Bioactive Saponins and Glycosides. IV.[1] Four Methyl-Migrated 16,17-*seco*-Dammarane Triterpene Glycosides from Chinese Natural Medicine, Hoveniae Semen Seu Fructus, the Seeds and Fruit of *Hovenia dulcis* THUNB.: Absolute Stereostructures and Inhibitory Activity on Histamine Release of Hovenidulciosides A_1, A_2, B_1, and B_2

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Four bioactive methyl-migrated 16,17-*seco*-dammarane type triterpene glycosides called hovenidulciosides A_1, A_2, B_1, and B_2 were isolated from a Chinese natural medicine, Hoveniae Semen Seu Fructus, the seeds and fruit of *Hovenia dulcis* THUNB. (Rhamnaceae) together with hoduloside III and (+)-gallocatechin. The absolute stereostructures of hovenidulciosides A_1, A_2, B_1, and B_2 have been elucidated by chemical and physicochemical evidence. All were found to inhibit the histamine release from rat peritoneal exudate cells induced by compound 48/80 and calcium ionophore A-23187.

Key words *Hovenia dulcis*; hovenidulcioside; hovenidulcigenin; methyl-migrated 16,17-*seco*-dammarane triterpene glycoside; Hoveniae Semen Seu Fructus; histamine release inhibitor

A Chinese natural medicine, Hoveniae Semen Seu Fructus (枳椇子), prepared from the seeds and fruit of *Hovenia dulcis* THUNB. (Japanese name "kenponashi", Rhamnaceae), has been prescribed as a diuretic and antidote in various Chinese traditional preparations. In regard to the chemical constituents of *Hovenia dulcis* THUNB., several dammarane and 16,17-*seco*-dammarane type triterpene glycosides and peptide alkaloids have been characterized from the root bark and leaves of this plant,[2] but no report on the seeds and fruit used for the above medicinal purposes, has been published to date as far as we know.

In the course of our studies on the bioactive principles of natural medicines,[3] we have found many olean-12-ene type triterpene oligoglycosides, which exhibit an inhibitory effect on ethanol absorption and hypoglycemic activity,

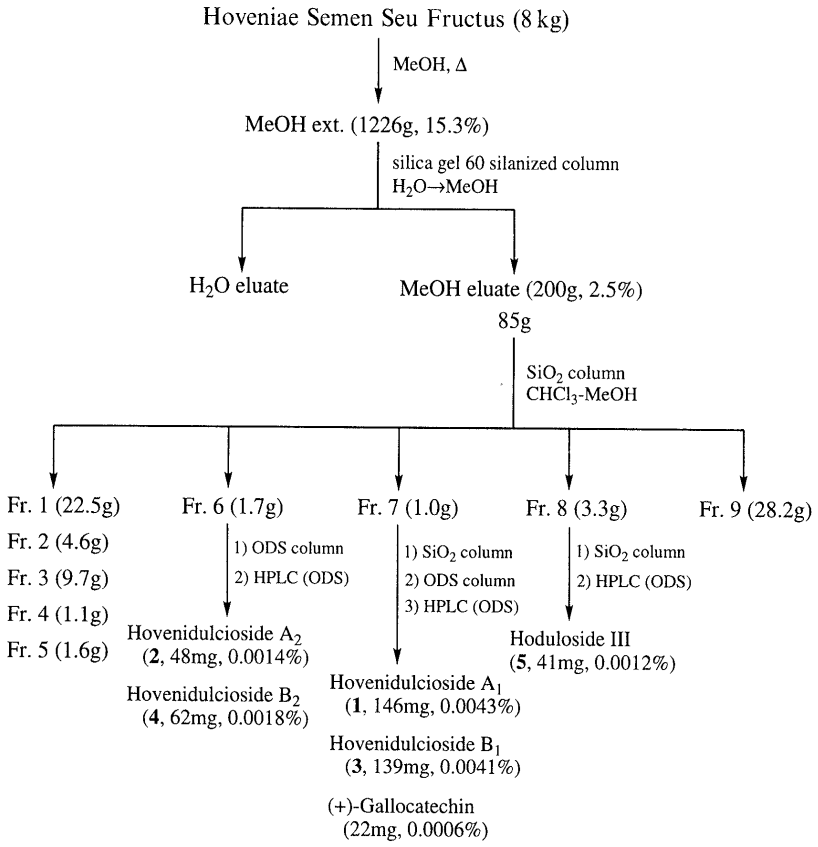

Chart 1

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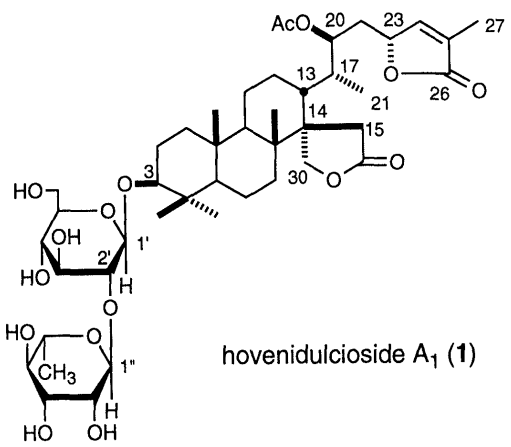
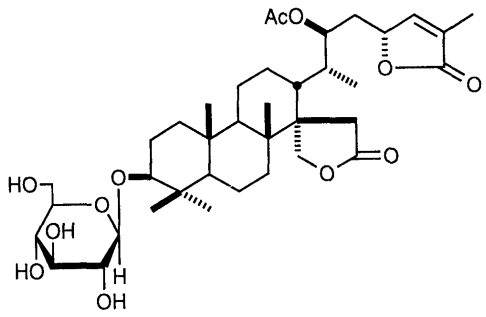
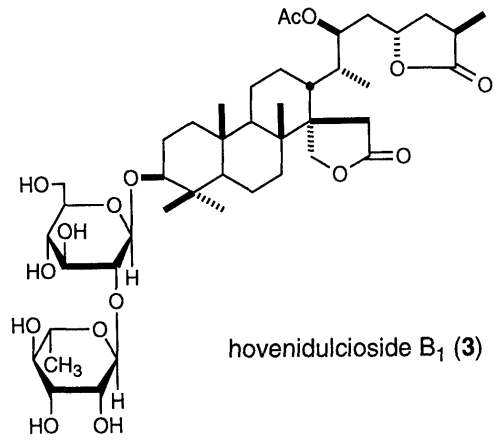


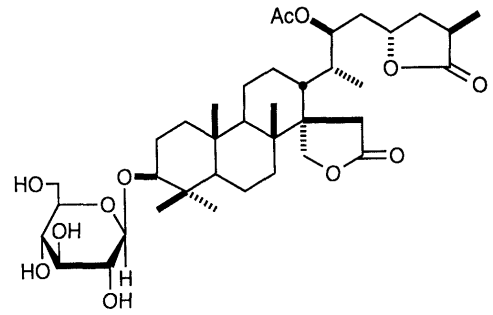
hovenidulcioside A_1 (**1**)



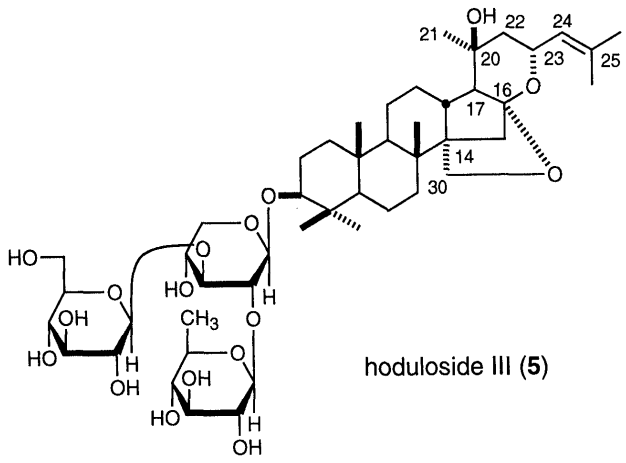
hovenidulcioside A_2 (**2**)



hovenidulcioside B_1 (**3**)



hovenidulcioside B_2 (**4**)



hoduloside III (**5**)

Chart 2

from *Aralia elata* SEEM. (root cortex, bark, and young shoot, Araliaceae),[4] *Camellia japonica* L. (seeds, Theaceae),[5] *Aesculus hippocastanum* L. (seeds, Hippocastanaceae),[1,6] *Polygala senega* L. var. *latifolia* TORREY *et* GRAY (roots, Polygalaceae),[7] *Beta vulgaris* L. (roots and leaves, Chenopodiaceae),[8] and *Gymnema sylvestre* R. BR. (leaves, Asclepiadaceae).[9] As a continuing part of our screening for bioactive saponin and glycoside constituents of natural medicines, four methyl-migrated 16,17-*seco*-dammarane type triterpene glycosides designated hovenidulciosides A_1 (**1**),[10] A_2 (**2**),[10] B_1 (**3**), and B_2 (**4**) were isolated from Hoveniae Semen Seu Fructus and were found to show inhibitory activity on histamine release from rat peritoneal exudate cells induced by compound 48/80 and calcium ionophore A-23187. In this paper, we provide a full account of the structure elucidation of the four hovenidulciosides (**1**—**4**) and their inhibitory activity on histamine release.[11]

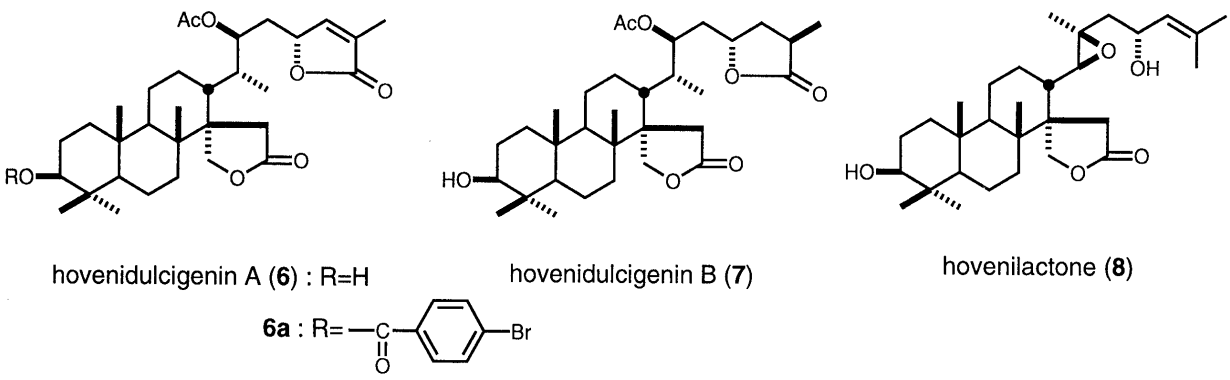
The triterpene glycoside constituents of Hoveniae Semen Seu Fructus were separated through the procedure shown in Chart 1. Thus, the methanolic extract from the natural medicine was first subjected to reversed-phase silica gel column chromatography. The methanol eluate was separated by normal-phase silica gel column chromatography to provide nine fractions. The reversed-phase silica gel column chromatography of fraction 6 followed by HPLC separation furnished hovenidulciosides A_2 (**2**, 0.0014%) and B_2 (**4**, 0.0018%). The normal and

reversed-phase silica gel column chromatography of fraction 7 followed by HPLC separation gave hovenidulciosides A_1 (**1**, 0.0043%) and B_1 (**3**, 0.0041%) and (+)-gallocatechin (0.0006%). A known dammarane triterpene glycoside hoduloside III (**5**, 0.0012%)[2i] was obtained from fraction 8 by normal-phase silica gel column and subsequent HPLC separation.

Hovenidulciosides A_1 (1) and A_2 (2) Hovenidulcioside A_1 (**1**) was isolated as colorless fine crystals of mp 183—186 °C from aqueous methanol. The IR spectrum of **1** showed absorption bands at 3453, 1765, 1751, 1735, 1602, and 1046 cm^{-1} due to hydroxyl, γ-lactone, butenolide, and ester groups. The UV spectrum of **1** showed absorption maximum at 224 nm ($\log\varepsilon$, 3.8), which suggested the presence of a butenolide group. In the negative-mode and positive-mode FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 851 $(M-H)^-$ and *m/z* 875 $(M+Na)^+$, respectively, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{44}H_{68}O_{16}$. Methanolysis of **1** with 9% hydrogen chloride in dry methanol liberated methyl glycosides of D-glucose and L-rhamnose in a 1 : 1 ratio.[12] On enzymatic hydrolysis of **1** with naringinase, a genuine aglycone called hovenidulcigenin A (**6**) was obtained as colorless fine crystals of mp 230—233 °C. The IR spectrum of **6** showed absorption bands assignable to hydroxyl, γ-lactone, butenolide, and ester groups. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **6**, which were assigned by various NMR analytical methods,[13] showed signals due to four tertiary methyls [δ 0.78 (s, 29-H_3), 0.82 (s, 19-H_3), 0.96 (s, 18-H_3), 0.99 (s, 28-H_3)], a secondary methyl [δ 0.89 (d, J=6.9 Hz, 21-H_3)], an acetyl methyl [δ 2.09 (s, 20-OAc)], a methine bearing hydroxyl group [δ 3.19 (dd, J=5.2, 11.2 Hz, 3-H)], a γ-lactone [δ 2.34, 2.59 (ABq, J=18.8 Hz, 15-H_2), 4.23, 4.38 (ABq, J=10.3 Hz, 30-H_2)], and an α-methyl butenolide [δ 1.91 (br s, 27-H_3), 4.88 (br s, 23-H), 7.09 (br s, 24-H)]. The carbon and proton signals assignable to the tricarbocyclic moiety of **6** were found to be very similar to those of hovenilactone (**8**), which was the common aglycone of various 16,17-*seco*-dammarane type triterpene glycosides obtained from the leaves of *Hovenia dulcis*.[2] The side chain structure with a secondary methyl, an acetoxyl, and an α-methyl-26,23-butenolide of **6** was confirmed from the heteronuclear mutiple bond connectivity (HMBC) experiment shown in Fig. 1. Namely, long-range correlations were observed between the following protons and carbons of **6**: 17-H and 20, 21-C; 21-H_3 and 13, 17-C; 22-H_2 and 20, 23-C; 24-H and 23, 25, 27-C; 27-H_3 and 24, 25, 26-C. On the basis of these findings, it appeared that **6** had the novel triterpene structure with the methyl group migrated from the 20-position to the 17-position in 16,17-*seco*-dammarane type triterpene. Finally, the absolute stereostructure of hovenidulcigenin A was established as **6** by the X-ray crystallographic analysis of the 3-*O*-*p*-bromobenzoylhovenidulcigenin A (**6a**),[10] which was prepared from **6** by condensation with *p*-bromobenzoic acid in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride and 4-dimethylaminopyridine in dichloromethane.

The ^{1}H-NMR (CD_3OD) spectrum of **1** showed signals due to the hovenidulcigenin A part [δ 0.92, 0.93, 1.06, 1.12 (all s, 29, 19, 18, 28-H_3), 0.97 (d, J=6.9 Hz, 21-H_3), 1.91 (dd, J=1.6, 1.7 Hz, 27-H_3), 2.12 (s, 20-OAc), 3.21 (m, 3-H), 4.75 (t-like, 20-H), 5.13 (br s, 23-H), 7.34 (br s, 28-H)], α-L-rhamnopyranosyl [δ 1.27 (d, J=5.9 Hz, 6″-H_3), 5.43 (br s, 1″-H)], and β-D-glucopyranosyl [δ 4.46 (d, J=7.3 Hz, 1′-H)] moieties. The carbon signals assignable to the sugar moiety in the ^{13}C-NMR (Table 1) spectrum of **1** were shown to be superimposable on those of several known glycosides having α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl moiety such as hovenoside E,[2f] saponin D,[2h] and hoduloside II.[2i] Furthermore, long-range correlations were observed between the 1′-proton of the β-D-glucopyranosyl moiety and the 3-carbon of the hovenidulcigenin A part and between the 1″-proton of the α-L-rhamnopyranosyl moiety and the 2′-carbon of the β-D-glucopyranosyl moiety. Consequently, the absolute stereostructure of hovenidulcioside A_1 was determined to be 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] hovenidulcigenin A (**1**).

Hovenidulcioside A_2 (**2**) was also obtained in the form of colorless fine crystals of mp 157—160 °C from aqueous methanol. The IR spectrum of **2** showed absorption bands assignable to hydroxyl, γ-lactone, butenolide, and ester, while its UV spectrum showed absorption maximum at 234 nm ($\log\varepsilon$, 3.8). The positive-mode FAB-MS of **2** showed a quasimolecular ion peak at *m/z* 729 $(M+Na)^+$ and the molecular formula $C_{38}H_{58}O_{12}$ was determined by high-resolution MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **2** showed signals due to the hovenidulcigenin A part [δ 0.84, 0.87, 0.99, 1.06 (all s, 29, 19, 18, 28-H_3), 0.92 (d, J=7.2 Hz, 21-H_3), 1.84 (br s, 27-H_3), 2.05 (s, 20-OAc), 2.43, 2.64



hovenidulcigenin A (**6**) : R=H

hovenidulcigenin B (**7**)

hovenilactone (**8**)

6a : R= —C(=O)— —Br

Chart 3

(ABq, $J=19.0$ Hz, 15-H_2), 3.18 (m, 3-H), 4.26, 4.47 (ABq, $J=10.5$ Hz, 30-H_2), 4.68 (m, 20-H), 5.06 (br s, 23-H), 7.27 (br s, 24-H)] and β-D-glucopyranosyl moiety [4.31 (d, $J=7.6$ Hz, 1′-H)]. Enzymatic hydrolysis of **2** with β-D-glucosidase liberated hovenidulcigenin A (**6**). Finally, comparison of the ^{13}C-NMR data for **2** with those for **1**, **6**, and methyl β-D-glucopyranoside led us to confirm the structure of hovenidulcioside A_2 as 3-*O*-β-D-glucopyranosylhovenidulcigenin A (**2**).

Hovenidulciosides B_1 (3) and B_2 (4) Hovenidulcioside B_1 (**3**), isolated as colorless fine crystals of mp 177—180 °C, lacked an UV absorption maximum at above 210 nm, while it showed absorption bands due to hydroxyl, γ-lactone, and ester groups at 3432, 1773, 1736, and 1048 cm^{-1} in its IR spectrum. The negative-mode and positive-mode FAB-MS of **3** showed quasimolecular ion peaks at *m/z* 853 $(M-H)^-$ and 877 $(M+Na)^+$, respectively. The molecular formula $C_{44}H_{70}O_{16}$ was determined from the quasimolecular ion peak $(M-H)^-$ and by high-resolution MS measurement. Methanolysis of **3** with 9% hydrogen chloride–dry methanol liberated methyl glucoside and methyl rhamnoside in a 1 : 1 ratio.[12] The enzymatic hydrolysis of **3** with naringinase gave a new genuine aglycone, hovenidulcigenin B (**7**), whose molecular formula $C_{32}H_{50}O_7$ was determined from the quasimolecular ion peaks [*m/z* 545 $(M-H)^-$, 547 $(M+H)^+$] and by high-resolution MS measurement. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **7** closely resembled those of hovenidulcigenin A (**6**) except for the signals due to the α-methyl-γ-lactone moiety. Namely, the ^{1}H-NMR spectrum of **7** lacked the signals due to a vinyl methyl and olefinic proton but instead showed a secondary methyl [δ 1.27 (d, $J=7.2$ Hz, 27-H_3), 2.67 (m, 25-H)]. This evidence presumed that **3** and **7** were the dihydro-analogs of **1** and **6** on the Δ^{24}-olefinic moiety. Furthermore, in the ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiment of **3** and **7**, NOE correlations were observed between the 23-proton and 27-methyl groups. Hydrogenation of **6** with 5% palladium–charcoal furnished 25-*epi*-hovenidulcigenin B (**9**), whose stereostructure in the α-methyl-γ-lactone moiety was identified by the observation of NOE correlation between the 23-proton and the 25-proton in the NOESY spectrum of **9**. Alkaline treatment of **9** with 5% sodium methoxide–methanol resulted in epimerization at the 25-position as well as deacetylation to give **10** and **11** in a 1 : 2 ratio. Both compounds (**10**, **11**) were also obtained by the same alkaline treatment of **7** in a 2 : 5 ratio. On the basis of this evidence, the absolute stereostructure of hovenidulcigenin B (**7**) was determined as shown.

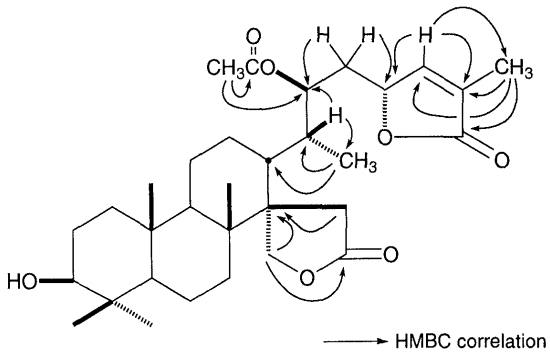


Fig. 1. Long-Range Correlations in the HMBC Spectrum of **6**

The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **3** showed signals due to the α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl moiety [δ 4.47 (d, $J=7.2$ Hz, 1′-H), 5.43 (br s, 1″-H)] together with the hovenidulcigenin B part. In the HMBC experiment of **3**, long-range correlations were observed between the 1′-H and the 3-C and between the 1″-H and the 2′-C. Hydrogenation of **1** with 5% palladium-charcoal yielded 25-*epi*-hovenidulcioside B (**12**). The stereostructure of **12** was determined on the basis of physicochemical evidence

Table 1. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **6**, **7**, **9**, **12**, and **13**

	1[a]	2[a]	3[a]	4[a]	6[b]	7[b]	9[a]	12[a]	13[a]
C-1	39.7	39.5	39.8	39.4	38.4	38.4	38.4	39.8	39.5
C-2	27.3	26.7	27.4	27.0	27.2	27.2	27.2	27.3	27.1
C-3	89.9	90.5	89.9	90.3	78.6	78.6	78.6	90.0	90.4
C-4	40.3	40.3	40.3	40.2	38.8	38.8	38.8	40.3	40.2
C-5	56.6	56.4	56.7	56.3	55.0	55.0	55.0	56.7	56.4
C-6	18.9	19.0	19.0	19.0	18.0	18.0	18.3	19.0	19.0
C-7	35.4	35.4	35.5	35.4	35.4	35.7	34.4	35.5	35.4
C-8	42.3	42.3	42.3	42.2	41.1	41.1	41.1	42.3	42.3
C-9	54.0	54.0	54.1	54.0	53.0	53.0	53.0	54.1	54.1
C-10	37.9	37.9	37.9	37.9	37.1	37.1	37.4	37.9	37.9
C-11	21.6	21.6	21.6	21.6	20.4	20.4	21.1	21.7	21.6
C-12	25.8	25.9	25.7	25.6	24.5	24.5	24.5	25.7	25.6
C-13	39.0	39.1	39.1	39.0	37.7	37.7	37.8	39.1	39.1
C-14	53.6	53.1	53.5	53.4	52.0	52.0	52.0	53.5	53.5
C-15	34.8	34.8	35.0	34.9	33.9	33.9	33.9	35.0	34.9
C-16	179.9	180.1	180.0	179.9	177.1	177.1	177.1	180.1	180.0
C-17	37.0	37.1	37.1	37.0	35.8	35.7	35.7	37.0	37.0
C-18	18.6	18.6	18.7	18.6	18.3	18.3	18.0	18.7	18.6
C-19	16.7	16.6	16.7	16.6	15.4	15.9	15.3	16.7	16.6
C-20	74.8	74.8	76.5	76.4	73.7	74.4	74.4	76.3	76.3
C-21	11.9	11.9	12.3	12.3	12.1	12.3	12.3	12.2	12.2
C-22	35.0	35.0	37.5	37.4	34.4	34.4	37.1	37.7	37.7
C-23	80.6	80.6	78.3	78.2	78.3	75.8	75.8	78.2	78.2
C-24	151.0	151.0	36.5	36.4	147.8	37.1	35.6	36.7	36.7
C-25	130.4	130.5	35.1	35.0	130.5	33.8	37.7	38.5	38.5
C-26	176.2	176.2	182.3	182.2	173.6	179.4	178.9	181.9	181.8
C-27	10.6	10.6	16.0	16.0	10.7	15.9	15.0	15.1	15.1
C-28	28.3	28.4	28.4	28.3	28.0	28.0	28.0	28.4	28.3
C-29	17.0	16.8	17.0	16.8	15.9	15.9	15.9	17.0	16.8
C-30	71.8	71.9	71.8	71.7	70.0	70.0	70.0	71.8	71.8
OAc	21.2	21.2	21.2	21.2	21.1	21.1	20.5	21.2	21.1
	172.4	172.5	172.8	172.4	170.7	170.9	170.9	172.7	172.7
Glc-1′	105.6	106.7	105.6	106.6				105.6	106.7
2′	78.9	75.7	78.9	75.6				78.9	75.7
3′	79.4	78.3	79.5	78.2				79.5	78.3
4′	72.0	71.7	72.1	71.6				72.1	71.7
5′	77.6	77.7	77.6	77.6				77.6	77.7
6′	62.8	62.8	62.8	62.8				62.8	62.8
Rha-1″	101.8		101.9					101.9	
2″	72.0		72.1					72.1	
3″	72.1		72.2					72.2	
4″	73.9		74.0					74.0	
5″	69.9		70.0					70.0	
6″	18.0		18.1					18.0	

The spectra were taken in *a*) CD_3OD or *b*) $CDCl_3$.

including the NOESY experiment, which showed correlation between the 23-proton and the 25-proton of **12**. Comparison of the spectral data for **3** with those for **1**, **7**, and **12** led us to formulate the absolute stereostructure of hovenidulcioside B_1 as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl] hovenidulcigenin B (**3**).

Hovenidulcioside B_2 (**4**), also obtained as colorless fine crystals of mp 133—136 °C, showed absorption bands due to hydroxyl, γ-lactone, and ester groups. Here again, the molecular formula $C_{38}H_{60}O_{12}$ was determined from its positive-mode FAB-MS [*m/z* 731 $(M+Na)^+$] and by high-resolution MS measurement. Enzymatic hydrolysis of **4** with β-D-glucosidase gave hovenidulcigenin B (**7**), while hydrogenation of hovenidulcioside A_2 (**2**) with 5% palladium–charcoal yielded 25-*epi*-hovenidulcioside B_2 (**13**), whose 25-configuration was determined by the NOESY experiment. The ^{1}H-NMR (CD_3OD) spectrum of **4** showed signals assignable to the β-D-glucopyranosyl moiety [δ 4.31 (d, *J*=7.2 Hz, 1′-H)] together with the hovenidulcigenin B part. By comparison of the ^{13}C-NMR (Table 1) for **4** with those for **3**, **7**, **13**, and methyl β-D-glucopyranoside, the stereostructure of hovenidulcioside B_2 was characterized as 3-*O*-β-D-glucopyranosyl hovenidulcigenin B (**4**).

Inhibitory Effects of Hovenidulciosides A_1 (1), A_2 (2), B_1 (3), and B_2 (4) on Histamine Release from Rat Peritoneal Exudate Cells Since Hoveniae Semen Seu Fructus has been used as an antidote in Chinese traditional medicine, the components of this natural medicine were expected to show antiallergic activity. As shown in Table 2, hovenidulciosides A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**) were found to inhibit the histamine release from rat peritoneal exudate cells induced by compound 48/80 and calcium ionophore A-23187. In the case of compound 48/80 induced histamine release, **2** and **3** showed a little more potent inhibitory activity than **1** and **4**. On the other hand, **2** and **4** showed inhibitory activity on histamine release induced by calcium ionophore A-23187, but **1** and **3** exhibited little effect.

The structures of hovenidulciosides (**1**—**4**) elucidated in this paper are characterized by a methyl-migrated 16,17-*seco*-dammarane type triterpene aglycone. To our knowledge, this is the first example of methyl-migrated 16,17-*seco*-dammarane type triterpene oligoglycosides confirming their absolute stereostructures. Since many dammarane type and 16,17-*seco*-dammarane type tri-

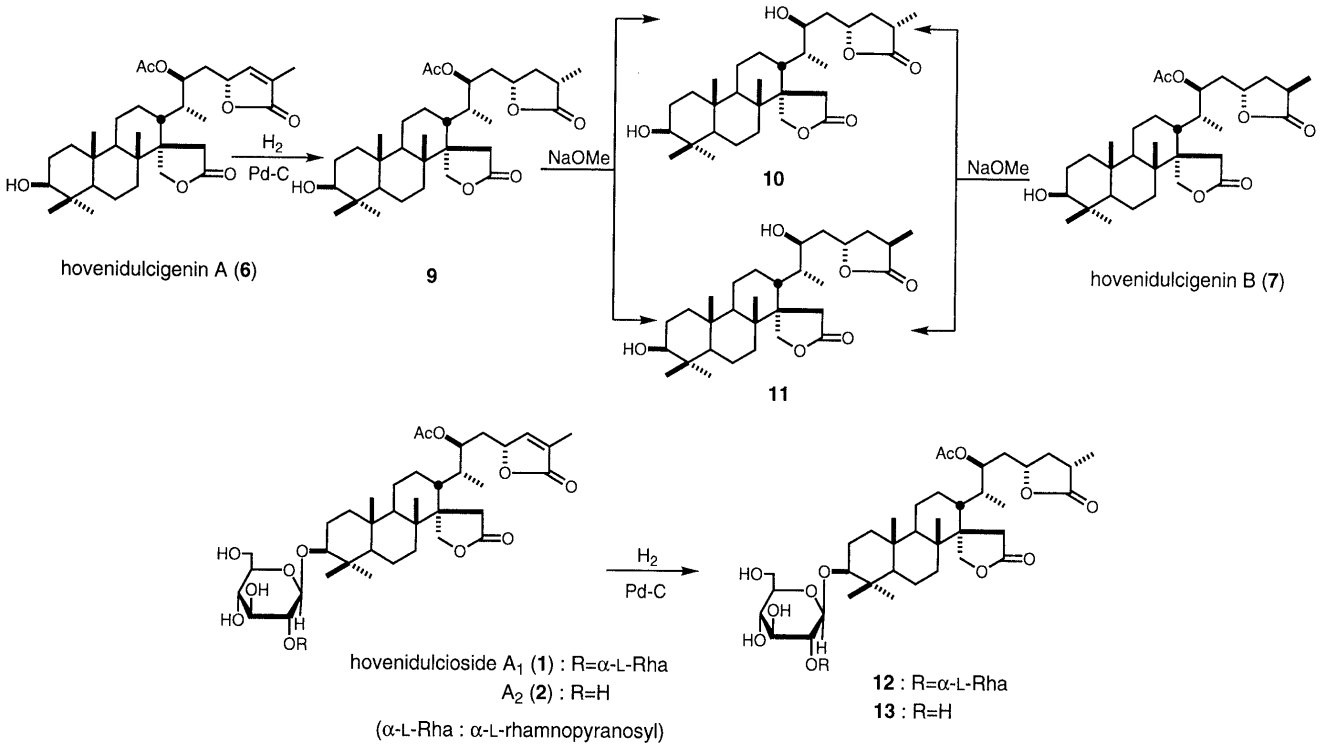


Chart 4

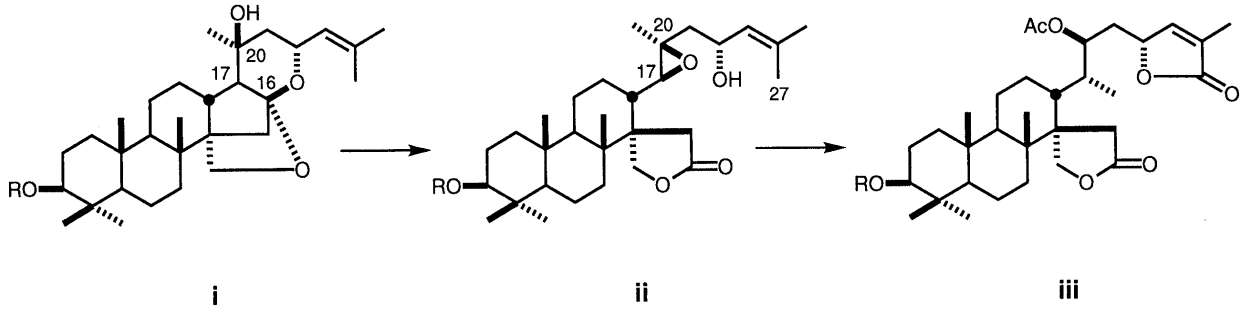


Chart 5

Table 2. Inhibitory Effects of Hovenidulciosides A_1 (**1**), A_2(**2**), B_1 (**3**), and B_2 (**4**) on Histamine Release from Rat Peritoneal Exudate Cells Induced by Compound 48/80 or Calcium Ionophore A-23187

	Compound 48/80	A-23187
Hovenidulcioside A_1 (**1**)	29.2±2.9	10.1±3.4
Hovenidulcioside A_2 (**2**)	53.2±1.1	48.2±2.3
Hovenidulcioside B_1 (**3**)	49.3±5.4	2.3±2.4
Hovenidulcioside B_2 (**4**)	33.2±5.8	42.4±2.4
Disodium cromoglycate	33.3±4.8	—
Tranilast	67.9±1.4	—

Each value represents the mean with standard error of 3—4 experiments. The numerical values denote the inhibition percentage of histamine release at 10^{-4} M.

terpene glycosides have been isolated from various Rhamnaceae plants including the leaves and roots of *Hovenia dulcis*, the carbon skeleton (iii) of hovenidulcigenins was presumed to be constructed from dammarane (i) type triterpene *via* 16,17-*seco*-dammarane type triterpene (ii) through cleavage of the 16,17-bond, migration of the 20-methyl group, and oxidation of the 26-methyl group; the existence of other triterpene glycosides with methyl-migrated 16,17-*seco*-dammarane structure is assumed.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[1,3]

Isolation of Hovenidulciosides A_1 (1), A_2 (2), B_1 (3), and B_2 (4) from Hoveniae Semen Seu Fructus Hoveniae Semen Seu Fructus (8 kg, purchased from Tochimoto Tenkaido Co., Ltd., Osaka) was cut finely and extracted with MeOH under reflux three times. Evaporation of the solvent from the extract under reduced pressure gave the MeOH extract (1226 g, 15.3% yield from the natural medicine). The MeOH extract (1226 g) was subjected to reversed-phase silica gel column chromatography [Silica gel 60 silanized (Merck, 4 kg), $H_2O \rightarrow$MeOH] followed by evaporation to furnish the MeOH eluate (200 g, 2.5%). Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 3 kg), $CHCl_3$–MeOH (10:1→5:1→1:1) MeOH] of the MeOH eluate (85 g) afforded nine fractions [fraction 1 (22.5 g), 2 (4.6 g), 3 (9.7 g), 4 (1.1 g), 5 (1.6 g), 6 (1.7 g), 7 (1.0 g), 8 (3.3 g), 9 (28.2 g)]. Fraction 6 (1.7 g) was purified by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical, Ltd.), MeOH–H_2O (v/v, 5:1→7:3)] and HPLC [YMC-Pack ODS-A (YMC Co. Ltd.), MeOH–H_2O (v/v, 7:3)] separation to give hovenidulciosides A_2 (**2**, 48 mg, 0.0014%) and B_2 (**4**, 62 mg, 0.0018%). Normal-phase [$CHCl_3$–MeOH (v/v, 7:1→3:1)] and reversed-phase silica gel column chromatography [MeOH–H_2O (v/v, 1:1)] of fraction 7 (1.0 g) followed by HPLC [YMC-Pack ODS-A, MeOH–H_2O (v/v, 65:35 or 15:85)] separation furnished hovenidulciosides A_1 (**1**, 146 mg, 0.0043%) and B_1 (**3**, 139 mg, 0.0041%) and (+)-gallocatechin (22 mg, 0.0006%). Fraction 8 (3.3 g) was purified by silica gel column chromatography [$CHCl_3$–MeOH (v/v, 2:1)] and subsequent HPLC [YMC-Pack ODS-A, MeOH–H_2O (v/v, 75:25)] separation to give hoduloside III (**5**, 41 mg, 0.0012%). The physical data for the known compounds were identical with reported values.[2i)]

Hovenidulcioside A_1 (**1**): Colorless fine crystals from aqueous MeOH, mp 183—186 °C, $[\alpha]_D^{26}$ −48.5° (c=0.5, MeOH). High-resolution negative-mode FAB-MS : Calcd for $C_{44}H_{67}O_{16}$ $(M-H)^-$: 851.4429; Found: 851.4442. UV λ_{max}^{MeOH} nm (log ε): 224 (3.8). IR (KBr) cm^{-1}: 3453, 2944, 1765, 1751, 1735, 1602, 1046. ^{1}H-NMR (CD_3OD) δ: 0.92, 0.93, 1.06, 1.12 (3H each, all s, 29, 19, 18, 28-H_3), 0.97 (3H, d, J=6.9 Hz, 21-H_3), 1.27 (3H, d, J=5.9 Hz, 6″-H_3), 1.91 (3H, dd, J=1.6, 1.7 Hz, 27-H_3), 2.12 (3H, s, Ac-2-H_3), 2.49, 2.71 (2H, ABq, J=19.0 Hz, 15-H_2), 3.21 (1H, m, 3-H), 4.34, 4.53 (2H, ABq, J=10.7 Hz, 30-H_2), 4.46 (1H, d, J=7.3 Hz, 1′-H), 4.75 (1H, t-like, 20-H), 5.13 (1H, br s, 23-H), 5.43 (1H, br s, 1″-H), 7.34 (1H, br s, 24-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (m/z): 851 $(M-H)^-$, Positive-mode FAB-MS (m/z): 875 $(M+Na)^+$.

Hovenidulcioside A_2 (**2**): Colorless fine crystals from aqueous MeOH, mp 157—160 °C, $[\alpha]_D^{27}$ −14.0° (c=0.1, MeOH). High-resolution positive-mode FAB-MS : Calcd for $C_{38}H_{58}NaO_{12}$ $(M+Na)^+$: 729.3826; Found: 729.3832. UV λ_{max}^{MeOH} nm (log ε): 234 (3.8). IR (KBr) cm^{-1}: 3432, 1768, 1750, 1734, 1600, 1034. ^{1}H-NMR (CD_3OD) δ: 0.84, 0.87, 0.99, 1.06 (3H each, all s, 29, 19, 18, 28-H_3), 0.92 (3H, d, J=7.2 Hz, 21-H_3), 1.84 (3H, dd-like, 27-H_3), 2.05 (3H, s, Ac-2-H_3), 2.43, 2.64 (2H, ABq, J=19.0 Hz, 15-H_2), 3.18 (1H, m, 3-H), 4.26, 4.47 (2H, ABq, J=10.5 Hz, 30-H_2), 4.31 (1H, d, J=7.6 Hz, 1′-H), 4.68 (1H, m, 20-H), 5.06 (1H, br s, 23-H), 7.27 (1H, br s, 24-H). ^{13}C-NMR : given in Table 1. Positive-mode FAB-MS (m/z): 729 $(M+Na)^+$.

Hovenidulcioside B_1 (**3**): Colorless fine crystals from aqueous MeOH, mp 177—180 °C, $[\alpha]_D^{27}$ −21.5° (c=0.3, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{44}H_{69}O_{16}$ $(M-H)^-$: 853.4571; Found: 853.4578. IR (KBr) cm^{-1}: 3432, 2944, 1773, 1736, 1048. ^{1}H-NMR (CD_3OD) δ: 0.92, 0.93, 1.06, 1.12 (3H each, all s, 29, 19, 18, 28-H_3), 0.98 (3H, d, J=6.9 Hz, 21-H_3), 1.27 (3H, d, J=5.6 Hz, 6″-H_3), 1.29 (3H, d, J=7.3 Hz, 27-H_3), 2.08, 2.27 (1H each, both m, 24-H_2), 2.15 (3H, s, Ac-2-H_3), 2.45, 2.75 (2H, ABq, J=18.8 Hz, 15-H_2), 2.72 (1H, m, 25-H), 3.23 (1H, m, 3-H), 4.38, 4.54 (2H, ABq, J=10.9 Hz, 30-H_2), 4.47 (1H, d, J=7.2 Hz, 1′-H), 4.66 (1H, m, 23-H), 4.88 (1H, m, 20-H), 5.43 (1H, br s, 1″-H). ^{13}C-NMR : given in Table 1. Negative-mode FAB-MS (m/z): 853 $(M-H)^-$, Positive-mode FAB-MS (m/z): 877 $(M+Na)^+$.

Hovenidulcioside B_2 (**4**): Colorless fine crystals from aqueous MeOH, mp 133—136 °C, $[\alpha]_D^{25}$ −6.0° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{38}H_{60}NaO_{12}$ $(M+Na)^+$: 731.3983; Found: 731.3954. IR (KBr) cm^{-1}: 3432, 1773, 1734, 1043. ^{1}H-NMR (CD_3OD) δ: 0.84, 0.86, 0.98, 1.06 (3H each, all s, 29, 19, 18, 28-H_3), 0.91 (3H, d, J=5.9 Hz, 21-H_3), 1.22 (3H, d, J=7.2 Hz, 27-H_3), 2.03, 2.19 (1H each, both m, 24-H_2), 2.08 (3H, s, Ac-2-H_3), 2.41, 2.66 (2H, ABq, J=18.8 Hz, 15-H_2), 2.73 (1H, m, 25-H), 3.19 (1H, m, 3-H), 4.30, 4.46 (2H, ABq, J=10.5 Hz, 30-H_2), 4.31 (1H, d, J=7.2 Hz, 1′-H), 4.58 (1H, m, 23-H), 4.86 (1H, m, 20-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (m/z): 731 $(M+Na)^+$.

Methanolysis of Hovenidulcioside A_1 (1) A solution of **1** (1.0 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the insoluble portion was removed by filtration. After evaporation 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, 0.02 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i) and methyl rhamnoside (ii). GLC conditions: column: CBR-M25-025, 0.25 mm (i.d.) × 25 m capillary column; column temperature: 140—280 °C; He flow rate 15 ml/min: t_R: i, 17.8, 18.2, 19.2, ii, 11.5, 13.9.

Enzymatic Hydrolysis of Hovenidulcioside A_1 (1) Giving Hovenidulcigenin A (6) A solution of **1** (57.6 mg) in 0.1 M acetate buffer (pH 3.8, 15.0 ml) was treated with naringinase (1.2 g, Sigma Chemical Co.) and the whole mixture was stirred at 40 °C for 72 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous NaCl and then dried over $MgSO_4$ powder. Removal of the solvent from the AcOEt extract under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [3 g, $CHCl_3$–MeOH (20:1)] to give hovenidulcigenin A (**6**, 34.2 mg, 96.2%).

Hovenidulcigenin A (**6**): Colorless fine crystals from $CHCl_3$–MeOH, mp 230—233 °C, $[\alpha]_D^{27}$ −23.1° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{32}H_{49}O_7$ $(M+H)^+$: 545.3478; Found: 545.3504. IR (KBr) cm^{-1}: 3439, 1771, 1736, 1655, 1042. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.82, 0.96, 0.99 (3H each, all s, 29, 19, 18, 28-H_3), 0.89 (3H, d, J=6.9 Hz, 21-H_3), 1.91 (3H, br s, 27-H_3), 2.09 (3H, s, Ac-2-H_3), 2.34, 2.59 (2H, ABq, J=18.8 Hz, 15-H_2), 3.19 (1H, dd, J=5.2, 11.2 Hz, 3-H), 4.23, 4.38 (2H, ABq, J=10.3 Hz, 30-H_2), 4.77 (1H, t-like, 20-H), 4.88 (1H, br s, 23-H), 7.09 (1H, br s, 24-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (m/z): 543 $(M-H)^-$. Positive-mode FAB-MS (m/z): 545 $(M+H)^+$.

***p*-Bromobenzoylation of Hovenidulcigenin A (6)** A solution of **6** (3.2 mg) in dry CH_2Cl_2 (0.5 ml) was treated with *p*-bromobenzoic acid (42.2 mg, 30 eq) in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (40.3 mg, 30 eq) and 4-dimethylaminopyridine (15.4 mg, 18 eq) and the whole mixture was stirred at room temperature (28 °C) for 4 h. It was poured into saturated aqueous NaCl and the whole was extracted with AcOEt. The AcOEt extract was

successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and NaCl, and then dried over $MgSO_4$ powder. Removal of the solvent from the AcOEt extract reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [1.5 g, hexane–AcOEt (2:1→1:1)] to give 3-*O*-*p*-bromobenzoyl hovenidulcigenin A (**6a**, 2.7 mg, 62.2%).

6a: A white powder, $[\alpha]_D^{25}$ −21.4° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{39}H_{51}BrNaO_8$ $(M+H)^+$: 749.2665; Found: 749.2715. ^{1}H-NMR ($CDCl_3$) δ: 0.90, 0.93, 0.98, 1.00 (3H each, all s, 29, 19, 18, 28-H_3), 0.90 (3H, d-like, 21-H_3), 1.91 (3H, dd, J=1.6, 2.0 Hz, 27-H_3), 2.09 (3H, s, Ac-2-H_3), 2.37, 2.60 (2H, ABq, J=18.7 Hz, 15-H_2), 4.25, 4.39 (2H, ABq, J=10.5 Hz, 30-H_2), 4.68 (1H, t-like, 3-H), 4.78 (1H, t-like, 20-H), 4.89 (1H, br s, 23-H), 7.08 (1H, br s, 24-H), 7.58 (2H, dd, J=1.7, 6.8 Hz, 2′, 6′-H), 7.89 (2H, dd, J=1.7, 6.8 Hz, 3′, 5′-H).

Enzymatic Hydrolysis of Hovenidulcioside A_2 (2) Giving Hovenidulcigenin A (6) A solution of **2** (10.5 mg) of 0.1 M acetate buffer (pH 4.4, 2.0 ml) was treated with β-D-glucosidase (10.0 mg, Wako Chemical Co., Ltd.) and the whole mixture was stirred at 38 °C for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$ powder and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by normal-phase silica gel column chromatography [1g, $CHCl_3$–MeOH (20:1)] to give hovenidulcigenin A (**6**, 6.8 mg), which was identified with an authentic sample obtained from **1** by spectral data.

Methanolysis of Hovenidulcioside B_1 (3) A solution of **3** (1.0 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. 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 BSTFA (0.02 ml) for 1 h. The reaction solution was analyzed by GLC (the same conditions as described above) to identify the trimethylsilyl (TMS) derivatives of i and ii.

Enzymatic Hydrolysis of Hovenidulcioside B_1 (3) Giving Hovenidulcigenin B (7) A solution of **3** (69.0 mg) in 0.1 M acetate buffer (pH 3.8, 15.0 ml) was treated with naringinase (2.0 g) and the whole mixture was stirred at 40 °C for 72 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous NaCl and then dried over $MgSO_4$ powder. Removal of the solvent from the AcOEt extract under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [3 g, $CHCl_3$–MeOH (20:1)] to give hovenidulcigenin B (**7**, 32.6 mg, 76.5%).

Hovenidulcigenin B (**7**): Colorless fine crystals from $CHCl_3$–MeOH, mp 205—208 °C, $[\alpha]_D^{27}$ +4.1° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{32}H_{51}O_7$ $(M+H)^+$: 547.3634; Found: 547.3605. IR (KBr) cm^{-1}: 3432, 1775, 1734, 1038. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.83, 0.95, 0.99 (3H each, all s, 29, 19, 18, 28-H_3), 0.99 (3H, d, J=7.2 Hz, 21-H_3), 1.27 (3H, d, J=7.2 Hz, 27-H_3), 2.10 (3H, s, Ac-2-H_3), 2.36, 2.60 (2H, ABq, J=18.6 Hz, 15-H_2), 2.67 (1H, m, 25-H), 3.19 (1H, dd, J=5.2, 11.2 Hz, 3-H), 4.24, 4.38 (2H, ABq, J=10.4 Hz, 30-H_2), 4.50 (1H, dd, J=6.3, 6.6 Hz, 23-H), 4.80 (1H, ddd, J=1.7, 9.3, 11.0 Hz, 20-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (m/z): 545 $(M-H)^-$. Positive-mode FAB-MS (m/z): 547 $(M+H)^+$.

Hydrogenation of Hovenidulcigenin A (6) Giving 25-*epi*-Hovenidulcigenin B (9) A solution of **6** (8.2 mg) in MeOH (2.0 ml) was stirred under an H_2 atmosphere in the presence of 5% Pd–C (30.0 mg) at room temperature (27 °C) for 2 h and the catalytic agent was removed by filtration. Removal of the solvent from the filtrate under reduced pressure provided 25-*epi*-hovenidulcigenin B (**9**, 6.6 mg).

25-*epi*-Hovenidulcigenin B (**9**): Colorless fine crystals from MeOH, mp 188—189 °C, $[\alpha]_D^{29}$ −23.3° (c=0.2, $CHCl_3$). High-resolution negative-mode FAB-MS: Calcd for $C_{32}H_{49}O_7$ $(M-H)^-$: 545.3478; Found: 545.3484. IR (KBr) cm^{-1}: 3453, 2930, 1775, 1734, 1032. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.83, 0.96, 0.99 (3H each, all s, 29, 19, 18, 28-H_3), 0.90 (3H, d, J=6.9 Hz, 21-H_3), 1.26 (3H, d, J=6.6 Hz, 27-H_3), 2.10 (3H, s, Ac-2-H_3), 2.35, 2.60 (2H, ABq, J=18.5 Hz, 15-H_2), 3.19 (1H, dd, J=5.3, 11.3 Hz, 3-H), 4.24, 4.38 (2H, ABq, J=10.5 Hz, 30-H_2), 4.51 (1H, m, 23-H), 4.80 (1H, m, 20-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (m/z): 545 $(M-H)^-$.

Enzymatic Hydrolysis of Hovenidulcioside B_2 (4) Giving Hovenidulcigenin B (7) A solution of **4** (9.8 mg) of 0.1 M acetate buffer (pH 4.4, 2.0 ml) was treated with β-D-glucosidase (10.0 mg, Wako Chemical Co., Ltd.) and the whole mixture was stirred at 38 °C for 12 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous NaCl and then dried over $MgSO_4$ powder. Removal of the solvent from the AcOEt extract under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [1 g, $CHCl_3$–MeOH (20:1)] to give hovenidulcigenin B (**7**, 6.0 mg, 84.5%). Thus obtained **7** was identified by comparison of the physical data with an authentic sample from **3**.

Alkaline Treatment of 25-*epi*-Hovenidulcigenin B (9) and Hovenidulcigenin B (7) Giving 10 and 11 A solution of **9** (3.0 mg) or **7** (2.5 mg) in 5% NaOMe–MeOH (0.5 ml) was stirred at room temperature (28 °C) for 1 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous NaCl and dried over Na_2SO_4. Evaporation of the solvent from the filtrate under reduced pressure gave a residue (2.7 mg from **9**, 2.6 mg from **7**), which was subjected to HPLC [YMC-Pack ODS-A-5, MeOH–H_2O (v/v, 85:15)] to give **10** (0.8 mg) and **11** (1.6 mg) from **9** and **10** (0.6 mg) and **11** (1.5 mg) from **7**.

10: Colorless fine crystals from MeOH–H_2O, mp 239—241 °C, $[\alpha]_D^{26}$ +23.0° (c=0.6, $CHCl_3$). High-resolution negative-mode FAB-MS: $C_{30}H_{47}O_6$ $(M-H)^-$: 503.3373; Found: 503.3374. IR (KBr) cm^{-1}: 3474, 2924, 1771, 1728, 1040. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.83, 0.99, 1.01 (3H each, all s, 29, 19, 18, 28-H_3), 0.87 (3H, d, J=6.9 Hz, 21-H_3), 1.30 (3H, d, J=7.2 Hz, 27-H_3), 2.38, 2.58 (2H, ABq, J=18.8 Hz, 15-H_2), 3.20 (1H, dd, J=4.6, 10.9 Hz, 3-H), 3.50 (1H, m, 20-H), 4.29, 4.39 (2H, ABq, J=10.1 Hz, 30-H_2), 4.70 (1H, m, 23-H). Negative-mode FAB-MS (m/z): 503 $(M-H)^-$.

11: Colorless fine crystals from MeOH–H_2O, mp 265—266 °C, $[\alpha]_D^{28}$ +11.9° (c=0.1, $CHCl_3$). High-resolution negative-mode FAB-MS: $C_{30}H_{47}O_6$ $(M-H)^-$: 545.3478; Found: 545.3460. IR (KBr) cm^{-1}: 3494, 2923, 1769, 1721, 1034. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.84, 0.99, 1.01 (3H each, all s, 29, 19, 18, 28-H_3), 0.88 (3H, d, J=7.0 Hz, 21-H_3), 1.28 (3H, d, J=6.9 Hz, 27-H_3), 2.38, 2.58 (2H, ABq, J=19.0 Hz, 15-H_2), 3.20 (1H, dd, J=5.3, 11.2 Hz, 3-H), 3.50 (1H, m, 20-H), 4.29, 4.39 (2H, ABq, J=10.1 Hz, 30-H_2), 4.51 (1H, m, 23-H). Negative-mode FAB-MS (m/z): 503 $(M-H)^-$.

Hydrogenation of Hovenidulciosides A_1 (1) and A_2 (2) by Palladium Charcoal (5%) A solution of **1** (10.3 mg) or **2** (10.0 mg) in MeOH (2.0 ml) was stirred under an H_2 atmosphere in the presence of 5% Pd–C (30.0 mg) at room temperature (28 °C) for 2 h and filtered. Then, removal of the solvent from the filtrate under reduced pressure yielded 25-*epi*-hovenidulcioside B_1 (**12**, 8.7 mg) from **1** and 25-*epi*-hovenidulcioside B_2 (**13**, 8.9 mg) from **2**.

25-*epi*-Hovenidulcioside B_1 (**12**): Colorless fine crystals from MeOH, mp 254—255 °C, $[\alpha]_D^{25}$ −27.6° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{44}H_{70}NaO_{16}$ $(M+Na)^+$: 877.4562; Found: 877.4557. IR (KBr) cm^{-1}: 3432, 2946, 1775, 1736, 1043. ^{1}H-NMR (CD_3OD) δ: 0.78, 0.83, 0.96, 0.99 (3H each, all s, 29, 19, 18, 28-H_3), 0.92 (3H, d, J=7.0 Hz, 21-H_3), 1.20 (6H, d, J=6.6 Hz, 27-H_3, 6″-H_3), 2.08 (3H, s, Ac-2-H_3), 2.41, 2.67 (2H, ABq, J=19.1 Hz, 15-H_2), 3.15 (1H, m, 3-H), 4.32, 4.48 (1H, ABq, J=10.0 Hz, 30-H_2), 4.41 (1H, d, J=6.9 Hz, 1′-H), 4.42 (1H, m, 23-H), 4.82 (1H, m, 20-H), 5.37 (1H, br s, 1″-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (m/z): 877 $(M+Na)^+$.

25-*epi*-Hovenidulcioside B_2 (**13**): Colorless fine crystals from MeOH–H_2O, mp 130—131 °C, $[\alpha]_D^{27}$ −14.3° (c=0.6, $CHCl_3$). High-resolution positive-mode FAB-MS: Calcd for $C_{38}H_{60}NaO_{12}$ $(M+Na)^+$: 731.3982. Found: 731.3981. IR (KBr) cm^{-1}: 3453, 2928, 1773, 1734, 1038. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.87, 0.99, 1.06 (3H each, all s, 29, 19, 18, 28-H_3), 0.92 (3H, d, J=7.0 Hz, 21-H_3), 1.20 (3H, d, J=7.0 Hz, 27-H_3), 2.08 (3H, s, Ac-2-H_3), 2.41, 2.66 (2H, ABq, J=18.8 Hz, 15-H_2), 3.18 (1H, m, 3-H), 4.31 (1H, d, J=7.9 Hz, 1′-H), 4.32, 4.47 (2H, ABq, J=10.6 Hz, 30-H_2), 4.43 (1H, m, 23-H), 4.82 (1H, m, 20-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (m/z): 731 $(M+Na)^+$.

Histamine Release from Rat Peritoneal Exudate Cells The method of bioassay was basically the same as described in the previous report.[3b,d,f] Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 300—400 g were exsanguinated and injected intraperitoneally with 10 ml of physiological solution consisting of NaCl (154 mM), KCl (2.7 mM), $CaCl_2$ (0.9 mM), glucose (5.6 mM), and HEPES (Dotite, 5 mM, pH 7.4). The abdominal region was gently massaged for 2 min and then the 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 (10^4 cells/ml) was mixed with test compounds (**1**, **2**, **3**, **4**) and the mixture was preincubated at 37 °C

for 15 min. Then, compound 48/80 or calcium ionophore A-23187 was added and the whole mixture was incubated at 37 °C for 10 min. After cooling, the amount of histamine released into the reaction mixture was measured by the method of Imada *et al.*[14] The results are summarized in Table 2.

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11) A preliminary communication[10] deals with the structure elucidation of hovenidulciosides A_1 (**1**) and A_2 (**2**) and the X-ray crystallographic data of *p*-bromobenzoyl hovenidulcigenin A (**6a**).
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13) The ^{1}H-NMR and ^{13}C-NMR data were assigned on the basis of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), homonuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA), NOESY, and HMBC.
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