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Triterpenoid Saponins from the Shells of *Argania spinosa* Seeds

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Two new oleanene saponins were isolated from the MeOH extract of the shell of *Argania spinosa*. They possess protobassic acid and 16α-protobassic acid as aglycons. The disaccharide moiety linked to C-3 of the aglycon is made up of two glucose units; the pentasaccharide moiety linked to C-28 is made up of arabinose, xylose, and three rhamnose units. Their structures were elucidated by 1D and 2D NMR experiments including $^{1}H-^{1}H$ (DQF-COSY, 1D TOCSY, and 2D HOHAHA) and $^{1}H-^{13}C$ (HSQC and HMBC) spectroscopy along with mass spectrometry.

KEYWORDS: ***Argania spinosa*; Sapotaceae; shell; saponins**

INTRODUCTION

The argan tree [*Argania spinosa* (L.) Skeels], of the family Sapotaceae, is endemic in southwestern Morocco. Argan fruits are nut-size, round (22.9 × 21.2mm), ovoid (32.5 × 19.6mm), or conical (38.4 × 18.9mm). Inside a milky pulp covered by a thick peel is a hard shell containing a kernel that affords a known edible oil. The oil has high dietetic value, unsaturated fatty acids being the major components. Oleic and linoleic acid make up 80% of the fatty acids, whereas linolenic acid is present only as traces (*1*). Oil from the kernel of *A. spinosa* is also considered as the best culinary oil by Moroccans, who are very familiar with this oil, which is also used in other countries, including the United States. Oil from *A. spinosa* is also used in cosmetics and may be considered as an interesting raw material for extraction of schotenol, which, in the form of a glycoside, is reported to be an anticancer agent (*2*). Many saponins have been reported from this tree (*3*, *4*). Toxicity (*5*) and anti-inflammatory and analgesic activities (*6*) of the cake saponins have been determined.

Continuing the studies on *A. spinosa*, we describe the isolation, from the shell of this species, of two new bidesmosidic oleanene saponins **3** and **4** showing at C-3 a sugar chain made up of two glucose units and at C-28 a sugar chain made up of five residues, along with two known saponins **1** and **2** (**Figure 1**).

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MATERIALS AND METHODS

Material. The fruits of *A. spinosa* were harvested at Tamanar (Essaouira, Morocco) in 2000. A voucher specimen was deposited in the Scientific Institut of Rabat under N RAB 65342.

All organic solvents were from Antibioticos S.p.A. (Rodano, Italy). HPLC grade water was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA).

Apparatus. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and at 150.6 MHz for ^{13}C, with the UXNMR software package, was used for NMR measurements in CD_3OD solutions. For two-dimensional (2D) experiments, $^{1}H-^{1}H$ DQF-COSY, inverse detected $^{1}H-^{13}C$ HSQC, and HMBC were obtained by employing the conventional pulse sequences as described previously. The selective excitation spectra, 1D TOCSY were acquired using waveform generator-based GAUSS shaped pulses, with mixing times ranging from 100 to 120 ms and a MLEV-17 spin−lock field of 10 kHz preceded by a 2.5 ms trim pulse.

HPLC separations were performed on an HP1100 series HPLC, equipped with a photodiode array detector, from Agilent Technologies (Palo Alto, CA).

Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm.

Electrospray ionization mass spectrometry (ESIMS) in the positive ion mode was performed using a Finnigan LCQ-Deca instrument from Thermoquest (San Jose, CA) equipped with Excalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 3 μL/min. The capillary voltage was at 5 V, the spray voltage at 5 kV, and the tube lens offset at 35 V. The capillary temperature was 220 °C. Data were acquired in MS1 scanning mode (*m*/*z* 700−2000).

Exact masses were measured by a Q-Star Pulsar (Applied Biosystems, Foster City, CA) triple-quadrupole orthogonal time-of-flight (TOF) instrument. Electrospray ionization was used in TOF mode at

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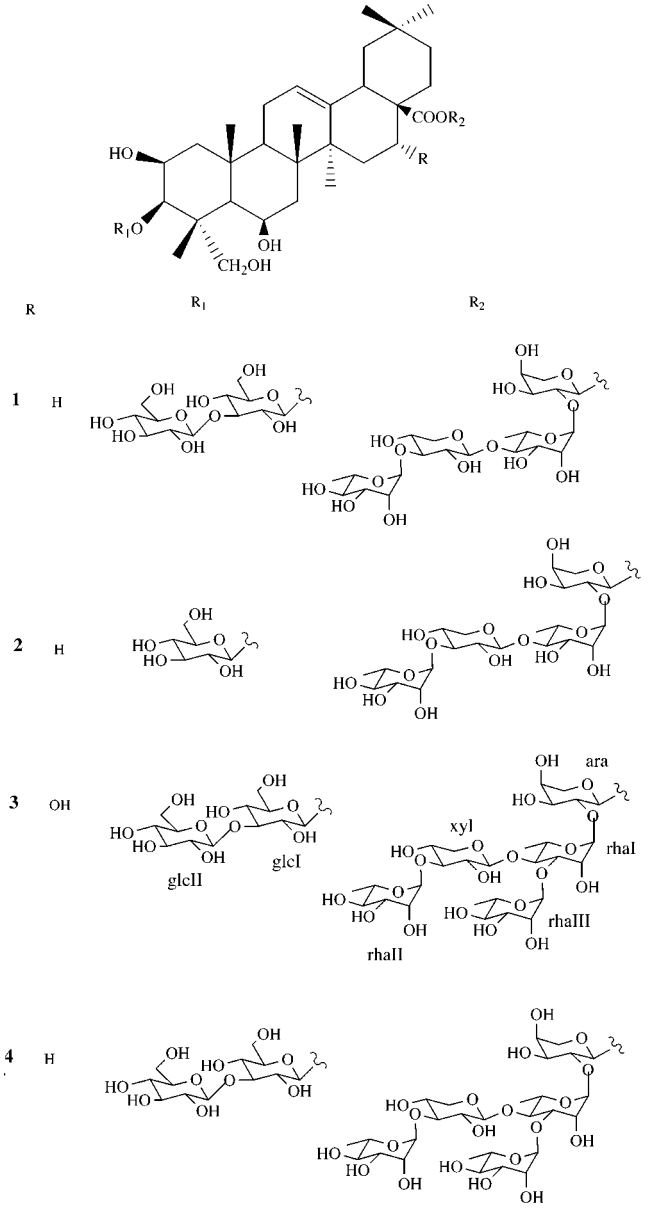

Figure 1. Structures of triterpenoid saponins **1**–**4**.

10.000 resolving power. Samples were dissolved in TFA 0.1% acetonitrile/water (1:1), mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Calibration was performed on the peaks of cesium iodide at *m*/*z* 1431.9550 and 1691.7649.

Extraction and Isolation of Saponins. Dried shells (800 g), cracked by hand for separation from the kernels, were ground and defatted with hexane and methanol. The methanol extract (100 g) was partitioned between water and chloroform; the water soluble portion was successively extracted with ethyl acetate and *n*-butanol. The *n*-butanol extract (9 g) was dissolved in water and chromatographed on Diaon HP 20 (150 × 3 cm), with water, 50% methanol, and MeOH. The methanol extract was purified on a Sephadex LH 20 column (30 × 2 cm) using MeOH as eluent. Fractions (8 mL) were collected and checked by TLC [silica gel plates, *n*-BuOH/AcOH/H_2O (12:3:5)]; fractions 1−5 (80 mg) containing the crude glycosidic mixture were purified by HPLC. The crude mixture (1 mg) was dissolved in 200 μL of MeOH and injected on an ODS Zorbax column (25 cm × 9.4 mm). The column was equilibrated with H_2O. After injection, a linear gradient to H_2O/CH_3-CN (72:28) was applied for 10 min, followed by isocratic elution during 20 min, a linear gradient to 70:30 for 5 min, a linear gradient to 65:35 for 10 min, a linear gradient to 30:70 for 5 min, a linear gradient to 5:95 for 5 min, and then isocratic elution during 10 min to afford **1** (80 μg, t_R 52.7 min), **2** (60 μg, t_R 54.3 min), **3** (40 μg, t_R 25.3 min), **4** (40 μg, t_R 50.8 min) after freeze-drying. Repeated injections allowed collection of 4 mg of **1**, 3 mg of **2**, 2 mg of **3**, and 2 mg of **4**.

Table 1. ^{1}H and ^{13}C NMR Data of the Sugar Portion of Compound **3** in CD_3OD[a]

sugar	position	δ_C	δ_H (*J* in Hz)
Ara (at C-28agl)	1	93.1	5.71 d (6.7)
	2	75.7	3.82 dd (6.7, 8.5)
	3	66.8	3.90 dd (8.5, 3.0)
	4	72.0	3.89 m
	5	63.2	3.53 dd (12.0, 3.0)
			3.96 dd (12.0, 2.0)
Rhal (at C-2ara)	1	102.4	5.01 d (1.5)
	2	72.0	3.97 dd (1.5, 2.5)
	3	81.2	3.93 dd (2.5, 8.9)
	4	77.6	3.75 t (8.9)
	5	69.1	3.86 m
	6	17.0	1.28 d (6.5)
RhalI (at C-3xyl)	1	101.2	5.20 d (1.5)
	2	72.0	3.97 dd (1.5, 2.5)
	3	72.1	3.73 dd (2.5, 8.9)
	4	73.8	3.42 t (8.9)
	5	69.8	4.05 m
	6	17.0	1.27 d (6.5)
Xyl (at C-4rhal)	1	105.0	4.54 d (7.5)
	2	75.3	3.32 dd (7.5, 9.0)
	3	84.0	3.45 t (9.0)
	4	69.0	3.57 m
	5	66.7	3.20 dd (10.0, 2.0)
			3.90 dd (10.0, 5.0)
RhalII (at C-3rhal)	1	104.5	5.02 d (1.5)
	2	71.9	4.09 dd (1.5, 2.5)
	3	72.2	3.75, dd (2.5, 8.9)
	4	73.5	3.42 t (8.9)
	5	69.9	3.85 m
	6	17.0	1.27 d (6.5)
Glcl (at C-3agl)	1	104.8	4.54 d (7.5)
	2	74.6	3.51 dd (7.5, 9.5)
	3	88.0	3.59 dd (9.5, 9.5)
	4	69.9	3.51 dd (9.5. 9.5)
	5	77.5	3.43 m
	6	61.8	3.75 dd (11.0, 5.0)
			3.84 dd (11.0, 2.0)
GlcII (at C-3Glcl)	1	104.8	4.59 d (7.5)
	2	75.1	3.30 dd (7.5, 9.6)
	3	77.7	3.40 dd (9.5, 9.5)
	4	71.2	3.30 dd (9.5, 9.5)
	5	77.9	3.34 m
	6	62.2	3.66 dd (11.0, 5.0)
			3.9 dd (11.0, 2.0)

[a] Assignments confirmed by 1D-TOCSY, DQF-COSY, HSQC, and HMBC.

Compound ***3****:* $[\alpha]^{25}_D$ −19.9° (*c* 0.01, MeOH); ^{1}H NMR data of the aglycon (CD_3OD, 600 MHz) δ 5.46 (1H, t, J = 3.7 Hz, H-12), 4.52 (1H, br m, $W_{1/2}$ = 7.0 Hz, H-16), 4.50 (1H, br m, $W_{1/2}$ = 8.5 Hz, H-6), 4.38 (1H, m, H-2), 3.76 (1H, d, J = 11 Hz, H-23), 3.62 (1H, d, J = 4.5 Hz, H-3), 3.45 (1H, d, J = 11.0 Hz, H-23), 2.30 (1H, br t, J = 13.0 Hz, H-19), 3.13 (1H, dd J = 13.3, 3.5 Hz, H-18), 2.14 (1H, m, H-11), 1.64 (3H, s, Me-25), 1.36 (3H, s, Me-27), 1.33 (3H, s, Me-24), 1.10 (H-19), 1.09 (3H, s, Me-26), 1.01 (3H, s, Me-30), 0.92 (3H, s, Me-29); ^{13}C NMR data of the aglycon (CD_3OD, 150 MHz) δ 177.0 (C-28), 144.2 (C-13), 124.2 (C-12), 83.6 (C-3), 74.4 (C-16), 71.2 (C-2), 68.3 (C-6), 65.0 (C-23), 48.9 (C-5), 48.4 (C-9), 48.2 (C-17), 47.6 (C-19), 46.8 (C-1), 44.0 (C-4), 43.7 (C-14), 41.9 (C-18), 41.4 (C-7), 40.0 (C-8), 37.2 (C-10), 36.4 (C-15), 36.4 (C-21), 33.5 (C-29), 31.6 (C-20), 31.3 (C-22), 27.4 (C-27), 25.1 (C-30), 23.9 (C-11), 19.3 (C-25), 19.0 (C-26), 16.3 (C-24); for ^{1}H and ^{13}C NMR of the sugar moieties, see **Table 1**; HRESIMS, *m*/*z* $[M + H]^+$ calcd for $C_{70}H_{114}O_{37}$ 1547.7117; found 1547.7121; ESIMS, *m*/*z* 1585 $[M + K]^+$, 1439 $[M + K - 146]^+$, 883 $[M + K - (146 + 146 + 146 + 132 + 132)]^+$.

Compound 4: $[\alpha]^{25}_{D}$ −11.3° (*c* 0.05, MeOH); ^{1}H NMR data of the aglycon (CD_3OD, 600 MHz) δ 5.35 (1H, t, J = 3.7 Hz, H-12), 4.50 (1H, br m, $W_{1/2}$ = 8.5 Hz, H-6), 4.33 (1H, m, H-2), 3.76 (1H, d, J = 11 Hz, H-23), 3.62 (1H, d, J = 4.5 Hz, H-3), 3.45 (1H, d, J = 11 Hz, H-23), 2.99 (1H, dd J = 13.3, 3.5 Hz, H-18), 2.14 (1H, m, H-11), 1.74 (1H, br t, J = 13.0 Hz, H-19), 1.64 (3H, s, Me-25), 1.36 (3H, s, Me-27), 1.33 (3H, s, Me-24), 1.18 (H-19), 1.09 (3H, s, Me-26), 1.01 (3H, s, Me-30), 0.92 (3H, s, Me-29); ^{13}C NMR data of the aglycon (CD_3-OD, 150 MHz) 177.0 (C-28), 144.2 (C-13), 124.2 (C-12), 83.6 (C-3), 71.2 (C-2), 68.3 (C-6), 65.0 (C-23), 48.9 (C-5), 48.4 (C-9), 48.2 (C-17), 47.6 (C-19), 46.8 (C-1), 44.0 (C-4), 43.7 (C-14), 41.9 (C-18), 41.4 (C-7), 40.0 (C-8), 37.2 (C-10), 36.4 (C-15), 36.4 (C-21), 33.5 (C-29), 31.6 (C-20), 31.3 (C-22), 27.4 (C-27), 24.7 (C-16), 24.5 (C-30), 23.9 (C-11), 19.3 (C-25), 19.0 (C-26), 16.3 (C-24); for ^{1}H and ^{13}C NMR of the sugar moieties, see **Table 1**; HRESIMS, *m/z* $[M + H]^+$ calcd for $C_{70}H_{114}O_{36}$ 1531.7168, found 1531.7221; ESIMS, *m/z* 1553 $[M + Na]^+$, 851 $[M + Na - (146 - 146 - 146 - 132 - 132)]^+$.

Compound 1: see Lavaud et al. (*7*).

Compound 2: see Kitagawa et al.(*8*).

RESULTS AND DISCUSSION

From the MeOH extract of the shell of *A. spinosa* saponins **1**−**4** were isolated.

Saponins **1** and **2** are known triterpene saponins, and their structures were established by analysis of 1D and 2D NMR experiments as 3-*O*-β-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] protobassic acid (*7*) and 3-*O*-β-D-glucopyranosyl-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]protobassic acid (*8*).

The HRESIMS of **3** showed an $[M + H]^+$ ion corresponding to a molecular formula of $C_{70}H_{114}O_{37}$. The ESIMS of **3** showed the $[M + K]^+$ ion at *m/z* 1585 and two peaks at *m/z* 1439 $[M + K - 146]^+$ due to the loss of a deoxyhexose and at *m/z* 883 $[M + K - (146 + 146 + 146 + 132 + 132)]^+$, corresponding to the loss of three deoxyhexoses and two pentoses.

The ^{1}H NMR spectrum of the aglycon moiety of **3** showed signals for six tertiary methyl groups (δ1.64, 1.36, 1.33, 1.09, 1.01, and 0.92). Also evident were signals of H-3ax at δ 3.62 (d, J = 4.5 Hz) due to the presence of a β-OH group at C-3 and H-2 at δ 4.38 (m) due to the presence of a β-OH group at C-2. Further features were signals at δ 5.46 (1H, t, J = 3.4 Hz), 4.52 (1H, br m, $W_{1/2}$ = 7.0 Hz), and δ 4.50 (1 H, br m, $W_{1/2}$ = 8.5 Hz) ascribable to an olefinic and two CHOH functions, respectively. The ^{13}C NMR spectrum showed 70 signals, of which 30 were assigned to a triterpenoid moiety and 40 to a saccharide portion. Analysis of ^{1}H and ^{13}C NMR data and comparison with literature (*3*) allowed identification of the aglycon of **3** as 2β,3β,6β,16α,23-pentahydroxyolean-12-en-28-oic acid, known as 16α-hydroxyprotobassic acid.

In the ^{1}H NMR spectrum (**Table 1**), the sugar portion of **3** contained seven anomeric proton signals at δ 5.71 (d, J = 6.7 Hz), 5.20 (d, J = 1.5 Hz), 5.02 (d, J = 1.5 Hz), 5.01 (d, J = 1.5 Hz), 4.54 (d, J = 7.5 Hz), 4.59 (d, J = 7.5 Hz), 4.54 (d, J = 7.5 Hz) and three methyl doublets (δ 1.28, 1.27, and 1.27), suggesting the occurrence of three deoxyhexose units. The other sugar signals were overlapped in the region between δ 3.07 and 4.09. The results of 1D TOCSY and DQF-COSY experiments allowed the sequential assignments of all the proton resonances to the individual monosaccharides as reported in **Table 1**.

Thus, the shifts of the sugar resonances, summarized in **Table 1**, were attributable to α-L-arabinopyranosyl (H-1, δ 5.71), β-D-xylopyranosyl (H-1, δ 4.54), two β-D-glucopyranosyl (H-1, δ 4.54; H-1, δ 4.59), and three α-L-rhamnopyranosyl (H-1, δ 5.02; H-1, δ 5.01; H-1, δ 5.20) units. An HSQC experiment allowed the assignments of the interglycosydic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methyl pyranosides. The absence of any ^{13}C NMR glycosidation shifts of the glucopyranosyl unit with H-1 at δ 4.59 (glucose II) and of the rhamnopyranosyl units with H-1 at δ 5.02 (rhamnose III) and H-1 at δ 5.20 (rhamnose II) suggested that these sugars were terminal units. Glycosidation shifts were observed for C-2 of arabinose (δ 75.7), C-3 of rhamnose I (δ 81.2), C-4 of rhamnose I (δ 77.6), C-3 of xylose (δ 84.0), and C-3 of glucose I (δ 88.0) (**Table 1**). Chemical shifts of H-1 arabinose (δ 5.71) and C-1 arabinose (δ 93.1) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group (*3*, *4*).

The positions of the sugar residues were unambiguously defined by the HMBC experiment. Cross-peaks due to long-range correlations were observed between C-3 of glucose I (δ 88.0) and H-1 of glucose II (δ 4.59), between C-2 of arabinose (δ 75.7) and H-1 of rhamnose I (δ 5.01), between C-3 of rhamnose I (δ 81.2) and H-1 of rhamnose III (δ 5.02), between C-4 of rhamnose I (δ 77.6) and H-1 of xylose (δ 4.54), and between C-3 of xylose (δ 84.0) and H-1 of rhamnose II (δ 5.20). Thus, rhamnose III and xylose were linked, respectively, to C-3 and C-4 of rhamnose I, which was linked to C-2 of arabinose; rhamnose II was linked to C-3 of xylose, and glucose II was linked to C-3 of glucose I.

On the basis of the above evidence, compound **3** was identified as 3-*O*-β-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-[(1→3)-α-L-rhamnopyranosyl]-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl}-16α-hydroxyprotobassic acid.

The HRESIMS of **4** showed the $[M + H]^+$ ion indicating a molecular formula of $C_{70}H_{114}O_{36}$, and the ESIMS showed $[M + Na]^+$ at *m/z* 1553 and a prominent fragment at *m/z* 851 $[M + Na - (146 - 146 - 146 - 132 - 132)]^+$ corresponding to the loss of three deoxyhexoses and two pentoses. Comparison of ^{1}H NMR data and ^{13}C NMR data of compound **4** with those of **3** indicated identical saccharide chains at C-3 and C-28 and revealed that the only difference between the two compounds was confined to the absence of the OH group at C-16 of the aglycon as suggested by the absence of the signal at δ 4.52 in the ^{1}H NMR spectrum and at δ 74.4 in the ^{13}C NMR spectrum, together with differences in the ^{13}C NMR values of rings D and E (*4*). Thus, compound **4** was determined to be 3-*O*-β-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-[(1→3)-α-L-rhamnopyranosyl]-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]protobassic acid.

LITERATURE CITED

(1) Farines, M.; Soulier, J.; Charrouf, M.; Soulier, R. Etude de l'huile des graines d'*Argania spinosa* (L.); Sapotaceae. La fraction glycéridique. *Rev. Fr. Corps Gras* **1984**, *31*, 283−286; CAN 102:23106.

(2) Maurin, R. Argan oil, *Argania spinosa* (L.) Skeels (Sapotaceae). *Rev. Fr. Corps Gras* **1992**, *39*, 3169−3172; CAN 118:5799.

(3) Charrouf, Z.; Wieruzeski, J. M.; Fkih-Tetouani, S.; Leroy, Y.; Charrouf, M.; Fournet, B. Triterpenoid saponins from *Argania spinosa*. *Phytochemistry* **1992**, *31*, 2079−2086.

(4) Oulad-Ali, A.; Kirchen, V.; Weniger, B.; Anton, R.; Guillaume, D.; Charrouf, Z. Structure elucidation of three glycosides from the trunk of *Argania spinosa*. *J. Nat. Prod.* **1996**, *59*, 193−195.

(5) Alaoui, K.; Belabbes, M.; Cherrah, Y.; Charrouf, Z.; Amarouch, H.; Roquebert, J. Acute and chronic toxicity of saponins of *Argania spinosa*. *Ann. Pharm. Fr.* **1998**, *56*, 231−219; CAN 129:327116.

(6) Alaoui, K.; Lagorge, J. F.; Cherrah, Y.; Hassar, M.; Amarouch, H.; Roquebert, J. Analgesic and anti-inflammatory activity of *Argania spinosa* saponins. *Ann. Pharm. Fr.* **1998**, *56*, 220−228; CAN 130:290.

(7) Lavaud, L.; Massiot, G.; Becchi, M.; Misra, G.; Nigam, K. Saponins from three species of *Mimusops*. *Phytochemistry* **1996**, *41*, 887−893.

(8) Kitagawa, I.; Inada, A.; Yosioka, I. Saponin and sapogenol−XII Misaponin A and misaponin D. Two major bidesmoides from the seed kernels of *Madhuca longifolia* (L.) Macbride. *Chem. Pharm. Bull*. **1975**, *23*, 2268−2278.

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