Neolignans and Phenylpropanoids from the Rhizomes of *Coptis japonica* var. *dissecta*

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Six new dihydrobenzofuran neolignans were isolated from the fresh rhizomes of *Coptis japonica* var. *dissecta*, together with (+)-isolariciresinol, (+)-lariciresinol glucoside, (+)-pinoresinol, (+)-pinoresinol glucoside, (+)-syringaresinol glucoside and ethyl ferulate. Their structures were determined on the basis of spectroscopic data and chemical evidence. Their absolute configurations were determined by means of CD studies.

Key words *Coptis japonica* var. *dissecta*, Ranunculaceae; neolignan; dihydrobenzofuran; dehydrodiconiferyl alcohol; woorenogenin; woorenoside

The rhizomes of *Coptis* species are used as a Chinese crude drug for digestive system problems. Previous phytochemical studies showed the occurrence of alkaloids (berberine, magnofiorine, sanguinarine, norsanguinarine, oxysanguinarine and 6-acetonyl-5,6-dihydrosanguinarin),[1,2] and phenolic constituents.[3-5] In this paper, we report the isolation and structural elucidation of six novel dihydrobenzofuran neolignans, woorenogenin (**1**) and woorenosides I—V (**2**—**6**) from the fresh rhizomes of *Coptis japonica* var. *dissecta*. Their structures were elucidated by chemical and spectral methods, two dimensional (2D)-NMR techniques being especially helpful.

The EtOH extract obtained from the fresh rhizomes of *Coptis japonica* var. *dissecta* was passed through an Amberlist 15 column to give a neutral and acid fraction. Repeated separation of this fraction by ordinary-phase (SiO_2) and reversed-phase (ODS) column chromatography furnished six new neolignans named woorenogenin (**1**) and woorenosides I (**2**), II (**3**), III (**4**), IV (**5**) and V (**6**), as well as six known compounds, (+)-isolariciresinol (**7**),[6] (+)-lariciresinol glucoside (**8**),[7] (+)-pinoresinol (**9**),[8] (+)-pinoresinol glucoside (**10**),[9] (+)-syringaresinol glucoside (**11**)[10] and ethyl ferulate (**12**).[11] ^1H–^1H correlation spectroscopy (^1H–^1H COSY), ^1H–^{13}C COSY, total correlation spectroscopy (TOCSY), heteronuclear multiple-bond correlation (HMBC) and rotating frame Overhauser enhancement spectroscopy (ROESY) experiments provided sufficient information to enable us to construct the complete structures of **1**—**6**, inclusive of the positions of attachment of the acyl moiety and the sugar chain to the aglycone.

Woorenogenin (**1**), $[\alpha]_D^{20}$ −9.0° (MeOH), $C_{22}H_{26}O_7$, showed a molecular ion peak at *m/z* 402.1662 in the high-resolution mass spectrum (HR-MS). The UV spectrum (λ_{max} 208 and 275) indicated a conjugated system similar to that of coniferyl alcohol. The ^{13}C-NMR distortionless enhancement by polarization transfer (DEPT) and ^1H-NMR spectra indicated the presence of one methine carbon, four methoxy carbons, two oxygen-bearing methylene carbons, one oxygen-bearing methine carbon and six tertiary and eight quaternary carbons due to aromatic ring (s) and/or olefinic bond (s). The ^1H–^1H COSY and TOCSY spectra of **1** revealed isolated spin systems (H-2′–6′, H-2–3–CH_2OH at C-3, H-4–6–8–9–10). The gross structure of **1** was determined by analysis of NMR data including ^1H–^1H COSY, ^1H–^{13}C COSY, HMBC and ROESY experiments, and by referring to the data for dihydrodehydrodiconiferyl alcohol[12] and dehydrodiconiferyl alcohol.[13] Thus, **1** is shown to be 2,3-dihydro-3-hydroxymethyl-5(3-hydroxypropenyl)-7-methoxy-2-(3′,4′,5′-trimethoxyphenyl)benzofuran. The relative stereochemistry of the dihydrofuran ring was elucidated by a ROESY experiment. The strong nuclear Overhauser effects (NOEs) between H-3 and H-2′/6′, and the very weak NOE between H-2 and H-3 indicated trans 2/3 configuration. The absolute configuration of the dihydrofuran ring was determined to be 2*R* and 3*S* from

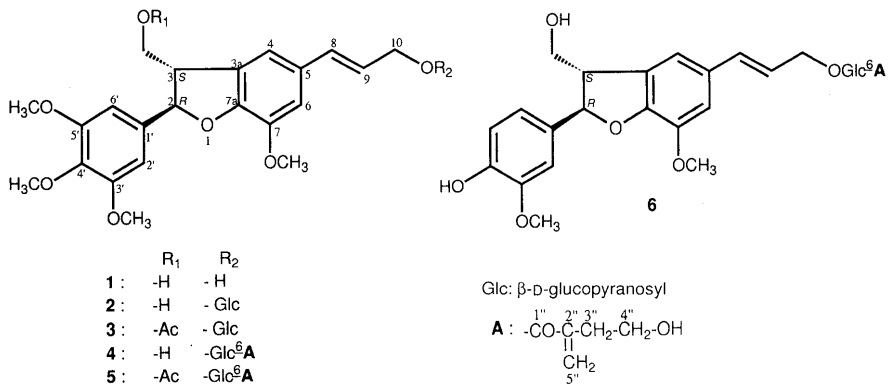

Chart 1

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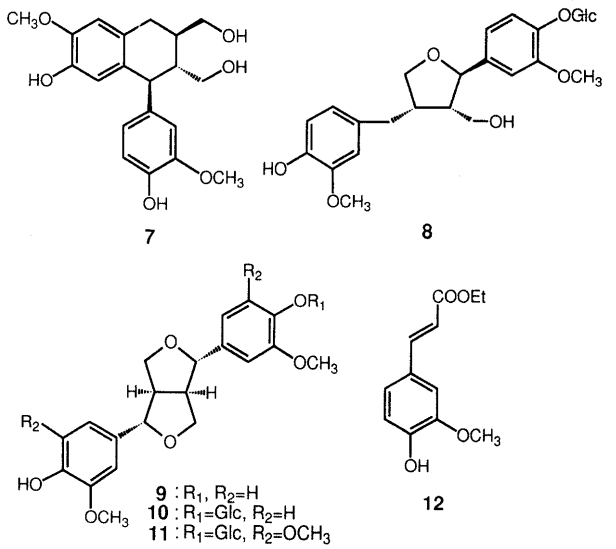

Chart 2

Table 1. ^{13}C-NMR Spectral Data for **1**—**6** (in CD_3OD)

Carbon	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
2	89.1	89.1	89.5	89.0	89.5	89.1
3	55.3	55.3	51.7	55.3	51.8	55.2
–CH_2OH	65.0	64.9	66.6	64.9	66.7	64.9
3a	130.0	130.0	129.0	130.0	129.1	130.2
4	116.5	116.6	116.3	116.7	116.4	116.7
5	132.8	132.4	132.6	132.4	132.7	132.3
6	112.2	111.2	112.3	112.1	112.4	112.2
7	145.6	145.4	145.6	145.4	145.6	145.5
7a	149.2	149.2	149.2	149.3	149.2	149.4
8	132.0	134.0	133.8	134.2	134.0	134.3
9	127.8	124.4	124.7	124.3	124.7	124.3
10	63.9	71.0	70.9	71.1	71.1	71.2
Ac			172.5		172.6	
			20.8		20.8	
–OMe	56.8	56.8	56.7	56.8	56.8	56.8
1′	139.4	139.1	138.2	139.2	138.3	135.9
2′	103.9	103.9	104.2	103.9	104.2	110.7
3′	154.7	154.6	154.6	154.6	154.7	150.3
4′	138.7	138.6	138.8	138.5	138.9	150.4
5′	154.7	154.6	154.6	154.6	154.7	112.9
6′	103.9	103.9	104.2	103.9	104.2	119.5
–OMe	56.6	56.6	56.7	56.6	56.7	56.4
	56.6	56.6	56.7	56.6	56.7	56.5
	61.2	61.0	61.1	61.2	61.2	
Glc-1		103.2	103.2	103.3	103.4	103.2
2		75.1	75.1	75.0	75.1	75.1
3		77.8	77.9	77.9	77.9	77.8
4		71.6	71.6	71.7	71.7	71.6
5		78.0	78.1	75.2	75.3	78.0
6		62.8	62.8	64.9	65.0	62.8
1″				168.2	168.3	168.3
2″				138.6	138.7	138.7
3″				36.3	36.4	36.4
4″				61.6	61.7	61.7
5″				128.0	128.0	128.0

a) 150 MHz. b) 50 MHz.

Table 2. ^{13}C–^{1}H Long-Range Correlations in the HMBC of Compound **1**

Carbons	H in **1**
2	H-3, H-3a, H-2′ (6′)
3	H-2, H-3a, H-4
–CH_2OH	H-2, H-3
3a	H-2, H-3
4	H-3, H-6, H-8
5	H-8, H-9
6	H-4, H-8
7	CH_3-*O*-C_7
7a	H-2, H-3, H-4, H-6
8	H-4, H-6, H-9, H-10
9	H-10
10	H-8
1′	H-2, H-3, H-2′ (6′)
2′ (6′)	H-2
3′ (5′)	H-2′ (6′), CH_3-*O*-$C_{3'(5')}$
4′	CH_3-*O*-$C_{4'}$

the positive Cotton effect at 265 nm ($\Delta\varepsilon = +1.00$) in the CD spectrum, with reference to the CD data ($\Delta\varepsilon = +5.06$ at 275 nm) for 2*R*,3*R*-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-7-methoxy-3-methyl-5(*E*)-propenylbenzofuran (licarin A).[14] Hence, **1** is shown to be 2*R*,3*S*-2,3-dihydro-3-hydroxymethyl-5(3-hydroxypropenyl)-7-methoxy-2-(3′,4′,5′-trimethoxyphenyl)benzofuran.

Woorenoside I (**2**), $[\alpha]_D^{20}$ −21.3° (MeOH), had the molecular formula $C_{28}H_{36}O_{12}$ (negative FAB-MS, *m/z* 563 $[M-H]^-$), *i.e.*, 162 more than that of **1**. The ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of one β-glucopyranosyl unit [H-1: δ 4.36 (d, J=7.8 Hz), C-1: δ 103.2]. On enzymatic hydrolysis with crude cellulase, **2** afforded **1** and D-glucose. In the ^{13}C-NMR spectrum of **2**, the C-10 signal showed a glycosylation shift to δ 71.0.[15,16] Furthermore, in the HMBC spectrum of **2**, a long-range correlation was observed between H-1 (δ 4.36) of the glucose and C-10 (δ 71.0) of the aglycone. Hence, the structure of woorenoside I was represented as **2**.

Woorenoside II (**3**), $[\alpha]_D^{20}$ −26.7° (MeOH), had the molecular formula $C_{30}H_{38}O_{13}$, *i.e.*, C_2H_2O more than that of **2** and showed the ion peaks *m/z* 605 $[M-H]^-$, 563 $[M-H-C_2H_2O]^-$ and 401 $[M-H-C_6H_{10}O_5-C_2H_2O]^-$ in the negative FAB-MS. The IR spectrum of **3** showed absorptions due to an ester function at 1745 and 1240 cm^{-1}. On mild alkaline hydrolysis, **3** afforded **2**. The ^{1}H- and ^{13}C-NMR spectra indicated that **3** was composed of 1 mol each of **1**, acetic acid and glucose. Comparison of the ^{13}C-NMR spectrum of **3** with that of **2** showed the hydroxymethyl at the C-3 position to be the acylation site in the former [+1.7 ppm, from δ 64.9 to 66.6 (hydroxymethyl at C-3), −3.6 ppm, from δ 55.3 to 51.7 (C-3)]. Accordingly, the structure of woorenoside II was represented as **3**.

Woorenoside III (**4**), $[\alpha]_D^{20}$ −24.2° (MeOH), had the molecular formula $C_{33}H_{42}O_{14}$, *i.e.*, $C_5H_6O_2$ more than that of **2**, and showed the fragment ion peaks *m/z* 563 $[M-H-C_5H_6O_2]^-$ and 401 $[M-H-C_6H_{10}O_5-C_5H_6O_2]^-$ in the negative FAB-MS. The IR spectrum of **4** showed absorptions due to an ester function at 1715 and 1240 cm^{-1}. On mild alkaline hydrolysis, **4** afforded **2**. The ^{1}H- and ^{13}C-NMR spectra indicated that **4** was acylated derivative of **2**. The ^{1}H-NMR spectrum of **4** showed six proton signals due to the acyl part at δ 6.25 (1H, t, J=1.5 Hz, H-5″), 5.68 (1H, t, J=1.5 Hz, H-5″), 3.67 (2H, t, J=6.7 Hz, H_2-4″) and 2.54 (2H, br t, J=6.7 Hz, H_2-3″)

(Chart 1), while the ^{13}C-NMR spectrum showed signals due to five carbons of the acyl moiety at δ 168.2 (s), 138.6 (s), 128.0 (d), 61.6 (t) and 36.3 (t). Analysis of the COSY spectrum indicated the presence of γ-hydroxy-α-methylenebutanoic acid (**A**). Comparison of the ^{13}C-NMR spectrum of **4** with that of **2** showed the C-6 position of glucose to be the acylation site in the former [+2.1 ppm, from δ 62.8 to 64.9 (C-6), −2.8 ppm, from δ 78.0 to 75.2 (C-5)]. Accordingly, the structure of woorenoside III was represented as **4**.

Woorenoside IV (**5**), $[\alpha]_D^{20}$ −17.6° (MeOH), had the molecular formula $C_{35}H_{44}O_{15}$, *i.e.*, C_2H_2O more than that of **4**. The negative FAB-MS of **5** showed the fragment ion peaks *m/z* 605 $[M-H-C_5H_6O_2]^-$, 563 $[M-H-C_5H_6O_2-C_2H_2O]^-$ and 401 $[M-H-C_6H_{10}O_5-C_5H_6O_2-C_2H_2O]^-$. On mild alkaline hydrolysis, **5** afforded **2**. The ^{1}H- and ^{13}C-NMR spectra indicated that **5** was composed of 1 mol each of **1**, glucose, **A** and acetic acid. The HMBC experiment revealed long-range coupling of H_2 (δ 4.32, 4.42) of the hydroxymethyl group at C-3 to the carbonyl (δ 172.6) of the acetyl group and 6-H_2 (δ 4.28, 4.51) of Glc to the carbonyl (δ 168.3) of **A**, demonstrating that the acetyl group is located at the hydroxymethyl at C-3 of the aglycone and **A** at 6-OH of Glc. Accordingly, the structure of woorenoside IV was represented as **5**.

Woorenoside V (**6**), $[\alpha]_D^{20}$ −11.8° (MeOH), had the molecular formula $C_{32}H_{40}O_{13}$, *i.e.*, CH_2O less than that of **4**. The negative FAB-MS of **6** showed ion peaks *m/z* 631 $[M-H]^-$, 533 $[M-H-C_5H_6O_2]^-$ and 371 $[M-H-C_6H_{10}O_5-C_5H_6O_2]^-$. Comparison of the ^{13}C-NMR spectra of **6** and **4** showed that **6** differs structurally from **4** only in its C-5′ substituent: a hydrogen in **6** instead of a methoxy group in **4**. In the ROESY experiment on **6**, NOE was observed between the H-5′ signal (δ 6.91, d, J=8 Hz) and H_3–O–C-4′ (δ 3.80). The strong NOEs between H-3 and H-2′/6′, and the very weak NOE between H-2 and H-3 indicated trans 2/3 configuration. The absolute configurations at C-2 and C-3 of **6** may be the same as that of **3**, because the CD spectrum of **6** showed a similar negative Cotton effect ($\Delta\varepsilon$ = −1.67 at 268 nm) to **3**.[17] Accordingly, the structure of woorenoside V was represented as **6**.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus, without correction. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. CD spectra were determined on a JASCO J-500C. IR and UV spectra were measured with JASCO FT/IR-5300 and Shimadzu UV-160 instruments. NMR spectra were recorded on Varian UNITY 200 and 600 spectrometers in CD_3OD solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H-COSY, ^{13}C–^{1}H-COSY, DEPT, HMBC (512×1024 data matrix size, 128 scans, recycle delay = 1.16 s), TOCSY and ROESY. Coupling constants (J values) are given in hertz (Hz). The HR-EI-MS and the FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as the matrix) were measured on JEOL JMS-HX-100 and JEOL JMS-PX303 mass spectrometers, respectively. For column chromatography, Kiesel gel 60 (230—400 mesh, Merck), and for TLC, Silica gel 60F-254 (Merck) were used. HPLC was carried out on a Waters ALC/GPC 244 instrument.

Plant Material The rhizomes of *Coptis japonica* var. *dissecta* cultivated in Sannan-cho, Hyougo prefecture, were collected in December 1993.

Extraction and Isolation The fresh rhizomes (5.0 kg) of *Coptis japonica* var. *dissecta* were extracted with absolute EtOH at room temperature for 3 weeks. The 20% EtOH solution obtained by adding water was passed through an Amberlist 15 column to give the non alkaloid fraction and then an Amberlite XAD-2 column to exclude water-soluble substances. The adsorbate of the Amberlite XAD-2 column was eluted with MeOH to give a neutral and phenolic eluate (23 g). The MeOH eluate was subjected to silica gel column chromatography with CH_2Cl_2–MeOH–H_2O (50:1:0→0.25:10:0.1) to give four fractions (frs. 1—4). Fraction 1 (3 g) was subjected to HPLC on ODS (Develosil Lop ODS, 15%→27% CH_3CN) to give woorenogenin (**1**, 30 mg), (+)-isolariciresinol (**7**, 100 mg), ethyl ferulate (**12**, 70 mg) and (+)-pinoresinol (**9**, 60 mg). Fraction 2 (8 g) was subjected to HPLC on ODS (Develosil Lop ODS, 10%→27% CH_3CN) to give woorenosides I (**2**, 70 mg), II (**3**, 285 mg), III (**4**, 160 mg), IV (**5**, 150 mg) and V (**6**, 25 mg), (+)-pinoresinol 4-*O*-β-D-glucopyranoside (**10**, 45 mg) and (+)-syringaresinol 4-*O*-β-D-glucopyranoside (**11**, 45 mg). Fraction 3 (9.5 g) was subjected to HPLC on ODS (Develosil Lop ODS, 6%→20% CH_3CN) to give woorenoside I (**2**, 100 mg) and (+)-lariciresinol 4′-*O*-β-D-glucopyranoside (**8**, 20 mg).

Woorenogenin (**1**): An amorphous powder, $[\alpha]_D^{20}$ −9.0° (c=0.6, MeOH). IR (film) cm^{-1}: 3400, 1595, 1500. UV λ_{max}^{EtOH} nm (log ε): 208 (4.64), 275 (4.21). CD (c=0.56, MeOH) $\Delta\varepsilon$ +1.00 (265 nm). HR-EI-MS obsd. for $[C_{22}H_{26}O_7]$ 402.1662, Calcd 402.1679, EI-MS *m/z* 402 $[M]^+$ (94%), 384 $[M-H_2O]^+$ (100%), 369 $[M-H_2O-CH_3]^+$ (69%), 353 $[M-H_2O-CH_3O]^+$ (69%), 337 $[M-2H_2O-CH_3O]^+$ (15%), 194 (21%), 181 (28%), 177 (33%), 164 (16%), 91 (21%). ^{1}H-NMR (600 MHz) δ: 3.47 (1H, ddd, J=7.5, 6.0, 5.3 Hz, H-3), 3.73 (3H, s, 4′-OMe), 3.78 (1H, dd, J=11.5, 7.5 Hz, H–CH_2OH at C-3), 3.79 (6H, s, 3′, 5′-OMe), 3.87 (1H, dd, J=11.5, 5.3 Hz, H–CH_2OH at C-3), 3.88 (3H, s, 7-OMe), 4.19 (2H, dd, J=6.0, 1.5 Hz, H-10), 5.57 (1H, d, J=6.0 Hz, H-2), 6.22 (1H, dt, J=15.8, 6.0 Hz, H-9), 6.53 (1H, dt, J=15.8, 1.5 Hz, H-8), 6.69 (2H, s, H-2′, H-6′), 6.95 (2H, br s, H-4, H-6). ^{13}C-NMR: Table 1.

Woorenoside I (**2**): An amorphous powder, $[\alpha]_D^{20}$ −21.3° (c=6.0, MeOH). IR (film) cm^{-1}: 3400, 1595, 1495. UV λ_{max}^{EtOH} nm (log ε): 212.5 (4.52), 275 (4.21). Negative FAB-MS *m/z*: 563 $[M-H]^-$, 401 $[M-H-C_6H_{10}O_5]^-$. *Anal.* Calcd for $C_{28}H_{36}O_{12}\cdot 1/2H_2O$: C, 58.63; H, 6.50. Found: C, 58.41; H, 6.62. CD (c= 0.87, MeOH) $\Delta\varepsilon$ −0.83 (271 nm). ^{1}H-NMR (200 MHz) δ: 3.68, 3.73, 3.73, 3.81 (each 3H, s, OMe), 4.36 (1H, d, J=7.8 Hz, H-1 of Glc), 5.52 (1H, d, J=5.9 Hz, H-2), 6.18 (1H, m, H-9), 6.55 (1H, d, J=16.0 Hz, H-8), 6.65 (2H, s, H-2′, H-6′), 6.91 (2H, s, H-4, H-6). ^{13}C-NMR: Table 1.

Woorenoside II (**3**): An amorphous powder, $[\alpha]_D^{20}$ −26.7° (c=9.9, MeOH). IR (film) cm^{-1}: 3550, 1745, 1495, 1260, 1175. UV λ_{max}^{EtOH} nm (log ε): 212.5 (4.68), 273 (4.37). Negative FAB-MS *m/z*: 605 $[M-H]^-$, 563 $[M-H-C_2H_2O]^-$, 401 $[M-H-C_6H_{10}O_5-C_2H_2O]^-$. *Anal.* Calcd for $C_{30}H_{38}O_{13}\cdot 2H_2O$: C, 56.07; H, 6.59. Found: C, 55.97; H, 6.32. CD (c=0.79, MeOH) $\Delta\varepsilon$ −1.20 (270 nm). ^{1}H-NMR (200 MHz) δ: 1.95 (3H, s, acetyl), 3.69, 3.73, 3.73, 3.80 (each 3H, s, OMe), 4.36 (1H, d, J=7.5 Hz, H-1 of Glc), 5.38 (1H, d, J=7.0 Hz, H-2), 6.19 (1H, m, H-9), 6.55 (1H, d, J=16.3 Hz, H-8), 6.64 (2H, s, H-2′ and H-6′), 6.93 (1H, s, H-4), 6.98 (1H, s, H-6). ^{13}C-NMR: Table 1.

Woorenoside III (**4**): An amorphous powder, $[\alpha]_D^{20}$ −24.2° (c=10.6, MeOH). IR (film) cm^{-1}: 3430, 1715, 1595, 1500, 1330 1240. UV λ_{max}^{EtOH} nm (log ε): 212.5 (4.55), 273.5 (4.20). Negative FAB-MS *m/z*: 563 $[M-H-C_5H_6O_2]^-$, 401 $[M-H-C_6H_{10}O_5-C_5H_6O_2]^-$. *Anal.* Calcd for $C_{33}H_{42}O_{14}\cdot H_2O$: C, 58.23; H, 6.52. Found: C, 58.50; H, 6.48. ^{1}H-NMR (200 MHz) δ: 2.54 (2H, br t, J=6.7 Hz, H-3″), 3.67 (2H, t, J=6.7 Hz, H-4″), 3.73, 3.79, 3.79, 3.87 (each 3H, s, OMe), 4.39 (1H, d, J=7.5 Hz, H-1 of Glc), 5.57 (1H, d, J=5.9 Hz, H-2), 5.68 (1H, t, J=1.5 Hz, H-5″), 6.20 (1H, m, H-9), 6.25 (1H, t, J=1.5 Hz, H-5″), 6.56 (1H, d, J=16.0 Hz, H-8), 6.70 (2H, s, H-2′, H-6′), 6.95 (2H, s, H-4, H-6). ^{13}C-NMR: Table 1.

Woorenoside IV (**5**): An amorphous powder, $[\alpha]_D^{20}$ −17.6° (c=3.2, MeOH). IR (film) cm^{-1}: 3550, 1750, 1715, 1595, 1500, 1330 1240. UV λ_{max}^{EtOH} nm (log ε): 210 (4.66), 273 (4.37). Negative FAB-MS *m/z*: 605 $[M-H-C_5H_6O_2]^-$, 563 $[M-H-C_5H_6O_2-C_2H_2O]^-$, 401 $[M-H-C_6H_{10}O_5-C_5H_6O_2-C_2H_2O]^-$. *Anal.* Calcd for $C_{35}H_{44}O_{15}\cdot 2H_2O$: C, 56.75; H, 6.53. Found: C, 56.69; H, 6.20. ^{1}H-NMR (600 MHz) δ: 2.54 (2H, tt, J=6.7, 1.5 Hz, H-3″), 3.23 (1H, dd, J=8.3, 7.8 Hz, H-2 of Glc), 3.35 (1H, dd, J=8.8, 8.3 Hz, H-4 of Glc), 3.38 (1H, dd, J=8.8, 8.8 Hz, H-3 of Glc), 3.50 (1H, ddd, J=8.8, 5.0, 2.0 Hz, H-5 of Glc), 3.67 (2H, t, J=6.7 Hz, H-4″), 3.70 (1H, ddd, J=7.5, 7.0, 6.8 Hz, H-3), 3.74 (3H, s, 4′-OMe), 3.79 (6H, s, 3′, 5′-OMe), 3.87 (3H, s, 7-OMe), 4.26 (1H, dd, J=12.5, 5.5 Hz, H-10), 4.28 (1H, dd, J=11.5, 5.0 Hz, H-6 of Glc), 4.32 (1H, dd, J=12.0, 7.0 Hz, H–CH_2OH at C-3), 4.37 (1H, d,

$J=7.8$ Hz, H-1 of Glc), 4.41 (1H, ddd, $J=12.5$, 5.4, 1.5 Hz, H-10) 4.42 (1H, dd, $J=12.0$, 7.5 Hz, H–CH_2OH at C-3) 4.51 (1H, dd, $J=11.5$, 2.0 Hz, H-6 of Glc), 5.48 (1H, d, $J=6.8$ Hz, H-2), 5.68 (1H, t, $J=1.5$ Hz, H-5″), 6.21 (1H, ddd, $J=15.9$, 5.4, 5.4 Hz, H-9), 6.25 (1H, t, $J=1.5$ Hz, H-5″), 6.57 (1H, dt, $J=15.9$, 1.5 Hz, H-8), 6.68 (2H, s, H-2′, H-6′), 6.93 (1H, br s, H-4), 6.97 (1H, br s, H-6). ^{13}C-NMR: Table 1.

Woorenoside V (**6**): An amorphous powder, $[\alpha]_D^{20}$ $-11.8°$ ($c=2.4$, MeOH). IR (film) cm^{-1}: 3550, 1715, 1595, 1500, 1330 1240. UV λ_{max}^{EtOH} nm ($\log\varepsilon$): 205 (3.81), 279 (3.23). Negative FAB-MS *m/z*: 631 $[M-H]^-$, 533 $[M-H-C_5H_6O_2]^-$, 371 $[M-H-C_6H_{10}O_5-C_5H_6O_2]^-$. *Anal.* Calcd for $C_{32}H_{40}O_{13}\cdot H_2O$: C, 59.10; H, 6.51. Found: C, 59.00; H, 6.71. CD ($c=0.78$, MeOH) $\Delta\varepsilon$ -1.67 (268 nm). ^{1}H-NMR (600 MHz) δ: 2.55 (2H, tt, $J=6.4$, 1.5 Hz, H-3″), 3.24 (1H, dd, $J=8.8$, 7.8 Hz, H-2 of Glc), 3.34 (1H, dd, $J=8.8$, 8.8 Hz, H-4 of Glc), 3.37 (1H, dd, $J=8.8$, 8.8 Hz, H-3 of Glc), 3.49 (1H, m, H-3), 3.50 (1H, ddd, $J=8.8$, 5.0, 2.0 Hz, H-5 of Glc), 3.65 (2H, t, $J=6.4$ Hz, H-4″), 3.68—3.75 (2H, m, H_2–CH_2OH at C-3), 3.78 (3H, s, 3′-OMe), 3.80 (3H, s, 4′-OMe), 3.87 (3H, s, 7-OMe), 4.26 (1H, ddd, $J=12.5$, 5.5, 1.5 Hz, H-10), 4.28 (1H, dd, $J=11.5$, 5.0 Hz, H-6 of Glc), 4.37 (1H, d, $J=7.8$ Hz, H-1 of Glc), 4.41 (1H, ddd, $J=12.5$, 5.5, 1.5 Hz, H-10), 4.51 (1H, dd, $J=11.5$, 2.0 Hz, H-6 of Glc), 5.56 (1H, d, $J=6.8$ Hz, H-2), 5.69 (1H, t, $J=1.5$ Hz, H-5″), 6.20 (1H, dt, $J=16.4$, 5.5 Hz, H-9), 6.26 (1H, t, $J=1.5$ Hz, H-5″), 6.57 (1H, dt, $J=16.4$, 1.5 Hz, H-8), 6.91 (1H, d, $J=8.0$ Hz, H-5′), 6.93 (1H, dd, $J=8.0$, 1.5 Hz, H-6′), 6.95 (1H, d, $J=1.5$ Hz, H-6), 6.955 (1H, d, $J=1.5$ Hz, H-4), 6.96 (1H, d, $J=1.5$ Hz, H-2′). ^{13}C-NMR: Table 1.

Enzymatic Hydrolysis of Woorenoside I (2) A solution of **2** (20 mg) in EtOH (0.2 ml) and 0.01 M NaH_2PO_4 buffer (pH 4.0, 1.8 ml) was incubated with crude cellulase (20 mg, Sigma) for 1 d at 37 °C. The reaction mixture was passed through a column of Amberlite XAD-2. From the water eluate, D(+)-glucose was detected by using RI detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 80% CH_3CN, 0.8 ml/min, 70 °C) by comparison with authentic sugars (10 mM each of D-Glc and L-Glc). The sugar part gave a positive peak at 13.40 min (D-Glc; 13.38 min). The crude hydrolysate (12 mg) obtained from the methanol eluate was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25 : 2 : 0.1) to give woorenogenin (**1**, 8 mg), the identity of which was examined by TLC, HPLC and ^{1}H-NMR.

Alkaline Hydrolysis of Woorenoside II (3) A 28% sodium methoxide (0.2 ml) solution was added dropwise to a solution of **3** (30 mg) in MeOH (1.0 ml) under an N_2 atmosphere. The mixture was stirred for 1 h at room temperature, then acidified with dilute HCl, and passed through an Amberlite XAD-2 column. The column was washed with water, and the absorbed materials were eluted with MeOH to obtain the crude hydrolysate. The crude hydrolysate was purified by HPLC (YMC, ODS S-5, 25% CH_3CN) to afford **2** (20 mg), the identity of which that was confirmed by TLC, HPLC and ^{1}H-NMR.

Alkaline Hydrolysis of Woorenoside III (4) and IV (5) Alkaline hydrolysis of **4** and **5** (each 10 mg) was carried out in the same way as described for **3** to give **2**, the identity of which was confirmed by TLC, HPLC and ^{1}H-NMR.

References and Notes

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17) The sign of the Cotton effect of the aglycone and its glucoside is reversed. Namely, compound **1** shows a positive Cotton effect at 265 nm, while its glucoside **3** shows a negative Cotton effect at 270 nm.