

Constituents of the Leaves of *Magnolia ovata*

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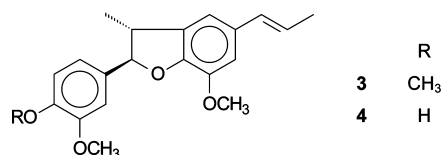
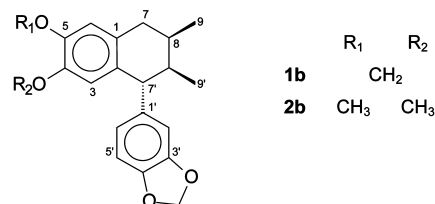
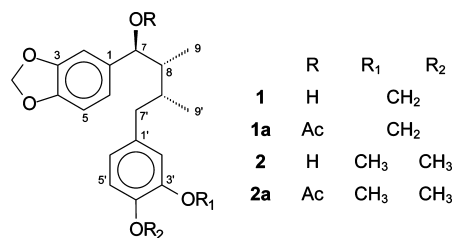
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Two new lignans, magnovatins A (**1**) and B (**2**), along with nine known compounds, were isolated from the leaves of *Magnolia ovata*. The known compounds were identified as acuminatin (**3**), licarin A (**4**), kadsurenin M, 4-*O*-demethylkadsurenin M, oleiferin A, oleiferin C, spathulenol, parthenolide, and 11,13-dehydrocompressanolide. In addition, compounds **1** and **2** yielded four new derivatives (**1a**, **1b**, **2a**, and **2b**). The structures of the new compounds were established on the basis of spectrometric data evaluation. Free-radical scavenging and antimicrobial activities of the major compounds **1**, **3**, and **4** were investigated.

Magnolia ovata (A. St.-Hil.) Spreng (formerly *Talauma ovata* A. St.-Hil.) (Magnoliaceae) is a medicinal plant that is widely distributed in Brazil. Its trunk bark has been used in the treatment of fever, while the leaves are considered useful in treating diabetes. However, preliminary pharmacological studies failed to demonstrate a hypoglycemic effect of crude extracts of the leaves of this plant.¹ On the other hand, an antipyretic activity was recently shown.² Early phytochemical investigations of the roots of *M. ovata* led to the isolation of the cytotoxic sesquiterpene lactone costunolide,³ while more recent studies of the trunk bark resulted in the isolation and characterization of alkaloids, sesquiterpenes, and neolignans.^{4,5} Crude extracts, from leaves and trunk bark, showed antibacterial activity, related with the presence of alkaloids.⁶ The composition and antimicrobial activity of the essential oils were also previously reported.^{7–9} The present work describes a phytochemical study of the leaves of *M. ovata* and evaluation of antioxidant and antimicrobial activities of the major compounds obtained.

Successive chromatographic fractionation of extracts in hexane and dichloromethane from the leaves of *M. ovata* yielded two new lignans, namely, magnovatins A (**1**) and B (**2**), along with nine known compounds. The structures of **1** and **2** were established by spectroscopic data interpretation.

Compound **1** was isolated as a colorless oil with the molecular formula C₂₀H₂₂O₅, as determined by GC-HRMS. The ¹H NMR spectrum (Table 1) showed signals due to the presence of two secondary methyl groups at δ 0.61 (d, *J* = 7.1 Hz) and 0.88 (d, *J* = 6.9 Hz), two methine protons at δ 1.82 (ddq, *J* = 9.5, 2.9, 7.1 Hz) and 2.32 (dddq, *J* = 11.6, 3.8, 2.9, 6.9 Hz), a benzylic methylene at δ 2.13 (dd, *J* = 13.1, 11.6 Hz) and 2.84 (dd, *J* = 13.1, 3.8 Hz), and an oxymethine proton at δ 4.41 (d, *J* = 9.5 Hz). The presence of two piperonyl groups was demonstrated by doublets in the ¹H NMR spectrum at δ 5.960 and 5.963 (d, *J* = 1.5 Hz) and δ 5.925 and 5.927 (d, *J* = 1.4 Hz) (methylenedioxyphenyl groups) and multiplets of six aromatic protons in the range δ 6.6–6.9. The ¹³C{¹H} NMR spectrum (Table 1) exhibited peaks for 20 carbon atoms. In particular, the carbons of methyl groups were observed at δ 11.4 and 17.7, while the oxymethine carbon was observed at δ 76.9. The coupling constant between the protons H-7 and H-8 of 9.5 Hz was consistent with a *threo* configuration between these



hydrogens, while the ¹H NMR chemical shifts of the methyl groups were compatible with an *erythro* relationship,¹⁰ which was confirmed by 1D NOE experiments. Selective irradiation of the resonance frequency of the H-9 proton at δ 0.61 caused a NOE enhancement of the signals of the protons H-9', H-7, H-8, and H-7' at δ 2.13. Moreover, selective irradiation of the resonance frequency of the H-9' at δ 0.88 showed a NOE enhancement of the signals of H-9, H-8', and H-7' at δ 2.13, while the irradiation of the H-8' at δ 2.32 caused a NOE enhancement mainly of the signals of the H-8, as well as H-9', but not for H-9. In addition, the coupling constant of 11.6 Hz observed for H-7' at δ 2.13 supported a *threo* relationship with H-8', in full accordance with the NOE results. The overall analysis of 1D NOE, ¹H–¹³C one-bond and long-range NMR correlation experiments permitted the unambiguous ¹H and ¹³C NMR chemical shift assignments for **1** to be made. Therefore, compound **1** was identified as *rel*-(7*S*,8*R*,8'*R*)-3,4:3',4'-bis(methylenedioxy)lignan-7-ol and named magnovatin A.

Compound **2** was isolated as a colorless oil with the molecular formula C₂₁H₂₆O₅, as determined by HRESIMS. The ¹H and

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Table 1. NMR Spectroscopic Data (400 MHz, CDCl₃) for Compounds **1**, **2**, **1a**, and **2a**^a

position	1		2		1a		2a	
	δ_C mult.	δ_H mult. (<i>J</i> in Hz)	δ_C	δ_H mult. (<i>J</i> in Hz)	δ_C	δ_H mult. (<i>J</i> in Hz)	δ_C	δ_H mult. (<i>J</i> in Hz)
1	138.5, qC		138.5		134.1		134.0	
2	107.0, CH	6.86 m	106.9	6.87 m	107.5	6.83 d (1.4)	107.5	6.85 d (1.6)
3	147.8, qC		147.8		147.7		147.7	
4	147.0, qC		147.0		147.2		147.2	
5	107.8, CH	6.76 m	107.9	6.79 m	107.9	6.76 d (7.8)	108.0	6.77 d (8.0)
6	120.4, CH	6.77 m	120.4	6.79 m	121.3	6.82 dd (7.8, 1.4)	121.3	6.79 m
7	76.9, CH	4.41 d (9.5)	77.0	4.42 d (9.6)	77.9	5.59 d (10.4)	77.9	5.67 d (10.4)
8	45.0, CH	1.82 ddq (9.5, 2.9, 7.1)	45.1	1.84 ddq (9.6, 2.9, 7.0)	43.0	2.02 m	43.2	2.02 m
9	11.4, CH ₃	0.61 d (7.1)	11.4	0.63 d (7.0)	11.3	0.61 d (7.2)	11.2	0.67 d (6.8)
1'	136.0, qC		134.8		135.5		134.4	
2'	109.5, CH	6.72 d (1.6)	112.4	6.76 m	109.4	6.61 d (1.6)	112.3	6.68 m
3'	147.4, qC		148.7		147.4		147.4	
4'	145.4, qC		147.0		145.5		145.5	
5'	107.9, CH	6.73 d (6.6)	111.1	6.79 m	108.0	6.73 d (8.0)	108.0	6.79 d (8.8)
6'	121.9, CH	6.65 dd (6.6, 1.6)	121.0	6.76 m	121.9	6.57 dd (8.0, 1.6)	121.0	6.68 m
7'	37.2, CH ₂	2.84 dd (13.1, 3.8) 2.13 dd (13.1, 11.6)	37.0	2.87 dd (13.2, 3.9) 2.17 dd (13.2, 10.5)	37.1	2.76 dd (12.8, 3.2) 2.11 dd (12.8, 10.8)	37.0	2.80 dd (13.2, 2.8) 2.08 dd (13.2, 3.2)
8'	35.2, CH	2.32 dddq (11.6, 3.8, 2.9, 6.9)	35.0	2.36 dddq (10.5, 3.9, 2.9, 6.9)	35.6	2.00 m	35.7	2.02 m
9'	17.7, CH ₃	0.88 d (6.9)	17.9	0.88 d (6.9)	17.5	0.88 d (6.4)	17.6	0.88 d (6.4)
OH-7		1.86 brs		1.76 brs				
OCH ₂ O-3,4	101.0	5.963 d (1.5) 5.960 d (1.5)	101.0	5.955 d (1.4) 5.953 d (1.4)	101.0	5.96 d (1.4) 5.95 d (1.4)	101.1	5.97 d (1.4) 5.96 d (1.4)
OCH ₂ O-3',4'	100.7	5.927 d (1.4) 5.925 d (1.4)			100.7	5.93 s		
CH ₃ O-3'			55.9	3.88 s			55.7	3.88 s
CH ₃ O-4'			55.8	3.87 s			55.9	3.87 s
CO-7					170.2		170.2	
CH ₃ CO-7					21.3	2.08 s	21.4	2.08 s

^a Unambiguous ¹H and ¹³C NMR chemical shifts were established by one-bond and long-range ¹H–¹³C correlation experiments. The exact signal multiplicities were obtained with the help of the first-order multiplet simulator/check FOMSC3.¹⁹

¹³C{¹H} NMR spectra (Table 1) were very similar to those of **1**, but with a 3,4-dimethoxyphenyl group replacing one methylenedioxyphenyl group. The location of the remaining methylenedioxyphenyl group was established by ¹H–¹³C long-range correlations. Moreover, the GC-EIMS exhibited a base peak at *m/z* 151, which represents the fragment [CH₂O₂C₆H₃CHOH]⁺, supporting a methylenedioxyphenyl group associated with a hydroxy group at C-7. The coupling constant between H-7 and H-8 (9.6 Hz) as well as the ¹H NMR chemical shifts of the methyl groups at δ 0.63 and 0.88 and the NOE results indicated that **2** has the same relative configuration as compound **1**. Therefore, compound **2** was identified as *rel*-(7*S*,8*R*,8'*R*)-3,4-methylenedioxy-3',4'-dimethoxylignan-7-ol and named magnovatin **B**.

Acetylation of **1** and **2** with acetic anhydride and pyridine yielded **1a** and **2a**, respectively. The ¹H NMR spectrum of **1a** (Table 1) was similar to that of **1**, including NOE observations, except for an additional acetyl signal at δ 2.08 (s) and the chemical shift change of the oxymethine proton from δ 4.41 to 5.59. Moreover, the ¹³C{¹H} NMR spectrum (Table 1) showed signals compatible with an acetyl derivative of **1**. The ¹H and ¹³C{¹H} NMR spectra (Table 1) of **2a** were very similar to those of **1a**, but showed signals for a 3,4-dimethoxyphenyl group replacing one methylenedioxyphenyl group.

In order to determine the absolute configuration,¹¹ compounds **1** and **2** were submitted to an acid-catalyzed cyclization, yielding the new lignans **1b** and **2b**, respectively. The cyclization was evident from the ¹H and ¹³C{¹H} spectra (Table 2), which showed signals of one dibenzylic methine in **1b** and **2b** (δ_H 3.61 and 3.67; δ_C 51.2 and 51.0, respectively), typical of 2,7'-cyclo lignans. The coupling constants between H-7' and H-8' (6.0 and 5.6 Hz for **1b** and **2b**, respectively) were not consistent for diaxial or diequatorial protons, but it is known that 2,7'-cyclo lignans with a *trans*-7',8'-*cis*-8,8' relative configuration exist as a mixture of conformers in equilibrium.¹² This fact was supported by the NOE experiments obtained by selective irradiation of the resonance frequency of the H-7' protons at δ 3.61 and 3.67 for **1b** and **2b**, respectively, which showed a strong NOE enhancement on the signal of the H-9' protons and a weak NOE enhancement of the signals for H-9. In the CD spectrum negative (around 280 nm) and positive (around 290 nm)

Cotton effects were observed for both compounds, showing that **1b** and **2b** had the absolute configuration 8*R*, 7'*R*, 8'*R*, as in the known 2,7'-cyclo lignan dimethylisoguaiaicin.¹² Therefore, since C-7 undergoes inversion of configuration during the cyclization process (S_N2 mechanism), the absolute configuration of **1** and **2** was assigned as 7*S*, 8*R*, 8'*R*.

The known compounds isolated from *M. ovata* were identified by comparison of their spectroscopic data with those reported in the literature as acuminatin (**3**),¹³ licarin A (**4**),¹⁴ kadsurenin M,¹⁵ 4-*O*-demethylkadsurenin M,¹⁵ oleiferin A,¹⁶ oleiferin C,¹⁶ spathulenol,¹⁷ parthenolide,¹⁸ and 11,13-dehydrocompressanolid.¹⁸

The major compounds **1**, **3**, and **4** were tested for free-radical scavenging activity using the DPPH test and for antimicrobial activity. Compound **4** showed a significant concentration-dependent free-radical scavenging activity with a SC₅₀ of 56.1 (1.20) μ g mL⁻¹, while compounds **1** and **3** were inactive under the test conditions used. The SC₅₀ values of the positive controls quercetin and Trolox were 13.0 (2.10) and 2.20 (1.25) μ g mL⁻¹, respectively. These three compounds were also screened for antimicrobial activity, but were inactive against all microorganisms tested (MIC > 100 μ g mL⁻¹).

Experimental Section

General Experimental Procedures. Optical rotations were measured in CHCl₃ on a Rudolph Research polarimeter. The UV spectra were obtained in MeOH on a Shimadzu UV-2401PC spectrophotometer. The circular dichroism (CD) spectra were measured in hexane–ethanol (1:1, v/v) on a JASCO CD-2095 Plus chiral detector. The IR spectra were recorded in KBr pellets on a Biorad FTIR spectrophotometer. 1D and 2D NMR experiments were acquired in CDCl₃ at 295 K on a Bruker AVANCE 400 NMR spectrometer operating at 9.4 T, observing ¹H and ¹³C at 400 and 100 MHz, respectively. The spectrometer was equipped with a 5 mm multinuclear direct detection probe with a *z*-gradient. All ¹H and ¹³C NMR chemical shifts are given in ppm (δ) and were related to the TMS signal at 0.00 ppm as internal reference, with the coupling constants (*J*) in Hz. Precise signal multiplicities were inferred or determined with the help of the first-order multiplet simulator FOMSC3.¹⁹ High-resolution mass spectra were obtained on a HP-5000 Shimadzu GC-HRMS and/or on a Micromass ESI-QqTof mass spectrometer. GC-MS analyses were performed using a HP5-MS column (30 m \times 0.25 mm \times 2.25 mm). Silica gel (Merck, 230–400

Table 2. NMR Spectroscopic Data (400 MHz, CDCl₃) for the Cyclolignans **1b** and **2b**^a

position	1b		2b	
	δ_C mult.	δ_H mult. (<i>J</i> in Hz)	δ_C	δ_H mult. (<i>J</i> in Hz)
1	129.3, qC		128.4	
2	130.7, qC		129.3	
3	110.1, CH	6.30 s	113.2	6.34 s
4	145.7, qC		147.1	
5	145.8, qC		147.4	
6	108.3, CH	6.56 s	111.2	6.59 s
7	35.4, CH ₂	2.85 dd (16.6, 5.4) 2.44 dd (16.6, 7.5)	34.6	2.84 dd (16.6, 5.5) 2.45 dd (16.6, 8.2)
8	28.8, CH	2.02 dddq (7.5, 6.9, 5.4, 3.1)	28.4	2.02 dddq (8.2, 6.9, 5.5, 3.1)
9	16.0, CH ₃	0.88 d (6.9)	16.6	0.90 d (6.9)
1'	141.1, qC		141.3	
2'	109.3, CH	6.48 d (1.7)	109.4	6.485 d (1.7)
3'	147.4, qC		147.3	
4'	145.6, qC		145.5	
5'	107.6, CH	6.70 d (7.9)	107.6	6.70 d (8.4)
6'	122.2, CH	6.51 dd (7.9, 1.7)	122.1	6.486 dd (8.4, 1.7)
7'	51.2, CH	3.61 d (6.0)	51.0	3.67 d (5.6)
8'	40.7, CH	1.90 ddq (6.9, 6.0, 3.1)	40.9	1.90 ddq (6.9, 5.6, 3.1)
9'	15.5, CH ₃	0.89 d (6.9)	15.3	0.91 d (6.9)
OCH ₂ O-4,5	100.5	5.855 d (1.4) 5.852 d (1.4)		
OCH ₂ O-3',4'	100.8	5.914 d (1.4) 5.908 d (1.4)	100.8	5.918 d (1.4) 5.916 d (1.4)
CH ₃ O-4			55.8	3.69 s
CH ₃ O-5			55.7	3.87 s

^a Unambiguous ¹H and ¹³C NMR chemical shifts were established by one-bond and long-range ¹H-¹³C correlation experiments. The exact signal multiplicities were obtained with the help of the first-order multiplet simulator/check FOMSC3.¹⁹

mesh) was used for column chromatographic separation, while silica gel 60 PF₂₅₄ (Merck) was used for analytical (0.25 mm) and preparative (1.0 mm) TLC. Compounds were visualized by exposure under UV_{254/366} light and spraying with 5% (v/v) H₂SO₄ in ethanol solution, followed by heating on a hot plate.

Plant Material. Leaves of *M. ovata* were collected in São Paulo, SP, Brazil, in January 2003, and were identified by one of the authors (R.M.-S.). A voucher specimen (Mello-Silva 1820) was deposited in the Herbarium of Universidade de São Paulo.

Extraction and Isolation. Dried and powdered leaves of *M. ovata* (442 g) were extracted, at room temperature, with hexane and dichloromethane, successively. The hexane extract (11.8 g) was submitted to partition between hexane and MeOH-H₂O (9:1). The MeOH-H₂O layer (2.9 g) was combined with the dichloromethane extract (9.1 g) and subjected to flash silica gel column chromatography (CC), eluted in a gradient system with increasing concentrations of EtOAc in petroleum ether, to give seven fractions. After TLC analysis, these fractions were combined into six groups (A-F). Group B (3.7 g) was subjected to silica gel CC, eluted with petroleum ether-EtOAc (7:3), to give 34 fractions. Fractions 1-7 (2.0 g) were subjected to further CC on silica gel, eluted with increasing amounts of EtOAc in hexane until hexane-EtOAc (7:3), to give 41 fractions, which were combined into seven groups (Ib-VIIb). Group Ib (65 mg) was purified by silica gel preparative TLC, eluted with hexane-EtOAc (9:1), to give spathulenol (7.3 mg). Group Vb (408 mg) was submitted to CC on silica gel, eluted with CH₂Cl₂-EtOAc (5:0.1), to give 17 fractions. Fractions 3-5 (36.0 mg) were purified by silica gel preparative TLC, eluted with hexane-acetone (8:2), to give **1** (20.0 mg). Group VIb (560 mg) was subjected to CC on silica gel, eluted with CH₂Cl₂-EtOAc (5:0.5), to give 54 fractions. Fractions 3-9 (110 mg) were subjected to repeated silica gel preparative TLC, eluted with CH₂Cl₂, to give acuminatin (13.0 mg) and licarin A (19.0 mg). Fractions 10-17 (94 mg) were submitted to CC on silica gel, eluted with hexane-acetone (8:2), to give 19 fractions. Fractions 5-8 (17 mg) were subjected to preparative TLC, eluted with hexane-acetone (8:2), to give licarin A (2.0 mg), kadsurenin M (1.5 mg), and oleiferin C (3.0 mg). Fractions 10-12 (6 mg) were purified by preparative TLC, eluted with hexane-acetone (7:3), to give 4-*O*-demethylkadsurenin M (1.2 mg). Group C (1.4 g) was submitted to CC on silica gel, eluted with hexane-acetone (8:2), to give 55 fractions, which were combined into five groups (Ic-Vc). Group Ic (158 mg) yielded parthenolide almost pure. Group IIc (128.0 mg) was submitted to CC on silica gel, eluted with CH₂Cl₂-acetone (9.8:0.2), to give 73 fractions. Fractions 15-45 (62.0 mg) were subjected to preparative TLC, eluted with hexane-EtOAc-MeOH (8:2:1), to give oleiferin A (10.4 mg) and **2** (8.2 mg).

Group IVc (8.0 mg) was purified by preparative TLC, eluted with hexane-acetone (8:2), to give 11,13-dehydrocompressanolide (1.6 mg).

Magnovatin A (1): colorless oil; [α]_D²⁵ +27.3 (*c* 0.5, CHCl₃); UV λ_{max} (MeOH) (log ϵ) 291 (3.8), 239 (3.9), 208 (4.6) nm; IR ν_{max} (KBr) 3434, 2962, 2889, 1491, 1248, 1034 cm⁻¹; ¹H and ¹³C and NMR data, see Table 1; GC-HRMS *m/z* 342.14658 (29) [M]⁺⁺ (calcd for C₂₀H₂₂O₅ 342.14672), 162.0696 (75), 151.03693 (100), 93.03689 (16).

Magnovatin B (2): colorless oil; [α]_D²⁵ +10.6 (*c* 0.5, CHCl₃); λ_{max} (MeOH) (log ϵ) 287 (3.8), 234 (4.1), 208 (4.7) nm; IR ν_{max} (KBr) 3436, 2960, 2925, 1519, 1247, 1031 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; GC-MS *m/z* 358 (5) [M]⁺⁺, 340 (10), 189 (20), 178 (22), 159 (21), 151 (100), 93 (25); HRESIMS *m/z* 381.1592 [M + Na]⁺ (calcd for C₂₁H₂₆O₅Na 381.1605).

Acetylation with Acetic Anhydride and Pyridine. Compounds **1** (10.0 mg) and **2** (4 mg) were treated with acetic anhydride and pyridine, in the usual manner, to yield **1a** (6.0 mg) and **2a** (3.0 mg).

Acetylmagnovatin A (1a): white powder; mp 50-52 °C; [α]_D²⁵ +34.3 (*c* 0.3, CHCl₃); λ_{max} (CHCl₃) (log ϵ) 291 (4.1), 240 (4.2), 208 (4.9) nm; IR ν_{max} (KBr) 2962, 2925, 1736, 1488, 1248, 1043 cm⁻¹; ¹H and ¹³C and NMR data, see Table 1; GC-MS *m/z* 384 (1) [M]⁺⁺, 324 (20), 238 (25), 162 (100), 151 (42), 135 (47), 77 (35); HRESIMS *m/z* 407.1471 [M + Na]⁺ (calcd for C₂₂H₂₄O₆Na 407.1471).

Acetylmagnovatin B (2a): white powder; mp 77-79 °C; [α]_D²⁵ +34.0 (*c* 0.2, CHCl₃); λ_{max} (CHCl₃) (log ϵ) 285 (3.8), 229 (4.2), 206 (4.7) nm; IR ν_{max} (KBr) 2960, 2923, 1722, 1247 cm⁻¹; ¹H and ¹³C and NMR data, see Table 1; HRESIMS *m/z* 401.2115 [M + H]⁺ (calcd for C₂₃H₂₉O₆ 401.1964).

Acid-Catalyzed Cyclization of 1 and 2. Compounds **1** (5.0 mg) and **2** (4.0 mg) were dissolved in CH₂Cl₂ (3.0 mL), and *p*-toluenesulfonic acid (0.5 mg) was added. The mixtures were kept for 2 h at room temperature and then were neutralized with 5% NaHCO₃. The organic layer was separated, washed with H₂O, and dried with Na₂SO₄. The solvent was evaporated to give **1b** (3.7 mg) and **2b** (2.0 mg).

8R,7R,8'R-4,5:3',4'-Bis(methylenedioxy)-2,7'-cyclo lignan (1b): colorless oil; [α]_D²⁵ -48 (*c* 0.1, CHCl₃); λ_{max} (MeOH) (log ϵ) 294 (3.7), 244 (3.8), 210 (4.5), nm; CD (*c* 0.3, hexane-EtOH) [θ] (nm) -1675 (277), +1810 (292); IR ν_{max} (KBr) 2960, 2923, 1486, 1232, 1040 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; GC-MS *m/z* 324 (76) [M]⁺⁺, 267 (55), 238 (100), 210 (35), 152 (40), 76 (92); HRESIMS *m/z* 325.1440 [M + H]⁺ (calcd for C₂₀H₂₁O₄ 325.1440).

8R,7R,8'R-4,5-Dimethoxy-3',4'-methylenedioxy-2,7'-cyclo lignan (2b): colorless oil; [α]_D²⁵ -11.7 (*c* 0.1, CHCl₃); λ_{max} (MeOH) (log ϵ) 289 (3.7), 235 (3.9), 208 (4.6) nm; CD (*c* 0.1, hexane-EtOH) [θ] (nm) -7350 (279), +3500 (288); IR ν_{max} (KBr) 2957, 2931, 1516,

1486, 1249 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 341.1732 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_4$ 341.1753).

Radical-Scavenging Activity Using the DPPH Method. The antiradical activity of isolated compounds **1**, **3**, and **4** was determined by the radical-scavenging method (DPPH assay) described by Blois.²⁰ The test was performed in 96-well microplates. Fifty microliters of a 250 μM DPPH solution in MeOH was added to a range of methanol solutions of different concentrations (seven serial 3-fold dilutions to give a final range of 100 to 1.6 $\mu\text{g mL}^{-1}$) of tested compounds (10 μL). Quercetin and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were used as positive controls. All samples were tested in triplicate. The antioxidant activity of each sample was expressed as an SC_{50} value, which is the concentration in $\mu\text{g mL}^{-1}$ that scavenged 50% of the DPPH radicals, mean (%RSD, relative standard deviation) of triplicate assays.

Antimicrobial Activity. Compounds **1**, **3**, and **4** were evaluated for antimicrobial activity using the broth microdilution method (96-well microtiter plates), as previously described by Salvador et al.,²¹ to give a concentration between 10 and 1000 $\mu\text{g/mL}$. The minimal inhibitory concentration (MIC) was calculated as the lowest concentration showing complete inhibition of a tested strain. In these tests, bacitracine (0.2 UI mL^{-1}) and ketoconazole (100.0 $\mu\text{g mL}^{-1}$) were used as experimental positive controls, while the solution DMSO–sterile distilled water (5:95, v/v) served as the negative control. Each sensitivity test was performed in duplicate for each microorganism evaluated and repeated three times. The following strains of microorganisms were utilized: Gram-positive bacteria (*Staphylococcus aureus* ATCC 14458, *Staphylococcus epidermidis* ATCC 12228); Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10799); yeasts (*Candida tropicalis* ATCC 157, *C. glabrata* ATCC 30070, *C. dubliniensis* ATCC 777, *C. dubliniensis* ATCC 778157).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**, **2**, **1a**, **1b**, **2a**, and **2b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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