Structure Elucidation of Three Triterpene Glycosides from the Trunk of *Argania spinosa*

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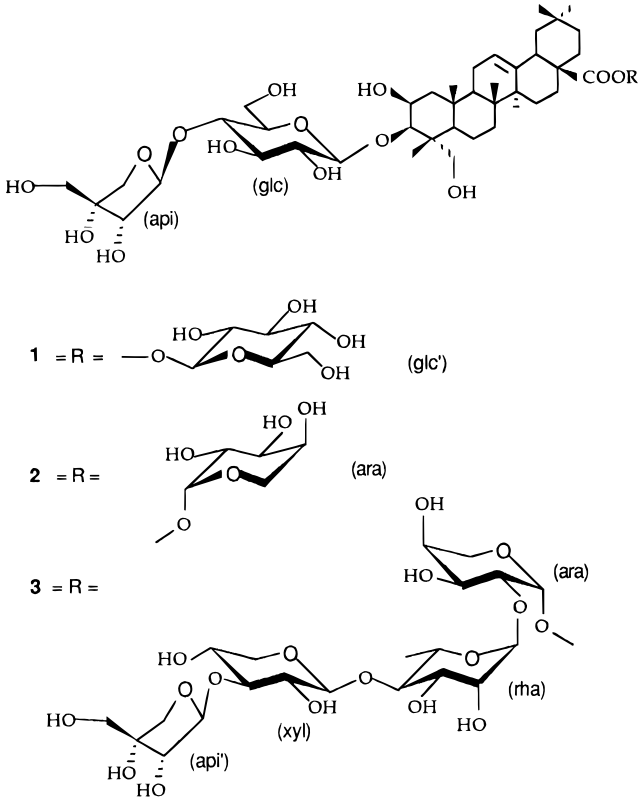
The structures of three novel saponins from *Argania spinosa*, named arganines G, H, and J, have been elucidated by MS and NMR techniques as 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranosylbayogenin (**1**), 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-α-L-arabinopyranosylbayogenin (**2**), and 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-[*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]bayogenin (**3**), respectively.

Argania spinosa (L.) Maire (Sapotaceae) is a slow-growing endemic tree of southwestern Morocco. Traditionally, its seeds are eaten by cattle and also furnish an oil used as a cosmetic or human food.[1] Currently, *A. spinosa* is still grown for its industrial cosmetic uses. As part of our continuing interest in the saponins from the Sapotaceae,[2−4] we studied the trunk of the title plant. We report herein the isolation and identification of three novel triterpene glycosides named arganines G (**1**), H (**2**), and J (**3**).

The methanolic extract of the dried and powdered trunk was partitioned between *n*-BuOH and water. The BuOH layer was further extensively chromatographed to give **1**−**3**. The FABMS of arganine G (**1**) displayed a quasimolecular ion peak at *m*/*z* 967 $[M + Na]^+$, allowing the determination of the molecular formula as $C_{47}H_{76}O_{19}$. The ^{13}C NMR spectrum of **1** showed 47 signals that were categorized by a DEPT experiment as six methyls, 14 methylenes, 18 methines, and nine quaternary carbons. Among the quaternary resonances, one could be directly assigned to a carbonyl signal (*δ* 178.0) and another to an olefinic signal (*δ* 144.9). The 14 methylene resonances included five CH_2O (*δ* 61.4, 62.4, 64.6, 65.5, 74.9) groups and nine more shielded signals (Table 1). The methyl groups were shown to be at angular positions from the 1D ^1H NMR spectrum (six singlets at *δ* 0.99, 1.08, 1.10 × 2, 1.34, and 1.46), indicating that they belonged to the aglycon of **1**. A 2D COSY spectrum rapidly established that C-2 and C-3 were both substituted by an oxygen atom [*δ* 4.50 (H-2), *δ* 3.72 (H-3)]. Comparison of the ^{13}C NMR data with literature values demonstrated that the aglycon of **1** was bayogenin substituted at positions C-3 and C-28.[5,6]

The ^{13}C and ^1H NMR spectra of **1** also displayed signals for three sugar residues (Table 1). One of these was easily identified from its quaternary carbon at *δ* 80.2 as a *β*-D-apiose (api) moiety (J_{H1-H2} = 3.6 Hz), as observed for sideroxylosides B and C.[3] The two other



sugar residues were identified as *β*-D-glucose (glc) units. The anomeric protons of these two glc residues appeared as two doublets at *δ* 5.54 (*J* = 7.7 Hz) and 4.62 (*J* = 7.8 Hz), with the former chemical shift indicating that this residue (glc′) was esterified at C-28. Analysis of the ^{13}C-NMR chemical shifts of this glc′ moiety, conducted by a combination of homo- and heteronuclear COSY spectra, indicated its unsubstituted nature.[7] A similar analysis made on the carbon atoms of the second glc residue showed that it was substitued at the C-4 position (δ_{C-4}

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Table 1. ^{13}C NMR Data for **1**−**3** in CD_3OD (50.2 or 75.4 MHz, ppm)

carbon	**1**		**2**		**3**		
aglycon							
1	44.4		44.4		44.4		
2	71.2		71.2		71.1		
3	83.9		83.9		83.8		
4	43.1		43.1		43.1		
5	49.0		49.7		49.6		
6	18.6		18.6		18.6		
7	33.2		33.5		33.5		
8	40.7		40.7		40.7		
9	48.0		49.2		49.1		
10	37.5		37.5		37.5		
11	25.2		25.3		25.2		
12	123.8		123.9		123.9		
13	144.9		145.0		144.9		
14	43.1		43.1		43.1		
15	28.8		28.8		29.0		
16	24.7		24.7		24.7		
17	49.0		49.0		48.9		
18	42.6		42.6		42.6		
19	47.2		47.1		47.2		
20	31.5		31.6		31.6		
21	34.9		34.9		34.9		
22	33.2		33.5		33.5		
23	65.5		65.4		65.1		
24	14.7		14.7		14.7		
25	17.5		17.5		17.5		
26	17.8		17.8		17.8		
27	26.9		26.4		26.4		
28	178.0		178.1		177.8		
29	33.5		33.5		33.5		
30	24.0		24.0		24.0		
3-*O*-sugar							
Glc-1	105.3	Glc-1	105.3	Glc-1	105.4		
Glc-2	75.1	Glc-2	75.0	Glc-2	75.7		
Glc-3	76.3	Glc-3	76.3	Glc-3	76.4		
Glc-4	79.3	Glc-4	79.3	Glc-4	79.4		
Glc-5	76.5	Glc-5	76.5	Glc-5	76.5		
Glc-6	61.4	Glc-6	61.3	Glc-6	61.4		
Api-1	111.1	Api-1	111.1	Api-1	111.1		
Api-2	77.7	Api-2	77.7	Api-2	77.8		
Api-3	80.2	Api-3	80.2	Api-3	80.3		
Api-4	74.9	Api-4	74.9	Api-4	75.0		
Api-5	64.6	Api-5	64.6	Api-5	64.7		
28-*O*-sugar							
Glc′-1	95.7	Ara-1	95.7	Ara-1	94.0	Xyl-1	106.6
Glc′-2	73.9	Ara-2	71.2	Ara-2	75.4	Xyl-2	76.0
Glc′-3	78.7	Ara-3	73.6	Ara-3	68.9	Xyl-3	86.0
Glc′-4	71.2	Ara-4	68.5	Ara-4	67.3	Xyl-4	69.9
Glc′-5	78.3	Ara-5	66.3	Ara-5	63.8	Xyl-5	66.8
Glc′-6	62.4			Rha-1	101.4	Api'-1	111.0
				Rha-2	72.1	Api′-2	77.9
				Rha-3	72.4	Api′-3	80.6
				Rha-4	84.1	Api′-4	75.4
				Rha-5	71.5	Api′-5	64.0
				Rha-6	18.1		

79.3). Thus, arganine G is 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranosylbayogenin (**1**).

The FABMS of **2** (arganine H) displayed a pseudomolecular ion peak at *m*/*z* 937 $[M + Na]^+$, establishing a molecular formula of $C_{46}H_{74}O_{18}$. Its ^{13}C NMR spectrum was similar to that of **1** (Table 1), allowing the identification of the aglycon of **2** as bayogenin. The major differences between **1** and **2** were the lack of a methine resonance corresponding to C-5 of the glc residue substituted at the aglycon C-28 and a shift of 4 ppm of a CH_2O signal. These data suggested that the 28-position of compound **2** was esterified by a pentose characterized as an α-L-arabinose moiety from its ^1H and ^{13}C pattern (δ 5.50, J = 4.4 Hz, H-1; see Table 1 for ^{13}C). Substitution at the C-4 position of the glucose residue by an apiose moiety as determined in the case of **1** (*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl) was further confirmed by the HMBC spectrum of **2** in which a correlation was displayed between H-1 of apiose and H-4 of glucose. This experiment also allowed the observation of correlations between C-28 and H-1 of apiose and between C-3 and H-1 of glucose. Thus, arganine H was assigned as 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-α-L-arabinopyranosylbayogenin (**2**).

The ^{13}C NMR spectrum of **3** displayed signals for 62 carbons, confirming a molecular formula of $C_{62}H_{100}O_{30}$ which was deduced from the FABMS spectrum in which the ion peak appeared at *m*/*z* 1347 $[(M + Na)^+]$. Assignment of the 62 carbon resonances provided evidence for the presence, within the structure of **3**, of one extra unit each of *β*-D-apiose (api′), α-L-rhamnose (rha), and *β*-D-xylose (xyl), when compared to **2**. The configurations of these sugar moieties were deduced by comparing the ^{13}C-NMR data of **3** with those of **1** and **2** and with literature values.[3,4,7] The interglycosidic linkages were deduced from an HMBC experiment. As expected, correlations were observed between H-1 of glucose and C-3, H-1 of arabinose and C-28, and one H-1 of apiose and H-4 of glucose. Moreover, correlations were observed between H-1 of apiose and C-3 of xylose, H-1 of xylose and C-4 of rhamnose, and H-1 of rhamnose and C-2 of arabinose. These data identified unambiguously arganine J (**3**) as 3-*O*-*β*-D-apiofuranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-[*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]bayogenin.

Arganines G, H, and J (**1**−**3**) are three new natural products.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH with a Perkin-Elmer Model 241 MC polarimeter. FABMS were run on a Kratos MS 80 spectrometer. ^1H NMR (200.13 or 300.13 MHz), ^{13}C NMR (50.2 or 75.4 MHz), and 2D NMR spectra were determined on a Bruker AC-200 or a AC-300-P spectrometer in CD_3OD.

Plant Material. A specimen of *A. spinosa* was collected near Agadir, Morocco, by H. Oulad-Ali, in February 1994. A voucher specimen (No. RAB 62064) is deposited in the herbarium of the Institut Scientifique de Rabat. The plant material was identified by Mrs. R. Nouïm, Département de Biologie, Faculté des Sciences d'Agadir, BP28/S Morocco.

Extraction and Isolation. The air-dried powdered material (490 g) was extracted in boiling MeOH. After filtration, the solvent was removed under reduced pressure to give a residue (43 g) that was dissolved in H_2O and extracted with saturated *n*-BuOH. The organic layers were combined, concentrated, and dissolved in a minimum of MeOH. Precipitation with Et_2O afforded a saponin fraction (28 g) isolated after centrifugation. A portion of the precipitate (10 g) was fractionated by flash column chromatography (CC) on silica gel 60, 0.040−0.063 mm, using a gradient CH_2Cl_2−MeOH−H_2O as eluent. Repeated flash CC yielded pure arganine G (**1**) (60 mg), arganine H (**2**) (20 mg), and arganine J (**3**) (50 mg).

Arganine G (1): amorphous powder (MeOH): $[\alpha]^{20}_D$ −5.1° (*c* 1.6, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 0.99, 1.08 (3H each, s, 2 × Me), 1.10 (6H, s, 2 × Me), 1.34, 1.46 (3H each, s, 2 × Me), 3.03 (1H, m, H-18), 4.33 (1H, d, J = 9.6 Hz, H-4-api), 4.50 (1H, m, H-2), 4.65 (1H, d, J = 7.3 Hz, H-1-glc), 5.28 (1H, d, J = 3.6 Hz, H-1-api), 5.45 (1H, m, H-12), 5.58 (1H, d, J = 8.0 Hz, glc′-H1); ^{13}C-NMR data, see Table 1; FABMS m/z $[M + Na]^+$ 967. Anal. Calcd for $C_{47}H_{76}O_{19}\cdot 4H_2O$: C, 55.48; H, 8.33. Found: C, 55.63; H, 8.58.

Arganine H (2): amorphous powder (MeOH): $[\alpha]^{20}_D$ +4° (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD) δ 1.00, 1.10 (3H each, s, 2 × Me), 1.13 (6H, s, 2 × Me), 1.35, 1.48 (3H each, s, 2 × Me), 3.10 (1H, m, H-18), 4.35 (1H, d, J = 9.8 Hz, H-4-api), 4.52 (1H, m, H-2), 4.64 (1H, d, J = 7.8 Hz, H-1-glc), 5.28 (1H, d, J = 3.6 Hz, H-1-api), 5.48 (1H, m, H-12), 5.62 (1H, d, J = 5.2 Hz, H-1-ara); ^{13}C-NMR data, see Table 1; FABMS m/z $[M + Na]^+$ 937 Anal. Calcd for $C_{46}H_{74}O_{18}\cdot 5H_2O$: C, 54.95; H, 8.43. Found: C, 54.84; H, 8.78.

Arganine J (3): amorphous powder (MeOH): $[\alpha]^{20}_D$ −19.7° (*c* 1.4, MeOH); ^{1}H NMR (CD_3OD) δ 0.92, 1.08, 1.09, 1.31 (3H each, s, 4 × Me), 1.48 (6H, s, 2 × Me), 1.10 (3H, d, J = 6.6 Hz, H-6-rha), 3.07 (1H, m, H-18), [4.28, (1H, d, J = 9.6 Hz), 4.33 (1H, d, J = 9.6 Hz), 2 × H-4-api], 4.48 (1H, m, H-2), 4.63 (1H, d, J = 7.6 Hz, H-1-glc), 4.68 (1H, d, J = 7.2 Hz), 5.25 (1H, brs, H-1-rha), 5.26 (1H, d, J = 3.4 Hz, H-1-api), 5.42 (1H, d, J = 3.1 Hz, H-1-api′), 5.46 (1H, m, H-12), 5.82 (1H, d, J = 3.5 Hz, H-1-ara); ^{13}C-NMR data, see Table 1; FABMS m/z $[M + Na]^+$ 1347. Anal. Calcd for $C_{62}H_{100}O_{30}\cdot 9H_2O$: C, 50.04; H, 8.00. Found: C, 49.94; H, 8.35.

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