

# Are Cannabis Derived Terpenes **Actually** Different Than Botanical Terpenes?

Chiral gas chromatography of three **Cannabis sativa** L. cultivars, four botanical essential oils and two commercial cannabis-mimic terpene products — **same molecules, opposite handedness.**

## ANALYTICAL DATA

Chiral GC analysis performed by **Laboratoire PhytoChemia**, Chicoutimi, Québec, under analyst **Alexis St-Gelais, Ph.D., chimiste** (Québec professional license 2013-174).

Documented in nine certificates of analysis and reproduced with the laboratory's written permission.

## Disclosure

Nexus Agriscience and its subsidiary Terpene Belt Farms manufacture and sell cannabis-derived terpene products. This paper is educational in intent and reflects the authors' chemistry and the analytical data described below. Readers should evaluate the commercial implications with that affiliation in mind. All chiral GC data presented here were generated by Laboratoire PhytoChemia (Chicoutimi, Québec), a third-party analytical laboratory commissioned by Nexus Agriscience, and are reproduced here with the laboratory's written permission.

### *Executive summary*

The term “isomer” is seldom heard in cannabis-product marketing. What you do hear are claims that botanically derived terpenes, limonene from oranges, linalool from lavender,  $\beta$ -pinene from turpentine, are chemically identical to the terpenes cannabis produces, and therefore interchangeable with them. On the surface, these claims have merit: the chemical compound called limonene does exist in both oranges and cannabis. Look closer, through the lens of stereochemistry, and the picture changes.

This paper presents chiral gas-chromatography data from three Nexus Agriscience *Cannabis sativa* L. cultivars, all from a single 2024 harvest and analyzed by one laboratory using a single chiral GC method, alongside two commercial “cannabis terpene” mimic products and four authentic botanical essential oils. For limonene and linalool the pattern is a straight opposition: the three cultivars tested carry one enantiomer in excess and both mimic products carry the other, matching the commodity botanical source. For  $\beta$ -pinene the contrast is one of degree rather than direction — cultivars, pine and mimics are all (S)-selective, but to very different extents. Taken together across the seven-compound panel, the cultivars occupy their own region of stereochemical space.

Principal component analysis of signed enantiomeric excess across the seven compounds detected in at least seven of the nine samples places the three cultivars in a single tight region of stereochemical space, resolved from every botanical oil and every mimic product tested.

These differences matter because stereoisomers, molecules with the same atoms and the same connectivity but a different three-dimensional arrangement, can interact differently with biological receptors and olfactory pathways. The chiral signature observed in these cultivars is not an arbitrary detail. It is most readily explained as a record of the terpene-synthase enzymes that assembled them, and it supports the broader case for chiral analysis as an authentication and quality tool.

### *Scope and limitations*

The dataset presented in this paper consists of three *Cannabis sativa* L. cultivars (2024 Fruit 135, 2024 Purple 103, 2024 Dessert 45) grown and harvested at a single Nexus Agriscience cultivation site and analyzed by a single laboratory using one chiral GC



method. The nine samples were submitted in three batches, analyzed on 25 April, 18 June and 26 June 2025; the batch structure is given with the certificate codes in the methods section. All three are hemp-type chemovars, grown under the federal THC threshold; they are used here because terpene biosynthesis is governed by the terpene synthase complement rather than by the cannabinoid pathway, so the enantiomeric ratios they produce are expected to be representative of *Cannabis sativa* L. more broadly. Cannabinoid content was not determined and is not relevant to the measurements reported. This dataset was compared against two commercial cannabis-mimic terpene products and four authentic botanical essential oils (orange, thyme, lavender, pine). Fifteen chiral compounds were resolved across the panel, but detection was uneven. Four were found in two or fewer samples and in none of the cannabis samples, and a further four in three to six samples. The seven detected in at least seven of the nine samples form the panel used for the multivariate analysis and are named in the Methods section, while the full fifteen-compound dataset is reported in the appendix. Each sample was analyzed once, so no analytical replicates are available and the principal component analysis is exploratory rather than confirmatory. No sequencing, gene-expression or enzyme-characterization work was performed on these cultivars; the enzymology and genomic organization discussed later in the paper are drawn from the published literature and are not results of this study. The findings describe the cultivars, mimic products and reference oils that were tested. Broader generalizations, across all cannabis cultivars, all harvests, all extraction methods, or all commercial terpene products, would require a substantially larger and more comprehensive study. The analytical method, batch structure, missing-value handling and the laboratory's own caveats are set out in the Methods section.

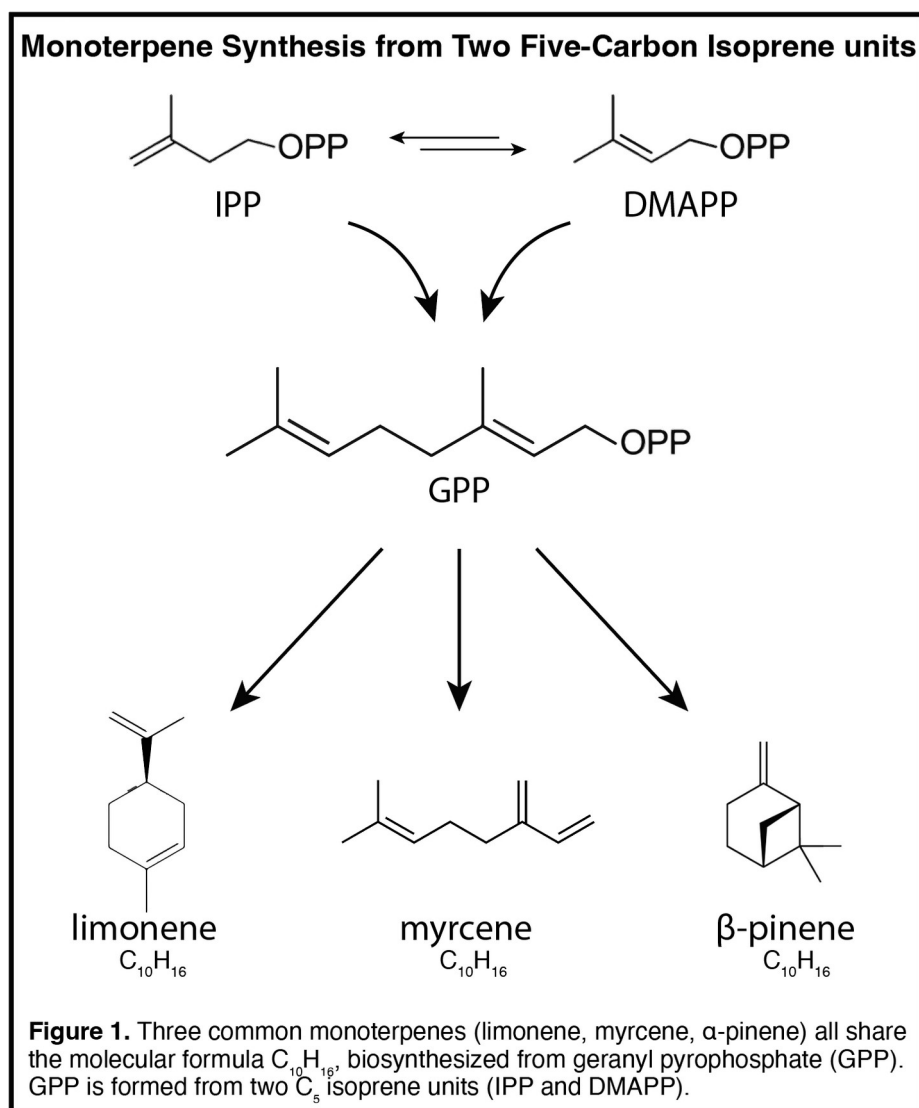
### *Introduction*

Cannabis essential oil is a chemical mosaic. The fragrance and flavor the plant is known for arise from a large number of biosynthesized volatile compounds present at concentrations spanning roughly four orders of magnitude. Cannabis volatile extracts contain a complex mixture of mono- and sesquiterpene hydrocarbons, oxygenated terpenoids, and other volatile constituents: monoterpenes such as limonene, myrcene and  $\beta$ -pinene, and sesquiterpenes such as  $\beta$ -caryophyllene, at percent-level abundance; oxygenated terpenoids such as linalool oxides, terpinen-4-ol and  $\alpha$ -terpineol at lower levels; and trace esters, aldehydes and ketones that can contribute disproportionately to perceived aroma.

Monoterpenes are assembled from two five-carbon isoprenoid diphosphates: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Geranyl diphosphate (GPP), the C<sub>10</sub> precursor of monoterpene biosynthesis, is formed by the enzyme-catalyzed head-to-tail condensation of one DMAPP unit with one IPP unit (see Figure 1). Because that single precursor can be folded, cyclized and quenched in many different ways, one starting material gives rise to a large family of products — some differing in connectivity, others only in the arrangement of the same atoms in space.



Botanical flavoring companies have built businesses on recreating the chemical profiles of popular cannabis cultivars using terpenes sourced from non-cannabis feedstocks: limonene from citrus peel, linalool from lavender,  $\beta$ -pinene from pine turpentine. The marketing claim underlying this industry is that any molecule called “limonene” is the same molecule regardless of where it came from. Although limonene from different sources shares the same molecular formula ( $C_{10}H_{16}$ ) and the same atom-to-atom connectivity, its enantiomeric composition can differ, and that difference can affect both odor and biological interactions. This paper examines why different forms of limonene exist, how those forms can differ in perceived aroma, and how chiral analysis distinguishes monoterpenes native to cannabis from those that are not.



### What is isomerism?

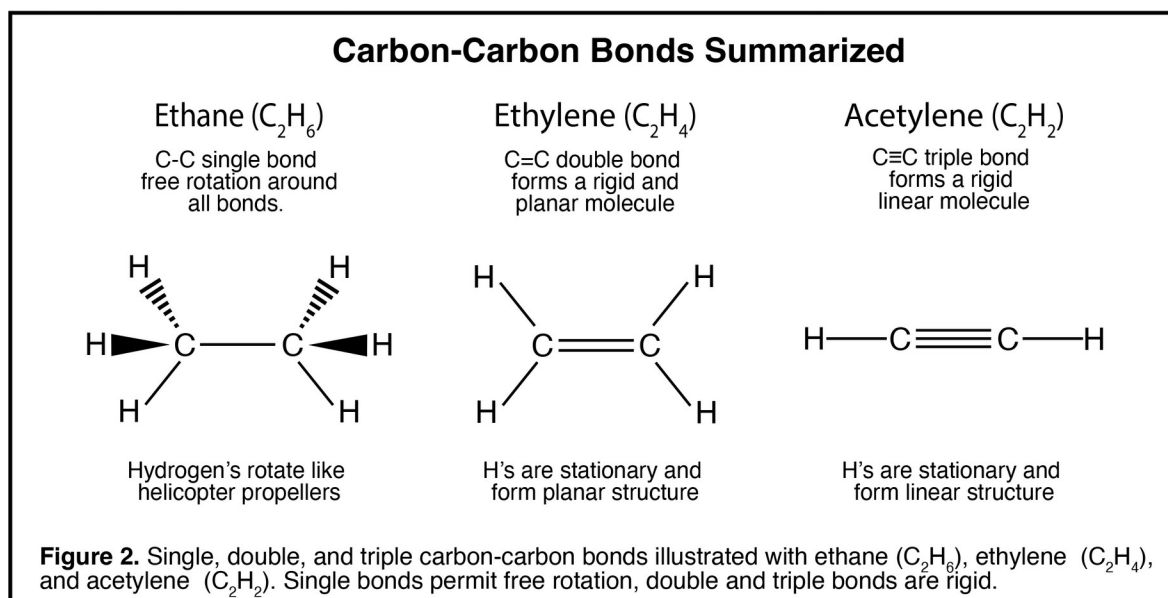
Designations such as (+)/(–), R/S, cis/trans or E/Z, and  $\alpha/\beta$  appear before compound names on certificates of analysis and in the scientific literature, but they do not all describe the same kind of isomerism. R/S specifies the absolute configuration at a



stereocenter. (+)/(-) reports the direction in which a sample rotates plane-polarized light, which is a measured property of a sample rather than a structural rule. cis/trans, and more formally E/Z, describes geometric configuration about a double bond or ring. The  $\alpha/\beta$  prefixes used for compounds such as pinene and caryophyllene are compound-specific naming conventions that distinguish structural isomers and carry no information about chirality at all. Understanding what each notation does and does not tell you requires a short detour through chemical bonding.

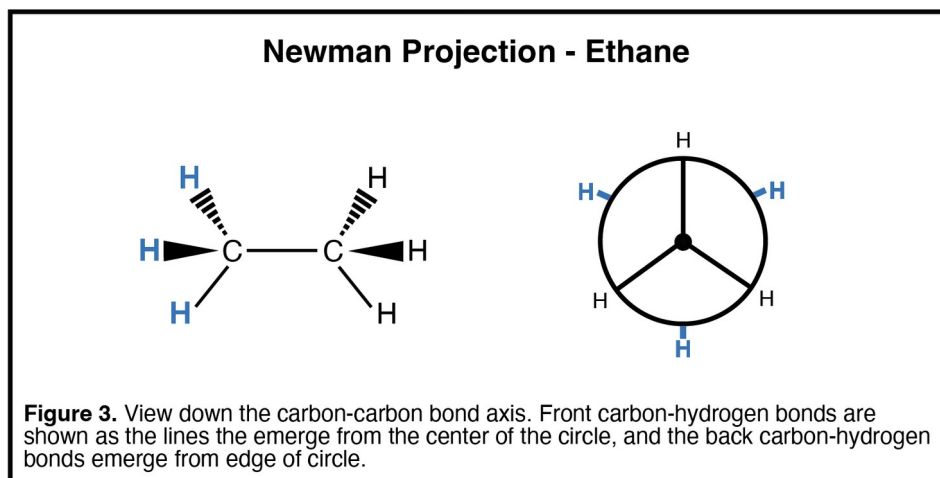
### *Carbon bonds, and how molecules are drawn*

Carbon is the backbone of every organic molecule found in *Cannabis sativa* L. Because a carbon atom has four valence electrons, it forms four covalent bonds, sharing an electron pair with each partner atom or group. A single bond is one shared electron pair, and rotation about it is generally possible, though steric and conformational barriers mean it is rarely completely free. A double bond consists of one  $\sigma$  bond and one  $\pi$  bond; the  $\pi$  bond prevents rotation and locks the attached groups in place. A triple bond consists of one  $\sigma$  bond and two  $\pi$  bonds, three shared electron pairs in total, and is likewise rigid. The number and type of bonds around each carbon therefore determine both the shape of a molecule and how freely its parts can move (Figure 2).



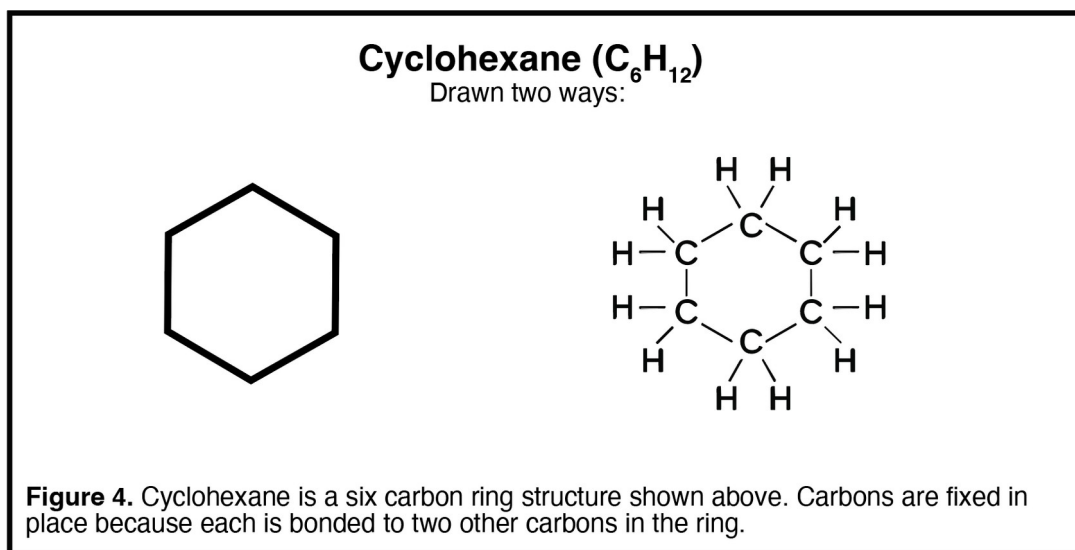
When carbons are connected by single bonds, the molecule can flex and rotate about each bond, subject to the steric and conformational barriers noted above. A useful way to visualise this rotation is the Newman projection, which looks straight down the axis of a single bond (Figure 3):





The Newman projection above is of ethane ( $C_2H_6$ ) in the staggered conformation, its lowest-energy arrangement. The lines from the center represent bonds on the front carbon; the lines from the outer circle represent bonds on the back carbon. Rotation about the bond between them is easy at room temperature, though it passes through a higher-energy eclipsed arrangement on the way.

Carbons can also form rings. Cyclohexane ( $C_6H_{12}$ ) is six carbons joined in a ring by single bonds. Ring closure prevents free rotation about those bonds, but the ring is not planar: it adopts a puckered chair conformation, and less stably a twist-boat, interconverting between equivalent chair forms at room temperature (Figure 4).



### *Structural (constitutional) isomers*

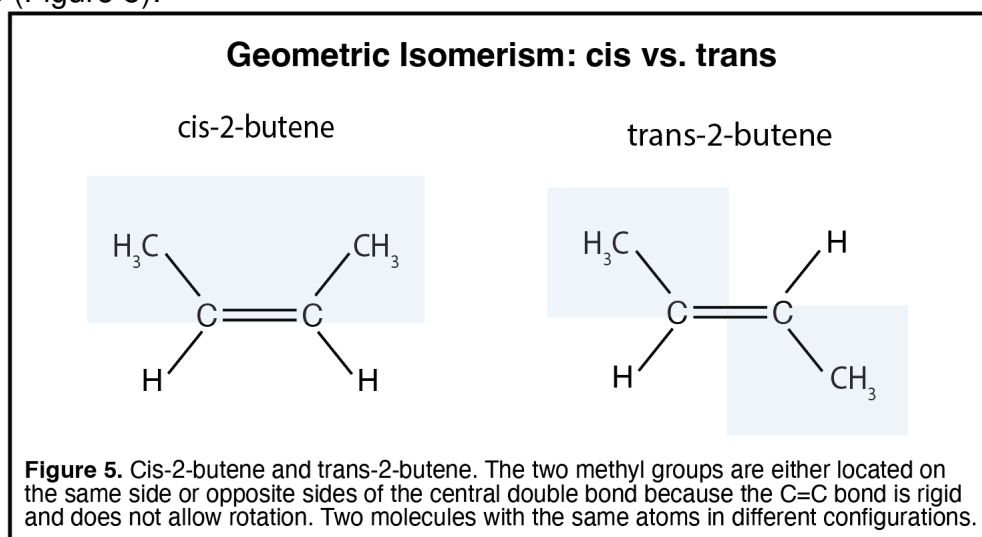
Structural isomers are molecules that share the same molecular formula but differ in the connectivity of their atoms. Many cannabis monoterpenes share the molecular formula  $C_{10}H_{16}$  and are therefore structural isomers of one another. Limonene has a six-carbon ring carrying a methyl substituent and an isopropenyl group. Myrcene is acyclic, a chain



with no ring at all.  $\alpha$ - and  $\beta$ -pinene are bicyclic, built on a bridged bicyclo[3.1.1]heptane framework rather than two fused rings (see Figure 1 above for examples of the structures).

#### *Geometric isomers (cis/trans)*

Geometric isomers, also called cis/trans isomers, arise around rigid structural features such as double bonds and rings. Because a double bond cannot rotate, the substituents on either side of it are locked either on the same side (cis, Latin for “on this side of”) or on opposite sides (trans, Latin for “across”). The smallest molecule that illustrates this is 2-butene (Figure 5):



#### *Stereoisomers, diastereomers and enantiomers*

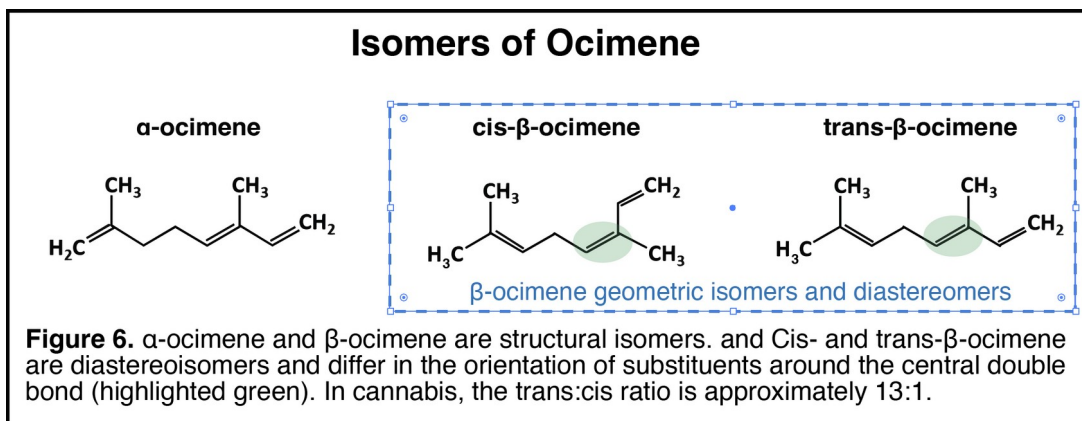
Stereoisomers share both molecular formula and atom-to-atom connectivity but differ in the arrangement of their atoms in space. They fall into two classes. Enantiomers are non-superimposable mirror images of one another. Diastereomers are stereoisomers that are not mirror images. The geometric isomers described above are a subtype of diastereomer rather than a third parallel category.

Diastereomers are uncommon among the major cannabis monoterpenes but occur among more complex terpenes.  $\beta$ -Caryophyllene, for example, carries two stereocenters, and its stereochemistry is functionally consequential: the (–)-trans form that predominates in cannabis is a selective CB2 receptor agonist (Gertsch et al., 2008), an activity not shared equally by its stereoisomers.

Ocimene exists in two main structural forms,  $\alpha$ -ocimene and  $\beta$ -ocimene, and  $\beta$ -ocimene occurs as cis- and trans- geometric isomers about its central double bond (Figure 6). Because they are not mirror images, cis- and trans- $\beta$ -ocimene are diastereomers. They differ in ordinary physical properties and are also reported to differ in odor, cis- $\beta$ -ocimene being commonly described as sweet, green and herbaceous and trans- $\beta$ -ocimene as more woody and resinous. These descriptors are drawn from flavor and

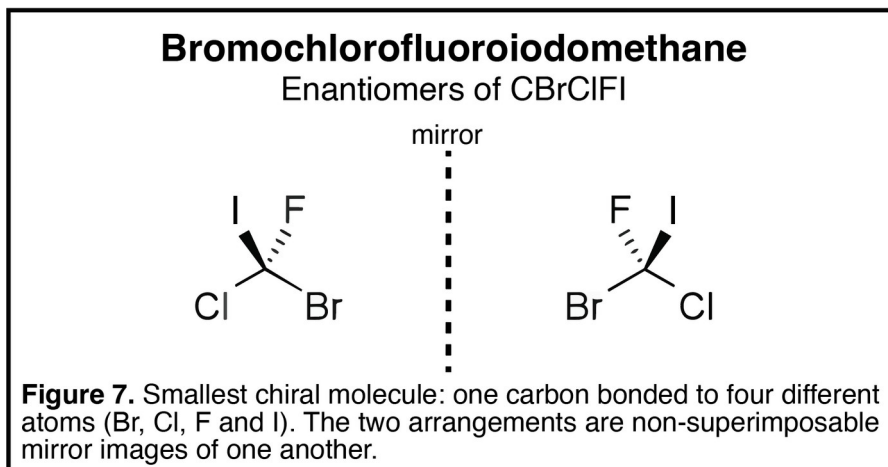


fragrance trade usage rather than a primary sensory study, and like all odor descriptors they depend on concentration and on the matrix in which a compound is presented, and should be read throughout this paper as reported characterizations rather than fixed properties of the molecule. The underlying point is that three-dimensional structure can influence perceived aroma, a theme returned to below.



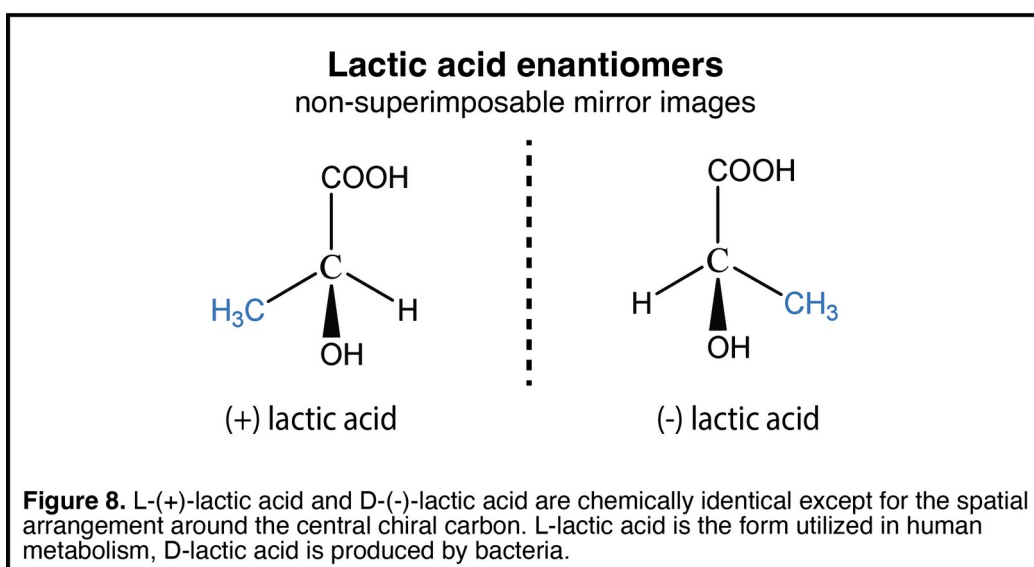
Enantiomers deserve a closer look. They share nearly all physical properties — the same boiling point, the same density, identical NMR spectra in achiral solvents — with two exceptions: they rotate plane-polarized light in opposite directions, and they interact differently with other chiral molecules. That second exception is what matters biologically, because it means enantiomers can bind receptors and enzymes differently.

A molecule is chiral when it cannot be superimposed on its own mirror image. The commonest cause is a carbon bearing four different substituents, which may be individual atoms or groups of atoms; such a carbon is called a stereocenter or chiral center. The simplest illustration is bromochlorofluoriodomethane (CBrClFI), in which a single carbon carries four different halogen atoms and no hydrogen, shown in Figure 7 below. There are exactly two non-superimposable arrangements of those four substituents around the central carbon, and the result is two different molecules:



The two arrangements are mirror images that cannot be superimposed. A familiar everyday analogy: a person's right and left hands are mirror images of each other but cannot be superimposed. This is why chirality is often called “handedness.”

Chirality is everywhere in living systems. Lactic acid exists as two enantiomers: (S)-(+)-lactic acid, conventionally written L-lactic acid, is produced by human metabolism, while (R)-(-)- or D-lactic acid is produced largely by bacteria (Figure 8, below). Proteins are built almost exclusively from L-amino acids, but D-amino acids are not simply biological rarities: they are structural components of bacterial cell walls and occur in mammalian tissue, D-serine and D-aspartate being well characterized in the brain. Sugars likewise occur predominantly in one configuration. And, critically for this paper, many terpenes are chiral, and the enantiomer that cannabis produces is often not the enantiomer that the global commodity supply chain delivers.

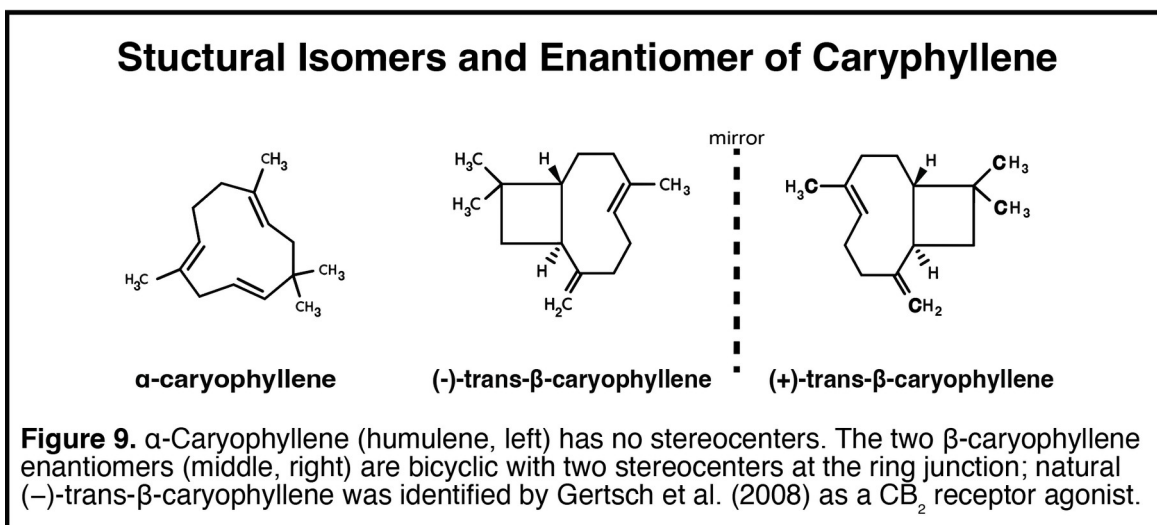


### *Isomeric sesquiterpenes in cannabis*

Cannabis essential oils also contain sesquiterpenes whose isomerism, whether geometric or structural, is in principle available for the same kind of comparison, although no sesquiterpene enantiomer data are presented here: the one sesquiterpene in the panel, caryophyllene oxide, was detected in too few samples to include. Ocimene served as the monoterpene example above (Figure 6); caryophyllene illustrates the same principle among the sesquiterpenes.

Caryophyllene is the most abundant sesquiterpene found in cannabis. It exists in two structural forms:  $\alpha$ -caryophyllene (humulene), an 11-membered ring with no stereocenters; and  $\beta$ -caryophyllene, a bicyclic structure with two stereocenters. Figure 9 distinguishes the difference between structural isomers ( $\alpha$ -caryophyllene and  $\beta$ -caryophyllene) and enantiomer pairs ((-)-trans- $\beta$ -caryophyllene and (+)-trans- $\beta$ -caryophyllene).





### *Naming conventions in stereochemistry*

Two naming systems matter for chiral molecules in essential-oil chemistry, and they answer different questions. One describes the three-dimensional configuration at each stereocenter; the other reports a measured optical property. They do not map onto one another in any fixed way, and this paper is explicit about which is being used.

### *The (+)/(-) system*

The plus and minus designators come from experimental optical rotation: a (+) sample rotates the plane of polarized light clockwise as seen by an observer looking back toward the source, and a (-) sample rotates it counterclockwise. This is the only one of the two systems based on measurement rather than on a structural rule; it describes a sample rather than a configuration, and it remains the convention most widely used in the flavor and fragrance industry. The older lowercase d- and l- prefixes are historical synonyms for dextrorotatory and levorotatory and mean exactly what (+) and (-) mean. They should not be confused with the uppercase D/L system, which describes configuration relative to glyceraldehyde and carries no information about the direction of optical rotation. Because lowercase d/l invites precisely that confusion, and because it is still common on commercial documentation, this paper uses R/S for configuration and (+)/(-) for measured rotation throughout, and avoids d/l as a label.

### *The R/S system*

The R (rectus, Latin for “right”) and S (sinister, Latin for “left”) designators come from a formal rule (the Cahn–Ingold–Prelog priority rules) applied to each chiral center. Each substituent around the chiral carbon is ranked by atomic priority, and the configuration is named R or S based on whether those priorities trace a clockwise or counterclockwise path. This is the modern systematic convention and the one published organic-chemistry literature uses.



A subtlety worth noting: the two systems do not map onto one another in any fixed way. (–)-Linalool is the R-enantiomer, but (–)- $\beta$ -pinene is the S-enantiomer. Which sign of rotation goes with which configuration depends on the substituent priorities at the chiral center, and those differ from compound to compound, so the direction of optical rotation cannot be predicted from R/S alone.

### *Chirality and biology*

Chirality matters in living systems because nearly all biological receptors, enzymes and signaling molecules are themselves chiral. They are built from L-amino acids that fold into chiral three-dimensional shapes, and like a hand fitting a glove, they interact selectively with the matching enantiomer of a small-molecule ligand.

A right-handed key opens a right-handed lock; the mirror-image key, otherwise identical, does not. In the same way a (+)-enantiomer may bind one receptor strongly while its (–)-counterpart binds it weakly, not at all, or binds a different receptor entirely.

Real-world examples are well documented in pharmaceutical chemistry. (R)-(–)-Albuterol carries essentially all of the bronchodilator activity in racemic albuterol, and the (S)-(+)-enantiomer contributes little or none. (S)-(+)-Ketamine is the more potent anesthetic of the two ketamine enantiomers, and the two have distinguishable psychoactive profiles. Drug development now routinely tracks and separates enantiomers, because the two forms can behave like quite different molecules in vivo.

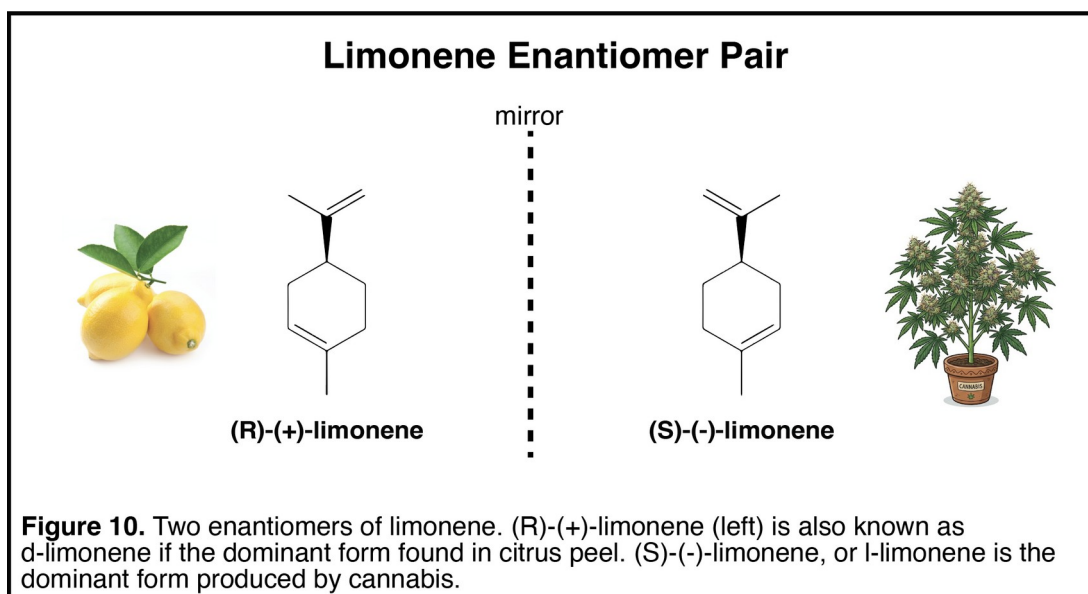
Configuration can affect smell as well as pharmacology, and among monoterpenes the olfactory differences are often the more immediately obvious of the two. One of the most striking examples is carvone, a useful comparison for cannabis because it is a  $C_{10}H_{14}O$  monoterpenoid found in aromatic plants. (R)-(–)-Carvone occurs in spearmint and defines the familiar scent of spearmint leaves, toothpaste and chewing gum, whereas (S)-(+)-carvone occurs in dill and gives pickles and culinary dill their pungent, grassy character.

The same principle applies to cannabis monoterpenes. Three key monoterpenes to examine are limonene, linalool and pinene. These are some of the most consequential chiral compounds in cannabis essential oil.

### *Limonene enantiomers*

Limonene is the chiral monoterpene most familiar to consumers, because the (R)-(+)-form gives orange peel its characteristic aroma. It exists as two enantiomers: (R)-(+)-limonene, with a sweet citrus character, and (S)-(–)-limonene, commonly described as sharper and more turpentine-like.





The structures of (S)-(-)-limonene and (R)-(+)-limonene differ only in the configuration at the chiral center bearing the isopropenyl group (Figure 10). The cannabis cultivars tested predominantly produced (S)-(-)-limonene, at 95.4–96.1% S, while the commercial orange oil tested was predominantly (R)-(+)-limonene at 99.3% R. Both mimic products were (R)-dominant on limonene, at 99.4% and 97.8% R, placing them with the citrus reference rather than with the cultivars. This is the sharpest single-compound contrast in the dataset.

Sun (2007) reviewed the bioactivity of (R)-(+)-limonene, the form that dominates citrus oils, noting chemopreventive and anticancer properties in rodent models. The mirror-image (S)-(-)-limonene, dominant in the cannabis cultivars tested, has received considerably less research attention, and we are not aware of published work that would establish whether the same outcomes apply to it.

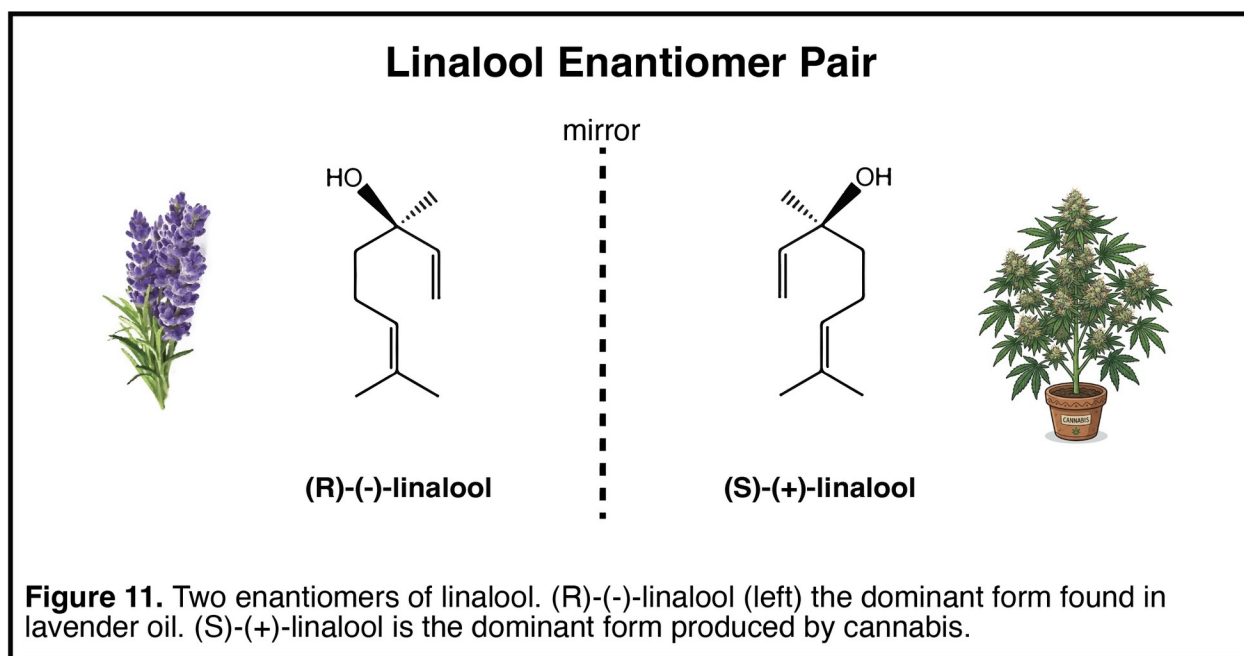
The aroma of (S)-(-)-limonene differs from that of (R)-(+)-limonene, much as the two carvones differ from one another. The sweet peel character associated with citrus tracks the (R)-(+)-form, while the (S)-(-)-form is commonly described as sharper and more turpentine-like; Bentley (2006) reviews the general phenomenon of enantioselective odor perception, including the carvones. As with all odor descriptors in this paper these are reported characterizations that vary with concentration and matrix, but the underlying point holds: changing configuration can change perceived aroma, which places chirality at the center of any attempt to reproduce cannabis flavor.

A note on cannabis COAs. Commercial cannabis terpene certificates of analysis routinely report “limonene” or “d-limonene” as the analyte, which can read as contrary to the finding above. Most cannabis terpene testing is performed on a non-chiral GC column. Such columns do not resolve enantiomers, and laboratories typically label the unresolved peak with the name of the reference standard used, most often d-limonene

because it is the dominant commercial form. A COA produced on a non-chiral column therefore reports the compound, not the enantiomer, and the same limitation applies to peer-reviewed work that reports d-limonene without a chiral separation. Chiral GC, as used in this study, is what makes the enantiomeric distinction visible. Readers reviewing a cannabis COA should look for explicit chiral-column methodology before drawing conclusions about which enantiomer is present.

### *Linalool enantiomers*

Linalool is the chiral monoterpene most associated with floral and lavender notes. (R)-(-)-Linalool, also known as licareol, is the major form in lavender and sweet basil. (S)-(+)-Linalool, also known as coriandrol, is a major constituent of coriander and sweet orange flower oils, and is the dominant form in the cannabis cultivars tested.



(R)-(-)-Linalool (Figure 11, left) is the dominant enantiomer in lavender oil, whereas (S)-(+)-linalool dominates in the cannabis cultivars tested. The aromas of the two enantiomers may also differ: (R)-(-)-linalool is commonly described as floral and lavender-like, and (S)-(+)-linalool as sweeter, with citrus and coriander character. As elsewhere, these descriptors depend on concentration and matrix; the linalool descriptors follow Aprotosoaie and colleagues (2014).

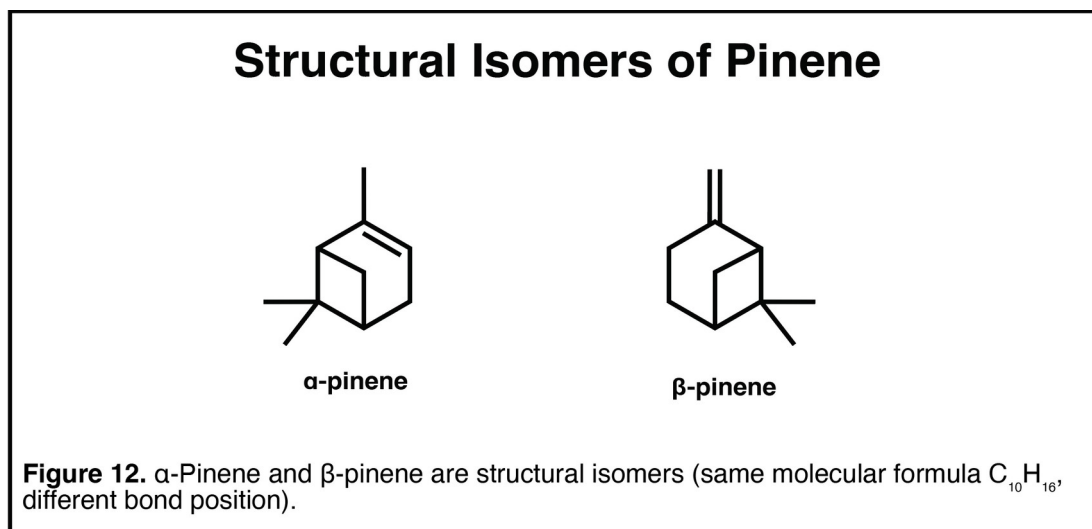
Höferl, Krist, & Buchbauer (2006) measured the effects of (+)- and (-)-linalool inhalation on physiological stress markers in human subjects. Both enantiomers reduced cortisol levels, but the cardiovascular effects diverged: (+)-linalool tended to increase heart rate and blood pressure, while (-)-linalool tended to reduce heart rate. Aprotosoaie and colleagues (2014), in a comprehensive review of linalool pharmacology, reported that (-)-linalool exhibits stronger sedative, stress-relieving, anticonvulsant and anti-

inflammatory effects than (+)-linalool, while (+)-linalool is generally less potent and can even produce mild activating cardiovascular effects.

The cannabis cultivars tested predominantly produced (S)-(+)-linalool, at 96.8–97.9% S. The lavender oil tested was predominantly (R)-(–)-linalool at 94.2% R. Synthetic linalool made by non-stereoselective routes is racemic or close to it, which follows from the general principle set out below rather than from any measurement made here. None of these commodity sources matched the enantiomeric profile observed in the cultivars tested. Where aroma or biological response depends on configuration, a substitution of this kind would not be expected to reproduce the cannabis profile, although that expectation remains to be tested directly.

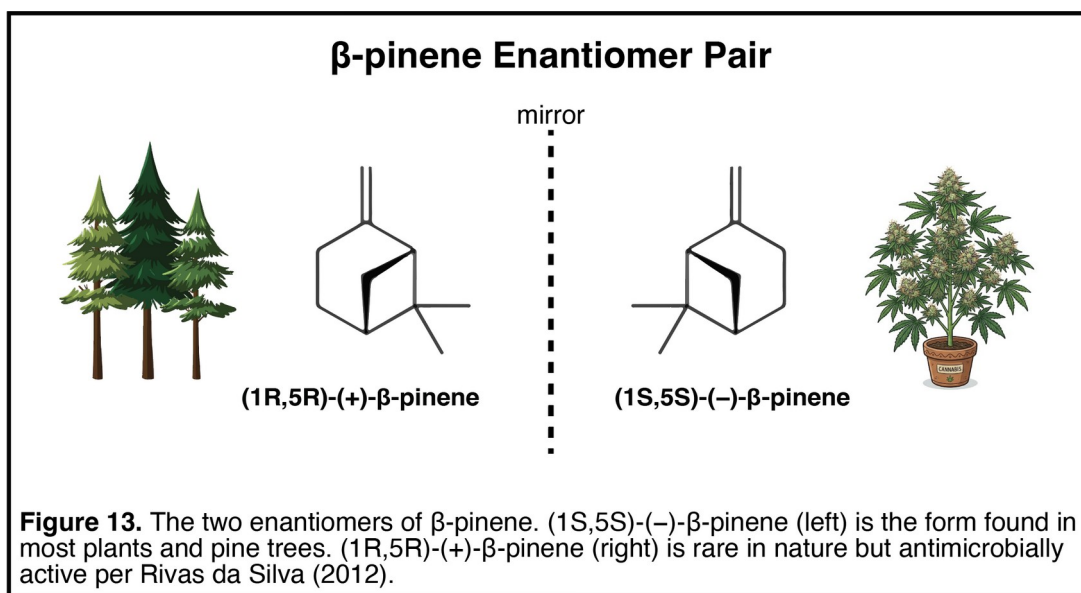
#### *Pinenes and the enantiomers of $\beta$ -pinene*

Pinene exists in two structural forms,  $\alpha$ -pinene and  $\beta$ -pinene, which differ in the position of the double bond within the bicyclic framework and are therefore structural isomers of one another rather than stereoisomers. Cannabis produces both, and each is chiral, so each occurs as its own pair of enantiomers. This study measured enantiomeric ratios rather than absolute abundance, and in any case the  $\alpha$ -pinene enantiomers were not resolved by the chiral column used, so the analysis that follows is confined to  $\beta$ -pinene (Figure 12).



$\beta$ -Pinene occurs as two enantiomers: (1S,5S)-(–)- $\beta$ -pinene, commonly the dominant form in pine-derived turpentine, and (1R,5R)-(+)- $\beta$ -pinene, generally the less abundant form in natural sources. Reported proportions vary substantially with botanical source and, for turpentine, with species, geographic origin and processing: surveys of *Pinus* species place the (R)-form across a wide range (Sjödín et al., 2000; Rodrigues et al., 2017; Ankney et al., 2022). The ranges quoted below should therefore be read as specific to the samples tested rather than as universal values (Figure 13).

$\beta$ -Pinene presents a more nuanced contrast than limonene or linalool, because here the difference lies in the ratio rather than in which enantiomer dominates. The cannabis cultivars tested and the pine turpentine tested are both (S)-selective, but not to the same degree. The pine sample was 98.0% S and 2.0% R, an enantiomeric ratio of roughly 49:1. The three cultivars ranged from 71.6% to 84.4% S, ratios of roughly 2.5:1 to 5.4:1, retaining between 15.6% and 28.4% of the (R)-enantiomer, which is some eight to fourteen times the (R) content of the pine sample. The botanical references did not share a common  $\beta$ -pinene signature: the thyme oil tested was strongly (R)-selective at 99.3% R, and the lavender oil was close to racemic at 55.0% S. Both mimic products were strongly (S)-selective, at 97.0% and 96.5% S, ratios of 32:1 and 28:1, placing them nearer to the pine turpentine than to any cultivar tested.



Rivas da Silva and colleagues (2012) compared the biological activities of both enantiomers of  $\alpha$ -pinene and of  $\beta$ -pinene. In their assays the (+)-enantiomers showed antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans* and methicillin-resistant *Staphylococcus aureus*, while the (-)-enantiomers showed no comparable activity even at substantially higher concentrations. In each pair the two compounds share a molecular formula and a connectivity, and differ only in configuration, yet only one was active. It is worth noting which way this cuts for the present dataset: the cultivars tested are (S)-(-)- $\beta$ -pinene dominant, so on this particular assay the enantiomer they favor is the less active one. Chirality determines behavior; it does not guarantee that the cannabis-native form is the more useful one for every endpoint.

#### *Chiral gas chromatography*

Enantiomers are notoriously difficult to distinguish using standard analytical chemistry. They have identical mass, identical NMR spectra in achiral solvents, identical infrared spectra, and (most relevantly for the flavor industry) they elute at exactly the same



retention time on a standard gas chromatography column. The usual way to resolve enantiomers chromatographically is a column with a chiral stationary phase. These columns are coated with chiral selectors that interact differentially with each enantiomer (Figure 14):

#### *Enantiomeric excess and enantiomeric ratio*

Enantiomeric excess (ee) is the difference between the percentages of the two enantiomers. A racemic mixture (50/50) has  $ee = 0\%$ , and a single pure enantiomer has  $ee = \pm 100\%$  under the signed convention adopted here. Because the central question in this paper is which enantiomer a given source produces, ee is reported here as a signed quantity,  $ee = \%R - \%S$ , so that a positive value denotes an excess of the (R)-form and a negative value an excess of the (S)-form. A sample that is 96% S and 4% R therefore has  $ee = -92\%$ . Unsigned magnitudes are avoided throughout, because  $|ee|$  records only how far a sample lies from racemic and would render a 96% R sample and a 96% S sample indistinguishable, collapsing the very contrast this analysis exists to detect; the comparison is shown with the results below.

Enantiomeric ratio (er) is the ratio of major to minor enantiomer. A racemic mixture has  $er = 1:1$ , and a sample that is 96% S and 4% R has  $er = 24:1$  in favor of S. Ratios are quoted alongside signed ee wherever the degree of selectivity, rather than its direction, is the point of comparison. For compactness the data are reported as %R and %S throughout; for compounds with more than one stereocenter, such as  $\beta$ -pinene, borneol and caryophyllene oxide, R and S denote the enantiomer pair resolved by the laboratory rather than the configuration at a single center.

#### Optical rotation (polarimetry)

Although enantiomers cannot be distinguished by ordinary spectroscopy, they can be distinguished by their interaction with polarized light. Polarimetry was not used in this study; all enantiomeric ratios reported here come from chiral GC. Where a (+) or (–) label is attached to a configuration in this paper it is taken from the published assignment for that compound, not from a rotation measured on these samples. A pure (+)-enantiomer rotates the plane of polarized light clockwise, a pure (–)-enantiomer rotates it counterclockwise, and a racemic mixture produces no net rotation.

#### Methods

Chiral GC analysis for this study was performed by Laboratoire PhytoChemia (Chicoutimi, Québec) under analyst Alexis St-Gelais, Ph.D., chimiste (Québec professional license 2013-174), and is documented in nine certificates of analysis: 25D11-TBP10 (orange), 25D11-TBP11 (thyme), 25F25-TBP01 (lavender), 25F25-TBP02 (pine), 25D11-TBP16 (2024 Fruit 135), 25D11-TBP17 (2024 Purple 103), 25F12-TBP10 (2024 Dessert 45) and 25F12-TBP08 and 25F12-TBP09 (the two mimic products). These fall into three analytical batches: 25D11 (orange, thyme, 2024 Fruit



135, 2024 Purple 103) analyzed 25 April 2025, 25F12 (2024 Dessert 45 and both mimic products) analyzed 18 June 2025, and 25F25 (lavender, pine) analyzed 26 June 2025. No bridging standards were run between batches, so batch is not formally controlled. It is worth noting which way this cuts for the central result: the three cultivars were not run together — two fall in the April batch and one in the June batch — yet they still group more closely with each other than with any sample run alongside them, which is the opposite of what a batch artefact would produce. Analyzes were run by GC-MS on a MEGA-DEX DET-Beta column (25 m × 0.25 mm × 0.25 µm; written “MEGA DEX-DET beta” on the certificates), a derivatized β-cyclodextrin chiral stationary phase in which the vendor’s DET designation denotes the diethyl-tert-butylsilyl derivatization.

Peak elution order was established by injecting authentic enantiomer reference standards and reference essential oils, and enantiomer ratios were determined by extracting compound-specific target ions from the GC-MS data.

Instrument conditions were supplied by the laboratory. The oven was programmed from 35 °C to 91 °C at 2 °C/min, then to 160 °C at 1.5 °C/min, then to 200 °C at 15 °C/min, held for 3 minutes. Carrier gas was helium at 1.2 mL/min under constant flow. Injection was split at 200:1, injector 220 °C, injection volume 0.1 µL. Samples were run neat or diluted up to 1:10 in pesticide-grade acetone, the dilution being used where the signal of interest was very intense, as with limonene in orange oil. Mass spectrometry used electron impact in scan mode over 40–550 amu, with the ion source at 230 °C, the quadrupole at 150 °C and the transfer line at 300 °C.

Enantiomer ratios were obtained from compound-specific extracted ions: linalool m/z 71, α-thujene m/z 77, borneol m/z 95, terpinen-4-ol m/z 111, camphene m/z 121, β-pinene m/z 93, limonene m/z 68, sabinene m/z 77, β-phellandrene m/z 77, Δ<sup>3</sup>-carene m/z 79, camphor m/z 108, α-terpineol m/z 59, caryophyllene oxide m/z 79 and linalyl acetate m/z 93. Elution order was established against authentic reference oils from the laboratory’s in-house collection — lavender, coriander seed, bergamot, dill weed, spearmint and neroli, according to the compound — together with commercial (+)-3-carene, commercial racemic camphor and commercial (–)-caryophyllene oxide, and with reference to Sciarrone et al. (2017). The laboratory did not supply an extracted ion for α-phellandrene, which is one of the compounds excluded from the multivariate panel.

On replication, the laboratory confirmed that no sample was run in duplicate, and gave its reasoning: an enantiomeric ratio is a ratio between two peaks of the same compound, which share an identical response factor in the detector because they ionise identically, so replication is not regarded as critical for a ratio in the way it would be for an absolute quantitation. That is a fair point and it is the reason the dataset can carry ratio comparisons at all. It does not, however, provide a precision estimate, which is why the multivariate analysis is treated as exploratory throughout.

On reporting limits, the laboratory works to a general limit of the first decimal place. Where a peak was visually very weak, as with the Δ<sup>3</sup>-carene signal, the decimal was

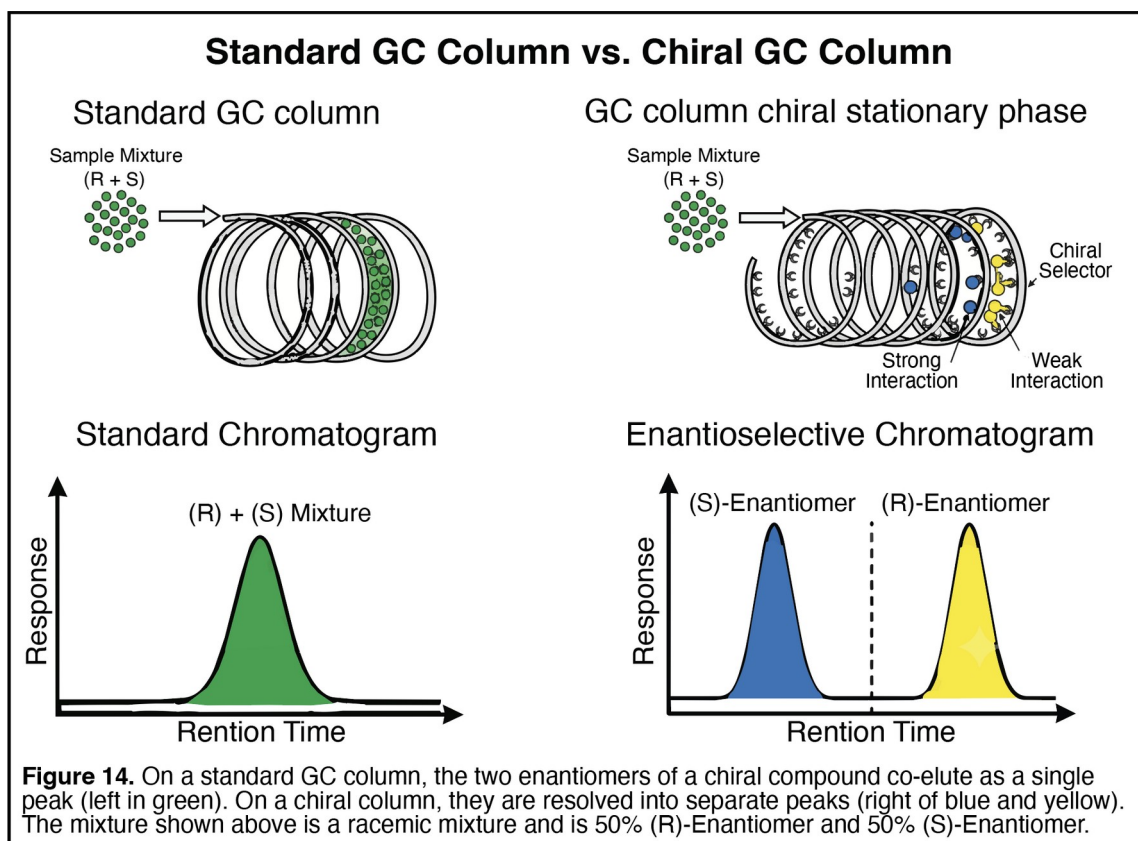


dropped because there was too little substance to warrant that precision. The presence or absence of a peak was likewise established by visual inspection against background noise rather than against a computed limit of detection. The laboratory's own view is that every value could be rounded to the nearest whole number without affecting the conclusions. Readers should therefore treat the final decimal place throughout the appendix as indicative rather than as a validated figure.

Three caveats from the laboratory apply. First, (+)-limonene coelutes with an ocimene isomer on this column, and with p-cymene in the thyme sample. The laboratory extracted a compound-specific ion to clean up the ratio, but ocimene is isomeric with limonene, so ion extraction cannot fully deconvolute the two. The interference sits on the (R) peak, which in the cultivars is the minor peak at only 3.9–4.6%, and the residual contribution was not quantified. The limonene ratios should therefore be read as carrying an unquantified positive bias on the minor enantiomer, and confirmation on a second column would be required before treating them as exact. Second, the enantiomers of  $\alpha$ -pinene were not separated, or were only marginally separated, on this column and were not assessed, which is why this paper makes no chiral claims about  $\alpha$ -pinene. Third, several values are reported by the laboratory as bounds rather than point estimates, and are reproduced as such in the appendix; where such a cell entered the multivariate analysis it was taken at the stated limit, so “>99” was read as 99.

Each sample was analyzed once and no analytical replicates were run. Fifteen compounds were resolved across the nine samples, but detection was uneven, and four compounds were detected in two or fewer samples and in none of the cannabis samples, and a further four in three to six samples. The multivariate analysis therefore uses the seven detected in at least seven of the nine samples — linalool, borneol, terpinen-4-ol, camphene,  $\beta$ -pinene, limonene and  $\alpha$ -terpineol. Within that panel four missing cells, 6% of the matrix, were replaced by the compound mean. All four fall in non-cannabis samples — borneol in orange and thyme, terpinen-4-ol in orange and in Cannabis Mimic 1 — so mean imputation was not applied to any cultivar value. It does, however, slightly deflate the variance of borneol and terpinen-4-ol, which affects the autoscaling denominators and therefore every distance in the plane, in a direction not fixed in advance. The full fifteen-compound dataset appears in the appendix.





### *Cannabis vs. botanicals vs. mimics*

Chiral GC analysis was performed on three Nexus Agriscience *Cannabis sativa* L. cultivars (2024 Fruit 135, 2024 Purple 103 and 2024 Dessert 45), two commercial cannabis-mimic terpene products (Cannabis Mimic 1 and Cannabis Mimic 2), and authentic essential oils of orange, thyme, lavender and pine. Fifteen chiral compounds were resolved across the panel; the full dataset appears in the appendix.

The three most consequential results, for the three primary chiral monoterpenes, are shown below. Each chart reports the percentage of each enantiomer as bar height. The two summary metrics used in this paper describe the same underlying data: a sample that is 97% S and 3% R has an enantiomeric ratio of about 32:1 and a signed enantiomeric excess of -94%.

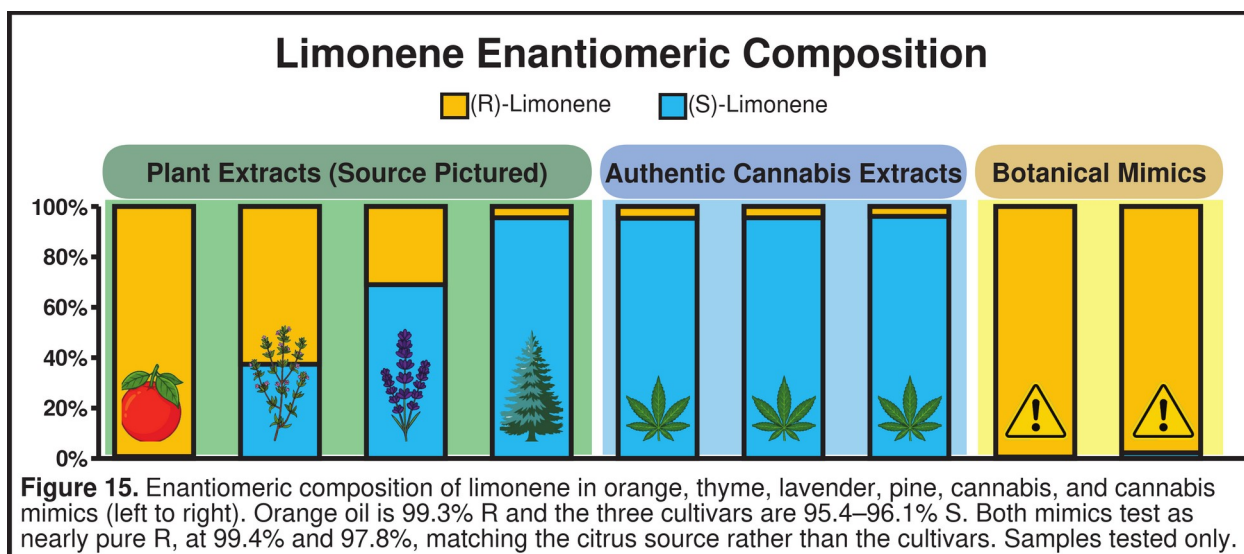


Figure 15 shows the cannabis cultivars producing (S)-limonene with high selectivity, while (R)-limonene dominates in orange oil and in both mimic products. On this compound the mimic products align with the citrus source rather than with the cultivars tested.

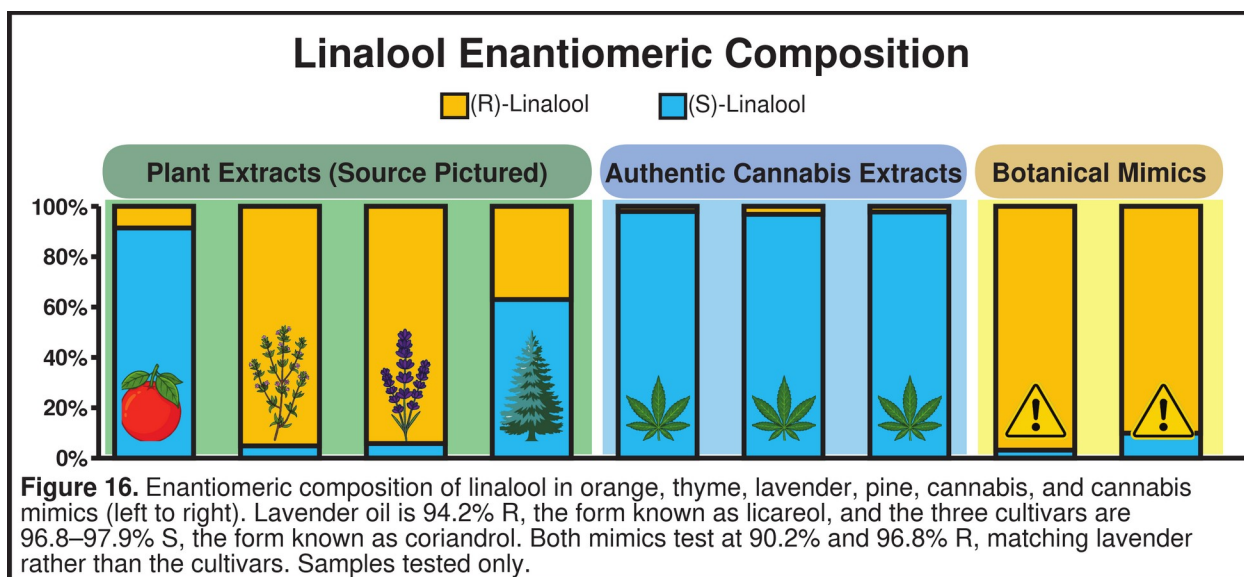


Figure 16 shows the same pattern for linalool: the cultivars are strongly (S)-selective at 96.8–97.9% S, while (R)-linalool dominates in the lavender reference at 94.2% R and in both mimic products, at 96.8% and 90.2% R. This is consistent with the linalool in these mimic products having been drawn from a lavender-type feedstock, but enantiomeric composition alone does not establish origin. Attributing a specific botanical source would require additional evidence, such as the accompanying minor-component profile, linalyl acetate content, or stable-isotope ratios.

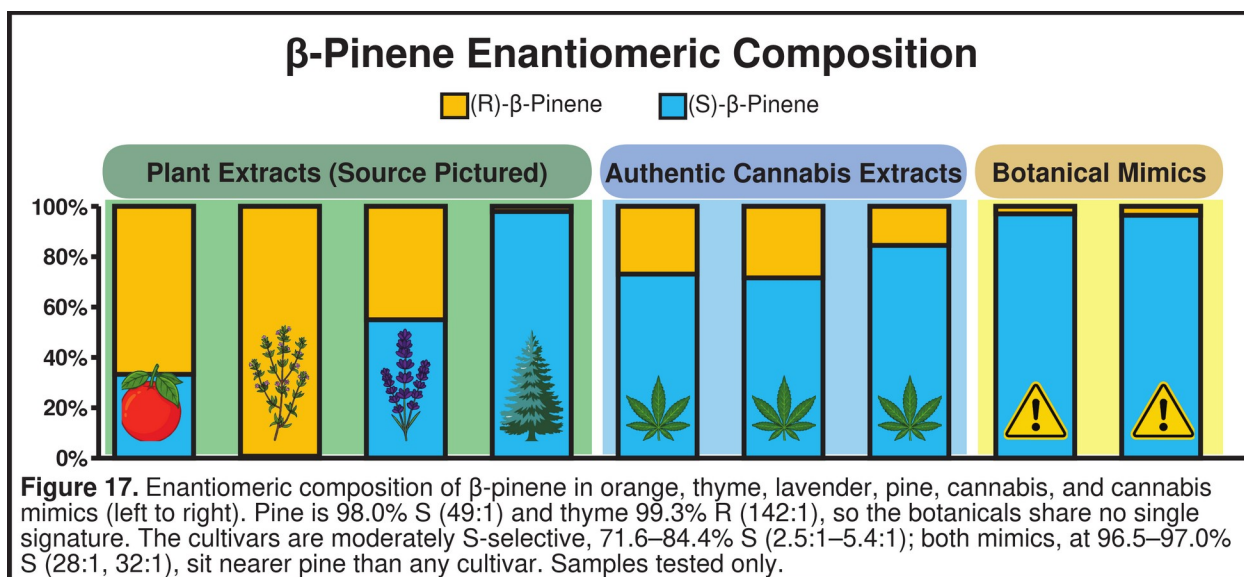
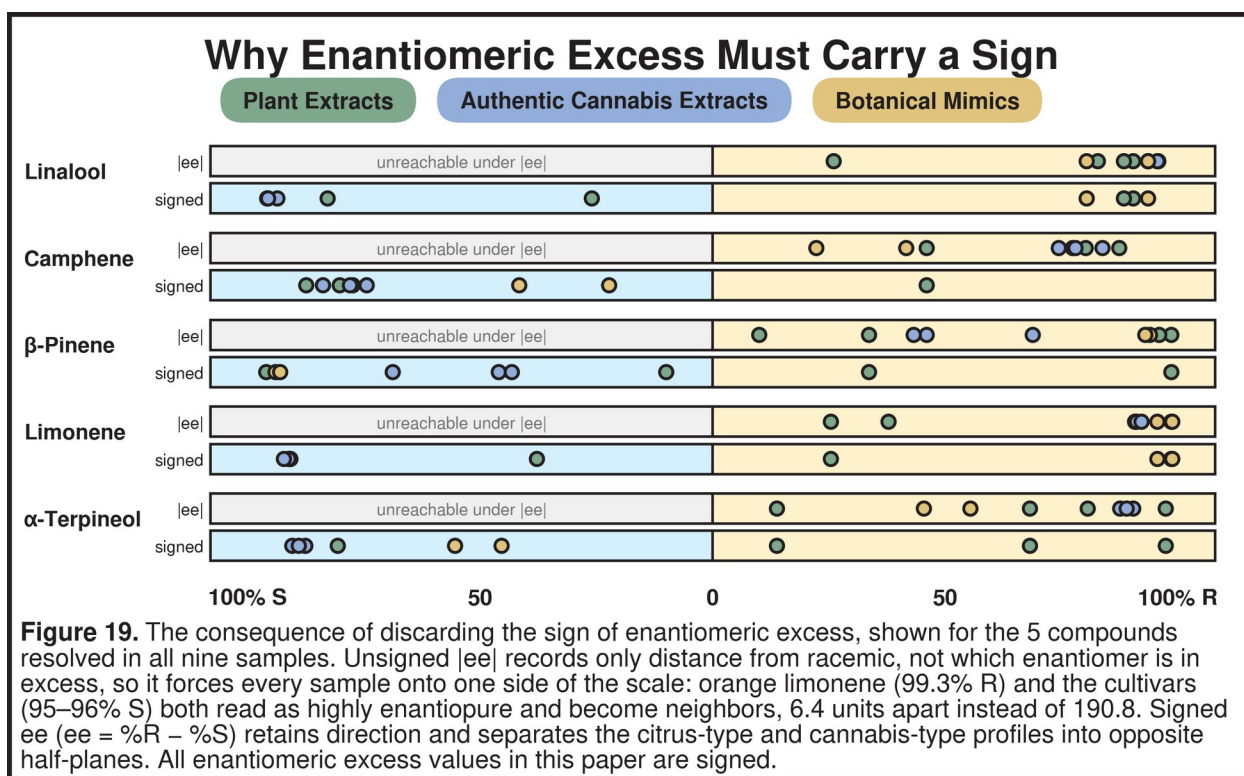
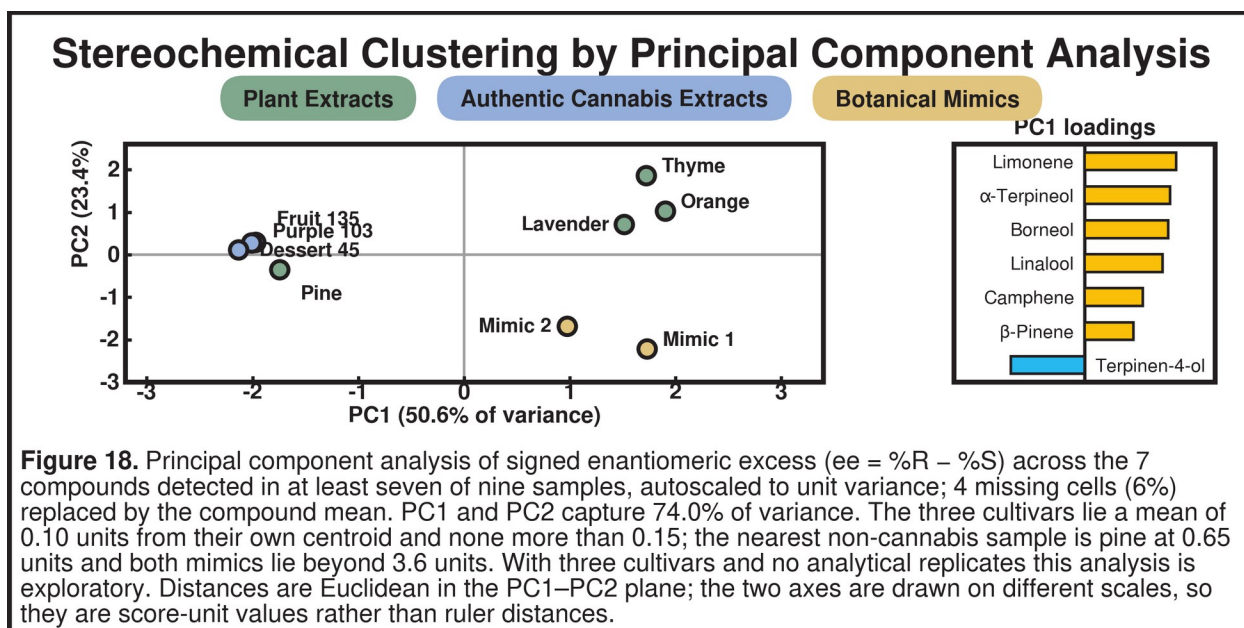


Figure 17 shows a more graded pattern for  $\beta$ -pinene. The cultivars are only moderately (S)-selective, at ratios of 2.5:1 to 5.4:1, whereas the pine turpentine reaches 49:1 and the two mimic products reach 32:1 and 28:1. Thyme, by contrast, is strongly (R)-selective at 142:1, and lavender is close to racemic, so the botanical oils tested do not share a single  $\beta$ -pinene signature. The mimic products sit considerably closer to pine than to any cultivar tested. This is a consistent trend across the samples analyzed rather than conclusive evidence of sourcing.

A pattern is visible across all three compounds: in each case the two mimic products sit with the botanical source whose enantiomeric signature they match, rather than with the cannabis samples: limonene with orange, linalool with lavender,  $\beta$ -pinene with pine. This is a statement about enantiomeric composition, not about market share — most commercial linalool, for instance, is synthetic rather than lavender-derived. Multivariate clustering across the seven-compound panel

A single-compound view tells one story; the multi-compound view tells a stronger one. Principal component analysis projects the signed enantiomeric excess data into two dimensions, positioning samples with similar overall stereochemical profiles close to one another. The analysis in Figure 18 uses the seven compounds detected in at least seven of the nine samples — linalool, borneol, terpinen-4-ol, camphene,  $\beta$ -pinene, limonene and  $\alpha$ -terpineol — autoscaled to unit variance, and is treated as exploratory given three cultivars and no analytical replicates. PC1 accounts for 50.6% of total variance and PC2 for 23.4%, 74.0% between them; the compound loadings on PC1 are shown alongside the score plot. Figure 19 shows what is lost if the sign of enantiomeric excess is discarded.



Within the samples in this study, essential oil from the three Nexus 2024 cultivars produced a stereochemical signature that was resolved from every commercial botanical source tested and from both commercial cannabis-mimic products tested. Measured as Euclidean distance in the PC1–PC2 plane, which carries 74.0% of the variance, the three cultivars lay a mean of 0.10 score units from their own centroid and none more than 0.15, while the nearest non-cannabis sample, pine, lay 0.65 units from



that centroid and both mimic products lay beyond 3.5 units. The separation was narrowest against pine, which shares the cultivars' (S)-limonene and (S)- $\beta$ -pinene dominance and is distinguished mainly by its linalool profile and its far more extreme  $\beta$ -pinene ratio. One nuance is worth stating: although each mimic matches a specific commodity source on each individual compound, in the combined seven-compound space the mimics form their own pair rather than sitting on top of any one botanical, with lavender their nearest neighbor. Generalization to all cannabis or all botanicals would require a larger and more diverse sample set.

Independent corroboration: Raeber et al. (2025)

These chiral GC findings track an independent investigation by Raeber and colleagues (2025), who developed four chromatographic methods to separate and quantify twenty-nine terpenes in *Cannabis sativa* L., among them thirteen enantiomers and five diastereomers. Their baseline, unstressed measurements report an excess of the (+)-enantiomer of linalool in cannabis flower of roughly 82% to 88%, reported by them as enantiomeric excess. In this paper the same dominance appears as ee –94% to –96%, a magnitude of 94–96%, because the convention used here is  $ee = \%R - \%S$  and (+)-linalool is the (S)-enantiomer; the magnitudes, not the signs, are the basis of comparison, and the two datasets agree on direction while differing somewhat in degree. Two of their conclusions bear directly on the argument made here. They observed cultivar- and growth-condition-specific enantiomeric ratios, which is the same finding this paper reports from a different laboratory and a different cultivar set. And under UV and heat stress they detected no enantiomeric conversion: compounds degraded, but enantiomers did not interconvert. That matters for authentication, because it means a chiral signature is not erased by ordinary aging even as the surrounding profile changes.

Raeber and colleagues also document something that this paper does not measure: terpene stability over time and under stress. They report compound-specific degradation, including oxidation and cyclization products, with p-cymene identified as a major aging marker derived from  $\gamma$ -terpinene and  $\alpha$ -terpinene. This is a useful caveat. A cannabis-derived terpene fraction that is native at extraction can drift toward an altered profile during storage, formulation and heating; “cannabis-derived” is not a stability claim. Authentication-by-chiral-GC therefore measures provenance at the point of analysis, not necessarily the chemistry of a finished consumer product weeks or months later.

### *Different sources give different products*

Why does cannabis produce one enantiomer of limonene while orange produces the other? Why do cannabis cultivars share a  $\beta$ -pinene signature that is distinctly different from pine? The answer is enzymology. Cannabis terpenes are not assembled by random chemistry; they are assembled by enzymes called terpene synthases, and those enzymes are stereoselective by design.



*Synthesis vs. enzymatic biosynthesis*

When terpenes are made in a chemical reactor, by hydration of myrcene, by isomerization of an existing terpene, or by total synthesis from smaller precursors, the outcome depends on the route. Non-stereoselective chemical routes commonly produce racemic or partially racemic mixtures unless an enantioselective step or a resolution is included. Chemical synthesis is not inherently racemic: enantioselective catalysts, chiral auxiliaries, kinetic resolution, biocatalysis and enantioenriched starting materials can all deliver non-racemic products. The relevant point for this paper is that the bulk commodity routes supplying the flavor market are generally not enantioselective, so the enantiomeric composition of the product is set by the feedstock rather than by the plant.

When nature synthesizes terpenes inside a plant cell, the situation is very different. The reactions are catalyzed by chiral enzymes, folded proteins whose active sites favor one binding orientation of the substrate and therefore yield one enantiomer of product preferentially. The preference is a strong bias rather than an absolute constraint, and its degree varies: in the cultivars tested limonene and linalool are 95–98% one-handed, while  $\beta$ -pinene is only 72–84% one-handed. Why one enzyme-derived compound should be so much less stereoselective than the others in the same plant is not answered by this dataset, and is worth pursuing.

*Terpene synthases*

A terpene synthase is a tiny biological machine. It takes a simple substrate, geranyl diphosphate (GPP) for monoterpenes, and rearranges its carbon skeleton through a series of conformational changes that fold the chain, cyclize it into a ring (or not), and place new bonds in specific stereochemical orientations.

Limonene synthase converts geranyl diphosphate (GPP) into limonene, and the enantiomer formed is determined by the shape of the enzyme's active site rather than by the substrate, which is identical in every species. *Cannabis sativa* expresses a trichome-specific (S)-(-)-limonene synthase, functionally characterized by Günnewich and colleagues (2007) and catalogued as UniProt A7IZZ1. Citrus species express the opposite activity: the (R)-(+)-limonene synthases of *Citrus sinensis* (A0A1C9J6A7), *Citrus limon* (Q8L5K3, Q8L5K1) and *Citrus unshiu* (Q6F5H3, Q6F5H2). Srividya and colleagues (2020) resolved how little separates the two. Comparing the crystal structures of the (S)-selective spearmint enzyme (PDB 2ONG) and the (R)-selective navel orange enzyme (PDB 5UV1), which is itself more than 95% (R)-selective, they identified four active-site residues that set the handedness of the bound intermediate, and showed that exchanging just those four in the spearmint enzyme (C321S, N345I, S453V, M458V) reverses its output to roughly 80% (R)-limonene. Enantiospecificity is therefore fixed at the point where the substrate folds into the active site, and a handful of amino acid differences is sufficient to invert it. Wiles and colleagues (2025) have since reported the structural basis of monoterpene cyclization by the *Cannabis sativa*

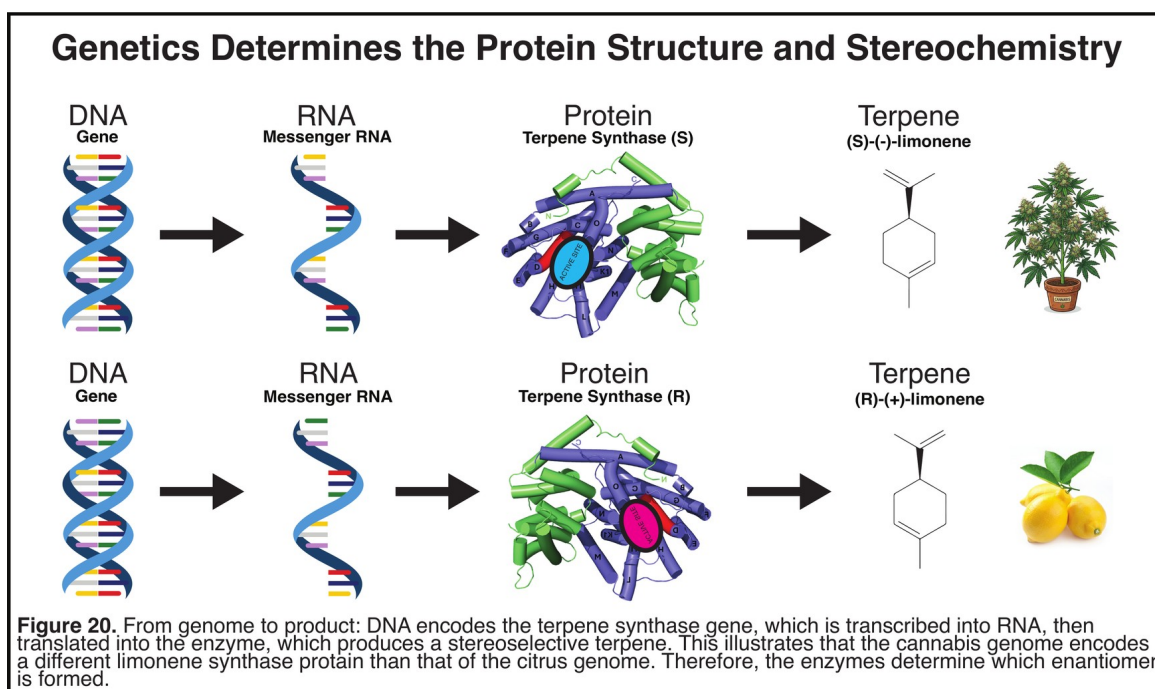


enzyme. This is the mechanistic reason a cannabis limonene and a citrus limonene are not interchangeable molecules in origin, even though they are the same compound.

A useful (if imperfect) analogy: imagine you are an enzyme assembling a molecule from a kit. If you hold the kit in your left hand, you might attach a particular component on the right side. If you hold it in your right hand, you might attach it on the left side. Either way, you end up with a recognizable molecule, but the two products are mirror images of each other.

### *Genetics drives chemistry*

A plant's genome encodes the terpene synthase enzymes it expresses. A cell's DNA is transcribed into RNA, which is translated into proteins, including the terpene synthases that build the terpene profile in the trichomes of cannabis flowers (Figure 20).



Terpene synthase gene families in cannabis are not only characterized but mapped. Lange and Srividya (2023) catalogued the twenty-two monoterpene- and sesquiterpene-synthase genes annotated in the CBDRx reference assembly — also designated cs10, NCBI accession GCF\_900626175.2, the assembly NCBI has adopted as the community reference genome for *Cannabis sativa* — and found them clustered rather than scattered. Eleven sit in a single array spanning roughly 1.2 Mb near the telomeric region of chromosome 5, each sharing between 60% and 100% sequence identity with monoterpene synthases of the TPS-b family that have been functionally characterized in other chemovars or species. Two loci in that array, NCBI 115716066 and 115716064, have limonene synthases as their closest characterized relatives, and a third locus in the same array, 115715884, corresponds most closely to an  $\alpha$ -pinene synthase.



Two cautions belong with that mapping. First, the functional assignments in CBDRx are inferred from sequence identity rather than measured. Lange and Srividya are explicit that none of the twenty-two genes in this reference chemovar has been functionally characterized and that no terpene analysis of CBDRx has been published; what the table records is the product of each locus's closest characterized relative, not a demonstrated activity. The evidence that cannabis expresses an (S)-(-)-limonene synthase comes from Günnewich and colleagues (2007), who expressed and characterized the enzyme from a different chemovar. Second, this study measured enantiomeric ratios and nothing else. No sequencing, gene-expression or enzyme work was performed on the three cultivars reported here, and those cultivars are not CBDRx, so their ratios cannot be attributed to particular loci. What can fairly be said is that the direction of the chemistry and the direction of the published enzymology agree: an (S)-selective limonene synthase is what the literature describes, and 95.4–96.1% (S)-limonene is what these cultivars contain.

One prediction is worth recording for future work. Günnewich and colleagues characterized the companion cannabis enzyme as an (R)-(+)- $\alpha$ -pinene synthase, and the chromosome 5 array contains a locus whose closest characterized relative is an  $\alpha$ -pinene synthase, so cannabis would be expected to favor (R)- $\alpha$ -pinene. The chiral column used in this study did not resolve the  $\alpha$ -pinene enantiomers, so that expectation remains untested here and no  $\alpha$ -pinene claim is made anywhere in this paper.

### *Conclusions*

Years of large-scale cultivation and essential-oil extraction have brought us up against a dimension of cannabis chemistry that receives little attention in the trade: isomerism and stereochemistry. The data presented in this paper make the case at three levels.

At the single-compound level: limonene, linalool and  $\beta$ -pinene in the three cultivars tested are stereochemically different from the same compounds as supplied by the commodity botanical market. The two mimic products tested match the commodity sources, not the cultivars.

At the multi-compound level: principal component analysis across the seven-compound panel places the cultivars tested in their own tight region of stereochemical space, resolved from every botanical source and commercial mimic product measured, with pine the nearest neighbor. With three cultivars and no analytical replicates this result is exploratory, and it describes the samples tested rather than cannabis in general.

At the mechanistic level: the published enzymology accounts for the direction of these differences, since cannabis expresses an (S)-selective limonene synthase and citrus an (R)-selective one. That account is drawn from the literature; this study did not measure enzyme activity or gene expression. For limonene, linalool and  $\beta$ -pinene the dominant enantiomer differs, or the ratio differs substantially, from that of the botanical sources tested. With one injection per sample this study cannot put a figure on analytical



precision, so the size of these differences is better judged against the spread among the three cultivars, which is small, than against a formal error estimate, which is not available.

Neither of the two mimic products tested shared the chemical signature of the cultivars tested across the major chiral monoterpenes. Whether that holds for other products on the market is not something two samples can settle. Differences in sensory and biological behavior between such products and authentic cannabis extracts are reasonable hypotheses that follow from the data, but they remain hypotheses until validated by sensory panels, GC-olfactometry, receptor assays, or comparable methods not performed in this study.

What this paper does not do is establish that cannabis-derived terpene fractions reconstruct the complete cannabis matrix. A volatile fraction does not contain the cannabinoids, cannabinoid acids, flavonoids, lipids, waxes or pigments that together make up the full chemistry of cannabis flower. What a cannabis-derived fraction can preserve is the native enantiomeric composition of the chiral terpenes measured here, together with the surrounding volatile constituents co-extracted with them. How much of that profile survives depends on extraction method, temperature, storage, oxidation and any fractionation applied, and this study did not quantify the complete volatile matrix, did not measure volatile sulfur compounds, and did not assess stability over time. Oswald and colleagues (2023) report that minor non-terpenoid volatiles, including volatile sulfur compounds, drive much of the aroma difference between cannabis varieties, which is a further reason not to read a chiral terpene signature as a complete account of aroma. The conclusions above are therefore restricted to the analytes resolved by the reported chiral GC method. Paper 2 of this series turns from the laboratory to the marketplace and examines the structural cost of cannabis-native stereochemistry across the global terpene supply chain.

### *Citations*

Ankney, E., Swor, K., Satyal, P., & Setzer, W. N. (2022). Essential oil compositions of *Pinus* species (*P. contorta* subsp. *contorta*, *P. ponderosa* var. *ponderosa*, and *P. flexilis*); enantiomeric distribution of terpenoids in *Pinus* species. *Molecules*, 27(17), 5658. <https://doi.org/10.3390/molecules27175658>

Aprotosoiaie, A. C., Hăncianu, M., Costache, I.-I., & Miron, A. (2014). Linalool: a review on a key odorant molecule with valuable biological properties. *Flavour and Fragrance Journal*, 29(4), 193–219. <https://doi.org/10.1002/ffj.3197>

Bentley, R. (2006). The nose as a stereochemist: enantiomers and odor. *Chemical Reviews*, 106(9), 4099–4112. <https://doi.org/10.1021/cr050049t>

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.-Z., Xie, X.-Q., Altmann, K.-H., Karsak, M., & Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid.



Proceedings of the National Academy of Sciences of the United States of America, 105(26), 9099–9104. <https://doi.org/10.1073/pnas.0803601105>

Günnewich, N., Page, J. E., Köllner, T. G., Degenhardt, J., & Kutchan, T. M. (2007). Functional expression and characterization of trichome-specific (–)-limonene synthase and (+)- $\alpha$ -pinene synthase from *Cannabis sativa*. *Natural Product Communications*, 2(3), 223–232.

Höferl, M., Krist, S., & Buchbauer, G. (2006). Chirality influences the effects of linalool on physiological parameters of stress. *Planta Medica*, 72(13), 1188–1192. <https://doi.org/10.1055/s-2006-947202>

Lange, B. M., & Srividya, N. (2023). Cannabis monoterpene synthases: evaluating structure–function relationships. *Phytochemistry Reviews*, 22, 1139–1163. <https://doi.org/10.1007/s11101-023-09861-4>

Oswald, I. W. H., Paryani, T. R., Sosa, M. E., Ojeda, M. A., Altenbernd, M. R., Grandy, J. J., Shafer, N. S., Ngo, K., Peat, J. R., Melshenker, B. G., Skelly, I., Koby, K. A., Page, M. F. Z., & Martin, T. J. (2023). Minor, nonterpenoid volatile compounds drive the aroma differences of exotic *Cannabis*. *ACS Omega*, 8(42), 39203–39216. <https://doi.org/10.1021/acsomega.3c04496>

Raeber, J., Bajor, B., Poetzsch, M., & Steuer, C. (2025). Comprehensive analysis of chemical and enantiomeric stability of terpenes in *Cannabis sativa* L. flowers. *Phytochemical Analysis*, 36(1), 205–217. <https://doi.org/10.1002/pca.3432>

Rivas da Silva, A. C., Lopes, P. M., Barros de Azevedo, M. M., Costa, D. C. M., Alviano, C. S., & Alviano, D. S. (2012). Biological activities of  $\alpha$ -pinene and  $\beta$ -pinene enantiomers. *Molecules*, 17(6), 6305–6316. <https://doi.org/10.3390/molecules17066305>

Rodrigues, A. M., Mendes, M. D., Lima, A. S., Barbosa, P. M., Ascensão, L., Barroso, J. G., Pedro, L. G., Mota, M. M., & Figueiredo, A. C. (2017). *Pinus halepensis*, *Pinus pinaster*, *Pinus pinea* and *Pinus sylvestris* essential oils chemotypes and monoterpene hydrocarbon enantiomers, before and after inoculation with the pinewood nematode *Bursaphelenchus xylophilus*. *Chemistry & Biodiversity*, 14(1), e1600153. <https://doi.org/10.1002/cbdv.201600153>

Sciarrone, D., Giuffrida, D., Rotondo, A., Micalizzi, G., Zoccali, M., Pantò, S., Donato, P., Rodrigues-das-Dores, R. G., & Mondello, L. (2017). Quali-quantitative characterization of the volatile constituents in *Cordia verbenacea* D.C. essential oil exploiting advanced chromatographic approaches and nuclear magnetic resonance analysis. *Journal of Chromatography A*, 1524, 246–253. <https://doi.org/10.1016/j.chroma.2017.10.007>

Sjödin, K., Persson, M., Fäldt, J., Ekberg, I., & Borg-Karlson, A. K. (2000). Occurrence and correlations of monoterpene hydrocarbon enantiomers in *Pinus sylvestris* and *Picea*



abies. *Journal of Chemical Ecology*, 26(7), 1701–1720.

<https://doi.org/10.1023/A:1005547131427>

Srividya, N., Lange, I., & Lange, B. M. (2020). Determinants of enantiospecificity in limonene synthases. *Biochemistry*, 59(18), 1661–1665.

<https://doi.org/10.1021/acs.biochem.0c00206>

Sun, J. (2007). D-Limonene: safety and clinical applications. *Alternative Medicine Review*, 12(3), 259–264.

Wiles, D., Roest, J., Vivian, J. P., & Beddoe, T. (2025). Structural insights into monoterpene cyclisation of limonene synthase from *Cannabis sativa*. *Biochemical and Biophysical Research Communications*, 777, 152271.

<https://doi.org/10.1016/j.bbrc.2025.152271>



### Appendix: full fifteen-compound stereochemical dataset

The table below presents the enantiomer percentages (%R / %S) for the fifteen compounds resolved across all samples, transcribed from the nine PhytoChemia certificates of analysis. Blank cells indicate that the compound was not detected in that sample. Values the laboratory reported as bounds rather than point estimates are shown with the inequality retained. Detection was uneven across the panel. Four compounds —  $\beta$ -phellandrene, linalyl acetate, camphor and sabinene — were found in two or fewer samples and in none of the cannabis samples, and four more — caryophyllene oxide,  $\alpha$ -thujene,  $\alpha$ -phellandrene and  $\Delta^3$ -carene — in three to six samples. Both groups are excluded from the multivariate analysis, which uses the seven compounds detected in at least seven of the nine samples (linalool, borneol, terpinen-4-ol, camphene,  $\beta$ -pinene, limonene and  $\alpha$ -terpineol).

Source data: chiral GC analysis performed by Laboratoire PhytoChemia (Chicoutimi, Québec) on the 2024 cultivar cohort and on the purchased reference oils and mimic products; certificates of analysis 25D11-TBP10, 25D11-TBP11, 25D11-TBP16, 25D11-TBP17, 25F12-TBP08, 25F12-TBP09, 25F12-TBP10, 25F25-TBP01 and 25F25-TBP02. Compiled by Nexus Agriscience.

| SOURCE                 | Orange |      | Thyme |      | Lavender |      | Pine |       | 2024 Fruit 135 |         | 2024 Purple 103 |      | 2024 Dessert 45 |      | Cannabis Mimic 1 |      | Cannabis Mimic 2 |      |
|------------------------|--------|------|-------|------|----------|------|------|-------|----------------|---------|-----------------|------|-----------------|------|------------------|------|------------------|------|
| Enantiomer chirality   | R      | S    | R     | S    | R        | S    | R    | S     | R              | S       | R               | S    | R               | S    | R                | S    | R                | S    |
| Caryophyllene oxide    |        |      |       |      |          |      |      |       | 94.6           | 5.4     | 92.2            | 7.8  | 91.5            | 8.5  | 77.4             | 22.6 | 80.5             | 19.5 |
| Linalool               | 8.6    | 91.4 | 95.2  | 4.8  | 94.2     | 5.8  | 37   | 63    | 2.1            | 97.9    | 3.2             | 96.8 | 2.2             | 97.8 | 96.8             | 3.2  | 90.2             | 9.8  |
| $\alpha$ -Thujene      | 60.7   | 39.3 | 50.9  | 49.1 |          |      | 25.2 | 74.8  | 21             | 79      | <1 (ND)         | >99  | 28              | 72   |                  |      |                  |      |
| Borneol                |        |      |       |      | 49.5     | 50.5 | 7.9  | 92.1  | 3.1            | 96.9    | 4.2             | 95.8 | 1.7             | 98.3 | >99.9            | <0.1 | 62.7             | 37.3 |
| Terpinen-4-ol          |        |      | 39.7  | 60.3 | 3.3      | 96.7 | 71.9 | 28.1  | 75             | 25      | 76.9            | 23.1 | 74              | 26   |                  |      | 59.3             | 40.7 |
| Camphene               | 73     | 27   | 6.3   | 93.7 | 11.3     | 88.7 | 9.9  | 90.1  | 12.8           | 87.2    | 11              | 89   | 8.1             | 91.9 | 38.9             | 61.2 | 29.2             | 70.8 |
| $\beta$ -Pinene        | 66.8   | 33.2 | 99.3  | 0.7  | 45       | 55   | 2    | 98    | 27             | 73      | 28.4            | 71.6 | 15.6            | 84.4 | 3                | 97   | 3.5              | 96.5 |
| Limonene               | 99.3   | 0.7  | 62.7  | 37.3 | 31.1     | 68.9 | 4.5  | 95.5  | 4.6            | 95.4    | 4.4             | 95.6 | 3.9             | 96.1 | 99.4             | 0.6  | 97.8             | 2.2  |
| $\alpha$ -Terpineol    | 98.7   | 1.3  | 84.1  | 15.9 | 56.9     | 43.1 | 9.7  | 90.4  | 6.2            | 93.8    | 4.8             | 95.2 | 5.5             | 94.5 | 27.3             | 72.7 | 22.3             | 77.7 |
| $\alpha$ -Phellandrene |        |      | 52    | 48   |          |      |      |       | 28             | 72      | 63              | 37   | 22.4            | 77.6 |                  |      | 43.1             | 56.9 |
| $\beta$ -Phellandrene  | <1     | >99  |       |      |          |      | 96.8 | 3.3   |                |         |                 |      |                 |      |                  |      |                  |      |
| $\Delta^3$ -Carene     | >99    | <1   | >99   | <1   |          |      | <0.1 | >99.9 | >99            | <1 (ND) |                 |      | >90             | <10  |                  |      | >90              | <10  |
| Linalyl acetate        |        |      |       |      | 99       | 1    |      |       |                |         |                 |      |                 |      |                  |      |                  |      |
| Camphor                |        |      |       |      | 41.5     | 58.5 | 92   | 8     |                |         |                 |      |                 |      |                  |      |                  |      |
| Sabinene               | 97.9   | 2.1  |       |      |          |      | 8.7  | 91.3  |                |         |                 |      |                 |      |                  |      |                  |      |

