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Short Report

Meroterpenes from *Peperomia oreophila* Hensch. and *Peperomia arifolia* Miq.

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Um novo meroterpeno, 4-[(2'*E*)-3',7'-dimetilocta-2',6'-dienil]-5-metil-2-(3''-metilbut-2''-enil)-benzeno-1,3-diol, além de oito substâncias conhecidas, foi isolado do extrato em MeOH das folhas de *Peperomia oreophila* Hesch. O fenol prenilado foi também isolado como principal componente do extrato em CH_2Cl_2:MeOH das folhas de *Peperomia arifolia* Miq. As estruturas das substâncias foram estabelecidas com base em dados espectrais e comparação com dados descritos na literatura.

One new meroterpene, 4-[(2'*E*)-3',7'-dimethylocta-2',6'-dien-1'-yl]-5-methyl-2-(3''-methylbut-2''-enyl)-benzene-1,3-diol, together with eight known compounds, was isolated from the MeOH extract from the leaves of *Peperomia oreophila* Hesch. The prenylated phenol was also isolated as main compound from the CH_2Cl_2:MeOH extract from leaves of *Peperomia arifolia* Miq. The structures of the substances were established on the basis of the spectral evidences and supported by literature data.

Keyword: *Peperomia arifolia*, *Peperomia oreophila*, polyketides, meroterpenes

Introduction

The species *Peperomia oreophila* Hensch. and *Peperomia arifolia* Miq. belong to the Piperaceae family in which the genus *Piper* (*ca.* 2000 species) and *Peperomia* (*ca.* 1500-1700 species) are the most abundant.[1] Comparatively, most of the phytochemical studies have been addressed to the *Piper* species while in the case of *Peperomia* only few species have been subjected to chemical or pharmacological scrutiny. The volatile compounds from several other *Peperomia* species were investigated by chromatography-mass spectrometry (GC-MS).[2,3] Nevertheless, in the case of *Peperomia oreophila* Hesch., two rare sesquiterpenes having the ishwarane skeleton were isolated from its essential oil.[4] Indeed, the chemical variability of *Peperomia* species became evident with the isolation of amides, benzoic acid/chromenes, flavonoids, lignoids and phenylpropanoids.[5-8] Additionally, the meroterpenes appear to be a noteworthy class of compounds among *Peperomia* species with the aromatic moiety resulting from orsellinic acid with a variable degree of prenylations such as those described from *P. obtusifolia*,[9,10] *P. galioides*,[11] *P. blanda*,[12] benzopyrans from *P. clusiifolia*,[13] *P. amplexicaulis*,[14] prenylated quinones from *P. galioides*,[15] and chromenes from *P. serpens*[16] and *P. villipetiola*.[6]

As a part of our continuing investigation aiming at the chemotaxonomic study of *Peperomia* species, *Peperomia oreophila* was selected due to the richness of signals in the ^{1}H nuclear magnetic resonance (NMR) spectrum. The phytochemical investigation led to the isolation of a new phenol (**1**), in addition to eight known compounds (**2**-**9**). The species *Peperomia arifolia* Miq. was also included in this study due to the occurrence of the meroterpene **1** in the extract from the leaves as the major compound.

Results and Discussion

The CH_2Cl_2:MeOH (2:1) extract from the leaves of *P. arifolia* Miq. was fractionated by column chromatography on Sephadex LH-20 to afford the new meroterpene **1** as the major compound (Figure 1). This new compound was also isolated from the MeOH extract from the

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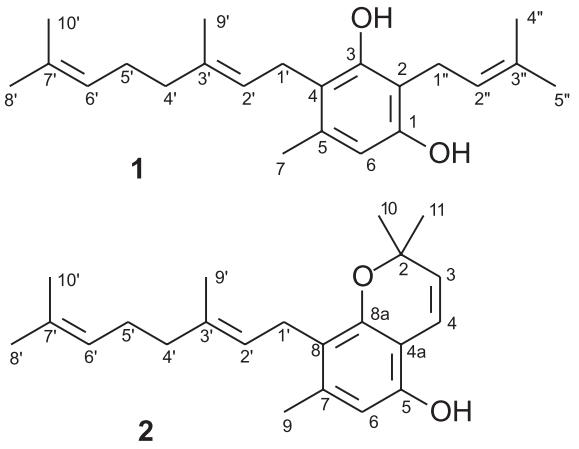

Figure 1. Compounds isolated from *Peperomia arifolia* Miq. (**1**) and from *Peperomia oreophila* Hensch. (**1** and **2**).

leaves of *P. oreophila*. The EtOAc phase, which was obtained from the partition of the crude extract, was fractionated by Sephadex LH-20 followed by silica prep. TLC (thin layer chromatography) to yield additional eight known compounds: the prenylated chromene 8-[(2'*E*)-3',7'-dimethylocta-2',6'-dien-1'-yl]-2,2,7-trimethyl-2*H*-chromen-5-ol (**2**),[16] two furofuran lignans (7*R*,8*R*,7'*R*,8'*R*)-3',4',5'-trimethoxy-3,4-methylenedioxy-8'.8-7'.O.9-9'.O.7-lignan (**3**) and (7',8'*R*,7*R*,8)-3',4',5'-trimethoxy-3,4,5-trimethoxy-8'.8-7'.O.9-9'.O.7-lignan (**4**),[17] three cinnamic acid derivatives methyl (2'*E*)-3'-(3,4,5-trimethoxyphenyl)acrylate (**5**), methyl (2'*Z*)-3'-(3,4,5-trimethoxyphenyl)acrylate (**6**)[18,19] and methyl (2'*E*)-3'-(5-methoxy-7,8-benzodioxol-1-yl)acrylate (**7**),[8] and two amides (2'*E*)-*N*-isobutyl-3'-(5-methoxy-7,8-benzodioxol-1-yl)acrylamide (**8**) and (2'*E*)-*N*-isobutyl-3'-(3,4,5-trimethoxyphenyl)acrylamide (**9**).[20]

Compound **1** was isolated as a brown oil with the molecular formula $C_{22}H_{32}O_2$ as indicated by the *quasi*-molecular ion at *m/z* 329.2485 $[M + H]^+$ in its HRESIMS (high resolution electron spray ionization mass spectrum). Its infrared (IR) spectrum showed absorption bands at 3449, 2967-2857, 1621 and 1450 cm^{-1} indicative of hydroxyl, methine, methylene, methyl and aromatic groups, respectively. The ^1H NMR displayed two signals at δ 6.26 (s) and 2.20 (s) assigned to aromatic hydrogen and to an aromatic methyl group, respectively. The spectrum also showed a set of characteristic signals of a prenyl group: two hydrogen at δ 3.28 (d, *J* 6.6 Hz) coupled with hydrogen at δ 5.13 (t, *J* 6.6 Hz) and, additionally, two methyl groups at δ 1.81 and 1.73 (s). A second set of signals was observed in this spectrum: two hydrogens at δ 3.39 (d, *J* 7.0 Hz) coupled with hydrogen at δ 5.25 (t, *J* 7.0 Hz), in addition to two multiplets at δ 2.05 and 2.09 (2H each) and three methyl groups at δ 1.80, 1.58 and 1.67 (s), characteristic of a geranyl group. The assignment of the prenyl and geranyl groups was supported either by coupling constants or by HMBC (heteronuclear multiple bond correlation) data, and the ^1H NMR data as a whole indicated that **1** has a similar structure to that of piperogalin (a prenylated phenol previously isolated from *P. galioides*).[11] Nevertheless, in spite of the similarities between ^1H and ^{13}C chemical shifts assigned to benzylic groups (C1' and C1"), significant differences were observed in the chemical shifts assigned to C5, C3' and C3''. In order to clarify this aspect, the HMBC experiment was carried out and the correlations observed from H1' to C3, C4 and C2', from H1" to C2, C3, C2" and C3" and from H7 to C4, C5 and C6 allowed the placement of methyl, prenyl and geranyl groups at C5, C2 and C4, respectively (Figure 2). Additional correlations from H6 to C1, C2, C4 and C7, and from O<u>H</u> to C2, C3 and C4 supported the placement of the aromatic hydrogen at C6 and of the O<u>H</u> at C3. Further confirmation for this substitution pattern on the aromatic ring was made using the NOESY (nuclear Overhauser effect spectroscopy) experiment (Figure 2). The compound **1** was thus deduced to be the new 4-[(2'*E*)-3',7'-dimethylocta-2',6'-dien-1'-yl]-5-methyl-2-(3"-methylbut-2"-enyl)benzene-1,3-diol, an isomer of piperogalin (Figure 1).

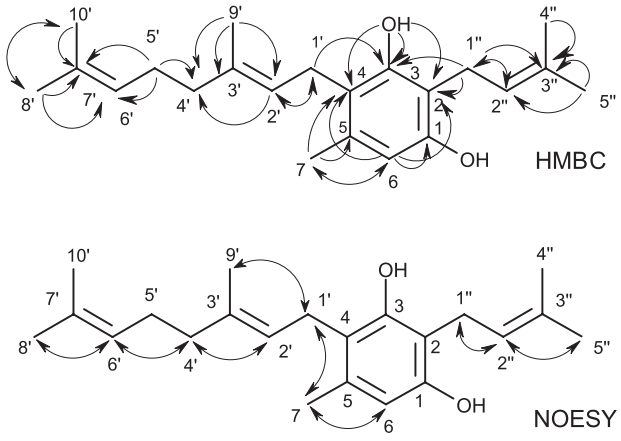

Figure 2. Key long-range correlations observed in the HMBC and NOESY of compound **1**.

Conclusions

The occurrence of the new phenol **1** (the chromene **2** in *P. oreophila*), as well of **1** in *P. arifolia* together with previous chemical studies made on *Peperomia* species,[5,9,11-15] suggests that these meroterpenes derived from orsellinic acid could be used as taxonomic markers for *Peperomia* species. The occurrence of **1** and **2** in these species indicates a specific biosynthetic pathway with regioselectivity at the prenylation and geranylation steps.

Experimental

General procedures

IR spectra were recorded on a Bomen MB-100 spectrometer. ^{1}H NMR (300 and 500 MHz), ^{13}C NMR (75 and 125 MHz), HMQC (heteronuclear multiple quantum coherence, 300 and 75 MHz), NOESY (125 MHz) and HMBC (500 and 125 MHz) spectra were measured in $CDCl_3$ on Bruker DPX300 and DRX500 instruments using TMS (tetramethylsilane) as the internal standard. LREIMS (low resolution electron impact mass spectrometry, 70 eV) spectra were obtained on Shimadzu QP-5050 spectrometer. HRESIMS and LRESIMS spectra were recorded on Bruker microTOF-QII and on Quattro II triple quadrupole equipment, respectively. HPLC (high performance liquid chromatographic) analysis were performed on a Shimadzu LC20A coupled to SPD20A detector or Bruker microTOF-QII mass spectrometer using a Phenomenex Luna C_{18} and MeOH:H_2O 3:2 (1% formic acid) to 1:0 (30 min) as eluent (at a flow rate of 0.5 mL min^{-1} with a delivery of 0.2 mL min^{-1} for mass spectrometer using a flow splitter). The chromatographic separations were based on gel filtration through Sephadex LH-20 and on prep. TLC over silica gel 60 F_{254} or 60 PF_{254} (Merck), the spots being visualized under a UV lamp (at 254 and/or 366 nm).

Plant material

Whole specimens of *Peperomia arifolia* Miq. were collected in Brotas County (São Paulo State, Brazil) in April of 2004, while *P. oreophila* Hensch specimens were collected in Serra da Piedade (Minas Gerais State, Brazil) in June of 2004. Plants were identified by Dr. Elsie Guimarães (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro). Voucher specimens of *P. arifolia* Miq. (Kato-395) and of *P. oreophila* Hensch (Kato-418) were deposited therein.

Extraction and isolation

Peperomia arifolia Miq.

Dried leaves (620 mg) were ground and extracted with CH_2Cl_2:MeOH (2:1) (2 × 100 mL, 12 h) at room temperature. The solutions were concentrated under vacuum yielding a crude extract (95 mg). The extract was chromatographed by Sephadex LH-20 using a gradient elution with *n*-hexane-CH_2Cl_2/CH_2Cl_2-Me_2CO mixtures to give 20 fractions. These fractions were pooled based on their similarities in TLC analysis to yield 5 groups (1-5). Groups 1 and 2 were constituted by fatty material while groups 3-4 (29 mg) were submitted to a second Sephadex LH-20 column, being eluted with *n*-hexane-CH_2Cl_2/CH_2Cl_2-Me_2CO mixtures to yield **1** (16 mg).

Peperomia oreophila Hensch.

Dried and powdered leaves (38.6 g) of plants were extracted with MeOH (4 × 600 mL, 12 h) at room temperature. The combined extracts were concentrated under vacuum resulting in a dark greenish and gummy crude material (4.6 g). Part of this extract (600 mg) was partitioned between EtOAc/H_2O. The organic fraction (after drying with Na_2SO_4 anhydrous) was concentrated and yielded 288 mg. The EtOAc fraction was chromatographed by Sephadex LH-20 using a gradient elution with *n*-hexane-CH_2Cl_2/CH_2Cl_2-Me_2CO mixtures yielding 28 fractions that were pooled on the basis of TLC analysis to F_1-F_7. F_1 (88.6 mg) was subjected to prep. TLC silica gel (*n*-hexane:EtOAc; 4:1) yielding **3** (58 mg) and **4** (21 mg). F_2 (14.2 mg) was subjected to silica gel prep. TLC (*n*-hexane:EtOAc; 4:1) and yielded **3** (3.8 mg), **4** (2.0 mg) and **5/6** (8.0 mg). Fraction F_3 (23 mg) was subjected to silica gel prep. TLC (*n*-hexane:EtOAc; 4:1) yielding **7** (5.4 mg), **8** (8.8 mg) and **9** (8.6 mg). F_4 (9.6 mg) yielded **1**, F_5 (45.4 mg) yielded **1** plus **2** and F_6 yielded **2** (6.8 mg).

4-[(2'*E*)-3',7'-Dimethylocta-2',6'-dien-1'-yl]-5-methyl-2-(3''-methylbut-2''-enyl)benzene-1,3-diol (**1**)

Brown oil; UV (MeOH) λ_{max}/nm (log ε) 208 (57.34) and 283 (3.38); IR (KBr) ν_{max}/cm^{-1} 3449, 2989, 2923, 2857, 1622, 1451, 1378, 1331, 1170, 1070; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.26 (s, H-6), 2.20 (s, H-7), 5.38 (br s, OH-3), 5.25 (t, *J* 7.0 Hz, H-2''), 5.13 (t, *J* 6.6 Hz, H-2'), 5.05 m (m, H-6'), 3.39 (d, *J* 7.0 Hz, H-1''), 3.28 (d, *J* 6.6 Hz, H-1'), 2.05 (m, H-4'), 2.09 (m, H-5'), 1.81 (s, H-4''), 1.80 (d, *J* 0.9 Hz, H-9'), 1.73 (s, H-5''), 1.67 (s, 8'), 1.58 s (s, H-10'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.6 (C-1), 153.5 (C-3), 137.5 (3'), 135.2 (C-5), 134.5 (3''), 131.8 (7'), 123.9 (6'), 122.5 (2'), 122.2 (C-2''), 118.0 (C-4), 111.6 (C-2), 109.7 (C-6), 39.6 (C-4'), 26.4 (C-5'), 25.7 (C-8', C-5''), 25.6 (C-1'), 22.7 (1''), 19.8 (C-7), 17.8 (10'), 17.6 (C-4''), 16.1 (C-9'); HMBC and NOESY, see Figure 2. HRESIMS *m/z* 329.2485 Da $[M+H]^+$; calculated *m/z* 329.4962; LREIMS *m/z* (rel. int.): 328 $[M]^+$ (18), 205 $[M-C_9H_{15}]^+$ (62), 203 (100), 189 (64), 149 (92).

8-[(2'*E*)-3',7'-Dimethylocta-2',6'-dien-1'-yl]-2,2,7-trimethyl-2*H*-chromen-5-ol (**2**)

LRESIMS, HRESIMS, ^{1}H and ^{13}C NMR data are similar to that previously described.[16]

Supplementary Information

Supplementary information are available free of charge at http://jbcs.sbq.org.br as PDF file.

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