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TRITERPENOID SAPONINS FROM *MIMOSA PIGRA*

JÜRGEN ENGLERT, BERNARD WENIGER, ANNELISE LOBSTEIN, ROBERT ANTON,*

Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université Louis Pasteur, B.P. 24, 67401 Illkirch Cedex, France

ELISABETH KREMPP,

Service de RMN, Faculté de Pharmacie, Université Louis Pasteur, B.P. 24, 67401 Illkirch Cedex, France

DOMINIQUE GUILLAUME,

Laboratoire de Chimie Thérapeutique URA 1310 du CNRS, Faculté de Pharmacie, Université R. Descartes, 4, Avenue de l'observatoire, 75270 Paris Cedex 06, France

and YVES LEROY

Laboratoire de Chimie Biologique, Service de spectrométrie de masse, bâtiment C 9, Université des Sciences de Lille Flandres-Artois, 59655 Villeneuve d'Ascq, France

ABSTRACT.—Two novel triterpene glycosides have been isolated from *Mimosa pigra*. The aglycone of these two compounds was identified as machaerinic acid by ^{1}H- and ^{13}C-nmr spectroscopy and by comparison with an authentic sample. This aglycone is substituted at position C-3 by an identical oligosaccharide chain in these glycosides, and at position C-21 by either a *Z*/*E*-methoxycinnamic [**1**] unit or an *E*-cinnamic acid [**2**] unit.

Recently, we reported the isolation from *Mimosa tenuiflora* L. (Mimosaceae) of two new saponins able to stimulate the proliferation of mouse thymocytes and splenocytes in vitro (1,2). These promising results prompted us to investigate the chemistry of other *Mimosa* species. We report herein the isolation and characterization by gc, gc-ms, ms, and 2D nmr spectroscopy, of two new saponins obtained from *Mimosa pigra* L.

The stem bark of *M. pigra* was extracted with MeOH. The dried MeOH extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was concentrated and the residue, dissolved in MeOH, was poured into Et_2O in order to precipitate the crude saponin mixture. Repeated cc of this mixture afforded saponins **1** and **2**.

The ^{1}H- and ^{13}C-nmr spectra of saponins **1** and **2** indicated that they each possess five sugar residues substituting the same aglycone. Their nmr spectral data displayed also additional signals corresponding to an aromatic moiety, olefinic functions and, in the case of **1**, a MeO group (Table 1).

Acid hydrolysis of **1** and **2** yielded the same aglycone identified as machaerinic acid from nmr data (3) and comparison with an authentic sample. Alkaline hydrolysis of **1** afforded **1a** whose negative-ion fabms displayed an ion peak at *m/z* 1205 (160 atomic mass units fewer than **1**), as well as a mixture of *Z* and *E* 4-methoxycinnamic acid, identified by uv and ^{1}H-nmr spectroscopy and comparison with authentic samples. COSY and a combination of DEPT 90 and 135 nmr spectra allowed the assignment of the 4-methoxycinnamoyl isomeric mixture signals in the ^{1}H- and ^{13}C-nmr spectra of **1** (Table 1). The location of this acyl function at C-21 was deduced from the shielding of H-21 in **1a** as compared to **1** ($\Delta\delta$=1.2 ppm).

The molecular formula of $C_{68}H_{102}O_{28}$ was deduced from fabms data for saponin **1**: the $[M-H]^-$ ion calculated at *m/z* 1365.6488 was verified by hrfabms measurement (found 1365.6472). Its negative-ion fabms showed, together with an $[M-H]^-$ ion at *m/z* 1365, prominent fragments at *m/z* 1233 $[(M-H)-132]^-$, 1205 $[(M-H)-160]^-$, 1059 $[(M-H)-160-146]^-$ and 897 $[(M-H)-160-146-162]^-$, suggesting the loss of a

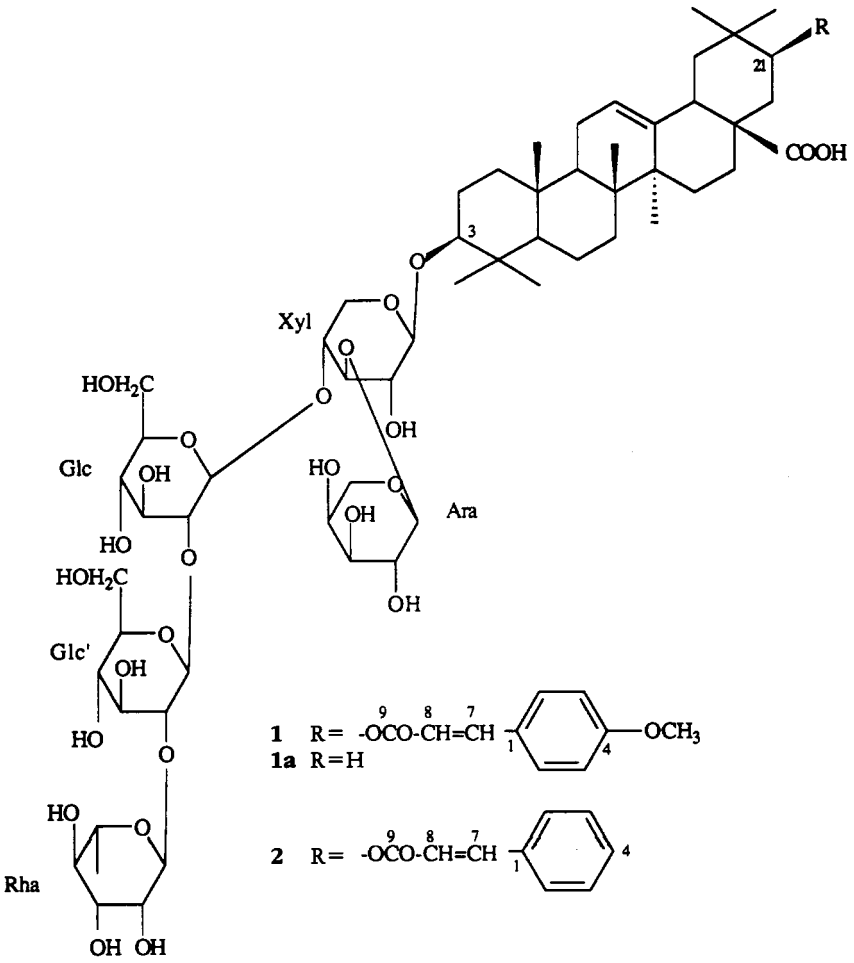

TABLE 1. Selected ^{13}C-Nmr Data for **1** in CD_3OD (125 MHz in ppm).

Aglycone		Sugar					
C-3	92.0	Xyl-1	105.8	Glc-1	107.1	Glc'-1	103.5
C-12	123.2	Xyl-2	71.1	Glc-2	81.8	Glc'-2	80.2
C-13	145.3	Xyl-3	79.8	Glc-3	78.5	Glc'-3	77.9
C-21	78.7	Xyl-4	83.2	Glc-4	73.0	Glc'-4	73.2
C-28	180.2	Xyl-5	65.2	Glc-5	78.2	Glc'-5	79.0
				Glc-6	62.8	Glc'-6	63.2
4-MeO-Cinnamoyl							
C-9	166.0	Ara-1	100.5	Rha-1	102.3		
C-8 (*E*,*Z*)	117.1; 118.9	Ara-2	74.0	Rha-2	73.0		
C-7 (*E*,*Z*)	146.3; 144.2	Ara-3	73.2	Rha-3	73.5		
C-1	135.2	Ara-4	66.9	Rha-4	74.9		
C-2; C-6	131.4	Ara-5	64.6	Rha-5	69.9		
C-3; C-5	116.2			Rha-6	18.2		
C-4	157.0						
O-Me (*E*,*Z*)	56.0; 55.8						

pentose, the *p*-MeO-cinnamoyl unit, and of deoxyhexose and hexose residues.

Methanolysis of **1** yielded a sugar component whose gc analysis (4) showed the presence of glucose (glc), xylose (xyl), arabinose (ara), and rhamnose (rha) in a respective molecular ratio of 2:1:1:1. Gc-ms analysis, carried out on the appropriate sugar derivatives (5), revealed the presence of two glucose units both substituted at C-2, one xylose unit substituted at C-3 and C-4, and one terminal rhamnose and arabinose unit. The COSY and HSQC (6) nmr spectra allowed the assignment of most of the protons and of all the carbons of the sugar residues of **1**, and a combination of ROESY (7) and HMBC (8) nmr experiments allowed the observation of the interglycosidic signals (Table 3). Chemical shifts of the sugar anomeric carbons and protons and the homonuclear coupling constants (Tables 1 and 2) were in full agreement with the presence of an α-L-rhamnose, an α-L-arabinose, a β-D-glucose, and a β-D-xylose. Thus, compound **1** is 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-β-D-xylopyranosyl}-21β-*Z*,*E*-4-methoxycinnamoyloxyolean-12-en-28-oic acid.

TABLE 2. ^{1}H-Nmr Chemical Shift (δ, ppm) and Coupling Constant (*J* in Hz) Values for the Anomeric Protons of **1** in CD_3OD.

Sugar	Chemical shift (δ)	*J* (Hz)
Xyl	4.44	7.6
Glc	4.55	7.5
Glc′	4.97	7.1
Ara	4.62	3.7
Rha	5.22	1.4

With the structure of **1** confirmed, that of compound **2** could be deduced easily. Its alkaline hydrolysis led to the isolation of **1a** and cinnamic acid. This latter residue, present only in the *E* form, was, as in the case of **1**, positioned at C-21 from the ^{1}H-nmr data. Thus, compound **2** is 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-β-D-xylopyranosyl}-21β-*E*-cinnamoyloxyolean-12-en-28-oic acid.

The isolation of acyl machaerinic derivatives has already been reported with no mention of biological activity (3); that of the two novel compounds **1** and **2** will be reported later.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For the nmr measurements, Bruker AC 200 or AMX 500 spectrometers were used. Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured at 500 MHz using phased sequences (TPPI) with a double quantum filter. They were obtained using a data set (t1×t2) of 2048×2048 points for a spectral width of 4464 Hz (relaxation delay 1 sec). The data matrix was processed using a shifted (π/6) square sine bell window function, followed by symmetrization (digital resolution in both F2 and F1 dimensions 1.36 Hz per point). The ROESY (7) experiment was performed in the phase-sensitive mode (TPPI). The spectral width (t2) was 4065 Hz; 1024 experiments of 32 scans each (relaxation delay 1 sec, mixing time 200 msec) were acquired

TABLE 3. Selected ROESY and HMBC Nmr Data of **1** in CD_3OD.

Proton	ROESY	HMBC correlation
H-3	H-1 xylose	
H-1 xylose		C-3 aglycone
H-3 xylose	H-1 arabinose	
H-4 xylose	H-1 glucose	C-1 glucose
H-2 glucose	H-1 glucose′	C-1 glucose′
H-2 glucose′	H-1 rhamnose	
H-1 rhamnose		C-2 glucose′

in 2K data points. For processing, a $\pi/2$ shifted square sine bell window function was applied in both dimensions before transformation (1.98 Hz/point). The HSQC (Heteronuclear Single Quantum Correlation) experiment (6) was performed on a data matrix (t1×t2), 512×1024, for a spectral width of 5000 Hz in the F2 and of 27907 Hz in the F1 dimension. The relaxation delay was 1 sec. The data matrix was processed using a $\pi/2$ shifted square sine bell window function before transformation (digital resolution 2.11 Hz/point in F2 and 17.13 Hz/point in the F1 dimension). The ^{1}H-^{13}C multiple bond correlation (HMBC) experiment was performed on a data matrix (t1×t2), 1024×2048, for a spectral width of 5555 Hz in the F2 and of 26315 Hz in the F1 dimension. The relaxation delay was 1 sec. The data matrix was processed using a $\pi/2$ shifted square sine bell window function before transformation (digital resolution 2.71 Hz/point in F2 and 321 Hz/point in the F1 dimension).

Optical rotations were measured on a Perkin-Elmer polarimeter using a sodium lamp operating at 589 nm. Uv spectroscopy was performed on a Shimadzu UV 1205 spectrophotometer between 200 and 500 nm. Fabms were recorded with a glycerol matrix in the negative-ion mode on a Kratos concept II HH instrument (Xe atoms at 7 kV). Hrfabms was performed with the concept II HH's decade box; γ-cyclodextrin taken as internal reference $(M-H)^-$: $C_{48}H_{79}O_{40}$ *m/z* 1295.4147. The gc-ms spectra were obtained using a Delsi DI 700 gas chromatograph fitted with a silicone OV-101 type capillary column (DB1, 0.32 mm×25 m from Scientific Glass Engineering, Villeneuve St. Georges, France) coupled to a Riber Mag R 10-10 H mass spectrometer working in the electron impact mode at 70 eV.

Authentic samples of *E*-4-methoxycinnamic and cinnamic acid were purchased from Fluka. *Z*-4-Methoxycinnamic acid was prepared by uv irradiation of the *E*-isomer.

PLANT MATERIAL.—*Mimosa pigra* plants were collected in the Mayagüez region of Puerto Rico in May 1991. A voucher sample is deposited at the herbarium of the Mayagüez University (No. 3473).

EXTRACTION AND ISOLATION.—The dried stem bark (150 g) was extracted with MeOH, yielding 15 g of dry residue after concentration. The residue was partitioned between an aqueous KOH solution (1%), and *n*-BuOH saturated with H_2O. The *n*-BuOH fraction was dried and dissolved in MeOH. The MeOH solution was poured into Et_2O to precipitate the crude saponins (4 g) which were sequentially chromatographed on Si gel [CH_2Cl_2-MeOH-H_2O (14:6:1)], RP-8 [MeOH-H_2O (6:4)] and Sephadex LH-20 columns (MeOH) to give 7 mg of **1** and 4 mg of **2**.

Alkaline hydrolysis of **1** *and* **2**.—Small amounts of saponin (1 mg of **1**, 0.3 mg of **2**) were separately hydrolyzed in 5% methanolic KOH at 80° for 2 h. The hydrolysates were partitioned between EtOAc and H_2O. The dried organic layers containing the cinnamic acid derivatives were identified by uv spectrometry and ^{1}H-nmr spectroscopy in comparison to authentic samples. The aqueous layers were neutralized with Ag_2CO_3, filtered and lyophilized. The resulting prosapogenol, **1a**, was identified by ^{1}H-nmr and negative-ion fabms techniques in comparison with literature data (3).

Compound **1**.—$[\alpha]^{25}D$ −8.3° (*c*=0.1, MeOH); uv (MeOH) λ max (log ε) 224 (4.12), 292 (4.38) nm; negative fabms *m/z* $[M-H]^-$ 1365, [(M−H)−ara] 1233, [(M−H)−$C_{10}H_8O_2$] 1205, [*m/z* 1205−rha] 1059, [*m/z* 1059−glc] 897; ^{1}H nmr (500 MHz, MeOD) aglycone: δ 1.01 (Me-23), 0.87 (Me-24), 0.98 (Me-25), 0.91 (Me-26), 1.21 (Me-27), 0.94 (Me-29), 1.12 (Me-30), 5.32 (H-12), 3.18 (1H, dd, *J*=4.5 and 10 Hz, H-3), 4.97 (1H, m, H-21); cinnamoyl residue *E*-isomer: δ 6.36 (1H, d, *J*=15.9 Hz, H-8), 7.61 (1H, d, *J*=15.9 Hz, H-7), 7.57 (2H, d, *J*=8.8 Hz, H-2, H-6), 6.98 (2H, d, *J*=8.8 Hz, H-3, H-5), 3.85 (MeO); *Z*-isomer: δ 5.82 (1H, d, *J*=12.7 Hz, H-8), 6.91 (1H, d, *J*=12.7 Hz, H-7), 7.62 (2H, d, *J*=8.8 Hz, H-2, H-6), 6.92 (2H, d, *J*=8.8 Hz, H-3, H-5), 3.84 (MeO); sugar signals: anomeric protons, see Table 2, δ 1.33 (Me-rha, d, *J*=6.1 Hz); ^{13}C-nmr data, see Table 1.

Compound **1a**.—Negative-ion fabms *m/z* $[M-H]^-$ 1205, [*m/z* 1205−rha] 1059, [*m/z* 1059−glc] 897; ^{1}H nmr (500 MHz, MeOD) aglycone: δ 1.01 (Me-23), 0.87 (Me-24), 0.98 (Me-25), 0.91 (Me-26), 1.21 (Me-27), 0.94 (Me-29), 1.12 (Me-30), 5.32 (H-12), 3.18 (H-3, m), 3.05 (H-21, m); sugar signals: identical to those of **1**.

Compound **2**.—$[\alpha]^{25}D$ −10.1° (*c*=0.1, MeOH); uv (MeOH) λ max (log ε) 223 (4.30), 285 (4.23) nm; hrfabms *m/z* 1336.644 (calcd for $C_{67}H_{100}O_{27}$, 1336.645); negative fabms *m/z* $[M-H]^-$ 1335, [(M−H)−ara] 1203, [(M−H)−C_9H_6O] 1205, [*m/z* 1205−rha] 1059, [*m/z* 1059−glc] 897; ^{1}H nmr (500 MHz, MeOD) aglycone: δ 1.01 (Me-23), 0.87 (Me-24), 0.98 (Me-25), 0.91 (Me-26), 1.21 (Me-27), 0.94 (Me-29), 1.12 (Me-30), 5.32 (H-12), 3.18 (1H, dd, *J*=4.5 and 10 Hz, H-3); cinnamoyl residue: δ 6.52 (1H, d, *J*=15.9 Hz, H-8), 7.68 (1H, d, *J*=15.9 Hz, H-7), 7.57 (2H, d, *J*=8.8 Hz, H-2, H-6), 6.98 (2H, m, H-3, H-5), 3.85 (MeO); sugar signals: anomeric protons: δ 4.45 (1H, d, *J*=7.6 Hz, xyl-1), 4.55 (1H, d, *J*=7.5 Hz, glc-1), 4.97 (1H, d, *J*=7.2 Hz, glc′-1), 4.62 (1H, d, *J*=3.7 Hz, ara-1), 5.22 (1H, d, *J*=1.4 Hz, rha-1); ^{13}C nmr (125 MHz, MeOD) aglycone: δ 29.0 (C-23), 17.4 (C-24), 16.4 (C-25), 18.8 (C-26), 26.9 (C-27), 30.0 (C-29), 19.6 (C-30), 74.9 (C-21), 92.0 (C-3), 123.2 (C-12), 145.3 (C-13), 182.7 (C-28);

cinnamoyl residue: δ 120.0 (C-8), 146.3 (C-7), 135.4 (C-1), 132.0 (C-2), 130.8 (C-3), 133.6 (C-4), 130.8 (C-5), 132.0 (C-6).

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