Progress in the Chemistry of Organic Natural Products

A. Douglas Kinghorn
Heinz Falk
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Jun'ichi Kobayashi *Editors*

103

Phytocannabinoids

Unraveling the Complex Chemistry and Pharmacology of *Cannabis sativa*

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Preface

In his 1974 paper on the "Structure–Activity Relationships in Man of Cannabis Constituents, and Homologs and Metabolites of Δ^9-Tetrahydrocannabinol," the late great Professor Leo E. Hollister stated "The more one studies cannabis chemically, the more complicated it becomes." Never have truer words been spoken about the science of cannabis generally, and it is likely that Professor Hollister knew precisely how complex, diverse, and far reaching the study of this intriguing plant would become. Cannabis science has exploded in recent years with new natural product chemistry, which may not have been predicted, new synthetic analogues as drugs of abuse, new uses and medicinal targets, and from the socioeconomic perspective, as an approved medicine in its purified state and as a decriminalized regulated material in its crude form, generating tax dollars for the US economy.

Indeed at the time of writing of this volume, more than half of the US states have decriminalized possession of cannabis for medicinal use, with some going further and allowing non-medicinal usage. This has presumably occurred due to the fact that many US citizens were already self-medicating illegally for pain, to reduce spasticity, and for joint inflammation for some considerable time, and the opportunity to generate taxable revenue was appealing to state governments and not distasteful to the electorate. However, there is a considerable body of evidence to suggest that both short- and long-time use of cannabis can lead to psychosis and schizophrenia, and the long-term mental health issues in the states that have allowed easier access to this material remain to be evaluated.

Despite potential harms associated with crude cannabis drug materials, the great medicinal potential of its components singly and in mixtures is profound. Sativex©, a product of the UK-based company GW Pharmaceuticals, has been approved for spasticity associated with multiple sclerosis in nearly 30 countries including the UK, Canada, Germany, Italy, and Spain, and its use in treating cancer-associated pain is under development. Excitingly, in April 2016, the US Food and Drug Administration (FDA) granted the GW Pharmaceuticals experimental cannabis-derived antiepilepsy drug Epidiolex© "orphan" status for patients affected by

Dravet and Lennox-Gastaut Syndrome, which are rare forms of epilepsy affecting children and adults, respectively. Epidiolex© is pure cannabis-derived cannabidiol, and this non-psychoactive compound has potential in a number of areas, particularly in inflammation and even as an anti-infective.

Cannabis science has also generated many synthetic cannabinoids that were initially lead molecules for pharmaceutical companies seeking analgesic products; unfortunately, some of these compounds are highly potent at the CB_1 receptor, and they consequently have undesirable psychoactivity. These compounds have been seized upon by chemists wishing to manufacture and market Novel Psychoactive Substances (NPS), and they are very common as additions to smoking mixtures, e.g., as "Spice" or even as single pure NPS. Unfortunately, they are highly potent, in some cases being 1000 times the strength of THC, and at the time of writing of this editorial, five deaths and four acute intoxications associated with the synthetic cannabinoid 5F-MDMB-PINACA (5F-ADB) have been reported by the EU Early Warning System.

Despite the toxicity associated with cannabinoids acting at the CB_1 receptor, there are a plethora of druggable targets that phytocannabinoids interact with, notably the glycine receptors, and this target, which is covered in this volume, has great potential in the treatment of neuropathic pain and inflammation. Indeed, it may be that some of the work surrounding cannabis and its constituents interacting with CB_1 and CB_2 may have been a pharmacological "red herring" to pain management and anti-inflammation, and many of the targets discussed in this volume are possibly responsible for these effects.

The endocannabinoid system is highly complex and is affected by cannabis natural products in many direct and indirect ways, and as a consequence, we have sought in this volume to focus on natural product cannabinoids: the phytocannabinoids, which have been demonstrated to act at specific receptor targets in the CNS. With this focus in mind, we have elicited contributions from experts in the fields of phytocannabinoid chemistry (ElSohly), synthesis of phytocannabinoids (Carreira), phytocannabinoid molecular pharmacology (Iversen and Whalley), and finally phytocannabinoid molecular targets (Reggio). We believe that this volume represents the current "state of the art" on phytocannabinoid chemistry and pharmacology and will be of much use to those wishing to understand the current landscape of the exciting and intriguing phytocannabinoid science.

Columbus, OH — Douglas Kinghorn
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Contents

Phytochemistry of *Cannabis sativa* L.

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Contents

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1 Introduction

Cannabis sativa L. (Cannabaceae) is found in a variety of habitats and altitudes, ranging from sea level to the alpine foothills of the Himalayas from which it possibly originated [1]. *Cannabis* cultivation and use are 5000 to 6000 years old, making it difficult to pinpoint the origin of this species [2]. Furthermore, it is one of the oldest plant sources of food and textile fiber [3]. Cultivation of *C. sativa* for textile fiber originated from Egypt and western Asia and was introduced subsequently to Europe between 1000 and 2000 B.C. and to South America (Chile) in 1545. Over 60 years later (1606), hemp cultivation was introduced to North America (Port Royal, Canada) [4]. Current federal laws in the United States prohibit the cultivation of *C. sativa*, including hemp.

Cannabis sativa has a rich history of medicinal use dating back to ancient times. The first account of its medicinal use came from the Middle East and Asia during the sixth century B.C. Its introduction into western medicine occurred much later, during the early nineteenth century [5, 6]. This species has been indicated in the treatment of pain, glaucoma, nausea, depression, and neuralgia [7–11]. The therapeutic value of phytocannabinoids has also been employed for HIV/AIDS symptom management and multiple sclerosis treatment [12, 13].

2 Nomenclature

2.1 Botanical Nomenclature

The taxonomic classification of *Cannabis sativa* is as shown below:

Kingdom:	Plantae (plants)
Subkingdom:	Tracheobionta (vascular plants)
Superdivision:	Spermatophyta (seed plants)
Division:	Magnoliophyta (flowering plants)
Class:	Magnoliopsida (dicotyledons)
Subclass:	Hamamelididae
Order:	Urticales
Family:	Cannabaceae
Genus:	*Cannabis*
Species:	*sativa*
Taxonomic authority abbreviation:	L.

The number of species in the genus *Cannabis* has been the subject of a long debate. Taxonomists have variously characterized the genus "*Cannabis*" based on its polytypic nature [14–16]. Schultes et al. divided this genus into three separate species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis* [17]. In contrast, several other researchers considered the genus to have two major species, *C. sativa* and *C. indica* [18, 19]. However, in spite of these different taxonomic interpretations, *Cannabis* is commonly treated as constituting only a single, highly diverse species, *C. sativa* L. [20–23]. *C. sativa*, *C. indica*, and *C. ruderalis* are now recognized as varieties of *C. sativa* L. (var. *sativa*, var. *indica*, and var. *ruderalis*, respectively). The *sativa* and *indica* varieties are more economically important and widespread, whereas *ruderalis* is considered a hardier variety grown in the northern Himalayas and southern states of the former Soviet Union that is characterized by a sparse, "weedy" growth, and is rarely cultivated for its drug content. Compared to *sativa* for which the average plant height ranges from 2.5 to 3.5 m, plants of the *indica* variety are generally shorter (average height ca. 1.8 m) and bushier with broader and darker green leaves that mature early when cultivated outdoors.

Field-grown *Cannabis sativa* L.: male plants (*left*), female plants (*right*)

2.2 Common Names

Around the world, local populations refer to *Cannabis sativa* by many common names, of which some are summarized below.

Arabic:	Al-Bhango; Al-Hashish; Al-Qanaap
Chinese:	Xian ma; ye ma
Danish:	Hemp
Dutch:	Hennep

English:	hemp; marihuana
Finnish:	Hamppu
French:	Chanvre; chanvre d'Inde; chanvre indien; chanvrier; chanvrier
German:	Hanf; Haschisch; indischer Hanf
Indian:	Bhang; charas; ganja
Japanese:	Mashinin
Nepalese:	Cares; gajiimaa; gaanjaa
Portuguese:	Cânhamo; maconha
Russian:	*Kannabis sativa*
Spanish:	Cáñamo; grifa; hachís; mariguana; marijuana
Swedish:	Porkanchaa

Cannabis sativa is normally a dioecious species, i.e. male and female flowers develop on separate plants. The sex is determined from the heteromorphic chromosomes, with males being heterogametic (XY) and females homogametic (XX). Morphologically, it is difficult to identify male and female plants at the vegetative stage. With sexual dimorphism occurring late in plant development, male plants can be differentiated from female plants after the onset of flowering. Molecular techniques can now be employed to differentiate male from female plants at an early stage [24–29]. The sexual phenotype of *Cannabis* occasionally shows flexibility leading to the formation of hermaphrodite flowers or bisexual inflorescences, i.e. a monecious phenotype.

2.3 Definition of Cannabis

According to the United States Code (USC), the term "marihuana/cannabis" is defined as "all parts of the plant *C. 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 term does not include the mature stalks of the 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 such plant which is incapable of germination" [30].

"Cannabis" is the drug prepared from the dicotyledonous (flowering plant with two leaves at germination), herbaceous (non-woody plant of which the aerial parts die after fruiting), diecious (the male plants are distinct from the female plants), apetalous (the flower has no corolla), annual herb, *C. sativa* L., and its variants (family: Cannabaceae), which biosynthesizes uniquely the terpenophenolic cannabinoids or phytocannabinoids, accumulating mainly in the glandular trichomes of the plant.

3 Cultivation of *Cannabis sativa*

Cannabis sativa is an annual crop that can be grown successfully under indoor and outdoor conditions, with each having its advantages and disadvantages. While it is possible with indoor cultivation to regulate the photoperiod to trigger the flowering and maturation (three to four crops per year are possible), the cultivation under outdoor conditions is normally limited to one crop per year (requiring five to seven months depending on the variety).

3.1 Outdoor Cultivation

Cultivation of a new crop from seeds usually starts in late March to early April. The crop usually lasts until November to early December in the northern hemisphere but this depends on the variety. Seeds are sown in small biodegradable pots, and the selected healthy seedlings are then transplanted in the field. Alternatively, seeds may be planted directly in the field. Male flowers and subsequently female flowers appear within two to three months (the middle of July) of seeding/transplanting. Culling of male plants is generally done, since male plants contain less Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**) (see the figure on page 14 of Sect. 6.1.1) compared to female plants, and this also avoids cross-pollination both among different varieties and within the same variety.

Although outdoor cultivated plants have more biomass compared to indoor-grown plants, depending on the weather and the space provided, it is difficult to maintain a constant chemical profile under outdoor conditions if grown from seeds, due to the allogamous (cross fertilization) nature of *C. sativa*. Therefore, vegetative propagation through cuttings is an appropriate method to maintain uniformity in the chemical profiles of the crops produced.

3.2 Indoor Cultivation

Indoor cultivation of *Cannabis sativa* under controlled environmental conditions allows for a complete control of the plant life cycle, resulting in management of the quality and quantity of biomass. This is important when producing *C. sativa* with a specific chemical profile for pharmaceutical use. The environmental parameters such as light level, photoperiod, humidity, temperature, CO_2 concentration, and circulation of air play a critical role in the indoor production of this plant.

Cannabis sativa requires high photosynthetic photon flux density (PPFD) for photosynthesis and growth [31]. In-house studies by the present authors have shown that *C. sativa* can utilize a fairly high level of PPFD and temperature for gas and water vapor exchange processes and can perform much better if grown at ca. 1500 μmol m^{-2} s^{-1} PPFD (comparable to a sunny summer day in northern

Mississippi) and around 25–30°C in temperature. 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. Separate ballasts are required to regulate MH and HPS bulbs. MH and HPS bulbs should be 0.9–1.2 m from the plants to avoid overexposure. However, when using indoor grow bulbs, it is difficult to achieve photosynthetically active radiation (PAR) levels up to 1500 μmol m^{-2} s^{-1} PPFD. Furthermore, photoperiods play an important role in regulating vegetative and flowering growth of *C. sativa*. These plants maintain a vegetative growth stage provided a photoperiod cycle consisting of $>$18-h light and $\leq$6-h dark is maintained. A 12-h photoperiod induces *C. sativa* to bloom [31].

In the indoor growing room, the level of humidity is crucial for all stages of plant growth, starting from seed germination or vegetative propagation to budding and harvesting. Juvenile plants require high humidity as compared to well-developed plants. Vegetative cuttings require a regular water spray on the leaves to maintain high humidity (ca. 70–75%) in its microclimate until the plants are well-rooted, while the active vegetative and flowering stages require 55–60% humidity. The amount and frequency of watering for *C. sativa* is dependent on numerous factors, such as growth stage, size of the plants and pots, temperature, humidity, and other parameters. During the early seedling or vegetative stage, it is recommended to keep the soil moist; however, the top layer of soil should be dry before established plants are watered.

Circulation of air is another important aspect of the indoor growing environment. Regulation of gas and water vapor exchange between the leaves and the microenvironment is achieved by having an airflow around the surfaces of the leaves. This affects the leaf boundary layer thermal conductance, energy budget, and ultimately, the physiology and growth of the plant. Electric fans can be employed to manage air circulation. Doubling the ambient CO_2 concentration in the environment is reported to stimulate the rate of photosynthesis and water use efficiency (WUE) in *C. sativa* by 50 and 111%, respectively, resulting in increased overall growth [31].

3.2.1 Propagation Through Seeds

Propagation starting from seeds is the most popular and conventional method of cultivating *C. sativa*. The seeds of this species need a moist environment, in addition to heat and air circulation for optimum germination. Several methods may be used for the germination of seeds. The most popular method is to plant the seeds in small biodegradable jiffy pots containing moist, aerated soil. Seedling heat mats may be placed underneath the pots to increase soil temperature and to enhance germination during the winter.

Seed germination starts after four to five days, with most of the viable seeds germinating within 15 days, depending on the seed variety, age, and storage conditions, as well as the soil and water temperatures. Seedlings should be exposed to cool