The Big Book of Terps

Understanding Terpenes, Flavonoids, and Synergy in Cannabis

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Never Stop Growing

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FOREWORD

By Mitch Earleywine, Ph.D.

(*Professor of Psychology, University at Albany, SUNY; Fmr. Chair of the Executive Board, National Organization for the Reform of Marijuana Laws* [*NORML*])

A few weeks after Colorado first legalized the recreational cannabis market, I walked down a Denver street with friends from the NORML board. We turned a corner and an unmistakable scent overwhelmed us all. Someone had obviously developed an elaborate home grow and we all would have guessed that the quality was outstanding. Of course, we could not have guessed the concentration of cannabinoids of any sort. We smelled the delightful plant molecules known as terpenes.

Years later, a month before the COVID pandemic shut down our laboratory, my graduate students and I finally got approval to administer terpenes one at a time in a real experiment. These phytochemicals have had their psychoactive effects confirmed in aromatherapy research, and we had hoped to make the most of any relaxation, stimulation, or joy that they might generate. We literally suggested that we would run around to various vape shops in town with a single terpene in a little vape pen. Then we would beg folks to fill out a survey on their moods before and after taking multiple hits from an oil that contained only one of the classics. One pen had limonene, that citrus scent common to any strain named "Lemon." Another had linalool, the predominant component of lavender. And of course, we had a control pen with nothing but oil.

But once we saw that the ethics board was willing, we somehow lost motivation for the project. Nobody in the real world uses one terpene at a time. Nature bundles these molecules in delightful ways that seem to serve some grand evolutionary purpose. Looking at them one at a time seemed a bit naïve. The entourage effect, that delightful impact that plant molecules have when they combine with each other and meet human physiology, is markedly more dramatic than the sum of its parts. Looking at them one at a time felt akin to taking a silvery metal like sodium and a toxic gas like chlorine and trying to explain table salt. The unique combination hardly compared to its components.

But that's often the scientific approach. Breaking things in pieces and understanding the pieces has helped some domains in some fields. Nevertheless, the approach is a far cry from appreciating the nature of novel aggregates that coalesce in unpredictable ways. As Russ Hudson details in this volume, the stories behind individual chemical compounds and their constituents can intrigue any student of plants, animals, history, ecology, psychology, or art. In a sense, these intriguing chunks of carbon and hydrogen reveal quite a bit about us and our world.

If all humans are siblings, then perhaps all living organisms are distant cousins. The idea that we evolved as a species just a bit behind the plant kingdom certainly has some intriguing implications. Although tens of thousands of organisms can survive with little more than sun and earth, ancient molecules, when given enough time, lined up to create mobile creatures. A few plants can move a bit, of course. The Venus Fly Trap and Bladderwort can capture small prey for their own ingestion thanks to some quick motion. Others toss pollen on flying insects in an effort to spread their offspring far and wide. But most movement is reserved for us animals. Those species who flourished around any edible organisms that could provide a nutrient or two had definite advantages. Those who couldn't function with the available foliage never had the chance to pass their genes along.

Although my mycologist friends would likely raise their hands to offer a caveat, I think it's fair to say that plants and humans literally grew up together. Humans benefitted from the available vegetation, and we eventually cultivated the species that we found most rewarding. But our explanations for these choices might be an example of what social psychologist Dick Nisbett called "telling more than we can know." The stories we recount about which plants we like and why we like them might be more myth than reality. Terpenes likely play a role outside our conscious awareness, an error that Russ Hudson is here to correct.

Few fans of cannabis ever question why our progenitors exerted effort to ensure that this genus never died, but the truth behind the narrative has more nuance than one might imagine. A great many unsaturated hydrocarbons sure seemed to play a role in this divine coincidence where plants and humans evolved together. These colorless, chiral molecules with strong and (frequently) pleasant scents just happened to have a superb impact on human physiology. The same class of substances that defended plants against insects and other herbivores also helped them thrive, in part, because humans found them so appealing. And as readers will note in each of these chapters, no single plant has the market cornered. No single molecule seems to be the lone contributor to one physiological effect.

This lesson about working together applies within our species as well. (We all benefit when we cooperate. No one of us can know what all of us know, and no one of us can do what we could all do together.) But we see that the same is true across multiple species and kingdoms. If we take care of our Earth, we can relish the fact that it will take good care of us. Alas, the contrapositive is also true. If we neglect our environs, they will return the favor.

So let me caution readers before they continue: This book will change you. I have no doubt you'll find the prose remarkable and the content informative. But more than that, you risk finding yourself a different person when you finish any section. The details on each terpene underscore the recurring theme that our planet is phenomenal. That fact might seem banal in the abstract, and you've likely thought it before, but this work will make you BELIEVE it. Suddenly you'll note with any whiff of lavender or scent of citrus that the terpenes are more than a simple source of delight, analgesia, or sedation. These are the molecules we literally evolved with, stimulating the neurons in the most primitive part of our brain to fire, reminding us that our environs are richer and more luxurious than we might remember.

And these thoughts might inspire a new appreciation for the cannabis plant (of course!), but also the entire plant kingdom, and the other kingdoms, and the planet, and the universe...So don't open this book. Don't turn a single page. Sure, you'll be entertained and instructed at first. (The first thrill is always free.) But down the line you'll find yourself eager to tell everyone the marvels of molecules. You'll note what a wild set of coincidences had to happen in order for our species and all these plants to develop into what we've become. And you'll have this nagging sense that you not only need to spread the word, but you also need to help take care of this planet, and the living things on it. I'm happy to join you, of course, but the task won't be effortless, or always enjoyable, even though we know it's the right thing to do.

—**Mitch Earleywine, Ph.D.**
August, 2021. Albany, NY

PROLOGUE

This book was written for researchers, scientists, educators, cannabis industry professionals, medicinal cannabis patients, and for everyone interested in terpenes, flavonoids, and/or cannabinoids. Significant efforts were expended to make the work presentable and sustainably interesting to all demographics, including laypeople. For this reason, all citations in the text appear in-line for immediate reference. This formatting allows readers to instantly reference and verify information without breaking continuity by referring to an independent index or bibliography. Readers are encouraged to review the studies and papers referenced in this book in their entirety where necessity or special interest compels.

Please note that most of the studies cited here are not specific to cannabis. This is primarily because we simply do not have sufficient research available after decades of prohibitionist and research-restriction policies around the world, particularly in the United States. However, it should be understood that the molecules discussed herein are the same from plant to plant; for instance, the alpha-pinene that occurs in cannabis is the same alpha-pinene molecule that occurs in pine trees. The geraniol that occurs in some cannabis strains is the same as the geraniol that occurs in roses. The flavonoid quercetin that occurs in hundreds of plants is the same molecule that occurs in cannabis.

We can reasonably presume that the majority of plants use these compounds in a comparable way, and for the same or similar purposes, with exceptions for specialist plants and anomalies across species. Nevertheless, this presumption should be short-lived; we must continue to fight for legalization and scientific/research freedoms related to cannabis. Future editions of this book will be updated with emerging research, and readers that have unique or new research to share should contact the author directly at TheBigBookOfTerps.com.

Finally, it is important to consider that many of the laboratory tests conducted as part of research for this book may be only partially accurate. This is because as of 2021, consistency in varieties or strains is sorely lacking. The Blue Dream acquired at a dispensary in California may be chemically entirely different than the Blue Dream acquired in Barcelona, Spain, or even in Oregon, Cali's northerly neighbor. Unfortunately, the power of strain name recognition often skews labeling and marketing efforts, making it extremely difficult to ascertain whether a particular batch of cannabis branded as any strain is in fact that stabilized strain. In most parts of the world, assurance of cannabis variety is challenging to impossible.

Because of these inconsistencies, breeders, geneticists, cultivators, dispensaries, legislators, investors, healthcare professionals, and all others involved in cannabis policy should advocate for more and better laboratory testing. It's not enough to only test for contaminants and cannabinoid content. Terpene and flavonoid content is important, and can directly impact the therapeutic effects of cannabinoid and other phytochemical content such as alcohols, ketones, and esters. Cannabis users need to know the phytochemical profile of a product to make sound decisions for their health and well-being. Testing and subsequent dissemination and education is the only method of obtaining this confidence.

INTRODUCTION

Terps, terps, everywhere; in the stain on wooded stair, in the shampoo in your hair,

In the magic of your bong, in the trichomes, thick and strong

Smell them here and taste them there; terps, terps, everywhere...

Terpenes are everywhere. In fact, chances are high that you've experienced, consumed, or otherwise ingested multiple terpenes—and likely flavonoids, too—at some point in the last 24 hours. But despite the worldwide pervasiveness of terpenes, most people have never heard of a 'terp'. Those who have likely learned about these compounds because of their experience with cannabis. But whether you're a 'toker or an abstainer, cannabis supporter or prohibitionist, we cannot escape terpenes and the effects that they have on us.

Your Nose Knows Terps

Did you smell roses, begonias, geraniums, or other flowers in the last day? Did you walk by a lawn filled with dandelions, clover, goldenrod, or buttercups? Perhaps you encountered the sweet smell of lilacs, lupine, or lilies? Flowering thistle or burdocks? Orange, cherry, or apple blossoms? Those fragrances are all caused by terpenes—thousands of different carbon-chain compounds that are found widely in the plant kingdom.

But terpenes aren't just something that you smell and/or taste, without meaning. These volatile molecules interact with the human body in ways that affect our mood, our health, and even our decisions. Think about that if there are pine, spruce, aspen, or fir trees near where you live or work. That fresh, minty evergreen scent that pervades these trees is caused by terpenes, and your olfactory senses are likely registering these aromatic molecules whether you're conscious of it or not.

Terps on the Tongue

Terpenes and flavonoids are also found in many of the foods we consume; so many foods, in fact, that it would be difficult to go more than a day without consuming some of these compounds.

For instance, in the last 24 hours:

- Did you eat an orange, or take lemon in your water or tea? The smell and taste of both oranges and lemons are largely due to terpenes, while the vibrant colors of these fruits can often be attributed to flavonoids.
- Did you choose between one variety or another of pears, apples, berries, or other fruits? You made that decision based partly on which terpenes and flavonoids your senses detected.
- Did you drink beer? The hops in your brew contained terpenes that impart potent sedative and relaxant effects to the beverage. These include myrcene, beta-caryophyllene, and, to a lesser degree, humulene.
- Did you use tobacco? Terpenes are present in these products, as well—especially if you smoke menthols—that minty flavor is derived from terpenes, usually eucalyptol and menthol.
- Are you the type of person that prefers to snack on candy, or do you prefer salad? Either way, you're consuming terpenes; in candy these compounds are often used as flavoring agents, and most salad items are rich in natural terpenes and flavonoids.
- Did it rain near you? The fresh, earthy aroma that is present after a rain is caused by terpenes.

Although terpenes are naturally occurring compounds, this doesn't mean that you'll only find them in natural or minimally processed foods. In fact, terpenes are used in a wide variety of food products, especially sweets like ice cream, puddings and gelatins, baked goods, candies, and even chewing gum. Terpenes are also used in enriched food products and beverages, often to improve the taste of less palatable ingredients.

Terpy Toiletries

Humans have been harnessing the power of terpenes through the production of essential oils and other extractions for thousands of years—so long, in fact that most people don't even realize there are terpenes in use everywhere around them. Many types of personal care products feature one or more terpenes in the ingredient list. This includes an astounding variety of products found in the bathrooms of most modernized countries; perfumes and after-shave, deodorants, hair gels and creams, toothpaste and mouthwash, moisturizers and lotions, lipstick and lip balms, shampoos, conditioners, body soaps, and many more toiletries and hygiene products often contain terpenes. Check the ingredient lists of the contents of your bathroom and kitchen for terpenes like limonene, linalool, cineole, geraniol, pinene, and others discussed later in this book—you might be surprised at how many of the products you use every day contain terpenes.

Terpenes in Industry & Medicine

Our use of terpenes extends far beyond food and personal care products. Terpenes are widely used in heavy industries; turpentine, a product used in thousands of industrial, agricultural, and medical applications, is comprised primarily of the terpenes alpha- and beta-pinene, as sourced from pine trees. Industries that work with turpentine, rubber, citronella, camphor, and many other extremely common base ingredients are all working directly with terpenes.

Other industrial and commercial uses for terpenes include as novel and experimental fuel sources, in paints, stains, solvents, and cleaning agents, in pesticides, herbicides, fungicides, and antibacterial applications, in the storage of grains and seeds, and in hundreds of household products.

One sector that is particularly dependent on terpenes is the fragrance and perfume industry, which has systematically categorized and characterized hundreds of aromatic compounds for use in thousands of different applications.

Beyond the fact that terpenes can improve our lives with the many products we use that are based on these compounds, terpenes can also be used to improve and even save human life. This is because many well-studied terpenes have been shown to exhibit antiparasitic, antimicrobial, antifungal, and antiviral properties. This means that terpenes can be used to fight parasites, bacteria, fungal infections, and viruses. These volatile plant compounds also reduce swelling—a common cause of secondary disease—and may even be effective at fighting certain types of cancer cells. Some terpenes have significant analgesic or pain-relieving properties that can be harnessed at home and in the clinic through the use of various plant essential oils and other therapeutic preparations.

Terpenes in Cannabis

Nearly all of the things that people love about cannabis and marijuana are actually a love of the plant's phytochemicals including terpenes, flavonoids, and cannabinoids. That nose-hair-curling fragrance when you open a bag of Zombie Kush? That's terps talking. That crisp, fruity flavor of Super Lemon Haze that causes you to shut your eyes and work your tongue and lips like a suckling fish? Terps talking again.

If you're talking about any of the properties of cannabis that make your eyes light up like a kid at Christmas, then you're talking about terpenes. Whether you're a full-time cannabis connoisseur or a part-time 'toker, your ability to distinguish a particular variety or strain depends mostly on the terpene and flavonoid profile of the plant or product you're evaluating. Although you may not know exactly what the compound is called, when you identify Chocolope because of the variety's distinctive choco-fruity aroma, what you've really done is recognize a particular terpene profile. Likewise, when you detect New York Diesel based on its unmistakable lemony-gasoline flavor, what you've really identified are terpenes.

Terpenes and Us

Human physiology responds differently to different terpenes, and among us, some are influenced more than others by these compounds. The reason one person might prefer the fragrance of the terpenes present in the Amnesia strain, while another person disdains that strain and instead prefers the aroma of Afghan Kush terpenes is based on individual physiology. While much more research is needed to comprehend why terpenes affect people differently, the ultimate analysis and elucidation of this may be at least partially out of the realm of current scientific understanding, which makes terpenes somewhat magical.

It's clear that the human senses interact with terpenes and flavonoids on a daily basis, completely unrelated to cannabis. So, for those of us who do consume cannabis or hemp as part of our lifestyle, we need to understand that our more intimate relationship with these compounds requires careful consideration. To advance our personal use of this plant and to continue the international trends toward legalization of cannabis, it is critical that we understand why cannabis affects people the way it does, and, even more particularly, why the same strain or variety affects people differently. The answers to these questions are partly found in terpenes and flavonoids, and perhaps more importantly, in the putative synergistic interactions between these compounds, and cannabinoids.

Let's Talk Terps

Until recently, the cannabis industry has largely been a supporter and consumer of 'silver bullet' theories, with THC and CBD considered the holy grail of the plant. However, there is substantial evidence to suggest that this single molecule approach—reminiscent of pharmaceutical industry practice—may not be the best option for all situations, products, and people. Instead, a more holistic method is required; one where we critically examine all of the significant cannabinoids, terpenes, flavonoids, and other compounds individually, and as combinations based on particular stabilized chemovars to test for additive, agonistic, antagonistic, modulatory, or other synergistic effects.

TERP TSARS

Leaders in Terpene Research

This chapter recognizes and summarizes the work of some of the most important terpene and phytochemical researchers and scientists in the world. The "Golden Era" of terpene research was in the mid to late 19th-century, when German scientists dominated the scene. Today, there are far fewer terpene researchers, and only a handful of these are dedicated to terpenes in cannabis. However, there will likely be a resurgence of work in this field in the coming years, as the therapeutic and medical value of terpenes and flavonoids become clearer to the public, and as the roles of these constituents in cannabis are elucidated.

TIMELINE OF TERP TSARS

- 1830s: *Jean-Baptiste Dumas*. One of the first chemists to seriously investigate essential oil constituents
- 1830s: *Friedrich Wöhler*. The first to refute 'vital force' theory, discovered and proved isomerism, first chemist to discover and advance functional groups
- 1850s: *August Kekulé*. Conceptualized and advanced the theory of chemical structure and the hexagonal benzene ring nature of aromatic compounds
- 1860s: *Pierre-Eugène-Marcellin Berthelot*. Extracted the odor of fresh soil, refuted vital force, advanced synthesis of organic compounds, characterized pinene
- 1880s: *Otto Wallach*. Isoprene Rule, Wallach's Rule, numerous structural determinations of terpenes
- 1890s: *David Prain*. Early hemp researcher, proposed synergy in the form of more than one active constituent in cannabis
- 1900s: *Friedrich Heusler*. Wrote *The Chemistry of the Terpenes*, appears to be the first to propose that terpene oxidation products should be classified as terpenes
- 1920s-1930s: *Leopold Ruzicka*. Carried out the total synthesis of many terpenes, expanded upon the isoprene rule, defined terpenes according to number of isoprene units
- 1930s-1950s: *Walter Siegfried Loewe*. Identified the activity of cannabinol, invented the isobologram analysis method of detecting and measuring synergy in compounds
- 1940s-1960s: *Robert Burns Woodward*. Development of Woodward Rules, total synthesis of reserpine and chlorophyll
- 1950s: *Wilhelm Treibs*. The first to isolate and elucidate the structure of beta-caryophyllene—the top terpene in cannabis
- 1950s-1960s: *František Šorm*. Synthesized and deduced the structures of sesquiterpenes, investigated terpenes as analogues of insect hormones
- 1950s-1970s: *Sir Derek Harold Richard Barton*. Developed the Barton-McCombie deoxygenation concept, confirmed the structure of beta-caryophyllene
- 1960s-Present: *Raphael Mechoulam*. Discoverer of THC, total synthesis of this molecule as well as CBD and CBG, first to suggest entourage effect

- 1980s-Present: *Mahmoud A. ElSohly*. Synthesis of cannabispiran, identification of genes in Δ9-tetrahydrocannabinol, various essential oil research, natural product structure determinations
- 1980s-Present: *John M. McPartland.* Researcher of synergy in cannabinoids and terpenes, cannabis botanical history researcher
- 1990s-Present: *Shimon Ben-Shabat*. Discovered 2-Arachidonoylglycerol, together with Mechoulam showed first evidence of the entourage effect in cannabis
- 1980s-Present: *Ethan Russo.* World's leading researcher on terpenes and synergy in cannabis
- 2000s-Present: *Sameksha Koul*. Author of *Comprehensive Treatise on the Terpenes*
- 2010-Present: *Colleen Quinn*. Clinical aromatherapist, terpene formulator
- 2000s-Present: *Susan Trapp*. Researcher; molecular evolution of terpenes, horizontal gene transfer of fungal terpenoids, founder of Terpedia.com

[Did we forget someone in this list? Let us know at www.TheBigBookOfTerps.com]

JEAN-BAPTISTE DUMAS 1800-1884

Important work: 1830's

One of the first chemists to systematically research essential oil constituents was Jean-Baptiste Dumas, a Frenchman whose name is inscribed among 72 other prominent scientists on the corners of the Eiffel tower.

In his early career, Dumas served as professor of chemistry at two prominent schools in Paris, then later served political posts under Charles-Louis Napoléon Bonaparte (Napoléon III) before returning to the field of chemistry.

Dumas investigated various hydrocarbons, but is perhaps best known for his work with nitrogen-containing constituents, including the development of a method for analyzing the content of nitrogen in many compounds. Dumas also used innovative techniques to establish molecular and atomic weights by measuring vapor densities.

FRIEDRICH WÖHLER 1800-1882

Important work: late 1820s to 1860

Friedrich Wöhler contributed more than 60 years of consistent, original scientific work to the field of organic chemistry. From Eschersheim, Germany, Wöhler studied under the famous Swedish chemist Jöns Jacob Berzelius, who later validated Wöhler's discovery of and work on isomerism.

Prior to Wöhler's discoveries, most chemists believed that organic compounds could not be created without a vital force or life force, which could only be formed or carried by a living thing. Wöhler disproved this longstanding theory by synthesizing urea in his laboratory, radically changing the future of organic chemistry.

Wöhler's synthesis of urea led to his discovery of isomers, chemical compounds that are identical in molecular content, but with slightly different arrangements of the atoms within. This work also led Wöhler to pioneer and advance the concept of functional groups, in particular the benzoyl group, and spurred other researchers to discover many more functional groups that have significantly enhanced our understanding of organic chemistry.

Wöhler's contributions extended beyond science and into academics. During Wöhler's time, the predominant practice for instruction in organic chemistry consisted primarily of lectures, and, to a much lesser degree, limited scientific demonstrations performed in laboratories thronged with students. Wöhler required all of his students to carry out prescribed experiments in the lab as part of his curricula. This practice has since been adopted in organic chemistry programs throughout the world, and today is the standard of education for nearly all stages of instruction in this field.

FRIEDRICH AUGUST KEKULÉ 1829-1896

Important work: 1850s

Terpenes were named such by August Kekulé, a German organic chemist who originally studied hydrocarbons that occurred in turpentine oil, among other essential oils. Just 5 years elapsed from the time Kekulé earned his doctorate in 1852, to the time when he founded the theory of chemical structure—a foundational concept in modern chemistry. This theory focused on the concept of a molecular skeleton to which carbon and other atoms could be attached or bonded together.

Kekulé's major work with terpenes also included the advancement of the theory that all aromatic compounds feature a hexagonal ring made of benzene; a fundamental concept in terpene science that remains undisputed.

Kekulé went on to work with Otto Wallach—arguably the "father" of terpene science—as mentioned below, and 3 of Kekulé's students earned Nobel Prizes in chemistry in the early part of the 1900s.

PIERRE EUGÈNE MARCELLIN BERTHELOT 1827-1907

Important work: 1860s

Marcellin Berthelot first came to scientific prominence when he studied the aroma of fresh soil, successfully extracting via steam distillation the organic compounds responsible for this characteristic odor[1].

Berthelot earned his doctorate in 1854 based on his work synthesizing natural fats, and it was his continued work in this area that dealt the final killing blow to the concept of vital force; work begun decades earlier by Friedrich Wöhler was finally completed by Berthelot.

Berthelot insisted that nearly all organic compounds could be synthesized, and successfully synthesized camphol and camphor from turpentine—a favorite essential oil of August Kekulé, who eventually invited Berthelot to work with him in the second half of the 19th century. However, Berthelot chose to continue his work alone, carrying out thousands of experiments and ultimately publishing 25 books and more than 1600 studies[2] before his death in 1907.

Berthelot's work modernized the classification of the terpenes, and illuminated the characterization of alpha- and beta- pinene. Additionally, some credit Berthelot as the first to advance the isoprene rule generally attributed to Otto Wallach.

Berthelot's influence in chemistry reverberated years after his death, with 2 of his students earning the Nobel Prize in chemistry in 1912.

OTTO WALLACH 1847-1931

Important work: 1880s—1910s

Otto Wallach studied at the University of Gottingen under Friedrich Wöhler, earning his doctorate in 1869. After various university appointments, Wallach went to work with August Kekulé in Bonn, Germany, where the men remained in close cooperation for nearly two decades. Wallach became a

[1] Berthelot M, et al., C R Acad Sci 1891, 112, 598.

[2] Bernadette Bensaude-Vincent Pierre-Eugène-Marcellin Berthelot—French chemist. From: https://www.britannica.com/biography/Pierre-Eugene-Marcellin-Berthelot, Accessed November 20, 2020.

professor at the University of Bonn in 1876, and it was during this time that he launched a series of investigations into the constituents of wormseed and other essential oils.

Wallach's work showed that terpenes were based on a unit of 5 carbon atoms, and could be derived from isoprene. Much of this work was focused on obtaining crystalline compounds and studying the associated rearrangement reactions to make structural determinations. This led Wallach to the conclusion that racemic crystals are denser than those of the base enantiomer, a discovery known as 'Wallach's Rule'.

Wallach successfully characterized the terpenes pinene, camphene, limonene, dipentene, phellandrene, terpinolene, fenchene, a-terpineol, and many others, and wrote a comprehensive book about this work titled *Terpene und Campher* in 1909. Additionally, of 179 original papers published by Wallach, more than 150 of them were on the subject of terpenes.

Wallach won the 1910 Nobel Prize for Chemistry, and the Davy Medal (Britain's highest award for chemists) in 1912, while more than 200 students earned their doctorate under his professorship[3] at the University of Bonn.

DAVID PRAIN 1857-1944

Important work: 1880s—1890s

David Prain was a famous botanist who served as the director of the Royal Botanic Gardens of Calcutta and Kew, India, and as the professor of Botany at Calcutta Medical College. Prain carried out significant research in Indian hemp, and produced a report for the government that—among other things—sought to determine the psychoactive compounds of the plant, in particular the active constituents of 'gánjá', concluding that "the active principle of gánjá is not a simple substance, but consists of at least two distinct things" (Prain, 1893.)

Prain's words from nearly 130 years ago serve as the first scientific claim that the active ingredients of cannabis cannot be relegated to a single compound, thus setting the stage for our case here, where we present the evidence and arguments for synergy in cannabis.

[3] Christmann, Mathias. Otto Wallach: Founder of Terpene Chemistry and Nobel Laureate 1910. Angewandte Chemie International Edition 49.50 (2010): 9580-9586.

FRIEDRICH HEUSLER 1866-1947

Important work: early 1900s

Friedrich Heusler, PhD, was the privatdocent of Chemistry at the University at Bonn. A German chemist who studied under Otto Wallach, Heusler was engaged in a major collaborative work where his section was included covering the terpenes, when he realized the scope of terpene information was so vast that it required its own text. This led him to write *The Chemistry of the Terpenes*, a book that scientists of the time hailed as "well-nigh indispensable for the scientific investigations of terpenes and their derivatives".

In *The Chemistry of the Terpenes*, Heusler appears to be the first to propose that terpene oxidation products should be classified as terpenes;

"In fact, it is impossible to develop the chemistry of the terpenes unless these oxygen-containing compounds are considered as members of the terpene series" (Heusler, 1902.)

Heusler was significantly influenced by Wallach, with Heusler dedicating his book to Wallach:

"To Professor O Wallach belongs the distinction of having elevated the methods of the terpene chemistry, by a series of superior experimental investigations, to such a plane, that the recognition and separation of the several terpene hydrocarbons have become relatively simple matters for the chemist" -Friedrich Heusler, PhD

LEOPOLD RUŽIČKA 1887-1976

Important work: 1920s—1930s

A Swiss scientist who received 8 honorary doctorates in various fields, Leopold Ružička stumbled upon terpineol while investigating constituents of the herb *Tanacetum cinerariifolium*. This work led him to publish a hypothesis that the molecular skeleton of terpenes and other large molecules were comprised of linked isoprene units, resurrecting and expanding upon the work of Otto Wallach.

In Ružička's work for some of the most important perfumeries of the time, he carried out the total synthesis of fenchone, linalool, nerolidol, farnesol, and a partial synthesis of pinene[4]. Ružička also developed the biogenetic isoprene rule, and was the first to define terpenes according to how many linked units of isoprene are in each molecule.

Ružička published nearly 600 original papers in his career, and was awarded the 1939 Nobel prize in chemistry for his work with higher terpenes, among other efforts.

WALTER LOEWE 1884-1963

Important work: 1930s—1950s

Walter Loewe continued the work of David Prain by delving deeper into studies on the synergy of cannabis and other plants. He is likely the first to identify cannabinol as an active compound, although it would be years before researchers discovered the compound is a degradation product of tetrahydrocannabinol.

Loewe, a German pharmacologist, developed a method of measuring synergy among chemical constituents called isobologram analysis. This method is also capable of measuring additivity and antagonism, and "has been mathematically proven and widely used to evaluate drug interactions"[5], according to leading terpene and drug interaction researchers.

ROBERT BURNS WOODWARD 1917-1979

Important work: 1940s to 1960s

Robert Burns Woodward intensively studied terpenes and made exemplary use of ultraviolet spectroscopy to determine their molecular structure, while also carrying out the synthesis of complex natural compounds. After studying as a young man at Massachusetts Institute of Technology, Woodward then went on to Harvard, where he remained for most of his career.

Woodward is most known for his 1956 total synthesis of reserpine; a substance derived from the Indian shrub Rauwolfia, as well as his 1960 synthesis of chlorophyll. His work led to the development of the Woodward Rules, which predict facets of stereochemistry and reactions in organic chemistry. He was awarded the Nobel Prize in Chemistry in 1965 for this and other work with natural products.

4 Leopold Ruzicka—Biographical. NobelPrize.org. Nobel Media AB 2020. From: https://www.nobelprize.org/prizes/chemistry/1939/ruzicka/biographical/ Accessed 11/20/2020

5 Huang, Ruo-Yue et al. "Isobologram Analysis: A Comprehensive Review of Methodology and Current Research." Frontiers in pharmacology vol. 10 1222. 29 Oct. 2019

WILHELM TREIBS 1890-1978

Important work: 1950s

Wilhelm Triebs was a German chemist who received his doctorate under Otto Wallach. From 1951 to 1961, he served as chair of Leipzig University, earning several high honors during his time at this esteemed school.

Although Triebs primarily worked with azulenes (a molecular skeleton found in only 2 terpenoids), his work with the sesquiterpene and dietary cannabinoid beta-caryophyllene has earned his place as a Terp Tsar. Triebs was the first to successfully isolate the crystalline form of caryophyllene, and elucidated its much-debated structure in 1952.

Triebs was a member of the German Academy of Sciences as well as the Saxon Academy of Sciences.

FRANTIŠEK ŠORM 1913-1980

Important work: 1950s

František Šorm worked extensively with terpenes and terpenoids; particularly sesquiterpenes and sesquiterpenoids, synthesizing a large number of these compounds while also elucidating the structures of many. Šorm also worked with farnesyl and farnesol, using these compounds as insecticidal control agents to mimic the hormones of juvenile Galleria Mellonella (great wax moth), against both this and other species of insects[6].

A Czech, Šorm founded the Institute for Organic Chemistry and Biochemistry arm of the Czechoslovak Academy of Sciences, and was professor of organic chemistry at the University of Prague in the early 1950s.

DHR BARTON 1918-1998

Important work: 1950s—1970s

Sir Derek Harold Richard Barton validated some of the work of František Šorm, particularly that regarding the structure of beta-caryophyllene. Although recognized worldwide for a variety of important discoveries, Barton is known among terpene researchers for developing what is referred

[6] Václav Jarolím, Karel Hejno, František Sehnal, František Šorm, Natural and synthetic materials with insect hormones activity 8. Juvenile activity of the farnesane-type compounds on Galleria Mellonella,

Life Sciences, Volume 8, Issue 16, Part 2, 1969, Pages 831-841

to as the Barton-McCombie deoxygenation, where a hydroxyl functional group is replaced by an alkyl group, changing the function of the molecule. Barton published a comprehensive text detailing this and other work titled *Reason and Imagination: Reflections on Research in Organic Chemistry*, a nearly 900-page collection of his papers meant to inspire originality in chemistry.

Barton served many appointments at universities in England and the United States, was awarded the Nobel Prize in Chemistry in 1969, and was knighted in 1972.

RAPHAEL MECHOULAM

Important work: 1960s to Present

Raphael Mechoulam is an Israeli scientist who discovered Δ9-tetrahydrocannabinol (THC)—the primary psychoactive constituent in cannabis—in 1964, paving the way for the later discovery of the endocannabinoid system. Mechoulam was successful in the first total synthesis of THC, cannabidiol (CBD), and cannabigerol (CBG), and discovered the cannabinoid anandamide, while his PhD student, Shimon Ben-Shabat discovered 2-arachidonoyl glycerol (2-AG).

The term 'entourage effect' was coined in 1988 by Mechoulam and his colleagues, although it was Ethan Russo who later correctly suggested that this synergistic action was likely due to the presence of terpenes and terpenoids, and, to a lesser but still important degree, flavonoids.

Mechoulam's work primarily focuses on cannabinoids, which are based on diterpenes.

Russ Hudson contacted Dr. Mechoulam and asked whether he thought cannabis research should continue to focus on individual cannabinoid compounds, or on the synergy between compounds. His response:

"We reported the entourage effect many years ago, but unfortunately there is limited research on this effect. I believe that some but not all cannabinoid effects are enhanced (or somewhat modified) by some terpenes, but we lack solid data. I wish we had. Anyway, cannabis users generally prefer cannabis extracts rather than pure THC" —Dr. Raphael Mechoulam, April, 2021.

MAHMOUD A. ELSOHLY

Important work: 1980—Present

Mahmoud A. ElSohly is a cannabis researcher based in the United States, where he is the director of the Marijuana Research Project at the University of Mississippi. Traditionally, this has been the only legal source of cannabis for medical research purposes in the U.S.

With a PhD from the University of Pittsburg, ElSohly's work has focused on determining the structure of various natural products and molecules in cannabis, the synthesis of cannabispiran, identification of genes in Δ9-tetrahydrocannabinol, and the investigation of various essential oils.

ElSohly is the professor of pharmaceutics at the Research Institute of Pharmaceutical Sciences, and the president and laboratory director of ElSohly Laboratories, Inc.

JOHN M. MCPARTLAND

Important Work: 1980s—Present

John M. McPartland has specialized in the botanical history of cannabis by studying fossil pollen, as well as studies involving synergy among cannabis oil constituents. McPartland has worked closely with Ethan Russo to determine the synergistic relationships between cannabinoids and terpenes.

McPartland founded GW Pharmaceuticals, a company that is "committed to the concept of synergy in whole plant extracts", producing one of the world's first cannabis-derived medicines to be approved for the public markets.

SHIMON BEN-SHABAT

Important Work: 1990s—Present

A student of Raphael Mechoulam, Shimon Ben-Shabat discovered an endogenous cannabinoid, 2-Arachidonoylglycerol (2-AG), and in the late 1990s was among the first scientists (together with Mechoulam) to demonstrate evidence of the entourage effect in cannabis[7].

Ben-Shabat has also studied the effect of flavone glycosides in the treatment of herpes[8].

7 Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z, Bisogno T, De Petrocellis L, Di Marzo V, Mechoulam R. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol. 1998 Jul 17;353(1):23-31.

8 Yarmolinsky L, Huleihel M, Zaccai M, Ben-Shabat S. Potent antiviral flavone glycosides from Ficus benjamina leaves. Fitoterapia. 2012 Mar;83(2):362-7.

ETHAN RUSSO

Important Work: 1980s—Present

Dr. Ethan Russo is the world's leading researcher of synergy in cannabis, particularly the synergy between cannabinoids and terpenes. While David Prain was the first to suggest more than one active constituent in cannabis, and Mechoulam et al coined the term entourage effect to describe the same concept, it was Russo who proposed that the entourage effect or synergistic action was primarily focused between cannabinoids and terpenes, particularly that between CBD and THC.

Russo has worked closely with John M. McPartland at GW Pharmaceuticals, researching synergy in cannabis and the therapeutic effects of various terpenes. Russo is a neurologist and psychopharmacology researcher, and the director of research and development for the International Cannabis and Cannabinoids Institute.

Russ Hudson contacted Russo and asked about his thoughts for the future of cannabis and terpene research. Russo provided the following quote:

"It is due in large part to cannabis that there has been a recent focus on the therapeutic potential of terpenoids and essential oils. Hopefully, this will translate into better research funding, as this group of substances has tremendous potential for contributing to improved human and animal health" —Dr. Ethan Russo, December, 2020.

SAMEKSHA KOUL

Important Work: 2000s—Present

Sameksha Koul is the assistant professor of pharmacognosy and phytochemistry at Bahrat Institute of Technology in Meerut, India. Koul's book, *A Comprehensive Treatise on Terpenes,* is a thorough overview of the terpenes, suitable for both researchers and laypersons.

COLLEEN QUINN

Important Work: 2010—Present

Colleen Quinn is a clinical aromatherapist specializing in the formulation of therapeutic terpene blends. Quinn trains the next generation of terpene blend formulators as an educator certifying professionals in the aromatherapy and terpene product formulations industry. Quinn has also worked extensively in oncology, providing therapy to cancer patients, and educating patients and their caregivers on the use of various terpene and essential oil formulations for therapies related to cancer treatment and general well-being.

Russ Hudson contacted Quinn and asked her thoughts on the therapeutic nature of terpenes. Quinn provided the following quote:

"Ultimately, the essence of plants that makes them therapeutic is terpenes, primarily because of the entourage effect or the theory that these phytochemicals work better together than as individual compounds" —Colleen Quinn, March 2021.

SUSAN TRAPP

Important Work: 2000s—Present

Susan Trapp has spent decades investigating terpenes, particularly the molecular evolution of terpenes and terpenoids, the horizontal gene transfer of trichothecenes, and in general the genomic architecture of plant terpenoids. Trapp is the founder of Terpedia.com, and, as of the summer of 2021, conducts bioassays and characterizations of cannabinoids, terpenes, and other phytochemicals for the Southwest College of Naturopathic Medicine.

Russ Hudson contacted Trapp and asked her thoughts on the viability of sourcing individual terpene constituents from cannabis. Trapp provided the following quote:

"Cannabis is certainly a viable industrial/medical source for individual terpene/enoids constituents, however, it is most likely not cost effective. There are other plant sources that may have higher quantities of individual terpene that will be much more cost effective and efficient to extract terpenes/enoid, certainly for the major abundant terpenes. For example, celery is high in myrcene and a much cheaper plant to grow than cannabis. Cannabis may be the only source for some of the minor terpenes/enoids being far less abundant and some potentially unique to cannabis.

Furthermore, given the field of bioengineering and biotechnology, it is a relatively straightforward problem to biosynthesize these compounds from microbes. This is already being pursued by a number of companies. Whether it is cost effective—is to be determined and individual terpene constituent dependent" —Susan Trapp, PhD, July 2021.

Terp Tsars Review

Answer the following questions to test your knowledge of this section.

Question #1: *Who was the first terpene scientist to refute vital force theory?*

Question #2: *Which German organic chemist named isoprene molecules 'terpenes?'*

Question #3: *Who advanced the theory that all aromatic compounds feature a hexagonal ring?*

Question #4: *Who studied the aroma of fresh soil?*

Question #5: *Which famous botanist first implied synergy in cannabis, stating: "...the active principle of gánjá is not a simple substance, but consists of at least two distinct things."?*

Question #6: *After studying under Otto Wallach, which German chemist went on to write The Chemistry of the Terpenes, where he proposed that terpene oxidation products should be classified as terpenes?*

Question #7: *Which German pharmacologist developed a method of measuring synergy among chemical constituents called isobologram analysis?*

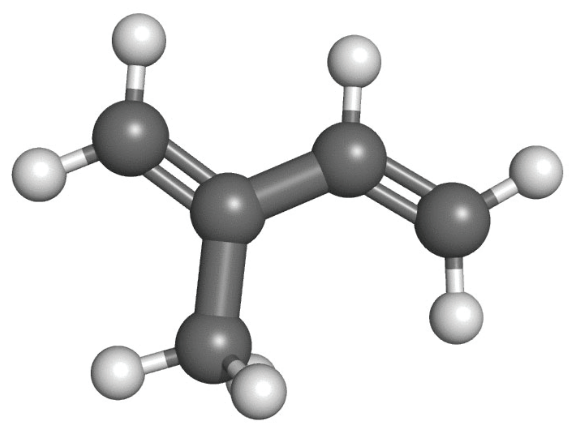
Question #8: *Which Israeli scientist discovered delta-9-THC, the endocannabinoid system, and coined the term 'entourage effect'?*

Question #9: *Which researcher is known for their work on the synergy between THC and CBD?*

For the answer key to Terp Tsars, please go here: http://thebigbookofterps.com/bbot-101-chapters-quiz-answer-key/

TERPENES 101

Isoprene



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Scientific Artwork by TheVisualThinker.

Figure 1: Isoprene is the basic building block of terpenes

Terpenes in the Plant Kingdom

Terpenes are small molecules that are produced primarily by plants (including marine plants), but also by fungi, some insects, nematodes, and microorganisms like bacteria. For the purpose of this book, we'll deal mostly with plant-based terpenes, and particularly those that are found in detectable concentrations in cannabis. Terpenes are found in the volatile aromatic or essential oils of plants, and serve an astounding array of functions. To begin, we will examine why and how plants use terpenes.

HOW DO PLANTS USE TERPENES?

Terpenes can be viewed as the tools of the plant world. Plants use these compounds as weapons of attack, implements of defense, to call for reinforcements, to heal and protect wounds, to promote propagation, as chemical weapons against insect, fungi, and bacteria attacks, to communicate

with other plants and organisms in their environment, and as an effective sunscreen, among other uses discussed in this text. Cannabis, in particular, likely uses terpenes for many of these purposes. However, as of the printing of this book, no studies have been conducted on how cannabis in particular uses specific terpenes. But because terpenes such as pinene, limonene, and linalool are the same from plant to plant, we can confidently surmise that similarities in function and purpose exist between plant species.

Studying how plants use terpenes invariably leads to a new perspective of the natural environment. For instance, consider the terpenes *pinene*, and *myrcene*, made by most varieties of conifer trees. These and several other terpenes that are also prominent in cannabis are used by evergreen trees to heal wounds. When a tree is wounded—perhaps by bear claws, insects, human activity, or storm damage—terpenes are produced rapidly in such quantity that a dense and viscous sap forms. This sap is exuded to cover the wound and protect it, especially from bacterial, fungal, and viral infection, but also as a barrier to prevent the incursion of insects or other pests.

Consider what we as humans might do when we are injured; we cover our wounds securely and apply antiseptics and/or antibacterial medications. We allow our plasma and blood to fill in the wound and protect it. Comparatively, in essence, a tree's sap is its plasma or blood, and that blood is comprised mostly of terpenes.

The scenario above is just one of the many ways that trees and plants use terpenes. In other cases, as described below, plants use terpenes for a fascinating and diverse variety of purposes, showing that the natural world is highly complex and advanced in ways that cannot be completely understood without an understanding of terpenes.

**Insect Traps*

Plants like cannabis sativa manufacture some terpenes that are designed to passively defend against insect attack. One of the most effective methods for managing pests is to physically immobilize them. Cannabis plants do this by producing copious amounts of extremely sticky glandular trichomes containing terpenes like pinene and myrcene; the reality is that the 'sticky icky' produced by cannabis was never actually meant for humans. Instead, those terpene-packed trichomes act as a dangerous type of glue trap, meant to immobilize and eventually kill insects.

In fact, this method of insect control has been so effective in the natural world that pest control companies have mimicked it perfectly, often using terpenes to do so. For instance, if we were to examine the composition of a glue trap meant to deal with mice or rats, we might see terpenes like eugenol, which is made by plants such as cinnamon, nutmeg, and basil. In 2018 the Tomcat variety of mouse glue traps featured eugenol for "enhanced stickiness". When we examine the composition of natural 'glue traps' made by plants like cannabis, we again see terpenes like pinene, myrcene, and eugenol, which are also used by conifer trees to protect and heal wounds with a thick sap as discussed previously.

Already, it's clear that the same terpene can have multiple functions.

Insect Repellants

Some terpenes can act as insect repellants. Much like the case of the glue traps presented above, humans learned to use terpenes as insect repellants by studying the natural world of plants. For example, consider the wide proliferation of citronella products, which are designed to repel mosquitos and other biting pests. Citronella candles, torches, and oils can be purchased readily in most modern countries for use in managing mosquitos and other pests in outdoor areas. The central ingredient in these products is citronellal, a terpene.

Other popular mosquito, tick, flea, and biting fly repellants include the OFF! brand of insect sprays, which makes use of the terpenes found in the oil of the lemon eucalyptus, including citronellal and geraniol, the latter of which is a terpene found in high concentrations in some varieties of cannabis, most notably Northern Lights.

In the natural world, many plants use terpenes to repel insect attacks, but some plants go beyond merely seeking to discourage insects with unpleasant aromas, textures, or flavors. Plants that manufacture a variation of farnesene—a terpene found commonly but in low concentrations in cannabis—were shown to trick aphids into dispersing. The terpene (E)-b-farnesene, released by plants like maize during herbivore attacks, mimics an alarm pheromone that is normally released by aphids to warn others nearby to flee[9].

Humans have sought to better understand how plants use terpenes as insect repellants. One interesting study genetically engineered a variety of Arabidopsis thaliana (thale cress or mouse-ear cress) to overexpress the monoterpene linalool, which is one of the most common terpenes in cannabis. The higher concentrations of linalool in the modified thale cress successfully repelled aphids (a bug that sucks the essential juices out of young plant leaves), whereas the unmodified plants did not enjoy such relief[10].

Other terpenes have been theorized to function merely as a result of individual preference—some insects just don't like the smell of some terpenes, just as some people don't. Not everyone enjoys the

9 Bernasconi ML, Turlings TCJ, Ambrosetti L, Bassetti P, Dorn S: Herbivore-induced emission of maize volatiles repel the corn leaf aphid Rhopalosiphum maidis. Entomologia Experimentalis et Applicata 1998, 87:133-142

10 Aharoni, A. et al. Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. Plant Cell 15, 2866–2884 (2003)

earthy smell of the myrcene found in Kush Valley cannabis varieties, or the sharp, citrusy scent of Super Silver Haze, which has a high concentration of terpinolene.

Insecticides

Plants can also produce terpenes that biologically attack insects and act as a direct pesticide. Limonene, pinene, cineole, linalool, myrcene, and pulegone (common terpenes in cannabis), have all been shown to possess fumigant properties[11].

In addition to toxic airborne terpenes, topical toxicity of monoterpenes such as limonene, pulegone, and variations of terpinene—all terpenoid molecules often found in cannabis—is strong in several insects including flies, corn borer larvae, and some mites[12]. This means that, for some pests, merely touching a plant that manufactures and releases these or other topically toxic terpene compounds can prove incapacitating or fatal.

Other natural terpene pesticides common to cannabis and many other plants include caryophyllene, myrcene, and pinene, which have been shown to be toxic to mosquitos, houseflies, some aphids, and tobacco cutworms[13].

Insect Attractants—Pollinators

Some terpenes are designed to attract plant pollinators like bees, wasps, butterflies, hummingbirds, etc. Found in the volatile essential oils of cannabis and other plants, most terpenes are strongly aromatic molecules, which explains why they evaporate so readily—a more volatile substance is easier to transfer from a plant to the air of the surrounding environment and into the scent receptors of beneficial pollinators.

For instance, insects that are attracted to terpenes like limonene, which has a strong citrus smell, are likely to land on cannabis, where these terpenes are sometimes found in abundance, depending on the variety. Pollinators that harvest or otherwise transport pollen from lemon flowers would also likely be interested in exploring cannabis flowers, as both have high concentrations of limonene. While

11 Dambolena, José S., María P. Zunino, Jimena M. Herrera, Romina P. Pizzolitto, Vanessa A. Areco, and Julio A. Zygadlo. Terpenes: Natural Products for Controlling Insects of Importance to Human Health—A Structure-Activity Relationship Study. Psyche: A Journal of Entomology 2016.2016 (2016).

12 Lee, Sang Kyun, Insecticidal properties of monoterpenoids and their derivatives as a new natural means of crop protection (1997). Retrospective Theses and Dissertations. 11478.

13 Giovanni Benelli, Roman Pavela, Riccardo Petrelli, Loredana Cappellacci, Giuseppe Santini, Dennis Fiorini, Stefania Sut, Stefano Dall'Acqua, Angelo Canale, Filippo Maggi. The essential oil from industrial hemp (Cannabis sativa L.) by-products as an effective tool for insect pest management in organic crops, Industrial Crops and Products, Volume 122, 2018, Pages 308-315.

cannabis propagates quite well by transferring genetic material via airborne pollen alone, insect pollinators can help pollen reach flowering female plants over larger distances, and in places not easily reachable by wind currents carrying pollen.

But most plants—especially those that flower and produce fruits—are much more dependent on attracting pollinators, which helps explain the wide variety of aromatic terpenes in the plant world. Aromatic terpenes help to lure beneficial pollinators via airborne scents, while flavonoids generally produce the colors that further draw these pollinators in (see more about flavonoids in the next chapter). Terpenes like farnesene, ocimene, and myrcene are just some examples of aromatic molecules that attract pollinators.[14]

**Insect Attractants—Predators & Indirect defense*

When a plant uses a terpene or other chemical that works by attracting the predator of an herbivore, this is called indirect defense. In simple terms, this means that a plant can call out to the enemies of its enemies and recruit assistance. The language spoken between plant and defender is terpenes—more on this below in the 'Terpenes as Communication' section.

One example of this is when the roots of maize (corn) plants release a large amount of a variation of the terpene caryophyllene in response to an attack by insect larvae. The rush of caryophyllene attracts nematodes (a type of worm) that prey on the insect larvae.[15] Bark beetles have also been shown to be predatorized upon by carnivorous insects responding to the release of volatile terpenes from trees[16] .

A variation of the sesquiterpene farnesene mentioned earlier—(E)-b-farnesene, the exact terpene that some plants use to trick aphids into fleeing in alarm—has also been shown to be used by pine trees in a form of indirect defense. In this case, the sesquiterpene is released into the air when a pine sawfly lays eggs on the extremities of a pine tree. The scent of the volatile terpenoid attracts the attention of a species of wasp that parasitizes the eggs[17].

14 Hartmann, Marie-Andrée. The way the dioecious plant Actinidia deliciosa attracts bees: critical role of volatile terpenes released from kiwifruit flowers of both genotypes. Journal of Experimental Botany 60.11 (2009): 2953-2954.

15 Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ: Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 2005, 434:732-737.

16 Marco Michelozzi (1999) Defensive roles of terpenoid mixtures in conifers, Acta Botanica Gallica, 146:1, 73-84.

17 Hilker, M., Kobs, C., Varama, M. & Schrank, K. Insect egg deposition induces Pinus sylvestris to attract egg parasitoids. J. Exp. Biol. 205, 455–461 (2002).

Interesting research is now showing that some plants might call birds to their aid when attacked. When white birch trees were attacked by nests of autumnal moth caterpillars, the trees released a blend of terpenes including a variation of ocimene and linalool. This terpenoid blend attracted several species of birds that attacked and fed on the caterpillars[18].

Defense against Competitors—Allelopathic Agents

Plants can use terpenes to influence the growth of other plants competing for the same resources. This can be accomplished by releasing terpenes and/or terpenoid mixtures into the ground via the root system, or by the evaporation of volatile terpene molecules into the atmosphere. When the chemicals released by one plant affect the growth or survival of competing plants, this is called allelopathy, and the terpenes and other chemicals involved are referred to as allelochemicals.

Two examples of common plants that use terpenes to influence the growth of cohabitant plant species include at least one variety of marigold from South America, and the sage brush of Southern California. In the case of the marigold, the plant uses a variation of the terpene ocimene to prevent the germination of other plants[19]. Sage brush also prevents the germination of competing or cohabitating plants, but it uses a variation of the terpene cineole for this purpose[20].

Examples of other plants that produce and use terpenes to influence cohabitant species include the date palm,[21] and salvia. In the case of salvia (specifically s. leucophylla and s. mellifera), the plant releases a terpene blend of cineole and camphor into the air, which inhibit the germination of the seeds of other plants[22].

Allelopathic properties are also attributable to cannabis, and it's likely that the chemicals responsible for this are terpenes, terpenoids, and flavonoids, although more research is needed to confirm this with specificity. One recent study showed that a water-based extract of cannabis sativa L. influenced the ability of rye, lupine, and rape plants to germinate, and subsequently affected the vigor of the

18 Mantyla E, Alessio GA, Blande JD, Heijari J, Holopainen JK, Laaksonen T, Piirtola P, Klemola T: From plants to birds: higher avian predation rates in trees responding to insect herbivory. PLoS ONE 2008, 3:e2832 2810.1371/Journal.pone.0002832.

19 M. Liza López, Norma E. Bonzani, Julio A. Zygadlo. Allelopathic potential of Tagetes minuta terpenes by a chemical, anatomical and phytotoxic approach, Biochemical Systematics and Ecology, Volume 36, Issue 12, 2008, Pages 882-890.

20 Jane Buckle PhD, RN, in Clinical Aromatherapy. Basic Plant Taxonomy, Basic Essential Oil Chemistry, Extraction, Biosynthesis, and Analysis (Third Edition), 2015.

21 Hadrami A.E., Daayf F., Hadrami I.E. (2011) Secondary Metabolites of Date Palm. In: Jain S., Al-Khayri J., Johnson D. (eds) Date Palm Biotechnology. Springer, Dordrecht.

22 Muller, C. H. Inhibitory terpenes volatilized from Salvia shrubs. Cornelius H. Muller Bull. Torrey Bot Club Bulletin of the Torrey Botanical Club 92(1):38 · January 1965.

cohabitant species[23]. An earlier study showed that cannabis sativa significantly inhibited the growth of the Parthenium hysterophorus[24] (Santa Maria plant).

**Egg, Ova, & Larvae Control*

Some terpenes can inhibit, delay, or retard the growth of insect eggs and/or larvae, in a similar way to the terpenes that inhibit the development of cohabitant plant species. In fact, some of the same terpenes serve both purposes.

Terpenes that have been shown to possess significant ovicidal (ability to kill eggs) properties include limonene, linalool, geraniol, terpineol, pinene, cineole, nerolidol[25], and variations of caryophyllene[26]—all of which are terpenes that commonly occur in cannabis.

Other terpenes work by killing insect larvae directly. One study found that many monoterpenes possess larvicidal properties, but that the toxicity of these terpenes increased in modified compounds[27], such as sesquiterpenes and diterpenes.

**Herbivore Repellants—Bitter Terpenes*

Terpenes that are bitter like humulene (found in hops) and limonene (found in citrus fruits) help to ward off attack by hungry herbivores, which can include both insects and animals. Bitterness is a natural indicator that a plant may not be suitable for consumption, or perhaps that it could be poisonous, and plants use this to their advantage by producing bitter-tasting compounds like terpenes and flavonoids—even if the plant isn't actually toxic. The bitterness is cause enough for doubt.

In cannabis, monoterpenes have been shown to repel insect herbivores, while sesquiterpenes—the most bitter of the terpenes—are meant primarily to deter grazing animals[28]. In humans, research has shown

23 Krzysztof Pudełko, Leszek Majchrzak, Dorota Narożna. Allelopathic effect of fibre hemp (Cannabis sativa L.) on monocot and dicot plant species. Industrial Crops and Products, Volume 56, 2014, Pages 191-199.

24 Singh, N.B. & Thapar, R. (2003). Allelopathic influence of Cannabis sativa on growth and metabolism of Parthenium hysterophorus. Allelopathy Journal. 12. 61-70.

25 Toloza, Ariel. (2010). Bioactividad y toxicidad de componentes de aceites esenciales vegetales, en Pediculus humanus capitis (Phthiraptera: Pediculidae) resistentes a insecticidas piretroides. 10.13140/RG.2.1.4342.2480.

26 Y.-C. Yang, S.-H. Lee, W.-J. Lee, D.-H. Choi, and Y.-J. Ahn, Ovicidal and adulticidal eAects of Eugenia caryophyllata bud and leaf oil compounds on Pediculus capitis, Journal of Agriculturaland Food Chemistry, vol. 51, no. 17, pp. 4884-4888, 2003.

27 Lee, Sang Kyun, Insecticidal properties of monoterpenoids and their derivatives as a new natural means of crop protection, (1997). Retrospective Theses and Dissertations. 11478.

28 S. Casano, G. Grassi, V. Martini and M. Michelozzi (2011) Variations in terpene profiles of different strains of Cannabis sativa L. Acta Horticulturae 925:115-121.

that bitterness is the most common cause of food rejection[29], suggesting that some of the very terpenes we seek in cannabis are the same that seek to repel us.

Raw cannabis leaves are bitter most likely because of terpenes like humulene, pinene, and limonene, but the bitterness isn't always enough to completely deter herbivores from munching on the plant. Given their preferred feed, most animals would likely leave cannabis alone. But if more desirable plants are not available, many animals will hungrily consume cannabis, including deer, elk, moose, cattle, horses, goats, and other ruminants, all of which can tolerate the bitterness of the plant's leaves. And because there is already a trend among humans to eat raw cannabis leaves in salads and juices, there is ample anecdotal evidence to suggest that the bitterness of cannabis leaves won't be enough to save the plant from salad bars of the future.

However, some varieties of cannabis may be better than others at repelling herbivores, depending on the terpene profile of the strain. For example, if we analyze the terpene profile of conifer trees such as the Sitka Spruce or the Twisted Pine, we see that the trees use monoterpenes to repel the massive Red Deer of Europe and Asia[30]. Cannabis with similar terpene profiles may prove just as resistant to red deer or other animals, including humans. Of course, humans can endeavor to breed a variety of cannabis that isn't bitter.

**Terpenes as Communication*

Chemical signaling is how much of the life on Earth communicates, and the medium of majority is terpenes[31]. This type of communication occurs between plants, insects, birds, fungi, bacteria, and some invertebrates. Terpene communication appears to be easily understood regardless of which entity is speaking it and which is receiving it.

For instance, when bark beetles attack conifer trees, a war occurs that is mostly based upon terpenes, as the tree attempts to amass enough volatile terpenes to repel or kill the invaders. Meanwhile, the beetles use these chemical signals to adjust their behavior, including cooperative behavior between beetles that can sometimes outpace the tree's ability to produce enough insecticidal terpenes[32] to defeat the insects. Both entities, regardless of the animosity and deadly intent between them, speak the same language.

29 Drewnowski, Adam, and Carmen Gomez-Carneros. "Bitter taste, phytonutrients, and the consumer: a review." The American Journal of Clinical Nutrition 72.6 (2000).

30 Marco Michelozzi (1999) Defensive roles of terpenoid mixtures in conifers, Acta Botanica Gallica, 146:1, 73-84.

31 Netherlands Institute of Ecology, Terpenes are the World's Most Widespread Communication Medium, from; https://phys.org/news/2017-04-terpenes-world-widespread-medium.html Accessed December 21, 2018.

32 Blomquist GJ, Figueroa-Teran R, Aw M, Song M, Gorzalski A, Abbott NL, Chang E, Tittiger C. Pheromone production in bark beetles. Insect Biochem Mol Biol. 2010 Oct;40(10):699-712.

Because of their sheer numbers, most of the world's communication in terpenes occurs between microorganisms. Recent research has demonstrated that bacteria and fungi communicate with each other in the language of terpenes[33], and it is already well understood that trees and other plants communicate with fungi and bacteria through the soil. Comparatively speaking, it is the languages of man that are odd and rare.

However, it's logical that this chemical communication extends to humans and plants—especially cannabis. Consider that when you smell a particular variety of cannabis that you enjoy, you are receiving communication from the plant in the medium of terpenes. This is more than just an arbitrary smell, however—it tells us something about the plant, and it makes us feel a certain way, although it's often not easy to articulate precisely how. When looked at in this light, and with more research and study, it will become clear that humans can indeed receive the communication of plants—including cannabis—through the language of terpenes.

The question that remains is, can we do more than just receive this communication?

**Terpenes as Sunscreen and Air Conditioning*

Terpenes can help to keep plants cool. When it comes to plant air conditioning, the science behind this is simple: as terpenes evaporate, the resulting airflow cools the plant and helps it retain more of its vital liquids, similarly to how "swamp coolers" work in the American Southwest.

Some terpenes, and, more importantly, flavonoids, can also act as sunscreen. In terpenes this is not yet well understood, but what is clear is that as some plants are exposed to UVB radiation, they produce significantly more terpenes via resin accumulation in leaves[34], leading some researchers to theorize that this increased production of resin and terpenes acts as a sunscreen and prevents cell damage[35].

One clue to the effectiveness of terpenes as sunscreen comes from a study conducted using mice, in which the terpene d-limonene protected the animals in the study from skin damage due to UVB radiation[36], indicating that plants might use this isoprene compound similarly.

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33 Schmidt, Ruth, Victor de Jager, Daniela Zühlke, Christian Wolff, Jörg Bernhardt, Katarina Cankar, Jules Beekwilder, Wilfred van Ijcken, Frank Sleutels, Wietse de Boer, Katharina Riedel, and Paolina Garbeva. Fungal volatile compounds induce production of the secondary metabolite Sodorifen in Serratia plymuthica PRI-2C. Scientific Reports 7 (2017).

34 Zavala, Jorge, and Damian Ravetta. The effect of solar UV-B radiation on terpenes and biomass production in Grindelia chiloensis (Asteraceae), a woody perennial of Patagonia, Argentina. Plant Ecology 161.2 (2004): 185-191.

35 Wen Jing Zhang, Lars Olof Björn, The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants, Fitoterapia, Volume 80, Issue 4, 2009, Pages 207-218.

36 Uddin, Ahmed. (2014). d -limonene prevents ultraviolet irradiation: Induced cyclobutane pyrimidine dimers in Skh1 mouse skin. World Journal of Dermatology. 3. 64. 10.5314/wjd.v3.i3.64.

What we do know for certain is that flavonoids offer distinctive protections against the sun's harmful rays; see more on this in the next chapter.

**Defense against Fungi & Bacteria*

If the number of terpene-based antibacterial and antifungal products on the market today are any indicator, humans are well aware of the use of terpenes to fight bacteria and fungi, although they might not always understand the mode of action.

Variations of cineole, cymene, limonene, linalool, pinene, terpinene, and terpinolene, among others, have all been found to possess antifungal activity[37], and the monoterpene geraniol in particular has been shown to be active against dermatophytes and yeasts[38]. These are among the top terpenes most commonly found in cannabis.

Terpenes can work effectively against bacteria as well. In a study involving P. uviferum (cypress trees), variations of caryophyllene and humulene were shown to protect the trees against microbial attack[39]. The authors of the study concluded that the tree's resistance to bacterial attack was entirely due to sesquiterpenes and diterpenes, although many of these do not occur in cannabis.

Other studies have concluded that the cell membrane of bacteria can be infiltrated and the cell's contents spilled (effectively killing the bacteria cell) by terpenes, including nerolidol and menthol[40,41].

WHEN DO PLANTS PRODUCE AND USE TERPENES?

Just as terpenes can be toxic to insects, fungi, and bacteria, they are also cytotoxic to plants—even the very ones that produce the terpenes in the first place. For this reason, terpenes are made and stored in protective enclosures within the plant, such as secretory idioblasts (isolated plant cells), and in

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37 Nazzaro, Filomena, Florinda Fratianni, Raffaele Coppola, and Vincenzo De Feo. "Essential Oils and Antifungal Activity." Pharmaceuticals 10.4 (2017).

38 Diogo Miron, Fernanda Battisti, Fernanda K. Silva, Aline D. Lana, Bruna Pippi, Bruna Casanova, Simone Gnoatto, Alexandre Fuentefria, Paulo Mayorga, Elfrides E.S. Schapoval, Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts, Revista Brasileira de Farmacognosia, Volume 24, Issue 6, 2014, Pages 660-667.

39 Solís, C. & Becerra, J. & Flores, C. & Robledo, J. & Silva, Mario. (2004). Antibacterial and antifungal terpenes from Pilgerodendron uviferum (D. Don) Florin. Journal of The Chilean Chemical Society—J CHIL CHEM SOC. 49.

40 Inoue, Yoshihiro, Akiko Shiraishi, Toshiko Hada, Kazuma Hirose, Hajime Hamashima, and Jingoro Shimada. The antibacterial effects of terpene alcohols on Staphylococcus aureus and their mode of action. FEMS Microbiology Letters 237.2 (2004): 325-331.

41 Trombetta, Domenico et al. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial agents and chemotherapy vol. 49,6 (2005): 2474-8.

trichomes[42]. If we take the pennyroyal plant as an example, we see that monoterpenes are formed in the young plant tissue, with more complex compounds like sesquiterpenes and diterpenes occurring in the older tissue[43].

Plants generally produce terpenes at higher temperatures—usually when the sun is shining—and terpenes tend to evaporate as the daytime temperature rises. In this way, plants are literally making and losing terpenes at the same time. For anyone that's cultivated cannabis outside, you probably noticed that your plants smelled more strongly in the morning, before the temperature rose. That's because the terpenes in your plants hadn't started to evaporate off yet, which meant that the overall concentration of terpenes was stronger and thus the smell was more potent. For this same reason, experienced cannabis cultivators recommend harvesting outdoor plants in the morning.

This phenomenon of daytime terpene emissions has also been observed by researchers studying pollinators of kiwifruit; the evaporation of volatile terpene compounds primarily occurred during the day, when pollinators were most active[44]. As discussed previously, pollinators rely at least partly on the release of terpenes to find their favorite flowers, thus the close association and timing makes sense.

CHEMICAL NATURE OF TERPENES

Acetic Acid

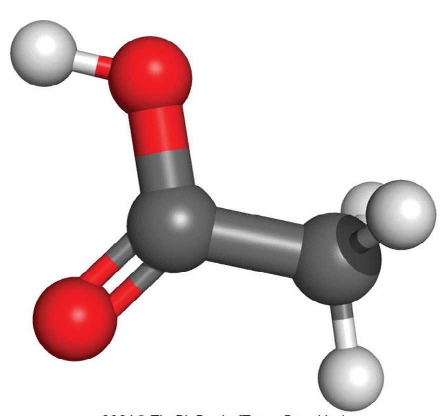

Figure 2: Isoprene, the basic building block of most terpenes, is formed by 2 acetic acid molecules.

[42] Marco Michelozzi (1999) Defensive roles of terpenoid mixtures in conifers, Acta Botanica Gallica, 146:1, 73-84.

[43] J. Battaile and W.D. Loomis, Biosynthesis of Terpenes, Biochim. Biophys. Acta, 51, 545 (1961).

[44] Hartmann, Marie-Andrée. The way the dioecious plant Actinidia deliciosa attracts bees: critical role of volatile terpenes released from kiwifruit flowers of both genotypes. Journal of Experimental Botany 60.11 (2009): 2953-2954.

Plants form terpenes by interlinking chains of 2-methylbutane—commonly called isoprene—an organic compound made when two acetic acid molecules combine to form isopentenyl pyrophosphate. This occurs within the cytosol or aqueous area of plant cells. Terpenes can occur as hydrocarbons, alcohols, ketones, esters, aldehydes, and other forms, depending on the functional groups contained within the molecule, and other features. Functional groups commonly predict and determine the activity of the molecule.

Each isoprene molecule is comprised of 5 carbon units, with molecules generally arranged in a head-to-tail fashion in most terpenes discussed here. The head of each molecule is typically comprised of isopropyl, while the tail part is ethyl. Classified as hydrocarbons, terpenes are made from the mother hydrocarbon, isoprene, which has a formula of C_5H_8. This denotes the fact that isoprene has 5 carbon atoms bound to 8 hydrogen atoms. To form a terpene, one or more of these units of isoprene must be joined together. This process of interlinking isoprene units to form terpenes is known as Wallach's Law, and was first developed by Otto Wallach and Leopold Ružička.

Different terpene classes are formed in different biosynthetic pathways in plants. For instance, monoterpenes are formed via the methyl erythritol phosphate pathway, while sesquiterpenes are formed via the mevalonate pathway. Terpene classes are determined by how many C_5H_8 isoprene units are in the terpene molecule:

- Hemiterpenes: One unit of isoprene has 5 carbon atoms and 8 hydrogen atoms.
- Monoterpenes: When 2 units of isoprene combine, the resulting $C_{10}H_{16}$ molecule (10 carbon atoms and 16 hydrogen atoms = 2 units of isoprene) is called a monoterpene.
- Sesquiterpenes: When 3 units of isoprene combine, the resulting $C_{15}H_{24}$ molecule (15 carbon atoms and 24 hydrogen atoms = 3 units of isoprene) is called a sesquiterpene.
- Diterpenes: When 4 units of isoprene combine, the resulting $C_{20}H_{32}$ molecule (20 carbon atoms and 32 hydrogen atoms = 4 units of isoprene) is called a diterpene.
- Sesterterpene: When 5 units of isoprene combine, the resulting $C_{25}H_{40}$ molecule (25 carbon atoms and 40 hydrogen atoms = 5 units of isoprene) is called a sesterterpene.

Continuing the above classification system, triterpenes have 6 isoprene units, tetraterpenes have 8, and so on. There can be dozens, hundreds, or even thousands of isoprene units in a terpene chain. The larger isoprene chains are generally referred to as polyterpenes.

In the cannabis plant, the majority of terpenes consist of monoterpenes, sesquiterpenes, and diterpenes, examples of which include:

- Myrcene, pinene, limonene, geraniol, eucalyptol, and linalool are all monoterpenes.
- Beta-caryophyllene, farnesol, and humulene are all sesquiterpenes.
- Phytol and some cannabinoids are diterpenes.

Cannabinoids are Terpenes, too

Interestingly, cannabinoids can be terpenes, and terpenes can be cannabinoids. For instance, the most common terpene by weight in cannabis, beta-caryophyllene, is also a dietary cannabinoid, while cannabidiol (CBD) is based on a diterpene molecular skeleton. THCA is a diterpenoid, while Delta-8- and Delta-9- tetrahydrocannabinol are tricyclic terpenoids. Terpenes and cannabinoids are both produced in the trichomes or secretory glands of cannabis, and in fact cannabinoids are known as terpenophenolic compounds, derived in the same biosynthetic pathways as terpenes[45].

This text will cover cannabinoids and their relationship with the human body and interactions with the other compounds found in cannabis separately in the coming chapters, even though many important cannabinoids are, technically speaking, a more-stable type of terpene.

Among the many monoterpenes, sesquiterpenes, and diterpenes, classifications of higher organizations of the basic isoprene structure are grouped into their own sub-classifications, based on arrangement and positions of the molecules in chains and hexagonal rings. At least one of these rings is comprised of benzene, while others can be formed from different substances.

The following are just some examples of these molecular arrangement sub-classifications:

- Acyclic monoterpenes: ocimene and linalool are acyclic monoterpenes, which are arranged in a linear fashion.
- Non-head-to-tail monoterpenes: the isoprene units are joined together not head to tail, but instead tail or head of one, to the central carbon atoms of the other.
- Monocyclic terpenes: limonene, a-phellandrene, and a-terpinene are monocyclic monoterpenes, which have a molecular structure with one ring.
- Bicyclic monoterpenes: pinene, borneol, and camphor are bicyclic monoterpenes, or those that have a molecular structure containing two rings.

[45] Turner, C.E., ElSohly, M.A., Boeren, E.G., 1980. Constituents of Cannabis sativa L. XVII. A review of the natural constituents. J. Nat. Prod. 43, 169–234.

- Cyclopentanoid monoterpenes: monoterpenes based on a cyclopentanoid carbon skeleton where five carbon atoms have been arranged in a ring.
- Acyclic sesquiterpenes: sesquiterpenes where the molecular structure is arranged linearly.
- Bicyclic sesquiterpenes: sesquiterpenes where the molecular structure features two rings.
- Linear diterpenes: an acyclic diterpene where the molecular structure is formed linearly.
- Diterpenes with rearranged skeletons: a diterpene where the original molecule has been rearranged as a structural isomer.

TERPENOIDS

The broadest categorization of a terpene depends on whether the molecule contains oxygen atoms. Terpenes without oxygen molecules are simply called 'terpenes,' while terpenes that do contain one or more oxygen atoms are called terpenoids, where the 'oid' specifically denotes that oxygen atoms are present. For instance, when we refer to cannabinoids, terpenoids, or flavonoids, we know that all of these compounds feature one or more oxygen atoms. Oxygen atoms can change the function of a molecule or the way it interacts with other molecules, or both.

GENERAL STABILITY OF TERPENES—VOLATILITY

Terpenes are constructed with differing levels of stability. As the number of isoprene units in each terpene structure increases, the resulting terpene class becomes less volatile, with diterpenes including cannabinoids being considerably stable compared to monoterpenes and sesquiterpenes This is important information for people who work with or consume cannabis, because it explains why the more volatile mono- and sesqui- terpenes like myrcene, pinene, linalool, and limonene, for example, vaporize at much lower temperatures than the diterpenes like cannabinoids. It's also worth noting that diterpenes are much less numerous in a given oil or extract than the mono- and sesqui-terpenes.

CONCENTRATION OF TERPENES IN PLANTS

As you can see, the terpene family is extremely large and complex, with many tens of thousands catalogued to date. The concentration of essential oils and the terpenes within them differs from plant to plant, and is based on numerous environmental factors. In most cannabis samples, each laboratory-tested variety contains 35 to 50 identified terpenes (based mostly on gas chromatography testing), although most labs only actually test for about 10 common terpenes. Researchers have reported yields of cannabis essential oils ranging from .05 to .29% in buds[46], with researchers Ross and ElSohly reporting concentration of essential oils after curing to 0.80% within the bud. This concentration occurs because the weight of the buds is reduced dramatically by drying, thereby increasing the total net weight of essential oil within the dried bud, as most of the bud weight is actually water.

Modern cannabis chemovars can produce 3.5% or more terpene content in inflorescences[47], or to 1.5% of inflorescence dry weight[48]. While the general content of these terpene profiles is largely inherited genetically, the total weight of essential oils and terpenes is much more dependent on environmental influences[49], including cultivation methods.

HOW DO HUMANS PERCEIVE TERPENES?

If—as many consumers and experts maintain—there are biochemical, pharmacological and phenomenological distinctions between available cannabis 'strains,' such phenomena are most likely related to relative terpenoid contents and ratios[50].

—Dr. Ethan Russo

How a terpene molecule is structured is important for human perception. Human olfactory receptors contain small depressions that are shaped to receive certain molecules. When those specifically shaped holes are filled with the correspondingly shaped volatile molecule, the human brain perceives this as a smell. In this way, humans are quite literally built to integrate with terpenes. In fact, the human nose is sensitive enough to detect even subtle differences in the same terpene molecule. One of the most recognizable examples of this is limonene, which is a top 5 terpene in cannabis. Limonene occurs as two different isomers or mirror versions; one is commonly known as d-limonene, while the other is l-limonene, where the d isomer is perceived by human olfactory receptors as citrusy, and the l version as fuel and/or pine. Other differences in the same molecule, such as functional group substitutions, are also detectable by some, but not all, humans.

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46 John M. McPartland DO, MS & Ethan B. Russo MD (2001) Cannabis and Cannabis Extracts, Journal of Cannabis Therapeutics, 1:3-4, 103-132.

47 Fischedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R. Metabolic fingerprinting of Cannabis sativa L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry. 2010 Dec;71(17-18):2058-73.

48 Giese MW, Lewis MA, Giese L, Smith KM. Development and Validation of a Reliable and Robust Method for the Analysis of Cannabinoids and Terpenes in Cannabis. J AOAC Int. 2015 Nov-Dec; 98 (6): 1503-22.

49 S. Casano, G. Grassi, V. Martini and M. Michelozzi (2011). Variations in terpene profiles of different strains of Cannabis sativa L. Acta Horticulturae 925:115-121.

50 Russo, Ethan B. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British journal of pharmacology vol. 163,7 (2011): 1344-64.

Terpenes 101 Review

Answer the following questions to test your knowledge of this section.

Question #1: *Name 3 ways that plants use terpenes:*

________________, ________________, ________________

Question #2: *Terpenes are based on interlinked chains of what compound?*

____

Question #3: *Terpene molecules are arranged:*

a. Tail-to-tail
b. Head-to-sidechain
c. Head-to-tail
d. Sidechain-to-tail

Question #4: *The chemical formula for monoterpenes is:*

a. C_5H_8
b. $C_{10}H_{16}$
c. $C_{15}H_{24}$
d. $C_{10}H_{18}$

Question #5: *The chemical formula for sesquiterpenes is:*

a. $C_{15}H_{24}$
b. $C_{15}H_{16}$
c. $C_{15}H_{22}$
d. $H_{10}C_{18}$

Question #6: *Bicyclic monoterpene molecules are defined by what key feature:*

____

Question #7: *What changes a terpene to a terpenoid?*

____

Question #8: *Describe how humans perceive terpenes:*

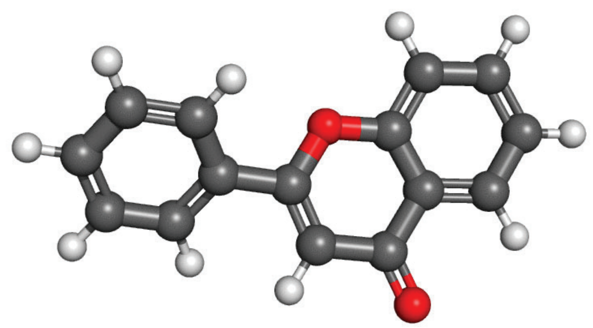
____

For the answer key to all '101' chapters, please go here:http://thebigbookofterps.com/bbot-101-chapters-quiz-answer-key/

FLAVONOIDS 101

Flavanoid

C6-C3-C6 Skeleton



2021© TheBigBookofTerps, Russ Hudson.
Scientific Artwork by TheVisualThinker.

Figure 3: This is the basic structure of most flavonoids present in cannabis.

Flavonoids in the Plant Kingdom

Flavonoids are phytochemicals made by plants, some bacteria[51], and some fungi[52]. Most edible plants produce flavonoids, and the human diet usually contains high amounts of these compounds. For the purpose of this book, we'll discuss primarily plant-derived flavonoids, particularly those found in cannabis.

Flavonoids are in many of the foods we eat, and it's generally thought that these compounds serve chiefly to attract pollinators by producing the yellow hues of some flowers and plant pollen. In fact,

[51] Wang Y, Chen S, Yu O. Metabolic engineering of flavonoids in plants and microorganisms. *Applied Microbiology and Biotechnology*. 2011;91(4):949–956.

[52] Du F, Zhang F, Chen F, et al. Advances in microbial heterologous production of flavonoids. *African Journal of Microbiology Research*. 2011;5(18):2566–2574.

the name alone suggests this, as the word is derived from the Latin 'flavus,' or 'yellow.' But flavonoids are like terpenes in that they serve a wide variety of functions, and have significant implications for human health and nutrition. Medical and nutritional value of flavonoids, of which the scientific literature is extensive, will be covered in each individual flavonoid section. This chapter discusses the use of flavonoids by plants, and the chemical nature of these compounds.

HOW DO PLANTS USE FLAVONOIDS?

Although flavonoids and terpenes are quite different chemically, the way that plants use these phytochemicals is often similar. For instance, both terpenes and flavonoids possess antibacterial properties. Terpenes and flavonoids can attract pollinators, and both substances are proven antifungals. But diphenylpropane-based flavonoids diverge from isoprene-based terpenes in other ways. This is especially true of the UV light protection, antioxidant, and seed germination properties of some flavonoids, as discussed below.

The most striking difference, however, is the visual beauty of flavonoids. These compounds are responsible for many of the yellow, orange, purple, red, and blue colors of some flowers, berries, fruits, and leaves. While terpenes fill our senses with diverse aromas and tastes, flavonoids fill our world with splashes of vibrant color. But don't let these bright displays deceive you—these are functional colors. Just as terpenes are much more than a collection of smells with no meaning, flavonoids are far more than a palette of attractive colors devoid of purpose.

**Antioxidants*

To understand how important antioxidants are, we need only look to the huge markets for products with antioxidant properties including vitamins, skin creams, beverages and sports-drinks, serums, lotions, shampoos, etc. In fact, many of these products contain flavonoids, precisely because they are effective natural antioxidants. Plants produce and use some flavonoids as antioxidants, and humans in turn get their flavonoids from plants—the ones we eat, and the antioxidant flavonoids that go into skin cream, vitamins, and other products.

Oxidation in plants occurs when unstable oxygen molecules damage cells. This can be caused by biotic stress: that caused by living things like bacteria, fungi, or insects, or by abiotic stress: that caused by sun, wind, temperature, rain, etc. Oxidation can interfere with a plant's ability to photosynthesize, so evolution has provided at least one way to fight this—antioxidants like flavonoids that inhibit oxidation.

Flavonoids have been shown to exhibit antioxidant activity when plants are exposed to stress[53], with flavonoid production focused on the site of reactive oxygen species[54], or the site of oxidation damage to plant tissue. In fact, the spectacular colors that we witness each autumn in some deciduous trees and other plants is caused at least in part by flavonoids responding to oxidation, which may offer protection to leaf cells against damage from photosynthetic activity or ultraviolet radiation[55]. In this case, flavonoids (together with carotenoids) can produce colors that aren't meant to attract pollinators; if anything, the colors have come to serve as a warning of impending winter.

**Attract Pollinators*

Perhaps the most visible role of flavonoids in plants is to attract pollinators. While flavonoids are well-known for producing yellow pigments in flowers and pollen that can be detected by insects and some animals, a variation of flavonoids called anthocyanidins produce a wide range of other colors in some flowers and berries[56,57]. The health-foods industry has capitalized on this information, touting foods like dark-colored berries, eggplant, pomegranate, and beets as potent sources of antioxidants.

But because cannabis doesn't rely on pollinators for propagation, it's not likely that it uses flavonoids in this manner. However, some varieties of cannabis do feature flowers that are deep purple, red, and sometimes even blue hues, and this is caused by the anthocyanidin group of flavonoids.

So why exactly would cannabis need to display bright colors?

The most likely answer is that the lively shades of anthocyanidins in some varieties of cannabis are related to UV protection or another response to abiotic stress, much in the same way as mentioned in the previous section, where the colors of autumn can be partly attributed to the protective and restorative efforts of antioxidant flavonoids. Considering that the most common pollinators such as bees, flies, wasps, butterflies, moths, and beetles don't show a preference for the color purple[58] anyway,

53 Kumar, Shashank, and Abhay K. Pandey. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World JOURNAL 2013.2013 (2013).

54 Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*. 2012; 196:67–76.

55 Feild T. S., Lee D. W., Holbrook N. M. (2001). Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol. 127, 566–574.

56 Falcone Ferreyra, María L., Sebastián P. Rius, and Paula Casati. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science 3 (2012).

57 Dudek B., Warskulat A.-C., Schneider B. The occurrence of flavonoids and related compounds in flower sections of Papaver nudicaule. Plants. 2016;5:28.

58 Reverté, Sara, Javier Retana, José M. Gómez, and Jordi Bosch. Pollinators show flower colour preferences but flowers with similar colours do not attract similar pollinators. Annals of Botany 118.2 (2016): 249-257.

it seems even less likely that the bright purples of cannabis varieties like Lavender, Purple Urkle, or Mendocino Kush were meant to attract pollinators.

**Symbiotic Relationships with Fungi and Bacteria*

One of the most interesting uses of flavonoids by plants is to develop symbiotic relationships with certain microorganisms. For instance, a number of plants—including cannabis—manufacture and release flavonoids via the root system to encourage beneficial soil bacteria to attach to the roots[59]. Other flavonoids—such as those found in legume roots—stimulate the germination of beneficial fungi spores[60].

But in an odd sort of twist, flavonoids can also be used by plants as antibacterial and antifungal agents.

**Antibacterial Agents*

While some flavonoids are made by plants to stimulate symbiotic relationships with bacteria, other flavonoids can be used as effective antibacterial agents when plants come under attack by harmful microorganisms. Several flavonoids have been found to possess robust antibacterial properties, including apigenin[61], a flavonoid often found in cannabis. Another flavonoid that commonly occurs in cannabis is quercetin, which has been attributed to the inhibition of the DNA gyrase of bacteria[62] (an essential bacterial enzyme).

The action of flavonoids on bacteria is potent, with some studies concluding that these compounds may inhibit the ability of bacteria to attach to other cells[63], turn off microbial enzymes, and alter microbial membranes[64], among other antibacterial functions. Impressively, some flavonoids may be able to overcome antibiotic tolerance of at least some bacteria[65].

As is the case with terpenes, it's likely that plants use flavonoids synergistically. One Algerian study appears to have confirmed this, concluding that while a variation of the flavonoid quercetin had

59 Dixon RA, Steele CL. Flavonoids and isoflavonoids a gold mine for metabolic engineering. Trends Plant Sci. 1999; 4(10):394-400.

60 Bagga S, Straney D. Modulation of cAMP and phosphodiesterase activity by flavonoids which induce spore germination of Nectriahaematococca MP VI (Fusarium solani).Physiol. Mol. Plant Path. 2000; 56(2):51-61.

61 Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*. 2005;26(5):343–356.

62 Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2006 Feb;27(2):181.

63 Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;12(4):564–582.

64 Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem. 2015;22(1):132-49.

65 Nobakht, Motahareh, Stephen J. Trueman, Helen M. Wallace, Peter R. Brooks, Klrissa J. Streeter, and Mohammad Katouli. Antibacterial Properties of Flavonoids from Kino of the Eucalypt Tree, Corymbia torelliana. Plants 6.3 (2017).

significant antibacterial properties, a synergistic blend of variations of quercetin, apigenin, and kaempferol was more effective against some strains of clinical bacteria[66].

*Antifungal Agents

Some flavonoids can protect plants from infestations of harmful fungi. Variations of kaempferol have been shown to exhibit antifungal activity against Fusarium soil pathogens[67], which sometimes attack both drug varieties and fiber varieties of cannabis. Fortunately, cannabis has at least some native protection from these potentially dangerous fungi, as many strains have been found to contain kaempferol.

Quercetin also exhibits antifungal properties, with recent research suggesting that the flavonoid might be able to modulate (and thereby inhibit) the fatty acid synthase of certain types of fungi that commonly infect humans[68], while other studies have shown variations of quercetin and other flavonoids to suppress fungal growth of some fungal species by up to 99%[69]. In fact, antifungal activities of 6 types of flavonoids were shown to be more potent antifungals than Fluconazole[70], one of the most common antifungal medications in the United States.

*UV Protection

As discussed previously in the section on antioxidants, flavonoids provide plants with significant protection from harmful ultraviolet and other types of radiation. This includes both preventative and restorative functions, as evidenced in a study that took place on the international space station, which concluded that flavonoids absorbed cellular damage from UV radiation[71]. Other research has confirmed this, showing that flavonoids can repair damage from UV-B and UV-A radiation by inhibiting ROS (reactive oxygen species), and repairing damaged tissue[72].

66 Akroum, Souâd & Bendjeddou, Dalila & Satta, Dalila & Lalaoui, Korrichi. (2009). Antibacterial Activity And Acute Toxicity Effect of Flavonoids Extracted From Mentha longifolia. Am. Eurasian J. Sci. Res. 4.

67 Francesco Galeotti, Elisa Barile, Paolo Curir, Marcello Dolci, Virginia Lanzotti. Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity, Phytochemistry Letters, Volume 1, Issue 1, 2008, Pages 44-48.

68 Bitencourt, Tamires Aparecida, Tatiana TakahasiKomoto, Mozart Marins, and Ana Lúcia Fachin. "Antifungal activity of flavonoids and modulation of expression of genes of fatty acid synthesis in the dermatophyte Trichophyton rubrum." BMC Proceedings 8.Suppl. 4 (2014): P53-P53.

69 Kanwal Q, Hussain I, Latif Siddiqui H, Javaid A. Antifungal activity of flavonoids isolated from mango (Mangifera indica L.) leaves. Nat Prod Res. 2010 Dec;24(20):1907-14.

70 Orhan DD, Ozçelik B, Ozgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res. 2010 Aug 20;165(6):496-504.

71 Takahashi A, Ohnishi T. The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the internal space station. Biol. Sci. Space2004; 18(4): 255-260.

72 Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*. 2012; 196:67–76.

*Seed Germination & Rooting

Most plant seeds contain flavonoids that serve multiple purposes, including regulating critical physiological functions throughout the seed's life, from dormancy period to maturation[73], and, later, to seedling and root development[74]. Additionally, higher seed flavonoid content has been associated with darker seed hull color in some plants[75], which corresponds neatly with other studies that found a positive relationship between antioxidant activity and darker seed hull color[76 77]. This should be of particular interest to the cannabis industry, where urban myths—now apparently confirmed—have circulated for decades that darker seeds contain better genetics. What makes those seeds darker? Higher antioxidant flavonoid content.

*Detoxification

Flavonoids are excellent detoxification agents, especially of metals. For instance, in Ginko biloba, flavonoids were shown to increase in plant tissue by up to 12 times the normal level in response to heavy metal stress[78]. Many of the flavonoids that are found in a large number of today's cannabis strains, such as quercetin, kaempferol, luteolin, and apigenin, have been shown to be effective at detoxifying both copper and iron[79]. However, in a true testament to the versatility of flavonoids, these phytochemicals can exhibit both antioxidant activity and prooxidant activity, depending on the concentration of the flavonoids and the concentration of the metal present[80].

73 Shirley BW, Flavonoids in seeds and grains: physiological function, agronomic importance, and the genetics of biosynthesis. Seed Sci. Res. 1998; 8: 415-422.

74 Nandakumar, Lakshmi, and S. N.Rangaswamy. Effect of some Flavonoids and Phenolic Acids on Seed Germination and Rooting. Journal of Experimental Botany 36.8 (1985).

75 O.S. Salawu, I.E. Oluwafemi, D. Oladipupo, B.O.S. Bukola. Effect of Callosobruchus maculatus infestation on the nutrient–antinutrient composition, phenolic composition and antioxidant activities of some varieties of cowpeas (Vigna unguiculata) Adv. J. Food Sci. Technol., 6 (2014), pp. 322-332.

76 B.D. Oomah, F. Caspar, L.J. Malcolmson, A.S. Bellido. Phenolics and antioxidant activity of lentil and pea hulls Food Res. Int., 44 (2011), pp. 436-441.

77 P. Polthum, A. Ahromrit GABA content and antioxidant activity of Thai waxy corn seeds germinated by hypoxia method. Songklanakarin J. Sci. Technol., 36 (2014), pp. 309-316.

78 Samanta Amalesh, Das Gouranga, Das, Sanjoy. (2011). Roles of flavonoids in Plants. International Journal of pharmaceutical science and technology. 6. 12-35.

79 Fernandez MT, Mira ML, Florêncio MH, Jennings KR. Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. J Inorg Biochem. 2002 Nov 11;92(2):105-11.

80 Cherrak, Sabri Ahmed, Nassima Mokhtari-Soulimane, Farid Berroukeche, Bachir Bensenane, Angéline Cherbonnel, Hafida Merzouk, and Mourad Elhabiri. In Vitro Antioxidant versus Metal Ion Chelating Properties of Flavonoids: A Structure-Activity Investigation. PLoS ONE 11.10 (2016).

*Regulation of Cells

Flavonoids play multiple roles in the regulation of plant cells. One such role is the modulation of the movement of the plant hormone auxin[81], which elongates cells and regulates plant growth. This is an important consideration for cannabis growers because the act of "tipping" or removing the topmost flowers of cannabis (or bending the top flowers to be level or lower than buds farther down the plant) is a method of manipulating auxin flow, whereby the topmost flowers inhibit the growth of flowers below them with a downward flow of auxins.

Plant cells contain further phytochemicals that act as flavonoid membrane transporters[82], while antioxidant flavonoids are proposed to regulate parts of cell growth and differentiation, and thus likely help to control the development of both individual plant parts, and the whole plant[83]. Flavonoids play these roles in the natural state, but they also respond similarly under manipulation in the laboratory setting, reacting to altered genes and suppressing growth of cultured cells[84].

While a great deal of research is ongoing, and more badly needed, the already seemingly prolific roles of flavonoids in the regulation of plant cells has led one of the world's leading flavonoid researchers, Brenda Winkel-Shirley, to conclude that one can "consider flavonoid biosynthesis, not as an assemblage of independent components, but as part of a large, complex, and tightly orchestrated metabolic network"[85].

Other scientists have echoed this complex nature of flavonoids, even pointing out that it can be difficult to determine whether a particular flavonoid or group of flavonoids are acting primarily as cell regulators, or as defense compounds against herbivores, as antioxidants, or other roles.

*Allelopathic Properties

Like terpenes, flavonoids possess allelopathic functions[86] in that they engage in chemical warfare with competing plants. For instance, when released from the roots of buckwheat the flavonoid

81 Wendy Ann Peer, Angus S. Murphy, Flavonoids and auxin transport: modulators or regulators? Trends in Plant Science, Volume 12, Issue 12, 2007, Pages 556-563.

82 Passamonti S, Terdoslavich M, Franca R, Vanzo A, Tramer F, Braidot E, Petrussa E, Vianello A. Bioavailability of flavonoids: a review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Curr Drug Metab. 2009 May;10(4):369-94.

83 Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. 2012 Nov;196:67-76.

84 Woo, Ho-Hyung, Byeong Jeong, and Martha Hawes. Flavonoids: from cell cycle regulation to biotechnology. Biotechnology Letters 27.6 (2005): 365-374.

85 Winkel-Shirley, Brenda. Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. 126.2 (2001)485.

86 Weston, Leslie, and Ulrike Mathesius. Flavonoids: Their Structure, Biosynthesis and Role in the Rhizosphere, Including Allelopathy. Journal of Chemical Ecology 39.2 (2013): 283-297.

quercetin strongly inhibited the growth of nearby lettuce seedlings[87]. Other examples include legumes, which exude the flavonoids quercetin and kaempferol, that are beneficial for seed germination at low concentrations, but inhibit the growth of seedlings at high concentrations[88]; and the flavonoid luteolin, which, as isolated from dogwood, inhibited both seed germination and seedling growth of nearby plants[89]. In fact, the success of some of the world's most tenacious weeds has been attributed to allelopathic properties of flavonoids[90]. One study has even concluded that a crude extract of flavonoids had a 100% inhibitory effect on the seed germination and plant growth for M. pigra[91] (the Giant Sensitive Plant), a staggering testament to the allelopathic properties of some flavonoids.

HOW DO PLANTS PRODUCE FLAVONOIDS?

Like terpenes, flavonoids are formed in the cytosol of plant cells from the derivatives of acetic acids. However, while this occurs in the methyl erythritol phosphate pathway for monoterpenes and the mevalonate pathway for sesquiterpenes, the final biosynthesis process occurs in the shikimic acid pathway for many flavonoids[92]. Found in the nucleus of mesophyll cells (plant leaf tissue specialized for photosynthesis) and within centers of reactive oxygen species (ROS) generation[93], flavonoids are one of the most widely occurring plant phenolic compounds, with more than 5,000 variations reported by late 2018.

Flavonoids are common in the human diet, and are largely responsible for the color, prevention of fat oxidation, and protection of vitamins and enzymes in many foods[94]. Flavonoids are also extremely phar-

87 Zahida Iqbal, Syuntaro Hiradate and Yoshiharu Fujii. Allelopathic flavonoids from buckwheat (Fagopyrum tataricum Gaertn.) (NIAES) J. Weed Sci. Tech. Vol. 48 (Sup.)

88 Mariana Palma-Tenango, Marcos Soto-Hernández and Eva Aguirre- Hernández. Flavonoids in Agriculture. InTechOpen Published: August 23rd 2017.

89 Zhang, H.Y. & Qi, Shan-Shan & Dai, Zhi-Cong & Zhang, M. & Sun, J.F. & Du, D.L.. (2017). Allelopathic potential of flavonoids identified from invasive plant Conyza canadensis on Agrostis stolonifera and Lactuca sativa. Allelopathy Journal. 41. 223-237.

90 Alford, Élan R., Jorge M. Vivanco, and Mark W. Paschke. The Effects of Flavonoid Allelochemicals from Knapweeds on Legume–Rhizobia Candidates for Restoration. Restoration Ecology 17.4 (2009): 506-514.

91 Tikamporn Yongvanich, Waraporn Juntarajumnong, Sittichoke Nakapong. Allelopathic effect of flavonoids from Typha angustifolia on seed growth of Mimosa pigra. Thai Journal of Agricultural Science (Thailand) 2002.

92 Wang TY, Li Q, Bi KS. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci. 2018 Jan;13(1):12-23.

93 Kumar, Shashank, and Abhay K. Pandey. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World JOURNAL 2013.2013 (2013).

94 Yao LH, Jiang YM, Shi J, et al. Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*. 2004;59(3):113–122.

macologically active, and there is a substantial amount of literature that has established the potential medical value of flavonoids, which we'll discuss in detail in each individual flavonoid chapter.

CHEMICAL NATURE OF FLAVONOIDS

Plants produce flavonoids initially in the phenylpropanoid metabolic pathway[95] (origination point for many key plant compounds), where an amino acid is used to create several substances leading to the development of a compound containing two benzene (phenyl) rings, referred to as Ring A and Ring B. These two rings are joined in the center by a 3-carbon ring of pyrane containing oxygen, called Ring C. This forms the classic 15 carbon skeleton structure of flavonoids, which can be abbreviated as C6-C3-C6 (see image of flavonoid skeleton at the beginning of this chapter).

Compounds consisting of this basic chemical structure can be divided into 6 groups; the first being the chalcones, which consist only of the two benzene rings, Ring A and Ring B. In this way, chalcones can be viewed as the base structure of higher flavonoids. Other compounds in this family include flavones, flavonols, flavandiols, anthocyanins and tannins—responsible for the bright purple, red, and blue hues of many plants, including cannabis.

When Ring B is attached at position 2 of Ring C, this formation comprises the compounds flavones, flavonols, flavnones, flavanonols, catechins, and anthocyanins. However, the rings can be attached in other positions:

- When Ring B is linked at position 3 on Ring C, these compounds are called isoflavones.
- When Ring B is linked at position 4 on Ring C, the resulting compounds are called neoflavonoids.

The activity or function of flavonoids depends on the chemical structure of the compound[96]. This modification and higher functioning is achieved via hydroxylation, glycosylation, or both. Hydroxylation refers to the addition of a functional group to the molecule consisting of a hydrogen atom covalently bonded to an oxygen atom, usually occurring at positions 3, 5, 7, 2, 3-1, 4-1, and 5-1. Glycosylation refers to the modification of the flavonoid by the addition of a sugar molecule, which can be placed in a wide variety of positions[97].

[95] Falcone Ferreyra Maria Lorena, Rius Sebastián, Casati Paula. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science VOLUME 3 2020.

[96] Kelly EH, Anthony RT, Dennis JB. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry. 2002;13(10):572–584.

[97] Davis, Barry, and Jennifer Brodbelt. Determination of the glycosylation site of flavonoid monoglucosides by metal complexation and tandem mass spectrometry. Journal of The American Society for Mass Spectrometry 15.9 (2011): 1287-1299.

Like terpenes, flavonoids can be chemically built in many different ways, with each modification serving one or more purposes. We have seen this in the preceding sections, where flavonoids have been shown to possess both antioxidant and prooxidant, antibacterial and pro-bacterial, antifungal and pro-fungal, and other conflicting properties. Slight variations in flavonoid structure can result in entirely different functions, making flavonoids among the most versatile of phytochemicals.

However, not all of these many variations occur in cannabis. Laboratory testing of cannabis in the United States typically shows that most strains contain some or all of just 14 flavonoids:

- Cannflavin A
- Cannflavin B
- Orientin
- Rutin
- Apigenin
- Quercetin
- Cannflavin C
- Silymarin
- Kaempferol
- Vitexin
- Isovitexin
- Apigenin
- Luteolin
- Wogonin
- Beta-sitosterol

Despite the fact that the beta-sitosterol compound is NOT a flavonoid and is in fact a plant sterol, many laboratories erroneously consider this compound a flavonoid and test for it in cannabis strains accordingly. However, because the compound is so prolific in cannabis, we have chosen to include it in this list, and have allocated the compound a seperate chapter.

A number of these flavonoids have been mentioned in the preceding sections, and it's likely that their functions in other plants are similar to their functions in cannabis. Nevertheless, it also appears that the unique ability of flavonoids to organize and act in thousands of different ways has lent cannabis some unique compounds in the form of cannflavin A, B, and C, which to date are known only to occur in cannabis[98].

Interestingly, the flavonoid profile of most types of cannabis, including 'indica,' 'sativa,' and 'ruderalis,' are similar enough that a 1971 study seeking to determine if cannabis is monotypic (a genus containing only one species) or polytypic (containing more than one species) concluded that cannabis has only one species, cannabis sativa L.[99], and further mused that all variations are phenotypes that can be bred into or out of existence in a single season. Most cannabis breeders know this to be true from experience, but these conclusions were based on the analysis of flavonoids extracted from cannabis from different regions of the world, using both feral varieties and cultivated varieties. The overall flavonoid profiles were not dissimilar enough to warrant the classification of 3 species of cannabis, though the debate in this regard continues in 2021.

This monotypic theory is also easily evidenced by the voracious interbreeding between varieties of cannabis; there are virtually no propagation barriers between strains or phenotypes, and resulting hybrid offspring do not experience reduced reproductive capacity. This further cements the theory that there is only one cannabis plant, but that the wide variety of strains is based on the concentration and arrangements of individual cannabinoid, terpene, and flavonoid profiles of each plant.

Concentration of Flavonoids in Plants

The concentration or total dry weight of flavonoids in plants varies widely, depending on the species. Among 91 different edible plant species, flavonoid content ranged from 0 mg to 254 mg / 100g fresh weight[100]. Leafy vegetables showed a higher flavonoid content than fruit crops, with parsley as an example containing 14.35 mg of quercetin per gram of dry weight[101], the highest for the leafy vegetables examined in the study. Another study found that onion leaves had the highest flavonoid content out of 62 edible tropical plants, containing 1497.5 mg/kg quercetin, 391.0 mg/kg luteolin, and 832.0 mg/kg kaempferol[102].

98 Brenneisen R. (2007) Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents. In: ElSohly M.A. (eds) Marijuana and the Cannabinoids. Forensic Science And Medicine. Humana Press.

99 Murray Nelson Clark. A Study of InfraSpecific Flavonoid Variation of Cannabis Sativa L. (Cannabaceae) Brandon University, Department of Botany, 1971.

100 Yang RY, Lin S, Kuo G. Content and distribution of flavonoids among 91 edible plant species. Asia Pac J Clin Nutr. 2008;17 Suppl 1:275-9.

101 Chandra, Suman, Shabana Khan, Bharathi Avula, Hemant Lata, Min Hye Yang, Mahmoud A. ElSohly, and Ikhlas A. Khan. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. Evidence-based Complementary and Alternative Medicine: eCAM 2014 (2014).

102 Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem. 2001 Jun;49(6):3106-12.

In the cannabis industry, it is generally accepted that flavonoids comprise around 2.5% of dry weight of cannabis sativa, although data concerning the total flavonoid content in trichomes is unavailable as of the publishing of this text.

Flavonoids 101 Review

Answer the following questions to test your knowledge of this section.

Question #1: *Flavonoids serve what primary biological role in plants:*

a. Attract pollinators by flavor or taste
b. Defend against predators with warning colors
c. Deter herbivores with bitter flavorings
d. Attract pollinators with colors

Question #2: *Name 3 ways that plants use flavonoids:*

______________________, ______________________, ______________________

Question #3: *How many variations of flavonoids have been discovered as of 2018?*

a. 15,000
b. 25,000
c. 7,500
d. 5,000

Question #4: *The basic flavonoid molecule contains how many rings?*

a. 4
b. 3
c. 2
d. 5

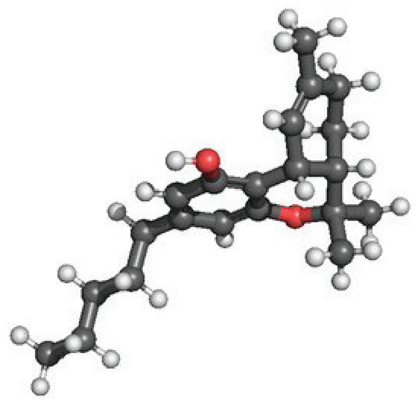
Question #5: *Name the 3 flavonoids that (as of 2021) have only been found in cannabis:*

______________________, ______________________, ______________________,

For the answer key to all '101' chapters, please go here: http://thebigbookofterps.com/bbot-101-chapters-quiz-answer-key/

CANNABINOIDS 101

D9-THC



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Scientific Artwork by TheVisualThinker.

The scientific body of work covering cannabinoids has grown significantly since Mechoulam discovered the THC molecule in 1964. Despite longstanding restrictions on cannabis research, there exists enough literature on these compounds that this text needn't cover the subject exhaustively, nor does it need to cover cannabinoids individually; there are other books, texts, and papers for this purpose. Rather, this book is meant to substantially supplement the existing body of cannabis research and general knowledge—which is focused on cannabinoids—in an effort to develop and disseminate a more robust phytochemical profile of therapeutic constituents in cannabis.

Where most researchers and scientists have focused on cannabinoids, this text focuses on terpenes, and to a lesser degree flavonoids, and the synergistic interactions between these compounds and cannabinoids. Additionally, as discussed below, many cannabinoids are actually terpenes. This section is meant to provide an overview of cannabinoids and the endocannabinoid system to correlate the significance of the occurrence of synergy between cannabinoids, terpenes, and flavonoids.

CANNABINOIDS IN THE PLANT KINGDOM

Of the cannabinoids that are often featured in news stories and cannabis industry publications, nearly all of them derive from the cannabis plant. However, some cannabinoids are also produced by other plants. The most obvious example of these is beta-caryophyllene, which is produced by dozens and perhaps hundreds of plant species. As discussed in the individual chapter for the compound, β-caryophyllene is a dietary cannabinoid, but it is also a bicyclic sesquiterpene that selectively binds to the CB2 cannabinoid receptor.

Other examples of non-cannabis plants that produce cannabinoids include black truffles, and cacao, which both produce anandamide, an endocannabinoid, the Acmella Oleracea (colloquially the toothache plant), which produces N-isobutylamides[103], helichrysum italicum (a member of the daisy family), which produces cannabigerol[104] (CBG), wairuakohu' (Radula marginate), which produces perrotentinenic acid, and rhododendron anthopogonoides, which produces cannabichromene (CBC), cannabicyclol (CBL), and cannabicitran (CBT)[105].

HOW DO PLANTS USE CANNABINOIDS?

The biological uses of cannabinoids in plants are not well researched as of 2021. However, if we again use the dietary cannabinoid β-caryophyllene as an example, research has shown that the compound is used by some plants for indirect defense (the compound attracts predatory wasps), as an antibacterial agent against both gram-positive and gram-negative bacteria, as insect repellant against spider mites and ants, as insecticide against weevils, beetles, and moths, and as an oviposition deterrent (discourages or stops insects from depositing eggs on the plant). The preceding uses are covered in more detail with citations in the "Uses in Plants" section in the individual chapter for β-caryophyllene.

Some research indicates that other cannabinoids might serve plants as insecticidal agents. For instance, cannabidiol (CBD) has been shown to significantly reduce growth and increase mortality in the tobacco hornworm[106]. Other work has indicated that cannabigerol (CBG) could protect plants

[103] Ramsewak, R S, A J Erickson, and M G Nair. Bioactive N-isobutylamides from the flower buds of Spilanthes acmella. Phytochemistry 51.6 (1999): 729-732.

[104] Bohlmann F, Hoffmann E. Cannabigerol-aehnliche Verbindunged aus Helichrysum umbraculigerum. Phytochemistry. 1979; 18:1371-1374.

[105] Iwata N, Kitanaka S. New cannabinoid-like chromane and chromene derivatives from Rhododendron anthopogonoides. Chem Pharm Bull (Tokyo). 2011;59(11):1409-12.

[106] Park, SH., Staples, S.K., Gostin, E.L. et al. Contrasting Roles of Cannabidiol as an Insecticide and Rescuing Agent for Ethanol–induced Death in the Tobacco Hornworm Manduca sexta. Sci Rep 9, 10481 (2019).

from bacterial infection[107]. Meanwhile, cannabis consumers the world over might be interested to know that THC has been theorized to act as a sunscreen against UV-B radiation in cannabis[108].

These scant few examples indicate that more research is needed to elucidate the biological uses of cannabinoids in plants, particularly in cannabis.

CHEMICAL NATURE OF CANNABINOIDS

Cannabinoids are formed when geranyl pyrophosphate combines with olivetolic acid to produce cannabigerolic acid (CBGA). From this point, nearly all the acid versions of classic cannabinoids are derived, most notably THCA, CBDA, and CBCA, with a small amount of the non-acid version of the parent compound remaining as cannabigerol (CBG).

Structurally related to tetrahydrocannabinol, classical cannabinoids may be diterpenes, triterpenes, or other types of terpenes (as in the case of beta-caryophyllene, a sesquiterpene), and are much more stable than monoterpenes and sesquiterpenes. These compounds are typically based on a molecular skeleton containing 21 carbon atoms:

- ***CBG (cannabigerol)*** $C_{21}H_{32}O_2$
- Boiling Point: 52˚C / 126˚F

Although CBG binds to both CB1 and CB2 cannabinoid receptors, the compound is non-psychoactive. CBG is derived directly from the acid version of the molecule.

- ***THCA (tetrahydrocannabinolic acid)*** $C_{22}H_{30}O_4$
- Boiling Point: 120°C / 248˚F

Derived from CBGA and the precursor of THC, THCA is a diterpenoid with hydroxy, methyl, and pentyl groups. This molecule occurs as two isomers that differ in the position of the carboxylic acid group.

- ***THCV (tetrahydrocannabivarin)*** $C_{19}H_{26}O_2$
- Boiling Point: 220°C / 428˚F

THCV is a propyl analogue of THC that interacts with both CB1 and CB2 receptors.

[107] Aqawi M, Sionov RV, Gallily R, Friedman M, Steinberg D. Anti-Bacterial Properties of Cannabigerol Toward Streptococcus mutans. Front Microbiol. 2021 Apr 22. 12:656471.

[108] Pate, David. Possible role of ultraviolet radiation in evolution of Cannabis chemotypes. Economic Botany 37.4 (2008): 396-405.

- ***D8/D9 THC (delta-8 / delta-9 tetrahydrocannabinol)*** $C_{21}H_{30}O_2$
- Boiling Point: 157°C / 315°F

Delta-8 and delta-9 are isomers of the tricyclic terpenoid THC, differing in the position of a carbon-carbon double bond.

- ***CBN (cannabinol)*** $C_{21}H_{26}O_2$
- Boiling Point: 185°C / 365°F

Cannabinol is a metabolite (in most cases a degradation product) of THC, which preferentially binds to the non-psychoactive CB2, but also activates CB1 receptors.

- ***CBDA (cannabidiolic acid)*** $C_{22}H_{30}O_4$
- Boiling Point: 130°C / 266°F

Cannabidiolic acid is the precursor to CBD much as THCA is the precursor to THC.

- ***CBDV (cannabidivarin)*** $C_{19}H_{26}O_2$
- Boiling Point: 165°C / 329°F

Cannabidivarin has 7 isomers and is non-psychoactive.

- ***CBD (cannabidiol)*** $C_{21}H_{30}O_2$
- Boiling Point: 180°C / 356°F

Widely considered non-psychoactive, CBD shows activity at both CB1 and CB2 receptors and other key pathways.

- ***CBC (cannabichromene)*** $C_{21}H_{30}O_2$
- Boiling point: 220˚C / 428˚F

Cannabichromene does not show activity at CB1 or CB1 receptor sites, instead acting on Transient Receptor Potential channels and preventing the scheduled breakdown of endocannabinoids.

HOW DO HUMANS PERCEIVE CANNABINOIDS?

Humans (and other mammals) primarily perceive cannabinoids through the endocannabinoid system (ECS) and the CB1 and CB2 cannabinoid receptors, but also through other receptors and pathways such as transient receptor potential (TRP) channels (ion channels that modulate temperature, taste, pressure, pain, and other senses).

ENDOCANNABINOIDS AND THE ENDOCANNABINOID SYSTEM (ECS)

The ECS is a cell-signaling neuromodulatory system discovered by Mechoulam and colleagues while researching THC. This system is theorized to regulate many physiological processes, including cognitive, locomotive, immune system, pain modulation, appetite, homeostasis, and other functions.

There are 3 parts of the ECS; Endocannabinoids, Endocannabinoid Receptors, and Enzymes:

Endocannabinoids:

Endogenous cannabinoids refer to those endocannabinoids that are produced by the human body; these are retrograde neurotransmitters that bind to cannabinoid receptors. There are at least 6 types of endocannabinoid ligands, the first two of which are well-studied, while the others are comparatively unknown:

- Arachidonoylethanolamine (Anandamide or AEA)
- 2-Arachidonoylglycerol (2-AG)
- 2-Arachidonyl glyceryl ether (noladin ether)
- N-Arachidonoyl dopamine (NADA)
- Virodhamine (OAE)
- Lysophosphatidylinositol (LPI)

Exogenous cannabinoids, on the other hand, consist of phytocannabinoids and synthetic cannabinoids. Endocannabinoid receptors have cavities or docking sites that match the structural shape of the endocannabinoid ligands listed above. Phytocannabinoids like CBG, CBD, THC, CBC, and synthetic cannabinoids closely match these shapes and corresponding cavities and therefore are able to activate the receptors, interrupt synaptic signaling, and/or interact with receptor sites and presynaptic and postsynaptic neurons in various ways that are not well understood. Cannabinoids bind reversibly to receptor sites, and different cannabinoids elicit different effects on these and other ECS pathways and channels. For instance, the effect of THC on these receptor sites is a feeling of euphoria or high.

Endocannabinoid Receptors:

Cannabinoid Receptor Type 1 (CB1)

CB1 receptors are G protein-coupled cannabinoid receptors (proteins that activate cellular responses involved in many physiological functions). Activated by endocannabinoids, phytocannabinoids, and synthetic cannabinoids, CB1 is encoded by the CNR1 gene, and appears to receive and interact primarily with the endocannabinoid anandamide (AEA)—the same chemical produced by cacao and black truffles mentioned previously. CB1 receptors are mostly found in the central and peripheral nervous system.

Cannabinoid Receptor Type 2 (CB2)

CB2 receptors are expressed in immune tissue, the gastrointestinal system, and peripheral tissue. CB2 receptors receive and interact primarily with the endocannabinoid 2-Arachidonoylglycerol (2-AG), and are encoded by the CNR2 gene.

Many scientists and researchers also theorize that there likely exist other cannabinoid receptors apart from CB1 and CB2.

Enzymes:

The third part of the ECS system features enzymes that break down endocannabinoids. In the classical ECS, the decomposition of endogenous cannabinoids is carried out by these enzymes automatically after the endocannabinoid has served its function. For instance, the decomposition of anandamide is carried out by fatty acid amino hydrolase, while several other enzymes—most notably monoacylglycerol acid lipase—carry out the destruction of 2-arachidonoylglycerol.

To summarize, the endocannabinoid system—which regulates many primal human processes—interfaces with cannabinoids produced by plants, particularly cannabis. One could speculate whether humans evolved with this design, or whether phytocannabinoids interface with the ECS by accident, merely because the molecules are shaped similarly to—and fit cavities seemingly meant for—endogenous endocannabinoids.

Whatever the case may be, the body of evidence supporting the therapeutic value of cannabinoids produced by cannabis is mounting, and the opportunity to use these compounds synergistically with terpenes, flavonoids, and other phytochemicals is one that should not be overlooked.

MEDICAL / THERAPEUTIC VALUE OF CANNABINOIDS

As mentioned previously, this text is not meant to comprehensively cover cannabinoids, nor is it intended to provide a review of the literature covering each compound and its medical or therapeutic uses. Rather, the information in this book will ideally lead to a realization of and/or commitment to the improvement of the existing and emerging therapeutic uses and related efficacy of cannabinoid-based medicines by using these compounds synergistically with terpenes, flavonoids, and other phytochemicals.

The therapeutic potential of cannabinoids is remarkable, but research in this field is in its infancy. Cannabinoids are actively researched and used in the treatment of:

- Addiction
- Alzheimer's disease
- Anorexia
- Anxiolytic disorders
- Arthritis
- Cancer (numerous types)
- Crohn's disease
- Epilepsy
- Glaucoma
- HIV / AIDS
- Huntington's disease
- Inflammation
- Irritable bowel syndrome
- Migraine
- Multiple sclerosis
- Nausea and vomiting
- Neurological conditions
- Ocular diseases
- Parkinson's disease
- Peripheral neuropathy
- PTSD
- Tourette syndrome

For each of these listed conditions where cannabinoids have been or could be used in treatment, it's likely that terpenes and/or flavonoids could sometimes be used synergistically to improve treatment efficacy.

Cannabinoids 101 Review

Answer the following questions to test your knowledge of this section.

Question #1: *Name 2 plants other than cannabis that produce cannabinoids:*

______________________, ______________________

Question #2: *Cannabinoids are formed from which 2 compounds:*

a. Geranyl pyrophosphate and olivetolic acid
b. Geranyl pyrane acid and oliveoil fraction
c. Geranyl acetate and ollitol
d. Geranyl acid and perrotentinenic acid

Question #3: *What is the base compound in the production of nearly all cannabinoids?*

a. THCV
b. CBNA
c. CBCA
d. CBNV
e. CBGA
f. THCA

Question #4: *Delta-8-tetrahydrocannabinol is a/an __________ of delta-9-tetrahydrocannabinol:*

a. Isotoner
b. Isopropyl
c. Analogue
d. Isomer
e. Homologue
f. Isologue

Question #5: *What are the 3 parts of the Endocannabinoid System (ECS)?*

________________, ________________, ________________

Question #6: *What is the difference between endogenous cannabinoids and exogenous cannabinoids?*

____

Question #7: *What is the function of enzymes in the ECS?*

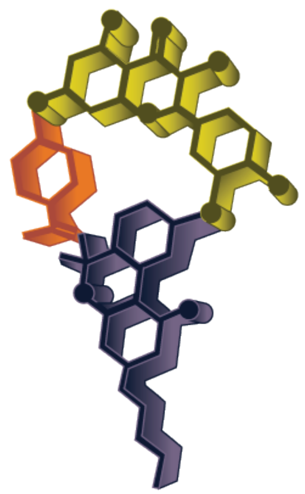
____

Question #8: *Name 3 medical conditions that could be treated using cannabinoid-based medicine:*

________________, ________________, ________________

For the answer key to all '101' chapters, please go here: http://thebigbookofterps.com/bbot-101-chapters-quiz-answer-key/

SYNERGY



Compounds that may affect the pharmacology of cannabinoids are abundant in nature, and so we may dangerously and mistakenly consider their presence to be trivial. If so, this could cause us to lose sight of the subtlety and efficiency of their design when applied in combination[109].

—Dr. Ethan Russo

OXFORD LANGUAGE DEFINITION OF SYNERGY:

"The interaction or cooperation of two or more organizations, substances, or other agents to produce a combined effect greater than the sum of their separate effects."

INTRODUCTION

Synergy as we define it today was first proposed as a concept by Shimon Ben-Shabat in 1998[110], who soon thereafter refined the theory together with Raphael Mechoulam[111] and mutual colleagues. At

109 Russo, Ethan & Marcu, Jahan. (2017). Cannabis Pharmacology: The Usual Suspects and a Few Promising Leads.

110 Ben-Shabat S., Fride E., Sheskin T., Tamiri T., Rhee M. H., Vogel Z., et al. (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur. J. Pharmacol. 353 23–31.

111 Mechoulam R., Ben-Shabat S. (1999). From gan-zi-gun-nu to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis. Nat. Prod. Rep. 16 131–143.

the time, Ben-Shabat referred to synergistic phenomena observed between compounds in the endocannabinoid system as the entourage effect, although it is only quite recently that this concept has been trending in the cannabis industry. However, evidence for synergy in plants has been known for at least a half-century, and this is particularly true of the evidence for synergy among phytochemicals like terpenes and other compounds.

Initially, Ben-Shabat and Mechoulam referred primarily to synergy between endogenous and exogenous cannabinoids (see "Cannabinoids 101" chapter for more information on the endocannabinoid system), and later Dr. Ethan Russo extended the concept to include terpenes, terpenoids, and potentially other compounds including flavonoids. The principal idea behind the entourage effect is that pure compounds in cannabis—particularly the primary psychoactive compounds like THC and CBD—are modulated or supplemented by other compounds, mostly in a positive or beneficial and additive way, resulting in therapeutic effects that are more potent than those elicited by single molecules like D9-THC.

In recent years, the cannabis industry has seized on the concept of the entourage effect, but with strikingly little understanding of the mechanisms behind it. Cannabis products and marketing materials in the United States, Canada, Europe, Israel, South America, and other places around the world tout the entourage effect as a proven, well-established, and unflinchingly positive aspect of their much-venerated plant. Unfortunately, many of the benefits claimed by these products, groups, and marketing materials are untested and disseminated without sound science—or even basic knowledge of chemistry—to support them. Perhaps worse is that potential negative or detrimental synergies are ignored. This chapter will discuss the evidence for and against synergy in cannabis, and in non-cannabis plants, the latter of which there is ample and well-established albeit neglected evidence for.

Understanding synergy and how it applies to cannabis is critical for the nascent medical and recreational markets, and especially for thousands of severely ill people and the healthcare workers who treat them, the latter of which often are not aware that cannabinoid monotherapies may not be as efficacious as blended preparations or formulations[112].

112 Sanchez-Ramos, Juan. The entourage effect of the phytocannabinoids. Annals of Neurology 77.6 (2015): 1083-1083.

SYNERGY 101

To begin, we must define three seemingly similar yet distinct concepts:

1. *Polypharmacology*

   In polypharmacology, individual molecules are applied therapeutically to affect multiple targets or pathways, or in some cases, multiple drugs to affect a specific target[113], but without the concept of implied synergy.

2. *The Entourage Effect*

   The entourage effect describes multiple components in cannabis all acting together, with one or two primary active compounds being supported by multiple inactive compounds, all taking place within the endocannabinoid system.

3. *Synergy*

   Synergy describes the enhanced effects caused by interactions between two or more compounds, with most compounds involved being considered active. This synergy can exist outside of the endocannabinoid system.

While the general concept of synergy in phytochemicals can be explained simply by the wisdom that it's nutritionally better to eat whole fruit than it is to take vitamins or supplements of singular compounds[114], the difference between this and the entourage effect needs clearer delineation. Apart from existing outside of the endocannabinoid system, perhaps the most significant difference is the active nature of the compounds involved in synergy, versus the inactive view of the compounds in the entourage effect. For instance, the terpenes geraniol, beta-pinene, alpha-humulene, and linalool have been shown to be cannabimimetic (cannabinoid-like), causing 75% of the classic CB1-response tetrad behaviors in mice, including antinociception, catalepsy, hypolocomotion, and hypothermia, while also selectively enhancing cannabinoid activity[115]. These actions indicate active versus inactive compounds.

Furthermore, consider that certain terpenes already have well-established actions outside of cannabis. Myrcene, for example, is a well-known and potent sedative, while beta-caryophyllene has affinity for the CB2 receptor, in both cases indicating active rather than inactive compounds.

113 Reddy, A Srinivas, and Shuxing Zhang. Polypharmacology: drug discovery for the future. Expert review of clinical pharmacology vol. 6,1 (2013): 41-7.

114 Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Adv. Nutr. Int. Rev. J. 4, 384S–392S.

115 LaVigne, Justin E., Ryan Hecksel, Attila Keresztes, and John M. Streicher. Cannabis sativa terpenes are cannabimimetic and selectively enhance cannabinoid activity. Scientific Reports 11.1 (2021).

Perhaps the most obvious example of synergy in cannabis—and the one that the majority of cannabis consumers can understand and relate to—is the aroma and flavor of each chemovar. This distinctive feature is caused mostly by the primary terpenes and terpenoids in the plant's inflorescences (flowers) volatilizing together, as opposed to being the product of a singular constituent.

Synergy Implications

The viewpoint that synergy is always positive or beneficial is relative, and often ignores negative synergies. Terpenes, flavonoids, cannabinoids, and many other compounds may interact synergistically, but whether this action is beneficial or detrimental depends on the individual situation and desired outcome.

Synergy Characters

Terpenes, cannabinoids, and flavonoids can potentially act synergistically with a virtually unlimited number of other compounds. These interactions can include activity and engagement between terpenes and chemotherapeutics, terpenes and cannabinoids, terpenes and antimalarial drugs, flavonoids and terpenes, flavonoids and anti-tumor agents, terpenes and neuroprotective agents, and many other combinations.

Mechanisms and Types of Synergy

As documented often in the individual compound chapters of this text, plants can produce specific blends of terpenes in response to a variety of stimuli. These blends appear to act synergistically, and can serve an astonishing number of complex functions.

Terpene formulations created in the pharmacological setting can also serve many functions, with synergy occurring via one or more of several different mechanisms including additive and competitive agonism or antagonism, regulation of cells, allosteric effects, multi-target effects, pharmacokinetic effects, modulation of adverse events[116], and other effects.

DIRECT EVIDENCE FOR NON-CANNABIS SYNERGY

Many studies have found evidence of synergy between terpenes, flavonoids, and other compounds, including man-made drugs. The following 63 quotes taken directly from peer-reviewed studies or research papers offer a distinct and fascinating glimpse into the synergistic nature of these compounds:

116 Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine. 2009 Mar;16(2-3):97-110.

"Based on the dose-dependent antennal and behavioural responses of E. obliqua to these bioactive compounds, myrcene, terpinene, linalool, cis-verbenol, camphor and verbenone were found to play a key role in repelling the moths, and the mixture that included all eight compounds was significantly more effective.[117]"

"However, significant cytotoxicity was observed when cells were cotreated with limonene and linalyl acetate whereas no other associations were effective. Only cotreatment, but not the single exposure to limonene and linalyl acetate, replicated distinctive morphological and biochemical changes induced by BEO (bergamot essential oil).[118]"

"Overall, the present investigation revealed that the major components of P. roseum and specially the whole essential oil could be helpful in developing novel and safe mosquito control tools and also offer an environmentally safe and cheap tool for reducing Cx. pipiens mosquito populations.[119]"

"The three compounds alone inhibited cell proliferation in a dose-dependent fashion, while their pairwise combination produced synergistic antiproliferative effects in both cell lines.[120]"

"In this study, diverse essential oils were initially investigated for anti-biofilm activity against C. albicans strains, and cascarilla bark oil and helichrysum oil and their components a-longipinene (a major constituent of both) and linalool were found to markedly inhibit biofilm formation without affecting planktonic cell growth. Moreover, a-longipinene and linalool were found to synergistically reduce biofilm formation.[121]"

"On the other hand, the nematicidal activity of T. mastichina EO did not correlate with that of its major components alone (1,8-cineole, terpineol, linalool and b-pinene) or in binary mixtures of 1,8-cineole with other compounds. Therefore, the contribution of each component to the activity of an

117 Zhang, Zhengqun, Lei Bian, Xiaoling Sun, Zongxiu Luo, Zhaojun Xin, Fengjian Luo, and Zongmao Chen. Electrophysiological and behavioural responses of the tea geometrid Ectropis obliqua (Lepidoptera: Geometridae) to volatiles from a non-host plant, rosemary, Rosmarinus officinalis (Lamiaceae). Pest Management Science 71.1 (2015): 96-104.

118 Russo R, Ciociaro A, Berliocchi L, Cassiano MG, Rombolà L, Ragusa S, Bagetta G, Blandini F, Corasaniti MT. Implication of limonene and linalyl acetate in cytotoxicity induced by bergamot essential oil in human neuroblastoma cells. Fitoterapia. 2013 Sep; 89:48-57.

119 Tabari MA, Youssefi MR, Esfandiari A, Benelli G. Toxicity of ß-citronellol, geraniol and linalool from Pelargonium roseum essential oil against the West Nile and filariasis vector Culex pipiens (Diptera: Culicidae). Res Vet Sci. 2017 Oct; 114:36-40.

120 Rodenak Kladniew, Boris, Mónica Polo, Sandra Montero Villegas, Marianela Galle, Rosana Crespo, and Margarita García de Bravo. Synergistic antiproliferative and anticholesterogenic effects of linalool, 1,8-cineole, and simvastatin on human cell lines. Chemico-Biological Interactions 214 (2014): 57-68.

121 Manoharan RK, Lee JH, Kim YG, Kim SI, Lee J. Inhibitory effects of the essential oils a-longipinene and linalool on biofilm formation and hyphal growth of Candida albicans. Biofouling. 2017 Feb;33(2):143-155.

EO follows a complicated pattern of interactions including synergistic and/or antagonistic interactions that contribute to the overall toxicity of the oil.[122]"

"In S. aureus, the linalool with Imipenem association showed a synergistic effect (FIC = 0.0625); while with ciprofloxacin, the linalool showed additivity (FIC = 0.75). In P. aeruginosa, the Imipenem/linalool association was synergistic for both the ATCC and clinical strains (FIC = 0.0625). We conclude that Linalool associated with existing standard antibiotics may increase antibacterial effectiveness, resulting in synergistic activity against bacterial strains of clinical importance.[123]"

"The isolated sesquiterpenes are the major active principles responsible for the potent mosquitocidal activity, but the synergistic action of other constituents of the oil cannot be disregarded, as the whole oil is more effective than the individual compounds.[124]"

"When the oils (from Laurus nobilis leaves and seeds) were added to a suboptimal concentration of the commercial drug, cytosine arabinoside, a clear synergic effect was observed.[125]"

"These results suggest that the TPL/DCF (terpinolene/diclofenac) association had a synergistic anti-inflammatory and analgesic effect without causing apparent gastric injury, and that the serotonergic system may be involved in the antinociceptive effect of this association.[126]"

"Compounds were also used as binary mixtures and tested for synergy, using toxicity and feeding inhibition parameters. The data showed that thymol and a-terpineol synergized the effects of both linalool and 1,8-cineole. The combination of compounds is more desirable because the insecticidal spectrum of some binary mixtures is increased.[127]"

122 Andrés, Maria, Azucena González-Coloma, Jesus Sanz, Jesus Burillo, and Paula Sainz. Nematicidal activity of essential oils: a review. Phytochemistry Reviews 11.4 (2012): 371-390.

123 Silva V.A., Sousa, J.P., Guerra F.Q.S., Pessôa H.L.F., Freitas A.F.R., Coutinho, H.D.M., Alves L.B.N, Lima E.O. Antibacterial Activity of the Monoterpene Linalool: Alone and in Association with Antibiotics Against Bacteria of Clinical Importance, International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1022-1026.

124 Ravi Kiran, S., and P. Sita Devi. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of Chloroxylon swietenia DC. Parasitology Research 101.2 (2007): 413-418.

125 Saab AM, Tundis R, Loizzo MR, Lampronti I, Borgatti M, Gambari R, Menichini F, Esseily F, Menichini F. Antioxidant and antiproliferative activity of Laurus nobilis L. (Lauraceae) leaves and seeds essential oils against K562 human chronic myelogenous leukaemia cells. Nat Prod Res. 2012;26(18):1741-5.

126 Macedo, E M A et al. Association of terpinolene and diclofenac presents antinociceptive and anti-inflammatory synergistic effects in a model of chronic inflammation. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas vol. 49,7 (2016): e5103.

127 Singh, Rajwinder, Opender Koul, Pushpinder Rup, and Jawala Jindal. Toxicity of some essential oil constituents and their binary mixtures against Chilo pattellas (Lepidoptera: Pyralidae). International Journal of Tropical Insect Science 29.2 (2009): 93-101.

"Limonene exhibited synergistic interaction with citral, caryophyllene oxide and Benznidazole (decreasing by 17 times its IC50) and was the most effective and selective treatment.[128]"

"None of the other compounds were toxic to adults, but caryophyllene oxide and sesamin exhibited moderate larvicidal effects (LD50>150 µg/ml). A mixture of the four compounds in the same ratio as in the hexane extract showed higher toxicity (LD50 34 ng/mg insect) towards adult insects than the pure compounds.[129]"

"The in vitro trypanocidal activity of a 1:4 mixture of lupenone and caryophyllene oxide confirmed a synergistic effect of the terpenoids against epimastigotes forms of T. cruzi.[130]"

"Oils with high sesquiterpene concentrations received a low rating, meaning that they smelled badly. In contrast, oils with high monoterpene percentages (but a low alpha-humulene or caryophyllene oxide concentration) got a high rating. Surprisingly, a mixed oil from different strains received the best rating.[131]"

"Comparison of the deterrent activity of 'full mixtures' with respective artificial blends missing individual constituents demonstrates that, for most oils, minor constituents in a mixture can be as important as major constituents with respect to the overall feeding deterrent effect. The rold of any individual compound is mixture-specific and depends on the interaction between the constituents of the mixture.[132]"

"Natural thyme oil showed greater toxicity than any single constituent or blend of constituents. The toxicity of blends of selected constituents indicated a synergistic effect among the putatively active and inactive constituents, with the presence of all constituents necessary to reach the highest toxicity.[133]"

128 Moreno, Érika, Sandra Leal, Elena Stashenko, and Liliana García. Induction of programmed cell death in Trypanosoma cruzi by Lippia alba essential oils and their major and synergistic terpenes (citral, limonene and caryophyllene oxide). BMC Complementary and Alternative Medicine 18.1 (2018): 1-16.

129 Moussavi, Nastaran, Karl Egil Malterud, Bertin Mikolo, Dag Dawes, Fabrice Chandre, Vincent Corbel, Daniel Massamba, Hans J. Overgaard, and Helle Wangensteen. Identification of chemical constituents of Zanthoxylum heitzii stem bark and their insecticidal activity against the malaria mosquito Anopheles gambiae. Parasites & Vectors 8 (2015).

130 Polanco-Hernández, Glendy, Fabiola Escalante-Erosa, Karlina García-Sosa, María E. Rosado, Eugenia Guzmán-Marín, Karla Y. Acosta-Viana, Alberto Giménez-Turba, Efraín Salamanca, and Luis M. Peña-Rodríguez. Synergistic Effect of Lupenone and Caryophyllene Oxide against Trypanosoma cruzi. Evidence-Based Complementary and Alternative Medicine 2013.

131 Vito Mediavilla and Simon Steinemann Essential oil of Cannabis sativa L. strains International Hemp Association from: http://www.internationalhempassociation.org/jiha/jiha4208.html Accessed April 10, 2019.

132 Akhtar, Yasmin, Emmanuelle Pages, Alex Stevens, Rod Bradbury, Claudio A. G. da Camara, and Murrat B. Isman, Effect of chemical complexity of essential oils on feeding deterrence in larvae of the cabbage looper. Physiological Entomology 37.1 (2012): 81-91.

133 Wu, Lipeng et al. Acaricidal Activity and Synergistic Effect of Thyme Oil Constituents against Carmine Spider Mite (Tetranychus Cinnabarinus (Boisduval)). Molecules (Basel, Switzerland) vol. 22,11 1873. 1 Nov. 2017.

"However, the observed activity might be due to synergism between compounds present in the plant extract. The synergism among these compounds which contribute to the cytotoxic activity, is not only dependent on the concentration of the compounds, but also on the structure and interaction(s) between the 27 compounds. This can explain the differences in the cytotoxic effect between crude extracts and isolated compounds against the same cell lines, as shown in our earlier report.[134]"

"The effectiveness of a binary 1:2 mixture of thymol and vanillin (0.05:0.1 microl per cm2) was found to be significantly more effective than thymol alone for a period of 120 min.[135]"

"Although none of these was active when presented individually at the levels present in the entrainment sample...the six-component blend and a blend of acetophenone, (Z)-3-hexenyl acetate, and 3-carene, in the same ratio and concentration as in a natural sample, was as attractive to female S. mosellana as the whole air entrainment sample.[136]"

"The slow onset of inhibition of butyrylcholinesterase was also shown by individual constituents, such as 3-carene and beta-pinene. Analyses of the chemical composition of the oils and anti-butyrylcholinesterase activity of their constituents revealed that none of the compounds tested would account for the total activity of the oils and that synergy is likely.[137]"

"Furthermore, the combination of borneol and menthol significantly enhanced the permeation rate in the same concentration, compared with their effects when used alone. Consequently, menthol combined with borneol can highly enhance fluconazole permeation through the ex vivo cornea.[138]"

"These results demonstrated the synergic effects between TMPP (tetramethylpyrazine phosphate) and BO (borneol) for treating ischemia-reperfusion injury in the cortex and hippocampus regions.[139]"

"These results suggested that the blend provided more useful information for female wasps than the individual compounds. ...the blend attracted the wasps at all of the tested concentrations, while the

134 Malek, Sri Nurestri Abdul et al. Cytotoxic components of Pereskia bleo (Kunth) DC. (Cactaceae) leaves. Molecules (Basel, Switzerland) vol. 14,5 1713-24. 6 May. 2009.

135 Park YU, Koo HN, Kim GH. Chemical composition, larvicidal action, and adult repellency of Thymus magnus against Aedes albopictus. J Am Mosq Control Assoc. 2012 Sep;28(3):192-8.

136 Birkett, Michael, Toby Bruce, Janet Martin, Lesley Smart, Jon Oakley, and Lester Wadhams. Responses of Female Orange Wheat Blossom Midge, Sitodiplosis mosellana, to Wheat Panicle Volatiles. Journal of Chemical Ecology 30.7 (2004): 1319-1328.

137 Savelev, Sergey U., Edward J. Okello, and Elaine K. Perry. Butyryl- and acetyl-cholinesterase inhibitory activities in essential oils of Salvia species and their constituents. Phytotherapy Research 18.4 (2004): 315-324.

138 Liu, Jingjing, Shaoying Fu, Nan Wei, Yongsheng Hou, Xiaoning Zhang, and Hao Cui. The effects of combined menthol and borneol on fluconazole permeation through the cornea ex vivo. European Journal of Pharmacology 688.1 (2012): 1-5.

139 Yu, Bin, Ming Ruan, Tao Liang, Shi-Wen Huang, Yun Yu, Hai-Bo Cheng, and Xiang-Chun Shen. The Synergic Effect of Tetramethylpyrazine Phosphate and Borneol for Protecting Against Ischemia Injury in Cortex and Hippocampus Regions by Modulating Apoptosis and Autophagy. Journal of Molecular Neuroscience 63.1 (2017): 70-83.

response windows for each pure compound were narrow. Additionally, at the 0.01 ng level, none of the individual compounds attracted A. ervi, while their mixtures attracted at a ten times lower level.[140]"

"Radical scavenging activity of Carum oil was than that of Ferula oil. These differential activities between essential oils could be reflected to the main components and strong synergism between them.[141]"

"Our third case of study, the mixture of (R)-pulegone and citronellal, showed quite different results from those predicted by MM, with observed LC50 values being markedly lower than expected. Moreover, CI [combination index] values were quite far below one, suggesting a real synergism between these two terpenes, which was supported also by the isobolograms.[142]"

"Terpinen-4-ol and c-terpinene exhibited a significant insecticidal activity against both insects, but c-terpinene was more toxic than terpinen-4-ol. When tested in a binary mixture with the synthetic insecticides profenofos and methomyl, it was found that both compounds enhanced the insecticidal activity of these insecticides by two- to threefold. These results show that terpinen-4-ol and c-terpinene have a synergistic effect on the insecticidal activities of synthetic insecticides profenofos and methomyl.[143]"

"Otherwise, sabinene and terpinen-4-ol were strongly repellent against T. castaneum as well as the essential oil, while c-terpinene exhibited weaker repellency against T. castaneum compared with the positive control, DEET (N, N-diethyl-3-methylbenzamide). Moreover, only the essential oil exhibited strong repellency against L. serricorne, the three compounds exhibited weaker repellency against L. serricorne relative to DEET.[144]"

"Our results showed imidacloprid treatment alone can significantly decrease aphid populations, and that combining insecticide with Eßf (farnesene) further reduced numbers of apterous aphids...

140 Takemoto, Hiroyuki, and Junji Takabayashi. Parasitic Wasps Aphidius ervi are More Attracted to a Blend of Host-Induced Plant Volatiles than to the Independent Compounds. Journal of Chemical Ecology 41.9 (2015): 801-807.

141 Kavoosi, Gholamreza, Asad Tafsiry, Ali Asghar Ebdam, and Vahid Rowshan. Evaluation of Antioxidant and Antimicrobial Activities of Essential Oils from Carum copticum Seed and Ferula assafoetida Latex. Journal of Food Science 78.2 (2013): T356-T361.

142 Esteban Scalerandi, Guillermo A. Flores, Marcela Palacio, Maria Teresa Defagó, María Cecilia Carpinella, Graciela Valladares, Alberto Bertoni, and Sara María Palacios. Understanding Synergistic Toxicity of Terpenes as Insecticides: Contribution of Metabolic Detoxification in Musca domestica. Front Plant Sci. 2018; 9: 1579.

143 Mostafa A. Abbassy, Samir A. M. Abdelgaleil, & Rasha Y. A. Rabie. Insecticidal and synergistic effects of Majoranahortensis essential oil and some of its majorconstituents. Entomologia Experimentalis et Applicata 131(3):225—232 · May 2009.

144 Y. Wang, C.X. You, K. Yang, Y. Wu, R. Chen, W.J. Zhang, Z.L. Liu, S.S. Du, Z. W. Deng, Z.F. Geng, and J. HanBioactivity of Essential Oil of Zingiber purpureum Rhizomes and Its Main Compounds against Two Stored Product Insects Journal of Economic Entomology; Lanham Vol. 108, Iss. 3, (Jun 2015): 925-932.

Our results demonstrate that imidacloprid can be effective in reducing the abundance of aphids in Chinese cabbage fields, but the degree of control can be even stronger in the presence of Eßf.[145]"

"The essential oil fraction of P. graveolens and its main components, geraniol and citronellol, exhibited additive effects with amphotericin B and with ketoconazole against both Aspergillus species. The FIC index calculated from these results was 0.562, which indicates the strong additive effects of geraniol and amphotericin B.[146]"

"With sorbitol, geraniol MIC was increased by 64-fold and citronellol by 32-fold. The drugs caused leakage of intracellular material and inhibited ergosterol biosynthesis.[147]"

"Overall, the present investigation revealed that the major components of P. roseum and specially the whole essential oil could be helpful in developing novel and safe mosquito control tools and also offer an environmentally safe and cheap tool for reducing Cx. pipiens mosquito populations"[148].

"Toxicity of these four major constituents in the same proportion as the natural oil, was greater than whole oil and each individual component. Removal of any four constituents produced a decreased in effectiveness.[149]"

"A strong synergism of DOX (doxorubicin) with NER (nerolidol) was found in the A2780 cells, while DOX acted synergistically with HUM (humulene) and CAO (b-caryophyllene) in the SKOV3 (ovarian cancer) cells. In the CCRF/CEM cells, a synergism of DOX with CAO and NER was observed.[150]"

"CAO, NER and VAL synergistically potentiated the efficacy of DOX in cancer cell killing.[151]"

145 Liang-LiangCui, Jie Dong, Frédéric Francis, Ying-Jie Liu, Stéphanie Heuskin, Georges Lognay, Ju-Lian Chen, Claude Bragard, John F.Tooker, Yong Liua. E-ß-farnesene synergizes the influence of an insecticide to improve control of cabbage aphids in China. Crop Protection Volume 35, May 2012, Pages 91-96.

146 Shin, Seungwon. Anti- Aspergillus activities of plant essential oils and their combination effects with ketoconazole or amphotericin b. Archives of Pharmacal Research 26.5 (2003): 389-393.

147 Pereira Fde O, Mendes JM, Lima IO, Mota KS, Oliveira WA, Lima Ede O. Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against Trichophyton rubrum involves inhibition of ergosterol biosynthesis. Pharm Biol. 2015 Feb;53(2):228-34.

148 Tabari MA, Youssefi MR, Esfandiari A, Benelli G. Toxicity of ß-citronellol, geraniol and linalool from Pelargonium roseum essential oil against the West Nile and filariasis vector Culex pipiens (Diptera: Culicidae). Res Vet Sci. 2017 Oct;114:36-40.

149 Gallardo A, Picollo MI, González-Audino P, Mougabure-Cueto G. Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against Pediculus humanus capitis (Phthiraptera: Pediculidae). J Med Entomol. 2012 Mar;49(2):332-5.

150 Ambrož, Martin et al. The Effects of Selected Sesquiterpenes from Myrica rubra Essential Oil on the Efficacy of Doxorubicin in Sensitive and Resistant Cancer Cell Lines. Molecules (Basel, Switzerland) vol. 22,6 1021. 20 Jun. 2017.

151 Ambrož, Martin et al. The Influence of Sesquiterpenes from Myrica rubra on the Antiproliferative and Pro-Oxidative Effects of Doxorubicin and Its Accumulation in Cancer Cells. Molecules (Basel, Switzerland) vol. 20,8 15343-58. 21 Aug. 2015.

"The presence of all constituents together in the artificial mixture caused a significant decrease in the number of eggs laid by females, at 0.26 mg liter(-1) (11 eggs), compared with the control (50 eggs). The toxicity of blends of selected constituents indicated that the presence of all constituents was necessary to reproduce the toxicity level of the natural oil.[152]"

"Indian cassia was non-toxi alone, but the mixture of India cassia + green cardamom showed toxicity, clearly demonstrating a synergistic effect.[153]"

"This study indicated that the combination of LM (l-menthol) and reboxetine, as well as LM and FLU (fluoxetine) showed synergistic effects.[154]"

"Our results indicate that two of the three geranium oil constituents tested exhibit excellent anti-Candida activity and significant synergistic activity with fluconazole.[155]"

"Our results indicated that EO's induced a selective antimicrobial activity. A synergistic effect of EO's and tetracycline (TET) was noticed with a reduction rate ranged from 2 to 8-fold (of bacteria).[156]"

"When thymol and cymene were applied simultaneously, it resulted in a greater antibacterial effect than when the compounds were applied separately.[157]"

"This study indicates a synergistic interaction between essential oils and PBO (piperonyl butoxide) in inhibiting the cytochrome P450 and GST detoxification enzymes in Ae. aegypti.[158]"

152 Attia, S., K. L. Grissa, G. Lognay, S. Heuskin, A. C. Mailleux, and T. Hance. Chemical Composition and Acaricidal Properties of Deverra scoparia Essential Oil (Araliales: Apiaceae) and Blends of Its Major Constituents Against Tetranychus urticae (Acari: Tetranychidae). Journal of Economic Entomology 104.4 (2011): 1220-1228.

153 Tripathi, Aran, Anil Singh, and Shikha Upadhyay. Contact and fumigant toxicity of some common spices against the storage insects Callosobruchus maculatus (Coleoptera: Bruchidae) and Tribolium castaneum (Coleoptera: Tenebrionidae). International Journal of Tropical Insect Science 29.3 (2009): 151-157.

154 Wang, Weidong, Yuanyuan Jiang, Enbo Cai, Bingchen Li, Yan Zhao, Hongyan Zhu, Lianxue Zhang, and Yugang Gao. L-menthol exhibits antidepressive-like effects mediated by the modification of 5-HTergic, GABAergic and DAergic systems. Cognitive Neurodynamics 13.2 (2018): 191-200.

155 Zore GB, Thakre AD, Rathod V, Karuppayil SM. Evaluation of anti-Candida potential of geranium oil constituents against clinical isolates of Candida albicans differentially sensitive to fluconazole: inhibition of growth, dimorphism and sensitization. Mycoses. 2011 Jul;54(4):e99-109.

156 Miladi, Hanene, Tarek Zmantar, Bochra Kouidhi, Yasir Mohammed A Al Qurashi, Amina Bakhrouf, Yassine Chaabouni, Kacem Mahdouani, and Kamel Chaieb. Synergistic effect of eugenol, carvacrol, thymol, p-cymene and -terpinene on inhibition of drug resistance and biofilm formation of oral bacteria. Microbial pathogenesis 112 (2018): 156-163.

157 Begoña Delgado, Alfredo Palop, Pablo S. Fernández & Paula M. Periago. Combined effect of thymol and cymene to control the growth of Bacillus cereus vegetative cells. European Food Research and Technology volume 218, pages188–193(2004).

158 Waliwitiya R, Nicholson RA, Kennedy CJ, Lowenberger CA. The synergistic effects of insecticidal essential oils and piperonyl butoxide on biotransformational enzyme activities in Aedes aegypti (Diptera: Culicidae). J Med Entomol. 2012 May;49(3):614-23.

"Data also suggest that ß-Elemene shows the synergistic effect when treated with hyperbaric oxygen in TBI (traumatic brain injury).[159]"

"The combination of ascorbic acid and rutin had higher antioxidant properties compared to the activity of the single compound alone, and showed a stronger effect against UV-induced reactive oxygen species generation.[160]"

"Subcutaneous and orthotopic xenograft studies also showed that tumor volumes were significantly lower in mice receiving combined TMZ/Rutin treatment as compared to TMZ or rutin alone treatment. Moreover, immunoblotting analysis showed that TMZ activated JNK activity to induce protective response autophagy, which was blocked by rutin, resulting in decreased autophagy and increased apoptosis, suggesting that rutin enhances TMZ efficacy both in vitro and in vivo.[161]"

"Compared with Quercetin and Rutin, FPL (an extract of persimmon leaves where these two compounds are the primary constituents) showed higher cytotoxicity at 12.5 and 25 µg/ml concentrations and also presented lower IC50 in PC-3 cells.[162]"

"Furthermore, the CD26 effect was enhanced when apigenin was paired with chemotherapeutic agents utilized in the treatment of advanced colorectal cancer including irinotecan, 5-fluorouracil and oxaliplatin. For irinotecan, apigenin caused a 4-fold increase in the potency of the drug.[163]"

"Apoptosis assay revealed that TRAIL or apigenin alone induced a marked apoptosis in RAFLS and their combination yielded a synergistic increase in RAFLS apoptosis.[164]"

159 Xiangen Meng, Na Li, Yu Zhang, Danfeng Fan, Chen Yang, Hang Li, Dazhi Guo, and Shuyi Pan. Beneficial Effect of ß-Elemene Alone and in Combination with Hyperbaric Oxygen in Traumatic Brain Injury by Inflammatory Pathway Translational Neuroscience 2018; 9: 33-37.

160 Gegotek, Agnieszka, Ewa Ambrozewicz, Anna Jastrzab, Iwona Jarocka-Karpowicz, and Elzbieta Skrzydlewska. Rutin and ascorbic acid cooperation in antioxidant and antiapoptotic effect on human skin keratinocytes and fibroblasts exposed to UVA and UVB radiation. Archives of Dermatological Research 311.3 (2019): 203-219.

161 Zhang, P., Sun, S., Li, N. et al. Rutin increases the cytotoxicity of temozolomide in glioblastoma via autophagy inhibition. J Neurooncol 132, 393–400 (2017).

162 Ding, Yan, Kai Ren, Huanhuan Dong, Fei Song, Jing Chen, Youtian Guo, Yanshan Liu, Weijie Tao, and Yali Zhang. Flavonoids from persimmon (Diospyros kaki L.) leaves inhibit proliferation and induce apoptosis in PC-3 cells by activation of oxidative stress and mitochondrial apoptosis. Chemico-Biological Interactions 275 (2017): 210-217.

163 Lefort, Émilie, and Jonathan Blay. The dietary flavonoid apigenin enhances the activities of the anti-metastatic protein CD26 on human colon carcinoma cells. Clinical & Experimental Metastasis 28.4 (2011): 337-349.

164 Sun, Q., Jiang, S., Yang, K. et al. Apigenin enhances the cytotoxic effects of tumor necrosis factor-related apoptosis-inducing ligand in human rheumatoid arthritis fibroblast-like synoviocytes. Mol Biol Rep 39, 5529–5535 (2012).

"When quercetin was combined with quercitrin, enhancement of anti-DENV-2 activity and reduced cytotoxicity were observed. However, the synergistic efficacy of the flavonoid combination was still less than that of the EA fraction.[165]"

"The combination of cisplatin with quercetin synergistically inhibits cell growth and triggers apoptosis in HepG2 cells. Our data revealed that the combination of quercetin and cisplatin was significantly (P<?0.05) effective in inducing growth suppression and apoptosis in HepG2 cells, when compared with single agent treatment.[166]"

"The experiment results suggest that quercetin can increase chemosensitivity of DDP and VC on the two cell lines (A549 human lung adenocarcinoma).[167]"

"We found that Quercetin synergistically enhanced rituximab-induced growth inhibition and apoptosis in DLBCL cell lines.[168]"

"Co-administration of luteolin and paclitaxel resulted in an increase in apoptosis compared with the treatment of paclitaxel alone..., immunoblotting analysis also showed that the co-administration of luteolin and paclitaxel activated caspase-8 and caspase-3 and increased the expression of Fas.[169]"

"Combined administration of luteolin and 5-FU in Solid Ehrlich Carcinoma model increased levels of p53, p21, caspase 3, DRAM and survivability...current results proved the antitumor therapeutic effects of luteolin alone or combined with 5-FU as a novel strategy for cancer therapy.[170]"

165 Chiow, K.H., M.C. Phoon, Thomas Putti, Benny K.H. Tan, and Vincent T. Chow. Evaluation of antiviral activities of Houttuynia cordata Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. Asian Pacific Journal of Tropical Medicine 9.1 (2015): 1-7.

166 Zhao, Ji-ling, Jing Zhao, and Hong-jun Jiao. Synergistic Growth-Suppressive Effects of Quercetin and Cisplatin on HepG2 Human Hepatocellular Carcinoma Cells. Applied Biochemistry and Biotechnology 172.2 (2013): 784-791.

167 Zhan, Xuejun, Runxiang Zhang, Yanping Xu, Shuhua Yang, Daze Xie, and Liwei Tan. Empirical studies about quercetin increasing chemosensitivity on human lung adenocarcinoma cell line A549. The Chinese-German Journal of Clinical Oncology 11.7 (2012): 380-383.

168 Li, Xin, Xinhua Wang, Mingzhi Zhang, Aimin Li, Zhenchang Sun, and Qi Yu. Quercetin Potentiates the Antitumor Activity of Rituximab in Diffuse Large B-Cell Lymphoma by Inhibiting STAT3 Pathway. Cell Biochemistry and Biophysics 70.2 (2014): 1357-1362.

169 Yang, Mon-Yuan, Chau-Jong Wang, Nai-Fang Chen, Wen-Hsin Ho, Fung-Jou Lu, and Tsui-Hwa Tseng. Luteolin enhances paclitaxel-induced apoptosis in human breast cancer MDA-MB-231 cells by blocking STAT3. Chemico-biological interactions 213 (2014): 60-68.

170 Soliman, Nema A, Rania N Abd-Ellatif, Amira A ELSaadany, Shahinaz M Shalaby, and Asmaa E Bedeer. Luteolin and 5-flurouracil act synergistically to induce cellular weapons in experimentally induced Solid Ehrlich Carcinoma: Realistic role of P53; a guardian fights in a cellular battle. Chemico-biological interactions 310 (2019).

"In particular, combined celecoxib and luteolin treatment significantly decreased the growth of MDA-MB-231 cancer cells in vivo compared with either agent alone.[171]"

"A combinational treatment of cisplatin and luteolin induced more effectively cell growth inhibition, compared to cisplatin treatment alone.[172]"

"In the majority of cases, luteolin, when combined with IFN-ß, had additive effects in modulating cell proliferation, IL-1ß, TNF-a, MMP-9 and TIMP-1.[173]"

"Luteolin enhanced anti-proliferation effect of cisplatin on cisplatin-resistant ovarian cancer CAOV3/DDP cells. Flow cytometry revealed that luteolin enhanced cell apoptosis in combination with cisplatin. Western blotting and qRT-PCR assay revealed that luteolin increased cisplatin-induced downregulation of Bcl-2 expression. In addition, wound-healing assay and Matrigel invasion assay showed that luteolin and cisplatin synergistically inhibited migration and invasion of CAOV3/DDP cells. Moreover, in vivo, luteolin enhanced cisplatin-induced reduction of tumor growth as well as induction of apoptosis.[174]"

"Vitexin can cooperate with HBO to sensitize the glioma radiotherapy...The present results showed that the combination of HBO and vitexin could synergistically sensitize the glioma radiotherapy.[175]"

"Furthermore, administration of N-acetylcysteine (NAC) and kaempferol significantly rescued more mice than a low dose of NAC only did when a lethal dose of propacetamol injected and therapized at a delayed time point.[176]"

171 Jeon, Ye, Young Ahn, Won Chung, Hyun Choi, and Young Suh. Synergistic effect between celecoxib and luteolin is dependent on estrogen receptor in human breast cancer cells. Tumor Biology 36.8 (2015): 6349-6359.

172 Wu, Bin, Qiang Zhang, Weiming Shen, and Jun Zhu. Anti-proliferative and chemosensitizing effects of luteolin on human gastric cancer AGS cell line. Molecular and Cellular Biochemistry 313.2 (2008): 125-132.

173 Sternberg, Zohara, Kailash Chadha, Alicia Lieberman, Allison Drake, David Hojnacki, Bianca Weinstock-Guttman, and Frederick Munschauer. Immunomodulatory responses of peripheral blood mononuclear cells from multiple sclerosis patients upon in vitro incubation with the flavonoid luteolin: additive effects of IFN-ß. Journal of Neuroinflammation 6 (2009): 28-28.

174 Wang, Haixia, Youjun Luo, Tiankui Qiao, Zhaoxia Wu, and Zhonghua Huang. Luteolin sensitizes the antitumor effect of cisplatin in drug-resistant ovarian cancer via induction of apoptosis and inhibition of cell migration and invasion. Journal of Ovarian Research 11.1 (2018): 1-12.

175 Xie, T., J.-R. Wang, C.-G. Dai, X.-A. Fu, J. Dong, and Q. Huang. Vitexin, an inhibitor of hypoxia-inducible factor-1a, enhances the radiotherapy sensitization of hyperbaric oxygen on glioma. Clinical and Translational Oncology 22.7 (2020): 1086-1093.

176 Tsai, Ming-Shiun, Ying-Han Wang, Yan-Yun Lai, Hsi-Kai Tsou, Gan-Guang Liou, Jiunn-Liang Ko, and Sue-Hong Wang. Kaempferol protects against propacetamol-induced acute liver injury through CYP2E1 inactivation, UGT1A1 activation, and attenuation of oxidative stress, inflammation and apoptosis in mice. Toxicology letters 290 (2018): 97-109.

"After the combined application of both phenols, a synergistic effect of kaempferol plus low but not high doses of (-)-epicatechin was observed.[177]"

"Of particular interest is that in combination, the two PACs [oridonin and wogonin] were synergistic in their cytotoxicity to five of six of the primary cultures and to both the cell lines.[178]"

"Wogonin significantly sensitized resistant HNC cells to cisplatin both in vitro and in vivo. Our study revealed that wogonin could act synergistically with cisplatin and thereby circumvent the resistance to cisplatin in HNC cells.[179]"

CANNABIS SYNERGY

Despite the prolific use of the term entourage effect incorrectly used interchangeably with synergy, the latter concept is distinct and generally poorly understood; in fact, it is a literal work in progress absconded with enthusiastic but underinformed consumers and businesspeople. The reality is that there is a significant and often contentious divide between scientists who support and those who doubt the existence of synergy. Nevertheless, if the phytochemical synergies listed previously can apply to many different plant-generated blends and many different terpene or flavonoid blends created by humans, then it's logical to assume that the same molecules as produced by cannabis can have similar effects.

THC and CBD

Most of the research concerning the existence of synergy in cannabis centers around THC and CBD, although the first mention of the entourage effect did not concern these cannabinoids. Interestingly, CBD and THC have been the subject of significant scientific debate, with some researchers attempting to classify cannabis species—proposed as indica, sativa, and ruderalis—using the ratios of these two cannabinoids as chemotaxonomic markers[180] (a naming system categorized by chemical content).

In 1998 Shimon Ben-Shabat and numerous colleagues including Raphael Mechoulam discovered that the endogenous ligand 2-Arachidonoyl-glycerol (2-Ara-Gl) was accompanied by several related esters

177 Escandón, R.A., del Campo, M., López-Solis, R. et al. Antibacterial effect of kaempferol and (-)-epicatechin on Helicobacter pylori . Eur Food Res Technol 242, 1495–1502 (2016).

178 Chen, Sophie, Matt Cooper, Matt Jones, Thumuluru Madhuri, Julie Wade, Ashleigh Bachelor, and Simon Butler-Manuel. Combined activity of oridonin and wogonin in advanced-stage ovarian cancer cells. Cell Biology and Toxicology 27.2 (2010): 133-147.

179 Kim, Eun, Hyejin Jang, Daiha Shin, Seung Baek, and Jong-Lyel Roh. Targeting Nrf2 with wogonin overcomes cisplatin resistance in head and neck cancer. Apoptosis 21.11 (2016): 1265-1278.

180 Small, E., Beckstead, H.D., 1973b. Cannabinoid phenotypes in Cannabis sativa. Nature 245, 147–148.

at the site of CB1 and CB2, significantly potentiating the binding of 2-Ara-Gl at the receptor sites, and thereby increasing the biological activity of the ligand[181]. This led the researchers to theorize that the compounds must interact synergistically, and thus the concept of the entourage effect was born.

With THC and CBD at the forefront of cannabis science in the late 90s and early 2000s, Russo expanded upon the work of Ben-Shabat and Mechoulam, proposing that the two cannabinoids acted synergistically. According to Russo, "CBD possesses the unique ability to counteract the intoxicating and adverse effects of cannabis, such as anxiety, tachycardia, hunger, and sedation in rats and humans". Subsequently, Russo has authored 3 important papers that have been seized by the cannabis industry as indisputable proof of the entourage effect and synergy in cannabis:

- **2006**: *A Tale of two Cannabinoids: the Therapeutic Rationale for Combining Tetrahydrocannabinol and Cannabidiol*

  In this paper, Russo asserts that CBD can mitigate undesirable side effects of THC, recommending that the two cannabinoids be combined in the therapeutic treatment of Multiple Sclerosis, cancer pain, rheumatoid arthritis, and several other conditions[182].

- **2011**: *Taming THC: Potential Cannabis Synergy and Phytocannabinoid-terpenoid Entourage Effects*

  This is Russo's quintessential paper, proposing that the entourage effect or synergy observed in cannabis could extend beyond THC and CBD to other compounds, including terpenes[183].

- **2019**: *The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No Strain, no Gain*

  In this paper, Russo again makes the case for the entourage effect, and discusses the chemotaxonomic classification of cannabis, recommending the cannabis industry refer to strains as the more accurate term 'chemovars'[184].

181 Ben-Shabat, Shimon & Fride, Ester & Sheskin, Tzviel & Tamiri, Tsippy & Rhee, Man & Vogel, Zvi & Bisogno, Tiziana & De petrocellis, Luciano & Di Marzo, Vincenzo & Mechoulam, Raphael. (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. European Journal of Pharmacology. 353. 23-31.

182 Russo E, Guy GW. A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses. 2006;66(2):234-46.

183 Russo, Ethan B. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British journal of pharmacology vol. 163,7 (2011): 1344-64.

184 Russo, Ethan B. The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No "Strain," No Gain. Frontiers in plant science vol. 9 1969. 9 Jan. 2019.

Readers are encouraged to review in their entirety the three articles mentioned above after studying Terpenes 101, Flavonoids 101, and Cannabinoids 101 in this book.

Prior to the work of Ben-Shabat, Mechoulam, and Russo, evidence of synergy in cannabis first came to light in 1974, when researchers demonstrated that 2 types of whole Brazilian cannabis induced psychological and other effects in human subjects that were 2 to 4 times as potent as THC alone[185]. Other early evidence of synergy in cannabis came in 1981, when experiments showed that whole cannabis elicited much higher anticataleptic activity in mice than those exposed to THC alone[186].

In 2001, McPartland and Russo published a review of the scientific literature on medical cannabis, finding again that non-cannabinoid compounds in cannabis enhanced the beneficial effects of THC, with the authors suggesting that terpenes and flavonoids play a role in the combined activity[187]. Two years later, Wilkinson et al found that a cannabis extract performed better than pure D9-THC in a mouse model of multiple sclerosis, and in a rat model of epilepsy[188]. The extract resulted in faster onset of therapeutic effects and a reduction in the time to achieve maximum effect, while also exhibiting unexpected anticonvulsant activity.

One particularly interesting account of in-plant use of synergy in compounds by cannabis was published in 2005 by Sirikantaramas, who noted an obvious synergy in trichomes between sticky terpenes which trap insects, and acidic cannabinoids which then act as insecticides[189]. The following year, an anonymously authored report found that terpenes including limonene and myrcene enhanced user experiences when combined with THC[190].

In 2010, Johnson et al published a multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of a blended THC and CBD extract, and a pure THC extract in patients with intractable cancer-related pain. The researchers found that the THC-only extract was comparable to placebo, while the blend containing THC and CBD relieved

185 Carlini EA, Karniol IG, Renault PF, Schuster CR. Effects of marihuana in laboratory animals and in man. Br J Pharmacol. 1974 Feb;50(2):299-309.

186 Fairbairn, J W, and J T Pickens. Activity of cannabis in relation to its delta'-trans-tetrahydro-cannabinol content. British journal of pharmacology vol. 72,3 (1981): 401-9.

187 McPartland, J.M., Russo, E.B., 2001. Cannabis and Cannabis extracts: greater than the sum of their parts? J. Cannabis Therap. 1, 103–132.

188 Wilkinson JD, Whalley BJ, Baker D, Pryce G, Constanti A, Gibbons S, Williamson EM. Medicinal cannabis: is delta9-tetrahydrocannabinol necessary for all its effects? J Pharm Pharmacol. 2003 Dec;55(12):1687-94.

189 Sirikantaramas, S., Taura, F., Tanaka, Y., Ishikawa, Y., Morimoto, S., and Shoyama, Y. (2005). Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. Plant Cell Physiol. 46, 1578–1582.

190 Name Withheld. (2006) Studying the Effects of Terpenes. O'Shaughnessy's Spring 2006. 2.

advanced cancer pain that no longer responded to opioids[191]. The same year, Fischedick et al proposed that synergistic effects observed in cannabis compounds could be due to processes unrelated to the CB1 receptor[192], while a year later Efferth and Koch theorized multiple synergistic mechanisms among various phytochemicals[193].

In 2015, Gallily, Yekhtin, and Hanus set out to find a way to overcome the biphasic dose response pure CBD produces when used in the treatment of pain (essentially, CBD is analgesic to a point, then stops working despite increased doses). They found that a full spectrum cannabis extract with CBD produced a linear analgesic effect with no observed ceiling[194] on increased doses. The authors also found that both pure cannabinoid compounds and cannabis extracts can induce anxiolytic (anxiety-relieving) and anxiogenic (anxiety-causing) effects, suggesting that the mechanism of action responsible for these opposing actions may include various entourage effects. The same year, Iseger and Bossong found that CBD could alleviate the negative effects of THC, including cognitive impairment, and reduce the risk of developing psychoses[195] after long-term use. In 2018, researchers found that a botanical drug preparation containing, among other compounds, small amounts of cannabigerol (CBG) and tetrahydrocannabinolic acid (THCA), was more potent than pure D9-THC in producing antitumor activity in several types of human breast cancer. The authors stated that "combination of cannabinoids with estrogen receptor- or HER2-targeted therapies (tamoxifen and lapatinib, respectively) or with cisplatin, produced additive antiproliferative responses in cell cultures"[196].

In a meta-analysis of clinical studies on epilepsy published the same year, Pamplona et al concluded that two thirds of patients treated with a CBD-rich extract reported reduced seizure frequencies, and lower average dose than patients treated with pure CBD, while also suffering fewer adverse events[197].

191 Johnson JR, Burnell-Nugent M, Lossignol D, Ganae-Motan ED, Potts R, Fallon MT. Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain. J Pain Symptom Manage. 2010 Feb;39(2):167-79.

192 Fischedick J, Van Der Kooy F, Verpoorte R. Cannabinoid receptor 1 binding activity and quantitative analysis of Cannabis sativa L. smoke and vapor. Chem Pharm Bull (Tokyo). 2010 Feb;58(2):201-7.

193 Efferth, T., and Koch, E. (2011). Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. Curr. Drug Target. 12, 122–132.

194 Gallily, R., Yekhtin, Z. and Hanuš, L.O. (2015) Overcoming the Bell-Shaped Dose-Response of Cannabidiol by Using Cannabis Extract Enriched in Cannabidiol. Pharmacology & Pharmacy, 6, 75-85.

195 Iseger, T. A., and Bossong, M. G. (2015). A systematic review of the antipsychotic properties of cannabidiol in humans. Schizophr. Res. 162, 153–161.

196 Blasco-Benito S, Seijo-Vila M, Caro-Villalobos M, Tundidor I, Andradas C, García-Taboada E, Wade J, Smith S, Guzmán M, Pérez-Gómez E, Gordon M, Sánchez C. Appraising the "entourage effect": Antitumor action of a pure cannabinoid versus a botanical drug preparation in preclinical models of breast cancer. Biochem Pharmacol. 2018 Nov;157:285-293.

197 Pamplona FA, da Silva LR, Coan AC. Potential Clinical Benefits of CBD-Rich Cannabis Extracts Over Purified CBD in Treatment-Resistant Epilepsy: Observational Data Meta-analysis. Front Neurol. 2018 Sep 12;9:759.

The authors theorized that these differences are caused by synergistic effects of the primary cannabinoids with other phytocompounds.

Also in 2018, Kamal made several observations: that pure THC causes more anxiety than whole cannabis, that THC and nerolidol are correlated with increased anxiolytic activity, and that guaiol, eucalyptol, terpinene, phellandrene, and other compounds were associated with decreased anxiolytic activity[198]. The authors suggested that these actions may be caused by modulatory effects of terpenes and other compounds.

Most recently, Anderson showed in 2021 that oral administration of a cannabidiolic acid (CBDA) extract in mice produced plasma CBDA concentrations that were 14 times higher than in mice administered pure CBDA[199].

ANTI-SYNERGY ARGUMENTS AND EVIDENCE

Anti-synergy arguments and evidence against the entourage effect have been presented in the last 2 years. In 2019, Santiago et al found that none of the 6 most common terpenes in cannabis activated CB1 or CB2, nor did these compounds modulate D9-THC[200]. A year later the same authors went on to find that the 7 most common terpenes in cannabis do not modulate the actions of cannabinoids on human transient receptor potential ankyrin 1 (hTRPA1) or human transient receptor potential vanilloid 1 (hTRPV1) channels[201], both of which had been proposed as alternative options for synergy targets.

Other work in 2020 addressed similar findings regarding CB1 and CB2 binding, with Finlay cautioning that "general lack of terpenoids effects on binding is not sufficient to completely rule out allosteric effects on function, as binding and functional modulation are separate in theory; receptor functional modulation may not necessarily be predicted by altered binding and vice versa"[202].

198 Kamal Brishna S., Kamal Fatima, Lantela Daniel E. Cannabis and the Anxiety of Fragmentation—A Systems Approach for Finding an Anxiolytic Cannabis Chemotype Frontiers in Neuroscience VOLUME 12 2018 Pg 730.

199 Anderson, Lyndsey L., Maia G. Etchart, Dilara Bahceci, Taliesin A. Golembiewski, and Jonathon C. Arnold. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. Scientific Reports 11.1 (2021).

200 Santiago, Marina, Shivani Sachdev, Jonathon C. Arnold, Iain S. McGregor, and Mark Connor. Absence of Entourage: Terpenoids Commonly Found in Cannabis sativa Do Not Modulate the Functional Activity of Δ9-THC at Human CB1 and CB2 Receptors. Cannabis and Cannabinoid Research 4.3 (2019): 165-176.

201 Heblinski, Marika, Marina Santiago, Charlotte Fletcher, Jordyn Stuart, Mark Connor, Iain S. McGregor, and Jonathon C. Arnold. Terpenoids Commonly Found in Cannabis sativa Do Not Modulate the Actions of Phytocannabinoids or Endocannabinoids on TRPA1 and TRPV1 Channels. Cannabis and Cannabinoid Research 5.4 (2020): 305-317.

202 Finlay, David B., Kathleen J. Sircombe, Mhairi Nimick, Callum Jones, and Michelle Glass. Terpenoids From Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors. Frontiers in Pharmacology 11 (2020).

The same year, Ferber studied the entourage effect in cannabinoids used for the treatment of mood and anxiety disorders, concluding only that "Further research is warranted to investigate the potential therapeutic value of adding terpenes to treatment with CBD, with or without additional THC... The effects of combining this original treatment with the conventional pharmacological approach also require further investigation"[203].

Other researchers have stated that, because of low levels of volatile monoterpenes in most cannabis products to begin with, and because of processes such as first-pass metabolism, high rates of clearance and short half-lives for elimination, phytonutrients like terpenes most likely could not accumulate to therapeutic levels[204].

Perhaps the strongest arguments and evidence casting doubt on synergy in cannabis originate from Peter S. Cogan, PhD (pharmaceutical sciences). In his March 2020 review article and meta-analysis titled *'The 'Entourage Effect' or 'Hodge-podge Hashish': the Questionable Rebranding, Marketing, and Expectations of Cannabis Polypharmacy*, Cogan methodically and ruthlessly attacks many of the articles and studies presented in the preceding "Cannabis Synergy" section of this book, concluding with his opinion on the entourage effect[205] (EE) in cannabis:

"In the opinion of this author, solicitations invoking the EE should be regulated as any other explicit therapeutic claim, as they are enticing the buyer to make a purchase based on the promise of benefit and...are leveraging assumptions of pharmacologic necessity (e.g. small molecule synergy) to do so.

Moreover, instead of treating entourage complexity with overarching assumptions regarding its clearly unpredictable effects, it seems more appropriate to test every proposed entourage product to see if it actually lives up to expectations."

Alternatives to Synergy

Interestingly, there are emerging alternatives to synergy theory. One fascinating alternative has been put forth after researchers working with houseflies observed that the insects processed and neutralized primary terpenes in an insecticidal essential oil blend, but not minor or secondary terpenes. This led researchers to theorize that, because the insects were unable to neutralize minor terpenes, these

203 Ferber, Sari Goldstein, Dvora Namdar, Danielle Hen-Shoval, Gilad Eger, Hinanit Koltai, Gal Shoval, Liat Shbiro, and Aron Weller. The "Entourage Effect": Terpenes Coupled with Cannabinoids for the Treatment of Mood Disorders and Anxiety Disorders. Current Neuropharmacology 18.2 (2020): 87-96.

204 Duggan, Peter J. The Chemistry of Cannabis and Cannabinoids. Australian Journal of Chemistry 74.6 (2021): 369-387.

205 Cogan PS. The 'entourage effect' or 'hodge-podge hashish': the questionable rebranding, marketing, and expectations of cannabis polypharmacy. Expert Rev Clin Pharmacol. 2020 Aug;13(8):835-845.

constituents then exhibited much more severe and potent action against the flies[206]. Essentially, it appeared that the flies were more affected by the minor terpenes because they had no defenses left against them, and not because of the synergistic action of the constituents.

As Raphael Mechoulam mentioned in his commentary in the "Terp Tsars" chapter of this text, not all terpene or flavonoid compounds are synergistic with all other compounds, in all other circumstances. However, although there is significant therapeutic value in individual compounds, when it comes to terpenes and flavonoids, the secondary general action of these compounds is often synergistic. Synergistic action of terpenes and other phytochemicals is logical and well-supported in the literature outside of cannabis, as documented herein.

Perhaps not curiously, the ever-contentious subject of cannabis lives up to this expectation even in the scientific journals. Nevertheless, the literature supporting synergy in plants other than cannabis is strong, and it seems logical to conclude that we can make at least some inferences about this body of evidence that subsequently cross over into the figurative and literal 'field' of cannabis.

WHAT'S NEXT FOR SYNERGY IN CANNABIS?

More Research / Better Dissemination / Better Oversight

While reasonable inferences made from non-cannabis phytochemicals will have to suffice for now, we must swiftly carry out more research into the combined or synergistic effects of cannabinoids, terpenes, and flavonoids, as well as other compounds. This research must be targeted and collaborative, and dissemination of results should be integrated in such a way that cannabis media and publications have ready access to it. Finally, as Cogan pointed out, we must also achieve better oversight of therapeutic claims and marketing materials, which must be based on sound science alone, and not the mythology that often swirls and clouds the cannabis industry.

[206] Scalerandi, Esteban et al. Understanding Synergistic Toxicity of Terpenes as Insecticides: Contribution of Metabolic Detoxification in Musca domestica. Frontiers in plant science vol. 9 1579. 30 Oct. 2018.

Synergy Review

Answer the following questions to test your knowledge of this section.

Question #1: *Match the term with the correct loose definition:*

d. Synergy	1. One compound affecting multiple targets
e. Polypharmacology	2. A nonsensical term
f. Sinergistic bonding	3. Inactive compounds supporting active ECS
g. Entourage Effect	4. 2 or more compounds with active synergy

Question #2: *Geraniol, beta-pinene, alpha-humulene, and linalool have been shown to be:*

a. Bicyclic
b. Tricyclic
c. Cylized
d. Cannabimimetic
e. Antispasmoidal

Question #3: *Synergy characters can include:*

a. Terpenes and flavonoids
b. Terpenes and cananbinoids
c. Terpenoids, flavones, and anticancer agents
d. Antibacterial agents, terpenoids, and cannabinoids
e. Flavonols, analgesics, terpenes, and benzodiazepines
f. All of the above

Question #4: *Name the endogenous ligan that led Ben-Shabat and Mechoulam to coin the term 'entourage effect':*

a. 2-Arachidonoyl-glycerol
b. 2-Delta-arachidonoyl glycol
c. Arachadonoyl-2-glycophosphate
d. 2-Arachidonoyl-geranoyl

Question #5: *Overall, cannabis consumers prefer whole plant products versus pure THC:*

a. True
b. False

Question #6: *While Russo, McPartland, and Mechoulam are in favor of the existence of synergy, these 3 scientists are against the concept:*

a. Fairborn, Childs, McDonald
b. Wendy, Rose, McLintock
c. Burger, Sorensen, Dumname
d. Cogan, Duggan, Santiago

Question #7: *Name one obvious example of synergy in cannabis:*

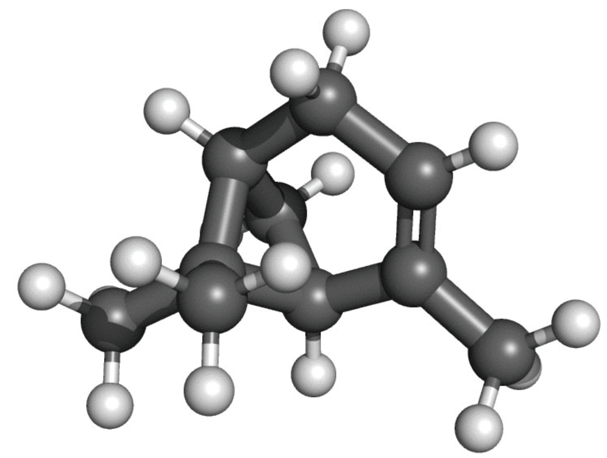
____

For the answer key to Synergy, please go here: http://thebigbookofterps.com/bbot-101-chapters-quiz-answer-key/

TERPENES AND TERPENOIDS

PINENE

Alpha Pinene



- **Type:** Bicyclic Monoterpene
- **Chemical Formula:** $C_{10}H_{16}$
- **Molecular Weight:** 136.23 g/mol
- **Boiling Point:** 155 to 156 °C (311 to 313 °F)
- **Flash Point:** 91.4 °F
- **Melting Point:** -67 °F
- **Solubility:** Insoluble in water
- **Biological Role:** Insecticide, allelopathic agent, fungicide
- **Therapeutic Role:** Anticancer agent, antioxidant, anti-inflammatory agent
- **Commercial Use:** Perfumes and fragrances, solvents, flavoring agent
- **Occurrence in Cannabis:** Top 3
- **Aroma/Flavor:** Turpentine, Pine, Woody, Balsam
- **Occurs in Cannabis Strains:** ACDC, Banana OG, Blue Dream, Bubba Kush, Cannatonic, Chem D, DJ Short Blueberry, Dutch Treat, Girl Scout Cookies, Gorilla Glue #4, Harlequin, Haze Berry, Island Sweet Skunk, Jack Herer, Obama Kush, OG Kush, Purps, Romulan, Royal Jack, Skywalker OG, Strawberry Cough, Trainwreck

INTRODUCTION

Found in high concentrations in the majority of cannabis strains, pinene is also the most widely distributed natural terpene on earth[207]. In fact, pinene is the major constituent of the aroma of coniferous forests, especially those with pine trees. In Japan, there is a rapidly growing practice of forest bathing, which began in the 1980s and has spread worldwide today, where the concept amounts to deliberately inhaling pinene and other terpene emissions in a natural forest environment. The idea behind forest bathing is that natural compounds like pinene, when inhaled, promote good health and healing in humans. There may be significant truth to this concept, as evidenced in the "Potential Uses in Medicine," and "Implications for Human Health & Nutrition" sections below.

Because pinene vapors are heavier than air, these emissions linger below the forest canopy, saturating the lower environment with scented air rich in monoterpenes and other phytochemicals. The amount of these emissions is staggering; in the United States, total emissions of alpha-pinene from forests were around 6.6 mega tons annually in the 1970s. Today, it's likely this number is smaller considering the rate of global deforestation.

Pinene is also the major constituent of pine sap and other coniferous resins, appearing as a clear to yellow or amber sap in the natural state, and a colorless, pungent liquid as a commercial product. This isoprene-based compound lends many cannabis varieties like Blue Dream, Gorilla Glue #4, Jack Herer, and OG Kush their terpenic, piney, woody, and sharp characteristics.

CHEMICAL STRUCTURE

Pinene is a hydrocarbon alkene containing 26 atoms; 10 carbon atoms, and 16 hydrogen atoms, notated as $C_{10}H_{16}$. This terpene is produced in the mevalonate pathway of plants, and is derived from isopentenyl pyrophosphate (isoprene). The 10 carbon atoms in this compound's skeleton denote the fact that pinene is comprised of two units of isoprene, each with 5 carbon atoms, which classifies this compound as a monoterpene.

Pinene is further classified as a bicyclic monoterpene because it has 2 rings fused together in the molecular structure, with methyl groups at positions 2, 6, and 6-1. Of the two rings, the four-membered ring is reactive, at least in part because of its contorted nature, which means that it is prone to skeletal rearrangements resulting in different functionality.

[207] Noma Y, Asakawa Y (2010). Biotransformation of monoterpenoids by microorganisms, insects, and mammals. In: Baser KHC, Buchbauer G (eds). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press: Boca Raton, Florida, pp. 585–736.

There are two structural isomers (the same compound, but with different arrangements of the atoms within the molecule) of this hydrocarbon, both of which occur commonly in nature; α-pinene (alpha-pinene), and β-pinene (beta-pinene). In some plants, both α-pinene and β-pinene occur in equal amounts in the same species; this is referred to as a racemic mixture.

FOUND IN WHICH PLANTS

Pinene can be found at some level in hundreds of different plant species. In fact, this terpene derived its name from pine trees, as it is the primary constituent of the sap or resin of many different varieties of pine. However, while the compound takes its name from the pine tree, it occurs in a wide variety of different types of plants, including:

Abies holophylla
Achillea tenuifolia lam.
Agathosma crenulata
Ajowan seed
Allspice
Angelica
Anise
Apple
Armoise
Artemisia
Aspen
Ayou
Bay
Black pepper
Basil
Basswood
Bay
Bell pepper
Bergamot
Blackberry
Black currant
Black walnut
Blueberry
Boldus
Buchu
Cajuput
Calamus
Cananga
Cannabis
Caraway
Cardamom
Carqueja
Carrot
Cascarilla
Cassia
Celery
Chamomile
Champaca
Cinnamon
Cilantro
Citronella
Clary sage
Clove
Coriander
Cotton
Croton
Cubeb
Cumin
Curry
Cypress
Dill
Echinophora cinerea
Eriocephalus punctulatus
Eucalyptus
Featherfew
Fennel
Fig
Fir
Fennel
Fleabane
Frankincense
Galangal
Geranium
Ginger
Grapefruit
Guava
Gum
Heracleum
Hinoki
Ho leaf
Horse chestnut
Horsemint

Hyssop
Ironwort
Jambu
Juniper
Kachur
Kewda
Labdanum
Laurel
Lavandin
Lavender
Lemon
Lemongrass
Lime
Lovage
Mace
Mandarin
Mango
Manuka
Marjoram
Makrut lime
Melissa
Microstrobos
Mikan
Mugwort
Myrtle
Narcissus absolute
Nepeta
Neroli
Nutmeg
Orange
Oregano
Origanum
Osmanthus
Parsley
Patchouli
Pennyroyal
Peppermint
Petitgrain
Pimenta
Pimento
Pine
Pinus
Plum
Pomelo
Prangos
Protium
Rosemary
Saffron
Sage
Salvia
Santolina
Savin
Savory
Snake root
Spearmint
Spruce
Strawberry
Tagete
Tamarind
Tangerine
Tansy
Tarragon
Taxodium distichum
Teatree
Thyme
Tomato
Turmeric
Valerian
Verbena
Water mint
Wild orange
Wormwood
Yarrow
Ylang ylang
Yuzu
Zanthoxylum
Zingiber
Zinnia

USES BY PLANTS / BIOLOGICAL ROLES

Insecticidal: José S. Dambolena, et al showed that pinene can act as a powerful toxic fumigant, while Giovanni Benelli, et al concluded that pinene also works as a contact toxin for some common insect pests. In Salvia tomentosa (balsamic sage), the essential oil of the plant featured β-pinene and α-pinene as the primary constituents, and caused complete mortality against Acanthoscelides (bean weevils), and nearly complete mortality against Tribolium castaneum (red flour beetles)[208].

Ovicidal agent: Pinene has been shown to act as an ovicidal agent (attacking or inhibiting the growth of insect ova) against Pediculus humanus capitis (head lice)[209], and likely acts similarly against some other insect larvae.

Nematicidal: As the primary constituent of the essential oil of Dryobalanops aromatica (borneo camphor) and Mentha haplocalyx var. piperascens (Japanese mint), α-pinene exhibited strong nematicidal properties and hatching inhibition activities against Meloidogyne icognita[210] (the southern root-knot nematode, a parasitic roundworm).

Antifungal agent: Filomena Nazzaro et al (2017) showed that pinene can act as an antifungal agent against several types of pathogenic or parasitic fungi. As a primary constituent of the essential oil of Liquidambar formosana hance (Formosan sweet gum), both α-pinene and β-pinene exhibited inhibitory activity against L. betulina (a type of mushroom that typically grows on birch trees) and L. sulphureus[211] (a bracket fungus known as chicken of the woods). As the primary constituent of the essential oil of Zanthoxylum bungeanum (Chinese-pepper), α-pinene controlled dry rot in potatoes inoculated with Fusarium sulphureum (a common fungal pathogen of cannabis), strongly inhibiting the growth of this fungus while significantly inhibiting spore germination, dropping germination rates by as much as 84% and decreasing cell membrane integrity[212]. In all tests conducted in the Chinese-pepper study, the EO was more effective than α-pinene alone, suggesting synergy.

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208 Ulukanli, Zeynep, Salih Karabörklü, Menderes Cenet, Osman Sagdic, Ismet Ozturk, and Mehmet Balcilar. "Essential oil composition, insecticidal and antibacterial activities of Salvia tomentosa Miller." Medicinal Chemistry Research 22.2 (2012): 832-840

209 Toloza, Ariel. (2010). Bioactividad y toxicidad de componentes de aceites esenciales vegetales, en Pediculus humanus capitis (Phthiraptera: Pediculidae) resistentes a insecticidas piretroides.

210 Kim, Soon-Il, Jae-Kook Lee, Young-Eun Na, Seong Tak Yoon, and Young Ju Oh. "Nematicidal and ovicidal activities of Dryobalanops aromatica and Mentha haplocalyx var. piperascens -derived materials and their formulations against Meloidogyne incognita second-stage juveniles and eggs." Nematology 16.2 (2014): 193-200

211 Chien, Shih-Chang, Jun-Hong Xiao, Yen-Hsueh Tseng, Yueh-Hsiung Kuo, and Sheng-Yang Wang. "Composition and antifungal activity of balsam from Liquidambar formosana Hance." Holzforschung 67.3 (2013)

212 Xing-dong, Li, and Xue Hua-li. Antifungal activity of the essential oil of Zanthoxylum bungeanum and its major constituent on Fusarium sulphureum and dry rot of potato tubers. Phytoparasitica 42.4 (2014): 509-517.

***Insect Repellant**: In the essential oil state, pinene has been shown to repel insects[213], including those that are not necessarily herbivores of the species in question. As a primary constituent in the essential oil extracted from the fruits of Evodia lenticellata, β-pinene showed fumigant and contact toxicity and was strongly repellant against the red flour beetle and liposcelis bostrychophila (the common booklouse), exhibiting repellent properties comparable to that of DEET[214], one of the most common commercial insect repellants in the world. Pinene, as the primary constituent of many types of coniferous tree sap, also acts as a direct insect repellant and trapping system.

***Allelopathic Agent:** α-pinene has been shown to exhibit allelopathic properties against Ipomoea triloba L. (little bell morning glory, considered a weed) by inducing oxidative stress during germination and the early stages of seedling growth[215]. α-pinene also inhibited the radicle growth (the first part of a seedling to emerge from the seed) of 5 common weeds: Cassia occidentalis, Amaranthus viridis, Triticum aestivum, Pisum sativum, and Cicer arietinum, while simultaneously inducing oxidative stress, enhancing production of ROS, and decreasing cell membrane integrity[216].

***Larvicidal:** As part of the essential oils of several different plants, β-pinene and α-pinene exhibited strong toxicity against the larvae of Culex pipiens[217], a type of mosquito that is a known disease vector.

USES IN INDUSTRY

Both α-pinene and β-pinene are used frequently in the foods, perfumes, and solvents industries. Most samples of industrial pinene are produced by fractional distillation of turpentine, presenting as a clear liquid, with up to 90%-95% purity.

Oxidation products of pinene, produced in the laboratory setting, are used extensively in the perfume and fragrance industry. In this industry, pinene is used in the same way as myrcene in that these terpenes are generally processed as intermediate or precursor chemicals, used to produce more

213 Nerio LS, Olivero-Verbel J, Stashenko E (2010). Repellent activity of essential oils: a review. Bioresour Technol. 101 (1): 372–378.

214 Cao, Ju-Qin, Shan-Shan Guo, Yang Wang, Xue Pang, Zhu-Feng Geng, and Shu-Shan Du. Toxicity and repellency of essential oil from Evodia lenticellata Huang fruits and its major monoterpenes against three stored-product insects. Ecotoxicology and Environmental Safety 160 (2018): 342-348.

215 Pergo, Érica, and Emy Ishii-Iwamoto. Changes in Energy Metabolism and Antioxidant Defense Systems During Seed Germination of the Weed Species Ipomoea triloba L. and the Responses to Allelochemicals. Journal of Chemical Ecology 37.5 (2011): 500-513.

216 Singh, Harminder P., Daizy R. Batish, Shalinder Kaur, Komal Arora, Radinder K. Kohli. α-Pinene Inhibits Growth and Induces Oxidative Stress in Roots. Annals of Botany 98.6 (2006): 1261-1269.

217 Michaelakis, Antonios, Dimitrios Papachristos, Athanasios Kimbaris, George Koliopoulos, Athanasios Giatropoulos, and Moschos Polissiou. "Citrus essential oils and four enantiomeric pinenes against Culex pipiens (Diptera: Culicidae)." Parasitology Research 105.3 (2009): 769-773

aromatic hydrocarbons like linalool, limonene, and geraniol. Pinene in also used as an intermediate in synthetic camphor manufacture, a product that is used in dozens of different applications.

Pinene is found in hundreds of essential oils available in natural preparations, or in blended oils containing other terpene constituents. The cosmetics industry uses essential oils containing pinene to add to cosmetic products, primarily to mask or enhance their aroma.

Used in the food industry as a flavoring agent, pinene is often added to sweets, baked goods, specialty beverages, and other value-added foodstuffs. Interestingly, as the primary constituents of Ferula oil, β-pinene (47.1%) and α-pinene (21.36%) exhibited significant antioxidant activity, with the study authors suggesting that the EO of Ferula could accordingly "be used as safe and effective natural antioxidants to improve the oxidative stability of fatty foods during storage and to preserve foods against food burn pathogens"[218].

As a highly flammable but reasonably stable liquid, pinene has also garnered the attention of special interest groups, including those in the hydrocarbon fuels industry. One study determined that pinene possesses a gravimetric energy density comparable to that of traditional hydrocarbon fuels (fuels consisting only of hydrogen and carbon atoms, for example; propane, butane, hexane, and methane gasses), and found that the monoterpene is suitable for operation in turbocharged SI (spark ignition) engines[219].

POTENTIAL USES IN MEDICINE

Pinene is well-studied and offers promise in several medical fields, and, with additional research, we will likely discover even more medical uses than detailed below. However, it should be noted that, while this substance is highly bioavailable with 60% pulmonary uptake and rapid metabolism[220], it is also potentially toxic in high concentrations, with as little as ½ ounce proving fatal to a child[221], and fatal doses for adults estimated at about 180 grams[222]. Toxicity figures are estimated based on turpentine consumption or contamination, where pinene is the primary constituent at up to 60%.

218 Evaluation of antioxidant and antimicrobial activities of essential oils from Carum copticum seed and Ferula assafoetida latex. Kavoosi G, Tafsiry A, Ebdam AA, Rowshan V. J Food Sci. 2013 Feb;78(2):T356-61

219 Raman, V., Sivasankaralingam, V., Dibble, R., and Sarathy, S., α-Pinene—A High Energy Density Biofuel for SI Engine Applications, SAE Technical Paper 2016-01-2171, 2016

220 Russo, E. B (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology. 163 (7): 1344–1364.

221 Gosselin, R.E., R.P. Smith, H.C. Hodge. Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams and Wilkins, 1984., p. III-393

222 The Merck Index. 9th ed. Rahway, New Jersey: Merck & Co., Inc., 1976., p. 969

Treatment for Stress: To say that stress is a killer is no longer cliché; it's a reality for millions of people. Related to this, the current growth of the aroma therapy industry is buoyed on the concept that aromatic compounds can help relieve, reduce, and even eliminate the negative physical effects of stress. As it turns out, there is scientific evidence to support these practices. In one study, 15 young men took part in an experiment where they breathed controlled emissions containing α-pinene, which lowered the systolic blood pressure of the participants[223], indicating relaxation. A follow-up study was conducted by one of the original study authors, but this time the participants were all young women. In the second study, olfactory stimulation by α-pinene significantly decreased the heart rate of the participants, again indicating physiological relaxation[224].

Insect Repellant: Because of the spread of severe mosquito-borne diseases like the Zika virus, Dengue fever, and Malaria, new types of natural and easily reproducible insect repellants are needed, and pinene is a good candidate. As a primary constituent of the essential oils of several plant species, α-pinene was shown to exhibit strong insect repellant activity[225], but researchers noted that, due to the volatile nature of this bicyclic monoterpene, re-application is needed frequently and thus more study is required before the compound could be a viable option as a commercial insect repellant.

Antioxidant: Pinene is a well-known antioxidant, and shows potential of being used in the treatment of oxidative stress-induced neurodegenerative disorders. In one study, hydrogen peroxide-induced oxidative stress in rat cells was treated with both 1,8-cineole and α-pinene, and the effects were significant, attenuating the loss of cell viability and changes in cell morphology, inhibiting intracellular ROS production, and markedly enhancing the expression of antioxidant enzymes[226]. In a separate study by the same authors, the essential oil of S. lavandulifoia (Spanish sage), which featured α-pinene and 1,8-cineole as the primary constituents, respectively, inhibited and decreased ROS production, and increased the endogenous antioxidant status, again while attenuating the loss of cell viability and morphology[227].

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223 Tsunetsugu Y, Park BJ, Miyazaki Y (2010) Trends in research related to "Shinrin-yoku" (taking in the forest atmostphere or forest bathing) in Japan. Environ Health Prev Med 15:27-37.

224 Ikei, Harumi, Chorong Song, and Yoshifumi Miyazaki. Effects of olfactory stimulation by α-pinene on autonomic nervous activity. Journal of Wood Science 62.6 (2016): 568-572.

225 Nerio, Luz Stella, Jesus Olivero-Verbel, and Elena Stashenko. Repellent activity of essential oils: A review. Bioresource Technology 101.1 (2010): 372-378.

226 Porres-Martínez, María, Elena González-Burgos, M. Emilia Carretero, and M. Pilar Gómez-Serranillos. In vitro neuroprotective potential of the monoterpenes α-pinene and 1,8-cineole against H 2 O 2 -induced oxidative stress in PC12 cells. Zeitschrift für Naturforschung C 71 (2016).

227 Porres-Martínez, M., E. González-Burgos, M. E. Carretero, and M. P. Gómez-Serranillos. Major selected monoterpenes α-pinene and 1,8-cineole found in Salvia lavandulifolia (Spanish sage) essential oil as regulators of cellular redox balance. Pharmaceutical Biology 53.6 (2015): 921-929.

Anti-parasitic: In a blinded, controlled study, an essential oil blend where the primary constituent was α-pinene was fed to pigs infected with Ascaris lumbricoides (large roundworms that infect humans), which resulted in significantly reduced total worm counts in the animals, while not causing any adverse reactions[228].

Anti-inflammatory: As part of an essential oil that featured α-pinene and 1,8-cineole as the primary constituents, these compounds have been shown to greatly reduce inflammation, in one case increasing the rate of skin flap survival in rats[229] (skin flaps in medicine refer to a section of healthy skin that is partly excised and used to cover a nearby wound). In another study, α-pinene and β-pinene were the primary constituents of the essential oil of Fructus Alpiniae zerumbet, which showed significant anti-inflammatory and analgesic effects in mice[230].

Antibacterial: The essential oil extracted from the leaves of Myracrodruon urundeuva, a tree native to South America, featured α-pinene and β-pinene as the primary compounds, which exhibited antibacterial activities against some of the most common strains of bacteria, including Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, and Salmonella Enteritidis[231]. Another study conducted last year echoed these findings regarding E. coli, where α-pinene eliminated the formation of bacterial colonies after just 2 hours of exposure[232].

Treatment for Liver Cancer: In a promising study of liver cancer, α-pinene obviously inhibited the growth of hepatoma carcinoma cells by 79.3% in vitro and 69.1% in vivo[233], an impressive effect against a deadly type of cancer.

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228 Kaplan RM, Storey BE, Vidyashankar AN, Bissinger BW, Mitchell SM, Howell SB, Mason ME, Lee MD, Pedroso AA, Akashe A, Skrypec DJ. Antiparasitic efficacy of a novel plant-based functional food using an Ascaris suum model in pigs. Acta Trop. 2014 Nov; 139:15-22.

229 İnce B, Dadacı M, Kılınç İ, Oltulu P, Yarar S, Uyar M. Effect of cineole, alpha-pinene, and camphor on survivability of skin flaps. Turk J Med Sci. 2018 Jun 14;48(3):644-652.

230 Xiao, Rui-Yao, Ling-Jing Wu, Xiao-Xiao Hong, Ling Tao, Peng Luo, and Xiang-Chun Shen. Screening of analgesic and anti-inflammatory active component in Fructus Alpiniae zerumbet based on spectrum–effect relationship and GC–MS. Biomedical Chromatography 32.3 (2018).

231 Rebouças de Araújo ÍD, Coriolano de Aquino N, Véras de Aguiar Guerra AC, Ferreira de Almeida Júnior R, Mendonça Araújo R, Fernandes de Araújo Júnior R, Silva Farias KJ, Fernandes JV, Sousa Andrade V. Chemical composition and evaluation of the antibacterial and Cytotoxic activities of the essential oil from the leaves of Myracrodruon urundeuva. BMC Complement Altern Med. 2017 Aug 22;17(1):419.

232 de Sousa Eduardo L, Farias TC, Ferreira SB, Ferreira PB, Lima ZN, Ferreira SB. Antibacterial Activity and Time-kill Kinetics of Positive Enantiomer of α-pinene Against Strains of Staphylococcus aureus and Escherichia coli. Curr Top Med Chem. 2018;18(11):917-924.

233 Weiqiang Chen, Ying Liu, Ming Li, Jianwen Mao, Lirong Zhang, Rongbo Huang, Xiaobao Jin, Lianbao Ye. Anti-tumor effect of α-pinene on human hepatoma cell lines through inducing G2/M cell cycle arrest. Journal of Pharmacological Sciences Volume 127, Issue 3, March 2015, Pages 332-338.

Protection against UVA Cellular Damage: α-pinene has been shown to prevent UVA-induced cytotoxicity, generation of ROS, lipid peroxidation and DNA stand breaks, and apoptosis in human skin cells[234].

Prevention of Aspirin Toxicity: Thanks mostly to its well-established antioxidant properties, α-pinene is able to protect small intestinal epithelial cells from aspirin-induced oxidative stress[235].

Treatment for Prostate Cancer: α-pinene has a profound impact on human prostate cancer cells, inducing apoptosis and cell cycle arrest, and inhibiting tumor progression in xenograft tumors in mice[236] (xenograft tumors are human tumor cells that have been introduced to immunocompromised mice that will not reject human cells).

Treatment for Melanoma: Lending further credibility to the potential power of aromatherapy, an impressive study with mice has shown that inhalation of environmental aromatic α-pinene reduced melanoma growth by about 40% over controls[237], which leads to the conclusion that people who have melanoma may benefit from forest bathing as mentioned earlier in this chapter.

Synergystic Treatment for Lung Cancer: To add to the already considerable potential for the use of pinene in cancer treatment, α-pinene and β-pinene showed synergistic effects against non-small-cell lung cancer cells when combined with Paclitaxel (Taxol), a common chemotherapy medication[238].

IMPLICATIONS FOR HUMAN HEALTH & NUTRITION

The easiest method of obtaining pinene is via a healthy diet. Consider regularly eating fruits like apples, grapefruit, guava, lemon, lime, mango, blackberries, blueberries,

oranges, pomelos, figs, strawberries, tangerines, or plums. Vegetables like bell peppers, carrots, celery, and tomatoes are rich in pinene and should be included as part of a regular diet. When cooking or preparing foods, consider working with spices like allspice, anise, black pepper, bay, ginger, caraway,

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nutmeg, oregano, parsley, rosemary, saffron, cilantro, coriander, dill, sage, tarragon, or thyme, which all have high concentrations of pinene.

Consider consuming cannabis strains that are rich in pinene such as ACDC, Banana OG, Blue Dream, Bubba Kush, Cannatonic, Chem D, DJ Short Blueberry, Dutch Treat, Girl Scout Cookies, Gorilla Glue #4, Harlequin, Haze Berry, Island Sweet Skunk, Jack Herer, Obama Kush, OG Kush, Purps, Romulan, Royal Jack, Skywalker OG, Strawberry Cough, or Trainwreck, for another way of obtaining biologically available pinene. Smoking or combustion of cannabis is not recommended, but oils and tinctures are good alternative options for consumption of this bicyclic monoterpene.

Use of or experimentation with essential oils containing pinene as the primary constituent should be undertaken with care considering the potentially toxic nature of the purified compound in high doses.