Antisweet Natural Products. VI.[1] Jujubasaponins IV, V and VI from *Zizyphus jujuba* MILL.

Kazuko YOSHIKAWA,* Naomi SHIMONO and Shigenobu ARIHARA

Faculty of Pharmaceutical Sciences, Tokushima-Bunri University, Tokushima-shi, Tokushima 770, Japan. *Received February 17, 1992*

Three new dammarane saponins, jujubasaponins IV—VI (1—3), in addition to zizyphus saponins I—III (4—6) and jujuboside B (7) have been isolated from the fresh leaves of *Zizyphus jujuba* MILL. (Rhamnaceae). Their structures were established on the basis of spectral and chemical evidence. On the bioassay of the sense of taste, all of them showed antisweet activities.

Keywords *Zizyphus jujuba*; Rhamnaceae; jujubasaponin; (20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol; trevoagenin D; jujubogenin; antisweet substance

As part of our studies on antisweet natural products, we reported the isolation and structure elucidation of new acyl dammarane saponins named jujubasaponins I—III as antisweet principles in the leaves of *Zizyphus jujuba* and the structural revision of ziziphin.[2] Further separation of the saponin fraction successively afforded three new dammarane saponins, jujubasaponins IV—VI (**1**—**3**), together with zizyphus saponins I—III (**4**—**6**) and jujuboside B (**7**). All of them showed sweet-reducing activity. This paper deals with the isolation and elucidation of their structures and activities.

The saponin fractions were repeatedly subjected to reversed-phase high-performance liquid chromatography (HPLC) developed with 22—29% CH_3CN to provide jujubasaponins IV (**1**, 110 mg), V (**2**, 45 mg) and VI (**3**, 65 mg), zizyphus saponins I (**4**, 35 mg), II (**5**, 65 mg) and III (**6**, 50 mg) and jujuboside B (**7**, 50 mg).

Based on analysis of the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra and the physical data, **4**—**7** were proved to be identical with zizyphus saponins I, II and III and jujuboside B, respectively, which have been isolated from the dried fruits of this plant.[3]

Jujubasaponin IV (**1**), amorphous powder, $[\alpha]_D$ −3.6° (MeOH), has the molecular formula $C_{48}H_{78}O_{18}$ based on elemental analysis. The negative fast atom bombardment mass spectrum (FAB-MS) of **1** exhibited a quasi-molecular ion peak at *m/z* 941 $[M-H]^-$ besides peaks at *m/z* 779 [941 − hexosyl]$^-$ and 633 [779 − deoxyhexosyl]$^-$. Treatment of **1** with 5% H_2SO_4 liberated D-galactose, D-glucose and L-rhamnose in a molar ratio of 1 : 1 : 1 as sugar components. The corresponding anomeric protons were observed at δ 4.79 (d, J = 7.7 Hz), 5.11 (d, J = 8.0 Hz) and 6.41 (s) in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum showed 18 signals due to three hexosyl moieties and 30 signals due to an aglycone moiety. The 30 carbons were readily separated to $CH_3 \times 7$, $CH_2 \times 9$, $CH \times 7$ and $C \times 7$ with the help of the various distortionless enhancement by polarization transfer (DEPT) experiment. The signals at δ 132.5 (C), 122.4 (CH), 118.3 (C), 94.3 (CH), 88.5 (CH), 75.6 (C), and 65.7 (CH_2) were assigned to a

TABLE I. ^{1}H-NMR Spectral Data of Sugar Moieties of Compounds **1**—**3** (in Pyridine-d_5, 600 MHz, δ-Values)

	1	2	3
Inner galactose or glucose			
H-1′	4.79 (d, 7.7)	4.86 (d, 7.7)	4.85 (d, 7.5)
H-2′	4.67 (dd, 9.8, 7.7)	4.31 (dd, 7.7, 9.0)	4.63 (dd, 9.5, 7.5)
H-3′	4.26 (dd, 9.8, 2.8)	4.24 (dd, 9.0, 9.0)	4.22 (dd, 9.5, 3.4)
H-4′	4.85 (d, 2.8)	4.10 (dd, 9.0, 9.5)	4.47 (d, 3.4)
H-5′	4.08 (dd, 6.2, 5.7)	3.91 (ddd, 9.5, 5.5, 2.5)	4.07 (dd, 6.0, 4.5)
H-6′	4.33 (dd, 11.0, 5.7)	4.29 (11.2, 5.5)	4.41 (dd, 12.0, 6.0)
	4.37 (dd, 11.0, 6.2)	4.52 (11.2, 2.5)	4.42 (dd, 12.0, 4.5)
Terminal rhamnose (1→2)			
H-1″	6.41 s	6.50 s	6.50 s
H-2″	4.81 (d, 3.0)	4.86 (d, 3.0)	4.84 (d, 3.4)
H-3″	4.61 (dd, 9.5, 3.0)	4.64 (dd, 9.5, 3.0)	4.67 (dd, 9.5, 3.4)
H-4″	4.24 (dd, 9.5, 9.5)	4.34 (dd, 9.5, 9.5)	4.30 (dd, 9.5, 9.5)
H-5″	4.69 (dt, 9.5, 6.0)	4.80 (dt, 9.5, 6.0)	4.74 (dt, 9.5, 6.0)
H-6″	1.57 (d, 6.0)	1.71 (d, 6.0)	1.60 (d, 6.0)
Terminal glucose (1→3)			
H-1‴	5.11 (d, 8.0)	5.14 (d, 8.0)	
H-2‴	3.92 (dd, 8.7, 8.0)	4.05 (dd, 8.7, 8.0)	
H-3‴	4.16 (dd, 8.7, 8.7)	4.21 (dd, 9.0, 8.7)	
H-4‴	4.09 (dd, 9.8, 8.7)	4.13 (dd, 9.5, 9.0)	
H-5‴	3.90 (ddd, 9.8, 5.0, 2.0)	4.06 (ddd, 9.5, 5.5, 2.5)	
H-6‴	4.22 (dd, 11.5, 5.0)	4.29 (dd, 11.2, 5.5)	
	4.44 (dd, 11.5, 2.0)	4.58 (dd, 11.2, 2.5)	

TABLE II. ^{13}C-NMR Spectral Data of Aglycone Moieties of Compounds **1**—**4** (in Pyridine-d_5, 150 MHz, δ-Values)

	1	2	3	4
C-1	39.0	39.0	38.8	38.9
C-2	26.9	26.8	26.9	26.8
C-3	88.5	88.6	88.5	87.9
C-4	39.6	39.6	39.7	39.6
C-5	56.3	56.2	56.3	56.3
C-6	18.3	18.2	18.2	18.3
C-7	36.0	36.0	36.1	36.0
C-8	37.7	37.7	37.6	37.5
C-9	52.6	52.5	52.6	53.0
C-10	37.1	37.1	37.1	37.3
C-11	21.4	21.4	21.3	21.7
C-12	27.5	27.5	27.4	28.5
C-13	37.3	37.3	37.9	37.1
C-14	56.7	56.4	56.6	53.8
C-15	39.0	38.9	39.0	36.9
C-16	118.3	118.2	119.7	110.6
C-17	62.5	62.5	61.4	54.0
C-18	18.9	18.8	18.7	18.9
C-19	16.5	16.5	16.4	16.4
C-20	75.6	75.8	87.4	68.5
C-21	24.1	24.1	21.3	30.1
C-22	94.3	94.3	94.5	45.5
C-23	28.7	28.7	36.3	68.6
C-24	122.4	122.4	85.8	127.1
C-25	132.5	132.5	70.7	134.2
C-26	25.8	25.8	26.9	25.6
C-27	17.9	17.9	27.0	18.3
C-28	27.9	27.9	28.0	27.9
C-29	16.8	16.9	17.0	16.6
C-30	65.7	65.7	65.5	65.7

TABLE III. ^{13}C-NMR Spectral Data of Sugar Moieties of Compounds **1**—**3** (in Pyridine-d_5, 150 MHz, δ-Values)

	1	2	3
Inner galactose or glucose			
3-*O*-C-1′	105.6	105.0	105.8
2′	74.8	76.8	76.0
3′	85.4	89.6	76.9
4′	70.3	69.9	71.0
5′	76.4	77.9	76.7
6′	62.4	62.3	62.2
Terminal rhamnose (1→2)			
C-1″	101.9	101.6	101.7
2″	72.5	72.6	72.6
3″	72.5	72.5	72.5
4″	74.0	73.9	74.2
5″	69.7	69.8	69.5
6″	18.6	18.6	18.6
Terminal glucose (1→3)			
C-1‴	105.4	103.9	
2‴	75.0	75.2	
3‴	78.4	78.4	
4‴	71.5	71.4	
5‴	78.5	78.7	
6‴	62.5	62.3	

TABLE IV. Selected Cross-Peaks from the HMBC and NOESY Experiments for Compounds **1**—**3** (in Pyridine-d_5, 600 MHz, δ-Values)

	HMBC	NOESY
1	H-13–C-14, C-17, C-20, C-30	H-13–H-15β, H-18
	H-15–C-13, C-14, C-16, C-17, C-30	H-17–H-12α, H-21, H-30α
	H-17–C-14, C-16	H-22–H-17, H-21, H-23
	H-21–C-22	
	H-22–C-20, C-24	
	H-23–C-22, C-24, C-25	
	H-24–C-26, C-27	
	H-26–C-24, C-27	
	H-27–C-24, C-26	
	H-1′(gal)–C-3, H-3–C-1′(gal)	H-3–H-1′(gal)
	H-1″(rha)–C-2′(gal), H-2′(gal)–C-1″(rha)	H-2′(gal)–H-1″(rha)
	H-1‴(glc)–C-3′(gal)	H-3′(gal)–H-1‴(glc)
2	H-1′(glc)–C-3	H-3–H-1′(glc)
	H-1‴(glc)–C-3′(glc)	H-2′(glc)–H-1″(rha)
	H-3′(glc)–C-1‴(glc)	
3	H-15–C-30	H-12α–H30α
	H-17–C-16	H-13–H-15β, H-18
	H-21–C-17, C-20, C-22	H-15β–H-18
	H-24–C-25, C-26, C-27	H-17–H-12α, H-21, H-22, H-30α
	H-26–C-24, C-25	H-21–H-24
	H-27–C-24, C-25	H-22–H-17, H-21, H-23α
	H-30–C-13	
	H-1″(gal)–C-3, H-3–C-1′(gal)	H-3–H-1′(gal)
	H-1′(rha)–C-2′(gal), H-2′(gal)–C-1″(rha)	H-2′(gal)–H-1″(rha)

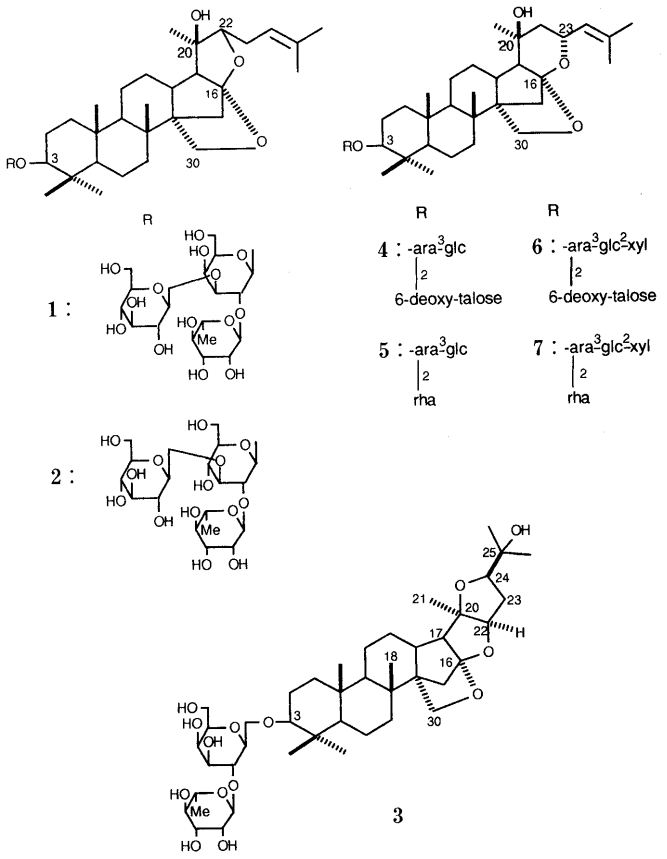

Chart 1

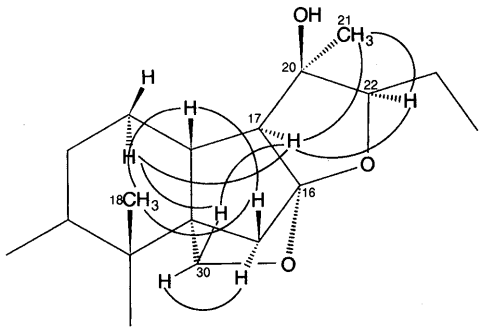

Fig. 1. Cross Peaks from the NOESY Experiment for Compound **1**

double bond, a ketal carbon, and the carbons bearing oxygen functions, respectively. By comparison of the ^{13}C-NMR spectra of **1** and **4**, the signals due to the aglycone of **1** were in good agreement with those of **4**, except for the D ring and the side chain (C-20–27). The ^{1}H–^{1}H correlation spectroscopy (COSY) and homonuclear Hartman–Hahn (HOHAHA) experiment of **1** revealed an isolated spin system[H-22–23–24–26(27)]. In the ^{1}H–^{1}H COSY spectrum of **1**, the methine proton at δ4.47 (dd, J=6.6, 3.2 Hz) was coupled with methylene protons at δ2.58 (ddd, J=12.0, 5.6, 3.2 Hz) and 2.81 (ddd, J=12.0, 6.6, 5.6 Hz), which were connected with a vinyl proton at δ5.47 (t, J=5.6 Hz). The vinyl proton at δ5.47 also showed a long-range coupling with two vinyl methyls at δ1.55 and 1.62. The methine proton at δ4.47 had a correlation to the carbon signal at δ94.3 in the ^{1}H–^{13}C COSY experiment, indicating the existence of other linkage between C-22 and C-16. The gross structure of the aglycone of **1** was elucidated by analysis of NMR data including ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments (Table IV). The cross peaks among H-13–H-15β, H-18 and among H-17–H-12α, H-21, H-30α, and among H-22–H-17, H-21, H-23 in the NOESY experiment indicated the absolute configurations at C-20 and 22 to be 20*R* and 22*R*, respectively. Accordingly, the aglycone of **1** was represented as (20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol. The sugar sequence at C-3 was determined by NMR experiments. A combination of the

^1H–^1H, ^1H–^{13}C COSY, HMBC, NOESY and HOHAHA experiments allowed a full assignment of the sugar moieties (Tables I, III and IV). The HMBC spectrum of **1** showed long-range correlations between H-1′ of galactose (gal) and C-3 of aglycone, H-1″ of rhamnose (rha) and C-2′ of gal, H-1‴ of glucose (glc) and C-3′ of gal, respectively. Reverse correlations [H-3–C′-1(gal), H-2′(gal)–C-1″(rha)] were also observed. Moreover, NOESY correlations were thus found between H-3–H-1′(gal), H-2′(gal)–H-1″(rha), H-3′(gal)–H-1‴(glc). Based on these findings and examination of NMR data, **1** was characterized as (20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-galactopyranoside.

The negative FAB-MS of jujubasaponin V (**2**), amorphous powder, $[\alpha]_D$ −14.2° (MeOH), exhibited the same quasi-molecular ion peak at *m/z* 941 $[M-H]^-$ together with peaks at *m/z* 779, 633, as in **1**. Acid hydrolysis of **2** provided D-glucose and L-rhamnose in a ratio of 2:1 as sugar components. The ^{13}C-NMR spectrum of **2** was almost superimposable on that of **1**, except for the sugar moieties. These data suggested **2** had the same aglycone as **1** and the galactopyranosyl unit in **1** was replaced by a β-D-glucopyranosyl unit in **2**. The sugar linkages at C-3 were confirmed by HMBC and NOESY experiments in the same way as **1**. The anomeric protons at δ 4.86 (inner glc) and 5.14 (terminal glc) showed long-range correlations with the ^{13}C signals at δ 88.6 (C-3) and 89.6 (C-3′ of glc), respectively in the HMBC spectrum. In addition, there were NOESY correlations between H-3–H-1′(glc), H-2′(glc)–H-1″(rha), H-3′(glc)–H-1‴(glc). Consequently, **2** was characterized as (20*R*,22*R*)-16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside.

Jujubasaponin VI (**3**), amorphous powder, $[\alpha]_D$ −28.1° (MeOH), provided D-galactose and L-rhamnose 1:1, upon acid hydrolysis. The negative FAB-MS of **3** exhibited quasi-molecular ion peaks at *m/z* 795 $[M-H]^-$, together with *m/z* 649 $[M-H-\text{deoxyhexosyl}]^-$, disclosing the molecular formula $C_{42}H_{68}O_{14}$. The ^1H-NMR spectrum of **3** showed the presence of the seven tertiary methyl groups, one β-galactopyranosyl [δ 4.85 (d, J=7.5 Hz) and α-rhamnopyranosyl unit (δ 6.50 s), whereas, a vinyl proton was not observed. The ^{13}C-NMR spectrum showed 12 signals due to two hexosyl moieties and 30 signals due to an aglycone moiety. The DEPT experiment disclosed that the aglycone of **3** consisted of seven methyls, nine methylenes, seven methines and seven quaternary carbons including one oxygen-bearing methylene (δ 65.5), three oxygen-bearing methines (δ 85.8, 88.5 and 94.5) and two oxygen-bearing quaternary carbons (δ 70.7, 87.4) and a ketal carbon (δ 119.7), respectively. In comparing of the ^{13}C-NMR spectrum of the aglycone moiety of **3** with that of **1**, the signals due to A—D rings were analogous, indicating C-3 of **3** was glycosylated; but the signals due to the side chain were saliently different, including the disappearance of a double bond and the appearance of two oxygen-bearing carbons. The gross structure of the aglycone of **3** was elucidated by analysis of NMR data including ^1H–^1H, ^1H–^{13}C COSY, HMBC, NOESY and HOHAHA experiments in the same way as **1**. The constructed structure of the aglycone of **3** was trevoagenin D isolated from the same family, *Trevoa trinervis* by Betancor *et al.*, the structure and stereochemistry of which were established by X-ray analysis.[4] Although we attempted to get an aglycone of **3** with enzymatic hydrolysis and/or mild acid hydrolysis, those hydrolyses failed. The HMBC and NOESY experiments of **3** led to the determination of the sugar sequence at the C-3 position. The long-range correlations between H-1′(gal) and C-3, and between H-1″(rha) and C-2′(gal) confirmed the sugar sequence (3-*O*-gal²rha) (Table IV). Accordingly, **3** was characterized as trevoagenin D 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside. It is notable that **3** is the first example of a naturally occurring trevoagenin D glycoside.

Application of a 1 mM solution of each of **1**—**3**, **6** and **7** led to the complete suppression of the sweet taste of 0.2 M sucrose. It corresponds to half of the activity of acylsaponins, jujubasaponins II, III and ziziphin. Compounds **4** and **5** suppressed the sweet taste of 0.1 M sucrose.

Kurihara *et al.* have reported ziziphin as the only antisweet substance in the leaves of *Z. jujuba*.[5] But, in our studies, all of the compounds obtained showed antisweet activities, though their potency was not as strong as ziziphin.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a Varian Unity 600 spectrometer in a C_5D_5N solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^1H–^1H-COSY, ^1H–^{13}C-COSY, NOESY, DEPT, HMBC and HOHAHA, Coupling constants (*J* values) are given in hertz (Hz). The FAB-MS was measured on a JEOL JMS-PX303 mass spectrometer. Kiesel gel 60 (230—400 mesh, Merck) and Silica gel 60F-254 (Merck) were used for column chromatography and for TLC, respectively. HPLC was carried out with a Waters ALC/GPC 244 instrument.

Isolation of Saponins The plant material was collected in Tokushima prefecture in October 1990. The fresh leaves (10 kg) of *Zizyphus jujuba* were extracted with 60% EtOH at room temperature for 2 weeks. After concentration *in vacuo*, the EtOH extract was partitioned with AcOEt and *n*-BuOH. The active *n*-BuOH layer was chromatographed on a silica gel column with CH_2Cl_2–MeOH–H_2O [25:6:0.5—65:35:10 (lower layer)] to give four fractions, frs. 1—4 in order of elution. Fraction 3 was passed through a Sephadex LH-20 column to give three fractions, frs. 3-1—3-3. Fraction 3-1 was repeatedly purified by HPLC (YMC, C_8, 22% CH_3CN and Nomura, ODS, 25% CH_3CN) to yield **3** (65 mg). Fractions 3-2 and 3-3 were also subjected to HPLC (Nomura, ODS, 26—29% CH_3CN) to isolate **1** (50 mg), **2** (45 mg), **4** (35 mg) and **6** (50 mg) from fr. 3-2, and **1** (60 mg), **5** (65 mg) and **7** (50 mg) from fr. 3-3.

Jujubasaponin IV (1) Amorphous powder, mp 185—187 °C, $[\alpha]_D$ −3.64° (*c*=5.0, MeOH). Negative FAB-MS *m/z*: 941 $[(M-H)^-]$. *Anal.* Calcd for $C_{48}H_{78}O_{18}\cdot 3H_2O$: C, 57.82; H, 8.49. Found: C, 57.56; H, 8.31. ^1H-NMR δ: 0.65 (C_{19}-H), 0.97 (C_{18}-H), 1.10 (C_{29}-H), 1.19 (C_{28}-H), 1.42 (C_{21}-H), 1.55 (C_{27}-H), 1.62 (C_{26}-H), *ca.* 1.73, 2.11 (each, d, *J*=8.5 Hz, H-15), 2.10 (d, *J*=6.1 Hz, H-17), 2.66 (m, H-13), 2.58 (ddd, *J*=12.0, 5.6, 3.2 Hz, H-23), 2.81 (ddd, *J*=12.0, 6.6, 5.6 Hz, H-23), 3.18 (dd, *J*=12.0, 5.5 Hz, H-3), 4.13, *ca.* 4.18 (each, d, *J*=7.7 Hz, H-30), 4.47 (dd, *J*=6.6, 3.2 Hz, H-22), 5.47 (br t, *J*=5.6 Hz, H-24). For other NMR data, see Tables I, II, III and IV.

Jujubasaponin V (2) Amorphous powder, mp 210—212 °C, $[\alpha]_D$ −14.2° (*c*=4.3, MeOH). Negative FAB-MS *m/z*: 941 $[(M-H)^-]$. *Anal.* Calcd for $C_{48}H_{78}O_{18}\cdot 3/2H_2O$: C, 59.43; H, 8.42. Found: C, 59.27; H, 8.69. ^1H-NMR δ: 0.70 (C_{19}-H), 1.02 (C_{18}-H), 1.14 (C_{29}-H), 1.22 (C_{28}-H), 1.46 (C_{21}-H), 1.60 (C_{27}-H), 1.67 (C_{26}-H), *ca.* 1.76, 2.14 (each, d *J*=8.5 Hz, H-15), 2.12 (d, *J*=6.1 Hz, H-17), 2.68 (m, H-13), 2.60 (ddd, *J*=12.0, 5.6, 3.2 Hz, H-23), 2.83 (ddd, *J*=12.0, 6.6, 5.6 Hz, H-23), 3.33 (dd, *J*=12.0, 5.5 Hz, H-3), 4.18, *ca.* 4.25 (each, d, *J*=7.7 Hz, H-30), 4.50 (dd, *J*=6.6, 3.2 Hz, H-22), 5.50 (br t, *J*=5.6 Hz, H-24). For other NMR

data, see Tables I, II, III and IV.

Jujubasaponin VI (3) Amorphous powder, mp 199—201 °C, $[\alpha]_D$ −28.1° (c=4.5, MeOH). Negative FAB-MS m/z: 795 $[(M-H)^-]$. *Anal.* Calcd for $C_{42}H_{68}O_{14}\cdot 3H_2O$: C, 59.28; H, 8.76. Found: C, 59.54; H, 9.01. ^{1}H-NMR δ: 0.71 (C_{19}-H), 0.96 (C_{18}-H), 1.13 (C_{29}-H), 1.21 (C_{28}-H), 1.30 (C_{21}-H), 1.47, 1.50 (C_{26} and C_{27}-H), *ca.* 1.61, 2.22 (each, d, J=8.8 Hz, H-15), 1.96 (d, J=6.1 Hz, H-17), 2.56 (m, H-13), 2.24 (ddd, J=14.0, 3.0, 3.0 Hz, H-23), 2.30 (ddd, J=14.0, 7.0, 7.0 Hz, H-23), 3.28 (dd, J=12.0, 5.5 Hz, H-3), 4.11, 4.14 (each, d, J=7.5 Hz, H-30), 4.09 (dd, J=7.0, 3.0 Hz, H-24), 4.91 (dd, J=7.0, 3.0 Hz, H-22). For other NMR data, see Tables I, II, III and IV.

Zizyphus Saponin I (4) Colorless needles from MeOH, mp 273—275 °C, $[\alpha]_D$ −39.3° (c=2.7, MeOH). Negative FAB-MS m/z: 911 $[(M-H)^-]$.

Zizyphus Saponin II (5) Colorless needles from MeOH, mp 288—290 °C, $[\alpha]_D$ ±0° (c=5.8, pyridine). Negative FAB-MS m/z: 911 $[(M-H)^-]$.

Zizyphus Saponin III (6) Amorphous powder, mp 205—207 °C, $[\alpha]_D$ −35.3° (c=5.1, MeOH). Negative FAB-MS m/z: 1043 $[(M-H)^-]$.

Jujuboside B (7) Colorless needles from MeOH, mp 223—225 °C, $[\alpha]_D$ −32.5° (c=4.3, MeOH). Negative FAB-MS m/z: 1043 $[(M-H)^-]$.

Acid Hydrolysis of Jujubasaponins IV—VI (1—3) Each solution of **1—3** (3—4 mg) in 5% H_2SO_4 was heated at 100 °C for 3 h. The reaction mixture was diluted with H_2O, neutralized with Amberlite IR-45 and evaporated *in vacuo* to dryness. The residue was checked by using refraction index detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 4.8 mm i.d. × 15 cm, 75% CH_3CN, 1 ml/min, 70 °C) by comparison with authentic sugars as standards.[6] These sugar peaks were as follows. t_R: L(−)-rhamnose, 4.8 min; D(+)-glucose, 7.38 min; D(+)-galactose, 8.0 min.

Bioassay of Antisweet Activity The antisweet activity of 1 mM solutions of **1—7** was tested on three volunteers. Each participant held the test solutions in the mouth for 3 min, spat, rinsed with distilled water and tasted sucrose solutions (0.1 and 0.2 M).

References

1) K. Yoshikawa, M. Nakagawa, R. Yamamoto, S. Arihara and K. Matsuura, *Chem. Pharm. Bull.*, **40**, 1779 (1992).
2) K. Yoshikawa, N. Shimono and S. Arihara, *Tetrahedron Lett.*, **32**, 7059 (1991).
3) N. Okamura, T. Nohara, A. Yagi and I. Nishioka, *Chem. Pharm. Bull.*, **29**, 676 (1981).
4) C. Betancor, M. Cortés, R. Hernández and E. Suárez, *Tetrahedron Lett.*, **23**, 1125 (1982).
5) Y. Kurihara, K. Ookubo, H. Tasaki, H. Kodama, Y. Akiyama, A. Yagi and B. Halpern, *Tetrahedron*, **44**, 61 (1988).
6) D. K. Lloyd, D. M. Goodall and H. Serivener, *Anal. Chem.*, **61**, 1238 (1989).