Chapter 15

Non-Phytocannabinoid Constituents of Cannabis and Herbal Synergy

John M. McPartland and Ethan B. Russo

15.1 Introduction

Therapeutic synergy is gaining respect in the medical world, and describes an interaction between two or more drugs whose combined effect is greater than the sum of their individual effects. Thanks to tractable research methods such as isobolographic analysis, the simultaneous administration of two or more drugs is no longer derided as "black box medicine." Polypharmacy has become the norm in clinical disciplines such as anesthesia, oncology, and infectious disease.

Medicinal plants are inherently polypharmaceutical. Turner et al. (1980) tallied 420 constituents in herbal cannabis. The tally is now over 530, of which 108 are phytocannabinoids (Hanuš 2008). Practitioners of traditional Chinese medicine (TCM) administer several medicinal plants at once, which exponentially increases the polypharmacy. TCM practitioners have used cannabis for centuries (see Russo, Chapter 2, this volume). One herbal remedy known as *má zǐ rén wán* ("hemp seed pill"), was prescribed by Zhāng Zhòngjǐng (*c.*150–219 CE). The exact same formulation is still in use today (Bensky et al. 1993).

Pharmacology as a science began with chemists isolating constituents from medicinal plants and testing them for physiological activity. In 1804, Friedrich Sertürner began analyzing opium extracted from poppy, *Papaver somniferum.* Not until 1817 did he unequivocally report the isolation of pure morphine (Huxtable and Schwarz 2001). Other early discoveries included caffeine from *Coffea arabica* in 1819, quinine from *Cinchona officinalis* in 1820, nicotine from *Nicotiana tabacum* in 1828, atropine from *Atropa belladonna* in 1831, cocaine from *Erythroxylum coca* in 1855, and digitoxin from *Digitalis purpurea* in 1869.

Cannabis drew attention early; Buchholz (1806) conducted the first analytical study, and he extracted a crude resin. The polypharmaceutical resin stymied chemists for over 150 years in their search for the "primary active ingredient." The recalcitrant substance turned out to be a terpenophenol, quite unlike the easy-to-isolate alkaloids listed earlier. Along the way, chemists suspected the primary active ingredient was a component of the resin, an essential oil, or even an alkaloid. Finally Raphael Mechoulam isolated and characterized delta-9-tetrahydrocannabinol (Δ^9-THC) as the primary psychoactive ingredient (Gaoni and Mechoulam 1964). But barely 6 years later Roger Pertwee noted that THC did not act alone in cannabis (Gill et al. 1970).

This chapter begins with an inventory of nonphytocannabinoid constituents of *Cannabis* (the plant) and cannabis (the plant product). We review the concepts of synergy, additivity, and antagonism, and their measurement. This is followed by a historical review of early research that demonstrated the impact of terpenoids upon phytocannabinoids and the endocannabinoid system. Lastly we highlight twenty-first-century research.

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

15.2 Non-phytocannabinoid constituents

Buchholz (1806) extracted 1.6% resin from cannabis with ethyl alcohol. His yield was low, but we wouldn't expect much more, because Bucholz analyzed cannabis *seed.* He extracted much more oil (19.1%) and protein (24.7%). Subsequently, Buchholz (1816) isolated "capsicin" (capsaicin) from Spanish pepper. His decision to work on cannabis and capsaicin was prophetic: nearly 200 years later, the receptor that binds capsaicin, known as TRPV1, would be named "the ionotropic cannabinoid receptor" (Di Marzo et al. 2002).

Tscheppe (1821) described *hanfblätter* (hemp foliage) as "narcotic." Tscheppe isolated a brown extract and a sweetish bitter extract, as well as chlorophyll, wood fiber, lignin, protein, and several salts and minerals. He could not isolate the psychoactive ingredient, possibly because he analyzed low-THC fiber-type German hemp. Schlesinger (1840) analyzed fresh flowering tops and isolated a green resinous ethanolic extract that Mechoulam (1973) characterized as "the first active extract." Schlesinger never described its activity beyond "bitter taste." In retrospect, the odds of finding the psychoactive ingredient were low because he also worked with hemp. O'Shaughnessy (1838–1840) extracted a strongly psychoactive ingredient by boiling Indian *gañjā* in alcohol under pressure. He subsequently utilized the ethanolic extract in many animal studies and clinical trials.

Bohlig (1840) extracted an essential oil from flowering tops of hemp. Essential oil is a volatile, aromatic, hydrophobic liquid derived from plants by steam distillation or solvent extraction. We now recognize the essential oil as a collection of terpenoid compounds. We use the term "terpenoid" broadly, to include terpenes and modified terpenes, where the methyl group has been moved or removed, or oxygen atoms added. The unique smell of *Cannabis* arises from its volatile terpenoids and not its phytocannabinoids. Bohling reasoned that the psychoactive ingredient was volatile, because he experienced somnolence in a field of flowering hemp. The essential oil was soporific, weakly anesthetic, and caused a headache when inhaled or taken internally.

Most terpenoids in cannabis are monoterpenoids ($C_{10}H_{16}$ template) and sesquiterpenoids ($C_{15}H_{24}$ template). Glandular trichomes secrete terpenoids, and they account for up to 10% of gland head contents (Potter 2009). No terpenoids are unique to *Cannabis*, but various types of *Cannabis* produce unique terpenoid profiles (Fischedick et al. 2010a; Hillig 2004; Mediavilla and Steinemann 1997; Nissen et al. 2010). Examples of terpenoids in cannabis are illustrated in (Fig. 15.1).

On an industrial scale, field-cultivated *Cannabis* yields 1.3 L of essential oil per ton of undried plants, or about 10 L ha^{-2} (Mediavilla and Steinemann 1997). Preventing pollination increases the yield. Meier and Mediavilla (1998) obtained 18 L ha^{-2} from *sinsemilla* crops, versus 8 L ha^{-2} from pollinated crops. In a greenhouse setting, Potter (2009) reported a much higher yield of 7.7 mL m^{-2}, equivalent to 77 L ha^{-2}.

Smith and Smith (1847a) analyzed an ethanolic extract of *gañjā*. They isolated the active principle in a resin that tasted "balsamic" and not bitter, like morphine. They determined that the active ingredient was neutral, "altogether destitute of basic properties" (i.e., not an alkaloid). The Smith brothers gave it the name "cannabine" (Smith and Smith 1847b). Its neutral properties were confirmed by de Courtive (1848), who obtained Algerian hashīsh from Jacques-Joseph Moreau. De Courtive named the active ingredient "cannabin."

Personne (in Robiquet 1857) acknowledged the discoveries of the Smith brothers, but reasoned that *another* active principle was volatile, because hashīsh fumes were psychoactive. Personne distilled the essential oil and isolated two fractions that produced psychoactive effects. Valente (1880, 1881) also searched for a volatile principle in hemp, reasoning that workers in Italian hemp fields became gay and giddy. Valente distilled a sesquiterpene (giving the formula as $C_{15}H_{24}$) from the

15-Pertwee-Chap15.indd 281 12/05/14 6:04 PM

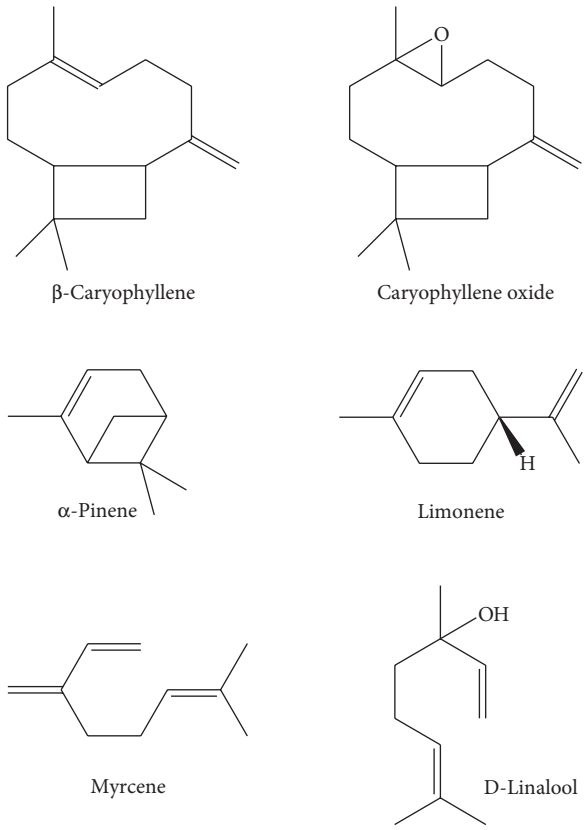

Fig. 15.1 Examples of terpenoids in cannabis: two sesquiterpenoids and four monoterpenoids.

essential oil. Valieri (1887) tested the essential oil on human subjects. Inhalation of the essential oil provided sedative effects, not unlike the essential oils distilled from other aromatic plants like lemon balm (*Melissa officinalis)* and mint (*Mentha* spp.). Valieri suggested its use immediately prior to treatment with "stronger" preparations made from cannabis resin.

Wood et al. (1896) extracted a monoterpenoid (identified as $C_{10}H_{16}$) and a sesquiterpenoid (identified as $C_{15}H_{24}$) from Punjabi *charas*. They described the physiological action of these substances: "In doses of 0.5 gram they have very little effect and produce none of the characteristic symptoms of cannabis action." Simonsen and Todd (1942) began to name individual terpenoids in *Cannabis*. They extracted *p*-cymene ($C_{10}H_{14}$) and humulene (α-caryophyllene, $C_{15}H_{24}$) from Egyptian hashīsh.

The list of terpenoids has steadily grown in modern studies that use utilize gas chromatography (GC). Dutt (1957) and Martin et al. (1961) established the presence of myrcene, limonene, α-caryophyllene, and β-caryophyllene in Indian cannabis and Canadian feral hemp, respectively. Nigam et al. (1965) isolated and identified 20 terpenoids from feral Kashmiri cannabis. They also *quantified* individual terpenoid fractions: they measured the areas of individual GC peaks as a percentage of the total area under all GC peaks. The essential oil consisted largely of β-caryophyllene (45.7%), followed by α-humulene (16.0%), with lesser percentages of other terpenoids in the single digits. Hendricks et al. (1975) listed 55 monoterpenoids and 33 sesquiterpenoids, eluted from *Cannabis sativa* strain X obtained from birdseed.

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

Hood et al. (1973) investigated *Cannabis* "headspace," the *odor* given off by Mexican cannabis, demonstrating a qualitative difference between terpenoids in the headspace and terpenoids in the essential oil. The headspace comprised mostly of monoterpenoids (α-pinene, β-pinene, myrcene, limonene) whereas the processed essential oil consisted of less-volatile oxygenated monoterpenoids (α-terpinenol, linalool, fenchyl alcohol, borneol) and sesquiterpenoids (β-caryophyllene, α-humulene, caryophyllene oxide). Stahl and Kunde (1973) tested seized hashīsh in which primarily the sesquiterpenoids remained. Seemingly, most of the monoterpenoids had out-gassed. They determined that caryophyllene oxide (the oxidation product of β-caryophyllene) was the volatile compound sensed by hashīsh detection dogs.

Ross and ElSohly (1996) measured the retention of essential oil in a "high potency hybrid." Freshly collected cannabis buds, yielded 0.29% v/w essential oil. Week-old buds air-dried at room temperature and stored in a paper bag yielded 0.20%, a loss of 31%. One-month-old buds yielded 0.16%, a loss of 45%. After 3 months the buds yielded 0.13%, a loss of 55%. Freshly-collected buds consisted of 92% monoterpenoids and 7% sesquiterpenoids. In 3-month-old buds the ratio shifted to 62% monoterpenoids and 36% sesquiterpenoids. Their study identified three new monoterpenoids and 14 new sesquiterpenoids not previously reported by Turner et al. (1980)—bringing the total to 60 monoterpenoids and 51 sesquiterpenoids.

Cannabis also produces about 20 flavonoids, which are aromatic, polycyclic phenols. Quercetin, apigenin, and cannaflavin A are anti-inflammatory, antioxidant, analgesic, and possibly prevent cancer (McPartland and Russo 2001). Flavonoids may retain activity in cannabis smoke (Sauer et al. 1983), but they do not vaporize at temperatures below combustion. Products created by combustion show anti-inflammatory activity (Burstein et al. 1976; Spronck et al. 1978), and resulting polycyclic aromatic hydrocarbons (PAHs) may be responsible for antiestrogenic effects (Lee et al. 2005). Other *Cannabis* compounds with pharmacological activity include phytosterols, glycoproteins, alkaloids, and compounds that remain completely unidentified (Gill et al. 1970).

15.3 Synergy, additivity, and antagonism

Polypharmacy gives rise to pharmacokinetic and pharmacodynamic drug interactions. *Pharmacokinetic* interactions arise when one drug alters the absorption, distribution, metabolism, or excretion of another drug. For example, the distribution of L-DOPA across the blood–brain barrier is enhanced by adding carbidopa, a combination drug called Sinemet®. Distribution in this case becomes a factor of metabolism, because carbidopa inhibits dopa decarboxylase activity in the periphery, thereby increasing the bioavailability of L-DOPA in the brain. *Pharmacodynamic* interactions arise when one drug potentiates or diminishes the effect of another drug by targeting different receptors or enzymes. For example, dry mouth caused by a sympathomimetic drug is potentiated by an anticholinergic drug.

Fischedick et al. (2010b) tested the binding affinity of compounds at the CB_1 receptor, and found no statistically significant difference between pure Δ^9-THC and cannabis smoke or vapor at equivalent concentrations of THC. Therefore synergy produced by other constituents in smoke or vapor must occur via pharmacokinetic mechanisms or via pharmacodynamic interactions at other targets. One likely target is the endocannabinoid system (ECS). Two well-known ECS ligands are *N*-arachidonylethanolamide (anandamide, AEA) and *sn*-2-arachidonoylglycerol (2-AG). AEA and 2-AG activate several receptors: CB_1, CB_2, GPR55, and several transient receptor potential ion channels (e.g., TRPV1, TRPV2, TRPA1, TRPM8). Other targets include the catabolic enzymes of AEA and 2-AG: fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), and cyclooxygenase 2 (COX2, prostaglandin-endoperoxide synthase). We detail these findings in section 15.4.

15-Pertwee-Chap15.indd 283 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

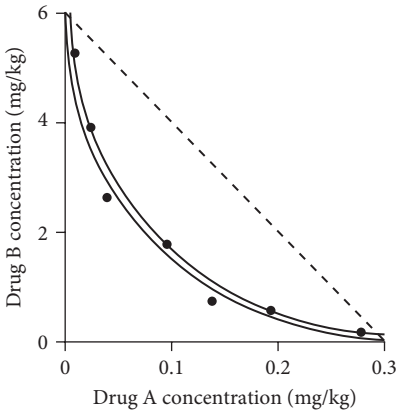

Fig. 15.2 Isobologram analysis: a "line of additivity" (dashed line) is drawn between the ED_{50} values of Drug A and Drug B when given individually. The isobole curve (double line) plots five ED_{50} values when Drug A and Drug B are coadministered at different doses. The isobole bows well below the line of additivity, indicating a synergistic interaction.

Interactions between drugs are usually additive. Departures from additivity are either synergistic ("greater than the sum of the parts") or antagonistic ("less than that expected" or infra-additive). Rector (1922) wrote about synergy, after defining the term, arising in combinations of analgesic drugs. Rector combined *Cannabis indica* with morphine sulfate and magnesium sulfate. Williamson (2001) reviewed the mathematical definitions of synergy. Pharmacologists such as Borisy et al. (2003) cite the pioneering efforts of Walter Siegried Loewe (1884–1963).

Loewe (1928) invented the isobologram to test drug combinations for synergy, additivity, and antagonism. The isobologram uses a two-coordinate graph of drug interactions (Fig. 15.2). The concentrations of single drug A and single drug B that produce x% drug effect (usually EC_{50}) are plotted on the *x*- and *y*-axes. A line that connects two points corresponding to the same x% drug effect becomes "the line of additivity." Then the concentrations of both drugs together that produced the same effect are plotted on the graph. The concentrations of drugs interacting synergistically will be less than the sum of the individual components, and the isobole curve is said to be "concave." The concentrations of drugs interacting antagonistically will be greater than expected, and produce a "convex" isobole (Fig. 15.2).

Loewe emigrated from Germany to the US in 1933, where he added cannabis to his studies of multicomponent medicines and synergy. He demonstrated synergy arising from coadministration of cannabis with butyl-bromallyl-barbituric acid (Loewe 1940). During Congressional hearings regarding the Marihuana Tax Act, Loewe stated to Anslinger, "nobody knows whether there is only one active principle or more than one active principle" (Bureau of Narcotics 1938). Loewe (1945) reported that cannabinol (CBN) exhibited 4% of the potency of "charas tetrahydrocannabinol" in the dog ataxia test. Therefore cannabinol must be included among the compounds having marihuana activity." Loewe served as the pharmacology director of the *LaGuardia Committee on Marihuana* (Loewe 1944), and he conducted the first human clinical trials with individual cannabinoids, including synthetic analogs (Loewe 1946).

15.4 **Combinatorial synergy within cannabis**

Well before synergy was defined, Prain (1893) demonstrated that more than one constituent contributed to cannabis psychoactivity. David Prain (FRS, University of Aberdeen 1857–1944) was a physician-botanist who worked in India. His publication is not well known, and extremely rare (four copies exist in libraries worldwide, according to WorldCat). Prain described "the distinctive

15-Pertwee-Chap15.indd 284 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

gánjá smell, a warm, aromatic, camphoraceous or peppermint-like smell." After 1 year the *gañjā* still smelled aromatic, but the camphoraceous or peppermint odor was gone. After 2 years the aromatic odor diminished. After 3 years the smell was entirely depleted.

Prain weighed fresh *gañjā*, dried it in various ways, and then rehydrated and reweighed it. Predictably, *gañjā* dried at 100°C lost more moisture than *gañjā* dried at room temperature. But rehydration showed that *gañjā* dried at 100°C lost something more: "the volatile constituents were driven off along with the moisture. Exposure to heat must therefore produce a permanent and deleterious change in gánjá."

He then extracted *gañjā* with a series of solvents including water, alcohol, ether, and petroleum-ether. He learned from the alcohol extract that "something is lost by *gánjá* during the first year of storage." Alcohol extracted the essential oil (i.e., terpenoids) that gave *gañjā* its characteristic odor. He calculated that 6.2% of fresh, dried *gañjā* consisted of essential oil. Prain surmised:

> It seems possible that to some extent the *exciting and exhilarating* effect of gánjá resides in an essential oil, which almost disappears by the time the drug has been kept in store for a year. There still, however, remains a considerable *narcotic* effect in gánjá of a year old, though it is much less marked than in fresh ganja. [Italics added for emphasis]

Prain conducted physiological testing of various extracts in cats and isolated the "narcotic fraction" of *gañjā* in a "fixed oil" from the petroleum ether extract. Surprisingly, a resin extracted with pure ether (not petroleum ether) was *not* active. Prain concluded:

> A fixed oil becomes converted into a resin by being oxidised. The quantity of resin increases as the age of the *gánjá* increases, and this increase can only happen at the expense of the substance that constitutes the active principle of the drug.

The petroleum ether contained what we now know as Δ^9-THC, the ether contained CBN, its less potent oxidation product.

Prain's work was continued by David Hooper (1858–1947), appointed as "analyzer" for the Indian Hemp Drugs Commission (IHDC) in 1892. Hooper tested samples of *gañjā* and *charas* obtained from around the subcontinent. Hooper (1908) expanded his earlier analysis of *charas*. He compared 24 samples, 15 from the IHDC report, plus five from Baluchistan, three from Kashgar, and one from Simla. A sample from Kashgar (in modern-day Xinjiang) contained the highest percentage of resin (48.1%). Hooper noted with curiosity that the perceived quality and the cost of three specimens from Kashgar did *not* correlate with resin content: Grade No. 1, 40.2%; Grade No. 2, 40.9%, Grade No. 3, 48.1%.

Hooper added an important analysis not reported in the IHDC report: percentage of essential oil. The Kashgar samples were highest. Intriguingly, the quality and cost of the Kashgar samples correlated with their essential oil content: Grade No. 1 12.7%; Grade No. 2 12.4%; Grade No. 3 12.0%.

Medieval literature indicates that Persian and Arabic physicians prescribed terpenoid-rich citrus fruits to counter the intoxication caused by excessive cannabis (reviewed by Russo 2011): Al-Rāzī (865–925 AD) wrote: "and to avoid these harms, one should drink fresh water and ice or eat any acid fruits." Ibn Sīnā (981–1037) and Ibn-al-Baitār (1197–1248) made similar recommendations. Citrus fruits and especially lemons have been used to treat cannabis overdoses by British physicians (Christison 1850), American homeopaths (Hamilton 1852), Italian physicians (Polli 1865), Āyurvedic practitioners (Shanavaskhan et al. 1997), as well as early American hashīsh littérateurs (Calkins 1871; Ludlow 1857; Taylor 1855) and Afro-Jamaican Rastafarians (Schaeffer 1975). Āyurvedic practitioners espoused calamus root, from *Acorus calamus*, for countering the

15-Pertwee-Chap15.indd 285 12/05/14 6:04 PM

side effects of cannabis (reviewed by McPartland et al. 2008). The use of pine nuts, pistachio nuts, and terebinth resin, from *Pinus* and *Pistacia* species with their pinene content, also have a rich tradition going back to medieval Arabic physicians and perhaps Pliny that suggest their use as antidotes or modulators of THC intoxication by cannabis (reviewed in Russo 2011).

15.5 Modern synergy research

Beginning in the 1970s, a handful of researchers have studied synergy in herbal cannabis. Since then, the pace of research has synergized (see the bottom row in Table 15.1). Gill et al. (1970) proposed that an acetylcholine-like component in whole cannabis extract potentiated the atropinic action of THC ("cotton mouth"). Mechoulam et al. (1972) suggested that THC activity was influenced by other compounds present in herbal cannabis. They proposed that the smell of volatile terpenoids caused a psychological conditioning that potentiated the effects of THC.

Kubena and Barry (1972) reported that rats trained to respond to THC actually showed a greater response to an ethanolic cannabis extract, "a synergistic action of Δ^9-THC with other compounds in the extract." Carlini et al. (1974) determined that cannabis extracts produced effects "two or four times greater than that expected from their THC content." Cannabis extracts were ten times more potent than THC at inhibiting MAO activity in porcine brain (Schurr and Livne 1976). A cannabis ethanol extract plus THC was more potent than an equal amount of THC (Truitt et al. 1976). Fairbairn and Pickens (1981) detected the presence of unidentified "powerful synergists," in cannabis extracts, causing 330% greater activity in mice than THC alone. Cannabis extracts provided greater analgesic activity than individual cannabinoids (Evans et al. 1987; Formukong et al. 1988).

Table 15.1 Interest in *Cannabis*, its constituents, and synergistic effects, estimated by counting the number of studies indexed by PubMed, binned by decade[a]

	1950s	1960s	1970s	1980s	1990s	2000s
Cannabis	40	401	3098	1456	1907	5187
Tetrahydrocannabinol	0	29	1549	1111	1115	1967
Cannabidiol	0	2	169	159	102	353
Cannabinol	0	2	136	103	76	99
Tetrahydrocannabivarin	0	0	2	1	2	16
Cannabichromene	0	5	14	21	8	11
Cannabigerol	0	1	3	12	6	13
Cannabis AND terpenoid[b]	0	0	1	1	0	1
Cannabis AND flavonoid[c]	0	0	2	4	1	14
Cannabis AND synergy[d]	0	0	5	3	2	17

[a] PubMed is a free database accessing life science and biomedical journals, accessible at http://www.ncbi.nlm.nih.gov/pubmed.

[b] Boolean combination of cannabis AND sesquiterpene OR monoterpene OR caryophyllene OR limonene OR linalool OR myrcene OR pinene.

[c] Boolean combination of cannabis AND flavonoid OR flavone OR flavonol OR cannaflavin.

[d] Boolean combination of cannabis AND synergy OR synergism OR synergistic OR isobologram OR isobolographic.

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

The essential oil of cannabis, devoid of cannabinoids, retained analgesic (Segelman et al. 1974), anti-inflammatory (Burstein et al. 1975), and perhaps even antidepressant effects (Hall 2008; Russo et al. 2000). Terpenoids improve THC pharmacokinetics by increasing vasodilatation of alveolar capillaries (which permits more absorption of THC by the lungs), and by increasing blood–brain barrier permeability (Agrawal et al. 1989).

Individual terpenoids in cannabis essential oil have been assessed for therapeutic properties. Russo (2011) listed some examples:

- β-myrcene is analgesic, anti-inflammatory, anti-convulsant, and a skeletal muscle relaxant.
- β-caryophyllene is analgesic and anti-inflammatory, eases gut muscle spasms, and is technically a cannabinoid because it binds to CB_2 receptors (but not the CB_1 receptor so it is not psychoactive).
- D-limonene is an antioxidant, antidepressant and anticonvulsant, and blocks carcinogenesis induced by benz[a]anthracene, one of the "tars" generated by the combustion of herbal cannabis.
- D-linalool is sedative, anxiolytic, analgesic and anti-inflammatory, and induces apoptosis in cancer cells.
- α-pinene is anti-inflammatory, aids memory as an acetylcholinesterase inhibitor, and causes bronchodilation.

Russo (2011) reviewed a dozen mechanistic studies that demonstrate the effects of individual terpenoids at clinically relevant dosages. For example, limonene is highly bioavailable with 70% human pulmonary uptake; and 60% of pinene is bioavailable in a similar assay. Inhaling the aroma of terpenoids decreases anxiety, imparts sedation, improves cognitive performance and EEG patterns of alertness in healthy volunteers.

Many studies have specifically identified cannabidiol (CBD) as an "entourage compound" in cannabis that modulates the effects of THC (Russo and Guy 2006). Although Pertwee and Cascio (Chapter 6, this volume) highlight CBD, we add some concepts here. CBD affects the pharmacokinetics of THC:

- *Absorption*—CBD is anti-inflammatory, which is one reason why inhaling cannabis smoke caused less airway irritation and inflammation than inhaling pure THC (Tashkin et al. 1977).
- *Distribution*—CBD is highly lipophilic, partitions into the lipid bilayer, and fluidizes membrane lipids (Howlett et al. 1989).
- *Distribution*—CBD fluidizes cell membranes, increasing the penetration of THC into muscle cells and thereby amplifying THC's muscle-relaxant effects (Wagner 2004).
- *Metabolism*—CBD inhibits the hepatic metabolism of drugs, including THC (Loewe 1944; Paton and Pertwee 1972).
- *Metabolism*—CBD inhibits two cytochrome P450 enzymes, 3A11 and 2C, that hydroxylate THC to its metabolite 11-hydroxy-Δ^9-THC (Bornheim et al. 1995, 1998).

CBD also alters endocannabinoid pharmacokinetics, by inhibiting FAAH hydrolysis of anandamide. Pharmacodynamically, CBD acts as a "synergistic shotgun," all by itself, by promiscuously targeting many receptors and signaling pathways. CBD enhances many benefits of THC (e.g., analgesic, anticarcinogenic, antiemetic, antiepileptic, anti-inflammatory, antispasmodic, and neuroprotective effects). The importance of CBD led GW Pharmaceuticals to formulate Sativex® as a 50:50 mixture of CBD and THC. 'Sativex can be considered a CBD product with some THC added" (G. Guy, personal communication, 2006).

15-Pertwee-Chap15.indd 287 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

The ability of CBD to decrease the adverse effects of THC permits the administration of higher doses of the latter, thereby increasing the clinical efficacy and safety of cannabis-based extracts. This "tale of two cannabinoids" (Russo and Guy 2006) is fascinating from an evolutionary perspective: Rottanburg et al. (1982) attributed a high incidence of cannabis-associated psychosis in South Africa to the virtual absence of CBD in plants from that region. Black market *Cannabis* breeders have selected plants for increased THC and decreased CBD, which may pose an increased risk to psychologically susceptible individuals (Potter et al. 2008). Dozens of animal studies and clinical trials have demonstrated CBD's antipsychotic effects, possibly by activating TRPV1 and attenuating dopaminergic effects (reviewed in Russo and Guy 2006) (Leweke et al. 2012; Morgan and Curran 2008).

However, panic reaction and not psychosis is the primary side effect of THC (Weil 1970). Animal studies and clinical trials have demonstrated the anxiolytic benefits of CBD, by suppressing tryptophan degradation, activating 5-HT_{1A} (Russo et al. 2005), and decreasing adenosine uptake (reviewed in Russo and Guy 2006).

15.6 The twenty-first century

The twenty-first century began early in the cannabis world: 1998 saw renewed interest in cannabis polypharmacy with a seminal review of therapeutic synergy (McPartland and Pruitt 1998). The same year, Geoffrey Guy and Brian Whittle founded GW Pharmaceuticals on the concept of synergy in whole cannabis extracts. If THC can be characterized as a "silver bullet," then cannabis can be considered a multicomponent "synergistic shotgun" (Izzo et al. 2009; McPartland and Mediavilla 2001; McPartland and Pruitt 1999; McPartland and Russo 2001; Russo 2011; Russo and Guy 2006; Russo and McPartland 2003). Many constituents in cannabis work by multiple mechanisms to modulate the therapeutic effects of THC and mitigate its side effects.

Studies on the combinational effects of THC and CBD have become quite nuanced. The effects of CBD on THC are dose related (Fadda et al. 2004; Vann et al. 2008; Varvel et al. 2006). Timing may be a factor: Zuardi (2008) proposed that preadministration of CBD potentiated the effects of THC via a pharmacokinetic mechanism, whereas coadministration of both compounds caused CBD to antagonize the effects of THC via a pharmacodynamic mechanism.

Williamson (2001) showed that THC reduced muscle spasticity in a mouse model of multiple sclerosis, but was significantly less effective than a cannabis extract containing the same amount of THC. A cannabis extract lacking THC inhibited epileptiform bursting in brain slices, more so than a cannabis extract with THC (Whalley et al. 2004). In an in vitro epilepsy model, the anticonvulsant effects of cannabis extracts were more potent and more rapidly acting than isolated THC (Wilkinson et al. 2003). In some cancer cell lines, a CBD-rich extract inhibited cell growth more potently than pure CBD (Ligresti et al. 2006). Calcium levels in cultured neurons and glia were elevated in a synergistic fashion by adding CBD to THC, but whole cannabis extracts raised calcium levels even more than pure CBD + THC (Ryan et al. 2006). Cannabis extracts provided better antinociceptive efficacy in rats than CBD given alone (Comelli et al. 2008). Cannabis extracts were more potent than pure cannabinoids at the receptors TRPV1, TRPA1, TRPM8 and at inhibiting the enzymes FAAH, DAGLα, and MAGL (De Petrocellis et al. 2011).

As can be seen in Table 15.1, interest in terpenoids still lags. King et al. (2009) demonstrated that pristimerin and euphol inhibit MAGL activity, although these terpenoids do not occur in cannabis. β-caryophyllene has become a focus of attention. It is a component of Sativex® (Guy and Stott 2005), and is the primary sesquiterpenoid in black pepper, *Piper nigrum*. It acts as a full

15-Pertwee-Chap15.indd 288 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

agonist at CB_2 with strong potency (100 nM), the first proven active phytocannabinoid beyond *Cannabis* (Gertsch et al. 2008).

Anonymous (2006) reported interactions between individual terpenoids and THC, apparently based on human bioassays. Drug interactions were assessed with a neuropsychological questionnaire, the Drug Reaction Scale. Limonene added to THC made the drug sensation more "cerebral and euphoric," whereas myrcene made the drug sensation more "physical, mellow, sleepy." Anonymous alleged that THC plus limonene and THC plus myrcene produced stronger cannabimimetic effects than THC alone.

Research in herbal synergy has elicited a predictable reaction—attempts to disprove it. Some scientists have contended that synthetic THC (which is legally available) accounts for all the effects of cannabis. Wachtel et al. (2002) observed no differences in human subjects ingesting or smoking THC versus herbal cannabis. Hart et al. (2002) reported that human subjects experienced "negative" subjective effects after smoking marijuana but not after oral THC consumption. Varvel et al. (2005) reported that THC accounts for all effects in mice subjected to the tetrad test. Ilan et al. (2005) compared the effects of cannabis with high or low CBD and CBC and found no differences in subjective reports and neurophysiological measures. The Wachtel study used cannabis with only 0.05% CBD, likely too low to modulate THC (Russo and McPartland 2003). The other studies share the same problem. Bloor et al. (2008) showed that black market cannabis contains 4.3–8.5 times more terpenoids than cannabis used in NIDA research.

The isobologram has been rediscovered by twenty-first-century cannabinoid researchers: Cichewicz and McCarthy (2003) demonstrated antinociceptive synergy between THC and opioids after oral administration in mice. Cox et al. (2007) showed synergy between THC and morphine in the arthritic rat. DeLong et al. (2010) found an additive effect when combining THC and cannabichromene in mice with LPS-induced inflammation. Four isobolographic studies of endocannabinoids (e.g., anandamide and *N*-arachidonoyl-dopamine) or drugs that block their breakdown (e.g., FAAH inhibitors) also show synergy with analgesics (Farkas et al. 2011; Guindon et al. 2006; Naidu et al., 2009; Sasso et al. 2012). Nearly a dozen isobolographic studies have also demonstrated synergy between synthetic cannabinoids, such as CP55,940 or WIN55,212-2, and a wide range of analgesics, anesthetics, and anticonvulsants, which is beyond the scope of this review on natural constituents.

15.7 Conclusion

Evolution (i.e., natural selection) over millions of years creates a phytochemical matrix around key constituents, so they can reach their biochemical targets. Many of our crop plants and medicinal plants exhibit this phenomenon (Spelman 2009). *Cannabis* is no exception. It has likely undergone two rounds of coevolution with animals: perhaps 30 million years of selection to fit mammalian herbivore physiology, followed by thousands of years of accelerated evolution by humans who selected plants for optimal benefit and minimal toxicity (McPartland and Guy 2004). Ehrlich's reductionist "silver bullet" philosophy is being replaced by Loewe's synergistic concepts (Borisy et al. 2003).

The data herein presented strongly support the therapeutic rationale for combining THC with other constituents present in cannabis. The impact of individual terpenoids upon THC requires further animal studies and clinical trials. The formal investigation of the effects of individual flavonoids and other constituents has not yet begun. Should positive outcomes result from such studies, phytopharmaceutical development may follow. Breeding work has already resulted in *Cannabis* chemotypes that produce 97% of monoterpenoid content as myrcene, or 77% as

15-Pertwee-Chap15.indd 289 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

limonene (E. de Meijer, personal communication, 2010). A better future via cannabis phytochemistry may be an achievable goal through further research of the entourage effect in this versatile plant that may help it fulfill its promise as a pharmacological treasure trove.

Conflict of interest statement

JM has been a consultant for GW Pharmaceuticals, and has received travel expenses and research support. ER is Group Senior Medical Advisor to GW Pharmaceuticals and serves as a full-time consultant.

References

Agrawal, A.K., Kumar, P., Gulati, A., and Seth, P.K. (1989). Cannabis-induced neurotoxicity in mice: effects on cholinergic (muscarinic) receptors and blood brain barrier permeability. *Research Communications in Substance Abuse*, **10**, 155–168.

Anonymous. (2006). Studying the effects of terpenes. *O'Shaughnessy's*, Spring, **2**.

Bensky, D., Gamble, A., and Kaptchuk, T. (1993). *Chinese Herbal Medicine: Materia Medica*. Seattle, WA: Eastland Press.

Bloor, R.N., Wang, T.S., Spanel, P., and Smith, D. (2008). Ammonia release from heated 'street' cannabis leaf and its potential toxic effects on cannabis users. *Addiction*, **103**, 1671–1677.

Bohlig, J.F. (1840). *Cannabis sativa* und *Urtica dioica* chemisch analysiert. *Jahrbuch für praktische Pharmacie und verwandte Fächer*, **3**, 1–58.

Borisy, A.A., Elliott, P.J., Hurst, N.W., *et al.* (2003). Systematic discovery of multicomponent therapeutics. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 7977–7982.

Bornheim, L.M. and Grillo, M.P. (1998). Characterization of cytochrome P450 3A inactivation by cannabidiol: possible involvement of cannabidiol-hydroxyquinone as a P450 inactivator. *Chemical Research in Toxicology*, **11**, 1209–1216.

Bornheim, L.M., Kim, K.Y., Li, J., Perotti, B.Y., and Benet, L.Z. (1995). Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain. *Drug Metabolism & Disposition*, **23**, 825–831.

Buchholz, C.F. (1806). Beiträge zur pflanzenchemie. Analyse des hanfsamens. *Neues allgemeines Journal der Chemie*, **6**, 615–630.

Buchholz, C.F. (1816). Chemische Untersuchung der trockenen reifen spanischen Pfeffers. *Almanach oder Taschenbuch für Scheidekünstler und Apotheker*, **37**, 1–30.

Bureau of Narcotics. (1938). *Marihuana conference held December 5 1938 in the United States Bureau of Internal Revenue Building (Room 3003)*. Washington, DC: Government Printing Office. Available at: http://www.globalhemp.com/1938/12/marihuana-conference.html.

Burstein, S., Taylor, P., El-Feraly, F.S., and Turner, C. (1976). Prostaglandins and *Cannabis*—V. Identification of p-vinylphenol as a potent inhibitor of prostaglandin synthesis. *Biochemical Pharmacology*, **25**, 2003–2004.

Burstein, S., Varanelli, C., and Slade, L.T. (1975). Prostaglandins and *Cannabis*—III. Inhibition of biosynthesis by essential oil components of marihuana. *Biochemical Pharmacology*, **24**, 1053–1054.

Cichewicz, D.L. and McCarthy, E.A. (2003). Antinociceptive synergy between delta(9)-tetrahydrocannabinol and opioids after oral administration. *Journal of Pharmacology and Experimental Therapeutics*, **304**, 1010–1015.

Calkins, A. (1871). *Opium and the Opium-Appetite: with Notices of Alcoholic Beverages, Cannabis Indica, Tobacco and Coca, and Tea and Coffee, in Their Hygienic Aspects and Pathologic Relationships*. Philadelphia, PA: J.B. Lippincott.

Carlini, E.A., Karniol, I.G., Renault, P.F., and Schuster, C.R. (1974). Effects of marihuana in laboratory animals and man. *British Journal of Pharmacology*, **50**, 299–309.

15-Pertwee-Chap15.indd 290 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

Christison, A. (1850). On *Cannabis indica*, Indian Hemp. *Transactions and Proceedings of the Botanical Society of Edinburgh*, **4**, 59–69.

Comelli, F., Giagnoni, G., Bettoni, I., Colleoni, M., and Costa, B. (2008). Antihyperalgesic effect of a *Cannabis sativa* extract in a rat model of neuropathic pain: mechanisms involved. *Phytotherapy Research*, **22**(8), 1017–1024.

Cox, M.L., Haller, V.L., and Welch, S.P. (2007). Synergy between delta9-tetrahydrocannabinol and morphine in the arthritic rat. *European Journal of Pharmacology*, **567**, 125–130.

de Courtive, M.E. (1848). *Hashisch. Étude Historique, Chimique et Physiologique*. Paris: Edouard Bautruche.

DeLong, G.T., Wolf, C.E., Poklis, A., and Lichtman, A.H. (2010). Pharmacological evaluation of the natural constituent of *Cannabis sativa*, cannabichromene and its modulation by Δ(9)-tetrahydrocannabinol. *Drug Alcohol Dependence*, **112**(1–2), 126–133.

De Petrocellis, L., Ligresti, A., Moriello, A.S., *et al.* (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology*, **163**, 1479–1494.

Di Marzo, V., De Petrocellis, L., Fezza, F., Ligresti, A., and Bisogno, T. (2002). Anandamide receptors. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, **66**, 377–391.

Dutt, S. (1957). Indian *Cannabis sativa* and essential oils derived from the same. *Indian Soap Journal*, **22**, 242–246.

Evans, A.T., Formukong, E.A., and Evans, F.J. (1987). Actions of cannabis constituents on enyzmes of arachidonate: anti-inflammatory potential. *Biochemical Pharmacology*, **36**(12), 2035–2037.

Fairbairn, J.W. and Pickens, J.T. (1981). Activity of *Cannabis* in relation to its Δ-tetrahydrocannabinol content. *British Journal of Pharmacology*, **72**, 401–409.

Fadda, P., Robinson, L., Fratta, W., Pertwee, R.G., and Riedel, G. (2004). Differential effects of THC- or CBD-rich cannabis extacts on working memory in rats. *Neuropharmacology*, **47**, 1170–1179.

Farkas, I., Tuboly, G., Benedek, G., and Horvath, G. (2011). The antinociceptive potency of N-arachidonoyl-dopamine (NADA) and its interaction with endomorphin-1 at the spinal level. *Pharmacology, Biochemistry and Behaviour*, **99**(4), 731–737.

Fischedick, J., Van Der Kooy, F., and Verpoort, R. (2010b). Cannabinoid receptor 1 binding activity and quantitative analysis of *Cannabis sativa* L. smoke and vapor. *Chemical & Pharmaceutical Bulletin*, **58**(2), 201–207.

Fischedick, J.T., Hazekamp. A., Erkelens, T., Choi, Y.H., and Verpoorte, R. (2010a). Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochemistry*, **71**, 2058–2073.

Formukong, E.A., Evans, A.T., and Evans, F.J. (1988). Inhibition of the cataleptic effect of tetrahydrocannabinol by other constituents of *Cannabis sativa* L. *Journal of Pharmacy and Pharmacology*, **40**, 132–134.

Gaoni, Y. and Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, **86**, 1646–1647.

Gertsch, J., Leonti, M., Raduner, S., *et al.* (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(26), 9099–9104.

Gill, E.W., Paton, W.D.M., and Pertwee, R.G. (1970). Preliminary experiments on the chemistry and pharmacology of *Cannabis*. *Nature*, **228**, 134–136.

Guindon, J., De Léan, A., and Beaulieu, P. (2006). Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. *Pain*, **121**, 85–93.

Guy, G.W. and Stott, C.G. The development of Sativex—a natural cannabis-based medicine. In: R. Mechoulam (ed.). *Cannabinoids As Therapeutics*. Basel: Birkhäuser Verlag, pp. 231–263.

Hall, B.P. (2008). *Structure Activity Relationships for Intracellular Loop 2 of the 5HT1a Serotonin Receptor*. Doctoral thesis, Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.

15-Pertwee-Chap15.indd 291 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

Hamilton, E. (1852). *Flora Homœopathica*, *Vol. 1*. London: H. Bailliere.

Hanuš, L. (2008). Pharmacological and therapeutic secrets of plant and brain (endo)cannabinoids. *Medicinal Research Reviews*, **29**, 213–271.

Hart, C.L., Ward, A.S., Haney, M., Comer, S.D., Foltin, R.W., and Fischman, M.W. (2002). Comparison of smoked marijuana and oral Delta(9)-tetrahydrocannabinol in humans. *Psychopharmacology*, **164**(4), 407–415.

Hendricks, H., Malingré, T.M., Batterman, S., and Bos, R. (1978). The essential oil of *Cannabis sativa*. *Pharmaceutisch Weekblad*, **113**, 413–424.

Hillig, K.W. (2004). A chemotaxonomic analysis of terpenoid variation in *Cannabis*. *Biochemical Systematics and Ecology*, **32**, 875–891.

Hood, L.V.S., Dames, M.E., and Barry, G.T. (1973). Headspace volatiles of marijuana. *Nature*, **242**, 402–403.

Hooper, D. (1908). Charas of Indian hemp. *Year-Book of Pharmacy*, 1908, 435–444.

Howlett, A.C., Scott, D.K., and Wilken, G.H. (1989). Regulation of adenylate cyclase by cannabinoid drugs. Insights based on thermodynamic studies. *Biochemical Pharmacology*, **38**(19), 3297–3304.

Huxtable, R.J. and Schwarz, S.K.W. (2001). The isolation of morphine—first principles in science and ethics. *Molecular Interventions*, **1**(4), 189–191.

Ilan, A.B., Gevins, A., Coleman, M., ElSohly, M.A., and de Wit, H. (2005). Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behavioural Pharmacology*, **16**, 487–496.

Izzo, A.A., Borrell, i F., Capasso, R., Di Marzo, V., and Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, **30**(10), 515–527.

King, A.R., Dotsey, E.Y., Lodola, A., *et al.* (2009). Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chemistry & Biology*, **16**, 1045–1052.

Kubena, R.K. and Barry, H. (1972). Stimulus characteristics of marihuana components. *Nature*, **235**, 397–398.

Lee, S.Y., Oh, S.M., Lee, S.K., and Chung, K.H. (2005). Antiestrogenic effects of marijuana smoke condensate and cannabinoid compounds. *Archives of Pharmacal Research*, **28**(12), 1365–1375.

Leweke, F. M., Piomelli, D., Pahlisch, F. *et al.* (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Translational Psychiatry*, **2**, e94.

Ligresti, A., Moriello, A.S., Starowicz, K., *et al.* (2006). Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *Journal of Pharmacology and Experimental Therapeutics*, **318**(3), 1375–1387.

Loewe, S. (1928). Die quantitative Problem der Pharmakologie. *Ergebnisse der Physiologie*, **27**, 47–187.

Loewe, S. (1940). Synergism of cannabis and butyl-bromallyl-barbituric acid. *Journal of the American Pharmaceutical Association* (*Scientific edition*), **29**, 162–163.

Loewe, S. (1944). Studies on the pharmacology of marihuana. In: LaGuardia Committee (eds.). *The Marihuana Problem in the City of New York*. Lancaster, PA: Jaques Cattell Press, pp. 149–212.

Loewe, S. (1945). Marihuana activity of cannabinol. *Science*, **102**, 615–616.

Loewe, S. (1946). Studies on the pharmacology and acute toxicity of compounds with marihuana activity. *Journal of Pharmacology and Experimental Therapeutics*, **88**, 154–164.

Ludlow, F.-H. (1857). *The Hasheesh Eater: Being Passages Form the Life of A Pythagorean*. New York: Harper.

Martin, L., Morison Smith, D., and Farmilo, C.G. (1961). Essential oil from fresh *Cannabis sativa* and its use in identification. *Nature* **191**, 774–776.

McPartland, J.M., Blanchon, D., and Musty, R.E. (2008). Cannabimimetic effects modulated by cholinergic compounds. *Addiction Biology*, **13**, 411–415.

15-Pertwee-Chap15.indd 292 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

McPartland, J.M. and Mediavilla, V. (2001). Non-cannabinoids in cannabis. In: F. Grotenhermen and E.B. Russo (eds.). *Cannabis and Cannabinoids*. Binghamton, NY: Haworth Press, pp. 401–409.

McPartland, J.M. and Pruitt, P.L. (1998). An herbal "synergistic shotgun" compared to a synthetic "silver bullet": medical marijuana versus tetrahydrocannabinol. *Proceedings 1998 Symposium on the Cannabinoids*. Burlington, VT: International Cannabinoid Research Society, p. 112.

McPartland, J.M. and Pruitt, P.L. (1999). Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. *Alternative Therapies in Health and Medicine* **5**, 57–62.

McPartland, J.M. and Russo, E.B. (2001). *Cannabis* and cannabis extracts: greater than the sum of their parts? *Journal of Cannabis Therapeutics* **1**(3–4), 103–132.

Mechoulam, R. (1973). Cannabinoid chemisty. In: R. Mechoulam (ed.). *Marijuana*. New York: Academic Press, pp. 1–87.

Mechoulam, R., Ben-Zvi, Z., Shani, A., Zemler, H., and Levy, S. (1972). Cannabinoids and cannabis activity. In: W.D.M. Paton and J. Crown (eds.). *Cannabis and its Derivatives*. London: Oxford University Press, pp. 1–13.

Mediavilla, V. and Steinemann, S. (1997). Essential oil of *Cannabis sativa* L. strains. *Journal of the International Hemp Association*, **4**(2), 82–84.

Meier, C. and Mediavilla, V. (1998). Factors influencing the yield and the quality of hemp (*Cannabis sativa* L.) essential oil. *Journal of the International Hemp Association*, **5**(1), 16–20.

Morgan, C.J. and Curran, H.V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry*, **192**, 306–307.

Naidu, P.S., Booker, L., Cravatt, B.F., and Lichtman, A.H. (2009). Synergy between enzyme inhibitors of fatty acid amide hydrolase and cyclooxygenase in visceral nociception. *Journal of Pharmacology and Experimental Therapeutics*, **329**, 48–56.

Nigam, M.C., Handa, K.L., Nigam, I.C., and Levi, L. (1965). Essential oils and their constituents. XXIX. The essential oil of marihuana: composition of the genuine Indian *Cannabis sativa* L. *Canadian Journal of Chemistry*, **43**, 3372–3376.

Nissen, L., Zatta, A., Stefanini, I., *et al.* (2010). Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). *Fitoterapia*, **81**, 413–419.

O'Shaughnessy, W.B. (1838–1840). On the preparations of the Indian hemp, or gunjah *(Cannabis indica)*; Their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *Transactions of the Medical and Physical Society of Bengal*, 71–102, 421–461.

Paton, W.D.M. and Pertwee, R.G. (1972). Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. *British Journal of Pharmacology*, **44**(2), 250–261.

Polli, G. (1865). Sull'antidoto dell'haschisch. *Annali di Chimica Applicata alla Medicina*, **40**(3), 343–345.

Potter, D. (2009). *The Propagation, Characterisation and Optimisation of Cannabis sativa L. as a Phytopharmaceutical*. Doctoral thesis, London: King's College.

Potter, D.J., Clark, P., and Brown, M.B. (2008). Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *Journal of Forensic Science*, **53**, 90–94.

Prain, D. (1893). *Report on the Cultivation and Use of Gánjá*. Calcutta: Bengal Secretariat Press.

Rector, J.M. (1922). Synergistic analgesia: clinical observations. *American Journal of Surgery*, **36**(10 Suppl.), 114–119.

Robiquet, E. (1857). *Rapport sur le concours relatif à l'analyse du chanvre présente au nom de la Société de Pharmacie. Journal de Pharmacie et de Chimie*, (Serie 3) **31**, 46–51.

Ross, S.A. and ElSohly, M.A. (1996). The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *Journal of Natural Products*, **59**, 49–51.

Rottanburg, D., Robins, A.H., Ben-Arie, O., Teggin, A., and Elk, R. (1982). Cannabis-associated psychosis with hypomanic features *Lancet*, ii, 1364–1366.

15-Pertwee-Chap15.indd 293 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

Russo, E.B. (2011). Taming THC: potential cannabis synergy and phytocannabinid-terpeoid entourage effects. *British Journal of Pharmacology*, **163**, 1344–1364.

Russo, E.B. and Guy, G.W. (2006). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Medical Hypotheses*, **66**, 234–246.

Russo, E., Macarah, C.M., Todd, C.L., Medora, R.S., and Parker, K.K. (2000). Pharmacology of the essential oil of hemp at 5-HT1a and 5-HT2a receptors. Abstract at the 41st Annual Meeting of the American Society of Pharmacognosy, July 22–26, Seattle, WA.

Russo, E.B. and McPartland, J.M. (2003). Cannabis is more than simply delta(9)-tetrahydrocannabinol. *Psychopharmacology*, **165**, 431–432.

Ryan, D., Drysdale, A.J., Pertwee, R.G., and Platt B. (2006). Differential effects of cannabis extracts and pure plant cannabinoids on hippocampal neurones and glia. *Neuroscience Letters*, **408**(3), 236–241.

Sasso, O., Bertorelli, R., Bandiera, T., *et al.* (2012). Peripheral FAAH inhibition causes profound antinociception and protects against indomethacin-induced gastric lesions. *Pharmacological Research*, **65**, 553–563.

Sauer, M.A., Rifka, S.M., Hawks, R.L., Cutler, G.B., and Loriaux, D.L. (1983). Marijuana: interaction with the estrogen receptor. *Journal of Pharmacy and Experimental Therapeutics*, **224**, 404–407.

Schaeffer, J. (1975). The significance of marihuana in a small agricultural community in Jamaica. In V. Rubin (ed.). *Cannabis and Culture*. The Hague: Mouton, pp. 355–388.

Schlesinger, S. (1840). Untersuchung der Cannabis sativa. *Buchner's Repertorium für die Pharmacie*, **21**, 190–208.

Schurr, A. and Livne, A. (1976). Differential inhibition of mitochondrial monoamine oxidase from brain by hashish compounds. *Biochemical Pharmacology*, **25**, 1201–1203.

Segelman, A.B., Sofia, R.D., Segelman, F.P., Harakal, J.J., and Knobloch, L.C. (1974). *Cannabis sativa* L. (marijuana) V: pharmacological evaluation of marijuana aqueous extract and volatile oil. *Journal of Pharmaceutical Sciences*, **63**, 962–964.

Shanavaskhan, A.E., Binu, S., Muraleedharan-Unnithan, C., Santhoshkumar, E.S., and Pushpangadan, P. (1997). Detoxification techniques of traditional physicians of Kerala, India on some toxic herbal drugs. *Fitoterapia*, **68**, 69–74.

Simonsen, J.L. and Todd, A.R. (1942). *Cannabis indica*, Part X. The essential oil from Egyptian hashish. *Journal of the Chemical Society (London)*, **1942**(1), 188–191.

Smith, T. and Smith, H. (1847a). On the resin of Indian hemp. *Pharmaceutical Journal*, **6**, 127–128.

Smith, T. and Smith, H. (1847b). Process for preparing cannabine, or hemp resin. *Pharmaceutical Journal*, **6**, 171–173.

Spelman, K., Wetschler, M.H., and Cech, N.B. (2009). Comparison of alkylamide yield in ethanolic extracts prepared from fresh versus dry *Echinacea purpurea* utilizing HPLC-ESI-MS. *Journal of Pharmaceutical and Biomedical Analysis*, **49**, 1141–1149.

Spronck, H.J., Luteijn, J.M., Salemink, C.A., and Nugteren, D.H. (1978). Inhibition of prostaglandin biosynthesis by derivatives of olivetol formed under pyrolysis of cannabidiol. *Biochemical Pharmacology*, **27**, 607–608.

Stahl, E. and Kunde, R. (1973). Die Leitsubstanzen der Haschisch-Suchhunde. *Kriminalistik*, **9**, 385–388.

Tashkin, D.P., Reiss, S., Shapiro, B.J., Calvarese, B., Olsen, J.L., and Lodge, W. (1977). Bronchial effects of aerosolized Δ^9-tetrahydrocannabinol in healthy and asthmatic subjects. *American Review of Respiratory Disease*, **115**, 57–65.

Taylor, B. (1855). *The Lands of the Saracens*. New York: G.P. Putnam & Sons.

Truitt, E.B., Kinzer, G.W., and Berlow, J.M. (1976). Behavioral activity in various fractions of marijuana smoke condensate in the rat. In: M.C. Braude and S. Szara (eds.). *Pharmacology of Marihuana*. Vol. 2. New York: Raven Press, pp. 463–474.

Tscheppe, F. (Schübler G, *präside*). (1821). *Chemische Untersuchung der Hanfblätter*. Dissertation, Tübingen.

15-Pertwee-Chap15.indd 294 12/05/14 6:04 PM

OUP-FIRST UNCORRECTED PROOF, May 12, 2014

Turner, C.E., ElSohly, M.A., and Boeren, E.G. (1980). Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *Journal of Natural Products*, **43**, 169–234.

Valente, L. (1880). Sull' essenza di canapa. *Gazzetta chimica italiana*, **10**, 479–481.

Valente, L. (1881). Sull' idrocarburo estratto dalla canapa. *Gazzetta chimica italiana*, **11**, 196–198.

Valieri, R. (1887). *Sulla canapa nostrana e suoi preparati in sostituzione della Cannabis indica*. Naples: Stabilimento tipografico dell'unione.

Vann, R.E., Gamage, T.F., Warner, J.A., *et al.* (2008). Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of delta(9)-tetrahydrocannabinol. *Drug and Alcohol Dependence*, **94**(1–3), 191–198.

Varvel, S.A., Bridgen, D.T., Tao, Q., Thomas, B.F., Martin, B.R., and Lichtman, A.H. (2005). Delta-9-tetrahydrocannbinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. *Journal of Pharmacology and Experimental Therapeutics*, **314**, 329–337.

Varvel, S.A., Wiley, J.L., Yang, R., *et al.* (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology (Berlin)*, **186**(2), 226–234.

Wachtel, S.R., ElSohly, M.A., Ross, R.A., Ambre, J., and de Wit, H. (2002). Comparison of the subjective effects of delta-9-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology*, **161**, 331–339.

Wagner, H. (2004). Natural products chemistry and phytomedicine research in the new millennium: new developments and challenges. *ARKIVOC Journal of Organic Chemistry*, **7**, 277–284.

Weil, A.T. (1970). Adverse reactions to marihuana, classification and suggested treatment. *New England Journal of Medicine*, **282**, 997–1000.

Whalley, B.J., Wilkinson, J.D., Williamson, E.M., and Constanti, A. (2004). A novel component of cannabis extract potentiates excitatory synaptic transmission in rat olfactory cortex in vitro. *Neuroscience Letters*, **365**(1), 58–63.

Wilkinson, J.D, Whalley, B.J., Baker, D., *et al.* (2003). Medicinal cannabis: is delta9-tetrahydrocannabinol necessary for all its effects? *Journal of Pharmacology and Pharmacotherapeutics*, **55**, 1687–1694.

Williamson, E.M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*, **8**, 401–409.

Wood, T.B., Spivey, W.T.N., and Easterfield, T.H. (1896). Charas, the resin of Indian hemp. *Journal of the Chemical Society*, **6**, 539–546.

Zuardi, A.W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, **30**, 271–280.

15-Pertwee-Chap15.indd 295 12/05/14 6:04 PM