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# Polarimetry equations and software for characterizing semiochemical enantiomers of insects

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Many compounds in nature are chiral such as various plant chemicals affecting herbivory, sex and aggregation pheromones, and allomones of insects. Each chiral semiochemical usually consists of 2 enantiomer structures that are non-superimposable mirror images. These contain at least one asymmetric carbon yielding absolute *R*- or *S*-stereo configurations that rotate plane-polarized light in a polarimeter in opposite directions to specific amounts. Most chiral insect semiochemicals consist of only one bioactive enantiomer functioning in communication with conspecifics (pheromones) or in interspecific interactions (allomones). Once the absolute configuration of each enantiomer is established, usually by chemical syntheses, and associated with the (±) optical rotations via polarimetry, then chiral gas chromatography-mass spectrometry (GC-MS) and polarimeters are used to identify the bioactive component in behavioral research. We present equations that use the optical rotations of compounds in a polarimeter to characterize the optical purity, specific rotation, enantiomeric excess, and percentage of each enantiomer in solution. We extend the polarimetry equations iteratively to solve for the specific rotation of a compound of interest in solutions of multiple chiral impurities with known proportions and specific rotations (from literature and analyzed by chiral GC-MS and polarimetry). The software allows for improved accuracy in measuring specific rotations of chiral semiochemicals in mixtures, as exemplified in previous literature and test samples. The equations implemented in Java software or webpages for personal computers and smartphones will aid research on the identification of volatile chiral molecules functioning in insect-insect and insect-plant interactions as well as in pest management.

*Keywords:* chirality, pheromone, plant volatile, monoterpene, essential oil



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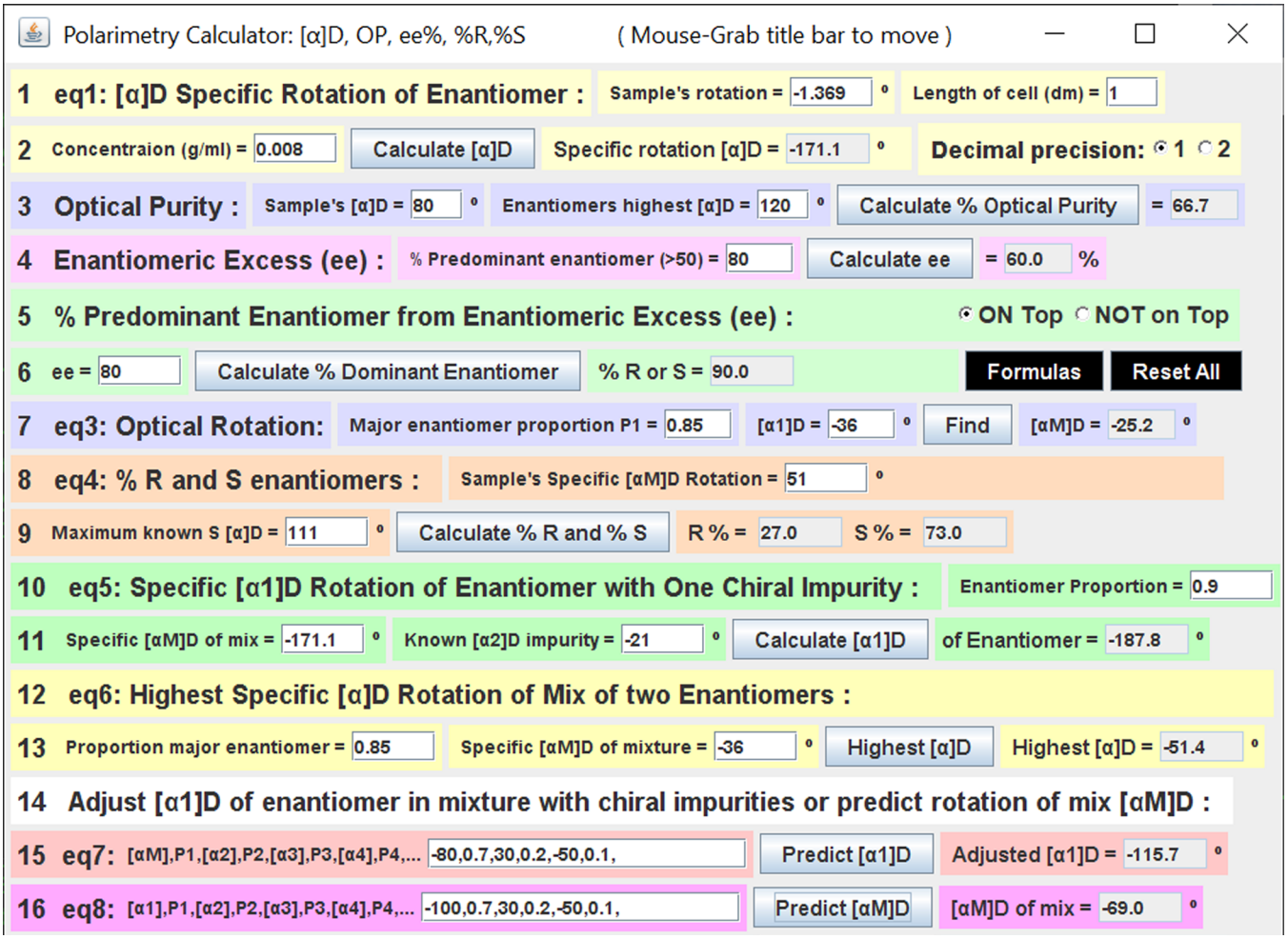
## Graphical abstract




## Introduction

Many compounds comprising volatile insect semiochemicals, such as those related to monoterpenes and sesquiterpenes, are chiral and commonly exist as 2 enantiomers each, having a distinctly different odor or taste perceived by animals. Pheromones, allomones, and kairomones of many animals, especially insects, are chiral and function in locating appropriate food resources, finding mates, and avoiding competitors and predators (Mori 1998, 2007, 2014, Wyatt 2010, El-Sayed 2024). Chiral compounds consist of one or more pairs of enantiomers, each rotating plane-polarized light a characteristic number of degrees either right (+) or left (–) as measured in a polarimeter (Morrison and Boyd 1973). Chiral sex pheromones have apparently evolved millions of years ago in the cockroach *Eurycotis floridana*, where males release chiral components to attract females (Farine et al. 1994). Males of many species of long-horned beetles (Coleoptera: Cerambycidae) release chiral aggregation pheromones that attract both sexes to the tree where they mate (Hanks and Millar 2016). Bark beetles (Coleoptera: Curculionidae) use attractive and inhibitory pheromone components that are typically chiral terpenoid alcohols and ketones or double/triple ring structures with one or two oxygen bridges (Silverstein et al. 1966a, 1966b, 1968, Wood et al. 1976, Borden et al. 1980, Silverstein 1981, Payne et al. 1982, Wood 1982, Byers 1983, 1989, Mori et al. 1983, Byers et al. 1989, Seybold 1993, Zada et al. 2004). In some bark beetle species, one of 2 possible enantiomers functions in (1) aggregation of conspecifics for mating and feeding on the host tree, (2) reducing intraspecific competition for bark phloem, or (3) avoiding interspecific competition from resident individuals of other species (Wood et al. 1976, Renwick et al. 1976, Byers 1989, Byers et al. 1989, Seybold 1993, Poland and Borden 1998, Mori 2007, 2014). Various chiral monoterpenes and sesquiterpenes and their alcohols, aldehydes, and ketones have been shown to be important for host plant recognition as well as for avoiding non-hosts by bark and ambrosia beetles (Byers 1995, Byers et al. 2000, 2018, 2020, Zhang et al. 2002).

Most moth species (Lepidoptera) use volatile sex pheromone components that are non-chiral; however, females of many species in the families Geometridae, Noctuidae, Arctiidae, and Lymantriidae employ long-chain epoxide enantiomers that are chiral sex pheromone components (Silverstein 1981, Millar 2000, Byers 2006, El-Sayed 2024). For example, males of the forest defoliator pest, *Lymantria dispar* (formerly 'gypsy moth,' now spongy moth), are only attracted by the epoxide (+)-disparlure enantiomer, while the (–)-disparlure is unattractive and even reduces the attraction to the (+)-enantiomer (Miller et al. 1977). Species of red harvester ants in the genus *Pogonomyrmex* use only (*S*)-4-methyl-3-heptanone as alarm pheromone, while the same enantiomer is used by a brachonid parasitoid wasp as

a defensive irritant expelled when biting (Byers and Levi-Zada 2011). The female olive fruit fly, *Bactrocera oleae*, releases a chiral pheromone with one enantiomer attracting males and the opposite enantiomer attracting females (Haniotakis et al. 1986). Other tephritid fruit fly pest species release sex-specific mixtures of chiral and non-chiral volatiles in circadian rhythms that likely play complex behavioral roles in lek formation and mating (Levi-Zada et al. 2020). In Hemiptera, Ho and Millar (2001) identified a chiral sex pheromone released by males of 2 *Chlorochoroa* stink bug species. Wingless female mealybugs (Hemiptera) release unique terpenoid sex pheromones that invariably are chiral-specific in attracting winged males (Zada et al. 2008, Zou et al. 2010, Ramesh et al. 2015, Zou and Millar 2015, Levi-Zada et al. 2019, 2021). Many terpene compounds and aromatic alcohols found in plant essential oils are chiral, and depending on the insect species are either attractive or repellent (Byers et al. 2000, 2018, 2020, Levi-Zada et al. 2024, 2025). Most plant essential oils contain monoterpenes and related terpenes in complex enantiomeric mixtures. Recently, the peach fruit fly, *Bactrocera zonata*, and the red palm weevil of dates, *Rhynchophorus ferrugineus*, were shown in the field to avoid essential oils and their components that may represent non-hosts (Levi-Zada et al. 2024, 2025). Thus, it is important in studies in chemical ecology to understand and have tools that characterize semiochemical enantiomers (Wood et al. 1976, Mori 1998, 2007, 2014).

Chiral chemicals are those having at least 1 asymmetric center (carbon connected by single bonds to 4 different molecular-weight components). Each asymmetric center can have a geometric configuration of *R* (rectus = right-handed) or *S* (sinister = left-handed) depending on a rule of priority to decide the absolute rotation. For each asymmetric center, the largest to smallest atoms (or extending to the smallest molecular weight groups) attached to this center are given priority ranks (high to low, respectively). A model of the molecule is rotated such that the asymmetric center's attachment with the lowest rank is pointed away from the viewer, and then stepping from the highest ranked attachment to the lowest will proceed either clockwise to the right-hand (*R*) or counterclockwise to the left-hand (*S*) of the viewer (Cahn et al. 1966, Morrison and Boyd 1973). Compounds with one or more asymmetric centers have stereoisomers that are either diastereomers (not mirror images and not superimposable) or enantiomers (mirror images that are not superimposable) or both (Byers et al. 1989). Straight chain compounds with several (*n*) chiral centers have up to $2^n$ enantiomers; if $n=2$ then there are 2 pairs of enantiomers (4 different structures): (*S*, *S*) and (*R*, *R*) are enantiomers of each other, and (*S*, *R*) and (*R*, *S*) are the other pair. However, ring structures with 2 chiral centers, such as α-pinene, have only 2 enantiomers due to conformational constraints of the rings.

Volatile semiochemicals, including those that are chiral, can be identified using gas chromatography-mass spectrometry (GC-MS). For those semiochemicals that have enantiomers, an appropriate chiral GC column is used to separate their enantiomers, giving the retention times and the proportion of each by peak area. This method cannot directly determine which enantiomer elutes first in the chiral column or the optical rotation of each enantiomer (Byers and Levi-Zada 2011, Levi-Zada et al. 2021). However, once an enantiomer is determined to be *R*- or *S*-configuration by either X-ray crystallography, chiral derivatizing agents with nuclear magnetic resonance spectroscopy (NMR) and chromatography, or enantioselective synthesis (chemical synthesis from known stereo-precursors) (Labuta et al. 2013), then the (+)- or (−)-rotation can be assigned by a polarimeter and their elution order on a chiral column by GC. Thereafter, in research studies, the identity of each enantiomer can be recognized by either polarimetry or chiral GC, which allows for identification of the behaviorally active enantiomer. The specific rotation of a chiral compound defines the degree of rotation of polarized light in a standardized way. The specific rotation, $[\alpha]_D^{20}$, is usually measured at 589 nm or D (sodium light) at 20 °C, thus in units of deg·ml·g$^{-1}$·dm$^{-1}$ (typically shortened to degrees), and hereafter we use $[\alpha]_D$. The specific rotation then depends on (i) the optical rotation (in degrees), (ii) the length of the tube containing the enantiomeric solution through which the polarized light travels (in dm), and (iii) the concentration of the chiral component (g/ml). The equation for the specific rotation presented subsequently can be solved for any of the 3 parameters and extended to more complicated equations.

Our objectives were to (i) present well-known formulas using the optical rotations measured in a polarimeter to calculate specific rotation, enantiomeric excess (ee), and percentage of *R* and *S* enantiomers, (ii) use the specific rotation equation and derive an equation that corrects the measured specific rotation of an enantiomer in mixture with a chiral impurity, (iii) extend this derived equation for any number of chiral impurities of known proportions and rotations to find the specific rotation of an enantiomer of interest, and (iv) solve the extended equation to predict the optical rotation of a mixture of chiral compounds when all specific rotations of components and their proportions are known. This calculated rotation should equal the mixture's optical rotation measured by polarimetry, unless there are errors reported in the literature for specific rotations of one or more of the components. We will confirm the extended equations by comparison with optical measurements of enantiomer mixtures of α-pinene, limonene, and camphor in a polarimeter. Finally, we use the extended equations to correct previously reported specific rotations of *trans*-verbenol, a pheromone of the western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae) (Byers 1983), and of α-necrodol, a sex pheromone precursor of the spherical mealybug *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae) (Levi-Zada et al. 2021). The equations are implemented in Java software that will aid researchers in the analysis of enantiomeric mixtures as well as characterizing commercial samples of dubious or unknown enantiomeric purity in preparation for behavioral experiments.

## Methods

A Java application was created in the Java language (Oracle, Redwood Shores, California) for Windows PC that allows user input to provide calculation outputs for optical purity, enantiomeric excess (ee), and enantiomer proportions from ee (Fig. 1). The software (polarimetry.jar) using Equations (1), (4), (5), (6), (7), and (8), presented subsequently, also can calculate (i) specific rotations, (ii) % of *R* and *S* enantiomers from a sample's specific rotation, (iii) the highest specific rotation from proportions of enantiomers, (iv) the specific rotation of an enantiomer with one or more chiral impurities of known proportions and specific rotations, and (v) the predicted specific rotation of a mixture of chiral compounds of known specific rotations and proportions in the mixture.

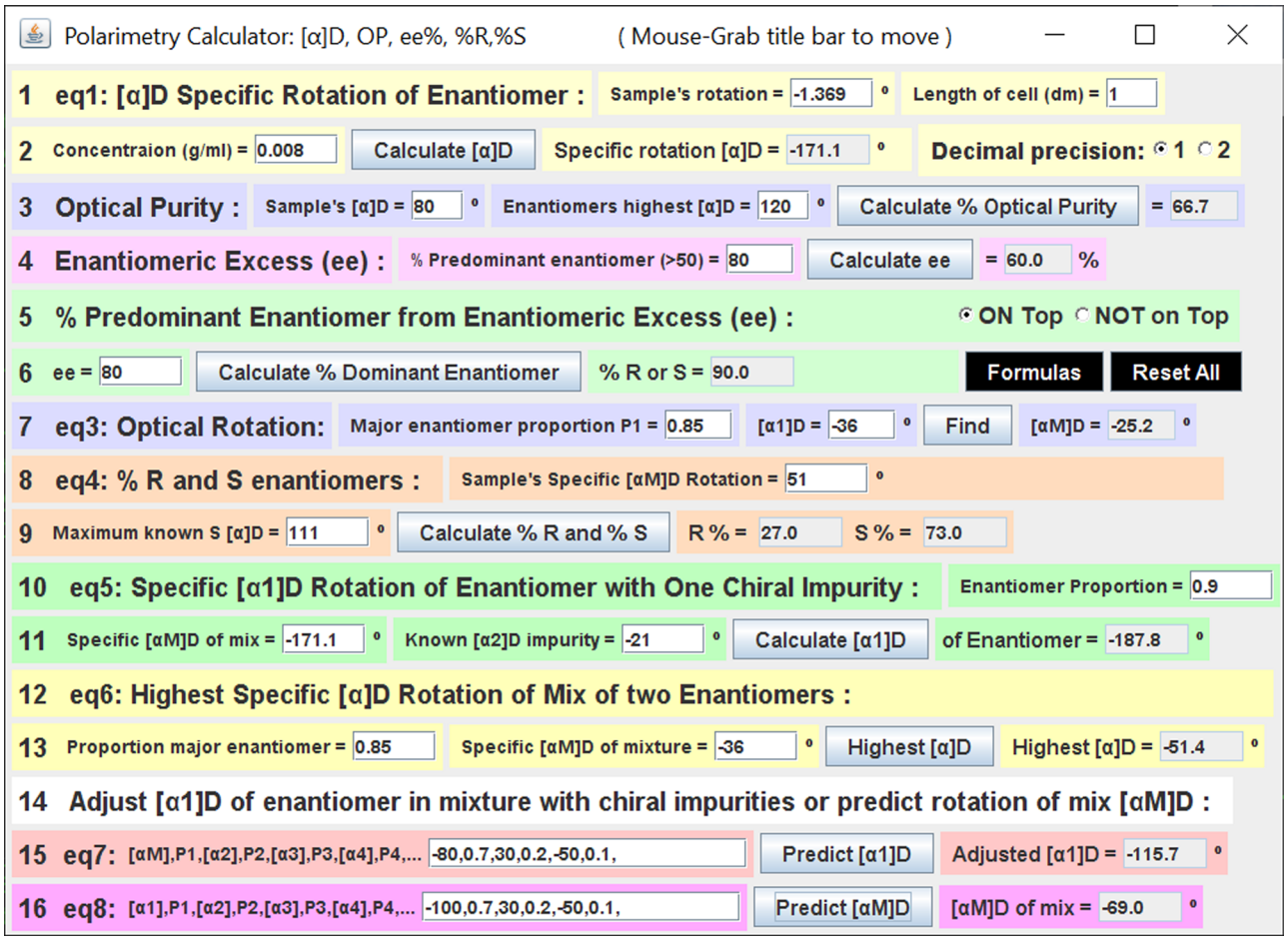



**Fig. 1.** Screen image of popup window in PC (Windows) of polarimetry.jar (an executable java program downloaded from https://www.chemical-ecology.net/java2/polarimetry.jar). The software is also available as an Internet webpage (https://www.chemical-ecology.net/polarimetry.htm). Lines 1 to 16 numbered at left are referenced in the text regarding various equations (eq# corresponds to Eq. # in text).

Calculation of a **specific rotation** $[\alpha]_D$ of an enantiomer is found from the well-known formula (Morrison and Boyd 1973, p. 119):

$$[\alpha]_D = \frac{\alpha}{L \cdot c}, \quad (1)$$

where α is the optical rotation of a solution in a polarimeter, $L$ is the length of the tube (dm) of polarimeter, and $c$ is the g/ml (concentration of the enantiomer in solvent). For example, if α = –1.369°, $L$ = 1 dm, and $c$ = 0.008 g/ml then $[\alpha]_D$ = –171.1° (Fig. 1, lines 1 and 2). Since $L$ and $[\alpha]_D$ are fixed for a polarimeter and chiral compound, the optical rotation of this compound increases linearly with its concentration ($\alpha = [\alpha]_D \cdot L \cdot c$). Equation (1) can be solved for $c$ when the concentration is desired based on the other three parameters:

$$c = \frac{\alpha}{L \cdot [\alpha]_D}. \quad (2)$$

The **optical purity** of a chiral compound is simply the sample's optical rotation divided by the highest known specific rotation of either enantiomer from the literature, or from a standard of only one enantiomer. For example, if the sample has a rotation of 80°, and the highest specific rotation known for an enantiomer is 120°, then the optical purity percentage of maximum is 66.7% (Fig. 1, line 3). **Enantiomeric excess** (ee) describes the excess of the dominant enantiomer in a mixture of unequal enantiomer proportions with the equation (Chen et al. 1982, Kennepohl et al. 2020): $ee = (D - 50) \cdot 100 / 50$, where $D$ is the percentage of the dominant enantiomer (≥50%). For example, if $S$-enantiomer is dominant with $D$ = 80% (thus $R$ = 20%), then the ee of this mixture is 60% $S$ (Fig. 1, line 4). If the ee is given instead, then solving for $D$ gives: $D$ = (ee/100) + 50. For example, if ee of $S$ is 60%, then there is 80% $S$ and 20% $R$ in the solution (Fig. 1, lines 5 and 6).

The **optical rotation** of a mixture of 2 enantiomers $[\alpha_M]_D$ such as (+)- and (–)-α-pinene can be calculated from the highest known specific rotation of one of the enantiomers (either $[+\alpha_1]_D$ or $[-\alpha_2]_D$ from the literature) as well as the proportion ($P$) of each enantiomer by chiral GC:

$$[\alpha_M]_D = P_1 \cdot [\alpha_1]_D + (1 - P_1) \cdot [\alpha_2]_D, \quad (3)$$

where $P_1$ is the proportion of $R$ enantiomer, $[\alpha_1]_D$ is the specific rotation of $R$ enantiomer, $P_2$ = 1—$P_1$ is the proportion of $S$ enantiomer, and $[\alpha_2]_D$ is the specific rotation of $S$ enantiomer (Kennepohl et al. 2020). For example, if $P_1$ = 0.85 (thus $P_2$ = 0.15) and if $[\alpha_1]_D$ = –36° then $[\alpha_2]_D$ = 36°, giving $[\alpha_M]_D$ = –25.2° (Fig. 1, line 7). Equation (3) was solved for $P_1$ (see "Results" section, Equation (4)). The equation can also be solved to

determine the highest specific rotation of an enantiomer when its opposite enantiomer is present in a mixture (see "Results" section, Equation (6)).

The calculated specific rotation of a predominant enantiomer in solution will be erroneous if there is a second chiral impurity present. However, the error can be corrected if the impurity is identified and has a known $[\alpha]_D$. We did not find such an equation in the literature, but Equation (3) can be solved for $[\alpha_1]_D$ assuming $[\alpha_M]_D$ is measured with a polarimeter to obtain the specific rotation of the mixture, $[\alpha_2]_D$ is known from the literature, and proportions $P_1$ and $P_2$ are known from GC separation on a chiral column. Less commonly, one might want to find $[\alpha_1]_D$ for an enantiomer of interest in a mixture of 3 or more chiral compounds. This equation can be extended from the two-enantiomer case where proportions of the compounds are known, and all specific rotations are known from literature except the enantiomer of interest (see "Results" section, Equation (4)).

The optical equations develop subsequently were tested by measuring the specific rotations of chiral terpenes (–)-α-pinene (96% by chiral GC), (–)-(*S*)-limonene (96%), and (+)-*R*-camphor (98%) at respective concentrations of g/ml chloroform (all compounds from Sigma Aldrich) in a polarimeter (Anton Paar OptoTec GmbH model MCP 5100, Seelze, Germany). A mixture of these 3 compounds (99% chemically pure) in unequal proportions of 0.26, 0.57, and 0.17, respectively, was made at a concentration of 0.01 g/ml and the optical rotation was obtained by polarimetry. GC-MS analyses were performed on an Agilent 7890 A/5975C instrument (Agilent Technologies, Santa Clara, California) with a chiral column (Rt-γDEXsa, 30 m × 0.32 mm ID × 0.25 µm, Restek, Bellefonte, Pennsylvania) temperature programmed starting at 60 °C (0 min hold), then heating 1 °C/min to 110 °C and held for 30 min. The column flow was split to both FID and MS detectors equally by an Agilent purged two-way effluent splitter, enabling qualitative and quantitative analyses simultaneously. Equations developed, subsequently (Equation (6), "Results" section), were used to find the highest specific rotation for each enantiomer of α-pinene. Assuming the specific rotation of (+)-*R*-camphor was unknown in the 3-chiral compound mixture above, but the specific rotations of (–)-α-pinene and (–)-limonene are known as well as the proportions (using chiral GC), then an extended equation can be used to obtain the specific rotation of camphor. The highest reported specific rotation reported in the literature for α-pinene is 52.4° (Comyns and Lucas 1957, Renwick et al. 1976), for limonene 123.8° (Lide and Milne 1994), and camphor 48° (Theimer et al. 1977, Ravid et al. 1993). The optical rotations of the solutions were then measured on our polarimeter and compared to the calculations from the equations above and derived subsequently in results.

To demonstrate the utility of the derived equations in research, we wanted to adjust the reported specific rotation of a Spanish lavender, *Lavandula luisieri* (Rozeira) (Lamiales: Lamiaceae), essential oil constituent, (–)-*trans*-α-necrodol ($[\alpha]_D^{20}$ = –168.95°). This chiral compound was used as a precursor to synthesize (–)-(*R*)-γ–necrodyl isobutyrate, a sex pheromone released by female spherical mealybugs *N. viridis* (Levi-Zada et al. 2021). The measured specific rotation of *trans*-α-necrodol was inexact because it was mixed with minor chiral impurities of 2% (–)-(*R*)–γ–necrodol ($[\alpha]_D$ = –7.04°, Levi-Zada et al. 2021) and 1% myrtenol ($[\alpha]_D$ = –50°, Renwick et al. 1976). In a second example, Byers (1983) exposed females of *D. brevicomis* to vapors of either +45.8° or –41.6° α-pinene ($[\alpha]_D$ = –52.4° the highest reported rotation) that were converted respectively to +104.9° or –91.5° *trans*-verbenol. We wanted to derive equations from Equation (3) above to calculate the proportions of each enantiomer of α-pinene in the 2 enantiomeric mixtures and use these with another derived equation to estimate the specific rotations of the (+)- and (–)-*trans*-verbenols produced from the respective enantiomers of α-pinene vapor.

## Results

### Derivative Equations of Specific Rotation and Enantiomeric Ratios

Equation (3) above was solved for $P_1$, the proportion of a binary mixture that is *R* enantiomer:

$$P_1 = \frac{[\alpha_M]_D - [\alpha_2]_D}{[\alpha_1]_D - [\alpha_2]_D}. \quad (4)$$

Hypothetically, if $[\alpha_M]_D$ = +51° (the measured $[\alpha]_D$ of the mixture), $[\alpha_1]_D$ = +111° (reported $[\alpha]_D$ of *S*-enantiomer), and $[\alpha_2]_D$ = –111° (opposite sign for *R*), then $P_1$ = 0.27 (27% *R*) and $P_2$ = (1 – $P_1$) = 0.73 (73% *S*) (see Fig. 1, lines 8 and 9).

A formula to calculate the specific rotation of an enantiomer in a solution with a known chiral compound impurity was not found readily in literature searches, but was derived from Equation (4) to give the following equation:

$$[\alpha_1]_D = \frac{[\alpha_M]_D - (P_2 \cdot [\alpha_2]_D)}{P_1}. \quad (5)$$

where $[\alpha_M]_D$ = specific rotation of the chiral mixture from Equation (3) or Equation (4), $P_1$ = proportion of enantiomer of interest, $P_2$ = proportion of chiral impurity, and $[\alpha_2]_D$ = known specific rotation of chiral impurity. For example, if $P_1$ = 0.9 and $P_2$ = 0.1 and $[\alpha_M]_D$ was measured as –171.1° and $[\alpha_2]_D$ is reported in literature to be –21°, then the corrected $[\alpha_1]_D$ = –187.8° (Fig. 1, lines 10 and 11). This specific rotation is more negative than $[\alpha_M]_D$ because the impurity did not rotate polarized light as much, which means that the enantiomer of interest must be even more negative to give the observed –171.1°. If $[\alpha_2]_D$ had been the (+)-enantiomer of +21° instead, then the enantiomer's specific rotation would need to be even more negative to counteract this, ie $[\alpha_1]_D$ = –192.4°.

Another problem is to determine the highest specific rotation of an enantiomer when its opposite enantiomer is present in the mixture. Chiral GC can separate 2 enantiomers of a compound and determine the proportions $P_1$ (eg 0.85) and $P_2$ (eg 0.15). It is then possible to isolate the 2 enantiomers together by column or GC fractionation and measure the specific rotation of the mixture with a polarimeter (eg $[\alpha_M]_D$ = –36°). These data are used to calculate the highest rotation of each enantiomer by substituting $P_2$ for (1—$P_1$) and –$[\alpha_1]_D$ for $[\alpha_2]_D$ in Equation (3) and solving for $[\alpha_1]_D$:

$$[\alpha_M]_D = P_1 \cdot [\alpha_1]_D - P_2 \cdot [\alpha_1]_D \rightarrow [\alpha_1]_D = \frac{[\alpha_M]_D}{P_1 - P_2}. \quad (6)$$

giving –51.4° for the dominant enantiomer ($P_1$) and the opposite enantiomer $[\alpha_2]_D$ = +51.4° (Fig. 1, lines 12 and 13).

More complicated situations are possible in which an enantiomer of unknown specific rotation is in solution with several chiral impurities that all have known $[\alpha]_D$ from the literature. In this case, the following iterative formula is appropriate:

$$[\alpha_1]_D = \frac{[\alpha_M]_D - \sum_{i=2}^{n} P_i \cdot [\alpha_i]_D}{P_1}. \quad (7)$$

For example, if there are 3 chiral compounds in a mixture giving $[\alpha_M]_D$ = –80° with $P_1$ = 0.7, $[\alpha_2]_D$ = 30° and $P_2$ = 0.2, $[\alpha_3]_D$ = –50° and $P_3$ = 0.1, then from Equation (7), the specific rotation of the enantiomer of interest $[\alpha_1]_D$ = –115.7° (Fig. 1, line 15). The more chiral impurities, the more error can be introduced by imprecision in the specific rotations reported in the literature, although the error is averaged. Equation (7) will adjust for even more than 2 chiral impurities providing these have known specific rotations (literature) and proportions (from chiral GC). While Equation (7) can be solved for $P_1$ algebraically, a result is not possible since the proportions $P_i$ cannot be determined without knowing $P_1$, which is being solved for.

If Equation (7) is used with known $[\alpha]_D$ for all *n* chiral compounds in the solution and the proportions are also known, then $[\alpha_M]_D$ can be predicted and compared to measurement by polarimetry according to:

$$[\alpha_M]_D = \sum_{i=1}^{n} P_i \cdot [\alpha_i]_D \quad (8)$$

For example, if the parameters above are used in Equation (8), except $[\alpha_1]_D$ = –100° rather than –115.7°, then the predicted $[\alpha_M]_D$ = –69° (Fig. 1, line 16), not –80° (as shown input on line 15, Fig. 1). In this case, the discrepancy (–69 vs –80) indicates that one or more $[\alpha]_D$ for the chiral compounds reported in the literature are incorrect.

## Confirmation of the Equations with Mixtures of Enantiomers and Polarimetry

The (–)-(1*S*, 5*S*)-α-pinene was 92% as determined by chiral GC, and using the highest reported rotation of –52.4° to input in Equation (8) (Fig. 1, line 16): –52.4, 0.92, 52.4, 0.08 predicts $[\alpha M]_D$ = –44.02°. Polarimetry showed that a solution of (–)-α-pinene (0.01212 g/ml, optical rotation of –0.536°) gave a specific rotation $[\alpha]_D$ = –44.22° (Equation (1)). Chiral GC showed the (–)-(*S*)-limonene was 94% enantiomer-pure and entry of: –123.8, 0.94, 123.8, 0.06 in Equation (8) gave –108.94°, nearly identical to the polarimeter measurement (0.01212 g/ml, –1.317°) of $[\alpha]_D$ = –108.66°. Chiral GC showed the (+)-camphor was 94.9% enantiomer-pure and entry of 48, 0.949, –48, 0.051 into Equation (8) gave $[\alpha]_D$ = 43.11° for (+)-camphor. In the polarimeter, (+)-camphor (0.01367 g/ml, optical rotation +0.589°) had $[\alpha]_D$ = +43.09° (Equation (1)).

The mixture of (–)-α-pinene, (–)-limonene, and (+)-camphor (in respective proportions 0.26, 0.57, and 0.17 totaling 0.01 g/ml) gave an optical rotation of –0.664° and $[\alpha_M]_D$ = –66.4°. Using the proportions and the specific rotations of our impure enantiomers of the two monoterpenes, then Equation (7) predicts that (+)-(*R*)-camphor has a specific rotation = +43.1° (same as our sample of +43.09°). Theoretically, if the enantiomers were enantiomerically pure and the highest known specific rotations of (–)-α-pinene and (–)-limonene are used in the same proportions are entered in Equation (8) (Fig. 1, line 16): –52.4, 0.26, –123.8, 0.57, 48, 0.17 then $[\alpha_M]_D$ = –76.03°. Entry of (–76.03, 0.17, –52.4, 0.26, –123.8, 0.57) into the software in Equation (7) (line 15) would give +48° for (+)-(*R*)-camphor (highest known specific rotation, Theimer et al. 1977). In practice, the specific rotations would be from the literature and the proportions measured by peak areas with chiral GC.

## Determining Specific Rotations From Data in the Literature

The sex pheromone of mealybug *N. viridis* was synthesized from (–)-*trans*-α-necrodol isolated from essential oil of Spanish lavender *L. luisieri*. The (–)-*trans*-α-necrodol was reported to have a specific rotation of –168.95° (Levi-Zada et al. 2021). This measurement, however, included minor chiral impurities consisting of 2% (–)-(*R*)–γ–necrodol and 1% myrtenol of unknown enantiomeric composition. A re-analysis of Spanish lavender oil using the methods of Levi-Zada et al. showed that the myrtenol was only the (1*S*, 5*R*)-(+)-enantiomer with no trace of the opposite enantiomer. Using Equation (7) with $[\alpha_M]_D$ = –168.95°, the proportions of the 3 compounds ($P_1$ = 0.97, $P_2$ = 0.02, and $P_3$ = 0.01), and corresponding $[\alpha_2]_D$ = –7.04° and $[\alpha_3]_D$ = +50° (highest rotation of myrtenol, Renwick et al. 1976) then $[\alpha_1]_D$ = –174.55°, is the corrected specific rotation of (–)-*trans*-α-necrodol (entry Fig. 1, line 15).

Female bark beetles *D. brevicomis* were exposed 18 h to vapors of either +45.8° or –41.6° α-pinene (predominately *R* or *S* mix of enantiomers), which were biosynthetically hydroxylated to *trans*-verbenol, respectively yielding specific rotations by polarimetry of +104.9° for (+)-(1*R*, 2*S*, 5*R*)-*trans*-verbenol or –91.5° for (–)-(1*S*, 2*R*, 5*S*)-*trans*-verbenol (Fig. 2) (Byers 1983). Based on the optical rotations of precursor (+)- or (–)-α-pinene, we calculated the proportions of each of the two enantiomers of α-pinene in the 2 mixtures above by using

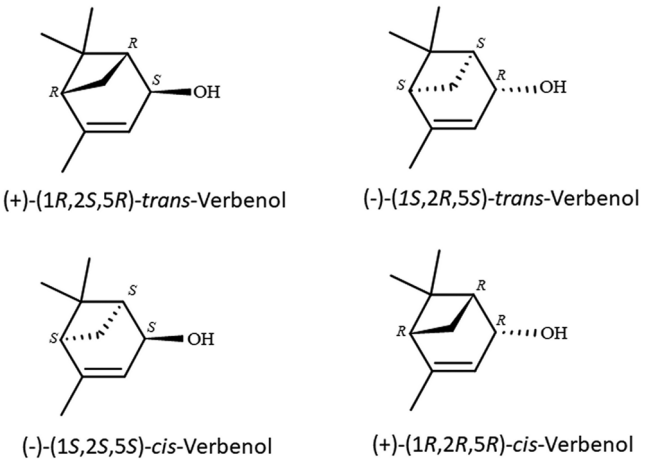



**Fig. 2.** Structures of enantiomers of *trans*-verbenol and *cis*-verbenol, pheromone components of pest bark beetles *Ips* and *Dendroctonus*, showing rotation of polarized light and absolute configurations of 3 chiral centers. (*S*)-*cis*-Verbenol (lower left) was reported to have a (+) optical rotation in methanol (+4° by Silverstein et al., 1966a, 1967; +5.9° by Renwick et al. 1976; or +11.4° by Mori 2007) but in chloroform the rotations reverse for each enantiomer with (*S*)-*cis*-verbenol being –9.8° (Mori 2007).

Equation (4). In this equation, $[\alpha_M]_D$ was either +45.8° or –41.6° (Byers 1983) and $[\alpha_1]_D$ and $[\alpha_2]_D$ are the respective +52.4° and –52.4° specific rotations for pure enantiomers. Thus, for +45.8° α-pinene, $P_1$ = (45.8–(–52.4))/(52.4–(–52.4)) = 98.2/104.8 = 0.937 for proportion of (+)-enantiomer and $P_2$ = 1–$P_1$ = 0.063 for proportion of (–)-enantiomer. Regarding the –41.6° α-pinene, $P_1$ = (–41.6 – (52.4))/(–52.4–52.4) = –94/–104.8 = 0.897 for proportion of (–)-enantiomer and $P_2$ = 1–$P_1$ = 0.103 for proportion of (+)-enantiomer. Using the measured specific rotations for either (+)- or (–)-*trans*-verbenol above for $[\alpha_M]_D$ and the calculated proportions for $P_1$ above, then Equation (4) was solved for $x = [\alpha_1]_D = [\alpha_2]_D$ in the following equation:

$$P_1 = \frac{[\alpha_M]_D - x}{(-x) - x} \rightarrow x = \frac{[\alpha_M]_D}{2 \cdot P_1 - 1}. \quad (9)$$

Thus, for $P_1$ = 0.937 and $[\alpha_M]_D$ = +104.9° $x$ = +120.02° is the predicted specific rotation of (*R*)-*trans*-verbenol. If $P_1$ = 0.897 and $[\alpha_M]_D$ = –91.5°, then $x$ = –115.2°, the predicted specific rotation of (*S*)-*trans*-verbenol. Of course, the higher rotation of 120.02° is the best estimate of the specific rotation of (+)-*trans*-verbenol, and the opposite rotation for the (–)-enantiomer.

## Discussion

Chemical ecologists since the 1960s have discovered that many insects biosynthesize and recognize chiral molecules in their olfactory communication systems, comprising sex and aggregation pheromones and allomones (Silverstein et al. 1966a, 1966b, 1967, 1968). Insects also respond toward, or are repelled by, chiral chemicals emanating from host plant resources (Byers et al. 2000, El-Sayed and Byers 2000). As mentioned earlier, chiral molecules comprised of ring structures with 2 or more chiral centers may have only 2 possible enantiomers (eg α-pinene and β-pinene) because of structural constraints of the rings. Some *Dendroctonus* bark beetle species use frontalin as an aggregation component that has two chiral centers and two enantiomers: (–)-(1*S*, 5*R*)-frontalin and (+)-(1*R*, 5*S*). *Exo*-brevicomin, is another aggregation pheromone component of *Dendroctonus* that has 3 chiral centers, but still only 2 enantiomers, (–)-(1*S*, 5*R*, 7*S*)- and (+)-(1*R*, 5*S*, 7*R*)-*exo*-brevicomin, the latter active for *D. brevicomis*. In the ambrosia beetle *Trypodendron lineatum*, its main pheromone component is lineatin with 4 chiral centers but just 2 enantiomers, (–)-(1*S*, 4*R*, 5*S*, 7*S*) and (+)-(1*R*, 4*S*, 5*R*, 7*R*)-lineatin, of which only the latter is attractive (Silverstein et al. 1967, Wood et al. 1976, Borden et al. 1980, Slessor et al. 1980, Byers 1989, Mori et al. 1983, Seybold 1993, Mori 1998, 2007, Hoover et al. 2000). Chalcogran, an aggregation pheromone component of bark beetle *Pityogenes chalcographus*, has 2 chiral centers and 2 pairs of enantiomers, but only 1 of the 4 compounds has significant bioactivity (Byers et al. 1989). Therefore, researchers need to associate the active enantiomer (the pheromone component) with either its absolute configuration or the optical rotation when attempting to monitor or control these pest beetles.

Due to this complexity, a pheromone component must be isolated and concentrated to determine its optical rotation by a polarimeter, while its absolute configuration would be unknown even when using chiral GC because the order of eluted enantiomers does not indicate either the absolute configuration or the rotation. However, the retention time of the pheromone component on a chiral GC column can be compared to a synthetic standard of primarily one enantiomer with known absolute configuration (eg *S*), and in a second chiral GC run as a racemic (or unbalanced) mixture of the enantiomers to reveal the elution order of the 2 enantiomers. After this, the pheromone enantiomer with assigned absolute configuration can be subjected to polarimetry to determine the optical rotation of each enantiomer. The polarimeter is not necessary if the configurations and optical rotations have already been associated in previous studies, but the instrument can be helpful in determining the enantiomeric purity of the standard, which cannot be done even with chiral GC columns unless the order of enantiomer elution has been associated with absolute configurations.

The specific rotation calculated from 3 variables, optical rotation, tube length, and concentration in solvent (Fig. 1, lines 1 and 2), is usually measured with sodium light (589 nm). However, other wavelengths may be used to increase sensitivity, but this can significantly alter rotations and make comparisons between studies difficult (DePalma 2024). Another less important variable is the temperature of the measurements, usually either 20° or 25 °C, which has a minor effect on rotation. The solvent used to dissolve the chiral compound, such as ethanol, methanol, hexane, chloroform, water, acetone, and DMSO, can have minor effects on rotations. The effects of different solvents are not well known; however, the effect is evident with compounds of small specific rotations (a few degrees) such as (*S*)- and (*R*)-*cis*-verbenol where the rotation is reversed depending on use of a polar or non-polar solvent (Fig. 2). The optical purity and enantiomeric excess percentage (ee%) are often used in chemical ecology. In the field or in laboratory bioassays, the behavior of an insect can vary dramatically depending on the ratio of enantiomers (Wood et al. 1976, Byers 1989), which can be calculated from the ee% (Fig. 1, line 6) or from the mixture's specific rotation $[\alpha_M]_D$ in Equation (4) (lines 8 and 9). In addition, enantiomeric proportions of pheromone components are known to vary geographically over North America in the bark beetle *Ips pini*. Its populations in Idaho produce and respond to (–)-ipsdienol and avoid the (+)-enantiomer, while New York populations produce (+) and (–) ipsdienol in a 65:35 ratio, with the (+)-enantiomer eliciting the most pheromonal response (Lanier et al. 1980).

Using chiral GC columns, one can determine the proportion of the major and minor enantiomer proportions of a mixture, and with the polarimeter one can determine the mixture's $[\alpha_M]_D$ (Equation (3)) to find the highest $[\alpha]_D$ of a pure enantiomer (Equation (6), Fig. 1, lines 12 and 13). The specific rotation of an enantiomer of highest purity can be determined with chiral GC to find the proportion of the enantiomer $P_1$ compared to the chiral impurity $P_2$ (where $P_1 + P_2 = 1$) and a polarimeter to find the specific rotation of the mixture [Equation (3) using $P_1$ and $P_2$, where $P_2$ replaces $(1 - P_1)$], and the known specific rotation of the chiral impurity (use Equation (5), Fig. 1, lines 10 and 11). Equation (7) (Fig. 1, line 15) can be used to find the specific rotation of an enantiomer of interest by using chiral GC to obtain its proportion, as well as several other chiral compounds in the mixture that have known specific rotations (from literature). Finally, the proportions of the chiral compounds determined by chiral GC can be input along with their

known specific rotations from literature to predict the specific rotation of the mixture (Equation (8), Fig. 1, line 16), which can be compared with a polarimeter to check for accuracy of the literature reports.

Silverstein et al. (1966a, 1967) identified the first multi-component aggregation pheromone for bark beetles in *Ips paraconfusus* Lanier, with one component synergist being (1*S*, 2*S*, 5*S*)-*cis*-verbenol, hereafter called (*S*)-*cis*-verbenol (Fig. 2). Renwick et al. (1976) exposed *I. paraconfusus* of either sex to –46.5° (–)-(*S*)-α-pinene vapors and found a selective accumulation of only (*S*)-*cis*-verbenol in their hindguts. Exposure of the beetle to +20.2° (+)-(*R*)-α-pinene vapors caused selective production of +127° (+)-(*R*)-*trans*-verbenol. Although the +20.2° (+)-(*R*)-α-pinene had 30.7% (–)-(*S*)-enantiomer (Fig. 1, lines 8 and 9), all the (–)-(*S*)-α-pinene was converted to (*S*)-*cis*-verbenol while the 69.3% (+)-(*R*)-α-pinene was converted only to (+)-(*R*)-*trans*-verbenol (Fig. 2). Thus, the +127° *trans*-verbenol is the highest known specific rotation because no (–)-α-pinene was converted to *trans*-verbenol (Renwick et al. 1976). The rotation of 127° is somewhat more than the adjusted 120.02° (Equation (9), "Results" section) found for the *trans*-verbenol converted from α-pinene by *D. brevicomis* in ethanol (Byers 1983). The discrepancy can be due to measurement errors in the polarimetry of low amounts of the beetle-produced *trans*-verbenol, as well as the solvent used for polarimetry. In any case, the adjusted value for the (+)- and (–)-*trans*-verbenols produced by *D. brevicomis* from each enantiomer of α-pinene (120.02°) shows a higher rotation than 104.9° reported by Byers (1983).

The use of polarimetry to characterize chiral semiochemicals is limited by the quantities utilized by various insect species. For example, defensive allomones, alarm pheromones (Byers and Levi-Zada 2011), and some pheromones that are produced in relatively large quantities (eg 10 µg/insect in *D. brevicomis*, Byers 1983) can be analyzed by concentrating repeated GC collections and quantified by comparison to dilutions of known amounts of synthetic compounds. Generally, the use of the software will aid in laboratory and field experiments with synthesized (commercial) compounds in which ratios of enantiomers are crucial for behavioral responses (Wood et al. 1976, Lanier et al. 1980, Byers 1989). Field testing and application of larger quantities of constituents of commercial essential oils (monoterpenes and terpenoids) as repellents in control of palm weevils, bark beetles, and fruit flies (Byers et al. 2000, 2018, 2020, Levi-Zada et al. 2024, 2025) is another area where the polarimetry software will aid entomologists.

Traditional polarimetry presented here uses polarized light vibrating in one plane. A newer, more complex method, termed circular dichroism (CD) spectroscopy, uses circularly polarized light rotating in opposite directions to study chiral macromolecules such as odorant binding proteins (Xu et al. 2021). They found that some sensory receptor proteins of a mealybug change conformation, as indicated by CD, when exposed to several plant volatiles. Chiral chemicals are not only important to the chemical ecology of insects but also to the human food, pharmaceutical, and cosmetic industries, with an estimated market value of over 59 billion USD in 2021 (Das 2025). As noted above, many insect species from primitive cockroaches (Blattodea) to Hemiptera (hemimetabolous) and more recently evolved holometabolous orders (Diptera, Coleoptera, Hymenoptera, Lepidoptera) use chiral semiochemicals in diverse ecological functions such as host plant selection, finding appropriate mates, defense, social alarm, and avoidance of intra- and interspecific competition for resources. The Java application and Internet webpage using polarimetric equations should aid chemical ecologists when characterizing chiral blends of pheromone components, preparing appropriate semiochemical blends in behavioral bioassays, and field applications of pest monitoring and control.

## Author Contributions

John A. Byers (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [lead], Project administration [equal], Software [lead], Supervision [equal], Validation [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Sara Steiner (Data curation [supporting], Investigation [supporting], Validation [supporting], Writing—review & editing [supporting]), Daniela Fefer (Data curation [supporting], Investigation [supporting], Validation [supporting], Writing—review & editing [supporting]), and Anat Levi Zada (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [lead], Investigation [equal], Methodology [equal], Project administration [equal], Resources [lead], Software [supporting], Supervision [equal], Validation [equal], Visualization [supporting], Writing—original draft [equal], Writing—review & editing [supporting])

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