Ethan Russo reviews the evidence

Terpenoids, ‘minor’ cannabinoids contribute to ‘entourage effect’ of Cannabis-based medicines

By Fred Gardner

The chemical structure of tetrahydrocannabinol (THC) was determined in 1964 by Raphael Mechoulam and Yechiel Gaoni at the Hebrew University in Jerusalem. Scientists were excited about the potential therapeutic effects of the molecule, but were dismayed about the “blatant psychoactivity of THC initiated scientists to define it as the active ingredient in the plant.”

Experienced marijuana smokers who tried the drug Marinol (pure, synthetic THC) when it became prescribable in the mid-1980s reported that the effects were dissimilar. But it wasn’t until the late 1990s that the research establishment acknowledged that another compound, cannabidiol (CBD), also exerted effects when present in significant amounts.

In 1999 a British start-up, GW Pharmaceuticals, began clinical trials of a whole-plant extract containing roughly equal amounts of THC and CBD. Multiple Sclerosis patients found the combination — dubbed “Sativex” — more effective in reducing pain and spasticity than a high-THC extract devoid of CBD, and less psychoactive. Sativex has now been approved for use by MS patients in England, Canada, New Zealand, and a growing list of European countries. CBD is no longer referred to as a “minor cannabinoid” at scientific conferences and in the literature.

Several cannabinoids still considered “minor” — tetrahydrocannabivarfin (THCV), cannabigerol (CBG) and cannabichromene (CBC) — also show therapeutic promise, according to recent studies. Plants with high levels of both terpenoids and cannabinoids are secreted inside the plant’s “essential oils” that create the fragrance. Terpenoids contain repeating units of a 5-carbon molecule called isoprene and are prevalent in smelly herbs and woods.

The aroma of a given plant depends on which terpenoids predominate. They tend to be volatile molecules that readily evaporate, and they’re very potent — all it takes is a few reaching the nose to announce their presence.

Evidence that “phytocannabinoid-terpenoid interactions” enhance the therapeutic effects of cannabis was presented by Ethan Russo, MD, at a conference in Israel last fall and published in the August 2011 British Journal of Pharmacology. Russo, a neurologist and ethnobotanist, is senior medical adviser at GW Pharmaceuticals.

Russo’s talk evoked the “entourage concept to phytocannabinoids (made by the plant) and endocannabinoids (made in the body) act in concert with other compounds to exert an ‘entourage effect.’” Russo, a senior medical advisor with GW Pharmaceuticals, applied the entourage concept to phytocannabinoids made by the plant.

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Plant cannabinoids —21-carbon molecules found only in Cannabis — are odorless. It’s the terpenoids — components of the plant’s “essential oils” — that create the fragrance. Terpenoids contain repeating units of a 5-carbon molecule called isoprene and are prevalent in smelly herbs such as mints and sage, citrus peel, some flowers, aromatic barks and woods.

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"Is this the best way we can grow our big orchards?"
—Woody Guthrie

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Project CBD Update — starts on page 7

• CBD-Rich Strains Abound
Some 35 strains containing more than 4% Cannabidiol have been identified by labs serving the medical cannabis industry in the U.S.

• SCC Launches Survey
The Society of Cannabis Clinicians has begun collecting patients’ responses to CBD-rich products.

• How CBD Works
Martin A. Lee lays out what scientists have learned about the mechanism of action by which CBD exerts its effects.

• "Sour Tsunami" Stabilized
Lawrence Ringo (below) has bred plants that produce seeds with a one-in-four chance of containing 10-11% CBD (and 6-7% THC!)

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The "Entourage Effect"

The conference at which Russo presented his paper was held at Hebrew University, Jerusalem, where Raphael Mechoulam directed a lab, in honor of Mechoulam’s 80th birthday. In 1999 Mechoulam co-authored a paper with Shimon Ben-Shabat suggesting that cannabinoids made in the body work by means of an “entourage effect.” They had found that the endocannabinoid 2-AG (2-arachidonoylglycerol), when administered by means of an "entourage effect." They concluded that cannabinoids made in the body work together with inactive 'entourage' compounds. Investigations of the effect of the active component in the presence of its 'entourage' compounds may lead to results that differ from those observed with the active component only.”

In 2001 John McPartland and Russo published a paper in the Journal of Cannabis Therapeutics applying the "entourage" concept to the plant itself. "Good evidence shows that secondary compounds in cannabis may enhance the beneficial effects of THC... and reduce THC-induced anxiety, cholinergic deficits, and immunosuppression," they wrote. “Cannabis terpenoids and flavonoids may also increase cerebral blood flow, enhance cortical activity, kill respiratory pathogens, and provide anti-inflammatory activity.”

A decade later, Russo is substantiating the molecular-teamwork hypothesis and expanding on it. His BIP paper, “Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects,” contains 304 citations. Although the paper takes the form of a review of the literature, Russo’s perspective is forward-looking and practical. The paper can be read as a strategic guide for breeding and/or blending Cannabis so as to maximize specific medical effects. Its structure is straightforward:

1. Russo cites studies documenting the beneficial effects of THC, CBD, CBC, THCV, CBGV, CBG and CBN (noting the adverse effects attributed to THC).

2. He cites studies documenting the beneficial effects of Limonene, α-Pinene, Myrcene, Linalool, β-Caryophyllene, Caryophyllene Oxide, Nerolidol, and Phytotol.

3. He notes which cannabinoid effects would be augmented by which terpenoids, and which terpenoid effects would be augmented by which cannabinoids.

There is a huge body of information to convey, and Russo’s style is compressed —documented fact after documented fact after documented fact, with insights positioned fittingly. The slides he showed in Israel have been integrated into two full-page tables for the BIP paper, listing the relevant studies and the cannabinoid-terpenoid combinations likely to produce a desired effect. The paper, online at http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5381.2011.01238.x/abstract, is well worth reading. Our summary and the disjointed highlights that follow cannot do justice to Russo’s carefully constructed thesis.

Phytocannabinoid Effects

**Δ^8-tetrahydrocannabinol (THC)**
- Analgesic via CB1, CB2
- Antipruritic
- Neuroprotective/Antioxidant
- 20 times the anti-inflammatory effect of aspirin
- Twice the anti-inflammatory effect of hydrocortisone
- Not a Cox-1 or Cox-2 inhibitor
- Reduces amyloid plaque build-up

**Cannabigerol (CBG)**
- GABA uptake inhibitor (more potent than THC or CBD)
- Modest anticonvulsant effect
- Antidepressant

**Cannabidiol (CBD)**
- Neuroprotective antioxidant, strongly inhibits glutamate excitotoxicity; more potent antioxidant than Vitamins C, E
- Inhibits uptake of anandamide, weakly inhibits its breakdown
- Alerting vs. THC
- Anticonvulsant
- Anti-anxiety
- Cytotoxic in breast cancer and many other cancer cell lines; cytoprotective for normal cells
- Antagonist at GPR55 and GPR18
- Antagonizes tumor necrosis factor alpha in rodent rheumatoid arthritis.
- Not Cox-1 or Cox-2 inhibitor
- Not a GABAA receptor (why it may counter anxiety)
- Reduces nausea
- Improved cognition in hepatic encephalopathy
- Enhances adenosine receptor A2A signaling via inhibition of an adenosine transporter, staving off anti-inflamma-tory and analgesic role
- Prevents prion accumulation and neuronal toxicity
- Powerful activity against MRSA

**Tetrahydrocannabinolic acid (THCA)**
- CB1 antagonist at low doses, but CB1 agonist at higher doses.
- Produces weight loss, decreased body fat, increased energy expenditure in obese mice.
- Anticonvulsant in rodent cerebellum and pyriform cortex.

**Cannabinol (CBN)**
- Sedative
- Anticonvulsant
- Anti-inflammatory
- Antibiotic, potent against MRSA
- TRPV2 agonist of interest in burns
- Inhibits keratinocyte proliferation (utility in psoriasis?)
- Stimulates recruitment of quiescent mesenchymal stem cells in marrow, promoting bone formation

**Tetrahydrocannabivarin (THCV)**
- Inhibited breast cancer resistance protein
- Produces weight loss, decreased body fat, increased energy expenditure in obese mice.
- Anticonvulsant in rodent cerebellum and pyriform cortex.

**Cannabidiolic acid (CBGV)**
- Produces weight loss, decreased body fat, increased energy expenditure in obese mice.
- Anticonvulsant in rodent cerebellum and pyriform cortex.

**Cannabinoxin (CBG)**
- Produces weight loss, decreased body fat, increased energy expenditure in obese mice.
- Anticonvulsant in rodent cerebellum and pyriform cortex.

**Cannabichromene (CBC)**
- Anti-inflamatory
- Analgesic (less than THC)
- Antibiotic/antifungal
- Cancer cytotoxic agent
- CBC extract antidiapressant in rodents
- Anandamide reuptake inhibitor

**Cannabigerolic acid (CBGV)**
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**Cannabidiolic acid (CBG)**
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**Cannabichromenic acid (CBCH)**
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**Cannabichromene (CBC)**
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**Cannabinolic acid (CBN)**
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The Cannabinoids
Formerly Known as Minor (CFKMs)

The extensive breeding program directed by GW Pharmaceuticals' Eileen de Meijer has yielded plants rich in CBD, CBC, CBG, and THCV.

Cannabinochrome (CBC) is produced early in the plant's life cycle according to a paper published by de Meijer in 2009. Citing de Meijer's co-worker David Potter, Russo notes that "An innovative technique employing cold water extraction of immature leaf matter from selectively bred cannabis chemotypes yields a high-CBC 'enriched trichrome preparation.'" Cannabigerolic acid (CBGA), the precursor of THC, CBD, and CBC in their acid forms, is usually found at low concentrations. "But recent breeding work has yielded cannabis chemotypes lacking in downstream enzymes that express 100% of their phytocannabinoid content as CBG," according to Russo. (More details are provided on GW Pharmaceuticals’ very informative website.)

Entourage Effect

Terpene Factoids

Whereas plant cannabinoids are found nowhere else in nature, terpenoids are produced by countless plant species. Some 20,000 terpenoids have been identified by chemists; they constitute the largest group of plant chemicals. More than 200 have been found in cannabis.

"Essential oil composition is much more genetically than environmentally determined."
Entourage Effect from previous page

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O'Shaughnessy’s antidepressants.) was provided by a Japanese study of se—

ing and, in many cases, well established.

α-pinene is the most common ter—

penoid in the plant world; limonene is second. Named for their strong presence in

pine needles and lemons, respectively, they are monoterpens, also prevalent in cannabis.

Terpenoids may account for only 7% of the weight when cannabis is tested but 10% of the weight within the trichome.

Monoterpenes evaporate more readily than the di- and sesquiterpenes during dry—

ing, storage, and production of extracts, which results in a relatively higher propor—

tion of any monoterpene in cannabis extracts.

Beneficial Effects

How do terpenoids exert effects within the body? Citing the relevant studies, Russo explains that they are “lipophilic, interact with cell membranes, neuronal and muscle ion channels, neurotransmit—

ter receptors, G-protein coupled (odorant) receptors, second messenger systems and enzymes.”

Limonene has been shown to decrease anxiety in mice via the serotonin receptors.

The beneficial effects are wide-rang—

ing and, in many cases, well established. Limonene, for example, has been shown to decrease anxiety in mice via the seroton—

in receptors. “Compelling confirma—

tory evidence in humans,” Russo writes, was provided by a Japanese study of se—

verely depressed hospital patients whose moods improved when exposed to citrus fragrance. (Nine of 12 were able to get off antidepressants.)

Linalool has sedative and anti—

convulsant properties.

Linalool, which is abundant in laven—

ders, affects serotonin neurotransmission and counters anxiety, according to a study cited by Russo. Linalool has sedative and anti—convulsant properties, and is also “the likely suspect in the remarkable therapeutic capa—

cities of lavender essential oil to alleviate skin burns without scarring.”

β-Caryophyllene, which is found in black pepper, Echinacea, and marigolds, “is frequently the predominant terpene in cannabis, and com—

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β-Caryophyllene is anti-inflammatory and, unlike other anti—inflammatories, protec—
tive of the stomach lining.

In 2008 Swiss investigators led by Jurg Gertsch showed that β—caryophyllene ac—
tivates the CB2 receptor — making it “the first proven phycannabinoid beyond the cannabis genus,” Russo proclaims. “Given the lack of psychoactivity attrib—
duted to CB2 agonists, caryophyllene offers great promise as a therapeutic compound, whether systemically or in dermatological applications.”

Other terpenoids with therapeutic po—
tential mentioned by Russo in his BHP pa—per are nerolidol (found in citrus, it may have sedative and anti—fungal effects, caryophyllene oxide (found in the herb lemon balm, it repels insects); and phytol (a breakdown product of chlorophyll with roles in plant protection, may have therapeutic effects, including green tea, despite its caffeine content, doesn’t jangle the nerves).”

In their landmark 2001 paper in the Journal of Cannabinoid Therapeutics, Russo and lead author John McPartland touched on the beneficial effects of euca—

typol, pulegone, sedative—terminals and oth—
er possibly efficacious terpenoids. These compounds were not discussed in Russo’s 2011 BHP paper.

Designer Extracts

Russo describes several mechanisms by which terpenoids and/or cannabinoids can

antidepressants. (See story

on next page.) ProjectCBD.org and can—
nabisclinicians.org will carry updates on what we, collectively, are about to learn.

RAPHAEL MECHOUAL in the fall of 2010, addressing scientists assembled in his honor. Ethan Russo paid homage to a 1999 paper in which Mechoulam used the term “entourage effect” to describe how compounds act in concert to activate receptors in the body, Mechoulam commented in that paper, “The type of synergism may play a role in the widely held (but not experimentally based) view that in some cases plants are better drugs than the natural products isolated from them.”

The Research Agenda

Cannabis designer extracts are likely to yield safe, effective new treatments for a wide range of conditions, and — in due course, it is hoped — to regulatory approval and sales. GW Pharmaceuticals has already bred cannabis chemotypes with very high fractions of myrcene and limonene, and we assume they’re working on plants high in pinene, limonene and other terpenoids with therapeutic potential. As Russo puts it in his BHP paper, “Selective cross—breeding of high—terpenoid— and high—psycho—
cannabinoid—specific chemotypes has... become a rational target.”

Meanwhile back in California, research—

minded doctors, cannabis cultivators, dis—

pensary and lab owners, have been think—
ing along similar lines. (The idea that can—
nabis can be bred to maximize production of more than one compound is as obvious as the association between aroma and ef—

fect.) We don’t have the resources to do high throughput pharmacological screen—
ing or animal studies involving radioactive labeling, but we do have access to labs that can identify the compounds in a cannabis bud, and we have our own senses to evalu—
ate effects.

As O’Shaughnessy’s goes to press in late August, we know of two labs in California that led by Russo and others are many plans to do so. (See story on next page.) ProjectCBD.org and can—
nabisclinicians.org will carry updates on what we, collectively, are about to learn.