Chemical Constituents from the Leaves of *Hydrangea macrophylla* var. *thunbergii* (III)[1]: Absolute Stereostructures of Hydramacrosides A and B, Secoiridoid Glucoside Complexes with Inhibitory Activity on Histamine Release

Hisashi MATSUDA, Hiroshi SHIMODA, Toshiaki UEMURA, Tomohiko UEDA, Johji YAMAHARA, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.
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Following the characterization of dihydroisocoumarin constituents, two secoiridoid glucoside complexes, called hydramacrosides A and B, were isolated from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO. The absolute stereostructures of hydramacrosides A and B were elucidated on the basis of chemical and physicochemical evidence, which included the application of the ^{13}C-NMR glycosylation shift rule of 1,1′-disaccharides and the modified Mosher's method. Hydramacrosides A and B exhibited an inhibitory effect on histamine release from rat mast cells induced by an antigen–antibody reaction.

Key words hydramacroside A; hydramacroside B; secoiridoid glucoside complex; *Hydrangea macrophylla* var. *thunbergii*; ^{13}C-NMR glycosylation shift; histamine release inhibitor

In the course of our studies on the bioactive constituents of natural medicine[2] and medicinal foodstuffs,[3] we have reported the isolation and structural elucidation of antiallergic and antimicrobial principles, thunberginols A,[4] B,[4] C,[5] D,[5] E,[5] and F,[4] thunberginol G 3′-*O*-glucoside,[5] and hydramacrophyllols A[6] and B[6] from Hydrangeae Dulcis Folium, the processed leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae).[7] Furthermore, we have characterized the detailed antiallergic activity and mechanism of thunberginol A, which showed more potent antiallergic activity against type I allergy than commercial antiallergic agents[4] and was easily synthesized from phyllodulcin, the principle component of this natural medicine.[6] In addition, ten dihydroisocoumarin glycosides,[1,8] 3*R*- and 3*S*-phyllodulcin 3′-*O*-glucosides, 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides, 3*R*- and 3*S*-hydrangenol 4′-*O*-apiosylglucosides, 3*R*- and 3*S*-thunberginol I 4′-*O*-glucosides, thunberginol I 8-*O*-glucosides, and 3*S*-phyllodulcin 8-*O*-glucoside, were isolated from the dried leaves of this plant and their absolute stereostructures were elucidated. As a continuing study, two new secoiridoid glucoside complexes called hydramacrosides A (**1**) and B (**3**) were also isolated from the dried leaves. In this paper, we present a full account of the structural elucidation of **1** and **3** and their inhibitory effects on histamine release from rat mast cells induced by an antigen–antibody reaction.[9]

Hydramacroside A (1) Hydramacroside A (**1**) was isolated as colorless fine crystals with a mp of 141—144 °C and negative optical rotation ($[\alpha]_D^{25}$ −129.5°). In the positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 565 $(M+H)^+$ and *m/z* 587 $(M+Na)^+$ and the molecular formula $C_{28}H_{36}O_{12}$ of **1** was confirmed by high-resolution MS measurement of the quasimolecular ion peak. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl, hydrogen bonded ketocarbonyl, and aromatic rings at 3400, 1700, and 1617 cm^{-1}, while its UV spectrum showed absorption maxima ascribable to an enone function and aromatic rings at 227, 240, and 280 nm. The ^{1}H-NMR spectrum dimethyl sulfoxide (DMSO)-d_6 of **1** showed signals due to the secoiridoid lactone moiety [δ 5.43 (d, J=1.3 Hz, 1-H),

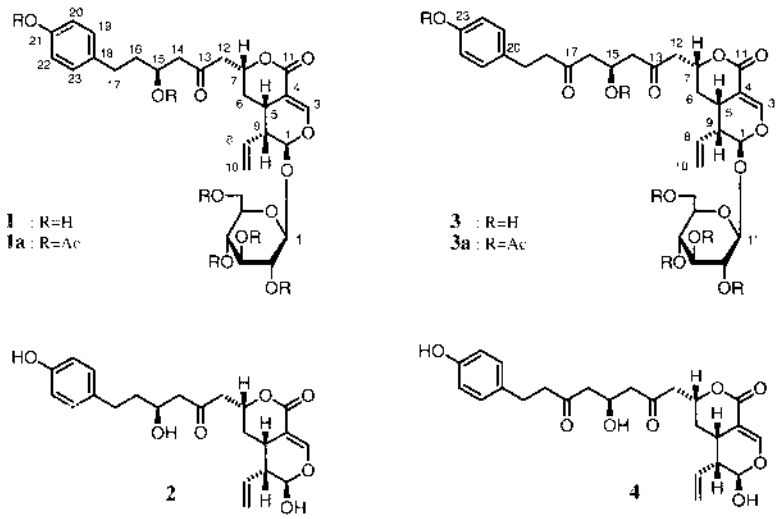

Chart 1

* To whom correspondence should be addressed.
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7.48 (d, J=2.3 Hz, 3-H), 3.12 (m, 5-H), 1.27, 1.82 (both m, 6-H_2), 4.75 (m, 7-H), 5.44 (m, 8-H), 2.64 (m, 9-H), 5.23 (dd, J=2.3, 9.9 Hz), 5.29 (dd, J=2.3, 17.2 Hz) (10-H_2)] and the side chain moiety (C-12-23) including a *p*-hydroxybenzene ring [δ 2.75 (dd, J=5.2, 17.1 Hz), 2.87 (dd, J=6.7, 17.1 Hz) (12-H_2), 2.51 (m, 14-H_2), 3.89 (m, 15-H), 1.57 (m, 16-H_2), 2.42, 2.58 (both m, 17-H_2), 6.97 (d, J=8.6 Hz, 19, 23-H), 6.65 (d, J=8.6 Hz, 20, 22-H)] together with a β-D-glucopyranoside part [δ 4.50 (d, J=7.7 Hz, 1′-H)]. In the ^{13}C-NMR spectrum (Table 1) of **1**, carbon signals due to the secoiridoid lactone glucoside moiety of **1** were superimposable on those of vogeloside (**6**)[10] and *epi*-vogeloside,[10] except for the signals around the 7-methoxyl group. The ^{1}H- and ^{13}C-NMR signals of **1** could be analyzed by use of distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy (COSY) experiments. Furthermore, the quaternary carbons of **1** were characterized by examination of the correlation *via* C–H long-range coupling (COLOC) spectrum, in which correlations were observed between the following carbons and protons of **1** (4-C and 3-H, 5-H, 6-H_2; 11-C and 3-H; 13-C and 12-H_2, 14-H_2; 18-C and 17-H_2) (Fig. 1). Acid hydrolysis of **1** with 5% aqueous sulfuric acid–dioxane (1 : 1) furnished D-glucose, which was identified by gas-liquid chromatography (GLC) analysis of the trimethylsilyl (TMS) thiazolidine derivative.[11] Enzymatic hydrolysis of **1** with β-D-glucosidase furnished the aglycone **2**, whose positive-ion FAB-MS showed a quasimolecular ion peak at *m/z* 425 $(M+Na)^+$, and the high-resolution MS measurement revealed the molecular formula of **2** to be $C_{22}H_{26}O_7$. The relative stereostructure of **2** was clarified by detailed comparisons of ^{1}H- and ^{13}C-NMR spectra with those for **1**, **6**, and **6a**. Acetylation of **1** with Ac_2O in pyridine furnished the hexaacetate (**1a**), whose ^{1}H-NMR spectrum (DMSO-d_6) showed signals indicative of a phenolic acetoxyl group (δ 2.24), and five alcoholic acetoxyl groups [δ 1.89, 1.95 (6H), 1.98, 2.02]. Comparison of the ^{13}C-NMR data (Table 1) for **1** with those for **1a** showed acetylation shifts around the C_{15} and C_{21} positions of its aglycone moiety. On the basis of the above evidence, the planar structure of **1** was clarified. The relative stereostructure of **1** was deduced by comparison of the ^{1}H- and ^{13}C-NMR data with those for the known secoiridoid glucosides such as **6**, *epi*-vogeloside and sweroside, and was finally determined by the nuclear Overhauser effect spectroscopy (NOESY) spectrum, in which nuclear Overhauser effect (NOE) enhancements were observed in several pairs of protons (1′-H and 1-H; 5-H and 7-H; 5-H and 9-H) (Fig. 1).

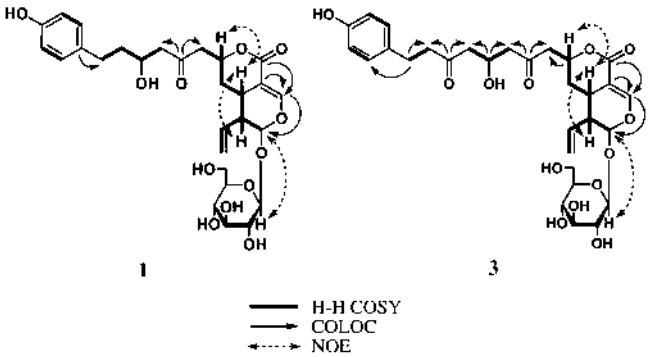


Fig. 1

The absolute configuration of the C_1 position in **1** has been determined by application of the ^{13}C-NMR glycosylation shift rule of 1,1′-disaccharide.[12] In order to confirm the applicability of the glycosylation shift rule for the dihemiacetal moiety of **1**, it was first tested on a known secoiridoid β-D-glucopyranoside, **6**. Thus, the aglycone (**6a**) was obtained from **6** by enzymatic hydrolysis with β-glucosidase, and the C_1 configuration of **6a** was found to be retained according to ^{1}H-NMR analysis, including NOE experiments. The glycosylation shifts [$\Delta\delta$ +1.5 ppm (1′-C) and +1.8 ppm (1-C)] were found to be characteristic of the *R*,*R*-dihemiacetal combination, which corresponded to the absolute stereostructure of **6** (Fig. 2). The glycosylation shifts of **1** also showed $\Delta\delta$ +1.9 ppm (1′-C) and +2.4 ppm (1-C), which were characteristic of the *R*,*R*-dihemiacetal combination, so that the absolute stereostructure of the C_1 position was determined to be an *S* configuration (Fig. 2). Finally, the absolute stereostructure of the C_{15} position in **1** was determined by means of the modified Mosher's method, as shown in Fig.

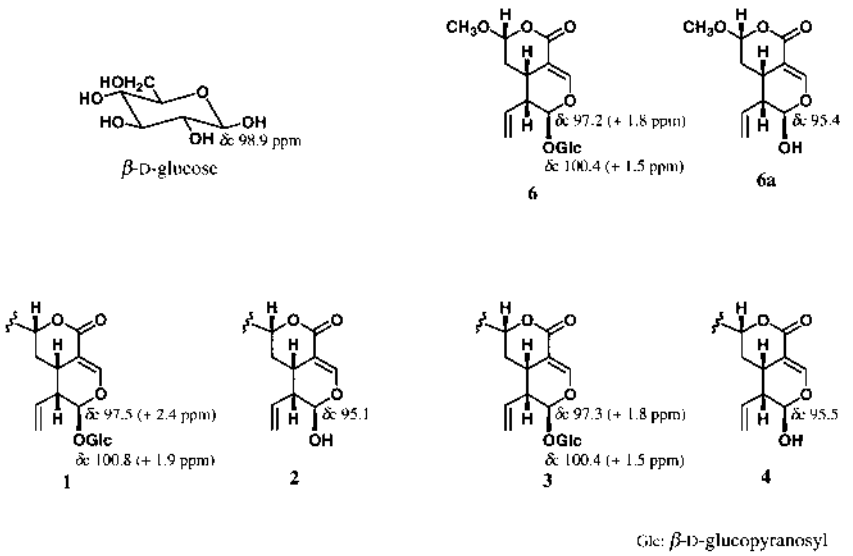
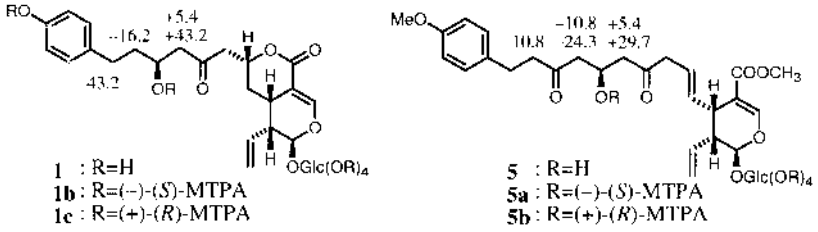


Fig. 2. ^{13}C-NMR Glycosylation Shift (68 MHz, Pyridine-d_5)



$\Delta\delta$ values in Hz (=δS–δR ; measured at 270 MHz)

Fig. 3

Table 1. ^{13}C-NMR Data for **1**, **1a**, **1b**, **1c**, **2**, **3**, **3a**, **4**, **5**, **5a**, **5b**, **6**, and **6a**

	1[a)]	**1**[b)]	**1a**[a)]	**1b**[c)]	**1c**[c)]	**2**[b)]	**3**[a)]	**3**[b)]	**3a**[a)]	**4**[b)]	**5**[a)]	**5**[c)]	**5a**[c)]	**5b**[c)]	**6**[b)]	**6a**[b)]
1	95.3	97.5	95.2	96.7	96.6	95.1	95.6	97.3	95.3	95.5	95.7	96.7	97.3	97.1	97.2	95.4
3	151.4	152.8	151.1	152.3	152.3	153.6	151.7	152.7	151.2	153.8	152.5	153.9	151.2	152.5	152.8	153.8
4	104.2	104.7	104.4	104.0	104.0	100.7	104.5	104.5	104.4	100.6	107.5	107.6	104.0	104.0	104.3	105.0
5	26.3	27.5	26.6	26.9	28.4	29.9	26.2	27.3	26.6	29.6	39.1	39.7	39.5	39.1	24.7	26.3
6	29.3	30.5	28.9	29.9	29.9	30.0	29.6	30.3	28.9	30.0	132.7	133.7	133.5	134.4	30.9	32.9
7	74.1	74.7	74.5	73.9	71.4	75.2	74.4	74.7	74.5	75.1	124.5	124.7	124.7	124.6	103.5	103.7
8	132.1	133.2	131.4	130.8	130.7	134.9	132.1	132.4	131.2	134.4	134.7	135.4	132.4	133.4	132.2	136.4
9	41.3	42.9	40.6	42.3	43.4	48.0	41.6	42.7	40.6	47.9	44.0	44.9	44.9	44.8	42.8	47.8
10	120.3	120.1	120.8	121.2	121.1	118.4	120.6	120.2	120.8	119.1	118.2	118.6	119.3	119.0	120.4	119.3
11	164.5	165.0	163.9	165.3	164.6	165.3	164.8	165.1	164.0	165.0	166.0	167.0	167.1	167.0	164.5	164.9
12	48.3	49.3	47.9	48.4	47.3	49.6	48.5	49.2	47.8	49.6	37.2	37.7	37.2	37.3		
13	206.7	207.0	204.8	203.4	203.3	206.8	206.5	206.5	204.6	206.6	197.0	198.5	200.8	200.9		
14	50.6	52.0	46.6	47.3	46.9	52.5	50.6	51.2	43.4	51.4	47.3	44.9	43.1	42.8		
15	65.8	67.1	69.0	71.3	72.0	67.2	63.5	64.1	65.7	64.2	64.2	65.7	70.5	70.5		
16	39.4	40.7	35.0	35.4	34.9	40.7	50.1	50.6	46.2	50.8	39.7	37.7	35.7	35.2		
17	30.3	31.7	30.0	30.3	31.9	32.1	208.8	209.0	206.8	208.9	204.2	206.2	209.1	208.7		
18	132.0	132.6	138.7	132.2	132.0	132.3	44.8	45.7	45.6	45.8	32.2	32.9	32.7	32.8		
19	129.0	130.0	129.0	129.8	128.5	130.0	28.3	29.0	28.1	29.1	32.2	32.7	32.7	32.8		
20	114.9	116.3	121.5	121.0	121.1	116.3	131.4	132.0	138.4	132.1	132.6	132.6	132.3	133.1		
21	155.1	157.3	148.5	148.3	147.8	157.2	129.2	129.8	129.1	129.9	129.1	129.3	129.3	129.2		
22	114.9	116.3	121.5	121.0	121.1	116.3	115.2	116.2	121.5	116.3	113.7	113.9	114.0	114.0		
23	129.0	130.0	129.0	129.8	128.5	130.0	155.6	157.0	148.5	157.2	157.5	158.1	158.1	158.2		
24							115.2	116.2	121.5	116.3	113.7	113.9	114.0	114.0		
25							129.2	129.8	129.1	129.9	129.1	129.3	129.3	129.2		
CO_2CH_3											54.9	55.3	55.3	55.3		
23-OCH_3											50.9	51.4	51.4	51.4	56.5	56.4
1′	97.8	100.8	96.4	98.2	96.6		98.1	100.4	96.4		98.9	99.1	100.6	99.5	100.4	
2′	73.0	74.9	70.3	71.7	70.4		73.3	74.7	70.3		72.9	73.2	71.3	71.9	74.9	
3′	76.1	78.4	70.8	74.3	74.1		76.4	78.1	70.8		76.5	76.0	73.8	73.9	78.4	
4′	69.9	71.4	67.8	68.8	68.6		70.2	71.2	67.7		69.6	70.2	68.2	68.6	71.3	
5′	77.2	79.8	71.3	74.4	74.4		77.5	78.7	71.3		77.1	76.1	76.1	75.7	79.7	
6′	60.9	62.5	61.3	64.1	63.7		61.2	62.4	61.3		60.8	62.2	63.6	65.1	62.5	

The spectra were taken with *a*) DMSO-d_6 or *b*) pyridine-d_5 or *c*) $CDCl_3$.

3. Thus, the treatment of **1** with (−)-(*S*)- and (+)-(*R*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in CH_2Cl_2 in the presence of 4-dimethylaminopyridine (DMAP) furnished the (−)-(*S*)-MTPA (**1b**) and (+)-(*R*)-MTPA esters (**1c**). Signals due to protons on the 16-C and 17-C of **1b** appeared at a higher field than those of **1c**, while the 14-proton signals of **1b** were observed at a lower field than those of **1c**, so that the absolute configuration at the C_{15} position is determined to be an *S* configuration. Consequently, the absolute stereostructure of **1** was determined as shown.

Hydramacroside B (3) Hydramacroside B (**3**) was isolated as colorless fine crystals with a mp of 154—157 °C and negative optical rotation ($[\alpha]_D^{25}$ −106.8°). In the FAB-MS of **3**, a quasimolecular ion peak was observed at *m/z* 607 $(M+H)^+$ and 629 $(M+Na)^+$, and the molecular formula $C_{30}H_{38}O_{13}$ of **3** was confirmed by high-resolution MS measurement of the quasimolecular ion peak. The IR and UV spectra of **3** were similar to those of **1**. The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **3** showed the presence of ketocarbonyl and methylene functions in addition to those of **3**.

The structure of **3** has been elucidated in the same way. Namely, **3** liberated D-glucose by acid hydrolysis, while the ordinary acetylation of **3** furnished the hexaacetate (**3a**). As shown in Fig. 1, the connectivities of the quaternary carbons were clarified by a COLOC experiment and ^{1}H–^{1}H COSY. Comparison of the NMR data for **3** and **3a** with those for **1** and **1a** led us to elucidate the planar structure of **1**. In the NOESY experiment of **3**, the observation of NOE enhancements between proton pairs in **3** (1′-H and 1-H; 5-H and 9-H; 5-H and 7-H) indicated the relative stereostructure of **3** (Fig.

Table 2. Inhibitory Effects of **1** and **3** on the Histamine Release from Rat Sensitized Peritoneal Exudate Cells Induced by an Antigen–Antibody Reaction.

	Conc. (μM)	Inhibition (%) Mean±S.E. (n=4)
Hydramacroside A (**1**)	10	9.1±11.4
	30	19.8± 4.0
	100	33.1± 4.2
	300	70.0± 3.5
Hydramacroside B (**3**)	10	21.3± 3.7
	30	21.3±21.8
	100	57.1± 2.6
	300	78.1± 9.5

Sensitized rat peritoneal exudate cells were preincubated with samples for 15 min at 37 °C prior to the antigen challenge with phosphatidyl-L-serine and dinitrophenylated bovine serum albumin (DNP-BSA), then incubation was continued for 15 min. Histamine was determined by HPLC.

1). The enzymatic hydrolysis of **3** yielded the aglycone (**4**), whose relative stereostructure was elucidated by detailed ^{1}H-NMR examination including NOE observation between proton pairs in **4** (1-H and 8-H; 5-H and 7, 9-H). By comparison of the chemical shift for **3** with those for **4** and β-D-glucopyranose, glycosylation shifts characteristic of the *R*,*R*-dihemiacetal linkage [$\Delta\delta$ +1.5 ppm (1′-C), +1.8 ppm (1-C)] were observed, so that the C_1-configuration of **3** was determined to be an *S* configuration. In order to determine the absolute configuration of the C_{15} position in **3**, the modified Mosher's method had been applied directly. But, the desired MTPA ester of **3** was not obtained because of preferential elimination of the 15-OH group. Finally, the following conversion has been carried out. Treatment of **3** with pig liver esterase in phosphate buffer (pH 7.0) followed by methylation with CH_2N_2 furnished the olefin methyl ester (**5**), which was converted to the (−)-(*S*)-MTPA ester (**5a**) and the (+)-(*R*)-MTPA ester (**5b**). The absolute configuration at the C_{15} position of **5** has been shown to be *S* by means of NMR analysis [$\Delta\delta$ values for the protons on C_{16} (−10.8, −24.3 Hz), C_{18} (−10.8 Hz) and C_{14} (+5.4, +29.7 Hz)]. Based on this evidence, the absolute stereostructure of **3** was determined as shown.

Inhibitory Effects of 1 and 3 on Histamine Release As a part of our studies characterizing the antiallergic components from the leaves of *Hydrangea macrophylla* var. *thunbergii*, we examined the inhibitory effects of **1** and **3** on histamine release. As shown in Table 2, **1** and **3** were found to inhibit the histamine release from rat peritoneal exudate cells induced by an antigen–antibody reaction in a concentration-dependent manner (10^{-5}—3×10^{-4} M).

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described previously.[1)]

Isolation of Hyramacrosides A (1) and B (3) As described in a previous report,[9)] the fraction 7—4 (422 mg) was subjected to HPLC [YMC-pack R&D-ODS-5A (250×10 mm i.d.), MeOH–H_2O (1 : 1, v/v)] followed by chiral column HPLC [Ceramospher Chiral RU-1 (Shiseido Ltd.), MeOH] to afford **1** (28.0 mg) and **3** (30.0 mg) together with 3*R*- (1.4 mg) and 3*S*-hydrangenol 4′-*O*-apiosylglucoside[1)] (4.8 mg), (+)-hydrangenol 4′-*O*-glucoside[5)] (1.7 mg), (−)-hydrangenol 4′-*O*-glucoside[5)] (3.4 mg).

Hydramacroside A (**1**): Colorless fine crystals, mp 141—144 °C, $[\alpha]_D^{25}$ −129.5° (c=0.516, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{28}H_{37}O_{12}$ $(M+H)^+$: 565.2285. Found: 565.2295. UV λ_{max}^{EtOH} nm (log ε): 227 (4.3), 240 (4.2), 280 (3.3). IR (KBr) cm^{-1}: 3400, 1700, 1617. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.27, 1.82 (2H, both m, 6-H_2), 1.57 (2H, m, 16-H_2), 2.42, 2.58 (2H, both m, 17-H_2), 2.51 (2H, m, 14-H_2), 2.64 (1H, m, 9-H), 2.75 (1H, dd, J=5.2, 17.1 Hz), 2.87 (1H, dd, J=6.7, 17.1 Hz) (12-H_2), 3.12 (1H, m, 5-H), 3.89 (1H, m, 15-H), 4.50 (1H, d, J=7.7 Hz, 1′-H), 4.75 (1H, m, 7-H), 5.23 (1H, dd, J=2.3, 9.9 Hz), 5.29 (1H, dd, J=2.3, 17.2 Hz) (10-H_2), 5.43 (1H, d, J=1.3 Hz, 1-H), 5.44 (1H, m, 8-H), 6.65 (2H, d, J=8.6, 20 Hz, 22-H), 6.97 (2H, d, J=8.6 Hz, 19, 23-H), 7.48 (1H, d, J=2.3 Hz, 3-H). ^{13}C-NMR (125 MHz, DMSO-d_6), (68 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 565 $(M+H)^+$, 587 $(M+Na)^+$.

Hydramacroside B (**3**): Colorless fine crystals, mp 154—157 °C, $[\alpha]_D^{25}$ −106.8° (c=0.309, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{39}O_{13}$ $(M+H)^+$: 607.2391. Found: 607.2415. UV λ_{max}^{EtOH} nm (log ε): 227 (4.2), 240 (4.1), 278 (3.5). IR (KBr) cm^{-1}: 3400, 1707, 1617. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.33, 1.81 (2H, both m, 6-H_2), 2.50 (4H, m, 14, 16-H_2), 2.64 (1H, m, 9-H), 2.65 (2H, m, 19-H_2), 2.68 (2H, m, 18-H_2), 2.75 (1H, dd, J=5.3, 17.5 Hz), 2.87 (1H, dd, J=7.3, 17.5 Hz) (12-H_2), 3.10 (1H, m, 5-H), 4.35 (1H, m, 15-H), 4.49 (1H, d, J=8.0 Hz, 1′-H), 4.77 (1H, m, 7-H), 5.23 (1H, dd, J=2.3, 9.9 Hz), 5.29 (1H, dd, J=2.3, 17.2 Hz) (10-H_2), 5.43 (1H, d, J=1.6 Hz, 1-H), 5.43 (1H, m, 8-H), 6.64 (2H, d, J=8.6 Hz, 22, 24-H), 6.97 (2H, d, J=8.6 Hz, 21, 25-H), 7.48 (1H, d, J=2.3 Hz, 3-H). ^{13}C-NMR (125 MHz, DMSO-d_6), (68 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 607 $(M+H)^+$, 629 $(M+Na)^+$.

Acid Hydrolysis of 1 and 3 A solution of hydramacroside (**1**, **3**, 2 mg each) in 5% aqueous H_2SO_4–dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent under reduced pressure from the filtrate, the residue was passed through a Sep-Pak C_{18} cartridge and eluted with H_2O and MeOH. The H_2O eluate was concentrated under reduced pressure and the residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) at 60 °C for 1 h. After the reaction was complete, the solution was treated with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivative of D-glucose from **1** and **3**. GLC conditions: column, Supelco SPR™-1, 0.25 mm i.d. ×30 m; column temperature, 230 °C; t_R, 24.2 min.

Acetylation of 1 A solution of **1** (3.3 mg) in pyridine (0.25 ml) was treated with Ac_2O (0.1 ml), and the reaction mixture was stirred at room temperature (20 °C) for 1 h. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, the hexaacetate (**1a**, 4.6 mg) was obtained.

Hydramacroside A Hexaacetate (**1a**): Colorless fine crystals, mp 55—58 °C, $[\alpha]_D^{25}$ −86.3° (c=0.130, $CHCl_3$). IR (KBr) cm^{-1}: 1757, 1736 (sh), 1615. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 1.89 (3H, $OCOCH_3$), 1.95 (6H, $OCOCH_3$×2), 1.98 (3H, $OCOCH_3$), 2.02 (3H, $OCOCH_3$), 2.24 (3H, $OCOCH_3$), 4.67 (1H, m, 7-H), 5.15 (1H, s, 1-H), 7.01 (2H, d, J=8.6 Hz, 20, 22-H), 7.22 (2H, d, J=8.6 Hz, 19, 23-H_2), 7.53 (1H, d, J=1.7 Hz, 3-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: given in Table 1.

Enzymatic Hydrolysis of 1 A solution of **1** (3.4 mg) in acetate buffer (pH 4.4, 0.7 ml) was treated with β-glucosidase (Oriental Yeast Co., Ltd., Japan, 3.4 mg) and the reaction mixture was left standing at 38 °C for 2.5 h. The reaction mixture was poured into H_2O and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, then dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography [1.0 g, $CHCl_3$–MeOH (10 : 1)] to give **2** (2.2 mg).

2: Colorless fine crystals, mp 154—157 °C, $[\alpha]_D^{25}$ +10.7° (c=0.118, $CHCl_3$). IR (KBr) cm^{-1}: 3453, 1713, 1619. ^{1}H-NMR spectrum (270 MHz, $CDCl_3$) δ: 4.29 (1H, m, 15-H), 4.78 (1H, m, 7-H), 5.35 (1H, s, 1-H), 6.75 (2H, d, J=8.5 Hz, 20, 22-H), 7.06 (2H, d, J=8.5 Hz, 19, 23-H), 7.63 (1H, d, J=2.4 Hz, 3-H). ^{13}C-NMR spectrum (68 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 425 $(M+Na)^+$.

Preparation of the MTPA Esters (1b, 1c) from 1 A solution of **1** (5.5 mg) in CH_2Cl_2 (2.0 ml) was treated with (*R*)-MTPA (23.4 mg, 0.1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (19.2 mg, 0.1 mmol) and DMAP (7.3 mg, 0.06 mmol), and the whole mixture was stirred at room temperature (25 °C) for 5 min. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, and then dried over $MgSO_4$ and filtered. Evaporation of the solvent from the filtrate under reduced pressure furnished a residue (14.0 mg), which was purified by silica gel column chromatography [2.0 g, *n*-hexane–AcOEt (3 : 2)] to

give a **1b** (4.0 mg). **1c** (4.3 mg) was also obtained from **1** (5.5 mg) by the same procedure described above.

1b: A white powder, $[\alpha]_D^{25}$ −8.4° (c=0.178, $CHCl_3$). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.25, 1.83 (2H, m, 6-H_2), 1.98 (2H, m, 16-H_2), 2.52, 2.87 (2H, m, 14-H_2), 2.53 (1H, m, 9-H), 2.65 (2H, m, 17-H_2), 2.67 (2H, m, 12-H_2), 2.89 (1H, m, 5-H), 4.77 (1H, m, 7-H), 4.78 (1H, d, J=7.9 Hz, 1′-H), 5.18, 5.27 (2H, m, 10-H_2), 5.22 (1H, m,8-H), 5.28 (1H, br s, 1-H), 5.49 (1H, m, 15-H), 7.04 (2H, d, J=8.6 Hz, 20, 22-H), 7.17 (2H, d, J=8.6 Hz, 19, 23-H), 7.40 (1H, d, J=3.0 Hz, 3-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δ_C: given in Table 1.

1c: A white powder, $[\alpha]_D^{25}$ −111.3° (c=0.372, $CHCl_3$). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.38, 1.78 (2H, m, 6-H_2), 1.92 (2H, m, 16-H_2), 2.49 (2H, m, 17-H_2), 2.56 (1H, m, 9-H), 2.63, 2.85 (2H, m, 12-H_2), 2.68, 2.89 (2H, m, 14-H_2), 2.96 (1H, m, 5-H), 4.78 (1H, d, J=8.0 Hz, 1′-H), 4.80 (1H, m, 7-H), 5.17, 5.24 (2H, m, 10-H_2), 5.22 (1H, m, 8-H), 5.24 (1H, d, J=2.3 Hz, 1-H), 5.53 (1H, m, 15-H), 7.00 (2H, d, J=8.9 Hz, 20, 22-H), 7.07 (2H, d, J=8.9 Hz, 19, 23-H), 7.46 (1H, d, J=3.3 Hz, 3-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δ_C: given in Table 1.

Acetylation of 3 A solution of **3** (4.1 mg, 0.0068 mmol) in pyridine (0.3 ml) was treated with Ac_2O (0.15 ml), and the reaction mixture was stirred at room temperature (20 °C) for 1 h. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, the hexaacetate (**3a**, 5.5 mg, quant.) was obtained.

Hydramacroside B Hexaacetate (**3a**): Colorless fine crystals, mp 82—85 °C, $[\alpha]_D^{25}$ −79.3° (c=0.091, $CHCl_3$). IR (KBr) cm^{-1}: 1757, 1726, 1624. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 4.66 (1H, m, 7-H), 5.15 (1H, s,1-H), 7.01 (2H, d, J=8.3 Hz, 22, 24-H), 7.22 (2H, d, J=8.3 Hz, 21, 25-H), 7.52 (1H, d, J=2.0 Hz, 3-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: given in Table 1.

Enzymatic Hydrolysis of 3 A solution of **3** (8.6 mg, 0.014 mmol) in acetate buffer (pH 4.4, 1.7 ml) was treated with β-glucosidase (8.6 mg) and the reaction mixture was left standing at 38 °C for 2.5 h. The reaction mixture was poured into H_2O, and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, then dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, a residue (10.8 mg) was purified by silica gel column chromatography [4.0 g, $CHCl_3$: MeOH (10 : 1)] to give **4** (5.5 mg, 91.7%).

4: Colorless fine crystals, mp 123—126 °C, $[\alpha]_D^{25}$ +13.9° (c=0.121, $CHCl_3$). IR (KBr) cm^{-1}: 3453, 1717, 1620. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 4.30 (1H, m, 15-H), 4.72 (1H, m, 7-H), 5.35 (1H, d, J=1.3 Hz, 1-H), 6.69 (2H, d, J=8.6 Hz, 22, 24-H), 6.95 (2H, d, J=8.6 Hz, 21, 25-H), 7.57 (1H, d, J=1.3 Hz, 3-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 467 $(M+Na)^+$.

Conversion from 3 to 5 A solution of **3** (11.8 mg, 0.019 mmol) in phosphate buffer (pH 7.0, 5.0 ml) was treated with pig liver esterase (40.0 mg), and the reaction mixture was stirred at 38 °C for 2 d. After removal of the solvent under reduced pressure, a residue (55.0 mg) was purified by reversed-phase silica gel column chromatography (4.0 g, H_2O→40% MeOH) to give the olefin derivative (11.1 mg, quant.). A solution of the olefin derivative (11.1 mg, 0.018 mmol) in MeOH (0.5 ml) was treated with $CH_2N_2 \cdot Et_2O$ (2.5 ml), and the reaction mixture was left standing at room temperature for 2 h. After removal of the solvent under reduced pressure, the olefin methyl ester (**5**, 11.2 mg, quant.) was obtained.

5: A white powder, $[\alpha]_D^{25}$ −38.2° (c=0.490, MeOH). IR (KBr) cm^{-1}: 3410, 1655, 1615 (sh). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.50, 2.62 (2H, m, 12-H_2), 2.51, 2.59 (2H, m, 14-H_2), 2.55, 2.66 (2H, m, 16-H_2), 2.66 (1H, m, 9-H), 2.68 (4H, m, 18, 19-H_2), 3.42 (1H, m, 5-H), 3.60 (3H, s, 11-CO_2CH_3), 3.76 (3H, s, 23-OCH_3), 4,15 (1H, m, 15-H), 4.72 (1H, d-like, 1′-H), 5.13, 5.18 (2H, m, 10-H_2), 5.57 (1H, d, J=9.0 Hz, 1-H), 5.68 (3H, m, 6, 7, 8-H), 6.80 (2H, d, J=8.6 Hz, 22, 24-H), 7.05 (2H, d, J=8.6 Hz, 21, 25-H), 7.57 (1H, s, 3-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 639 $(M+Na-H_2O)^+$.

Preparation of the MTPA Esters (5a, 5b) from 5 A solution of **5** (5.8 mg, 0.0091 mmol) in CH_2Cl_2 (2.9 ml) was treated with (R)-MTPA (21.1 mg, 0.09 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (17.3 mg, 0.090 mmol) and DMAP (6.6 mg, 0.054 mmol), and the whole mixture was stirred at room temperature (25 °C) for 1 h under N_2 atmosphere. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$ and filtered. Evaporation of the solvent from the filtrate under reduced pressure furnished a residue (11.3 mg), which was purified by silica gel column chromatography [1.5 g, n-hexane–AcOEt (3 : 2→1 : 1)] to give **5a** (3.1 mg). **5b** (3.9 mg) was obtained from **5** by the same procedure.

5a: A white powder, $[\alpha]_D^{25}$ −15.3° (c=0.533, $CHCl_3$). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.52, 2.62 (2H, m, 12-H_2), 2.54 (1H, m, 9-H), 2.56, 2.71 (2H, m, 14-H_2), 2.62, 2.70 (2H, m, 16-H_2), 2.66 (4H, m, 18, 19-H_2), 3.47 (1H, m, 5-H), 3.64 (3H, s, 11-CO_2CH_3), 3.78 (3H, s, 23-OCH_3), 4.78 (1H, d, J=7.9 Hz, 1′-H), 5.13, 5.18 (2H, m, 10-H_2), 5.30 (1H, d, J=8.3 Hz, 1-H), 5.40 (1H, m, 15-H), 5.58 (1H, m, 8-H), 5.85 (1H, m, 7-H), 5.87 (1H, m, 6-H), 6.82 (2H, d, J=8.6 Hz, 22, 24-H), 7.05 (2H, d, J=8.6 Hz, 21, 25-H), 7.54 (1H, s, 3-H). ^{13}C-NMR spectrum (68 MHz, $CDCl_3$) δ_C: given in Table 1.

5b: A white powder, $[\alpha]_D^{25}$ −75.1° (c=1.066, $CHCl_3$). ^{1}H-NMR spectrum (270 MHz, $CDCl_3$) δ: 2.46 (1H, m, 9-H), 2.53, 2.59 (2H, m, 12-H), 2.53, 2.66 (2H, m, 16-H_2), 2.62 (4H, m, 18, 19-H_2), 2.67, 2.73 (2H, m, 14-H_2), 3.46 (1H, m, 5-H), 3.65 (3H, s, 11-CO_2CH_3), 3.78 (3H, s, 23-OCH_3), 4.76 (1H, d, J=7.9 Hz, 1′-H), 5.13, 5.20 (2H, m, 10-H_2), 5.26 (1H, d, J=8.2 Hz, 1-H), 5.43 (1H, m, 15-H), 5.59 (1H, m, 8-H), 5.86 (2H, m, 6, 7-H), 6.81 (2H, d, J=8.6 Hz, 22, 24-H), 7.03 (2H, d, J=8.6 Hz, 21, 25-H), 7.53 (1H, s, 3-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δ_C: given in Table 1.

Enzymatic Hydrolysis of 6 A solution of **6** (3.2 mg, 0.0079 mmol) in acetate buffer (pH 4.4, 0.7 ml) was treated with β-glucosidase (Oriental Yeast Co., Japan, 3.2 mg), and the reaction mixture was left standing at 38 °C for 2 h. The reaction mixture was poured into H_2O and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, then dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, the residue (9.6 mg) was purified by silica gel column chromatography [4.0 g, $CHCl_3$: MeOH (5 : 1)] to give **6a** (2.0 mg, quant.).

6a: Colorless fine crystals, mp 79—82 °C, $[\alpha]_D^{25}$ −80.7° (c=0.132, $CHCl_3$). IR (KBr) cm^{-1}: 3453, 1686, 1619. UV λ_{max}^{EtOH} nm (log ε): 231 (3.9), 313 (3.2). ^{1}H-NMR spectrum (270 MHz, $CDCl_3$) δ: 2.64 (1H, ddd, J=1.7, 4.0, 11.9 Hz, 9-H), 3.59 (3H, s, 7-OCH_3), 5.20 (1H, dd, J=2.7, 9.9 Hz, 7-H), 5.23 (1H, dd, J=1.3, 18.6 Hz), 5.28 (1H, dd, J=1.3, 10.1 Hz, 10-H_2), 5.43 (1H, d, J=1.7 Hz, 1-H), 5.70 (1H, ddd, J=10.1, 11.9, 18.6 Hz, 8-H), 7.62 (1H, d, J=2.0 Hz, 3-H). ^{13}C-NMR spectrum (68 MHz, pyridine-d_5) δ_C: given in Table 1.

Bioassay Test for the Inhibitory Activity on Histamine Release The methods of bioassay testing are the same as described previously.[4c,g)]

References and Notes

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