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Studies on the Constituents of Zizyphi Fructus. III.[1] Structures of Dammarane-type Saponins

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Zizyphus saponins I, II, III and jujuboside B were isolated from Zizyphi Fructus. On Smith-de Mayo degradation, zizyphus saponins I, II and III yielded jujubogenin as a genuine aglycone. The configuration of the sugar linkages in each saponin was determined on the basis of PMR and CMR examinations and application of Klyne's rule on molecular rotation, and the structures of zizyphus saponins I, II and III were established as (I), (II) and (III), respectively. 6-Deoxy-L-talose was isolated as a sugar component of zizyphus saponins I and III.

Keywords——*Zizyphus jujuba*; Rhamnaceae; zizyphus saponin I; zizyphus saponin II; zizyphus saponin III; jujuboside B; jujubogenin; 6-deoxy-L-talose

As part of our studies on pharmacological active principles in Zizyphi Fructus, the previous paper described the isolation of eleven pentacyclic triterpenoids.[1] Further examination provided four dammarane-type saponins, zizyphus saponins I, II, III and jujuboside B (compound I). This paper deals with the structure elucidation of these saponins.

The dried fruits were treated as described in the experimental sectoin to yield zizyphus saponins I, II and III, together with compound I. On acid hydrolysis, saponins I, II III and compound I yielded ebelin lactone[3] (IV'). However, since the infrared (IR) and the ultraviolet (UV) spectra of each saponin showed no evidence for the presence of a lactone ring and conjugated double bond, ebelin lactone must be an artifact produced by acid treatment. On Smith-de Mayo degradation[4] (which was performed twice), saponins I, II, III and compound I afforded jujubogenin (IV)[5] as a genuine sapogenin which was almost quantitatively converted into ebelin lactone on treatment with sulfuric acid. Thus, it was demonstrated that jujubogenin is a genuine aglycone of zizyphus saponins I, II, III and compound I.

Zizyphus saponin I, mp 269—272°, $[\alpha]_D^{18}$ −48.5° (c=1.00, MeOH), $C_{47}H_{76}O_{17}\cdot H_2O$ (I), provided L-arabinose, D-glucose and an unidentified sugar (V) as sugar moieties in the molar ratio of 1:1:1. The unidentified sugar was analyzed as follows. In the mass (MS) spectrum, the methyl glycoside triacetate (Va) of V gave fragment ion peaks at m/e 305 ($M^+ +1$) and 273. In the proton nuclear magnetic resonance (PMR) spectrum, signals due to a methyl group and an anomeric proton of Va appeared at δ 1.23 (3H, d, J=6.5 Hz) and 4.73 (1H, br.s), respectively suggesting V to be a 6-deoxyhexose. The above results and comparative analysis of the physical data for the 6-deoxyhexose led to the conclusion that Va is methyl 2,3,4-tri-O-acetyl-6-deoxy-α-L-talopyranoside. Authentic methyl 2,3,4-tri-O-acetyl-6-deoxy-α-L-talopyranoside was synthesized from L-rhamnose according to the literature[6], and was found to be identical with Va. Thus, V was determined to be 6-deoxy-L-talose.

Per-O-methyl derivatives (Ia) of I prepared by Hakomori's method[7] exhibited fragment ion peaks due to terminal permethylated glucose (m/e 219 and 187) and 6-deoxytalose (m/e 189 and 157) residues in the mass spectrum, and the PMR signals of three anomeric protons appeared at δ 4.30 (1H, d, J=5.5 Hz), 4.37 (1H, d, J=7.0 Hz) and 5.34 (1H, br.s).

Comparative studies on the carbon 13 nuclear magnetic resonance (CMR) (Table I), the molecular rotation difference (Table II) and the PMR of Ia indicated that the L-arabinose possesses an α-linkage and D-glucose a β-linkage in the $^4C_1$ conformation. 6-Deoxy-L-talose

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was determined to have an α-linkage in $^1C_4$ conformation by comparison of the direct bonded C–H coupling constant in the C-1 signal with that of methyl 6-deoxy-α-L-talopyranoside.

On methanolysis, zizyphus saponin I permethyl ether (Ia) yielded methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-6-deoxy-L-talopyranoside and methyl 4-mono-O-methyl-L-arabinopyranoside, which were identified by comparison with authentic samples by means of gas chromatography (GLC).

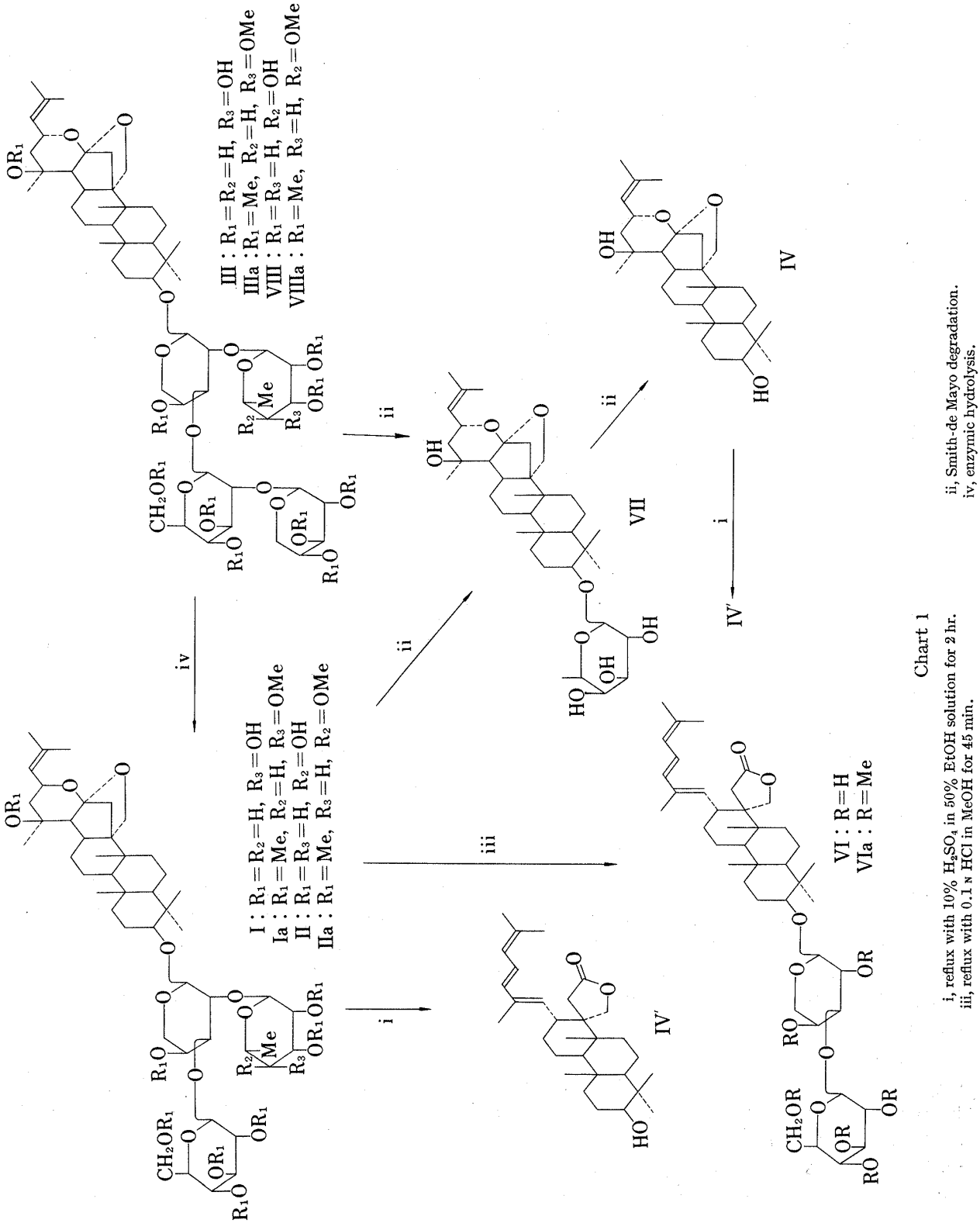

Chart 1

i, reflux with 10% H_2SO_4 in 50% EtOH solution for 2 hr.
ii, Smith-de Mayo degradation.
iii, reflux with 0.1 N HCl in MeOH for 45 min.
iv, enzymic hydrolysis.

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Table I. CMR Spectral Data

Compd.	Jujubogenin[a]	Jujubogenin 3-O-α-L-arabinoside	Compd. II[b]	Z. saponin I	Z. saponin III	Z. saponin II	Compd. I	Methyl 6-deoxy-α-L-talopyranoside
C-3	78.0	88.2	88.2	87.7	87.8	87.8	88.0	
20	68.6	68.3	68.3	68.3	68.3	68.3	68.3	
21	30.0	30.0	29.9	29.9	29.9	30.0	30.0	
22	45.2	45.3	45.3	45.5	45.2	45.3	45.3	
Arabinose								
C-1		107.1	106.9	105.0	104.3	104.4	103.8	
($^1J_{C1-H1}$)				(158.8)	(157.4)	(155.9)		
2		72.6	71.6	74.5	74.2	74.6	74.6	
3		74.3	83.8	83.6	82.1	82.1	83.2[c]	
4		69.2	68.9	68.3	67.4	68.3	67.5	
5		66.4	66.6	65.7	64.5	64.7	63.1	
Glucose								
C-1			105.9	105.0	103.1	104.4	103.4	
($^1J_{C1-H1}$)				(158.8)	(158.8)	(155.9)		
2			75.3	74.5	82.1	74.6	81.9[c]	
3			78.0	78.2	77.9	78.0	77.8	
4			71.2	71.1	71.0	71.2	71.0	
5			78.2	78.2	78.2	78.2	78.2	
6			62.4	62.4	62.2	62.3	61.5	
6-Deoxy-talose or Rhamnose								
C-1				101.6	101.5	101.6	101.2	102.8
($^1J_{C1-H1}$)				(173.5)	(173.5)	(173.5)		(167.7)
2				71.7	71.7	72.2	72.1	71.6
3				67.5	67.4	72.2	72.1	67.3
4				73.6	74.0	73.6	73.6	73.7
5				67.0	66.8	69.7	69.7	67.0
6				17.2	17.2	18.5	18.4	17.3
Xylose								
C-1					105.5		106.0	
($^1J_{C1-H1}$)					(163.2)			
2					75.6		75.9	
3					78.2		78.0	
4					70.6		70.6	
5					67.4		67.5	

a) See the literature[11]
b) Compound II: jujubogenin 3-O-β-D-glucopyranosyl(1→3)-α-L-arabinopyranoside, isolated from Zizyphus flowers. Mp 292—294°(MeOH), $[\alpha]_D^{15}$ −16.9° (c=0.86, MeOH). $C_{41}H_{66}O_{13}\cdot 1.5H_2O$.
c) Assignments may be reversed.

Prosapogenin A (VI) obtained by partial hydrolysis of zizyphus saponin I was methylated by Hakomori's method to give prosapogenin A permethyl ether (VIa). Methanolysis of VIa yielded methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,4-di-O-methyl-L-arabinopyranoside, which were identified by comparison with authentic samples by means of GLC. Consequently, prosapogenin A is represented by the structure (VI) and zizyphus saponin I is by the structure (I).

Zizyphus saponin II, mp 268—269°, $[\alpha]_D^{14}$ −43.3° (c=0.67, MeOH), $C_{47}H_{76}O_{17}\cdot H_2O$ (II), provided L-arabinose, D-glucose and L-rhamnose as sugar moieties in the molar ratio of 1 : 1 : 1. The per-O-methyl derivative (IIa) of II prepared by Hakomori's method exhibited fragment ion peaks due to terminal permethylated glucose (m/e 219 and 187) and rhamnose (m/e 189 and 157) residues in the mass spectrum, and in the PMR spectrum signals due to three anomeric protons appeared at δ 4.39 (1H, d, J=5.5 Hz), 4.40 (1H, d, J=7.0 Hz) and 5.22 (1H, br.s).

Comparative studies on the CMR (Table I), the molecular rotation difference (Table II)

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Table II. Molecular Rotation Differences [M]D in MeOH

Compound	[α]D(°)	[M]D(°)	Δ[M]D(°)	
Jujubogenin[a]	−36.0	−169.9		
			+ 24.9	
Jujubogenin 3-O-α-L-arabinoside	−24.0	−145.0		
			+ 15.5	
Compound II	−16.9	−129.5		
			−312.8	
Zizyphus saponin I	−48.5	−442.3		−265.4
			− 43.2	
Zizyphus saponin II	−43.3	−394.9		
				− 77.0
Zizyphus saponin III	−46.5	−485.5		
Compound I	−45.2	−471.9		

The following [M]D values were used to determine the configurations of glycosidic linkages.[b] Methyl pyranosides of α-L-arabinose +17.3°; β-L-arabinose +245°; α-L-rhamnose −111°; β-L-rhamnose +170°; α-D-glucose +309°; β-D-glucose −66°; α-D-xylose +252°; β-D-xylose −108°; 6-deoxy-α-L-talose −209°.

a) See the literature[9]

b) W. Klyne, *Biochem. J.*, **47**, xli (1950).

and the PMR of IIa indicate that the L-arabinose possesses an α-linkage and D-glucose a β-linkage in the $^4C_1$ conformation. L-Rhamnose was demonstrated to have an α-linkage in the $^1C_4$ conformation by comparison of the direct bonded C–H coupling constant of the C-1 signal with those of methyl α and β-L-rhamnopyranosides.[8]

On methanolysis, zizyphus saponin II permethyl ether (IIa) yielded methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 4-mono-O-methyl-L-arabinopyranoside, which were identified by comparison with authentic samples by means of GLC. Furthermore, prosapogenin obtained by partial hydrolysis of zizyphus saponin II was identical with prosapogenin A (VI). The above findings indicated zizyphus saponin II to be prosapogenin III obtained as an enzymic hydrolysate of *Zizyphus jujuba* var. *spinosus*.[9] The CMR spectrum of zizyphus saponin II was superimposable on that of prosapogenin III. Therefore, zizyphus saponin II is represented by the structure (II).

Zizyphus saponin III, mp 229—233°, $[\alpha]_D^{28}$ −46.5° (c=0.98, MeOH), $C_{52}H_{84}O_{21}\cdot 2.5H_2O$ (III), provided L-arabinose, D-glucose, 6-deoxy-L-talose and D-xylose as sugar moieties in the molar ratio of 1:1:1:1. The enzymic hydrolysis of III with crude hesperidinase yielded D-xylose and zizyphus saponin I. Comparison of the CMR spectral data of III with those of zizyphus saponin I revealed that the signals due to C-2 and C-1 of the glucose moiety were shifted by +7.6 ppm and −1.9 ppm, respectively.

Therefore, it is suggested that β-D-xylose is linked at C-2 of D-glucose in III.[10] The per-O-methyl derivative (IIIa) of III prepared by Hakomori's method exhibited fragment ion peaks due to terminal permethylated xylose (*m/e* 175 and 143), 6-deoxytalose (*m/e* 189 and 157) and a disaccharide consisting of xylose and glucose (*m/e* 379 and 347) residues in the mass spectrum, and in the PMR spectrum four anomeric protons appeared at δ 4.36 (1H, d, J=7.5 Hz), 4.61 (1H, d, J=7.0 Hz), 4.69 (1H, d, J=6 Hz) and 5.36 (1H, br.s,).

Comparative studies on the CMR (Table I), the molecular rotation difference (Table II) and the PMR of IIIa indicate that D-glucose, D-xylose and L-arabinose possess β, β and α-linkages in $^4C_1$ conformation, respectively. 6-Deoxy-L-talose was determined to have an α-linkage in $^1C_4$ conformation by comparison of the direct bonded C–H coupling constant in the C-1 signal with that of methyl 6-deoxy-α-L-talopyranoside.[8] On methanolysis, zizyphus saponin III permethyl ether (IIIa) yielded methyl 3,4,6-tri-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-6-deoxy-L-talopyranoside, methyl 2,3,4-tri-O-methyl-D-xylopyranoside and 4-mono-O-methyl-L-arabinopyranoside, which were identified by comparison with authentic samples by means of GLC.

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On the basis of the above results, zizyphus saponin III is represented by the structure (III).

Compound I, mp 256—259°, $[\alpha]_D^{21}$ −45.2° (c=1.00, MeOH), $C_{52}H_{84}O_{21}\cdot 2H_2O$ (VIII), provided L-arabinose, D-glucose, L-rhamnose and D-xylose as sugar moieties in the molar ratio of 1:1:1:1. The enzymic hydrolysis of VIII yielded D-xylose and zizyphus saponin II. Comparison of the CMR spectral data of VIII with those of II revealed that the signals due to C-2 and C-1 of the glucose moiety were shifted by +7.3 ppm and −1.0 ppm, respectively. Therefore, it is suggested that β-D-xylose is linked at C-2 of D-glucose in VIII. The above findings indicate compound I to be identical with jujuboside B which was isolated from *Zizyphus jujuba* var. *spinosus*.[9] The CMR spectrum of VIII was superimposable on that of jujuboside B.[11] Consequently, compound I is represented by the structure (VIII).

The application of CMR spectroscopy to determine sugar linkage positions in saponin has been investigated. Data on glycosylation shifts provided valuable evidence for the structure elucidation of zizyphus saponins. The results obtained from our CMR studies are in good agreement with those of an earlier report.[9] 6-Deoxy-L-talose which was isolated as a sugar component in zizyphus saponins I and III, had been identified as a sugar component in a cardiac glycoside, sarmentoside A,[12] and in glycolipid produced by *Mycobacterium avium*[13] and *M. marianum*.[14] The existence of 6-deoxy-L-talose is the first reported example of the presence of this sugar in a saponin. Pharmacological studies on the saponins are in progress.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. IR spectra were obtained with a KOKEN DS-301 machine and UV spectra (in MeOH) were recorded with a Hitachi ESP-3T automatic recording spectrophotometer. NMR spectra were taken with a JEOL PS-100H spectrometer at 100 MHz in $CDCl_3$ (^1H) and a JEOL FX-100 spectrometer at 25.15 MHz in pyridine-d_5 (^{13}C); chemical shifts are given in δ (ppm) scale with tetramethylsilane as an internal standard and coupling constants (J) in Hz. The abbreviations used to describe the splittings are as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 4.5—6.7 kV and an ionizing potential of 75 eV. Field desorption (FD) mass spectra were recorded on a JEOL D300 machine with an emitter heating current of 21—24 mA. GLC was run on a Shimadzu GC-48M with a flame ionization detector using a glass column (2×3 mm) packed with 5% 1,4-butanediol succinate on Shimalite W (60—80 mesh): a) column temperature, 166°; N_2 65 ml/min; b) column temperature, 179°; N_2 40 ml/min. Thin-layer chromatography (TLC) of sugars was conducted on Avicel SF (Funakoshi) with the upper layer of *n*-BuOH–pyridine–H_2O (6:2:3)+pyridine (1) as the solvent and aniline hydrogenphthalate as the spraying reagent. TLC of saponin and sapogenin was performed on Kieselgel G (Merck) and detection was done by spraying diluted H_2SO_4 reagent followed by heating. Column chromatography was carried out with Kieselgel (0.05—0.5 mesh, Merck) and Sephadex LH-20 (25—100 μ) (Pharmacia Fine Chemicals). The ratios of solvent and reagent are given in v/v.

Isolation of Saponins——The dried commercial fruits (50 kg) of *Zizyphus jujuba* were extracted three times with boiling EtOH for 2 hr each. The EtOH extract (25.2 kg) suspended in H_2O was applied to an Amberlite XAD-2 column and eluted with H_2O, EtOH–H_2O (1:1) and $CHCl_3$–MeOH (2:1) successively. The $CHCl_3$–MeOH fraction (370 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (7:3:0.5)] to give the fraction containing saponins (21 g). This fraction was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (10:2:0.1)] to give fraction A (4.84 g) and B (2.94 g). Fraction A (4.84 g) was subjected to repeated chromatography [$CHCl_3$–MeOH–H_2O (10:2:0.1)] to give zizyphus saponin I (50 mg) and II (100 mg). Fraction B (2.94 g) was subjected to droplet counter current chromatography (DCC) [$CHCl_3$–MeOH–H_2O (5:6:4)] by the ascending method to separate the fraction of zizyphus saponin III from the fraction of compound I. The fraction of zizyphus saponin III was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (10:2:0.2)] to afford pure zizyphus saponin III (28 mg) as a colorless powder. The fraction of compound I was recrystallized from MeOH to give colorless compound I (20 mg).

Zizyphus Saponin I (I)——Colorless needles, mp 269—272° (MeOH), $[\alpha]_D^{18}$ −48.5° (c=1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400. FD-MS m/e: 935 $(M+Na)^+$, *Anal.* Calcd for $C_{47}H_{76}O_{17}\cdot H_2O$: C, 60.62; H, 8.23. Found: C, 60.14; H, 8.44. A solution of I (100 mg) dissolved in EtOH (10 ml) and 20% H_2SO_4 (10 ml) was heated for 2 hr under reflux. The solution was diluted with water and extracted with ether to yield ebelin lactone (37.5 mg), $[\alpha]_D^{14.5}$ −20.0° (c=0.6, MeOH), UV λ_{max} nm (log ε): 270 (4.59), 279 (4.61), 288 (4.58). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1770, 1642, 1602, 1450. MS m/e: 454 (M^+). The aqueous layer neutralized with ion exchange resin

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Amberlite IRA 400 was evaporated to dryness *in vacuo* and the sugar portion was found to consist of glucose, arabinose and 6-deoxytalose (Avicel TLC). The sugar mixture was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.1)] and then over Sephadex LH-20 (MeOH) to give three kinds of sugars: colorless syrup (9.6 mg), *Rf* 0.32, $[\alpha]_D^{22}$ +53.0° (24 hr later) (c=0.96, H_2O) D-glucose; colorless needles (3 mg), *Rf* 0.35, $[\alpha]_D^{21}$ +112.2°→+100.2° (24 hr later) (c=0.4, H_2O), L-arabinose; colorless plates (5.1 mg), *Rf* 0.67, $[\alpha]_D^{21}$ −13.0°→−17.0° (24 hr later) (c=0.51, H_2O) 6-deoxy-L-talose.

Permethylate (Ia) of I——According to Hakomori's method, 50% NaH (250 mg) washed with *n*-hexane was added to dimethylsulfoxide (5 ml) and the reaction mixture was heated for 1 hr at 60°. A solution (4 ml) of methylsulfinylmethanide was added to a stirred solution of I (40 mg) in dimethylsulfoxide (2 ml), and the reaction mixture was stirred at room temperature for 1 hr. Methyl iodide (4 ml) was added and the reaction was continued for 1 hr. The reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with water and evaporated to dryness. The residue was chromatographed over silica gel [*n*-hexane–acetone (4: 1)] to give the nona-O-methyl derivative (Ia) as a colorless powder (16 mg), $[\alpha]_D^{15.5}$ −50.5° (c=0.7, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no hydroxyl group. MS *m/e*: 1024 $(M-CH_2)^+$, 553 (permethylated trisaccharide residue), 219 (terminal permethylated hexose residue), 189 (terminal permethylated methylpentose residue), 187 (219−MeOH), 157 (189−MeOH). PMR δ: 3.13 (3H, s, C_{20}–OMe), 4.30 (1H, d, J=5.5), 4.37 (1H, d, J=7.0), 5.34 (1H, br.s), FD-MS *m/e*: 1039 $(M+H)^+$, 219 (terminal permethylated hexose residue), 189 (terminal permethylated methylpentose residue). *Anal.* Calcd for $C_{56}H_{94}O_{17}$: C, 64.71; H, 9.12. Found: C, 64.99; H, 9.10.

Methanolysis of Ia——A solution of Ia (4 mg) dissolved in 10% HCl in MeOH (2 ml) was refluxed for 2 hr. The reaction mixture was neutralized with Ag_2CO_3, then the precipitate was filtered off and the filtrate was evaporated to dryness. The residue was subjected to GLC under the condition (a) to give the following methylated sugars. t_R (min): Methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose 2.7; 2,3,4-tri-O-methyl-6-deoxy-α-L-talose 2.9; 4-mono-O-methyl-β-L-arabinose 11.0; Ref. Methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose 3.7; 2-mono-O-methyl-β-L-arabinose 15.1; 3-mono-O-methyl-β-L-arabinose 11.0.

Partial Hydrolysis of I——A solution of I (100 mg) in 0.1 N HCl–MeOH (10 ml) was heated for 1 hr under reflux. After the reaction mixture has been neutralized with Ag_2CO_3, the precipitate was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed over silica gel [$CHCl_3$–MeOH (12: 1)] to give prosapogenin A, VI (22 mg),[9] ebelin lactone 3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinoside (IR ν_{max}^{KBr} cm^{-1}: 1770 MS *m/e*: 832 permethyl M^+) and methyl 6-deoxy-α-L-talopyranoside (12 mg). VI was hydrolyzed in the usual way to give ebelin lactone as an aglycone, and glucose and arabinose were detected as sugar moieties on Avicel TLC. Methyl 6-deoxy-α-L-talopyranoside (12 mg) dissolved in pyridine (0.3 ml) was acetylated with Ac_2O (0.3 ml) at room temperature overnight, then the reaction mixture was evaporated to dryness *in vacuo*. The product was chromatographed over silica gel [*n*-hexane–EtOAc (2: 1)] to give methyl 2,3,4-tri-O-acetyl-6-deoxy-α-L-talopyranoside as colorless plates (Va, 8 mg, petroleum ether).

Permethylate (VIa) of VI and Methanolysis of VIa——The methylation product of VI (8 mg) was worked up in the same way as for I to give the per-O-methyl derivative (VIa, 1.5 mg). MS *m/e*: 832 (M^+), 454 and 219 (terminal permethylated hexose residue). VIa (1 mg) was refluxed with 10% HCl in MeOH (0.5 ml) for 2 hr and the reaction mixture was worked up in the same way as for Ia. The residue was examined by GLC under the condition (a) to give the following methylated sugars. t_R (min): Methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose 2.7; 2,4-di-O-methyl-β-L-arabinose 5.7. Ref. Methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose 3.7; 3,4-di-O-methyl-β-L-arabinose 5.2; 2,3-di-O-methyl-β-L-arabinose 4.8.

Smith-de Mayo Degradation of I——Sodium metaperiodate (2 g) was added to a solution of I (1 g) in EtOH (200 ml) and H_2O (200 ml). The solution was kept for 12 hr at room temperature in the dark, then the alcohol was evaporated off. The product was extracted with *n*-BuOH. The organic layer was washed with H_2O and evaporated to dryness *in vacuo*. KOH (12.5 mg) was added to the residue dissolved in EtOH (125 ml) and H_2O (125 ml). After refluxing of the solution for 1 hr, the reaction mixture was carefully neutralized with dilute HCl. The alcohol was evaporated off *in vacuo* and the residue was extracted with *n*-BuOH. The organic layer was washed with H_2O and evaporated to dryness, and the residue was chromatographed over silica gel [$CHCl_3$–MeOH (25: 1)] to give the prosapogenin (VII), jujubogenin 3-O-α-L-arabinopyranoside (120 mg),[15] as colorless needles, mp 275—277° (MeOH), $[\alpha]_D^{16}$ −24.0° (c=1.0, MeOH), PMR (pyridine-d_5) δ: 4.77 (1H, d, J=7). *Anal.* Calcd for $C_{35}H_{56}O_8 \cdot 1/2H_2O$: C, 68.48; H, 9.20. Found: C, 68.05; H, 9.28. The oxidation and saponification procedures used for VII were repeated, and purification by chromatography over silica gel [C_6H_6–acetone (10: 1)] gave the main sapogenin (IV), jujubogenin, as colorless needles (34 mg), mp 250—253° (MeOH), $[\alpha]_D^{19}$ −32.3° (c=0.8, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3500, 3270. *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.01; H, 10.44. IV was identified jujubogenin by mixed melting point determination and comparison of physical data with those of an authentic sample.

Synthesis of Methyl 2,3,4-Tri-O-acetyl-6-deoxy-α-L-talopyranoside (Va)——According to the literature,[12] methyl 2,3-O-isopropyldene-α-L-rhamnopyranoside (X, 1.1 g) was oxidized with phosphorus pentoxide/dimethyl sulfoxide to give a crude oil. The oil was chromatographed over silica gel [*n*-hexane–EtOAc (6: 1)] to give methyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose (XI, 667 mg), $[\alpha]_D^{16}$ −90.6° (c=1.26, EtOH), IR ν_{max}^{KBr} cm^{-1}: 1740. $NaBH_4$ (130 mg) was added in portions to a solution of XI (500 mg) dissolved in MeOH with vigorous stirring at room temperature during 10 min. The reaction mixture was

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neutralized with AcOH and then evaporated to dryness *in vacuo*. The residue was purified by chromatography on Sephadex LH-20 [MeOH–$CHCl_3$ (1: 1)] and silica gel [*n*-hexane–EtOAc (7: 1)] to give methyl 6-deoxy-2,3-O-isopropylidene-α-L-talopyranoside (XII), 343 mg), $[\alpha]_D^{15.5}$ −12.0° (c=1.03, EtOH). A solution of XII (293 mg) in MeOH (0.5 ml) was treated with 0.3 N HCl (3 ml) and the reaction mixture was kept for 40 min at room temperature. The mixture was neutralized with 3% KOH–MeOH and evaporated to dryness. The residue was passed through Sephadex LH-20 to give methyl 6-deoxy-α-L-talopyranoside (XIII, 190 mg), $[\alpha]_D^{17.5}$ −106.5° (c=1.05, H_2O), $[\alpha]_D^{23}$ −117.4° (c=0.9, MeOH). After the hydrolysis of XII (40 mg) with 2 N HCl for 2 hr, the reaction mixture was neutralized with 3% KOH–MeOH and evaporated to dryness. The residue was purified by chromatography on Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.2)] to give 6-deoxy-L-talose (V) as colorless plates (15 mg), mp 136—137° (acetone–EtOH), $[\alpha]_D^{23}$ −14.4°→−17.7° (24 hr later) (c=1.0, H_2O), FD-MS m/e: 164 (M^+). *Anal.* Calcd for $C_6H_{12}O_5$: C, 43.9; H, 7.37; Found: C, 43.86; H, 7.36. Methylation of XII (40 mg) was carried out in the same way as for I to give methyl 2,3,4-tri-O-methyl-6-deoxy-α-L-talopyranoside (Vb) as colorless needles (8 mg), mp 102—104° (petroleum ether), $[\alpha]_D^{23}$ −4.7° (c=0.6, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: no hydroxyl group. *Anal.* Calcd for $C_{10}H_{20}O_5$: C, 54.53; H, 9.15. Found: C, 54.12; H, 9.01. XIII (30 mg) dissolved in pyridine (1.5 ml) was acetylated with Ac_2O (1.5 ml) at room temperature overnight to give methyl 2,3,4-tri-O-acetyl-6-deoxy-α-L-talopyranoside (Va) as colorless plates (23 mg), mp 93—94° (petroleum ether), $[\alpha]_D^{13}$ −76.3° (c=0.9, MeOH),[14] IR ν_{max}^{KBr} cm^{-1}: no hydroxyl group. MS m/e: 305 $(M+1)^+$, 273. PMR δ: 1.23 (3H, d, J=6.5, C_6–Me), 2.00, 2.15, 2.17 (3H, each, Ac×3), 3.38 (3H, s, OMe), 4.11 (1H, doublet (J=1.0) of doublet (J=1.5) of quartet (J=6.5), C_5H), 4.73 (1H, br.s, C_1–H), 5.08 (1H, m, C_2–H), 5.13 (1H, m, C_4–H), 5.28 (1H, t, C_3–H_3).

Zizyphus Saponin II (II)——Colorless needles, mp 268—269° (MeOH), $[\alpha]_D^{14}$ −43.3° (c=0.67, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400. FD-MS m/e: 935 $(M+Na)^+$. *Anal.* Calcd for $C_{47}H_{76}O_{17}\cdot H_2O$: C, 60.62; H, 8.23. Found: C, 60.68; H, 8.39. Hydrolysis of II (100 mg) with 10% H_2SO_4 in EtOH (20 ml) gave ebelin lactone, and the sugar portion was found to consist of glucose, arabinose and rhamnose (Avicel TLC). The sugar mixture was treated in the same way as in the case of I to give three kinds of sugars: D-glucose, L-arabinose and L-rhamnose, colorless syrup (8.0 mg), Rf 0.55, $[\alpha]_D^{21}$ +5.0°→+7.0° (24 hr later) (c=0.8, H_2O).

Permethylate (IIa) of II——Methylation of II (50 mg) was carried out in the same way as for I to give the nona-O-methyl derivative (IIa) as colorless needles (20 mg), mp 224—227° (H_2O–MeOH), $[\alpha]_D^{18}$ −36.4° (c=1.36, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no hydroxyl group. MS m/e: 1024 $(M-CH_2)^+$, 553 (permethylated trisaccharide residue), 219 (terminal permethylated hexose residue), 189 (terminal permethylated methylpentose residue), 187 (219−MeOH), 157 (189−MeOH). PMR δ: 3.15 (3H, s, C_{20}–OMe), 4.39 (1H, d, J=5.5), 4.40 (1H, d, J=7.0), 5.22 (1H, br.s). FD-MS m/e: 1039 $(M+H)^+$, 219 (terminal permethylated hexose residue), 189 (terminal permethylated methylpentose residue). *Anal.* Calcd for $C_{56}H_{94}O_{17}$: C, 64.71; H, 9.12. Found: C, 64.28; H, 9.14.

Methanolysis of (IIa)——Methanolysis of IIa (5 mg) was carried out in the same way as for Ia to give the following methyl sugars, which were identified by GLC under the condition (*a*). t_R (min): Methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose 2.7; 2,3,4-tri-O-methyl-α-L-rhamnose 1.3; 4-mono-O-methyl-β-L-arabinose 11.0. Ref. Methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose 3.7.

Partial Hydrolysis of II——Partial hydrolysis of II (60 mg) was carried out in the same way as for I to give a main prosapogenin (15 mg) which was identified as prosapogenin A (VI) by direct comparison (TLC, IR and MS).

Smith-de Mayo Degradation of II——II (500 mg) was oxidized with periodate as in the case of I and the product was chromatographed over silica gel to give a main sapogenin (19 mg) as colorless needles, mp 250—253° (MeOH); this was identified as jujubogenin (IV) by direct comparison (mixed melting point, TLC and IR).

Zizyphus Saponin III (III)——Colorless plates, mp 229—233° (pyridine–H_2O), $[\alpha]_D^{28}$ −46.5° (c=0.98, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400. FD-MS m/e: 1083 $(M+K)^+$, 1067 $(M+Na)^+$. *Anal.* Calcd for $C_{52}H_{84}O_{21}\cdot 2.5H_2O$: C, 57.31; H, 8.19. Found: C, 57.55; H, 8.39. Hydrolysis of III (80 mg) with 10% H_2SO_4 in 50% EtOH (20 ml) was carried out in the same way as for I to give ebelin lactone, and the sugar portion was found to consist of glucose, arabinose, xylose and 6-deoxy-talose (Avicel TLC). The sugar mixture was treated in the same way as in the case of I to give four kinds of sugars: D-glucose, L-arabinose, 6-deoxy-L-talose, and D-xylose, colorless syrup (6.0 mg), Rf 0.42, $[\alpha]_D^{20}$ +19.5° (c=0.8, H_2O).

Hydrolysis of III with Crude Hesperidinase——A solution of III (5 mg) in H_2O (1.5 ml) was incubated with crude hesperidinase (10 mg) at 37° for 4 days and the hydrolysate was extracted with *n*-BuOH. The organic layer was evaporated to dryness *in vacuo* and the residue was examined by TLC to give I. The aqueous layer was evaporated to dryness *in vacuo* and the residue was shown by Avicel TLC to contain D-xylose.

Permethylate (IIIa) of III——Permethylation of III (50 mg) was carried out in the same way as for I to give undeca-O-methyl derivative (IIIa) as a colorless powder (21 mg), $[\alpha]_D^{20}$ −49.7° (c=1.45, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no hydroxyl group. MS m/e: 379 (permethylated disaccharide residue), 347 (379−MeOH), 189 (terminal permethylated methylpentose residue), 175 (terminal permethylated pentose residue), 157 (189−MeOH), 143 (175−MeOH). PMR δ: 3.15 (3H, s, C_{20}–OMe), 4.36 (1H, d, J=7.5), 4.61 (1H, d, J=7.0), 4.69 (1H, d, J=6.0); 5.36 (1H, br.s). *Anal.* Calcd for $C_{63}H_{106}O_{21}$: C, 63.08; H, 8.91. Found: C, 62.70; H,

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8.97.

Methanolysis of IIIa——Methanolysis of IIIa (5 mg) was carried out in the same way as for Ia and the product was found by GLC under the condition (*b*) to contain the following methylated sugars. t_R (min): Methyl pyranosides of 2,3,4-tri-O-methyl-β-D-xylose 1.7; 2,3,4-tri-O-methyl-6-deoxy-α-L-talose 3.1; 4-mono-O-methyl-β-L-arabinose 10.4; 3,4,6-tri-O-methyl-α-D-glucose 8.8. Ref. Methyl pyranosides of 2-mono-O-methyl-β-L-arabinose 14.2; 3-mono-O-methyl-β-L-arabinose 10.4; 2,4,6-tri-O-methyl-α-D-glucose 13.2; 2,3,6-tri-O-methyl-α-D-glucose 12.9; 2,3,4-tri-O-methyl-α-D-glucose 10.3.

Compound I (VIII)——Colorless needles, mp 256—259° (MeOH), $[\alpha]_D^{21}$ −45.2° (c=1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400. FD-MS m/e: 1067 $(M+Na)^+$, 1049 $(M-H_2O+Na)^+$. *Anal.* Calcd for $C_{52}H_{84}O_{21}\cdot 2H_2O$: C, 57.76; H, 7.83. Found: C, 57.99; H, 8.12. Hydrolysis of VIII (100 mg) was carried out in the same way as for I to give ebelin lactone, and D-glucose, L-arabinose, D-xylose and L-rhamnose as sugar moieties.

Hydrolysis of (VIII) with Crude Hesperidinase——A suspension of VIII (5 mg) in H_2O (2 ml)–EtOH (0.03 ml)–Tween 80 (0.03 ml) was incubated with crude hesperidinase (15 mg) at 37° for 3 days and the hydrolysate was extracted with *n*-BuOH. The organic layer was evaporated to dryness *in vacuo* and the residue was shown by TLC to contain II. The aqueous layer was evaporated to dryness *in vacuo* and the residue was shown on Avicel TLC to contain D-xylose.

Permethylate (VIIIa) of VIII——The permethylation product of VIII (76 mg) was worked up in the same way as for I to give the undeca-O-methyl derivative (VIIIa) as colorless needles (29 mg), mp 142—147° (H_2O–acetone), $[\alpha]_D^{22}$ −64.9° (c=1.10, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no hydroxyl group. MS m/e: 379 (permethylated disaccharide residue), 347 (379−MeOH), 189 (terminal permethylated methyl pentose residue), 175 terminal permethylated pentose residue), 157 (189−MeOH), 143 (175−MeOH). PMR δ: 3.15 (3H, s, C_{20}–OMe), 4.36 (1H, d, J=7.5), 4.55 (1H, d, J=7.0), 4.73 (1H, d, J=6.0), 5.35 (1H, br.s). *Anal.* Calcd for $C_{63}H_{106}O_{21}$: C, 63.08; H, 8.91. Found: C, 62.62; H, 8.89.

Methanolysis of VIIIa——Methanolysis of VIIIa (5 mg) was carried out in the same way as for Ia to give the following methanolysate, which was analyzed by means of GLC under the condition (b). t_R (min): Methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose 1.7; 2,3,4-tri-O-methyl-β-D-xylose 1.7; 3,4,6-tri-O-methyl-α-D-glucose 8.8; 4-mono-O-methyl-β-L-arabinose 10.4.

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