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Further monoterpene chromane esters from *Peperomia obtusifolia*: VCD determination of the absolute configuration of a new diastereomeric mixture

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ABSTRACT

A reinvestigation of the monoterpene chromane ester enriched fraction from *Peperomia obtusifolia* using chiral chromatography led to the identification of a minor peak, which was elucidated by NMR and HRMS as 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 same structure assigned to two other fenchyl esters described previously, pointing out a stereoisomeric relationship among them. Further NMR analysis revealed that it was actually a mixture of two compounds, whose absolute configurations were determined by VCD measurements. Although, almost no vibrational transitions could be assigned to the chiral chromane, the experimental VCD spectrum was largely opposite to that obtained for the average experimental VCD [(2*S*,1‴*R*,2‴*R*,4‴*S* + 2*R*,1‴*R*,2‴*R*,4‴*S*)/2] for fenchol derivatives. These results allowed us to assign the putative compounds as a racemic mixture of the chiral chromane esterified with the monoterpene (1*S*,2*S*,4*R*)-fenchol, which had not been identified in our early work.

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Introduction

Peperomia obtusifolia (Piperaceae) is a well known foliage plant which grows from Mexico to South America.[1] In spite of its main ornamental use, some Central American communities utilize this species to treat insect and snake bites or even as a skin cleanser.[2] Previous bioactivity-guided studies resulted in the isolation of racemic chromanes with potent trypanocidal activity and low unspecific cytotoxicity, among other secondary metabolites.[3,4] Moreover, a recent report from our research group[5] described the isolation, structure elucidation, and absolute configuration of six novel isomeric monoterpene chromane esters from *P. obtusifolia*. Four compounds esterified with borneol and two with fenchol were identified and their absolute configuration, involving four chiral centers, was unambiguously determined by using vibrational circular dichroism (VCD) and density functional theory (DFT) calculations. The finding of both enantiomers of the bornyl moiety, but only a sole enantiomeric form of the fenchyl moiety, that is, 1*R*,2*R*,4*S*, tethered to the chiral chromane prompted us to reinvestigate the chiral chromatogram of the chromane ester enriched fraction.

As a result, a minor unresolved peak was purified and elucidated as a novel diastereomeric mixture containing racemic proportion of the enantiomers of the chiral chromane 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 both esterified with the monoterpene (1*S*,2*S*,4*R*)-fenchol, which had not been identified in our early work. Therefore, we herein report the capability of VCD to determine the absolute configuration of complex natural products even in mixtures and without the aid of further DFT calculations. Moreover, the isolation of secondary metabolites with new stereochemical composition opens a new avenue in understanding the biosynthetic pathways involved in the formation of monoterpene chromane esters in *P. obtusifolia*.

Materials and methods

General

NMR spectra were obtained with a Varian INOVA NMR spectrometer at 500 MHz for ^{1}H using TMS as chemical shift reference. The HRESIMS spectra were obtained on a Bruker ultrOTOF-Q-ESI-TOF Mass Spectrometer. Analytical HPLC analyses were performed using stainless-steel Phenomenex Lux Cellulose-1 (250 × 4.6 mm,

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5 μm) column, as well as Daicel Chiralcel OD-RH (150 × 4.6 mm, 5 μm), Chiralcel OC-H (250 × 4.6 mm, 5 μm), and Chiralpak IC (250 × 4.6 mm, 5 μm) columns. Semi-preparative enantioseparations were performed using Daicel Chiralcel OD-H column (250 × 10 mm, 5 μm). Mobile phase for chromatography was prepared from HPLC grade *n*-hexane 95%, ethanol, methanol, and acetonitrile purchased from Tedia. Water was purified in-house with a Millipore Milli-Q system. HPLC separations were carried out using a Shimadzu LC-20AT pump system, a SIL-20A auto-sampler, and a SPD-M20A UV-PDA detector. The IR and VCD spectra were recorded with a modified BioTools Dual-PEM[6] ChiralIR™ FT-VCD spectrometer using a resolution of 4 cm^{-1} and a collection time of 11 h. The optimum retardation of the two ZnSe photoelastic modulators (PEMs) was set at 1400 cm^{-1}. An optical band-pass filter was used to allow only the spectral region 800–2000 cm^{-1} to reach the detector. Spectra were calibrated automatically, using the standard calibration files. VCD spectra were recorded in $CDCl_3$ solution (3.0 mg in 100 μL of $CDCl_3$ in a BaF_2 cell with 100 μm path length). Baseline offsets were eliminated by subtracting the VCD spectrum of the sample from that obtained for the solvent under identical conditions.

Plant material, extraction, and isolation

Leaves and stems from *Peperomia obtusifolia* A. Dietr. (Piperaceae) 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 Institute of Chemistry – UNESP in Araraquara, SP, Brazil. The procedure for obtaining the monoterpene chromane ester enriched fraction was previously described.[5] This fraction (2.0 mg/mL in *n*-hexane) was then successively subjected to normal phase semi-preparative chiral HPLC using Chiralcel OD-H column (250 × 10 mm; 5 μm) with isocratic elution of 100% *n*-hexane 95% over 30 min at a flow rate of 2.0 mL/min and with injection volume of 250 μL. The peak eluting at t_R = 11.7 min corresponded to the novel diastereomeric mixture (**1**).

Results and discussion

The reinvestigation of the monoterpene chromane ester enriched fraction using chiral chromatography resulted in the identification of a minor peak (**1**) with shorter retention time when compared with the other compounds previously isolated (Fig. 1). Peaks 2 and 3 in the chromatogram presented in Figure 1 correspond to (−)-(2*S*,1‴*R*,2‴*R*,4‴*S*)- and (+)-(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, respectively, while the unnumbered peaks from 17 to 27 min correspond to (−)-(2*S*,1‴*S*,2‴*R*,4‴*S*)- and (+)-(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 along with their enantiomers. Peak 1 was then further purified and yielded an identical UV spectrum and the same HRESIMS ($[M+H]^+$ obsd *m*/*z* 507.3468, Calcd for $C_{33}H_{47}O_4$ *m*/*z* 507.3474; $[M+Na]^+$ obsd *m*/*z* 529.3324, Calcd 529.3293) as the six monoterpene chromane esters recently reported.[5] Additionally, the subsequent fragmentation by MS–MS of the *quasi*-molecular ion $[M+H]^+$ = 507.3468 gave rise to the fragment ions *m*/*z* 371.2232 and 353.2133 indicating the loss of the monoterpene moiety followed by the loss of a water molecule. The ^1H NMR data were compared with literature values and confirmed the new structure as 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 (**1**), the same structure assigned to the other fenchol derivatives[5] described recently, pointing out a stereoisomeric relationship among them. However, from the ^1H NMR spectrum it was possible to observe some duplicated signals, particularly in the region from 5.4 to 6.3 ppm where three hydrogen resonances (H-1′, H-2′, and H-3′), which arise from the diene directly bound to the stereogenic center at C-2, are lo-

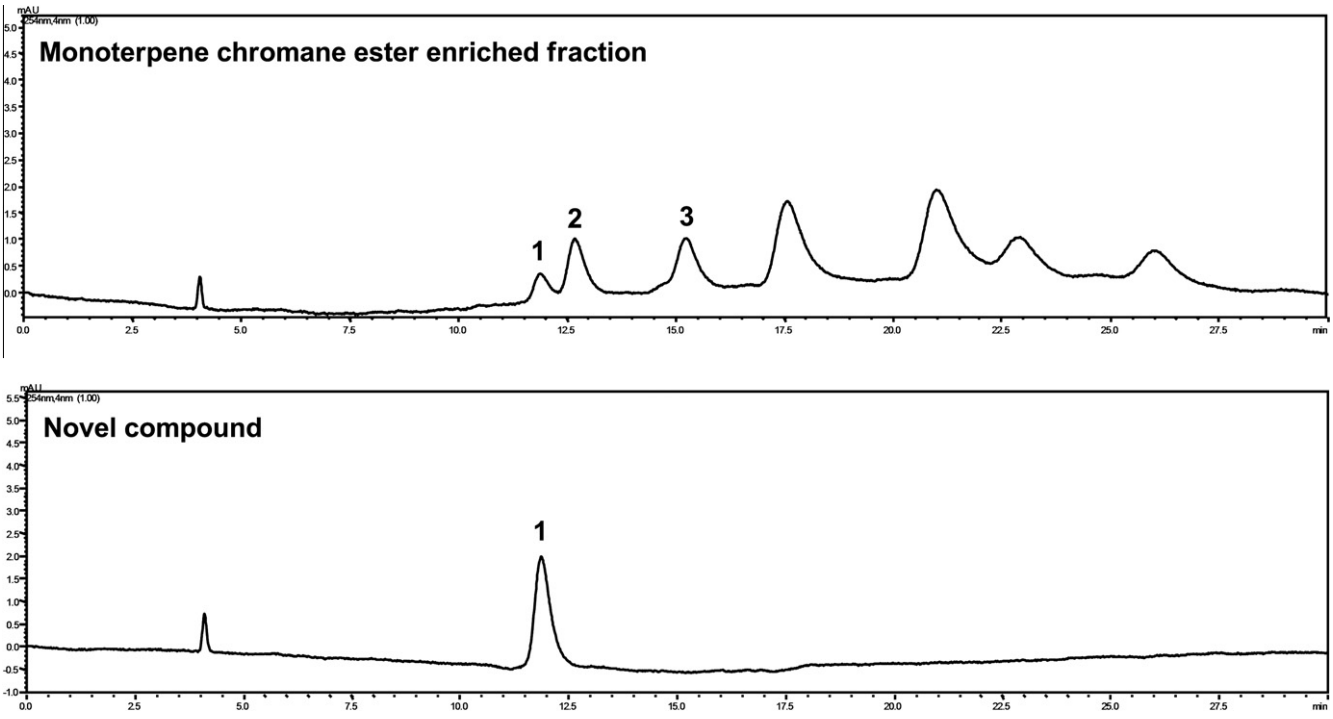

Figure 1. Analytical chiral chromatograms of the monoterpene chromane ester enriched fraction and the novel compound **1**. Conditions: Phenomenex Lux Cellulose-1 (250 × 4.6 mm, 5 μm) column, isocratic elution using 100% *n*-hexane 95%, flow rate of 0.8 mL/min, and UV detection at 254 nm.

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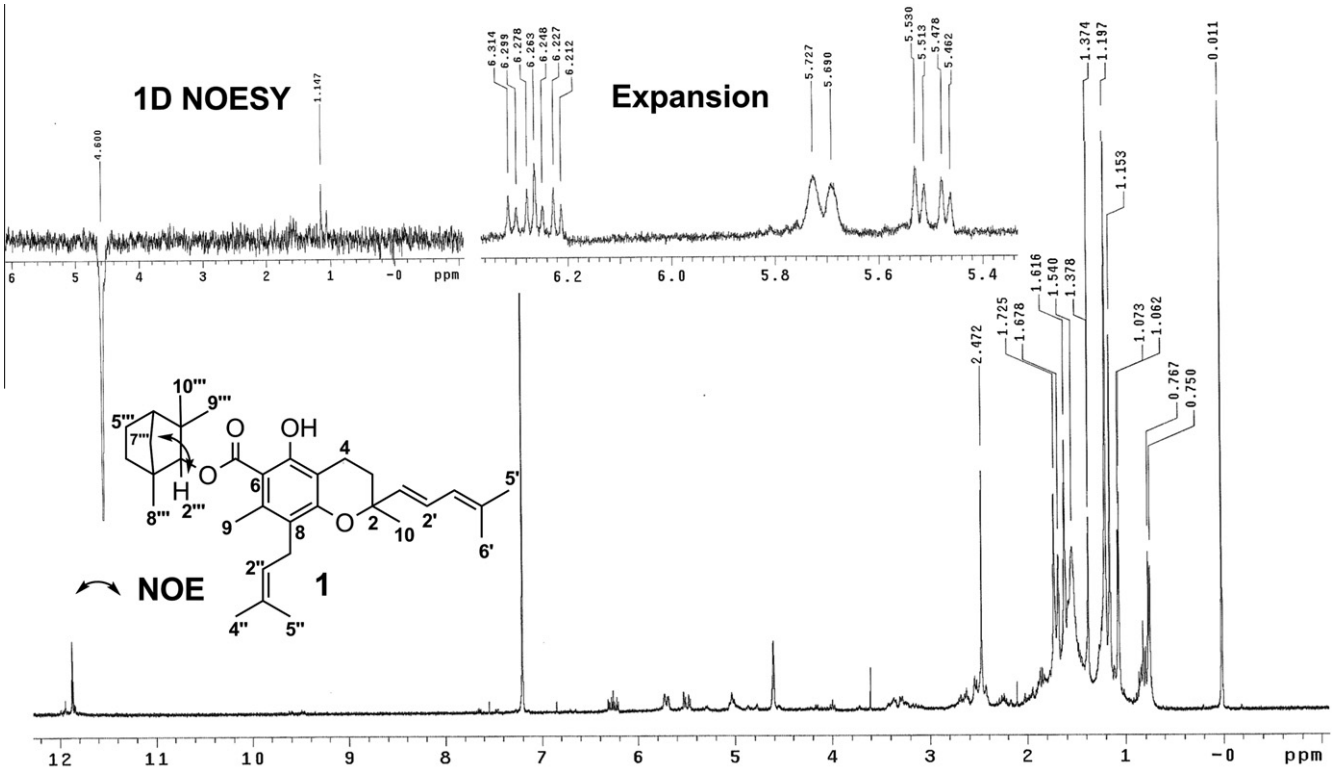

Figure 2. ^{1}H NMR and 1D NOESY spectra of compound **1**.

cated (Fig. 2). This finding could indicate the presence of a mixture of compounds rather than a single isomer. Further chromatographic studies using distinct chiral stationary phases were then carried out in an effort to resolve the putative mixture **1**. However, none of the four different commercial chiral columns employed, namely, Lux Cellulose-1, Chiralcel OD-RH, Chiralcel OC-H, and Chiralpak IC, was able to separate it. The analysis of the 1D NOESY spectrum of 1 demonstrated it to have H-2‴ as exo and the chromane group at the endo side of the fenchyl moiety, the same relative configuration assigned to the other two fenchyl esters isolated from *P. obtusifolia*. Upon irradiation of H-2‴, a NOE enhancement was observed for H-7‴ (δ 1.14) (Fig. 2).

In order to assess the absolute configuration of **1**, IR and VCD measurements (Figs. 3 and 4) were carried out. From the IR spectrum we could see basically the same vibrational transitions (975, 1040, 1090, 1200, 1275, 1350, 1400, 1450, 1650, and 1725 cm^{-1}) as those observed in the unpolarized absorption spectra for the other isomers that corroborate the structure assigned. From the VCD spectrum, however, almost no vibrational transitions could be assigned to the chiral chromane. Only those transitions previously designated as markers for the monoterpene stereochemistry were predominantly observed, namely, 975, 1040, and 1090 cm^{-1}. More interestingly, the experimental VCD spectrum of **1** was largely opposite to that obtained for the average experimental VCD [(2*S*,1‴*R*,2‴*R*,4‴*S* + 2*R*,1‴*R*,2‴*R*,4‴*S*)/2] for the fenchol derivatives **2** and **3**, respectively, described in our early work[5] (Fig. 4). Compounds **2** and **3** shared the same absolute configuration within the fenchyl moiety, but presented inverted configurations for the chiral chromane. Upon averaging their experimental spectra one gets rid of the signals with opposite sign, which come predominantly from the vibrational transitions of the enantiomeric chromanes and ends up with a spectrum reflecting most of the transitions of the chiral terpene, despite some overlapped normal modes.

These results allowed us to assign the structure of **1** as a racemic mixture of the chiral chromane esterified with the monoterpene (1*S*,2*S*,4*R*)-fenchol, which had not been described in our previous work. Additionally, this is the first time these two compounds are described in nature. As advocated in our former paper,[5] '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', and this statement is clearly demonstrated herein. Nevertheless, it is important to stress that despite the relative success of this approach, any assignment of absolute configuration involving comparison of experimental VCD data only is highly dependent on the correct absolute configuration of the model compound.

Even though this is not the first example of VCD spectral arithmetic operations[5,7] used to solve stereochemical problems, this

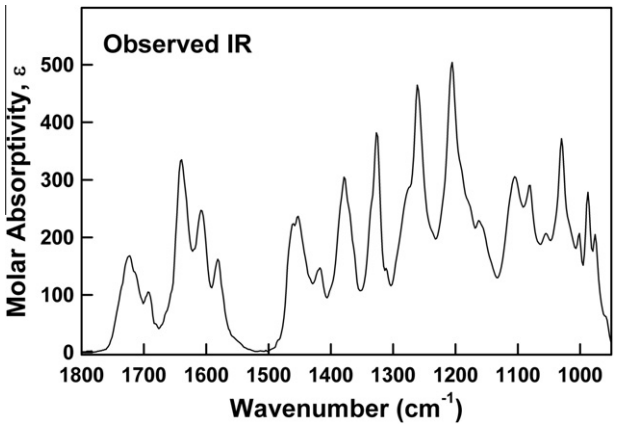

Figure 3. Observed IR spectrum of **1**.

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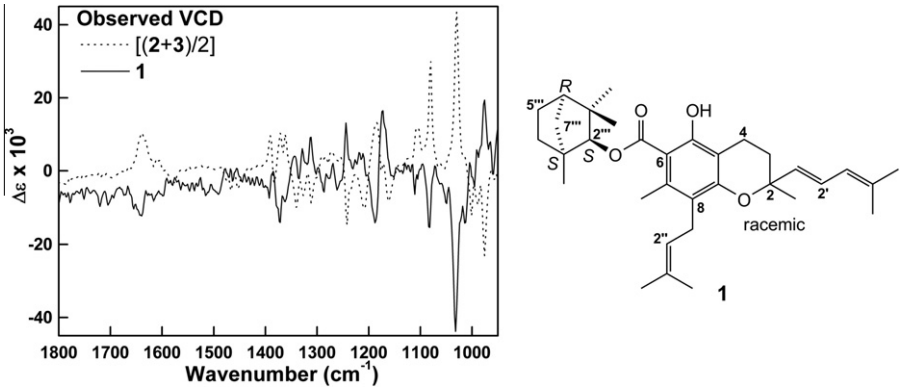

Figure 4. On the left: comparison of the observed VCD for **1** with the average experimental VCD [(2*S*,1‴*R*,2‴*R*,4‴*S* + 2*R*,1‴*R*,2‴*R*,4‴*S*)/2] for the fenchol derivatives **2** and **3**. On the right: chemical structure and absolute configuration of the novel diastereomeric mixture **1**.

work demonstrates the potentiality of VCD to determine unambiguously the absolute configuration of natural product molecules with multiple chiral centers even in mixtures. Particularly for this case, none of the classical approaches such as X-ray crystallography, NMR, and electronic circular dichroism (ECD) could be used to tackle this stereochemical challenge. In particular, ECD would be of no value since for all the other monoterpene chromane esters their ECD spectra were identical to that obtained for the chiral chromane alone. Besides this fact, for the mixture described in this work the chromane chromophore is racemic and, as expected, yielded an null ECD spectrum.

Finally, the stereochemistry of the secondary metabolites provides some insights about their biosynthesis. From this work we learn that *P. obtusifolia* biosynthesizes both enantiomers of the chiral chromane and both enantiomers of fenchol, probably as racemates, nevertheless attaches them stereoselectively. The presence of a racemic chromane may indicate the lack of enzymatic control in the final ring closure step, while racemic monoterpenes may indicate two separate enzyme systems each capable of producing a single enantiomer.[8] Further biosynthetic and proteomic studies on *P. obtusifolia* are under way.

Conclusion

Analysis of the IR and VCD spectra of an unresolved chiral chromatography peak allowed the assignment of its structure and absolute configuration, directly in solution, without derivatization, and without the aid of further DFT calculations. Such a peak was characterized as a diastereomeric mixture of two novel monoterpene chromane esters with (*rac*-2,1‴*S*,2‴*S*,4‴*R*) absolute configuration. It is also noteworthy that these two compounds are the enantiomers of **2** and **3**, both described earlier. This work expands our knowledge about composition and stereochemistry of monoterpene chromane esters within *P. obtusifolia* and provides another example of how powerful and versatile vibrational optical activity can be for the stereochemical analysis of complex chiral natural product molecules even in mixtures.

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References and notes

1. Tanaka, T.; Asai, F.; Iinuma, M. *Phytochemistry* **1998**, *49*, 229–232.
2. Lentz, D. L.; Clark, A. M.; Hufford, C. D.; Meurer-Grimes, B.; Passreiter, C. M.; Cordero, J.; Ibrahimi, O.; Okunade, A. L. *J. Ethnopharmacol.* **1998**, *63*, 253–263.
3. Mota, J. S.; Leite, A. C.; Batista, J. M.; López, S. N.; Ambrósio, D. L.; Passerini, G. D.; Kato, M. J.; Bolzani, V. S.; Cicarelli, R. M. B.; Furlan, M. *Planta Med.* **2009**, *75*, 620–623.
4. Batista, J. M.; López, S. N.; Mota, J. S.; Vanzolini, K. L.; Cass, Q. B.; Rinaldo, D.; Vilegas, W.; Bolzani, V. S.; Kato, M. J.; Furlan, M. *Chirality* **2009**, *21*, 799–801.
5. Batista, J. M.; Batista, A. N. L.; Mota, J. S.; Cass, Q. B.; Kato, M. J.; Bolzani, V. S.; Freedman, T. B.; López, S. N.; Furlan, M.; Nafie, L. A. *J. Org. Chem.* **2011**, *76*, 2603–2612.
6. Nafie, L. A. *Appl. Spectrosc.* **2000**, *54*, 1634–1645.
7. Cichewicz, R. H.; Clifford, L. J.; Lassen, P. R.; Cao, X.; Freedman, T. B.; Nafie, L. A.; Deschamps, J. D.; Kenyon, V. A.; Flanary, J. R.; Holman, T. R.; Crews, P. *Bioorg. Med. Chem.* **2005**, *13*, 5600–5612.
8. Dewick, P. M. *Medicinal Natural Products—A Biosynthetic Approach*; John Wiley & Sons: West Sussex, 2009.