Constituents of the Root Wood of *Austroplenckia populnea* var. *ovata*

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The root wood of *Austroplenckia populnea* var. *ovata* was extracted successively with chloroform and methanol. Lapachol and dehydro-*β*-lapachone were isolated from the chloroform extract, and euonine, alatusinine, wilfordine, 2-*O*-deacetyleuonine (**1**), 7-*O*-deacetyleuonine (**2**), and austronine (**3**) from the methanol extract. The structures of the new compounds **1**−**3** were elucidated by spectroscopic data interpretation. Lapachol, dehydro-*β*-lapachone, euonine, alatusinine, and wilfordine are known compounds that are newly identified from root wood of *Austroplenckia populnea*.

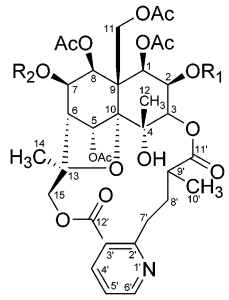
Austroplenckia populnea (Reiss.) Lundell var. *ovata* (Celastraceae) is widely distributed in the northern and central parts of Brazil. This plant has been used in Brazilian folk medicine as an antidysenteric[1] and antirheumatic[2] agent. It has been found to contain pyridine alkaloids having a highly oxygenated dihydro-*β*-agarofuran unit on a sesquiterpene moiety and evoninoate or wilforate esters on the alkaloid moiety.[3] In addition, extracts from the leaves of this tree exhibit a larvicidal activity against *Strongyloides stercoralis* and hookworms.[4] Sesquiterpene pyridine alkaloids have drawn considerable attention because of their potential anti-HIV activity,[5,6] their cytotoxicity against several human tumor cell lines,[7] and their insect antifeedant and insecticidal activity.[8,9]

As part of a study on the constituents of extracts from *A. populnea* var. *ovata*, the root wood meal was extracted successively with chloroform and methanol at room temperature to obtain chloroform and methanol extracts. Particular attention was paid to the occurrence of sesquiterpene pyridine alkaloids of the *β*-dihydroagarofuran type, since compounds of this class were identified in the leaves from this same tree species.[3]

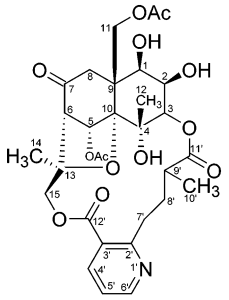
From the chloroform-soluble extract, lapachol,[10] 3,4-dehydro-*β*-lapachone,[11] populonic acid,[12] 3-hydroxy-2-oxofriedelan-3-ene-20-α-carboxylic acid,[13] maytenfolic acid,[14] and abruslactone A[14] were isolated by chromatography on a silica gel column and were identified by comparing their mp and IR, ^{1}H NNR, or mass spectra with those of authentic samples. Both lapachol[10] and 3,4-dehydro-*β*-lapachone[11] are known compounds, but were identified for the first time from the root wood of *A. populenea* var. *ovata*. In addition, six sesquiterpene pyridine alkaloids were isolated from the methanol extract of this species.

The IR spectra of the alkaloids exhibited absorption bands at 1750−1730 cm^{-1} (C═O of OAc and esters) and 1588, 1570 cm^{-1} (doublet for −C═C− and −C═N− stretch), indicating that they are sesquiterpene pyridine alkaloids. In addition, the mass spectra of the alkaloids suggested that they were sesquiterpene alkaloids of the euonine type, since their EIMS exhibited intensive peaks at *m*/*z* 206 and 93 but not at *m*/*z* 107 (data not shown). By contrast, the mass spectra of euonymine alkaloids show characteristic ion peaks at *m*/*z* 206 and 107.[15] Moreover, the ^{1}H and ^{13}C NMR spectroscopic data of these alkaloids also confirmed that they are euonine alkaloids (Table 1). The structures for the new alkaloids **1**−**3** were elucidated on the basis of their spectroscopic data.

Euonine gave a molecular weight of 805 ([M + H]$^+$, *m*/*z* 806) as determined by its CIMS. The identity of the compound was established by comparison of its ^{1}H and ^{13}C NMR spectra with those of published data.[16−18] Alatusinine showed a molecular weight of 821 ([M + H]$^+$, *m*/*z* 822), as determined by CIMS, and identified by comparison of its ^{1}H NMR spectrum with published data.[19] Wilfordine had a molecular weight of 883 ([M + H]$^+$, *m*/*z* 884), as determined by CIMS, and again was established by comparison of its NMR data with those of published data.[19−21]



2-*O*-Deacetyleuonine (**1**)
R_1 = H; R_2 = Ac
7-*O*-Deacetyleuonine (**2**)
R_1 = Ac; R_2 = H



Austronine (**3**)

Both compounds **1** and **2** had molecular weights of 763, as determined by elemental analyses and CIMS ([M + H]$^+$, *m*/*z* 764), with the elemental composition of $C_{36}H_{45}NO_{17}$, corresponding to elimination of a ketene group from **1**. This could be achieved by eliminating one molecule of ketene from one of the six acetoxyl groups from **1** to the corresponding hydroxyl group. Indeed, both the ^{1}H and ^{13}C NMR spectra of these compounds exhibited signals for five acetoxyl groups (Table 1). In addition, the ^{1}H NMR spectrum of **2** exhibited a doublet of doublets proton signal centered at *δ* 4.10 with coupling constants of 3.4 and 2.4 Hz, which was assigned to H-2, while that of **2** exhibited a doublet of doublets proton signal centered at *δ* 3.99 with coupling constants of 3.6 and 6.0 Hz, which was assigned to H-7 (Table 1). As compared to the chemical shift of the corresponding proton of **1**, ^{1}H NMR chemical shifts of H-2 in **2** and H-7 in **3** were shielded by Δ*δ* 1.05 and 1.53, respectively, indicating that the C-2 carbon of **1** and the C-7 carbon of **2**, respectively, were substituted by a hydroxyl group rather than by an acetoxyl group. The chemical shifts for the other protons were similar to those of the corresponding ^{1}H NMR values of **1** (Table 1). In addition, the ^{13}C NMR spectrum of **3** showed the C-2 signal at *δ* 70.2, while that of **2** showed the C-7 signal at *δ* 70.4. As compared to the corresponding ^{13}C nuclei of euonine, the ^{13}C NMR chemical shifts of C-2 in **1** and C-7 in **2** were deshielded by Δ*δ* 0.8 and 1.3, respectively (Table 1). Consequently, compounds **1** and **2** were elucidated as 2-*O*-deacetyleuonine and 7-*O*-deacetyleuonine, respectively.

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Table 1. ^{1}H and ^{13}C NMR Data ($CDCl_3$) for 2-*O*-Deacetyleuonine (**1**), 7-*O*-Deacetyleuonine (**2**), and Austronine (**3**)

	1			**2**			**3**		
position	δ_H (ppm)	multiplicity (J_1, Hz)	δ_C (ppm)	δ_H (ppm)	multiplicity (J_1, Hz)	δ_C (ppm)	δ_H (ppm)	multiplicity (J_1, Hz)	δ_C (ppm)
1	5.64	d (3.4)	73.0	5.54	d (3.2)	73.8	4.51	d (4.8)	74.2
2	4.10	dd (3.4, 2.9)	70.2	5.16	dd (3.2, 2.4)	69.0	4.29	dd (4.8, 2.8)	70.4
3	5.10	d (2.4)	75.5	5.04	d (2.4)	75.6	5.02	d (2.8)	75.3
4			69.9			69.7			69.6
5	6.39	br s	73.9	6.90	br s	71.3	6.58	br s	72.4
6	2.35	d (4.3)	50.7	2.34	d (3.6)	51.0	3.09	s	58.1
7	5.57	dd (3.9, 5.4)	69.2	3.99	dd (3.6, 6.0)	70.4			191.2
8_{ax}	5.35	d (5.4)	70.8	5.32	d (5.4)	70.6	2.30	ABd (12.8)	54.8
8_{eq}							2.55	ABd (12.8)	
9			51.9			52.4			48.9
10			93.4			94.0			92.2
11	4.46	AXd (12.7)	62.9	4.60	AXd (13.2)	60.5	3.89	ABd (12.8)	57.1
11	5.26	AXd (12.7)		5.38	AXd (13.2)		4.01	ABd (12.8)	
12	1.54	s	23.4	1.59	s	22.8	1.59	s	21.9
13			84.3			84.1			84.0
14	1.66	s	17.9	1.67	s	18.0	1.68	s	17.9
15	3.89	AXd (11.7)	70.4	3.76	AXd (11.6)	70.2	3.99	AXd (12.0)	70.3
15	5.75	AXd (11.7)		5.71	AXd (11.6)		5.49	AXd (12.0)	
OH-4									
2′			162.9			163.8			163.7
3′			123.9			122.7			122.9
4′	8.30	dd (7.9, 1.6)	138.9	8.30	dd (8.0, 2.0)	138.7	8.27	dd (8.0, 1.6)	139.0
5′	7.27	dd (7.9, 4.8)	121.2	7.28	dd (8.0, 5.2)	121.1	7.25	dd (8.0, 4.6)	121.1
6′	8.74	dd (4.8, 1.6)	152.8	8.74	dd (5.2, 2.0)	153.0	8.71	dd (4.6, 1.6)	152.5
7′	2.87−3.92	m	32.9	2.98−3.89	m	33.4	3.09−3.58	m	34.2
8′	1.90−2.18	m	33.0	1.96−2.16	m	33.2	2.09−2.43	m	32.8
9′	2.36	m	38.8	2.41	m	38.5	2.22	m	39.3
10′	1.22	d (6.9)	18.2	1.15	d (6.8)	18.6	1.22	d (6.8)	18.6
11′			174.9			175.6			175.3
12′			167.1			166.8			168.2
AcO-1	1.88	s	20.5	1.97	s	20.5			
C═O			169.7			169.7			
AcO-2				2.18	s	21.4			
C═O						169.8			
AcO-5	2.28	s	21.6	2.28	s	21.6	2.16	s	21.0
C═O			169.6			169.7			169.0
AcO-7	2.00	s	21.0						
C═O			170.3						
AcO-8	1.89	s	20.5	2.01	s	20.6			
C═O			168.6			169.0		170.03	
AcO-11	2.19	s	21.0	2.18	s	21.0	2.16	s	20.5
C═O			170.1			170.1			

Compound **3** gave a molecular weight of 619, as determined by elemental analysis and CIMS ($[M + H]^+$, 620), with an elemental composition of $C_{30}H_{37}NO_{13}$, corresponding to the elimination of four molecules of ketene and one molecule of water from euonine. This suggested that four among the six acetoxyl groups were *O*-deacetylated to the corresponding hydroxyl groups, followed by elimination of one molecule of water from a resulting 1,2-diol structural moiety that was converted to an enol structure [−C(OH)═CH−] via enol−keto tautomerism to give a corresponding keto structural moiety [−(C═O)−CH_2−]. The ^{1}H NMR spectrum of **3** exhibited two AB doublet proton signals centered at δ 2.30 and 2.55 with coupling constants of 12.8 Hz (Table 1). As compared to the H-8_{ax} signal of euonine, appreciable shielding of these signals indicated that they were assignable to the two ^{1}H nuclei of a methylene group adjacent to a carbonyl group. In addition, the fact that these protons constituted a simple AB system (J = 14.8 Hz) indicated further that they are part of a (C═O)−CH_2−C_3− unit in the decalin moiety of a β-dihydroagarofuran type structure. Thus, two AB ^{1}H nuclei of the CH_2− group were assigned to H-8_{ax} and H-8_{eq}, respectively; the more shielded one centered at δ 2.26 was axial, while the less shielded one centered at δ 2.58 was equatorial. Moreover, in the spectrum, a doublet ^{1}H signal at δ 4.51 (J = 4.8 Hz) was coupled with a doublet of doublets proton signal centered at δ 4.29 with coupling constants of 4.8 and 2.8 Hz, which was in turn coupled with a doublet proton signal at δ 5.02 (J = 2.8 Hz). These three ^{1}H nuclei constituted an AMX system and could be assigned to H-1, H-2, and H-3 (Table 1). As compared to the chemical shift of the corresponding proton of euonine, the ^{1}H NMR chemical shifts of H-1 and H-2 in **3** were shielded by $\Delta\delta$ 1.1 and 0.86, respectively, indicating C-1 and C-2 of **3** were each substituted by a hydroxyl group rather than by an acetoxyl group. The chemical shift for H-3 of **3** and the wilfordic acid diester structural part of the spectrum were both similar to enonine. In addition, the ^{13}C NMR spectrum of **3** showed that the chemical shifts of C-1, C-2, and C-6 were deshielded by $\Delta\delta$ 0.6, 0.94, and 18.5, respectively, as compared to the corresponding ^{13}C nuclei of euonine. The deshielding of the ^{13}C NMR chemical shifts of the C-1 and C-2 nuclei supported the conclusion that the C-1 and C-2 of **3** were each substituted by a hydroxyl group, as observed in the ^{1}H NMR spectrum of **3**. Moreover, the appreciable deshielding of C-6 showed that the C═O group was at C-7, with a ^{13}C NMR chemical shift of δ 191.2. The ^{13}C NMR chemical shift at δ 54.8 could be then assigned to the CH_2 group at C-8. Thus, compound **3** was elucidated as 7-oxo-1α,2α,3β,4β,15α-pentahydroxy-5β,11-diacetoxyl-β-dihydroagrofuran, bonded at the 3β- and 15α-hydroxyl groups with wilfordic acid in the form of a cyclic diester, and was ascribed the trivial name austronine.

Experimental Section

General Experimental Procedures. All melting points are uncorrected. IR spectra were recorded on a Shimadzu Model IR-408 spectrometer, and ^{1}H and ^{13}C NMR spectra on a JEOL model α-400

NMR spectrometer with operating frequency of 400.12 MHz for ^{1}H nuclei and 100.3 MHz for the ^{13}C nuclei with the broadband-noise decoupling at 25 °C. Mass spectra were determined on a Varian mass spectrometer model 1200. Column chromatography was performed on Merck silica gel (70−230 mesh), Merck alumina (90, active, neutral), or a Sigma Sephadex (25−100 μm) column. TLC was conducted on Merck silica gel 60G or Merck alumina 60G neutral (Type E) plates.

Plant Material. The root wood of *Austroplenckia populnea* was collected in Nova Lima Region, Minas Gerais State, Brazil. A voucher specimen is deposited at the Herbarium of the Natural History Museum of Universidade Federal de Minas Gerais (Belo Horizonte, Minas Gerais, Brazil) under collection no. 10473.

Extraction and Isolation. The air-dried root wood chips were ground to pass a 60 mesh screen. The wood meal (300 g) was extracted successively with $CHCl_3$ and MeOH at room temperature by steeping in these solvents individually for 24 h, to obtain solutions that were concentrated on a rotavapor, furnishing $CHCl_3$ and MeOH extracts. The $CHCl_3$ extract (15 g) was chromatographed on a silica gel column using CH_2Cl_2, $CHCl_3$, and then a $CHCl_3$−EtOAc mixture to give lapachol (21 mg) and dehydro-β-lapachone (8.5 mg) (CH_2Cl_2), populnonic acid[12] (49 mg) ($CHCl_3$), abruslactone A[14] (7 mg) ($CHCl_3$−EtOAc, 19:1, v/v), 3-hydroxy-2-oxofriedelan-3-en-20α-carboxylic acid[13] (16 mg) ($CHCl_3$−EtOAc, 9:1, v/v), and maytenfolic acid[14] (5 mg) ($CHCl_3$−EtOAc, 8:2, v/v). Lapachol: yellow needles (from *n*-hexane); mp 139−141 °C (lit.[10] 139−140 °C). Dehydro-β-lapachone: red rhombic crystals (from $CHCl_3$−MeOH); mp 142−145 °C (lit.[11] 142.5−143.5 °C). The MeOH extract (16 g) was chromatographed on a silica gel column using successively $CHCl_3$, $CHCl_3$−EtOAc, and EtOAc, as eluents, to give populnonic acid[12] (yield: 54 mg; eluent: $CHCl_3$), 3-hydroxy-2-oxofriedelan-3-en-20α-carboxylic acid[13] (yield: 53 mg), abruslactone A[14] (yield: 16 mg; eluent: $CHCl_3$−EtOAc, 9:1, v/v), and a colorless powder (yield: 39 mg; eluent: EtOAc). This colorless powder from the MeOH extract was chromatographed on a Sephadex column using MeOH as eluent to give an alkaloid mixture. This mixture was further chromatographed on a neutral alumina column using a $CHCl_3$−EtOAc (9:1, v/v) mixture as eluent to isolate six alkaloids with decreasing order of R_f values on silica gel TLC using CH_2Cl_2−MeOH (19:1, v/v) as solvent. MS analyses of these compounds indicated that they were sesquiterpene pyridine alkaloids. Euonine: white, amorphous solid (from $CHCl_3$−MeOH); mp 149−154 °C (lit.[22] 149−153 ° C). Alatusinine: white, amorphous solid (from $CHCl_3$−MeOH); mp 147−149 °C (lit.[23] 149−153 °C). Wilfordine: white, amorphous solid (from $CHCl_3$−MeOH); mp 147−149 °C (lit.[23,24] 149−153 °C).

2-*O*-Deacetyleuonine (1): white, amorphous solid (from $CHCl_3$−MeOH); mp 147−149 °C; IR ($CHCl_3$) ν_{max} 3640 (H_2O; OH), 1760−1725 (C═O of OAc and esters), 1588, 1570 (doublet for −C═C− and −C═N− stretch) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS (moderating gas CH_4) *m*/*z* 765 (13), 764 ([M + H]$^+$, 43), 705 (7), 746 (6, [M + H]$^+$ − 18), 722 (5; [M + H]$^+$ − 42), 704 (14, [M + H]$^+$ − 60), 662 (8), 644 (5, [M + H]$^+$ − 2 × 60), 584 (1, [M + H]$^+$ − 3 × 60); *anal*. calcd for $C_{36}H_{45}NO_{17}$ (763.76) C% 56.61, H% 5.94, N% 1.83; found C% 56.58, H% 5.85, N% 1.76.

7-*O*-Deacetyleuonine (2): white, amorphous solid (from $CHCl_3$−MeOH); mp 153−155 °C; IR ($CHCl_3$) ν_{max} 3400 (H_2O; −OH), 1760−1725 (C═O of OAc and esters), 1588 and 1570 (doublet for −C═C− and −C═N− stretch) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS (moderating gas CH_4) *m*/*z* 765 (3), 764 ([M + H]$^+$, 7), 705 (7), 704 (5, [M + H]$^+$ − 60), 644 (2, [M + H]$^+$ − 2 × 60), 620 (6), 584 (1, [M + H]$^+$ − 3 × 60); *anal*. calcd for $C_{36}H_{45}NO_{17}$ (763.76) C% 56.61, H% 5.94, N% 1.83; found C% 56.56, H% 5.86, N% 1.78.

Austronine (3): white, amorphous solid (from $CHCl_3$−MeOH); mp 169−172 °C; IR ($CHCl_3$) ν_{max} 3400 (H_2O; −OH), 1740−1725 (C═O of OAc and ester), 1715 (sh; C═O of ketone), 1586 and 1578 (doublet for −C═C− and −C═N− stretch) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS (moderating gas CH_4) *m*/*z* 621 (35), 620 ([M + H]$^+$, 100), 603 (7), 602 (5, [M + H]$^+$ − 18), 779 (16), 178 (49, [M + H]$^+$ − 42), 561 (17), 560 (56, [M + H]$^+$ − 60); *anal*. calcd for $C_{30}H_{37}NO_{13}$ (619.64) C% 58.15, H% 6.02, N% 2.26; found C% 58.06, H% 5.96, N% 2.17.

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Note Added after ASAP Publication. The name of compound **2** was misspelled in Table 1 in the version posted on Aug 1, 2006. The correct spelling appears in the version posted on Aug 2, 2006.

References and Notes

(1) Corrêa, M. P. *Dicionário das Plantas ×c5teis do Brasil e das Plantas Exóticas Cultivatas*; Imprensa National: Rio de Janeiro, 1926−1978.
(2) Brüning, R.; Wagner, H. *Phytochemistry* **1978**, *17*, 1821−1858.
(3) Conzalez, G.; Bazzocchi, I.. L.; Domingues, X. A.; Ravelo, A. G.; Luis, J. G.; Veasquez, G.; Cano, G. *Rev. Latinamer.-Quim.* **1987**, *18*, 83−84.
(4) Vichenwski, W.; Siva Prasad, J.; Herz, W. *Phytochemistry* **1984**, *23*, 1655−1657.
(5) Dauan, H. Q.; Takaishi, Y.; Imahura, Y.; Jia, Y.; F.; Li, D.; Consentino, M.; Lee, K. H. *J. Nat. Prod.* **2000**, *63*, 357−361.
(6) Dauan, H. Q.; Takaishi, Y.; Morita, H.; Ohomoto, Y.; Taki, T.; Jia, Y. F.; Li, D. *J. Nat. Prod.* **2001**, *64*, 582−587.
(7) Kuo, Y. H.; King, M. l.; Chen, C. F.; Chen, H. Y.; Chen, K.; Lee, K. H. *J. Nat. Prod.* **1994**, 57, 263−269.
(8) Shirota, O.; Otsuka, A.; Morita, H.; Takeya, K.; Itokawa, H. *Heterocycles* **1994**, *38*, 2219−2229.
(9) Núñez, M.; Guadaño, A.; Jeménez, I. A.; Ravelo, A. G.; Conzález-Coloma, A.; Bazzocchi, I. L. *J. Nat Prod.* **2004**, *67*, 14−18
(10) Burnett, A. R.; Thomson, R. H. *J. Chem. Soc.* **1967** (C), 2100−2104.
(11) Gasinovi, C. G.; Marini-Bettolo, G. B.; da Mala, O. G.; Lima, M. H. D.; D'Albuguerque, I. L. *Ann. Chim.* **1962**, *52*, 1184−1189.
(12) Ramajah, P. A.; Devi, P. U.; Frolow, F.; Lavie, D. *Phytochemistry* **1984**, *23*, 2251−2255.
(13) De Sousa, J. R.; Jannotti, N. K.; Silva, G. D. F.; Pinheiro, J. A. *Gazz. Chem. Ital.* **1988**, *118*, 821−822.
(14) Nozaki, H.; Suzuki, H.; Lee, K.-H.; McPhail, A. T. *Chem. Commun.* **1982**, 1048−1051.
(15) Klásek, A.; ×b3Šantavý, F.; Duffield, A. M.; Reichstein, T. *Helv. Chem. Acta* **1971**, *54*, 2144−2158.
(16) He, Z.-S.; Li, Y.; Fang, S.-D.; Hong, S.-H. *Acta Chem. Sin.* **1987** *45*, 510−513.
(17) Li, Y.; Strunz, G. M.; Calhoun, L. A. *Can. J. Chem.* **1990**, *68*, 371−374.
(18) Han, B. H.; Ryu, J. H.; Han, Y. H.; Park M. K.; Park, J. H.; Naoki, H. *J. Nat. Prod.* **1990**, *53*, 909−914.
(19) Yamada, K.; Shizuri, Y.; Hirata, Y. *Tetrahedron* **1978**, *34*, 1915−1920.
(20) Shizuri, Y.; Yamada, K.; Hirata, Y. *Tetrahedron Lett.* **1973**, *14*, 741−744.
(21) Li, Y.; Strunz, G. M.; Calhoun, L. A. *Phytochemistry* **1991**, *30*, 719−721.
(22) Sugiura, K.; Yamada, K.; Hirata, Y. *Tetrahedron Lett.* **1973**, *14*, 113−116.
(23) Ishiwata, H.; Shizuri, Y.; Yamada, K. *Phytochemistry* **1983**, *22*, 2839−2841.
(24) Beroza, M. *J. Am. Chem. Soc.* **1951**, *73*, 3656−3659.

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