ABSOLUTE STEREOSTRUCTURES OF HYDRAMACROSIDES A AND B, NEW BIOACTIVE SECOIRIDOID GLUCOSIDE COMPLEXES FROM THE LEAVES OF *HYDRANGEA MACROPHYLLA* SERINGE VAR. *THUNBERGII* MAKINO

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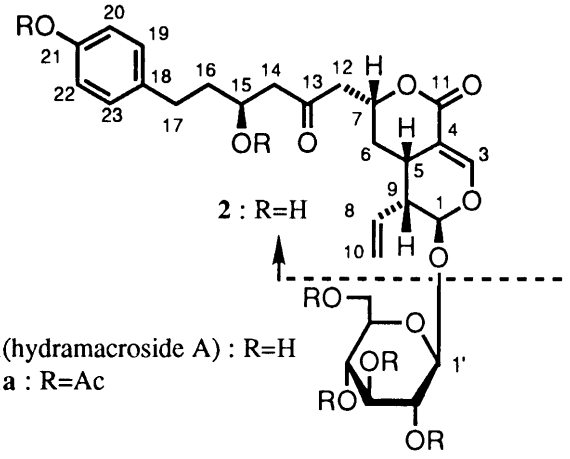
Two new bioactive secoiridoid glucoside complexes named hydramacrosides A and B were isolated from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO. The absolute stereostructures of hydramacrosides A and B were elucidated on the basis of chemical and physicochemical evidence which 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 inhibitory effect on the histamine release from rat mast cells induced by antigen-antibody reaction.

KEYWORDS hydramacroside A; hydramacroside B; secoiridoid glucoside complex; *Hydrangea macrophylla* var. *thunbergii*; ^{13}C NMR glycosylation shift; histamine release inhibitator

During the course of chemical characterization studies of natural medicine processing,[1] we have investigated the bioactive constituents of Hydrangeae Dulcis Folium (Amacha in Japanese), the fermented and then dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae). We have so far isolated from the natural medicine a number of new antiallergic and antimicrobial constituents, namely two isocoumarins, thunberginols A[2] and B,[2] three dihydroisocoumarins, thunberginols C,[3] D,[3] and E,[3] three phthalides, thunberginol F,[2] hydramacrophyllols A[4] and B,[4] and two dihydroisocoumarin glucosides, thunberginol G 8-*O*-glucoside[3] and (-)-hydrangenol 4'-*O*-glucoside.[3] We have also developed a HPLC quantitative analysis method of dihydroisocoumarins, and, by use of this HPLC method, chemical processing of this natural medicine was investigated.[5] In a continuing study, we isolated novel secoiridoid complexes, hydramacrosides A(**1**) and B(**3**), together with several new dihydroisocoumarin glycosides from the unprocessed leaves of *Hydrangea macrophylla* var. *thunbergii*. In this paper, we describe the absolute stereostructures of hydramacrosides A(**1**) and B(**3**) and their inhibitory effects on the histamine release from rat mast cells induced by antigen-antibody reaction.

The MeOH extract of the leaves was partitioned into a mixture of AcOEt and water; then the water-soluble portion was further extracted with 1-BuOH. The 1-BuOH-soluble portion was subjected to ordinary and reversed-phase SiO_2 column and HPLC separation to afford **1** (0.0041% from the natural medicine) and **3** (0.0063%) together with vogeloside (**6**, 0.0059%),[6] *epi*-vogeloside (**7**, 0.0170%),[6] citroside A(0.0021%), flavonoid glycosides, and isocoumarin glycosides.

Hydramacroside A(**1**), colorless fine crystals, mp 141-144°C, $[\alpha]_D$ -129.5° (MeOH), $C_{28}H_{36}O_{12}$, UV(EtOH, log ε): 227(4.3) 240(4.2), 280(3.3) nm, IR(KBr): 3400, 1700, 1617 cm^{-1}, positive FAB-MS : m/z 565(M+H)$^+$, 587(M+Na)$^+$, liberated D-glucose by acid hydrolysis. The ^1H NMR spectrum (500MHz, DMSO-d_6) of **1** showed the signals due to the secoiridoid lactone moiety [δ 5.43(d, J=1.3, 1-H), 7.48(d, J=2.3, 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), 5.29(dd, J=2.3, 17.2)(10-H_2)] and the side chain moiety including a *p*-hydroxybenzene ring [δ 2.75(dd, J=5.2, 17.1), 2.87(dd, J=6.7, 17.1)(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, 19, 23-H), 6.65(d, J=8.6, 20, 22-H)] together with a β-D-glucopyranoside part [δ 4.50(d, J=7.7, 1'-H)]. In the ^{13}C NMR spectrum (Table I) of **1**, the carbon signals due to the secoiridoid lactone glucoside



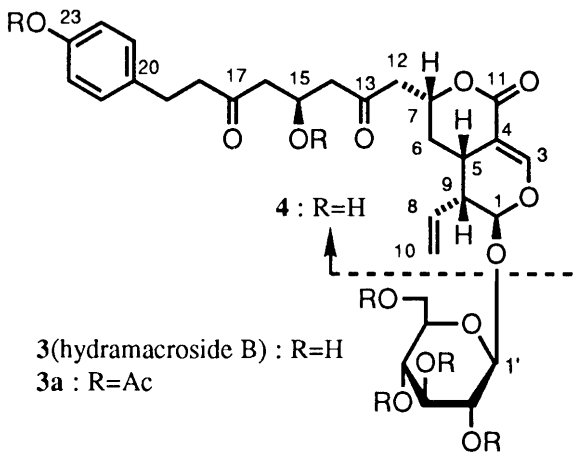


Table I. ^{13}C NMR Data for **1**, **1a**, **2**, **3**, **3a**, **4**, and **5**

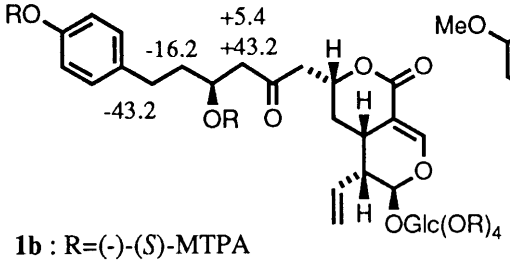
	1[a]	1[b]	1a[a]	2[b]	3[a]	3[b]	3a[a]	4[b]	5[a]
1	95.3	97.5	95.2	95.1	95.6	97.3	95.3	95.5	95.7
3	151.4	152.8	151.1	153.6	151.7	152.7	151.2	153.8	152.5
4	104.2	104.7	104.4	100.7	104.5	104.5	104.4	100.6	107.5
5	26.3	27.5	26.6	29.9	26.2	27.3	26.6	29.6	39.1
6	29.3	30.5	28.9	30.0	29.6	30.3	28.9	30.0	132.7
7	74.1	74.7	74.5	75.2	74.4	74.7	74.5	75.1	124.5
8	132.1	133.2	131.4	134.9	132.1	132.4	131.2	134.4	134.7
9	41.3	42.9	40.6	48.0	41.6	42.7	40.6	47.9	44.0
10	120.3	120.1	120.8	118.4	120.6	120.2	120.8	119.1	118.2
11	164.5	165.0	163.9	165.3	164.8	165.1	164.0	165.0	166.0
12	48.3	49.3	47.9	49.6	48.5	49.2	47.8	49.6	37.2
13	206.7	207.0	204.8	206.8	206.5	206.5	204.6	206.6	197.0
14	50.6	52.0	46.6	52.5	50.6	51.2	43.4	51.4	47.3
15	65.8	67.1	69.0	67.2	63.5	64.1	65.7	64.2	64.2
16	39.4	40.7	35.0	40.7	50.1	50.6	46.2	50.8	39.7
17	30.3	31.7	30.0	32.1	208.8	209.0	206.8	208.9	204.2
18	132.0	132.6	138.7	132.3	44.8	45.7	45.6	45.8	32.2
19	129.0	130.0	129.0	130.0	28.3	29.0	28.1	29.1	32.2
20	114.9	116.3	121.5	116.3	131.4	132.0	138.4	132.1	132.6
21	155.1	157.3	148.5	157.2	129.2	129.8	129.1	129.9	129.1
22	114.9	116.3	121.5	116.3	115.2	116.2	121.5	116.3	113.7
23	129.0	130.0	129.0	130.0	155.6	157.0	148.5	157.2	157.5
24					115.2	116.2	121.5	116.3	113.7
25					129.2	129.8	129.1	129.9	129.1
1'	97.8	100.8	96.4		98.1	100.4	96.4		98.9
2'	73.0	74.9	70.3		73.3	74.7	70.3		72.9
3'	76.1	78.4	70.8		76.4	78.1	70.8		76.5
4'	69.9	71.4	67.8		70.2	71.2	67.7		69.6
5'	77.2	79.8	71.3		77.5	78.7	71.3		77.1
6'	60.9	62.5	61.3		61.2	62.4	61.3		60.8

The spectra were taken with DMSO-d_6[a] or pyridine-d_5.[b]

moiety of **1** were superimposable to those of vogeloside(**6**) and *epi*-vogeloside(**7**) except for the signals around the 7-methoxyl group. The ^1H and ^{13}C NMR signals of **1** could be analyzed completely by use of DEPT, ^1H-^1H and ^1H-^{13}C COSY experiments. Furthermore, the quart. carbons of **1** were characterized by examination of the COLOC spectrum, in which correlations were observed between the following carbons and protons of **1** [4-C & 3-H, 5-H, 6-H_2;11-C & 3-H; 13-C & 12-H_2, 14-H_2; 18-C & 17-H_2]. Acetylation of **1** with Ac_2O in pyridine furnished the hexaacetate(**1a**),[7] whose ^1H NMR spectrum (DMSO-d_6) showed the 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 I) for **1** with those for **1a** showed the acetylation shifts around the C_{15} and C_{21} positions of its aglycone moiety. Based on this evidence, the plane structure of **1** was clarified. The relative stereostructures of **1** were deduced by comparison of the ^1H and ^{13}C NMR data with those for the known secoiridoid glucosides such as **6**, **7**, and sweroside(**8**), and finally determined by the NOESY spectrum, in which the NOE enhancements were observed in several pairs of protons [1'-H & 1-H; 5-H & 7-H; 5-H & 9-H].

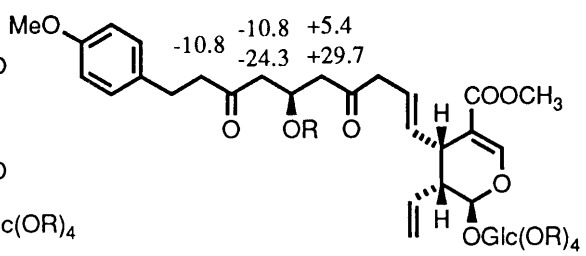
The absolute configuration of the C_1 position in **1** has been determined by application of the ^{13}C NMR glycosylation shift rule of 1,1'-disaccharide.[8] In order to confirm the applicability of the glycosylation shift rule for the dihemiacetal moiety of **1**, it was first tested on the known secoiridoid β-D-glucopyranoside, vogeloside(**6**). Thus, the aglycone(**6a**) was obtained from **6** by the enzymatic hydrolysis with β-D-glucosidase, and the C_1 configuration of **6a** was found to be retained by the ^1H NMR analysis including NOE experiments. The glycosylation shifts [Δδ +1.5 ppm(1'-C) and +1.9ppm(1-C)] were found to be characteristic of the *R*, *R*-dihemiacetal combination which was corresponding to the absolute stereostructure of **6**.[9]

Enzymatic hydrolysis of **1** with β-D-glucosidase furnished the aglycone **2**,[10] $C_{22}H_{26}O_7$, IR(KBr): 3453, 1713, 1619cm^{-1}, positive FAB-MS : m/z 425(M+Na)$^+$, whose relative stereostructure was clarified by the detailed comparisons of ^1H and ^{13}C NMR spectra with those for **1**, **6**, and **6a** along with NOE experiments. The glycosylation shifts of **1** showed Δδ +1.9ppm(1'-C) and +2.4ppm(1-C), which were characteristic of the *R*, *R*-dihemiacetal combination, so that the absolute

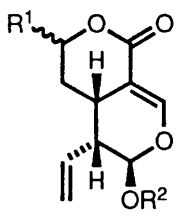


1b : R=(-)-(*S*)-MTPA
1c : R=(+)-(*R*)-MTPA

Δδ values in Hz (=δ*S*-δ*R* ; measured at 270MHz)



5 : R=H
5a : R=(-)-(*S*)-MTPA
5b : R=(+)-(*R*)-MTPA



6 : R^1=α-OCH_3, R^2=Glc
6a: R^1=α-OCH_3, R^2=H
7 : R^1=β-OCH_3, R^2=Glc
8 : R^1=H, R^2=Glc

Table II. Inhibitory Effects of Hydramacrosides A and B on the Histamine Release Induced by Antigen-Antibody Reaction

	Conc.(M)	Inhibition (%) mean ± S.E. (n=4)
Hydramacroside A(**1**)	$3x10^{-4}$	70.0±3.5
	10^{-4}	33.1±4.2
	$3x10^{-5}$	19.8±4.0
	10^{-5}	9.1±11.4
Hydramacroside B(**3**)	$3x10^{-4}$	78.1±9.5
	10^{-4}	57.1±2.6
	$3x10^{-5}$	21.3±21.8
	10^{-5}	21.3±3.7

stereostructure of the C_1 position was determined to be *S* configuration. Finally, the absolute stereostructure of the C_{15} position in **1** was determined by means of the modified Mosher's method. Thus, treatment of **1** with (-)-(*S*)- and (+)-(*R*)-α-methoxy-α-trifluoromethylphenylacetic acid(MTPA) and 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride(EDC•HCl) in CH_2Cl_2 in the presence of dimethylaminopyridine (DMAP) furnished the (-)-(*S*)-MTPA(**1b**) and (+)-(*R*)-MTPA esters(**1c**). The signals due to protons on the 16-C and 17-C of **1b** appeared at higher field than those of **1c**, while the 14-proton signals of **1b** was observed at lower field than those of **1c**, so that the absolute configuration at the C_{15} position has been determined to be *S* configuration. Consequently, the absolute stereostructure of hydramacroside A(**1**) was determined as shown.

The absolute stereostructure of hydramacroside B(**3**)[11] has been elucidated in the same way. Namely, **3** liberated D-glucouse by acid hydrolysis, while ordinary acetylation of **3** furnished the hexaacetate(**3a**).[12] The observation of NOE enhancements between proton pairs in **3** (1'-H & 1-H; 5-H & 9-H; 5-H & 7-H) indicated the relative stereostructure of **3**. The enzymatic hydrolysis of **3** yielded the aglycone (**4**)[13] whose relative stereostructure was elucidated by detailed ^1H NMR examination including NOE observation between proton pairs in **4** (1-H & 8-H; 5-H & 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 [Δδ +1.5ppm(1'-C), +1.8ppm(1-C)] were observed, so that the C_1-configuration of **3** was determined to be *S* configuration. Finally, in order to determine the absolute configuration of the C_{15} position in **3**, the following conversion has been carried out. First, treatment of **3** with pig liver esterase in phosphate buffer (pH 7.0) followed by methylation with CH_2N_2 furnished **5**,[14] 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 [Δδ values for the protons on C_{16}(-10.8, -24.3Hz), C_{18}(-10.8Hz) and C_{14}(+5.4, +29.7Hz)]. Based on this evidence, the absolute stereostructure of hydramacroside B was determined to be **3**.

As shown in Table II, hydramarosides A(**1**) and B(**3**) showed inhibitory activity on the histamine release from the rat mast cells induced by antigen-antibody reaction. Further examination of the antiallergic activity of **1** and **2** is in progress in our laboratory.

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(Received May 23, 1994; accepted June 22, 1994)