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Four New Dammarane Saponins from *Zizyphus lotus*

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Five dammarane-type saponins were isolated by means of centrifugal partition chromatography from the leaves of *Zizyphus lotus*. Their structures were elucidated using a combination of 1D and 2D ^{1}H and ^{13}C NMR spectra and mass spectroscopy. One of these glycosides is the known jujuboside B (**5**). Three are new jujubogenin glycosides, identified as 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyljujubogenin-20-*O*-(2,3,4-*O*-triacetyl)-α-L-rhamnopyranoside (**1**), 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyljujubogenin-20-*O*-α-L-rhamnopyranoside (**2**), and 3-*O*-α-L-rhamnopyranosyl-(1→2)-[(4-sulfo)-*β*-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyljujubogenin (**3**). The last is a new sulfated derivative of jujubasaponine IV, identified as 3-*O*-α-L-rhamnopyranosyl-(1→2)-[(4-sulfo)-*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranosyl-(20*R*,22*R*)-16*β*,22:16α,30-diepoxydammar-24-ene-3*β*,20-diol (**4**).

Plants of the *Zizyphus* genus (Rhamnaceae) are commonly used as edible or medicinal plants in warm and tropical regions.[1] As for most species from this genus, the chemical analysis of the root bark and leaves of *Z. lotus* (L.) Lam., a Mediterranean tree or shrub used in Maghreb as an antidiabetic, has led to the identification of cyclopeptide alkaloids,[2−5] flavonoids,[6] and saponins.[7] Acetylated triterpene saponins were reported as the most active antisweet saponins from *Zizyphus jujuba*.[8] Antifungal and molluscicidal activities of root bark extracts of *Z. lotus* have been reported.[9] As a continuation of our work on *Z. lotus*, we report here the isolation and structural elucidation of four new dammarane saponins (**1**−**4**) from the leaves, along with the known jujuboside B (**5**). Among them two show sulfated sugars, which are not very common in plants, and one is acetylated. These saponins differ from the root bark, confirming the relevance of analyzing different plant parts. Centrifugal partition chromatography has been used to fractionate the crude extract.

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Results and Discussion

The methanol extract of a defatted powder of leaves of *Zizyphus lotus* (L.) Lam. (Rhamnaceae) was purified by precipitation with acetone and diethyl ether. The precipitate was further dialyzed and the retained part lyophilized to give a crude saponin mixture. This mixture was fractionated by centrifugal partition chromatography using an EtOAc/1-BuOH/H_2O biphasic system in a gradient elution mode followed by medium-pressure liquid chromatography on normal silica gel and preparative TLC, yielding the saponins **1**, **2**, **3**, and **4** along with the known jujuboside B (**5**).[10] Acid hydrolysis of the crude saponin mixture yielded four sugars analyzed by TLC and identified by comparison with authentic samples as D-glucose, D-galactose, L-rhamnose, and L-arabinose. Their absolute configurations were determined by the measurement of optical rotation after separation by preparative TLC.

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{54}H_{84}O_{20}$ from the HRMS. The positive mode ESIMS of **1** showed a quasimolecular ion peak at *m*/*z* 1075 $[M + Na]^+$ and the negative-ion mode exhibited a quasimolecular ion peak at *m*/*z* 1051 $[M - H]^-$. A significant ion peak detected at *m*/*z*

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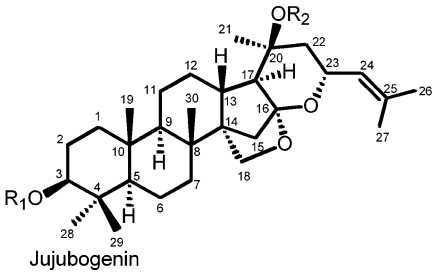
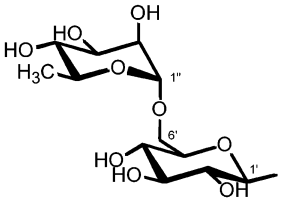
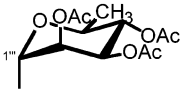
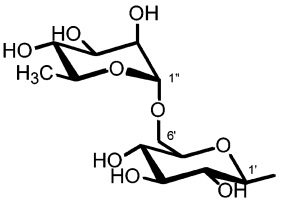
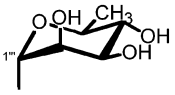
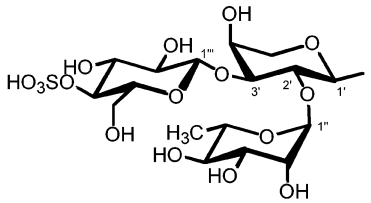
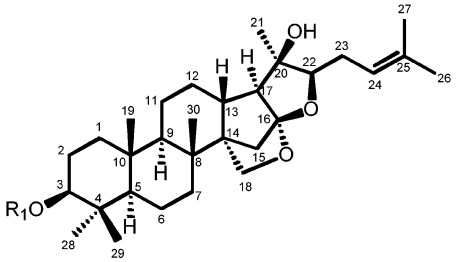


Jujubogenin

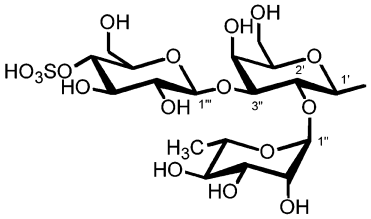
1 R_1 =  R_2 = 

2 R_1 =  R_2 = 

3 R_1 =  R_2 = H



(20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol

4 R_1 = 

767 [(M + Na) − 308]$^+$ suggested the loss of a desoxyhexose-hexose chain. Analysis of the MS−MS spectrum suggested the successive loss of three acetate units with ion peaks visible at *m*/*z* 991 [(M − H) − 60]$^-$, 931 [(M − H) − 60 − 60]$^-$, and 871 [(M − H) − 60 − 60 − 60]$^-$. The aglycone (Agly) structure was assigned as jujubogenin on the basis of the ^{1}H and ^{13}C NMR spectra and homo- and heteronuclear correlations observed in ^{1}H−^{1}H COSY, HSQC and HMBC experiments. Most of the ^{13}C NMR signals were assigned through $^2J_{H-C}$ and $^3J_{H-C}$ couplings of the seven methyls and are in agreement with literature data (Table 1).[7,11−15] The downfield shifts of C-3 at δ_C 90.8 and C-20 at δ_C 78.1 suggested a bidesmosidic nature for the saponin **1**.[16] The ROE correlations between H-17, Me-21, and H-22α and between H-22β and H-23 and the magnitude of the coupling constants between H-13 and H-17 (J = 6 Hz) and H-23 and H-22α (J = 11 Hz) are in agreement with the configuration of rings D−F of jujubogenin.[7,17]

The presence of three sugars was revealed by three anomeric signals in the ^{1}H NMR spectrum at δ_H 4.31 (d, J = 8 Hz), 4.76 (d, J = 1.5 Hz), and 5.14 (d, J = 2 Hz), which correlated with carbon signals in the HSQC spectrum at δ_C 106.8, 102.2, and 93.5, respectively. Complete assignment of each sugar proton system was achieved by considering ^{1}H−^{1}H COSY and TOCSY spectra, while glycosidic carbons were assigned from the HSQC spectrum. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-glucopyranosyl (Glc′) and two α-rhamnopyranosyl (Rha″ and Rha‴) moieties, respectively (Tables 1 and 2). The HMBC correlations between δ_H 4.31 (Glc-1′) and δ_C 90.8 (Agly-3) and between δ_H 4.76 (Rha-1″) and δ_C 68.3 (Glc-6′) showed that the diglycosidic chain α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl was linked to jujubogenin at C-3. The linkage of the second rhamnosyl unit (Rha‴) on C-20 of jujubogenin was deduced from the HMBC correlation between δ_H 5.14 (Rha-1‴) and δ_C 78.1 (Agly-20). These linkages were confirmed by ROE correlations between proton signals of Glc-1′ and Agly-3, of Rha-1″ and Glc-6′, and of Rha-1‴ and Agly-CH_3-21, respectively. By comparison of the values of proton chemical shifts of the two rhamnosyl units, an acylation shift was observed for H-2‴ [$\Delta\delta_H$ + 1.35 ppm], H-3‴ [$\Delta\delta_H$ + 1.72 ppm], and H-4‴ [$\Delta\delta_H$ + 1.70 ppm] of Rha‴. The HMBC correlations between these protons and the three carbonyls of acetates at δ_C 172.0, 171.7, and 171.6, respectively, suggested that the positions 2, 3, and 4 of the rhamnosyl moiety at C-20 were acetylated.[14] Thus, the structure of **1** was established as 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyljujubogenin-20-*O*-(2,3,4-*O*-triacetyl)-α-L-rhamnopyranoside, a new natural compound.

Compound **2** had the elemental composition $C_{48}H_{78}O_{17}$ from its HRMS. The structure of **2** was deduced from ^{1}H, ^{13}C, COSY, TOCSY, HMQC, HMBC, and ROESY experiments, which allowed the complete assignments of ^{1}H and ^{13}C resonances. The NMR spectroscopic data of **2** were similar to that of **1** except for the signals of protons for the rhamnose linked at C-20 and the absence of three acetates. By comparison with **1**, shielded values where observed for H-2‴ [$\Delta\delta_H$ −1.38 ppm], H-3‴ [$\Delta\delta_H$ −1.62 ppm], and H-4‴ [$\Delta\delta_H$ −1.66 ppm], respectively, suggesting that **2** is the nonacetylated analogue of **1**. Thus, the new compound **2** is 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyljujubogenin-20-*O*-α-L-rhamnopyranoside.

The negative-ion mode ESIMS of compound **3** showed a quasimolecular ion peak at *m*/*z* 991 (M − H)$^-$ corresponding to a molecular formula of $C_{47}H_{76}O_{20}S$ obtained from HRMS. The presence of a sulfur atom suggested a sulfate group in the molecule. The structure of the aglycone was assigned as jujubogenin on the basis of ^{1}H and ^{13}C NMR spectra and homo- and heteronuclear correlations observed in ^{1}H−^{1}H COSY and HSQC experiments. The comparison of the genin signals of **3** with the literature data of monodesmosidic jujubogenin saponins showed superimposable ^{13}C signals.[7,11−15]

The presence of three sugars was revealed by three anomeric proton signals at δ_H 4.49 (d, J = 5.3 Hz), 4.57 (d, J = 7.8 Hz), and 5.26 (brs), which gave correlations with ^{13}C NMR signals on the HSQC spectrum at δ_C 103.9, 102.7, and 100.6, respectively. Complete assignment of each sugar proton was achieved by considering ^{1}H−^{1}H COSY and TOCSY spectra, while carbons were assigned from the

Table 1. ^{13}C NMR Data for Compounds **1**−**4** (CD_3OD)[a]

C	1	2	3	4	C	1	2	3	4
1	39.6	39.6	38.5	38.6		β-D-Glc	β-D-Glc	α-L-Ara	β-D-Gal
2	27.3	27.3	25.9	25.9	1′	*106.8*	*106.8*	*103.9*	*104.4*
3	*90.8*	*90.7*	*88.1*	*88.4*	2′	75.6	75.6	73.8	73.8
4	40.4	40.3	39.1	39.0	3′	78.3	78.2	81.3	84.5
5	57.3	57.2	56.0	56.1	4′	71.8	71.7	67.3	68.7
6	19.1	19.1	17.6	17.6	5′	76.4	76.4	63.6	74.4
7	36.8	36.7	35.4	35.4	6′	68.3	68.2		60.8
8	38.3	38.3	36.8	37.2					
9	54.1	53.9	52.7	52.3		α-L-Rha	α-L-Rha	α-L-Rha	α-L-Rha
10	38.5	38.4	37.0	37.2	1″	*102.2*	*102.2*	*100.6*	*100.4*
11	22.4	22.5	21.1	20.7	2″	72.3	72.3	70.6	70.5
12	29.2	29.0	27.7	26.8	3″	72.4	72.4	70.6	70.5
13	38.3	37.8	36.6	36.7	4″	74.0	74.0	72.3	72.4
14	54.9	54.8	53.2	56.1	5″	69.8	69.8	68.7	68.5
15	37.8	37.7	35.7	37.0	6″	18.2	18.1	16.5	16.5
16	110.8	110.9	109.9	116.9					
17	55.3	55.5	52.9	61.5		α-L-Rha	α-L-Rha	β-D-Glc	β-D-Glc
18	66.9	66.9	65.4	65.1	1‴	*93.5*	*96.6*	*102.7*	*103.6*
19	16.8	16.8	15.6	15.6	2‴	*72.3*	73.6	73.5	73.6
20	78.1	76.9	68.0	74.7	3‴	*70.8*	72.5	75.1	75.4
21	24.5	28.4	27.0	22.0	4‴	*71.8*	73.6	75.8	75.8
22	41.0	41.4	43.9	94.1	5‴	69.3	71.5	74.8	74.7
23	69.6	69.5	68.2	27.2	6‴	18.0	18.2	60.6	60.6
24	125.9	125.9	124.8	120.6					
25	137.3	138.0	135.3	132.1	Ac 2‴	172.0			
26	25.8	25.7	24.3	24.4		20.6			
27	18.8	19.0	16.9	16.5	Ac 3‴	171.7			
28	28.4	30.8	28.1	26.9		20.6			
29	19.5	16.7	15.4	15.5	Ac 4‴	171.6			
30	20.7	19.7	17.7	17.7		20.6			

[a] ^{13}C NMR chemical shifts of substituted residues are italicized.

Table 2. ^1H NMR Data of the Sugar Moieties of Compounds **1**−**4** (CD_3OD)[a]

	1	2	3	4
H	δ_H (ppm) *J* (Hz)	δ_H (ppm) *J* (Hz)	δ_H (ppm) *J* (Hz)	δ_H (ppm) *J* (Hz)
	β-D-Glc	β-D-Glc	α-L-Ara	β-D-Gal
1′	*4.31 (d, J = 8)*	4.31 (d, *J* = 7.6)	*4.49 (d, J = 5.3)*	*4.43 (d, J = 7.6)*
2′	3.20 (dd, *J* = 9, 8)	3.19 (t, *J* = 7.9)	*3.88 (m)*	*3.85 (dd, J = 9.5, 7.7)*
3′	3.34 (t, *J* = 9)	3.34 (t, *J* = 7.9)	*3.88 (m)*	*3.77 (dd, J = 9, 2.5)*
4′	3.26 (t, *J* = 9)	3.27 (t, *J* = 9.4)	4.05 (brs)	4.15 (brd, *J* = 3)
5′	3.41 (ddd, *J* = 9, 6, 2)	3.40 (m)	3.89 (dd, *J* = 12, 4)	3.53 (t, *J* = 6.3)
5′			3.53 (dd, *J* = 12, 2)	
6′	*3.97 (dd, J = 11, 2)*	3.61 (dd, *J* = 11.2, 6.4)		3.72−3.74 (m)
6′	*3.61 (dd, J = 11, 6)*	3.97 (brd, *J* = 11)		3.72−3.74 (m)
	α-L-Rha	α-L-Rha	α-L-Rha	α-L-Rha
1″	*4.76 (d, J = 1.5)*	4.75 (brs)	*5.26 (brs)*	*5.40 (d, J = 1.3)*
2″	3.83 (dd, *J* = 3, 1.5)	3.83 (brs)	3.94 (dd, *J* = 3.3, 1.5)	3.98 (dd, *J* = 3.3, 1.5)
3″	3.69 (dd, *J* = 9.5, 3)	3.68 (dd, *J* = 9.4, 5)	3.74 (dd, *J* = 9.5, 3.3)	3.77 (dd, *J* = 9.6, 3.2)
4″	3.39 (t, *J* = 9.5)	3.39 (t, *J* = 9.3)	3.41 (t, *J* = 9.5)	3.41 (t, *J* = 9.7)
5″	3.70 (m)	3.69 (m)	3.90 (m)	4.02 (m)
6″	1.28 (d, *J* = 6)	1.28 (d, *J* = 6.5)	1.22 (d, *J* = 6.2)	1.22 (d, *J* = 6)
	α-L-Rha	α-L-Rha	β-D-Glc	β-D-Glc
1‴	*5.14 (d, J = 2)*	5.0 (brs)	*4.57 (d, J = 7.8)*	*4.57 (d, J = 7.8)*
2‴	*5.18 (brt, J = 2)*	3.80 (brs)	3.42 (dd, *J* = 9, 7.8)	3.42 (dd, *J* = 9.4, 7.9)
3‴	*5.41 (dd, J = 10, 3)*	3.79 (brd, *J* = 10)	3.71 (t, *J* = 9)	3.69 (t, *J* = 9.3)
4‴	*5.09 (t, J = 10)*	3.43 (t, *J* = 9.3)	*4.19 (t, J = 9.3)*	*4.18 (t, J = 9.4)*
5‴	4.00 (m)	3.69 (m)	3.49 (ddd, *J* = 9.5, 5.2, 2.2)	3.47 (ddd, *J* = 9.8, 5, 2)
6‴	1.20 (d, *J* = 6)	1.25 (d, *J* = 6)	3.78 (dd, *J* = 12.3, 5.2)	3.77 (dd, *J* = 12.3, 5.5)
6‴			3.88 (brd, *J* = 12.3)	3.89 (dd, *J* = 12.3, 2)
Ac 2‴	2.17 (s)			
Ac 3‴	2.09 (s)			
Ac 4‴	2.03 (s)			

[a] ^1H NMR chemical shifts of substituted residues are italicized.

HSQC spectrum and from comparison with literature values.[7,11,18] Evaluation of spin−spin couplings of protons and chemical shifts of carbons allowed the identification of an α-arabinopyranosyl (Ara′), a β-glucopyranosyl (Glc‴), and an α-rhamnopyranosyl (Rha″), respectively. The ROESY experiment showed homonuclear spatial correlations between signals at δ_H 4.49 (Ara-1′) and 3.15 (Agly-3), between δ_H 5.26 (Rha-1″) and 3.88 (Ara-3′), and between δ_H 4.57 (Glc-1‴) and 3.88 (Ara-3′ and 2′) and 4.05 (Ara-4′), indicating that the glycosidic chain was the β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl and linked to jujubogenin at C-3. In the HMBC, a weak correlation observed between H-1″ of rhamnopyranosyl and C-2′ of arabinopyranosyl confirmed partially the sequencing of the triglycosidic chain. The signals of position 4 of glucose at δ_C 75.8 and δ_H 4.19 were deshielded when compared with the values of glucose in saponin **1**, indicating a substitution. The IR spectrum of

compound **3** showed absorption bands at ν 1382, 1258, 1072, and 832 cm^{-1}, characteristic of a sulfate group.[19] Thus, **3** is 3-*O*-α-L-rhamnopyranosyl-(1→2)-[(4-sulfo)-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyljujubogenin, a new natural compound corresponding to the sulfated derivative of zizyphus saponin II.[20]

Compound **4** also contained a sulfate group from the characteristic absorption bands at ν 1383, 1234, 1068, and 831 cm^{-1} in the IR spectrum.[19] The negative-ion mode ESIMS showed a quasimolecular ion peak at *m/z* 1021 $[M - H]^-$, indicating a molecular weight of 1022, and the HRMS indicated the molecular formula $C_{48}H_{78}O_{21}S$. The aglycone structure was assigned as the known (20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β-20-diol on the basis of ^1H and ^{13}C NMR spectra. Most of the ^{13}C NMR signals were assigned through $^2J_{H-C}$ and $^3J_{H-C}$ couplings of the seven methyls and are in agreement with literature data although the reported values were recorded in pyridine.[13] The terminal tetrahydrofuran ring structure was assumed on the basis of the deshielded dioxygenated quaternary C-16 at δ_C 116.9, of the presence of the methine C-22 at δ_C 94.1 showing, in the HSQC spectrum, a correlation with a proton at δ_H 4.14 (t, $J = 7.0$ Hz), and of the methylene C-23 at δ_C 27.2 bearing two equivalent protons at δ_H 2.30 (t, $J = 6.5$ Hz).[7] The spatial correlations in the ROESY spectrum observed between Me-21 (δ_H 1.20, s), H-17 (δ_H 1.81, d, $J = 8$ Hz), and H-22 (δ_H 4.14, t, $J = 7$ Hz) and between H-17 and the two H-18 (δ_H 3.95, m and 3.97, m) confirmed the configuration at C-16, C-20, and C-22.[13] The presence of three sugars was revealed by three anomeric ^1H NMR signals at δ_H 4.43 (d, $J = 7.6$ Hz), 4.57 (d, $J = 7.8$ Hz), and 5.40 (d, $J = 1.3$ Hz), which gave correlations with ^{13}C NMR signals in the HSQC spectrum at δ_C 104.4, 103.6, and 100.4, respectively. A combination of the COSY, HOHAHA, and HSQC experiments allowed the identification of a β-galactopyranosyl (Gal′), a β-glucopyranosyl (Glc‴), and a α-rhamnopyranosyl (Rha″) (Tables 1 and 2). The sugar sequence was deduced from the correlations detected in the ROESY and HMBC spectra. The ROESY experiment showed homonuclear spatial correlations between H-1′ of galactose (δ_H 4.43) and H-3 of aglycone (δ_H 3.18), between H-1″ of rhamnose (δ_H 5.40) and H-2′ of galactose (δ_H 3.85), and between H-1‴ of glucose (δ_H 4.57) and H-3′ of galactose (δ_H 3.77). Moreover, HMBC correlations were found between H-1′ of galactose and C-3 of aglycone, H-1″ of rhamnose and C-2′ of galactose, and H-1‴ of glucose and C-3′ of galactose, indicating that the trisaccharide chain α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl was linked to the genin at C-3. When compared to jujubasaponin IV,[13] the NMR signals of glucose at position 4 showed deshielded values (δ_C 75.8 and δ_H 4.18), indicating a substitution by a sulfate group, as for saponin **3**.[19] Thus, **4** is 3-*O*-α-L-rhamnopyranosyl-(1→2)[(4-sulfo)-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-(20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol, a new natural compound corresponding to the sulfated derivative of jujubasaponin IV.[13]

Experimental Section

General Experimental Procedures. The 1D and 2D NMR spectra ($^1H-^1H$ COSY, HSQC, HMBC, HOHAHA, and ROESY) were performed using an Avance DRX 500 Bruker spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C spectra). All chemical shifts (δ) are given in ppm, and the samples were solubilized in MeOH-d_4. Electrospray ionization (ESIMS) was conducted on a Bruker Esquire-LC-MS (**1**) or a ZABSPEC-T Micromass apparatus (**2**−**5**). High-resolution mass spectra (HRMS) were measured by MALDI-TOF on a Voyager-DE STR PerSeptive Biosystems spectrometer using trihydroxyacetophenone (THAP) as matrix. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra (anhydrous KBr disk) were recorded on a Midac série M IRTF spectrometer. TLC employed precoated Si gel plates 60 F_{254} 250 μm (Whatman). The TLC solvent systems were as follows: for saponins (a) $CHCl_3$/MeOH/H_2O (65:35:6); for monosaccharides (b) MEK/^{i}PrOH/Ac_2O/H_2O (40:20:14:12). Spray reagents were as follows: for the saponins, sulfuric acid 50%; for the sugars, ethanolic α-naphthol 15%/H_2SO_4 conc/EtOH/H_2O (18:10:66:6) followed by butanolic aniline 2 N/butanolic H_2PO_4 2 N (1:2). Liquid−liquid fractionation was carried out using an HPCPC Sanki Series fitted with a column presenting a total internal volume of 240 mL, connected to a ELSD II A Varex Type light-scattering detector. Further isolations were performed on a Lobar MPLC column (B-type, 310 × 25 mm, Si 60 40−63 μm) or precoated Si gel plates 60 F_{254} 250 μm (Whatman).

Plant Material. The leaves of *Z. lotus* (L.) Lam. were collected in Cherahil, near Monastir, Tunisia, in September 1994 and identified by Prof. M. A. Nabli, University of Tunis. A voucher specimen (No. KG/ZL/F-010) is deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy in Monastir, Tunisia.

Extraction and Isolation. Dried powdered leaves of *Z. lotus* (300 g) were defatted successively with *n*-hexane and $CHCl_3$ by Soxhlet extraction and then extracted with hot MeOH. The vacuum-dried MeOH extract (43 g) was dissolved in MeOH (130 mL) and precipitated in Et_2O (650 mL), yielding 18 g of a crude saponin fraction, which was dialyzed under agitation for 2 days. From the resulting extract (6 g), three batches of 1 g were submitted to ascending mode centrifugal partition chromatography, using an EtOAc/*n*-BuOH/H_2O ternary biphasic system, applying a gradient from 95:1:4 to 46:40:14 in 560, 480, and 580 min, respectively. Rotation was set at 1300 rpm, flow-rate 2 mL/min, fraction size 2 min, and pressure drop 27 bar. Similar fractions were pooled, and final purification was performed by preparative TLC using $CHCl_3$/MeOH/H_2O (70:30:5) (**2**−**4**) as eluent or normal phase-MPLC (**1**) using a gradient from CH_2Cl_2/MeOH 1% to CH_2Cl_2/MeOH 10% to give compounds **1** (7 mg), **2** (6 mg), **3** (15 mg), and **4** (6 mg) and jujuboside B (**5**) (9 mg).

Compound 1: white amorphous powder; TLC R_f 0.75 (system a); $[\alpha]^{20}_D$ −31.8° (*c* 0.58, MeOH); ^{13}C NMR of genin (CD_3OD, 125 MHz), Table 1; ^{13}C NMR (CD_3OD, 125 MHz) and ^1H NMR (CD_3OD, 500 MHz) of sugar moieties, Table 2; positive ESIMS *m/z* 1075 $[M + Na]^+$, negative ESIMS *m/z* 1051 $[M - H]^-$; positive HRMS *m/z* 1075.5429 $[M + Na]^+$ (calcd for $C_{54}H_{84}O_{20}Na$ 1075.5453).

Compound 2: white amorphous powder; TLC R_f 0.58 (system a); $[\alpha]^{20}_D$ −10.0° (*c* 0.12, MeOH); ^{13}C NMR of genin (CD_3OD, 125 MHz), Table 1; ^{13}C NMR (CD_3OD, 125 MHz) and ^1H NMR (CD_3OD, 500 MHz) of sugar moieties, Table 2; positive ESIMS *m/z* 949 $[M + Na]^+$, *m/z* 486 $[M + 2Na]^{2+}$; positive HRMS *m/z* 949.5133 $[M + Na]^+$ (calcd for $C_{48}H_{78}O_{17}Na$ 949.5136).

Compound 3: white amorphous powder; TLC R_f 0.39 (system a); $[\alpha]^{20}_D$ −29.0° (*c* 0.5, MeOH); ^{13}C NMR of genin (CD_3OD, 125 MHz), Table 1; ^{13}C NMR (CD_3OD, 125 MHz) and ^1H NMR (CD_3OD, 500 MHz) of sugar moieties, Table 2; negative ESIMS *m/z* 991 $[M - H]^-$; negative HRMS *m/z* 991.4600 $[M - H]^-$ (calcd for $C_{47}H_{75}O_{20}S$ 991.4572).

Compound 4: white amorphous powder; TLC R_f 0.28 (system a); $[\alpha]^{20}_D$ −11.2° (*c* 0.17, MeOH); ^{13}C NMR of genin (CD_3OD, 125 MHz), Table 1; ^{13}C NMR (CD_3OD, 125 MHz) and ^1H NMR (CD_3OD, 500 MHz) of sugar moieties, Table 2; negative ESIMS *m/z* 1021 $[M - H]^-$; negative HRMS *m/z* 1021.4704 $[M - H]^-$ (calcd for $C_{48}H_{77}O_{21}S$ 1021.4677).

Jujuboside B (5): white amorphous powder; TLC R_f 0.45 (system a); the physical characteristics and spectroscopic data were in full agreement with previously published data.[10]

Acid Hydrolysis. A solution of 330 mg of dried, dialyzed extract in 0.02 N aqueous H_2SO_4 (10 mL) and $HClO_4$ 6.5% (10 mL) was heated at 140 °C in a sealed glass tube for 2 h. The supernatant was lyophilized and dissolved in H_2O (4 mL) and

filtered through a Millex HV 0.45 μm cartridge. The resulting solution containing monosaccharides was submitted to preparative TLC (solvent b) to give glucose (5.8 mg), galactose (5.8 mg), rhamnose (4.5 mg), arabinose (6.5 mg), and xylose (6.2 mg), which were compared to authentic samples and used to measure the optical rotation in MeOH. Rhamnose: $[\alpha]^{20}_D$ +3.5° (*c* 0.34, H_2O); arabinose: $[\alpha]^{20}_D$ +8.3° (*c* 0.54, H_2O); glucose: $[\alpha]^{20}_D$ +19.8° (*c* 0.45, H_2O); galactose: $[\alpha]^{20}_D$ +8.1° (*c* 0.48, H_2O).

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