

LIGNANS FROM *PROTIUM TENUIFOLIUM*

JANE B.G. SIQUEIRA, MARIA DAS G.B. ZOGHBI*, JOSÉ A. CABRAL, and WILSON W. FILHO

Coordenação de Pesquisas em Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia,
Alameda Cosme Ferreira 1756, CEP 69083-083 Manaus, Brazil

ABSTRACT.—An ether extract of the wood of *Protium tenuifolium* furnished two lignans characterized as (+)-(2*S*,3*S*)-2-(3'',4''-methylenedioxybenzyl)-3-(3',4'-methylenedioxyacetophenone)-butyrolactone [**1**] and a mixture of (–)-cubebin epimers. This is the first isolation of **1** as a natural product.

Species of the genus *Protium* (Burseraceae) are known for the production of oleoresin exudates that occur as a result of insect stings, broken branches, or other acts injurious to their bark. Triterpenes of the oleanane and euphane series are known to be produced, in addition to essential oils (1). The occurrence of lignans in this genus was previously recorded with the isolation of a coumarinolignan from *Protium opacum* (2). The isolation from the wood of *Protium tenuifolium* (Engl.) Engl. of two lignans, (+)-(2*S*,3*S*)-2-(3'',4''-methylenedioxybenzyl)-3-(3',4'-methylenedioxyacetophenone)-butyrolactone [**1**] and (–)-cubebin (as an epimeric mixture) is reported herein.

Chromatographic separation of the Et₂O extract of the wood of *P. tenuifolium* resulted in the isolation of compound **1** as white needles, mp 105–106°. The eims exhibited a molecular ion peak at *m/z* 368, suggesting a molecular formula of C₂₀H₁₆O₇. The ir spectrum indicated the presence of a lactone ring

(1770 cm⁻¹), an aromatic nucleus (1600, 1480 cm⁻¹), and a methylenedioxy group (920 cm⁻¹). Compound **1** exhibited uv absorption bands at λ max (EtOH) 233, 281, and 314 nm. The ¹H-nmr spectrum of **1** (Table 1) showed two triplets, 1H each, centered at δ 4.14 and δ 4.39, characteristic of lactone methylene protons. The singlet at δ 6.06 (2H) and doublets at δ 5.90 (*J*=1.4 Hz) and δ 5.88 (*J*=1.4 Hz), 1H each, were indicative of two aromatic methylene dioxy groups. A group of signals, representative of six protons, that appeared between δ 7.26 and δ 6.52, was assigned to aromatic protons. Detailed assignments of these signals indicated the presence of two 1,3,4-trisubstituted aromatic rings. Signals for benzylic protons were observed at δ 2.92 and δ 3.06, and for methine protons at δ 4.00 and δ 3.46 as a triple doublet and doublet of double doublets, respectively. ¹H-¹H COSY nmr data corroborated the proposed assignments. These data, and the fact that the eims of **1** exhibited peaks at *m/z* 192 and 135, attributable to [(OCH₂O)C₆H₃C₂H₃CO₂]⁺ and [(OCH₂O)C₆H₃CH₂]⁺ fragments, respectively, indicated that **1** is a dibenzylbutyrolactone lignan. The trans relationship between the two benzylic groups was established from the nonequivalence of the H-4 protons in the ¹H-nmr spectrum (3); the coupling constants *J*_{3-4α} and *J*_{3-4β} were identical, which lent further support to a trans relationship between the two benzylic groups (4). The ¹³C-nmr chemical shifts were assigned on the basis of proton-noise

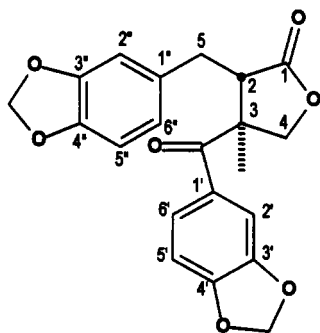
**1**

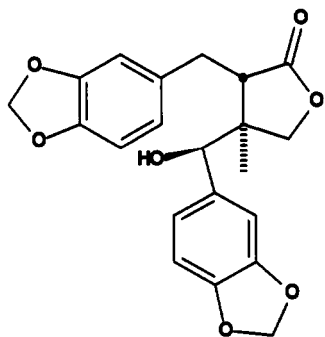
TABLE 1. ¹H-Nmr Spectral Data for Compound 1.^a

Proton	δ	(mult.)	(J, Hz)
2	3.46	(1H, ddd)	$J_{2-3}=8.6, J_{2-6\alpha}=7.4, J_{2-6\beta}=5.5$
3	4.00	(1H, td)	$J_{3-2}=J_{3-4\alpha}=J_{3-4\beta}=8.6$
4 α	4.14	(1H, t)	$J_{4\alpha-4\beta}=J_{4\alpha-3}=8.6$
4 β	4.39	(1H, t)	$J_{4\beta-4\alpha}=J_{4\beta-3}=8.6$
6 α	2.92	(1H, dd)	$J_{6\alpha-6\beta}=14.3, J_{6\alpha-2}=7.4$
6 β	3.06	(1H, dd)	$J_{6\beta-6\alpha}=14.3, J_{6\beta-2}=5.5$
2'	7.20	(1H, d)	$J_{2'-6'}=1.8$
5'	6.80	(1H, d)	$J_{5'-6'}=8.2$
6'	7.26	(1H, dd)	$J_{6'-5'}=8.2, J_{6'-2'}=1.8$
2''	6.60	(1H, d)	$J_{2''-6''}=1.8$
5''	6.62	(1H, d)	$J_{5''-6''}=8.0$
6''	6.52	(1H, dd)	$J_{6''-5''}=8.0, J_{6''-2''}=1.8$
OCH ₂ O	5.88	(1H, d)	$J=1.4$
	5.90	(1H, d)	$J=1.4$
OCH ₂ O	6.06	(2H, s)	

^aRecorded at 300 MHz in CDCl₃. Chemical shifts were determined by HETCOR and COSY experiments.

decoupled and APT spectra. Because the specific rotation ($[\alpha]^{25}_D +36.6^\circ$) is opposite in sign to that of (-)-hinokinin ($[\alpha]^{20}_D -26.3^\circ$) (3), and from the above discussion, **1** was characterized as (+)-(2*S*,3*S*)-2-(3'',4''-methylenedioxybenzyl)-3-(3',4'-methylenedioxyacetophenone)-butyrolactone.

Previously, a compound given the name of "oxo-parabenzlactone" and having the same structure as **1**, was obtained as an oxidation product of (-)-*para*-benzylactone [2] (5). However, it was characterized only by mp (113–115°), elemental analysis, and ir and uv spectral data (5). This is the first report of **1** as a natural product, and is the first observation of the presence of dibenzylbutyrolactones in the genus *Protium*.



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Analysis of the physical and spectroscopic properties of the second isolate showed it to be a dibenzylbutyrolactol lignan. Furthermore, these data were consistent with those reported for (-)-cubebin (6) and allowed us to determine that the isolate was an epimeric mixture.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps are uncorrected. Uv spectra (EtOH) were recorded on a Beckman DB-6 instrument and ir spectra on a Perkin-Elmer 298 spectrophotometer. Nmr spectra were recorded on a Varian VXR 300 spectrometer operating at 75 MHz for ¹³C and 300 MHz for ¹H, and were measured using TMS as internal standard. Mass spectra were recorded on a Finnigan 3200 gc-ms spectrometer coupled to a Teknivent Vector One data system, operating at 70 eV. Specific rotations were measured on a Jasco digital polarimeter model DIP-370, using CHCl₃ as solvent.

PLANT MATERIAL.—The wood of *P. tenuifolium* was collected near Rio Moa, in the State of Acre, Brazil, and identified by Dr. Douglas Daly of the New York Botanical Garden. A voucher specimen (No. 140,206) has been deposited at the Herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA).

EXTRACTION AND ISOLATION.—The wood (2.35 kg) was ground and extracted at room temperature with Et₂O. The solvent was evaporated to give 70.7 g of extract. Part of this (20 g) was subjected to cc on Si gel, giving compound **1** (50 mg) and (-)-cubebin (548 mg), eluted with CHCl₃ and CHCl₃-MeOH (95:5), respectively.

Compound 1.—Obtained as white needles (CHCl₃): mp 105–106°; $[\alpha]_D^{25} + 36.66^\circ$ ($c=0.476$, CHCl₃); uv (EtOH) λ max (log ϵ) 233 (4.60), 281 (4.12), and 314 (3.10) nm; ir (KBr) ν max 1770 (lactone), 1606, 1600–1480 (aromatic nucleus), 1440, 1370, 1250, 1110, 1020, 920 (methylenedioxy), 810, 790 cm⁻¹; ¹H-nmr data (CDCl₃, 300 MHz), see Table 1; ¹³C nmr (CDCl₃, 75.4 MHz) δ 177.0 (s, C-1), 46.7 (d, C-2), 44.8 (d, C-3), 68.1 (t, C-4), 194.5 (s, C-5), 34.6 (t, C-6), 130.4 (s, C-1'), 109.7 (d, C-2'), 148.5 (s, C-3'), 148.5 (s, C-4'), 108.0 (d, C-5'), 122.5 (d, C-6'), 130.7 (s, C-1''), 110.0 (d, C-2''), 147.9 (s, C-3''), 147.9 (s, C-4''), 108.3 (d, C-5''), 124.8 (d, C-6''), 102.1 (OCH₂O), 101.0 (OCH₂O); ms m/z 368 [M]⁺ (18), 219 (9), 193 (10), 192 (100), 176 (13), 175 (18), 149 (60), 135 (52), 121 (18).

(-)-*Cubebin epimers.*—C₂₀H₂₀O₆, mp 130–132° (CHCl₃); spectroscopic data are consistent with those reported in the literature (6).

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