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THE STRUCTURE OF JUJUBOSIDES A AND B, THE SAPONINS ISOLATED FROM THE SEEDS OF *ZIZYPHUS JUJUBA*

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Key Word Index—*Zizyphus jujuba* (*Z. vulgaris* var. *spinosus*); Rhamnaceae; jujuboside A; jujuboside B.

Abstract—Stepwise chemical and enzymatic hydrolyses of jujubosides A and B, the saponins of the seeds of *Zizyphus jujuba*, afforded prosapogenins I, II and III. The sequence and configuration of the sugar linkages in the saponins and the pro-sapogenins were determined from the PMR data and the application of Klyne's rule of molecular rotations and structures were assigned to jujubosides A and B.

INTRODUCTION

The isolation of jujubosides A and B in crystalline form from the saponin fraction of the seeds of *Zizyphus jujuba* (=*Z. vulgaris* Lamarck var. *spinosus* Bunge) (Chinese drug: Suan-tsao-jen) and the structure elucidation of their sapogenin, jujubogenin (**4**), including an X-ray crystallographical study were reported previously [1–3].

RESULTS AND DISCUSSIONS

Jujuboside A (**1a**) $C_{58}H_{94}O_{26}\cdot 2H_2O$, colourless needles, mp 211–216°, $[\alpha]_D^{24°}$ − 50.1° (*c* = 0.43, MeOH), afforded on acid hydrolysis a secondary sapogenin, ebelin lactone (**3**), and arabinose, xylose, rhamnose and glucose (1:1:1:2) which were isolated from the water-soluble portion of the hydrolysate.

On acid hydrolysis, jujuboside B (**2a**), $C_{52}H_{84}O_{21}\cdot 3H_2O$, colourless needles, mp 222–225, $[\alpha]_D^{24°}$ − 42.0° (*c* = 0.50, MeOH), yielded **3** along with the same sugar components as those obtained from **1a**, but in a different proportion, 1:1:1:1. On treatment with snail enzyme, naringenase, or hesperidinase, **1a** was readily converted into **2a** with the release of one mole of glucose. On the Smith–de Mayo degradation [4, 5], **1a** and **2a** as well as hovenoside G, a saponin of *Hovenia dulcis* [2], afforded in the first step of the reaction prosapogenin-I (**5**), $C_{35}H_{56}O_8\cdot H_2O$, mp 237–241°, $[\alpha]_D^{24°}$ − 21.2°, which possessed one molecule of arabinose as the sugar component. According to Klyne's rule [6], the difference of molecular rotation of **4** and **5** (Δ*C*: +41.9°) indicated that α-L-arabinose was combined with the aglycone (Table 1). The PMR spectrum of **5** gave a signal at δ4.34 (1*H*, *d*, *J* = 5.5 Hz) revealing the C-1 conformation of α-L-arabinopyranose [7–9] in the sugar moiety.

On refluxing with dioxane–0.1 N H_2SO_4 **1a** was partially hydrolysed to give prosapogenin II (**6a**) which was converted into hexamethyl ether **6b** by the Kuhn method [10]. Methanolysis of **6b** with 5% HCl–MeOH afforded methyl-2,4-di-*O*-methyl-arabinopyranoside and methyl-2,3,4,6-tetra-*O*-methyl-glucopyranoside which were determined by means of GLC. Treatment with acid converted the aglycone into an ebelin lactone-type structure. TLC of the products obtained by the enzymatic hydrolysis of **1a** with naringinase or hesperidinase showed the formation of prosapogenin-III (**7a**) in addition to **2a**. Prosapogenin-III (**7a**), colourless powder, $C_{47}H_{76}O_{17}\cdot 4H_2O$, mp 243–246°, $[\alpha]_D^{24°}$ − 26.0° (*c* = 1.00, MeOH), which was separated by means of droplet counter current chromatography yielded on acid hydrolysis glucose, rhamnose and arabinose in a ratio of 1:1:1 which showed that **7a** was obtained from jujuboside B (**2a**) by loss of one molecule of xylose. Methylation of **7a** by the Hakomori method [11] yielded nona-*O*-methyl ether (**7b**) which gave MS fragments at *m/e* 219, 187 (219-MeOH), 189 and 157 (189-MeOH),

Table 1. Molecular rotation values of the saponins of *Zizyphus jujuba*

Saponins	Found $[M]_D$ in MeOH	Δ*C*
4	−169.9°	+41.9°
5	−128.0°	
7a	−237.1°	−109.1°
2a	−438°	−201.1°
1a	−506°	−68°

The following $[M]_D$ values were used to determine the configurations of glycosidic linkages. Me α-L-arabinopyranoside + 17.3°, Me β-L-arabinopyranoside + 245°, Me α-L-rhamnopyranoside −111°, Me β-L-rhamnopyranoside + 170°, Me β-D-glucopyranoside −66°, Me α-D-glucopyranoside + 309°, Me β-D-xylopyranoside −108°, Me α-D-xylopyranoside + 252°.

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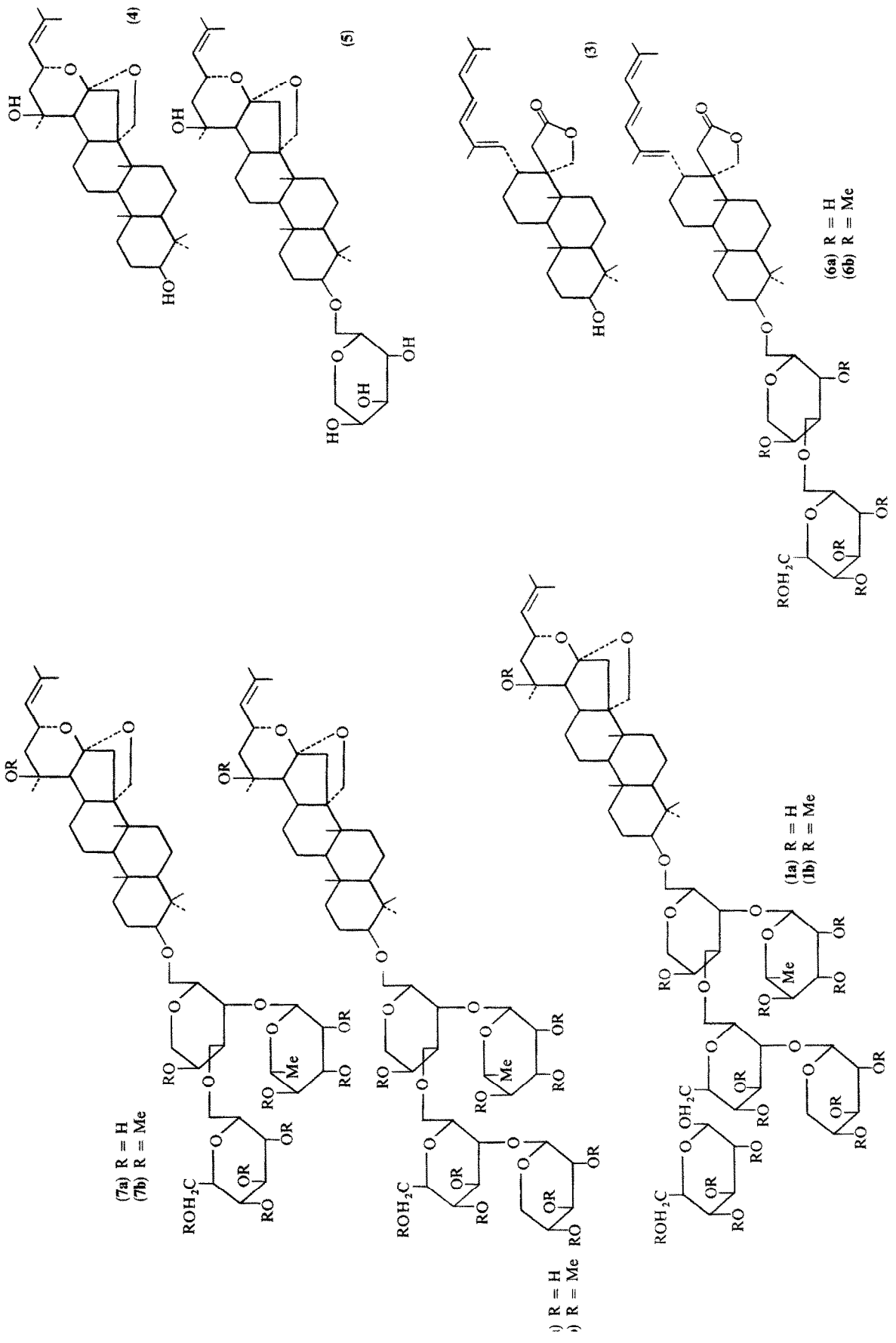
(4)
(5)
(3)
(6a) R = H
(6b) R = Me
(1a) R = H
(1b) R = Me
(7a) R = H
(7b) R = Me

indicating the presence of glucose and rhamnose as the terminal groups of the sugar moiety of **7a**. The PMR spectrum of **7b** gave the signals of 3 anomeric protons at δ4.39 (1H, *d*, *J* = 7.5 Hz), 4.69 (1H, *d*, *J* = 5.5 Hz) and 5.20 (1H, *br.s.*) whose coupling constants indicated that D-glucose possessed a β-linkage and L-arabinose an α-linkage. The configuration of the linkage of L-rhamnose was determined as α in accord with Klyne's rule [6] by the difference of molecular rotations observed between prosapogenins **5** and **7a** (Δ*C* −109.1°).

On methanolysis, prosapogenin-III methyl ether (**7b**) yielded methyl 2,3,4-tri-*O*-methyl-rhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, and methyl 4-mono-*O*-methyl-arabinopyranoside, which were identified with authentic samples by means of GLC.

Jujuboside B (**2a**) was methylated by the Hakomori method [11] to give undeca-*O*-methyl ether (**2b**), $C_{63}H_{106}O_{21}\cdot 2H_2O$, mp 131–134°, the MS of which showed peaks corresponding to terminal xylose (*m/e* 175, 143), terminal rhamnose (*m/e* 189, 157), and a disaccharide consisting of xylose and glucose (*m/e* 379, 347). The PMR signals of four anomeric protons in **2b** appeared at δ4.37 (1H, *d*, *J* = 7.5 Hz), 4.53 (1H, *d*, *J* = 7.5 Hz), 4.73 (1H,*d*, *J* = 5.0 Hz) and 5.34 (1H, *br.s*). In comparison with the PMR signals given by **7b**, an additional anomeric proton signal was observed at δ4.53 in the PMR spectrum of **2b**, the coupling constant of which was assigned to a β-linkage of D-xylose. This assignment was also supported by the difference of the molecular rotations of **2a** and **7a**. Methanolysis of **2b** yielded methyl 2,3,4-tri-*O*-methyl-xylopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside and methyl 4-mono-*O*-methyl-arabinopyranoside, which were identified with authentic samples by means of GLC.

Based on the above mentioned results, jujuboside B should be represented by the structure **2a**. Tetradeca-*O*-methyl ether (**1b**) prepared from jujuboside A (**1a**) by the Hakomori method [11] showed in the MS additional fragment ions at *m/e* 219 and 187 in comparison with that of **2b** to indicate the presence of a terminal glucose moiety in **1a**. The PMR spectrum of **1b** gave a signal at δ 1.23 (3H, *d*, *J* = 6.0 Hz) to reveal the presence of a rhamnosyl methyl group, and five anomeric protons at δ 4.20 (1H, *d*, *J* = 7.5 Hz), 4.42 (2H, *d*, *J* = 7.5 Hz), 4.68 (1H, *d*, *J* = 5.5 Hz) and 5.42 (1H, *br.s*). An anomeric proton signal at δ 4.42 given by **1b** and not by **2b** was assigned to that of a terminal glucose moiety whose linkage configuration was deduced to be β from the coupling constant of the PMR signal and the comparison of the molecular rotations of **1a** and **2a**. Methanolysis of **1b** yielded methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 4-mono-*O*-methylarabinopyranoside and methyl 3,4-di-*O*-methylglucopyranoside, which were identified with authentic samples on GLC.

On the basis of the above results, jujuboside A is represented by the structure **1a**.

EXPERIMENTAL

Mps are uncorr. The IR spectra were measured in KBr pellets. The PMR spectra were recorded at 100 MHz.

Isolation of jujubosides A and B. The seeds (10 kg) of *Zizyphus jujuba* Mill. (*Z. vulgaris* Lamarck var. *spinosus* Bunge) were extracted first with C_6H_6 to remove lipids and then with MeOH. The concd MeOH extract was added to H_2O and extracted with H_2O-satd *n*-BuOH. The BuOH layer was separated and treated with aq. 1% KOH. On evapn a saponin fraction (17.9 g, 0.18%) was obtained.

Acid hydrolysis of jujubosides A (**1a**) *and B* (**2a**). (1) Jujuboside A or B (5 mg each) was dissolved in a mixture of 10% H_2SO_4 in EtOH (5 ml) and refluxed for 5 hr. The reaction mixture was filtered to remove crystals and the filtrate was evapd. H_2O was added to the residue and the soln was neutralized with CG-4B(OH^-) and then evapd to dryness. The sample was dissolved in 0.13 M borate buffer, pH 7.5 (20 ml) and subjected to sugar analysis using a Recording JEOL-6AH sugar analyser to show the sugar components of jujuboside A (Glc:Rha:Xyl:Ara, 2:1:1:1) and jujuboside B (Glc:Rha:Xyl:Ara, 1:1:1:1). (2) Jujuboside A (5 mg) was dissolved in H_2O (1 ml) and added to dioxane (1 ml) and 2N H_2SO_4 (2 ml). After refluxing the soln at 100° for 5 hr an oily substance which separated out was removed by extraction with Et_2O, and the aq. layer was neutralized with IR-45 (OH^-) and then evapd. The residue was dissolved in C_5H_5N and trimethylsilylated with TMSC and HMDS. The reaction mixture was evapd *in vacuo* to obtain a residue which was extracted with *n*-hexane to provide a sample for GLC analysis. By GLC (2%OV-1 Chromosorb W 2 m × 3 mm; column temp. 160°; N_2 flow rate 70 ml/min) arabinose, xylose, rhamnose and glucose were identified by comparison with authentic samples. (3) Jujuboside B (5 mg) was hydrolysed as above to determine the sugar components: arabinose, xylose, rhamnose and glucose.

The Smith–de Mayo degradation of Jujuboside B. To an aq. soln of jujuboside B (50 mg), $NaIO_4$ (75 mg) was added and stirred for 4 days to complete oxidation. The reaction mixture was refluxed for 4 hr with 5% aq. KOH in Ar. After treatment with H_3PO_4 pH 3, the reaction mixture was chromatographed over a prepacked column (Merck) to obtain a prosapogenin (a few mg), needles, mp 237–241° (from $CHCl_3$–MeOH) identical with an authetic sample obtained from hovenoside G, $[\alpha]_D^{24°}$ − 21.2° (*c* = 0.50, MeOH. δ4.34 (1H, *d*, *J* = 5.5 Hz). (Found: C, 67.80; H, 9.32. Calcd. for $C_{35}H_{56}O_8\cdot H_2O$: C, 67.50; H, 9.39%)

Partial hydrolysis of jujubosides A and B. Jujuboside A or B (100 mg each) dissolved in dioxane and 0.1 N HCl (1:3) was warmed at 90° for 2.5 hr. The reaction mixture was extracted with *n*-BuOH, and the BuOH layer was washed with H_2O and concd. The soln was chromatographed on a Si gel column using $CHCl_3$–MeOH (10:1) to obtain prosapogenin II (**6a**) (2.7 mg). **6a** (2.7 mg) was methylated by the Kuhn method with Ag_2O (150 mg) and MeI (1 ml) in DMF at 100° for 5 hr to obtain a permethylate (**6b**), colourless powder, mp 111–113°; $\nu_{max}^{KBr}cm^{-1}$: 1768 (C = 0); MS *m/e*: 816, (M^+) 219, 187. **6b** was refluxed in 5% HCl–MeOH for 2 hr, and the reaction mixture was evapd *in vacuo*. The residue was analysed by GLC (10% DEGS on Chromosorb W 2 m × 3 mm); column temp. 160°; N_2 flow rate 70 ml/min.; 5%NPGs on Chromosorb W 2 m × 3 mm; column temp. 165°; N_2 flow rate 40 ml/min to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,4-di-*O*-methylarabinopyranoside by comparison with authentic samples.

Transformation of jujuboside A into jujuboside B by the action of snail enzyme. Jujuboside A(100 mg) dissolved in H_2O (30 ml) was added to snail enzyme (1 ml). (Suc D'*helix pomatia* (Industrie biologique Française)), and the mixture was incubated at 30° for 24 hr. The disappearance of jujuboside A and formation of jujuboside B in the soln was detected on TLC. Jujuboside B was isolated by the extraction of the reaction mixture with BuOH, and by purification with droplet counter-current chromatography (DCC) (900 theoretical plates) using $CHCl_3$–MeOH–H_2O(5:6:4) by the ascending method.

Transformation of jujuboside A into jujuboside B by the action of naringinase. Jujuboside A(300 mg) dissolved in H_2O was added to naringenase (200 mg) and incubated at 38° for 5 days. The reaction mixture was checked by TLC to monitor the

formation of jujuboside B and prosapogenin I(**7a**) and to follow the disappearance of jujuboside A. The reaction mixture was extracted with *n*-BuOH and the extracts were chromatographed over a Si gel (20 g) column using the lower layer of a solvent system $CHCl_3$–MeOH–H_2O (7:3:1) to obtain jujubogenin B (120 mg) and prosapogenin III (**7a**) (18 mg).

Transformation of jujuboside B with hesperidinase. Jujuboside B (43 mg) was dissolved in citrate–phosphate buffer pH 3.9 (40 ml) and added to hesperidinase (50 mg) followed by incubation at 38° for 3 days. The reaction mixture was monitored by TLC to find the quantitative formation of prosapogenin III (**7a**) The reaction mixture was extracted with *n*-BuOH and separated with DCC(800 theoretical plates) using $CHCl_3$–MeOH–H_2O (7:13:8) by the ascending method to obtain prosapogenin III(**7a**), mp 243–246° (15.6 mg). (Found: C, 57.26; H, 8.21. Calcd. for $C_{47}H_{76}O_{17}\cdot H_2O$: C, 57.30; H, 8.59%) $[\alpha]_D^{24°}$ − 26.0° (*c* = 1.00, MeOH).

Transformation of jujuboside B with naringinase. Jujuboside B (60 mg) dissolved in HOAc–AcONa pH 5.1 (60 ml) was incubated with naringinase (300 mg) (Sigma Chem. Co.) at 30° for 8 days. It was estimated by TLC that jujuboside B was transformed into prosapogenin III (**7a**) in a ratio of 5:3. The reaction mixture was extracted with *n*-BuOH, and the extracts were separated by DCC(800 theoretical plates) using $CHCl_3$–MeOH–H_2O (7·13·8) by the ascending method to obtain **7a** (16.0 mg).

Acid hydrolysis of prosapogenin III (**7a**). Prosapogenin III(**7a**) (5 mg) dissolved in H_2O (1 ml) was mixed with dioxane (1 ml) and 2N H_2SO_4 (2 ml), and refluxed at 100° for 5 hr. The mixture was treated by the same procedure described in the case of acid hydrolysis of jujuboside A to determine glucose, rhamnose and arabinose (1:1:1) as the hydrolytic products.

Prosapogenin III nona-O-methyl ether (**7b**). 50% NaH (800 mg) washed with petrol was added to DMSO (25 ml) in an atmosphere of Ar, and the mixture was heated for 1 hr at 50–60° on an oil bath. The above soln of carbanion (10 ml) was added to a soln of **7a** (35 mg) in DMSO (10 ml), and the mixture was kept for 4 hr with stirring and then MeI (5 ml) was added dropwise into the soln with continuous stirring for 24 hr. The reaction mixture was poured into ice H_2O, and extracted with $CHCl_3$. The $CHCl_3$ soln was washed with H_2O and then evapd to remove the solvent. The residue was chromatographed over a Si gel column (7 g) using *n*-hexane–Me_2CO (7:1). **7b** (8.7 mg) was obtained as colourless needles from *n*-hexane–Me_2CO. $\nu_{max}^{CCl_4}$: no absorption of OH: PMR $CDCl_3$: δ3.15 [3H, *s*, C-20 (*S*) OMe].

Methanolysis of **7b**. **7b** (5 mg) was refluxed with 5% HCl–MeOH for 2 hr. The reaction mixture was treated by the procedure as used for **6b**. On GLC analysis the formation of methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 4-mono-*O*-methylarabinopyranoside were determined by comparison with authentic specimens.

Undeca-O-methyljujuboside B (**2b**). 50% NaH (1 g) washed with petrol was added to DMSO (59 ml) in Ar, and the mixture was heated at 50–60° in an oil bath for 1 hr to form carbanion. To the soln of jujuboside B(155 mg) in DMSO (10 ml) the carbanion soln was added, and the mixture was kept for 4 hr with stirring, and then MeI(10 ml) was gradually added with continuous stirring for 14 hr. The reaction mixture was poured into ice H_2O and extracted with $CHCl_3$. The $CHCl_3$ soln was washed with H_2O and the $CHCl_3$ distilled off. The residue (116 mg) was chromatographed over a column of Si gel (8 g) using *n*-hexane–Me_2CO (6:1) to obtain **7b**, colourless needles, mp 131–134° (74 mg) from *n*-hexane–Me_2CO. (Found: C, 61.54; H, 8.86. Calcd. for $C_{63}H_{106}O_{21}\cdot 2H_2O$: C, 61.24; H, 8.96%) $[\alpha]_D^{24°}$ −43.4° (*c* = 2.3, $CHCl_3$). IR $\nu_{max}^{CCl_4}$: no OH absorption; PMR $CDCl_3$: δ3.14 [3H, *s*, C-20 (*S*) OMe]: MS *m/e*: 379, 347, 189 (base peak) 175, 157, 143.

Methanolysis of jujuboside B permethylate (**2b**). **2b** (10 mg) was refluxed with 5% HCl–MeOH for 2 hr. The products were treated by the procedure as used for **6b**. The formation of methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside and methyl 4-mono-*O*-methylarabinopyranoside was detected on GLC by comparison with authentic samples.

Tetradeca-O-methyljujuboside A (**1b**). Jujuboside A (100 mg) dissolved in DMSO (2 ml) was treated for 5 hr with stirring with carbanion soln (10 ml) which was prepared from 50% NaH (2 g) and DMSO (50 ml) under Ar with heating at 50–60°. The reaction mixture was poured into ice H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evapd to obtain a residue which was chromatographed over a Si gel column (10 g) using *n*-hexane–Me_2CO (5:1) to isolate an oily substance (39.5 mg), $[\alpha]_D^{24°}$ −50.1° (*c* = 1.97, $CHCl_3$). IR $\nu_{max}^{CCl_4}$: no OH absorption; PMR $CDCl_3$: δ3.14 [3*H*, *s*, C-20 (*S*) OMe]; MS *m/e*: 219, 189, 187, 175, 157, 143.

Methanolysis of jujuboside A permethylate (**1b**). **1b** (10 mg) was refluxed with 5% HCl–MeOH (2 ml) for 2 hr. Among the products methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl mono-*O*-methylarabinopyranoside were detected by GLC.

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