# Bioactive Saponins and Glycosides. X.[1] On the Constituents of Zizyphi Spinosi Semen, the Seeds of *Zizyphus jujuba* MILL. var. *spinosa* HU (1): Structures and Histamine Release-Inhibitory Effect of Jujubosides $A_1$ and C and Acetyljujuboside B

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**New dammarane-type triterpene oligoglycosides, jujubosides $A_1$ and C and acetyljujuboside B, were isolated from Zizyphi Spinosi Semen, the seeds of *Zizyphus jujuba* MILL. var. *spinosa* HU, together with three known saponins. The structures of jujubosides $A_1$ and C and acetyljujuboside B were determined on the basis of chemical and physicochemical evidence.**

**Jujubosides $A_1$ and C and acetyljujuboside B were found to inhibit the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction.**

**Key words** jujuboside $A_1$; jujuboside C; acetyljujuboside B; *Zizyphus jujuba* var. *spinosa*; Zizyphi Spinosi Semen; histamine release inhibitor

The seeds of *Zizyphus jujuba* MILL. var. *spinosa* HU (Rhamnaceae) have been used as a Chinese natural medicine, Zizyphi Spinosi Semen [Sansounin (酸棗仁) in Japanese],[2] which is prescribed for tonic and sedative purposes and treatment of insomnia in Chinese traditional preparations. As chemical constituents of *Zizyphus jujuba* MILL. var. *spinosa* HU, flavonoids, triterpenes, saponins, peptides, and cyclic nucleosides have been reported from the leaves and seeds of this plant.[3] As the saponin constituents, three dammarane-type triterpene oligoglycosides, jujubosides A (**4**), B (**5**), and $B_1$ (**6**), were characterized from the seeds of *Zizyphus jujuba* MILL. var. *spinosa* HU.[4]

In the course of our studies on the bioactive saponins and glycosides of natural medicines[1,5] and medicinal foodstuffs,[6] we have isolated four methyl-migrated 16,17-*seco*-dammarane-type triterpene glycosides, hovenidulciosides $A_1$, $A_2$, $B_1$, and $B_2$, from *Hovenia dulcis* THUNB. (Rhamnaceae)[7] and two oleanene-type triterpene ketone oligoglycosides, sandosaponins A and B, from *Phaseolus vulgaris* L. (Leguminosae)[8]; these triterpene oligoglycosides were also found to show an inhibitory effect on histamine release. As a continuing part of our screening for antiallergic saponins of natural medicines, three new dammarane-type triterpene oligoglycosides called jujubosides $A_1$ (**1**) and C (**2**) and acetyljujuboside B (**3**) were

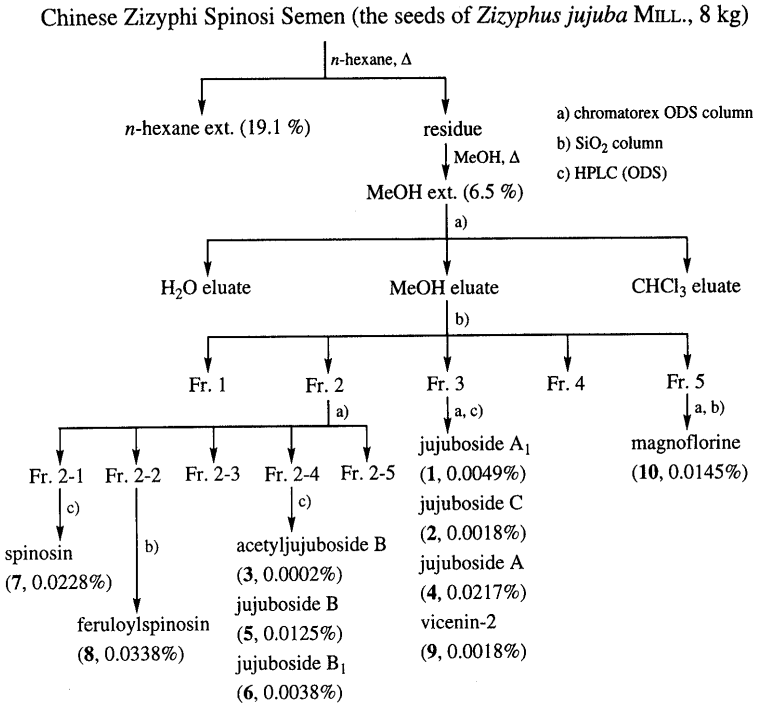


Chart 1

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isolated from Chinese Zizyphi Spinosi Semen, the seeds of *Zizyphus jujuba* MILL. var. *spinosa* HU, and were found to inhibit the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction. In this paper, we elucidate the structure of jujubosides $A_1$ (**1**) and C (**2**) and acetyljujuboside B (**3**)[9] and the inhibitory activity of the saponin constituents from this natural medicine on the histamine release.

Chinese Zizyphi Spinosi Semen was defatted with *n*-hexane and then extracted with methanol under reflux. The methanolic extract was first subjected to reversed-phase silica-gel column chromatography. The methanol eluate was separated by normal-phase silica-gel column chromatography to provide five fractions. Each fraction was separated through the procedure shown in Chart 1 using reversed-phase and normal-phase silica-gel column chromatography and HPLC to give jujubosides $A_1$ (**1**, 0.0049%) and C (**2**, 0.0018%) and acetyljujuboside B (**3**, 0.0002%) together with three known saponins[4] [jujubosides A (**4**, 0.0217%), B (**5**, 0.0125%), and $B_1$ (**6**, 0.0038%)], three flavonoid glycosides[10,11] [spinosin (**7**, 0.0228%), feruloylspinosin (**8**, 0.0338%), and vicenin-2 (**9**,

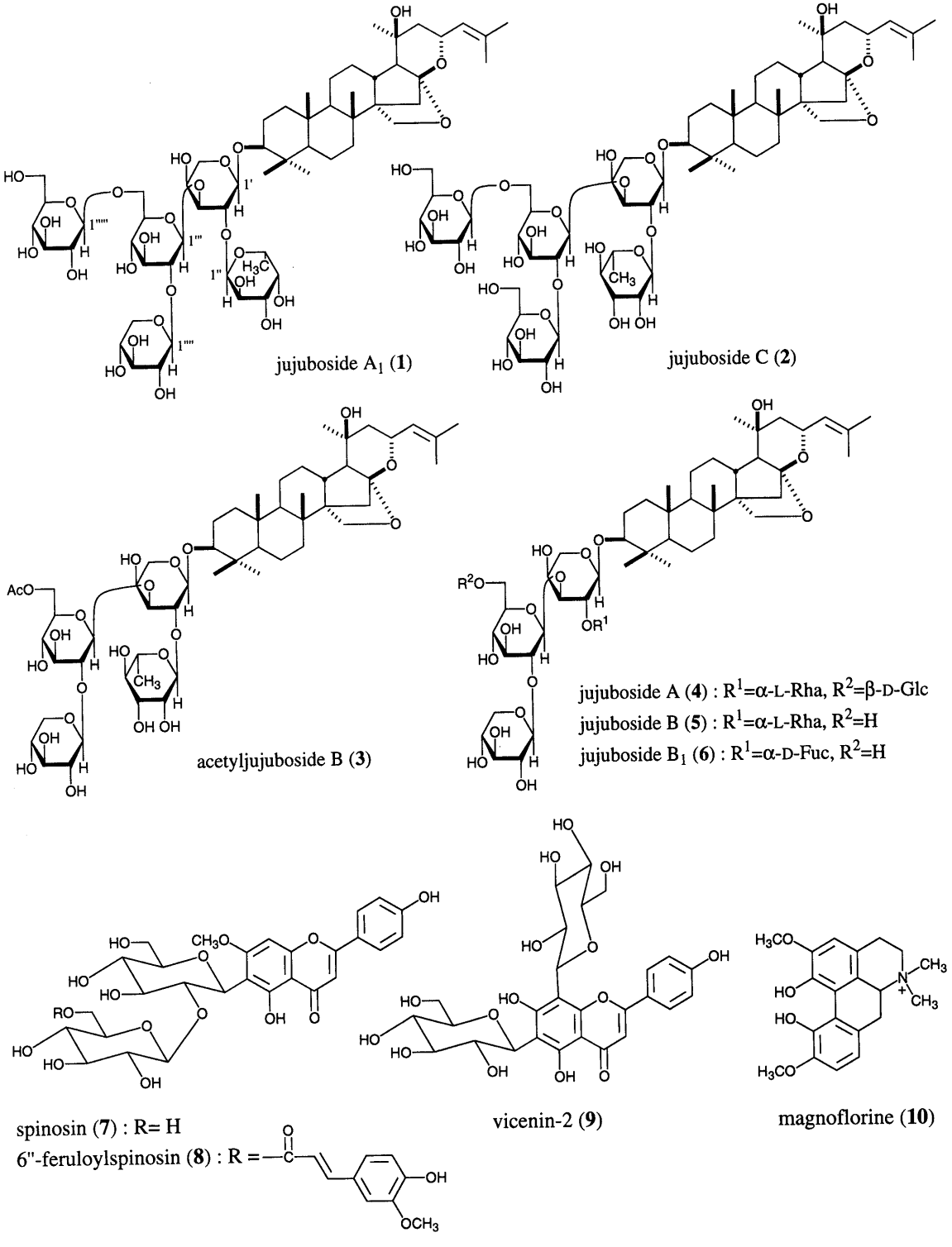



Chart 2

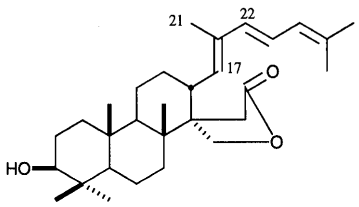
0.0018%)], and an alkaloid [magnoflorine (**10**, 0.0145%)]. Among those known compounds, magnoflorine (**10**), which was one of the principal ingredients of Zizyphi Spinosi Semen and known to have various pharmacological activities, was isolated for the first time from natural medicines originating in the Rhamnaceae plant.[12]

**Structures of Jujubosides $A_1$ (1) and C (2) and Acetyljujuboside B (3)** Jujuboside $A_1$ (**1**) was isolated as colorless fine crystals of mp 223—225 °C. The IR spectrum of **1** showed an absorption band at 1637 $cm^{-1}$ due to the olefin group and broad bands at 3432 and 1047 $cm^{-1}$ suggestive of an oligoglycosidic structure. In the negative-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1205 $(M-H)^-$, while the positive-ion FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 1229 $(M+Na)^+$ and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{58}H_{94}O_{26}$. Methanolysis of **1** with 9% hydrogen chloride in dry methanol liberated ebelin lactone (**11**)[13] and another aglycone designated 17(*Z*)-ebelin lactone (**12**) in *ca.* 1 : 1 ratio together with the methyl glycosides of arabinose, fucose, glucose, and xylose in *ca.* 1 : 1 : 2 : 1 ratio.[14]

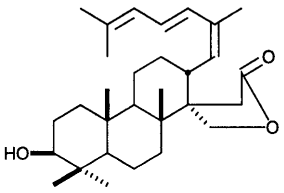
The EI-MS of **12** showed a molecular ion peak at *m/z* 454 $(M^+)$ and its molecular formula $C_{30}H_{46}O_3$, identical with that of **11**, was determined by high-resolution MS analysis. The UV spectrum of **12** showed an absorption maximum at 285 nm (log ε, 4.1), suggesting the presence of a triene function, while its IR spectrum showed absorption bands at 3461 and 1775 $cm^{-1}$ due to hydroxyl and γ-lactone groups. The $^1$H-NMR ($CDCl_3$) and $^{13}$C-NMR (Table 2) spectra[15] of **12** showed signals assignable to four tertiary methyls [δ 0.79, 0.87, 1.00, 1.06 (all s, 29, 19, 28, 18-$H_3$)], a triene side chain [δ 1.81, 1.83, 1.88 (all s, 27, 26, 21-$H_3$), 5.05 (d, *J*=9.5 Hz, 17-H), 5.93 (d, *J*=10.7 Hz, 24-H), 6.36 (d, *J*=15.0 Hz, 22-H), 6.50 (dd, *J*=10.7, 15.0 Hz, 23-H)], a γ-lactone [δ 2.14, 2.43 (ABq, *J*=18.0 Hz, 15-$H_2$), 4.28, 4.37 (ABq, *J*=10.4 Hz 30-$H_2$)], and a hydroxyl bearing methine [δ 3.21 (dd, *J*=4.6, 12.2 Hz, 3-H)]. Comparison of the $^1$H-NMR and $^{13}$C-NMR data for **12** with those for **11** led us to presume that **12** was the 17-geometric isomer of **11**. Namely, the $^{13}$C-NMR ($CDCl_3$) spectra of **11** showed the signal of *cis*-orientated 20-methyl carbon at $\delta_C$ 13.3, whereas the signal due to the *trans*-orientated 20-methyl carbon was observed at $\delta_C$ 18.1 in the $^{13}$C-NMR ($CDCl_3$) spectra of **12**. Furthermore, in the $^1$H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiments of **11** and **12**, NOE correlations were observed between the 17-proton and the 22-proton of **11** and between the 17-proton and 21-methyl proton of **12**. Finally, the acid hydrolysis of jujubogenin oligoglycoside, jujuboside A (**5**), under previous reported conditions[4,13] liberated **11** and **12** in *ca.* 1 : 1 ratio. On the basis of this evidence, the structure of 17(*Z*)-ebelin lactone (**12**) was determined, so that the aglycone of **1** was presumed to be jujubogenin (**13**).

The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR (Table 1) spectra[15] of **1** indicated the presence of the jujubogenin part [δ 0.71, 0.96, 1.39, 1.67, 1.70 (all s, 19, 29, 21, 25, 26-$H_3$), 1.09 (s, 18, 28-$H_3$), 3.18 (dd-like, 3-H), 5.19 (m, 23-H), 5.51 (d, *J*=7.9 Hz, 24-H)], an α-L-arabinopyranosyl moiety [δ 4.86 (d-like, 1′-H)], a α-D-fucopyranosyl moiety [δ 1.55 (d, *J*=5.6 Hz, 6″-$H_3$), 6.12 (br s, 1′-H)], two β-D-glucopyranosyl moieties [δ 5.01 (d, *J*=7.6 Hz, 1‴-H), 4.93 (d, *J*=7.6 Hz, 1⁗-H)], and a β-D-xylopyranosyl moiety [δ 5.44 (d, *J*=7.0 Hz, 1⁗-H)]. The proton and carbon signals of the aglycone moiety in the $^1$H-NMR and $^{13}$C-NMR spectra of **1** were superimposable on those of jujubogenin glycoside (**4**, **5**, **6**), while the proton and carbon signals of the oligoglycoside moiety were similar to those of **4** and **6**, except for the signals due to the 2′-*O*-α-D-fucopyranosyl moiety (for **4**) and the 6‴-*O*-β-D-glucopyranosyl moiety (for **6**). The pentaglycosidic structure bonding to the 3-hydroxyl group of jujubogenin (**13**) in **1** was characterized by means of a heteronuclear multiple bond correlation (HMBC) experiment, which showed long-range correlations between the following protons and carbons: Glc-1⁗-H and Glc-6‴-C, Xyl-1⁗-H and Glc-2‴-C, Glc-1‴-H and Ara-3′-C, Fuc-1″-H and Ara-2′-C, Ara-1′-H and jujubogenin-3-C as shown Fig. 1. Consequently, the structure of jujubogenin $A_1$ has been elucidated as jujubogenin 3-*O*-{[β-D-glucopyranosyl(1→6)][β-D-xylopyranosyl(1→2)]-β-D-glucopyranosyl(1→3)}[α-D-fucopyranosyl(1→2)]-α-L-arabinopyranoside (**1**).

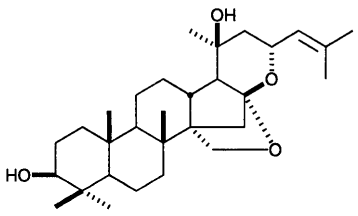
Jujuboside C (**2**) was also isolated as colorless fine crystals of mp 229—231 °C and its IR spectrum showed absorption bands at 3418, 1639, and 1075 $cm^{-1}$ due to hydroxyl and olefin groups. The molecular formula $C_{59}H_{96}O_{27}$ was determined from the negative-ion and positive-ion FAB-MS and by high-resolution MS measurement. That is, in the negative-ion FAB-MS of **2**, a quasimolecular ion peak was observed at *m/z* 1235 $(M-H)^-$, while the positive-ion FAB-MS showed a quasimolecular ion peak at *m/z* 1259 $(M+Na)^+$. The methanolysis of **2** liberated **11** and **12** (*ca.* 1 : 1 ratio) together with the methyl glycosides of arabinose, rhamnose, and glucose in *ca.* 1 : 1 : 3 ratio. The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR (Table 1) spectra[15] of **2** indicated the presence of the jujubogenin moiety [δ 0.72, 1.08, 1.14, 1.17, 1.39, 1.68, 1.70 (all s, 19, 18, 29, 28, 21, 25, 26-$H_3$), 3.18 (dd-like, 3-H), 5.19 (m, 23-H), 5.56 (d, *J*=8.6 Hz, 24-H)], an




ebelin lactone (**11**)




17(*Z*)-ebelin lactone (**12**)




jujubogenin (**13**)

Chart 3

Table 1. $^{13}$C-NMR Data of Jujubosides $A_1$ (**1**) and C (**2**) and Acetyljujuboside B (**3**)

| | **1**[a] | **2**[a] | **3**[b] | | **1**[a] | **2**[a] | **3**[b] |
|---|---|---|---|---|---|---|---|
| C-1 | 38.8 | 39.0 | 38.9 | Ara-1′ | 104.5 | 105.1 | 103.7 |
| C-2 | 26.6 | 26.8 | 26.6 | 2′ | 74.6 | 74.8 | 75.1 |
| C-3 | 88.1 | 88.2 | 88.4 | 3′ | 83.1 | 83.6 | 83.3 |
| C-4 | 39.5 | 39.7 | 39.7 | 4′ | 68.5 | 69.7 | 67.9 |
| C-5 | 56.2 | 56.4 | 56.2 | 5′ | 64.5 | 66.1 | 63.7 |
| C-6 | 18.3 | 18.3 | 18.3 | Rha (Fuc)-1″ | 101.7 | 101.0 | 101.7 |
| C-7 | 36.0 | 36.1 | 36.0 | 2″ | 67.7 | 72.4 | 72.4 |
| C-8 | 37.5 | 37.6 | 37.5 | 3″ | 72.1 | 72.4 | 72.6 |
| C-9 | 53.0 | 53.1 | 53.0 | 4″ | 74.2 | 73.9 | 74.0 |
| C-10 | 37.2 | 37.3 | 37.3 | 5″ | 67.0 | 69.8 | 70.1 |
| C-11 | 21.7 | 21.8 | 21.8 | 6″ | 17.3 | 18.3 | 18.6 |
| C-12 | 28.5 | 28.5 | 28.5 | Glc-1‴ | 103.6 | 103.2 | 104.2 |
| C-13 | 37.1 | 37.1 | 37.1 | 2‴ | 82.1 | 84.4 | 82.5 |
| C-14 | 53.7 | 53.8 | 53.8 | 3‴ | 78.1 | 78.3 | 78.1 |
| C-15 | 36.9 | 36.9 | 36.9 | 4‴ | 71.4 | 71.3 | 71.2 |
| C-16 | 110.6 | 110.6 | 110.6 | 5‴ | 76.7 | 76.8 | 74.8 |
| C-17 | 54.0 | 54.0 | 54.0 | 6‴ | 70.3 | 70.6 | 64.5 |
| C-18 | 18.9 | 18.9 | 18.9 | Ac-1 | | | 170.9 |
| C-19 | 16.3 | 16.5 | 16.4 | 2 | | | 20.8 |
| C-20 | 68.5 | 68.5 | 68.6 | Xyl (Glc)-1⁗ | 105.9 | 106.3 | 106.5 |
| C-21 | 30.0 | 30.0 | 30.1 | 2⁗ | 75.9 | 76.2 | 76.2 |
| C-22 | 45.4 | 45.4 | 45.5 | 3⁗ | 78.1 | 78.1 | 78.1 |
| C-23 | 68.6 | 68.6 | 68.5 | 4⁗ | 70.8 | 70.4 | 70.8 |
| C-24 | 127.1 | 127.1 | 127.1 | 5⁗ | 67.7 | 78.8 | 67.9 |
| C-25 | 134.2 | 134.2 | 134.2 | 6⁗ | | 61.8 | |
| C-26 | 25.6 | 25.6 | 25.6 | Glc-1⁗′ | 105.2 | 105.5 | |
| C-27 | 18.3 | 18.4 | 18.3 | 2⁗′ | 75.3 | 75.3 | |
| C-28 | 28.0 | 28.0 | 28.1 | 3⁗′ | 78.4 | 78.4 | |
| C-29 | 16.7 | 17.0 | 17.0 | 4⁗′ | 71.4 | 71.6 | |
| C-30 | 65.8 | 65.8 | 65.8 | 5⁗′ | 78.4 | 78.4 | |
| | | | | 6⁗′ | 62.5 | 62.6 | |

*a*) 125 MHz, *b*) 68 MHz, pyridine-$d_5$.

Table 2. $^{13}$C-NMR Data of Ebelin Lactone (**11**) and 17 (*Z*)-Ebelin Lactone (**12**)

| | **11** | **12** | | **11** | **12** |
|---|---|---|---|---|---|
| C-1 | 38.5 | 38.5 | C-16 | 179.4 | 179.1 |
| C-2 | 29.3[a] | 29.3[a] | C-17 | 130.2 | 128.5 |
| C-3 | 78.7 | 78.7 | C-18 | 17.9 | 17.9 |
| C-4 | 39.0 | 38.9 | C-19 | 16.0 | 16.0 |
| C-5 | 55.2 | 55.1 | C-20 | 137.2 | 137.1 |
| C-6 | 20.0 | 20.0 | C-21 | 13.3 | 18.1 |
| C-7 | 34.3 | 34.4 | C-22 | 134.3 | 130.2 |
| C-8 | 51.8 | 51.8 | C-23 | 124.6 | 125.7 |
| C-9 | 52.7 | 52.7 | C-24 | 125.5 | 126.0 |
| C-10 | 37.2 | 37.1 | C-25 | 135.6 | 136.0 |
| C-11 | 27.3[a] | 27.3[a] | C-26 | 26.2 | 26.2 |
| C-12 | 29.7[a] | 29.7[a] | C-27 | 18.5 | 18.6 |
| C-13 | 39.0 | 38.9 | C-28 | 28.0 | 28.0 |
| C-14 | 40.1 | 40.1 | C-29 | 15.4 | 15.4 |
| C-15 | 34.6 | 34.8 | C-30 | 69.7 | 69.5 |

125 MHz, $CDCl_3$. *a*) May be interchangeable.

α-L-arabinopyranosyl moiety [δ 4.75 (d-like, 1′-H)], a α-L-rhamnopyranosyl moiety [δ 1.70 (d-like, 6″-$H_3$), 6.36 (br s, 1″-H)], and three β-D-glucopyranosyl moieties [δ 5.02 (d, $J$=7.6 Hz, 1‴-H), 5.29 (d, $J$=7.6 Hz, 1⁗-H), 4.93 (d, $J$=7.6 Hz, 1⁗′-H)]. The proton and carbon signals in the $^1$H-NMR and $^{13}$C-NMR spectra of **2** significantly resembled to those of jujuboside A (**4**), except for the signals due to the 2″-*O*-β-D-glucopyranosyl moiety. Finally, the oligoglycosidic structure of **2** was characterized from the HMBC experiment, which showed long-range correlations between the following protons and carbons: 1⁗′-H and 6‴-C, 1⁗-H and 2‴-C, 1‴-H and 3′-C, 1″-H and 2′-C, 1′-H and 3-C. On the basis of the above evidence, the structure of jujuboside C has been determined as jujubogenin 3-*O*-{[β-D-glucopyranosyl(1→6)][β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl(1→3)}[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranoside (**2**).

Acetyljujuboside B (**3**), obtained as colorless fine crystals of 207—210 °C, showed absorption bands at 3424, 1736, 1638, and 1047 cm$^{-1}$ ascribable to hydroxyl, acetyl, and olefin groups in its IR spectrum. The molecular formula $C_{54}H_{86}O_{22}$ of **3** was obtained from the quasimolecular ion peaks [$m/z$ 1085 $(M-H)^-$ and 1109 $(M+Na)^+$] in the negative-ion and positive-ion FAB-MS of **3**. The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR spectra[15] of **3** showed signals assignable to the jujubogenin part, a α-L-arabinopyranosyl moiety [δ 4.90 (d-like, 1′-H)], a α-L-rhamnopyranosyl moiety [δ 1.67 (br s, 6″-$H_3$), 5.95 (br s, 1″-H)], an 6‴-acetyl-β-D-glucopyranosyl moiety [δ 2.10 (s, acetyl methyl), 5.12 (d, $J$=7.6 Hz, 1‴-H), 4.24, 4.48 (both m, 6‴-$H_2$)], and a β-D-xylopyranosyl moiety [δ 5.37 (d, $J$=7.3 Hz, 1⁗-H)]. Alkaline hydrolysis of **3** with 5% aqueous potassium carbonate furnished jujuboside B (**5**). Comparison of the $^{13}$C-NMR (pyridine-$d_5$) data for **3** with those for **5** revealed an acetylation shift around the 6‴-position of the D-glucopyranosyl moiety [**3**: $\delta_C$ 74.8 (C-5‴), 64.5 (C-6‴); **5**: $\delta_C$ 78.5 (C-5‴), 62.4 (C-6‴)]. Furthermore, the HMBC experiment of **3** showed a long-range correlation between the 6‴-methylene and the acetyl carbonyl carbon (Fig. 1). Consequently, the structure of acetyljujuboside B has been elucidated as jujubogenin 3-*O*-{[β-D-xylopyranosyl(1→2)-6-*O*-acetyl-β-D-glucopyranosyl(1→3)]}[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranoside (**3**).

**Inhibitory Effects of Jujubosides A (4), $A_1$ (1), B (5), and C (2) and Acetyljujuboside (3) on Histamine Release from Rat Peritoneal Exudate Cells** Since methyl-migrated dammarane-type triterpene glycosides, hovenidulciosides $A_1$, $A_2$, $B_1$, and $B_2$, were found to show inhibitory activity on histamine release from rat peritoneal exudate cells induced by calcium ionophore A-23187 and compound 48/80, dammarane-type triterpene oligoglycosides, jujubosides A (**4**), $A_1$ (**1**), B (**5**), and C (**2**) and acetyljujuboside B (**3**), were also expected to inhibit the histamine release. As is apparent from Table 3, all jujubosides (**1**—**5**) showed inhibitory activity on the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction.

## Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[1,5]

**Isolation of Jujubosides $A_1$ (1) and C (2) and Acetyljujuboside B (3) from Chinese Zizyphi Spinosi Semen** Chinese Zizyphi Spinosi Semen (8.0 kg, purchased from Tochimoto Tenkaido Co., Ltd., Lot. No. 406-C211, 1995) was crushed and extracted three times with *n*-hexane under reflux. After removal of the solvent by filtration, the defatted residue was further extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure from the *n*-hexane extracted portion and the MeOH-extracted portion yielded 1.5 kg and 517 g of extract,

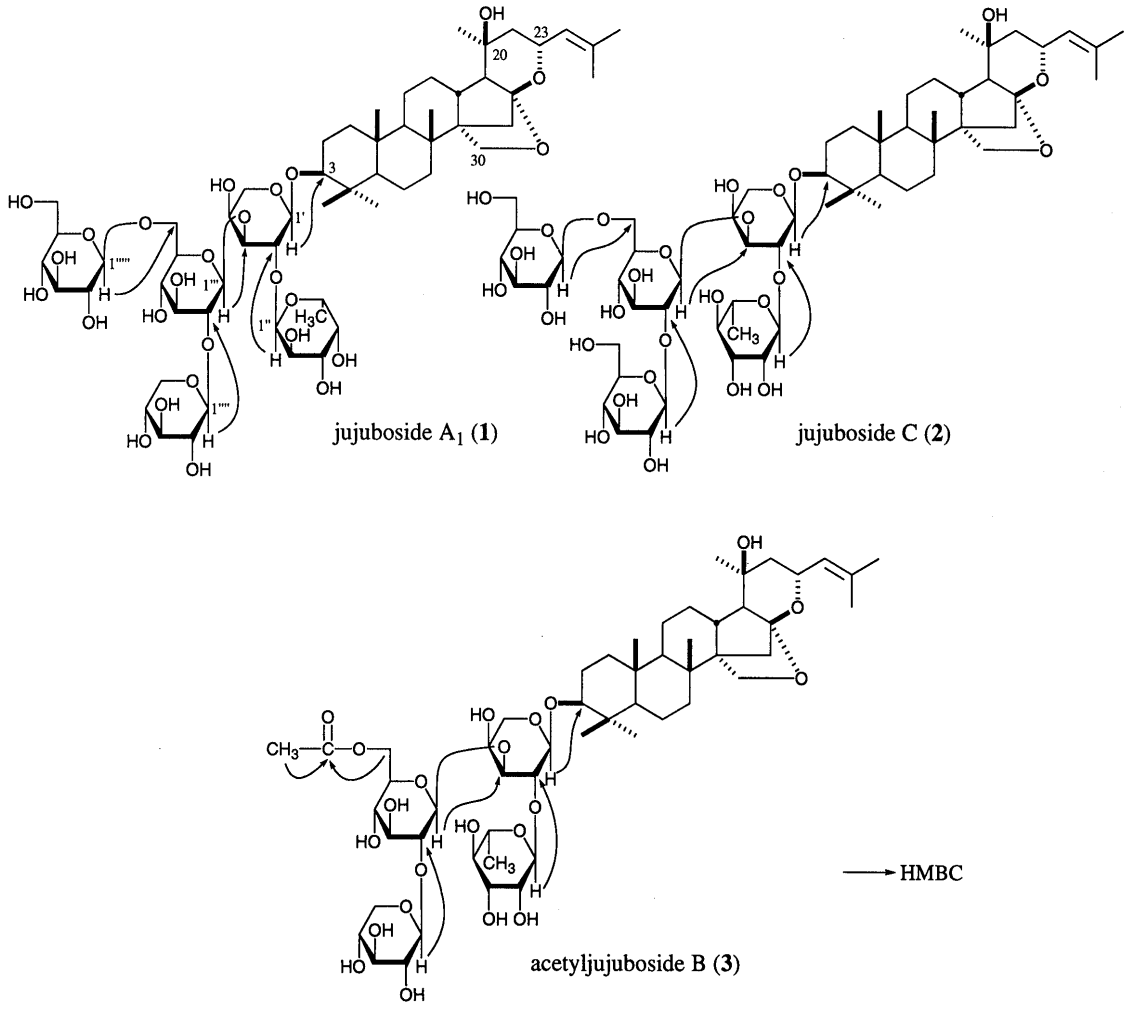



Fig. 1. HMBC Correlations of Jujuboside $A_1$ (**1**) and C (**2**) and Acetyljujuboside B (**3**)

Table 3. Inhibitory Effects of Jujubosides A (**4**), $A_1$ (**1**), B (**5**), and C (**2**) and Acetyljujuboside B (**3**) on the Histamine Release from Rat Peritoneal Exudate Cells Induced by Antigen–Antibody Reaction

| Compound | Concentration (M) | $n$ | Inhibition (%) |
|---|---|---|---|
| Jujuboside $A_1$ (**1**) | $10^{-4}$ | 4 | 30.3± 4.7 |
| Jujuboside C (**2**) | $10^{-4}$ | 4 | 71.4±29.9 |
| Acetyljujuboside B (**3**) | $10^{-4}$ | 4 | 14.5±18.0 |
| Jujuboside A (**4**) | $10^{-4}$ | 4 | 46.9±16.4 |
| Jujuboside B (**5**) | $10^{-5}$ | 4 | 32.0± 8.6 |
| Amlexanox | $10^{-5}$ | 4 | 11.8± 3.4 |
| | $3\times10^{-5}$ | 4 | 33.8± 1.8 |
| | $10^{-4}$ | 4 | 61.2± 3.1 |

respectively. The MeOH extract (510 g) was subjected to reversed-phase silica-gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 3 kg), $H_2O\rightarrow MeOH\rightarrow CHCl_3$] to give the MeOH eluate (188.7 g) and the $CHCl_3$ eluate (196.9 g). Ordinary-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 3 kg), $CHCl_3$–MeOH (40:1→20:1→10:1)→$CHCl_3$–MeOH–$H_2O$ (7:3:1, lower phase→6:4:1→5:5:1)] of the MeOH eluate (188.7 g) afforded five fractions [fr. 1 (91.5 g), fr. 2 (32.5 g), fr. 3 (12.4 g), fr. 4 (6.5 g), and fr. 5 (3.5 g)]. Fraction 2 (30 g) was separated by reversed-phase silica-gel column chromatography to give five fractions [fr. 2-1 (9.4 g), fr. 2-2 (3.2 g), fr. 2-3 (10.4 g), fr. 2-4 (1.8 g), fr. 2-5 (3.2 g)]. HPLC [YMC-Pack R&D ODS-5 (250×20 mm i.d.), MeOH–$H_2O$ (45:55, v/v), flow rate 9.0 ml/min] of fraction 2-1 (300 mg) furnished spinosin (**7**, 53 mg, 0.0228%). Fraction 2-2 (100 mg) was purified by ordinary-phase silica-gel column chromatography [10 g, AcOEt–MeOH–$H_2O$ (60:10:7)] to give feruloylspinosin (**8**, 77 mg, 0.0338%). Repeated HPLC [i) MeOH–$H_2O$ (75:25, v/v); ii) MeOH–$H_2O$ (65:35, v/v)] of fraction 2-4 (1.8 g) yielded jujubosides B (**5**, 912 mg, 0.0125%) and $B_1$ (**6**, 249 mg, 0.0038%) and acetyljujuboside B (**3**, 14 mg, 0.0002%). Fraction 3 (2.5 g) was subjected to reversed-phase silica-gel column chromatography [75 g, MeOH–$H_2O$ (40:60→70:30, v/v)→MeOH] followed by repeated HPLC [MeOH–$H_2O$ (70:30, v/v) to give jujubosides A (**4**, 343 mg, 0.0217%), $A_1$ (**1**, 78 mg, 0.0049%), and C (**2**, 29 mg, 0.0018%) and vicenin-2 (**9**, 28 mg, 0.0018%). Fraction 5 (1 g) was purified by reversed-phase silica-gel column chromatography [120 g, MeOH–$H_2O$ (50:50, v/v)→MeOH] and HPLC [MeOH–1% aq. trifluoroacetic acid (65:35, v/v)] to yield magnoflorine (**10**, 327 mg, 0.0145%). The physical data for the known compounds (**4**—**9**) were identified by comparison of their physical data with reported values [jujubosides A (**4**),[4] B (**5**),[4] and $B_1$ (**6**),[4] spinosin (**7**),[10] feruloylspinosin (**8**),[10] and vicenin-2 (**9**)[16]]. Magnoflorine (**10**)[17] was derived to the picrate salt which was identical with the authentic sample on the basis of mp, IR (KBr), $^1$H-NMR (acetone-$d_6$), and $^{13}$C-NMR (acetone-$d_6$) comparisons.

Jujuboside $A_1$ (**1**): Colorless fine crystals from aqueous MeOH, mp 223—225 °C, $[\alpha]_D^{29}$ −47.6° ($c$=0.3, MeOH). High-resolution positive-ion FAB-MS ($m/z$): Calcd for $C_{58}H_{94}O_{26}Na$ $(M+Na)^+$: 1229.5940; Found: 1229.5936. IR (KBr, $cm^{-1}$): 3432, 1637, 1047. $^1$H-NMR (pyridine-$d_5$, $\delta$): 0.71, 0.96, 1.39, 1.67, 1.70 (3H each, all s, 19, 29, 21, 25, 26-$H_3$), 1.09 (6H, s, 18, 28-$H_3$), 1.55 (3H, d, $J$=5.6 Hz, Fuc-6″-$H_3$), 2.82 (1H, m, 13-H), 3.18 (1H, dd-like, 3-H), 4.86 (1H, d-like, Ara-1′-H), 4.93 (1H, d, $J$=7.6 Hz, Glc-1‴″-H), 5.01 (1H, d, $J$=7.6 Hz, Glc-1‴-H), 5.19 (1H, m, 23-H), 5.44 (1H, d, $J$=7.0 Hz, Xyl-1‴″-H), 5.51 (1H, d, $J$=7.9 Hz, 24-H), 6.12 (1H, br s, Fuc-1″-H). $^{13}$C-NMR (pyridine-$d_5$, $\delta_C$): given in Table 1. Negative-ion FAB-MS ($m/z$): 1205 $(M-H)^-$. Positive-ion FAB-MS ($m/z$): 1229 $(M+Na)^+$.

Jujuboside C (**2**): Colorless fine crystals from aqueous MeOH, mp 229—231 °C, $[\alpha]_D^{29}$ −32.8° ($c$=0.3, MeOH). High-resolution positive-ion

FAB-MS (*m/z*): Calcd for $C_{59}H_{96}O_{27}Na$ $(M+Na)^+$: 1259.6034; Found: 1259.6024. IR (KBr, $cm^{-1}$): 3418, 1639, 1075. $^1$H-NMR (pyridine-$d_5$, $\delta$): 0.72, 1.08, 1.14, 1.17, 1.39, 1.68 (3H each, all s, 19, 18, 29, 28, 21, 25-$H_3$), 1.70 (6H, br s, 26-$H_3$, Rha-6″-$H_3$), 2.82 (1H, m, 13-H), 3.18 (1H, dd-like, 3-H), 4.75 (1H, d-like, Ara-1′-H), 4.93 (1H, d, $J$=7.6 Hz, Glc-1‴″-H), 5.02 (1H, d, $J$=7.6 Hz, Glc-1‴-H), 5.19 (1H, m, 23-H), 5.29 (1H, d, $J$=7.6 Hz, Glc-1⁗-H), 5.56 (1H, d, $J$=8.6 Hz, 24-H), 6.36 (1H, br s, Rha-1″-H). $^{13}$C-NMR (pyridine-$d_5$, $\delta_C$): given in Table 1. Negative-ion FAB-MS (*m/z*): 1235 $(M-H)^-$. Positive-ion FAB-MS (*m/z*): 1259 $(M+Na)^+$.

Acetyljujuboside B (**3**): Colorless fine crystals from aq. MeOH, mp 207—210 °C, $[\alpha]_D^{28}$ −42.8° ($c$=0.3, MeOH). High-resolution negative-ion FAB-MS (*m/z*): Calcd for $C_{54}H_{85}O_{22}$ $(M-H)^-$: 1085.5532; Found: 1085.5548. IR (KBr, $cm^{-1}$): 3424, 1736, 1638, 1047. $^1$H-NMR (pyridine-$d_5$, $\delta$): 0.71, 1.08, 1.11, 1.16, 1.39, 1.70 (3H each, all s, 19, 18, 29, 28, 21, 26-$H_3$), 1.67 (6H, br s, 25-$H_3$, Rha-6″-$H_3$), 2.10 (3H, s, OAc), 2.81 (1H, m, 13-H), 3.18 (1H, dd-like, 3-H), 4.24, 4.48 (1H each, both m, Glc-6‴-$H_2$), 4.90 (1H, d-like, Ara-1′-H), 5.12 (1H, d, $J$=7.6 Hz, Glc-1‴-H), 5.20 (1H, m, 23-H), 5.37 (1H, d, $J$=7.3 Hz, Xyl-1⁗-H), 5.53 (1H, d, $J$=8.2 Hz, 24-H), 5.95 (1H, br s, Rha-1″-H). $^{13}$C-NMR (pyridine-$d_5$, $\delta_C$): given in Table 1. Negative-ion FAB-MS (*m/z*): 1085 $(M-H)^-$. Positive-ion FAB-MS (*m/z*): 1109 $(M+Na)^+$.

**Methanolysis of Jujubosides $A_1$ (1) and C (2)** 1) A solution of **1** and **2** (10 mg each) in 9% HCl–dry MeOH (1.0 ml) was heated under reflux for 1 h. After cooling, the reaction solution was poured into ice-water and the whole mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After removal of the solvent from the $CHCl_3$ extract under reduced pressure, the residue was subjected to ordinary-phase silica-gel column chromatography [1.0 g, *n*-hexane–AcOEt (5 : 1, v/v)] and HPLC [YMC-Pack R&D ODS-5, MeOH–$H_2O$ (85 : 15, v/v)] to give ebelin lactone (**11**, 1.2 mg from **1**; 1.1 mg from **2**), which was identified by comparison of their physical data ($[\alpha]_D$, IR, $^1$H-NMR, $^{13}$C-NMR, MS) with reported values,[12] and 17(*Z*)-ebelin lactone (**12**, 1.0 mg from **1**; 1.0 mg from **2**).

17(*Z*)-Ebelin lactone (**12**): A white powder, $[\alpha]_D^{29}$ −17.9° ($c$=0.1, $CHCl_3$). High-resolution positive-ion EI-MS (*m/z*): Calcd for $C_{30}H_{46}O_3$ ($M^+$): 454.3447; Found: 454.3409. UV $\lambda_{max}$ $CHCl_3$ nm (log $\varepsilon$): 285 (4.1). IR (KBr, $cm^{-1}$): 3461, 1775. $^1$H-NMR ($CDCl_3$, $\delta$): 0.79, 0.87, 1.00, 1.06, 1.81, 1.83, 1.88 (3H each, all s, 29, 19, 28, 18, 27, 26, 21-$H_3$), 2.14, 2.43 (2H, ABq, $J$=18.0 Hz, 15-$H_2$), 2.80 (1H, ddd, $J$=3.7, 9.5, 13.1 Hz, 13-H), 3.21 (1H, dd, $J$=4.6, 12.2 Hz, 3-H), 4.28, 4.37 (2H, ABq, $J$=10.4 Hz, 30-$H_2$), 5.05 (1H, d, $J$=9.5 Hz, 17-H), 5.93 (1H, d, $J$=10.7 Hz, 24-H), 6.36 (1H, d, $J$=15.0 Hz, 22-H), 6.50 (1H, dd, $J$=10.7, 15.0 Hz, 23-H). $^{13}$C-NMR ($CDCl_3$, $\delta_C$): given in Table 1. EI-MS (*m/z*): 454 ($M^+$).

2) A solution of **1** and **2** (1 mg each) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized with $Ag_2CO_3$ powder and filtered. After removal of the solvent under reduced pressure from the filtrate, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.01 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl arabinoside (i), methyl fucoside (ii), methyl rhamnoside (iii), methyl xyloside (iv), and methyl glucoside (v). GLC conditions: column CBR1-M25-025 [0.25 mm (i.d.) × 25 m] capillary column; Injector temperature: 140 °C; Detector temperature: 280 °C; Column temperature: 140—240 °C, 5 °C/min; Initial time: 5 min; He flow rate: 15 ml/min; $t_R$: i: 13.2, 13.3, 13.6, 14.4 min, ii: 14.8, 15.4 min, iii: 14.1; 14.5 min, iv: 16.0, 16.5 min, v: 21.3, 21.7 min.

**Deacetylation of Acetyljujuboside B (3)** A solution of **3** (0.8 mg) in 5% aqueous $K_2CO_3$ (0.2 ml) was stirred at room temperature for 1 h. The reaction solution was neutralized with Dowex HCR W × 2 ($H^+$ form) and the resin was removed by filtration. Evaporation of the solvent under reduced pressure from the filtrate furnished jujuboside B (**5**, 0.7 mg), which was identified by TLC, $[\alpha]_D$, IR, and $^1$H-NMR spectra comparisons with an authentic sample.

**Histamine Release from Rat Peritoneal Exudate Cells** The method of bioassay testing was basically the same as described in the previous report.[8] Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 350—500 g were killed by exsanguination and injected intraperitoneally with 10 ml of physiological solution consisting of NaCl (150 mM), KCl (2.7 mM), $CaCl_2$ (0.9 mM), glucose (5.6 mM) and HEPES (5 mM) (pH 7.4). The abdominal region was gently massaged for 2 min and then peritoneal exudate was collected. The cell suspension was centrifuged (100 × $g$, 4 °C, 10 min) and washed several times with the physiological solution. The peritoneal exudate cells were sensitized with diluted anti-DNP IgE ( × 100) at 37 °C for 1 h. The cell suspension ($10^4$/1.62 ml) and 180 $\mu$l of test compound were preincubated for 15 min; 200 ml of phosphatidyl-L-serine (1 mg/ml) and 222 $\mu$l of DNP-BSA (1 mg/ml) were added at the same time and the incubation was continued for 20 min. The test tube was dropped into an ice-cold bath to stop the reaction. The supernatant was obtained by centrifugation for 10 min at 100 × $g$, 4 °C, and the histamine concentration was measured by the method of Imada *et al.*[18]

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