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METABOLITES FROM *PRUMNOPITYS EXIGUA* DE LAUBENF

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ABSTRACT

A chemical investigation of the chloroform extract of the leaves of *Prumnopitys exigua* afforded two methylenedioxybenzene compounds. The structure and relative configuration of the isolated compounds, of which one is known and one is new, were established by NMR spectroscopy and HRMS.

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RESUMEN

La investigación química del extracto de cloroformo de las hojas de *Prumnopitys exigua* proporcionó dos compuestos metilenedioxibenzénicos. La estructura química y configuración relativa de los compuestos aislados fueron establecidas por espectroscopía de NMR y HRMS.

INTRODUCTION

Bolivia occupies 6% of the surface of South America. Its geographical position combined with the many different environments and landscapes has given Bolivia an extremely rich biodiversity. Bolivia is considered to be one of the most forest-rich countries of the world [1] and to have more than 20 000 species of plants [2], and it is estimated that between 20 and 25% of the plants from Bolivia are endemic [3]. As part of our interest in the secondary metabolites of Bolivian plants, a phytochemical investigation of *Prumnopitys exigua* was performed. *P.exigua* is a conifer evergreen tree belonging to the Podocarpaceae family, it grows in the Sub-Andean Amazonian Forest and is known as “pino colorado” by the natives of the region [4, 5]. The only current known use is as energy source and timber [6, 7], and literature survey showed that no phytochemical study of this plant was ever reported. From a chloroform extract of the leaves two methylenedioxybenzene derivatives were isolated, and characterized by spectroscopic techniques.

RESULTS AND DISCUSSION

The structures of compounds **1** and **2** (Figure 1) were elucidated by ¹H NMR and ¹³C NMR spectra and 2D COSY, NOESY, HMQC and HMBC experiments, as well as by HRMS-ESI experiments.

Compound **1** was isolated as a yellow powder. The HRMS-ESI and the 1D NMR data established that the elemental composition of **1** is C₁₇H₁₂O₇, and that it has twelve degrees of unsaturation. The ¹H NMR spectrum recorded in DMSO-*d*₆ (see Table 1 for 1D NMR data) displays a singlet at δ 8.36, that is characteristic for 2-H in an isoflavone [8, 9]. This gives HMBC correlations to C-4, C-9 and C-1'. The sharp signal at δ 12.89 is a chelated hydroxyl proton, a common proton signal of 5-hydroxyisoflavones [10], assigned as 5-OH by the HMBC correlations from the hydroxyl proton to C-5, C-6 and C-10. A sharp singlet at δ 6.19 integrating for two protons attached to the carbon at δ 102.9, was attributed to a methylenedioxy group [11, 12]. The HMBC correlations between these protons and C-6 as well as C-7 establish the position of the methylenedioxy group. The singlet at δ 6.92 represents consequently the only aromatic proton in ring A, its position at C-8 was demonstrated by the HMBC couplings from 8-H to C-6, C-7, C-9 and C-10. With the above-mentioned HMBC correlation between 2-H and C-9, the chromenone part of the molecule is now established, and C-1' must be part of the B-ring. This contains three aromatic protons, which signals unfortunately overlap at δ 6.82 in the ¹H NMR spectrum recorded in DMSO-*d*₆, a methoxy group (δ 3.68) and a hydroxyl group (exchangeable proton at δ 9.03). HMBC correlations were observed between the hydroxyl proton and

C-1', C-2' and C-3', demonstrating that the hydroxylated B-ring carbon is next to C-1'. The methoxy protons give a long-range correlation to C-5'. Additional information was obtained from the ^1H NMR spectrum recorded in chloroform- d with 10% methanol- d_4 (Table 1), as the spectrum allowed the identification of the aromatic spin system of the B-ring. Besides the singlet assigned to C-8, the three proton signals of the B-ring are now resolved at δ 6.86 (1H, dd, $J=8.8$ and 2.9 Hz), δ 6.94 (1H, d, $J=8.8$ Hz), and δ 6.70 (1H, d, $J=2.9$;Hz). The B-ring is consequently 1,2,5-trisubstituted, and a NOESY correlation between 2-H and δ 6.70 (6'-H) determines the B-ring as shown in Figure 1. In addition, NOESY correlations between the methoxy protons and 4'-H as well as 6'-H confirm the suggested structure of compound **1**. **1** is consequently the isoflavone 5,2'-dihydroxy-5'-methoxy-6,7-methylenedioxy isoflavone (tetranin B), that recently was reported from *Salsola tetrandra* [13].

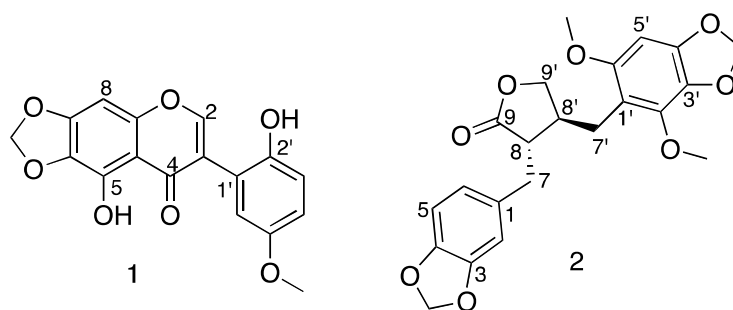


Figure 1. Structures of compounds **1** and **2**.

Compound **2** was isolated as a yellowish oil. The HRMS-ESI suggested that the elemental composition is $\text{C}_{22}\text{H}_{22}\text{O}_8$ which was confirmed by ^1H NMR and ^{13}C NMR data (see Table 1) displaying signals for 22 protons and 22 carbons, and **2** consequently has twelve degrees of unsaturation. The ATR-IR spectrum suggested the presence of a γ -lactone (1767 cm^{-1}) and a methylenedioxy group (932 cm^{-1}). The NMR data show that **2** in fact have two methylenedioxy groups, with two singlets at δ 5.28 and 5.31 both integrating for two protons and correlated with the carbon signals at δ 100.6 and 100.8 in the HMQC spectrum. The NMR data also suggest the presence of six carbon-carbon double bonds, or two benzene rings, and one carbonyl group, which makes **2** pentacyclic, as well as two methoxy groups, three additional methylene groups, and two saturated methine groups.

The methylenedioxy moiety giving rise to the ^1H NMR signal at 5.31 ppm (2H, m; δ_c 100.8) is attached to an aromatic ring, specifically to C-3 and C-4, which was demonstrated by HMBC correlations. Three of the unsaturated protons, 2-H, 5-H and 6-H, are obviously part of an aromatic spin system, as indicated by the ^1H - ^1H coupling constants as well as the COSY correlations. Both 2-H and 6-H give HMBC signals to the benzylic carbon C-7, while 7-H₂ give HMBC correlations to C-1, C-2 and C-6. Additional HMBC correlations from 6-H to C-2 and C-4, from 2-H to C-3, C-4 and C-6, and from 5-H to C-1, C-3 and C-4 determine the structure of the first benzene. The ^1H spin system 7-H₂ – 8-H – 8'-H – 7'-H₂ and 9'-H₂ is demonstrated by the COSY correlations as well as the ^1H - ^1H coupling constants. C-9' (δ_c 70.9) is oxygenated and the HMBC correlations from 9'-H₂ to the carbonyl group C-9 as well as from 7-H₂, 8-H and 8'-H to C-9 settle this part of the molecule as a lactone. Also the second methylenedioxy group at δ 5.28 (2H, m; δ_c 100.6) is located at an aromatic ring attached to C-3' and C-4'. 7'-H₂ give HMBC correlations to C-1', C-2' and C-6' while the protons of the two methoxy groups give HMBC correlations to C-2' and C-6'. 5'-H gives HMBC correlations with C-1', C-3', C-4' and C-6', and C-3', C-4' and C-5' are consequently the remaining constituents of the second benzene.

The relative configuration at the lactone ring was assigned by the analysis of the NOESY correlations. Strong correlations were observed from 7'-H₂ to 9'-H β and 8-H β protons, favoring the *trans* configuration. Additionally, correlations between 7-H₂ and 8'-H α , and from H-2/H-6 to 8'-H α were observed, suggesting again the *trans* configuration. The absence of a NOESY correlation between 7-H₂ and 7'-H₂ (as expected in a *cis* configuration) confirmed the *trans* configuration. Consequently, the structure of compound **2** was shown to be (8 β ,8' α)-3,4:3',4'-bis-methylenedioxy-2',6' dimethoxy lignano-9,9'-lactone, a new natural product.



Table 1: 1D NMR data of compounds **1** and **2**. See Experimental section for details.

Compound 1				Compound 2		
Position	1 ^a		1 ^b	Position	2 ^c	
	¹³ C	¹ H	¹ H		¹³ C	¹ H
1				1	132.4	
2	156.2	8.36 s	8.02 s	2	110.6	6.70 d (1.1)
3	120.2			3	148.0	
4	180.7			4	146.7	
5	141.3			5	108.3	6.59 d (8.1)
6	129.6			6	123.2	6.61 dd (1.1, 8.1)
7	154.0			7	34.3	2.88 dd (6.1, 14.0) 2.77 dd (5.1, 14.0)
8	89.6	6.92 s	6.55 s	8 β	47.0	2.28 ddd (9.7, 6.1, 5.1)
9	153.0			9	178.0	
10	107.5			1'	110.7	
1'	118.1			2'	142.4	
2'	149.3			3'	130.3	
3'	116.3	6.82	6.94 d (8.8)	4'	148.5	
4'	115.1	6.82	6.86 dd (2.9, 8.8)	5'	88.6	6.04 s
5'	151.8			6'	153.3	
6'	116.9	6.82	6.70 d (2.9)	7'	25.7	2.61 dd (6.4, 13.0) 2.47 dd (7.7, 13.0)
CH ₃ O (5')	55.5	3.68 s	3.75 s	8' α	39.4	2.36 m
OCH ₂ O (6, 7)	102.9	6.19 s	6.09 s	9' α	70.9	3.71 dd (8.7, 7.8)
2'-OH		9.03 s		9' β		3.59 dd (8.7, 8.7)
5-OH		12.89 s		CH ₃ O (2')	59.0	3.72 s
				CH ₃ O (6')	55.4	3.16 s
				OCH ₂ O (3, 4)	100.8	5.31 s
				OCH ₂ O (3', 4')	100.6	5.28 s

Spectra recorded in: ^aDMSO-*d*₆, ^bCDCl₃ with 10% CD₃OD, and ^cBenzene-*d*₆. Assignments were based on COSY, HMQC, HMBC and NOESY experiments.

EXPERIMENTAL

General experimental procedures

The optical rotation was measured with a Perkin-Elmer 341 polarimeter at 20°C. The ATR-IR spectrum was obtained with a Bruker-Alpha-P spectrometer. The HRMS-ESI spectra were recorded with a Waters Q-TOF Micro system, using H₃PO₄ for calibration and as internal standard. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) as well as 2D spectra were measured with a Bruker DRX500 spectrometer; in DMSO-*d*₆ (solvent residual signals at δ 2.50 and 39.52), in mixture of 10% methanol-*d*₄ in chloroform-*d* (solvent residual signals at δ 7.26 and 77.16) and in benzene-*d*₆ (solvent residual signals at δ 7.16 and 128.06). Chemical shift (δ) are given in ppm, and coupling constants (*J*) in Hertz. Vacuum liquid chromatography (VLC) was carried out using TLC grade silica gel (Merck), while column chromatography (CC) was run on silica gel 60 (230-400 mesh, Merck). TLC analyses were carried out on silica gel GF₂₅₄ pre-coated plates (Merck); chromatograms were visualized under a UV lamp (254 nm) and by spraying with vanillin (6%)-sulfuric acid (1.5%)-ethanol solution, followed by heating.

Plant material

The whole aerial part of *Prumnopitys exigua* de Laubenf was collected in April 2010 at the region of "Monte Punco", which belongs to the Carrasco province, this region is located at 2930 meters above sea level, and 180 km to the south of Cochabamba city (Bolivia). The collecting and identification of the plant was made by Lic. Modesto Zárate, a research scientist at the National Herbarium "Herbario Nacional Martin Cardenas" (Cochabamba-Bolivia). A voucher specimen (MZ-3336) has been stored at the "Herbario Nacional Martin Cardenas".



Extraction and isolation

The air-dried powdered plant material (200g) was successively extracted with hexane during 12 h and subsequently with chloroform for another 12 h, at room temperature. The extracts were concentrated under reduced pressure. Part of the chloroform extract (2.5g) was submitted to VLC on silica gel eluted with a stepwise gradient of heptane-ethyl acetate (90:10, 80:20 and 70:30) to afford six main fractions (1-6). Fraction 6 was submitted to VLC eluted with toluene-ethyl acetate (30:1 and 90:10) to give ten subfractions (A-J). Subfraction D was precipitated with methanol to give a yellow precipitate to yield compound **1** (3mg). Subfraction A (250mg) was subjected to VLC using mixtures of heptane-ethyl acetate (30:1, 90:10 and 80:20) to give 3 fractions. The third of these (60 mg) was filtered through a mixture of silica gel and activated charcoal (100:3), and eluted with toluene-ethyl acetate (80:20) to give two fractions, of which the first fraction was subjected to CC eluted with heptane-ethyl acetate (80:20) to give five fractions. Fraction 5 consisted of pure compound **2** (6 mg).

5,2'-dihydroxy-5'-methoxy-6,7-methylenedioxy isoflavone 1. Compound **1** was obtained as a yellowish powder; mp 218-220 °C. ^1H (at 500 MHz) and ^{13}C NMR (at 125 MHz) 1D NMR data are shown in Table 1. HRMS-ESI, calculated for $\text{C}_{17}\text{H}_{12}\text{O}_7$ (M+H) $^+$: 329.0661. Found: 329.0659.

(8 β ,8' α)-3,4:3',4' -bis-methylenedioxy-2',6' dimethoxy lignano-9,9'-lactone 2. Compound **2** was obtained as a yellowish oil; $[\alpha]_{\text{D}}^{20}$ -29 (c 0.22, CHCl_3); ATR-IR ν_{max} (cm^{-1}): 932, 1039, 1075, 1124, 1192, 1247, 1445, 1482, 1629, 1767, 2778, 2909; ^1H (at 500 MHz) and ^{13}C NMR (at 125 MHz) 1D NMR data are shown in Table 1. HRMS-ESI, calculated for $\text{C}_{22}\text{H}_{22}\text{O}_8$ (M+H) $^+$: 415.1393. Found: 415.1373.

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