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# TRITERPENOID GLYCOSIDES FROM THE BARK OF *MIMOSA TENUIFLORA*

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**Key Word Index**—*Mimosa tenuiflora*; Mimosaceae; bark; saponins; oleanolic acid; mimonosides A and B.

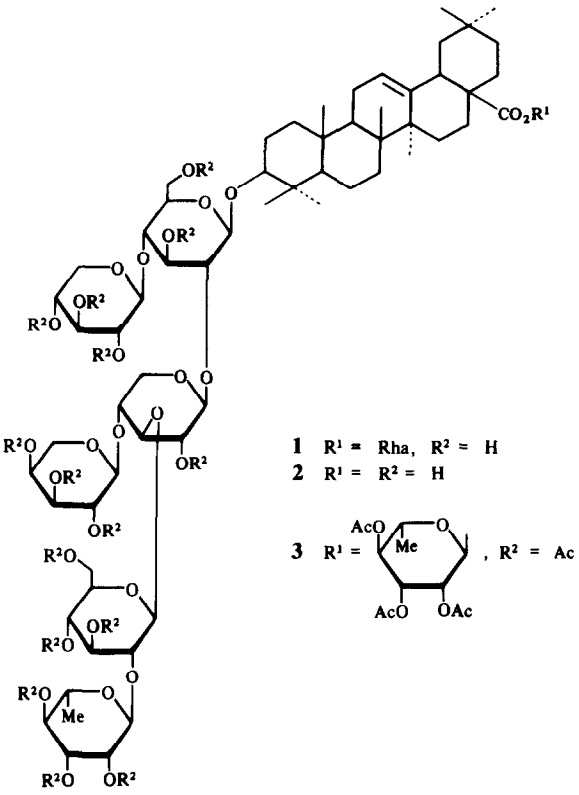
**Abstract**—Two new saponins were isolated from *Mimosa tenuiflora* and their structures established as 3-*O*-{[(α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4))-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyl oleanolic acid and 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} oleanolic acid.

## INTRODUCTION

*Mimosa tenuiflora* (Willd) Poiret (Mimosaceae) is widely distributed in Mexico and South America [1] where its bark is popularly used as a remedy to treat skin lesions. In Mexico, this material has recently been used to alleviate the suffering of burn victims following the 1985 earthquake and the San Juanico natural gas explosion in 1984. The clinical use indicates that application of properly prepared bark powder to burned tissue facilitates skin regeneration and prevents scarring [2]. Pharmacological studies of the water and alcoholic extracts, prepared with the dried and powdered bark demonstrated *in vitro* antimicrobial properties [3] and phytochemical investigations on the small branches led to the isolation of two new chalcones, kukulkanin A and B [4]. We now report the isolation and the structural elucidation of two new triterpenoid glycosides, designated as mimonosides A (**1**) and B (**2**), from the bark of this plant.

## RESULTS AND DISCUSSION

The methanol extract of dried and powdered bark was partitioned between *n*-butanol and water containing 1% NaOH. The butanol layer was repeatedly subjected to CC to give two new saponins, **1** and **2**. Acid hydrolysis of **1** and **2** afforded the same aglycone, identified as oleanolic acid, by comparison with an authentic sample (TLC, MS and $^1$H NMR). The sugars obtained from the saponin hydrolysates were identified by GC as glucose, xylose, rhamnose and arabinose for both **1** and **2**. On basic hydrolysis, saponin **1** afforded **2**.




**1** $R^1$ = Rha, $R^2$ = H
**2** $R^1$ = $R^2$ = H
**3** $R^1$ = , $R^2$ = Ac

The $^{13}$C NMR spectrum of **1** exhibited seven anomeric signals at δ106.2, 105.4, 105.2, 102.7, 102.5, 99.8 and 95.0, indicating the presence of seven sugar moieties: the last signal showed that one sugar residue is attached to the aglycone by an ester bond. The $^{13}$C NMR spectrum of **2** exhibited six anomeric signals at δ106.3, 105.8, 104.8, 102.7, 101.4 and 99.4, indicating the presence of six sugar moieties. The disappearance of the anomeric signal at δ95.0 in the $^{13}$C NMR spectrum of **2** suggested that only one sugar moiety is attached to the aglycone by an ester bond in saponin **1**. The $^1$H NMR and 2D COSY spectra

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showed that this sugar moiety is a rhamnose. This suggested that saponin **1** is composed of saponin **2** and of a rhamnose moiety attached to C-28 of the aglycone by an ester bond.

The $M_r$ of the saponins was established by FAB mass spectrometry. The FABMS (thioglycerol matrix, positive ion mode) of compound **1** showed a quasi-molecular ion $[M+H+Na]^+$ at *m/z* 1492. The FAB mass spectrum of compound **2** presented a quasi-molecular ion $[M+Na]^+$ at *m/z* 1345, supporting the suggestion that the difference between **1** and **2** is a rhamnose unit. The FAB mass spectrum (thioglycerol matrix, negative ion mode) of **2** exhibited a quasi-molecular ion, $[M-H]^-$, at *m/z* 1321 and the fragments at *m/z* 1189 $[M-H-Pen]^-$, 1175 $[M-H-Rha]^-$, 1013 $[M-H-Rha-Glc]^-$, 881 $[M-H-Rha-Glc-Pen]^-$, 749 $[M-H-Rha-Glc-2Pen]^-$, 455 $[M-H-Rha-2Glc-3Pen]^-$. This sequence suggested that one rhamnose, or a rhamnose-glucose disaccharide, is in the terminal position. These results were supported by the FAB mass spectrum of compound **3** which was obtained by acetylation of saponin **1** with acetic anhydride–pyridine. The FAB mass spectrum (thioglycerol matrix, positive ion mode) of **3** exhibited a quasi-molecular ion $[M+Na+H]^+$ at *m/z* 2248, and very intense signals at *m/z* 259, 273, 561, 993 which corresponded to terminal pentose and rhamnose, to a disaccharide composed of rhamnose and of glucose and to a tetrasaccharide composed of rhamnose, of glucose and of two pentoses, respectively.

The sugar sequence and the interglycosidic linkage of saponins were determined by permethylation/$NaBH_4$/GC-MS analysis [5–7] and by analysis of the NMR spectra of the peracetylated saponin [8–10]. The GC-MS of the methylated alditolacetate derivatives of **1** indicated the formation of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpentitol, 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylpentitol, 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol, 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methylhexitol which correspond to 1-substituted pentose, 1,3,4-trisubstituted pentose, 1-substituted rhamnose, 1,2-disubstituted glucose and 1,2,4-trisubstituted glucose, respectively. The $^1H$ NMR spectrum of **3** showed seven anomeric protons which gave rise to separated resonances at $\delta$6.55 (*d*, $J=1.6$ Hz), 5.26 (*d*, $J=1.9$ Hz), 5.03 (*d*, $J=5.8$ Hz), 4.75 (*d*, $J=7.4$ Hz), 4.46 (*d*, $J=6.9$ Hz), 4.33 (*d*, $J=6.3$ Hz), 4.22 (*d*, $J=7.2$ Hz).

The two sugars corresponding to the two first anomeric protons have their H-2, H-3 and H-4 in the $\delta$5.5–5.8 area. Their coupling patterns are typical of α-L-rhamnopyranoses. According to the chemical shifts of H-2, H-3 and H-4, the two sugars are terminal rhamnoses. The Heteronuclear Multiple-Bond Connectivity (HMBC) experiment [11] showed a correlation between the first rhamnose H-1 and C-28 of the aglycone. This confirmed that the rhamnose is attached to C-28 of the aglycone. The anomeric doublets at $\delta$4.75 and 4.46 belong to two glucose residues, according to 2D COSY where H-2, H-3, H-4, H-5 and $H_2$-6 were detected. All the coupling constants were large and correspond to the axial protons of two β-D-glucopyranose residues. In one of the sugars, H-2 ($\delta$3.95) and H-4 ($\delta$3.61) were shielded, which showed that these positions are substituted. In the second glucose, H-2 ($\delta$3.75) was shielded indicating a substitution at the position C-2.

The sugar whose anomeric proton is a doublet at $\delta$4.22 has a deshielded H-2 ($\delta$5.53), H-3 (5.12), H-4 (5.27) and $H_2$-5 shielded at $\delta$3.73, 2.97, suggesting that it is a terminal pentose. H-1 appeared as a doublet ($J=7.2$ Hz), H-2 as a double doublet ($J=7.2$, 10.0 Hz), H-3 as a double doublet ($J=10.0$, 3.8 Hz) and H-4 as a double doublet ($J=3.8$, 2.0 Hz). These couplings are typical of α-L-arabinopyranose. The two remaining anomeric protons were doublets at $\delta$4.33 and 5.03, which were used as starting points for the analysis of the 2D COSY experiments. They correspond to two sugars, one of which deshields H-2, H-3, H-4 and two shield H-5; while the other deshields H-2 and shield H-3, H-4 and $H_2$-5. In both of them, H-1, H-2, H-3 and H-4 showed large coupling constants of axial protons of β-D-xylopyranoses. These results suggested that one is a terminal β-D-xylose, and the other a β-D-xylose substituted at positions C-3 and C-4.

Despite the fact that FAB mass spectral data provided information on the gross shape of the sugar chain, this technique did not allow recognition of degenerate residues (xylose from arabinose, for example). A more precise sequencing was obtained by measuring inter-residue Overhauser effects. To overcome the $\omega\tau_c$ problem, it was chosen to run a ROESY experiment [12] with a 200 msec mixing time. Gross peaks were observed between H-3 of the aglycone and trisubstituted glucose H-1, between this glucose H-2 and inner xylose H-1, between this glucose H-4 and terminal xylose H-1, between inner xylose H-3 and disubstituted glucose H-1 and between disubstituted glucose H-2 and terminal rhamnose H-1. The missing correlation is between terminal arabinose H-1 and inner xylose in the ROESY experiment; but the correlation between arabinose H-1 and

Table 1. $^1H$ NMR chemical shift values ($\delta$) of the sugar moieties of compound **3** (in $C_6D_6$)

| | H | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sugar moieties | 1 | 2 | 3 | 4 | 5 | 5′ | 6 | 6′ |
| Rha (ester) | 6.55 | 5.68 | 5.76 | 5.64 | 4.31 | | 1.34 | |
| Rha (terminal) | 5.26 | 5.53 | 5.67 | 5.60 | 4.42 | | 1.54 | |
| Glc (attached to genin) | 4.46 | 3.95 | 5.41 | 3.61 | 3.38 | | 4.18 | 4.50 |
| Glc (attached to xylose) | 4.75 | 3.75 | 5.44 | 5.28 | 3.38 | | 4.42 | 4.20 |
| Xyl (inner) | 5.03 | 5.33 | 4.26 | 4.22 | 3 54 | 4.33 | | |
| Xyl (terminal) | 4.33 | 5.12 | 5.33 | 4.94 | 3.89 | 3.02 | | |
| Ara | 4.22 | 5.53 | 5.12 | 5.27 | 3.72 | 2.97 | | |

Table 2. $^{13}C$ NMR chemical shift values ($\delta$) of the sugar moieties of compounds **1–3**

| C | | 1 | 2 | 3* | C | | 1 | 2 | 3* |
|---|---|---|---|---|---|---|---|---|---|
| Rha (ester) | 1 | 95.0 | | 90.9 | Xyl (inner) | 1 | 102.7 | 103.0 | 100.4 |
| | 2 | 73.1[a] | | 69.8[a] | | 2 | 76.0 | 76.1 | 71.9[b] |
| | 3 | 72.2[b] | | 69.6[a] | | 3 | 80.1 | 80.3 | 77.2 |
| | 4 | 72.1[b] | | 68.9 | | 4 | 78.0 | 78.5 | 74.4 |
| | 5 | 70.9 | | 69.4[a] | | 5 | 64.5 | 64.6 | 62.5 |
| | 6 | 18.1 | | 18.6 | | | | | |
| Rha (terminal) | 1 | 99.8 | 99.4 | 98.4 | Xyl (terminal) | 1 | 105.4 | 105.8 | 102.0 |
| | 2 | 73.4[a] | 73.5 | 69.4[a] | | 2 | 75.0 | 75.2 | 70.7 |
| | 3 | 72.4[b] | 72.5[a] | 68.9 | | 3 | 76.6[c] | 76.1 | 72.1[b] |
| | 4 | 71.4 | 71.8 | 69.2[a] | | 4 | 73.1 | 73.0 | 69.6[a] |
| | 5 | 69.8 | 69.8 | 67.2 | | 5 | 67.2 | 67.5 | 62.7 |
| | 6 | 18.1 | 18.1 | 18.6 | | | | | |
| Glc (attached to genin) | 1 | 106.2 | 106.3 | 103.2 | Ara | 1 | 102.5 | 103.7 | 99.7 |
| | 2 | 80.0 | 79.4 | 76.2 | | 2 | 74.1 | 74.3 | 71.7[b] |
| | 3 | 79.1 | 79.1 | 75.3 | | 3 | 76.0 | 76.1 | 72.1[b] |
| | 4 | 81.0 | 81.2 | 78.4 | | 4 | 66.3 | 65.6 | 68.0 |
| | 5 | 76.6[c] | 76.1 | 72.1[b] | | 5 | 62.6 | 62.0 | 63.8 |
| | 6 | 62.1 | 62.0 | 62.1 | | | | | |
| Glc (attached to xylose) | 1 | 105.2 | 104.8 | 101.5 | | | | | |
| | 2 | 82.6 | 82.1 | 78.5 | | | | | |
| | 3 | 78.0 | 78.5 | 74.4 | | | | | |
| | 4 | 72.4[b] | 73.0[a] | 69.4[a] | | | | | |
| | 5 | 77.0[c] | 77.2 | 72.1 | | | | | |
| | 6 | 62.1 | 62.0 | 62.1 | | | | | |

*The assignment was based on the HMBC, HMQC and DEPT experiments.
[a–c]Signals may be interchangeable in each vertical column.

inner xylose C-4 was observed in the HMBC experiment. The ROESY experiment also afforded confirmations of α-L-arabinose, β-D-xylose and β-D-glucose: ROEs were found between arabinose H-1 and H-3, between H-1 and H-5 for xylose and for glucose. α-L-rhamnose was confirmed from H-1 to H-5 long range coupling (COSY LR) [13]. These data allowed proposal of the structure, 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 oleanolic acid for **1** and 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} oleanolic acid for **2**.

## EXPERIMENTAL

*General.* Mps: uncorr. IR were measured in KBr discs. EIMS were obtained at 70 eV. $^1H$ and $^{13}C$ NMR spectra: 300 and 75 MHz in $CDCl_3$, and $C_6D_6$. GC conditions: fused silica column 0.22 mm × 25 m with packed DB1-60, injection temp. 250°, column temp. 160–320° (4° $min^{-1}$) (25 min). Silica gel 60 F 254 precoated plates (Merck) and silica 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. Dried and powdered bark (2 kg) was treated respectively by $CHCl_3$, EtOAc and MeOH; it afforded 643 g of MeOH extract. The MeOH extract (187 g) was partitioned between *n*-BuOH and $H_2O$ containing 1% NaOH. The BuOH layer was washed with $H_2O$ and dried with $Na_2SO_4$ and concd *in vacuo* to dryness (27 g). The residue was dissolved in a minimal vol. of MeOH and poured into $Et_2O$. The ppt. was subjected to Sephadex LH 20 column chromatography using MeOH as solvent and then to silica gel CC using a gradient of $CHCl_3$–MeOH as solvents. The saponin frs were rechromatographed over LiChroprep RP-8 column with MeOH–$H_2O$ (3:2). The final purification was performed over a silica gel column using $CHCl_3$–MeOH–$H_2O$ (14:6:1) as solvents to afford saponin **1** (200 mg) and saponin **2** (60 mg).

*Saponin* **1**. Powder, mp 243.9–245.2° (dec.). $[\alpha]_D$ −29.2° (MeOH; *c* 0.75), IR $\nu_{max}^{KBr}$ $cm^{-1}$ 3400 (OH), 1720 (ester). FABMS (positive ion mode) *m/z*: 1492 $[M+H+Na]^+$, 1469 $[M+H]^+$, 1361 $[M+2H+Na-132]^+$, 1216 $[M+3H+Na-132-146]^+$, 1017 $[M+3H-2\times146-162]^+$, 867 $[M+3H-2\times146-162-132-18]^+$, 735 $[M+3H-2\times146-162-2\times132-18]^+$, 437 $[M+2H+Na-2\times146-2\times162-3\times132-44]^+$. $^1H$ NMR (200 MHz, $CD_3OD$): δ 5.94 (*d*, *J* = 1.0 Hz, ester Rha H-1), 5.18 (*br s*, Rha H-1), 4.62 (*d*, *J* = 3.0 Hz, Ara H-1), 4.94 (*d*, *J* = 7.8 Hz), 4.60 (*d*, *J* = 7.6 Hz), 4.45 (*d*, *J* = 8.5 Hz), 4.38 (*d*, *J* = 7.8 Hz) (4 anomeric H: 2Glc + 2Xyl). $^{13}C$ NMR (50 MHz, $CD_3OD$) see Table 1.

*Saponin* **2**. Powder, mp 237.4–240.2° (dec.). $[\alpha]_D$ −28.4° (MeOH; *c* 0.78). FAB-MS (positive ion mode) *m/z*: 1345 $[M+Na]^+$, FAB-MS (negative ion mode) *m/z*: 1321 $[M-H]^-$, 1189 $[M-H-132]^-$, 1175 $[M-H-146]^-$, 1013 $[M-H-146-162]^-$, 881 $[M-H-146-162-132]^-$, 749 $[M-H-146-162-2\times132]^-$, 455 $[M-H-146-2\times162-3\times132]^-$. $^1H$ NMR (400 MHz, $CD_3OD$): δ 5.17 (*br s*, Rha H-1), 4.66 (*d*, *J* = 3.1 Hz, Ara H-1), 4.60 (*d*, *J* = 8.2 Hz), 4.45 (*d*, *J* = 7.6), 4.77 (*d*, *J*

= 7.65 Hz) (3 anomeric H), 2D COSY spectrum showed that an anomeric H signal was hidden under residual $CD_3OH$ signals. $^{13}C$ NMR (50 MHz, $CD_3OD$) see Table 1.

*Acid hydrolysis of saponin* **1**. Saponin **1** (10 mg) was hydrolysed with 10% HCl at 100° for 4 hr and worked-up in the usual way. The residue was purified over silica gel column using $CHCl_3$–MeOH (49:1) as solvent and was followed by crystallization from MeOH, to afford oleanolic acid (2 mg). EIMS *m/z* 456 $[M]^+$ (1.8), 248 (100), 203 (75). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1695 ($CO_2H$). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$0.76, 0.78, 0.92 (× 2), 0.94, 1.0, 1.14 (*s*, 7 × Me), 2.87 (*dd*, *J* = 3.8, 13.4 Hz, H-3), 5.26 (*t*, *J* = 3.0 Hz, H-12). The filtrate from the hydrolysate was concentrated to dryness. The residue was identified by GC as glucose, xylose, rhamnose and arabinose after trimethylsilylation with pyridine and BSTFA + 1% TMSC.

*Acid hydrolysis of* **2**. As described above.

*Basic hydrolysis of* **1**. A soln of saponin **1** (30 mg) in 0.5 M KOH (25 ml) was refluxed for 3 hr. The pH of the reaction mixt. was adjusted to 5 with 1 M HCl and the mixt. was extracted with *n*-BuOH saturated with $H_2O$. The BuOH soln was concd to dryness and the residue subjected to silica gel CC using $CHCl_3$–MeOH–$H_2O$ (14:6:1) as solvent to afford prosapogenol (17 mg) which was characterized as saponin **2** from its physical and spectral characteristics.

*Permethylation of saponin* **1** *and preparation of the partially methylated alditolacetates*. To a soln of saponin **1** (5 mg) in DMSO (0.5 ml) was added a saturated soln of *t*-BuOK in DMSO (0.5 ml) and the soln was sonicated at room temp. for 1 hr. To this soln was added $CH_3I$ (1.5 ml) under cooling and the mixt. was further sonicated at room temp. for 1 hr. Excess $CH_3I$ was distilled off at 40°, and the mixt. was diluted with $H_2O$, dried and concd to dryness. The residue was hydrolysed with 90% $HCO_2H$ (1 ml) and 1 M $CF_3CO_2H$ (1 ml) for 2 hr at 100°. The soln was evapd to remove the acid; the residue was dissolved in $H_2O$ (2 ml) containing 2 drops of $NH_4OH$ and 25 mg of $NaBH_4$ were added after standing at room temp. for 2 hr. The mixt. was acidified with $MeCO_2H$ to pH 3.5 and the soln was evapd to dryness. $H_3BO_3$ in the residue was removed by repeated co-distillation with MeOH. The resulting methylated alditol mixt. was acetylated with $Ac_2O$–pyridine (1:1, 1 ml) at 100° for 1 hr. Excess reagent was removed by co-distillation with toluene. The methylated alditol acetate mixt. obtained was subjected to GC-MS.

*Acetylation of* **1**. Saponin **1** (10 mg) was dissolved in $Ac_2O$–pyridine (1:1, 2 ml) and left at room temp. for 24 hr The reagent in the reaction mixt. was removed by co-distillation with toluene The residue was dissolved in MeOH and was subjected to Sephadex LH 20 CC using MeOH as solvent to afford the peracetate **3**.

*Compound* **3**. Powder, $[\alpha]_D$ −13.2° (MeOH; *c* 1.06), FAB-MS *m/z*: 2248 $[M + Na + H]^+$, 993 $[Rha - Glc - 2Pen(Ac)_{10}]^+$, 561 $[Rha - Glc(Ac)_6]^+$, 273 $[Rha(Ac)_3]^+$, 259 $[Pen(Ac)_3]^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 5.95 (*d*, *J* = 1.1 Hz, ester Rha H-1), 4.82 (*br s*, Rha H-1), 4.61 (*d*, *J* = 7.4 Hz, Glc H-1); 4.50 (*d*, *J* = 7.9 Hz, Xyl H-1), 4.41 (*d*, *J* = 6.8 Hz, Ara H-1), 4.36 (*d*, *J* = 7.0 Hz, Xyl H-1), 4.35 (*d*, *J* = 7.0 Hz, Glc H-1). $^1H$ NMR ($C_6D_6$): see Table 1. $^{13}C$ NMR ($C_6D_6$): see Table 2.

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