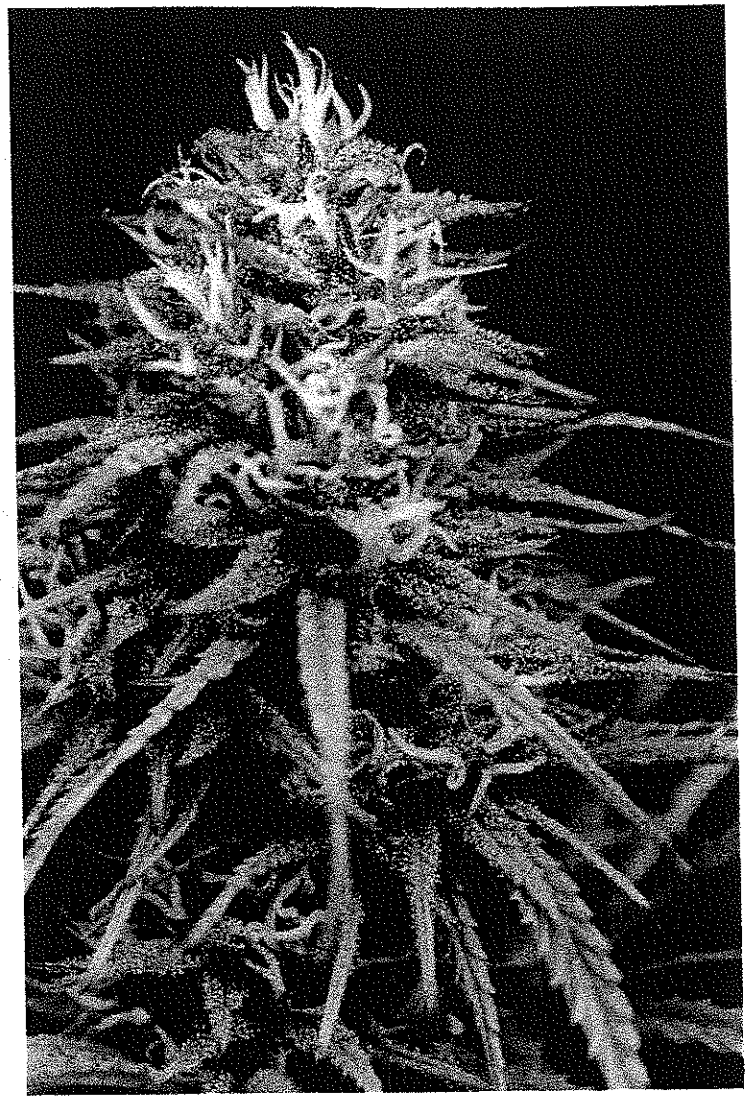


2c.

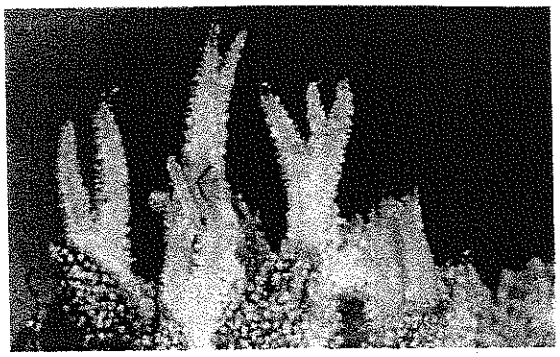
2d.

Figure 2 **Botanical characteristics of cannabis inflorescences**

2a. Full view of mature high-THC-producing female (pistillate) plant.
2b. Full view of mature high-CBD-producing female (pistillate) plant.
2c. Long dense raceme of a high THC-producing female plant bearing pistillate (female) flowers.
2d. Inflorescence of a high CBD-producing (CBD:THC ratio 30:1) female plant (note long slender leaves).



2e.



2f.

2g.

2h.

Figure 2 (continued) Botanical characteristics of cannabis inflorescences

2e. Maturing female inflorescence showing young yellow styles and stigmas (often referred to as "pistils").

2f. Close-up of maturing female inflorescence showing young yellow styles and stigmas senescing brown and shriveling and an abundance of glandular trichomes.

2g. Female inflorescence with senesced reddish-brown styles and stigmas, an indicator of inflorescence maturity.

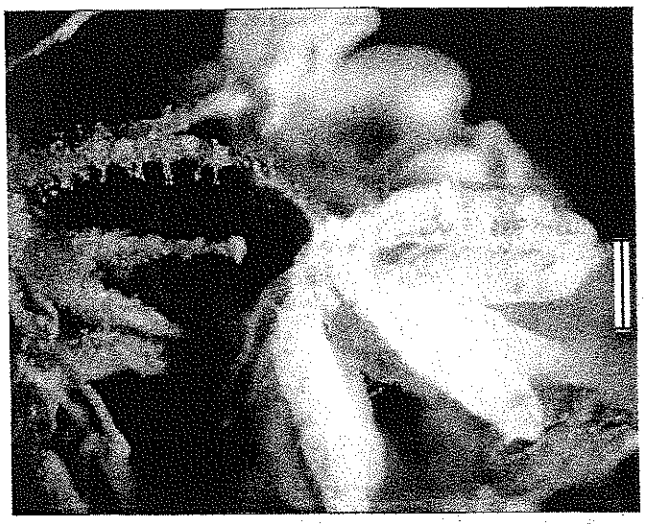
2h. Close-up of female inflorescence with senesced reddish-brown styles and stigmas.

(*C. sativa* L. and *C. ruderalis* Janisch.), *Flora of Pakistan* lists one, *Flora of Missouri* lists one, *Flora of North America* lists one, and *Flora of Taiwan Checklist* lists one.

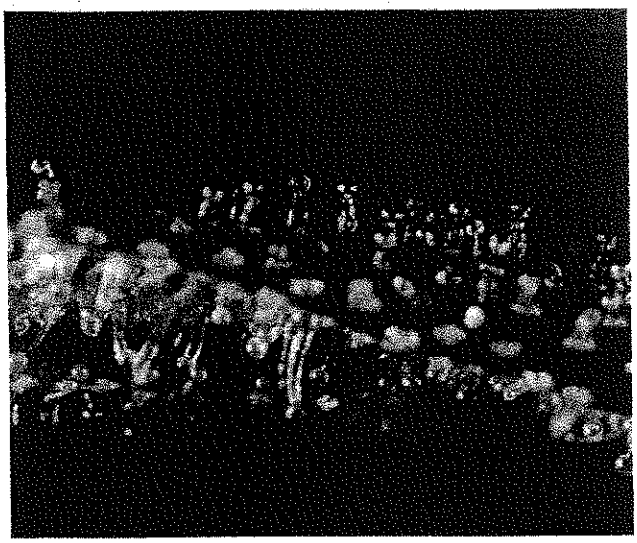
Extensive co-cultivation and crossbreeding practices have effectively crossed the boundaries between the various taxonomic categories within *Cannabis*. Although outside of the strictly botanical classification, nomenclature of cultivated plants, governed by the International Code of Nomenclature for Cultivated Plants (Brickell et al. 2009), may be more applicable to further differentiation of *Cannabis* plants cultivated today. Such nomenclature is not taxonomic, but cultonomic, and recognizes cultivars and groups based on economically important characteristics, without an appeal to the phylogenetic hierarchy. With this approach, *Cannabis* plants that satisfy selected criteria might be assigned to any number of groups, depending on the use emphasized, e.g., THC-drug group, CBD-drug group, mixed THC-CBD-drug group, fiber-hemp group, seed-oil group, etc.

Cannabis is a member of the *Cannabaceae* family, together with another well-known member of the family, hops (*Humulus*). The family has recently been expanded to contain 9 other genera (Stevens 2001). The following

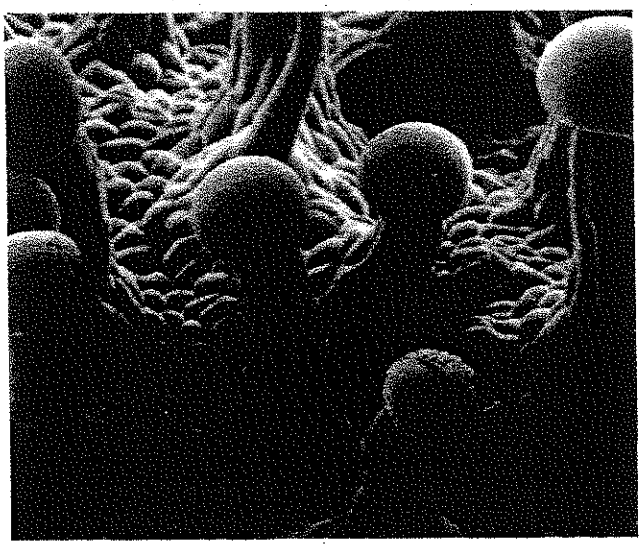
2i.



2j.



2k.



2l.

Figure 2 (continued) Botanical characteristics of cannabis inflorescences

2i. Trichomes along anther scale.

2j. Trichomes along the pedicel of a male flower.

2k. Close-up of glandular trichomes.

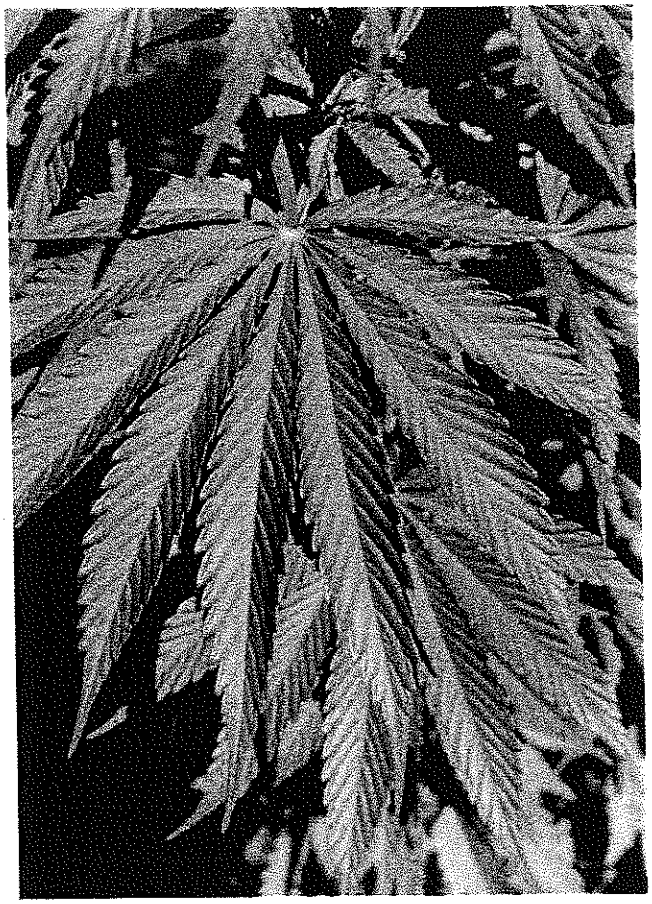
2l. Magnification of multicellular glandular trichomes with electron microscopy.

Photographs courtesy of: (2a–b, e, f) The Wo/Men's Alliance for Medical Marijuana (WAMM), Santa Cruz, CA; (2c, h) Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy; (2d, g, i–k) © David J Potter, Salisbury, UK; (2l) University of Mississippi, University, MS.

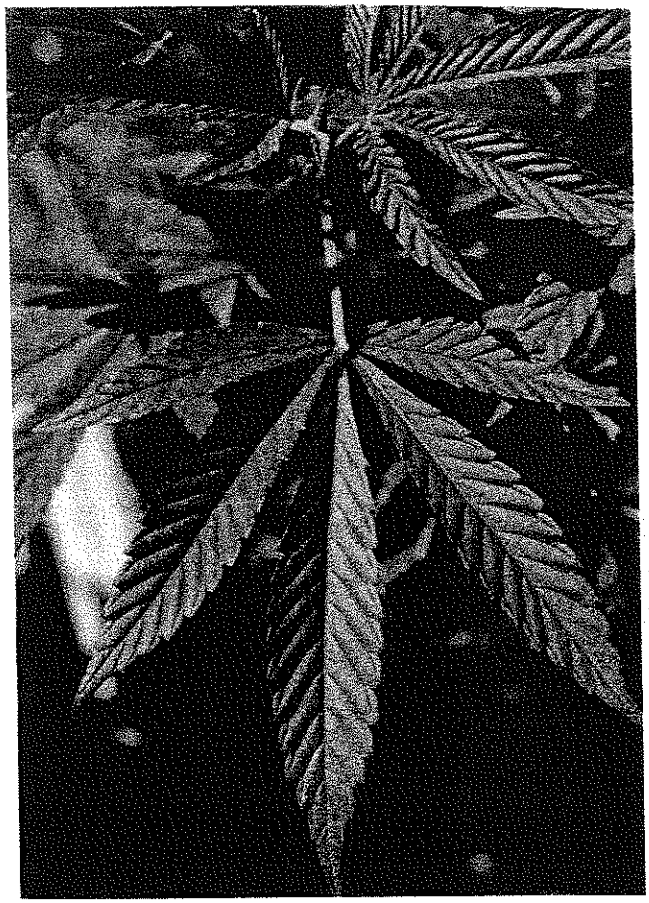
describes the published range of morphological diversity within plants recognized as *Cannabis* spp.

Morphological Characterization of *Cannabis* L.

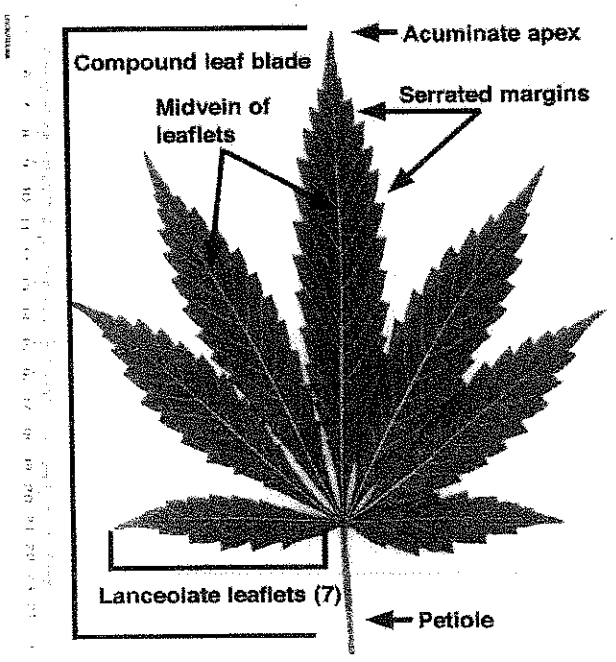
Herbaceous annual, taprooted (taproot not developed on vegetatively propagated/cloned plants). Plants dioecious (male and female flowers occur on separate plants) and rarely monoecious (male and female flowers occur on the same plant). Monoecious plants are often referred to as "hermaphrodites." True hermaphrodites bear bisexual flowers and are less common, whereas monoecious plants bear unisexual male and female flowers at different locations on the plant. Staminate (male) plants tend to be taller but less robust than pistillate (female) plants. Height and degree of branching depends on both genetic and environmental factors (UNODC 2009). **Root:** A laterally branched taproot, generally 30–40 cm deep in loose soil, and up to 2.5 m deep; the horizontal spread of lateral roots also depends on the soil type, up to 80 cm in width. **Stem:** Erect, furrowed, round to obtusely hexagonal in cross-section often hollow, 0.2–6 m (usually 1–3 m) tall, simple to well branched; branchlets densely pubescent; staminate (male) plants usually taller and less robust, compared with pistillate (female) plants (Raman 1998); stipules linear, lateral, acute, persistent, 2–5 mm. **Leaves:** Alternate or opposite basally on



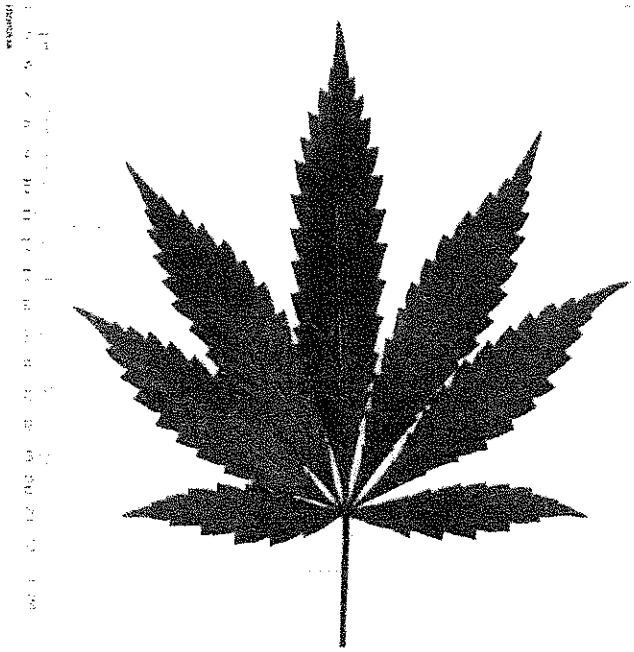
3a.



3b.



3c.



3d.

Figure 3 Botanical characteristics of cannabis leaf

3a. Adaxial (upper) surface of a typical cannabis leaf (9 leaflets).
3b. Adaxial (upper) surface of a typical cannabis leaf (5 leaflets).
3c. Abaxial (lower) surface of a typical cannabis leaf.
3d. Adaxial (upper) surface of a typical cannabis leaf.

3e.

3f.

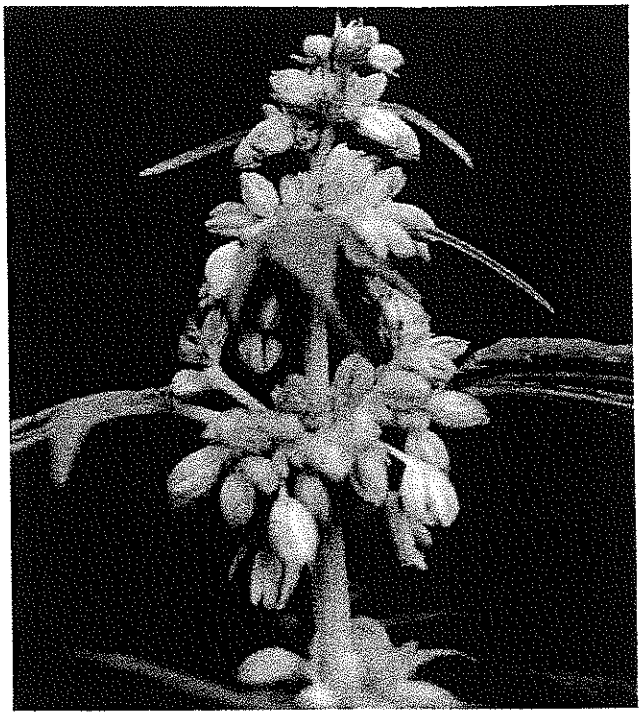
Figure 3 (continued) Botanical characteristics of cannabis leaf

3e. Upper and lower surface of broad-leaf strain ("indica" type).
3f. Upper and lower surface of narrow-leaf strain ("sativa" type).
Photographs courtesy of: (3a–d) WAMM, Santa Cruz, CA; (3e–f) Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy.

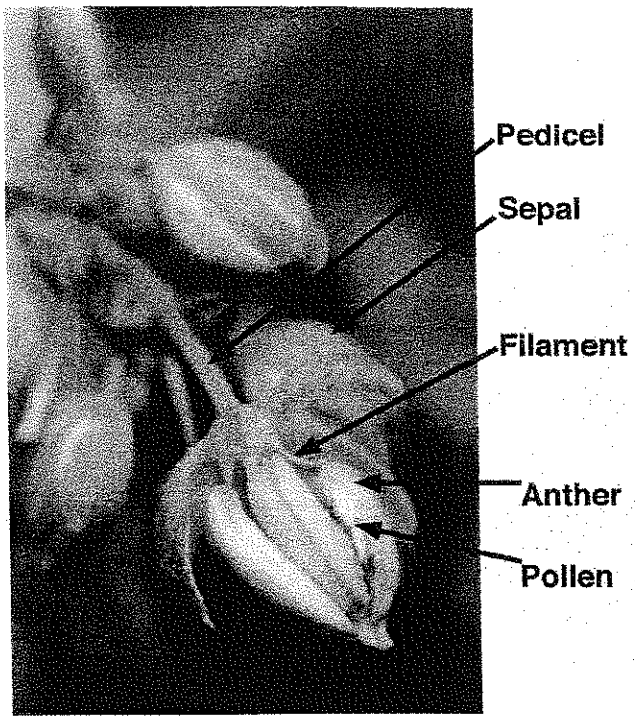
stem, with the longest in the middle, palmately compound, basally with (3)5–11(13) leaflets, apically with 1–3 leaflets. **Leaflet**: Usually lanceolate, sometimes oblanceolate to linear, uneven in size, (3)7–15 x (0.2)0.5–1.5(2) cm, ; margin serrate, with fine, very acute to coarser, almost blunt serrations; apex acuminate; petiole 2–7 cm; leaf blade abaxially whitish-green, strigose, and rarely whitish-clear to opaque to brownish glandular trichomes, adaxially dark green with cystolithic trichomes. Blade surfaces abaxially sparsely to densely pubescent. **Staminate (male) inflorescences:** Axillary or terminal, erect, up to ca. 25 cm, a lax panicle or a compound cyme. **Male flowers:** Yellowish green, nodding; pedicel 2–4 mm, thin; sepals imbricate, ovate to lanceolate, 2.5–4 mm, membranous, with sparse prostrate trichomes; petals absent; filament 0.5–1 mm, straight in bud; anthers oblong; rudimentary pistil small. **Pistillate (female) inflorescences:** Pseudospikes, congested, erect to spreading, among leaf-like bracts and bracteoles. **Female flowers:** Green, sometimes purple to red and/or mottled or streaked, sessile; bract (subtending floral leaves) proximal upper surfaces are densely covered by capitate stalked trichomes, with serrate or entire margins (Potter 2009); bracteole (alternately called a calyx, perigonium, or perigonal bract) usually refers to a small (4–8 mm long), fused, conically-shaped sheath that completely envelopes the ovary and loosely encloses mature fruit, densely hispid or pilose, covered with resinous glandular trichomes; perianth thin, papery, undivided, closely appressed to the ovary and mature fruit, often reduced or absent in cultivated forms, often marbled with light and dark areas; ovary superior, sessile, subglobose, one-locular with one pendulous anatropous ovule; styles 2, long-linear, caduceus, emerging from the apex of the bracteole. **Achenes (fruits):** Solitary, usually green-brown but also white or gray, with a pale, fine reticulation pattern on the smooth surface (in cultivated forms), or with brown or purple mottling (in strains retaining wild-type morphology and a persistent perianth), ovoid to oblong in outline, somewhat compressed (lenticular) in cross section, 2–5 mm; endosperm fleshy and oily; embryo strongly curved; cotyledon fleshy.

The upper leaves, unfertilized (female) flower heads, and flower bracts of the female plant are the primary source of cannabinoids in *Cannabis*. The cannabinoids are enclosed in tiny (just visible to the eye) glandular trichomes occurring in several different forms: sessile glands (trichomes without a stalk); small bulbous glandular trichomes with one-celled stalks; and long, multicellular-stalked glandular trichomes mainly present on bracts and bracteoles surrounding female flowers (Hammond and Mahlberg 1977; Raman 1998; Starks 1990; see Table 2). Numerous unicellular non-glandular trichomes are located on both surfaces of the leaves, bracts, and bracteoles. Those on the upper (adaxial) epidermal surface frequently bear calcium carbonate crystals (cystoliths) at the base. The presence and distribution of the rigid, curved cystolithic non-glandular trichomes on the upper leaf surfaces and of the fine, slender non-cystolithic non-glandular trichomes on both upper and lower surfaces are characteristic of *Cannabis* and enable positive identification of even fragmented material (UNODC 2009).

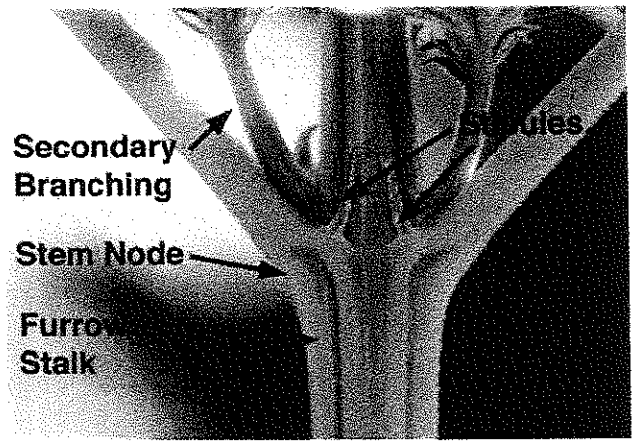
Although some selections of cannabis are day-neutral (flower under any day-length; sometimes referred to as "autoflowering"), most are short-day-length plants (needing



4a.



4b.



4c.

4d.

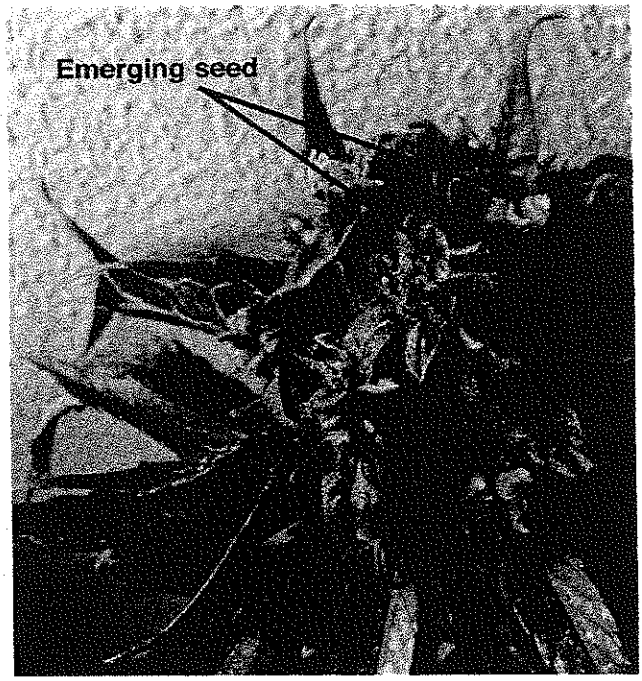
Figure 4 Botanical characteristics of cannabis staminate (male) flowers and stem

4a. Male (staminate) flowers.
4b. Close-up of male flowers showing primary floral characteristics.
4c. Vegetative stem of mature plant showing node, furrows, stipules, and axillary branching.
4d. Stalk of purple variety.
Photographs courtesy of: WAMM, Santa Cruz, CA.

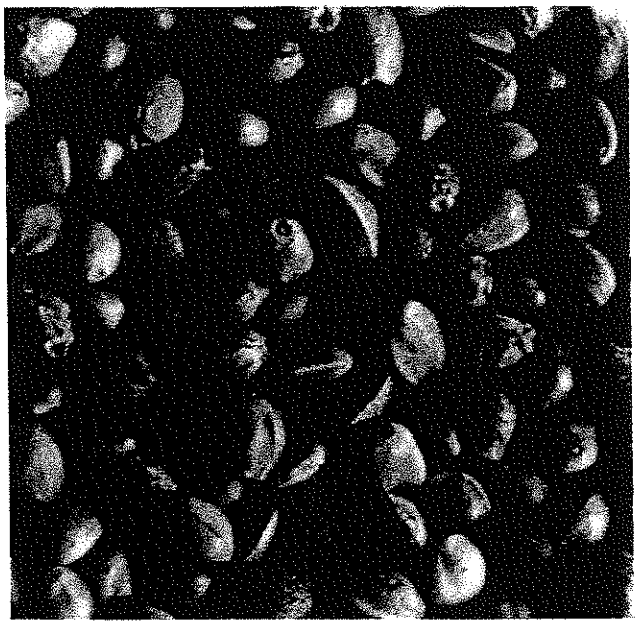
a long, usually ≥ 14 hours dark period) and shift from vegetative to reproductive growth upon exposure to short day-length conditions. With the change to reproductive growth, the leaf pair arrangement changes from opposite to an alternate, spiral arrangement (Potter 2004). Distinguishing male and female plants during vegetative growth is difficult, although the female plant tends to be stockier and to flower later than the male plant (Raman 1998). Occasionally, one or few individual flowers are produced in lower leaf axils to allow the determination of the plant's sex during the vegetative phase of growth.

Distribution: Humans have dispersed cannabis worldwide over the past 10,000 years from probable origins in Central Asia, the Northwestern Himalayas, and China to a variety of habitats throughout the temperate and tropical regions of the world. Within the purported native range, the plant occurs in open, disturbed habitats, such as along riverbanks, bottomlands, and hillsides. In North America, C. *sativa* subsp. *sativa* is reported naturalized or ruderal in most all states and provinces across the Northeast, Midwest, and Eastern Plains, occurring at altitudes 0–2000 m (USDA-NRCS 2014). The plant can be observed in fertile, moist farmlands, in open habitat, in waste areas, and, occasionally, in fallow fields and open woodlands (Small 1997). In addition to the habitats in which the

5a.



5b.



5c.

Figure 5 Botanical characteristics of cannabis seed (achene)

5a. Developing seed (fruit; formally known as "achene").

5b. Seed emerging from and surrounded by the sac-like fused calyx.

5c. Mature seeds.

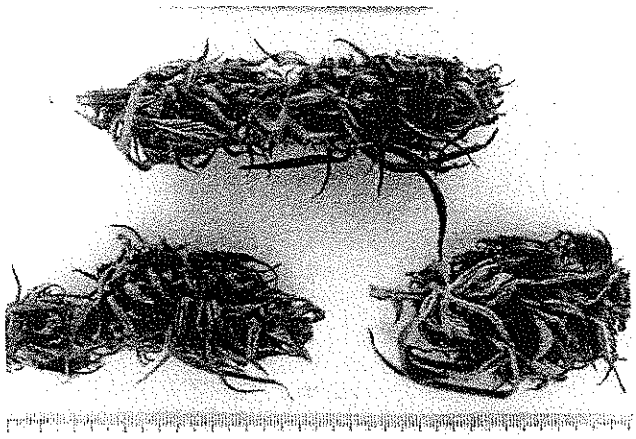
Photographs courtesy of: Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy.

plants would otherwise be naturalized if growth were not actively curtailed, cannabis is widely cultivated outdoors and indoors for both recreational and medicinal purposes.

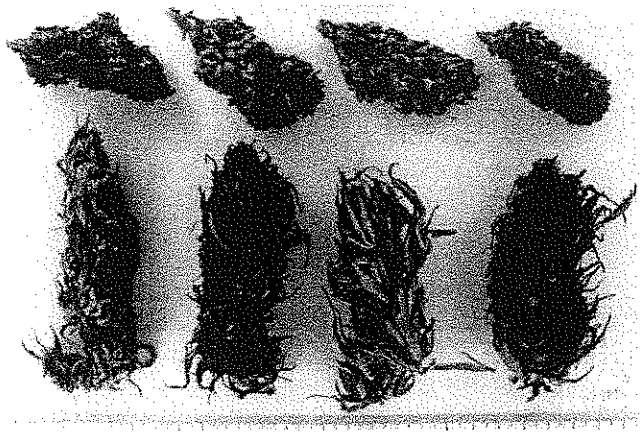
Macroscopic Identification

Cannabis raw material is most often supplied as variously sized, 1.5–15 cm or longer, branches and branchlets, sometimes broken up, of the dried inflorescences of pistillate plants. These inflorescence segments, colloquially known as "buds," are often closely trimmed by hand or machine, sometimes leaving portions of the leaf bases and stiff petioles. The segments are generally light to dark green, various shades of purple to dark purple, or from green-brown to brown and may include whole, or fragments of, reduced upper leaves, stems, bracts, bracteoles, rudimentary calyx, immature ovules, styles, and glandular and non-glandular trichomes. Cannabis selections vary to the extent of the length of the internodes within the inflorescence. Those of short length have a denser cluster of flowers so that the segment pieces appear more rounded; those of a longer length have a greater distance between individual flowers. Variation exists between selections in the size and prominence of the various parts. Morphological characteristics and variation in color of cannabis products are influenced by the variety as well as environmental factors including light, water, nutrients, and methods of cultivation, harvesting, handling, and curing. For macroscopic examination of material that is stuck together, soak the material in strong alcohol (70%) to dissolve the resin, pour off the alcohol, and then soak in water. The leaves, stems, bracts, flowers, and fruit can then be separated. However, material prepared in this manner should not be used for quantitative analysis due to constituent loss.

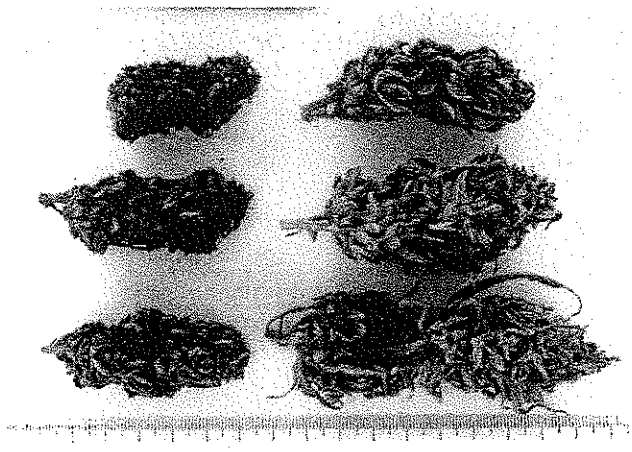
Stems: Light brown, pale green, or variously mottled or entirely purple in color. Stems within inflorescences are



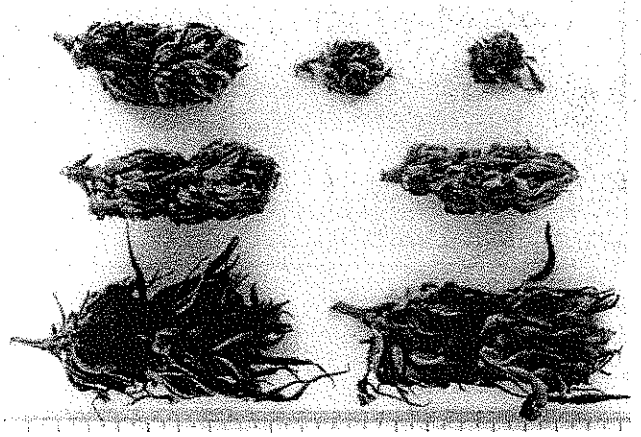
6a.



6b.

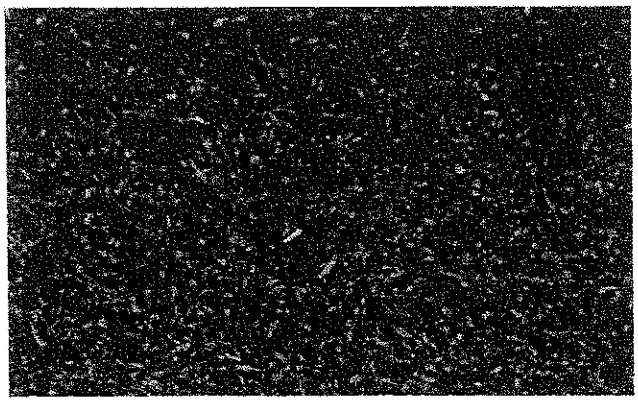


6c.



6d.

6e.



6f.

Figure 6 Macroscopic characteristics of cannabis inflorescence

6a. Dried, untrimmed pistillate inflorescences of morphological type "sativa."

6b. Dried pistillate inflorescences of morphological type "sativa" (bottom – untrimmed; top – trimmed).

6c. Storage effects on color of cannabis material (left – 1-year-old; right – new harvest).

6d. Dried pistillate inflorescences of morphological type "indica" (bottom – untrimmed; middle and top – trimmed).

6e. Close-up of a dried pistillate inflorescence (note the visible glandular trichomes).

6f. Powdered dry cannabis material (leaves and pistillate inflorescences).

Photographs courtesy of: (6a–e) WAMM, Santa Cruz, CA; (6f) University of Mississippi, University, MS.

often cut just below the node. Stems branch freely and repeatedly but the extent of branching is dependent on environmental and hereditary factors, and the method of cultivation. Nodes and internodes are distinct, with alternate branches, and can be of varying length. Stem texture is fibrous and the surface is longitudinally furrowed with short stiff hairs. The cortex and wood are thin with the pith white and porous. Larger diameter (≥ 3 mm) branch pieces are often sourced from terminal shoots. Material with thinner stems is most often from lateral inflorescence branches or from side branches cut from terminal inflorescences.

Upper leaves: Rarely present in cultivated plants as these are often removed through mechanical or hand trimming. When present, the upper leaves are light to dark green, sometimes purple or mottled purple in color, or brown, dried and shriveled, and sometimes clasping the inflorescence. After trimming, only the base of the petioles is typically left as stiff remnants at the nodes.

Bracts: Light to dark green or brownish-green. Numerous, alternate, with narrow stipules at the base; some are simple and others tri-partite, but in both cases the segments are lanceolate with an entire margin. Bracts subtending the spikes are often divided into 5 linear leaflets. Those subtending the individual flowers usually have 3 minute leaflets. Bracts and stipules both show a marked tendency to shrivel upon drying, and in some cases only the veins of the bracts remain intact. With magnification (10x) numerous glandular and non-glandular trichomes are seen.

Bracteoles: Light to dark green or brownish-green; formed in pairs in the axil of a bract. Ovate with an acute apex and incurved at the base to enclose the flower or fruit. With magnification (10x) numerous glandular and non-glandular trichomes are observed.

Flowers: A single flower is formed in the axil of each bracteole. Calyx is light to dark green or brownish, pubescent, and somewhat folded around the ovary or fruit. Ovary is single-chambered containing a single campylotropous ovule, surrounded by the thin hairy perianth. Attached to the flower are 2 slender, long, pubescent styles and stigmas, spreading at the apex, and of a dark reddish-brown to orange color. Plants are dioecious. Male flowers have stamens; female flowers do not.

Fruit: The fruit of cannabis is an achene and, together with the enclosed seed, is commonly referred to as the "seed." Unless specifically desired, seeds should be lacking from properly harvested material. Achenes separate easily from dry samples. The achene is 2–5 mm in diameter and enclosed within an enlarged persistent perianth surrounded by bracts; solitary, somewhat compressed (lenticular) ovoid, glossy, off-white, green, brown-green, or yellowish-green often mottled in purple. The thin wall of the ovary tightly covers the shell of the seed. The pericarp is dry and brittle and finely reticulate. The endosperm and cotyledons are fleshy. The embryo is curved.

Trichomes: Two primary categories of trichomes are present; glandular, cannabinoid-producing trichomes, and non-

Table 1 **Microscopic characteristics of cannabis inflorescence powder**

Bracts	Polygonal upper epidermis cells with faintly striated cuticle and few trichomes; sinuous-walled lower epidermis cells with anomocytic stomata and abundant trichomes; small clusters of calcium oxalate in the mesophyll cells.
Bracteoles	Polygonal upper epidermis cells with beaded walls; sinuous lower epidermis cells with slightly beade walls, anomocytic stomata, and tapering, unicellular covering trichomes; mesophyll similar to that of bracts, containing calcium oxalate clusters.
Trichomes and glands	Glandular and non-glandular trichomes.
Stigma	Fragments occurring as epidermal cells with reddish-brown papillae.
Seed	Fragments mainly visible as thick-walled sclereids of the epicarp.
Stem	Occurring as epidermis with large cystolith trichomes, parenchyma containing clusters of calcium oxalate, fibers, which are normally unlignified, vessels lignified with reticulate or annular thickening, and lactiferous tissue containing red-brown content.
Bracts	Polygonal upper epidermis cells with faintly striated cuticle and few trichomes; sinuous-walled lower epidermis cells with anomocytic stomata and abundant trichomes; small clusters of calcium oxalate in the mesophyll cells.
Leaflet	Upper epidermis cells wavy-walled with striated cuticle, stomata absent, sessile glandular trichomes and cystolith trichomes abundant; lower epidermis wavy-walled cells with anomocytic stomata and all trichomes characteristic of cannabis.

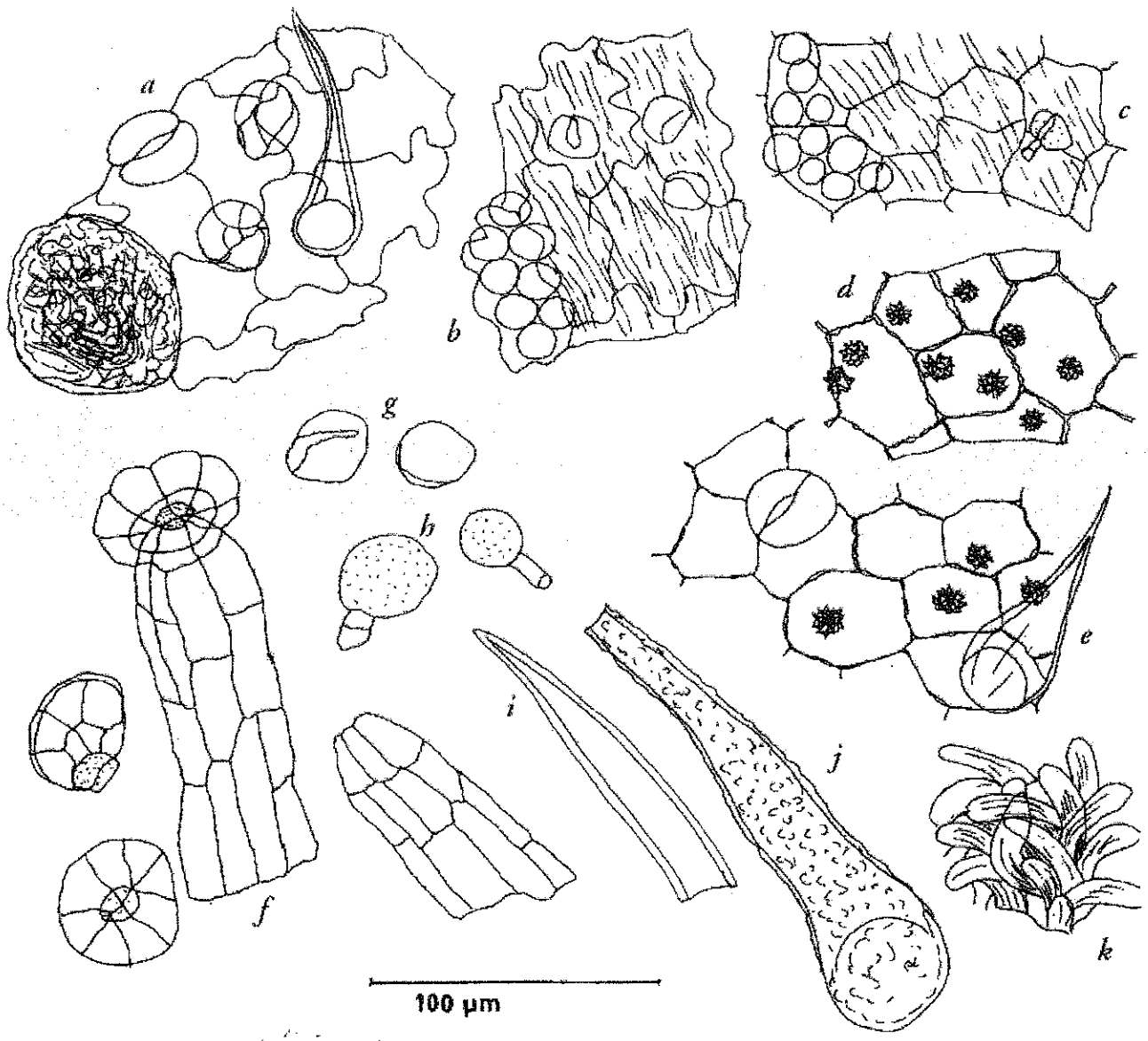


Figure 7 Microscopic characteristics of cannabis (illustrations)

7a. Lower epidermis of leaf showing cystolithic trichome.
7b. Upper epidermis of leaf with underlying palisade.
7c. Upper epidermis of bract with underlying palisade.
7d. Upper epidermis of bracteole with underlying calcium oxalate.
7e. Lower epidermis of bracteole with stoma and underlying calcium oxalate crystal.
7f. Fragments of multicellular glandular trichomes.
7g. Detached sessile glands.
7h. Small glandular trichomes.
7i. Part of a covering trichome.
7j. Part of large warty covering trichome from stem.
7k. Fragment of stigma.
Microscopic images courtesy of Elizabeth Williamson, University of Reading, Reading, UK.

glandular, non-cannabinoid-producing trichomes. Both can be observed with 10–20x magnification (see Table 2).

Powder: Dull light to dark green, to brown; sometimes purplish. When viewing coarsely ground material under 20x magnification, fragments of lower epidermis of leaves contain wavy vertical walls and oval stomata, while upper epidermis pieces have straight vertical walls and no stomata (see Table 1). Most of these characters require higher magnification if viewing finely ground powder.

Organoleptic Characterization

Aroma: Historically, the aroma of cannabis was described as agreeably aromatic, strong and heavy, peculiar, and narcotic. In recent decades, breeding and selection have produced a wide variety of aromas within cannabis strains. Commercial marketing of cannabis has led to the use of numerous comparative terms to describe the aromas of can-

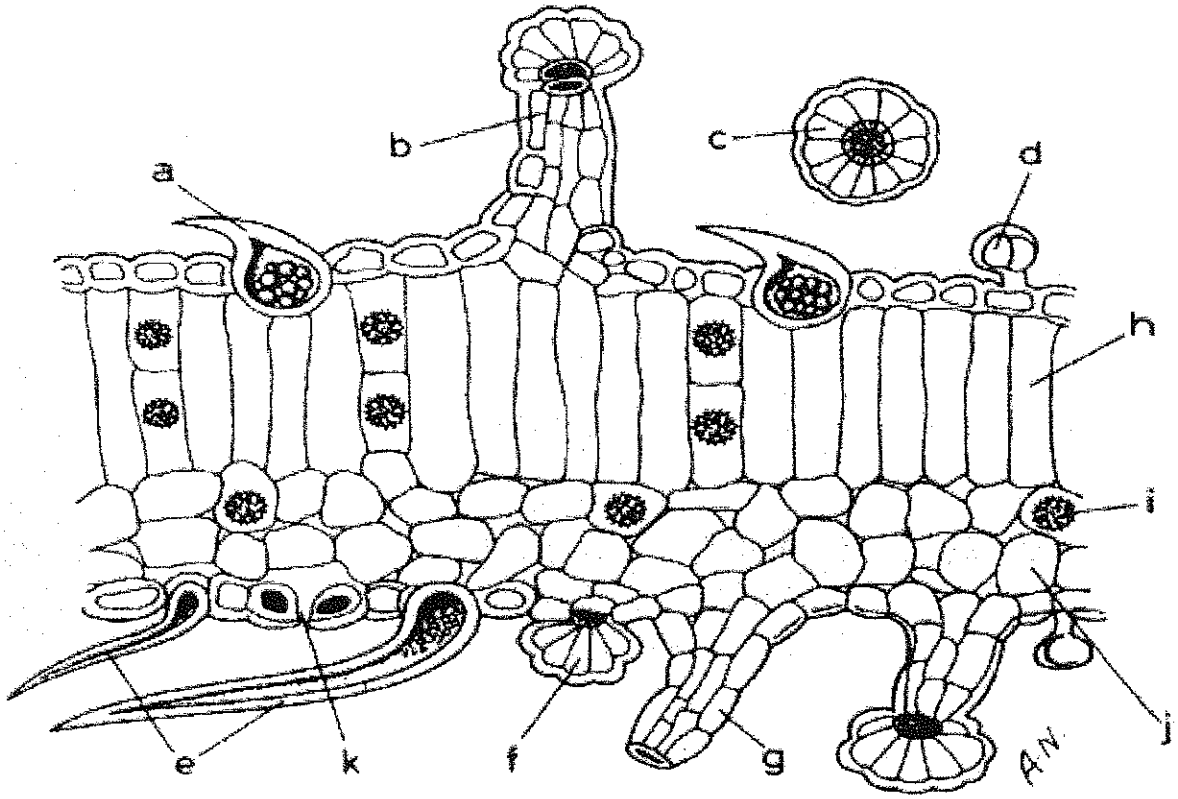


Figure 8 Microscopic characteristics of cannabis; cross section of a fruiting bract

8a. Cystolithic trichome.
8b. Glandular multicellular trichome.
8c. Surface view of large glandular trichome head.
8d. Glandular trichome with bicellular head and unicellular stalk.
8e. Thick-walled conical trichome.
8f. Developing glandular trichome.
8g. Stalk of a glandular trichome.
8h. Palisade cell.
8i. Cluster crystal of calcium oxalate.
8j. Parenchymal cell.
8k. Stoma.

Source: *The botany and chemistry of cannabis.* Joyce CRB and Curry SH (eds.) (1970) J & A Churchill, London.

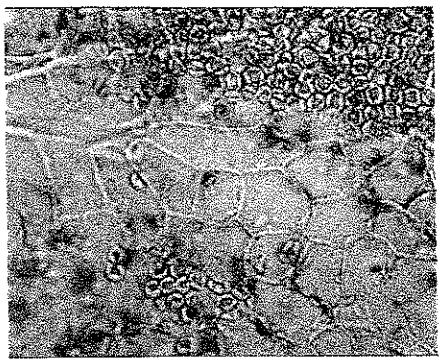
nabis strains. The aromas as described in modern advertising include: peculiar, narcotic, strong, sweet to sour, fruity to pungent, agreeable, aromatic, fresh and sweet, euphoric, spicy citrusy, musty, skunky, acrid, juniper, floral, sour, diesel, vanilla, complex, blueberry, pineapple, perfumed, piney, sandalwood, mango, skunky-cheese, and more.

Color: Color is influenced by variety and mode of cultivation, handling, harvest, and curing. Pistillate inflorescence parts vary in color from bright, light green to deeper, dark green through dark purple to light yellow-gold to brown, sometimes with flowers having long reddish-orange to brown styles and stigmas. Indoor grown material is often lighter green to bright purple, while material cultivated outdoors tends to be darker green to green-brown to dark purple. The color should be consistent throughout each sample and should not show signs of gray or black, which are indicators of fungal infection. Inflorescence parts with a high density of glandular and non-glandular trichomes can appear bright whitish and crystalline.

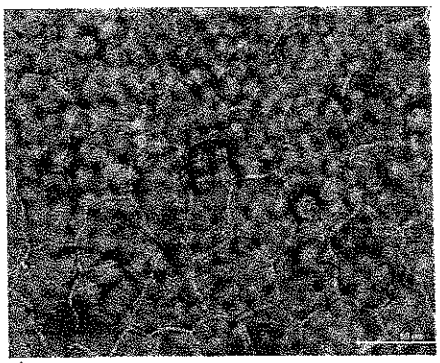
Taste and mouth feel: Bitter, somewhat acrid, resinous, sticky, and pungent.

Microscopic Identification

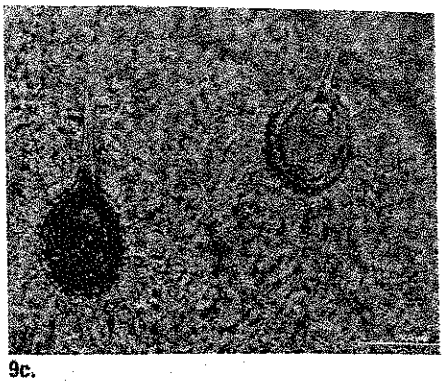
Bracts and Leaves: Microscopically, transverse sections of the leaflets and bracts show a dorsiventral structure. The palisade consists of a single layer (rarely 2 layers) of cylindrical cells and the spongy tissue of 2–4 layers of rounded parenchyma; cluster crystals of calcium oxalate are present in all parts of the mesophyll. The upper epidermis cells bear unicellular, sharply pointed, curved conical trichomes, approximately 150–220 µm long, with enlarged bases containing cystoliths of calcium carbonate; the lower epidermis bears conical trichomes, which are longer, approximately 340–500 µm, and more slender, but without cystoliths. Both upper and lower epidermises bear numerous glandular trichomes, and on the underside glandular trichomes are especially abundant over the midrib. The glandular trichomes are of 3 types: (1) a long multicellular stalk and a multicellular head with approximately 8 radiating club-shaped cells; (2) a short unicellular stalk and a bicellular, rarely 4-cell, head; (3) sessile (without stalk) with a multicellular head. Both upper and lower epidermises in the midrib region are followed by a few layers of collenchyma. The vascular bundle is composed of phloem, made up of small cells, and xylem vessels arranged in radial rows. The lower epidermis displays numerous trichomes of 3 types: non-glandular, non-glandular cystolithic,



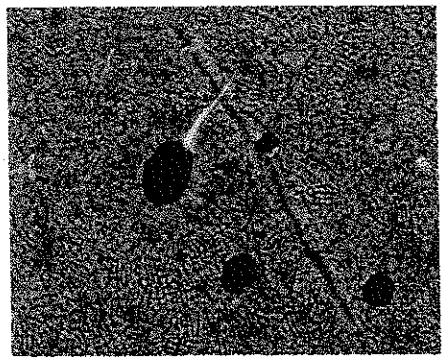
9a.



9b.



9c.

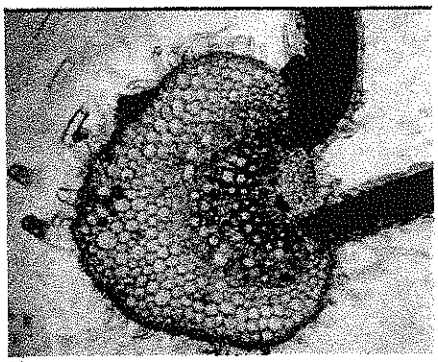


9d.

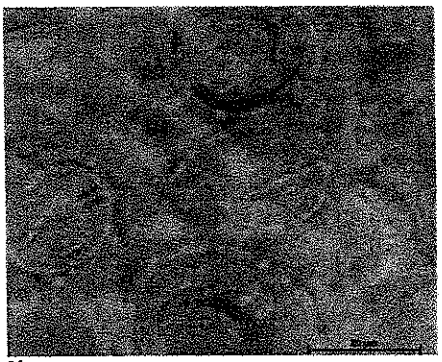
9e.

9f.

9g.



9h.

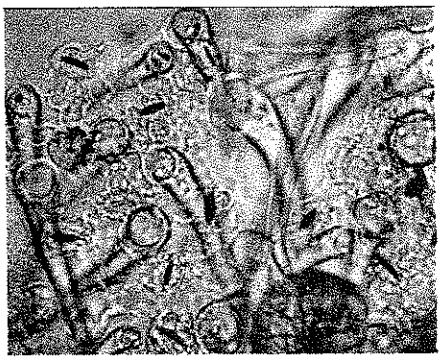


9i.

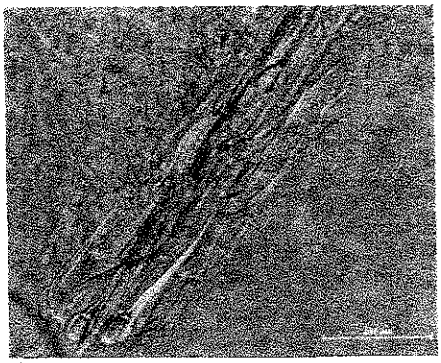
Figure 9 Microscopic characteristics of cannabis

9a. Upper (adaxial) leaflet surface showing epidermal cells with anticlinal walls.
9b. Upper (adaxial) leaflet epidermis showing curved anticlinal walls.
9c. Cystolithic trichomes on the upper surface of the leaflet (surface view).
9d. Cystolithic trichomes on the upper surface of the leaflet (surface view; polarized light).
9e. Cystolithic trichomes on the upper surface of female flower bract (surface view; polarized light).
9f. Cystolithic trichome (lateral view; polarized light).
9g. Cystolithic trichomes on the leaflet margin (lateral view; polarized light).
9h. Transverse section at the leaflet midrib.
9i. Stomata on the lower (abaxial) surface of the leaflet (surface view).
9j. Lower (abaxial) leaflet surface showing long unicellular non-cystolithic trichomes

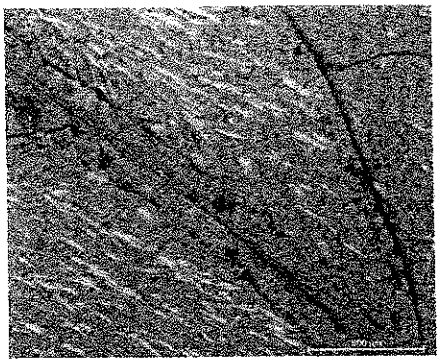
Microscopic images courtesy of: (9a-e; g-l) University of Mississippi, University, MS; (9f, m) Reinhard Länger, AGES PharmMed, Vienna, Austria; (9n) ©2013 David J. Potter, Salisbury, UK; (9o–u) Elan Sudberg, Costa Mesa, CA.



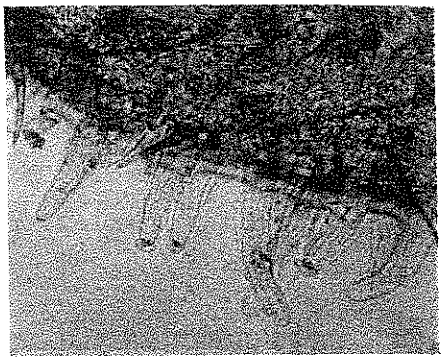
9j.



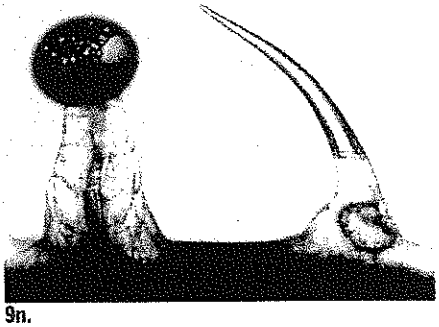
9k.



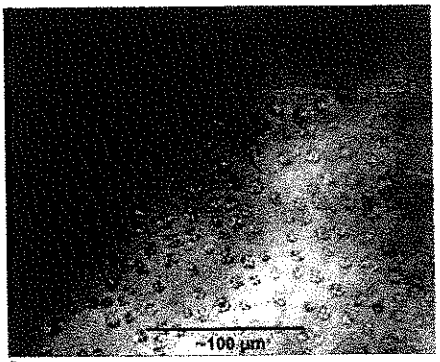
9l.



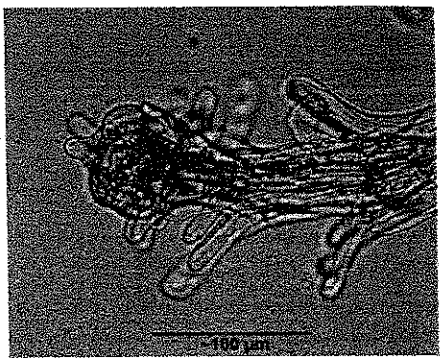
9m.



9n.

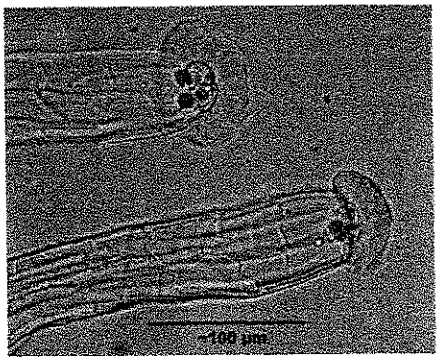


9o.

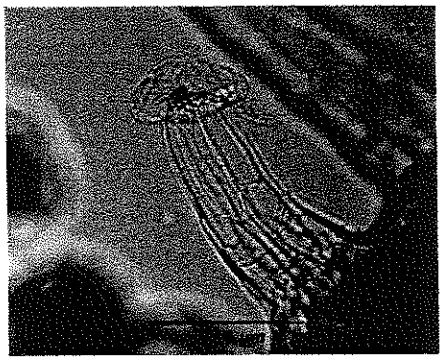


9p.

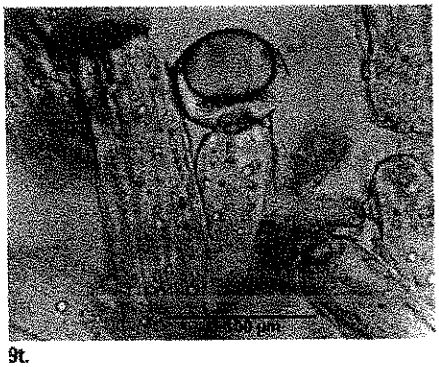
9q.



9r.



9s.



9t.

9u.

Figure 9 (continued) Microscopic characteristics of cannabis

9k. Non-cystolithic trichomes on the lower leaflet surface (polarized light).

9l. Non-cystolithic trichomes on the lower surface of the bract (polarized light).

9m. Multicellular-stalked glandular trichomes on bract.

9n. Multicellular-stalked glandular (left) and non-glandular cystolithic trichomes (right).

9o. Upper epidermis of bracteole showing underlying calcium oxalate cluster crystals in the mesophyll.

9p. Terminal end of a senesced stigma.

9q. Mid-section of a senesced stigma showing unicellular trichomes with rounded ends.

9r. Multicellular glandular trichomes.

9s. Multicellular glandular trichome showing orange-brown resin-oil deposits.

9t. Glandular trichome showing orange-brown resin-oil deposits.

9u. Sessile glandular trichome showing orange-brown resin-oil deposits either exuding or retracting through the stalk.

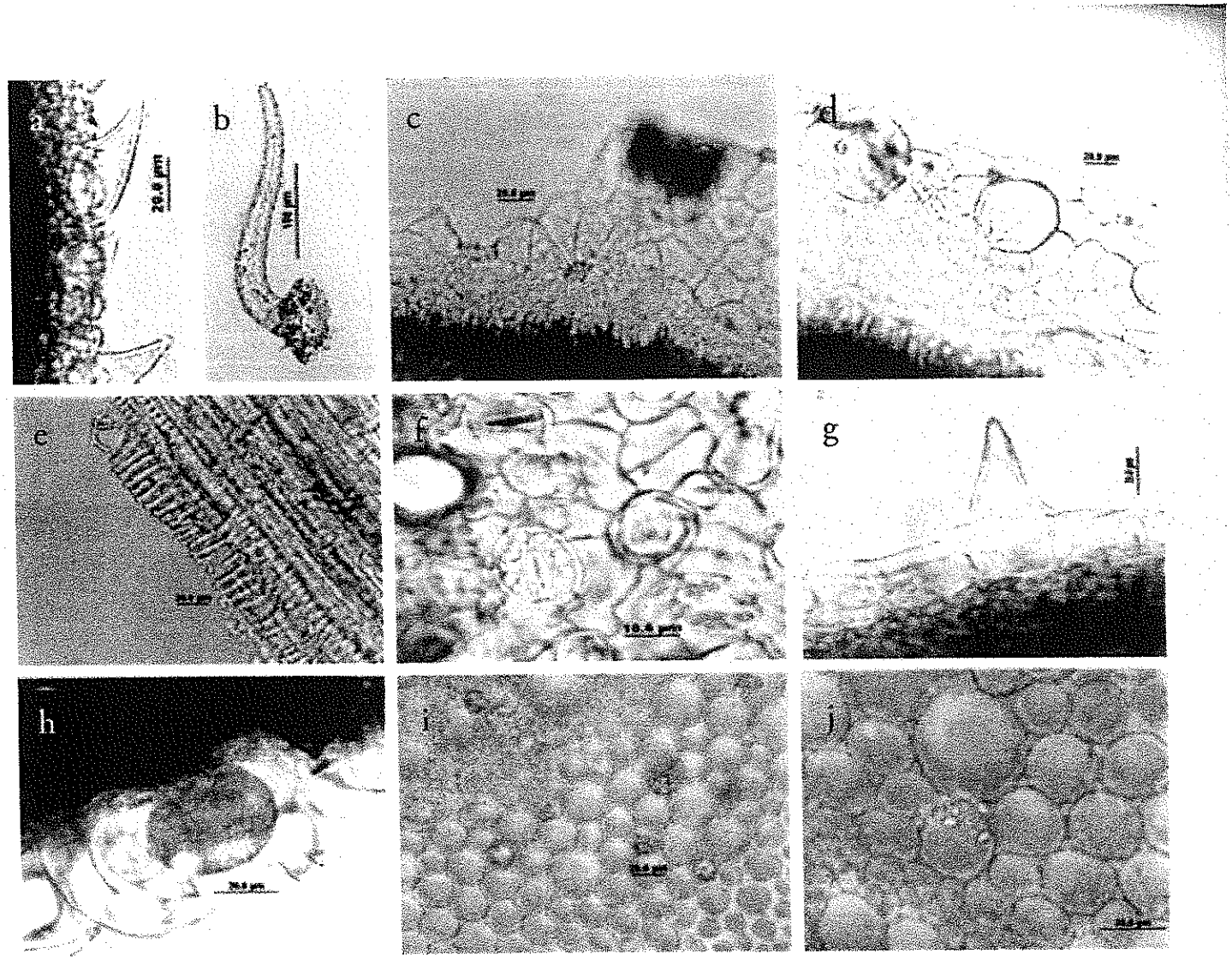
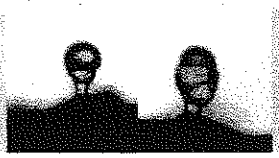
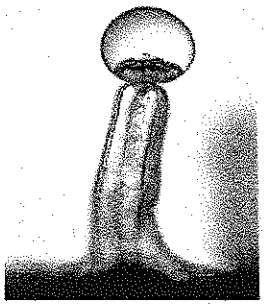


Figure 10 Microscopic characteristics of cannabis inflorescence powder

10a. Non-glandular conical trichomes on the upper epidermis of leaflet.
10b. Cystolithic trichome with warty cuticle.
10c. Head of glandular trichome showing cells radiating from basal cells.
10d. Surface view of epidermis showing trichomes and cystoliths.
10e. Fragments of vessel elements showing spiral wall thickenings.
10f. Lower epidermis showing anomocytic stomata.
10g. Non-glandular conical trichome with cystolith.
10h. Head of a glandular trichome covered with cuticle.
10i. Cortical parenchyma showing crystals of calcium oxalate.
10j. Cortical parenchyma showing simple starch grains.
Photographs courtesy of: University of Mississippi, University, MS.

Table 2 **Types and distribution of cannabis trichomes**

Trichome type		Cannabinoid-producing	Distribution	Image
Glandular	**Sessile (unstalked) glands**	Yes	All aerial epidermal tissues. Especially abundant on the underside (abaxial) surface of leaves and bracts and outer surface of bracteoles.	
	Bulbous (one-cell-stalked) trichomes	Possibly	All aerial epidermal tissues.	
	Multicellular-stalked trichomes	Yes	Bracts, bracteoles, and (rarely) on uppermost leaves; both surfaces.	
Non-glandular	**Cystolithic trichomes**	No	Leaves, bracts; mostly upper epidermis only.	
	Non-cystolithic trichomes	No	All aerial epidermal surfaces.	

Photographs © 2013 David J. Potter, Salisbury, UK.

and glandular. Clusters of calcium oxalate crystals are scattered in the ground tissue. The simultaneous presence of cystolithic trichomes on the upper surface and non-cystolithic trichomes and sessile glandular trichomes on the lower surface of the leaflets is characteristic of cannabis (UNODC 2009).

Bracteoles: Bracteoles have an undifferentiated mesophyll of about 4 layers of cells, the lower hypodermal layer having a cluster crystal of calcium oxalate in almost every cell. The abaxial surface bears numerous bulbous, sessile, and stalked glandular trichomes as well as unicellular conical trichomes. These trichomes are most numerous where the bracteole curves in to enclose the ovary or fruit.

Flowers: In the stigmatic epidermis, nearly every cell has an extended papilla about 90–180 µm long with a rounded apex.

Stem: The stem epidermis bears very few trichomes similar to those of the leaves. In cross-section of the stem, large, unbranched laticiferous tubes can be seen in the phloem. Well-developed bundles of pericyclic fibers are present to the interior of the phloem. Both pith and cortex contain calcium oxalate cluster crystals, about 25–30 µm in diameter.

For microscopic examination, leaves, bracts, and twigs can be mounted in alcohol, water, or chloral hydrate solution. Some compounds may be diluted or lost when prepared in this manner so these samples should not be used for quantitative analysis

Commercial Sources and Handling

In commerce, cannabis generally refers to the dried inflorescences and subtending leaves and stems of the female plant, commonly referred to as the bud. Considerable efforts in breeding and selection have produced cannabis strains that are uniquely suited for either fiber (hemp; rich in bast phloem cells in the stem) or drug production (cannabinoid-

containing resin secreted by epidermal glands) (Small and Marcus 2002).

The most important cannabinoid in this context is the psychoactive molecule THC. Fiber types are economically important in China, Europe, Canada and many other territories, and grown in subtropical and temperate climates. Drug types however are more typically acclimatized to semi-tropical zones. In Canada, most Western US states, and Northern Europe, the climate is not optimal for most drug strains, encouraging indoor or greenhouse cultivation. Since the 1970s, in the US and Canada, a law enforcement crackdown and large-scale eradication efforts may have inadvertently encouraged more indoor growers. Breeding for high THC strains (predominantly for recreational purposes) has occurred historically and very aggressively over the past 40 years, with growers in California, the Pacific Northwest and British Columbia, and Holland crossing plants of Afghan, Columbian, and Mexican origin in order to increase THC yields well above 10% THC. Potency is especially high when only female plants are grown. Unfertilized female cannabis plants, (widely known as sinsemilla, a Spanish term meaning without seeds) utilized no energy in seed production and diverted more to total THC biosynthesis. Later changes obtained through breeding and controlled indoor growing conditions led to strains with increased total THC potency.

In the 1960s and 1970s, the average percentage of THC in herbal cannabis was less than 1%, although anomalous samples reaching 9.5% were reported (reviewed in Mikuriya and Aldrich 1988). In 1980 (Turner 1983) average total THC concentrations were less than 1.5%, but rose to approximately 3.3% in 1983 and 1984, fluctuated around 3% until 1992, and increased to 4.7% (average) in 1997. Since 1997, due to the increasing prevalence of strains grown using a variety of techniques, samples have been found to contain a mean of 8.8% and anomalous samples have contained as high as 29% total THC. In the same time period, other cannabinoid concentrations (e.g., CBD) remained relatively stable (ElSohly et al. 2000; Mehmedic et al. 2010).

In the European Union (EU) as a whole, total THC potency of crude cannabis has not had the same steady upward trend as in the US. For example, between 1998 and 2002 EU supplies ranged from a low of 1.1% [Hungary 2002], a high of 16.9% [Italy 1998], and a mean of approximately 7.7% total THC (EMCDDA 2004). In most European countries the current upper legal limit for cultivated cannabis for industrial purposes is 0.2% THC (for comparison Canada: 0.3%) with a ratio of CBD to THC greater than one (UNODC 2009). There are currently no minimum or maximum THC-CBD concentrations legally mandated.

Comparison of total THC values, as well as interpretation of trends in most countries, should be taken as relative numbers due to intraspecies differences, inconsistent sampling, and variance in analytical techniques, among other factors affecting total THC concentration and yields. Reported US values can be taken as more, but still relatively, consistent, as they are predominantly based on analyses through NIDA's Marijuana Potency Monitoring Program.

In comparison with THC-predominant strains, fiber strains contain < 1% total THC and have a very low level of psychoactivity (De Backer et al. 2009; Galal et al. 2009). Additionally, due to putative therapeutic effects of CBD, CBD-predominant strains are being developed both domestically and internationally.

Sourcing

Cannabis is cultivated in at least 172 countries (EMCDDA 2008). North America is the largest self-supplying market for herbal cannabis. Europe is the largest consumer market for cannabis resin, which is predominantly supplied by Morocco (EMCDDA 2012).

There are 3 primary sources of indoor and outdoor cultivated cannabis in the United States: 1. Federally legal material; 2. Material that is regulated by select states; 3. Material that is traded illegally according to state or federal law. Sources in other countries vary, with some (e.g., the Netherlands) exerting national controls on the production of cannabis. Despite such national controls, illegal supplies still exist. Sources in the US are briefly described below.

Federally Legal Cannabis: Because cannabis is classified as a Schedule I controlled substance, its growth, transport, possession, and use are stringently restricted. The Coy W. Waller Laboratory Complex of the University of Mississippi is the only source of cannabis for research and medicinal purposes that is legally approved federally. Since 1968, the National Institute on Drug Abuse, and its predecessor agency, has contracted with the University of Mississippi (UM) to grow, harvest, and process cannabis and to provide material to licensed facilities across the country for federally approved research purposes. UM also receives and collects samples of cannabis seized by law enforcement to determine the potency of confiscated samples and to document national drug trends. The federal government continues to legally provide cannabis grown by UM for medicinal use to the few remaining patients in the Compassionate Investigational New Drug program started in 1978.

According to federal regulations, transfer of cannabis requires that material originate from a Drug Enforcement Administration (DEA)-registered facility and be sent directly to another DEA-registered facility. DEA-registered facilities that receive or transfer cannabis from or to a non-registered source, risk loss of their DEA registration and criminal penalties.

State-Regulated Cannabis: Numerous states have adopted initiatives allowing the medicinal use of cannabis and provide provisions for growing, accessing, possessing, and using

cannabis. Additionally, Colorado and Washington approved the non-medical use of cannabis in 2014. Regarding medical use, state regulations vary greatly, often varying between counties and municipalities, and often changing. Sometimes, cannabis may be grown by a patient who, based on a physician's recommendation, has been given approval to use cannabis medicinally. In other cases, designated caregivers cultivate cannabis and supply products to individual patients, or to members of a collective. Often, cannabis products are made available to patients through dispensaries. In all cases, the amount that can be grown or possessed is limited, with a variety of restrictions. Federal regulators have formally stated they will only take action against those not complying with state regulations governing the medicinal use of cannabis (Cole 2013), while maintaining their authority to respond when actions are deemed outside of compliance with state regulations. Additionally, Federal policies contend that states do not have the legal right to regulate cannabis. Thus, current exercise of federal policy is inconsistent with state policy and also inconsistently enforced.

Illegally Traded Cannabis: By far the overwhelming majority of cannabis used and traded in the United States is from illegal sources. Most of this material is traded for recreational purposes and lesser amounts are used for medical purposes, either with some basis of legal sanction or for unapproved medicinal use. Federal regulators actively work to curtail the illicit trade of cannabis.

In the United States, it is estimated that 17% of the domestic cultivation of cannabis occurs indoors under controlled conditions (Gettman 2006). Cannabis is grown in substantial quantities in every state within the US. Illicit imports predominantly originate in Mexico and, to a lesser extent, in Canada. This illegal supply primarily fulfills the illicit recreational market, but may find its way into medicinal use (UNODC 2011).

State-regulated or illegally traded cannabis is supplied from material produced either outdoors (in temperate, subtropic, or tropical zones) or indoors throughout all climates. Indoor production of cannabis is concentrated in developed countries, such as in North America, Europe, and Oceania.

Cultivation

There is a plethora of information regarding the cultivation of cannabis. The following information provided is specifically relevant to the development of material to be used medicinally. This information does not take into consideration any of the production methods specifically used to enhance total THC content for recreational purposes, the large number of strains that are bred, or practices employed for fulfilling various recreational desires (e.g. differing organoleptic profiles).

The Ministry of Health, Welfare, and Sport in the Netherlands developed a set of guidelines for the cultivation of cannabis specifically for purposes of medicinal use, all of which is grown indoors. The key guidelines are relevant to outside growing as well and are provided below along with additional information that can contribute to making a quality medicinal product. There are advantages and disadvantages with both indoor and outdoor growing.

Seed and Clone Selection

Selection of seed and clones is based upon both the strain desired and growing environment. Growing from seed results in a portion of the crop being male plants. This can be avoided by starting with clones. Cross breeding of *indica* and *sativa* strains has resulted in the hybrid commonly known as "skunk" which is reportedly 75% *sativa* and 25% *indica* and combines the high THC concentration of *C. sativa* with the growth and yield of *C. indica* (UNODC 2009).

Plant Selection

All material to be propagated, whether from seed or clone, must be identified to genus, species, variety, and chemotype. Plants should be traceable to origin and be free of pests and disease as is practically attainable to ensure healthy growth. Cuttings of female plants are typically used as propagation material for the production of cannabis in order to avoid male plants. Restricting male plants prevents seed fertilization, which allows the female plants to produce more flowers and increased production of resin and cannabinoids. Additionally, plants showing an anomalous concentration of yellow coloring, reflecting a lack of chlorophyll, will not be robust. This can result in misshapen leaves that can curl and turn into each other, and interfere with the growth of the plant. During the entire production process (cultivation, harvest, drying, packaging), the presence of male plants as well as different species, strains, or different plant parts must be monitored and removed if present.

Soil and Fertilization

Cannabis prefers neutral to alkaline loamy and sandy soils, with good water-holding capacity that is not subject to water logging, and an optimum pH of 6.5–7.2. In hydroponic growing, the nutrient solution is best at 5.8–6.0 (Cervantes 2006). Growing mediums for medicinal cannabis should be free of contaminants, such as those introduced from sludge, metals, pesticides, and waste products not required for appropriate growing. If manure is used, it should be thoroughly composted and must be devoid of human feces. Fertilizers should be used in such a way that leaching is kept to a minimum.

Irrigation

Irrigation should be controlled and only applied according to the actual needs of the cannabis plant to prevent over watering. The water used must contain as few contaminants as possible, such as fecal contamination, metals, herbicides,

pesticides and toxicologically hazardous substances (see Limit Tests).

Sexing

Under outdoor growing conditions, plants display all sexual characteristics at approximately 8 weeks, and to maturation from seed. These early, fully formed and receptive pre-flowers are used to determine the sex of the plants, to select seed parents for breeding and for culling if desired. Flowering can occur as early as 4 weeks and is dependent on strain and environmental conditions (UNODC 2009).

Male plants are generally culled, because of the relatively low total THC content of the leaves compared to the inflorescences of the female plant and to prevent pollination of all plants (Chandra et al. 2013). Male plants can be tested for their concentrations of specific cannabinoids (e.g., high or low-yielding THC or CBD strains) and those plants used for breeding depend on the class of cannabinoids desired.

Outdoor Cultivation, Planting, and Maturity

In the Northern Hemisphere, outdoor cultivation of seeds normally begins late March to early April (depending on environment). Full maturity of the plant is typically reached by 6–8 months (depending on variety). The THCA content increases as the plant matures, typically reaching its maximum at full budding stage, maintaining maximum levels for 2–3 weeks after budding, and declining with the onset of senescence. When grown from seeds outdoors, it is difficult to maintain a constant chemical profile due to changing environmental conditions (Chandra et al. 2013), and so some growers (e.g., the Netherlands) only produce approved medicinal products from material cultivated indoors where all conditions can be controlled. Autoflowering strains mature from seed to harvest in approximately 75 days.

There are numerous advantages to outdoor cultivation. Cannabis is relatively resistant to pests so pesticides are seldom needed (McPartland et al. 2000). Growing plants in well cared for soil allows for a more natural growing environment, provides stresses that the plant would experience in a natural environment, allows for natural light cycles, does not require the intensive investment in equipment needed for indoor cultivation, and, when done properly, is more ecologically sound. The primary disadvantages of cultivating cannabis outdoors is the inability to control all growing conditions, many of which affect the chemical profile, purity, and quality, potential for mold, and logistics of harvest and processing. For example, changes in weather may make it unfavorable to harvest when the plant material has reached desired maturity level and cannabinoid profile desired (Potter 2009), or may introduce moisture from rain or fog that could result in damage to the plants when harvest is anticipated. According to one report, cannabinoid and terpenoid profiles of outdoor and indoor cultivated plants were similar if the crops were harvested at the same stage of maturity, as denoted by complete style and stigma senescence. However, as outdoor cultivation requires a longer growing season than plants cultivated indoors, there is greater chance for fungal development (e.g., *Botrytis* spp.), especially in regions with autumn rain or fog (McPartland et al 2000; Potter 2009).

Indoor Cultivation

Indoor cultivation occurs in a variety of locations (basements, warehouses, converted grow houses, etc). The primary advantage of indoor cultivation is that it allows for control of environmental conditions that would otherwise influence cannabinoid profile. However, there are numerous disadvantages to indoor growing. Due to lack of insect predators normally abundant in outdoor growing environments, cannabis grown indoors can be subject to insect infestation, primarily spider mites. This leads to growers utilizing a host of pesticides that can contaminate the medicinal material. Soil composition and nutrient content and distribution in purchased commercial soil mixes may have significantly varied nutrient density that can lead to nutrient deficiencies or excesses that negatively affect the plant. With indoor growing, artificial lighting conditions may also cause burning of the plant. The following parameters are considered critical for indoor cultivation (Chandra et al. 2013).

Light: Cannabis requires high photosynthetic photon flux density (PPFD) for photosynthesis and growth. Because photosynthesis prefers certain wavelengths, PPFD is a more accurate metric than simple irradiance (measured in W/m^2) or light intensity (measured in Lux or Lumens). Chandra et al. 2008 report photosynthesis leveling off at 1500 $\mu mol/m^2/s$ PPFD. Different light sources can be used for indoor propagation, namely, fluorescent light bulbs for juvenile cuttings, and metal halide (MH) and/or high pressure sodium (HPS) bulbs for established plants. MH bulbs impart less PPFD than HPS bulbs per watt. Separate ballasts are required to regulate MH and HPS bulbs. MH and HPS bulbs should be placed 3–4 feet from the plants to avoid overexposure. Photoperiods of 12 and 18 hours are optimum for initiation of flowering and vegetative growth, respectively. Ultraviolet (UV_B) light increases THC yields, although Potter and Duncombe (2012) conclude that the small increase does not warrant human exposure to UVB.

Humidity and moisture: Humidity plays a crucial role in plant growth, starting from seed germination or vegetative propagation/reproduction through budding and harvesting. Juvenile plants require high humidity (ca. 75%), vegetative cuttings require a regular water spray on the leaves to maintain a high humidity in the microclimate until the plants are well rooted, while the active vegetative and flowering stages require 55–60% humidity (Chandra et al. 2013).

Temperature: The optimal temperature for growing any given plant depends on its genetic origin and original growth habitat. However, the photosynthetic maximum for strains of tropical origin is 25–30 °C with a lower maximum of 25 °C for plants of temperate origin (Bazzaz et al. 1975; Chandra et al. 2008; 2011a).

Carbon dioxide: Increased (doubled) ambient carbon dioxide levels stimulate both photosynthesis (50%) and water use efficiency (111%) in cannabis, resulting in increased growth (Chandra et al. 2008; Chandra et al. 2011b).CO_2 enrichment has been used in cannabis glasshouses for more than 35 years.

Irrigation: The optimal amount and frequency of watering needed depends on a variety of factors including environment, variety, and growth stage. Soil should be kept evenly moistened during the early seedling and vegetative stage. In established plants the top layer of soil should be allowed to dry out before watering (Chandra et al. 2013).

Air circulation: Regulation of gas and water vapor exchange affects thermal conductance and energy budget of the leaf and overall growth and physiology of the plants. Electric fans can be used to facilitate the circulation of air (Chandra et al. 2013). Plants exposed to oscillating fans produce stronger stems, which lessens lodging in varieties with heavy apical colas.

Seed Propagation

Seeds are typically planted in moist aerated soil. Germination usually begins after 4 days with all seeds generally germinating within 15 days. For enhanced winter germination, seedling heating mats can be placed under pots. A photoperiod of 18 h of cool fluorescent lights should be used for seedlings. When transferred to larger pots, cool fluorescent lights should be exchanged for full spectrum lights. At the end of the vegetative growth, the photoperiod can be reduced to 12 h to initiate flowering. Flowers should emerge within 3 weeks (Chandra et al. 2013).

Soil Propagation Through Vegetative Cuttings

Cuttings from the lower branches of select female plants can be used for vegetative propagation using a fresh segment of branch (6–10 cm long) that contains at least 3 nodal segments and planted in soil, a liquid hydroponic medium, or for in vitro micropropagation (Chandra et al. 2013).

For soil propagation, cut a soft apical branch at a 45° angle immediately below a node, immediately dip in distilled water to avoid any air bubble formation in the stem, then dip in rooting hormone (e.g., Green Light, US), and plant in pots of a coco natural growth medium with equal parts of sterile potting soil and fertilome (e.g., Canna Continental, US). Cover at least one of the nodes with soil. Irrigate regularly; rooting occurs in 2–3 weeks; after 6 weeks, transplant into larger pots. These can be maintained in a constant vegetative state with 18 h light exposure (Chandra et al. 2013; Potter 2009).

Hydroponics

A small branch consisting of a growing tip with 2 or 3 leaves is cut and immediately dipped in distilled water. Prior to dipping the cutting in a rooting compound, a fresh cut is made just above the first cut. The cuttings are inserted one inch deep into a rockwool cube or a hydroton clay ball supporting medium. Plants are supplied with vegetative fertilizer formula (e.g., Advanced Nutrients, Canada) and exposed to a diffused light: dark cycle (18:6) for vegetative growth. Rooting initiates in 2–3 weeks, followed by transplantation to a larger hydroponic system.

Micropropagation

Seed raised plants are highly heterozygous due to the allogamous nature of cannabis, while vegetative propagation of a selected mother plant can only produce a certain number of cuttings at a time, thus presenting difficulties when large scale cultivation of cannabis is needed. Micropropagation and tissue culture techniques have tremendous potential to overcome these problems. Direct organogenesis using nodal segments and axillary buds is the most reliable method for clonal propagation since it upholds genetic uniformity among progenies (Hartsel el al. 1983; Mandolino and Ranalli 1999; Slusarkiewicz-Jarzina et al. 2005). An efficient micropropagation protocol for mass propagation of drug-type strains using apical nodal segments containing axillary buds has been reported (Lata et al. 2009a; 2009b) as well as the micropropagation of hemp using shoot tips (Wang et al. 2009). Somaclonal variation produced by formation of calli is a fundamental step for the genetic manipulation and improvement in crops (Lata et al. 2002). Micropropagation of cannabis through callus production has been reported, including production of roots through cannabis calli (Fisse et al. 1981), occasional shoot regeneration (Mandolino and Ranalli 1999), and high frequency plant regeneration from leaf tissue derived calli (Lata et al. 2010).

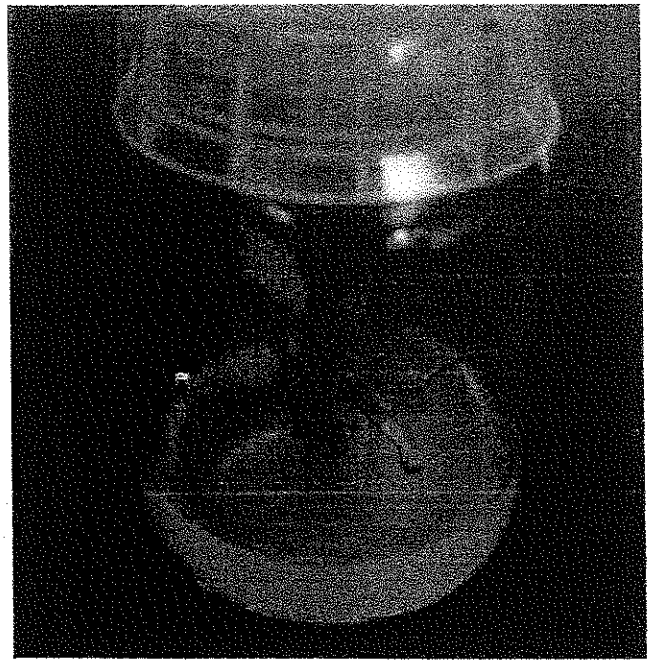
Genetic Integrity

Micropropagation of shoot tips, axillary buds, and nodal cuttings generally maintain their genetic fidelity. However, use of plant growth regulators and prolonged cultivation of the plant can result in somaclonal variation (Chandra et al. 2013).

Diseases and Pests Associated with Cannabis Cultivation

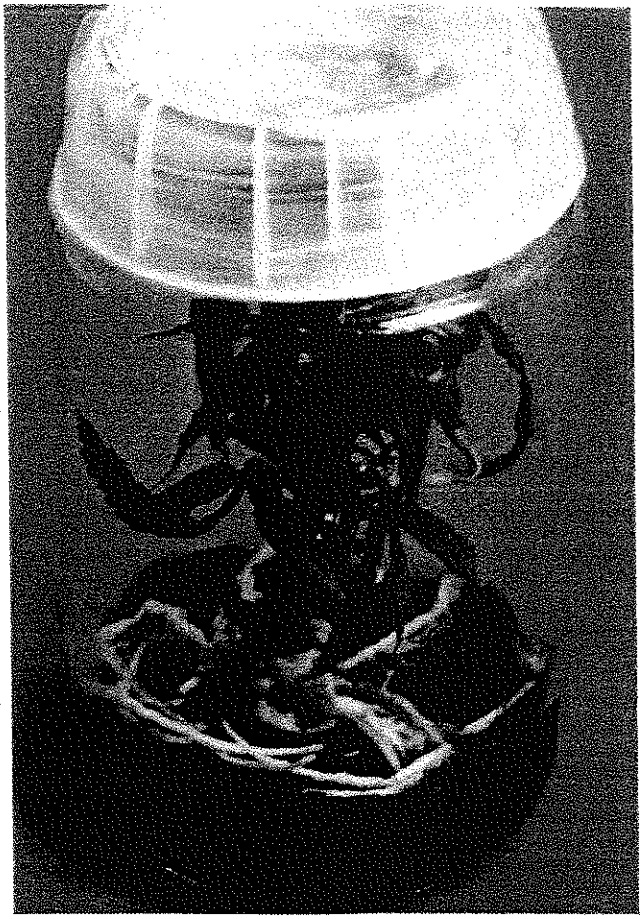
There are a host of pests, bacteria, and fungi associated with both indoor and outdoor cultivation of cannabis. Generally speaking, plants cultivated outdoors in a healthy environment are relatively resistant to pests, so commercial pesticides are often not needed (EMCDDA 2012; McPartland et al 2000), and with indoor cultivation, most conditions can be controlled.

In outdoor cultivation, small animals, such as birds and rabbits can eat sown seeds and emerging greenery. Insects and nematodes are not a significant problem in healthy growing environments that maintain healthy populations



11a.

11c.



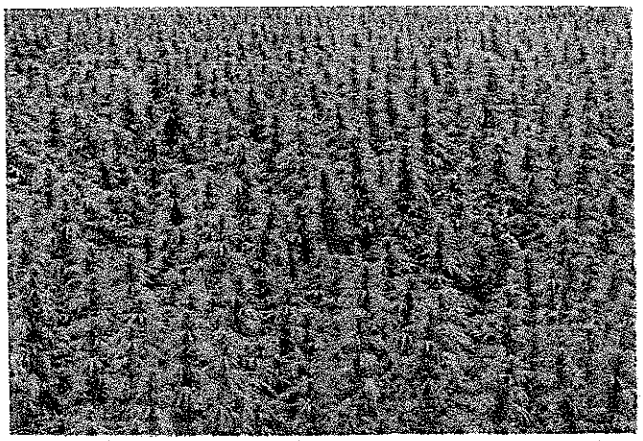
11b.

11d.

Figure 11 **Cultivation of cannabis at the University of Mississippi**

11a–b. Micropropagated cannabis plants.
11c. Rooted plant.
11d. Field-grown cannabis.
Photographs courtesy of: University of Mississippi, University, MS.

11e.



11f.

Figure 11 (continued) Cultivation of cannabis at the University of Mississippi

11e. Partially grown plants.

11f. Fully grown plants.

Photographs courtesy of: University of Mississippi, University, MS.

Figure 12 Common fungal contamination of cannabis

Photographs courtesy of: WAMM, Santa Cruz, CA

of natural predators. The primary concern with outdoor growing, in addition to security and weather, are mold and fungi, which have been addressed in detail by McPartland (1996), among others.

In indoor cultivation systems, the primary pests of concern are spider mites, thrips, aphids and white flies. Growers can use a host of natural (e.g., copper or sulfer sprays, garlic [*Allium sativum*] and neem [*Azadirachta indica*] solutions) or synthetic pest controls, while some companies growing for medicinal preparations (e.g., GW Pharmaceuticals, UK) control indoor pests with natural predators. Application of any treatment has to be timed in a manner that allows the treatment agent to be cleared prior to harvest, as use of commercial pesticides at time of harvest can pose a health risk to consumers, and all treatments can affect the organoleptic profile of the material.

Outbreaks of hepatitis associated with cannabis use have been reported in Germany (Cates and Warren 1975) and Mexico (Alexander 1987), where human excrement was used as a fertilizer.

Harvest

Pre-harvest Considerations

Cannabinoids and terpenoids are predominantly biosynthesized and stored in the trichomes of the plant (Mahlberg et al. 1984; Malingre et al. 1975; Turner et al. 1980a), which are at their highest densities on mature inflorescences. Timing of harvest can be determined based on chemical analysis of the inflorescences, specifically for those compounds that are

Physical Evaluation for Determining Optimal Harvest Time

1. When the largest percentage of styles and stigmas turn reddish brown and shrivel (senesce) (Chandra et al. 2013; Potter 2009); the higher the percentage of senesced and stigmas and styles, the greater the maturity. Appropriate harvest times based on percent of senesced stigmas and styles are given as 75% (UNODC 2009) and 95% (Clarke 1981), varying according to the variety and the grower's personal preference.
2. Firmness of the inflorescence, which should display a relatively firm resistance when pressed.
3. Some growers suggest that the ideal harvest timing is indicated by the color of the glandular trichomes. The resin head on newly formed trichomes is crystal clear, but eventually turns more cloudy and then almost opaque white before finally turning brown with age. In some cases, trichomes turn brown without experiencing a white phase. Although peak potency and harvest timing is often associated with the clouding of the trichome, research has shown that peak potency is achieved in plants exhibiting clear trichomes (UMiss 2013 personal communication to AHP, unreferenced).
4. Organoleptic profile. At maturity, the aromatic terpenoid composition of the inflorescence matures to the pungent, often unique, strain-specific aroma. Over-maturity can be observed as the inflorescences begin to develop leaves (Corral 2012, personal communication to AHP, unreferenced). The timing of harvest affects the total cannabinoid content (potency), its psychoactive effects, and medicinal benefits.

13a. **13b.** **13c.**

Figure 13 Maturation of cannabis inflorescences

13a. Maturing female inflorescence showing no styles and stigmas.
13b. Semi-mature female inflorescence showing light-colored styles and stigmas.
13c. Matured female inflorescence showing shriveled reddish-brown styles and stigmas.
Photographs courtesy of: (13a) WAMM, Santa Cruz, CA; (13b & c) © 2013 David J Potter, Salisbury, UK.

most desired (e.g., tetrahydrocannabinolic acid [THCA] and cannabidiolic acid [CBDA]) or more usually through observation and organoleptic evaluation. Cannabinoid ratios of a particular strain of cannabis are genetically determined (de Meijer et al. 2003), while cannabinoid levels (potency), which are determined by biosynthetic pathways, are subject to the influence of age and environmental factors, particularly temperature, light, and humidity. In general, cannabinoid content reaches a maximum when inflorescences are fully ripe and remain at this level until the onset of plant senescence (Chandra et al. 2013; Potter 2009).

When using analytical techniques for determining optimal harvest times, high performance liquid chromatography (HPLC) or gas chromatography (GC) are the most appropriate tools for quantitation of desired compounds. These 2 methodologies can give different quantitative values for the same plant, so consistent baselines with either method should be established to determine the time of maximal potency. Thin layer chromatography (TLC), promoted by commercial testing laboratories, provides a qualitative comparison of cannabinoids, but the method is not quantitative. High performance TLC (HPTLC), can provide more accurate quantitative data than standard TLC, but remains secondary to more accurate methodologies.

All stages of maturity are often present within an inflorescence with mature flowers occurring at the base of the inflorescences and younger, less mature flowers at the apices or tips. Towards the end of the flowering process, plant growth slows and fewer new florets are formed within the inflorescence.

Time of cannabis harvest depends upon which class of compounds is desired (Potter 2009). Total THC content varies widely with the particular strain and part. Analyses by the United Nations (UNODC 2009) report total THC values as highest in the inflorescences (10–12%) followed

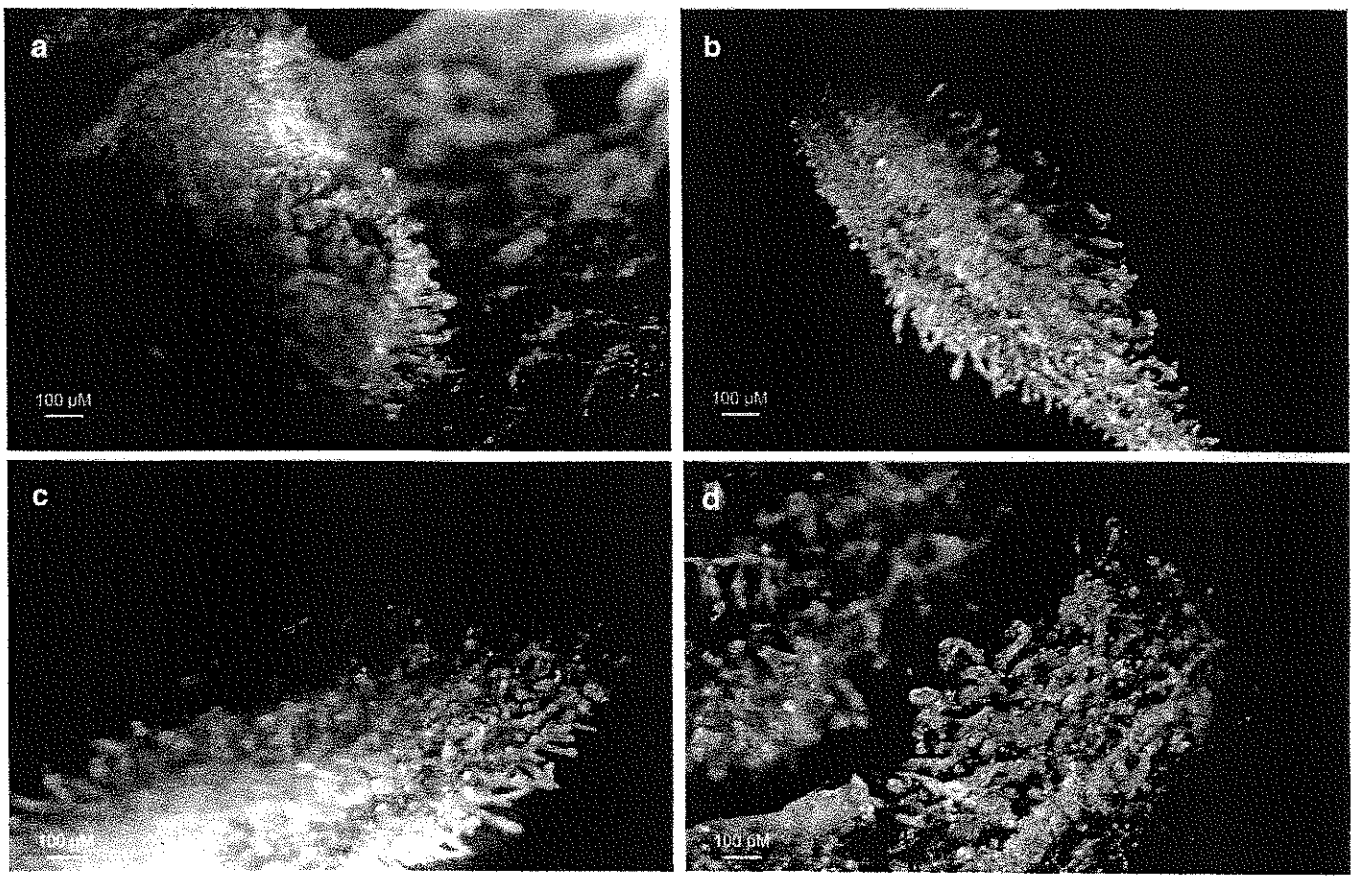
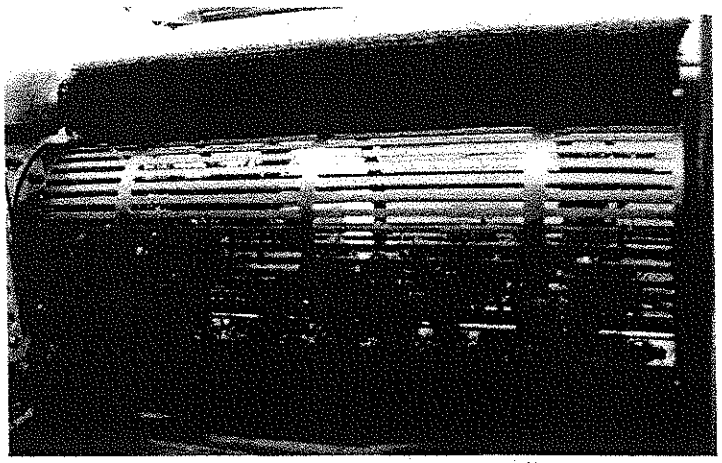
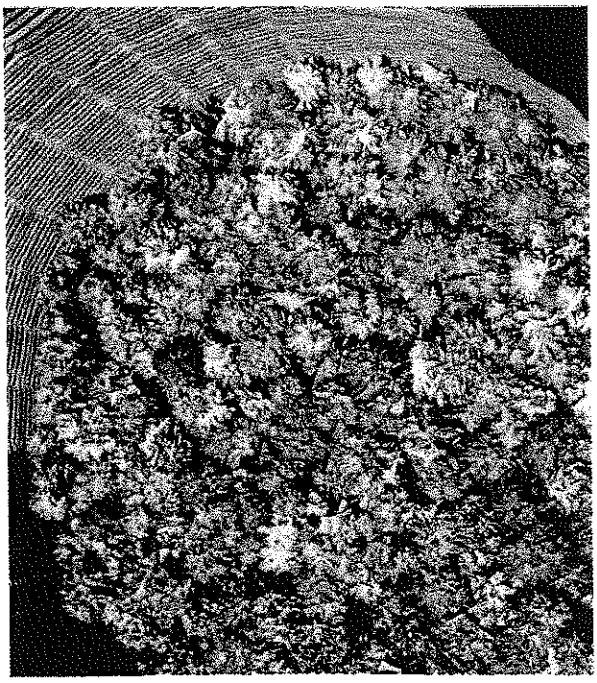


Figure 14 Glandular trichomes of *Cannabis sativa* showing THC-containing ducts at various stages of flowering
14a. 2 weeks.
14b. 4 weeks.
14c. 6 weeks.
14d. 8 weeks post flowering.
Photographs courtesy of: University of Mississippi, University, MS.

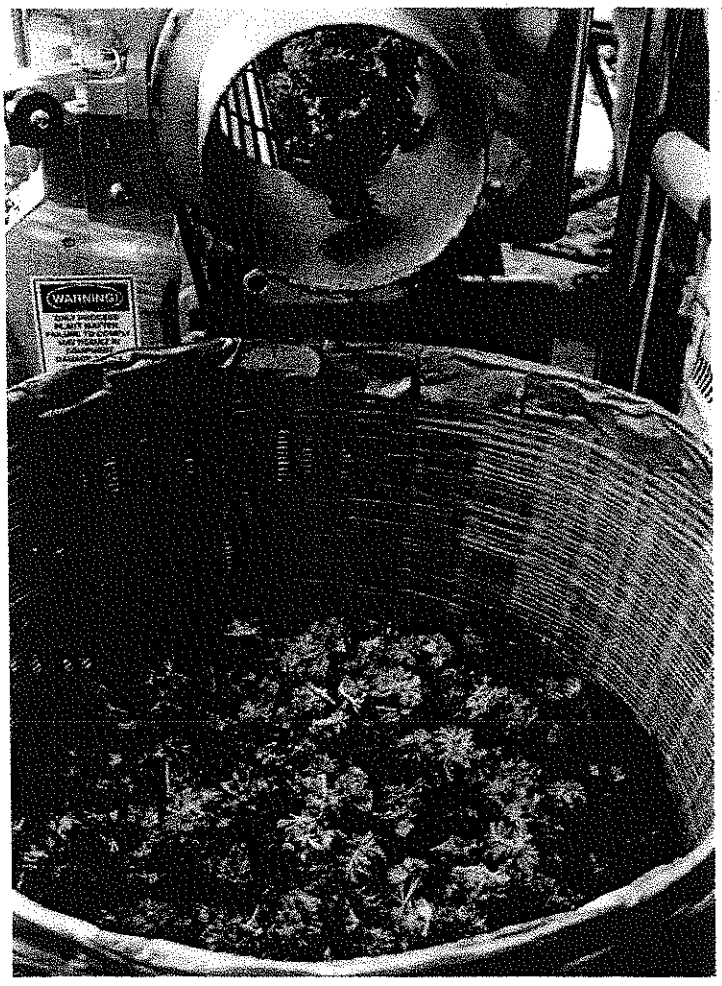
15a.



15b.



15c.



15d.

Figure 15 Mechanical trimming of freshly harvested cannabis inflorescences

15a. Hopper feeding freshly harvested inflorescences into the trimmer.

15b. Leaves extend through the openings and are trimmed by blades below the spinning chamber, removing ~90% of subtending leaves.

15c. Trimmed inflorescences awaiting visual inspection before final trimming by hand.

15d. Trimmed inflorescences tumble into catch basin.

Photographs courtesy of: WAMM, Santa Cruz, CA.

by the leaves (1–2%), stalks (0.1–0.3%), and roots (< 0.03%). Cannabinoids are almost completely absent in clean seeds.

The ratio of THCA and CBDA is under strict genetic control. Research suggests that the production of THCA or CBDA, from the common precursor cannabigerolic acid (CBGA), is closely controlled by 2 co-dominant alleles at a single locus (de Meijer et al. 2003). As a result, cannabis plants can be identified as belonging to any one of 3 chemotypes. These can be THCA dominant (homozygous for the THCA synthase allele), CBDA dominant (homozygous for the CBDA synthase allele), or contain an approximately equal mixture of the 2 (heterozygous condition). Cannabis today is almost entirely derived from the THCA dominant chemotype. The majority of seeds sold commercially for the cultivation of recreational cannabis in Europe have been found to be of the homozygous THCA chemotype, with a small minority being the heterozygous mixed profile THCA+CBDA chemotype (EMCDDA 2012).

Over the past 3 decades worldwide, optimization of growing techniques, domestic production versus imported material, and selective breeding and cloning, among other parameters, have focused on the development of increasingly potent THC-yielding strains. Production of high cannabinoid CBDA strains has been of more limited interest, but breeding of CBDA-rich strains has been achieved (de Meijer et al. 2003). An increasing number of heterozygous mixed THCA/CBDA strains are being produced to provide users with material with different pharmacological activity than the pure THCA type; however, this is an exception not a rule in both legal and illegal cannabis production.

Optimal Harvest Times

The optimal harvest time depends on the level of constituents desired and environmental conditions of the crop. Some growers (e.g., University of Mississippi) perform analyses of their raw material daily to determine the optimal time of harvest for peak THCA concentrations. Generally, optimal harvest time is when the inflorescences reach full maturity (Chandra et al. 2013). Optimal harvest time can also be determined visually when at least ~75% of the stigmas turn brown and shrivel (senesce) (UNODC 2009). With higher degrees of maturity, higher concentrations of THC will be produced. However, when resin heads shift from a clear or cloudy color to brown, this indicates the conversion of THC to CBN (Potter 2009)

There are 2 primary ways to harvest inflorescences: harvesting individual buds or branches as they ripen, and harvesting the entire plant. When harvesting individual buds or branches, the mature upper buds are harvested first, usually by cutting approximately 38 cm (15-inch) long branch sections, while the lower branches are given more time to develop (Chandra et al. 2013). Collecting when buds ripen allows other buds hidden in the canopy to ripen, a process that takes approximately 10 days. Buds closest to the outer edges, capturing the most light, typically ripen first.

Harvest is done in 4 primary steps: clipping a bud-filled stem from a plant; clipping the bud from the stem; removing large leaves from the bud; removing small leaves from the bud. Alternatively to harvesting individual ripened inflorescences or branches, whole plants can be harvested and hung upside down in a drying room. The large leaves are removed while the plant is hung and is followed by a manicuring as described below. Drying or storage in unclean barns and other such areas can lead to significant microbial contamination.

Post-harvest Handling

Directly after harvesting, plant material must be processed in a manner that protects it from pests and contaminants, packaged in a manner that prevents damage, dried as soon as possible to prevent chemical degradation, and protected from excess exposure to light and humidity.

Manicuring (trimming): After harvesting the inflorescences, the leaves immediately subtending the buds as well as any dead leaves or stems are trimmed or removed. Manicuring is best accomplished when the inflorescences are fresh for maximum preservation of the trichomes, which when fresh, are pliable rather than brittle: dry trichomes break off easily. Manicuring can be accomplished by hand trimming, machine trimming, or a combination of both.

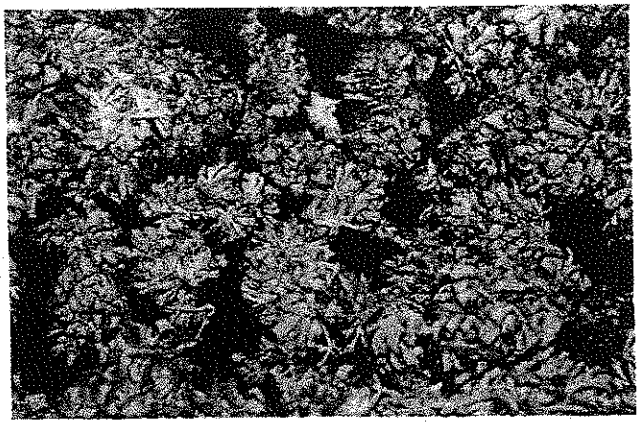
Budding branches (rather than the entire plant) are harvested and the buds are removed manually and the subtending leaves removed either by hand or with a trimming machine. Machine trimming removes approximately 90% of subtending leaves. If desired, the rest can be removed by hand after the buds are fully dry.

At UM, buds are carefully rubbed through different sized screens (e.g., mesh of ~100 strands per square inch) to separate small stems and seeds. Automated plant processing machines can also be used to separate large stems from the useable biomass.

Manicuring is sometimes done by working over a screen (mesh of ~100 strands per square inch) to allow for collection of the trichomes that fall off in the manicuring process, a technique also used in the processing of hops. The loose trichomes (commonly known as "kief"), have very little vegetation, contain high concentrations of cannabinoids, and can be used in a variety of medicinal preparations. The multi-fingered leaves surrounding the inflorescence are often removed (commonly referred to as "trim"), have more glands than larger lower leaves, and yield a higher concentration of cannabinoids. This post-harvest processing should be conducted in cool temperatures with good air circulation to prevent molding.

Drying

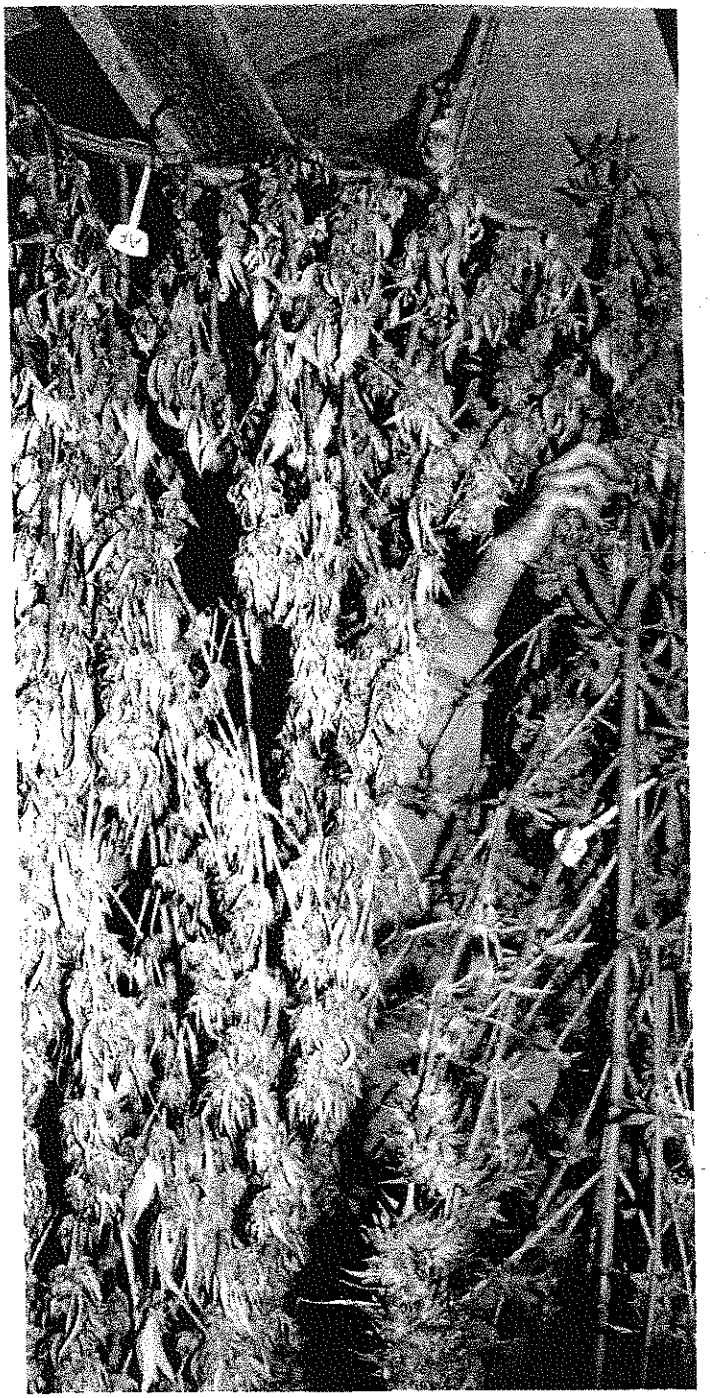
When drying medicinal plants, great care must be taken in the drying process (Chen and Mujumdar 2006), both for preservation of putative medicinally relevant compounds



16a.

16b.

16c.



16d.

Figure 16 **Drying cannabis**

16a. Freshly harvested inflorescences drying on screens.

16b. Drying leaves to be used in tincture and edible preparations.

16c. Moldy leaves.

16d. Hang-drying whole plants.

Photographs courtesy of: WAMM, Santa Cruz, CA.

and to reduce the risk of molding. Drying is usually done by either cutting the flowering tops from the plant or by hanging the entire plant upside down in shaded areas.

In the production of medicinal cannabis at UM, a commercial tobacco drying barn (e.g., BulkTobac, Gas-Fired Products, Inc., US), is used and material is dried at 40 °C for 12–15 h. Prior to drying, larger leaves and stems are removed from the mature buds. The buds can be dried whole or halved or quartered for quicker drying. The material is fully dried when the central stem of the floral cluster snaps, when bent, rather than remaining pliable (Chandra et al. 2013).

Varying drying practices are employed by state-approved growing facilities. These practices are predominantly designed to preserve maximum cannabinoid content and a myriad of organoleptic characteristics. Numerous references (e.g., Cervantes 2006; Clarke 1981; Rosenthal 2010; among others) describe a multi-step process of curing and drying in much the same way that tobacco leaves are prepared. When drying by hanging, drying is complete when the leaves next to the flowering tops are brittle and the central stem snaps. This takes from 24–72 hours, depending on temperature and humidity. The moisture content of such plants is usually 8–13% (UNODC 2009). Many growers use fans and or heaters to maintain some control over the drying environment. Following are commonly employed drying practices.

Initial Drying: After the inflorescences are harvested and initially processed (trimmed), they are typically placed in single layers in boxes, on breathable trays, or screens that allow for steady airflow in a well-ventilated area. The initial drying is done for approximately 3 days at a temperature of approximately 15–21 °C and a humidity of approximately 35% until the inflorescences reach 25% of their original weight. Heaters are typically required to maintain a consistent temperature, fans are typically used to maintain a constant airflow, and sometimes dehumidifiers are used to remove moisture. Buds that are dried too quickly retain a greater amount of chlorophyll, which changes the qualitative organoleptic characteristics of the material (Corral 2012, personal communication to AHP, unreferenced). Excess humidity encourages molding.

Reports from state-approved markets indicate a preference for all but the tiniest leaves to be completely removed. However, some state-approved growers (e.g., in California) consider it advantageous to keep the surrounding leaves intact until the material is to be used. This creates a protective covering that shields the trichomes from damage in storage (Corral 2013, personal communication, unreferenced).

Final Drying: After the initial drying process, the inflorescences are often placed in plastic bags or glass containers, are initially closed and then opened every 12–24 h for 1–2 weeks until the material is completely dried. This allows the moisture that remains inside the buds to evaporate. Drying is sufficient when the small stem attached to the inflorescence snaps easily. If the stem bends, too much moisture remains. When completely dried, the inflorescences contain approximately 10% moisture (Clarke 1981). If not properly dried, mold can form over a several month period and is evident by smell, graying of color, a slippery feel, and loss of firmness. If dried completely in papsmither bags or on open trays, the outside of the inflorescence turns brittle, while the inside remains moist, increasing the potential to mold.

When drying, plants should be protected from light and should be minimally handled as the inflorescences bruise easily during handling. Bruised tissue will turn dark green or brown upon drying (Clarke 1981). At 45–55% humidity, buds will dry gradually over 1–2 weeks depending on inflorescence size. Humidity can be lowered to 20–40% to hasten drying times. Proper drying maintains the terpenoids, which give cannabis its characteristic organoleptic qualities. Improper drying, such as at high temperatures, dramatically alters the organoleptic profile.

Over time, decarboxylation of the cannabinoid acids occurs in dried flowers. The process is expedited by heat. During this period, cannabinoid acids decarboxylate into the psychoactive cannabinoids, and terpenoid isomerize to create new polyterpenes with tastes and aromas different from fresh floral clusters (Clarke 1981).

According to the Dutch Office of Medicinal Cannabis (OMC 2003), the moisture content of cannabis prior to packaging must be between 5–10%. Dutch consumers have reported a more pleasant flavor when the moisture content of buds is approximately 8%.

In the UK, in one investigation, plants were spread evenly on the floor of a well-ventilated drying room at a depth of approximately 15 cm. Gas burners maintained a constant temperature of 40 °C to a moisture content of approximately 15% (+/- 2%) and took 24 h. In another experiment, plants were hung from wires in the same drying room at 30, 40, and 50 °C. Mean times to achieve a finished moisture content of 15% were approximately 36, 18, and 11 h, respectively. Alternatively, the same cultivars dried in a glasshouse crop drying facility at 25 °C, but with different ventilation, took 4.5–5 days to reach the same moisture level. These latter prolonged drying conditions resulted in fungal and bacterial growth. Additionally, plants initially showing preliminary signs of fungal or bacterial damage further deteriorated under these conditions (Potter 2009).

Packaging

In the Netherlands, packaging of medicinal materials is done according to the European Pharmacopoeia Chapter 5.1.4 Microbiological Quality of Pharmaceutical Preparations and Substances For Pharmaceutical Use. These guidelines are specific to medicinal preparations used for inhalation, specifically to prevent microbial exposure. To reduce microbial loads, Netherlands cannabis may be subjected to gamma irradiation (dose < 10 kGy). Use of irradiation for ingredients in the US requires specific approval. Ungerleider et al. (1982), demonstrated that 15-20 kGy killed bacterial contaminants (*Klebsiella*, *Enterobacter*, and *Enterococcus*

spp.) in NIDA-sourced cannabis. In comparison, packaged meat and poultry may be irradiated with 70 kGy.

Treatment with irradiation of other medicinal plants (e.g., *Digitalis*, *Ephedra*, etc.) has been shown to negatively effect constituent profiles (Samuelsson 1992) and in other plant material to specifically lower terpenoid levels (e.g., cilantro, oranges) (Fan and Gates 2001; Fan and Sokarai 2002). Thus irradiation may similarly negatively affect the general composition and specific terpenoid profile of cannabis.

Storage

Once cannabis is properly dried, degradation of the primary cannabinoids is negligible, if protected from air and light and the material can remain active for many years. The UM produced material is stored in FDA approved polyethylene bags placed in sealable fiber drums. If stored for short periods of time, a storage temperature of 18–20 °C is used; for long-term storage a temperature of -20 °C is used. However, some sources (eg., Clarke 1981) suggest that freezing damages trichomes.

THC is especially sensitive to degradation by oxygen and light (Chandra et al. 2013) and decarboxylation of THCA to the active THC occurs in storage (Hazekamp 2007). Over time, the concentration of THC in cannabis products decreases slowly, while the concentration of CBN increases (Chandra et al. 2013; Ross and ElSohly 1999). In one experiment, approximately 90% of the THC content of dried plant material was still present after storage for 1 year at room temperature in the dark (Fairbairn et al. 1976). According to the same experiment, storage temperatures of up to 20 °C had little effect on stability of THC. Further evidence of cannabinoid stability was provided in an analysis of 3 dried samples from the turn of the 20th century, which were stored at room temperature with some possible exposure to light. The analysis detected trace amounts of THC, THC acid (1.39-1.79%), traces of other cannabinoids, and significant amounts of CBN (17.26–44.51%) and CBN acid (7.19–10.95%) (Harvey 1990).

A number of popular sources (e.g., Clarke 1981) recommend against freezing, which can cause the trichomes to become brittle and break off with handling. For the same reason, handling of dried material should be kept to a minimum. Additionally, according to Fairbairn et al. (1976), excessive handling of the inflorescences causes them to rupture exposing the cannabinoids to oxidation even when protected from light.

The stability of a 140-year-old ethanolic cannabis extract has been investigated (Harvey 1985). Using gas chromatography, it was shown that, while traces of THC, CBD, and CBC were present, most of the THC had decomposed to CBN. Additionally, cannabitriols were also present.

Natural Contaminants and Adulterants

Due to its widespread cultivation, there is little concern regarding adulteration of the plant itself. However, the large economic potential and illicit aspect of cannabis has given rise to a number of reported potentially hazardous natural contaminants or artificial adulterants in crude cannabis and cannabis preparations.

Natural contaminants: Several plant species have morphological characteristics comparable to *Cannabis* species, e.g., *Hibiscus cannabinus* (kenaf), *Acer palmatum* (Japanese maple), *Urtica cannabina* (an Asian species of nettle), *Dizygotheca elegantissima* (false aralia), *Potentilla recta* (sulphur cinquefoil, rough-fruited cinquefoil), and *Datisca cannabina* (false hemp), leading to occasional contamination of cannabis internationally (UNODC 2009). However, these plants can be readily differentiated from cannabis by inspection of their macroscopic and microscopic characteristics. More commonly, natural contaminants consist of degradation products, microbial (fungi and bacteria) contamination, and heavy metals. These contaminants are usually introduced during cultivation and storage (McLaren et al. 2008; McPartland 2002).

Adulterants: Growth enhancers and pest control chemicals, introduced during cultivation and storage, are possible risks to the producer and the consumer (McPartland and Pruitt 1999). There are anecdotal reports of the use of banned substances such as daminozide (Alar), the degradation product of which is the highly toxic hydrazine. Cannabis can also be contaminated for marketing purposes. This usually entails adding substances, e.g., tiny glass beads, to increase the weight of the cannabis product, or adding psychotropic substances, e.g., tobacco, calamus (*Acorus calamus*), and other cholinergic compounds, to enhance the efficacy of low-quality cannabis or to alleviate the side effects of cannabis (McPartland et al. 2008; McPartland 2008).

In the Netherlands, chalk and sand have been used to make cannabis appear to be of higher quality, the sand giving the appearance of trichomes. In the UK, similar adulterations have been made by adding glass beads with a similar diameter to trichome resin heads to cannabis (Randerson 2007). In Germany, lead has intentionally been added to street cannabis to increase its weight. Lead is readily absorbed upon inhalation and this adulteration resulted in lead intoxication in at least 29 users (Busse et al. 2008). Additionally, in the Netherlands, 2 chemical analogs of sildenafil (Viagra) were found in cannabis samples. In the UK, other contaminants including turpentine, tranquilizers, boot polish, and henna, have been reported (Newcombe 2006).

In recent years, various products laced with synthetic cannabinoids have appeared on the market. These are believed to mimic the effects of cannabis. These products are known by various names (e.g., "Spice" and "K2") and might be sold as "incense" or "natural smoking blends".

Like cannabis, some of these synthetic cannabinoids are Schedule I restricted substances. The Spice blend is reported to contain synthetic cannabinoids with a mixture of otherwise legal, safe, and non-psychotropic herbal dietary supplement ingredients including: damiana (*Turnera diffusa*), Chinese motherwort (*Leonurus japonicus*), and water lily (*Nymphaea* spp.). According to the National Institute on Drug Abuse (NIDA 2012), those using some of these various blends have been admitted to Poison Control Centers and report "rapid heart rate, vomiting, agitation, confusion, and hallucinations. Spice can also raise blood pressure and cause reduced blood supply to the heart (myocardial ischemia), and in a few cases it has been associated with heart attacks. Regular users may experience withdrawal and addiction symptoms."

Qualitative Differentiation

Cannabis used for medicinal purposes should be as free from foreign matter as practically possible (see Limit Tests). Medicinal material should be free of molds and bacteria that have a high likelihood of pathogenicity (e.g. *Aspergillus* spp., *E. coli* (O157:H7). Visible mold should be absent, material should be free of stems greater than 1.5 cm, only subtending leaves should be present, material should be free of metals to the degree allowed by a naturally occurring growing substrate, and free of pesticides and fungicides that could present a health hazard to the consumer. Microbial standards should be adopted based on those required for non-sterile pharmaceutical preparations for use by inhalation (see European Pharmacopoeia 8.0: section 5.1.4). Color should be consistent throughout each sample and should not show signs of gray or black, which are indicators of fungal infection.

For medical users of cannabis, there is a balance sought between organoleptic qualities (taste and aroma) and medicinal effect, as well as a balance between THC- and CBD-yielding cultivars. Many cultivators select, breed, and process for these varying qualities. For medicinal purposes an optimal ratio between total THC, Δ^9-THC, and/or CBD has not been definitively determined. Different health conditions may respond differently to plants containing different ratios of the 2 primary cannabinoids. For example, evidence indicates that CBD is responsible for some of the putative anxiolytic and anti-schizoprenic effects of the plant (Mechoulam et al. 2002; Zuardi et al. 2002) while THC has been associated with appetite stimulation (Dejesus et al. 2007; Nelson et al. 1994). The process of trimming is done both for yielding higher concentrations of THC and for yielding more desirable, organoleptic qualities, since the leaves possess a sharp and bitter organoleptic characteristic. A better organoleptic profile may enhance compliance.

Dispensaries should maintain strict quality control practices to ensure the purity and quality of their material by contracting for testing with independent labs that apply independently verified testing methodologies and transparent testing standards. Individual growers and care givers producing medical cannabis for personal use should employ good agricultural practices (GAPs) to the extent possible in all aspects of growing, harvesting, drying, and storage.

Documentation Guidelines (WHO 2003)

a. Location of cultivation and the name of the supervising cultivator.

b. Details on crops previously grown at that location.

c. Nature, origin and quantity of the herbal starting materials.

d. Chemicals and other substances used during cultivation, such as fertilizers, pesticides, and herbicides.

e. Standard cultivation conditions, if applicable.

f. Particular circumstances which occurred during cultivation, harvesting, and production that may affect the chemical composition, such as plant diseases or temporary departure from standard cultivation conditions, particularly during the harvesting period

g. Nature and quantity of the yield.

h. Date or dates and time or times of day when harvesting occurred.

i. Drying conditions.

j. Measures for pest control.

Sustainability and Environmental Impact

As all cannabis is derived from cultivated sources, there is little risk of the plant becoming environmentally threatened unless aggressive eradication programs are implemented worldwide. However, without development, implementation, and enforcement of Good Agricultural Practices (GAPs), both indoor and outdoor cannabis cultivation can have significant negative environmental and social impacts (Montford and Small 1999). Environmentally, the illegal diversion of water, clear cutting of trees, dumping of chemicals, misappropriation of state and federal lands, and disruption of sensitive ecosystems are associated with outdoor cultivation, while high carbon emissions are associated with indoor production. In North America, especially with crops grown indoors, part of this environmental impact is driven by the illegality of cannabis cultivation that requires growers to hide crops. Others may choose indoor growing for greater control over crops and higher yields. The high-energy intensive processes associated with controlling all aspects of the indoors growing environment has been estimated to consume 1% of the national electricity use (Mills 2011). Whether by regulation or choice, growers should apply GAPs to cannabis cultivation.

In addition to the impacts of cannabis cultivation, the manufacture of butane extracts poses significant risks. A number of explosions and fires associated with home cannabis extract production have been reported, some that have included injury. Industrial grade butane contains

Table 3 **Cannabis plant groups and typical Δ⁹-THC/CBD concentration and ratios**

		THC	CBD	THC:CBD Ratio
I	**Drug**	0.5–15%	0.01–0.16%	50:1
II	**Intermediate**	0.5–5%	0.9–7.3%	0.25/~ 2
III	**Fiber**	0.05–0.70%	1.0–13.6%	< 1:5
IV	**CBG**	< 0.05%	< 0.5%	-
V	**Non-cannabinoid**	0	0	-

Source: Modified from Galal et al. (2009). Note: THCA-predominant strains can yield in excess of 25% Δ⁹-THC; specially selected CBDA clones can yield up to 20% CBD.

compounds that may not be desirable in finished products. Extraction with CO_2 (sub- or super-critical) is preferred by some and is one environmentally safe extracting option.

Documentation of Supply

For cannabis that is to be used in medicinal preparations, every aspect of cultivation, harvest, processing, and storage should be documented to the fullest extent possible. Various county and state ordinances require adherence to specific regulations that differ between locations for trade of cannabis among growers, dispensaries, and collectives. The Dutch OMC provides the following guidelines for documentation as follows (also see inset page 32).

Security (modified from OMC 2003)

The buildings in which cannabis is cultivated, processed, packaged and stored must be sufficiently secured, only allowing authorized personnel access to the buildings. Personnel involved in the production process of cannabis must be authorized for that purpose by the employer. Waste must be stored in such a way that the potential for theft is minimized.

Suppliers and Dispensaries

Cannabis products supplied by dispensaries should be as fully characterized as possible with traceability and a verifiable chain of custody to type of material, whether the plants were cultivated conventionally or organically, or were indoor or outdoor cultivated. Procedures should be implemented to ensure the absence of pesticides and raw material and finished product should be characterized as to its basic chemical profile (e.g., THC and/or CBD content). This information should be made available to patients upon request. Dispensary personnel should be appropriately trained in how to process and handle cannabis to ensure purity, maintain quality, and to morphologically identify material. The cannabis committee of the American Herbal Products Association (AHPA) has developed a set of draft guidelines outlining recommended practices for dispensaries and cultivators to follow (AHPA 2013a), and Americans for Safe Access (ASA) has developed an industry certification program for dispensaries and cultivators (ASA 2013).

Constituents

To date, more than 750 different constituents have been identified in cannabis. The diversity of constituents encompasses numerous phytochemical classes, notably, cannabinoids, and a host of other secondary metabolites. These other compound classes include terpenoids, non-cannabinoid phenols, nitrogenous compounds, as well as other more common plant compounds, all of which are non-psychotropic. Cannabinoids are the most studied and well-known chemical constituents of cannabis. Of these, THC has received the most attention, since it is the principal psychoactive component of the plant. Cannabinoid acids lack psychoactivity. Therapeutic activity is not limited to cannabinoids. Emerging research suggests that other minor compounds (e.g., terpenoids) may also play a role in the complex pharmacology of this botanical, either directly or through modulation of cannabinoid responses (reviewed in Russo 2011) (see Table 6). Additionally, research on the non-psychoactive acid cannabinoids has been limited due to the overriding interest in decarboxylated THC (Mechoulam 2013, personal communication, unreferenced).

Cannabinoids

Cannabinoids (CBs) are a class of more than a hundred terpenophenolic compounds, most commonly associated with the pharmacological activity of cannabis. Several main structures are distinguished (Tabel 6). The term "phytocannabinoids" (Pate 1994) has been used to designate naturally occurring cannabinoids in cannabis; however, the discovery of compounds from other plants (e.g., *Echinacea* spp.) also have CB-receptor activity and, thus, can be named "phytocannabinoids." A synonymous term "exocannabinoids" is used to distinguish phytocannabinoids from endocannabinoids, the endogenous ligands to cannabinoid receptors. "Classical" and "non-classical" cannabinoids refer to synthetic cannabinoid receptor agonists (Makriyannis et al. 2005) and indicate the relative degree of structural similarity with phytocannabinoids.

Cannabinoids mainly exist in the plant as carboxylic acids and are converted to neutral analogs by light and heat while in storage (Veress et al. 1990) or when combusted. The alkyl group at the third carbon atom (C-3) is considered an important site in substrate-receptor interactions (Loewe

1944; Pertwee et al. 2010). This group is typically a pentyl—for example, in Δ^9-THC, cannabigerol (CBG), cannabidiol (CBD), and cannabinol (CBN)—but can also be a propyl, in which case the compounds are named by attaching the suffix -varin to the name of the pentylated analog, e.g., tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabigerovarin (CBGV), and cannabivarin (CBV).

Cannabis plants typically exhibit one of 3 main distinctly different chemotypes based on the absolute and relative concentrations of Δ^9-THCA and CBDA (after conversion from the respective acids). Small and Beckstead (1973) refer to these as drug-type, intermediate type, and fiber-type plants. Plants with more rare chemical profiles have been identified, including those predominant in cannabigerol (CBG) (de Meijer and Hammond 2005) or tetrahydrocannabivarin (THCV), and those lacking any cannabinoids (de Meijer et al. 2009), for a total of 5 general types (Table 3).

The cannabinoid profile is affected most by the plant's sex, genotype (de Meijer et al. 1992; 2003), and maturity (Small et al. 1976), followed by environmental and other factors, such as light intensity, light cycle (Valle et al. 1978), temperature (Chandra et al. 2008), and fertilization (Bocsa et al. 1997). Cannabinoids are produced in glandular trichomes distributed across all epidermal surfaces of the plant's aerial parts in varying degrees. The distribution of glandular trichomes and, hence, phytocannabinoids varies widely, from the lowest concentrations found in stems to increasing amounts in large leaves, subtending leaves of the inflorescences, the inflorescences, and to the highest concentrations found in female flower bracts.

Cannabinoids are highly lipophilic, permeate cell membranes, and have the ability to cross the blood-brain barrier both when inhaled and ingested.

Following is a review of major and minor cannabinoids primarily associated with the psychoactive and pharmacological effects of cannabis. Not all compounds will be found in every plant sample, and the ratios of the compounds will vary. THC is generally the most abundant cannabinoid in contemporary horticulture of cannabis, due to the focus of growers on high THC yielding strains, specifically for enhanced psychoactive effects.

Cannabinoid Acids

Cannabinoids occur in living plants mainly in carboxylated form. Cannabigerolic acid (CBGA), derived from olivetolic acid and geranyl pyrophosphate (Fellermeier and Zenk 1998), is the precursor of all other major cannabinoid acids—THCA, CBDA, and cannabichromic acid (CBCA)—as well as their analogs and biogenic derivatives (Yamauchi et al. 1968). Two THC acids are present in cannabis and differ in the position of the carboxyl group THC acid-A (Korte et al. 1965) and THC acid-B (Mechoulam et al. 1969). Both are non-psychotropic and their pharmacology is almost unknown (Mechoulam 2013, personal communication, unreferenced). In fresh, unheated plant material, virtually no neutral (non-carboxylated) compounds have been found (Verhoeckx et al. 2006), despite cannabinoid acids being readily thermo- and photolabile (Hazekamp 2007; Johnson et al. 1984). The THCA-Δ^9-THC ratio in leaves and flowers of the female plants has been reported to be from 2:1 (these days rarely) to 20:1 and higher, depending on the variety (strain) tested (e.g., Brenneisen 1984; Pitts et al. 1992, among others). Higher THCA-Δ^9-THC ratios are more typical and are often found even in dried, one-year-old plant material (Wurzer and Dixon 2013, personal communication, unreferenced). Heating for 5 minutes (min) at 200–210 °C has been reported to be effective for conversion to occur, but slow decarboxylation occurs also at room temperature (Brenneisen 1984). An aqueous decoction of cannabis (simmered for 15 min) retained a large THCA-Δ^9-THC ratio (Hazekamp 2007).

Cannabinoid acids, including THCA, are devoid of psychotropic effects (Burstein 1999; Dewey 1986). Medical users report health benefits from modes of cannabis consumption that do not use combustion or high temperatures (certain kinds of foods, capsules, infusions, juices), thus preserving most of the cannabinoids in their acid forms. Little specific pharmacological investigation of THCA has been published to date, but immunomodulatory activity of THCA has been reported (Verhoeckx et al. 2006).

Major Cannabinoids

Δ^9-Tetrahydrocannabinol (Δ^9-THC) Type Phytocannabinoids

Δ^9-THC was isolated in 1964 (Gaoni and Mechoulam 1964a), and additional THCs were identified by 1980 (e.g., reviewed in Suurkuusk 2010, among others), followed by almost 3 decades before a series of 8 Δ^9-THCA terpenoid esters were isolated from high-potency cannabis (Ahmed et al. 2008b). THCA, commonly the primary cannabinoid of this group existing in the plant, is synthesized from CBGA by THCA synthase, which is abundantly present in glandular trichomes. Δ^9-THC is a product of THCA decarboxylation, usually formed via degradation (such as during storage) or heating (vaporization or combustion). THCA is typically the predominant cannabinoid in cannabis strains that exhibit psychoactivity, but it also occurs in low levels in fiber-type plants (Table 3).

THC has a high affinity for cannabinoid receptors of both type 1 (CB_1) and type 2 (CB_2), and is thought to behave as their partial agonist, similar to the endocannabinoid anandamide (Howlett et al. 2010; Pertwee 2008). The primary natural isomer, (−)-trans-Δ^9-THC, displays a higher potency compared to the other isomers (e.g., (+)-trans-) or enantiomers (e.g., (3R,4R)-Δ^1-THC) (Mechoulam et al. 1990) and is used preferentially in clinical trials. THC has been used as an antiemetic in chemotherapy-associated nausea and emesis; as an appetite promoter, especially for AIDS and cancer patients who are prone to severe weight loss due to anorexia and anorexia-cachexia, respectively; as an analgesic, e.g., for cancer, damaged nerves, migraine, spinal cord injury, postoperative, dental, and phantom limb pain; for treatment and symptom management of neurological disorders such as

multiple sclerosis (Fox and Zajicek 2002; Rog et al. 2005). Its utility for treating glaucoma is limited by the high dosage needed to lower intraocular pressure, and its short duration of action in this condition (Buys and Rafuse 2010).

Tetrahydrocannabivarin (THCV) is the propyl homolog of Δ^9-THC and usually occurs in cannabis in minor amounts, although THCV-rich strains (up to about 16% dry weight in selected inflorescences) have been developed (de Meijer 2013, personal communication to AHP, unreferenced). This cannabinoid is a CB_1 neutral antagonist at low doses (Gill 1971; Thomas et al. 2005) and agonist at both CB_1 and CB_2 receptors at high doses (Bolognini et al. 2010; Thomas et al. 2005). Anticonvulsant, anti-inflammatory, and analgesic properties have been reported for THCV (Bolognini et al. 2010; Hill et al. 2010). A recent study reported antioxidant and potential neuroprotective effects of THCV in an experimental Parkinson's disease model in mice, suggesting utility in the amelioration of Parkinsonian symptoms, in part via activation of CB_2 (Garcia et al. 2011).

Cannabidiol (CBD) Type Phytocannabinoids

Cannabidiol (CBD) and cannabidiolic acid (CBDA) are the main non-psychotropic cannabinoids in cannabis and are the most abundant cannabinoids in European hemp. Cannabidiol was isolated in 1940 (Adams et al. 1940b), with its structure determined in later studies (Mechoulam and Shvo 1963; Mechoulam and Gaoni 1967; Petrzilka et al. 1969). Cannabidiolic acid, cannabigerolic acid, and cannabinolic acid were first isolated by Mechoulam and Gaoni (1965). To date, 8 CBD-type phytocannabinoids have been identified (Shoyama et al. 1972a; Sirikantaramas et al. 2007).

Cannabidiol lacks the cognitive and psychoactive properties of THC and displays a very low affinity for cannabinoid receptors (Thomas et al. 2007). Research has focused on identifying CB_1- and CB_2-independent mechanisms of CBD action. Cannabidiol is known to be an agonist at serotonin (5-HT1A) receptors (Mishima et al. 2005; Russo et al. 2005) and transient receptor potential vanilloid type 1 (TRPV1) receptors (Bisogno et al. 2001; McHugh et al. 2010). Cannabidiol can also enhance adenosine receptor signaling by inhibiting adenosine inactivation, suggesting a potential therapeutic role in pain and inflammation (Carrier et al. 2006). Some of the pharmacological actions of CBD include anticonvulsive, anti-inflammatory, antioxidant, antipsychotic, hypnotic, and sedative (at very high doses). The antioxidant and anti-inflammatory properties account for the neuroprotective actions of CBD (Scuderi et al. 2009), which could potentially be utilized for the treatment and symptom relief of a number of neurological disorders, e.g., epilepsy and seizures (Hofmann and Frazier 2013; Jones et al. 2010), psychosis (Zuardi et al. 2006), anxiety (Bergamaschi et al. 2011), movement disorders (e.g., Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis) (de Lago and Fernandez 2007; Iuvone et al. 2009), Alzheimer's disease (Martin-Moreno et al. 2011), and multiple sclerosis (Lakhan and Rowland 2009). Cannabidiol has demonstrated an exceptional tolerability in humans, making it a potential candidate for clinical application or as a lead compound for the development of cannabimimetic drugs (Mechoulam and Hanus 2002).

Cannabigerol (CBG) Type Phytocannabinoids

Cannabigerolic acid (CBGA) is a direct precursor to THCA, CBDA, and cannabichromenic acid (CBCA) (Gaoni and Mechoulam 1964b, 1966; Taura et al. 1995a, 1995b, 1996). It is typically present in cannabis only in minute amounts, though in some cannabis this class of cannabinoids may be dominant (de Meijer et al. 1992), and cannabis plants that produce CBG as the primary cannabinoid have been cultivated (de Meijer and Hammond 2005). To date, 16 CBG-type cannabis constituents have been identified (DeBacker et al. 2009; ElSohly and Slade 2005; Turner et al. 1980b), including cannabigivarin (CBGV). CBGV is the biosynthetic precursor of THCV and is reputedly found in higher concentration in some feral accessions from India (Hillig and Mahlberg 2004; Vollner et al. 1969). While there is little research to date on CBGV, there are indications of anti-inflammatory action associated with THCV (Tubaro et al. 2010) and activation of CB_2 receptors on mesenchymal cells (Izzo et al. 2009).

CBG-type cannabinoids are non-psychoactive cannabinoids that generally act as weak ligands at both CB_1 and CB_2 receptors (Costa 2007; Fisar 2009; Eisenstein et al. 2007; Gaoni and Mechoulam 1964b; Pollastro et al. 2011). Cannabigerol is a GABA uptake inhibitor with more potent effects than THC or CBD (Banerjee et al. 1975). It is a potent alpha$_2$-adrenocorticotropic receptor agonist (Cascio et al. 2010), a potent antagonist of transient receptor potential cation channel subfamily M member 8 (TRPM8) (De Petrocellis et al. 2008), and has been shown to have some uptake-inhibitory activity at 5-HT1A receptors (Banerjee et al. 1975; Rock et al. 2010). This latter action is responsible for countering the anti-emetic effects of CBD (Rock et al. 2010). Additionally, this cannabinoid has demonstrated antimicrobial activity (Appendino et al. 2008), inhibited proliferation of keratinocytes (Wilkinson and Williamson 2007) and cancer cells (Ligresti et al. 2006), and was shown to have greater analgesic activity than THC (Cascio et al. 2010; Evans 1991). These actions suggest that CBG may have a therapeutic potential, e.g., as an antidepressant or for the treatment of psoriasis (Wilkinson and Williamson 2007). The presence of CBG has also been found in *Helichrysum umbraculigerum* (Woelkart et al. 2008).

Minor Cannabinoids

Δ^8-Tetrahydrocannabinol (Δ^8-THC) Type Phytocannabinoids

This group has only 2 compounds, namely, (–)-Δ^8-THC and (–)-Δ^8-tetrahydrocannabinolic acid A ((–)-Δ^8-THCA A). Δ^8-THC is stable in air, and is less psychotropic than Δ^9-THC, making it a viable option as a therapeutic alternative to Δ^9-THC. At low doses, Δ^8-THC (0.001 mg/kg po) is capable of inducing appetite stimulation without psy-

chotropic effects such as alterations in cognitive function (Avraham et al. 2004).

Cannabielsoin (CBE) Type Phytocannabinoids

To date, 5 compounds of this type have been identified, including cannabielsoin (Bercht et al. 1973), cannabielsoic acids (CBEA) A and B (Shani and Mechoulam 1970, 1974), and 2 additional isomers (Hartsel et al. 1983). Cannabielsoic acids and CBE are not always found in natural sources and can be obtained by photooxidation or pyrolysis of naturally occurring CBDAs and CBDs (Kueppers et al. 1973). Rather, these compounds are found in processed cannabis products such as hashish and may be artifacts of other naturally occurring phytocannabinoids (Bercht et al. 1973; Grote and Spiteller 1978a; Kueppers et al. 1973; Shani and Mechoulam 1974). Cannabielsoin is found in mammals as a metabolite of CBD (Yamamoto et al. 1988).

Cannabitriol (CBT) Type Phytocannabinoids

(–)-Cannabitriol was isolated from cannabis grown in Japan (Obata and Ishikawa 1966). Other related cannabitriols (e.g., 6α,7,10α-trihydroxytetrahydrocannabinol, 9,10-epoxycannabitriol) were identified in pollen grains (Ross et al. 2005). Cannabidiolate, 9-O-CBT, was isolated from hashish (Von Spulak et al. 1968).

Cannabichromene (CBC) Type Phytocannabinoids

In the 1970s, CBC was reported to be the second most abundant cannabinoid in some strains of cannabis growing in the United States (Holley et al. 1975), but this may be attributed to past difficulties distinguishing CBC from CBD. To date, a total of 8 CBC-type phytocannabinoids have been identified (Radwan et al. 2009). Usually CBC is present in minor amounts due to its biosynthetic enzyme being produced by a recessive gene (de Meijer and Hammond 2005), although high CBC plants have been selectively bred. This compound is also present in a higher concentration in juvenile cannabis plants, and may be concentrated into an "enriched trichome product" (Potter 2009). More recently, cannabinoids of this type were isolated from high-potency cannabis (Radwan et al. 2009) (concentrations not reported).

Cannabichromene interacts with TRPV channels, having a strong affinity for TRPV1, but has poor affinity for the CB_1 receptor (Booker et al. 2009; DeLong et al. 2011; De Petrocellis et al. 2011). The compound is known to produce anti-nociceptive and anti-inflammatory effects in rodents (Davis and Hatoum 1983; Turner and ElSohly 1981; Wirth et al. 1980). Three cannabinoids of this type have been reported to have antimicrobial and moderate anti-leishmanial activities, while lacking cytotoxicity against African green monkey kidney fibroblast cell line Vero (Radwan et al. 2009).

Table 4 Content ranges of major and minor cannabinoids in cannabis and their degradation products

Compound	% dry weight
Δ^9-THC	0.1–25
CBD	0.1–7.98
CBN	0.0–1.6
THCV	0.0–1.36
CBG	0.03–1.15
CBC	0.0–0.65
Δ^8-THC	0.0–0.1

Source: Modified from McPartland and Russo (2001) with additional data from Fischedick et al. (2010); Fournier et al. (1987); Pitts et al. (1992); Small (1979); and Veszki et al. (1980).

Degradation Products and Artifacts

Cannabinol (CBN) Type Phytocannabinoids

Cannabinoids of this type are fully aromatized derivatives of THC, and, although they have been isolated from different cannabis extracts (Bercht et al. 1973; Harvey 1976; Mechoulam and Gaoni 1965; Wood et al. 1896), they are believed to be artifacts (Bowd et al. 1975) obtained by non-enzymatic oxidation of THC. Some of the reported levels in dry plant material are summarized in Table 4. There are 10 known CBN-type cannabinoids (Adams et al. 1940a; Cahn 1932; Ghosh et al. 1940). The concentration of CBN in cannabis products (marijuana, hashish, and hash oil) has been reported to increase during storage, while the THC concentration decreases, but at a different rate (Ross and ElSohly 1999).

Cannabicyclol (CBL) Type Phytocannabinoids

This group has 3 known compounds: cannabicyclol (Claussen et al. 1968; Korte and Sieper 1964a, 1964b), cannabicyclolic acid A (CBLA) (Shoyama et al. 1972b), and cannabicyclovarin (CBLV) (Claussen et al. 1968; Vree et al. 1972). The photochemical conversion of CBC into CBL has been demonstrated (Crombie et al. 1968). Larger amounts of CBLA were observed in cannabis harvested earlier, during the vegetative phase, and stored for prolonged periods of time, compared with that harvested later, in the reproductive phase (Shoyama et al. 1968). These observations prompted the conclusion that CBL and CBLA are not genuine cannabinoids but artifacts produced by natural irradiation of CBC and CBCA during storage (Shoyama et al. 1972b).

Cannabinodiol (CBND) Type Phytocannabinoids

Cannabinoids of the CBND type are the fully aromatized derivatives of CBDs (Lousberg et al. 1977; Van Ginneken et al. 1972).

Table 5 **Content of major terpenoids in the volatile oil freshly extracted from cannabis inflorescences, as determined by GC-MS by various research groups**

Compound	Content, % oil
Monoterpenoids	
α-Pinene	1.11–31.0
β-Pinene	0.6–7.95
β-Myrcene	8.23–67.11
Limonene	0.2–16.38
Terpinolene	0.12–23.8
cis-Ocimene	0.04–10.28
Linalool	0.09–2.8
Sesquiterpenoids	
β-Caryophyllene	1.33–28.02
Humulene	0.28–12.61
β-Eudesmol	0.02-1.56
Caryophyllene oxide	0.3–11.3
trans-Nerolidol	0.09–1.72

Source: Compiled from Bertoli et al. (2010); Mediavilla and Steinemann (1997); and Ross and ElSohly (1996).

Benzoquinone Type and Other Phytocannabinoids

Two geranyl-*n*-pentyl-1,4-benzoquinones were isolated from high-potency cannabis (Radwan et al. 2008b, 2009). Cannabicitran was first synthesized (Crombie and Ponsford 1971) and subsequently isolated from Lebanese hashish (Bercht and Paris 1974). Its structure was described by Bercht et al. (1974). The isolation and identification of cannabichromanone, dehydrocannabifuran, cannabifuran, and 10-oxo-$\Delta^{6a(10a)}$-THC from hashish (Friedrich-Fiechtl and Spiteller 1975) was followed by the isolation of cannabichromanone-C3 (Grote and Spiteller 1978a) and cannabicoumaronone (Grote and Spiteller 1978b). *cis*-Δ^9-THC was found in samples of confiscated cannabis (Smith and Kempfert 1977). Cannabiripsol was isolated from South African-grown cannabis (Boeren et al. 1979). Cannabis grown in Thailand (Meao strain) provided (±)-*cis*-Δ^7-isotetrahydrocannabivarin (Shoyama et al. 1981). Cannabiglendol was isolated from an Indian cannabis variety grown in Mississippi (Turner et al. 1981). A polyhydroxylated cannabinoid, cannabitetrol, was was also isolated from natural sources and identified (ElSohly et al. 1984). The GC-MS analysis of hash oil (Morita and Ando 1984) led to the identification of *trans*-(1*R*,3*R*,6*R*)-Δ^7-*iso*-THCV and *trans*-(1*R*,3*R*,6*R*)-Δ^7-*iso*-THC. Three cannabichromanone derivatives (Ahmed et al. 2008a) and cannabicoumarononic acid A (Radwan et al. 2009) were isolated from high-potency cannabis.

Terpenoids

The essential oil (volatile oil) of cannabis is a blend of terpenoids, a term that encompasses terpenes and modified terpenes (where the methyl group has been moved or removed, or oxygen atoms added). Approximately 200 terpenoids have been extracted from cannabis, primarily monoterpenoids ($C_{10}H_{16}$ template) and sesquiterpenoids ($C_{15}H_{24}$ template), as well as di-, and triterpenoids, megastigmanes, and apocarotenoids. No terpenoids are unique to cannabis, but various types of cannabis biosynthesize unique terpenoid profiles (Brenneisen and ElSohly 1988; Hillig 2004; Mediavilla and Steinemann 1997). The qualitative and quantitative profile of terpenoids may vary between different batches of the same seed source (Fischedick et al. 2010).

Ester conjugates of terpenoid alcohols with cannabinoid acids have been reported as minor constituents in cannabis extracts (Ahmed et al. 2008b). The biological profile of these compounds is currently unknown, despite their potential to act as pro-drugs of pre-cannabinoids.

Terepenoids are primarily respnsible for the aroma of cannabis, while cannabinoids, despite their terpenoid origins, are odorless.

Terpenoids produce a wide range of biological activity, possibly including modulation of the effects of THC via their own anxiolytic, sedative, analgesic, antinociceptive, and anti-depressant effects (reviewed in McPartland and Pruitt 1999; Russo 2011). Other actions of terpenoids include anti-inflammatory, acetylcholinesterase (AChE) inhibition, antioxidant, antibiotic, and anti-mutagenic (Maffei et al. 2011).

Terpenoids, together with cannabinoids, alkanes, and other compounds, are synthesized inside glandular trichomes via a common precursor, geranyl pyrophosphate. Yields of cannabis essential oil obtained from fresh plants through steam distillation range from 0.05–0.29% v/w and may represent 10% of trichome content, varying greatly with growing, drying, and harvest conditions (Hazekamp 2008–2009; McPartland and Mediavilla 2001; Potter 2009). Ross and ElSohly (1996) demonstrated the ephemeral nature of terpenoids in stored flowering tops. Freshly-collected material yielded 0.29% v/w essential oil; 1-week-old material air-dried at room temperature and stored in a paper bag yielded 0.20%, a loss of 31%; 1-month-old cannabis yielded 0.16%, a loss of 45%; 3-month-old cannabis yielded 0.13%, a loss of 55%.

Monoterpenoids

Monoterpenoids typically predominates in cannabis, comprising 47.9–92.48% of essential oil extracted from fresh plant material (Mediavilla and Steinemann 1997; Ross and ElSohly 1996 (see Table 5). β-myrcene usually dominates the monoterpenoid fraction in all types of cannabis. Limonene or terpinolene predominate in some drug-type plants (Fischedick et al. 2010, terpinolene and α-pinene predominate in some European fiber-type plants (Bertoli et al. 2010), and α-inene predominates in some Chinese fiber-type plants (Hillig 2004). Other common monoterpenoids

Table 6 **Structure and activity of primary phytocannabinoids**

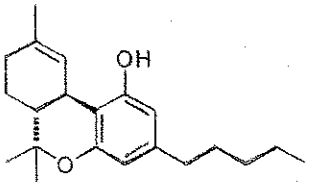
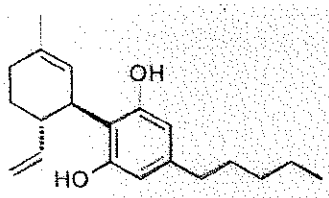
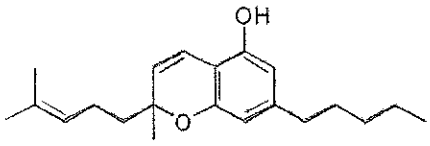
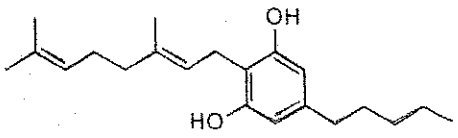
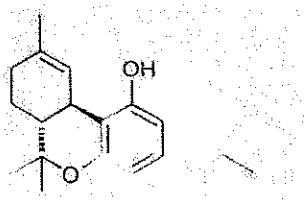
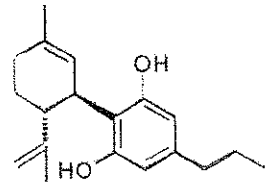
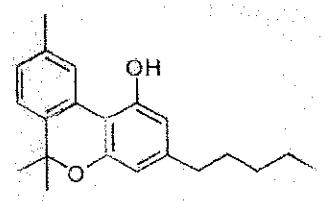
Compound	Putative Medicinal Action
 Δ⁹-Tetrahydrocannabinol (Δ⁹-THC)	**Primary psychotropic cannabinoid** Activates PPAR-γ and TRPA1 at nano- and micromolar concentrations, respectively (Pertwee 2008). Analgesic via CB_1 and CB_2 agonism (active at ~20–40 nM) (Rahn and Hohmann 2009). Antiemetic (Haney et al. 2007; Hollister 1971; Machado et al. 2008). Anti-inflammatory, antioxidant (Hampson et al. 1998). Antipruritic, cholestatic jaundice (Neff et al. 2002). Benefits duodenal ulcers (Douthwaite 1947). Bronchodilatory (Williams et al. 1976). Muscle relaxant (Kavia et al. 2010). Reduces Alzheimer's symptoms (Eubanks et al. 2006; Volicer et al. 1997).
 Cannabidiol (CBD)	**Non-psychotropic cannabinoid** Anandamide (AEA) reuptake inhibitor (De Petrocellis et al. 2011). Analgesic (Davis and Hatoum 1983). Anticonvulsant (Jones et al. 2010). Antidepressant in rodents (Deyo and Musty 2003). Anti-emetic (5HT1A agonist; 5 mg/kg ip) (Rock et al. 2010). Antifungal (ElSohly et al. 1982). Anti-inflammatory (Booz 2011). Antagonizes effects of THC in humans (Pertwee 2008). Antioxidant (Hampson et al. 1998). Anxiolytic via 5HT1A agonism (Campos and Guimaraes 2008; Resstel et al. 2009; Russo et al. 2005). Decreases sebum/sebocytes proliferation (Biro et al. 2009). Effective against methicillin-resistant *Staphylococcus aureus* (MRSA) (Appendino et al. 2008). Increases adenosine A2A signaling (Carrier et al. 2006). Pro-apoptotic against breast cancer cell lines (Ligresti et al. 2006). Treatment of addiction (Xi et al. 2010). Treatment of psychosis (Russo et al. 2007).
 Cannabichromene (CBC)	**Non-psychotropic cannabinoid** Analgesic (weak) (Turner et al. 1980b). Anandamide reuptake inhibitor (weak) (De Petrocellis et al. 2008; Ligresti et al. 2006). Anti-inflammatory (Davis and Hatoum 1983). Antimicrobial (Turner and ElSohly 1981). TRPA1 agonist (De Petrocellis et al. 2008; Ligresti et al. 2006).

Table 6 (continued) **Structure and activity of primary phytocannabinoids**

Compound	Putative Medicinal Action
 Cannabigerol (CBG)	**Non-psychotropic cannabinoid** Analgesic via α-2 adrenergic blockade (Cascio et al. 2010). Anandamide reuptake inhibitor (low micromolar range) agonist (De Petrocellis et al. 2008; Ligresti et al. 2006). Anti-fungal (ElSohly et al. 1982). Anti-inflammatory, anti-hyperalgesic (Bolognini et al. 2010). Effective against MRSA (Appendino et al. 2008). GABA uptake inhibitor (Banerjee et al. 1975). Reduces keratinocytes proliferation in psoriasis (Wilkinson and Williamson 2007). 5HT1A antagonist; counters antiemetic effects of CBD (Rock et al. 2010). TRPM8 antagonist (De Petrocellis et al. 2011). TRPV1, TRPA1, and cannabinoid agonist (De Petrocellis et al. 2008; Ligresti et al. 2006).
 Δ^9-Tetrahydrocannabivarin (THCV)	**Non-psychotropic cannabinoid** Antagonizes Δ^9-THC at low doses (< 3 mg/kg); acts as CB_1 agonist at higher doses (10 mg/kg) in mice (Pertwee et al. 2007, 2008). Anticonvulsant (Hill et al. 2010). Reduced food intake in mice (Cawthorne et al. 2007). Improved glucose tolerance, insulin sensitivity, and insulin signalling in vivo (Wargent et al. 2013).
 Cannabidivarin (CBDV)	**Non-psychotropic cannabinoid** Anticonvulsant in vitro and in vivo (Hill et al. 2010, 2012).
 Cannabinol (CBN)	**Non-psychotropic cannabinoid; minor by-product of Δ^9-THC oxidation** Decreases breast cancer resistant protein (Holland et al. 2008). Effective against MRSA (Appendino et al. 2008). Reduces keratinocytes proliferation in psoriasis (Wilkinson and Williamson 2007). Sedative (Musty et al. 1976). TRPV2 agonist for burns (Qin et al. 2008).
 β-Caryophyllene	**Non-psychotropic sesquiterpene of the essential oil** Common compound of many aromatic plants. Anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, and local anaesthetic activities (Leandro et al. 2012).

Source: Modified from Izzo et al. (2009); Russo (2011).

in cannabis include β-pinene, *cis*-ocimene, *trans*-ocimene, and linalool.

β-Myrcene is recognized to have sedative, muscle-relaxant (do Vale et al. 2002), anti-inflammatory, and analgesic activities (Lorenzetti et al. 1991; Rao et al. 1990). Limonene, a precursor to other monoterpenoids and fairly ubiquitous in nature (Noma and Asakawa 2010), is highly bioavailable and has been suggested to be anxiolytic (do Vale et al. 2002), anticarcinogenic (Elson et al. 1997), and radical-scavenging (Malhotra et al. 2009), while also used to treat gastro-esophageal reflux and gallstones (Sun 2007). α-Pinene is one of the most widely encountered terpenoids in nature, being especially common to coniferous trees (Chalchat et al. 1985; Persson et al. 1996). This terpenoid is reported to have anti-inflammatory (Gil et al. 1989), bronchodilatory (Falk et al. 1990), and antibiotic (anti-MRSA) (Kose et al. 2010) activities, and is an AChE inhibitor that may be of use as a memory aid (Perry et al. 2000). Terpinolene has been reported to be a sedative (Ito and Ito 2013) and antispasmodic (Riyazi et al. 2007) agent. Linalool, common to lavender (*Lavandula* spp.) and coriander (*Coriandrum sativum*), has anxiolytic (Souto-Maior et al. 2011), local anaesthetic, analgesic (Peana et al. 2004), sedative, and anticonvulsant (Karlagania 2002) properties, and is used as a topical treatment for burns (Gattefosse 1993). Pulegone is a minor terpenoid in cannabis (Turner et al. 1980b), and is also found in rosemary (*Rosmarinus officinalis*), and possesses sedative (Miyazawa et al. 1997) and anti-pyretic (Ortiz de Urbina et al. 1989) properties. Turner et al. (1980b) report *p*-cymene in cannabis; it has anti-microbial properties (Kisko and Roller 2005) and is able to effect AChE inhibition (Perry et al. 1996).

As with cannabis flavonoids, many of these proposed uses are extrapolated from the same compounds in other medicinal plants, with their relevance to cannabis effects equally unknown.

Monoterpenoids are exceptionally volatile and particularly susceptible to loss during drying and storage. As demonstrated by Ross and ElSohly (1996), the relative percentage of monoterpenoids in the essential oil fraction gradually reduced from 92.48 to 62.02% after cannabis was dried and stored at room temperature in closed paper bags for 3 months. Specifically, for example, the content of β-myrcene gradually decreased from 67.11% to 32.88% of the oil, while linalool increased from 2.80% to 5.07%, and α- and β-pinenes and limonene remained seemingly unaffected (no statistical analysis was reported in the study). However, none of the major (> 0.1% of the total) compounds decreased to unquantifiable levels.

Sesquiterpenoids

Sesquiterpenoids comprise 6.84%–47.5% of the essential oil extracted from fresh plant material (Mediavilla and Steinemann 1997; Ross and ElSohly 1996). The primary sesquiterpenoid in cannabis is usually β-caryophyllene. This sesquiterpenoid surpasses β-myrcene as the overall predominate terpenoid in some fiber-type plants (Bertoli et al. 2010; Mediavilla and Steinemann 1997). Caryophyllene oxide, reportedly the volatile compound sensed by drug detection dogs (Stahl and Kunde 1973), is common to all cannabis strains. Other common sesquiterpenoids in cannabis include α-humulene (ie., α-caryophyllene), trans-nerolidol, α-guaine, elemene, and isomers of farnesene and bergamotene (Bertoli et al. 2010; Fischedick et al. 2010; Hillig 2004; Mediavilla and Steinemann 1997; Ross and ElSohly 1996).

β-Caryophyllene is a dominant constituent in black pepper (*Piper nigrum*) and clove (*Syzygium aromaticum*). It reportedly has anti-inflammatory (Basile et al. 1988), gastrocytoprotective (Tambe et al. 1996), analgesic (Chelardini et al. 2001), and anti-malarial properties (Campbell et al. 1997). This terpenoid was demonstrated to be a selective CB_2 receptor agonist (Gertsch et al. 2008). β-caryophyllene and α-humulene, which along with monoterpenoids myrcene and β-farnesene, predominates in hops (*Humulus lupulus*), imparts its cannabis-like odor.

The relative levels of sesquiterpenoids may increase after drying and in storage, due to loss of the more volatile monoterpenoids. Prolonged storage of the material characterized by Ross and ElSohly (1996) resulted in the sesquiterpenoids content of 35.63%, compared with 6.84% in the oil extracted from the fresh plant. The content of β-caryophyllene increased from 1.33% to 5.45% after 3 months of storage of dried plant material, compared with fresh plant (Ross and ElSohly 1996).

Flavonoids

To date, more than 29 flavonoids have been identified in cannabis (Clark and Bohm 1979; ElSohly and Slade 2005; Ross et al. 2005; Vanhoenacker et al. 2002). cannabis flavonoids belong mainly to 2 classes: flavones (e.g., vitexin, apigenin, isovitexin, luteolin, and orientin and their O-glucosides) and 3-hydroxyflavones, or flavonols (e.g., kaempferol and quercetin). Clark (1978) examined 9 cannabis accessions grown in a common garden, and a canonical analysis of flavonoid profiles separated drug-type plants from fiber-type plants (see also Clark and Bohm 1979). Flavones act as phytoestrogens; Sauer et al. (1983) report that a cannabis extract and cannabis smoke condensate showed affinity for estrogen receptors in a heterologous competition assay. The displacement of [^{3}H]estradiol was *not* due to THC, rather apigenin was implicated.

Cannabis also biosynthesizes 3 unique prenylated aglycone flavanones, cannflavins A, B, and C, (Crombie et al. 1980; Radwan et al. 2008a). The cannflavins have only been reported in studies of drug-type plants (Barrett et al. 1985; Crombie et al. 1980; Radwan et al. 2008a;) and appear to be absent in fiber-type plants (Vanhoenacker et al. 2002). Cannflavins are potent inhibitors of cyclooxygenase enzymes and prostaglandin E2 production (Barrett et al. 1985). The cannflavins are structurally related to 8-prenylnaringenin, a potent phytoestrogen from hops. The pharmacology of cannabis flavonoids was reviewed in McPartland and Russo (2001), who propose many potential uses, predominantly

extrapolating from research on numerous other medicinal plants. Whether these uses have clinical relevance to cannabis is unknown.

Other Constituents

To date, 527 compounds have been isolated from cannabis (Appendino et al. 2011; ElSohly and Slade 2005). These other compounds occurring in cannabis include carbohydrates (monosaccharides, disaccharides, polysaccharides, sugar alcohols, cyclitols, and amino sugars), amino acids, amines (e.g., piperidine, hordenine, ammonia), non-cannabinoid phenols (spiro-indan-type, dihydrostilbene-type, cannabidihydrophenanthrene derivatives, simple phenols, simple phenolic glycosides, and phenol methyl esters), simple alcohols, aldehydes, ketones, acids, esters, lactones, steroids (phytosterols and brassinosteroides), vitamins, xanthones, coumarins, and pigments. Two unique spermidine-type C21-alkaloids, (+)-cannabisativine (Turner et al. 1976) and anhydrocannabisativine (ElSohly et al. 1978), have been found in cannabis and are reviewed in Mechoulam (1988).

Among the 527 compunds, some predominate in achenes or roots, and are marginally relevant to flowering tops. These include amides, fatty acids and their esters (oxylipins), quaternary bases (e.g., choline, trigonelline), and proteins.

Pharmacological effects have been established for many of these compounds. Notably, β-sitosterol, a phytosterol ubiquitous in the plant kingdom and found in cannabis (Mole and Turner 1974) and cannabis smoke (Adams and Jones 1975; Foote and Jones 1974), was shown to reduce topical inflammation and chronic edema in skin models (Gomes et al. 2008). A group of unique stilbenoids, canniprene and its spiranized (cannabispirans) and quinoid (denbinobin) derivatives (Turner et al. 1980b), were shown to have anti-inflmmatory, antibacterial, and antifungal activities (Flores-Sanchez and Verpoorte 2008; Pagani et al. 2011). Whether these actions are of clinical relevance remains to be determined.

Analytical

There are a number of analytes of interest in cannabis. Historically and presently, the quantitation of THC has been the focus of greatest interest. In recent decades, other cannabinoids have gained interest (e.g., CBD, THCV) due to their therapeutic benefits, as have terpenoids. Gas chromatography (GC) has been the primary methodological technique used for federal regulatory and toxicology purposes (e.g., ElSohly et al. 2000; Mehmedic et al. 2010, among others). Generally, there are a host of non-standardized, non-validated methods across several analytical platforms being used that give a wide range of total or THC values with unknown reliability. Thus, there is a need for standardized and validated testing methodologies.

THC is present only at very low levels in fresh or dry plant material. This compound is derived by decarboxylation of the naturally occurring non-psychoactive THCA during storage (small amounts) and heating (e.g., more complete decarboxylation when smoked) (Sirikantaramas et al. 2004; Yamauchi et al. 1967). In absence of a specific legislative directive regarding THC quantification, it is most common to quantify "total THC" (THCA + THC), as this best represents the potential activity associated with THC. Total THC content more closely reflects the amount of THC potentially yielded when smoked. Because of this, many legal systems consider total THC content as the primary quantitative value desired.

Decarboxylation from THCA to total THC can be achieved prior to and during analysis. Decarboxylation prior to analysis can be accomplished by placing a plant sample that has been extracted into a solvent into a heating block at 150 °C in an open glass vial. When the extraction solvent has evaporated, decarboxylation can occur within 5 min; however, individual analysts need to validate this process in their own laboratories (UNODC 2009).

During GC analysis, a sample elutes through a column within an oven, which decarboxylates most of the THCA into THC. Therefore, GC typically measures total THC. However, if the goal of the analysis is to quantify both THCA and THC by GC, prior derivatization is required (UNODC 2009). Additionally, varied degrees of decarboxylation can occur during injection in some GC systems, and high injection temperatures in the liner may cause a decomposition of THC. Decarboxylation may be partial, complete, or inconsistent depending on the temperature and geometry of the injector. Therefore, if decarboxylation is not performed prior to analysis, the specific gas chromatograph system and analysis conditions must be validated to ensure that complete decarboxylation of THCA is attained without undue decomposition of THC (Dussy et al. 2005; UNODC 2009).

High Performance Liquid Chromatography (HPLC) is also applicable for the quantification of cannabinoids. HPLC allows for the quantitation of the naturally occurring acid compounds, as well as the neutral forms, as both acids and neutrals are detected, and the peaks for both compounds can be added together for "total THC" or individual cannabinoids can be quantified. HPLC is therefore the optimal testing methodology for quantifying the authentic plant compounds prior to decarboxylation.

Thin-layer chromatography (TLC) is predominantly of value for the identification of cannabis. Currently, there are no validated TLC or high performance TLC (HPTLC) methods for the quantitation of THC that give results equal to those obtained from LC or GC analyses, although, some commercial laboratories are attempting to do so.

Some US states that have legalized the use of cannabis for either medicinal or non-medical use have proposed mandates requiring quantitative analysis. Both growers and dispensers are making claims of varying quantitative values of THC, other cannabinoids, and terpenoids in herbal cannabis and associated products. However, as cannabinoids are closely related in structure and molecular weight,

adequate chromatographic separation of these molecules is requisite to accurately report quantitative values. For example, Debruyne et al. (1994) compared TLC and HPLC to their gold standard: capillary column GC-MS. Analysis of a single cannabis specimen produced different quantitative peak sizes using these three methods. With GC-MS, THC=CBD>CBN; with HPLC, CBN>THC=CBD; with TLC, CBN>THC=CBD.

With appropriate sample preparation, analytical methods can be applied to a variety of cannabis preparations (foods or topicals), extractions (tinctures or oils), or concentrates; however method extensions must be performed for various matrices. To aid laboratories in the analysis of cannabis, the cannabis committee of the American Herbal Products Association (AHPA) developed a set of draft guidelines outlining recommended practices for labs to follow (AHPA 2013b), and Americans for Safe Access (ASA) has developed a laboratory certification program (ASA PFC 2013).

Lastly, and of significant importance in the analysis of cannabis, is to employ a formal sampling protocol (e.g. [OMC] BMC 2010; WHO 1998 among others) to assure the sample being tested is representative of an entire batch. This is critical, as dosing decisions either for medical or non-medical use can be based on claimed potencies, and there can be significant variation in constituent concentration between plants and even within a single plant itself. For cannabis, the sampling program being applied may differ between products being tested (e.g., raw material versus extracts). For crude cannabis, specific guidance is provided by the Bureau voor Medicinale Cannabis (BMC) monograph of the Netherlands ([OMC] BMC 2010).

Thin-Layer Chromatography Characterization of Cannabis and Its Major Cannabinoids

The following method was developed by the University of Mississippi and provides a characteristic fingerprint that can be used for the identification of cannabis and its primary cannabinoids as well as distinguish between THC-dominant, CBD-dominant, and fiber types. Two different reagents for visualization can be used. Both identify the primary cannabinoids, and either of them can be used for purposes of basic identification of crude cannabis plant material. Additionally, some different bands are visible with the 2 reagents. Therefore, examination using the 2 reagents allows for a more complete visualization of cannabis compounds.

Sample Preparation

Weigh approximately 100 mg of dried powdered cannabis, and extract by maceration with sonication in 10 mL dichloromethane for 1 h. Filter the extract and evaporate the solution under nitrogen. Redissolve the residue in methnol, adjusting the concentration to 10 mg/mL.

Decarboxylation of Cannabinoid Acids (optional)

To decarboxylate cannabinoid acids (e.g., to convert THCA to THC), heat the dried plant extract at 120 °C for 2 h* and adjust the concentration to 10mg/mL as indicated above.

* Alternatively, heating at 210 °C for 15 min can facilitate sufficient decarboxylation.

Table 7 R_f values for cannabinoid standards

Phytocannabinoid	R_f
CBC	0.21
Δ^9-THC	0.26
CBN	0.29
CBG	0.33
CBD	0.40
THCV	0.42
Δ^9-THCA	0.61
CBDA	0.77

Note: Due to its relatively high concentration in drug type samples, Δ^9-THC can overlap with CBN. CBN is a degradation compound of Δ^9-THC.

Standards Preparations

Cannabinoid standards are dissolved in methanol at a concentration of 1 mg/mL.

Note: All cannabinoid standards utilized in the development of this method were isolated at the University of Mississippi. There is limited availability of commercially prepared cannabinoid standards.

Standards Solution Stability

CBD, CBG, and CBN are stable in methanol, both at room temperature and with freezing. THC, THCV, and CBC methanolic solutions are stable only when frozen and acid compounds are only stable in a freezer. Due to their instability, acid compounds should be prepared cool and stored and shipped frozen.

Reagent Preparation

Fast Blue reagent: Dissolve 0.5 g Fast Blue B salt (MP Biochemicals, LLS) in 100 mL distilled water.

Vanillin/H2SO4: Dissolve 6 g vanillin in 90 mL ethanol (95%). Add 10 mL of 98% H_2SO_4. This reagent is relatively unstable and is best to use fresh each time.

Chromatographic Conditions

Stationary Phase:

C18 (UV 254) TLC plates 150 µm, 10 cm × 10 cm (Sorbent Technologies).

Figure 17a **TLC chromatogram of cannabis and its primary cannabinoids (Fast Blue reagent; white light)**

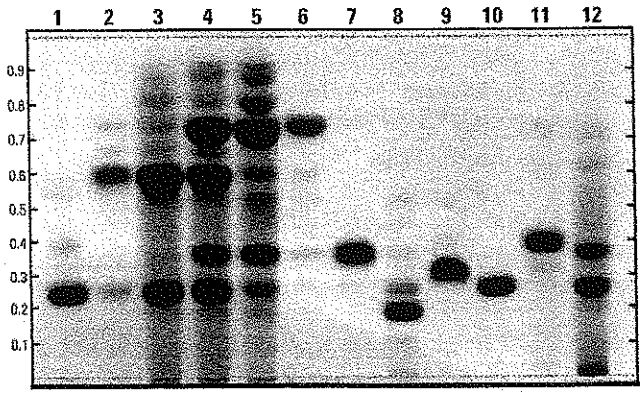


Figure 17b **TLC chromatogram of cannabis and its primary cannabinoids (Vanillin/H2SO4 reagent; white light)**

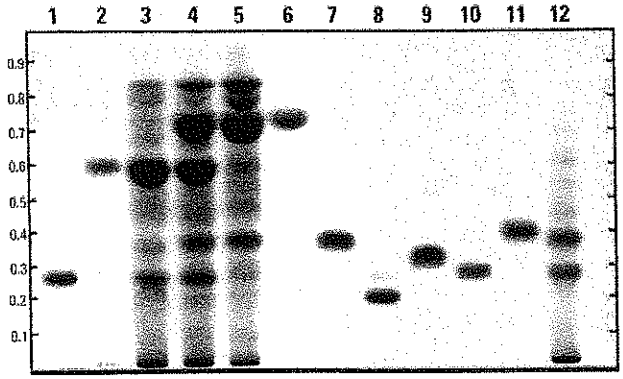
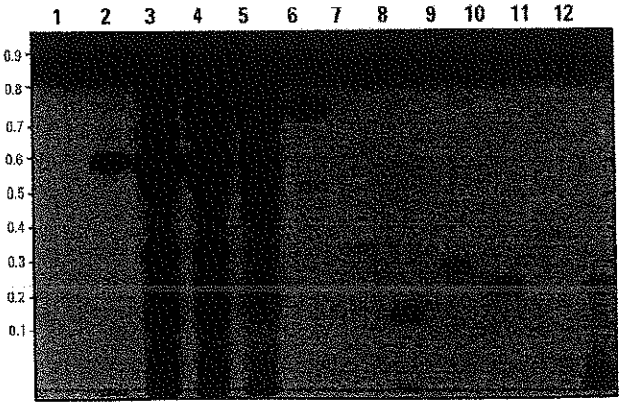


Figure 17c **TLC chromatogram of cannabis and its primary cannabinoids (UV 254 nm)**



Discussion of chromatograms

Observations (Fast Blue reagent; white light)

17a) In the cannabis THC drug type, the most prominent visible bands are those for Δ^9-THC, and THCA with 4 primary bands in the upper Rf region, including CBDA. In the intermediate type, the most prominent visible bands are those for Δ^9-THC, CBD, THCA, and CBDA with additional bands showing for CBC in the lower Rf; unknown bands in the middle R_f and 3 bands in the upper Rf, including CBDA. In the cannabis fiber type, the pattern of banding is very similar to the intermediate type, but reflects a much lower concentration of THCA and a similar concentration of CBD and CBDA. When subjected to decarboxylation, a degradation of a number of the original cannabinoid acids occurs, leaving characteristic bands for Δ^9-THC and CBD and a faint band for CBC. THCA-dominant types are most often notably lacking in CBD, while fiber types yield very low concentrations of Δ^9-THC and relatively high concentrations of CBDA. Thus, these 3 clearly delineated types can be readily distinguished. However, other materials, which are highly crossed, may not be readily distinguished.

17b) All standards (Lanes 1, 2, 6-11) appear as purple bands with varying intensities. In the cannabis drug type (Lane 3), the most prominent visible bands are those for THCA (R_f 0.61) and Δ^9-THC (R_f 0.26). In the intermediate type (Lane 4), the most prominent visible bands are those for CBDA, THCA, CBD, and Δ^9-THC. In the cannabis fiber type (Lane 5), the strongest bands are seen for CBDA and CBD. In the decarboxylated intermediate cannabis type (Lane 12), the only visible bands are for Δ^9-THC and CBD due to decarboxylation of the cannabinoid acids by heating.

17c) All cannabinoids are of varying intensities. THCA (Lane 2), CBDA (Lane 6), CBC (Lane 8), and CBN (Lane 10) are more intense than the others. In the cannabis drug type (Lane 3), a strong band is seen at the position of THCA. In the intermediate type (Lane 4), the most prominent visible bands are those for THCA and CBDA, while in the cannabis fiber type (Lane 5), the band for CBDA is most prominent. In the decarboxylated intermediate cannabis type (Lane 12), a band corresponding to CBN occurs in the lower third R_f (0.3).

Figure 17a-c lane assignments

Lane 1: Δ^9-THC
Lane 2: THCA
Lane 3: THC-type cannabis
Lane 4: Intermediate-type cannabis
Lane 5: Fiber-type cannabis
Lane 6: CBDA
Lane 7: CBD
Lane 8: CBC
Lane 9: CBG
Lane 10: CBN
Lane 11: THCV
Lane 12: Cannabis intermediate type decarboxylated (UM)

Mobile Phase:

75:25 (v:v) methanol/water with 0.1% glacial acetic acid.

Sample Application

Apply 5 µL of the sample preparations and 2 µL of the standards preparations on the plate as 5 mm bands 2 mm apart from each other. The application position should be 8 mm from the lower edge of the plate and at least 15 mm from the left and right edges of the plate. For visualization using both reagents, separate plates should be prepared.

Development

Line a flat bottom chamber (14 cm x 14 cm x 8 cm) with a filter paper or chromatography paper. Add a sufficient amount (~25 mL) of the Mobile Phase solution to ensure that the filter paper is covered to a a height of at least 5 mm, and let saturate for 15 min. Measure and mark on the plate the developing distance 60 mm from the application position. Introduce the plate into the chamber, and allow the developing solvent to reach the mark. Remove the plate and dry for 2 min at 70 °C in an oven.

Detection

Visualize the plates under UV 254 nm, then spray one set of the plates with the Fast Blue reagent and the other set of plates with the vanillin/H_2SO_4 reagent, followed by visualization under white light. For basic identification of the primary cannabinoids, either reagent can be used.

Results

See Table 7 and refer to the chromatograms provided (Figure 17a–c).

High-Performance Liquid Chromatography (HPLC) for the Determination of Major Phytocannabinoids in Cannabis

This HPLC method was adapted from Swift et al. (2013) and can be used for quantitation of THCA-A, Δ^9-THC, CBDA, CBD, CBCA, CBG, and CBN in cannabis preparations. The method was adapted from an earlier method developed by DeBacker et al. (2009), which also quantified Δ^8-THC. The original method of DeBacker et al. (2009) was validated for cannabis raw material and fully validated using total error approach in accordance with ISO17025 and the guidelines of the French Society of Pharmaceutical Sciences and Techniques (SFSTP). This modified and optimized method of Swift et al. (2013) was subjected to validation for selectivity, linearity, accuracy, precision, and recovery according to the US Food and Drug Administration (FDA) guidance for bioanalytical method validation (FDA 2001).

With appropriate modifications in sample preparations, the same chromatography can be used for the analysis of other cannabis materials (i.e. concentrates, extracts, foods). However, the robustness of this chromatography when applied to various matrices requires further validation (e.g., recovery, spiking experiments).

Sample Preparation

Crude Cannabis

Test samples are dried for 24 h in a 35 °C forced ventilation oven. Dried samples are ground to a fine powder. 200 mg of the sample is weighed in a glass vial and extracted with 10 mL of a mixture of methanol/chloroform (v/v: 9:1) by sonication for 30 min. The extract is filtered into an amber vial and diluted with methanol/chloroform solution (v/v: 9:1) to a concentration of 1:10. A 100-µL aliquot of the dilution is evaporated under a gentle stream of nitrogen and re-dissolved in 100 µL of a mixture of water/acetonitrile (v/v: 5/5).

Note: For analysis, the UNODC (2009) recommends that crude cannabis be dried to a finished moisture content of 8–13%, pulverized, and sieved through a 1 mm sieve. The UNODC provides the following sample preparations for different matrices. This specific method was not validated with these matrices, but these guidelines may be useful to the analyst.

Sample Preparation of Cannabis Resin

Grate into small pieces to a particle size of approximately 1 mm, or if sticky, cool with liquid nitrogen, pulverize, and sieve through a 1 mm sieve (UNODC 2009). Dissolve 50 mg in 10 mL of a mixture of methanol/ chloroform (v/v: 9:1) by sonication for 30 min.

Sample Preparation of Cannabis Oil

For HPLC analysis, cannabis oil requires no prior preparation. Dissolve 50 mg in 10 mL of a mixture of methanol/ chloroform (v/v: 9:1) by sonication for 30 min.

Standards Preparation

The availability of cannabinoid reference materials varies due to federal legal restrictions. A variety of cannabinoids are sold pre-diluted at concentrations of one mg/mL or less. Stock solutions for the standard curves are prepared across a broad range of concentrations to account for variable concentrations of cannabinoids. For accuracy, it is necessary to include at least 4 points in the standard curve. Standards should be run with every sample set and a relative bias not greater than 10% should be achieved. Limits of quantitation (LOQ) should be established using a calibration curve covering a range from 0.5 µg/mL to 100 µg/mL.

Internal Standard

Diazepam (50 mg/L). Diazepam is a schedule IV controlled substance. Use of an alternative internal control, such as methyl or propylparaben (e.g., 30 mg/L), should be validated for acceptable recovery and chromatographic separation.

Standard Stability

CBD, CBG, and CBN are stable in methanol, both at room temperature and with freezing. Δ^9-THC, THCV, and CBC methanolic solutions are stable only when frozen and acid compounds are only stable in a freezer. Due to their instability, acid compounds should be prepared cool and stored and shipped frozen.

Linearity Range

Compound	r^2	LOQ (%)	LOD (%)
THCA	0.9969	0.05	0.025
Δ^9-THC	0.9940	0.05	0.025
CBDA	0.9939	0.05	0.05
CBD	0.9951	0.075	0.075
CBGA	0.9948	0.05	0.05
CBG	0.9959	0.15	0.1
CBN	0.9917	0.05	0.025

r^2=coefficient determination; LOQ=Limit of Quantitation; LOD=Limit of Detection

Note: This method was not validated for quantitation of Δ^8-THC.

Storage of Reference Standards

For long-term storage of reference standards, store at -20 C protected from light and air. When properly stored, reference standards are stable for up to 12 months.

Chromatographic Conditions

Apparatus:

Validation was performed on a Shimadzu ADVP module (Kyoto, Japan) equipped with a SIL-10 autoinjector with sample cooler and LC-10 in-line vacuum degassing solvent delivery unit.

Column:

Waters X-Bridge C18 (4.6 mm x 150 mm, 3.5 µm) reverse-phase column (Waters, Australia) coupled with a 1-mm Opti-Guard C18 pre-column (Optimize Technologies, Alpha Resources, Thornleigh, Australia).

Column Temperature:

30 °C.

Injection Volume:

30 µL.

Mobile Phase:

A. 50 mM ammonium formate (adjusted to pH 3.75 with 10% acetonitrile)

B. 90% acetonitrile.

Time (min) B in A (%)

0 70

15 90

30 90

31 70

40 70

Flow Rate:

1 mL/min.

Detection (diode array detector):

Full spectra monitoring from 190-370 nm is recommended. Non-acidic cannabinoids are typically detected at approximately 228 nm and acidic cannabinoids at approximately 270 nm. Note: The validation was performed using a photodiode array detector. For routine use, a standard UV detector is suitable.

Run Time:

30 min.

Post-run Time:

6 min.

Note: CBD and CBC peaks may slightly overlap if present in high concentrations (> 10%).

Quantitation

Inject each standard preparation and generate a standard curve based on the peak area vs. concentration, as a ratio of standard to internal standard.

Cannabinoid contents in the sample are quantified using the linear equation based on least squares regression for each cannabinoid compound: $(y = mx + c)$

where:

- x = concentration of the individual cannabinoid in the sample (µg/mL);
- y = peak area of the invidivual cannabinoid;
- c = calculated y-intercept of the calibration curve;
- m = calculated slope of the calibration curve.

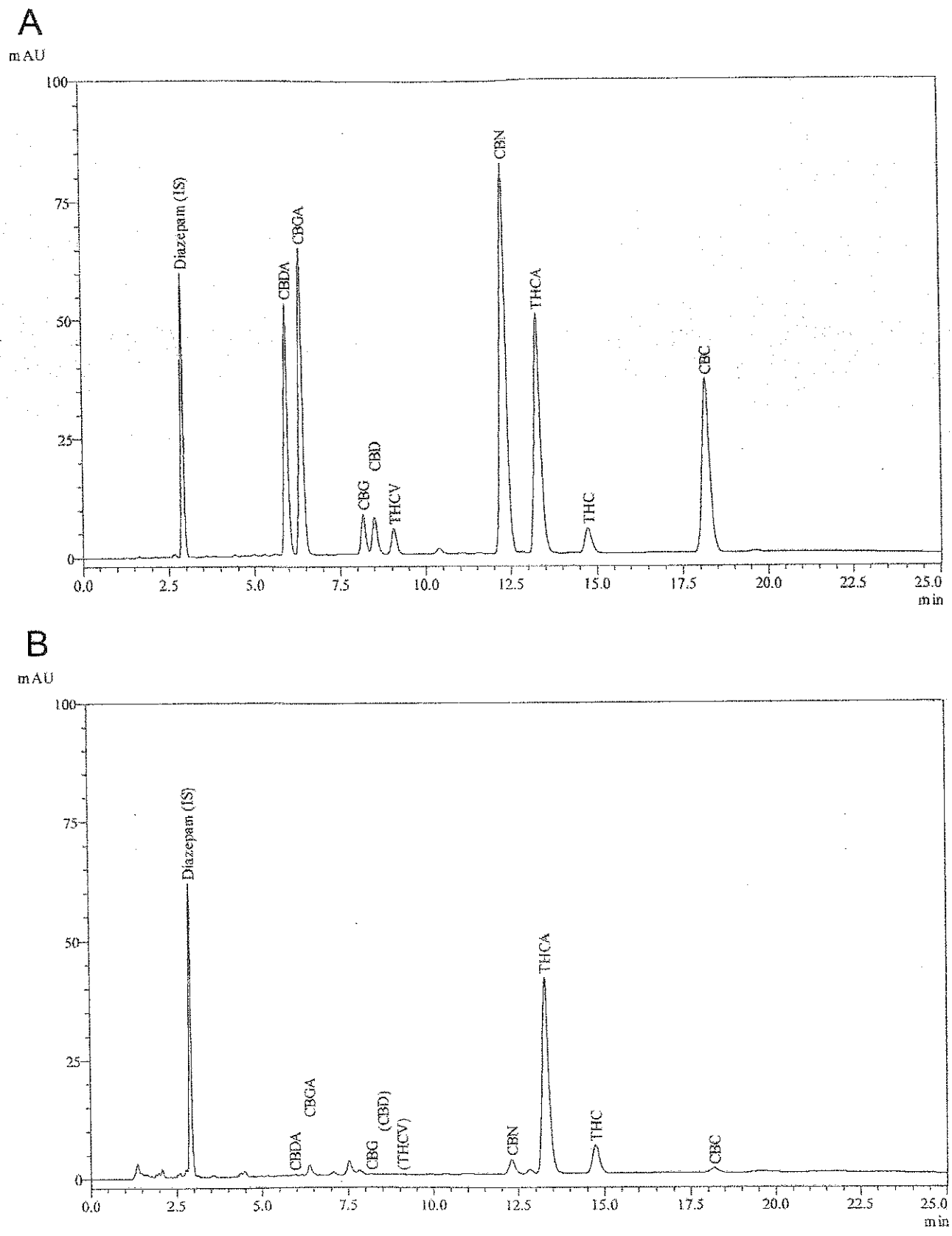
Using the concentration from the equation $(y = mx + c)$, total content (C_{CBXT}) in the sample can be calculated as a sum of the concentrations of the neutral (C_{CBX}) and the acidic (C_{CBXA}) components. A conversion factor of 0.877 is used for adjustment of the molar masses of THCA-A and CBDA; a conversion factor of 0.878 is used for CBGA; both after decarboxylation. These conversion factors may not apply for other cannabinoids:

$$C_{CBXT} = C_{CBX} + C_{CBXA} \times 0.877$$

The individual cannabinoid content in the material is then calculated according to the following equation:

$$W_{CBX(T)} = \frac{C_{CBX(T)} \times V_{sample} \times D}{m_{sample} \times 10^6} \times 100\%$$

Figure 18 **Representative HPLC chromatograms of cannabinoid standards (A at 11 µg/mL) and cannabis raw material (B)**



where:

$W_{CBX(T)}$ = (total) cannabinoid content in the material (% weight);

$C_{CBX(T)}$ = (total) cannabinoid content in the sample (µg/mL);

V_{sample} = sample volume (mL);

D = dilution factor;

m_{sample} = sample mass (g).

Calibration Range

Linear from 2 µg/mL to 100 µg/mL. Extrapolations from this curve should not be made; however, cannabinoid concentrations in samples greater than 100 µg/mL can be appropriately diluted, or the curve can be extended out to 1000 µg/mL (with 7 or more points in the curve) to ensure the reading is within the calibration range.

Gas Chromatography with Flame Ionization Detection (GC-FID) for the Quantitation of Phytocannabinoids

The following GC-FID method is used for the quantitation of the major phytocannabinoids of confiscated cannabis material submitted to the University of Mississippi by the DEA and other United States law enforcement agencies as part of NIDA's Marijuana Potency Monitoring Program (ElSohly et al. 2000; Mehmedic et al. 2010). Due to the high temperature of the GC injector port, in situ decarboxylation of the acidic cannabinoids occurs upon injection. This method, therefore, quantifies total cannabinoids (acidic and neutral) simultaneously. If quantitation of free (neutral) and acidic compounds is required for a specific cannabinoid, a non-destructive method, e.g., HPLC, or derivatization, e.g., silylation or formation of the alkylboronates, should be employed and validated.

Sample Preparation

Crude cannabis and hashish: To 100 mg of dried, powdered cannabis material with seeds and stems removed, add 3 mL of the internal standard solution (see below on the preparation instructions). Macerate for 1 hour at room temperature Sonicate for 5 min. Filter the extract into GC vials, and cap the vials.

Hash oil: To 100 mg of hash oil, add 4 mL of hash oil extraction solution (see below). Macerate for a minimum of 2 h at room temperature. Sonicate for 5 min. Add 20 mL of absolute ethanol, and sonicate again for 5 min. Filter the extract into GC vials, and cap the vials.

Internal Standard Preparation (use for extraction of cannabis and hashish)

Dissolve 100 mg of 4-androstene-3,17-dione in 100 mL of 1:9 v/v chloroform/methanol mixture.

Hash Oil Extraction Solution: Dissolve 50 mg of 4-androstene-3,17-dione in 50 mL of absolute ethanol.

Chromatographic Conditions

Column:

DB-1MS: 15 m x 0.25 mm id x 0.25 µm film (J&W Scientific, Inc, US [Agilent Technologies]).

Mobile Phase:

Helium.

Column\Head Pressure:

14 psi (1.0 mL/min).

Traps:

Moisture and oxygen traps for the purification of the helium.

Injection Volume:

1 µL.

Injection Mode:

Split (can be selected based on the sensitivity needed and analytical goal).

Injector Temperature:

240 °C.

Temperature Program (Column Control):

170 °C (hold 1 min) to 250 °C (hold 3 min) at 10 °C/min, 12 min total run time.

Detection Temperature:

260 °C.

Make-up Gas:

Helium (UHP): 20 psi, 20 mL/min (nitrogen may be used as an alternative make-up gas).

Combustion Gases:

Hydrogen (UHP): 30 psi, 30 mL/min and compressed air (suitably purified) at 30 psi, 400 mL/min.

Split Flow:

50 mL/min.

Split Ratio:

50:1.

Septum Purge:

5 mL/min (will vary on different systems).

Detection (FID):

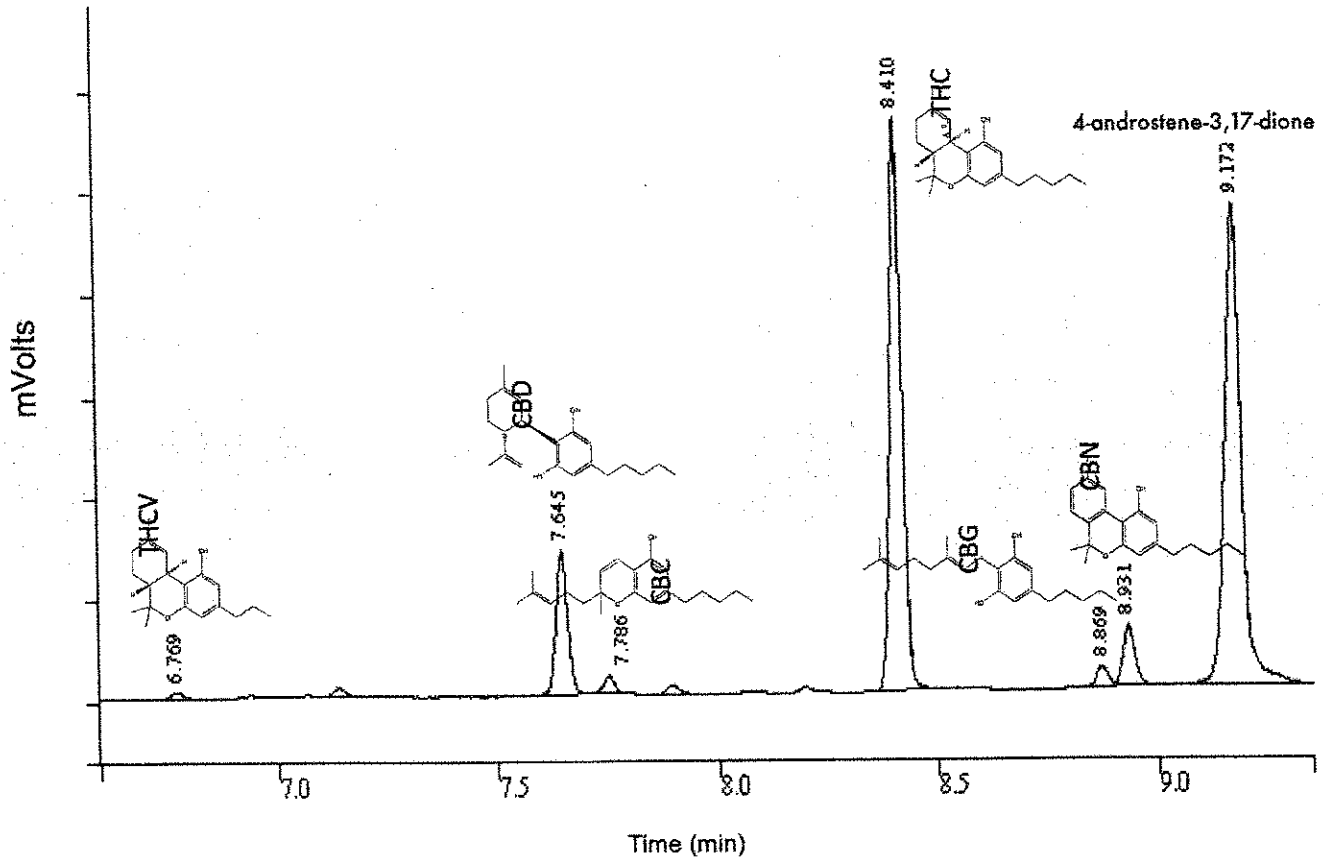
Relative retention times are provided in Table 8.

Calculations:

Cannabinoid potency is calculated as shown in the following equation:

$$W_C = (I_C \times m_{is}) / (I_{is} \times m_{simple}) \times 100\%$$

Figure 19 **Characteristic gas chromatography (GC) chromatogram of cannabis with an internal standard**



where:

W_C = relative cannabinoid content of the material, % weight;

I_C = integrated area of the cannabinoid peak from GC-FID chromatogram;

I_{is} = integrated area of the peak of the internal standard from GC-FID chromatogram;

m_{is} = mass of internal standard

Table 8 Relative retention times of phytocannabinoids and 4-androstene-3,17-dione, as observed using GC-FID

Cannabinoid	Time (min)	
	Without internal standard	With internal standard
THCV	6.772	6.769
CBD	7.649	7.645
CBC	7.786	7.786
Δ^9-THC	8.420	8.410
CBG	8.869	8.869
CBN	8.930	8.931
4-androstene-3,17-dione	–	9.172

Limit Tests

Limits that are applicable to cannabis include those that are generally applied to herbal materials, such as tolerance levels of microbial and fungal contamination, content of certain metals, as well as limits of solvent and pesticide residues. With exception to loss on drying and moisture content of dry material, the following limits are based on general recom-

mendations for botanical ingredients established by various national and international bodies. Tests can be performed according to standard pharmacopoeial instructions (e.g., European Pharmacopoeia, United States Pharmacopeia, among others).

Foreign Organic Matter (crude cannabis material): Not more than 5.0% of stems 3 mm or more in diameter; not more than 2.0% of other foreign matter.

Total Ash (crude cannabis material): Not more than 20.0%.

Acid-insoluble Ash (crude cannabis material): Not more than 4.0%.

Loss on Drying (crude cannabis material): Not more than 10.0% of its weight, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C for 2 h (BMC 2010).

Moisture content of dry material (crude cannabis after packaging): Not more than 15% (BMC 2010).

Microbial and Fungal Limits

The presence of microbes is typical for all natural products. Unless carefully cultivated, illegal supplies may not meet the prescribed specifications. Conversely, reports in which a causal association between microbial exposure through cannabis use and infections has been established (e.g., Carod Artal 2003) appear to be rare considering the prevalence of use and exposure.

Tolerance limits for microbial and fungal contamination in cannabis and its products should be consistent with applicable state, federal, and international regulations, whenever applicable. Recommended tolerance limits for cannabis products are provided in Table 9 and were based on a review of national and international recommendations for botanical products as well as discussion with a variety of stakeholders (e.g., Washington State). Additional guidance for botanical products is provided in national and international compendia based on oral consumption of finished botanical products. Additionally, more restrictive limits may be adopted for medical use of cannabis, most notably when used by immune compromised individuals. Microbes such as *Aspergillus* spp., for example, can be transmitted through inhalation and are of specific concern in those with specific medical conditions (e.g. chronic granulamatous disease and cystic fibrosis) and when employing specific medical treatments (e.g., immunosuppressive therapies). Reducing total microbial risk may require specific microbial reduction treatment to the greatest level possible without compromising the putative medicinal activity. Appropriate methods for testing microbial loads can be found in the *Bacteriological Analytical Manual* (FDA 2013a).

It is important to note that microbial and fungal values do not typically represent pass or fail criteria. Rather they are recommended levels when plants are produced under normal circumstances and growing conditions. Individual herbs, such as mints (*Mentha* spp.), which have a high concentration of trichomes, are prone to higher levels of molds than crops with fewer trichomes. As cannabis also possesses high concentrations of trichomes, this may be a factor and recommended limits may require adjustment over time. Higher levels of molds can also occur in seasons of heavy rain without undue damage to the crop and may justify a material exceeding the proposed limits as long as there is no visible damage to the plant and other qualitative specifications are met. Limits must also be appropriately applied to the various preparations being made. Typical microbial and fungal limits may not be relavant to materials that are to

Table 9 Microbial and fungal limits recommended for orally consumed botanical products in the US (CFU/g)

	Total viable aerobic bacteria	Total yeast and mold	Total coliforms	Bile-tolerant gram-negative bacteria	*E. coli* (pathogenic strains) and *Salmonella* spp.
Unprocessed materials*	10^5	10^4	10^3	10^3	Not detected in 1 g
Processed materials*	10^5	10^4	10^3	10^3	Not detected in 1 g
CO_2 and solvent-based extracts	10^4	10^3	10^2	10^2	Not detected in 1 g

* Unprocessed materials include minimally processed crude cannabis preparations such as inflorescences, accumulated resin glands (kief), and compressed resin gland (hashish). Processed materials include various solid or liquid infused edible preparation, oils, topical preperations, and water-processed resin glands ("bubble hash"). Significant microbial contamination can occur during post-harvesting hadling.

Table 10 **Pesticides commonly used in cannabis cultivation**

Pesticide	Use	Residue Analytical Methods (RAM) Environmental Protection Agency (EPA)[1] or Literature[2]
Abamectin (Avermectins B1a and B1b)	Insecticide/acaricide	LC-FLD[1]; LC-MS/MS[2]
Acequinocyl	Insecticide/acaricide	LC/MS/MS[1]
Bifenazate	Acaricide	LC[1]; LC-MS/MS[2]
Bifenthrin (synthetic pyrethroid)	Insecticide	GC-ECD[1]; GC-MS/MS[2]
Chlormequat chloride	Plant growth regulator (PGR)	IC, LC-MS/MS[2]
Cyfluthrin (synthetic pyrethroid)	Insecticide	LC[2] (WHO 2004); GC-MS/MS[2]
Daminozide (Alar)	Plant growth regulator (PGR)	UV Spectroscopy[1]; LC-MS/MS[2]
Etoxazole	Acaricide	GC-MS(/MS)[1]
Fenoxycarb	Insecticide	LC/UV[1]; LC-MS/MS[2]
Imazalil	Fungicide	GC-ECD[1]; LC-MS/MS[2]
Imidacloprid	Insecticide	LC-MS/MS[2]
Myclobutanil	Fungicide	GC-ECD; GC-NPD[1]; GC-MS/MS)[2]; LC-MS/MS[2]
Paclobutrazol	Plant growth regulator (PGR); fungicide	LC-MS/MS[2]
Pyrethrins*	Insecticide	GC-ECD[1]
Spinosad	Insecticide	LC-MS/MS; immunoassay[1]
Spiromesifen	Insecticide	GC-MS[1]; LC-MS/MS[2]
Spirotetramat	Insecticide	LC/LC-MS/MS[2]
Trifloxystrobin	Fungicide	GC-NPD[1]; GC-MS/MS[2]; LC-MS/MS[2]

ECD = Electron capture detector; FLD = Fluorescence detector; GC = Gas chromatography; LC = Liquid chromatography; IR = Infrared spectroscopy; MS = Mass spectrometry; NMR = Nuclear magnetic resonance; NPD = Nitrogen phosphorous detector.

* Natural pyrethrins are tolerance exempt; synthetic pyrethrins are not.

be subjected to processing, such as infusing, decocting, or extracting with heat, alcohol, or other processes that introduce a microbial reduction step prior to consumption.

Metal Limits

When grown in contaminated soil, cannabis accumulates heavy metals to the extent that it has been proposed as a candidate for bioremediation of toxic waste sites (Shi and Cai 2009). Siegel et al. (1988) measured 440 ng mercury per gram of cannabis in Hawaii, whose volcanic soil contains naturally high levels of mercury. Siegel notes that mercury is absorbed 10 times more efficiently by the lungs than by the gut. He calculated that smoking 100 g of volcanic cannabis per week could lead to mercury poisoning. The American Herbal Products Association (AHPA) provides manufacturers of herbal products with general recommendations for maximum heavy metals levels in herbal products, based on the daily product intake amount (Table 11). The most appropriate method for quantification of metals in medicinal products is an inductively coupled plasma-mass spectrometry (ICP-MS) method of the US Food and Drug Administration (FDA), which analyzes arsenic, cadmium, chromium, lead, and mercury (FDA 2011). The cannabis monograph of the Netherlands BMC (2010) considers the risk of metal contamination of cannabis grown under controlled conditions to be low.

Pesticide Limits

In the US, pesticides are regulated by the Environmental Protection Agency (EPA), which registers or licenses pesticides for use in the United States, and by individual states (usually, by that state's department of agriculture), which may regulate pesticides more stringently than EPA. Pesticide tolerances are approved on an individual or crop group basis, so that the approval of a pesticide for use on one commodity does not confer the approval of its use on another. Where no limits are specifically established for a specific crop or class of crops, the limit is zero (0), generally considered as < 0.01 ppm or 10 ppb according to analytical methods set forth in the *Pesticide Analytical Manual* (PAM; available from the US Food and Drug Administration) (FDA 2013b).

To date, there are no pesticides specifically approved for use on cannabis in North America on the federal level. However, some pesticides with tolerance exempt ingredi-

Table 11 Metal limits recommended for herbal products in the US

Contaminating metal	Limit, µg/daily dose
Inorganic arsenic	10
Cadmium	4.1
Lead	6
Methyl mercury	2.0

Source: AHPA (2008).

ents have broad use sites that could allow for their use on cannabis. Additionally, some states, (e.g., Massachusetts, Washington, and Colorado) are formulating guidelines for pesticide use in cannabis cultivation, whose ingredients are approved in that state for organic production, or are listed by the Organic Materials Review Institute (OMRI). Use of unapproved pesticides in those states that allow for OMRI-listed or exempt pesticides represents a public safety license violation and can result in the cancellation of a cannabis producer's license. State allowance for pesticide use on cannabis may be in conflict with federal pesticide regulations.

Presence and Testing of Pesticides in Cannabis

Specialty agricultural supply stores for the cannabis industry, have proliferated across the US, many of which are categorized as "hydroponic". This aspect of the industry lacks any meaningful regulation or guidance. Products found in such stores have been reported to contain banned substances, and often fail to accurately disclose ingredients or provide adequate information for proper use. For example, the California Department of Food and Agriculture (CDFA) in 2011 issued cease and desist orders against the sale of a number of popular cannabis cultivation products due to their inclusion of a number of banned plant growth regulators including daminozide (Alar) and paclobutrazol (CDFA 2011). A number of these products are labeled as "organic" though they may not be compliant under the National Organics Program of the United States Department of Agriculture (USDA).

The use of such agents on cannabis crops is widespread. Daley et al. (2013) compiled a list of 148 pesticide products used in cannabis cultivation, based on a survey of California growers. Insecticides and miticides are often used on cannabis grown indoors, while fungicides are used on both indoor and outdoor crops. Inappropriate use of insecticides, miticides, and fungicides (such as improper product selection, application rate, concentration, and/or timing) can lead to pests becoming resistant and/or medical users being exposed to inappropriate residue levels.

Appropriate testing methodologies, as recommended by the Environmental Protection Agency (EPA Residue Analytical Methods [RAM]) or those of the Food and Drug Administration (FDA Pesticide Analytical Manual [PAM]), should be employed when appropriate. However, as these tests were developed for commodity food products, the amount of sample needed may be prohibitive to apply to the cannabis industry. Alternatively, The food testing QuEChERS screen uses smaller quantities and may be more applicable to a variety, though not all, of cannabis products (Schoen 2013, personal communication to AHP, unreferenced).

In the cannabis industry today, the most commonly used screening technology for organophosphates, organochlorines, carbamates, and ethylenediaminetetraacetic acid (EDTA) are immunoassays (e.g., enzyme-linked immunosorbent assays [ELISA]) and broad spectrum field tests that may or may not be validated for use on cannabis. Similarly, immunoassays for a broad range of PGRs and fungicides commonly used in cannabis cultivation are not available. Because of their relative inexpense, immunoassays are routinely used by analytical labs specializing in cannabis testing and are at high risk of not detecting pesticide residues and reporting samples to be "pesticide-free" or "non-detected". Before commercial use, any immunoassay should be validated against a standard testing methodology.

Table 10 provides a list of the most common pesticides (including acaricides, insecticides, fungicides, and plant growth regulators) used in cannabis production.

Solvent Residues

Limits on solvents used in the manufacture of botanical products are established by the International Conference on Harmonization (ICH) (ICH 2011), with exceptions made for ethanol and acetic acid in products formulated to contain these substances (e.g., tinctures and vinegars). According to the ICH guideline, solvents are categorized in 3 classes. Class 1 includes known carcinogens, toxic substances, and environmental hazards such as benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, and 1,1,1-trichloroethane. These are to be avoided in the manufacture of herbal and/or pharmaceutical products. Class 2 and 3 solvents (Table 12) are distinguished based on their relative toxicity level. Limits established for permissible daily exposures (PDE) are determined individually for Class 2 solvents. Limits for Class 3 solvents are set at a general limit of 50 mg/day. In addition, the ICH guideline lists solvents for which no adequate toxicological data was found (Table 13) and requires manufacturers of pharmaceutical products that choose to use these solvents to supply justification for residual levels of these solvents in their final products. Petroleum ether, found in this group, is reportedly used in the production of hash oil (UNODC 2009).

Solvent extracted products made with Class 3 or other solvents, are not to exceed 0.5% residual solvent by weight or 5000 parts per million (PPM) per 10 gram of solvent-based product and are to be quantified according to the United States Pharmacopeia (USP <467>), Residual Solvents, Option 1. Higher concentrations may also be acceptable provided they are realisti in relation to safety, manufacturing, and good manufacturing practices.

Table 12 **Permissable and restricted solvents in the manufacture of cannabis preparations**

Class 2 solvents		Class 3 solvents
Solvent	Permissible daily exposure: mg/day	Permissible daily exposure: 50 mg/day
Acetonitrile	4.1	Acetic acid†
Chlorobenzene	3.6	Acetone
Chloroform*	0.6	Anisole
Cyclohexane	38.8	1-Butanol
1,2-Dichlorothene	18.7	2-Butanol
Dichloromethane*	6.0	Butyl acetate
1,2-Dimethoxyethane	1.0	tert-Butylmethylether
N,N-Dimethylacetamide*	10.9	Cumene*
N,N-Dimethylformamide	8.8	Dimethyl sulfoxide
1,4-Dioxane*	3.8	Ethanol*†
2-Ethoxyethanol	1.6	Ethyl acetate
Ethyleneglycol	6.2	Ethyl ether
Formamide	2.2	Ethyl formate
Hexane	2.9	Formic acid
Methanol*	30.0	Heptane
2-Methoxyethanol	0.5	Isobutyl acetate
Methylbutyl ketone	0.5	Isopropyl acetate
Methylcyclohexane	11.8	Methyl acetate
N-Methylpyrrolidone*	5.3	3-Methyl-1-butanol
Nitromethane*	0.5	Methylethyl ketone
Pyridine*	2.0	Methylisobutyl ketone
Sulfolane	1.6	2-Methyl-1-propanol
Tetrahydrofuran	7.2	Pentane
Tetralin	1.0	1-Pentanol
Toluene*	8.9	1-Propanol
1,1,2-Trichloroethene	0.8	2-Propanol
Xylene	21.7	Propyl acetate

* Listed as chemicals known to the state of California to cause cancer or reproductive toxicity under Proposition 65 (CAEPA 2013).
Source: AHPA (2008); CAEPA (2013); ICH (2011); United States Pharmacopeia (USP 30-NF 25 2007).

Table 13 **Solvents for which no adequate toxicological data was found**

1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

Source: ICH (2011).

International Status

Definitions and regulations of what constitutes a "controlled substance" and medically useful substance differ greatly between countries. There are also varying levels of tolerance for use of mind-altering substances such as alcohol and cannabis. Internationally and domestically, regulations regarding the medical and recreational use of cannabis are changing rapidly. In the US, individual states have enacted their own rights, regulations, and prohibitions regarding both medical and recreational cannabis use, which conflict with federal law. Similarly, a number of countries (e.g., the US, Canada, Israel, the Netherlands, and others) provide an official source of medicinal-grade cannabis to certain chronically ill patients. Additionally, several countries (e.g., Canada, Denmark, Germany, Spain, New Zealand, United Kingdom) have approved pharmaceutical preparations made from cannabis extracts (e.g., Sativex®) as prescription-only medicines (MHRA 2010).

The regulation of cannabis is a subject of international treaties that include the US as a signatory (United Nations 1973). The US Controlled Substances Act (CSA) was designed to fulfill the country's treaty obligations under the United Nations' Single Convention on Narcotic Drugs (1961). This treaty restricts cannabis to appropriate medical use only, and places strict controls on cannabis cultivation in a manner similar to those imposed on opium poppies. The treaty does not apply to cannabis plants grown exclusively for industrial (fiber and seed) or horticultural purposes. As of 2013, there were 61 signatories to the Convention (United Nations 2013a) and 54 signatories to the Protocol that amended the convention in 1972 (United Nations 2013b). Following is a brief review of the manner in which cannabis is regulated domestically and internationally. Due to the rapidly changing regulatory environment, interested readers must refer to primary regulatory policies in various states and countries as well as expected requirements under international treaties.

United States

The term "marihuana" is defined in the United States Code (USC) as "all parts of the plant Cannabis sativa L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin. Such "marihuana" term does not include the mature stalks of such plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of cannabis, such plant which is incapable of germination" (USC 2010). This language remains essentially unchanged from the Marihuana Tax Act of 1937.

Drug (Federal): "Marihuana," "tetrahydrocannabinols" and CBD are classified by the Drug Enforcement Administration (DEA) as Schedule I controlled substances (DEA 2011a). The findings required to place a substance on Schedule I of the Controlled Substances Act are: (a) the drug or other substance has a high potential for abuse; (b) the drug or other substance has no currently accepted medical use in treatment in the United States; and (c) there is a lack of accepted safety for use of the drug or other substance under medical supervision. Several formal petitions for the rescheduling of cannabis have been denied (DEA 2011b).

Rescheduling to Schedule II by the DEA requires for the following 5-part test to be fulfilled: 1) the drug's chemistry must be known and reproducible; 2) there must be adequate safety studies; 3) there must be adequate and well-controlled studies proving efficacy; 4) the drug must be accepted by qualified experts; and 5) the scientific evidence must be widely available. Alternatively, rescheduling could occur by Executive Order of the President or by Congress. The DEA rescheduled synthetic THC (dronabinol, Marinol®) to Schedule II in 1985, and Schedule III in 1999.

An exception is made for the "Compassionate Use" Investigational New Drug (IND) Program. In 1976, the DC Superior Court found a defendant suffering from glaucoma not guilty of possession of marijuana based on the Common Law Doctrine of Necessity (US v Randall). The defendant successfully argued that inhalation of marijuana smoke had a beneficial effect, normalizing intraocular pressure and lessening visual distortions (DC Superior Court 1976). In 1978, the same glaucoma patient brought a lawsuit against the federal government (Randall v US) for its role in disrupting his legal access to marijuana. An outcome of the lawsuit settlement by the Department of Health and Human Services (HHS), which became the basis for the Food and Drug Administration (FDA) Compassionate IND Program, was that the National Institute on Drug Abuse (NIDA) would begin supplying cannabis to patients whose physicians applied for and received use permits from the FDA. The NIDA provides funding to the University of Mississippi for growing, harvesting and storage of cannabis as well as potency monitoring and other services for the DEA (NIDA 1988). The NIDA is responsible for shipping the marijuana to registered patients. Medical diagnosis of Compassionate IND Program patients have included (ProCon.org 2014)

- AIDS
- Glaucoma
- Multiple Congenital Cartilaginous Exostoses
- Multiple sclerosis
- Nail Patella Syndrome

Drug (State): To date, medical cannabis laws have been enacted in 22 states and the District of Columbia (Stroup 2014). These laws exist in conflict with federal laws leaving discretion to US Attorneys on when to enforce federal law against participants in state-sanctioned programs. To date, however, there has been no attempt by the federal government to overturn such state laws. In August 2013 the Department of Justice issued a memo to US attorneys advising that individuals and companies following state laws should not be priorities for prosecution but ultimately

left the decision of whether or not to prosecute up to US Attorneys (Cole 2013).

Recreational (State): In 2012, Washington and Colorado, both of which allow for the medical use of cannabis, through ballot initiative, approved the controlled recreational use of cannabis, limiting its use to legal-age adults and with specific restrictions.

In 2000–2007, there have been approximately 7.9 million cannabis-related arrests in the US (US Bureau of Justice Statistics) making cannabis-related crimes one of the most frequently enforced crimes in the country. In 2012, there were a total of 749,825 marijuana arrests, of which 91,593 were trafficking/sale arrests and 658,231 were for possession (FBI Uniform Crime Report 2012).

Canada

Canadians currently have access to the widest representation of cannabinoid drugs in the world, including dronabinol (Marinol®), nabilone (Cesamet®), Sativex®, and crude cannabis. Canada also re-legalized industrial hemp cultivation in 1998. Cannabis for medical use is regulated under the Marihuana for Medical Purposes Regulations (MMPR), which came into force on June 7, 2013. Under the MMPR, marihuana for one's own medical purposes or for those of another person for whom they are responsible may be obtained only from a) a licensed producer In accordance with a medical document (signed by a licensed health care practitioner), b) from a health care practitioner in the course of treatment, or c) from a hospital in accordance with Narcotic Control Regulations. An individual may obtain up to 30 times the daily quantity from a licensed producer or from a hospital. Individuals must register to become clients of a licensed producer. Adults who reside in Canada and/or corporations with a head office or branch office in Canada are eligible to apply for a producer's license (Government of Canada 2014).

Indications: Potential therapeutic uses are outlined in Health Canada's information for health care professionals on cannabis and the cannabinoids. The listed uses include the following:

- Alzheimer's disease and dementia
- Arthritides and Musculoskeletal Disorders
- Asthma
- Chemotherapy-induced nausea and vomiting
- Epilepsy
- Gastrointestinal system disorders (irritable bowel syndrome, inflammatory bowel disease, hepatitis, pancreatitis, metabolic syndrome/obesity)
- Glaucoma
- Hypertension
- Inflammation (Inflammatory skin diseases [dermatitis, psoriasis, pruritus])
- Movement disorders (dystonia, Huntington's Disease, Parkinson's Disease, Tourette's syndrome)
- Multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury
- Pain (acute and chronic)
- Palliative care (relief from pain and other distressing symptoms, and the enhancement of quality of life)
- Psychiatric disorders (alcohol and opioid withdrawal symptoms [drug withdrawal symptoms], anxiety and depression, sleep disorders, schizophrenia and psychosis
- Wasting syndrome (cachexia, e.g., from tissue injury by infection or tumor) and loss of appetite (anorexia) in AIDS and cancer patients, and anorexia nervosa (Health Canada 2013).

European Union (EU)

In the EU, rules regarding the commerce of cannabis are not harmonized. Possession of small amounts for medical or personal use has been decriminalized or liberalized to varying degrees in several countries including Belgium, the Czech Republic, Estonia, Germany, Italy, the Netherlands, Poland, Portugal, and Spain, as well as in some non-EU European countries like Switzerland (Reuter 2010; Rosmarin and Eastwood 2012). The Netherlands and the Czech Republic have enacted programs for access to dried cannabis flowers for medical use. The Netherlands represents the most liberal state in terms of access to cannabis for both medicinal and recreational use; France, in contrast, has prohibited cannabis drugs since 1925, but never outlawed fiber-type plants (France was the only country in Western Europe that grew hemp between 1982 and 1985). Spain, because of its proximity to Morocco, leads the world in hashish seizures, accounting for 26% of global seizures with 356 tons seized in 2011 and 326 tons in 2012 (UNODC 2014).. Concerning the cultivation of industrial hemp, the current upper legal limit is 0.2% THC with a ratio of CBD to THC greater than one in most European countries (UNODC 2009).

In November 2013, a European citizens' initiative proposing the legalization of cannabis and the EU to adopt a common policy on the control and regulation of cannabis production, use and sale, was registered with the European Commission. Citizens' initiatives have one year to collect one million signatures of EU citizens old enough to vote. If the requisite number of signatures is obtained, the Commission has 3 months to examine the initiative, meet with the initiative organizers, hold a public hearing, and prepare a formal response. The Commission is not obliged to propose legislation as a result of an initiative. If the Commission decides to put forward a legislative proposal, the normal legislative procedure kicks off, i.e. the Commission proposal is submitted to the legislator (generally the European Parliament and the Council, or, in some cases, only the Council) and, if adopted, it becomes law (European Commission 2013).

India

India enacted the Narcotics Drugs and Psychotropic Substances Act in 1985, which brought India into compliance with the UN's Single Convention on Narcotic Drugs. Ganja (flowering tops) and charas (hashish) are illegal. Bhang (the dried leaf of cultivated or wild-collected Cannabis sativa,) when used in traditional medicine preparations and products, is regulated as an active ingredient of traditional medicines used in the Indian Systems of Medicine (Ayurveda, Siddha, and Unani). Quality standards monographs are published in the Ayurvedic Pharmacopoeia of India (API), Siddha Pharmacopoeia of India (SPI), and Unani Pharmacopoeia of India (UPI).

Indications: Ayurveda: Agnimandya (digestive impairment), anidra (insomnia), atisara (diarrhea), klaibya (male impotence), grahani roga (malabsorption syndrome) (API 1989); Siddha: Kakkirumal (whooping cough), mikupaci (excessive appetite), narampuvali (neuralgia), orraittalaivali (hemicranias/migraine), perumpatu (menorrhagia), vantipeti (vomiting and diarrhea) (SPI 2008). Unani: Ishal (diarrhea), kasrat-e-tams (polymenorrhagia), bawaseer (piles), sual (bronchitis), waj-ul-kabid (hepatalgia), qulanj (colic) (UPI 2007).

Israel

In July 2011, the Israeli Cabinet approved arrangements and supervision regarding the supply of cannabis for medical and research uses in recognition that the medical use of cannabis is necessary in certain cases. The Health Ministry, in coordination with the Israel Police and the Israel Anti-Drug Authority, is responsible for supplies from imports and local cultivation (State of Israel Prime Minister's Office 2011).

In December 2013, the Israeli Cabinet amended the medical marijuana regulations by increasing the pool of physicians allowed to prescribe cannabis to their patients from 21 to 31. The new rule also changes the way in which marijuana can be grown, packaged and distributed in Israel. As of early 2014, approximately 14,000 patients have been given prescriptions to use medicinal marijuana.

Quality: There are currently 10 different strains of marijuana being grown by 8 authorized growers and distributed to patients with a prescription (Israeli Medical Association 2014).

Indications: Cannabis prescriptions are available for these conditions (and others on a case-by-case basis):

- AIDS wasting syndrome
- Asthma
- Chronic pain due to a proven organic etiology
- HIV patients with significant loss of body weight or a CD4 cell count below 400
- Inflammatory bowel disease (but not Irritable Bowel Syndrome)
- Malignant cancerous tumor in various stages
- Multiple sclerosis
- Orphan diseases (i.e., diseases and conditions that affect only a small percentage of the population and for which few, if any, pharmaceutical drugs are developed)
- Parkinson's Disease
- Vomiting and pain associated with chemotherapy for cancer (Stafford Mader 2013).

Netherlands

The Office for Medicinal Cannabis (OMC) is responsible for the production of cannabis (dried flower tips harvested from female Cannabis sativa plants) for medical and scientific purposes and is the exclusive supplier of medicinal cannabis to pharmacies, and on its import and export.
Quality: Medicinal cannabis provided by the OMC is of pharmaceutical quality, produced under controlled cultivation according to Good Agricultural Practices (GAPs). Three types of medicinal cannabis are available through pharmacies: Bedrocan, Bedrobinol, and Bediol. The recommended modes of administration are by making tea or through inhalation.

Indications: According to OMC, there is sufficient reason to believe that medicinal cannabis can help in cases of:

- Pain and muscle spasms or cramps associated with multiple sclerosis or spinal cord damage;
- Nausea, loss of appetite, weight loss, and debilitation due to cancer or AIDS;
- Nausea and vomiting associated with chemotherapy or radiotherapy used in the treatment of cancer, hepatitis C or HIV infection and AIDS;
- Chronic pain; primarily pain associated with the nervous system, (e.g., damaged nerve, phantom pain, facial neuralgia or chronic pain which remains after the recovery from shingles);
- Gilles de la Tourette syndrome;
- Therapy-resistant glaucoma (OMC 2011).

Switzerland

Medical use: Obtaining marketing authorization from Swissmedic for Complementary and Herbal Medicinal Products (KPAV) that contain preparations made from Cannabis sativa as an active ingredient is possible (Swissmedic 2013a). In November 2013 the first cannabis product received marketing authorization, Sativex® Spray (Cannabis sativae folii cum flore extractum spissum) (Swissmedic 2013b).

Indications: Treatment for symptom improvement in adult patients with moderate to severe spasticity due to multiple sclerosis who have not responded adequately to other antispasticity medication and who demonstrate clinically sig-

nificant improvement in spasticity related symptoms during an initial trial of therapy (Almirall AG 2013).

Recreational use: In 2012, the Federal Assembly amended the federal law on narcotics and psychotropic substances, ostensibly decriminalizing possession of up to 10 grams of cannabis with the implementation of a simplified procedure for imposing a flat fee fine in the amount of 100 Swiss Francs (Federal Assembly of the Swiss Confederation 2012).

Uruguay

In December 2013, Uruguay became the first country to legalize the growing, sale, and smoking of cannabis. The government-sponsored bill that led to this approval provides for regulation of the cultivation, distribution, and consumption of cannabis. The primary stated motivation of the legislation was to fight drug trafficking of cannabis (Uruguay, Law No. 19.172). The law allows for Uruguayan residents over the age of 18 to become a registered user and to purchase up to 40 grams (1.4 ounces) per month from licensed pharmacies. A government database will monitor consumer monthly purchases. Additionally, Uruguayans will be able to grow 6 cannabis plants in their homes a year, or as much as 480 grams (approximately 17 ounces), and form smoking clubs of 15–45 members that can grow up to 99 plants per year. Regional leaders in Latin America consider legalization as a way to help curb the criminal activity and violence associated with the illegal drug trade.

Under the law, a drug control board will be convened that will regulate cultivation standards, fix prices, and monitor consumption of registered users. The use of cannabis is legal in Uruguay, but until this law, cultivation and sale of the drug was not.

Select Countries with Severe Penalties for Cannabis Possession or Trafficking

Indonesia

Penalties for possession, use, or trafficking in illegal drugs in Indonesia are severe, and convicted offenders can expect long jail sentences and heavy fines. A life sentence or the death penalty can be given in cases of drug trafficking (US Department of State 2014).

Iran

Iran executes many people each year on drug-related charges (US Department of State 2014). Under the 2011 Anti-Narcotics Law the term "narcotic" (for certain offenses) refers to bhang (preparation of the leaves and flower tops of Indian hemp), Indian hemp juice, opium, opium juice or residue, or synthetic non-medical psychotropic substances listed by Parliament. The drugs cocaine, heroin, GHB, LSD, and MDMA, among others, fall under a separate "narcotic" definition with different punishments. Offenses that carry the death penalty include fourth conviction for cultivation of cannabis; third conviction for purchase, possession, concealment or transport of 5–20 kg of cannabis; and import, export, production, manufacture, distribution, sale, or supply of more than 5 kg of cannabis. The death penalty is commuted for first-time offenders when distribution or sale was not accomplished and the amount was less than 20 kg (Amnesty International 2011).

Malaysia

Malaysian legislation provides for a mandatory death penalty for convicted drug traffickers. Those arrested with possession of 200 grams (7 ounces) of cannabis will be presumed by law to be trafficking in drugs (US Department of State 2014). The majority of those sentenced to death in Malaysia were convicted of marijuana or hashish offenses with an estimated 77 executions during 2008–2010 (Gallahue 2011).

Saudi Arabia

Those convicted of the import, manufacture, possession, and/or consumption of illegal drugs in Saudi Arabia can expect long jail sentences, heavy fines, public floggings, and/or deportation. The penalty for drug trafficking in Saudi Arabia is death. Saudi officials make no exceptions (US Department of State 2014).

Singapore

Singapore has a mandatory death penalty for many narcotics offenses including trafficking cannabis. Police have the authority to compel both residents and non-residents to submit to random drug analysis (US Department of State 2014). Any person having in his/her possession more than 15 grams of cannabis, 30 grams of cannabis mixture (any mixture of vegetable matter containing THC and CBD in any quantity), or 10 grams of cannabis resin (any substance containing resinous material and in which THC and CBD are found in any quantity) shall be presumed to have had that drug in possession for the purpose of trafficking. The punishment for trafficking in cannabis where the quantity is not less than 330 grams and not more than 500 grams is maximum 30 years or imprisonment for life and 15 strokes of the cane. The minimum punishment is 20 years and 15 strokes of the cane. The penalty for trafficking more than 500 grams is death (AGC Singapore 2008).

United Arab Emirates (UAE)

Legislation enacted in January 1996 imposes the death sentence for convicted drug traffickers. Since January 2006, possession of even trace amounts of illegal drugs, which include cannabis, has resulted in lengthy prison sentences for foreign citizens transiting the UAE. It is possible to be convicted for drug possession based on the result of a drug test even if no other evidence exists, regardless of when or where the consumption originally occurred (US Department of State 2014).

References

Adams R, Baker BR, Wearn RB. 1940a. Structure of cannabinol. III. Synthesis of cannabinol, 1-hydroxy-3-amyl-6,6,9-trimethyl-6-dibenzopyran. J Am Chem Soc 62:2204-7.

Adams R, Hunt M, Clark JH. 1940b. Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp. J Am Chem Soc 62:196-200.

Adams TC, Jones LA. 1975. Phytosterols of cannabis smoke. J Agr Food Chem 23:352-3.

[AGC Singapore] Attorney General's Chambers Singapore. 2008. Misuse of Drugs Act (Chapter 185) Revised Edition 2008. [Internet]. Access Date: 2014 Jul 2. Available from: http://statutes.agc.gov.sg/aol/search/display/view.w3p;ident=cf935cfb-467e-4307-9b86-85956a72c254;page=0;query=Id%3A%226d983371-cfd6-47d9-9c06-ab644ebdfc9f%22%20Status%3Ainforce;rec=0#legis

Ahmed SA, Ross SA, Slade D, Radwan MM, Khan IA, ElSohly MA. 2008a. Structure determination and absolute configuration of cannabichromanone derivatives from high potency Cannabis sativa. Tetrahedron Lett 49:6050-53.

Ahmed SA, Ross SA, Slade D, Radwan MM, Zulfiqar F, ElSohly MA. 2008b Cannabinoid ester constituents from high-potency Cannabis sativa. J Nat Prod 71:536-42.

[AHPA] American Herbal Products Association. 2008. AHPA adopts new trade recommendation; guidance on heavy metal, microbiological limits. Silver Spring (MD): American Herbal Products Association. Available from: http://www.ahpa.org

[AHPA] American Herbal Products Association. 2013a. Recommendations to regulators: Cannabis dispensing operations. Silver Spring (MD): American Herbal Products Association. p. 12.

[AHPA] American Herbal Products Association. 2013b. Recommendations to regulators: Cannabis laboratory operations. Silver Spring (MD): American Herbal Products Association. p. 12.

Alexander T. 1987. Hepatitis outbreak linked to imported pot. Sinsemilla Tips 7:22.

Almirall AG. 2013. Sativex®. [Internet]. Access Date: 2014 Jul 14. 6 p. Available from: http://www.kompendium.ch/mpro/mnr/24719/pdf/de?start=1#page1

Amnesty International. 2011. Addicted to death – executions for drug offences in Iran. London (UK): Amnesty International. [Internet]. Access Date: 2014 Jul 2. Available from: https://doc.es.amnesty.org/cgi-bin/ai/BRSCGI/EXECUTIONS%20FOR%20DRUGS%20OFFENCES%20IN%20IRAN?CMD=VEROBJ&MLKOB=30179594646

Anderson LC. 1980. Leaf variation among *Cannabis* species from a controlled garden. Bot Museum Leaf Harv Univ 28:10.

[API] Ayurveda Pharmacopoeia India. 1989. The Ayurveda Pharmacopoeia of India. New Delhi: Ayurveda Pharmacopoeia Committee; Ministry Health Family Welfare Government India.

Appendino G, Chianese G, Taglialatela-Scafati O. 2011. Cannabinoids: occurrence and medicinal chemistry. Curr Med Chem 18:1085-99.

Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, Smith E, Rahman MM. 2008. Antibacterial cannabinoids from *Cannabis sativa*: a structure-activity study. J Nat Prod 71:1427-30.

[ASA] Americans for Safe Access. 2013. Patient Focused Certification. Washington (DC) Americans for Safe Access. [Internet] Access Date: 2013 Dec 2. Available from: PatientFocusedCertification.org

Avraham Y, Ben-Shushan D, Breuer A, Zolotarev O, Okon A, Fink N, Katz V, Berry EM. 2004. Very low doses of Δ^9-THC increase food consumption and alter neurotransmitter levels following weight loss. Pharmacol Biochem Behav 77:675-84.

Banerjee SP, Snyder SH, Mechoulam R. 1975. Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. J Pharmacol Exp Ther 194:74-81.

Barrett ML, Gordon D, Evans FJ. 1985. Isolation from Cannabis sativa L. of cannflavin—a novel inhibitor of prostaglandin production. Biochem Pharm 34:2019-24.

Basile AC, Sertie JA, Freitas PC, Zanini AC. 1988. Anti-inflammatory activity of oleoresin from Brazilian Copaifera. J Ethnopharmacol 22:101-9.

Bazzaz FA, Dusek D, Seigler DS, Haney AW. 1975. Photosynthesis and cannabinoid content of temperate and tropical populations of Cannabis sativa. Biochem Syst Ecol 3:15-18.

Berchi CAL, Lousberg RJJ, Küppers FJEM, Salemink CA. 1974. Cannabis. IX. Cannabicitran. New naturally occurring tetracyclic diether from Lebanese Cannabis sativa. Phytochemistry 13:619–21.

Bercht CAL, Lousberg RJJ, Küppers FJEM, Salemink CA, Vree TB, Van Rossum JM. 1973. *Cannabis*. VII. Identification of cannabinol methyl ether from hashish. J Chromatog B 81:163–6.

Bercht CAL, Paris MR. 1974. Oil of *Cannabis sativa*. Bull Technique [Gattefosse] 68:87-90.

Bergamaschi MM, Queiroz RH, Chagas MH, de Oliveira DC, De Martinis BS, Kapczinski F, Quevedo J, Roesler R, Schroder N, Nardi AE, et al. 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. Neuropsychopharmacology 36:1219-26.

Bertoli A, Tozzi S, Pistelli L, Angelini LG. 2010. Fibre hemp inflorescences: from crop-residues to essential oil production. Ind Crops Prod 32:329-37.

Biro T, Toth BI, Hasko G, Paus R, Pacher P. 2009. The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. Trends Pharmacol Sci 30:411-20.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V. 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 134:845-52.

[BMC] Bureau voor Medicinale Cannabis. 2010. Monografie voor analyse van de grondstof *Cannabis* flos (hennepbloemen): analytical monograph. The Netherlands: Bureau voor Medicinale *Cannabis*. 21 p.

Bocsa I, Mathe P, Hangyel L. 1997. Effect of nitrogen on by means of population genetics in a monoecious hemp stand. tetrahydrocannabinol (THC) content in hemp (*Cannabis sativa* L.) leaves at different positions. J Int Hemp Assoc 4:80–1.

Boeren EG, ElSohly MA, Turner CE. 1979. Cannabitripsol: a novel *Cannabis* constituent. Experientia 35:1278-9.

Bolognini D, Costa B, Maione S, Comelli F, Marini P, Di Marzo V. 2010. The plant cannabinoid Δ^9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. Br J Pharmacol 160:677-87.

Booker L, Naidu PS, Razdan RK, Mahadevan A, Lichtman AH. 2009. Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception. Drug Alcohol Depend 105:42-7.

Booz GW. 2011. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Rad Biol Med 51:1054-61.

Bowd A, Swann DA, Turnbull JH. 1975. Photochemical transformations of cannabinol. J Chem Soc Chem Comm 19:797–8.

Brenneisen R. 1984. Psychotropic drugs. II. Determination of cannabinoids in Cannabis sativa L. and in cannabis products with high pressure liquid chromatography (HPLC). Pharmaceut Acta Helv 59:247-59.

Brenneisen R, ElSohly MA. 1988. Chromatographic and spectroscopic profiles of Cannabis of different origins: Part 1. J Forensic Sci 33:1385-1404.

Brickell CD, Alexander C, David JC, Hetterscheid WLA, Leslie AC, Malecot V, Jin X, Cubey JJ, Editors. 2009. New edition of the International Code of Nomenclature for cultivated plants. Belgium: International Society Horticultural Science. 204 p.

Burstein SH. 1999. The cannabinoid acids: nonpsychoactive derivatives with therapeutic potential. Pharmacol Ther 82:87-96.

Busse FP, Fiedler GF, Leichtle A, Hentschel H, Stumvoll M. 2008. Lead poisoning due to adulterated marijuana in Leipzig. Dtsch Arztebl Int 105:757-62.

Buys YM, Rafuse PE. 2010. Canadian Ophthalmological Society policy statement on the medical use of marijuana for glaucoma. Can J Ophthalmol 45:324-326.

[CAEPA] California Environmental Protection Agency. 2013. Chemicals known to the state of California to cause cancer or reproductive toxicity. Sacramento (CA): California Environmental Protection Agency. 22 p.

Cahn RS. 1932. Cannabis indica resin. III. Constitution of cannabinol. J Chem Soc 1342-53.

Campbell WE, Gammon DW, Smith P, Abrahams M, Purves TD. 1997.

Composition and antimalarial activity in vitro of the essential oil of Tetradenia riparia. Planta Med 63:270-2.

Campos AC, Guimaraes FS. 2008. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology 199:223-30.

Carod-Artal FJ. 2003. Neurological syndromes associated with the ingestion of plants and fungi with a toxic component (II). Hallucinogenic fungi and plants, mycotoxins and medicinal herbs. Rev Neurol 36:951-60.

Carrier EJ, Auchampach JA, Hillard CJ. 2006. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc Natl Acad Sci U S A 103:7895-900.

Cascio MG, Gauson LA, Stevenson LA, Ross R, Pertwee RG. 2010. Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. Br J Pharmacol 159:129-41.

Cates WC, Warren JW. 1975. Hepatitis B in Nuremberg, Germany. Epidemiology of a drug-associated epidemic among US Army soldiers. JAMA 234:930-4.

Cawthorne MA, Wargent E, Zaibi M, Stott C, Wright S. 2007. The CB1 antagonist, Δ^9-tetrahydrocannabivarin (THCV) has antiobesity activity in dietary-induced obese (DIO) mice. Saubt-Sauveur (QC): Inter Cann Res Soc. 141 p.

[CDFA] California Department of Food and Agriculture. 2011. Notice of quarantine and removal from sale. [Internet]. Access Date: 2014 Jul 23. Available from: http://www.cdfa.ca.gov

Cervantes J. 2006. Marijuana horticulture: the indoor/outdoor medical grower's bible. Vancouver (WA): Van Patten Pub. 512 p.

Chalchat JC, Garry RP, Michet A, Remery A. 1985. The essential oil of two chemotypes of *Pinus sylvestris* (oil of type A). Phytochem 24:24-43.

Chandra S, Lata H, Khan IA, ElSohly MA. 2008. Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO2 conditions. Physiol Mol Biol Plants 14:299-306.

Chandra S, Lata H, Khan IA, ElSohly MA. 2011b Photosynthetic response of *Cannabis sativa* L., an important medicinal plant, to elevated levels of CO2. Physiol Mol Biol Plants 17:291–95.

Chandra S, Lata H, Khan IA, ElSohly MA. 2013. The role of biotechnology in *Cannabis sativa* propagation for the production of phytocannabinoids. Berlin: Springer Verlag. 123-48 p.

Chandra S, Lata H, Khan IA, MA. E. 2011a. Temperature response of photosynthesis in different drug and fiber varieties of *Cannabis sativa* L. Physiol Mol Biol Plants 17:297–303.

Chen GH, Majumdar AS. 2006. Drying of herbal medicines and tea. In: Mujumdar AS, editor. Handbook of industrial drying. 3rd ed. Boca Raton: CRC p. 1312.

Clark MN. 1978. A study of infraspecific flavonoid variation of *Cannabis sativa* L. (Cannabaceae). [Masters Thesis]. Vancouver (Can): University of British Columbia.

Clark MN, Bohm BA. 1979. Flavonoid variation in *Cannabis*. Bot J Linnean Soc 79:249-57.

Clarke RC. 1981. Marijuana botany: an advanced study, the propagation and breeding of distinctive cannabis. Berkeley (CA): Ronin 197 p.

Clarke RC, Merlin MD. 2013. Cannabis: evolution and ethonobotany. Berkeley (CA): Univ Calif Pr. 434 p.

Claussen U, Von Spulak F, Korte F. 1968: Hashish. XIV. Components of hashish. Tetrahedron 24:1021–3.

Cole JM. 2013. Guidance regarding marijuana enforcement. Washington (DC): US Department Justice.

Costa B. 2007. On the pharmacological properties of Δ^9-tetrahydrocannabinol (THC). Chem Biodiv 4:1664–77.

Crombie L, Crombie WML, Jamieson SV. 1980. Extractives of Thailand Cannabis: synthesis of canniprene and isolation of new geranylated and prenylated chrysoeriols. Tetrahedron Lett 21:3607-10.

Crombie L, Ponsford R. 1971. Synthesis of cannabinoids by pyridine catalyzed citral-olivetol condensation: synthesis and structure of cannabicyclol, cannabichromene (hashish extractives), citrylidene-cannabis, and related compounds. J Chem Soc Org 4:796-804.

Crombie L, Ponsford R, Shani A, Yagnitinsky B, Mechoulam R. 1968. Hashish components. Photochemical production of cannabicyclol from cannabichromene. Tetrahedron Lett 55:5771-2.

D.C. Superior Court. 1976. Criminal law and procedure: medical necessity. Washington (DC). [Internet]. Access Date: 2014 Jul 23. p. 2249-54. Available from: http://www.drugpolicy.org/docUploads/randall.pdf

Daley P, Lampach D, Squerra S. 2013. Testing *Cannabis* for contaminants. Botec Analysis Corporation. Lafayette,CA: Alexander Shulgin Research Institute. [Internet] No. I-50 Project #430-1a. Access Date: 2014 Jul 2. 67 p. Available from: http://lcb.wa.gov/publications/Marijuana/BOTEC%20reports/1a-Testing-for-Contaminants-Final-Revised.pdf

Davis WM, Hatoum NS. 1983. Neurobehavioral actions of cannabichromene and interactions with Δ^9-tetrahydrocannabinol. Gen Pharmacol 14:247-52.

[DEA] Drug Enforcement Agency. 2011a. Schedules of controlled substances. In: Code of Federal Regulations, Title 21, Part 1308. Washington (DC): U.S. Government Printing Office. [Internet]. Access Date: 2014 Jul 2. Available from: http://www.gpo.gov/fdsys/pkg/CFR-2011-title21-vol9/pdf/CFR-2011-title21-vol9-part1308.pdf

[DEA] Drug Enforcement Agency. 2011b. 21 CFR Chapter II. Denial of petition to initiate proceedings to reschedule marijuana. Federal Register. [Internet]. Access Date: 2014 Jul 2. p. 40552-89. Available from: http://www.gpo.gov/fdsys/pkg/FR-2011-07-08/pdf/2011-16994.pdf

De Lago E, Fernandez-Ruiz J. 2007. Cannabinoids and neuroprotection in motor-related disorders. CNS Neurol Dis Drug Target 6:377-87

de Meijer EP, Bagatta M, Carboni A, Crucitti P, PM. E, Cristiana M, Paolo R, Mandolino G. 2003. The inheritance of chemical phenotype in *Cannabis sativa* L. Genetics 163:335-46.

de Meijer EP, Hammond K. 2005. The inheritance of chemical phenotype in *Cannabis sativa* L. (II): Cannabigerol predominant plants. Euphytica 145(1):189-98.

de Meijer EP, Hammond KM, Sutton A. 2009. The inheritance of chemical phenotype in *Cannabis sativa* L. (IV): cannabinoid-free plants. Euphytica 168:95-112.

de Meijer EP, Van Der kamp HJ, Van Eeuwijk FA. 1992. Characterization of Cannabis accessions with regard to cannabinoid content in relation to other plant characters. Euphytica 62:187-200.

De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S, Stott CG, Di Marzo V. 2011a. Effects of cannabinoids and cannabinoid-enriched cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol 163:479-94.

De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, Di Marzo V. 2008. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J Pharmacol Exp Ther 325:1007-15.

DeBacker B, Debrus B, Lebrun P, Theunis L, Dubois N, Decock L, Verstraete A, Hubert P, Charlier C. 2009 Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. J Chromatogr B Anal Technol Biomed Life Sci 877:4115-24.

Debruyne D, Albessard F, Bigot MC, Moulin M. 1994. Comparison of three advanced chromatographic techniques for Cannabis identification. Bull Narc 44:109-121.

Dejesuse, Rodwick BM, Bowers D, Cohen CJ, Pearce D. 2007. Use of dronabinol improves appetite and reverses weight loss in HIV/AIDS-infected patients. J Int Assoc Phys AIDS Care (Chic) 6:95-100.

DeLong GT, Wolf CE, Poklis A, Lichtman A. 2011. Cannabichromene and tetrahydrocannabinol determination in mouse blood and brain by gas chromatography-mass spectrometry. J Anal Toxicol 35:496-500.

Dewey WL. 1986. Cannabinoid pharmacology. Pharmacol Rev 38:151-78.

Deyo AR, Musty RE. 2003. Cannabichromene (CBC) extract alters behavioral despair on the mouse tail suspension test of depression. Burlington (VT): International Cannabinoid Research Society. p. 146.

Do Vale TG, Furtado EC, Santos JG Jr, Viana GS. 2002. Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from Lippia alba (Mill.) NE Brown. Phytomedicine 9:709-14.

Douwaite AH. 1947. Choice of drugs in the treatment of duodenal ulcer. BMJ 2:43-7.

Dussy FE, Hamberg C, Luginbuhl M, Schwerzmann T, Briellmann TA. 2005. Isolation of Δ^9-THCA-A from hemp and analytical aspects concerning the determination of Δ^9-THC in *Cannabis* products. Forensic Sci Inter 149(1):3-10.

[EC] European Commission. 2013. Weed like to talk [Internet]. Access Date: 2014 Jul 23. Available from: http://ec.europa.eu/citizens-initiative/public/initiatives/

ongoing/details/2013/000008/en

Eisenstein TK, Meissler JJ, Wilson Q, Gaughan JP, Adler MW. 2007. Anandamide and Δ⁹-tetrahydrocannabinol directly inhibit cells of the immune system via CB2 receptors. J Neuroimmunol 189:17-22.

Elsohly HN, Turner CE, Clark AM, Elsohly MA. 1982. Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. J Pharm Sci 71:319-23.

ElSohly MA, Holley JH, Lewis GS, Russel MH, Turner CE. 1984. Constituents of *Cannabis sativa* L. XXLV. The potency of confiscated marijuana, hashish, and hash oil over a ten-year period. J Forensic Sci:500-14.

ElSohly MA, Ross SA, Mehmedic Z, Arafat R, Yi B, Banahan BF 3rd. 2000. Potency trends of delta-9-THC and other cannabinoids in confiscated marijuana from 1980-1997. J Forensic Sci 45:24-30.

ElSohly MA, Slade D. 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci 78:539–48.

Elsohly MA, Turner CE, Phoebe CH J, Knapp JE, Schiff PL J, Slatkin DJ. 1978 Anhydrocannabisativine, a new alkaloid from *Cannabis sativa* L. J Pharmaceut Sci 67:124.

Elson CE, Maltzman TH, Boston JL, Tanner MA, Gould MN. 1997. Anti-carcinogenic activity of d-limonene during the initiation and promotion/progression stages of DMBA-induced rat mammary carcinogenesis. Carcinogenesis 9:331-2.

[EMCDDA] European Monitoring Centre for Drugs and Drug Addiction. 2004. An overview of *Cannabis* potency in Europe. Luxembourg: Office Official Publications European Communities. 71 p.

[EMCDDA] European Monitoring Centre for Drugs and Drug Addiction. 2008. A cannabis reader: global issues and local experiences. Lisbon: European Monitoring Centre for Drugs and Drug Addiction. 364 p.

[EMCDDA] European Monitoring Centre for Drugs and Drug Addiction. 2012. Cannabis production and markets in Europe. Luxembourg: Office for Official Publications of the European Communities. 268 p.

Eubanks LM, Rogers CJ, Beuscher AE, Koob GF, Dickerson TJ, Janda KD. 2006. A molecular link between the active component of marijuana and Alzheimer's disease pathology. Mol Pharmacol 3:773-7.

Evans FJ. 1991. Cannabinoids: the separation of central from peripheral effects on a structural basis. Planta Med 57:S60-67.

Fairbairn JW, Liebman JA, Rowan MG. 1976. The stability of *Cannabis* and its preparations on storage. J Pharm Pharmacol 28 1-7.

Falk AA, Hagberg MT, Lof AE, Wigaeus-Hjelm EM, Wang ZP. 1990. Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. Scand J Work Environ Health 16:372-8.

Fan X, Gates RA. 2001. Degradation of monoterpenes in orange juice by gamma radiation. J Agric Food Chem 49:2422-6.

Fan X, Sokorai KJ. 2002. Changes in volatile compounds of gamma irradiated fresh cilantro leaves during cold storage. J Agric Food Chem 50:7622-6.

[FBI] Federal Bureau of Investigation. 2013. Uniform crime reports: crime in the United States 2012 [Internet]. Washington (DC): US Department of Justice; Access Date: 2014 Jul14. Available from: http://www.fbi.gov/about-us/cjis/ucr/crime-in-the-u.s/2012/crime-in-the-u.s.-2012/persons-arrested/persons-arrested

[FDA] Food and Drug Administration. 2001. Guidance for industry – bioanalytical method validation. Rockville (MD): US Department Health and Human Services. [Internet]. Access Date: 2013 Oct 23. 25 p. Available from: http://www.fda.gov/cder/guidance/index.htm

[FDA] Food and Drug Administration. 2011. Drug enforcement administration 21 CFR chapter II [docket No. DEA–352N] denial of petition to initiate proceedings to reschedule marijuana: proposed rules. Washington (DC): United States Gov Printing. [Internet]. Access Date: 2013 Jan 3. 38 p. Available from: http://r20.rs6.net/tn.awkwbab&et=1106521392982&s=1809&e=001G5iPdsWQryU6nh1GnSYKh8T51yEb14L1L8ASx3MJqpCTi0O82d7YTvqHRIfsa8KbJt2Q86uHT2h13vl53Finy5qxIbhI7TnfczuRBUIz36-yVVwEb4kMgBBVfl4JLSPhg-tnfaWydsoGgX72R519ulTmF_3XOr9rToyMlmwrVosQ=

[FDA] Food and Drug Administration. 2013a. Bacteriological analytical manual (BAM). [Internet]. Access Date: 2014 Jul 24. Available from: http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm

[FDA] Food and Drug Administration. 2013b. Pesticide analytical manual (PAM). [Internet]. Access Date: 2014 Jul 24. Available from: http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006955.htm

Federal Assembly of the Swiss Confederation. 2012. Loi fédérale sur les stupéfiants et les substances psychotropes (Loi sur les stupéfiants, LStup) - Modification du 28 septembre 2012. Bern The Swiss Parliament. Access Date: 2014 Jul 2. Available from: http://www.admin.ch/ch/f/ff/2012/7539.pdf

Fellermeier M, Zenk MH. 1998. Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. FEBS Lett 427:283-5.

Fisar Z. 2009 Phytocannabinoids and endocannabinoids. Curr Drug Abuse Rev 2:51-75.

Fischedick JT, Hazekamp A, Erkelens T, Hae Choi Y, Verpoorte R. 2010. Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry 71:2058-73.

Fisse J, Braut F, Cosson L, Paris M. 1981. In vitro study of the organogenetic capacity of *C. sativa* L. tissues: effect of different growth substances. Plantes Med Phytother 15:217–23.

Flores Sanchez IJ, Verpoorte R. 2008. Secondary metabolism in Cannabis. Phytochem Rev 7:615–39.

Foote RS, Jones LA. 1974. Analysis of the phytosterols of two varieties of *Cannabis*. J Agric Food Chem 22:534-5.

Fournier GC, Richez-Dumanois J, Duvezin JP, Paris M, Paris M. 1987. Identification of a new chemotype in *Cannabis sativa*: cannabigerol-dominant plants, biogenetic and agronomic prospects. Planta Med 53:277-80.

Fox PJ, Zajicek JP. 2002. Cannabis for multiple sclerosis. Prac Neurol 3:154-60.

Friedrich-Fiechtl J, Spiteller G. 1975. New cannabinoids. T. Tetrahedron 31:479-87.

Galal AM, Slade D, Gul W, El-Alfy AT, Ferreira D, Elsohly MA. 2009. Naturally occurring and related synthetic cannabinoids and their potential therapeutic applications. Recent Pat CNS Drug Discov 4(2):112-36.

Gallahue P. 2011. The death penalty for drug offences global overview 2011. London Harm Reduction International. [Internet]. Access Date: 2014 Jul 2. 44 p. Available from: http://www.ihra.net/files/2011/09/14/IHRA_DeathPenaltyReport_Sept2011_Web.pdf

Gaoni Y, Mechoulam R. 1964a. Hashish. III. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646–7.

Gaoni Y, Mechoulam R. 1964b. Structure and synthesis of cannabigerol, a new hashish constituent. Proceedings of the Chemical Society: 82.

Gaoni Y, Mechoulam R. 1966. Cannabichromene, a new active principle in hashish. Chem Comm 1:20-1.

García C, Palomo-Garo C, García-Arencibia M, Ramos J, Pertwee R, Fernández-Ruiz J. 2011. Symptom-relieving and neuroprotective effects of the phytocannabinoid Δ⁹-THCV in animal models of Parkinson's disease. Br J Pharmacol 163:1495-506.

Gattefosse RM. 1993. Gatefosse's aromatherapy. Essex (MD): CW Daniel Ltd. 176 p.

Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, Altmann KH, Karsak M, Zimmer A. 2008. Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 105:9099-104.

Gettman J. 2006. Marijuana production in the United States (2006). Bull Cannabis Reform. [Internet]. Access Date: 2014 Jul 28. p. 25. Available from: http://www.drugscience.org/Archive/bcr2/MJCropReport_2006.pdf

Ghelardini C, Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A. 2001. Local anaesthetic activity of β-caryophyllene Il Farmaco 56 387-9.

Ghosh R, Todd AR, Wilkinson S. 1940. *Cannabis indica*. V. Synthesis of cannabinol. J Chem Soc:1393-6.

Gil MT., Jimenez J, Ocete MA, Zarzuelo A, Cabo MM. 1989. Comparative study of different essential oils of Bupleurum gibraltaricum Lamarck. Die Pharmazie 44:284-7.

Gill EW. 1971. Propyl homolog of tetrahydrocannabinol: isolation from *Cannabis*, properties, and synthesis. J Chem Soc C: Organic 3:579-82.

Gomes A, Fernandes E, Lima JLFC, Mira L, Corvo ML. 2008. Molecular mechanisms of anti-inflammatory activity mediated by flavonoids. Curr Med Chem 15:1586–605.

Government of Canada. 2014. Consolidation: marihuana for medical purposes regulations. Ottowa: Minister of Justice. [Internet] No. SOR/2013-119. Access Date: 2014 Jul 24. 117 p. Available from: http://www.laws-lois.justice.gc.ca/PDF/SOR-2013-119.pdf

Grote H, Spiteller G. 1978a. New cannabinoids. II. J Chromatog 154:13-23.

Grote H, Spiteller G. 1978b. New cannabinoids. The structure of cannabicoumaronone and analogous compounds. Tetrahedron 34:3207–13.

Hammond C, Mahlberg P. 1977. Morphogenesis of capitate glandular hairs of Cannabis sativa L. (Cannabaceae). Amer J Botany 65:1023-31.

Hampson AJ, Grimaldi M. Axelrod J, Wink D. 1998. Cannabidiol and (-)Δ⁹-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci 95:8268-73.

Haney M, Gunderson EW, Rabkin J, Hart CL, Vosburg SK, Comer SD, Foltin RW. 2007. Dronabinol and marijuana in HIV-positive marijuana smokers. Caloric intake, mood and sleep. JAIDS 45:545–54.

Hartsel SC, Loh WH, Robertson LW. 1983. Biotransformation of cannabidiol to cannabielsoin by suspension cultures of *Cannabis sativa* and Saccharum officinarum. Planta Med 48:17-9.

Harvey DJ. 1976. Characterization of the butyl homologues of Δ¹-tetrahydrocannabinol, cannabinol and cannabidiol in samples of *Cannabis* by combined gas chromatography and mass spectrometry. J Pharm Pharmacol 28:280-5.

Harvey DJ. 1985. Examination of a 140 year old ethanolic extract of *Cannabis*: identificaton of a new cannabitriol homologues and the ethyl homologue of cannabinol. In:

Harvey DJ, Paton W, Nahas GG, editors. Marihuana '84 Proceedings of the Oxford Symposium on Cannabis, 9th International Congress of Pharmacology, 3rd Satellite Symposium on Cannabis. England: IRL Pr. p. 22-30.

Harvey DJ. 1990. Stability of cannabinoids in dried samples of Cannabis dating from around 1896-1905. J Ethnopharmacol 28:117-28.

Hazekamp A. 2007. *Cannabis*; extracting the medicine. [Doctoral]. Leiden: Universiteit Leiden. p. 181.

Hazekamp A. 2008-2009. Medicinal use of *Cannabis*: a review. Leiden (Netherlands): Hazekamp. 101 p.

Health Canada. 2005. Drugs and health products. Frequently asked questions – medical use of marihuana. Ottawa (ON): Health Canada. [Internet]. Access Date: 2014 Jul 2. Available from: http://www.hc-sc.gc.ca/dhp-mps/marihuana/about-apropos/faq-eng.php

Health Canada. 2012. Marihuana medical access regulations. Ottawa (ON): Minister of Justice. Access Date: 2014 Jul 2. Available from: http://www.hc-sc.gc.ca/ahc-asc/media/nr-cp/_2012/2012-193bka-eng.php

Hill AJ, Mercier MS, Hill TD, Glyn SE, Jones NA, Yamasaki Y, Futamura T, Duncan M, Stott CG, Stephens GJ. et al. 2012. Cannabidivarin is anticonvulsant in mouse and rat in vitro and in seizure models Br J Pharmacol doi: 10.1111/j.1476-5381.2012.02207.X.

Hill AJ, Weston SE, Jones NA, Smith I, Bevan SA, Williamson EM, Stephens GJ, Williams CM, Whalley BJ. 2010. Δ⁹-tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats. Epilepsia 51:1522-32.

Hillig KW. 2004. A chemotaxonomic analysis of terpenoid variation in *Cannabis*. Biochem System Ecol 32:875-91.

Hillig KW. 2005. Genetic evidence for speciation in *Cannabis* (Cannabaceae). Genet Resour Crop Evol 52:161-80.

Hillig KW, Mahlberg PG. 2004. A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). Am J Bot 91:966-75.

Hofmann ME, Frazier CJ. 2013. Marijuana, endocannabinoids, and epilepsy: Potential and challenges for improved therapeutic intervention. Exper Neurol 244:43-50.

Holland ML, Allen JD, Arnold JC. 2008. Interaction of plant cannabinoids with the multidrug transporter ABCC1 (MRP1). Eur J Pharmacol 591:128-31.

Holley JH, Hadley KH, Turner E. 1975. Constituents of *Cannabis sativa* L. XI: cannabidiol and cannabichromene in samples of known geographical origin. J Pharm Sci 64:892-4.

Hollister LE. 1971. Hunger and appetite after single doses of marihuana, alcohol, and dextroamphetamine. Clin Pharmacol Ther 12:44-9.

Howlett AC, Blume LC, Dalton GD. 2010. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem 17:1382-93.

[ICH] International Conference on Harmonization. 2011. Impurities: guidelines for residual solvents: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. No. Q3C(R5). 25 p.

[IMA] Israeli Medical Association. 2014. Medical marijuana regulations approved by Israeli Cabiner. Tel Aviv. [Internet]. Access Date: 2014 Jul 24. 1 p. Available from: http://www.ima.org.il/ENG/ViewNew.aspx?NewId=1352

Ito K, Ito M. 2013. The sedative effect of inhaled terpinolene in mice and its structure–activity relationships. J Nat Med 67:833-7.

Iuvone T, Esposito G, De Filippis D, Scuderi C, Steardo Luca. 2009. Cannabidiol: a promising drug for neurodegenerative disorders? CNS Neur Ther 15:65-75.

Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. 2009. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 30:515-27.

Izzo AA, Piscitelli F, Capasso R, Aviello G, Romano B, Borrelli F, Petrosino S, Di Marzo V. 2009. Peripheral endocannabinoid dysregulation in obesity: relation to intestinal motility and energy processing induced by food deprivation and re-feeding. Br J Pharmacol 158:451-61.

Johnson JM, Lemberger L, Novotny M, Forney RB, Dalton WS, Maskarinec MP. 1984. Pharmacological activity of the basic fraction of marihuana whole smoke condensate alone and in combination with Δ⁹-tetrahydrocannabinol in mice. Toxicol App Pharmacol 72:440-8.

Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, Stephens GJ. 2010. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther 332:569-77.

Karlaganis G. 2002. SIDS initial assessment report for SIAM 14: Linalool. Berne: UNEP No. CAS N°: 78-70-6. p. 157.

Kavia R, De Ridder D, Constantinescu CS, Stott CG, Fowler CJ. 2010. Randomised controlled trial of Sativex to treat detrusor over activity in multiple sclerosis. Mult Scler 16:1349-59.

Kisko G, Roller S. 2005. Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple juice. BMC 5:36.

Korte F, Haag M, Claussen U. 1965. Tetrahydrocannabinolcarboxylic acid, a component of hashish. Angew Chem Int 4(872).

Korte F, Sieper H. 1964a. On the chemical classification of plants. XXIV. Investigation of hashish constituents by thin-layer chromatography. J Chromatog 13:90-8.

Korte F, Sieper H. 1964b. On the chemical classification of plants. XXV. Quantitative determination of hashish components by thin-layer chromatography. J Chromatog 14:78-83.

Kose EO, Deniz IG, Sarikurkcu C, Aktas O, Yavuz M. 2010. Chemical composition, antimicrobial and antioxidant activities of the essential oils of Sideritis erythrantha Boiss. and Heldr. (var. erythrantha and var. cedretorum PH Davis) endemic in Turkey. Food Chem Toxicol 48:2960-5.

Kueppers FJEM, Lousberg RJJ, Bercht CAL, Salemink CA, Terlow JK, Heerma WM, Laven A. 1973. Cannabis VIII. Pryolysis of cannabidiol. Structure elucidation of the main pyrolytic product. Tetrahedron 29:2797-802.

Lakhan SE, Rowland M. 2009. Whole plant *Cannabis* extracts in the treatment of spasticity in multiple sclerosis: a systematic review. BMC Neurology 9:59.

Lamarck JB. 1785. Encyclopedique methodique, Botanique I (part 2). Paris: Panchoucke. Unknown p.

Lata H, Bedir E, Hosick A, Ganzera M, Khan I, Moraes RM. 2002. In vitro plant regeneration from leaf-derived callus in black cohosh (*Cimicifuga racemosa*). Planta Med 68:912–5.

Lata H, Chandra S, Khan I, ElSohly MA. 2009b. Propagation through alginate encapsulation of axillary buds of *Cannabis sativa* L.–an important medicinal plant. Physiol Mol Biol Plants 15:79–86.

Lata H, Chandra S, Khan IA, ElSohly MA. 2009a. Thidiazuron induced high frequency direct shoot organogenesis of *C. sativa* L. In vitro Cellular Dev Biology-Plant 45:12–9.

Lata H, Chandra S, Khan IA , ElSohly MA. 2010. High frequency plant regeneration from leaf derived callus of high Δ⁹-tetrahydrocannabinol yielding *Cannabis sativa* L. Planta Med 76:1629-33.

Leandro LM, Vargas FDE S, Barbosa PC, Neves JK, da Silva JA, da Veiga-Junior VF. 2012. Chemistry and biological activities of terpenoids from copaiba (*Copaifera* spp.) oleoresins. Molecules 17:3866-89.

Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, Laezza C, Portella G, Bifulco M, Di Marzo V. 2006. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. J Pharmacol Exp Ther 318:1375-87.

Linnaeus C. 1753. Species Plantarum Stockholm: Salvius [facsimile edition, 1957–1959. Ray Society, London, UK]. 1027 p.

Loewe S. 1944. Studies on the pharmacology of marihuana. In: Committee L, editor. The marihuana problem in the city of New York. Lancaster (PA) Jaques Cattell Pr. p. 149-212

Lorenzetti B, Souza G, Sarti S, Filho

D, Ferreira S. 1991. Myrcene mimics the peripheral analgesic activity of lemongrass tea. J Ethnopharmacol 34:43-8.

Lousberg RJJ, Bercht CAL, Van Ooyen R, Spronck HJW. 1977. Cannabinodiol: conclusive identification and synthesis of a new cannabinoid from *Cannabis sativa*. Phytochemistry 16:595-7.

Machado Rocha FC, Stefano SC, De Cassia Haiek R, Rosa Oliveira LM, Da Silveira DX. 2008. Therapeutic use of *Cannabis sativa* on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. Eur J Can Care 17:431-43.

Maffei ME, Gertsch J, Appendino G. 2011. Plant volatiles: production, function and pharmacology. Nat Prod Rep 28:1359-80.

Mahlberg PG, Turner J, Hemphill J, Hammond C. 1984. Ultrastructure, development and composition of glandular trichomes of *Cannabis*. In: Rodriguez P, Healey P, Mehta I, editors. Biology and chemistry of plant trichomes. NY: Pergamon p. 23-51.

Makriyannis A, Tian X, Guo J. 2005. How lipophilic cannabinergic ligands reach their receptor sites. Prostag Other Lipid Med 77:210-8.

Malhotra S, Suri S, Tuli R. 2009. Antioxidant activity of citrus cultivars and chemical composition of *Citrus karna* essential oil. Planta Med 75:62-4.

Malingré T, Hendriks H, Batterman S, Bos R, Visser J. 1975. The essential oil of *Cannabis sativa*. Planta Med 28:56-61.

Mandolino G, Ranalli P. 1999. Advances in biotechnological approaches for hemp breeding and industry. In: Ranalli P, editor. Advances in hemp research. New York: Haworth. p. 185–208.

Martin-Moreno AM, Reigada D, Ramirez BG, Mechoulam R, Innamorato N, Cuadrado A, de Ceballos ML. 2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol Pharmacol 79:964-73.

McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB. 2010. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11:44.

McLaren J, Swift W, Dillon P, Allsop S. 2008. *Cannabis* potency and contamination: a review of the literature. Addiction 103:1100-9.

McPartland J. 2002. Contaminants and adulterants in herbal *Cannabis*. Binghamton (NY): Haworth 337 p.

McPartland JM. 1996. A review of *Cannabis* diseases. J Int Hemp Assoc 3:19-23.

McPartland JM. 2008. Adulteration of *Cannabis* with tobacco, calamus, and other cholinergic compounds. Addict Biol 13:411-5.

McPartland JM, Blanchon DJ, Musty RE. 2008. Cannabimimetic effects modulated by cholinergic compounds. Addict Biol 13:411–5.

McPartland JM, Clarke RC, Watson DP. 2000. Hemp diseases and pests - management and biological control. Wallingford (UK): CABI 272 p.

McPartland JM, Hillig KW. 2006. Host-parasite relationships in *Cannabis*. J Indus Hemp 10:85-104.

McPartland JM, Mediavilla V. 2001. Non-cannabinoids in cannabis. In: Grotenhermen F, Russo EB, editors. Cannabis and cannabinoids. Binghamton (NY): Haworth Pr.

McPartland JM, Pruitt PP. 1999. Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. Altern Therap 5:57-62.

McPartland JM, Russo EB. 2001. *Cannabis* and *Cannabis* extracts: greater than the sum of their parts? J Cann Ther 1:103-32.

Mechoulam R. 1988. Alkloids in Cannabis sativa L. Academic Pr. 77-93 p.

Mechoulam R, Ben-Zvi Z, Yagnitinsky B, Shani A. 1969. New tetrahydrocannabinolic acid. Tetrahedron Lett. p. 2339–41.

Mechoulam R, Gaoni Y. 1965. Hashish. IV. Isolation and structure of cannabinolic, cannabidiolic, and cannabigerolic acids. Tetrahedron 21:1223-9.

Mechoulam R, Gaoni Y. 1967. Recent advances in the chemistry of hashish. Fortschr Chem Org Naturst 25:175-213.

Mechoulam R, Hanuš L. 2002. Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. Chem Physics Lipids 121:35-43.

Mechoulam R, Naftali L, Breuer A, Zahalka J. 1990. Synthesis of the individual, pharmacologically distinct, enatiomers of a tetrahydroccannabinol derivative. Tetrahedron Asymm 1:315-8.

Mechoulam R, Parker LA, Gallily R. 2002. Cannabidiol: an overview of some pharmacological aspects. J Clin Pharmacol 42(11 Suppl):11S-19S.

Mechoulam R, Shvo Y. 1963. Hashish. I. Structure of cannabidiol. Tetrahedron Lett 19:2073–8.

Mediavilla V, Steinemann S. 1997. Essential oil of *Cannabis sativa* L. strains. J Int Hemp Assoc 4:80-2.

Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, Ross SA, Khan IA, ElSohly MA. 2010. Potency trends of Δ^9-THC and other cannabinoids in confiscated *Cannabis* preparations from 1993 to 2008. J Foren Sci 55:1209-17.

[MHRA] Medicines and Healthcare Products Regulatory Agency. 2010. Public assessment report: Sativex oromucosal spray UK/H/2462/001/DC. [Internet]: MHRA. Access Date: 2014 Jul 2. 114 p. Available from: http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con084961.pdf

Mikuriya TH, Aldrich MR. 1988. *Cannabis* 1988. Old drug, new dangers. The potency question. J Psych Drugs 20:47-55.

Mills E. 2011. Energy up in smoke. The carbon footprint of indoor cannabis production. [Internet]. Access Date: 2014 Jul 2. 14 p. Available from: http://evan-mills.com/energy-associates/Indoor.html.

Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K, Fujiwara M. 2005. Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. Stroke 36:1077-82.

Miyazawa M, Watanabe H, Kameoka H. 1997. Inhibition of acetylcholinesterase activity by monoterpenoids with a p-methane skeleton. J Agricult Food Chem 45:677-9.

Mole ML, Turner CE. 1974. Phytochemical screening of *Cannabis sativa* L. I. Constituents of an Indian variant. J Pharmaceut Sci 63:154-6.

Montford S, Small E. 1999. A comparison of the biodiversity friendliness of crops with special reference to hemp (*Cannabis sativa* L.). J Intern Hemp Assoc 6:53-63.

Morita M, Ando H. 1984. Analysis of hashish oil by gas chromatography/mass spectrometry. Kagaku Keisatsu Kenkyusho Hokoku, Hokagaku-hen 37:137-40.

Musty RE, Karniol IG, Shirikawa I, Takahashi RN, Knobel E. 1976. Interactions of Δ^9-tetrahydrocannabinol and cannabinol in man. In: Braude MC, Szara S, editors. The pharmacology of marihuana. New york: Raven Pr. p. 559-63.

Neff GW, O'Brien CB, Reddy KR, Bergasa NV, Regev A, Molina E, Amaro R, Rodriguez MJ, Chase V, Jeffers L. et al. 2002. Preliminary observation with dronabinol in patients with intractable pruritus secondary to cholestatic liver disease. Am J Gastroenterol 97:2117–9.

Nelson K, Walsh D, Deeter P, Sheehan F. 1994. A phase II study of Δ^8-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. J Palliat Care 10:14-8.

Newcome R. 2006. Dr Russell Newcome on the ACMD report on *Cannabis*. [Internet]. Access Date: 2014 Jul 14. Available from: http://www.lifelineproject.co.uk/Dr-Russell-Newcome-on-the-ACMD-report-on-cannabis_25.php

[NIDA] National Institute Drug Abuse. 1998. Provision of marijuana and other compounds for scientific research - recommendations of The National Institute on Drug Abuse National Advisory Council. Rockville (MD): National Institute of Health. [Internet]. Access Date: 2014 Jul 24. p. 6. Available from: http://archives.drugabuse.gov/about/organization/nacda/MarijuanaStatement.html

[NIDA] National Institute Drug Abuse. 2012. Spice (synthetic marijuana). [Internet]. Access Date: 2014 Jul 10. p. 1-2. Available from: www.drugabuse.gov

Noma Y, Asakawa Y. 2010. Biotransformation of monoterpenoids by microorganisms, insects, and mammals. In: Baser KHC, Buchbauer G, editors. Handbook of essential oils: science,technology, and applications. Boca Raton (FL): CRC Press. p. 585–736.

Obata Y, Ishikawa Y. 1966. Constituents of hemp plant (*Cannabis sativa*) III. Isolation of a Gibbs-positive compound from Japanese hemp. Agr Biol Chem 30: 619-20.

[OMC] Office of Medicinal Cannabis. 2003. Guidelines for cultivating *Cannabis* for medicinal purposes [Voorschriften voor de Verbouw van Cannabis voor Medicinale Doeleinden] J Cann Ther 3:51-61.

[OMC] Office of Medicinal Cannabis. 2011. Medicinal cannabis information for patients. The Hague (The Netherlands): Institute for Responsible Medicine Use and the Office of Medicinal *Cannabis* of the CIBG, Ministry of Health, Welfare and Sport. [Internet]. Access Date: 2014 Jul 24. p. 16 Available from: http://www.cannabisbureau.nl/en/doc/pdf/5089-A5-BMC-Pat-ENG-web_25097.pdf

Ortúz de Urbina AV, Martín ML, Montero MJ, Morán A, San Román L. 1989. Sedating and antipyretic activity of the essential oil of *Calamintha sylvatica* subsp. *ascendens*. J Ethnopharmacol 25:165-71.

Pagani A, Scala F, Chianese G, Grassi G, Appendino G, Tagliateli-Scafati O. 2011. Cannabioxepane, a novel

tetracyclic cannabinoid from hemp, *Cannabis sativa* L. Tetrahedron 67:3369-73.

Pate D. 1994. Chemical ecology of *Cannabis*. J Inter Hemp Assoc 2:32-7.

Peana AT, De Montis MG, Sechi S, Sircana G, D'Aquila PS, Pippia P. 2004. Effects of (-)-linalool in the acute hyperalgesia induced by carrageenan, L-glutamate and prostaglandin E2. Eur J Pharmacol 497:279-84.

Perry N, Court G, Bidet N, Court J, Perry E. 1996. European herbs with cholinergic activity: potential in dementia therapy. Inter J Geriatr Psych 11:1063-9.

Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK. 2000. In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes. J Pharm Pharmacol 52:895-902.

Persson M, Sjodin K, Borg-Karlson AK, Norin T, Ekberg I. 1996. Relative amounts and enantiomeric compositions of monoterpene hydrocarbons in xylem and needles of *Picea abies* Phytochemistry 42:1289-97.

Pertwee RG. 2008. The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. Br J Pharmacol 153:199-215.

Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K. et al. 2010. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacol Rev Pharmacol Rev 62:588–631.

Pertwee RG, Thomas A, Stevenson LA, Ross RA, Varvel SA, Lichtman AH, Martin BR, Razdan RK. 2007. The psychoactive plant cannabinoid, Δ^9-tetrahydrocannabinol, is antagonized by Δ^8- and Δ^9-tetrahydrocannabivarin in mice in vivo. Br J Pharmacol 150:586-94.

Petrzilka T, Haefliger W, Sikemeier C. 1969. Synthesis of hashish components. IV. Helvet Chim Acta 52:1102-34.

Pitts JE, Neal JD, Gough TA. 1992. Some features of Cannabis plants grown in the United Kingdom from seeds of known origin. J Pharm Pharmacol 44:947-51.

Pollastro F, Taglialatela-Scafati O, Allara M, Munoz E, Di Marzo V, De Petrocellis L, Appendino G. 2011. Bioactive prenylogous cannabinoid from fiber hemp (*Cannabis sativa*). J Nat Prod 74:2019-22.

Potter D. 2004. Growth and morphology of medicinal Cannabis. In: Guy GW, Whittle BA, Robson PJ, editors. The medicinal uses of *Cannabis* and cannabinoids. London: Pharmaceut Pr. p. 17-54.

Potter D. 2009. The propagation, characterisation and optimisation of *Cannabis sativa* L. as a phytopharmaceutical. London: King's College London. p. 110.

Potter DJ, Duncombe P. 2012. The effect of electrical lighting power and irradiance on indoor-grown cannabis potency and yield. J Forensic Sci 57:618-22.

[ProCon.org]. 2014. Who are the patients receiving medical marijuana through the federal government's Compassionate IND Program? [Internet]. Access Date: 2014 Jul 24. Available from: http://medicalmarijuana.procon.org/view.answers.php?questionID=000257

Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM. 2008. TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 28:6231-8.

Radwan MM, ElSohly MA, Slade D, Ahmed SA, Khan IA, Ross SA. 2009. Biologically active cannabinoids from high-potency *Cannabis sativa*. J Nat Prod 72:906-11.

Radwan MM, ElSohly MA, Slade D, Ahmed SA, Wilson L, El-Alfy AT, Khan IA, Ross SA. 2008a. Non-cannabinoid constituents from a high potency *Cannabis sativa* variety. Phytochemistry 69:2627–33.

Radwan MM, Ross SA, Slade D, Ahmed SA, Zulfiqar F, ElSohly MA. 2008b. Isolation and characterization of new *Cannabis* constituents from a high potency variety. Planta Med 74:267–72.

Rahn EJ, Hohmann AG. 2009. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. Neurotherapeutics 6:713-37.

Raman A. 1998. The *Cannabis* plant: botany, cultivation, and processing for use. In: Brown DT, editor. *Cannabis* – The genus *Cannabis* Amsterdam: Harwood Acad. p. 32-57.

Randerson J. 2007. Warning issued over *Cannabis* adulterated with glass beads. [Internet]. Access Date: 2014 Jul 28. Available from: http://www.theguardian.com/society/2007/jan/12/drugsandalcohol.drugs

Rao VS, Menezes AM, Viana GS. 1990. Effect of myrcene on nociception in mice. J Pharm Pharmacol 42:877-8.

Resstel LB, Tavares RF, Lisboa SF, Joca SR, Correa FM, Guimaraes FS. 2009. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 156:181-8.

Reuter PH. 2010. Marijuana legalization: What can be learned from other countries? Santa Monica (CA): Rand Drug Policy Research Center. [Internet]. Access Date: 2014 Jul 28. Available from: http://www.rand.org/content/dam/rand/pubs/working_papers/2010/RAND_WR771.pdf

Riyazi A, Hensel A, Bauer K, Geissler N, Schaff S, EJ V. 2007. The effect of the volatile oil from ginger rhizomes (*Zingiber officinale*), its fractions and isolated compounds on the 5-HT3 receptor complex and the serotoninergic system of the rat ileum. Planta Med 73:355-62.

Rock EM, Goodwin JM, Limebeer CL, Breuer A, Pertwee RG, Mechoulam R, Parker LA. 2010. Interaction between non-psychotropic cannabinoids in marihuana: effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. Psychopharmacology 215:505-12.

Rog DJ, Nurmiko T, Friede T, Young C. 2005. Randomized controlled trial of *Cannabis* based medicine in central neuropathic pain due to multiple sclerosis. Neurology 65:812-9.

Rosenthal E. 2010. Ed Rosenthal's marijuana grower's handbook. Oakland (CA): Quick American 510 p.

Rosmarin A, Eastwood N. 2012. A quiet revolution: drug decriminalisaton policies in practice across the globe. UK: Release. [Internet]. Access Date: 2014 Jul 2. 44 p. Available from: http://www.release.org.uk/sites/release.org.uk/files/pdf/publications/Release_Quiet_Revolution_2013.pdf

Ross SA, ElSohly MA. 1996. The volatile oil composition of fresh and air-dried buds of *Cannabis sativa* L. J Nat Prod 59:49-51.

Ross SA, ElSohly MA. 1999. CBN and Δ^9-THC concentration ratio as an indicator of the age of stored marijuana samples. Bull Narc 49-50:139–47.

Ross SA, ElSohly MA, Sultana GNN, Mehmedic Z, Hossain CF, Chandra S. 2005. Flavonoid glycosides and cannabinoids from the pollen of *Cannabis sativa* L. Phytochem Anal 16:45-8.

Russo E. 2004. History of Cannabis as a medicine. In: Guy GW, Whittle BA, Robson PJ, editors. The medicinal uses of *Cannabis* and cannabinoids. London: Pharmaceutical. p. 1-16.

Russo EB. 2011. Taming THC: potential *Cannabis* synergy and phytocannabinoid-terpenoid entourage effects. Brit J Pharmacol 163:1344-64.

Russo EB, Burnett A, Hall B, Parker KK. 2005. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res 30:1037-43.

Russo EB, Guy GW, Robson PJ. 2007. *Cannabis*, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. Chem Biodiver 4:1729-43.

Samuelsson G. 1992. Drugs of natural origin. Stockholm: Swedish Pharm Pr. 320 p.

Schultes RE, Klein WT, Plowman T, Lockwood TE. 1974. Cannabis: An example of taxonomic neglect. Harv Univ Bot Mus Leaf. 23:337-67.

Scuderi C, De Filippis D, Iuvone T, Blasio A, Steardo A, Esposito G. 2009. Cannabidiol in medicine: a review of its therapeutic potential in CNS disorders. Phytother Res 23:597-602.

Shani A, Mechoulam R. 1970. A new type of cannabinoid. Synthesiss of cannabielsoic acid A by a novel photo-oxidative cyclization. J Chem Soc D:273-4.

Shani A, Mechoulam R. 1974. Cannabielsoic acids. Isolation and synthesis by a novel oxidative cyclization. Tetrahedron 30 2437-46.

Shi G, Cai Q. 2009. Cadmium tolerance and accumulation in eight potential energy crops. Biotechnology Advances 27:555-561.

Shoyama Y, Fujita T, Yamauchi T, Nishioka I. 1968. Cannabis. II. Cannabichromenic acid, a genuine substance of cannabichromene. Chem Pharmaceut Bull 16:1157-8.

Shoyama Y, Kuboe K, Nishioka I, Yamauchi T. 1972a. *Cannabis*. VII. Cannabidiol monomethyl ether. New neutral cannabinoid. Chem Pharmaceut Bull 20:2072.

Shoyama Y, Motimoto S, Nishioka I. 1981. *Cannabis*. XIV. Two new propyl cannabinoids, cannabicyclovarin and Δ^7-cis-iso-tetrahydrocannabivarin, from Thai *Cannabis*. Chem Pharmaceut Bull 29:3720–3.

Shoyama Y, Oku R, Yamauchi T, Nishioka I. 1972b. *Cannabis*. VI. Cannabicyclolic acid. Chem Pharmaceut Bull 20:1927-30.

Siegel BZ, Garnier L, Siegel SM. 1988. Mercury in marijuana. BioScience 38:619-623.

Sirikantaramas S, Morimoto S, Shoyama Y, Ishikawa Y, Wada Y, Shoyama Y, Taura F. 2004. Enzyme catalysis and regulation: The gene controling marijuana

psychoactivity; Molecular cloing and heterologous expression of Δ^1-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. J Biol Chem 279:39767-74.

Sirikantaramas S, Taura F, Morimoto S, Shoyama Y. 2007. Recent advances in *Cannabis sativa* research: biosynthetic studies and its potential in biotechnology. Curr Pharmaceut Biotechnol 8:237-43.

Slusarkiewicz-Jarzina A, Ponitka A, Kaczmarek Z. 2005. Influence of cultivar, explant source and plant growth regulator on callus induction and plant regeneration of *Cannabis sativa* L. Acta Biol Craco Ser Bot 47:145–51.

Small E. 1979. The species problem in *Cannabis*. Toronto: Corp Infor Serv Lim. 218 p.

Small E. 1997. *Cannabis*. New York: Oxford Univ Pr. 616 p.

Small E. 2007. *Cannabis* as a source of medicinals, nutraceuticals, and functional foods. In: Acharya SN, Thomas JE, editors. Advances in medicinal plant research. Trivandrum (India): Research Signpost. p. 1-39.

Small E, Beckstead HD. 1973. Common cannabinoid phenotypes in 350 stocks of *Cannabis*. Lloydia 36:144-165.

Small E, Cronquist A. 1976. A practical and natural taxonomy for Cannabis. Taxon 25:405-35.

Small E, Marcus D. 2002. Hemp: A new crop with new uses for North America. In: Janick J, Whipkey A, editors. Trends in new crops and new uses. Alexandria (VA): ASHS Pr. p. 284-326.

Smith RM, Kempfert KD. 1977. Δ^1-3,4-cis-Tetrahydrocannabinol in *Cannabis sativa* Phytochemistry 16:1088-9.

Souto-Maior FN, de Carvalho FL, de Morais LC, Netto SM, de Sousa DP, de Almeida RN. 2011. Anxiolytic-like effects of inhaled linalool oxide in experimental mouse anxiety models. Pharmacol Biochem Behav 100:259-63.

[SPI] Siddha Pharmacopoeia India. 2008. The Siddha Pharmacopoeia of India. New Delhi Siddha Pharmacopoeia Committee, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health & Family Welfare, Government of India.

Stafford ML. 2013. The quiet giant: Israel's discreet and successful medicinal *Cannabis* program. HerbalGram 97:38-45.

Stahl E, Kunde R. 1973. Die Leitsubstanzen der Haschisch-Suchhunde. Leading substances for hashish narcotic dogs. Kriminalistik 9: 385-8.

Starks M. 1990. Marijuana chemistry – genetics, processing, and potency. Berkeley: Ronin. 191 p.

State of Israel Prime Minister's Office. 2011. Cabinet approves arrangements for the use of cannabis for medical purposes. [Internet]. Access Date: 2014 Jul 28. Available from: http://stopthedrugwar.org/chronicle/2011/aug/07/israel_cabinet_approves_medical

Stevens PF. 2001. Cannabaceae [Internet]. Access Date: Nov 22 2013. Available from: http://www.mobot.org/mobot/research/apweb/orders/rosalesweb.htm#Cannabaceae

Stroup K. 2014. Despite legalization, thousands of Americans still go to jail–and need lawyers to defend them [Internet]. Marijuana.com. Available from: http://marijuana.com/news/2014/07/despite-legalization-thousands-of-americans-still-go-to-jail-and-need-lawyers-to-defend-them/

Sun J. 2007. D-Limonene: safety and clinical applications. Altern Med Rev 12:259-64.

Suurkuusk G. 2010. Validation of the gas chromatographic method for THC, CBD and CBN determination. [Masters]. Tartu, Estonia: University of Tartu. p. 40.

Swift W, Wong A, Li KM, Arnoldd JC, McGregor LS. 2013. Analysis of *Cannabis* seizures in NSW, Australia: *Cannabis* potency and cannabinoid profile. PLOS One 8:e70052.

Swissmedic. 2013a. The way to the *Cannabis* medication. Bern: Swissmedic, Division Complementary Herbal Medicines. [Internet]. Access Date: 2014 Jul 11. 17 p. Available from: http://www.stcm.ch/files/presentation_tschuemperlin.pdf

Swissmedic. 2013b. Zulassung eines Arzneimittels mit neuem Wirkstoff Sativex®, spray zur Anwendung in der Mundhöhle (Cannabis sativae folii cum flore extractum spissum). Swissmedic J:945-1065.

Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. 1996. Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, beta-caryophyllene. Planta Med 62:469-70.

Taura F, Morimoto S, Shoyama Y. 1995a. Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. Phytochemistry 39:457–8.

Taura F, Morimoto S, Shoyama Y. 1996. Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. J Biol Chem 271:17411-6.

Taura F, Morimoto S, Shoyama Y, Mechoulam R. 1995b. First direct evidence for the mechanism of Δ1-tetrahydrocannabinolic acid biosynthesis. J Am Chem Soc 117:9766-7.

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. 2007. Cannabidiol displays unexpectedly high potency as an antagonist of CB_1 and CB_2 receptor agonists in vitro. Br J Pharmacol 150:613-23.

Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA, Pertwee RG. 2005. Evidence that the plant cannabinoid Δ^9-tetrahydrocannabivarin is a cannabinoid CB_1 and CB_2 receptor antagonist. Br J Pharmacol 146:917-26.

Tubaro A, Giangaspero A, Sosa S, Negri R, Grassi G, Casano S, Della Loggia R, Appendino G. 2010. Comparative topical anti- inflammatory activity of cannabinoids and cannabivarins. Fitoterapia 81:816-9.

Turner CE. 1983. *Cannabis*: the plant, its drugs, and their effects. Aviat Space Environ Med:363-8.

Turner CE, Elsohly MA. 1981. Biological activity of cannabichromene, its homologs and isomers. J Clin Pharmacol 21(8-9 Suppl):283S-291S.

Turner CE, Elsohly MA, Boeren EG. 1980b. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J Nat Prod 43:169-304.

Turner CE, Hsu MH, Knapp JE, Schiff PL Jr, Slatkin DJ. 1976. Isolation of cannabisativine, an alkaloid, from *Cannabis sativa* L. root. J Pharm Sci 65:1084-5.

Turner CE, Mole ML, Hanus L, Elsohly HN. 1981. Constituents of *Cannabis sativa* L. XIX. Isolation and structure elucidation of cannabiglendol. A novel cannabinoid from an Indian variant. J Nat Prod 44:27-33.

Turner J, Hemphill J, Mahlberg PG. 1980a. Trichomes and cannabinoid content in developing leaves and bracts of *Cannabis sativa* L. (Cannabaceae) Amer J Bot 67:1397-406.

U.S. Department of State. 2014. Learn about your destination. [Internet]. Access Date: 2014 Jul 2. Available from: http://travel.state.gov/content/passports/english/country.html

United Nations. 1973. Single convention on narcotic drugs 1961 (as amended by the 1972 protocol amending the Single Convention on Narcotic Drugs, 1961). Geneva: United Nations. [Internet]. Access Date: 2014 Jul 2. Available from: http://www.unodc.org/pdf/convention_1961_en.pdf

United Nations. 2013a. Status as at: 15-01-2013: Single convention on narcotic drugs, 1961. Herndon (VA): United Nations. [Internet]. Access Date: 2014 Jul 2. Available from: http://treaties.un.org/Pages/ViewDetails.aspx?src=TREATY&mtdsg_no=VI-15&chapter=6&lang=en

United Nations. 2013b. Status as at: 15-01-2013: Protocol amending the Single Convention on Narcotic Drugs 1961. Herndon (VA): United Nations. Access Date: 2014 Jul 2. Available from: http://treaties.un.org/Pages/ViewDetails.aspx?src=TREATY&mtdsg_no=VI-17&chapter=6&lang=en

[UNODC] United Nations Office on Drugs and Crime. 2006. World Drug Report. United Nations Office on Drugs and Crime. Vienna: Vienna International Center.

[UNODC] United Nations Office on Drugs and Crime. 2009. Recommended methods for the identification and analysis of *Cannabis* and *Cannabis* products Vienna: United Nations Office on Drugs and Crime. [Internet]. Access Date: 2014 Jul 2. 60 p. Available from: http://www.unodc.org/documents/scientific/ST-NAR-40-Ebook.pdf

[UNODC] United Nations Office on Drugs and Crime. 2011. World Drug Report 2011. New York: United Nations. 266 p.

[UNODC] United Nations office on drugs and crime. 2014. World drug report. New York: United Nations. [Internet]. Access Date: 2014 Jul24. 128 p. Available from: http://www.unodc.org/documents/wdr2014/World_Drug_Report_2014_web.pdf

[UPI] Unani Pharmacopoeia India. 2007. The Unani Pharmacopoeia of India. New Delhi (India): The Unani Pharmacopoeia Committee, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health & Family Welfare, Government of India

Uruguay. 2013. Poder Legislativo. Uruguay. [Internet] No. Ley No. 19.172. Access Date: 2014 Jul 28. 24 p. Available from: http://archivo.presidencia.gub.uy/sci/leyes/2013/12/cons_min_803.pdf

[USC] United States Code. 2010. Title 21 food and drugs, Chapter

13 drug abuse prevention and control, subchapter I control and enforcement, Part A introductory provisions, section 802 definitions. Washington (DC): Office of the Law Revision Counsel of the U.S. House of Representatives. [Internet]. Access Date: 2014 Jul 24. Available from: http://www.gpo.gov/fdsys/pkg/USCODE-2010-title21/pdf/USCODE-2010-title21-chap13-subchapI-partA-sec802.pdf

[USP 30-NF 25] United States Pharmacopoeia 30- National Formulary 25. 2007. Residual solvents. Rockville (MD): United States Pharmacopoeial Convention.

Valle JR, Vieira JEV, Aucélio JG, Valio IFM. 1978. Influence of photoperiodism on cannabinoid content of *C. sativa* L.. Bull Narc 30:67–8.

Van Ginneken CAM, Vree TB, Breimer DD, Thijssen HWH, Van Rossum JM. 1972. Cannabinodiol, a new hashish constituent, identified by gas chromatography-mass spectrometry. In: Frigerio A, editor. Proceedings of the International Symposium on Gas Chromatography Mass Spectrometry. Isle of Elba Milano (Italy): Tamburini. p. 109-29.

Vanhoenacker G, Van Rompaey P, De Keukeleire D, Sandra P. 2002 Chemotaxonomic features associated with flavonoids of cannabinoid-free *Cannabis* (*Cannabis sativa* subsp. *sativa* L.) in relation to hops (*Humulus lupulus* L.). Nat Prod Lett 16:57-63.

Veress T, Szanto JI, Leisztner L. 1990. Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation in an open reactor. J Chromatogr A 520:339-47.

Verhoeckx KCM, Korthout HAAJ, van Meeteren-Kreikamp AP, Ehlert KA, Wang M, van der Greef J, Rodenburg RJT, Witkamp RF. 2006. Unheated *Cannabis sativa* extracts and its major compound THC-acid have potential immuno-modulating properties not mediated by CB1 and CB2 receptor coupled pathways. Int Immunopharmacol 6:656-65.

Veszki P, Verzár-Petri G, Mészáros S. 1980. Comparative phytochemical study on the cannabinoid composition of the geographical varieties of Cannabis sativa L. under the same condition. Herba Hungarica 19:95-102.

Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ. 1997. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. Int J Geriatr Psychiatry 12(9):913-9.

Vollner L, Bieniek D, Korte F. 1969. Hashish. XX. Cannabidivarin, a new hashish constituent. Tetrahedron Lett 3:145-7.

Von Spulak F, Claussen U, Fehlhaber HW, Korte F. 1968. Hashish. XIX. Tetrahydrocannabitriol cannabidiolcarboxylic acid ester, a new constituent of hashish. Tetrahedron 24:5379-83.

Vree TB, Breimer DD, Van Ginneken CA, Van Rossum JM. 1972. Identification of cannabicyclol with a pentyl or propyl side-chain by means of combined gas chromatography-mass spectrometry. J Chromatog 74:124-7.

Wang R, He LS, Xia B, Tong JF, Li N, Peng F. 2009. A micropropagation system of cloning of hemp (*Cannabis sativa* L.) by shoot tip culture. Pak J Bot 41:603-8.

Wargent ET, Zaibi MS, Silvestri C, Hislop DC, Stocker CJ, Stott CG, Guy GW, Duncan M, Di Marzo V, Cawthorne MA. 2013. The cannabinoid Δ^9-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in tw muse models of obesity. Nutr Diabetes doi: 10.1038/nutd.2013.9.

Wilkinson JD, Williamson EM. 2007. Cannabinoids inhibit human keratinocyte proliferation through a non-CB_1/CB_2 mechanism and have a potential therapeutic value in the treatment of psoriasis. J Dermatol Sci 45:87–92.

Williams SJ, Hartley JPR, Graham JDP. 1976. Bronchodilator effect of Δ^1-tetrahydrocannabinol administered by aerosol to asthmatic patients. Thorax 31:720-3.

Wirth PW, Watson ES, ElSohly M, Turner CE, Murphy JC. 1980. Anti-inflammatory properties of cannabichromene. Life Sci 26:1991-5.

Woelkart K, Salo-Ahen OM, Bauer R. 2008. CB receptor ligands from plants. Curr Top Med Chem 8(3):173-86.

Wood TB, Spivey WTN, Easterfield TH. 1896. Charas. The resin of Indian hemp. J Chem Soc Transactions 69:539-46.

Xi ZX, Peng XQ, Li X, Zhang H, Li JG, Gardner EL. 2010. Brain cannabinoid CB2 receptors inhibit cocaine self-administration and cocaine-enhanced extracellular dopamine in mice. Proceedings 20th Annual Symposium on the Cannabinoids. Lund: Internatioanl Cannabinoid Research Socity. p. 32.

Yamamoto I, Gohda H, Narimatsu S, Yoshimura H. 1988. Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea-pig hepatic microsomal enzymes, and its pharmacological activity in mice. J Pharmacobiodyn 11:833-8.

Yamauchi T, Shoyama Y, Matsuo Y, Nishioka I. 1968. *Cannabis*. III. Cannabigerol monomethyl ether, a new component of hemp. Chem Pharm Bull 16:1164-5.

Yanauchi T, Shoyama Y, Aramaki H, Azuma T, Nishioka I. 1967. Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol. Chem Pharm Bull 15:1075-6.

Zuardi AW, Guimaraes FS, Guimaraes VM, Del Beni EA. 2002. Cannabidiol: possible therapeutic application. In: Grotenhermen F, Russo E, editors. *Cannabis* and cannabinoides: pharmacology, toxicology and therapeutic potential. New York: Psychology Pr. p. 359-69.

Zuardi AW, Hallak JE, Dursun SM, Morais SL, Sanches RF, Musty RE, Crippa JA. 2006. Cannabidiol monotherapy for treatment-resistant schizophrenia. J Psychopharmacol 20:683-86.

Female cannabis plant
Source: Elizabeth Blackwell, Herbarium Blackwellianum (1757). Courtesy of the Lloyd Library and Museum, Cincinatti, OH.

American Herbal Pharmacopoeia®

PO Box 66809
Scotts Valley, CA 95067
Tel: 831-461-6318
Fax: 831-438-2196
Website: www.herbal-ahp.org
Email: ahp@herbal-ahp.org

ISBN 978-1-929425-37-2

No. T-2030-13

FEDERAL COURT

BETWEEN:

NEIL ALLARD
TANYA BEEMISH
DAVID HEBERT
SHAWN DAVEY

PLAINTIFFS

AND:

HER MAJESTY THE QUEEN IN RIGHT OF CANADA

DEFENDANTS

CERTIFICATE CONCERNING CODE OF CONDUCT FOR EXPERT WITNESSES

I, Caroline Ferris, having been named as an expert witness by the Plaintiffs, certify that I have read the Code of Conduct for Expert Witnesses set out in the schedule to the Federal Courts Rules before the commissioning of my Affidavit and agree to be bound by it.

Dated: December 18th, 2014

Caroline Ferris
Expert Witness
Surrey North Community Health Centre
10697 135A St
Surrey BC V3T 4E3

This is Exhibit "B" referred to in the affidavit of Caroline Ferris sworn before me at Abbotsford BC this 18th day of Dec. 2014

A commissioner for taking affidavits for British Columbia