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Chromenes from *Peperomia serpens* (Sw.) Loudon (Piperaceae)

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Abstract

Chromatographic separation of the CH_2Cl_2 extract from leaves of *Peperomia serpens* yielded two chromenes [5-hydroxy-8-(3′,7′-dimethylocta-2′,6′-dienyl)-2,2,7-trimethyl-2*H*-1-chromene (**1**) and 5-hydroxy-8-(3′-methyl-2′-butenyl)-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylic acid (**2**)], besides the known chromene [methyl 5-hydroxy-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylate (**3**)] and the flavonoid, dihydrooroxylin (**4**). Their structural elucidation were achieved by spectroscopic analyses. The antifungal activities of the CH_2Cl_2 extract and the isolated chromenes were measured bioautographically against *Cladosporium cladosporioides* and *C. sphaerospermum*, when it was found that the crude extract showed higher activity as compared to the pure compounds.

Keywords: *Peperomia serpens*; Piperaceae; Chromenes

1. Introduction

The genus *Peperomia* is the second largest in Piperaceae, and consists of about 1000 herbaceous species (Lei and Liang, 1999). Despite the large number of *Peperomia* species only a few chemical studies on them have been carried out. Two major classes of aromatic compounds have been reported which include the secolignans from *Peperomia dindigulensis* (Govindachari et al., 1998), *P. glabela* (Monache and Compagnone, 1996) and *P. pellucida* (Bayma et al., 2000) as well as prenylated phenolic compounds of polyketide origin from *P. clusiifolia* (Seeram et al., 1998), *P. vulcanica* (Mbah et al., 2002) and *P. obtusifolia* (Tanaka et al., 1998). In the case of *P. sui* (Cheng et al., 2003) and *P. villipetiola* (Salazar et al., 2005) significant cytotoxicity and antifungal potential was observed for their phenolic compounds.

The CH_2Cl_2 extract from leaves of *P. serpens* showed significant antifungal potential (in a bioautographic assay) against *Cladosporium cladosporioides* and *C. sphaerospermum*. Chromatographic separation of this extract led to isolation of two new chromenes: 5-hydroxy-8-(3′,7′-dimethylocta-2′,6′-dienyl)-2,2,7-trimethyl-2*H*-1-chromene (**1**) and 5-hydroxy-8-(3′-methyl-2′-butenyl)-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylic acid (**2**) along with two known compounds [methyl 5-hydroxy-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylate (**3**) and dihydrooroxylin (**4**)] (Salazar et al., 2005; Agrawal, 1989). This work describes the isolation, structural elucidation and antifungal potential of the isolated metabolites.

2. Results and discussion

The bioactive CH_2Cl_2 extract from leaves of *P. serpens* was subjected to chromatographic separation procedures on silica gel and Sephadex LH-20 to afford two new chromenes **1** and **2**, along with the known compounds methyl 5-hydroxy-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylate (**3**) and dihydrooroxylin (**4**) (Salazar et al., 2005; Agrawal, 1989).

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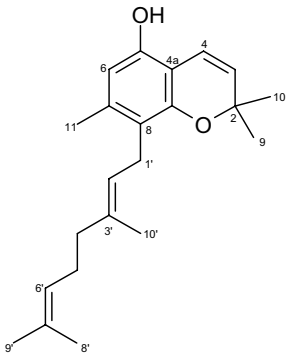

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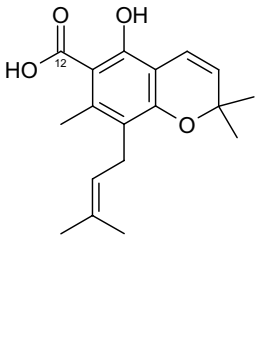

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Compound **1** was obtained as a colorless oil. The LRE-IMS showed a molecular ion peak at *m/z* 326, which in association with its ^{13}C NMR spectroscopic data and elemental analysis, was compatible with the molecular formula $C_{22}H_{30}O_2$. Its IR spectrum indicated the presence of an hydroxyl group at 3444 cm^{-1} and an aromatic ring at 1452 cm^{-1}. The ^1H NMR spectrum of **1** showed an AB system at δ 5.52 (*d*, $J = 10$ Hz, 1H) and δ 6.57 (*d*, $J = 10$ Hz, 1H), an intense singlet at δ 1.39 (6H), referring to two methyl groups, one aromatic hydrogen at δ 6.13 (*s*, 1H), and one singlet at 2.17 (3H), characteristic of an aromatic methyl group (Tanaka et al., 1998). These data suggested a polyketide chromene skeleton with a penta-substituted aromatic ring similar to those previously isolated from *P. villipetiola* (Salazar et al., 2005). Additional signals at δ 1.56 (*s*, 3H), 1.64 (*s*, 3H), 1.75 (*s*, 3H), at δ 1.9–3.2 (*m*) and at δ 5.05 (*m*) could be assigned to a geranyl moiety linked to an aromatic ring. The ^{13}C NMR spectra (BBD and DEPT 135°) showed signals corresponding to a carbinolic carbon at δ 76.6 (C), two methine sp^2 carbons at δ 128.1 and 116.7, two equivalent methyl groups at δ 27.6, one methyl group bearing an aromatic ring at δ 19.7 and six aromatic carbons at δ 107.3 (C), 109.0 (CH), 120.6 (C), 137.7 (C), 148.9 (C) and 151.3 (C), with the latter two signals indicative of oxygenated sp^2 carbons. The remaining resonances, as shown in Table 1, could be assigned to a geranyl moiety in agreement with the literature data (Tanaka et al., 1998). Analyses of the HMBC spectra showed a ^1H-^{13}C long-range coupling between H-6 (6.13) and C-4a (107.3), C-5 (148.9), C-8 (120.6) and C-11 (19.7), between H-1′ (3.23) and C-7 (137.7), C-8 (120.6), C-8a (151.3), C-2′ (123.1) and C-3′ (134.0) thereby positioning the methyl group at C-7 and the geranyl moiety at C-8. Strong correlations were also observed between H-11 (2.17) and C-6 (109.0), C-7 (137.7) and C-8 (120.6). Thus, the structure of chromene **1** was deduced as 5-hydroxy-8-(3′,7′-dimethyl-octa-2′,6′-dienyl)-2,2,7-trimethyl-2*H*-1-chromene.

Compound **2** had a molecular formula of $C_{18}H_{22}O_4$ as indicated by analysis of LRESIMS which showed the $[M + H]^+$ peak at *m/z* 303 and which was subsequently supported by elemental analysis. The IR spectrum exhibited bands assignable to an hydroxyl group (3393 cm^{-1}), a chelated carboxyl group (1648 cm^{-1}) and an aromatic ring (1593 cm^{-1}). The ^1H NMR spectrum of **2** (Table 1) showed two doublets at δ 6.70 (1H, $J = 10$ Hz) and δ 5.53 (1H, $J = 10$ Hz) and one intense singlet at δ 1.41 (6H), these being characteristic of a chromene moiety (Salazar et al., 2005). This spectrum also had one singlet at δ

Table 1
^1H and ^{13}C (500 and 125 MHz, δ ppm, $CDCl_3$) spectroscopic data for **1** and **2** isolated from *Peperomia serpens*

Position	**1**			**2**		
	^1H [*m*, *J* (Hz)]	^{13}C[a]	HMBC (H → C)	^1H [*m*, *J* (Hz)]	^{13}C[a]	HMBC (H → C)
2	–	76.6 (C)	–	–	77.3 (C)	–
3	5.52 (*d*, 10)	128.1 (CH)	C_2, C_{4a}	5.53 (*d*, 10)	127.3 (CH)	C_2, C_{4a}
4	6.57 (*d*, 10)	116.7 (CH)	C_2, C_{8a}	6.70 (*d*, 10)	116.7 (CH)	C_2, C_5, C_{8a}
4a	–	107.3 (C)	–	–	107.1 (C)	–
5	–	148.9 (C)	–	–	158.3 (C)	–
6	6.13 (*s*)	109.0 (CH)	C_{4a}, C_5, C_8, C_{11}	–	104.4 (C)	–
7	–	137.7 (C)	–	–	141.5 (C)	–
8	–	120.6 (C)	–	–	121.6 (C)	–
8a	–	151.3 (C)	–	–	156.6 (C)	–
9	1.39 (*s*)	27.6 (CH_3)	C_2, C_3, C_{10}	1.41 (*s*)	28.1 (CH_3)	C_2, C_3, C_{10}
10	1.39 (*s*)	27.6 (CH_3)	C_2, C_3, C_9	1.41 (*s*)	28.1 (CH_3)	C_2, C_3, C_9
11	2.17 (*s*)	19.7 (CH_3)	C_6, C_7, C_8	2.49 (*s*)	18.6 (CH_3)	C_6, C_7, C_8
12	–	–	–	–	175.5 (C)	–
1′	3.23 (*d*, 6.9)	24.5 (CH_2)	C_7, C_8, C_{8a}, $C_{2'}$, $C_{3'}$	3.31 (*m*)	24.7 (CH_2)	C_7, C_{8a}, $C_{2'}$, $C_{3'}$
2′	5.05 (*m*)	123.1 (CH)	C_4, C_8	4.99 (*m*)	122.7 (CH)	C_8, $C_{3'}$, $C_{4'}$, $C_{5'}$
3′	–	134.0 (C)	–	–	131.0 (C)	–
4′	1.96 (*m*)	39.8 (CH_2)	$C_{2'}$, $C_{5'}$	1.76 (*s*)	18.0 (CH_3)	$C_{2'}$, $C_{3'}$, $C_{5'}$
5′	2.05 (*m*)	26.7 (CH_2)	$C_{3'}$, $C_{7'}$	1.66 (*s*)	25.7 (CH_3)	$C_{2'}$, $C_{3'}$, $C_{4'}$
6′	5.06 (*m*)	124.4 (CH)	$C_{4'}$, $C_{7'}$	–	–	–
7′	–	131.2 (C)	–	–	–	–
8′	1.56 (*s*)	17.6 (CH_3)	$C_{6'}$, $C_{7'}$, $C_{9'}$	–	–	–
9′	1.64 (*s*)	25.6 (CH_3)	$C_{6'}$, $C_{7'}$, $C_{8'}$	–	–	–
10′	1.75 (*s*)	16.2 (CH_3)	$C_{2'}$, $C_{3'}$, $C_{4'}$	–	–	–

[a] Multiplicities obtained from ^{13}C NMR DEPT 135° spectrum.

2.49 (3H), characteristic of a methyl group attached to a benzene ring. Thus, in accordance with the absence of aromatic ring protons, the benzene ring was hexa-substituted. These data thus suggested that **2** possess a similar structure to that of the polyketide chromene **1**, however containing an additional substituent in the aromatic ring. The presence of a 2,2-dimethyl-2*H*-1-chromene moiety was confirmed by analysis of the ^{13}C NMR spectra (BBD and DEPT 135°) which revealed signals corresponding to tertiary carbinolic carbon at δ 77.3, two sp^2 carbons at δ 127.3 (CH) and δ 116.7 (CH) besides two magnetically equivalent methyl groups at δ 28.1. This spectrum also contained six non-substituted aromatic carbon resonances at δ 104.4, 107.1, 121.6, 141.5, 156.6, 158.3, one carboxyl group at δ 175.5 and one methyl group linked to an aromatic ring at δ 18.6. Additional resonances at δ 18.0 (CH_3), 24.7

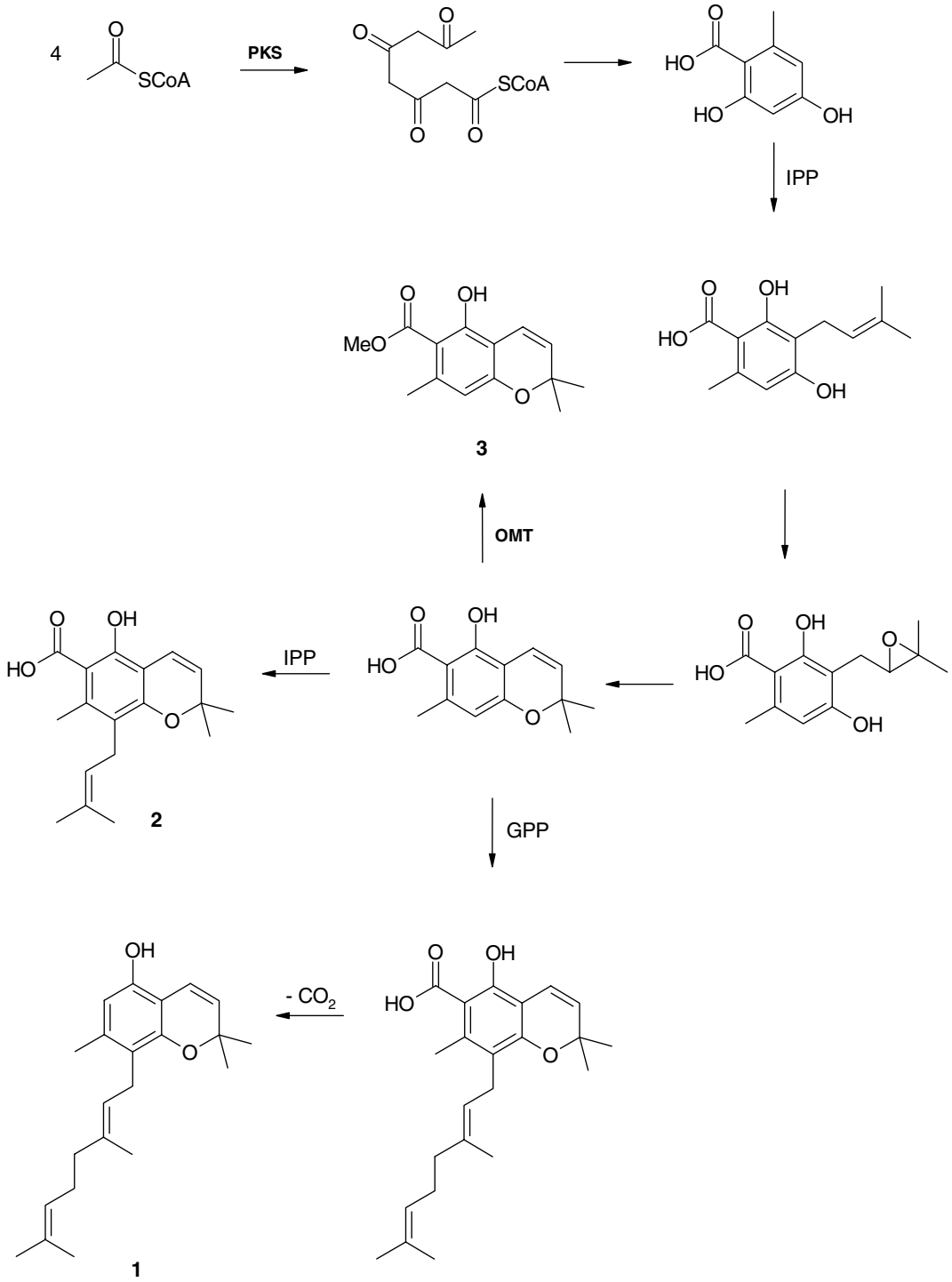

Fig. 1. Possible biogenetic route to isolated chromenes **1**, **2** and **3** in *Peperomia serpens*.

(CH_2), 25.7 (CH_3), 122.7 (CH) and 131.0 (C) indicated the presence of a prenyl moiety in the molecular structure. The connectivity of the carbons in the molecular structure was determined by analysis of the HMQC and HMBC spectra. The latter spectrum showed correlations between H-11 (2.49) and C-6 (104.4), C-7 (141.5) and C-8 (121.6) and between H-1′ (3.31) and C-7 (141.5), C-8a (156.6), C-2′ (122.7) and C-3′ (131.0), which placed the methyl group at C-7 and the prenyl group at C-8. Therefore, the carboxyl group could only be positioned at C-6. Other correlations observed in HMBC spectrum (Table 1) supported the proposed structure of **2** as 5-hydroxy-8-(3′-methyl-2′-butenyl)-2,2,7-trimethyl-2*H*-1-chromene-6-carboxylic acid.

Compounds **3** and **4** were identified by analysis of their $^{1}H/^{13}C$ NMR, IR and MS spectrometric data and by comparison with the literature data (Salazar et al., 2005; Agrawal, 1989).

The biogenetic route to chromenes **1**, **2** and **3** (Fig. 1), similarly to that of the chromenes isolated from *P. villipetiola*, may involve orsellinic acid (2,4-dihydroxy-6-methyl benzoic acid) as precursor (Salazar et al., 2005). Action of a prenyltranferase/IPP and addition of methyl group by an *O*-methyltransferase could yield compounds **2** and **3**, respectively. A parallel pathway with GPP followed by decarboxylation would give compound **1** (Salazar et al., 2005; Mann, 1994).

The crude extract from leaves of *P. serpens* showed good antifungal activity and was subjected to dereplication procedures using chromatographic techniques. The detection limits to crude extract and to compounds **1**–**4** were determined by means of bioautography on TLC plates. As shown in Table 2, compound **2** was twice as active than **3**, suggesting that the presence of a prenyl group and/or free carboxyl group may be associated with the toxicity of this compound in the tested fungi. However, the activities of pure compounds were weaker than the crude extract and decreased during the fractionation procedures. Therefore, this behavior could be associated to a possible synergistic action of these compounds, or to other minor metabolites not isolated from the crude CH_2Cl_2 extract in the present study.

Table 2
Antifungal activity of compounds **1**–**4** against *Cladosporium cladosporioides* and *C. sphaerospermum*

Compound	Antifungal activity[a] (μg)	
	Cladosporium cladosporioides	*C. sphaerospermum*
1	i	i
2	10.0	10.0
3	20.0	20.0
4	i	i
Crude CH_2Cl_2 extract	5.0	5.0
Nystatin	1.0	1.0
Miconazole	1.0	1.0

i: inactive at 100.0 μg.
[a] Minimum amount required for the inhibition of fungal growth on thin-layer chromatographic plates (TLC).

3. Experimental

3.1. General procedures

Silica gel (Merck 230–400 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for CC separation while silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) and prep. TLC (1.0 mm). NMR spectra were recorded on Bruker DRX-500 operating at 500 MHz to ^{1}H and at 125 MHz to ^{13}C, in $CDCl_3$ with TMS as internal standard. IR spectra were obtained on a Perkin–Elmer model 1750 spectrometer. LREIMS and LRESIMS were measured, respectively, on HP 5990/5988A (70 eV) and Platform II spectrometers. UV spectra were recorded on a UV/Visible Shimadzu UV-1601PC spectrophotometer. Elemental analyses were obtained on a Perkin–Elmer Elemental Analyzer model 2400 CHN.

3.2. Plant material

Leaves of *P. serpens* (Sw.) Loudon were collected in November 2004 in Trindade District, Rio de Janeiro, Brazil. A voucher specimen (K-557) was deposited in the Herbarium of the Instituto de Botânica (SEMA/SP), São Paulo – SP, Brazil.

3.3. Antifungal bioassay

Ten microliters of the solutions of the crude extracts, fractions and pure compounds were prepared, in different concentrations, corresponding to 20, 10, 5, and 1 μg for pure compounds and 100 μg for the crude extracts or fractions. The samples were applied to TLC plates, these being eluted with $CHCl_3$:MeOH 99:1 followed by complete removal of the solvent at room temperature. The chromatographic plates were sprayed with spores suspension of *C. sphaerospermum* and *C. cladosporioides* which have been maintained at the Instituto de Botânica (SEMA/SP), Brazil. After incubation, clear inhibition zones appeared against a dark background chromatogram. Nystatin and miconazole were used as positive controls, whereas ampicillin and chloramphenicol were used as negative controls (Lago et al., 2005; Homans and Fuchs, 1970; Rahalison et al., 1994).

3.4. Extraction and isolation of constituents

Dried leaves of *P. serpens* (3.4 g) were powdered and extracted with CH_2Cl_2 (4 × 150 mL) at room temperature. The CH_2Cl_2 extract was filtered and concentrated *in vacuum* to afford a crude extract (0.42 g). The CH_2Cl_2 extract was subjected to on silica gel CC eluted with a step gradient of *n*-hexane and EtOAc to give 120 fractions (10 mL each). These fractions were combined based on their similarities on TLC analysis to yield 15 groups (1–15). Groups 1 (15 mg) and 2 (7 mg) were composed by fatty material. Group 3 (24 mg) was subjected to silica gel CC eluted with a step gradient CH_2Cl_2 and EtOAc to yield **1** (5 mg). Group

7 was composed of pure **2** (17 mg). Groups 8 (19 mg) and 9 (17 mg) were combined and purified by prep. TLC (CH_2Cl_2:EtOAc 7:3 + 1% AcOH, twice) to give **3** (6 mg). Group 14 (37 mg) was subjected to CC on Sephadex LH-20 using $CHCl_3$:MeOH 1:1 as eluent to afford **4** (12 mg).

*3.5. 5-Hydroxy-8-(3′,7′-dimethylocta-2′,6′-dienyl)-2,2,7-trimethyl-2H-1-chromene (**1**)*

Colorless oil. IR (KBr) ν_{max} cm^{-1}: 3444, 2973, 2923, 2856, 1636, 1614, 1452, 1124. UV λ_{max} (MeOH) nm ($\log\varepsilon$): 336 (3.81), 272 (4.12), 232 (4.33). LREIMS *m/z* (rel. int.): 326 (17) [$M^{+\cdot}$], 311 (100), 283 (1), 257 (11), 203 (20), 187 (77), 175 (7), 161 (5), 145 (5), 128 (4), 115 (6), 91 (9); for NMR spectroscopic data (^1H, ^{13}C, HMQC and HMBC), see Table 1. Found C, 72.21%; H, 8.03%, requires C, 72.88%; H, 8.35%.

*3.6. 5-Hydroxy-8-(3′-methyl-2′-butenyl)-2,2,7-trimethyl-2H-1-chromene-6-carboxylic acid (**2**)*

Colorless oil. IR (KBr) ν_{max} cm^{-1}: 3393, 2853, 1648, 1606, 1593, 1200, 1125, 758. UV λ_{max} (MeOH) nm ($\log\varepsilon$): 251 (4.36), 259 (4.37). LREIMS *m/z* (rel. int.): 258 (20) [$M - CO_2$], 243 (100), 217 (6), 199 (7), 187 (54), 175 (4), 159 (5), 145 (4), 128 (7), 115 (7), 91 (7). LRESIMS *m/z*: 303 [M + H]; for NMR spectroscopic data (^1H, ^{13}C, HMQC and HMBC), see Table 1. Found C, 72.01%; H, 7.16%, requires C, 71.49%; H, 7.34%.

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