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.

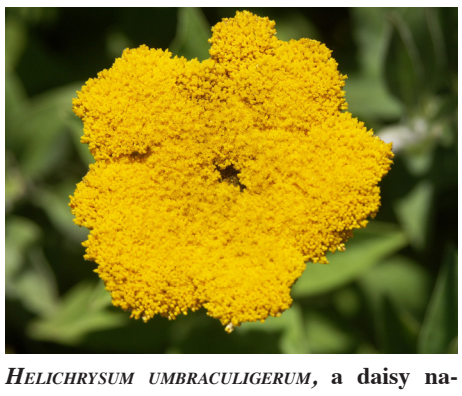
Appendino, a professor at the Università del Piemonte Orientale, noted proudly that he is from Carmagnola, a northern Italian town renowned for its fiber hemp variety of the same name.

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..."

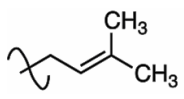
LEAVES THAT RESEMBLE *CANNABIS SATIVA* are (top row, left to right): *Acer japonicus, Aconitum vulparia, Geranium pratense*. Bottom row, left to right: *Hibiscus cannabinum, Vitex agnus-castus, Cannabis sativa*. Graphic from *"Plantes interdites. Une histoire des plantes politiquement incorrectes,"* 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.

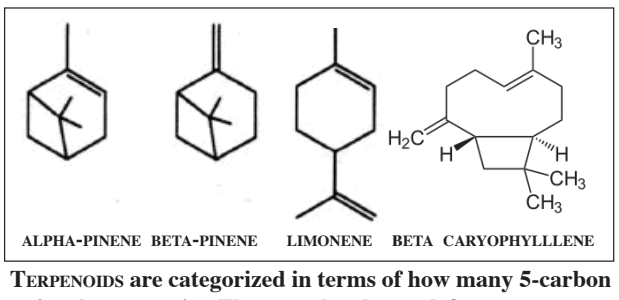
Helichrysum is used in African ethnopharmacology, Appendino explains, "like hemp, to make fumes in ritual ceremonies" and that a "psychotropic effect... similar to cannabinoids," might ensue.

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

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

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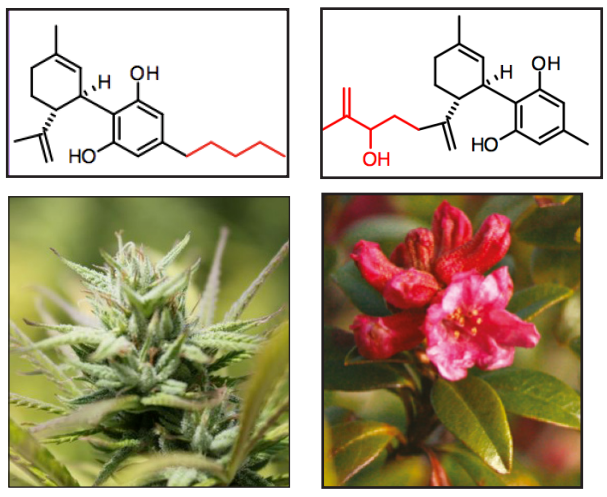
large sums investigating proprietary synthetic CB2-selective compounds that end up showing little clinical efficacy. "But β-caryophyllene is a special lottery ticket," said Appendino.

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.

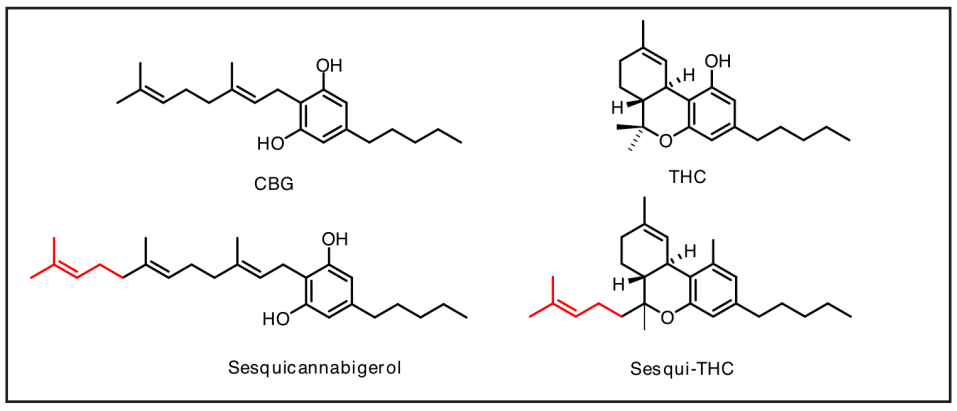


CBD ***(top left)*** **and Ferruginene C (top right) have similar molecular structures. Ferruginene C is produced by *Rhododendron ferrugineum*, an Alpine evergreen shrub (photo at right). Photo at left is of a CBD-rich variety called "ACDC," grown and photographed by Lawrence Ringo.**

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.

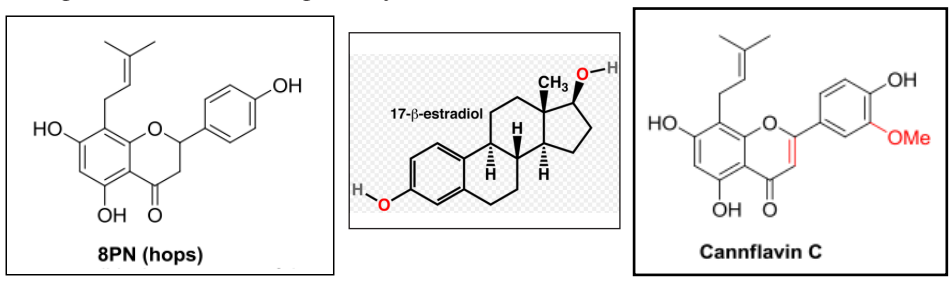


Cannabigerol (CBG, top left) is the compound in *Cannabis* from which other plant cannabinoids are synthesized. Molecule at bottom left is sesqui-CBG, which has been identified in fiber hemp It consists of CBG plus a five-carbon pentyl tail (at right in illustration). Appendino posits the existence of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

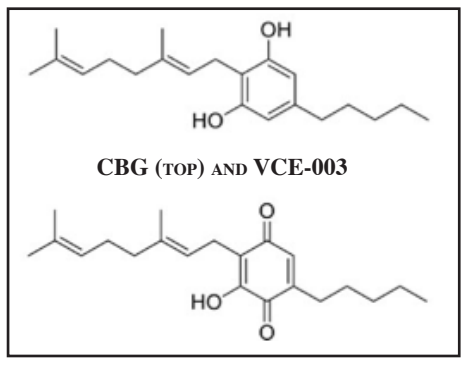
Drugs like VCE-003, made by adding side chains to naturally occurring molecules, are known as "semi-synthetics."

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."



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