Phytochemistry Letters xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Structure and absolute configuration of a secolignan from *Peperomia blanda*

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ARTICLE INFO

Article history:
Received 1 March 2011
Received in revised form 6 April 2011
Accepted 11 April 2011
Available online xxx

Keywords:
Peperomia blanda
Piperaceae
Secolignan
Absolute configuration
Electronic circular dichroism
Density functional theory

ABSTRACT

A secolignan, (−)-2-methyl-3-[bis(3′,4′-methylenedioxy-5′-methoxyphenyl) methyl]butyrolactone (**1**), with a rare *cis* configuration was isolated from the aerial parts of *Peperomia blanda* (Piperaceae). The structure of this compound was elucidated by a combination of spectroscopic methods, including ultraviolet, infrared, 1D- and 2D- nuclear magnetic resonance as well as high resolution mass spectrometry data. The absolute configuration of (−)-**1** was determined as (2*R*,3*S*) by the comparison of experimental electronic circular dichroism (ECD) spectroscopy and time-dependent density functional theory (TDDFT) calculations.

1. Introduction

Peperomia blanda (Jacq.) H.B. & K., a perennial herb that typically grows in wet rock crevices, is found from northeast to the south of Brazil (Guimarães and Giordano, 2004). Chemical studies of the aerial parts of *P. blanda* showed the presence of two chromenes (Velozo et al., 2006), two C-glycosylflavones with antioxidant activity (Velozo et al., 2009), and five tetrahydrofuran lignans with trypanocidal activity (Felippe et al., 2008).

Species of *Peperomia* have revealed the presence of a special class of secolignans known as peperomins, which showed important biological activities, such as antifeedant (Govindachari et al., 1998), cytotoxic (Cheng et al., 2003; Wu et al., 2006; Xu et al., 2006), anti-HIV (Zhang et al., 2007), and anti-inflammatory (Tsutsui et al., 2009). It is also noteworthy that these secolignans show structural features similar to the podophyllotoxin skeleton (Sibi et al., 2001) used for the clinical treatment of warts and malign neoplasms (Castro et al., 2003). In this work we report the structure elucidation and absolute configuration of a new secolignan, the (−)-2-methyl-3-[bis(3′,4′-methylenedioxy-5′-methoxyphenyl) methyl]butyrolactone (**1**), with a rare *cis* configuration at the γ-butyrolactone ring, isolated from the aerial parts of *P. blanda*. A racemic mixture of this compound was synthesized by Zee and Chou (1990), however this is the first time that this isomer is reported as a natural product.

Although three levorotatory secolignans with *cis* configuration at the γ-butyrolactone ring have been described from *P. dindygulensis* (Wu et al., 2006) their absolute configuration remained undetermined. Herein, a combination of electronic circular dichroism (ECD) spectroscopy and time-dependent density functional theory (TDDFT) calculations was successfully used to determine the absolute configuration of (−)-**1** as (2*R*,3*S*).

2. Results and discussion

Compound **1** (Fig. 1) was isolated from the CH_2Cl_2 soluble portion of the EtOAc extract of the aerial parts of *P. blanda*. Its molecular formula was established as $C_{22}H_{22}O_8$ by HRMS ($[M + Na]^+$ obsd *m*/*z* 437.1209, calcd 437.1212) in combination with extensive NMR analysis. The ^1H NMR spectrum exhibited resonances of four meta-coupled aromatic hydrogens at δ 6.28 (H-2′, *J* = 1.5 Hz), 6.31 (H-6′, *J* = 1.5 Hz), 6.35 (H-2″, *J* = 1.5 Hz) and 6.41 (H-6″, *J* = 1.5 Hz), two methylenedioxy groups at δ 5.83 (d, *J* = 1.5 Hz), 5.85 (d, *J* = 1.5 Hz), 5.86 (d, *J* = 1.5 Hz), and 5.87 (d, *J* = 1.5 Hz), and two *O*-methyl singlets at δ 3.82 and 3.83. This set of signals characterized two 3,4-methylenedioxy-5-methoxy-

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doi:10.1016/j.phytol.2011.04.007

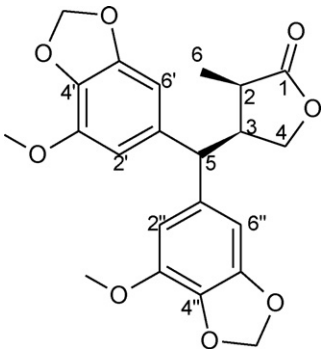

Fig. 1. Structure of (2*R*,3*S*)-secolignan from the aerial parts of *Peperomia blanda*.

phenyl rings with magnetically non-equivalent methylenedioxy protons resulting from the anisotropy of the aromatic ring (Rezende and Kato, 2002). A doublet at δ 1.04 (H-6, d, J = 8.0 Hz) was assigned to the methyl group, multiplets at δ 3.96 (H-4a) and 3.84 (H-4b) due to the methylene group of the butyrolactone moiety, and resonances at δ 2.63 (H-2, dq, J = 7.5, 8.0 Hz), 3.25 (H-3, m), and 3.59 (H-5, d, J = 12.0 Hz), due to three methine groups, were observed. A γ-butyrolactone ring was confirmed by the HMBC and HMQC cross-peaks between H-2, H-4, and H-6 and the lactone carbonyl carbon [δ 179.9 (C-1)]. The ^{13}C NMR data corroborated the presence of the butyrolactone system, and all signals were accordingly assigned based on the HMBC data (Table 1). The *cis* configuration at the γ-butyrolactone ring was established by 1D NOESY experiments. Upon irradiation of H-2 an NOE enhancement was observed for H-3. Based on the relative configuration assigned to **1**, there were only two possible absolute configuration, namely (2*S*,3*R*) or (2*R*,3*S*).

For compound **1**, the configuration (2*R*,3*S*) was arbitrarily chosen, and after conformational analysis and geometry optimization, the seven lowest-energy conformers were identified. The main conformational changes were related to rotations about the C-5–C-1′, C-5–C-1″, C-3′–O and C-3″–O single bonds (Fig. 2). Owing to the symmetrical pattern of the substituents linked to C-5 the effects of rotation about the C-3–C-5 bond could not be probed.

The overall patterns of the gas phase calculated weighted ECD spectra were consistent with those of the experimental one, i.e., a negative Cotton effect (CE) near 275 nm, a positive CE near 260 nm, and a negative CE near 230 nm (Fig. 3). The experimentally observed negative CE around 275 nm may result from two high-amplitude negative rotational strengths common to the seven conformers considered, while the positive CE around 260 nm is contributed by three positive rotational strengths at around 250, 255, and 260 nm. The negative CE near 230 nm may arise from a series of predominantly negative low-amplitude rotational strengths since the two high-amplitude ones predicted at around 233 and 237 nm are expected to cancel each other. Additionally, the presence of a negative bisignate CD couplet at around 265 nm is suggestive of exciton coupling (Berova et al., 2007) between the two identical chromophores, e.g. 3,4-methylenedioxy-5-methoxyphenyl rings, which are located near in space and have their transition moments oriented with a negative angle. The negative CE near 230 nm is due to electronic transitions of the asymmetric lactone system. Thus, by comparing ECD measurements with the results of TDDFT calculations carried out at the B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) level, the absolute configuration of (−)-**1** was unambiguously determined as (2*R*,3*S*). Also, as the experimental measurements were carried out in polar solvents, the self-consistent reaction field (SCRF) with polarizable continuum model (PCM) was employed to simulate solution-state ECD spectra of **1** in acetonitrile at the B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) level. However, the calculated ECD spectra of each conformer were largely similar to the ones obtained by B3LYP/6-311++G(2d,2p) in the gas phase. The suitability of B3LYP/6-31G(d) for the optimization of geometry

Table 1
NMR data of compound **1** ($CDCl_3$, 11.7 T).

Position	^{1}H (δ)[a]	^{13}C (δ)	HMBC[b]
1		179.9 (q)[c]	
2	2.63 dq (7.5, 8.0)	43.0 (t)	1, 4, 6
3	3.25 m	37.2 (t)	
4a, 4b	3.96 m, 3.84 ov[d]	70.3 (s)	1, 3, 2
5	3.59 d (12.0)	50.2 (t)	2, 4, 1′, 1″, 2′, 2″, 6′, 6″
6	1.04 d (8.0)	10.4 (p)	3, 2
1′		136.3 (q)	
2′	6.28 d (1.5)	107.4 (t)	5,4′, 6′
3′		149.5 (q)	
4′		134.3 (q)	
5′		143.6 (q)	
6′	6.31 d (1.5)	101.0 (t)	5, 2′, 4′
1″		136.5 (q)	
2″	6.35 d (1.5)	107.4 (t)	5, 4″, 6″
3″		149.5 (q)	
4″		134.3 (q)	
5″		143.7 (q)	
6″	6.41 d (1.5)	101.1 (t)	5, 2″, 4″
OCH_2O	5.87 d (1.5)	101.4 (s)	3′, 4′
	5.86 d (1.5)	101.4 (s)	3″, 4″
	5.85 d (1.5)	101.4 (s)	3″, 4″
	5.83 d (1.5)	101.4 (s)	3″, 4″
5′-OCH_3	3.82 s	56.9 (p)	5′
5″-OCH_3	3.83 s	57.0 (p)	5″

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY experiments, J in Hz.
[b] HMBC correlations from H to C.
[c] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.
[d] Overlapped.

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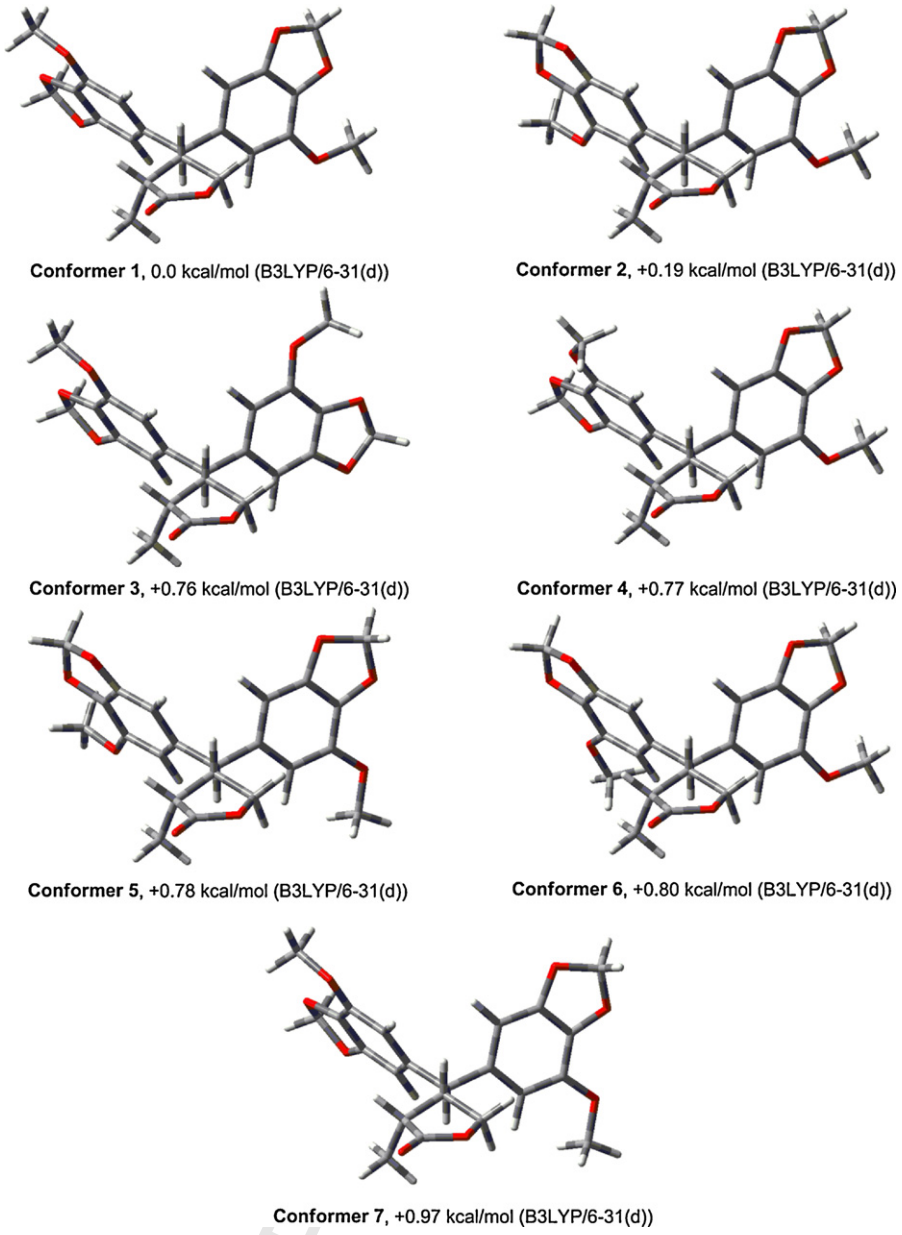

Fig. 2. Optimized structures and relative energies of the seven lowest-energy conformers of (2*R*,3*S*)-**1** used for ECD calculations at the B3LYP/6-311G++(2d,2p) level.

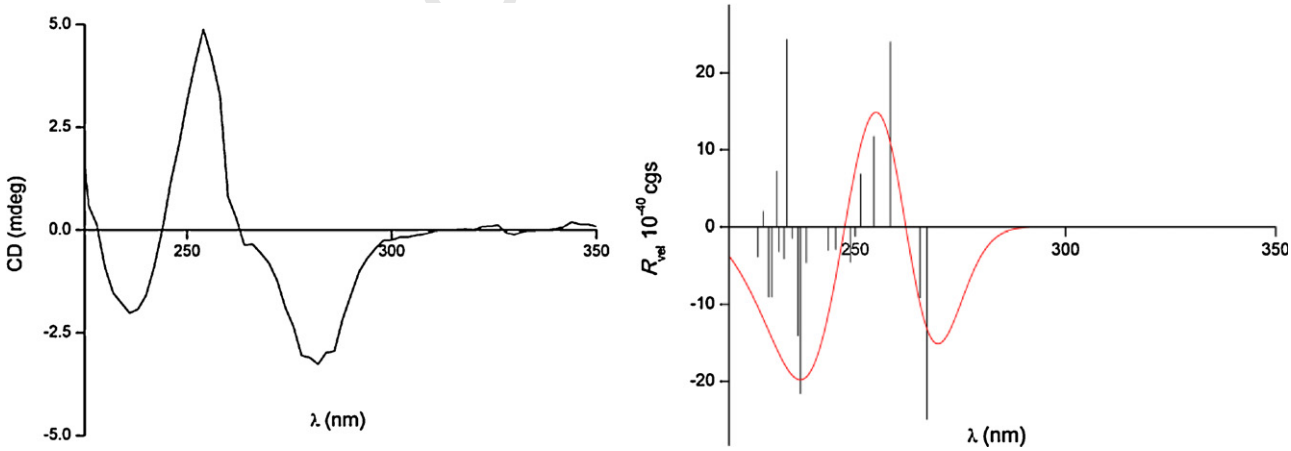

Fig. 3. On the left, experimental ECD spectra of **(−)-1** assigned as (2*R*,3*S*). On the right, calculated ECD spectra [B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d)] of the Boltzmann average of the seven lowest-energy conformers of the corresponding (2*R*,3*S*)-1 The bars represent the rotational strengths for the weighted ECD spectrum.

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as well as B3LYP/6-311++G(2d,2p) for ECD calculations have been previously demonstrated in our group (Batista et al., 2010, 2011).

3. Conclusion

Comparison of experimental and TDDFT-calculated ECD spectra of the new secolignan (−)-**1** isolated from *P. blanda* established its absolute configuration as (2*R*,3*S*). Such structural and stereochemical information is vital to the studies of the biological activity of **1** and the biosynthetic pathways involved in its formation, both of which are to be investigated further in the near future.

4. Experimental

4.1. General

One-dimensional (^{1}H, ^{13}C, DEPT, and gNOESY) and two-dimensional (*g*HMBC and *g*HMQC) were recorded on a Varian Inova-500 (11.7 T) spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) using $CDCl_3$ as solvent and TMS as reference. HRESIMS was measured using a Bruker Daltonics model ultrOTOF$_Q$ ESI-TOF instrument. Separations by column chromatography (CC) were carried out using silica gel (230–400 mesh; Merck). All solvents were redistilled prior to use. HPLC separations were performed on a Varian PrepStar model SD-1 LC/UV/VIS chromatograph equipped with a Phenomenex C-18 reversed phase column (250 × 21.2 mm). The IR spectrum was measured as KBr pellets on a Perkin-Elmer Infrared Spectrometer FTIR series 1600, the optical rotation in $CHCl_3$ at 589 nm in a digital polarimeter JASCO model DIP-370, and the UV spectrum recorded in MeOH using an HP 8452 A spectrophotometer. The enantiomeric excess (ee > 99%) for **1** was determined by HPLC (Jasco 2000 HPLC equipped with PU-2089 Plus pump, a MD-2010 Plus photodiode array detector and a CD-2095 Plus circular dichroism detector) using the commercial analytical chiral column Chiralcel OD-RH (250 × 4.6 mm, 5 μm, 50% ACN/H_2O with 0.5% formic acid, 0.7 mL/min, 280 nm). The ECD spectrum of (−)-**1** eluting from the chiral HPLC was measured in the Jasco CD-2095 detector by trapping in a 1.0 cm quartz cell through a switching valve. The spectra were average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg). The ECD spectra were corrected by baseline subtraction obtained from a measurement of the same solvent used.

4.2. Plant material

The aerial parts of *P. blanda* (Jacq.) H.B. & K. were collected at the Reserva da Ripasa, Ibaté - SP, Brazil in January of 2008 and identified by Dra. Elsie Franklin Guimarães. A voucher specimen (Kato-547) has been deposited at the Herbarium of the Instituto de Biociências, Universidade de São Paulo, São Paulo – SP, Brazil.

4.3. Isolation of compounds

Dried aerial parts (86 g) of *P. blanda* were milled, extracted with EtOAc, and the extract concentrated under vacuum to yield 8.5 g. The extract was resuspended in MeOH:H_2O (4:1) and partitioned with hexanes, CH_2Cl_2 and EtOAc successively. The portion soluble in CH_2Cl_2 (1.95 g) was subjected to CC over silica gel and eluted with a gradient of hexanes–EtOAc to yield fractions 1–14. Fraction 5 (630 mg) was subjected to CC over silica gel and eluted with a gradient of hexanes–EtOAc providing fractions 5–1 to 5–40. Fraction 5–36 (215 mg) was subjected to preparative HPLC and eluted with isocratic MeOH:H_2O (60:40) to afford compound 1 (53.4 mg).

4.4. (2R,3S)-2-methyl-3-[bis(3′,4′-methylenedioxy-5′-methoxyphenyl)methyl]butyrolactone (1)

Pale yellow oil, $[\alpha]_D^{21}$ −54.7 (CHCl3; *c* 0.14). UV (MeOH) λ_{max}: 220, 248, 276 nm. IR (KBr) ν_{max} cm^{-1}: 1775, 1632, 1514, 1464. ^{1}H and ^{13}C NMR see Table 1. HRMS/ESI-TOF *m*/*z* (rel. int.): 437.1209 $[M + Na]^+$ (98), (calcd for $C_{22}H_{22}O_8Na$, 437.1212).

4.5. Computational methods

All DFT and TDDFT calculations were carried out at 298 K both in the gas phase and SCRF/PCM (acetonitrile) with Gaussian 09. Calculations were performed for the arbitrarily chosen (2*R*,3*S*)-**1**. Conformational searches were carried out at the molecular mechanics level of theory employing MM+ and MMFF force fields incorporated in Hyperchem 7 and Spartan 08 software packages, respectively. For compound **1**, 17 conformers with relative energy (rel *E*.) within 6 kcal/mol of the lowest-energy conformer were selected and further geometry optimized at the B3LYP/6-31G(d) level. Among the 12 conformers with rel *E*. < 1.4 kcal/mol, the seven lowest-energy conformers, which correspond to 85% of the total Boltzmann distribution, were selected for ECD spectra calculation. The Boltzmann factor for each conformer was calculated based on Gibbs free energy. Vibrational analysis at the B3LYP/6-31G(d) level of theory resulted in no imaginary frequencies, confirming the considered conformers as real minima. TDDFT was employed to calculate excitation energy (in nm) and rotatory strength *R* in dipole velocity (R_{vel} in cgs units: 10^{-40} erg esu cm Gauss^{-1}) form, at the B3LYP/6-311++G(2d,2p) level. The calculated rotatory strengths from the first 20 singlet → singlet electronic transitions were simulated into an ECD curve using Gaussian band shapes and 10 nm half-width at 1/*e* of peak height. The predicted wavelength transitions are used as such without any scaling.

Uncited references

Ding et al., 2009. Q1

Acknowledgements

This work was supported by the State of São Paulo Research Foundation (FAPESP) with grants 03/11524-9 and 05/57042-0, and also within the Biodiversity Virtual Institute Program (BIOTA/FAPESP – www.biotasp.org.br). This research was also supported by resources supplied by the Center for Scientific Computing (NCC/GridUNESP) of the São Paulo State University (UNESP). MF, MJK, and VSB are grateful to CNPq for research fellowships. DCB and JMBJ thank FAPESP for the provision of fellowships, and LGF thanks CAPES for providing scholarship.

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