Laevisines A and B: Two New Sesquiterpene−Pyridine Alkaloids from *Maytenus laevis*

Sonia Piacente, Nunziatina De Tommasi, and Cosimo Pizza*

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, P.zza V. Emanuele 9, Penta di Fisciano (SA), Italy

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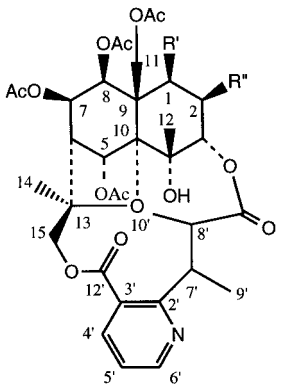
Two new sesquiterpene−pyridine alkaloids, laevisines A (**1**) and B (**2**), have been isolated from the bark of *Maytenus laevis*, along with seven known alkaloids (**3**−**9**). Their structures were elucidated by FABMS analysis and 1D and 2D NMR spectroscopy including DQF-COSY, HSQC, and HMBC.

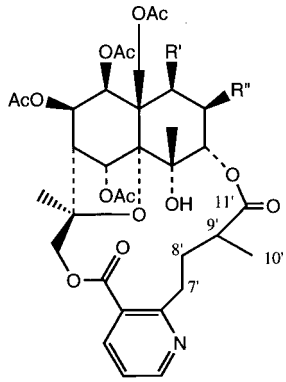
Species belonging to the genus *Maytenus* (Celastraceae) are widely used in the Amazonian region as folk remedies against cancer, rheumatism, and skin ailments.[1,2] Many characteristic and bioactive compounds, such as maytansinoids with antitumor activity,[3] quinoid triterpenes with cytotoxic activity,[4−6] and sesquiterpene polyesters[7] and sesquiterpene pyridine alkaloids with insecticidal, insect antifeedant, and antitumor activities,[8−10] have been reported from these species. Recently, immunosuppressive activity of sesquiterpene−pyridine alkaloids has also been reported.[11]

During our studies on new potentially bioactive compounds from South American medicinal plants, we have investigated the $CHCl_3$:MeOH (9:1) extract of the bark of *Maytenus laevis*, known by the vernacular name of "chuchuasi". Gonzalez et al.[2] reported on the isolation of polyphenols and tingenone derivatives from the MeOH extract of *M. laevis*. We have undertaken a systematic investigation of the $CHCl_3$:MeOH (9:1) extract of this species to verify the occurrence of other classes of metabolites, in particular sesquiterpene−pyridine alkaloids, which represent useful taxonomic markers. Our study led to the isolation of laevisines A (**1**) and B (**2**), which are new natural compounds, along with ebenifoline E-1 (**3**), euojaponine F (**4**), euojaponine I (**5**), euonine (**6**), euonymine (**7**), mayteine (**8**), and wilforine (**9**), which were isolated previously from other species belonging to the family Celastraceae.[12−16]

Laevisina A (**1**) had a molecular formula $C_{41}H_{51}NO_{18}$, as suggested by ^{13}C, ^{13}C DEPT NMR data, and the FABMS showed a quasi molecular anion at *m*/*z* 844. The ^1H NMR spectrum of **1** showed two methyl signals at δ 1.54 and 1.69, two sets of isolated oxymethylene protons at δ 4.52 and 5.24 (H_2-11), and at δ 3.70 and 5.95 (H_2-15), and six oxymethine protons at δ 4.75, 5.21, 5.32, 5.54, 5.71, and 7.02. On the basis of a DQF-COSY experiment, signals at δ 5.71, 5.21, and 4.75 were assigned to H-1, H-2, and H-3, while signals at δ 7.02, 2.33, 5.54, and 5.32 were attributed to H-5, H-6, H-7, and H-8 of an euonyminol sesquiterpene skeleton.[12] Also evident in the ^1H NMR spectrum were five acetyl singlets at δ 1.82, 2.13, 2.15, 2.22, and 2.32 and signals at δ 1.70 (3H, s), 1.73 (3H, d, J = 8.5 Hz), and 6.62 (1H, q, J = 8.5 Hz), which were correlated by a HSQC experiment to the corresponding carbon signals at δ 11.7, 14.2, and 137.5. On the basis of the HMBC correlations observed between the carbon signal at δ 165.7 and the proton signal at δ 6.62, and between the carbon signal at δ 128.0 and the proton signals at δ 1.70 and 1.73, the presence of a 2-methylbut-2-enoyl moiety was deduced.[10] The dibasic acid moiety of **1** was determined to be evoninic acid[12] on the basis of two secondary methyl proton signals



	R'	R"
LAEVISINE A (**1**)	$OCOC(CH_3)=CHCH_3$	OAc
EBENIFOLINE E-I (**3**)	OBz	OH
EUOJAPONINE I (**5**)	ONic	OAc
EUONYMINE (**7**)	OAc	OAc
MAYTEINE (**8**)	OBz	OAc



	R'	R"
LAEVISINE B (**2**)	ONic	OAc
EUOJAPONINE F (**4**)	OBz	OAc
EUONINE (**6**)	OAc	OAc
WILFORINE (**9**)	OAc	OBz

* To whom correspondence should be addressed. Phone: ++ 39 89 968954. Fax: ++ 39 89 968937.

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at δ 1.21 (d, J = 7.0 Hz, Me-10′) and 1.38 (d, J = 7.0 Hz, Me-9′) and three aromatic proton signals at δ 7.27 (dd, J = 4.9 and 8.0 Hz), 8.08 (dd, J = 1.8 and 8.0 Hz), and 8.68 (dd, J = 1.8 and 4.9 Hz). The location of the acyl groups was deduced unambiguously from the HMBC experiment which showed three-bonded long-range correlations between carbonyl carbons and oxymethine protons and two-bonded long-range correlations of the same carbonyl carbons with the acyl protons. Long-range correlation between the carbonyl carbon signal at δ 165.7 and the proton signal at δ 5.71 (H-1) suggested the 2-methyl-but-2-enoyl moiety to be located at C-1. The HMBC experiment allowed the five acyl groups to be placed at C-2, C-5, C-7, C-8, and C-11 and their ^{1}H and ^{13}C chemical shifts to be assigned as reported in Table 1.

Laevisine B (**2**) had a molecular formula $C_{42}H_{48}N_2O_{18}$, as indicated by ^{13}C, ^{13}C DEPT NMR, and FABMS showed a quasi molecular anion at m/z 867. NMR analysis again suggested the occurrence of euonyminol as the dihydro-β-agarofuran sesquiterpene core. The nicotinate unit in the macrocycle was wilfordic acid,[12] as suggested by one secondary methyl signal at δ 1.22 (d, J = 7.1 Hz, Me-10′), one methine proton at δ 2.41 (m, H-9′), and two sets of methylenes at δ 1.98 and 2.30 (each m, H_2-8′) and at δ 2.95 (ddd, J = 5.6, 6.6, and 13.0 Hz, H-7′a) and 4.00 (ddd, J = 6.0, 9.9, and 13.0 Hz, H-7′b). Both ^{1}H and ^{13}C NMR spectra showed strong similarities to those of euonine (**6**);[12] comparative analysis indicated that one of the acetyl group in **6** was replaced by a nicotinoyl moiety, as indicated by the signals at δ 7.37 (dd, J = 4.6 and 8.0 Hz, H-5″), 8.14 (ddd, J = 1.5, 1.5, and 8.0 Hz, H-4″), 8.76 (dd, J = 1.5 and 4.6 Hz, H-6″), and 9.00 (d, J = 1.5 Hz, H-2″) in the ^{1}H NMR spectrum and at δ 123.3, 125.1, 137.0, 150.1, and 164.0 in the ^{13}C NMR spectrum.

The nicotinoyl moiety was located at C-1 on the basis of the long-range correlation observed in the HMBC spectrum between the carbon signal at δ 164.0 and the proton signal at δ 5.98 (H-1). In good agreement with this finding was the diamagnetic shift observed for the methyl group of the acetate at C-8 (δ 1.50); in fact, as previously described for several polyhydroxy agarofuran derivatives, the unusual diamagnetic effect arises when an equatorial acetate on C-8 is shielded by an aromatic ester on C-1.[12] DQF-COSY, HSQC, and HMBC led to the assignments of ^{1}H and ^{13}C NMR data as reported in Table 1.

Laevisine B (**2**) is closely related to wilfornine, characterized by the occurrence of a nicotinoyl moiety at C-2 and an acetyl group at C-1; wilfornine was found to exert immunosuppressive activity in mice.[11]

Compounds **3**−**9** were identified on the basis of their NMR data in comparison with data from the literature.[12−16]

Experimental Section

General Experimental Procedures. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.86 for ^{13}C using the UXNMR software package was used for NMR measurements in $CDCl_3$ solutions. 2D experiments: ^{1}H−^{1}H DQF-COSY,[17] inverse detected ^{1}H−^{13}C HSQC,[18] and HMBC[19]-were obtained using UX-NMR software. Melting points were determined using a Bausch & Lomb apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. Fast atomic bombardment mass spectra (FABMS) were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2−6 kV). UV measurements were performed on a Perkin-Elmer Lambda 7 spectrophotometer. HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a model 401 refractive index detector.

Table 1. ^{1}H and ^{13}C NMR Data of Laevisines A (**1**) and B (**2**)[a]

	1		**2**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	5.71, d (4.2)	72.5	5.98, d (4.0)	74.0
2	5.21, dd (2.2, 4.2)	68.9	5.25, dd (2.5, 4.0)	69.4
3	4.75, d (2.2)	74.3	5.00, d (2.5)	75.5
4	4.48 (OH)	71.2	4.42 (OH)	71.3
5	7.02, br s	73.6	6.91, br s	73.4
6	2.33, d (4.2)	50.2	2.36, d (4.2)	50.9
7	5.54, dd (4.2, 5.9)	68.6	5.52, dd (4.2, 5.9)	68.8
8	5.32, d (5.9)	69.0	5.42, d (5.9)	71.3
9		52.3		52.3
10		94.1		93.9
11	4.52, d (13.0)	59.8	4.61, d (13.0)	59.9
	5.24, d (13.0)		5.41, d (13.0)	
12	1.54, s	22.5	1.58, s	22.6
13		84.1		84.7
14	1.69, s	18.2	1.68, s	17.8
15	3.70, d (11.5)	69.6	3.78, d (11.6)	70.1
	5.95, d (11.5)		5.76, d (11.6)	
2′		165.4		164.0
3′		124.7		124.9
4′	8.08, dd (1.8, 8.0)	137.3	8.33, dd (1.6, 8.0)	138.5
5′	7.27, dd (4.9, 8.0)	121.0	7.28, dd (4.9, 8.0)	121.3
6′	8.68, dd (1.8, 4.9)	151.3	8.76, dd (1.6, 4.9)	153.7
7′	4.66, q (7.0)	36.2	2.95, ddd (5.6, 6.6, 13.0)	33.2
			4.00, ddd (6.0, 9.9, 13.0)	
8′	2.55, q (7.0)	44.6	1.98, m	33.0
			2.30, m	
9′	1.38, d (7.0)	11.7	2.41, m	38.2
10′	1.21, d (7.0)	9.5	1.22, d (7.1)	18.7
11′		174.0		175.0
12′		168.5		166.9
Mebu (1)				
1		165.7		
2		128.0		
3	6.62, q (8.5)	137.5		
4	1.73, d (8.5)	14.2		
5	1.70, s	11.7		
Nic (1)				
C=O				164.0
2			9.00, d (1.5)	150.1
3				125.1
4			8.14, ddd (1.5, 1.5, 8.0)	137.0
5			7.37, dd (4.6, 8.0)	123.3
6			8.76, dd (1.5, 4.6)	153.7
Ac (2)				
C=O		168.3		168.5
Me	2.15	20.8	2.15	20.8
Ac (5)				
C=O		169.9		169.9
Me	2.22	21.4	2.20	21.5
Ac (7)				
C=O		170.0		169.9
Me	2.13	20.8	2.13	21.1
Ac (8)				
C=O		168.7		168.9
Me	1.82	20.0	1.50	19.8
Ac (11)				
C=O		170.3		170.1
Me	2.32	21.4	2.32	21.2

[a] Assignments confirmed by DQF-COSY, HSQC, and HMBC experiments.

Plant Material. *Maytenus laevis* was collected in the Pastaza Region, Ecuador, in July 1995. A voucher sample of the plant (DF 001024) is deposited at the herbarium of Dipartimento di Scienze Farmaceutiche, University of Salerno.

Extraction and Isolation. The air-dried bark (550 g) was defatted with petroleum ether and $CHCl_3$ and then extracted with $CHCl_3$−MeOH (9:1) to give 8 g of residue. Part of the $CHCl_3$−MeOH (9:1) extract (3.5 g) was chromatographed on a silica gel column using $CHCl_3$ and increasing amounts of MeOH (up to 20%).

After monitoring on TLC [Si gel plates, $CHCl_3$−MeOH (19:1)] the fractions were combined to give A (650 mg), B (250 mg), and C (95 mg) containing crude alkaloid mixtures. Fractions A−C were submitted to HPLC on a μ-Bondapak C-18 column (30 cm × 7.8 mm i.d., flow rate 2.0 mL/min) using MeOH:H_2O

in the ratio 3:2 for A and 13:7 for B and C (isocratic conditions). Pure **1** (8 mg, t_R = 60 min), **7** (52 mg, t_R = 30 min), and **8** (62 mg, t_R = 80 min) were obtained from A; **3** (8 mg, t_R = 22 min), **4** (7.3 mg, t_R = 18 min), **5** (28.5 mg, t_R = 14 min), **6** (38 mg, t_R = 9 min), and **9** (8 mg, t_R = 38 min) were obtained from B; and **2** (6.5 mg, t_R = 18 min) was obtained from C.

Laevisine A (**1**): white amorphous solid; mp 171−173 °C; $[\alpha]_D^{25}$ = +97.3, (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 223 (3.61) and 2.65 (3.24) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 600 MHz), Table 1; FABMS *m*/*z* 844 $[M - H]^-$.

Laevisine B (**2**): white amorphous solid; mp 148−150 °C; $[\alpha]_D^{25}$ = +31.2, (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (4.20) and 2.62 (3.74) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 600 MHz), Table 1; FABMS *m*/*z* 867 $[M - H]^-$.

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