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Structure Elucidation and Absolute Stereochemistry of Isomeric Monoterpene Chromane Esters

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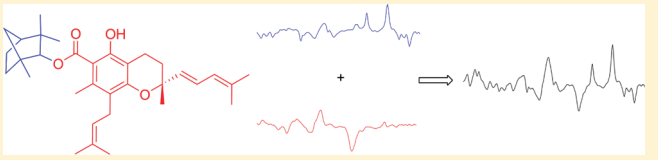
Supporting Information

ABSTRACT: Six novel monoterpene chromane esters were isolated from the aerial parts of *Peperomia obtusifolia* (Piperaceae) using chiral chromatography. This is the first time that chiral chromane esters of this kind, ones with a tethered chiral terpene, have been isolated in nature. Due to their structural features, it is not currently possible to assess directly their absolute stereochemistry using any of the standard classical approaches, such as X-ray crystallography, NMR, optical rotation, or electronic circular dichroism (ECD). Herein we report the absolute configuration of these molecules, involving four chiral centers, using vibrational circular dichroism (VCD) and density functional theory (DFT) (B3LYP/6-31G*) calculations. This work further reinforces the capability of VCD to determine unambiguously the absolute configuration of structurally complex molecules in solution, without crystallization or derivatization, and demonstrates the sensitivity of VCD to specify the absolute configuration for just one among a number of chiral centers. We also demonstrate the sufficiency of using the so-called inexpensive basis set 6-31G* compared to the triple-ζ basis set TZVP for absolute configuration analysis of larger molecules using VCD. Overall, this work extends our knowledge of secondary metabolites in plants and provides a straightforward way to determine the absolute configuration of complex natural products involving a chiral parent moiety combined with a chiral terpene adduct.



INTRODUCTION

Piperaceae is a basal angiosperm family comprising more than 4000 species. The structural richness of its members is exemplified by the large variety of secondary metabolites isolated, such as phenylpropanoids, lignan/neolignans, pyrones, aliphatic and aromatic amides, alkaloids, polyketides, benzoic acids derivatives and benzopyrans.[1] A recent survey of biologically active metabolites from Piperaceae species pointed out the *Peperomia* genus as the second most abundant source of bioactive compounds within this family, contributing to 15% of the total.[1] This number could be higher given that only few studies have been carried out on this genus, mainly due to its predominantly ornamental use. The same study also showed that prenylated benzopyrans are among the best biologically active compounds found in *Peperomia* species, with cytotoxic and antiprotozoal activities being the most frequently reported.[1]

Recently, two prenylated benzopyrans, peperobtusin A and 3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3′′-methyl-2′′-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylic acid (**1**), were isolated as racemates from *P. obtusifolia* and showed potent trypanocidal activity as well as low unspecific cytotoxicity.[2] These two compounds had their enantiomers resolved and the absolute configuration determined by VCD and DFT calculations.[3] The enantiomers of **1** are reported here as building blocks for the novel compounds described in this work, which are esterified with the monoterpenes borneol and fenchol (Figure 1).

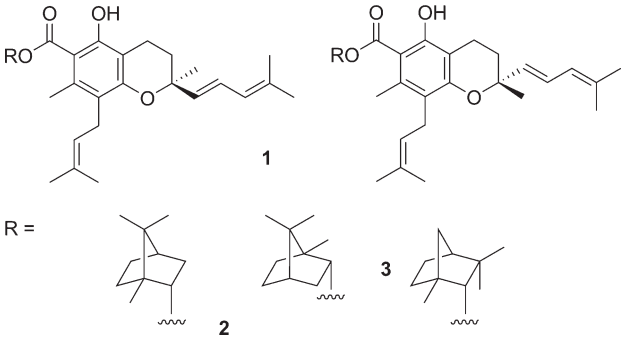


Figure 1. Structure of the six novel monoterpene chromane esters isolated from *P. obtusifolia*.

From the hexane extract of the aerial parts (leaves and stems) of *P. obtusifolia*, a stereisomeric mixture of four compounds esterified with borneol (**2**) and two with fenchol (**3**) were isolated. This is the first time that chromane esters of this kind have been isolated in nature.

It is well-known that different stereoisomers can trigger very distinct biological activities. This feature is extremely important in drug-like molecules and is exemplified by the tragic case of

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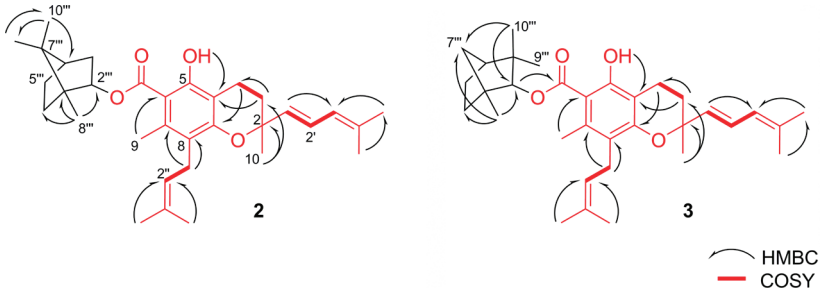

Figure 2. Selected *g*HMBC (H → C) and $^{1}H-^{1}H$ COSY correlations common to compounds **2** and **3**.

teratogenesis caused by thalidomide. This drug caused a large number of birth defects when administered to pregnant women as antiemetic. The cause for this tragedy was found in the fact that only the dextrorotatory isomer had the desired effect, whereas the levorotatory one was teratogenic.[4] In addition, the stereochemistry of secondary metabolites plays an important role in understanding their biosynthetic pathways since, as in the case of monoterpenes, the presence of both enantiomers in the same plant[5−13] may indicate two separate enzyme systems each capable of producing a single enantiomer.[14]

Among all the methods capable of determining the absolute configuration of organic compounds, directly or indirectly, e.g., X-ray diffraction analysis, NMR spectroscopy, enzymatic transformations, optical rotation measurements (OR and ORD), Raman optical activity as well as circular dichroism (vibrational and electronic),[15] the use of chiroptical methods has lately faced a renaissance.[16] The increased confidence in the use of these methods results from the development of quantum mechanical software programs for predicting both vibrational and electronic spectra that can be reliably compared with experimental results.

VCD was first reported in 1974[17] and confirmed in 1975.[18] It is the extension of ECD into infrared and near-infrared regions of the spectrum where vibrational transitions occur within the ground electronic state of the molecule.[19] This technique has many advantages over other methods widely used since there is no need of either single crystals, chromophores or derivatizations, and due to the wealth of bands and sensitivity of VCD to molecular conformations, not only is absolute stereochemistry determination feasible but also conformational analysis in solution.[19−22]

In this work we describe the isolation, structure determination and absolute configuration of six novel compounds from *P. obtusifolia* (Piperaceae). Due to the flexible chemical structure of these molecules, it has not been possible to obtain single crystals and there are no UV−vis chromophores present within the monoterpene moieties. Furthermore, even if derivatization were a possibility, NMR methods[23] would not be useful since the only site for reaction is a sterically hindered phenolic OH group, far away from the chiral centers and strongly hydrogen bonded with the carbonyl group nearby.[24] Therefore, VCD, along with DFT calculations, is seen as a powerful and relatively straightforward methodology for addressing this and related stereochemical challenges.

RESULTS AND DISCUSSION

The molecular formula of compounds **2.1**−**2.4** as well as **3.1** and **3.2** was established as $C_{33}H_{46}O_4$ by high-resolution electrospray ionization mass spectrometry (HRESIMS) measurements ($[M + H]^+$ obsd *m*/*z* 507.3468, calcd 507.3474, Δ −0.6 mmu; $[M + Na]^+$ obsd *m*/*z* 529.3278, calcd 529.3293, Δ −1.5 mmu) in combination with extensive NMR analyses.

Additionally, the subsequent fragmentation by MS−MS of the quasi-molecular ion $[M + H]^+$ = 507.3468 gave rise to the fragment ions *m*/*z* 371.2215 and *m*/*z* 353.2114 indicating the loss of the monoterpene moiety followed by the loss of a water molecule.

Compound **2.1** was obtained as pale yellow oil. Analyses of the ^{13}C, ^{1}H NMR and *g*HMQC data revealed 33 carbon resonances, including an upfield shifted carboxyl group (δ 173.0) suggestive of an ester, four olefinic methines (δ_H/δ_C 5.02/123.1, 5.46/133.7, 5.68/124.4, 6.24/125.2) four aliphatic methyls (δ_H/δ_C 0.86/13.7, 0.84/19.7, 0.89/18.9, 1.36/27.3), four olefinic methyls (δ_H/δ_C 1.60/18.2, 1.60/25.7, 1.66/25.8, 1.71/18.0), one aromatic methyl (δ_H/δ_C 2.45/18.9), six methylenes, one aliphatic methine (δ_H/δ_C 1.68/44.8), one oxymethine (δ_H/δ_C 5.06/82.3), eight nonprotonated olefinic or aromatic carbons, two bonded to oxygen (δ 159.8 and 156.3), and an oxygen-bearing quaternary aliphatic carbon (δ 77.0). Of 33, 23 signals were in accordance with those for the 3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylic acid (**1**),[24] corroborating the 10 additional signals to belong to a monoterpene moiety attached to it. Further *g*HMQC, *g*HMBC and *g*COSY experiments suggested the monoterpene moiety to be borneol. The key *g*HMBC and $^{1}H-^{1}H$ COSY correlations are presented in Figure 2. Compounds **2.2**−**2.4** were also isolated as oily compounds and presented NMR data almost identical to those for **2.1** (Table 1) which, in addition to the same high resolution molecular mass spectra, allowed us to assign them as stereoisomers of bornyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate. The analysis of the 1D NOESY spectra of these compounds indicated that all have the H-2‴ (δ 5.06) as exo and the chromane group at the endo side of the bornyl moiety. Upon irradiation of H-2‴, a NOE enhancement was observed for -CH_3-8‴ (δ 0.86) and -CH_3-10‴ (δ 0.89) as well as H-3‴ (δ 2.44). Thus, the bornyl group had two likely absolute configurations: (1‴*R*,2‴*S*,4‴*R*) or (1‴*S*,2‴*R*,4‴*S*); however, the relative stereochemistry at C-2 remained unknown. The main NOE correlations for compound **2** are presented in Figure 3. Furthermore, although no *g*HMBC correlation between the oxymethine H-2‴ and the carbonyl group was observed, NOE correlations between the monoterpene and chromane moieties confirm their point of attachment. Irradiation of the methyl group -CH_3-8‴ (δ_H 0.86) resulted in NOE enhancement for the aromatic methyl group -CH_3-9 at δ_H 2.45. Finally, the presence of an IR absorption band at 1650 cm^{-1} (ester C═O), common to all compounds, supports the identification of **2.1** through **2.4** as esters.

The structure of **3.1** was deduced as follows. Analyses of the ^{13}C, ^{1}H NMR and *g*HMQC data also revealed 33 carbon resonances, including an upfield shifted carboxyl group (δ 173.0) suggestive of an ester, four olefinic methines (δ_H/δ_C

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Table 1. ^{1}H and ^{13}C NMR Data for 2.1−2.2 and 3.1−3.2 in $CDCl_3$

	2.1		2.2		3.1		3.2	
no.	$^{13}C^{a}$	$^{1}H,^{b}$ m, J (Hz)	$^{13}C^{a}$	$^{1}H,^{b}$ m, J (Hz)	$^{13}C^{a}$	$^{1}H,^{b}$ m, J (Hz)	$^{13}C^{a}$	$^{1}H,^{b}$ m, J (Hz)
2	77		77.2		77		77	
3(a)	31.5	1.85 ddd 5.5, 5.5, 13.5	31.5	1.86 m	31.5	1.86 ddd 5.5, 5.5, 13.0	31.5	1.85 m
(b)		1.72 m		1.72 m		1.74 m		1.71 m
4(a)	17	2.64 ddd 5.5, 5.5, 17.0	17	2.66 ddd 5.5, 5.5, 17.0	17	2.64 ddd 5.5, 5.5, 17.5	17.2	2.64 ddd 5.5, 5.5, 17.0
(b)		2.45 m		2.45 m		2.44 m		2.45 m
4a	106.9		106.9		106.9		106.5	
5	159.8		159.8		160.1		160.1	
6	105.4		105.4		105		104.5	
7	136.8		136.8		136.8		136.5	
8	120.6		120.6		120.5		120	
8a	156.3		156.3		156.3		156.3	
9	18.9	2.45 s (3H)	18.9	2.45 s (3H)	19.1	2.47 s (3H)	18.8	2.47 s (3H)
10	27.3	1.36 s (3H)	27.2	1.36 s (3H)	27.3	1.38 s (3H)	26.8	1.37 s (3H)
1′	133.7	5.46 d 15.0	133.7	5.46 d 15.0	133.7	5.45 d 15.5	133.4	5.49 d 15.5
2′	125.2	6.24 dd 11.0, 15.0	125.2	6.22 dd 11.0, 15.0	125.2	6.22 dd 11.0, 15.5	125.2	6.24 dd 11.0, 15.5
3′	124.4	5.68 d 15.0	124.4	5.68 d 15.0	124.4	5.66 d 11.0	124.2	5.70 d 11.0
4′	135.4		135.4		135.4		135.2	
5′	25.8	1.66 s (3H)	25.8	1.66 s (3H)	25.8	1.67 s (3H)	25.6	1.68 s (3H)
6′	18.2	1.60 s (3H)	18.2	1.59 s (3H)	18.2	1.60 s (3H)	17.8	1.61 s (3H)
1″(a)	24.9	3.36 dd 7.0, 15.0	24.9	3.38 dd 7.0, 15.0	24.9	3.39 dd 6.5, 15.0	24.9	3.37 dd 6.5, 15.0
(b)		3.25 dd 7.0, 15.0		3.26 dd 7.0, 15.0		3.25 dd 6.5, 15.0		3.26 dd 6.5, 15.0
2″	123.1	5.02 m	123.1	5.02 m	123.1	5.01 m	123.1	5.03 m
3″	130.8		130.8		130.8		130.5	
4″	25.7	1.60 s (3H)	25.7	1.60 s (3H)	25.8	1.62 s (3H)	25.6	1.61 s (3H)
5″	18	1.71 s (3H)	18	1.71 s (3H)	18	1.72 s (3H)	17.4	1.72 s (3H)
1‴	48.8		48.7		48.3		48.2	
2‴	82.3	5.06 m	82.3	5.07 m	89	4.58 d 2.0	89	4.60 d 2.5
3‴(a)	37.1	2.44 m	37.2	2.42 m	39.6		39.2	
(b)		1.06 dd 3.5, 14.0		1.05 m				
4‴	44.8	1.68 m	44.7	1.67 m	48.4	1.68 m	48.2	1.70 m
5‴(a)	28	1.70 m	28	1.70 m	25.7	1.20 m (2H)	25	1.19 m (2H)
(b)		1.20 m		1.20 m				
6‴(a)	27.9	1.98 ddd 4.0, 11.0, 13.5	27.8	1.98 m	27.5	1.80 m (2H)	27	1.80 m (2H)
(b)		1.34 m		1.34 m				
7‴(a)	47.8		47.8		41.5	1.58 m	41.5	1.60 m
(b)						1.15 m		1.18 m
8‴	13.7	0.86 s (3H)	13.7	0.86 s (3H)	19.7	1.07 s (3H)	19.7	1.06 s (3H)
9‴	19.7	0.84 s (3H)	19.7	0.84 s (3H)	20.5	0.75 s (3H)	20.2	0.76 s (3H)
10‴	18.9	0.89 s (3H)	18.9	0.89 s (3H)	29.5	1.15 s (3H)	29.5	1.15 s (3H)
C═O	173		173		173.2		172.8	
O−H		11.7 s		11.6 s		11.8 s		11.8 s

[a] 125 MHz. [b] 500 MHz. Chemical shifts in ppm.

5.01/123.1, 5.45/133.7, 5.66/124.4, 6.22/125.2) four aliphatic methyls (δ_H/δ_C 0.75/20.5, 1.07/19.7, 1.15/29.5, 1.38/27.3), four olefinic methyls (δ_H/δ_C 1.60/18.2, 1.62/25.8, 1.67/25.8, 1.72/18.0), one aromatic methyl (δ_H/δ_C 2.47/19.1), six methylenes, one aliphatic methine (δ_H/δ_C 1.68/48.4), one oxymethine (δ_H/δ_C 4.58/89.0), eight nonprotonated olefinic or aromatic carbons, two bonded to oxygen (δ 160.1 and 156.3), and an oxygen-bearing quaternary aliphatic carbon (δ 77.0). Of these 33, 23 signals were again in accordance with those for **1**[24] corroborating the 10 additional signals to belong to a monoterpene moiety attached to it. Additionally, the *g*HMBC spectrum of **3.1** displayed correlation between H-2‴ (δ 4.58) and the carbonyl group confirming a monoterpene ester. Further *g*HMQC, *g*HMBC and *g*COSY experiments indentified the monoterpene moiety as fenchol. The key *g*HMBC correlations are presented in Figure 2 as well. Compounds **3.1** and **3.2** were isolated as oily compounds and presented almost identical NMR data (Table 1) which, in addition to the same high resolution molecular mass spectra, allowed us to assign them as stereoisomers of fenchyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate. The analysis of

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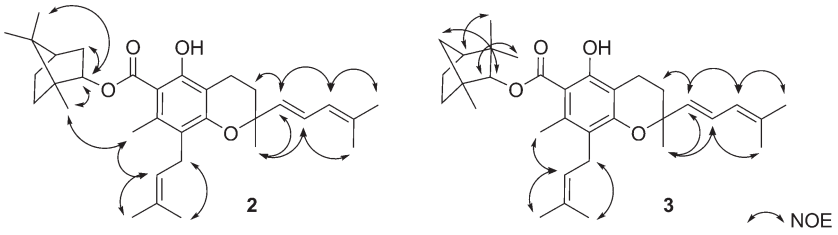

Figure 3. Selected NOE interactions for **2** and **3**.

the 1D NOESY spectra of these two compounds indicated them to have H-2‴ as exo and the chromane group at the endo side of the fenchyl moiety. Upon irradiation of H-2‴, a NOE enhancement was observed for -CH_3-8‴ (δ 1.07), -CH_3-9‴ (δ 0.75), -CH_3-10‴ (δ 1.15) as well as H-7‴ (δ 1.59). Thus, the fenchyl group had two likely absolute configurations: (1‴*R*,2‴*R*,4‴*S*) or (1‴*S*,2‴*S*,4‴*R*); however, the relative stereochemistry at C-2 remained unknown. The main NOESY correlations for compound **3** are presented in Figure 3. The NMR data for all compounds are in accordance with literature values for monoterpene esters.[25]

Once the relative stereochemistry was assessed, at least for the monoterpene parts, the next step was the determination of the absolute configuration. By comparing IR and VCD measurements with the output of DFT calculations, the absolute configuration of each compound was unambiguously determined in solution. Compounds within the **2** and **3** series had their experimental data subtracted from solvent ($CDCl_3$) in order to correct the baseline. This procedure was adopted because the stereosiomeric relations among them were unknown.

For the calculations, two different combinations of hybrid functional and basis set were used, B3LYP/6-31G*, which has been by far the most used for VCD,[19,21] although sometimes considered less accurate,[4,22] and B3PW91/TZVP, which uses a Gaussian basis set of triple ζ valence quality augmented by polarization functions,[26] claimed to give better results, at least for some small molecules.[27]

The experimental and calculated IR and VCD data for all stereoisomers of **2** are presented in Figure 4. From that it is possible to observe a very good agreement between the spectra of (−)-**2.1** and those calculated for (2*S*,1‴*S*,2‴*R*,4‴*S*) using both levels of theory. It is also possible to observe that (+)-**2.4** has a VCD spectrum fully opposite to that for (−)-**2.1**, which allowed us to assign it as the enantiomer of the latter, therefore (2*R*,1‴*R*,2‴*S*,4‴*R*). For these first two compounds, the output of the Confidence Level algorithm was as follows: ESI = 70.4 and Confidence Level of 100% for B3LYP/6-31G* and ESI = 72.2 and Confidence Level of 100% for B3PW91/TZVP. These values of ESI lie at the 70th and 76th percentiles in the database for correct assignments, respectively.

Still from Figure 4 it is possible to observe a very good agreement between the spectra of (+)-**2.2** and those calculated for (2*R*,1‴*S*,2‴*R*,4‴*S*) using both levels of theory. It is also possible to observe that (−)-**2.3** has a VCD spectrum completely opposite to that for (+)-**2.2**, which allowed us to assign it as the enantiomer of the latter, therefore (2*S*,1‴*R*,2‴*S*,4‴*R*). For these two compounds, the output of the Confidence Level algorithm was as follows: ESI = 66.7 and Confidence Level of 100% for B3LYP/6-31G* and ESI = 70.5 and Confidence Level of 100% for B3PW91/TZVP. These values of ESI lie at the 61st and 70th percentiles in the database for correct assignments, respectively.

It is noteworthy that **2.1** and **2.2** have the same monoterpene moiety, namely, bornyl (1‴*S*,2‴*R*,4‴*S*), attached to one of the enantiomers of the chromane **1**. For compounds **2.3** and **2.4**, the same relation is observed except that they carry the antipode of the given monoterpene. Moreover, from the chiral chromatogram obtained for **2** (Supporting Information), we learned that **2.1** and **2.2** account for approximately 66% of the relative peak area (33% each), while **2.3** and **2.4** account for 34% (17% each). These findings suggest that *P. obtusifolia* produces both enantiomers of chromane **1** and the referred bornyl moiety as racemates. The relatively weak stereoselectivity (2:1) regarding the formation of these diastereomeric esters may be explained by a stereorandom process where the free energy of each esterification is assumed to be different.

Regarding the stereisomers of **3**, the good agreement between experimental and calculated data for (−)-**3.1** allowed us to assign it as (2*S*,1‴*R*,2‴*R*,4‴*S*) (Figure 5). Compound (+)-**3.2**, on the other hand, was found to have the same configuration within the fenchyl moiety (region between 950 and 1250 cm^{-1}) however presented an inversion in the chiral center at C-2. Therefore, (+)-**3.2** was assigned as (2*R*,1‴*R*,2‴*R*,4‴*S*) (Figure 5).

The output of the Confidence Level algorithm for these two diastereoisomers was as follows. For **3.1**, ESI = 70.4 and Confidence Level of 100% for B3LYP/6-31G* and ESI = 75.4 and Confidence Level of 100% for B3PW91/TZVP. These values of ESI lie at the 70th and 84th percentiles in the database for correct assignments, respectively. As for **3.2**, the ESI was 64.3 and the Confidence Level 100% using B3LYP/6-31G* and 68.7 and 100%, respectively using B3PW91/TZVP. These values of ESI lie at the 56th and 65th percentiles in the database for correct assignments, respectively.

From the chiral chromatogram obtained for **3** it is possible to observe that **3.1** accounts for 53% of the relative peak area while **3.2** accounts for about 47%. These results corroborate that chromane **1** is produced as a racemic mixture and then each enantiomer is attached to the monoterpene available. In this case only one enantiomer of the fenchyl moiety was identified.

Although the use of B3PW91/TZVP generated better results compared to B3LYP/6-31G* to all compounds analyzed, the latter proved to be enough for an unambiguous assignment with the same level of confidence (100%). Additionally, the calculations with the larger basis set were about seven times as long as those with B3LYP/6-31G*. Moreover, the lowest-energy conformers obtained using both levels of theory were almost identical except for the Boltzmann population distribution (Figures 6 and 7). Therefore, no practical advantages arise from the use of a larger basis set for this compounds of 83 atoms and 276 electrons as far as time and computational power are concerned.

Regarding the VCD spectra of the stereoisomers of **2** and **3**, presented in Figures 4 and 5, it is clear that there are some particular features that can be assigned to the monoterpene and

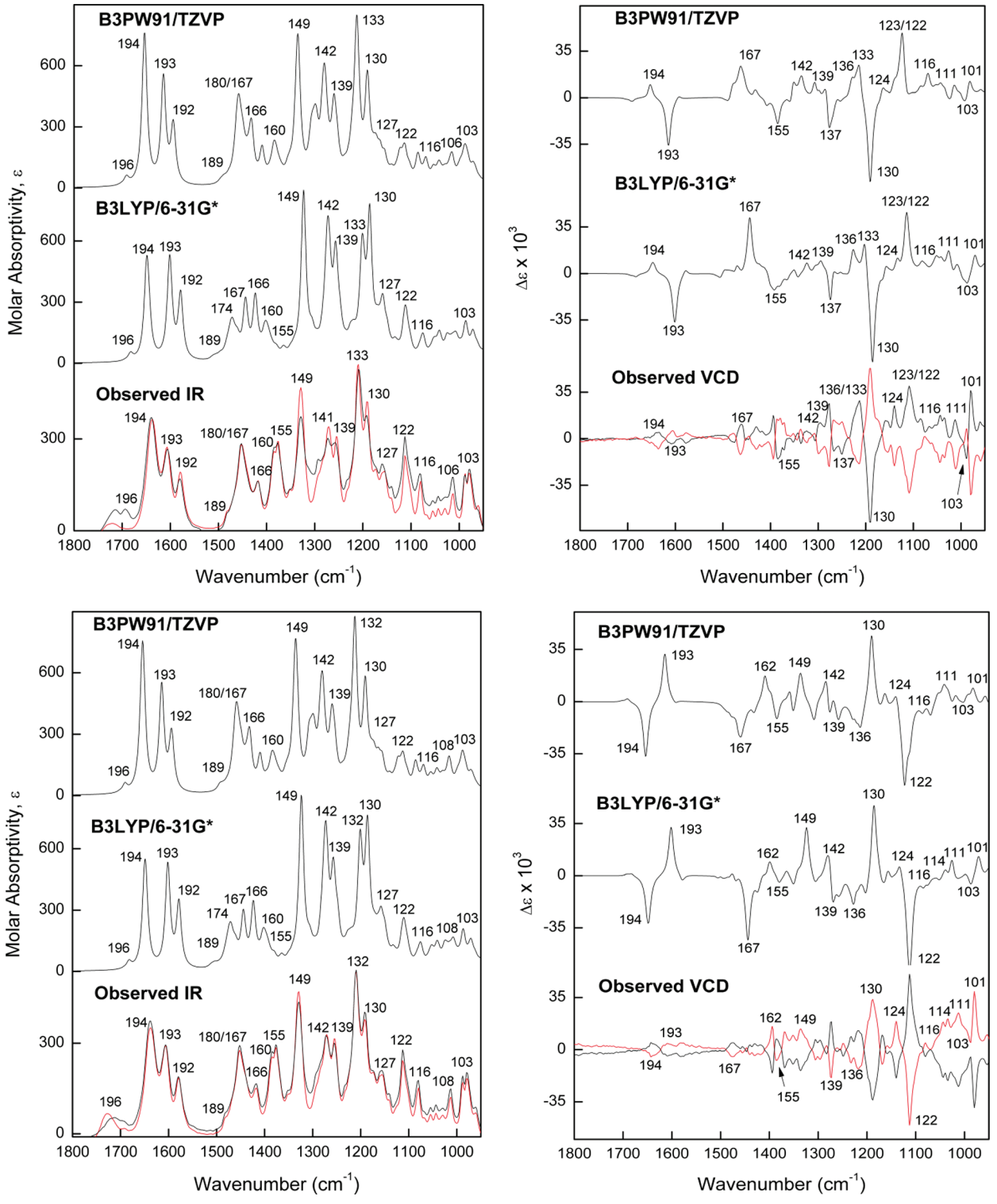

Figure 4. Upper frame: experimental IR and VCD for (−)-**2.1** (black) and (+)-**2.4** (red) and calculated data for the borneol derivative with configuration (2*S*,1‴*S*,2‴*R*,4‴*S*). Lower frame: experimental IR and VCD for (+)-**2.2** (red) and (−)-**2.3** (black) and calculated data for (2*R*,1‴*S*,2‴*R*,4‴*S*). Numbers represent fundamentals.

chromane motifs separately, while others predominantly result from vibrations involving most of the molecular frame.

Fundamentals 101, 103, 111, and 116 are due to vibrations within the monoterpenes and permit the identification of the same or opposite configurations among the title molecules. On the other hand, the signal of fundamentals 122/123 and 130, which are always opposite to each other, is a precise mark for the configuration at C-2. A negative and positive combination from low to high frequency accounts for the (*R*) configuration, whereas the opposite corresponds to (*S*).

In order to gain even more confidence regarding the assignments described above, another approach was applied to those molecules with diastereomeric relations, namely, **2.1** and **2.2** as well as **3.1** and

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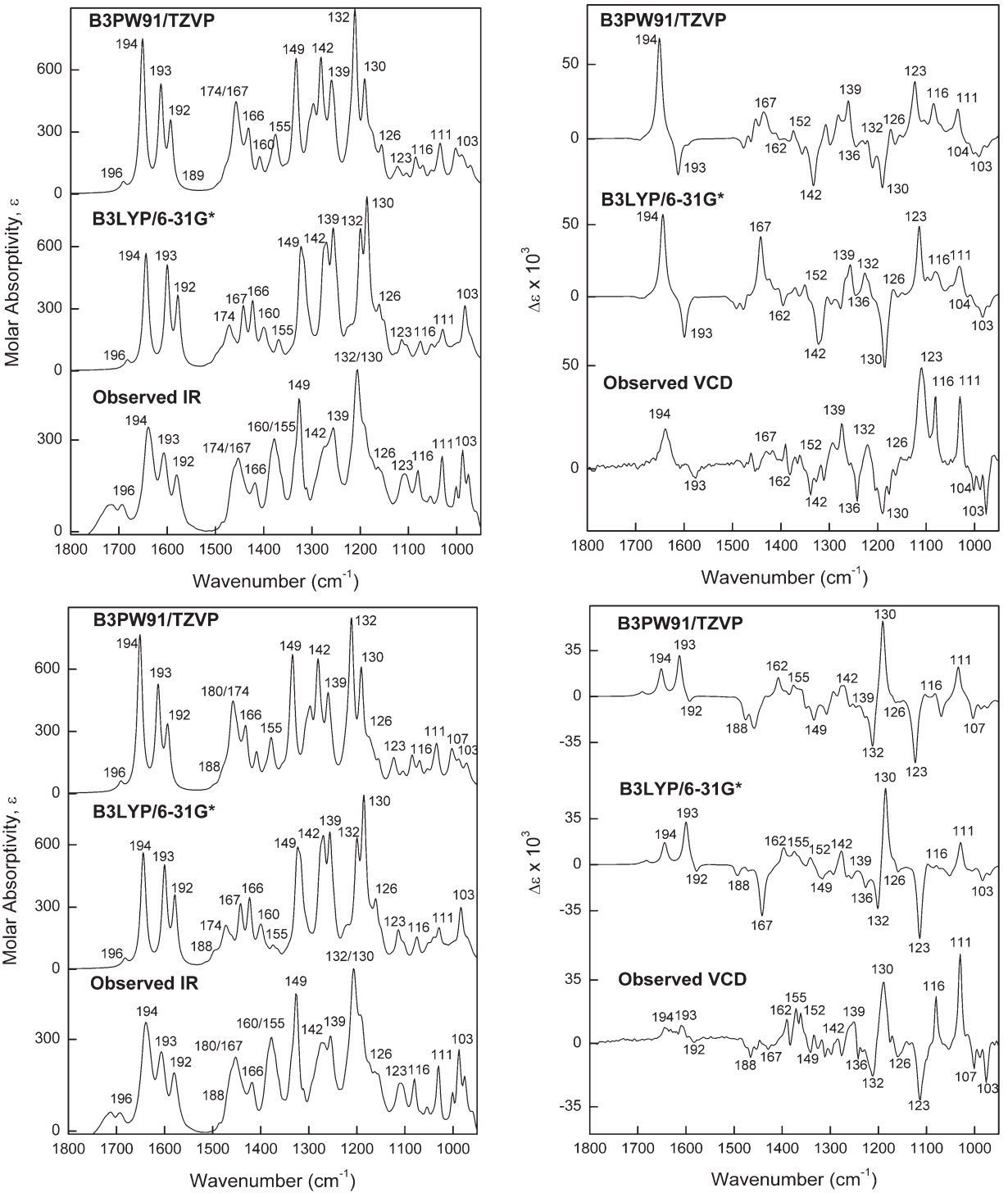

Figure 5. Upper frame: experimental IR and VCD for (−)-**3.1** and calculated data for the fenchol derivative with configuration (2*S*,1‴*R*,2‴*R*,4‴*S*). Lower frame: experimental IR and VCD for (+)-**3.2** and calculated data for (2*R*,1‴*R*,2‴*R*,4‴*S*). Numbers represent fundamentals.

3.2. Their experimental spectra were both averaged and subtracted, followed by division by 2,[28] to give the measured VCD spectral signatures of the monoterpene and the chromane moieties. The two resulting spectra of each series were then compared to the corresponding calculated spectra, obtained by averaging and subtracting, and then dividing by 2, for the (2*R*,1‴*S*,2‴*R*,4‴*S*) and (2*S*,1‴*S*,2‴*R*,4‴*S*) borneol derivatives and the (2*R*,1‴-*R*,2‴*R*,4‴*S*) and (2*S*,1‴*R*,2‴*R*,4‴*S*) fenchol derivatives. The results are presented in Figure 8 and further support our assignments of the absolute configuration for each of the chiral centers. In addition, these comparisons confirm the bands previously designated as markers for determining de absolute configuration of the monoterpene and chromane moieties.

Most of the terpenes that have been isolated from *Piper*, the most significant genus of Piperaceae family, are either monoterpene or sesquiterpenes.[29] However, only few reports exist regarding

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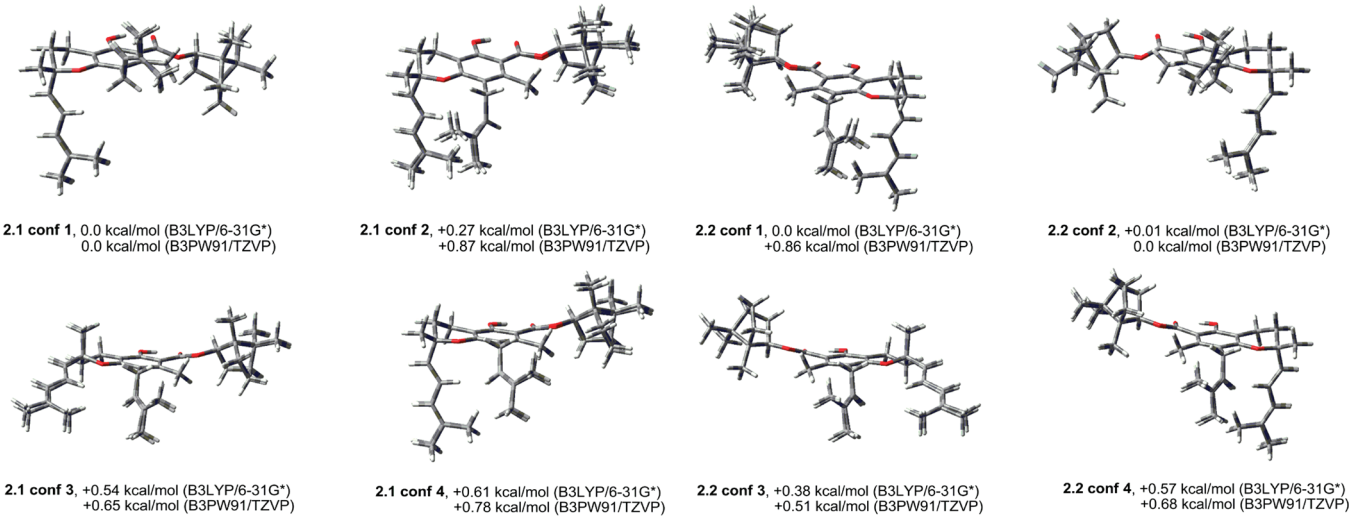

Figure 6. Merged optimized structures and relative energies of the four lowest-energy conformers found for borneol derivatives with configurations (2*S*,1‴*S*,2‴*R*,4‴*S*) (left) and (2*R*,1‴*S*,2‴*R*,4‴*S*) (right) using both B3LYP/6-31G* and B3PW91/TZVP levels of theory.

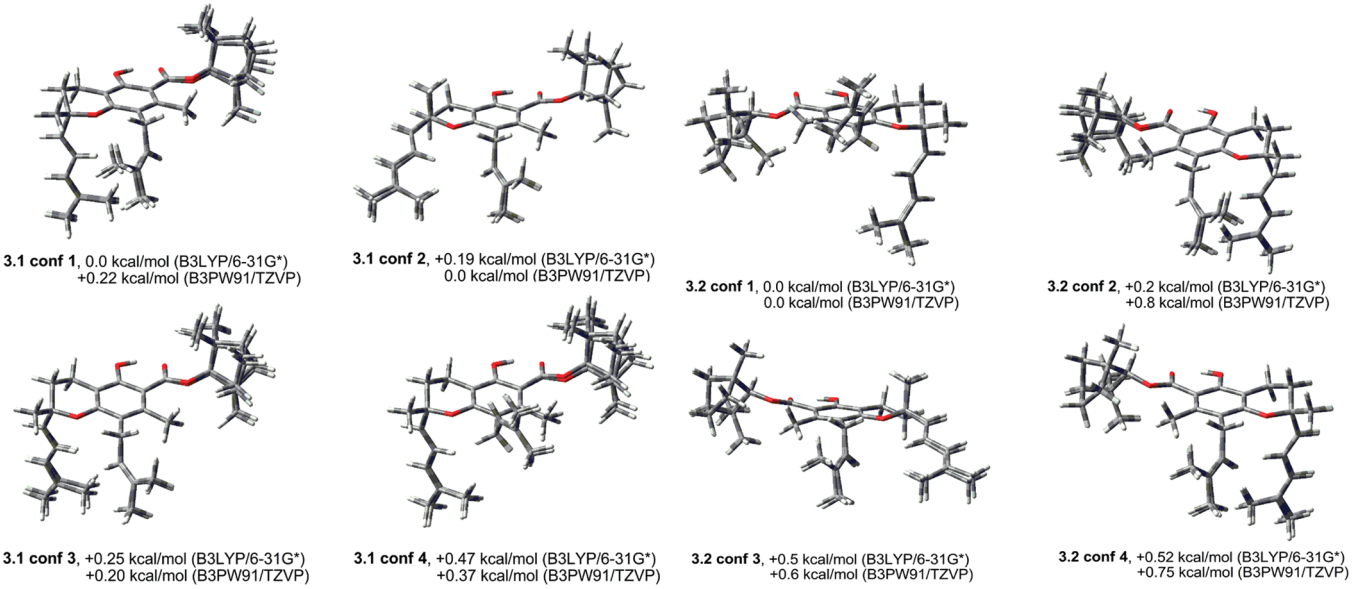

Figure 7. Merged optimized structures and relative energies of the four lowest-energy conformers found for fenchol derivatives with configurations (2*S*,1‴*R*,2‴*R*,4‴*S*) (left) and (2*R*,1‴*R*,2‴*R*,4‴*S*) (right) using both B3LYP/6-31G* and B3PW91/TZVP levels of theory.

monoterpene esters, none of them ever with chromanes. Some borneol esters with small carboxylic acids molecules were isolated from *Piper philippinum*,[30] *Piper aff. pedicellatum*,[31] *Piper caninum*,[32] and *Piper methysticum*,[33] and to the best of our knowledge, never has a fenchyl ester with any molecule been isolated from Piperaceae. Therefore, this work contributes to our growing knowledge of new secondary metabolites in plants and provides a straightforward way to determine the absolute configuration of complex natural products involving a chiral parent moiety combined with a terpene adduct. Interestingly, the NMR spectra of those compounds that differ only at the C-2 stereo center, namely, **2.1** and **2.2**, as well as **3.1** and **3.2**, presented in Table 1, are basically indistinguishable even though a diastereomeric relationship takes place. If it were not for the VCD analysis, based only on the optical rotation values and relative abundance in the chiral chromatogram, they might have been erroneously considered as enantiomers, despite some discrepancies in the optical rotation magnitude. Finally, due to the presence of fundamentals assigned as markers both for monoterpene and chromane stereochemistry, the absolute configuration of related molecules could be assessed in the future using VCD spectroscopy even without the aid of DFT calculations.

CONCLUSIONS

Comparison of experimental and calculated IR and VCD spectra of six novel isomeric monoterpene chromane esters isolated from the aerial parts of *P. obtusifolia* (Piperaceae) by chiral chromatography established their absolute configuration and conformer distribution directly in $CDCl_3$ solution. The six compounds were assigned as follows: (−)-**2.1** (2*S*,1‴*S*,2‴*R*,4‴*S*), (+)-**2.2** (2*R*,1‴*S*,2‴*R*,4‴*S*), (−)-**2.3** (2*S*,1‴*R*,2‴*S*,4‴*R*), (+)-**2.4** (2*R*,1‴*R*,2‴*S*,4‴*R*), (−)-**3.1** (2*S*,1‴*R*,2‴*R*,4‴*S*), and (+)-**3.2** (2*R*,1‴*R*,2‴*R*,4‴*S*). Two levels of theory were used in the DFT calculations, B3LYP/6-31G* and

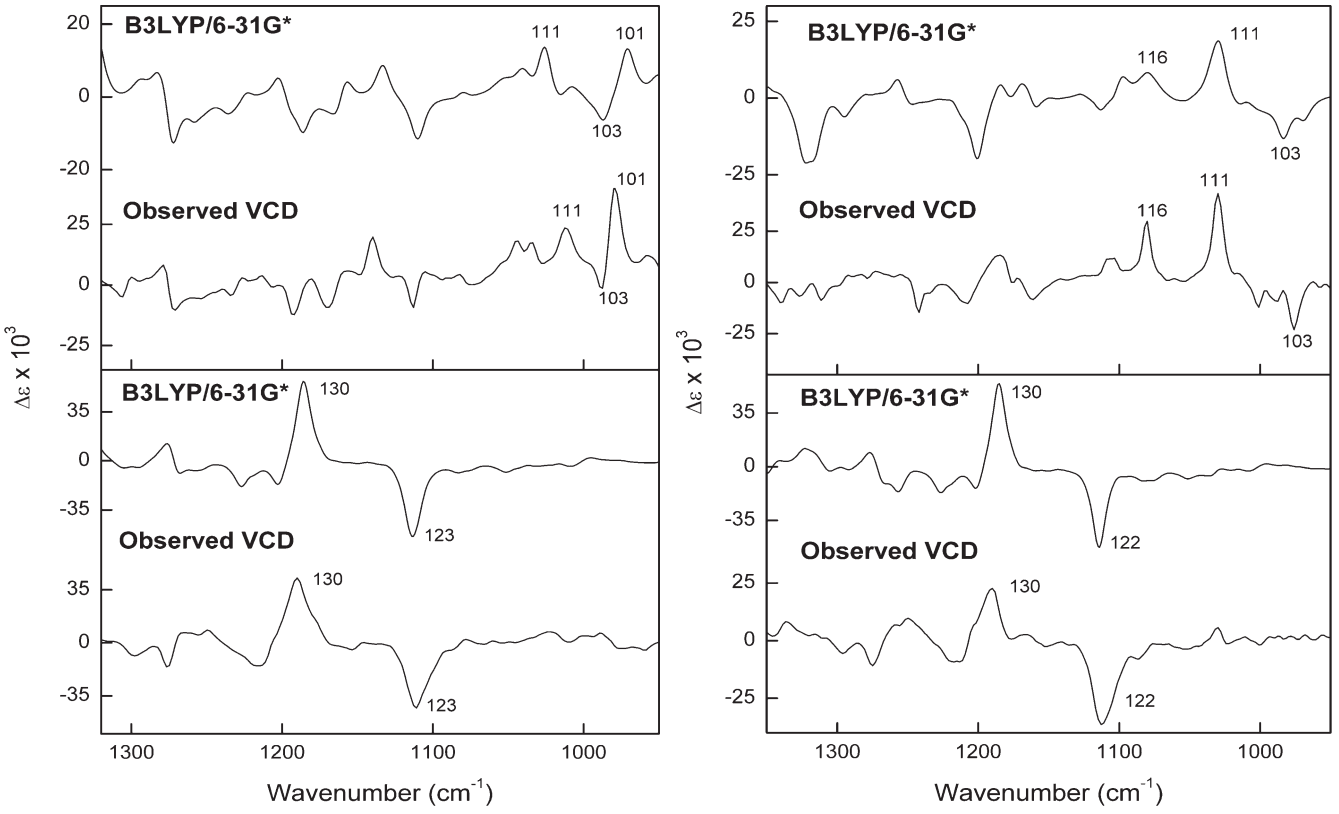

Figure 8. Left lower frame: observed difference VCD [(**2.2** − **2.1**)/2] versus calculated difference VCD [(2*R*,1‴*S*,2‴*R*,4‴*S* − 2*S*,1‴*S*,2‴*R*,4‴*S*)/2] for borneol derivatives. Left upper frame: observed average VCD [(**2.1** + **2.2**)/2] versus calculated average VCD [(2*S*,1‴*S*,2‴*R*,4‴*S* + 2*R*,1‴*S*,2‴*R*,4‴*S*)/2] for borneol derivatives. Right lower frame: observed difference VCD [(**3.2**-**3.1**)/2] versus calculated difference VCD [(2*R*,1‴*R*,2‴*R*,4‴*S* - 2*S*,1‴*R*,2‴*R*,4‴*S*)/2] for fenchol derivatives. Right upper frame: observed average VCD [(**3.1**+**3.2**)/2] versus calculated average VCD [(2*S*,1‴*R*,2‴*R*,4‴*S* + 2*R*,1‴*R*,2‴*R*,4‴*S*)/2] for fenchol derivatives.

B3PW91/TZVP, which gave very similar results including the same Confidence Level. As the calculations using the larger triple-ζ basis set took seven times longer when compared to the inexpensive 6-31G*, this work points out the advantage of using the latter for large molecules, as far as the accuracy-to-time ratio is concerned. Once again, VCD arises as a reliable, powerful methodology for the unambiguous assignment of absolute configuration directly in solution, without derivatization, without the requirement for the presence of UV−vis chromophores for electronic CD, and without the requirement of single crystals for X-ray analysis. The application of this methodology in natural products chemistry has increased over the past few years[21] and although chiral monoterpenes are usually used to test VCD devices, papers dealing with vibrational study of terpenoids are still scarce.[34]

EXPERIMENTAL SECTION

Plant Material, Extraction, and Isolation. Leaves and stems from *P. obtusifolia* A. Dieter were collected in Araraquara, SP, Brazil and identified by Dr. Inês Cordeiro (Instituto de Botânica, São Paulo, SP, Brazil). The voucher specimen (KATO 070) was deposited at the Herbário do Estado "Maria Eneyda P. Kaufmann Fidalgo" (São Paulo, SP, Brazil). Specimens were cultivated under greenhouse conditions at the Instituto de Química-UNESP, Araraquara.

The powdered air-dried leaves and stems (900 g) were extracted at room temperature with EtOH for 72 h. The crude EtOH extract (60 g) was dissolved in 200 mL of MeOH/H_2O (80:20, v/v) and partitioned using hexanes (Hex) and EtOAc (3 × 60 mL, each), which were later evaporated under reduced pressure. The Hex-soluble fraction was subjected to column chromatography (40 × 12 cm) over silica gel (0.063−0.200 mm) eluted with a gradient of Hex/EtOAc and EtOAc/MeOH yielding 12 fractions. Subfraction 3 (10 g) was then subjected to column chromatography (21 × 8 cm) over silica gel (0.063−0.200 mm) eluted with a gradient of 100−80% of Hex in EtOAc yielding 60 fractions. Subfractions eluted with 97% of Hex were pooled together (3 g) and submitted to solid phase extraction (SPE) column (10 × 5 cm), under reduced pressure, over C-18 silica gel, eluted with 100% of MeOH. The cleaned-up fraction (300 mg) was then successively subjected to silica gel coated preparative thin layer chromatography (TLC, 20 × 20 cm plates, 50 mg of sample each) developed in Hex/toluene (80:20, v/v) affording **2** (80 mg, R_f = 0.75) and **3** (30 mg, R_f = 0.6).

Both **2** and **3** were further subjected to normal phase semipreparative chiral HPLC using Chiralcel OD-H column (250 × 10 mm; 5 μm) with an isocratic elution of 100% *n*-hexane 95% over 30 min at a flow rate of 2.0 mL/min. Compound **2** afforded four compounds: **2.1** (30 mg, t_R 11.6 min), **2.2** (20 mg, t_R 13.3 min), **2.3** (6 mg, t_R 14.8 min) and **2.4** (7 mg, t_R 16.2 min). Compound **3**, on the other hand, afforded compounds with shorter retention time, **3.1** (6 mg, t_R 9.0 min) and **3.2** (7 mg, t_R 10.4 min).

(2*S*,1‴*S*,2‴*R*,4‴*S*)-Bornyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (2.1). Pale yellow oil; $[\alpha]^{25}_D$ −20 (*c*.1.0, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR data, see Table 1.

(2*R*,1‴*S*,2‴*R*,4‴*S*)-Bornyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (2.2). Pale yellow oil; $[\alpha]^{25}_D$ +10

(*c*0.9, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR data, see Table 1.

(2*S*,1‴*R*,2‴*S*,4‴*R*)-Bornyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (2.3). Pale yellow oil; $[\alpha]^{25}_D$ −9.0 (*c*0.4, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR spectra, see Supporting Information.

(2*R*,1‴*R*,2‴*S*,4‴*R*)-Bornyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (2.4). Pale yellow oil; $[\alpha]^{25}_D$ +19 (*c*0.5, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR spectra, see Supporting Information.

(2*S*,1‴*R*,2‴*R*,4‴*S*)-Fenchyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (3.1). Pale yellow oil; $[\alpha]^{25}_D$ −10 (*c*0.4, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR data, see Table 1.

(2*R*,1‴*R*,2‴*R*,4‴*S*)-Fenchyl-3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2*H*-1-benzopyran-6-carboxylate (3.2). Pale yellow oil; $[\alpha]^{25}_D$ +20 (*c*0.5, $CHCl_3$); UV (Hex) λ_{max} 228, 270, and 313 nm; HRESIMS $[M + H]^+$ *m*/*z* 507.3468 (calcd for $C_{33}H_{47}O_4$ 507.3474, Δ −0.6 mmu), ^1H and ^{13}C NMR data, see Table 1.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and molecular modeling; ^1H NMR, ^{13}C NMR, *g*COSY, *g*HMQC, *g*HMBC, and 1D NOESY spectra of **2.1**, **2.2**, **3.1**, and **3.2**; ^1H NMR spectra of **2.3** and **2.4**. HRESIMS and MS−MS spectra of **2.1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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