Novel Triterpene Saponins from *Zizyphus joazeiro*

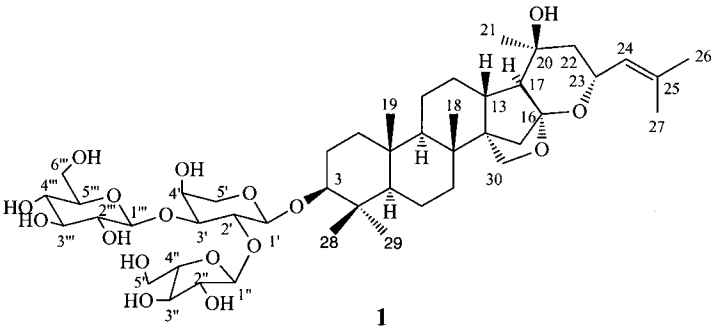
by **Wolfgang Schühly**[a]), **Jörg Heilmann**[a]), **Ihsan Çalis**[b]), and **Otto Sticher***[a])

[a]) Department of Applied BioSciences, Institute of Pharmaceutical Sciences, ETH-Zürich, CH-8057 Zürich
[b]) Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara

Two dammarane-type saponins with a novel aglycone derived from the parent 16,22-epoxy-24-methylidenedammarane and lotoside A, a new lotogenin derivative, were isolated from the MeOH extract of the stem bark of the Brazilian medicinal plant *Zizyphus joazeiro*, in addition to the known saponin 3*β*-{{*O*-[*O*-[*α*-L-arabinofuranosyl-(1→2)]-*O*-[*β*-D-glucopyranosyl-(1→3)]]-*α*-L-arabinopyranosyl}oxy}jujubogenin (**1**). The structures of the new compounds were determined as 16,22-epoxy-3*β*-[(*β*-D-glucopyranosyl)oxy]-24-methylidenedammarane-15*α*,16*α*,20*β*-triol (**2**), 16,22-epoxy-3*β*-{{*O*-[*O*-[*β*-D-glucopyranosyl-(1→2)]-*O*-[*β*-D-apiofuranosyl-(1→3)]]-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl}oxy}-24-methylidenedammarane-15*α*,16*α*,20*β*-triol (**3**), and 3*β*-{{*O*-[*O*-[*β*-D-glucopyranosyl-(1→2)]-*O*-[*β*-D-apiofuranosyl-(1→3)]]-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl}oxy}lotogenin (**4**) by means of 1D- and 2D-NMR spectroscopy, as well as FAB mass spectrometry. For the novel aglycone, we propose the name *joazeirogenin* and, for the new saponins, *joazeiroside A* (**2**) and *B* (**3**). Joazeirogenin was found to be 16,22-epoxy-24-methylidenedammarane-3*β*,15*α*,16*α*,20*β*-tetrol.

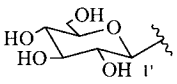
1. Introduction. – The genus *Zizyphus* (Rhamnaceae) is well-known for its content of triterpenes, *e.g.*, betulinic and alphitolic acid, triterpene saponins like jujubosides and dammaranes, as well as cyclopeptide alkaloids like amphibines [1–4]. Many species of the genus are used in traditional folk medicine, like *Z. jujuba* MILLER, which is listed in the Chinese Pharmacopoeia for its tonic and sedative properties [5]. *Zizyphus joazeiro* C. MART. (raspa de juá) is used in traditional Brazilian folk medicine as a remedy for, *e.g.*, fever, bronchitis, and gastric ulcers [6]. In a previous work, we reported the isolation of betulinic, alphitolic, and ursolic acid, as well as the isolation of three new betulinic acid derivatives 7*β*-*O*-(4-hydroxybenzoyl)betulinic acid, 7*β*-*O*-(4-hydroxy-3-methoxybenzoyl)betulinic acid, and 27-*O*-(4-hydroxy-3-methoxybenzoyl)-betulinic acid from the CH_2Cl_2 extract of the stem bark of *Z. joazeiro* [7]. In continuation of our work, we investigated the MeOH and the MeOH/H_2O 70:30 extract of its stem bark. This paper deals with the isolation and structure elucidation of the known 3*β*-{{*O*-[*O*-[*α*-L-arabinofuranosyl-(1→2)]-*O*-[*β*-D-glucopyranosyl-(1→3)]]-*α*-L-arabinopyranosyl}oxy}jujubogenin (**1**) and three new saponins **2**–**4**. Jujubogenin-derived saponins (jujubosides), which are also found in other species of the Rhamnaceae like *Hovenia*, show partly antisweet properties [8–10], and, therefore, we tested the antisweet properties of the isolated compounds.

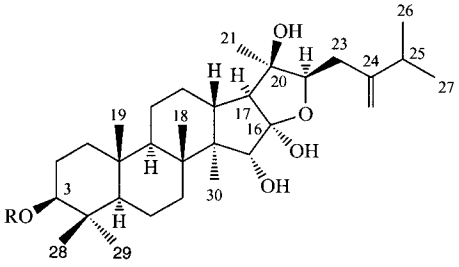
2. Results and Discussion. – Sequential percolation of 850 g of the powdered stem bark of *Z. joazeiro* with hexane, CH_2Cl_2, MeOH, and MeOH/H_2O 70:30 yielded the crude extracts. After TLC analysis, the MeOH and MeOH/H_2O extracts were combined and preseparated by vacuum liquid chromatography (VLC). Subsequent



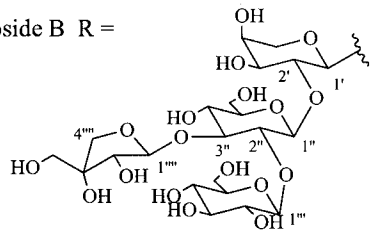
1

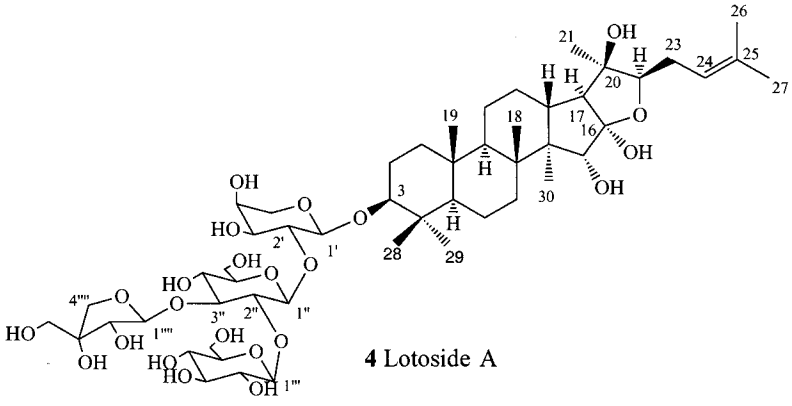
2 Joazeiroside A R =





3 Joazeiroside B R =





4 Lotoside A

open column chromatography and HPLC yielded the pure compounds, which were identified by mass spectrometry and NMR spectroscopy. Compound **1**, which is a well-known saponin isolated by *Higuchi et al.* [2], was found to be the major saponin in the

bark (0.025%). The amounts of compounds **2**–**4** were *ca.* 3–5 times lower. All compounds were tested for their antisweet properties according to [9].

3*β*-{{*O*-[*O*-[*α*-L-arabinofuranosyl-(1→2)]-*O*-[*β*-D-glucopyranosyl-(1→3)]]-*α*-L-arabinopyranosyl}oxy}jujubogenin (**1**) was obtained as transparent crystals from MeOH. The molecular formula was determined as $C_{46}H_{74}O_{17}$ by FAB-MS and ^{13}C-NMR. Acid hydrolysis and subsequent TLC revealed the presence of *α*-arabinose and *β*-glucose. All NMR and MS data were identical with those given in [2].

Saponin **2** was obtained as a white amorphous powder. The molecular formula was determined as $C_{37}H_{62}O_{10}$ by FAB-MS and ^{13}C-NMR. The ^{13}C-NMR spectra (including DEPT 135) resembled those of the A–E rings of lotogenin [4], but showed an additional signal of a methylidene C-atom (*δ* 108.6), as well as differences concerning shifts and multiplicities of the side-chain C-atoms (see *Table 1*). After extensive NMR analysis by COSY, HSQC and HMBC and in accordance with the data given for lotogenin [4], the aglycone of **2** was established to be 16,22-epoxy-24-methylidene-

Table 1. *^{13}C-NMR Data* (125 MHz) *of Compounds* **1**–**4** *in (D_4)Methanol*[a]). Chemical shifts *δ* in ppm, *J* in Hz.

	Aglycone of **1**	Aglycone of **2** and **3**	Aglycone of **4**		Sugars of **1**	Sugar of **2**	Sugars of **3** and **4**
C(1)	39.8 (*t*)	40.4, 40.5 (*t*)	40.5 (*t*)		Ara*p*	Glc*p*	Ara*p*
C(2)	27.3 (*t*)	27.2 (*t*)	27.3 (*t*)	C(1′)	106.0 (*d*)	106.6 (*d*)	105.1 (*d*)
C(3)	90.3 (*d*)	90.7, 91.3 (*d*)	91.3 (*d*)	C(2′)	77.2 (*d*)	75.6 (*d*)	81.2 (*d*)
C(4)	40.6 (*s*)	40.3, 40.6 (*s*)	40.6 (*s*)	C(3′)	83.7 (*d*)	78.2 (*d*)	78.5 (*d*)
C(5)	57.4 (*d*)	57.5 (*d*)	57.5 (*d*)	C(4′)	69.2 (*d*)	71.6 (*d*)	70.9 (*d*)
C(6)	19.1 (*t*)	19.1 (*t*)	19.1 (*t*)	C(5′)	65.9 (*t*)	77.6 (*d*)	67.0 (*d*)
C(7)	36.9 (*t*)	37.0 (*t*)	37.0 (*t*)	C(6′)	–	62.8 (*t*)	–
C(8)	38.5 (*s*)	42.2 (*s*)	42.2 (*s*)				
C(9)	54.1 (*d*)	52.3 (*d*)	52.3 (*d*)		Ara*f*		Glc*p*
C(10)	38.3 (*s*)	38.1 (*s*)	28.1 (*s*)	C(1″)	110.3 (*d*)		102.9 (*d*)
C(11)	22.5 (*t*)	22.4 (*t*)	22.4 (*t*)	C(2″)	83.7 (*d*)		82.1 (*d*)
C(12)	29.2 (*t*)	27.0 (*t*)	27.0 (*t*)	C(3″)	77.9 (*d*)		87.4, 87.3 (*d*)
C(13)	38.0 (*d*)	36.8 (*d*)	38.9 (*d*)	C(4″)	84.6 (*d*)		70.6 (*d*)
C(14)	54.6 (*s*)	54.0 (*t*)	54.0 (*t*)	C(5″)	62.1 (*t*)		77.8 (*d*)
C(15)	36.7 (*t*)	77.6, 77.7 (*d*)	77.7 (*t*)	C(6″)	–		62.9 (*t*)
C(16)	111.4 (*s*)	111.0, 110.9 (*s*)	111.0 (*s*)				
C(17)	54.4 (*s*)	63.1 (*d*)	63.4 (*d*)		Glc*p*		Glc*p*
Me(18)	19.2 (*q*)	16.6 (*q*)	16.7 (*q*)	C(1‴)	104.9 (*d*)		105.5 (*d*)
Me(19)	16.8 (*q*)	17.1 (*q*)	17.1 (*q*)	C(2‴)	75.2 (*d*)		75.3 (*d*)
C(20)	69.4 (*s*)	79.1, 79.2 (*s*)	78.8 (*s*)	C(3‴)	77.7 (*d*)		77.6 (*d*)
Me(21)	29.6 (*q*)	26.4 (*q*)	26.7 (*q*)	C(4‴)	71.1 (*d*)		71.1 (*d*)
C(22)	45.5 (*t*)	87.7 (*d*)	89.4 (*d*)	C(5‴)	77.8 (*d*)		77.3 (*d*)
C(23)	69.7 (*d*)	34.0, 34.1 (*t*)	28.2 (*t*)	C(6‴)	62.3 (*t*)		63.1 (*t*)
C(24)	126.3 (*d*)	154.4, 154.5 (*s*)	122.7 (*s*)				
CH_2=C(24)	–	108.6 (*t*)	–				Api*f*
C(25)	136.7 (*s*)	34.8, 34.9 (*d*)	133.4 (*s*)	C(1⁗)			111.5 (*d*)
Me(26)	25.8 (*q*)	22.2 (*q*)	25.9 (*q*)	C(2⁗)			77.7 (*d*)
Me(27)	18.4 (*q*)	22.2 (*q*)	17.8 (*q*)	C(3⁗)			80.2 (*s*)
Me(28)	28.3 (*q*)	28.3 (*q*)	28.3 (*q*)	C(4⁗)			74.9 (*t*)
Me(29)	16.8 (*q*)	16.8 (*q*)	16.8 (*q*)	C(5⁗)			64.4 (*t*)
Me(30)	66.9 (*t*)	9.9 (*q*)	10.0 (*q*)				

[a]) Ara*p* = Arabinopyranose, Ara*f* = arabinofuranose, Glc*p* = glucopyranose, Api*f* = apiofuranose.

dammarane-3β,15α,16α,20β-tetrol. To the best of our knowledge, this is a novel aglycone skeleton, which we named joazeirogenin.

The ^{1}H-NMR spectrum of **2** confirmed the presence of an aglycone structure similar to that of the lotosides I and II [4], but the H–C(24) signal, which in lotosides is a *m* near δ 5.20, was replaced by two protons at δ 4.86 and 4.80 (see *Table 2*). In place of the olefinic Me(26) and Me(27) groups, which resonate in lotogenin as two broad *s*, **2** exhibited two upfield shifted *d* at δ 1.05 and 1.06 (each $J = 6.2$ Hz, 6 H), indicating an i-Pr group (see *Table 2*). HSQC Analysis revealed that the signal of H–C(24) was absent and replaced by a quaternary C-atom at δ 154.5. Due to HMBC correlations to the neighboring atoms C(23), C(24), and C(25), the thus established CH_2= group was found to be directly bound to the side chain of the lotogenin skeleton. This was confirmed by a NOE signal between H-atom of CH_2=C(24) *cis* to C(23) and H–C(22). The configuration of the aglycone was confirmed by the NOESY correlations H–C(13)/H–C(15) and Me(18), Me(21)/H–C(17) and H–C(22), H–C(22)/1 H of CH_2=C(24). The glycosidation position in **2** was found to be at O–C(3) according to the downfield shift of C(3) at δ 90.7 and the HMBC correlation between C(3) of the aglycone and H–C(1′) of the sugar moiety. The presence of a β-D-glucose moiety was determined by COSY and NOESY spectra and confirmed by TLC analysis of the hydrolysate, which was compared to authentic sugar. Thus, the structure of **2** was established as 16,22-epoxy-3β-[(β-D-glucopyranosyl)oxy]-24-methylidenedammarane-15α,16α,20β-triol.

Saponin **3** displayed a pseudomolecular ion peak $[M + Na]^+$ at *m*/*z* 1115.4 in the FAB-MS. ^{13}C-NMR Experiments (including DEPT 135) sorted the signals into eight C, 24 CH, 13 CH_2, and eight Me C-atoms, corresponding to the molecular formula $C_{53}H_{88}O_{23}$. The ^{13}C-NMR data of the aglycone moiety were superimposable on those of **2**, indicating an identical aglycone part and pointing to the presence of a sugar moiety consisting of four monosaccharides linked at C(3). The NMR data, acid hydrolysis, and TLC comparison of the hydrolyzed components with authentic compounds led to the identification of β-D-glucose and α-L-arabinose; the presence of apiose was not as well-established as that of the other sugars. The assignment of the sugar signals of **3** was based on HSQC, HMBC, and TOCSY experiments, and their linkage was established on the basis of NOE and HMBC correlations. Acetylation of **3** with Ac_2O/pyridine led to the dodecaacetate **3a** with a FAB-MS pseudomolecular ion $[M + K]^+$ at *m*/*z* 1619.6. The fragment of the undeca-*O*-acetyl-sugar moiety appeared at *m*/*z* 1051.2, as well as tri-*O*-acetyl-apiose at *m*/*z* 259.0 and tetra-*O*-acetylated outer glucose at *m*/*z* 331.0. The ^{1}H-NMR analysis of the acetyl derivative **3a** confirmed the sugar identification.

The ^{1}H- and ^{13}C-NMR data of **3** indicated the presence of one α-L-arabinopyranose unit with H–C(1′) at δ 4.44 (*d*, $J = 7.8$ Hz) and C(1′) at δ 105.1, two β-D-glucopyranose units with H–C(1″) at δ 4.84 (*d*, $J = 6.8$ Hz), H–C(1‴) at δ 4.65 (*d*, $J = 7.7$ Hz), C(1″) at δ 102.9, C(1‴) at δ 105.5, and one β-D-apiofuranose unit with H–C(1⁗) at δ 5.24 (*d*, $J = 4.3$ Hz) and C(1⁗) at δ 111.5. HMBC Correlations were observed between H–C(2′) of the arabinose and C(1″) of the inner glucose, between H–C(2″) and C(1‴) of the peripheral glucose, and between H–C(3″) and C(1⁗) of the apiose, as well as between H–C(1′) of the arabinose and C(3) of the aglycone. The downfield shift of *ca.* 0.6–1 ppm observed for **3a** due to acetylation does not exist for the sugar atoms H–C(2′) (δ 3.81) of arabinose, and H–C(2″) and H–C(3″) (δ 3.71 and 3.75, resp.) of the inner glucose (see *Table 3*). This confirms that these positions are the linking positions in the sugar moiety. These results were also confirmed by an HMBC experiment performed with **3a** (see *Table 3*).

Saponin **4** was obtained as white amorphous powder. The FAB-MS provided a pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1101.3, corresponding to the molecular formula $C_{52}H_{86}O_{23}$. Acid hydrolysis, comparison of the ^{1}H- and ^{13}C-NMR data (including those of the peracetylated compound; see *Table 1*), as well as 2D-NMR experiments revealed the same glycosidation pattern for compounds **3** and **4**. Multiplicities and shift values of

Table 2. *^{1}H-NMR Data* (500 MHz) *of Compounds* **1**–**4** *in (D_4)Methanol*[a]). Chemical shifts δ in ppm, J in Hz.

H	Aglycone of **1**	Aglycone of **2** and **3**	Aglycone of **4**
CH_2(1)	1.67, 0.92 (2*m*)	1.70, 0.97 (2*m*)	1.71, 0.99 (2*m*)
CH_2(2)	1.85, 1.70 (2*m*)	1.95, 1.75 (2*m*)	1.95, 1.73 (2*m*)
H–C(3)	3.11 (*dd*, *J* = 12.3, 4.3)	3.17 (*dd*[b]))	3.20 (*dd*, *J* = 12.3, 4.8)
H–C(5)	0.74 (*d*, *J* = 9.1)	0.75 (*d*, *J* = 11.8)	0.78 (*d*, *J* = 10.8)
CH_2(6)	1.59, 1.52 (2*m*)	1.52 (*m*)	1.54, 1.45 (2*m*)
CH_2(7)	1.56, 1.49 (2*m*)	1.58 (*m*)	1.55 (*m*)
H–C(9)	0.88 (*m*)	1.31 (*m*)	1.34 (*m*)
CH_2(11)	1.64, 1.50 (2*m*)	1.50, 1.40 (2*m*)	1.54, 1.45 (2*m*)
CH_2(12)	1.86, 1.67 (2*m*)	1.70, 1.25 (2*m*)	1.71, 1.27 (2*m*)
H–C(13)	2.48 (*ddd*, *J* = 12.5, 6.7, 6.4)	2.02 (*m*)	2.05 (*m*)
CH_2(15) or H–C(15)	2.06, 1.18 (2*d*, *J* = 8.7)	3.95 (*s*)	3.94 (*s*)
H–C(17)	1.00 (*d*, *J* = 6.7)	1.88 (*d*, *J* = 11.2)	1.88 (*d*, *J* = 11.2)
Me(18)	1.14 (*s*)	1.15 (*s*)	1.15 (*s*)
Me(19)	0.88 (*s*)	0.90 (*s*)	0.91 (*s*)
Me(21)	1.14 (*s*)	1.24 (*s*)	1.24 (*s*)
CH_2(22) or H–C(22)	1.47, 1.38 (2*m*)	4.02 (*dd*, *J* = 7.6, 4.1)	3.82 (*m*)
H–C(23) or CH_2(23)	4.68 (*ddd*, *J* = 11.3, 8.1, 1.3)	2.28 (*m*)	2.26 (*dd*, *J* = 15.2, 7.9)
H–C(24)	5.15 (*m*)	–	5.22 (*m*)
CH_2=C(24)	–	4.86, 4.80 (2*s*)	–
H–C(25)	–	2.33 (*m*)	–
Me(26)	1.72 (*s*)	1.06 (*d*, *J* = 6.2)	1.68 (*s*)
Me(27)	1.70 (*s*)	1.05 (*d*, *J* = 6.2)	1.64 (*s*)
Me(28)	1.05 (*s*)	1.05 (*s*)	1.06 (*s*)
Me(29)	0.85 (*s*)	0.86 (*s*)	0.85 (*s*)
CH_2(30) or Me(30)	4.03, 3.94 (2*d*, *J* = 12.6)	0.95 (*s*)	0.96 (*s*)
H	Sugars of **1**	Sugar of **2**	Sugars of **3** and **4**
	Ara*p*	Glc*p*	Ara*p*
H–C(1′)	4.39 (*d*, *J* = 6.4)	4.30 (*d*, *J* = 7.8)	4.44 (*d*, *J* = 7.8)
H–C(2′)	3.75 (*dd*[b]))	3.22 (*dd*[b]))	3.54 (*dd*[b]))
H–C(3′)	4.05 (*dd*, *J* = 5.1, 2.6)	3.32 (*dd*[b]))	3.54 (*dd*[b]))
H–C(4′)	4.03 (*ddd*[b]))	3.30 (*dd*[b]))	3.52 (*dd*[b]))
CH_2–C(5′) or H–C(5′)	3.85, 3.54 (2*dd*[b]))	3.27 (*ddd*[b]))	3.95, 3.23 (2*dd*, *J* = 10.8, 5.3)
CH_2(6′)	–	3.88 (*d*, *J* = 3.8)	–
	Ara*f*		Glc*p*
H–C(1″)	5.30 (*d*, *J* = 2.6)		4.84 (*d*, *J* = 6.8)
H–C(2″)	4.09 (*dd*, *J* = 5.0, 2.6)		3.52 (*dd*[b]))
H–C(3″)	3.93 (*dd*[b]))		3.59 (*dd*[b]))
H–C(4″)	3.97 (*ddd*[b]))		3.24 (*dd*[b]))
CH_2–C(5″) or H–C(5″)	3.71, 3.60 (2*dd*, *J* = 12.3, 2.9)		3.28 (*ddd*[b]))
CH_2(6″)	–		3.86, 3.66 (2*dd*, *J* = 11.9, 5.3)
	Glc*p*		Glc*p*
H–C(1‴)	4.51 (*d*, *J* = 7.6)		4.65 (*d*, *J* = 7.7)
H–C(2‴)	3.28 (*dd*[b]))		3.26 (*dd*[b]))
H–C(3‴)	3.92 (*dd*, *J* = 7.6, 4.9)		3.28 (*dd*[b]))
H–C(4‴)	3.33 (*dd*[b]))		3.32 (*dd*[b]))
H–C(5‴)	3.35 (*ddd*[b]))		3.36 (*ddd*[b]))
CH_2(6‴)	3.83, 3.67 (2*dd*, *J* = 8.2, 5.2)		3.84, 3.59 (2*d*[b]))
			Api*f*
H–C(1⁗)			5.24 (*d*, *J* = 4.3)
H–C(2⁗)			4.02 (*d*, *J* = 4.3)
CH_2(4⁗)			4.16, 3.81 (2*d*, *J* = 9.8)
CH_2(5⁗)			3.55 (*s*)

[a]) Ara*p* = Arabinopyranose, Ara*f* = arabinofuranose, Glc*p* = glucopyranose, Api*f* = apiofuranose. [b]) Coupling constants not assignable due to overlapping signals.

Table 3. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz) *of the Sugar Moiety of* **3a** *in $CDCl_3$*[a]). Chemical shifts δ in ppm, J in Hz.

		$\delta(^{13}C)$	$\delta(^{1}H)$	HMBC Correlations
Ara*p*	H–C(1′)	103.56	4.54 (*d*, *J* = 7.3)	C(1′)/*H*–C(3), *H*–C(2′)
	H–C(2′)	76.28	3.81 (*dd*, *J* = 8.0, 7.3)	C(2′)/*H*–C(3′), *H*–C(1″)
	H–C(3′)	75.00	5.28 (*dd*[b]))	C(3′)/*H*–C(4′)
	H–C(4′)	77.94	5.02 (*dd*[b]))	–
	CH_2(5′)	62.44	4.15, 3.37 (2*dd*, *J* = 10.7, 8.2)	C(5′)/*H*–C(4′)
Glc*p*	H–C(1″)	100.76	4.50 (*d*, *J* = 7.8)	C(1″)/*H*–C(2′), *H*–C(2″)
	H–C(2″)	77.94	3.71 (*dd*, *J* = 8.8, 7.8)	C(2″)/*H*–C(3″)
	H–C(3″)	80.92	3.75 (*t*, *J* = 8.8)	C(3″)/*H*–C(2″), *H*–C(4″), *H*–C(1‴)
	H–C(4″)	68.60	4.93 (*t*[b]))	C(4″)/*H*–C(3″)
	H–C(5″)	71.46	3.51 (*m*)	C(5″)/*H*–C(4″)
	CH_2(6″)	62.26	4.20, 4.03 (2*d*[b]))	–
Glc*p*	H–C(1‴)	99.75	4.83 (*d*, *J* = 7.6)	C(1‴)/*H*–C(2‴), *H*–C(6‴)
	H–C(2‴)	72.48	4.98 (*dd*, *J* = 9.2, 7.6)	C(2‴)/*H*–C(3″)
	H–C(3‴)	72.36	5.27 (*t*, *J* = 9.2)	C(3‴)/*H*–C(4‴)
	H–C(4‴)	70.07	5.00 (*dd*[b]))	C(4‴)/*H*–C(3‴)
	H–C(5‴)	71.08	3.86 (*m*)	C(5‴)/*H*–C(4‴), *H*–C(6‴)
	CH_2(6‴)	62.45	4.32, 4.11 (2*dd*, *J* = 12.6, 5.3)	–
Api*f*	H–C(1⁗)	106.92	5.30 (br. *s*)	C(1⁗)/*H*–C(3″), *H*–C(4⁗)
	H–C(2⁗)	76.10	5.44 (br. *s*)	C(2⁗)/*H*–C(1⁗), *H*–C(4⁗), *H*–C(5⁗)
	C(3⁗)	83.39	–	C(3⁗)/*H*–C(1⁗), *H*–C(4⁗), *H*–C(5⁗)
	CH_2(4⁗)	72.93	4.20, 4.12 (2*d*[b]))	C(4⁗)/*H*–C(5⁗)
	CH_2(5⁗)	62.52	4.72, 4.50 (2*d*, *J* = 12.3)	C(5⁗)/*H*–C(4⁗)

[a]) Ara*p* = Arabinopyranose, Ara*f* = arabinofuranose, Glc*p* = glucopyranose, Api*f* = apiofuranose.
[b]) Coupling constants not assignable due to overlapping signals.

the aglycone were identical to published data of O–C(3) glycosylated lotogenins [4]. Therefore, compound **4** (lotoside A) was identified as 3β-{{*O*-[*O*-[β-D-glucopyranosyl-(1 → 2)]-*O*-[β-D-apiofuranosyl-(1 → 3)]]-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl}oxy}lotogenin.

3. Antisweet Properties. – Taste modifiers such as sweetness inhibitors are reported from several plants. Since some of them are saponins from plants of the Rhamnaceae family, we performed a test for antisweet properties according to [9]. Like other jujubosides mentioned in [11], compound **1** showed a remarkable sweetness-inhibiting effect on sucrose solution (0.1M). Compound **2** was without significant sweetness inhibition. Compounds **3** and **4** exhibited an extremely bitter taste but did not derange sweetness sensation. Moreover, **3** showed a remarkable anaesthetic effect.

Experimental Part

General. TLC: silica gel *60 F_{254}* precoated plates, *RP-18 $F_{254}S$* precoated plates (both *Merck*, Darmstadt, Germany); detection by vanillin/H_2SO_4 and 30% H_2SO_4 in EtOH. Vacuum liquid chromatography (VLC): silica gel *60* (40–60 μm; *Merck*, Darmstadt). Open column chromatography (CC): silica gel *60* (63–200 μm; *Merck*, Darmstadt). HPLC: *Merck-Hitachi L-6200* pump, fraction collector *L-5200*, UV detector *L-4000*, and chromatointegrator *L-2500*; column: *Spherisorb S5 ODS 2* (10 μm; 250 × 16 mm; *Knauer*, Berlin, Germany); reversed phase-cartridge *Sep-Pak RP-18* (*Waters*, Milford, MA, USA). M.p. *Mettler F5* apparatus. Optical rotation: *Perkin-Elmer 241* polarimeter; at 20°. NMR Spectra (^{1}H, ^{13}C, ^{1}H,^{1}H-COSY, ^{1}H,^{1}H-TOCSY, ^{1}H,^{13}C-

HMQC, and ^{1}H,^{13}C-HMBC): *Bruker DRX-500* spectrometer at 500 and 125.77 MHz, resp.; all spectra in CD_3OD with residual MeOH peak as reference; chemical shifts δ in ppm, J in Hz. FAB-MS: *VG ZAB2-SEQ*; matrix = 3-nitrobenzyl alcohol; in m/z.

Plant Material. Z. joazeiro was collected and identified by Dr. *Albert Schnarwiler*, Ballwil, Switzerland, in northeastern Brazil. A voucher specimen (No. DP-ETH 96/1) is deposited at the Institute of Pharmaceutical Sciences, ETH-Zürich.

Extraction and Isolation. The dried and powdered bark (850 g) of *Z. joazeiro* was first extracted with hexane, then with CH_2Cl_2, MeOH, and MeOH/H_2O 70:30 (percolation with each solvent lasted 5 d with *ca.* 10 to 20 l). The MeOH and MeOH/H_2O extracts were combined and dried after TLC analysis: 54 g of residue. From this, 20.00 g were submitted to VLC (silica gel, $CHCl_3$/MeOH/H_2O 90:10:0 → 40:60:8, 12 steps, each step *ca.* 250 ml) to give *ca.* 20 fractions. *Jujuboside* (**1**; 70 mg) was mainly found in the VLC *Fr. 11* and *12* ($CHCl_3$/MeOH/H_2O 75:25:2.5) and purified by CC (silica gel, $CHCl_3$/MeOH/H_2O 80:20:1 → 50:50:5). *Joazeiroside A* (**2**; 20 mg) was isolated as main compound from VLC *Fr. 7* ($CHCl_3$/MeOH/H_2O 80:20:1) by CC (silica gel, $CHCl_3$/MeOH/H_2O 80:20:0.5 → 50:50:5, 40 fractions) and subsequent VLC (*RP-18*, MeOH/H_2O 50:50 → 70:30). *Joazeiroside B* (**3**; 42 mg) and *lotoside A* (**4**; 40 mg) were isolated from VLC *Fr. 13–15* ($CHCl_3$/MeOH/H_2O 70:30:3) by CC (silica gel, $CHCl_3$/MeOH/H_2O 80:20:2 → 55:45:5, 200 fractions) and subsequent prep. HPLC of the *Fr. 85–93* ($CHCl_3$/MeOH/H_2O 60:40:4) (*RP-18* silica gel, MeOH/H_2O 70:30, t_R(**3**) 18.5 min, t_R (**4**) 14.5 min).

Sugar Analysis. Each saponin (1–2 mg) was dissolved and hydrolyzed in dioxane (1 ml) and 2N H_2SO_4 (1 ml). After dilution with H_2O, the non-polar products were extracted with Et_2O, and the aq. phase was neutralized with ion-exchange resin (*Dowex 2 × 4*, *Fluka*, Buchs, Switzerland) and dried. The residue was dissolved in MeOH and applied to a TLC plate with cellulose coating (*Merck*, Darmstadt); as references, authentic sugars were used. Eluent: BuOH/pyridine/AcOH/H_2O 36:36:7:21. Detection (spraying with aniline phthalate (2 g of phthalic anhydride and 1 g of aniline in 100 ml of H_2O-sat. BuOH)) and heating according to [12].

Acetylation. To the saponin (5 mg) in pyridine (1 ml), Ac_2O (1 ml) was added. After mixing for 12 h at r.t., H_2O (2 ml) was added and the acetylated compound purified by HPLC (*SP* cartridge).

Evaluation of the Antisweet Properties. A 0.001M aq. sol. of each saponin (1 ml) was tested on three human volunteers. The test soln. was kept in the mouth for 2–3 min and then spat, and the sucrose solns. (0.1 and 0.2M) were tested immediately after rinsing with distilled H_2O.

Jujuboside (= *3β-[{*O-*[*O-*[α-*L*-Arabinofuranosyl-(1 → 2)]*-O-*[β-*D*-glucopyranosyl-(1 → 3))]-α-*L*-arabinopyranosyl}oxy]jujubogenin* = *(3β,16β,20*S*,23*R*)-16,23:16,30-Diepoxy-20-hydroxydammar-24-en-3yl* O-*α*-*L*-*Arabinopyranosyl-(1 → 2)*-O-*[β-*D*-glucopyranosyl-(1 → 3)]-α-*L*-arabinofuranoside*; **1**). Transparent crystals (from MeOH). M.p. >250°. $[\alpha]_D^{20} = -41$ (MeOH, $c = 1.0$). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 921.4 ($[M(C_{46}H_{74}O_{17}) + Na]^+$).

Joazeiroside A (= *16,22-Epoxy-3β-[(β-*D*-glucopyranosyl)oxy]-24-methylidenedammarane-15α,16α,20β-triol* = *(3β,15α,16α,20*R*,22*R*)-16,22-Epoxy-15,16,20-trihydroxy-24-methylidenedammaran-3-yl* O-*β*-*D*-*Glucopyranoside*; **2**). White powder. M.p. 248–252°. $[\alpha]_D^{20} = +2$ (MeOH, $c = 1.0$). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 689.0 ($[M(C_{37}H_{63}O_{10}) + Na]^+$).

Joazeiroside B (= *16,22-Epoxy-3β-[{*O-*[*O-*[β-*D*-glucopyranosyl-(1 → 2)]*-O-*[β-*D*-apiofuranosyl-(1 → 3)]]-β-*D*-glucopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl}oxy]-24-methylidenedammarane-15α,16α,20β-triol* = *(3β,15α,16α,20*R*,22*R*)-16,22-Epoxy-15,16,20-trihydroxy-24-methylidenedammaran-3-yl* O-*β*-*D*-*Apiofuranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 2)]-β-*D*-glucopyranosyl-(1 → 2)-α-*L*-arabinofuranoside*; **3**). White powder. M.p. >250° (dec.). $[\alpha]_D^{20} = -18$ (MeOH, $c = 1.0$). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1115.4 ($[M(C_{53}H_{88}O_{23}) + Na]^+$).

Lotoside A (= *3β-[{*O-*[*O-*[β-*D*-Glucopyranosyl-(1 → 2)]*-O-*[β-*D*-apiofuranosyl-(1 → 3)]]-β-*D*-glucopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl}oxy]lotogenin* = *(3β,15α,16α,20*R*,22*R*)-16,22-epoxy-15,16,20-trihydroxydammar-24-en-3-yl* O-*β*-*D*-*Apiofuranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 2)]-β-*D*-glucopyranosyl-(1 → 2)-α-*L*-arabinofuranoside*; **4**). White powder. M.p. >255° (dec.). $[\alpha]_D^{20} = -22$ (MeOH, $c = 1.0$). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1101.3 ($[M(C_{52}H_{86}O_{23}) + Na]^+$).

Dodeca-O-*acetyljoazeiroside B* **3a**. Amorphous powder. ^{1}H-NMR: *Table 3*. FAB-MS: 1619.6 ($[M(C_{77}H_{112}O_{35}) + Na]^+$).

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