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An Unprecedented Neolignan Skeleton from *Chimarrhis turbinata*

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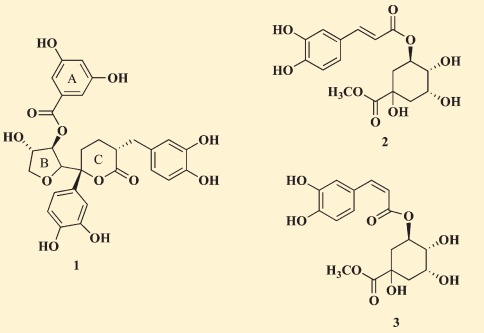
Supporting Information

ABSTRACT: A lignan with a new skeleton named chimarrhinin (**1**) was isolated from an extract of the leaves of *Chimarrhis turbinata*, a Rubiaceae plant species. ^{13}C NMR spectrometric techniques including 1D and 2D experiments and HRESIMS provided unequivocal structural confirmation of this new $C_6.C_3$ skeleton type. The relative configuration of **1** was established by 2D $^{1}H-H$ analysis and *J* couplings, while its conformation was evaluated through molecular modeling using the RM1 semiempirical method, with the aid of coupling constants obtained by NMR analysis. The antioxidant activity of the new derivative **1** and two known and previously isolated phenolic derivatives (**2** and **3**) was investigated. An IC_{50} value of 7.50 ± 0.5 μmol L^{-1} was obtained for the new derivative **1**, while **2** and **3** showed IC_{50} values of 18.60 ± 0.4 and 18.50 ± 0.6 μmol, respectively.



As part of our research project focusing on the phytochemical investigation of new biologically active compounds from Amazonian plant species, the constituents of the leaves and barks from *Chimarrhis turbinata* DC Prodr. (Rubiaceae) have been examined.[1−5] Previous phytochemical studies of *C. turbinata* established the presence of indole alkaloids with weak anticholinesterase activity[2] as well as several phenolic compounds with antioxidant properties.[3,5] Herein, the isolation, structural elucidation, and conformational analysis of a new $C_6.C_3$ lignan skeleton type (**1**) are reported.

We have previously described the isolation of the two known phenolic compounds **2** and **3**.[4,6] Herein, their antioxidant properties along with those of compound **1** are reported and compared with those of the commercially available antioxidant BHT. Evaluation of the parameters related to the antioxidant activity was accomplished by measuring the free radical scavenging ability of the compounds toward DPPH.[7]

The liquid−liquid partitioning of the EtOH extract of air-dried leaves of *C. turbinata,* followed by a sequence of size exclusion chromatography of the EtOAc-soluble fraction on Sephadex LH-20 and further purification using C18 HPLC, eluted with a CH_3CN/H_2O (18:88 v/v) isocratic elution using a Phenomenex Luna column (25.0 mm × 21.20 mm × 5 μm) at a flow rate of 10 mL min^{-1} and UV detection at 280 nm, afforded compound **1**.

Compound **1** was obtained as an amorphous, pale brown powder, $[\alpha]_D^{25}$ −204 (*c* 0.01, MeOH), which was analyzed for $C_{29}H_{28}O_{12}$ by a combination of HRESIMS and ^{13}C NMR data. The molecular formula of **1** was determined from the $[M - H_2O]^+$ peak at *m/z* 550.6433 (calcd for $C_{29}H_{26}O_{11}$ 550.1475). The IR spectrum with absorption bands at 3403, 1705, 1657, and 1627 cm^{-1} suggested the presence of phenolic and carboxylic groups. The ^{1}H NMR spectrum of **1** (Table 1) displayed signals for nine aromatic hydrogens, which indicates the presence of three aromatic ring systems with different substitution patterns. The signals at δ 7.31 (d, *J* = 2.0 Hz), 7.33 (ddbr, *J* = 2.0; 2.0 Hz), and 7.23 (s) corresponded to the aromatic hydrogens of a 1,3,5-trisubstituted ring. Additionally, the signals at δ 6.78 (d, *J* = 2.0 Hz), 6.69 (d, *J* = 8.0 Hz), 6.61 (*J* = 8.0 Hz), 6.67 (dd, *J* = 8.0; 2.0 Hz), 6.55 (sbr), and 7.32 (d, br, *J* = 8.0 Hz) were attributed to six aromatic hydrogens from 1,3,4-trisubstituted rings. These ^{1}H NMR data clearly confirm the presence of three distinct aromatic moieties in **1**.

The ^{13}C NMR spectrum of **1** showed signals for 29 different carbons: 11 quaternary (sp^2), two of which are carbonyls (δ 176.5, C-9; 173.6, C-7a), one quaternary (sp^3); nine sp^2 methines; four sp^3 methines, three of which are oxymethine; and four methylenes, one of which is a sp^3 methines. The signals at δ_C 146.0, 146.5, 116.8, 115.7, 123.8, 146.7, 146.0, 115.2, 116.5, and 121.7 were ascribed to two 1,3,4-trisubstituted aromatic systems characteristic of a catechol moiety.[8] The signals at δ_C

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117.7, 151.3, 115.7, 151.3, and 117.7 evidenced the presence of one 1,3,5-substituted aromatic ring.[9] The signals at δ_C 63.3, 70.2, 71.2, and 81.5 were assigned to a dihydroxytetrahydrofuran moiety. The signal at δ_C 91.7 revealed a peculiar downfield shifted carbon, which, analyzed together with the signals at δ_C 28.5, 41.3, 43.2, and 173.6, allowed us to attribute one part of the structure of **1** as being a trisubstituted lactone moiety (Table 1).

Detailed analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectra demonstrated that **1** bears a different skeleton, with two $C_6.C_3$ units similar to those found in lignoids. Correlations between H-7 (2H, δ 3.06, d, J = 13.0; 17.5 Hz), H-8 (δ 4.22, dd, J = 13.0; 9.0 Hz), H-9′ (2H, δ 2.70 and 2.75, J = 8.5 Hz), and H-8′ (δ 3.33, m) were observed by $^1H-^1H$ COSY. The hydrogen at δ 3.06 (H-7) has HMBC correlations with the aromatic carbon at δ 125.6 (C-1) and with a quaternary carbon at δ 176.5 (C-9), as well as a correlation with a methine carbon at δ 43.2 (C-8). Correlations of the methylene hydrogen resonating at δ 3.33 (H-8′) with C-1′ and C-9′ and correlations of H_2-9′ with C-8′ and C-7′ are also evident. The hydrogen at δ 4.66 (H-2b) correlates with C-7′ and C-3b. From these observations and the analysis of all ^1H and ^{13}C NMR data (Table 1), it is evident that the two caffeic acid units and a dihydroxytetrahydrofuran system are connected to the δ-lactone moiety.

These data suggest the presence of a δ-lactone moiety linked at C-1′ (ring C) and at C-2b (ring B), Figure 2. The anisotropic unshielded signals of the quaternary carbon C-7 (δ 91.7) might be explained by its ligands: oxygen, the aromatic carbon C-1′, and C-2b. The correlations between H-2b (δ 4.66, d, J = 8.5 Hz), H-3b (δ 3.68, dd, J = 2.5; 8.5 Hz), H_2-4b (δ 3.57, ddd, J = 2.5; 6.0; 6.5 Hz and δ 3.41, dd, J = 6.5; 11.0 Hz), and H_2-5b (δ 3.32, m) indicate that ring B consists of a dihydroxytetrahydrofuran system, biogenetically derived from a tetrose sugar unit (Figure 2). HMBC correlations from the aromatic hydrogens at δ 7.33 (H-2a) and 7.31 (H-6a) of the third 1,3,5-trisubstituted aromatic ring (A) with C-7a (δ 176.5) showed that the third aromatic system is the 3,5-dihydroxylbenzoic ester moiety at C-3 of the tetrose ring (B) (Figure 2).

These observations and the presence of two caffeic acid moieties, which could be biogenetically defined as a neolignan, determined a skeleton formed by unusual couplings C-7−C-9′ and HOC-9−C8′. The spectroscopic evidence and a literature survey of lignan types (neolignans and lignans) indicated that compound **1** has a new neolignan-type skeleton.

The relative configuration of the sterocenters at C-2b, C-3b, C4-b, C-7′, and C-8 and the conformation of **1** were determined on the basis of coupling constants and conformational analysis by the semiempirical RM1 method in the gas phase. For C-2b and C-3b, the coupling constants of H-2b (δ 4.66 d, J = 8.5 Hz) and H-3b (δ 3.68 dd, J = 8.5, 2.5 Hz) indicate a vicinal H-$2b_{ax}$−H-$3b_{ax}$ position, which is in good agreement with the presence of the gallate moiety in the β-position at C-3b. For C-4b, the coupling constants of H-4b (δ 3.56 ddd, J = 6.5, 6.75, 2.5 Hz) also confirm an α-hydroxy group at this position. The ^1H NMR data recorded in methanol-d_4 clearly show all the coupling constants of H-4b with H_{ax} and H_{eq}-5b (Table 1) and confirmed the tetrose moiety as part of this molecule. The coupling constants of H-8 (δ 4.22 dddbr, J = 9.0, 13.0 Hz) suggest the α-positioned 8-R, which is in accordance with the relative configuration of C-8. The relative configuration is assigned as 2R*, 3R*, 4S*, 7′S*, and 8R*.

To confirm the relative configuration of C-2b, C-3b, C-4b, and C-8 and in order to establish the conformation of **1**, this structure was submitted to conformational analysis by the semiempirical RM1 method in the gas phase. The obtained minimum energy conformations enable estimation of $^3J_{HH}$ coupling constants by means of the Haasnoot−Altona parametrization of the Karplus equation,[10] which considers the electronegativity of the substituents and the geometry of the H−C−C−H dihedral angle. A comparison between the theoretical and experimental coupling constants permitted the characterization of the conformation of chimarrhinin, represented in Figure 3, with the respective theoretical coupling constants (Table 1), considering the most stable envelope conformation of the tetrahydrofuran ring and half-chair conformation of the tetrahydro-2-pyranone ring. Consistent with previous conformational analyses of natural products with diverse skeletons,[1,11,12] the employed semiempirical calculations agreed with experimental data more satisfactorily when compared with older Hamiltonians such as AM1 and PM3 and *ab initio* HF/6-31G** calculations.

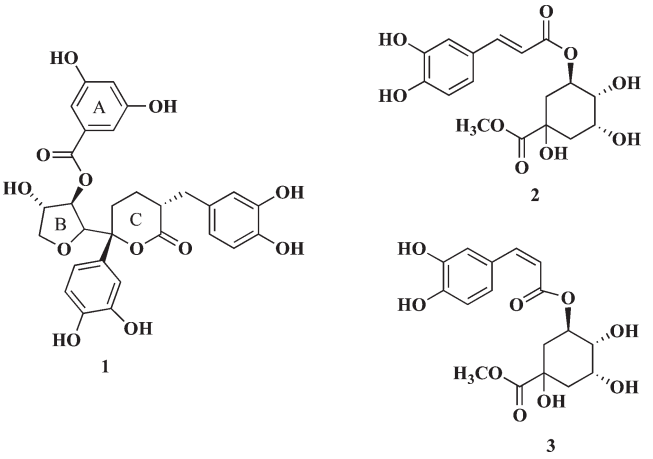

Figure 1. Compounds from *C. turbinata*.

The radical scavenging effects observed for compounds **1**−**3** assayed with DPPH are summarized in Table 2, the standard antioxidant BHT (IC_{50} 62.50 ± 0.6 μmol L^{-1}), and chlorogenic acid (IC_{50} 20.0 ± 0.2 μmol L^{-1}) used as reference. The results indicate that the free radical scavenging activity of these molecules is due to their hydrogen-donating ability, provided by the stabilization of the phenoxy radical after DPPH reduction. Stabilization is enhanced by the presence of catechol groups and, in the case of the phenylpropanoids (**2**, **3**), the α,β-unsaturated carbonyl moiety, as evidenced by their IC_{50} values. Compound **1** gave the best results, with an IC_{50} value lower than that of the standard BHT, a well-known food antioxidant. This result may be related to the presence of two catechol groups in this molecule.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation measurements were conducted on a Perkin-Elmer 241 polarimeter using a quartz cuvette (length 1 cm). IR spectra were measured on either a Perkin-Elmer 1600 or a Nicolet EMACT-40 FTIR spectrophotometer. NMR spectra were recorded on a Varian INOVA 500 NMR spectrometer operating at 500 MHz for ^1H and at 125 MHz for ^{13}C. TMS was used as internal standard. Mass spectra were obtained at high resolution on a Micromass Q-TOF spectrometer. For chromatographic procedures, Sephadex LH-20 (Pharmacia Biotech) was employed. Preparative HPLC separations were performed on a Varian Prep LC 4000 System, and C18 Luna (Phenomenex) columns and precolumns were utilized.

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for 1 in Methanol-d_4[a]

		gHMQC				
position	δ_C	δ_H (J in Hz)	J (Hz)$^{b}_{theor}$	gHMBC	COSY	TOCSY
1	125.6			H-7		
2	116.8	6.78 d (2.0)	n.d.[c]		H-5	
3	146.7			H-2		
4	146.0			H-5, H-6		
5	115.7	6.69 d (8.0)	n.d.	H-6	H-6/H-2	H-6
6	123.8	7.32 dbrl (8.0, -)	n.d.	H-5	H-5	H-5
7	34.9	3.06 dd (13.0, 17.5)	13.0	H-8	H-9′/H-8	H-8
8	43.2	4.22 dddbr(9.0, 13.0)	8.8, 13.0	H-7	H-9′/H-7	H-7/H-9′
9	176.5			H-7, H-8		
1′	132.0*			H-8′, H-9′		
2′	115.2	6.55 sbr			H-5′	
3′	146.5			H-5′		
4′	146.0			H-6′		
5′	116.5	6.61 d (8.0)	n.d.		H-2′/H-6′	H-6′
6′	121.7	6.67 dd (2.0, 8.0)	n.d.		H-5′	H-5′
7′	91.5			H-2b, H-9′		
8′	41.2	3.33 m		H-9′	H-9′	H-9′/H-7
9′	28.5	2.70 dddbr (9.0)	8.8	H-8′, H-7	H-7/H-8′/H-8	H-8′/H-8/H-7
		2.75 dddbr (9.0, 17.5)				
1a	123.8			H-2[b]		
2a	117.7	7.33 dd (2.0, 2.0)	n.d.		H-6a	
3a	151.3			H-2[b]		
4a	115.7	7.23 s			H-6a	
5a	151.3			H-6a		
6a	117.7	7.31 d (2.0)	n.d.		H-4a/H-2a	
7a	173.6			H-2[b], H-6a		
2b	71.2	4.66 d (8.5)	8.9		H-3b	H-3b
3b	81.5	3.68 dd (2.5, 8.5)	2.4, 8.9	H-2b	H-2b/H-4b	H-2b
4b	70.3	3.57 ddd (6.5, 2.5, 6.75)	5.5, 2.4, 6.8	H-5b	H-3b/H-5b/	
5b	63.3	a: 3.32 dd (6.75,11.0)	6.8	H-4b	H-4b/H-5b/	H-5b/
		b: 3.42 dd (6.5, 11.0)	5.5			

[a] Internal standard: TMS. Spectra measured at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Assignments are based on 2D ^{1}H NMR and HMQC correlations. Multiplicity and J-values in Hz are given in parentheses. [b] Theoretical vicinal coupling constants for the H−C−C−H dihedral angle obtained from RM1 minimum energy conformation. [c] Not determined.

Peaks were detected using a Varian model 320-chromatointegrator connected to a UV detector. All solvents (Merck or Mallinckrodt) were analytical or HPLC grade. The theoretical study was carried out using the semiempirical RM1 method on MOPAC 7.0 software.

Plant Material. *Chimarrhis turbinata* was collected in Reserva do Viro, Belém, PA, Brazil, in February 2000, and identified by Dr. Marina Thereza V. do A. Campos. A voucher specimen, Lopes-51, was deposited in the Herbarium of the Botanic Garden, São Paulo.

Extraction and Isolation. Dried and powdered leaves of *C. turbinata* (1.2 kg) were exhaustively extracted with EtOH at room temperature to afford 57.1 g of dry residue, which was dissolved in MeOH/H_2O (8:2, v/v) and extracted with *n*-hexane. The aqueous alcoholic fraction was evaporated to roughly MeOH/H_2O (6:4, v/v) and then extracted successively with CH_2Cl_2, EtOAc, and *n*-BuOH. All fractions were screened with β-carotene and DPPH assays, aiming at selecting potential antioxidant compounds. The EtOAc layer was active in these assays, and this extract was selected for guided fractionation. The dried EtOAc extract (4.16 g) was dissolved in MeOH (5 mL) and submitted to gel filtration over Sephadex LH-20. A total of 25 fractions of 25 mL each were collected and pooled according to their TLC profiles

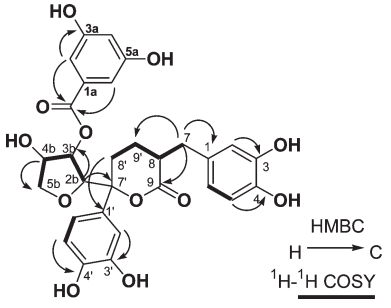

Figure 2. Selected HMBC and COSY correlations for chimarrhinin (**1**).

($CHCl_3$/MeOH/H_2O, 65:30:5). Fractions A-6,7 and A-8 to A-12 exhibited antioxidant activity and were further purified by HPLC. Fractions A-8 to A-12 yielded the compounds described elsewhere.[3,4]

Fraction A-6,7 (0.699 g) was purified by gel filtration over Sephadex LH-20 CC (88.0 cm × 2.0 cm), which was eluted with MeOH, giving 36

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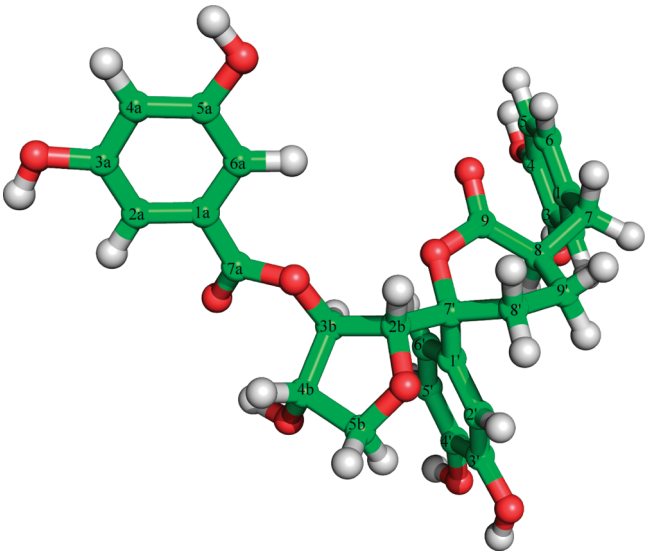

Figure 3. Conformation of compound **1** predicted by the semiempirical conformational analysis using the RM1 method, according to the obtained methanol-d_4 vicinal coupling constants.

Table 2. Radical Scavenging Activity with Respect to the DPPH Radical for Compounds 1−3

compound	IC_{50} (μmol L^{-1})[a]
1	7.50 ± 0.5
2	18.60 ± 0.4
3	18.50 ± 0.6
BHT	62.5 ± 0.6
chlorogenic acid	20.0 ± 0.2

[a] Concentration in μmol L^{-1} effective for scavenging 50% of DPPH free radical; mean values from triplicate ± SD.

fractions of 10.0 mL each. The fractions B-20−30 (0. 387 g) tested positively in the β-carotene and DPPH assays, and they were further combined and subjected to preparative HPLC separation using CH_3CN/H_2O isocratic elution (18:88) [Phenomenex C18 Luna column 25.0 mm × 21.20 mm × 5 μm, at 10 mL.min^{-1}, with UV detection at 280 nm], to afford 10 subfractions. From those, C-3 (97.2 mg) was separated by preparative HPLC using the same column described above [isocratic elution; H_2O/MeCN/HOAc (84.5:15:0.5), UV detection at 237 nm, 12.0 mL min^{-1} flow rate], to give compound **1** (4.3 mg).

Molecular Modeling. The semiempirical calculations were carried out using the RM1 Hamiltonian[13] within the MOPAC 7.0 program.[14] The MOLDEN program was employed as a graphic interface for construction and visualization of the structure of compound **1**.[15] Hessian matrix analyses were used to unequivocally characterize the obtained conformations as true potential energy surface minima. Additionally, the minimum energy achieved by RM1 was further refined through *ab initio* calculations, using the GAMESS program[16] at the HF/6-31G** level. In order to obtain theoretical $^3J_{HH}$ coupling constants for the minimum energy conformation achieved by conformational analysis, the Haasnoot−Altona parametrization of the Karplus equation[15] was applied to the H−C−C−H dihedral angle geometries.

Bleaching Experiments Using β-Carotene. After being developed and dried, TLC plates were sprayed with 0.02% β-carotene solutions (Aldrich) in CH_2Cl_2. Plates were placed under natural light until discoloration of the background. The remaining yellow spots indicated the presence of antioxidant substances.[7]

Determination of the Radical Scavenging Activity. The DPPH radical was used in MeOH (100 μmol L^{-1}). The reagent (2.0 mL) was added to a 1.0 mL aliquot of the compounds previously dissolved in MeOH, to yield final concentrations of 100, 80, 40, 20, 10, and 5 μmol L^{-1}. Each mixture was shaken and kept at room temperature for 30 min, in the dark. Chlorogenic acid and BHT were employed as standards. A DPPH solution (2.0 mL) in MeOH (1.0 mL) served as a control. Absorbances of the resulting solutions were measured at 517 nm using a Milton Roy 20 D spectrophotometer, and the percent inhibition was determined by comparison with a MeOH-treated control group.

Chimarrhinin (***1***)*:*. amorphous, pale brown powder, $[\alpha]^{25}_D$ −204 (*c* 0.01, MeOH); IR (KBr) 3410 (OH), 1656, and 1604 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 550.6433 $[M - H_2O]^+$ (calcd for $C_{29}H_{26}O_{11}$ 550.1475).

■ ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Koji Nakanishi for his pioneering work on bioactive natural products.

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