Prospects for New Cannabis-Based Prescription Medicines

Brian A. Whittle
Geoffrey W. Guy
Philip Robson

SUMMARY. Cannabis is now emerging from a period of prohibition and being revisited as a potential source of treatments for conditions ill served by synthetic substances. Previous research focussed primarily on effects produced by synthetic cannabinoids such as THC, or cannabis of unknown cannabinoid content. Chemovars of cannabis characterized by high content of specific cannabinoids (primarily, but not only THC and CBD) have been developed. Clinical research using defined extracts from these chemovars is now underway in the UK.

Many diseases are multifactorial; a variety of receptors need to be targeted to produce a therapeutic effect. A defined botanical may better achieve this than a single synthetic compound as the components can act synergistically. A new generation of cannabis based medicinal products takes advantage of increasing understanding of the mode of action of cannabinoids, evidence-based research on clinical uses and new technology for realization of products, in anti-diversionary presentations. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>*

Brian A. Whittle, Geoffrey W. Guy and Philip Robson are affiliated with GW Pharmaceuticals Ltd., Porton Down Science Park, Salisbury, Wiltshire, UK SP4 0JQ.

[Haworth co-indexing entry note]: "Prospects for New Cannabis-Based Prescription Medicines." Whittle, Brian A., Geoffrey W. Guy, and Philip Robson. Co-published simultaneously in *Journal of Cannabis Therapeutics* (The Haworth Integrative Healing Press, an imprint of The Haworth Press, Inc.) Vol. 1, No. 3/4, 2001, pp. 183-205; and: *Cannabis Therapeutics in HIV/AIDS* (ed: Ethan Russo) The Haworth Integrative Healing Press, an imprint of The Haworth Press, Inc., 2001, pp. 183-205. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST). E-mail address: getinfo@haworthpressinc.com].

KEYWORDS. Cannabinoids, cannabis, CB receptors, new chemovars, clinical research, multiple sclerosis, spinal cord injury, neurogenic pain, botanical extracts, secure dispensing, alternative delivery systems, harm reduction

PROSPECTS FOR NEW CANNABIS-BASED PRESCRIPTION MEDICINES

There is nothing new about cannabis as a prescription medicine. The use of cannabis by mankind is probably as old as the need for pain relief but the use of cannabis as a prescription medicine is complicated by its alternate use as a recreational drug, and attempts to regulate that practice. The novelty described in this article arises from a re-examination of some historical uses of cannabis in the light of new technology. Research on possible modes of action of cannabinoids, and novel methods of administration have prompted a re-evaluation of the therapeutic benefit of cannabis and cannabinoids.

A distinction has to be made between the cultivation of hemp, which is primarily used for textile fibre and oil seed on the one hand, and cannabis, which is used for medicinal purposes on the other. Botanists are still debating whether *Cannabis sativa* and *Cannabis indica* are two species within the family Cannabaceae, or whether there is only one species with great diversity. The various uses of *Cannabis* spp. arise from crosses and selective breeding of varieties from various landraces. The plasticity of the cannabis genome provides opportunities for rational investigation of cannabis by providing defined chemovars. The availability of specific chemovars (varieties distinguished by the active constituents which they contain rather than fine differences in morphology) provides the test materials to cater for the current resurgence of interest in the therapeutic benefit of cannabis-based medicines.

The first edition of Merck's Manual (1899) reflects the important place that cannabis-based medicines had in the armamentarium of physicians at that time. At the end of the 19th century the majority of active drug substances were *materia medica* of plant origin. It is interesting that cannabis-based medicines provided treatments for conditions which, during the pharmaceutical revolution over the last half-century, have come to be catered for by synthetic drugs such as the benzodiazepines and potent synthetic analgesics. In Merck's Manual, preparations of cannabis are recommended as a hypnotic sedative which is very useful for the treatment of hysteria, delirium, epilepsy, nervous insomnia, migraine, pain and dysmenorrhoea. It is worth remembering that at that time, the available hypnotics were bromides, extracts of valerian and opium. Apparently, extracts of cannabis were prescribed for Queen Victoria, and in Victorian times, cannabis was a respectable and useful component of prescribed medicines. It continued in use until the middle of the 20th Century

but social abuse caused a re-think on the risks perceived to attend its use. Its use as a prescription medicine was reduced and finally prohibited by legislation.

The renewal of interest in cannabis-based medicines may lead to treatments for conditions which cannot be adequately treated by the best available medicines based on synthetic compounds. It is therefore vital that introduction of cannabis-based medicines is justified on the grounds of evidence-based medicine.

The use of cannabis as a recreational substance has resulted in the classification of cannabis as a Schedule I drug in the USA. Corresponding proscriptive legislation has been enacted by other signatories to the United Nations Single Convention. This reflects the regulatory attitude that it is a drug of potential abuse with no therapeutic benefit. In order to show that cannabis-based medicines have therapeutic benefit it is necessary to carry out clinical research. In order to carry out clinical research it is necessary to have a license to possess cannabis for research purposes. Although such licensing is theoretically possible, it has not until now been considered expedient to support research into the clinical usefulness of cannabis. Since 1971, the type of research that has received most support in the USA has been directed towards demonstrating the risks and hazards of taking marijuana. Research into its therapeutic benefit has not been practically possible or politically correct until very recently.

The House of Lords Science and Technology Sub Committee report (2001) gave very positive encouragement to carry out clinical research in the UK. With the support of the UK Home Office and the Medicines Control Agency (MCA) clinical research is now underway in the UK in patients with pain and associated with Multiple Sclerosis (MS), other neuropathic pain, and cancer pain unresponsive to opioids.

During the last three decades the main avenues of research have been preclinical investigations into the mode of action of cannabinoids and cannabinoid-like compounds. Clinical research has focused on the effects (mostly adverse events), which follow from use of marijuana either in smoked or orally ingested form. The majority of work on cannabis in the USA has employed a variety of cannabis that contains delta-9 tetrahydrocannabinol (THC) as the principle cannabinoid. In America and the Caribbean, there has been selection of plant varieties that produce maximum psychotropic effects, and this material contains only a small proportion of cannabidiol (CBD). In contrast, street material in Europe contains proportionately more CBD (about equal quantities of THC and CBD). This type of cannabis is referred to as "Moroccan"; it is grown in many European and Mediterranean countries.

Cannabidiol was formerly regarded as an inactive constituent of cannabis (Merck Index 1996), but there is now evidence that it has pharmacological activity, which is different from that of THC in several respects. In some cases it

appears that the pharmacological effects are different in sign, and that a combination of the two cannabinoids has therapeutic benefit not evidenced by either cannabinoid alone. In the case of cannabis there is evidence from clinical studies, and a strong patient perception that the ceiling of effect produced by extracts is greater than the effect produced by the corresponding amount of THC as a pure chemical substance (Price and Notcutt 1998).

In a regulatory climate where the emphasis is on new chemical entities, it is refreshing to see that the idea of using cannabis as a "botanical" extract is attracting serious attention. It means that clinical investigators can use a defined extract rather than a mixture of synthetic cannabinoids as the test object. Many of the reports of early work on cannabis are based on observations of subjects who smoked marijuana. Unfortunately, in the majority of these reports a clear understanding of the content of the major cannabinoids is lacking. The availability of cannabis with a pedigree and provenance allows for this variable to be controlled rigorously.

There are therefore a number of issues that need to be addressed in rehabilitating cannabis, first of all for some of its historic indications, and also for newer indications especially those areas where cannabis has a unique contribution. Among these are its use in the field of opioid-resistant pain in neurological conditions such as multiple sclerosis, cancer pain and migraine, and as an appetite stimulant in AIDS syndrome. In order to do this it is necessary to revisit the regulatory and statutory requirements for cannabis-based medicines. The major issues are:

- the concept of cannabis-based medicines as botanicals as opposed to pure cannabinoids;
- selective breeding of high yielding chemovars that produce an abundance of one particular cannabinoid;
- investigation of the pharmacological properties of various cannabinoids, i.e., cannabis is not just THC;
- variability of composition of cannabis. The geographical and genetic basis for variation in cannabinoid content of cannabis biomass and its control to give a standardized product;
- the quality aspects of cannabis biomass production;
- routes of administration and optimization of formulations to achieve particular pharmacokinetic profiles;
- regulatory issues, including health registration, and international legal requirements;
- security packaging and anti-diversionary devices which can be used in connection with cannabis-based medicines in order to satisfy statutory requirements.

THE RATIONALE FOR USE OF CANNABIS EXTRACTS

The pharmaceutical revolution in the 20th Century has been built on the concept of treating disease as a target that can be hit with a defined chemical compound. The discovery of receptors in tissues to which drugs bind, and in which they initiate a biological effect gave support to this idea. It is ironic that the concept of the "magic bullet" came to be understood in terms of targeting "receptors." With the advent of cloned receptor proteins it is now possible to show that a variety of targets may need to be hit in order to effect a therapeutic benefit. It seems likely that what is needed is a broadside rather than a sniper's bullet to despatch the pathological lesion. It is true that some diseases are the result of a single biochemical defect, for example the congenital absence of a particular enzyme system in phenylketuronia, but the majority of disease processes are multifactorial and have to be tackled holistically. Pathological lesions, where they can be considered as causative, require a number of therapeutic agents, or a single chemical with several properties, to achieve an improvement in the patient's condition. Plants contain a variety of active and adjuvant substances, and by a process of selection those that are clinically beneficial have become accepted as *materia medica*. Humans have been exposed to these complex mixtures for millennia. Those that are useful have been selected, and it is hypothesized that they present less of a metabolic shock than synthetic new chemical entities. In practice it has been found that extracts of cannabis provide greater relief of pain than the equivalent amount of cannabinoid given as a single chemical entity.

POLYPHARMACY

In medical education over the last half century, polypharmacy has been frowned upon. It was considered desirable in an age when new chemical entities were discovered with precise effects on biological systems and even particular receptors that these "clean" chemicals could be used to treat pathological lesions with surgical precision. Unfortunately, human disease has multiple pathologies and is rarely treatable with a single chemical agent. By default, a number of new chemical entities are used in order to treat different aspects of the patient's condition holistically. In plant extracts accessory constituents may produce an effect that is synergistic with the principal one. Others may mitigate side effects produced by one drug. By repeated use and empirical observation, these processes have selected the *materia medica* that are most useful and safe. Polypharmacy is a defining characteristic of most systems of traditional medicine, but it is sometimes overlooked that combination of agents in complex prescriptions was common in the UK until the third quarter of the 20th Century. The reasons for using combinations of *materia medica* as

active ingredients in Western prescriptions were four-fold. The prescription typically contained a principal active ingredient, a secondary ingredient, which was thought to have an adjuvant effect, a substance that antagonized some of the adverse events, and excipients, which had a physical function in ensuring the stability and patient acceptability of the total prescription. This style of prescription was phased out in the second half of the 20th Century, with the end of extemporaneous dispensing in the UK. However, when a botanical extract is tested in a well designed trial it is possible to justify clinical research on the extract as it is. It may contain more than one active ingredient. However, if it is presented as a well-defined botanical drug it can be used as a drug substance in its own right. The requirements from a regulatory point of view are that the medication should have the same composition each time it is prepared and be stable. In the case of cannabis, the limited stability of some of the older galenic preparations may have been a factor in their falling out of use. Improved methods of selection of plants, care in growing and improved methods of analysis now provide materials which can be used confidently as drug substances in their own right.

EFFICACY

Mechoulam (1976) showed that many of the characteristic effects of cannabis are produced by ^{9}-tetrahydrocannabinol. The availability of synthetic THC prompted the investigation of this chemical entity as the active ingredient in pharmaceutical preparations. Dronabinol (Marinol) is available as soft gelatin capsules containing 2.5, 5 and 10 mg of THC. As a synthetic compound it falls outside the Single Convention on Narcotics and is available as a prescription medicine. It has been progressively moved from schedule I and is now in Schedule III. Dronabinol capsules have an indication as an anti-emetic and have also been shown to stimulate appetite in patients with AIDS. The oral route of administration for cannabinoids leads to slow and irregular absorption. Some of the variability in response and low therapeutic window may be inevitable consequences of giving the drug by this route. Perhaps of greater significance is the pharmacokinetic profile after administration. Once the capsule is swallowed it is committed, and one of the unfortunate adverse events following the use of Marinol is that the patients who are heavily sedated ('stoned'), have to wait until the effect wears off. Comparisons of the effect of preparations containing dronabinol with those containing an equivalent amount of THC in the form of a cannabis extract (Iversen 2000) have shown that the maximal therapeutic effect and incidence of adverse events is lower when the cannabinoid is given as extract.

A number of explanations have been offered for the lower incidence of side effects and the higher ceiling of cannabis extracts over synthetic cannabinoids. In cannabis-based medicines, the presence of other cannabinoids such as cannabidiol (CBD) is thought to have an antagonistic effect to some of the effects of THC, although CBD may sum with other THC effects. The basis for this explanation is illustrated in Table 1, which shows, in broad outline, the different pharmacological effects of THC and CBD. It is clear from this table that all of the effects of cannabis cannot be explained in terms of just one cannabinoid. It is equally true that combination of THC and CBD in the correct proportions can offer a product with a tailored pharmacological and therapeutic profile, and possibly a lower cost in terms of adverse events.

In a pilot pharmacological screening test (In-house report, GPA 002/000159 2000), CBD gave a positive effect in a maximal electroshock test, showed antinociceptive activity, *in vitro* inhibition of 5HT-induced contractions of guinea pig ileum, anti-inflammatory action in the rat paw oedema test (rat), antimicrobial activity (*in vitro*) and potentiation of hexabarbitone sleeping time. These pharmacological activities support the proposed use of a CBD-

TABLE 1. Comparison of Some Pharmacological Effects of THC and CBD

Effect	THC	CBD	Reference
CB1 (Brain receptors)	++		Pertwee et al., 1998
CB2 (Peripheral receptors)	+	−	
CNS Effects			
Anticonvulsant†	−−	++	Carlini et al., 1973
Muscle Relaxant	−−	++	Petro, 1980
Antinociceptive	++	+	
Catalepsy	++	++	
Psychotropic	++	−	
Antipsychotic	−−	++	Zuardi, 1997
Neuroprotective Antioxidant Activity*	+	++	Hampson A J et al., 1998
Antiemetic	++	−	
Sedation (reduced spontaneous activity)	+	+	Zuardi, 1997
Cardiovascular Effects			
Bradycardia	−−	+	Smiley et al., 1976
Tachycardia	+	−	
Hypertension§	+	−	
Hypotension§	−	+	Adams et al., 1977
Anti-inflammatory			Brown, 1998

* Effect is CB1 receptor independent.
† THC is pro convulsant.
§ THC has a biphasic effect on blood pressure; in naïve patients it may produce postural hypotension and it has also been reported to produce hypertension on prolonged usage.

rich cannabis extract in the treatment of severe arthritis (Burstein and Raz 1972).

QUALITY ISSUES

There is a compelling case for development of cannabis-based medicines using defined extracts as the active substance. GW Pharmaceuticals has developed a growing system that builds in quality by excluding the majority of adventitious factors, which normally have to be monitored and tolerated in field grown crops. Standardization and high quality have been achieved by growing specific chemovars under controlled conditions. However compelling the case for botanical extracts, it is essential that quality be built into the product. In order to do this it has been necessary to examine critically every aspect of the growing and production process. Field grown crops are subject to a range of factors adversely affecting quality. Recently, guidelines have been proposed for Good Agricultural Practice, expected to be incorporated into European Union (EU) legislation. These guidelines address many of the issues applicable to field grown crops. However, a more radical approach, giving an even higher degree of regulatory assurance, is to protect the plants from as many adventitious factors as possible by growing under glass in a controlled environment.

BOTANICAL SOURCE OF MEDICINAL CANNABIS

Hortapharm BV and GW Pharmaceuticals have produced a range of cannabis chemovars, which express a high proportion of their cannabinoid content as a specific chemical entity. This library contains chemovars that produce predominantly either THC, CBD, one of their precursors or congeners. This opens up the exciting prospect of using chemovars that produce some of the less well-studied cannabinoids such as tetrahydrocannabivarin (THCV) and cannabinodivarin (CBDV), which are characteristic of cannabis grown in South East Asia. The use of extracts from specific chemovars makes it possible to examine the effects of single extracts, or by blending extracts, to achieve a ratio of cannabinoids which may be optimal for a particular therapeutic condition. Initially, extracts from a high THC and a high CBD chemovar have been used to produce medicinal cannabis products. These contain predominantly THC, predominantly CBD or a defined ratio of THC and CBD.

The high THC chemovar is a stable hybrid of *Cannabis sativa*, subtype *indica* crossed with *Cannabis sativa*, subspecies *indica*. The principal cannabinoid produced (typically more than 94% of the total cannabinoid) is ^{9}-tetrahydrocannabinol with approximately 1.5% of cannabidiol.

The high CBD chemovar is a stable hybrid of *Cannabis sativa*, subtype *sativa* that has been heavily crossed and inbred with other varieties of *Cannabis sativa*. Typically, the principal cannabinoids produced in this chemovar have more than 90% of total cannabinoid as CBD, with approximately 3% present as THC. The exact details of the pedigree of these chemovars are the subject of Plant Breeders' Rights.

After selection for cannabinoid content, a group of chemovars is produced, but not all of them are equally hardy and suitable for volume production of cannabinoids. The plants are further selected for vigour and robustness. The production of standardized cannabis is from cuttings prepared from "mother" plants. This ensures that the genotype is fixed and there is consistency in the proportion of cannabinoids in each chemovar. Stability of cannabis biomass is also improved in these chemovars. Production quantities of cannabis are produced from seedless female plants.

Cannabis is a dioecious plant, and it is thus typical for male and female flowers to appear in separate plants. The male plant bears staminate flowers and the female plant carries pistillate flowers, which develop into the fruit and seeds. The content of useful cannabinoid is greatest in the flowering heads, particularly in the female plants. Monoecious plants may occur bearing both male and female flowers on different branches of the same plant. The appearance of male flowers results in early fertilization of female plants, with loss of yield. To optimize cannabinoid content it is essential to remove these "rogue" male flowers before they mature. Monoecious plants appear spontaneously in medicinal cannabis plants but are more frequent in varieties intensively bred for hemp production. In production of cannabis, the appearance of male flowers will result in fertilization of female plants, and reduction in yield of cannabinoids. For this reason plants bearing male flowers are removed as soon as they are detected. Raman (1998) has reviewed the process of masculinization of female plants in order to produce "self-varieties." Masculinization of female plants can be induced by chemical agents in order to produce self varieties for selective breeding (Ram and Sett 1982). A number of agents are known to induce masculinization including irradiation, treatment with streptovaricin and exposure to low levels of carbon monoxide.

Using a variety of techniques, De Meijer and Keizer (1996) have produced specific chemovars that have a very high content of total cannabinoids expressed as either THC or CBD. This programme of work has resulted in other chemovars that predominantly express cannabinoid content as specific cannabinoids other than THC and CBD.

In addition to fixation of the chemovars in terms of cannabinoid expression, it is necessary to further select for vigour. This has resulted in a series of chemovars that have the necessary robustness for large-scale production of cannabis in controlled conditions of lighting and temperature. This is the es-

sence of the technology that has been developed by Hortapharm BV and GW Pharmaceuticals, after selection from accessions of material obtained from around the world.

The original (mother) plants are maintained in long day length to produce non-flowering, vegetative growth. The mother plants are used as a source of genetically identical cuttings (also referred to as clones). The clones are then grown on, and by manipulation of day length they are induced to flower and produce plants from which the product is prepared. A percentage of the young clone plants, when established, are retained under vegetative conditions (not allowed to flower) to produce further clones. The two chemovars used in the production of cannabis-based medicinal extract were selected from the range of varieties produced in this programme.

In the plant, cannabis resin is present in glandular trichomes. It is possible to obtain fractions containing a high concentration of resin by collecting these. Fractions rich in these trichomes constitute hashish, which may contain up to 60% of cannabis resin. However, for volume production it is more economic to harvest whole plants when the flowers are beginning to senesce, and to extract cannabinoids from the entire aerial parts of the plant.

HARVESTING

Field grown cannabis, if allowed to fall on the ground, is subject to fungal attack and contamination from birds and vermin. When grown on the small scale it is possible to hand pick the flowering tops from cannabis, but volume production demands mechanized means of harvesting and processing. When harvested, cannabis has moisture content of approximately 25%. In order to have a stable product it is necessary to reduce the moisture content to under 12%. Biomass stored away from light and heat is relatively stable. In the dried plant, a significant proportion of total cannabinoid is present as the cannabinoid acids (THCA and CBDA). These acids are not known to have cannabinergic activity and conversion of cannabinoid acids to free cannabinoids, the biologically active form, occurs spontaneously over time and is accelerated by heating. Smoking effectively decarboxylates cannabinoid acids. In other methods of preparation for medicinal use, it is necessary to ensure that this change is effected, as the cannabinoid acids do not have biological activity.

PREPARATION OF EXTRACTS

Historically, extracts of cannabis were prepared (BPC 1934) by percolation with 90% ethanol. Various galenical preparations have been used, including tinctures (ethanolic extracts). Solid extracts (solvent-free) have been used for

preparation of finished dosage forms after the optional removal of solvent. The extract of cannabis is an oily resinous material, which is virtually insoluble in water.

Other solvents that have been used in an attempt to produce a purified extract of cannabis include fluorinated solvents such as heptafluropropane (HFA 227) and norflurane (HFA 134a). These solvents produce extracts that contain waxes and colouring agents, and a small amount of terpenes in addition to the cannabinoids.

A cleaner extract is produced by extraction with supercritical CO_2. In this process the majority of colouring matter and chlorophyll are left behind. The extract contains principally the cannabinoids but also some high molecular weight waxy ballast and sufficient terpenes to produce the characteristic scent of cannabis. Most of the ballast can be removed by chilling an alcoholic solution, a process referred to as "winterization." The winterized extract is an accessible material for production of liquid dosage forms using pharmaceutically acceptable solvent systems.

CHOICE OF DOSAGE FORM

Cannabis preparations have been administered by most routes commonly employed in the pharmaceutical industry. Historically, it was given in mixtures prepared from tinctures, and in the form of pills. In Victorian times plasters prepared from powdered drug were applied locally to relieve pain and with ointments represent the first attempts at transdermal application. Oily eye drops were also used for the treatment of glaucoma. Smoking is probably the fastest way of producing the pharmacological effects of cannabis in humans after intravenous injection. However effective as a mode of recreational use, smoking as a route of dosing for a prescription product can no longer be justified on ethical, medico-legal or safety grounds.

The sublingual route administration has shown a rate of absorption intermediate between that achieved by smoking and the oral (swallowed) route. Selection of a dose presentation based on extracts containing THC and CBD has produced a medicine that is organoleptically acceptable to the majority of patients. More importantly, the ability to take the medicine in small sub-doses has been invaluable in the investigation of efficacy and safety. The time course is such that the patient is able to take account of cognitive cues in timing the next dose increment. This allows patients to titrate the dose to a level where they achieve benefit and minimize unwanted side effects.

Recreationally, smoking is the commonest route of administration, closely followed by oral ingestion (brownies). Some patients with multiple sclerosis who smoke cannabis report relief of spasm and pain after the second or third

puff of a cannabis cigarette. This implies very rapid transit to, and absorption into the central nervous system. The time involved is seconds rather than minutes. The oro-pharyngeal, buccal, sublingual and respiratory mucosae have venous drainage directly into the *vena cava* and the left side of the heart. Material absorbed through the mucosae of these areas is therefore not exposed to the liver during its first circuit into the systemic circulation. The drainage from the rest of the gastrointestinal tract (other than for the distal part of the rectal mucosae) perfuses the liver, the major detoxifying organ of the body. In addition to protecting the organism from ingested toxins, the liver also metabolizes medicaments, which are subject to the same chemical processes. Blood from the liver subsequently returns to the left side of the heart and reaches the rest of the systemic circulation. This first pass through the liver may result in the removal of a substantial proportion of an ingested medicament. In the case of cannabinoids, more than 95% of an ingested dose is metabolized during this first pass. This may contribute to the variability and timing to achieve maximal plasma concentration (C_{max}) and the time to achieve this maximum (T_{max}). In the case of cannabis there is a very wide variation in the values of C_{max} and T_{max} observed. A further complication is the rapid metabolism of tetrahydrocannabinol to 11-hydroxy-THC, which is also psychoactive. This occurs during the first pass through the liver and possibly through other tissues involved in the chain of absorption before cannabinoids reach the left side of the heart.

The areas of the respiratory/alimentary system having venous drainage into the systemic circulation, thus avoiding the first pass effect, are the mucous membrane of the buccal cavity, the sublingual area, the oro-pharynx, the respiratory tract, and the distal part of the rectum. The avoidance of the first pass effect is the rationale for the use of buccal, nasal, sublingual and suppository formulations. Each has advantages and disadvantages.

- Suppositories are subject to hygienic and patient compliance restriction.
- Formulations intended for administration to the nasal mucosae may cause pain or reflex sneezing, and in extreme cases cause irritation (Tashkin et al. 1973) and damage to the mucosae.
- Preparations intended for administration by inhalation have the advantage of speed of onset, but there is a direct irritant effect of THC *per se*, in addition to the irritant effect of the products of pyrolysis. Opinion is divided on the direct irritant effect of cannabinoids and it is possible that formulations that contain particles capable of being hydrated in their transit of the respiratory tract have lower irritancy.
- Sublingual formulations may stimulate the flow of saliva, and it is difficult for patients to avoid swallowing when substantial amounts of saliva are produced. If drugs applied to the sublingual mucosae are swallowed

the cannabinoids will be subject to the first pass effect and will therefore be less effective. This will result in proportionately higher levels of metabolic products.

- Buccal formulations where the product is held in contact with the parietal buccal membrane may be subject to the same limitations. Both sublingual and buccal formulations depend on the efficient transfer of medicament from a hydrophilic vehicle to the cell membrane of the sublingual or buccal mucosae. It is likely that absorption of cannabinoids takes place through the interstices in the membranes or by transfer into the epithelial cells. This transfer is governed principally by the lipid solubility of medicaments, and the partitioning of a lipid solid drug through an aqueous surface layer into a lipophilic absorption mechanism is an area for investigative research.

There are therefore a number of physical and biological limitations on the routes of administration of cannabinoids, but also opportunities for innovation in devising presentations to optimize administration. With the development of sensitive and specific methods of analysis, it is now possible to produce the kinetic profile that is best suited to treatment of specific therapeutic indications. Sublingual administration gives slower absorption than the respiratory route. However it is fast enough. The interval between doses allows time for subjects to become aware of the onset of cognitive changes in relation to wanted effects. Patients are thereby able to titrate doses to exploit the window between wanted therapeutic effects and unwanted side effects.

CLINICAL STUDIES

Initial phase 1 studies were carried out using a glycoalcoholic solution of cannabis extract, which was applied to the sublingual mucosae (Guy, Whittle and Grey 2000a and Whittle and Guy 2001). Fractional doses were given so that 2.5 mg was applied at intervals of 10 minutes.

The first human exposure to GW Pharmaceuticals (GWP) preparations of THC and CBD took place in healthy volunteers late in 1999. This placebo-controlled, six period, crossover study in six healthy volunteers assessed pharmacodynamic effects, pharmacokinetic profile, safety and tolerability including examination of routine clinical laboratory results and continuous monitoring of ECG and vital signs, cognitive effects, adverse events and subjective effects of a single dose of three cannabis based medicinal extracts (CBME) administered sublingually, and one formulation via aerosol and nebulizer.

CBME tested were High THC, High CBD, THC:CBD 1:1 mixture, and matching placebo. Maximum permitted dose was THC 20 mg and/or CBD 20

mg given incrementally at 10-minute intervals. All six subjects successfully completed the six periods of the study without giving rise to safety concerns. Pharmacokinetic profiles showed reliable absorption of CBME with peaks at 5 minutes following inhalation and approximately 2 h sublingually. Well-recognized effects of THC such as psychoactivity, conjunctival reddening, and intermittent tachycardia were observed. Overall, the cognitive effects were modest. Adverse effects reported by the subjects included vivid dreams, conjunctival injection, tachycardia, postural hypotension, hunger, pallor and sweating. No serious adverse effects were noted.

The design chosen by GWP for the initial clinical research is a series of double-blind, crossover, placebo-controlled single case studies. After an initial two week run-in period on open label THC:CBD 1:1 mixture, subjects enter a four way double blind crossover study comparing the 1:1 THC:CBD mixture, High THC, High CBD and placebo. After completion of this, patients are given the option of entering a long-term safety and tolerability follow-up study.

Sixty-four patients with a range of medical diagnoses including multiple sclerosis, spinal cord injury and other serious neurological conditions, have so far been titrated on to sublingual CBME. Of these, over 80% have chosen to continue receiving the medication in the long-term extensions. Between them, these subjects have now generated more than 950 patient-treatment weeks.

Virtually all the subjects remaining on treatment have experienced significant alleviation of at least one key symptom, and in some cases the improvement has been sufficient, in the patients own words, to transform lives by dramatically reducing pain. These improvements are particularly notable in that an inclusion criterion is intractability of symptoms in the face of available standard therapy. Among the positive effects recorded are relief of neuropathic pain, spasms, spasticity and bladder-related symptoms; at least partial alleviation of tremor; and improvements in mood and measures of overall well-being. Intoxication is the most frequent dose-limiting effect for the THC-containing medicines.

Because so little is known of optimal dose patterns for CBME, patients have been allowed to establish dose level and frequency by self-titration, with defined upper limits (no more than eight 2.5 mg doses within any 3 h period and no more than 120 mg/24 h). Many subjects chose to take small doses at more frequent intervals than the usual three or four times a day pattern. A wide range of individual daily dose requirements has been noted, but once a pattern is established very little variation within subject seems to occur. No evidence of tolerance to therapeutic effects has been noted so far. Most patients can achieve symptom relief at a sub-intoxication dose, although the margin between the two thresholds is often narrow.

A range of adverse effects has been reported, most of which seem to occur early in the treatment and diminish as a suitable dose is arrived at by self-titration. The most commonly occurring effects (i.e., those reported on 3 or more occasions) in descending order of frequency were headaches, nausea, burning in the mouth, intoxication, sweating, flu-like symptoms, vomiting, falls, and chest pain of unknown origin. Almost all of these effects have been transient, of only mild or moderate intensity, and well tolerated by the patients. One event defined as severe has been reported, but this ended in complete recovery following supportive treatment.

These pilot studies have provided important information which will inform future randomized, placebo-controlled cohort studies, including appropriate doses and dosing patterns, selection of CBME content for different conditions, identification of target symptoms and outcome measures. They have uncovered opportunities for optimization of the formulations (e.g., volume, solvents, taste, and blinding) and types of presentation, which can be incorporated into larger studies. With such small numbers, it is difficult to interpret the ultimate significance of adverse events. However, these preliminary studies have provided the investigators with invaluable hands-on experience of using cannabis-derived medicines in a therapeutic setting. They supplied further reassuring evidence of the safety and tolerability of these medicines in patients, often middle-aged or elderly, with serious medical disorders.

NON-SMOKING INHALATION

Non-smoking inhalation of cannabis is a fast and attractive route of administration for the new generation of cannabis-based medicines, which have been developed. The question arises, why not use smoking as a method of administration for a prescription product? The reasons are self-evident but are worth re-stating as this proposal periodically re-surfaces.

There are medico-legal implications involved in recommending smoking in any form. Cannabis, like other cellulosic materials, produces particulates and tar when burnt. These contain polyaromatic hydrocarbons (PAHs) known to be carcinogenic. The pattern of bronchial pre-carcinogenic cytological changes in habitual chronic cannabis smokers is similar to that of tobacco smokers. It is difficult to dissect out the contribution made by cannabis alone in this regard, since many cannabis smokers also smoke tobacco, and many study designs do not allow this variable to be assessed. Other factors that militate against the use of smoking as a route of administration for a prescription drug are the dislike of some patients for smoke and the perceived sociological disincentives expressed by some patients who do not wish to be seen smoking a street drug. Reports of an irritant effect of cannabis smoke are also a factor to be taken into

account. Recreational smoking is a personal decision and is vigorously defended by users as a personal right. However, in the present climate of opposition to smoking in general, drug developers are unwilling to shoulder the moral and legal responsibility for adverse events resulting from recommending it as method of administration. There is, therefore, a search for non-smoking methods of administering cannabinoids via the respiratory tract. A number of methods of administration currently in use for other drugs have been examined for their applicability to administration of cannabinoids or extracts of cannabis.

The physical properties of medicaments given by inhalation are important. When air is inhaled through the nose it passes through the naso-pharynx and past the epiglottis into the trachea. The naso-pharynx acts as a filter to prevent the entry of large particles and has a role in warming (or cooling in the case of smoking) and humidifying the stream of air and particles. Air passing into the trachea enters the lung via the bronchi, bronchioles and alveoli. The bronchi walls contain rings of cartilage linked together with smooth muscle. Their inner surface is lined by cilia, which beat and assist the upward and outward movement of unwanted fine particles, which are then swallowed. The bronchioles are narrower versions of the bronchi, which do not have cartilage but are elastic; they constrict and dilate to modify the resistance to passage of air. Deeper within the lung the bronchioles branch repeatedly giving rise to terminal bronchioles, and the end outgrowths of the bronchioles are the alveoli. The walls of the respiratory bronchioles and alveoli are thin, covered in a network of fine capillaries and are the sites for gaseous and drug exchange. Products given by inhalation usually deliver the active ingredient in the form of aerosol droplets or as solid particles. In the case of cannabis, some of these particles may be condensed from vaporized cannabinoid that have subsequently become hydrated in the high relative humidity within the bronchial tree. The site at which droplets or particles are deposited in the lung depends largely on their aerodynamic diameter. This measure is the diameter of the perfect sphere that would fall through air at the same speed as the particle. Particles with an aerodynamic diameter greater than 10 micrometers tend to be deposited in the upper regions of the respiratory tract where they are quickly removed by the ciliated epithelium. Only particles approximately 2 micrometers or less are capable of reaching the alveoli. In the case of conventional drug particle inhalers, it is estimated that only 5-20% of the delivered dose reaches its site of action.

The relative humidity of the respiratory tract is approximately 99.5%, and inhaled particles, may hydrate and grow in size. Aerosolized liquid droplets may behave similarly. An equilibrium is attained with this type of particle when vapour pressure on the surface of the particle and in the respiratory tract are the same. This process may take only milliseconds to complete. Particles with an aerodynamic diameter of less than 1 micrometer may also be re-expired. Particles with a diameter of less than 0.5 micrometers display Brownian

motion and a small fraction may be re-expired and lost. These factors are important in designing novel presentations of cannabis-based medicines for inhalational use.

The technology for producing aerosolized metered dose inhalers (MDIs) and drug-particle inhalers is well described, and attempts have been made to adapt this type of inhaler for delivery of cannabinoids. THC and CBD are virtually insoluble in physiological saline, but are soluble in high concentrations of ethanol. There are limits on the quantity of ethanol that can be taken into the respiratory tract. Vaporization of ethanol also produces both cooling and irritant effects. This greatly limits the amount of cannabinoid that can be administered per actuation of a pressurized device. Co-solvents such as propylene glycol and glycerol and surfactants produce marginal improvements in the concentration of cannabinoid, but a typical quantity that can be delivered (25-125 micro litres) using commercially available spray buttons is a trade off between solubility, volume and the irritant effect of the solvent.

VAPORIZERS

The challenge is to devise a vaporizer that produces the rapid effects of cannabis without the disadvantages of pyrolysed material and the consequent tar production. On the World Wide Web there are a number of sites where designs for vaporizers are posted. These consist of a source of heat, which is applied to a portion of cannabis herb, and a means for containing the volume of vapour, which is produced so that it can be inhaled in the stream of inspired air. Typically, the heat is applied in the form of an electrical heating element (soldering iron bit) or radiant heat from an incandescent light bulb. The fluidised bed principle has been applied in a device recently patented by Pate (1997). In this device a portion of cannabis herb is entrapped between two screens, and heated air is passed through the fluidised bed of cannabis and distills off the cannabinoids. The vapour so produced can then be inspired into the respiratory tract substantially free of particulates and smoke. Careful regulation of the temperature is necessary to ensure that distillation is carried out at a temperature below that at which cannabis pyrolyses.

Vaporizers, in which a concentrated extract of cannabis is heated to produce vapor, are under development. Electronic control of the energy applied to the heater ensures that the concentrated extract of cannabis is efficiently vaporized, without the production of pyrolysis products. The device generates the vapour during the course of a single inspiratory cycle in a manner intended to produce a profile of absorption in the patient similar to that obtained from a cannabis cigarette. The device is a portable self-contained unit, powered by rechargeable batteries and is controlled electronically. The control device has an

algorithm which computes the energy required to produce vaporization in the dosage form, and switches current to effect vaporization at a temperature below that causing pyrolysis. The generation of vapour from the portion of medicament is done in the course of a single inspiration. The equipment also has provision for recording the date and time of use.

Control of and recording of the pattern of use are important from the standpoint of security. The recording of data on usage is an important factor in giving confidence to enforcement agencies, and monitoring of the supply by the pharmacist. The technology also provides an opportunity for data collection in the context of clinical trials monitoring and patient compliance. The method of secure dispensing and the device are the subject of UK and overseas patents (Guy, Whittle and Grey 2000b). The secure dispensing features of the equipment include a tamper-evident membrane, matching of the patient with prescription and a frangible linear link. This ensures that if the device is improperly used it locks up completely and cannot be operated. In addition to the recording and read-out of data relating to use, a simple and robust exchange scheme has been set up with pharmacist-supervised control of replacement and extension therapy.

NEBULIZERS

Nebulizers are in use for the administration of antimicrobial drugs, bronchodilators, corticosteroids and asthma treatments such as sodium chromoglycate. Nebulizers rely for their efficacy on provision of doses of medicament over a relatively prolonged period at correspondingly low concentration. A feature of nebulization is that the nebulisate is carried on a mist of water particles. Many types of nebulizer are in use, and a characteristic of the designs is that a fine mist of particles is generated in a flow of gas that is saturated with water vapour. This last feature facilitates breathing by ensuring that mucous is thinned and the epithelium of the respiratory tract does not dry out. Considerable ingenuity has been exercised in devising methods for generating a mist of particles. These include devices using a Venturi effect. Essentially, this consists of a jet of gas blowing across the end of a dip tube, the other end of which rests in a reservoir of liquid. The reduction in pressure draws water up the tube that is then converted into a spray. Smaller particles are separated from those with a high mass and kinetic energy, which are returned to the reservoir for recycling.

In the Halolite™ nebulizer, medicament solution is drawn up the central part of a rotating hollow-stemmed "T" bar, which throws off particles centripetally. Particles with a high mass hit the wall of the chamber, drain down into the reservoir and are recycled. Smaller particles are aspirated in the stream of

inhaled air. This model of nebulizer has an algorithm that ensures that a portion of nebulisate is injected into the inspired air at a determined time. The amount of nebulisate retained within the respiratory tree by the use of this algorithm is in excess of 93%. Where the vehicle used contains volatile components it is necessary to make allowance for this as some fractionation may occur with variation in the amount of drug made available as nebulisate. The extent of lung deposition from different types of nebulizer is reviewed by Hardy, Newman and Knoch (1993).

Many commercial nebulizers are intended to provide a relatively low concentration of medicament, in a saturated atmosphere over a prolonged period of time. This is not the ideal for administration of cannabis. Smoked cannabis is rapidly effective and patients with MS report relief starting with the second or third draw. Davis, McDaniel et al. (1984), have described the complex changes occurring in a smoked marijuana cigarette. The amount of THC entering the respiratory system is probably in the range 5-10 mg of THC. An important characteristic of this type of administration is the pulsatile presentation of a discreet quantity of cannabinoid that allows the patient to discern cognitive effects following each draw. The patient can then regulate both the amount absorbed and rate of absorption. Patients with disabling pain claim that they can titrate the dose to obtain relief with the minimum unwanted cognitive effects.

The ability to achieve this window of therapeutic benefit without adverse events is also a difference between patients and recreational users. The object of non-smoking alternative methods of administration must be to mimic this pattern of delivery. A number of designs of nebulizer have been devised to increase the concentration of medicament in the nebulisate. Piezoelectric oscillators have been used to generate fine particles by forcing liquid through perforated plates into a stream of air or oxygen. This method of production results in a population of particles with a more uniform mass distribution. Ultrasonic energy can be used to produce a dense cloud of particles of uniform size, and there are proprietary devices based on this principle. A feature of this type of device is the rapid response made possible by switching electrical energy to the ultrasonic generator. This gives what is essentially a square wave function and the algorithm described in the secure dispensing patents (Guy, Whittle and Grey 2000b) can be used to control the rate and quantity of cannabinoid delivered during each respiratory cycle.

TRANSDERMAL ADMINISTRATION

Theoretically, the use of transdermal delivery systems for cannabinoids is attractive. The active constituents are lipid soluble, non-ionized, and of a molecular mass which is at the top end of the range normally considered feasible

for transdermal absorption. However, in practice the results have to date been rather disappointing.

One traditional and spectacular way of collecting hashish reported by Samuelsson (1992) is for the operative to run through a crop of flowering cannabis and to allow resin to adhere to clothing and skin. Cannabis resin, which adheres easily to the body, is then scraped from the clothing and skin. Exposure of skin to resin containing high concentrations of cannabinoids has not been reported to produce intoxication. This would indicate that absorption of cannabinoids transdermally without further formulation is not extensive. Nevertheless, there are a number of patents that claim that significant plasma levels can be achieved by transdermal administration. This illustrates the contribution made by presentation and formulation.

To speed absorption, a number of systems have been designed to enhance transdermal delivery by energizing transport. These include:

- the use of ultrasonic stimulation of the skin
- iontophoresis
- incorporation of cannabinoids into liposomes, which are then incorporated into transdermal patches.

A function of skin is to protect the internal organs from the environment, and in the case of cannabis and cannabinoids it appears to do this well. The routes of entry through the skin are migration:

- through the epidermis
- into the dermis which is well served with capillaries
- diffusion into the interstitial cement surround cells
- diffusion into the lipophilic secretion in sebaceous glands and hair follicles.

Cannabinoids are very lipophilic. Although the principal cannabinoids are not ionized and have a molecular mass of around 300 and a high milligram potency, the transdermal flux is low in human skin. Touitou, Fabin, Danny and Almog (1988) compared permeation kinetic parameters through human and rat skin *in vitro*. Rat skin was found to be about 13 times more permeable to ^{8}-THC than human skin. Autoradiographs of horizontal sections showed that 24 hours after application the drug was concentrated in the *stratum corneum*, in the upper dermis and around hair follicles, indicating that THC penetrates the skin through lipophilic pathways. These studies also examined the effect of oleic acid as a permeation enhancer, and in rats a serum level of approximately 50 ng/ml of THC and metabolites (measured as cross-reacting cannabinoids) was maintained for about 24 hours.

Compensation for the lower transdermal flux in human skin can be made to some extent by increasing the effective area of the patch. However, a patch with an area of 50 cm^2 with a drug reservoir concentration of 26.5 mg/g was calculated to provide a blood concentration of 12 ng/ml for THC. A number of later patents, many of which are probably speculative, are based on reservoir, drug in matrix types of composition. Penetration enhancers include DMSO, azone and oleic acid, which are well-tried substances to produce this effect.

One factor, which does not appear to be addressed in patch patents, is the practical point of disposal. In order to achieve linear diffusion of any agent into the skin it is necessary to have a steep concentration gradient. This means that the patch must contain relatively large amounts of cannabinoids to ensure linearity of transfer through the skin initially, and the kinetics of absorption are such that the used patch may contain as much as 90-95% of the original dose. This presents a real problem of how the spent dosage unit is to be disposed. A spent patch is likely to contain sufficient cannabinoid for several doses by the oral route, or by smoking.

The formulation of cannabinoids for topical application to the skin in the form of liposomes has been proposed by Touitou (1996). The essential components of the liposomes are a phospholipid, a lower aliphatic alcohol (C2-4), with aqueous propylene glycol as the solvent. It is claimed that the hydro/alcoholic/glycolic phospholipid system increases the permeation rate of a range of active compounds including cannabinoids through the skin. Transdermal application produces an approximately constant plasma level of cannabinoids, but it remains to be seen whether the pharmacokinetic profile produced by such devices is clinically effective.

Current research into cannabinoids has revealed that the body possesses an endocannabinoid system. The system, which is present in nearly all phyla from *Hydra* upwards is characterized by cannabinergic mediators such as anandamide and 2-arachidonyl glycerol (2-AG) that are derived from substances quite different chemically from the plant cannabinoids. The analogy between cannabinoids, vanilloids and opioids is striking. In each case there is a plant material, which appears to bind to the same receptors as endogenous ligands. Research into novel cannabinergic compounds has followed a similar pattern to that employed in the case of opioids. It has polarized into a search for synthetic agonists, antagonists, reverse agonists or partial agonists using the paradigms familiar to the pharmaceutical industry in the development of analgesics to replace morphine. It is approximately 200 years since Setürner isolated morphine from opium and about 30 years since the discovery of different types of opioid receptors and the endorphins. The search for novel cannabinergic compounds should not take as long. In the meantime there is an alternative route to novel cannabis based medicines. It depends on a renewed search for novelty in the clinical application of cannabis extracts containing known combinations of

cannabinoids. Not all of the actions of cannabis are based on receptors that are currently characterized, leaving open the possibility of further cannabinoid receptors. There are also other cannabinoids that have not been studied in the same detail as THC and CBD that may have clinical benefit.

In finding new cannabis-based medicines, an alternative to the pharmaceutical industry research approach is to build on the knowledge of receptor and non-receptor pharmacology and to explore the clinical benefit of these known compounds. It is probable that they will provide surprises in efficacy, but because man has already been exposed to them for thousands of years, they may not present so many problems in metabolism and toxicity.

REFERENCES

Adams, M.D., J.T. Earnhardt, B.R. Martin, L.S. Harris, W.L. Dewey, and R.K. Razdan. 1977. A cannabinoid with cardiovascular activity but no overt behavioral effects. *Experientia* 33:1204-1205.

Burstein, S. and A. Raz. 1972. Inhibition of prostaglandin E2 biosynthesis by D1-tetrahydrocannabinol. *Prostaglandins* 2:369-375.

Brown, D.T. 1998. *Cannabis: The genus Cannabis*. Amsterdam: Harwood Academic Publishers.

Carlini E.A., J.R. Leiter, M. Tannhauser, and A.C.Berardi. 1973. Cannabidiol and *Cannabis sativa* extract protect mice and rats against convulsive agents. *J Pharm Pharmacol* 25:664-665.

Davis, K.H., I.A. McDaniel, Jr., et al. 1984. Some smoking characteristics of marijuana cigarettes. In Stig, W.L. Dewey, R.E. Agurell. *The cannabinoids*: *Chemical*, *pharmacologic and therapeutic aspects*. San Diego, CA: Academic Press.

De Meijer, E.P.M., and L.C.P. Keizer. 1996. Patterns of diversity in *Cannabis*. *Genet Resourc Crop Evolut* 43:41-52.

Guy, G.W., B.A. Whittle and M.J. Grey. 2000a. Dose dispensing Apparatus. GB Pat Application 25809.5, Oct 20, 2000.

Guy, G.W., B.A. Whittle and M.J. Grey. 2000b. Secure dispensing of materials. GB Patent Application 25811.1, Oct 20, 2000.

Hampson, A.J., M. Grimaldi, J. Axelrod, and D. Wink. 1998. Cannabidiol and (−) 9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Nat Acad Sci* 95: 8268-8273.

Hardy, J.G., S.P. Newman, and M. Knoch. 1993. Lung deposition from four nebulizers. *Resp Med* 87: 461-465.

House of Lords Science and Technology Sub Committee report. 2001. The development of prescription cannabis-based medicines (Jan 2001).

In-house Report GPA 002/000159. 2000. CBD Primary Screening Program.

Iversen, L.L. 2000. *The science of marijuana*. Oxford: Oxford University Press.

Mechoulam, R. (ed.) 1976. *Cannabinoids as therapeutic agents*. Boca Raton, FL: CRC Press.

Merck index: *An encyclopedia of chemicals*, *drugs*, *and biologicals*. 1996. S. Budavari, and M.J. O'Neil (Eds.), 12th Edition, Boca Raton, FL: CRC Press.

Merck's manual. 1899. Part 1, p. 26.

Pate, D. 1997. US Patent Application Number 08/919317, 28 August 1997.

Pertwee, R.G. 1998. Advances in cannabinoid receptor pharmacology in cannabis. pp. 125-174, in D.T. Brown (Ed.). *Cannabis*: *The genus Cannabis*. Amsterdam: Harwood Academic Publishers.

Petro, D.J. 1980. Marijuana as a therapeutic agent for muscle spasm or spasticity. *Psychosom* 21(1):81-85.

Price, M.A.P., and W.G. Notcutt. 1998. Cannabis in pain relief. pp. 223-246. In D.T. Brown (Ed.). *Cannabis*: *The genus Cannabis*. Amsterdam: Harwood Academic Publishers.

Raman, A. 1998. The cannabis plant: Botany, cultivation and processing for use. pp. 29-54. In D.T. Brown (Ed.). *Cannabis*: *The genus Cannabis*. Amsterdam: Harwood Publishers.

Ram, H.Y.M., and R. Sett. 1982. Reversal of ethephon-induced feminization in male plants of cannabis by ethylene antagonists. *Zeitschrift fur pflanzenphysiologie* 107(1):85-89.

Samuelsson, G. 1992. *Drugs of natural origin*. Stockholm, Sweden: Swedish Pharmaceutical Press.

Smiley, K.A., R. Karber, and S.A. Turkanis. 1976. Effect of cannabinoids on the perfused rat heart. *Res Comm Chem Pathol Pharmacol* 14:659-675.

Tashkin, D.P., B.J. Shapiro, and I.M. Frank. 1973. Acute pulmonary and physiological effects of smoked marijuana and oral delta-9-THC in healthy young men. *N Eng J Med* 289(7):336-341.

Touitou, E. 1996. US Patent 5,540,934 (July 30, 1996).

Touitou, E., B. Fabin, S. Danny, and S. Almog. 1988. Transdermal delivery of tetrahydrocannabinol. *Int J Pharmaceut* 43:9-15.

Whittle, B.A., and G.W. Guy. 2001. Formulations for sublingual delivery. GB Patent Application 103638.3, Feb 14, 2001.

Zuardi, A.W., and F.S. Guimarães. 1997. Cannabidiol as an anxiolytic and antipsychotic. In M.L. Mathre (Ed.), *Cannabis in medical practice*. Jefferson, NC and London: McFarland & Co. pp. 133-141.