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J. Nat. Prod., **1991**, 54 (5), 1247-1253• DOI:
10.1021/np50077a002 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 5, pp. 1247-1253, Sep-Oct 1991

STRUCTURE OF A NEW SAPONIN FROM THE BARK OF *MIMOSA TENUIFLORA*

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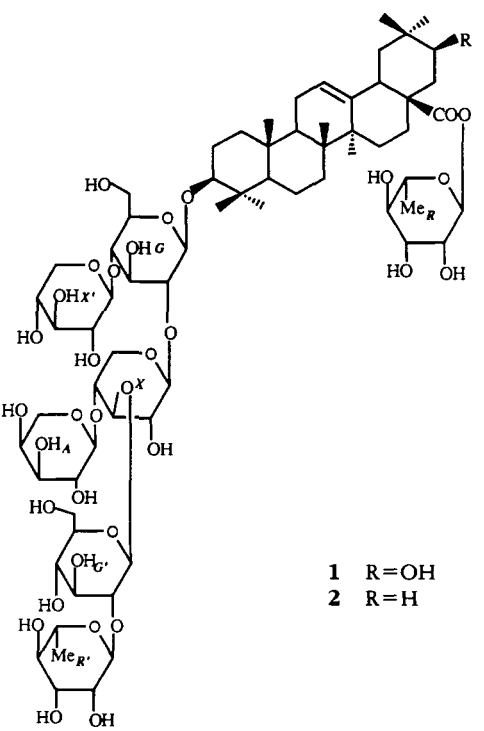
ABSTRACT.—A new saponin was isolated from *Mimosa tenuiflora*, and its structure was established as 3-*O*-{[(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4))-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyl machaerinic acid on the basis of nmr and degradation studies.

Mimosa tenuiflora (Willd.) Poiret (Mimosaceae) is a medicinal plant used in traditional medicine for the treatment of burns and the prevention of inflammation (1,2). In a preceding paper (3), we reported the isolation and structural elucidation of two main saponins: mimonosides A [**2**] and B. As a continuation of studies on this plant, we present here the isolation and the structural elucidation of a minor saponin, which we named mimonoside C [**1**].

RESULTS AND DISCUSSION

The MeOH extract of the bark of *M. tenuiflora* was partitioned between *n*-BuOH and H_2O containing 1% NaOH. The *n*-BuOH extract was repeatedly subjected to cc to



1 R=OH
2 R=H

afford mimonoside C [**1**]. The fabms (thioglycerol matrix, positive ion mode) of **1** showed an $[M+Na+H]^+$ ion at *m/z* 1508, indicating its mol wt to be 1484.

The ^{1}H-nmr spectrum showed the signals of seven tertiary methyl groups (δ 0.78, 0.82, 0.90, 0.92, 0.94, 1.06, 1.14), one trisubstituted olefinic proton (δ 5.36), and six anomeric protons [δ 4.36 (d, J = 7.7 Hz), 4.45 (d, J = 7.6 Hz), 4.60 (d, J = 7.6 Hz), 4.46 (d, J = 3.0 Hz), 5.16 (d, J = 1.4 Hz), 5.93 (d, J = 1.8 Hz)]; the COSY experiment showed that another anomeric proton (δ 4.92) was hidden under the signal of residual CD_3OH. The ^{13}C-nmr spectrum revealed the presence of six SP^3 quaternary carbon atoms (δ 36.2, 37.9, 39.9, 40.3, 40.6, 49.4), a pair of olefinic carbons (δ 124.4, 144.7), one sugar-substituted methine (δ 91.0), one ester carbonyl (δ 176.9), and seven anomeric carbon atoms (δ 95.1, 99.6, 102.5, 102.7, 105.2, 105.4, 106.2). The chemical shift of the first anomeric carbon signal showed that the sugar residue was attached to the aglycone by an ester bond. These spectral data suggested that **1** was a bidesmosidic saponin of an oleanolic-type triterpene (4). Comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** with those of mimonoside A [**2**] showed great similarity.

Acid hydrolysis of **1** gave an aglycone and arabinose, rhamnose, xylose, and glucose, which were identified by gc. The mol wt of the aglycone was determined by gc-ms and was equal to 472. The usual retro-Diels-Alder fragmentation showed that an OH was present in ring A and another in ring D or E. Examination of heteronuclear multiple-bond connectivity (HMBC) (5) of peracetylated **1** indicated that one hydroxyl was at C-3 and the other at C-21 (^3J correlations with Me-23 and -24 and with Me-29 and -30, respectively) (Figure 1). Therefore the aglycone was identified as machaerinic acid.

Sequencing of the sugar chain was done on the basis of ^{1}H- and ^{13}C-nmr on the

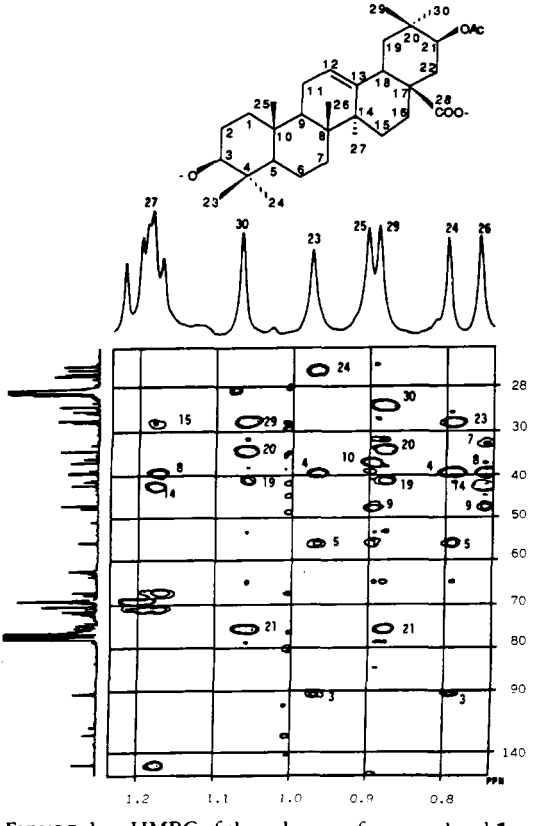


FIGURE 1. HMBC of the aglycone of peracetylated **1**.

peracetylated material (6). Acetylation deshields all the C*H* α to acetates in the 4.5–5.5 ppm area and leaves unaffected all the protons α to branching points (α to ether functions). Thus ^{1}H chemical shift examination allows the determination of the branching of the sugars as well as the identification of the terminal sugars, which were fully acetylated. Sequencing of the sugar chain requires the observation of the correlations between elements of different sugar residues; this is done using the HMBC experiment which correlates protons with carbon atoms separated by two or three bonds. The glycosidic bond offers two such C-H correlations.

High field nmr is now used more and more frequently to determine the sequence of glycosidic chains. We are aware of several examples of saponins whose structures were established by nmr and independently by degradation techniques (7–9). The structures of chrysantellines, for example, were based on degradation and on partially assigned ^{1}H-nmr spectra. This led to wrong configurational assignments which were later revised using nmr techniques (10).

^{1}H- and ^{13}C-nmr are broadly accepted as tools for structural elucidation of natural products; this should be also true for saponins and there is no reason that two demonstrations of structure should be mandatory for this particular class of compounds.

The ^{1}H-nmr spectrum of peracetylated **1** showed seven anomeric protons that gave rise to separated resonances at δ 6.00 (d, J = 1.8 Hz), 4.90 (br s), 4.68 (d, J = 7.4 Hz), 4.58 (d, J = 7.0 Hz), 4.48 (d, J = 6.8 Hz), 4.44 (d, J = 6.9 Hz), and 4.41 (d, J = 7.5 Hz). The spin systems corresponding to those protons were analyzed by means of relayed COSY and HOHAHA experiments and by comparison with the spectrum of compound **2**.

The sugars corresponding to the two first anomeric protons showed similar coupling patterns with H-1 as broad doublets (J<2 Hz), H-2 double doublets (J = 2, 3 Hz on the average), H-3 double doublets (J = 3 and 9 Hz), H-4 triplets (J = 9 Hz), and H-5 double quartets (J = 9 and 6 Hz). These couplings are typical of α-L-rhamnopyranosyl. The chemical shifts of protons 2, 3, and 4 (δ 5.5–5.8 ppm) indicate that the two rhamnoses are fully acetylated and are thus terminal. The HMBC experiment showed a correlation between the first rhamnose H-1 and C-28 of the aglycone (Figure 2), confirming that a terminal rhamnose was attached to C-28 of the aglycone. The six other sugars therefore form the second chain at C-3 of the aglycone.

The anomeric doublets at δ 4.41 and 4.68 belong to two glucose residues, according to COSY and relayed COSY experiments, which allowed detection of H-2, H-3, H-4, H-5, and 2H-6. All the coupling constants except H-5 and 2H-6 were large and corresponded to the axial protons of two β-D-glucopyranose residues. In one of the sugars,

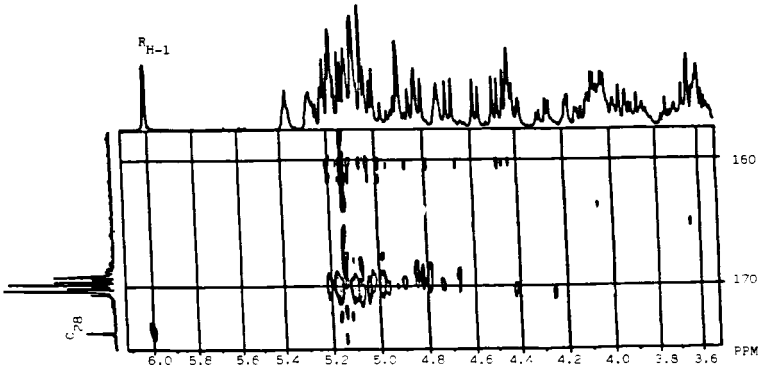


FIGURE 2. HMBC of the rhamnose moiety attached to C-28 in the structure of peracetylated **1**.

H-2 (δ 3.64) and H-4 (δ 3.59) were shielded, which showed that these positions were substituted. In the second glucose, H-2 (δ 3.60) was shielded, indicating that position 2 was substituted.

The sugar whose anomeric proton is a doublet at δ 4.48 had deshielded H-2 (δ 5.12), H-3 (5.04), H-4 (5.26), and two shielded H-5 at δ 4.40, 3.59, suggesting that it is a terminal pentose. H-1 appeared as a doublet ($J = 6.8$ Hz), H-2 as double doublet (dd, $J = 6.8$, 9.0 Hz), H-3 (dd, $J = 9.0$, 3.4 Hz) and H-4 (dd, $J = 3.8$, 2.0 Hz). These couplings were typical of α-L-arabinopyranose.

The remaining two anomeric doublets at δ 4.58 and 4.44, were used as starting points for the analysis of the 2D COSY experiments. They corresponded to two sugars, one having deshielded H-2, H-3, H-4, and two shielded H-5, while the other had deshielded H-2 and shielded H-3, H-4, and 2H-5. In both, H-1, H-2, H-3, and H-4 showed the large coupling constants of axial protons of β-D-xylopyranoses. These results suggest that one is a terminal β-D-xylose, and the other a β-D-xylose substituted at positions C-3 and C-4.

Having assigned all proton resonances of the oligosaccharide part, we determined the ^{13}C shift values by DEPT, HMBC, and HMQC (11) experiments (Tables 1, 2).

TABLE 1. ^{13}C-nmr Chemical Shifts of the Aglycone Moiety of Peracetylated **1**.

Carbon	δ	Carbon	δ	Carbon	δ
C-1	38.6	C-11	23.4	C-21	76.4
C-2	25.7	C-12	123.6	C-22	35.9
C-3	90.5	C-13	142.8	C-23	27.6
C-4	39.3	C-14	42.0	C-24	16.2
C-5	55.7	C-15	27.8	C-25	15.4
C-6	18.2	C-16	23.4	C-26	17.5
C-7	32.8	C-17	46.9	C-27	25.3
C-8	39.1	C-18	41.2	C-28	174.6
C-9	47.5	C-19	40.8	C-29	27.3
C-10	36.7	C-20	34.1	C-30	24.2

The HMBC experiment showed cross peaks between C-3 of the aglycone and 2,4-disubstituted glucose H-1, which demonstrated that this sugar was linked to the aglycone at position 3. We also observed the correlations between 2,4-disubstituted glucose C-2 and 3,4-disubstituted xylose H-1, between this glucose C-4 and terminal xylose H-1, between terminal arabinose H-1 and 3,4-disubstituted xylose C-4, between the xylose H-3 and 2-substituted glucose C-1, and between 2-substituted glucose C-2 and terminal rhamnose H-1. This allowed the establishment of a sugar sequence identical with that of mimonoside A [**2**].

The fabms of peracetylated **1** (thioglycerol matrix, positive ion mode) exhibited a quasi-molecular ion $[M + Na]^+$ at *m/z* 2305 and intense signals at *m/z* 259, 273, 561, 993, which corresponded to terminal pentose, to terminal rhamnose, to a disaccharide composed of rhamnose and of glucose, and to a tetrasaccharide composed of rhamnose, of glucose, and of two pentoses, respectively. These results confirmed the results obtained by nmr analysis.

Based on the above results, mimonoside C [**1**] was established as 3-*O*-{[(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4))-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyl machaerinic acid.

TABLE 2. ^{13}C-nmr Chemical Shifts (δ) of Sugar Moieties of Peracetylated **1** and **2**.[a]

Carbon	Compound		Carbon	Compound		Carbon	Compound		Carbon	Compound	
	Peracetylated **1**	Peracetylated **2**		Peracetylated **1**	Peracetylated **2**		Peracetylated **1**	Peracetylated **2**		Peracetylated **1**	Peracetylated **2**
G-1	103.1	103.2	G′-1	100.8	101.5	A-1	99.7	99.7	X′-1	101.5	102.2
G-2	77.9	76.2	G′-2	77.9	78.5	A-2	71.2	71.7	X′-2	71.8	70.7
G-3	75.4	75.3	G′-3	74.1	74.4	A-3	71.8	72.1	X′-3	72.1	72.1
G-4	80.9	78.4	G′-4	70.7	69.4	A-4	67.5	68.0	X′-4	69.4	69.6
G-5	77.4	72.1	G′-5	72.1	72.1	A-5	63.4	63.8	X′-5	62.6	62.7
G-6	62.2	62.1	G′-6	62.2	62.1						
X-1	100.2	100.4	R-1	90.7	90.9	R′-1	98.0	98.4			
X-2	71.4	71.9	R-2	70.2	69.8	R′-2	70.2	69.4			
X-3	77.4	77.2	R-3	69.4	69.6	R′-3	69.4	68.9			
X-4	74.6	74.4	R-4	69.1	68.9	R′-4	68.8	69.2			
X-5	62.4	62.5	R-5	69.4	69.4	R′-5	67.0	67.2			
			R-6	17.0	18.6	R′-6	17.1	18.6			

[a]G = glucose, X = xylose, R = rhamnose, A = arabinose.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Fabms were obtained on a ZAB HF in the positive ion mode. ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AC-300, 300 MHz and 75 MHz in $CDCl_3$ for peracetylated saponin, 400 MHz and 100 MHz in CD_3OD for saponin. Gc-ms was obtained on a Perkin-Elmer Sigma 3B with VG 7070F spectrometer. Si gel 60 F 254 precoated plates (Merck) and Si gel (70–230 mesh, Merck) were used for tlc and cc, respectively.

EXTRACTION AND ISOLATION.—Plant material was collected in Mexico and supplied to us by UPSA Company, Rueil, France, where a voucher specimen is kept. Dried and powdered bark (2 kg) was successively treated with $CHCl_3$, EtOAc, and MeOH; it afforded 643 g of MeOH extract. Part of the MeOH extract (187 g) was partitioned between *n*-BuOH and H_2O containing 1% NaOH. The *n*-BuOH layer was washed with H_2O, dried with Na_2SO_4, and concentrated in vacuo to dryness (27 g). The residue was dissolved in a minimal volume of MeOH and poured into Et_2O. The precipitate was subjected to Sephadex LH 20 cc using MeOH as solvent and then to Si gel cc using a gradient of $CHCl_3$/MeOH as solvents. The saponin fractions were chromatographed again over a LiChroprep RP-8 column with MeOH-H_2O (6:4). Final purification was performed over Si gel column using $CHCl_3$-MeOH-H_2O (14:6:1) as solvent to afford **1** (30 mg).

Saponin **1**.—White powder: $[\alpha]_D$ −27.2 (MeOH, c = 0.36); fabms *m/z* $[M+Na+H]^+$ 1508; ^{1}H nmr (400 MHz, CD_3OD) δ 5.93 (d, J = 1.8 Hz, ester rhamnose H-1), 5.16 (d, J = 1.4 Hz, rhamnose H-1), 4.46 (d, J = 3.0 Hz, arabinose H-1), 4.60 (d, J = 7.6 Hz), 4.45 (d, J = 7.6 Hz), 4.36 (d, J = 7.7 Hz) (3 anomeric H), 5.36 (t, J = 3.0, H-12), 3.02 (dd, J = 3.2, 13.7 Hz, H-3), 1.22 (d, J = 6.2 Hz), 1.28 (d, J = 6.2 Hz, two rhamnose Me), 1.14, 1.06, 0.94, 0.92, 0.90, 0.82, 0.78 (s, 7 × Me); ^{13}C nmr (50 MHz, CD_3OD) δ 176.9 (C-28), 144.7 (C-13), 124.4 (C-12), 91.0 (C-3), 76.6 (C-21), 95.1 (rhamnose C-1), 99.6 (rhamnose C-1), 102.5 (arabinose C-1), 102.7 (xylose C-1), 105.2 (glucose C-1), 105.4 (xylose C-1), 106.2 (glucose C-1).

ACID HYDROLYSIS OF **1**.—A solution of **1** (2 mg) in 10% HCl (5 ml) was refluxed for 4 h and worked up in the usual way. The residue was purified on tlc with $CHCl_3$-MeOH (98:2) as solvent and was subjected to gc-ms. Eims *m/z* $[M]^+$ 472 (2.8), 454 (3.0), 436 (5.8), 390 (35.5), 264 (65.8), 246 (41.0), 207 (35.3), 201 (92.4), 133 (26.2).

The filtrate from the hydrolysate was concentrated to dryness. The residue was analyzed by gc as glucose, xylose, rhamnose, and arabinose after trimethylsilylation with pyridine and BSTFA + 1% TMSC.

ACETYLATION OF **1**.—Compound **1** (10 mg) was dissolved in Ac_2O-pyridine (1:1) (2 ml) and left at room temperature for 24 h. The solvent and reagent were removed by codistillation with toluene. The residue was subjected to Si gel cc using $CHCl_3$-MeOH (120:1) as solvent to afford peracetylated **1** (10 mg).

Peracetylated **1**.—White powder: fabms *m/z* $[M+Na]^+$ 2305, $[Rha-Glu-2Pen(Ac)10]^+$ 993, $[Rha-Glu(Ac)6]^+$ 561, $[Rha(Ac)3]^+$ 273, $[Pen(Ac)3]^+$ 259; ^{1}H nmr see Table 3; ^{13}C nmr see Tables 1 and 2.

TABLE 3. ^{1}H-nmr Chemical Shift Values (δ) of the Sugar Moieties of Peracetylated **1**.

Sugar moiety	Proton							
	H-1	H-2	H-3	H-4	H-5	H-5′	H-6	H-6′
Rhamnose (ester)	6.00	5.17	5.20	5.08	4.06		1.18	
Rhamnose (terminal)	4.90	5.08	5.17	5.10	3.90		1.22	
Glucose (2,4-disubstituted)	4.41	3.64	5.04	3.59	3.55		4.40	4.05
Glucose (2-substituted)	4.68	3.60	5.18	5.00	3.69		4.25	4.18
Xylose (inner)	4.44	4.82	3.95	3.83	3.24	4.06		
Xylose (terminal)	4.58	4.82	5.10	4.91	3.31	4.06		
Arabinose	4.48	5.12	5.04	5.26	4.40	3.59		

ACKNOWLEDGMENTS

Financial support has been provided by the UPSA Company, Rueil, France. The authors are grateful to Dr. J.C. Quirion (ICSN/CNRS Gif/Yvette, France) for nmr facilities.

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Received 7 *November* 1990