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

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Received July 9, 1999; accepted August 23, 1999

Following the characterization of dihydroisocoumarin constituents, two secoiridoid glucoside complexes, called hydramacosides A and B, were isolated from the leaves of *Hydrangea macrophylla* *Seringe* var. *thunbergii* Makino. The absolute stereostructures of hydramacosides A and B were elucidated on the basis of chemical and physicochemical evidence, which included the application of the 13C-NMR glycosylation shift rule of 1,1′-disaccharides and the modified Mosher’s method. Hydramacosides A and B exhibited an inhibitory effect on histamine release from rat mast cells induced by an antigen–antibody reaction.

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

In the course of our studies on the bioactive constituents of natural medicine and medicinal foodstuffs, we have reported the isolation and structural elucidation of antiallergic and antimicrobial principles, thunberginols A, B, C, D, E, and F, thunberginol G 3′-O-glucoside, and hydramacyrophyllols A and B from *Hydrangea* Dulcis Folium, the processed leaves of *Hydrangea macrophylla* *Seringe* var. *thunbergii* Makino (Saxifragaceae). 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 and was easily synthesized from phyllodulcin, the principle component of this natural medicine. In addition, ten dihydroisocoumarin glycosides, 3R- and 3S-phyllodulcin 3′-O-glucosides, 3R- and 3S-thunberginol H 8-O-glucosides, 3R- and 3S-hydrangenol 4′-O-apiosylglucosides, 3R- and 3S-thunberginol I 4′-O-glucosides, thunberginol 1 8-O-glucosides, and 3S-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 hydramacosides 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.

**Hydramacoside A (1)** Hydramacoside A (1) was isolated as colorless fine crystals with a m.p. of 141–144 °C and negative optical rotation ([α]D20 = −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 C28H35O12 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 1H-NMR spectrum dimethyl sulfoxide (DMSO)-d6 of 1 showed signals due to the secoiridoid lactone moiety [δ 5.43 (d, J=1.3 Hz, 1-H),

![Chart 1](image-url)

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7.48 (dd, J=2.3 Hz, 3-H), 3.12 (m, 5-H), 1.27, 1.82 (both m, 6-H), 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), 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), 2.51 (m, 14-H), 3.89 (m, 15-H), 1.57 (m, 16-H), 2.42, 2.58 (both m, 17-H), 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 13C-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 1H- and 13C-NMR signals of 1 could be analyzed by use of distortionless enhancement by polarization transfer (DEPT), 1H-1H and 1H-13C 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; 11-C and 3-H; 13-C and 12-H, 14-H, 18-C and 17-H) (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 C22H36O5. The relative stereostructure of 2 was clarified by detailed comparisons of 1H- and 13C-NMR spectra with those for 1, 6, and 6a. Acetylation of 1 with Ac2O in pyridine furnished the hexacacetate (1a), whose 1H-NMR spectrum (DMSO-d6) showed signals indicative of a phenolic acetyl group (δ 2.24), and five alcoholic acetyl groups (δ 1.89, 1.95 (6H), 1.98, 2.02). Comparison of the 13C-NMR data (Table 1) for 1 with those for 1a showed acetylation shifts around the C15 and C21 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 1H- and 13C-NMR data with those for the known secoiridoid glucosides such as 6, epi-vogeloside and xerovogside, 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).

The absolute configuration of the C1 position in 1 has been determined by application of the 13C-NMR glycosylation shift rule for the dihedral moieties 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 C1 configuration of 6a was found to be retained according to 1H-NMR analysis, including NOE experiments. The glycosylation shifts [Δδ +1.5 ppm (1'C) and +1.8 ppm (1-C)] were found to be characteristic of the R,R-dihemicetal combination, which corresponded to the absolute stereostructure of 6 (Fig. 2). The glycosylation shifts of 1 also showed Δδ +1.9 ppm (1'C) and +2.4 ppm (1-C), which were characteristic of the R,R-dihemicetal combination, so that the absolute stereostructure of the C1 position was determined to be an S configuration (Fig. 2). Finally, the absolute stereostructure of the C15 position in 1 was determined by means of the modified Mosher's method, as shown in Fig.

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**Fig. 1.** 1H-H COSY, COLOC, and NOE

**Fig. 2.** 13C-NMR Glycosylation Shift (68 MHz, Pyridine-d5)
$\Delta \delta$ values in Hz (=\delta--\delta'; measured at 270 MHz)

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

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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$_2$Cl$_2$ 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$_{16}$ 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 mp 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$_{13}$H$_{20}$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 o-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 I and 3 on the Histamine Release from Rat Sensitized Peritoneal Exudate Cells Induced by an Antigen–Antibody Reaction.

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<th>Conc. (μM)</th>
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<td>Mean±S.E.</td>
<td>(n=4)</td>
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<td>78.8±1.95</td>
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Sensitized rat peritoneal exudate cells were preincubated with samples for 15 min at 37 °C prior to the antigen challenge with phosphoryl-d-serine and dimethylaminophenylboronic acid 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 1H-NMR examination including NOE observation between proton pairs in 4 (1-H and 4-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 [Δδ = 1.5 ppm (1'-C), +1.8 ppm (1-C)] were observed, so the C-4 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 CH2N2, 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 [Δδ values for the protons on C(15) (-10.8, -24.3 Hz), C(18) (-10.8 Hz) and C(16) (+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 antilipemic 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 (10⁻³ − 3×10⁻⁴ μM).

Experimental

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

Isolation of Hydramacrosides A (1) and B (3)

As described in a previous report, the fraction 7−4 (422 mg) was subjected to HPLC [YMC-pack R&D-ODS-5A (250×10 mm i.d.), MeOH-H2O (1:1, v/v) followed by chiral column HPLC (Cerebral Chiral RU-1 (Shiseido Ltd.), MeOH) to afford 1 (28.0 mg) and 3 (30.0 mg) together with 3R-(1.4 mg) and 3S-hydramacrolone 4'-O-apiosylglucose (2.8 mg), (-)-hydramacrolone 4'-O-glucoside (3.4 mg).

Hydramacroside A (1): Colorless fine crystals, mp 141−144 °C, [α]D20 +129.5° (c=0.516, MeOH). High-resolution positive-ion FAB-MS: Calcd for C36H45O29SNa (M+Na⁺) 558.2169. Found: 556.2156. 1H-NMR (500 MHz, DMSO-d6) δ 1.27, 1.82 (2H, both m, 6-H), 1.57 (2H, m, 16-H), 2.42, 2.58 (2H, both m, 17-H), 2.51 (2H, m, 14-H), 2.64 (1H, m, 9-H), 2.75 (1H, dd, J=5.2, 7.1 Hz), 2.87 (1H, dd, J=6.7, 17.1 Hz), 12.32 (1H, m, 5-H), 3.89 (1H, m, 15-H), 4.50 (1H, dd, J=7.7, 14.3 Hz), 4.75 (1H, m, 7-H), 5.23 (1H, dd, J=2.3, 9.9 Hz), 5.20 (1H, dd, J=2.3, 17.2 Hz) (10-H), 5.43 (1H, dd, J=1.3, 13.1 Hz), 5.44 (1H, m, 8-H), 6.65 (2H, d, J=8.6, 20 Hz), 6.97 (2H, d, J=8.6 Hz, 19, 23-H), 7.48 (1H, d, J=2.3 Hz, 3-H).

1C-NMR (125 MHz, DMSO-d6, 68 MHz, pyridine-d5) δ: given in Table 1. Positive-ion FAB-MS m/z: 565 (M+H⁺), 557 (M+Na⁺).

Hydramacroside B (3): Colorless fine crystals, mp 154−157 °C, [α]D20 +10.7° (c=0.118, CHCl₃), IR (KBr) cm⁻¹: 3453, 1713, 1619. 1H-NMR spectrum (270 MHz, CDCl₃) 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). 13C-NMR spectrum (68 MHz, pyridine-d5) δ: given in Table 1. Positive-ion FAB-MS m/z: 628 (M+H⁺), 644 (M+Na⁺) (M+Na⁺).
Acetylation of 3 A solution of 3 (4.1 g, 0.0068 mmol) in pyridine (0.3 ml) was treated with Ac2O (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 NaHCO3, and brine, then dried over MgSO4 and filtered. After removal of the solvent under reduced pressure, the hexaacetate (3a, 5.5 g, quant.) was obtained.

Hydramoacridine B Hexaacetate (3a): Colorless fine crystals, mp 62–80°C (in CH2Cl2); [α]20D +113° (c = 0.372, CHCl3); 1H-NMR (20 MHz, CDCl3) δ 5.28, 7.23 (2H, 2H-15, 2H-16), 4.92 (2H, 2H-17, 2H-18), 2.49 (2H, 2H-17, 2H-18), 2.56 (1H, 1H-9, 2H-9, 2.63, 2.85 (2H, 2H-12, 2H-13, 2.68, 2.89 (2H, 2H-14, 2.96) (1H, 1H-5, 4.78 (1H, δ = 8.0 Hz, 1H-1'), 4.80 (1H, 1H-7), 5.17, 5.24 (2H, 2H-10, 2.52 (1H, 1H-8, 5.24 (1H, δ = 2.3 Hz, 1H-12, 5.53 (1H, 1H-15, 7.02 (2H, δ = 8.9 Hz, 22, 20), 7.07 (2H, δ = 8.9 Hz, 19, 23-2H), 7.46 (1H, δ = 7.3 Hz, 3-H). 13C-NMR (68 MHz, CDCl3); δ 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 ml) was treated with β-glucoisidase (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 water and the whole was extracted with CH2Cl2. The CH2Cl2 extract was washed with brine, then dried over MgSO4 and filtered. After removal of the solvent under reduced pressure, the residue (9.6 g) was purified by silica gel column chromatography [40 g, CHCl3, MeOH: 1:10 to 4 (5.5 g, 91.7%)].

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 mg). The reaction mixture was stirred at 38°C for 2 h. 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, H2O–40%MeOH) to give 5 (11.1 mg, quant.) on the basis of the NMR spectrum of the total 13C and 1H NMR signals. After evaporation of the solvent, CH2Cl2 (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.

A white powder, [α]20D -38.2° (c = 0.400, MeOH). IR (KBr cm-1): 3410, 1655, 1615 (sh). 1H-NMR (270 MHz, CDCl3) δ: 2.50, 2.62 (2H, m-15), 2.51, 2.50 (2H, m-14, 2.52, 2.66 (2H, 1H-16), 2.66 (1H, 1H-9, 2H-9), 2.68 (4H, m-18, 19-2H), 3.42 (1H, 1H-5, 3.60 (3H, 3H-11, 3.76 (3H, s-23-OCH3), 4.15 (1H, m-15, 15-H), 4.72 (1H, d-like, 1'-H), 5.13, 5.18 (2H, 2H-10), 5.57 (1H, δ = 9.0 Hz, 1'-H), 5.68 (3H, 1H-6, 7, 8-H), 6.80 (2H, δ = 8.6 Hz, 22, 24-H), 7.05 (2H, δ = 8.6 Hz, 21, 25-H), 7.57 (1H, s-3-H). 13C-NMR (68 MHz, CDCl3); δ given in Table 1. Positive-ion FAB-MS m/z: 467 [M+Na]+.

Preparation of the MTPA Esters (5a, 5b) From a solution of 5 (5.8 mg, 0.011 mmol in CDCl3, 2.0 ml) was treated with (BIP-TMPA (21.1 mg, 0.090 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (17.2 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 N2 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 NaHCO3, and brine, then dried over MgSO4 and filtered. Evaporation of the solvent from the filtrate under reduced pressure furnished an oil (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.

Biassic Test for the Inhibitory Activity on Histamine Release The methods of bioassay testing are the same as described previously.

References and Notes


