Appendino's Advice to Cannabinoid Researchers: Consider 'New Targets, Chemistry, and Plant Sources'

By Ryan Lee and O'S News Service

The International Cannabinoid Research Society held its 24th annual meeting at a lakeside hotel in Baveno, Italy, in June 2014. ICRS members are mainly —but not exclusively— university-connected biochemists and pharmacologists investigating how things work at the sub-cellular level.

Baveno is a resort town on big, beautiful Lake Maggioro, with the Alps visible to the north. There were four days of talks describing recent studies, and sessions at which investigators answered questions about their findings as summarized on posters.

When the ICRS was founded in 1990, its original name was "International *Cannabis* Research Society." In 1995 —after the body's own cannabinoid receptor system had been discovered and elucidated by ICRS members— the group changed the *C*-word in its name to "Cannabinoid." As pharmacologist Dale Deutsch explained in 1998, "The field is moving away from the plant."

The 2014 ICRS meeting marked the return of the plant to the forefront of the field. Neurologist Ethan Russo was serving as ICRS president (the job is held for a year), and he invited the Italian natural product chemist Giovanni Appendino to give the featured talk at the meeting in Baveno.

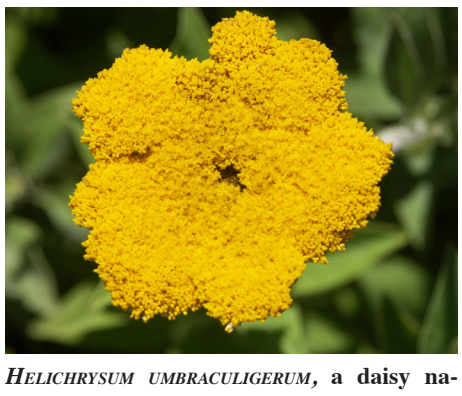
Appendino, a professor at the Università del Piemonte Orientale, noted proudly that he is from Carmagnola, a northern Italian town renowned for its fiber hemp variety of the same name.

Appendino first published research in the cannabinoid field in 2002, when he was co-author of a paper on "Noladin ether —a putative endocannabinoid." (The lead authors were Raphael Mechoulam and Vincenzo DiMarzo.) But Appendino's "relationship with cannabis as fiber hemp" goes much further back: "My grandfather was growing it and the odor of hemp retting tanks was filling the air around Carmagnola during the Fall."

By defining cannabinoids as drugs that work at the CB1 and CB2 receptors, researchers may be overlooking beneficial compounds in Cannabis *that work by other mechanisms.*

Researchers have focused almost exclusively on THC, CBD, CBC (cannabichromene) and CBG (cannabigerol, precursor to the other three), Appendino said, while not investigating the therapeutic potential of related molecules present in *Cannabis* —and other plants as well.

Similarly, by defining cannabinoids as drugs that work at the CB1 and CB2 receptors, researchers may be overlooking beneficial compounds in *Cannabis* that work by other mechanisms. "Nature has varied on the cannabinoid structure," Appendino reminded his ICRS audience.



HELICHRYSUM UMBRACULIGERUM, a daisy native to South Africa, produces cannabigerol (CBG). It was identified by Ferdinand Bohlmann and Evelynn Hoffmann in 1978.

"Natural Selection Works Like a Tinkerer..."

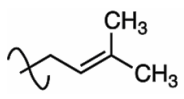
LEAVES THAT RESEMBLE *CANNABIS SATIVA* are (top row, left to right): *Acer japonicus, Aconitum vulparia, Geranium pratense*. Bottom row, left to right: *Hibiscus cannabinum, Vitex agnus-castus, Cannabis sativa*. Graphic from *"Plantes interdites. Une histoire des plantes politiquement incorrectes,"* by Jean-Michel Groult. Appendino quoted the French scientist Francois Jacob in connection with this slide:: "Natural selection works like a tinkerer who does not know exactly what he is going to produce, but uses whatever he finds around him to produce some kind of workable object. None of the material at the tinkerer's disposal has a precise and definite function. Each can be used in different ways. Novelty comes from previously unseen association of old material. To create is to recombine."

In the course of screening more than 200 varieties of fiber hemp, Appendino and colleagues have found significant quantities of obscure compounds whose medical potential he considers "worthy of investigation."

Cannabinoids are not unique to Cannabis *—they have been found in other plants.*

He touched briefly on canniprene, the cannflavins, cannabinoid esters, and "sesqui-CBG," which Appendino's group isolated from a fiber hemp variety.

Appendino has encountered a hemp variety containing two percent canniprene —a compound he called "the *Cannabis* version of resveratrol" (a beneficial compound present in red grapes).



From others varieties he isolated the prenylated version of cannabigerol —meaning CBG attached to a prenyl group *(illustration at left)*. There is no reason, Appendino said, that marijuana should not also produce the prenylated version of THC —which would have distinct biological activity.

Cannabinoids not unique to Cannabis

Cannabinoids are not unique to cannabis —they have been found in other plants. Appendino reported that a large amount of CBG and its carboxylic precursor had been isolated from a specific *Helichrysum* variety found only in South Africa.

Studying how *Helichrysum* makes "non-cannabis" CBG and its related compounds has been difficult for Appendino and his colleagues, because strict South African bio-piracy laws prohibit the collection and export of native species or their seeds. These laws, designed to prevent foreign corporate exploitation of the country's unique genetic resources, also impede legitimate scientific research. After two years of bureaucratic red tape, Appendino was only able to obtain a small vial of extract from the plant. Being unable to obtain seeds themselves has limited his ability to investigate the biosynthetic pathways by which *Helichrysum* produces cannabinoids.

Appendino discovered that cannabinoid-like compounds are made by plants "apart from the normal cannabinoid biosynthetic route. There is a new pathway that starts from an aromatic acid." Referred to as the "*Helichrysum* cannabinoids," these compounds also have been detected in liverwort.

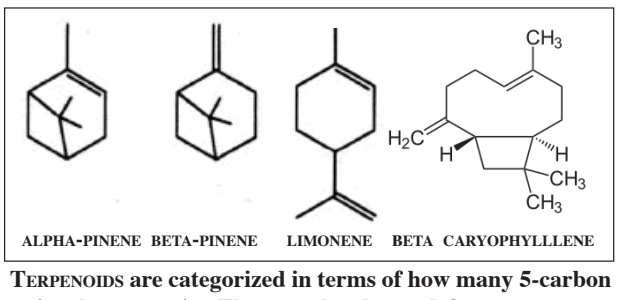
Helichrysum is used in African ethnopharmacology, Appendino explains, "like hemp, to make fumes in ritual ceremonies" and that a "psychotropic effect... similar to cannabinoids," might ensue.

Beta-caryophyllene

Terpenoids, the largest class of naturally occurring compounds on the planet, are the chemicals that give plants their unique smells and flavors. Found in high concentrations in many culinary herbs and spices, terpenes not only provide flavor and scent, they are also important signaling chemicals that plants use to communicate with insects.

Terpenes are synthesized by the plant from five-carbon isoprene units, two of which come together in specialized cellular compartments to form the 10-carbon monoterpenes (limonene, pinene, linalool, terpinolene, et al).

The 15-carbon sesquiterpenes such as beta-caryophyllene, differ from the monoterpenes by the incorporation of an extra isoprene unit. (β is the Greek letter *beta.)*



TERPENOIDS are categorized in terms of how many 5-carbon units they contain. Three molecules at left are monoterpenes —each contains 10 carbon atoms. Larger molecule at right, β-caryophyllene, is a sesquiterpene with 15 carbon atoms. Because β-caryophyllene is heavier than the monoterpenes, it evaporates less readily and is often present in relatively large amounts in dried *Cannabis*. (But not all *Cannabis* produces large amounts of β-caryophyllene.)

When Cannabis is dried, stored for periods of time, or made into extracts, the monoterpenes are generally first to evaporate. The sesquiterpenes like β-caryophyllene are more likely to remain.

Monoterpenes are more volatile —they evaporate at lower temperatures— so when *Cannabis* is dried, or stored for periods of time, or made into extracts, the monoterpenes are generally first to evaporate. The sesquiterpenes like β-caryophyllene are more likely to remain.

β-caryophyllene seems like the *Cannabis* plant's own perfect key for nature's CB2 lock. Plants use β-caryophyllene to defend themselves against predators. Some species up-regulate specific terpenes when attacked by herbivores to render the plant less palatable to the attacking insect.

In a beautiful demonstration of the web that Mother Nature has created, these same terpenes have been shown to recruit parasitic bugs that themselves attack the herbivores that are eating the plant!

The drive to breed high-yielding varieties of corn for intensive commercial agriculture sacrificed the ability of the plant to produce β-caryophyllene .

Appendino recounted how the wild, ancestral relative of corn, *teosinte,* grown by the Mayan and Incan farmers in pre-European Central and South America, produced significant amounts of β-caryophyllene before modern breeders selected towards high yielding corn with an increased sugar content. The drive to breed high-yielding varieties of corn for intensive commercial agriculture sacrificed the ability of the plant to produce β-caryophyllene .

That β-caryophyllene binds specifically to the CB2 receptor (which is found mainly outside the central nervous system) was reported by Jürg Gertsch at the 2007 ICRS meeting.

The CB2 receptor

The CB2 receptor has yet to be successfully exploited by the pharmaceutical industry, Appendino said. "If drug discovery is a sea, then CB2 is a rock that is surrounded by shipwrecked-projects," he commented poetically.

Pharmaceutical companies have spent

continued on next page

***TEOSINTE*, THE ANCESTOR OF CORN was tiny but rich in beta-caryophyllene. The cob in this photo is two inches tall.**

ICRS *from previous page*

large sums investigating proprietary synthetic CB2-selective compounds that end up showing little clinical efficacy. "But β-caryophyllene is a special lottery ticket," said Appendino.

β-caryophyllene is known to be anti-inflammatory and easy on the stomach lining. A special lottery ticket, indeed! So grind some black pepper on your next salad, and order those Echinacea and marigold seeds now —they all contain β-caryophyllene.

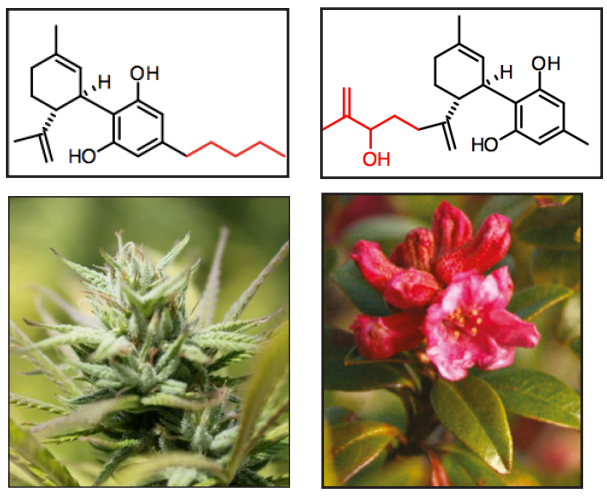
Appendino described how the β-caryophyllene molecule interacts with the CB2 receptor. It's an unusual physical relationship for cannabinoid-type agonists. β-caryophyllene does not look like any other molecule that binds to the cannabinoid receptors.

Extracts from plants high in β-caryophyllene have shown some analgesic effect in clinical trials. "Maybe the interaction of β-caryophyllene with CB2 is an echo of an ancient dialog between plants and insects," Appendino said.

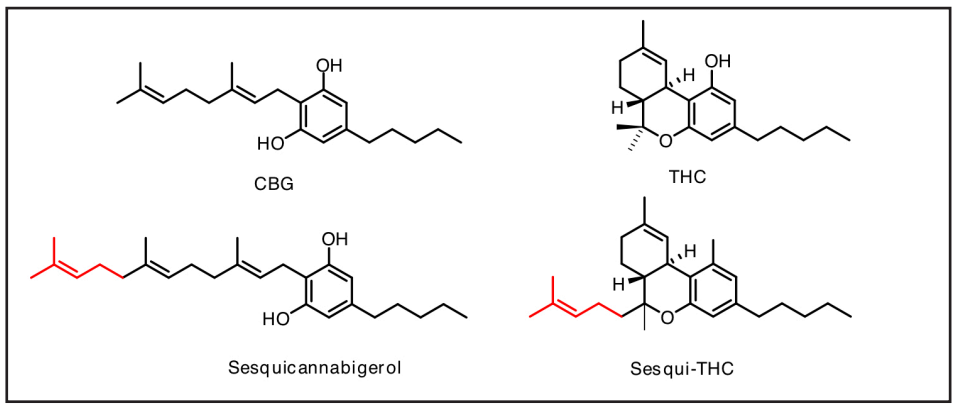
Expanded-Definition Cannabinoids

Just as natural selection tinkers with compounds, so do scientists, hoping to find a useful modification that evolutionary pressure hasn't induced nature to come up with. Research is underway into some of the unorthodox cannabinoids Appendino discussed.

For example, a Spanish biotech company called VivaCell has developed a drug, VCE-003, which outperforms CBG in activating PPAR receptors. VCE has shown efficacy in studies using mouse models of Multiple Sclerosis and Encephalomyelitis.



CBD ***(top left)*** **and Ferruginene C (top right) have similar molecular structures. Ferruginene C is produced by *Rhododendron ferrugineum*, an Alpine evergreen shrub (photo at right). Photo at left is of a CBD-rich variety called "ACDC," grown and photographed by Lawrence Ringo.**

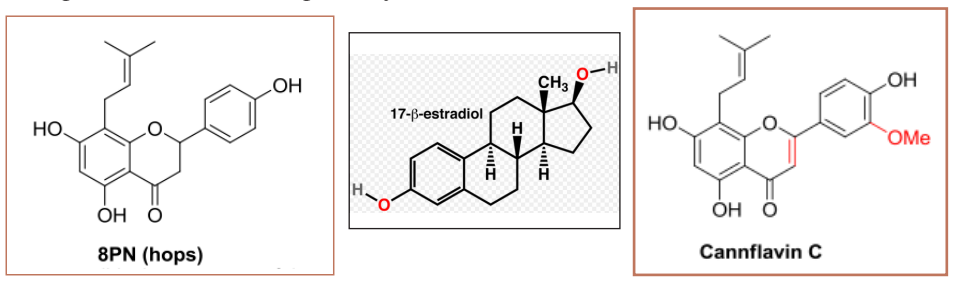


Cannabigerol (CBG, top left) is the compound in *Cannabis* from which other plant cannabinoids are synthesized. Molecule at bottom left is sesqui-CBG, which has been identified in fiber hemp It consists of CBG plus a five-carbon pentyl tail (at right in illustration). Appendino posits the existence of Sesqui-THC, a plant compound consisting of THC plus a pentyl group.

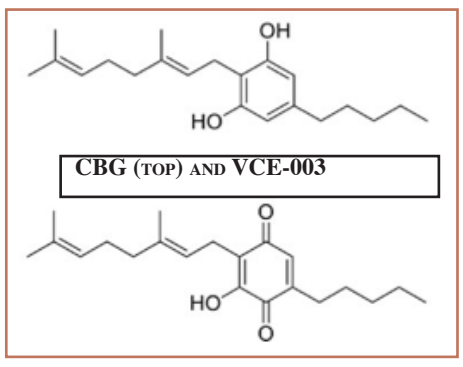
Drugs like VCE-003, made by adding side chains to naturally occurring molecules, are known as "semi-synthetics."

Hydrocodone and bupenorphine, which have replaced codeine and morphine and most opioid analgesics now sold in the U.S., are well-known semi-synthetics.

Appendino's expanded definition of cannabinoid drugs involves an expanded concept of the endocannabinoid system. In addition to CB1 and CB2, the biological targets of the expanded-definition cannabinoids include the GPR55 receptor; TRPs (pronounced "trips"), which are tiny ion channels with gates that open and close to transmit signals; and transcription factors in the mitochondria that switch genes on and off.



8PN (8-Prenylnaringenin, left), a flavonoid prevalent in hops, is the most potent estrogenic compound found in plants. Its effects are similar to, but weaker than the hormone estradiol (center). Pointing out the similar structure of flavonoids found in *Cannabis*, Appendino asked, "Could Cannflavin be the estrogenic principle of *Cannabis*?" Chemist Matt Giese adds, "These type of flavonoids can form isomers, where the methoxy (OMe) and hydroxy(OH) groups have switched positions. This can greatly affect binding and functionality, which is why A and B have such different activities."



ICRS coverage continued on next page.

The Flavonoids Unique to *Cannabis*

Flavonoids are compounds produced by many plants that influence the color of flowers, among other things. Flavonoids are defined by a 15-carbon backbone that includes two phenyl groups. Like terpenoids, they are "secondary metabolites," advantageous to the plant (attracting a pollinator, inhibiting growth of a mold, etc.) but not "primary" components like the proteins, lipids, and carboydrates needed for life itself.

In the 1980s, Dr. Marilyn Barrett identified two diprenylated flavonoids in *Cannabis* which were previously unknown. She named them "Cannflavin" A and B.

In 2013 Mahmoud ElSohly and colleagues at the University of Mississippi identified a third, Cannflavin C.

As noted by Giovanni Appendino at the 2014 ICRS meeting, Cannflavins are now being studied for anti-inflammatory activity, and hemp cultivars with unusually high cannflavin content (c. 2%) are being grown in Italy.

We sought some background info from Barrett, who is based in Mill Valley.

Barrett describes her discovery as "classic pharmacognosy." She was a PhD student at the School of Pharmacy, University of London, looking for compounds that would counter the activity of an inflammatory mediator, prostaglandin E2 (PGE2), present in synovial cells cultured from the knee joints of patients undergoing surgery for rheumatoid arthritis.

Barrett and her co-workers found that a cannabinoid-free alcohol extract of *Cannabis* was inhibiting the release of these inflammatory prostaglandins from the cultured cells. To determine which component of the extract was having the anti-inflammatory effect, they divided the extract into fractions (using preparative thin layer chromatography) and measured the activity of each fraction in the cell culture assay. The most active fraction, in turn, was divided into fractions and its most active fraction selected, and the process repeated until a pure compound was isolated.

"Once you get down to a pure compound," Barrett explains, "then you can work on identifying the structure of that compound. We used mass spectrometry (MS) to measure the molecular weight along with proton- and carbon- nuclear magnetic spectroscopy to get a picture of the structure. Ultraviolet spectroscopy confirmed we were working with a flavonoid, belonging to the class of flavones

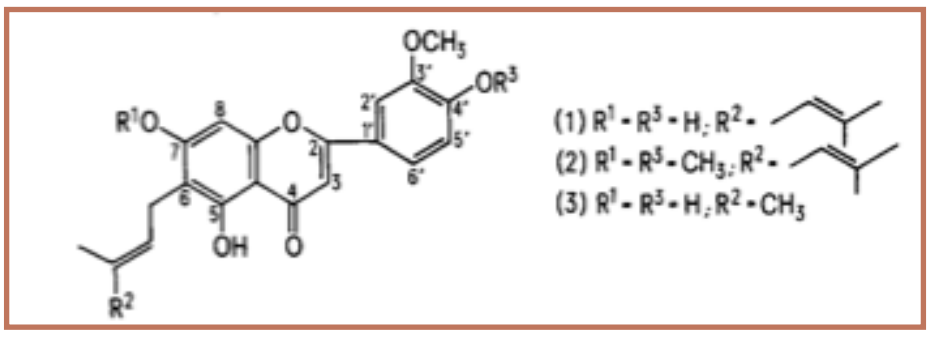
Previous work by Barrett's colleagues (Fairbairn and Pickens) at the School of Pharmacy used a model of catalepsy in mice to measure the psychoactive properties of the cannabinoids, particularly THC. They were extracting the cannabinoids from dried plant material using petroleum spirit until the remaining plant material was cannabinoid-free. The spent plant material was then extracted with alcohol.

Fairbairn and Pickens determined that this alcoholic extract of *Cannabis,* which was free of cannabinoids, had the ability to counteract the cataleptic activity of THC in mice. They suspected that the inhibition of prostaglandins was important to this effect, and in confirmation of this idea, inhibitors of cyclooxygenase also demonstrated this activity in mice. It was this work that led to Barrett's search for an anti-inflammatory agent in the alcoholic extract.

Barrett first published her account of isolating Cannflavin in Biochemical Pharmacology, June 1985. Details of the structure elucidation were published in a second paper in Experientia 42, April 1986.

Although Barrett's team found Cannflavin to be 30 times more potent than aspirin as an anti-inflammatory in the cell culture assay, it was 18 times weaker than Indomethacin —which is perhaps why no effort was made to develop Cannflavin as a drug.

"Especially interesting, from a scientific point of view," Barrett notes, "might be that the *Cannabis* plant contains substances that both cause and reduce a cataleptic effect in mice." This finding was duplicated in the synovial cell assay, in which the cannabinoids stimulated the production of PGE2 and Cannflavin had an inhibitory effect. ***—O'S News Service***



Cannflavin A (structure 1) and cannflavin B (structure 3) were isolated from *Cannabis* in the 1980s by Barrett et al. These are prenylated flavones—compounds that have a prenyl group (3-methyl-but-2-en-1-yl) attached to their flavonoid backbone. Cannflavin B is different from A, lacking the five carbon alkyl unit at C-4. (Structure 2 was included for purposes of structure identification; it is not a compound found in *Cannabis*.)

ISOLATION FROM *CANNABIS SATIVA* L. OF CANNFLAVIN—A NOVEL INHIBITOR OF PROSTAGLANDIN PRODUCTION

M. L. Barrett,* D. Gordon and F. J. Evans†

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN, U.K. and †Department of Pharmacognosy, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, U.K.

(*Received* 15 *October* 1984; *accepted* 20 *December* 1984)

Abstract—The isolation from *Cannabis sativa* L. of an inhibitor of prostaglandin (PG) E_2 production by cultured rheumatoid synovial cells is described. This agent, for which the name Cannflavin has been coined, is distinct from cannabinoids on the basis of isolation procedure, preliminary structural analysis and biological properties. The activity of Cannflavin has been compared with several established anti-inflammatory drugs and the major cannabinoids.

Paper by Marilyn Barrett and colleagues in "Biochemical Pharmacology," June 1985 described the isolation of Cannflavin, the first in a new group of diprenylated flavones.

Table 3. Comparison of Cannflavin with established anti-inflammatory drugs in inhibiting TPA-induced PGE_2 release from cultured synovial cells

Drug	IC 50 (ng/ml) mean (range)	Relative potency
Cannflavin	31 (4.4–58)	1
Aspirin	840 (460–1500)	0.037
Indomethacin	1.7 (0.3–4.0)	18
Dexamethasone	0.27 (0.036–0.5)	115

The IC 50 values are the means of four assays of each drug; the range of values observed in the assays is also given. Cannflavin was arbitrarily assigned a potency of 1 for comparison of potency with other drugs.

ICRS *from previous page*

The 2014 ICRS meeting was attended by 328 people —a record. Major pharmaceutical companies that used to send scientists to present papers and monitor the latest research—Abbott, Allergan, AstraZeneca, Bayer, Bristol-Myers Squibb, GlaxoSmith-Kline, Merck, Pfizer, Eli Lilly and Sanofi-Aventis—were not represented in Baveno. At least for now, Big PhRMA seems to have surrendered to the plant itself as a source of cannabinoid drugs.

The one exception was Hoffman-La-Roche. Researchers employed by the Basel-based giant have synthesized a drug with an extra-strong affinity for the CB2 receptor. It was found to greatly reduce the build-up of collagen (which obstructs the ureter) in a mouse model of kidney disease. "CB2 agonists might have beneficial effects in both acute and chronic kidney disease," the presentation by Jean-Michel Adam concluded.

The trend towards studying plant compounds and their possible therapeutic applications is largely attributable to GW Pharmaceuticals, a British company, founded in 1998, that has been developing plant-based medicines to treat various conditions.

The ICRS scientists' Holy Grail —even GW Pharmaceuticals'— is a drug that exerts the beneficial effects of cannabis without psychoactivity.

Thanks to GW providing materials and funding for studies of cannabidiol (CBD) and other so-called "minor cannabinoids," the virtual monopoly of the U.S. National Institute on Drug Abuse as sponsor of cannabinoid research has ended. But NIDA is still the biggest ICRS backer, and quite a few presentations in Baveno were devoted to "abuse potential" and other elusive adverse effects.

NIDA funds basic research

Mostly, NIDA provides funding for scientists doing important basic research in physiology and pharmacology. Over the years NIDA-funded scientists have figured out the mechanisms of action by which plant cannabinoids and endocannabinoids (made in the body) exert their effects and get metabolized (broken down).

This research continues in ever-finer detail at the molecular level. For example, in the late 1990s Dale Deutsch and colleagues at Stony Brook University identified fatty acid amide hydrolase (FAAH) as the enzyme that breaks down the endogenous cannabinoid anandamide within the cell. In recent years Deutsch's lab has focused on the fatty acid binding proteins (FABPs) that bring endocannabinoids from the cell membrane to the endoplasmic reticulum (where FAAH does its stuff).

Dale Deutsch reported that the molecules that transport anandamide and 2-AG from the receptor to the nucleus of brain cells — certain fatty-acid binding proteins— perform the same function for THC and CBD.
PHOTO BY ISTVAN UJVARY

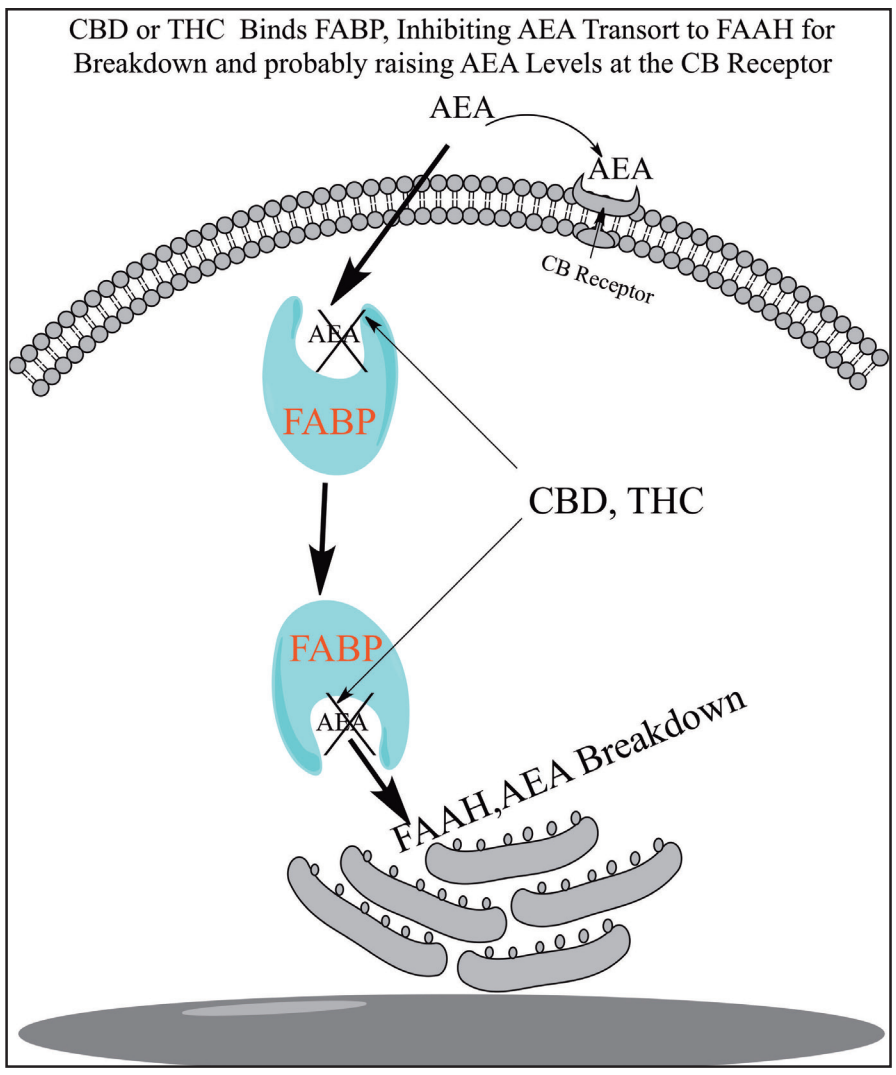
When plant cannabinoids are ingested by people smoking or eating marijuana, THC and CBD molecules are carried in the blood into the brain, "presumably by albumin and lipoproteins, which carry fats," says Deutsch. "But there's no albumin inside those cells in the brain."

In 2012 Deutsch's lab identified the molecules that transport anandamide (and perhaps 2-AG) in cells inside the brain: fatty-acid binding proteins —FABPs 3, 5, and 7 to be precise.

To determine if these very same fatty-acid binding proteins transport the plant cannabinoids THC and CBD, Deutsch reported this year in Baveno, his team did three kinds of experiments.

Simulations of molecular shapes, done by computational analysis, showed that THC and CBD "fit very nicely inside the fatty-acid-binding-protein carriers."

Binding studies using FABPs synthesized and purified in the lab showed that THC and CBD bind to these molecules as readily as anandamide and 2-AG do.



ANANDAMIDE (AEA) INACTIVATION results when CBD or THC targets fatty-acid binding proteins within the cell. At top, anandamide crosses the membrane by diffusion at the cannabinoid (CB) receptor. Once inside the cell, AEA requires fatty-acid binding proteins (FABPs) for transport through the cytoplasm to the endoplasmic reticulum (canoe-shaped structures), where it gets broken down by fatty acid amide hydrolase (FAAH). FABP inhibitors prevent anandamide from being delivered to FAAH for breakdown, resulting in increased anandamide levels at the receptor. Dale Deutsch and collaborators at Stony Brook University have identified the enzyme SB-FI-26 as a potent inhibitor of the FABP transporters.

Cell cultures confirmed that adding THC and CBD inhibited the uptake of anandamide and 2-AG —meaning they were binding to the same transporter molecules.

Deutsch also cited two human studies in which ingestion of THC or CBD was shown to increase anandamide levels in the blood because they act as anandamide -transport inhibitors. (See illustration above).

Deutsch's identification of FAAH as the enzyme that metabolizes anandamide inspired drug developers to create compounds that inhibit production of FAAH, resulting in elevated cannabinoid levels without ingestion of exogenous ("from without") THC. Fatty-acid binding proteins could also be drug targets, and their efficacy the topic of future ICRS talks.

Gender Distinctions

Cannabinoids are more potent analgesics in female rats than in male rats.

Rebecca Craft and MD Leitl reported in 2008 that the sex hormone estradiol enhances the analgesic effects of THC in females whose ovaries had been removed, whereas testosterone blocks the motion-reducing effects of THC in males.

At the 2013 meeting Aaron Haas, a post-doc in Craft's lab at Washington State University, presented a poster showing that estradiol increases sensitivity to THC's anti-pain effects, but testosterone does not.

A team of Israeli researchers led by Sharon Anavi-Goffer found a marked difference in the way male and female mice respond to postnatal administration of HU-267, a cannabinoid drug developed by co-author Raphael Mechoulam. ("HU" stands for "Hebrew University.") HU-267 is described as "a novel synthetic compound whose structure resembles that of ajulemic acid." The researchers gave the drug to mice of both sexes 24 hours after birth. They found that by 25 days of age, the males were more hyperactive while the females where more hypoactive compared with their control litter mates.

Now the researchers' goal is to figure out why this drug exerted gender-related effects. Its pharmacology is being elucidated in collaboration with Roger Pertwee.

Sexist Science Surpassed

At the 2014 meeting, Chris Breivogel, a pharmacologist at Campbell University in North Carolina, presented a paper called "Beta-arrestin2 appears to mediate the activity of cannabinoids in female mice in a manner that differs from males."

Beta-arrestin2 is a protein inside the cell that interacts with an activated cannabinoid receptor, and —it was thought by the scientists who isolated the molecule and put arrest in its name— blocks or dampens the signal.

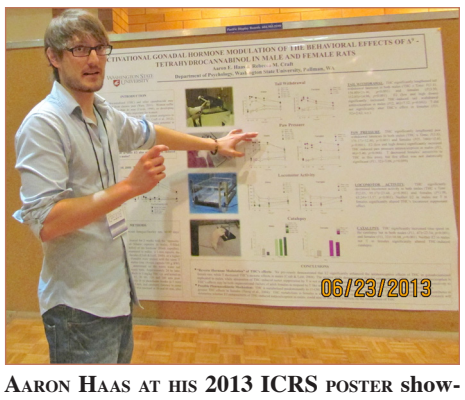
Breivogel had previously shown that male beta-arrestin2 knockout mice (bred to lack the gene that encodes for beta-arrestin2) respond to THC more strongly than wild-type males.

Traditionally, experiments with rodents have been conducted with males.

His more recent studies, using female mice, "have shown very different effects from what was seen in males. The antinociceptive (but not rectal temperature) effects of THC obtained in wild-type females were nearly absent in beta-arrestin2 knockouts."

Traditionally, experiments with rodents

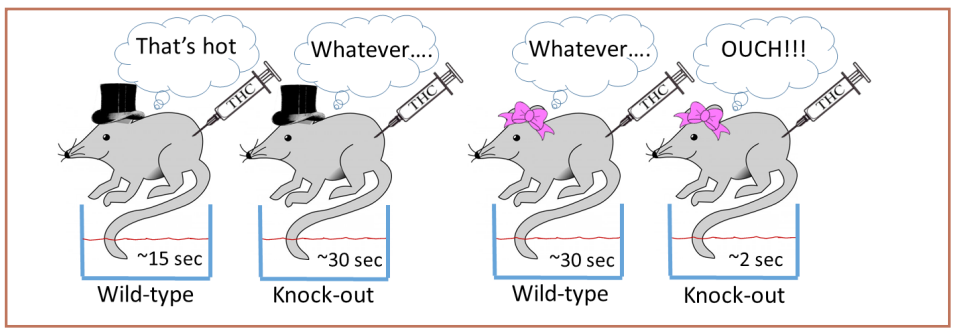
continued on next page.



Aaron Haas at his 2013 ICRS poster showing that estradiol increases sensitivity to THC but testosterone does not.

Changing of the Guard among ICRS sponsors is reflected on page from the conference abstract book, although the U.S. National Institute on Drug Abuse remained the biggest backer of the organization (and of sanctioned cannabinoid research). A decade ago ICRS sponsors included major pharmaceutical companies. In 2014 GW Pharmaceuticals and Otsuka (a Japanese company allied with GW) were next, followed by Tilray, one of 13 Canadian companies licensed to cultivate.

ICRS *from previous page*



Different responses to pain are seen in wild-type and beta-arrestin2 knock-out mice on THC. In the tail-flick test, mice are treated and held with the tip of the tail in a warm water bath. Untreated mice will remove their tails in about two seconds, but THC will dull the uncomfortable sensation so that they leave their tails in the water longer —about 15 seconds for wild-type males, and up to 30 seconds, the limit of the test, for the knock-out males.

Knocking out beta-arrestin2 in female mice practically eliminates the effect of THC. Wild-type females typically endured close to 30 seconds; the knockouts flicked out after about two seconds.

have been conducted with males. Researchers have known that "there are slight differences" in test results from male and female animals, according to Breivogel, but these were attributed to differences in rates of metabolism by the liver, differences in the amount of muscle mass and/or body fat, or perhaps changes in receptor level or sensitivity during the estrus cycle.

It was assumed that at the receptor level, there were no differences in mechanism of action.

Breivogel explains, "Males are simpler [to use in experiments] because researchers don't have to worry about changes during the estrus cycle, and how that would affect your results... And so it just got to be the habit where everybody just looked at males.

In the experiments Breivogel described in Baveno, male and female beta-arrestin2 knockout mice, and male and female wild-type mice were given THC by intraperitoneal injection (the most common route used in rodents).

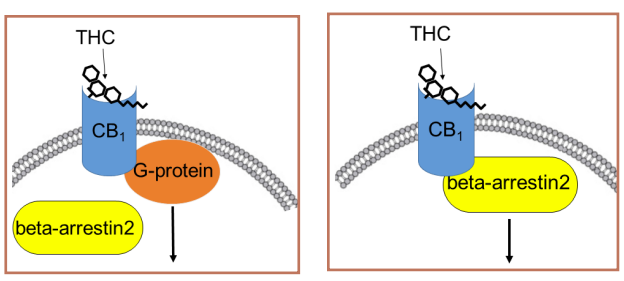
In the wild types, the males and females showed classic symptoms of cannabinoid activation: a drop in body temperature, and greater tolerance for pain as measured by a tail-flick test.

Deleting beta-arrestin2 in the males increased the effect of THC, so the knock-out males showed a greater response.

"What was so surprising when we did the females," Breivogel recounted, "was that it went in the opposite direction. Instead of enhancing the effect of THC, knocking out beta-arrestin2 decreased it to practically nothing.

In female mice, beta-arrestin2 might be involved in helping to mediate the signal instead of blocking the signal.

"The implication is that in a male when THC activates the cannabinoid receptor, beta-arrestin2 will bind to the receptor on the inside of the cell, and interact with other proteins to actually block the signal. It decreases the effect of THC on the cell.



Possible Explanation of different responses to THC by male and female mice observed by Breivogel is illustrated. In both males (cell at left) and females (cell at right) THC activates the CB1 receptor on the outside of the cell membrane. CB1 can couple to *either* a G-protein *or* a beta-arrestin at any given time. Both molecules couple to the same part of the receptor, so when one is there, the other is blocked.

Male CB1 receptors activated by THC produce an antinociceptive (and temperature depressing) effect via G-proteins. When beta-arrestin2 is removed, THC is more effective. In females, Breivogel proposes, "THC may activate CB1 to couple beta-arrestin2 to produce antinociception, so when you knock out beta-arrestin2, you lose the effect of THC."

"In the knock-out males, when you remove the beta-arrestin2, you get an enhanced effect.

'In the knock-out females, at least in one kind of assay, the effect practically goes away. This suggests that the beta-arrestin2 might be involved in helping to mediate the signal instead of blocking the signal."

Beta-arrestin knockout mice had been engineered/created and bred by Robert Lefkowitz, a Nobel Prize-winning scientist at Duke University, who supplied Breivogel with 12 animals to start breeding for his experiments in 2003.

The Lefkowitz lab —working only with males—had determined that beta-arrestin2 knockout mice had a stronger response to morphine's anti-pain effect than wild-types.

Breivogel recently tested the response to morphine of beta-arrestin2 knockout females and attempted to reproduce the effects previously seen in males. "There was no difference between males and females in the effect of morphine upon deletion of beta-arrestin2," he reports, which implies that the sex-differences for beta-arrestin2 seen with THC may not be universal, and may occur only with some drugs and their receptors, and may even be limited to only a few receptor systems. That all still needs to be investigated.

The differences in activation of beta-arrestin2 by THC in male and female mice are probably also present for anandamide, Breivogel says, because the differences are also brought on by a FAAH inhibitor, URB597, which works by augmenting anandamide.

"We just sort of stumbled on it one day. We were doing an experiment with a lab course, and I always do something I haven't done yet that might be interesting. 'You know we've never looked at the female mice...'"

The National Institutes of Health announced a policy in May, 2014, mandating that researchers state the sex of the animals (including people) used in their experiments, or the sex of the animal the tissue or cells came from when publishing data. Basic, pre-clinical research grant applications must "address the influence of sex in the design and analysis of biomedical research with animals and cells."

"Biased Signaling"

Drug developers hope to exploit the discovery that different ligands activate signaling pathways inside the cell with varying efficacy. Drugs (like THC) that activate the same receptor in males and females but then activate different signaling pathways inside the cell are said to exhibit "biased signaling." Such drugs might have different effects in males and females, or at least might vary in the ability to cause certain effects (therapeutic and/or side effects). "Differences in signaling, mediated by beta-arrestin2 and possibly other proteins, may be why certain drugs have somewhat different effects in men and women," says Breivogel.

An olive orchard in Crete.

All About EVOO

People in Greece, Southern Italy, and Spain have lower rates of colon, breast, prostate, and ovarian cancer than Northern Europeans. This is attributed to differences in diet. In the Mediterranean diet, the primary source of fat is extra-virgin olive oil (EVOO); the Northerners use butter and lard.

Extra-virginity is important because the polyphenols in freshly pressed olive oil degrade with aging and refining.

The EVOO benefit is dramatic —an almost 50% lower rate of colon cancer, for example. Extra-virginity is important because the polyphenols in freshly pressed olive oil degrade with aging and re-processing.

Andrea Di Francesco and colleagues in Mauro Maccarrone's lab have been studying EVOO's mechanism of action. Exposing colon cancer cells (Caco-2) to EVOO or an extract of its phenolic compounds resulted in "a selective increase in CB1 gene expression" and "inhibited proliferation of Caco-2 cells and arrested their cycle."

The researchers also fed healthy rats with a standard diet and an EVOO supplement, then looked for changes in cells lining the colon. Ten days of EVOO supplement led to "a significant increase in CB1 gene expression levels in colon."

This was due to "epigentic mechanisms." As explained by Maccarrone, "We found that CB1 is less expressed in cancer cells because it is more methylated at the promoter level. The gene is there, but it is not expressed."

A "promoter" is a region of DNA that initiates transcription of a particular gene. It's where DNA is turned into RNA. Methylation refers to the addition of methyl groups (CH_3) to a molecule.

Catalyzed by specific enzymes, methylation is involved in regulating gene expression and protein function. In normal cells, the promoter region is not highly methylated and the gene is expressed. But in cancer cells, the promoter region is highly methylated and the gene is silenced. More methylation of the gene means less CB1 expression and weaker endocannabinoid tone.

Aberrant methylation appears to be a precipitating factor in the development of cancer. But methylation doesn't just happen on its own. If a gene is inappropriately methylated, then some process in the body is causing this to happen.

Psychological trauma and high level activation of the body's stress system, especially in early childhood, are known to trigger abnormal methylation that changes DNA and disables genes. So, too, in animals. There have been studies that show differences in maternal care during the first six days of a rat's life result in different methylation patterns in promoter regions, thereby influencing gene expression.

Poor diet and exposure to environmental toxins can also skew gene expression.

What's in a Name?

A presentation by Dr. John McPartland challenged the widespread notion that there are three species of Cannabis —indica, sativa, and ruderalis.

McPartland used a novel approach involving "DNA barcodes."

There are 10 chromosome pairs in the nucleus of every typical cannabis cell. In every generation mutations occur.

Unlike human or plant 'nuclear' genomes, which are inherited from both an individual's male and female parents, chloroplast genomes are inherited only from the mother. Thus chloroplast genomes experience fewer mutations and evolve much more slowly

By focusing on regions of the chloroplast genome that are present in all plants, McPartland and co-author Geoffrey Guy were able to calculate the degree of relatedness between 'indica' and 'sativa' genetic sequences described in the academic literature.

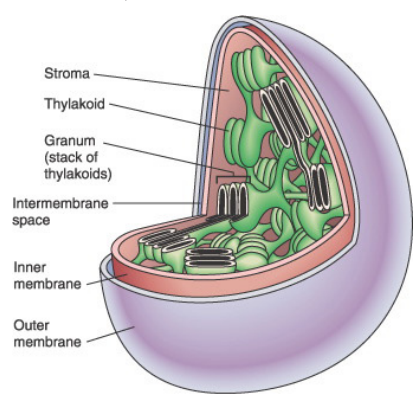
McPartland was able to create baselines that expressed the genetic differences as a numerical value.

Chloroplasts contain the genes responsible for key pieces of the photosynthetic machinery of the plant, in addition to other genes required for the plant to survive. Because these genes are so crucial for proper plant development and function, they mutate or "evolve" at a much slower rate than nuclear DNA. Comparing these conserved genetic sequences between both related and unrelated species, in addition to varieties or cultivars of the same species, McPartland was able to create baselines that expressed the genetic differences as a numerical value.

McPartland selected genetic sequences from *Cannabis* chloroplasts published in the academic literature, and used these same methods to calculate the degree of relatedness between the plants from which the samples were derived. The genetic differences show that the degree of variation between sequences was far less than those between unrelated species. In fact, they resembled the distance between different varieties as seen in other species.

The conclusion, therefore: *Cannabis sativa* and *indica* belong to different varieties of the same species. The evidence is corroborated by the ability of all varieties of cannabis —indica, sativa, and ruderalis— to inter-mate and produce fertile offspring.

McPartland also called on his audience to use correct terminology when referring to *Cannabis indica* (misnamed *"sativa"* in the current vernacular), *Afghanica* (misnamed *"indica"*), and *sativa* (misnamed *"ruderalis")*.



Chloroplast structures

ICRS *from previous page*

Exome Sequencing

It's unusual for a single case study to represent a breakthrough, but that was how Kevin McKernan's presentation —"Exome Sequencing of Familial Atrial Fibrillation Informs Positive Treatment With Cannabidiol"—was received by the ICRS.

McKernan was the lead researcher behind Medicinal Genomic's draft sequence of the *Cannabis sativa* genome in 2011. With co-workers at Courtagen Life Sciences in Woburn, Massachusetts, he has been using a sophisticated exome sequencing technique —targeting the "panel" of genes that encode for proteins, about 1% of the whole genome— to determine if certain disorders may be treatable by cannabinoids.

McKernan described a patient with an inherited form of atrial fibrillation who had not been helped by the conventional pharmaceutical treatments —beta blockers and calcium channel blockers. By comparing the exome sequences of six family members, it was determined that the patient inherited rare mutations in calcium-channel genes *RYR2* and *CACNA1C*. Knowing that cannabidiol regulates intracellular calcium homeostasis led to treatment with CBD. And sure enough, sprayed in the mouth, 75mg once a day, CBD promoted a regular heartbeat.

Atrial fibrillation returned with doses below 25mg given three times a day. "This case presents a private scenario," McKernan aptly noted. But targeting gene panels to identify underlying disorders treatable by cannabinoids is widely applicable.

McKernan's team has been exome sequencing the DNA of pediatric epilepsy patients involved in the clinical trial of G.W. Pharmaceuticals' Epidiolex.

The family with A-Fib was sequenced in two steps. First 500 genes, then all 18,000 genes —"and even this 18,000 genes represents only 1% of the genome," McKernan notes.

As of June 2015, he adds, "For GW we are still in the 500 gene phase, and even though there is some exciting data starting to emerge, the IRBs may not approve sequencing the whole exomes of the children without substantial increases to the genetic counseling budget. Genes like APOE and BRCA1 (associated with Alzheimers and breast cancer risks, respectively) are unrelated to pediatric epilepsy but present an ethical dilemma sequencing children with Epilepsy. Discovery of variants in these genes can produce stress and harm to the family and our study is designed to have no harm to the family. But even with the 500 genes, we are seeing some impressive signals. We need more patients sequenced to improve the signal."

G.W. Pharmaceuticals' Strategy

G.W.'s drug development strategy was outlined in a talk by James Brodie. The prevailing approach in the pharmaceutical industry involves "screening synthesized molecular libraries to identify those most potent and selective at a single receptor/disease target."

G.W.'s approach involves figuring out the mechanism of the disease and deciding which cannabis extract is best suited to treating it. "Disease causation is multifactorial," Brodie said, "and a 'broadside' approach may be more successful in overcoming the redundancy and multifunctionality that are inherent compensatory mechanisms in biological systems. "In other words, hitting one mechanism of a neurodegenerative disease or a cancer is less likely to work than a multipronged attack on the physiology of the disorder.

G.W.'s flagship product, Sativex, a plant extract formulated for spraying under the tongue, has been approved by regulators in 27 countries (starting with Canada in 2005) for treating pain and spasticity in Multiple Sclerosis. Sativex is in phase 3 clinical trials in the U.S. as a treatment for intractable cancer pain.

G.W.'s Epidiolex, an almost-pure-CBD extract, is in clinical trials as a treatment for two rare pediatric epilepsy syndromes *(See story on page 1)*.

Brodie said that advances in high-throughput screening, ever-declining sequencing costs, and the profusion of online databases —plus "proprietary in-house data"— enables G.W. to assess the role of "receptors, enzymes, genes, organelles and more" in a disease of interest.

One such disease is Duchenne Muscular Dystrophy (DMD), a hereditary condition that causes irreversible degeneration of muscle tissue and is usually fatal before age 15 due to respiratory failure.

Earlier in the conference it had been reported by F.A. Ianotti that in a mouse model of DMD, certain genes belonging to the endocannabinoid system were upregulated at the time of disease onset.

Treating the mice with Rimonabant to reverse the effect of CBD resulted in "a marked increase in locomotor activity... These findings indicate a novel role for CB1 in the development of dengerative muscle disease, perhaps by affecting muscle differentiation and repair processses, thus making this receptor a potential therapeutic target for the treatment of such disorders."

Presumably the extract GW would deploy as a treatment for DMD would be high in CBDV, which —like synthetic Rimonabant — is an inverse agonist at the CB1 receptor.

DMD is one of the so-called "orphan diseases" —defined by the Food and Drug Administration as affecting fewer than 200,000 Americans— that G.W. is focused on developing drugs to treat. Others include Dravet Syndrome and Lennox-Gastaut Syndrome.

A drug that is beneficial in treating the most severe forms of epilepsy is likely to be beneficial in treating most seizure disorders.

By developing extracts and natural compounds with specified ratios, Brodie said, "you can form a matrix of intellectual property that will be safe... It is our belief and the belief of our commercial partners that you cannot genericize Sativex."

Help for Acute Pancreatitis?

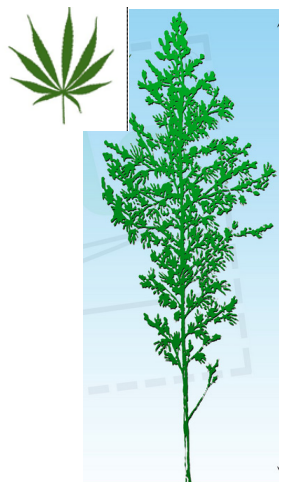
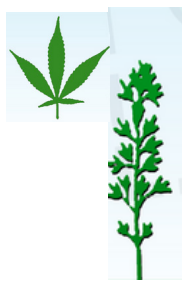
• Acute pancreatitis is a very painful disease in which digestive cells created by acinar cells in the pancreas for use in the small intestine start digesting the pancreas itself. There are no drugs to treat it —only painkillers.

Cannabinoid receptors are expressed in the pancreas and appear to prevent acinar cell pathogenesis (possibly by modulating interacellular calcium-ion signals). Using a mouse model, Huang et al tested GW 13542, an extract that targets CB2, as a treatment for acute pancreatitis and concluded that it "eliminates intracellular Ca2+ signaling in pancreatic acinar cells, which may provide a new therapeutic strategy."

continued on next page

From John McPartland's ICRS Presentation

Correct(ed) Vernacular Nomenclature

	Indica (formerly "Sativa")	**Afghanica (formerly "Indica")**	**Sativa (formerly "Ruderalis")**
			
Original provenance:	India	Central Asia (Afghanistan, Turkestan, Pakistan)	Usually feral or wild *C. sativa* from Europe, but sometimes of Asian provenance.
Morphology:	Relatively tall (ca. ≥1.5 m), laxly branched, with narrowly lanceolate leaflets, and relatively sparse flowering tops.	Relatively short (ca. 0.6-1.5 m), densely branched, with broad leaflets often oblanceolate, and dense flowering tops.	Variable, depending on provenance.
Physiology:	Flowering time (seed germination to intiation of reproduction structures under natural conditions) long, 9-14 weeks; no frost tolerance, moderate resin production.	Flowering time short, 7-9 weeks; frost tolerance, high resin production, susceptible to mold.	Flowering time relatively short but variable, sometimes autoflowering; moderate frost tolerance, relatively low resin production.
Chemistry:	THC much greater than CBD; uniquely prominent terpenoids: sabinene, α-terpinolene, trans-β-ocimene, trans-β-farnesene, imparting a flowery fragrance.	Cannabinoid profile variable (THC greater than or roughly equal to CBD); uniquely prominent terpenoids: camphene, β-myrcene, guaiol, β- and γ-eudesmol, imparting an acrid fragrance.	CBD>THC; prominent terpenoids: β-caryophyllene, myrcene, imparting a flowery fragrance.
Psychoactivity:	"Stimulating."	"Sedating."	Usually lacking.
Medical indications:	Lethargic depression, nausea, appetite stimulation, migraine headaches, and chronic pain. Relative contraindications: insomnia, anxiety, and schizophrenia.	Insomnia, anxiety, chronic pain, joint stiffness and inflammation, muscle spasms, tremors (from multiple sclerosis and Parkinson's disease), and epilepsy. Relative contraindications: lethargic depression, somnolence, and schizophrenia.	Chronic pain, joint stiffness and inflammation, epilepsy. Relative contraindications: allergy to cannabis.

Revised nomenclature was proposed by John McPartland at the 2014 meeting of the International Cannabinoid Research Society. His paper, co-authored by Geoffrey Guy, used "DNA barcodes" to determine whether or not *Cannabis indica* and *Cannabis sativa* are separate species. The answer was not. *C. indica* and *C. sativa* are subspecies —separate varieties of one *Cannabis* species. McPartland traced the confusion that prevails today among plant breeders and the pot-loving masses to the 1970s, when a *C. afghanica* plant collected by botanist Richard Evans Schultes was incorrectly identified as *C. indica*.

***ICRS** from previous page*

Cannabis, the anti-drug

Ever since he started running a cannabis dispensary in Vancouver, B.C. in 2002, Philippe Lucas realized that many people were using the herb as a substitute for alcohol and other drugs.

In the spirit of Tod Mikuriya's "Marijuana as a Substitute for Alcohol" (O'Shaughnessy's 2003), Lucas continued collecting data from patients who used cannabis as an alternative to harder drugs. He has been updating his findings at ICRS meetings since 2005, in ever more impressive posters.

In Baveno he gave a talk based on a survey to which 628 Canadians had responded. Consisting of 414 questions [that's a lot of questions], it was distributed online and in hardcopy to patients. Lucas found:

"Overall, 86.6% of patients reported substituting cannabis for at least one other substance: 80.3% (n=504) of patients stated that they used cannabis as a substitute for prescription drugs, 51.7% used it as a substitute for alcohol, and 32.6% used it as a substitute for illicit substances.

"The main reasons cited included 'better symptom management' and 'less adverse side effects.' Patients who listed a greater number of symptoms were more likely to report cannabis substitution, and younger patients (below 30) were far more likely to substitute cannabis for prescription drugs, alcohol and illicit substances than older patients (50 and over)."

Lucas ended with a call for "research into cannabis as a treatment for problematic substance use in non-patient populations."

Bringing it all back home

The 80th and final oral presentation in Baveno was by Jahan Marcu, PhD, who has gone from lab research to auditing cannabis production and distribution by U.S. growers and dispensaries as Senior Scientist for Americans for Safe Access.

The U.S. contingent included California practioners (Jeffrey Hergenrather, Michelle Sexton, William Courtney, R. Stephen Ellis), lab directors (Jeffrey Raber, Justin Hartzell), and political organizers (Steph Sherer, Martin Lee, Kristen Peskuski). There were also about a dozen entrepreneurs seeking out scientists with products to market and/or expertise to tap.

"With popularity of a name," Raber noted, "comes the greater potential for its abuse by those simply seeking to capitalize on a transaction involving the cultivar."

Raber gave an oral presentation, showing with data from his lab, the Werc Shop, the extent to which cannabis sold in California and Washington dispensaries is inaccurately named. "With popularity of a name," Raber noted, "comes the greater potential for its abuse by those simply seeking to capitalize on a transaction involving the cultivar."

Raber also found lamentable inconsistency in the way cultivars were dubbed *indica* and *sativa*. "A specifically named cultivar at one disepnsary is not necessarily the same product in the package at another dispensary simply because it posesses the same name. This may also be the case even week to week at the same dispensary, leading towards many different reports of the physiological impacts for a specific name and considerable numbers of frustrated patients seeking to find relief with a specific varietal."

Raber foresees more accurate identification of cultivars based on terpene content.

CBD Activates Serotonin

The evidence now seems conclusive that CBD works in part by activating the 5HT1A (serotonin) receptors, as suggested by Ethan Russo in a 2005 poster. Spanish researchers reported that CBD administered in the first six hours after hypoxic-ischemia had been induced in newborn piglets had strong neuroprotective effects. But if given along with a compound that blocks 5HT1A, there is no beneficial effect.

CBD Protects the RPE

Macular degeneration is a leading cause of blindness in the elderly. It is caused by accumulation in the retinal pigment epithelium (RPE) of a compound called A2E, which down-regulates a compound produced in the RPE called MCP-1

Shimon Ben-Shabat and colleagues at the University of the Negev have determined that cannabinoids (HU-210, HU-308 and CBD) counter the down-regulation of MCP-1 and provide neuroprotection.

THREE DISCOVERERS

IN ORDER OF THEIR LANDMARK FINDINGS: WILLIAM ANTHONY DEVANE (RIGHT), molecular pharmacologist. Working in the lab of Allyn Howlett at St. Louis University in 1988, he discovered the cannabinoid receptor (dubbed the CB1 receptor when a second one was found in 1992).

LUMÍR ONDŘEJ HANUŠ (LEFT) analytical chemist. Working with Devane in the lab of Raphael Mechoulam at Hebrew University in Jerusalem, he isolated the first endocannabinoid on March 24, 1992. Devane named it "anandamide," incorporating the Sanskrit word for "bliss."

SHIMON BEN-SHABAT (CENTER), medicinal chemist. Working in Mechoulam's lab in 1994, he was the first to isolate the endocannabinoid 2-arachidonoyl glycerol (2-AG).

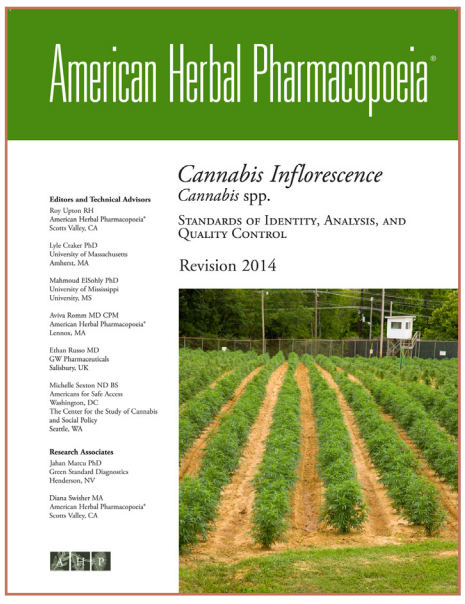
This photo was taken at the 2014 ICRS meeting with Dr. Ben-Shabat's cellphone by a passerby. Hanuš describes it as "a unique picture," the first of the three discoverers together. "It is for us pleasant to see how these three discoveries influenced science," he says. "This ICRS conference is basically on cannabinoid receptors and endocannabinoids. Around the world there are whole laboratories on this subject."

Will the industry accept?

ASA's Audacious Audit Offer

As the cannabis industry burgeoned in recent years, naturopath Michelle Sexton urged ASA's executive director, Steph Sherer, to push for safety standards that would protect patients' interests. Sexton suggested involving the American Herbal Products Association —AHPA, a trade association for the natural-products industry— and the American Herbal Pharmacopeia —AHP, which publishes monographs defining "standards of identity, purity, and analysis for botanicals."

Sherer pursued them. AHPA agreed to create guidelines on proper manufacturing, processing, dispensing, and lab procedures.



AHP MONOGRAPH COVER features a photo of the garden at the University of Mississippi where *Cannabis* is grown legally under federal law (surrounded by a 10-foot tall barbed wire fence, with cameras and a guard tower for extra security). The herb is for distribution by NIDA to government-sanctioned researchers. The grower, Mahmoud ElSohly, is one of the Monograph editors.

The AHP set to work on a monograph establishing standards of purity for cannabis that could be incorporated into regulations by state legislators. (AHP standards are widely used by companies that make and distribute licensed herbal supplements.)

Sherer raised funds for the mongraph's publication in December, 2013, a revised edition issued in the fall of 2014, and a second volume —a "Therpaeutic Compendium" citing all the medical literature, coming soon.

Auditing the producers

At the 2014 ICRS meeting, ASA's Jahan Marcu described how an "audit" is conducted to confirm that a facility —a farm, dispensary, or lab— meets AHP standards, complies with state and local regulations, and qualifies for "Patient-Focused Certification."

"I go to the site," says Marcu. "I make sure that the place is clean. I go through all their paperwork —staff training manuals, documentation. Is there a logbook? Do they have policies and procedures stating how they do things? When was the last time they applied a pest-management product? How much do they apply? What batch number of what pesticide product did you use? When was the last test on the water you're using from your well? When was your last soil test? Where did the last batch you cultivated go and could you initiate a recall? Is the facility processing on site? Are solvents stored properly and labeled? Are fertilizers and fuels stored properly and labeled?"

Marcu typically sees problems that can be readily resolved. Most common at the dispensary level, he says, is "the lack of a plan to record and report adverse events."

Waste disposal by labs and dispensaries

Marijuana prohibition can be seen as part of a broader war on botanical medicine.

is another area that often calls for improvement. "The rules and requirements differ from state to state," Marcu says, "but you don't throw away moldy cannabis or outdated products into an easily accessible trash can." Marcu says AHP standards are not costly to comply with.

Facilities that pass the audit get a label vouching for the quality of their operation, be it cultivation, manufacturing, dispensing, or lab reports. "Doctors can't tell people where to get cannabis," Marcu notes, "but they *can* remind patients to look for the PFC seal. If you have this seal on your product, you know that it's following basic safety and handling protocols for botanical medicine, or that the readings from the lab are going to be within acceptable limits of accuracy."

Certification by a third party is common in U.S. industries. "FDA does not certify manufacturing facilities and labs," Marcu points out. "They punt it to third parties that are often established by the very industry they're trying to regulate." Marcu compares his role to that of a rabbi certifying a food product as kosher.

Marcu's presentation to the ICRS in July 2014 framed the ASA audit as an experiment, and as this issue goes to press in December, 2015, the results are inconclusive. "Everyone really seems to like the audit and certification except for businesses," he said. "The patients like it, regulators, researchers love it... We'll just have to see about the industry."

Steph Sherer had come to Baveno for the ICRS meeting and gave us the back story on the audit project. Researching the origins of marijuana prohibition, she came to see it in the context of "scientific medicine" wiping out alternative approaches —including herbal medicine— in the first part of the 20th century.

In 1910 the Carnegie Foundation funded a crtique of U.S. medical schools by a layman named Abraham Flexner (who also had connections to the Rockefeller family and Johns Hopkins University). It was a blueprint for transforming the profession.

The American Medical Association became the mechanism for driving out competition from herbalists, homeopaths, and all other practitioners who had not been trained at elite medical schools (whose labs and hospitals required underwriting by the wealthy, and whose tuition fees effectively excluded working-class students).

'Scientific medicine' glorified technology and research. Historian Richard Brown attributes its credibility to the work of European bacteriologists who had "identified discrete, external, and specific agents of disease. This perspective encouraged the idea of specific therapies to cure specific pathological conditions, and it diverted attention from the social and economic causes of disease."

Scientific medicine made possible the cannabis prohibition of 1937 (and its continuation to this day) by disrespecting "crude" herbs. Scientific medicine recognizes that certain plants contain specific active ingredients that can be isolated, synthesized, and marketed as medicine.

Marijuana prohibition can be seen as part of a broader war on botanical medicine.