Tetrahydrofuran Lignans from *Nectandra megapotamica* with Trypanocidal Activity[⊥]

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The phytochemical investigation of the crude ethanol extract of the leaves of *Nectandra megapotamica* afforded three new (**2d**, **3b**, and **3c**) and eight known (**1a**−**d**, **2a**−**c**, and **3a)** tetrahydrofuran lignans. Regarding the in vitro trypanocidal activity, the lignan-rich fraction, at a concentration of 2.0 mg/mL, was 100% active. Compound **1a** was the most active, showing an IC_{50} value of 2.2 μM and lysis of 94% of the parasites at 32 μM. Compounds **1b**, **1d**, **2a**, **2c**, and **3a** displayed moderate activity, while compound **1c** was inactive.

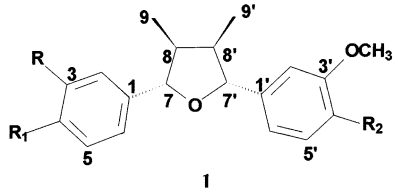
Nectandra megapotamica (Spreng) Chodat et Hassler (Lauraceae), popularly known as "canela-do-mato", is used in folk medicine as an antirheumatic and to relieve pain.[1,2] This woody plant is found in the southern and central parts of Brazil.[3] Previous phytochemical investigation reported the presence of two indole alkaloids.[1] However, this genus is mainly characterized by the presence of lignoids,[4] from which several lignans and neolignans are well known to possess important biological activities.[5] Moreover, the occurrence of 7,7′-diaryl-8,8′-dimethyltetrahydrofuranoid lignans had been previously reported in the *Nectandra* sp.[6] but not in such numbers as in this investigation.

Chagas' disease affects more than 18 million people in Latin America, leading to approximately 400 000 deaths per year.[7] *Trypanosoma cruzi*, the etiologic agent of the disease, causes a pathology of which blood transfusion is the most important mechanism of transmission, and gentian violet is the only effective compound available that eliminates the parasite in the blood prior to its transfusion.[8]

It is also important to point out that Lopes et al. (1998)[9] had previously reported the trypanocidal activity of the tetrahydrofuran lignans grandisin and veraguensin (**2b**). Both compounds were active at concentrations as low as 5 μg/mL.[10] Also, Bastos et al. (1999)[11] had reported the highly significant trypanocidal activity of seven lignans belonging to the dibenzylbutyrolactone group.

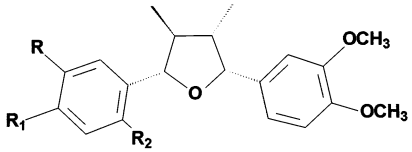
Results and Discussion

In a preliminary biological assay for trypanocidal activity, the crude ethanol extract of the leaves of *N. megapotamica* displayed weak activity. Nevertheless, the trypanocidal bioassay-guided fractionation of the crude ethanol extract of its leaves led to the isolation of eight known tetrahydrofuran lignan type compounds: machilin G (**1a**),[12] galgravin (**1b**),[13] nectandrin A (**1c**),[6,13] nectandrin B (**1d**),[6,13] calopiptin (**2a**),[14] veraguensin (**2b**),[14,15] aristolignin (**2c**),[13] and ganschisandrine (**3a**),[16] along with three new compounds, nectandrin C (**2d**), nectandrin D (**3b**), and nectandrin E (**3c**), several of which displayed significant trypanocidal activity. The chemical structures of known compounds **1a**−**d**, **2a**−**c**, and **3a** were established by ^{1}H and ^{13}C NMR data analysis in agreement with the literature.



1

	R	R_1	R_2
a	OCH_2O		OCH_3
b	OCH_3	OCH_3	OCH_3
c	OCH_3	OH	OCH_3
d	OCH_3	OH	OH



2

	R	R_1	R_2
a	OCH_2O		H
b	OCH_3	OCH_3	H
c	OCH_3	OH	H
d	OCH_3	OCH_3	OH

The ^{1}H NMR spectrum of **3b** (Table 1) suggested a nonsymmetric tetrahydrofuran lignan, through signals corresponding to two methyl groups at δ 1.00 (d, J = 6.3 Hz) and 0.62 (d, J = 7.0 Hz) in addition to two oxybenzyl methines at δ 5.46 (d, J = 4.3 Hz) and 4.66 (d, J = 9.3 Hz). As previously reported,[17,18] the coupling constant of 9.3 Hz for the doublet at δ 4.66 for H-7 indicates that this hydrogen is in *trans* configuration with the adjacent H-8 and that the coupling constant of 4.3 Hz of H-7′ suggests its *cis* relationship with H-8′. The differential NOE experiments confirmed the relative stereochemistry at the tet-

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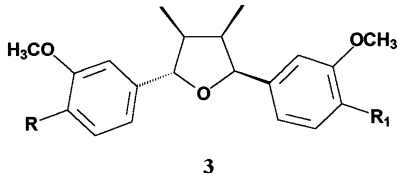
Table 1. ^{1}H NMR Spectral Data of **2d**, **3b**, and **3c** (400 MHz, $CDCl_3$)[a]

position	2d	3b	3c
H-7	4.57 d (9.6)	4.66 d (9.3)	4.64 d (9.1)
H-7′	5.15 d (8.6)	5.46 d (4.3)	5.46 d (4.5)
H-8	2.05−1.97 m	2.47−2.41 m	2.49−2.40 m
H-8′	2.31−2.21 m	2.47−2.41 m	2.49−2.40 m
H-9	1.17 d (6.3)	1.00 d (6.3)	1.00 d (6.6)
H-9′	0.71 d (7.1)	0.62 d (7.0)	0.62 d (7.0)
Ar-OH	8.17 s	5.53 s	5.56 s
OCH_3	3.88; 3.85; 3.83; 3.82 s (3H each)	3.90; 3.89; 3.88 s (3H each)	3.90; 3.88; 3.87 s (3H each)
H-2	6.57 s	6.96 d (1.8)	6.95 d (1.8)
H-2′	6.77 d (2.0)	6.94 d (2.0)	6.92 d (1.3)
H-5	6.51 s	6.85 d (8.1)	6.89 d (8.1)
H-5′	6.85 d (8.1)	6.91 d (8.8)	6.87−6.85 m
H-6		6.92 dd (1.8; 8.1)	6.87−6.85 m
H-6′	6.78 dd (2.0; 8.1)	6.78 dd (2.0; 8.8)	6.87−6.85 m

[a] The assignments are based on HMQC, HMBC, and NOE-DIFF.

rahydrofuran ring as *trans* (C-7/C-8), *cis* (C-8/C-8′), and *cis* (C-8′/C-7′). These spectroscopic data allowed the establishment of a *cis* relationship between H-7 and the two methyl groups, since enhancement of the hydrogens H-9 and H-9′ was observed after irradiation of the H-7 resonance at δ 4.66. A similar NOE effect was observed on H-7 and H-9′ (δ 0.62) after irradiation of the methyl hydrogens at 1.00 (H-9), which allowed the assignment of a *cis* configuration for them. Thus, a *cis* configuration was established between the aromatic group at C-7′ and methyl groups at C-9 and C-9′.



3

R	R_1
a OCH_3	OCH_3
b OCH_3	OH
c OH	OCH_3

The IR absorption band near 3400 cm^{-1} displayed for **3b** and the ^{1}H NMR chemical shift observed at δ 5.53 (s, 1H), suggested the presence of a hydroxyl group. The ^{1}H NMR showed six aromatic hydrogens as two systems, one at δ 6.96 (1H, d, J = 1.8 Hz), 6.85 (1H, d, J = 8.1 Hz), and 6.92 (1H, dd, J = 1.8, 8.1 Hz) and another at δ 6.94 (1H, d, J = 2.0 Hz), 6.91 (1H, d, J = 8.8 Hz), and 6.78 (1H, dd, J = 2.0, 8.8 Hz), indicating the presence of two 1,3,4-trisubstituted benzene rings. The chemical shifts observed for aromatic hydrogens along with the presence of three singlets corresponding to *O*-methyl hydrogens at δ 3.90, 3.89, and 3.88 (s, 3H each) indicated the presence of both the veratryl (3,4-dimethoxyphenyl) and the guaiacyl (3-methoxy-4-hydroxyphenyl) groups in this compound. The ^{13}C NMR spectrum (Table 2) corroborated the assignments made for the structural determination of both aromatic rings.

The point of attachment of each aromatic group to the tetrahydrofuran ring was determined by HMQC and HMBC experiments. In the HMQC experiment, mutual cross-peaks were observed between the aromatic hydrogens at δ 6.94 (H-2′), 6.91 (H-5′), and 6.78 (H-6′) to the carbons at δ 108.7 (C-2′), 113.9 (C-5′), and 118.8 (C-6′), respectively. Also, in the HMBC spectrum (Table 2), cross-peaks were

Table 2. NMR Spectral Data of **2d**, **3b**, and **3c** [^{13}C (100 MHz) and HMBC (100/400 MHz), $CDCl_3$][a]

	2d		3b		3c	
C	δ_C	HMBC (C→H)	δ_C	HMBC (C→H)	δ_C	HMBC (C→H)
1	114.2	H-5, H-8, ArOH	138.1	H-5	135.1	H-5
2	111.2	H-7	109.7		108.4	H-7
3	142.1	H-5	148.4	H-5	146.7	
4	148.4		151.5		145.1	H-2, H-6
5	101.7	ArOH	110.9		114.0	ArOH
6	150.1	H-2, H-7, ArOH	118.4	H-7	119.3	H-2, H-7
7	87.8	H-2, H-8, H-9	85.7	H-2, H-9	85.8	H-2, H-6, H-9
8	46.9		43.4	H-2	43.5	
9	15.6	H-7, H-8	11.9		11.8	
1′	131.8	H-5′, H-8′	132.6	H-5′	133.3	H-5′
2′	110.1	H-6′, H-7′	108.7	H-6′	109.5	
3′	148.8		147.0	H-5′	148.8	
4′	149.7		144.3	H-2′, H-6′	147.8	H-2′
5′	110.8		113.9	ArOH	110.9	
6′	119.3	H-2′, H-7′	118.8		118.1	H-2′
7′	84.4	H-8′, H-9′	84.8	H-2′, H-9′	84.7	H-2′, H-9′
8′	45.2		47.5		47.6	
9′	14.9	H-8′	10.3		9.4	
OMe	55.8		55.9		55.9	
OMe	55.8		55.9		55.9	
OMe	55.9		55.9		55.9	
OMe	55.9					

[a] ^{13}C assignments are based on HMQC, HMBC, and NOE-DIFF.

observed between H-2′/C-7′and C-4′ and between ArOH/C-5′. Additionally, a mutual correlation was observed between H-6′/C-4′ and C-2′ and between H-2/C-7. These data indicate the presence of the guaiacyl and veratryl groups at C-7′ and C-7, respectively. The HRMS data using electrospray ionization (ESI) in the negative ion mode showed an $[M - H]^-$ ion at m/z 357.1707, consistent with the proposed molecular formula: $C_{21}H_{26}O_5$.

These data permitted the establishment of the structure of the new tetrahydrofuran lignan **3b** as *rel*-(7*S*,8*S*,7′*S*,8′*R*)-3,4,3′-trimethoxy-4′-hydroxy-7,7′-epoxylignan.

The chemical shifts, coupling constants, and NOE-DIFF data of compound **3c** were quite similar to those of compound **3b**, differing only at the positions of the aromatic groups at the carbinolic carbons C-7 and C-7′ in **3c**. The veratryl and guaiacyl groups are bound to C-7′ and C-7, respectively, as observed in the HMQC and HMBC spectra. In the HMQC experiments, mutual cross-peaks between the aromatic hydrogens H-7 (δ 4.64), H-5 (δ 6.89), and H-2 (δ 6.95) respectively to carbons at δ 85.8 (C-7), 114.0 (C-5), and 108.4 (C-2) were observed. In the HMBC (Table 2), correlations between H-2/C-7, C-4, and C-6 were observed. In addition, a mutual correlation was observed between the Ar-OH at δ 5.56 and the signal corresponding to C-5 (δ 114.0). Additional evidence for the presence of both aromatic rings was also provided by analysis of its HRMS data, which showed an $[M - H]^-$ ion at m/z 357.1791, corresponding to a molecular formula $C_{21}H_{26}O_5$ and confirming **3c** as an isomer of **3b**. Therefore, the structure of the new lignan **3c** was established as *rel*-(7*S*,8*R*,7′*S*,8′*S*)-3,4′,3′-trimethoxy-4-hydroxy-7,7′-epoxylignan.

Compound **2d** was isolated as a colorless oil. The HRMS showed an $[M - H]^-$ ion at m/z 387.1913, consistent with the formula $C_{22}H_{28}O_6$. The IR spectrum revealed the presence of a hydroxyl group at 3336 cm^{-1}.

Analysis of the ^{1}H NMR spectrum suggested that **2d** is a lignan, exhibiting chemical shifts for the oxybenzylic hydrogens H-7 (δ 4.57, d, J = 9.6 Hz) and H-7′ (5.15, d, J = 8.6 Hz) and for methyl hydrogens H-9 (1.17, d, J = 6.3 Hz) and H-9′ (0.71, d, J = 7.1 Hz), reminiscent of a tetrahydrofuran moiety.

Table 3. In Vitro Trypanocidal Activity of the Crude Extract and Pure Compounds from *N. megapotamica* against the Y Strain of *T. cruzi*[a]

	concentration (μM) × % of lysis (±SD)			
compound	2.0	8.0	32.0	IC_{50} (μM)
1a	54.8 ± 4.1	53.6 ± 5.2	94.0 ± 3.2	2.2
1b	44.0 ± 9.7	56.3 ± 1.4	59.9 ± 2.5	4.4
1c	10.2 ± 8.9	13.5 ± 3.6	18.0 ± 9.4	17 407.0
1d	23.0 ± 3.6	25.0 ± 4.4	48.0 ± 3.0	47.3
2a	21.4 ± 4.9	49.2 ± 9.9	60.3 ± 5.5	12.6
2c	34.2 ± 3.1	46.8 ± 5.5	47.6 ± 8.2	34.8
3a	36.5 ± 2.0	49.2 ± 7.3	55.6 ± 7.3	12.2
crude extract (4.0 mg/mL) 30.6 ± 9.3				
$CHCl_3$ fraction (2.0 mg/mL) 100 ± 0				

[a] Positive control: gentian violet at 250 μg/mL (IC_{50} = 76 μM) displayed 100% lysis. Negative control: infected blood plus 1% DMSO.

The chemical shifts and coupling constants of oxybenzylic and methyl hydrogens indicated the configuration of the tetrahydrofuran ring as for veraguensin.[14,15] The differential NOE spectra confirmed the relative stereochemistry as *trans*(C-7/C-8)−*trans*(C-8/C-8′)−*cis*(C-8′/C-7′), since it showed enhancement of signals for H-7, H-8′, and H-9 when the hydrogen H-7′ (δ 5.15) was irradiated. As was previously reported for veraguensin type tetrahydrofuran lignans,[19,20] the coupling constants of oxybenzylic hydrogens indicate dihedral angles of approximately 150° and 0° between H-7/H-8 and H-7′/H-8′, respectively. This conformation of the tetrahydrofuran ring may be devoid of the mutual steric repulsion of the three *cis* substituents at C-7 (Ar), C-8′ (Me), and C-7′ (Ar′).[16,19]

The ^{1}H NMR spectrum exhibited chemical shifts for four *O*-methyl groups at δ 3.82, 3.83, 3.85, and 3.88 (each 3H, s) and five aromatic proton signals, two observed as singlets at δ 6.57 (H-2) and 6.51 (H-5), indicating that one of the aryl groups was tetrasubstituted.

The HMQC experiment showed correlations between the signals at δ 4.57 (H-7) and 87.8 (C-7) as well as between the multiplet at δ 2.26 (H-8′) and 45.2 (C-8′). In addition, correlations between H-2 (δ 6.57)/C-7 (δ 87.8) and between H-5′ (δ 6.85)/C-1′ (δ 131.8) were observed in the HMBC experiment (Table 2). These data indicated that the 1,2,4,5-tetrasubstituted aromatic ring is bound to C-7 and that the 1,3,4-trisubstituted one is bound to C-7′. Additionally, a mutual correlation between hydrogen at δ 8.17 (1H, s, Ar-OH) and signals corresponding to C-5 (δ 101.7) and C-1 (δ 114.2) was observed. Thus, the structure for the new tetrahydrofuran lignan **2d** was determined as *rel*-(7*S*,8*S*,7′*R*,8′*S*)-3,3′,4,4′-tetramethoxy-6-hydroxy-7,7′-epoxylignan.

Regarding the trypanocidal assay, on one hand it was observed that the crude *N. megapotamica* extract at a concentration of 4.0 mg/mL was inactive (30.6 ± 9.3), but on the other hand the $CHCl_3$ fraction, at a concentration of 2.0 mg/mL, was fully active (100.0 ± 0.0) (Table 3). Therefore, for the crude extract there might be either antagonist compounds or low lignan content, which could lead to its inactivity. On the other hand, for the chloroform fraction there might be either additive or synergistic effects, since the lignans are concentrated in this fraction. For the pure isolated compounds, it was observed that compound **1a** was the most active, showing an IC_{50} value of 2.2 μM and lysis of 94% of the parasites at 32 μM. Compound **2a** is quite similar to compound **1a**, as both bear a methylenedioxy and two methoxy groups, differing only by the C-8′ stereochemistry, which may interfere with its trypanocidal activity, since compound **2a** showed an IC_{50} value of 12.6 μM and lysis of 63%, which is in accordance with the findings of Abe et al.[21] for related lignans. A similar situation could be observed for compounds **1b** and **3a**. Both compounds possess four aromatic *O*-methyl groups, but differ only by the C-7′ stereochemistry, and show similar percentage of parasite lysis and IC_{50} values of 4.4 and 12.2 μM, respectively. These results indicate that not only the nature of the aromatic substituents but also the stereochemistry of the furan ring play important roles in the activity against trypomastigote forms of *T. cruzi*. This became clear with the presence of aromatic hydroxy groups, which is suggested to reduce its trypanocidal activity, as observed for compounds **1c**, **1d**, and **2c** (IC_{50} = 17 407.0, 47.3, and 34.8 μM, respectively). Therefore, a greater number of compounds of this class, bearing different patterns of substituent groups at the aromatic rings, as well as possessing different stereochemistry at carbons 7, 7′ and 8, 8′, should be evaluated to determine its quantitative structural activity relationship (QSAR). Also, this work ratifies the potential of this class of lignan, as observed by Lopes et al. (1998),[9] for the development of new chemoprophylactic trypanocidal agents to replace gentian violet, which displayed 100% activity at 250 μg/mL (IC_{50} = 76 μM) against trypomastigote forms of the parasite in the blood at 4 °C. Despite its good activity, gentian violet causes alteration of blood color,[8] is mutagenic,[22] and is rejected by the patients. Moreover, Zhang et al. (2001)[23] demonstrated that grandisin, a tetrahydrofuran lignan, showed low cytotoxicity, which was similar to that observed for chloroquine in antimalarial assays. Therefore, this class of compounds should be considered for further studies.

Experimental Section

General Experimental Procedures. Optical rotations were measured at λ 589 nm on a Shimidt-Haenseh polartronic HH8 polarimeter using 1.0 cell. IR spectra were recorded on a Nicolet FT-IR Protegé 520 instrument. NMR spectra were recorded on a Brucker ARX 400 spectrometer. Samples were dissolved in $CDCl_3$, and the spectra were calibrated at the solvent signals at δ 7.26 (^{1}H) or δ 77.0 (^{13}C). ESIMS data were obtained using a Micromass Quattro LC system. HRMS data were obtained in a Micromass QTOF hybrid quadrupole orthogonal time-of-flight mass spectrometer operating at 7.000 mass resolution. Vacuum-liquid chromatography (VLC) was carried out with silica gel 60H 100−200 mesh ASTM (Merck), in glass columns with 5−10 cm i.d. Flash chromatography was carried out with silica gel 230−400 mesh (Merck) in a 450 × 25 mm glass column at 5 mL/min. Semipreparative HPLC separation analyses were carried out on a Shimadzu SCL-10 AVP liquid chromatography system equipped with a SPD-M10AVP Shimadzu UV-DAD detector (the channel was set at 281 nm) and a Shimadzu column (ODS, 250 × 20 mm, 15 μm).

Plant Material. The leaves of *N. megapotamica* (Spreng) Chodat et Hassler were collected by Ademar A. S. Filho in August of 1999 in Ribeirão Preto, SP, Brazil. The plant was kindly authenticated by Ida de Vattimo, and a voucher specimen was deposited in both the Herbarium of the Botanical Department of the Biology Institute, Federal University of Rio de Janeiro-UFRJ, under number 8634, and the Herbarium of the Biology Department of the University of São Paulo-Ribeirão Preto, under number SPFR 05655.

Extraction and Isolation. Air-dried, powdered leaves (1450 g) were exhaustively extracted with EtOH−H_2O (9:1) at room temperature. The filtered extract was concentrated under vacuum to furnish 240 g of crude extract, which was dissolved in MeOH−H_2O (7:3), followed by sequential partition with hexane (16.0 g), $CHCl_3$ (12.0 g), and *n*-BuOH (94.0 g). The $CHCl_3$ fraction was chromatographed over silica gel under a VLC system, using hexane−EtOAc mixtures in increasing

proportions as eluent, to afford six fractions, based on their trypanocidal activity obtained at 2.0 mg/mL that led to total lysis of *T. cruzi*. The resulting fractions III (hexane−EtOAc, 1:1; 1.38 g) and IV (hexane−EtOAc, 4:6; 0.51 g) were submitted to flash column chromatography over silica gel, using hexane−EtOAc (9:1) as mobile phase, followed by semipreparative HPLC (MeOH−H_2O, 75:25), furnishing the following compounds: machilin-G **1a** (29 mg) and calopeptin **2a** (22 mg) from fraction III and galgravin **1b** (60 mg), veraguensin **2b** (70 mg), and ganschisandrine **3a** (14 mg) from fraction IV. Fraction V (0.52 g) was submitted to a VLC system over silica gel 60H, eluted with hexane−EtOAc at increasing proportions to furnish six additional subfractions. Subfractions V.3 (hexane−EtOAc, 7:3; 90 mg), V.4 (hexane−EtOAc, 1:1; 160 mg), and V.5 (hexane−EtOAc, 3:7; 40 mg) were submitted to analytical HPLC (ODS column, 250 × 4.6 mm, 5 μm; MeOH−MeCN−H_2O, 60:5:35) to afford the following compounds: nectandrin-A **1c** (3 mg) and aristolignin **2c** (2 mg) from fraction V.3; nectandrin-B **1d** (4 mg), **3b** (2 mg), and **3c** (3 mg) from fraction V.4; and **2d** (4 mg) from fraction V.5.

Parasites. The crude extract, chromatographic fractions, and pure compounds were tested against the Y strain of *T. cruzi*, which is maintained in the Parasitology Laboratory of the Faculty of Pharmaceutical Sciences−USP, Ribeirão Preto, SP, Brazil.[24]

Trypanocidal Activity. The bioassays were carried out using trypomastigote forms of the parasite obtained from mouse macrophage culture cells. Approximately 2×10^6 parasites/mL were incubated in RPMI 1640 medium (Sigma) in 96-well microtiter plates containing the test compounds. The compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted in blood to give 2.0, 8.0, and 32.0 μM, respectively, as final concentrations of the tested compounds, as well as 1% of DMSO. The crude extract was evaluated at 4.0 mg/mL and the $CHCl_3$ fraction at 2.0 mg/mL. The plates were incubated at 4 °C, and the lysis percentage was determined by an MTT colorimetric method.[25] The bioassays were performed in triplicate. Infected blood added with the same volume of pure DMSO was used as negative control, and gentian violet was used as positive control (250 μg/mL).

***rel*-(7*S*,8*S*,7′*R*,8′*S*)-3,4,3′,4′-Tetramethoxy-6′-hydroxy-7,7′-epoxylignan (2d):** colorless oil; $[\alpha]^{26}_D$ +1.34° (*c* 0.010), IR ($CHCl_3$) ν_{max} (cm^{-1}) 3336, 2922, 1514, 1458, 1261, 1200, 1129, 1025; ^1H, ^{13}C NMR and HMBC data, see Tables 1 and 2; ESIMS *m*/*z* 387 $[M - H]^-$ (100), 305 (16), 249 (80), 62 (11); HRMS *m*/*z* $[M - H]^-$ 387.1913 (calcd for $C_{22}H_{27}O_6$, 387.1808).

***rel*-(7*S*,8*R*,7′*S*,8′*S*)-3,4,3′-Trimethoxy-4′-hydroxy-7,7′-epoxylignan (3b):** colorless oil; $[\alpha]^{26}_D$ +1.87° (*c* 0.008, $CHCl_3$); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3441, 2959, 2928, 1603, 1514, 1459, 1266, 1235, 1153, 1029; ^1H, ^{13}C NMR and HMBC data, see Tables 1 and 2; ESIMS *m*/*z* 357 $[M - H]^-$ (70), 305 (24), 249 (100), 181 (14), 137 (4); HRMS *m*/*z* $[M - H]^-$ 357.1707 (calcd for $C_{21}H_{25}O_5$, 357.1702).

***rel*-(7*S*,8*R*,7′*S*,8′*S*)-3,4′,3′-Trimethoxy-4-hydroxy-7,7′-epoxylignan (3c):** colorless oil; $[\alpha]^{26}_D$ +1.09° (*c* 0.011, $CHCl_3$); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3440, 2955, 2931, 1613, 1514, 1458, 1261, 1235, 1161, 1136, 1029; ^1H, ^{13}C NMR and HMBC data, see Tables 1 and 2; ESIMS *m*/*z* 357 $[M - H]^-$ (89), 283 (28), 249 (100), 181 (45), 167 (25), 137 (11), 89 (57); HRMS *m*/*z* $[M - H]^-$ 357.1791 (calcd for $C_{21}H_{25}O_5$, 357.1702).

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