ABSOLUTE STEREOSTRUCTURES OF HOVENIDULCIOSIDES A_1 AND A_2, BIOACTIVE NOVEL TRITERPENE GLYCOSIDES FROM HOVENIAE SEMEN SEU FRUCTUS, THE SEEDS AND FRUIT OF *HOVENIA DULCIS* THUNB.

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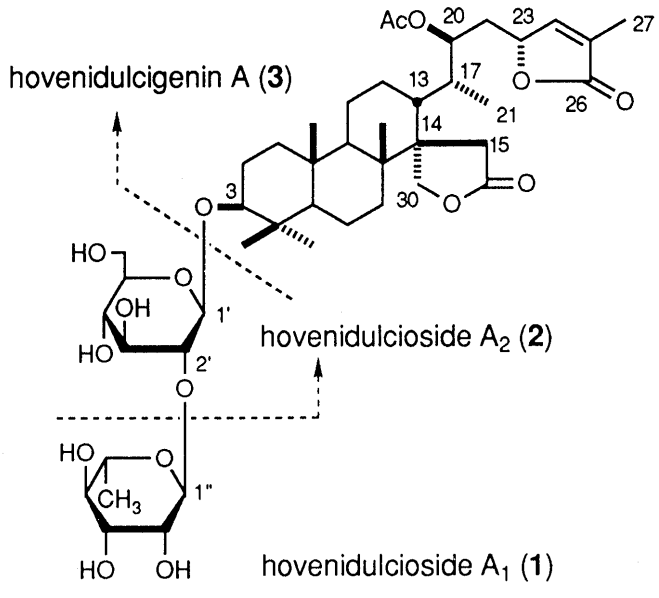
Two bioactive novel triterpene glycosides named hovenidulciosides A_1 and A_2 have been isolated from a Chinese natural medicine, Hoveniae Semen Seu Fructus, the seeds and fruit of *Hovenia dulcis* Thunb. (Rhamnaceae). The absolute stereostructures of hovenidulciosides A_1 and A_2 with a migrated 16,17-*seco*-dammarane skeleton have been determined on the basis of chemical and physicochemical evidence which included the X-ray crystallographic analysis of the *p*-bromobenzoate of their common aglycone, hovenidulcigenin A. Hovenidulciosides A_1 and A_2 exhibited inhibitory activity on the histamine release from rat mast cells induced by compound 48/80 or calcium ionophore A-23187.

KEY WORDS hovenidulcioside ; hovenidulcigenin A ; migrated 16,17-*seco*-dammarane triterpene ; Hoveniae Semen Seu Fructus ; *Hovenia dulcis* ; histamine release inhibitor

Hoveniae Semen Seu Fructus (Japanese name "Kigushi"), the seeds and fruit of *Hovenia dulcis* Thunb. (Japanese name "Kenponashi," Rhamnaceae), has been used as a diuretic and antidote in Chinese traditional medicine. In the chemical study of *Hovenia dulcis*, several dammarane and 16,17-*seco*-dammarane type triterpene glycosides have been characterized from the root cortex and leaves of this plant.[1] In the course of our studies on the bioactive constituents in natural medicine,[2] we have found many oleanene type triterpene glycosides which exhibit potent inhibitory effect on ethanol and sugar absorption in the digestive tract.[3] As a continuing part of our screening for bioactive saponins, novel triterpene glycosides named hovenidulciosides A_1 (**1**) and A_2 (**2**) were isolated from Hoveniae Semen Seu Fructus. This communication deals with the absolute stereostructures of **1** and **2** and their inhibitory effect on the histamine release from the rat mast cells induced by compound 48/80 or calcium ionophore A-23187.[4]



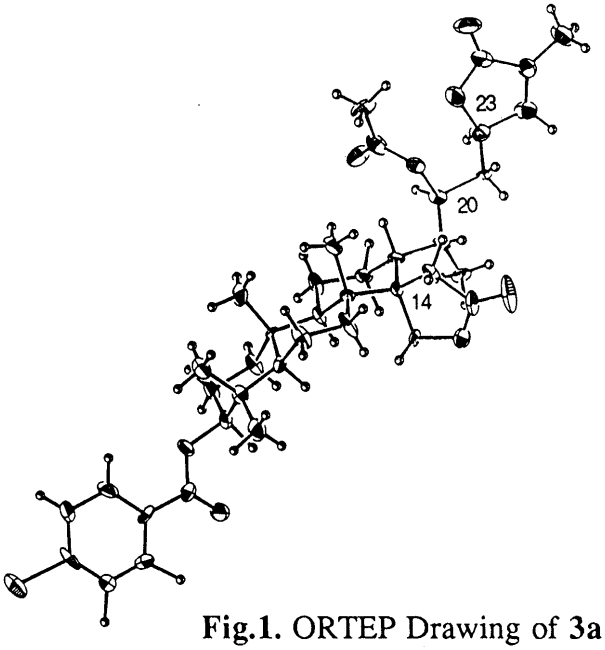


Fig.1. ORTEP Drawing of **3a**

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Table I. ^{13}C NMR Data for Hovenidulciosides A_1 (**1**) and A_2 (**2**), and Hoveniadulcigenin A (**3**) (68MHz)

	1[a]	2[a]	3[b]		1	2	3
C-1	39.7	39.5	38.4	C-23	80.6	80.6	78.3
C-2	27.3	26.7	27.2	C-24	151.0	151.0	147.8
C-3	89.9	90.5	78.6	C-25	130.4	130.5	130.5
C-4	40.3	40.3	38.8	C-26	176.2	176.2	173.6
C-5	56.6	56.4	55.0	C-27	10.6	10.6	10.7
C-6	18.9	19.0	18.0	C-28	28.3	28.4	28.0
C-7	35.4	35.4	35.4	C-29	17.0	16.8	15.9
C-8	42.3	42.3	41.1	C-30	71.8	71.9	70.0
C-9	54.0	54.0	53.0	OAc	21.2	21.2	21.1
C-10	37.9	37.9	37.1		172.4	172.5	170.7
C-11	21.6	21.6	20.4	Glu-1	105.6	106.7	
C-12	25.8	25.9	24.5	2	78.9	75.7	
C-13	39.0	39.1	37.7	3	79.4	78.3	
C-14	53.6	53.1	52.0	4	72.0	71.7	
C-15	34.8	34.8	33.9	5	77.6	77.7	
C-16	179.9	180.1	177.1	6	62.8	62.8	
C-17	37.0	37.1	35.8	Rha-1	101.8		
C-18	18.6	18.6	18.3	2	72.0		
C-19	16.7	16.6	15.4	3	72.1		
C-20	74.8	74.8	73.7	4	73.9		
C-21	11.9	11.9	12.1	5	69.9		
C-22	35.0	35.0	34.4	6	18.0		

The spectra were taken in CD_3OD[a] or $CDCl_3$[b].

The MeOH extract of Hoveniae Semen Seu Fructus was subjected to reversed phase SiO_2 column (silica gel 60 silanised, H_2O-MeOH) chromatography; then the MeOH-eluted fraction was purified by repeated ordinary SiO_2 ($CHCl_3$-MeOH) and reversed phase SiO_2 (chromatorex-ODS DM1020T, H_2O-MeOH) column chromatography and finally HPLC (YMC-Pack ODS-A, H_2O-MeOH) to give hovenidulciosides A_1 (**1**, 0.0043% from the natural medicine), A_2 (**2**, 0.0014%), B_1 (0.0041%), and B_2 (0.0018%) together with hoduloside III (0.0012%).[1d]

Hovenidulcioside A_1 (**1**), colorless fine crystals, mp 183~186°C, $[\alpha]_D$ -48.5° (MeOH), $C_{44}H_{68}O_{16}$, UV [MeOH (log ε)] : 224 nm (3.8), IR (KBr) : 3453, 2944, 1765, 1751, 1735 cm^{-1}, negative FAB-MS (m/z) : 851 $(M-H)^-$, positive FAB-MS (m/z) : 875 $(M+Na)^+$, liberated methyl D-glucoside and methyl L-rhammnoside upon methanolysis with 9% HCl-MeOH. On the enzymatic hydrolysis of **1** with naringinase, a genuine aglycon, hovenidulcigenin A (**3**), colorless fine crystals, mp 230~233°C, $[\alpha]_D$ -23.1° (MeOH), $C_{32}H_{48}O_6$, IR (KBr) : 3439, 2962, 1771, 1736 cm^{-1}, was obtained in 97% yield.

The ^1H NMR (**1** : CD_3OD ; **3** : $CDCl_3$) and ^{13}C NMR (Table I) spectra of **1** and **3**, which were assigned by COSY (^1H-^1H, ^1H-^{13}C), HMBC, and HOHAHA (^1H-^1H, ^1H-^{13}C), indicated the presence of four *tert.* methyls [**1** : δ 0.92 (29-H_3), 0.93 (19-H_3), 1.06 (18-H_3), 1.12 (28-H_3) ; **3** : δ 0.78 (29-H_3), 0.82 (19-H_3), 0.96 (18-H_3), 0.99 (28-H_3)], a *sec.* methyl [**1** : δ 0.97 (d, J=6.9) ; **3** : δ 0.89 (d, J=6.9) (21-H_3)], an acetyl methyl [**1** : δ 2.12 ; **3** : δ 2.09 (20-OAc)], a methine-bearing glycosyl residue or hydroxyl group [**1** ; δ 3.21 (m) ; **3** : δ 3.19 (dd, J=5.2, 11.2) (3-H)], a γ-lactone [**1** : δ 2.49, 2.71 (ABq, J=19.0, 15-H_2), 4.34, 4.53 (ABq, J=10.7, 30-H_2) ; **3** : δ 2.34, 2.59 (ABq, J=18.8, 15-H_2), 4.23, 4.38 (ABq, J=10.3, 30-H_2)], and α-methyl butenolide [**1** : δ 1.91 (dd, J=1.6, 1.7, 27-H_3), 5.13 (br s, 23-H), 7.34 (br s, 24-H) ; **3** : 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 moieties of **1** and **3** were found to be very similar to those of hovenolactone,[1] which was the common aglycone of various 16,17-*seco*-dammarane type triterpene glycosides obtained from the leaves of *Hovenia dulcis*, excepting the signals due to the side chain moiety.

The side chain moiety with a *sec.* methyl, an acetoxyl, and α-methyl-26,23-butenolide group of **1** was confirmed from the HMBC experiment. Namely, long-range correlations were obtained between the following carbons and protons of **1** (17-H & 20, 21-C; 21-H_3 & 13, 17-C; 22-H_2 & 20, 23-C; 24-H & 23, 25, 27-C; 27-H_3 & 24, 25, 26-C). Based on these findings, it appeared that **1** had the novel triterpene structure with the methyl group migrated from 20-C to 17-C in the 16,17-*seco*-dammarane triterpene.[5]

In order to substantiate the presumption and to establish the absolute stereostructure of the aglycon part of **1**, the X-ray crystallographic analysis of 3-*O*-*p*-bromobenzoyl hovenidulcigenin A (**3a**), which was prepared from **3** by the condensation with *p*-bromobenzoic acid in the presence of 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride and 4-dimethylaminopyridine in CH_2Cl_2, was carried out (Fig. 1). The crystal data are as follows :

Table II. Inhibitory Effects of Hovenidulciosides A_1 (**1**) and A_2 (**2**) on Histamine Release from Rat Mast 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

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

$C_{39}H_{51}O_8Br$, M=727.73, monoclinic, a=14.059 (3), b=7.683 (4), c=16.767 (4)Å, β=95.78 (2)°, V=1802 (1)Å^3, Z=2, space group $P2_1$ (#4), Dc=1.341g/cm^3.[6]

Finally, based on comparison of the ^{13}C NMR spectra for the oligosaccharide moiety of **1** with those for the saponins having α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl moiety[1] and observation of the HMBC correlation between the following protons and carbons of **1** (1"-H & 2'-C; 1'-H & 3-C), the total structure of hovenidulcioside A_1 (**1**) was determined as shown.

Hovenidulcioside A_2 (**2**), colorless fine crystals, mp 157~160°C, $[\alpha]_D$ -14.0° (MeOH), $C_{38}H_{58}O_{12}$, UV [MeOH (log ε)] : 234 nm (3.8), IR (KBr) : 3432, 1768, 1750, 1734 cm^{-1}, liberated hovenidulcigenin A (**3**) on enzymatic hydrolysis with β-D-glucosidase. Comparison of the ^1H NMR [7] and ^{13}C NMR (Table I) for **2** with those of **1** and **3** led us to determine the structure of hovenidulcioside A_2 (**2**).

Inhibitory effects of hovenidulciosides A_1 (**1**) and A_2 (**2**) on the histamine release from rat peritoneal mast cells induced by compound 48/80 and calcium ionophore A-23187 are summarized in Table II. Both hovenidulciosides A_1 (**1**) and A_2 (**2**) were found to exhibit activity inhibiting histamine release.

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5) a) Recently a methyl migrated 16,17-*seco*-dammarane triterpene was obtained from the stems of *Gouania lupuloides* and, based on the NMR analysis, the relative stereostructure was deduced excepting the side chain structure [5b]; b) E. J. Kennelly, W. H. Lewis, R. E. K. Winter, S. Johnson, M. E. Lewis, J. Gossling, *J. Nat. Prod.*, **56**, 402 (1993).

6) All data were collected on Rigaku AFC5R diffractometer with MoKa radiation and a graphite monochrometer. The structure was solved by direct methods and refined by full-matrix least-squares to an R factor of 0.043 for 4436 reflections.

7) The ^1H NMR (CD_3OD) data of **2** : 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, 21-H_3), 1.84 (3H, br s, 27-H_3), 2.05 (3H, s, OAc), 2.43, 2.64 (2H, ABq, J=19.0, 15-H_2), 3.18 (1H, m, 3-H), 4.26, 4.47 (2H, ABq, J=10.5, 30-H_2), 4.68 (1H, m, 20-H), 5.06 (1H, br s, 21-H), 7.27 (1H, br s, 24-H).

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