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# MACROPYLLANOSIDES A – D, SECOIRIDOID GLYCOSIDES FROM *HYDRANGEA MACROPHYLLA* SUBSP. *SERRATA*

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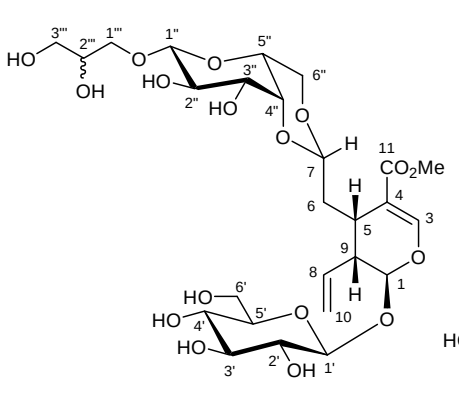
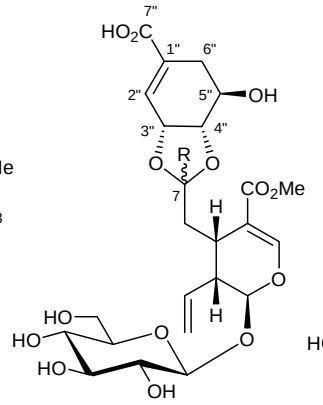
**Abstract** – Four new secoiridoid glycosides, named macrophyllanosides A – D, were isolated from the leaves of *Hydrangea macrophylla* subsp. *serrata*. Their structures have been determined by spectroscopic and chemical means.

## INTRODUCTION

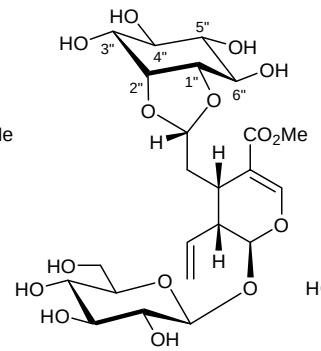
Plants of the genus *Hydrangea* (Saxifragaceae) are known to contain secoiridoid glycosides[1-4] and dihydroisocoumarins,[5,6] and some of them possess antiallergic and antimicrobial activities.[4,6] The constituents of the leaves of *H. macrophylla* subsp. *serrata* (*yama-ajisai* in Japanese) have been previously investigated and shown to contain dihydroisocoumarin glycosides.[7] In previous papers, we reported the isolation and structural elucidation of secoiridoid glycosides,[8-10] dihydroisocoumarin glycoside[10] and megastigmane glycoside[10] from the leaves of the plant. Here, we report the isolation and structure elucidation of four new secoiridoid glycosides, named macrophyllanosides A (**1**) – D (**4**) from the leaves of the plant.



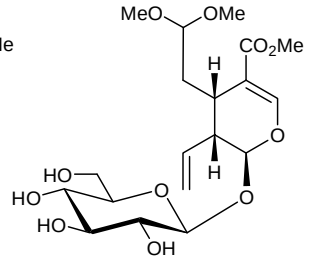
**1**



**2** R = β-H
**3** R = α-H



**4**

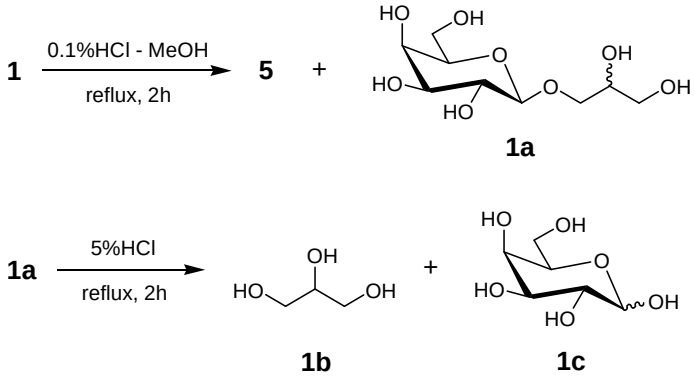


**5**

This paper is dedicated to Prof. Dr. Ryoji Noyori on the occasion on his 70th birthday.

## RESULTS AND DISCUSSION

Macrophyllanoside A (**1**) was isolated as an amorphous powder, $[\alpha]_D$ -81.5° (MeOH). The molecular formula was determined to be $C_{26}H_{40}O_{17}$ by HR-FAB-MS. The $^1H$ and $^{13}C$ NMR spectra (Experimental) were similar to those of secologanin dimethyl acetal (**5**) isolated from the same plant,[8] except for the presence of an additional hexosyl [$\delta_H$ 4.27 (1H, d, $J$ = 7.6 Hz, H-1″); $\delta_C$ 68.2 (C-5″), 69.7 (C-6″), 72.1 (C-2″), 73.1 (C-3″), 77.0 (C-4″), 104.9 (C-1″)] and a glycerol [$\delta_C$ 64.0 (C-3‴), 71.9 (C-1‴), 72.1 (C-2‴)] moieties. Methanolysis of **1** afforded **5** and 1-*O*-glyceryl-β-D-galactopyranoside (**1a**)[11] (Scheme 1). Furthermore, acid hydrolysis of **1a** furnished glycerol (**1b**) and D-galactose (**1c**) (Scheme 1).




Scheme 1

Interpretation of the HMBC spectrum of **1** revealed correlations from H-7 to C-4″ and C-6″ (Figure 1). As a result, C-7 carbon is linked to two oxygen atoms at C-4″ and C-6″ to establish a six-membered acetal ring. In the difference NOE experiment, irradiation at $\delta_H$ 4.72 (H-7) caused NOE enhancement in the signals of H-4″ and *axial*-H-6″. These NOEs implied that H-7 was β with respect to the six-membered acetal ring with the chair conformation and that H-7 and each H-4″ and *axial*-H-6″ were in 1,3-diaxial arrangements (Figure 1).

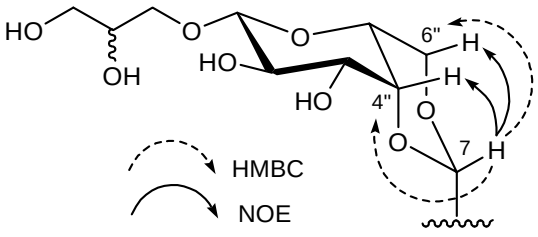
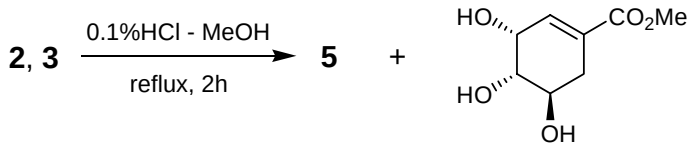



Figure 1

Stereochemistry at C-2‴ remain undetermined. Therefore, the structure of macrophyllanoside A was determined as the acetal between secologanin and 1-*O*-glyceryl-β-D-galactopyranoside.

Macrophyllanoside B (**2**) was isolated as an amorphous powder, $[\alpha]_D$ -41.6° (MeOH). The molecular formula was determined to be $C_{24}H_{32}O_{14}$ by HR-FAB-MS. The $^1H$ and $^{13}C$ NMR spectra were similar to

those of **5**, except for the presence of an additional signals, corresponding to a shikimic acid moiety [$\delta_H$ 2.19 (1H, dd, *J* = 17.6, 7.0 Hz, H-6″β), 2.67 (1H, dd, *J* = 17.6, 4.4 Hz, H-6″α), 3.75 (1H, m, H-5″), 3.96 (1H, t, *J* = 7.1 Hz, H-4″), 4.60 (1H, m, H-3″), 6.73 (1H, br.s, H-2″); $\delta_C$ 31.5 (C-6″), 71.6 (C-5″), 74.7 (C-3″), 79.5 (C-4″), 129.4 (C-1″), 131.6 (C-2″), 169.3 (C-7″)]. Methanolysis of **2** provided **5** and shikimic acid methyl ester (**2a**) (Scheme 2).




**2a**

Scheme 2

The NOESY cross peaks observed between H-3″ and H-4″, H-7 and H-3″, and H-7 and H-4″ implied that C-7 carbon is linked to two oxygen atoms at C-3″ and C-4″ to establish a five-membered acetal ring and that H-7 was β with respect to the five-membered acetal ring (Figure 2).

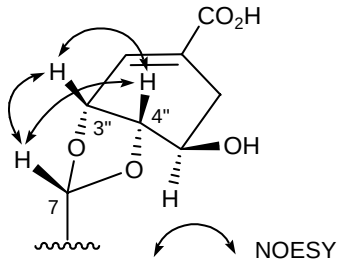



Figure 2

Accordingly, the structure of macrophyllanoside B was determined as the acetal between secologanin and shikimic acid.

Macrophyllanoside C (**3**) was isolated as an amorphous powder, $[\alpha]_D$ -48.6° (MeOH). The molecular formula was determined to be $C_{24}H_{32}O_{14}$ by HR-FAB-MS. The $^1H$ and $^{13}C$ NMR spectral data of **3** closely resembled of **2**, except for the signals due to the shikimic acid moiety. Methanolysis of **3** afforded **5** and **2a** (Scheme 2). In the difference ROE experiment, irradiation at $\delta_H$ 5.14 (H-7) caused ROE enhancement in the signals of H-5″. This ROE implied that H-7 was α with respect to the five-membered acetal ring (Figure 3). Thus, macrophyllanoside C was a C-7 epimer of **2** as shown in formula **3**.

Macrophyllanoside D (**4**) was isolated as an amorphous powder, $[\alpha]_D$ -89.2° (MeOH). The molecular formula was determined to be $C_{23}H_{34}O_{15}$ by HR-FAB-MS. The $^1H$ and $^{13}C$ NMR spectra were similar to those of **5**, except for the presence of an additional signals, corresponding to a *myo*-inositol moiety [$\delta_H$ 3.14 (1H, dd, *J* = 10.0, 9.3 Hz, H-5″), 3.46 (1H, dd, *J* = 10.0, 7.3 Hz, H-6″), 3.55 (1H, dd, *J* = 9.3, 9.3 Hz, H-4″), 3.69 (1H, dd, *J* = 9.3, 4.1 Hz, H-3″), 3.90 (1H, dd, *J* = 7.3, 5.1 Hz, H-1″), 4.16 (1H, dd, *J* = 5.1, 4.1

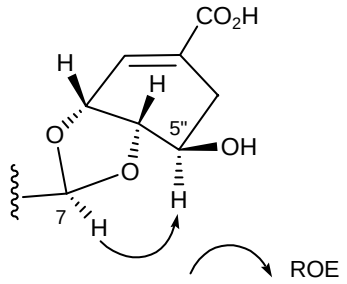
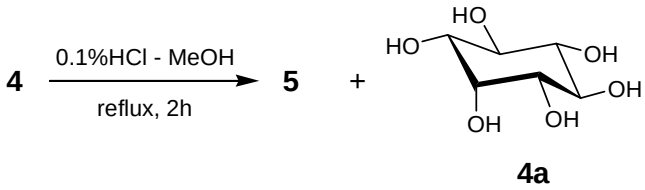



Figure 3

Hz, H-2″); $\delta_C$ 71.7 (C-3″), 74.0 (C-4″), 75.2 (C-5″), 77.4 (C-6″), 79.8 (C-1″), 80.0 (C-2″)]. Methanolysis of **4** furnished **5** and *myo*-inositol (**4a**) (Scheme 3).




**4a**

Scheme 3

In the $^{13}$C NMR spectrum of **4**, the downfield shifts due to the β-effect of alkyl group[12] were observed for the signals of C-1″ (+7.4 ppm) and C-2″ (+7.8 ppm) as compared with the signals of **4a**. The NOESY cross peaks were observed between H-1″ and H-2″, H-2″ and H-3″, and H-7 and H-2″ (Figure 4). These data indicated that C-7 carbon is linked to two oxygen atoms at C-1″ and C-2″ to establish a five-membered acetal ring and that H-7 was β with respect to the five-membered acetal ring.

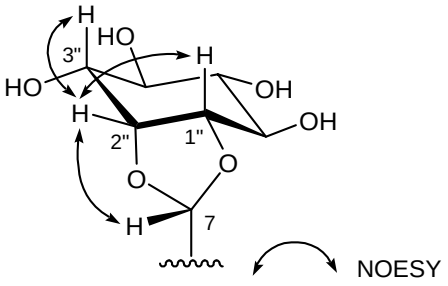



Figure 4

Therefore, the structure of macrophyllanoside D was determined as the acetal between secologanin and *myo*-inositol.

Macrophyllanosides A (**1**) – D (**4**) might be artifacts during the extraction and isolation processes. Compounds **1** – **4** are a rare type of secoiridoid glycosides that connects secologanin and 1-*O*-glyceryl-β-D-galactopyranoside, shikimic acid or *myo*-inositol by acetal structure. Similar acetal derivatives of secologanin were reported from the plant species, *Adina racemosa*,[13] *Gentiana verna*,[14] *Lonicera caerulea*,[15] *L. korolkovii*[16] and *L. japonica.*[17]

## EXPERIMENTAL

**General** Optical rotations were taken with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. The $^{1}H$ and $^{13}C$ NMR spectra were recorded with JEOL JNM-LA 400 (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. HR-FAB-MS were recorded on a JEOL JMS-700 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230 – 400 mesh) and Diaion HP-20 (Mitsubishi-Chemical). HPLC was performed by using a system comprised of CCPS pump (Tosoh), a UV-8020 detector (Tosoh), a RI-8020 detector (Tosoh) and a JASCO OR-2090 plus chiral detector. GLC was carried out on a GC-7A gas chromatograph (Shimadzu).

**Plant Material** The leaves of *Hydrangea macrophylla* subsp*. serrata* were collected in the Aizu region in Fukushima Prefecture, Japan, in August of 2003, and identified by one of the authors (M.K.). A voucher specimen (HMS-2003-01) was deposited in our laboratory.

**Extraction and Isolation** The leaves of *H. macrophylla* subsp*. serrata* (1.2 kg) were extracted with MeOH at rt. The MeOH extract was concentrated under reduced pressure. The MeOH extract (110 g) was suspended in water, and this suspension was extracted with $CHCl_3$, AcOEt, *n*-BuOH and $H_2O$. The $H_2O$ soluble fraction was passed through a Diaion HP-20 column, and adsorbed material was eluted with $H_2O$ and MeOH. The MeOH elute fraction was concentrated. The residue (8 g) was chromatographed on a silica gel column using $CHCl_3$ – MeOH – $H_2O$ (30 : 10 : 1) to afford 10 fractions. Fraction 4 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40℃; mobile phase, MeOH – $H_2O$ (1 : 4); flow rate, 1.0 ml/min; UV detector, 230 mn] to give macrophyllanoside A (**1**, 3.4 mg), macrophyllanoside B (**2**, 9.6 mg), macrophyllanoside C (**3**, 0.6 mg) and macrophyllanoside D (**4**, 0.8 mg).

**Macrophyllanoside A (1)** Amorphous powder. $[\alpha]_D^{22}$ -81.5° (c 0.34, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 203 (4.1), 229 (4.1). HR-FAB-MS *m*/*z*: 625.2334 ($[M + H]^+$, $C_{26}H_{41}O_{17}$, calcd for 625.2344). $^{1}H$ NMR ($CD_3OD$) δ: 1.82 (1H, m, H-6A), 2.18 (1H, m, H-6B), 2.69 (1H, m, H-9), 3.03 (1H, m, H-5), 3.19 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 3.35 (1H, t, *J* = 8.8 Hz, H-3′), 3.45 (1H, br.s, H-5″), 3.65 (1H, dd, *J* = 10.2, 6.1 Hz, H-6′A), 3.70 (3H, s, $CH_3O$-11), 3.80 (1H, m, H-2‴), 3.84 (1H, br.d, *J* = 11.0 Hz, *axial*-H-6″), 3.90 (1H, dd, *J* = 10.2, 2.2 Hz, H-6′B), 3.93 (1H, br.s, H-4″), 4.01 (1H, br.d, *J* = 11.0 Hz, *equatorial*-H-6″), 4.27 (1H, d, *J* = 7.6 Hz, H-1″), 4.68 (1H, d, *J* = 7.8 Hz, H-1′), 4.72 (1H, dd, *J* = 6.8, 3.9 Hz, H-7), 5.24 (1H, dd, *J* = 10.5, 1.7 Hz, H-10A), 5.32 (1H, dd, *J* = 17.3, 1.7 Hz, H-10B), 5.52 (1H, d, *J* = 5.9 Hz, H-1), 5.74 (1H, m, H-8), 7.42 (1H, s, H-3). The signals of H-4′ and H-5′ were overlapped with the

residual solvent signal. $^{13}$C NMR ($CD_3OD$) δ: 29.8 (C-5), 35.4 (C-6), 45.1 (C-9), 51.8 ($CH_3O$-11), 62.8 (C-6′), 64.0 (C-3‴), 68.2 (C-5″), 69.7 (C-6″), 71.6 (C-4′), 71.9 (C-1‴), 72.1 (C-2″, C-2‴), 73.1 (C-3″), 74.6 (C-2′), 77.0 (C-4″), 78.0 (C-3′), 78.4 (C-5′), 97.6 (C-1), 100.0 (C-1′), 102.1 (C-7), 104.9 (C-1″), 111.6 (C-4), 119.7 (C-10), 135.8 (C-8), 153.2 (C-3), 169.3 (C-11).

**Methanolysis of 1.** Compound **1** (1.0 mg) was refluxed with 0.1% HCl – MeOH (50 μL) for 2h. The reaction mixture was neutralized with $Ag_2CO_3$ and filtered. The filtrate was subjected to preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40°C; mobile phase, MeOH – $H_2O$ (1 : 1); flow rate, 1.0 mL/min; RI detector] to give secologanin dimethyl acetal [**5**, 0.2 mg; $[\alpha]_D^{23}$ -108.0° (c 0.02, MeOH)] and 1-*O*-glyceryl-β-D-galactopyranoside (**1a**, 0.3 mg). **5** was identified with authentic sample by $[\alpha]_D$ and $^1$H NMR spectral data comparisons. **1a** was identified by comparison of the spectroscopic data (MS, $^1$H and $^{13}$C NMR) with reported values.[11]

**Acid hydrolysis of 1a.** Compound **1a** (0.1 mg) was refluxed with 5% HCl (100 μL) for 2h. The reaction mixture was neutralized with $Ag_2CO_3$ and filtered. The filtrate was evaporated *in vacuo* to dryness. The residue was analyzed by GLC [column, 3% SE-52 on Chromosorb W (AW) 2 mm i.d.×1.5 m; column temperature, 120°C for 20 min, from 120°C to 220°C (8°C/min), 220°C to the end the run; carrier gas, $N_2$ (40 ml/min); detector, FID]. Identification of glycerol (**1b**, $t_R$ 3.8 min) and galactose ($t_R$ 15.5, 16.0, and 16.5 min) as these TMSi derivatives were carried out by the comparison of these retention times with those of authentic samples. Next, a part of the acid hydrolysis product was analyzed by HPLC [column, TSKgel Amide-80 (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40°C; mobile phase, MeCN – $H_2O$ (5 : 1); flow rate, 1.0 mL/min; chiral detection]. Identification of D-galactose (**1c**) was carried out by the comparison of its retention time and optical rotation with that of an authentic sample; $t_R$ 51.0 min (positive optical rotation).

**Macrophyllanoside B (2)** Amorphous powder. $[\alpha]_D^{22}$ -41.6° (c 0.96, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 224 (3.9); HR-FAB-MS *m/z*: 567.1680 ($[M + Na]^+$, $C_{24}H_{32}O_{14}Na$, calcd for 567.1690). $^1$H NMR ($CD_3OD$) δ: 1.81 (1H, m, H-6A), 2.11 (1H, m, H-6B), 2.19 (1H, dd, *J* = 17.6, 7.0 Hz, H-6″β), 2.67 (1H, dd, *J* = 17.6, 4.4 Hz, H-6″α), 2.78 (1H, m, H-9), 3.05 (1H, m, H-5), 3.18 (1H, dd, *J* = 9.1, 7.8 Hz, H-2′), 3.64 (1H, m, H-6′A), 3.68 (3H, s, $CH_3O$-11), 3.75 (1H, m, H-5″), 3.90 (1H, dd, *J* = 10.7, 1.7 Hz, H-6′B), 3.96 (1H, t, *J* = 7.1 Hz, H-4″), 4.60 (1H, m, H-3″), 4.65 (1H, d, *J* = 8.1 Hz, H-1′), 5.13 (1H, dd, *J* = 5.1, 4.9 Hz, H-7), 5.23 (1H, dd, *J* = 10.5, 1.7 Hz, H-10A), 5.28 (1H, dd, *J* = 18.8, 1.7 Hz, H-10B), 5.52 (1H, d, *J* = 5.1 Hz, H-1), 5.71 (1H, m, H-8), 6.73 (1H, br.s, H-2″), 7.42 (1H, d, *J* = 1.5 Hz, H-3). The signals of

H-3′, H-4′ and H-5′ were overlapped with the residual solvent signal. $^{13}$C NMR ($CD_3OD$) δ: 29.9 (C-5), 31.5 (C-6″), 35.1 (C-6), 45.3 (C-9), 51.8 ($CH_3O$-11), 62.8 (C-6′), 71.6 (C-4′, C-5″), 74.7 (C-2′, C-3″), 78.1 (C-3′), 78.4 (C-5′), 79.5 (C-4″), 97.9 (C-1), 100.2 (C-1′), 104.8 (C-7), 111.4 (C-4), 119.5 (C-10), 129.4 (C-1″), 131.6 (C-2″), 135.6 (C-8), 153.4 (C-3), 169.3 (C-11, C-7″).

**Macrophyllanoside C (3)** Amorphous powder. $[\alpha]_D^{22}$ -48.6° (c 0.06, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 226 (4.1). HR-FAB-MS *m*/*z*: 567.1677 ($[M + Na]^+$, $C_{24}H_{32}O_{14}Na$, calcd for 567.1690). $^1$H NMR ($CD_3OD$) δ: 1.80 (1H, m, H-6A), 2.10 (1H, m, H-6B), 2.20 (1H, dd, *J* = 17.1, 8.8 Hz, H-6″β), 2.66 (1H, dd, *J* = 17.1, 3.7 Hz, H-6″α), 2.78 (1H, m, H-9), 3.05 (1H, m, H-5), 3.19 (1H, dd, *J* = 8.8, 8.1 Hz, H-2′), 3.64 (1H, m, H-6′A), 3.68 (3H, s, $CH_3O$-11), 3.76 (1H, m, H-5″), 3.89 (1H, dd, *J* = 10.2, 1.7 Hz, H-6′B), 3.97 (1H, t, *J* = 7.1 Hz, H-4″), 4.60 (1H, m, H-3″), 4.66 (1H, d, *J* = 7.8 Hz, H-1′), 5.14 (1H, dd, *J* = 5.1, 4.9 Hz, H-7), 5.24 (1H, dd, *J* = 10.7, 1.7 Hz, H-10A), 5.28 (1H, dd, *J* = 18.5, 1.7 Hz, H-10B), 5.52 (1H, d, *J* = 5.4 Hz, H-1), 5.71 (1H, m, H-8), 6.76 (1H, br.s, H-2″), 7.42 (1H, d, *J* = 1.5 Hz, H-3). The signals of H-3′, H-4′ and H-5′ were overlapped with the residual solvent signal. $^{13}$C NMR ($CD_3OD$) δ: 30.0 (C-5), 30.4 (C-6″), 35.1 (C-6), 45.3 (C-9), 51.8 ($CH_3O$-11), 62.8 (C-6′), 71.7 (C-4′, C-5″), 74.7 (C-2′, C-3″), 78.0 (C-3′), 78.4 (C-5′), 79.4 (C-4″), 97.9 (C-1), 100.2 (C-1′), 104.8 (C-7), 111.4 (C-4), 119.4 (C-10), 129.1 (C-1″), 132.8 (C-2″), 135.7 (C-8), 153.4 (C-3), 169.3 (C-11, C-7″).

**Methanolysis of 2 and 3.** Methanolysis of **2** (1.0 mg) and **3** (0.5 mg) gave **5** (0.2 mg from **2**; 0.1 mg from **3**) and shikimic acid methyl ester [**2a**, 0.2 mg from **2**, 0.1 mg from **3**; $[\alpha]_D^{20}$ -76.9° (c 0.01, MeOH)] in the above manner. **2a** was identified with authentic sample by $[\alpha]_D$ and $^1$H NMR spectral data comparisons.

**Macrophyllanoside D (4)** Amorphous powder. $[\alpha]_D^{22}$ -89.2° (c 0.08 MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 205 (4.0), 230 (4.1). HR-FAB-MS *m*/*z*: 551.1949 ($[M + H]^+$, $C_{23}H_{35}O_{15}$, calcd for 551.1976). $^1$H NMR ($CD_3OD$) δ: 1.86 (1H, m, H-6A), 2.24 (1H, m, H-6B), 2.85 (1H, m, H-9), 3.12 (1H, m, H-5), 3.14 (1H, dd, *J* = 10.0, 9.3 Hz, H-5″), 3.21 (1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 3.46 (1H, dd, *J* = 10.0, 7.3 Hz, H-6″), 3.55 (1H, dd, *J* = 9.3, 9.3 Hz, H-4″), 3.67 (1H, dd, *J* = 10.2, 5.6 Hz, H-6′A), 3.69 (1H, dd, *J* = 9.3, 4.1 Hz, H-3″), 3.69 (3H, s, $CH_3O$-11), 3.89 (1H, dd, *J* = 10.2, 2.0 Hz, H-6′B), 3.90 (1H, dd, *J* = 7.3, 5.1 Hz, H-1″), 4.16 (1H, dd, *J* = 5.1, 4.1 Hz, H-2″), 4.65 (1H, d, *J* = 7.8 Hz, H-1′), 5.13 (1H, dd, *J* = 5.9, 4.9 Hz, H-7), 5.27 (1H, dd, *J* = 10.5, 1.7 Hz, H-10A), 5.33 (1H, dd, *J* = 17.3, 1.7 Hz, H-10B), 5.54 (1H, d, *J* = 4.6 Hz, H-1), 5.72 (1H, m, H-8), 7.45 (1H, d, *J* = 1.7 Hz, H-3). The signals of H-3′, H-4′ and H-5′ were overlapped with the residual solvent signal. $^{13}$C NMR ($CD_3OD$) δ: 28.5 (C-5), 35.5 (C-6), 45.1 (C-9), 51.7 ($CH_3O$-11), 62.7 (C-6′), 71.5 (C-4′), 71.7 (C-3″), 74.0 (C-4″), 74.6 (C-2′), 75.2 (C-5″), 77.4 (C-6″),

78.0 (C-3′), 78.4 (C-5′), 79.8 (C-1″), 80.0 (C-2″), 97.9 (C-1), 100.1 (C-1′), 105.6 (C-7), 111.0 (C-4), 120.3 (C-10), 135.6 (C-8), 153.6 (C-3), 169.2 (C-11).

**Methanolysis of 4.** Methanolysis of **2** (0.5 mg) gave **5** (0.1 mg) and *myo*-inositol (**4a**, 0.1 mg) in the above manner. **4a** was identified with authentic sample by $^{1}H$ and $^{13}C$ NMR spectral data comparisons.

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