Studies on the Constituents of *Cimicifuga* Species. XIV.[1) A New Xyloside from the Aerial Parts of *Cimicifuga simplex* WORMSK.

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A new xyloside (I), mp 252—253 °C, $C_{37}H_{58}O_{11}$, $[\alpha]_D$ −51.4°, obtained from the methanolic extract of the aerial parts of *Cimicifuga simplex* was characterized as 7β-hydroxy-23-*O*-acetylshengmanol 3-*O*-β-D-xylopyranoside. A new triterpene (V), 7β-hydroxycimigenol, was also obtained as an aglycone of a glycoside mixture.

Keywords *Cimicifuga simplex*; Ranunculaceae; cycloartane xyloside; 7β-hydroxycimigenol; two dimensional-NMR

Although we and another group have isolated and characterized cimicifugoside,[1)] cimigenol xyloside,[2)] 25-*O*-acetyl- and 25-*O*-methylcimigenol xyloside,[2)] 24-*O*-acetylhydroshengmanol xyloside,[3)] 12-hydroxycimigenol xyloside,[3)] and acetylshengmanol xyloside[4)] from the roots of some Japanese *Cimicifuga* species (Ranunculaceae), these species contain many additional uncharacterized glycosides. In this study, we isolated from the aerial parts of *C. simplex* a new xyloside, 7β-hydroxy-23-*O*-acetylshengmanol 3-*O*-β-D-xylopyranoside (I). We also obtained a new triterpene, 7β-hydroxycimigenol (V), as an aglycone, besides 7β-hydroxy-23-*O*-acetylshengmanol (III), by enzymatic hydrolysis of a glycoside mixture.

The new xyloside (I) was obtained as described in the experimental section after repeated chromatographies on octadecyl silyl silicic acid (ODS) and silica gel columns of the butanol-soluble portion of the methanolic extract from the aerial parts of *C. simplex*.

The xyloside (I), mp 252—253 °C, showed $[\alpha]_D$ −51.4°, $[M]_{290}$ +10111.9°, $[M]_{334}$ −9530.7° (MeOH), and its molecular formula was determined as $C_{37}H_{58}O_{11}$ by fast-atom bombardment mass spectroscopic (FAB-MS) measurement ($M^+ + 1$, m/z 679.3625). The infrared (IR) spectrum of I exhibited strong hydroxyl absorption bands at 3400—2700 cm^{-1}, and ester carbonyl and five-membered ketone bands at 1730 cm^{-1}.

In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in pyridine-d_5), I showed a pair of doublets (J=4.0 Hz) due to cyclopropane methylene protons at δ 0.37 and 0.70 ppm, six singlets due to tertiary methyl groups at 1.07, 1.22, 1.23, 1.35, 1.38, and 1.40 ppm, a doublet (J=6.5 Hz) due to a secondary methyl group at 1.28 ppm, and a singlet due to an acetyl group at 2.09 ppm, along with signals due to other protons (Table I and Fig. 1).

The ^{1}H- and ^{13}C-NMR signals were assigned by the use of ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H-COSY) coupled with ^{1}H–^{13}C-COSY, indicating the presence of the partial structures shown in Fig. 1.

The long-range ^{1}H–^{13}C-COSY of I clarified the connectivities of these partial structures. The methyl signal at 1.22 ppm (28-H_3) showed long-range correlations with the carbons at 41.673 (quaternary carbon (s), C-13), 46.108 (s, C-14), 55.988 (methine carbon (d), C-8), and 81.834 ppm (d, C-15), and the methyl signal at 1.40 ppm (18-H_3) showed correlations with the carbons at 41.673 (s, C-13), 46.108 (s, C-14), 32.678 (methylene carbon (t), C-12) and 60.079 ppm (d, C-17). Furthermore, the methyl signals at 1.23 and 1.38 ppm (26-H_3 and 27-H_3) showed long-range correlations with the carbons at 58.449 (s, C-25) and 65.015 ppm (d, C-24).

Additionally, the methyl signal at 1.07 (30-H_3) showed correlations with the carbons at 19.225 (q, C-29), 46.309 (d, C-5), 40.993 (s, C-4) and 88.029 ppm (d, C-3). Similarly,the methyl signal at 1.35 ppm(29-H_3) showed correlations with the carbons at 15.256 (q, C-30), 40.993 (s, C-4), 46.309 (d, C-5) and 88.029 ppm (d, C-3). The cyclopropane methylene protons signals at 0.37 and 0.70 ppm showed long-range correlations with the carbons at 19.030 (s, C-9), 25.891 (t, C-11), 27.230 (s, C-10), 46.309 (d, C-5), 32.087 (t, C-1) and 55.988 (d, C-8).

The anomeric carbon (d) at 107.492 ppm showed long-range correlations with the hydrogens at 3.75 (double doublet (dd), 5′-H), 4.37 (dd, 5′-H), 4.04 (dd, 2′-H). The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum suggested an interaction between the anomeric hydrogen and 3α-H along with closings due to geminal and antiperiplanar hydrogens (Fig. 2). Acetylation of I provided a pentaacetate (II), mp 120—121 °C, $C_{47}H_{68}O_{16}$, the ^{1}H-NMR spectrum of which showed an acetylation shift of 7α-H, while the signal of 3α-H remained at 3.15 ppm. These data established the structure (I) for the new xyloside, excluding the stereostructure of the side chain, as shown in Fig. 3.

The xyloside (I) was hydrolyzed with cellulase and an aglycone (III), mp 194—196 °C, $C_{32}H_{50}O_7$, $[\alpha]_D$ −27.0°, was obtained. The high-resolution mass spectrum (HR-MS) of III showed an ion peak at m/z 546.3546 (M^+), supporting the above molecular formula. The ^{1}H-NMR spectrum showed the presence of a 1,1,2,2-tetrasubstituted cyclopropane ring, six tertiary methyl groups, a secondary methyl group, an acetoxy group and three secondary hydroxy groups (Table I). These data suggested that the compound (III) is the genuine aglycone of the xyloside (I).

The xyloside (I) was hydrolyzed with 0.5 N HCl (MeOH : H_2O = 1 : 1) and two triterpenic products (IV, V) and D-xylose, $[\alpha]_D$ +17.0°, were obtained . Compounds

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Table I. Characteristic ^{1}H-NMR Chemical Shifts (ppm) and Coupling Constants (in Hz, in Parentheses) of I—VIIa

	I[a]	II	III	IV	V	Va	Vb	VI	VII	VIIa
3-H	3.53 dd (12.0, 4.0)	3.15 dd (12.0, 4.0)	3.32 dd (9.5, 4.7)	3.31 ddd (9.5, 4.5)	3.34 dd (9.5, 4.5)	4.60 dd (9.5, 4.5)	4.57 dd (9.5, 4.5)	3.30 dd (9.5, 4.5)	3.32 dd (9.5, 4.5)	4.61 dd (9.0, 4.0)
7-H	3.75	4.75 ddd (10.0, 10.0, 3.0)	3.52 ddd (10.2, 10.2, 2.8)	3.50[b]	3.52[b]	3.50[b]	4.97 ddd (11.0, 7.0, 6.5)	n	n	n
15-H	4.48 s	5.31 s	4.07 s[c]	4.04 s[c]	4.08 s	4.07[c]	3.97 s[c]	3.94 s[c]	3.93 s[c]	3.85 s[c]
17-H	2.39 d (4.2)	2.55 d (4.5)	n	n	n	n	n	n	n	n
19-H_2	0.37 d (4.0) 0.70 d (4.0)	0.05 d (4.0)	0.52 d (4.0) 0.76 d (4.0)	0.45 d (4.0) 0.74 d (4.0)	0.48 d (4.0) 0.78 d (4.0)	0.47 d (4.0) 0.77 d (4.0)	0.14 d (4.8)	0.37 d (4.0) 0.63 d (4.0)	0.38 d (4.0) 0.65 d (4.0)	0.27 d (4.0) 0.66 d (4.0)
21-H_3	1.28 d (6.5)	1.07 d (6.5)	1.12 d (6.7)	0.91 d (6.5)	0.95 d (6.5)	0.92 d (6.5)	0.91 d (6.5)	0.90 d (6.0)	0.90 d (6.0)	n
23-H	5.38 ddd (8.8, 8.5, 3.0)	4.97 ddd (8.8, 8.5, 3.0)	4.95 ddd (8.6, 8.4, 2.5)	4.48 dd (9.5, 2.5)	4.52 dd (9.5, 3.0)	4.48 dd	4.52 d (8.3)	4.44 dd (9.3, 3.0)	4.47 dd (9.5, 3.0)	4.28 d (8.5)
24-H	3.02 d (8.2)	2.77 d (8.4)	2.79 d (8.2)	3.47 s	3.50 s	3.49 s	3.45 s	3.47 s	3.47 s	3.43 s
1′-H	4.87 d (7.7)	4.52 d (8.0)					8-H: 2.24 d (7.0)			
2′-H	4.04 dd (8.8, 7.7)	5.19 dd (8.0, 8.0)								
3′-H	4.17 dd (9.0, 8.8)	4.97 dd (8.0, 8.0)								
4′-H	4.24 ddd (10.0, 9.0, 5.0)	4.97 ddd (8.0, 8.5, 5.0)								
5′-H	4.37 dd (11.2, 5.0)	4.11 dd (11.0, 5.5)								
	3.75 dd (11.2, 10.0)	3.22 dd (8.5, 8.0)								
$OCOCH_3$	2.09 s	1.92, 20.2, 2.04, 2.05, 2.08, 2.12 (s)	2.09 s			2.06 s	1.99 s, 2.05 s			2.07
OCH_3				3.49 s				3.50 s		

I: 7β-Hydroxy-23-*O*-acetylshengmanol 3-β-D-xylopyranoside. II: I-pentaacetate. III: 7β-Hydroxy-23-*O*-acetylshengmanol. IV: 25-*O*-Methyl-7β-hydroxycimigenol. V: 7β-Hydroxycimigenol. Va: 3-*O*-Acetyl-7β-hydroxycimigenol. Vb: 3,7-Di-*O*-acetyl-7β-hydroxycimigenol. VI: 25-*O*-Methylcimigenol. VII: Cimigenol. VIIa: 3-*O*-Acethylcimigenol. Abbreviations: d, doublet; dd, double doublet; s, singlet; n, not detected. Data were obtained in $CDCl_3$ unless otherwise stated. *a*) Obtained in pyridine-d_5. *b*) Overlapping with *O*-methyl signal or 24-H signal. *c*) The signal observed after addition of D_2O.

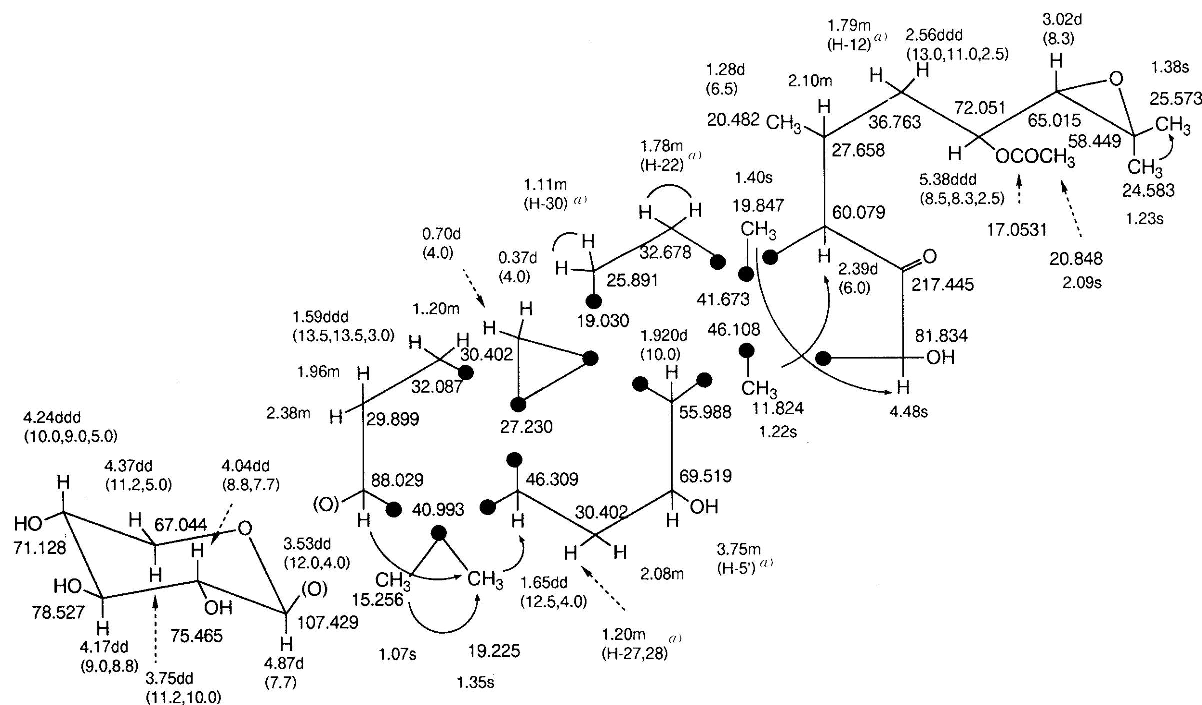

Fig. 1. Partial Structures of I Deduced from the ^{1}H- and ^{13}C-NMR Data

Arrows show NOE observed. *a*) Duplicated signals with the protons in parenthesis.

IV, mp 213—214 °C, $C_{31}H_{50}O_6$, and V, mp 255—256 °C, $C_{30}H_{48}O_6$ showed similar ^{1}H-NMR spectra except for the *O*-methyl signal of the former (Table I).

The spectra were also similar to those of 25-*O*-methylcimigenol (VI)[5] and cimigenol (VII),[6] except for an additional signal (3.50 ppm, 1H, partially overlapping the *O*-methyl signal, for IV; 3.52 ppm, 1H, partially overlapping the 24-H signal, for V), respectively. Acetylation of V with acetic anhydride in pyridine provided a monoacetate (Va), mp 227—228 °C, $C_{32}H_{50}O_7$, and a diacetate (Vb), mp 283—285 °C, $C_{34}H_{52}O_8$. The ^{1}H-NMR spectrum ($CDCl_3$) of Vb was similar in part to that of 3-*O*-acetylcimigenol (VIIa) , and in particular, a pair of signals due to 15-OH (2.67 ppm, d, $J=8.2$ Hz, exchangeable with D_2O) and 15-H (3.97 ppm, d, $J=8.2$ Hz, changed to a singlet by D_2O addition), and a pair of signals due

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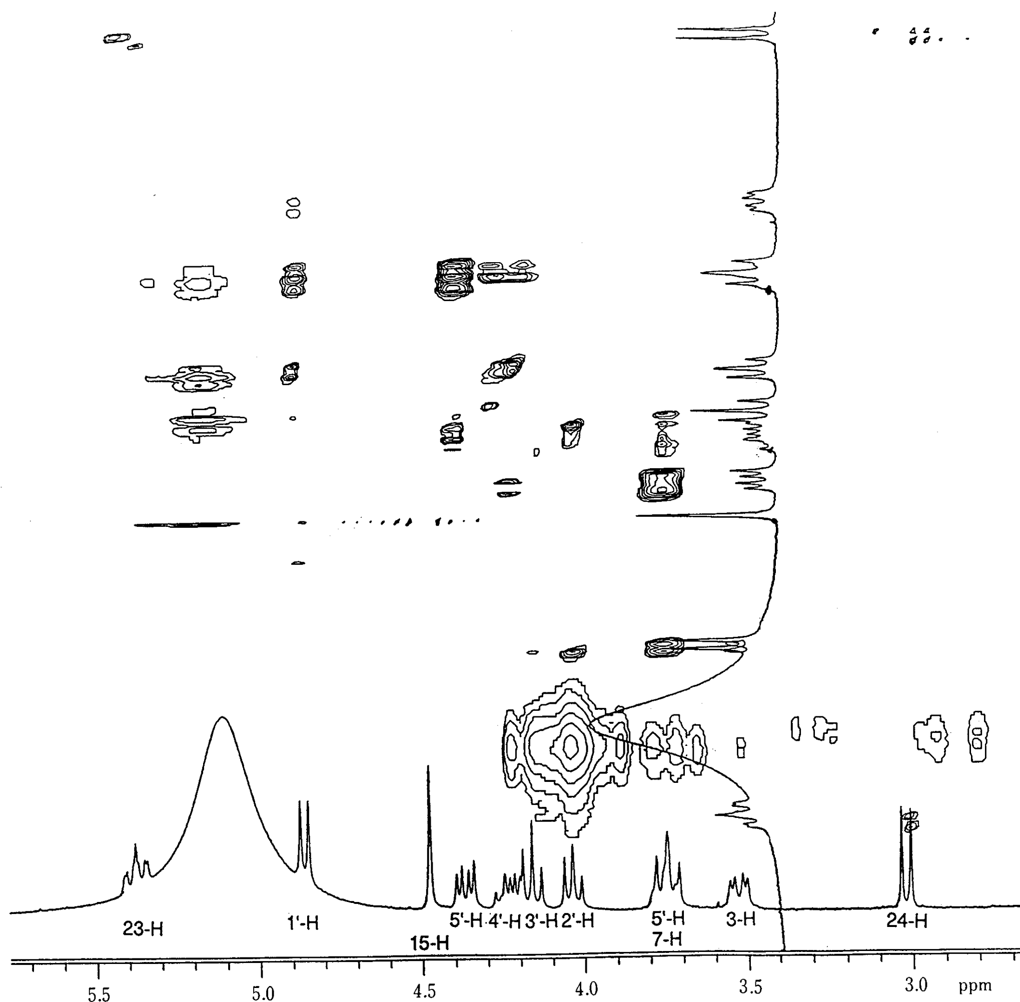

Fig. 2. NOESY Spectrum (in Pyridine-d_5) of I

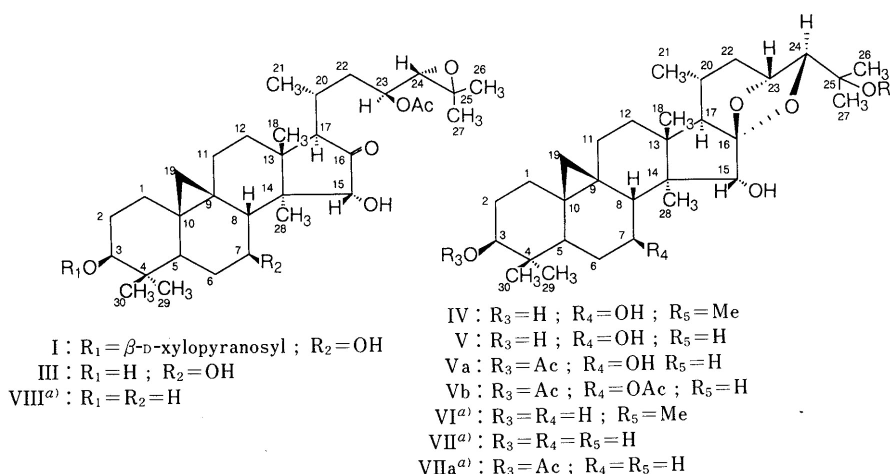

Fig. 3. Structures of I and Products Derived (III, IV, Va, Vb) from I

a) Related compounds (VI, VII, VIIa and VIII) cited in this report.

to 23-H (4.52 ppm, d, $J=8.3$ Hz) and 24-H (3.45 ppm, s) were similar to the corresponding signals of VIIa, indicating the same partial structures. The chemical conversion of I to IV and V was thought to proceed as shown in Fig. 4, suggesting the stereostructure (15*R*, 23*R*, 24*S*) in I to be the same as that in acetylshengmanol (VIII).[4]

Furthermore, the ^{1}H-NMR spectrum of Vb showed one of a pair of doublets due to the cyclopropane methylene group at 0.14 ppm (d, $J=4.8$ Hz), while the other was included in the methyl–methylene envelope, although in the spectrum of VIIa they appeared at 0.27 and 0.66 ppm

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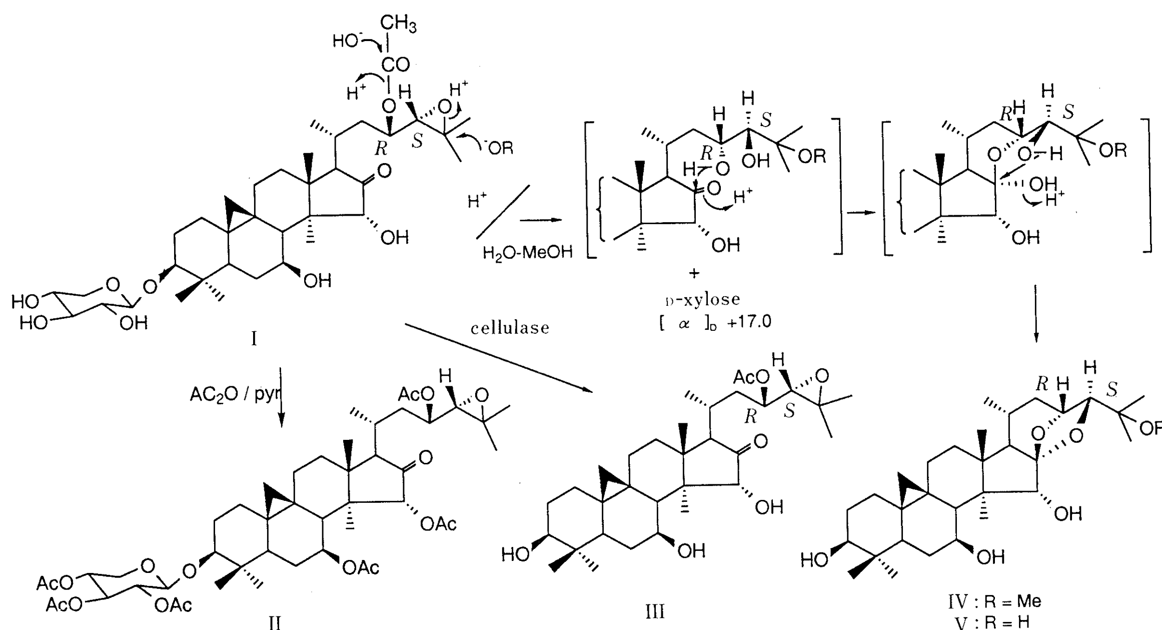

Fig. 4. Conversion of I to II, III, IV and V

as a pair of characteristic doublets (J=4.0 Hz).[5] It also showed signals of an acetyl methyl (2.05 ppm, H_3, s) and an acetoxylic carbinyl hydrogen (4.97 ppm, ddd, J_1=11.0, J_2=7.0, J_3=6.5 Hz, 7-H). In a decoupling experiment, the doublet at 2.24 ppm (d, J=7.0 Hz, 8-H) was correlated with the ddd signal (7-H). We concluded that compound V is 7β-hydroxycimigenol and the genuine aglycone (III) is 7β-hydroxy-23-*O*-acetylshengmanol.

Thus, the new xyloside (I) should be 7β-hydroxy-23-*O*-acetylshengmanol 3-*O*-β-D-xylopyranoside, as shown in Fig. 3. 7β-Hydroxycimigenol (V) was also obtained as a product of enzymatic hydrolysis of a mixture of glycosides obtained during isolation of the new xyloside (I).

Experimental[7]

Extraction and Isolation of I The dried aerial parts (6.07 kg) of *Cimicifuga simplex*, which were collected in August, 1991 in Sendai, Japan and identified by G. Kusano (representative samples have been deposited at the Department of Pharmacognosy, Osaka University of Pharmaceutical Sciences) were extracted with MeOH (80 l × 3) at room temperature overnight. The MeOH solution was subjected to activated charcoal column chromatography (250 g, i.d. 6.7 cm × 37 cm) and the passed fraction was concentrated *in vacuo* to yield a gummy extract (500 g). The concentrated extract was shaken with *n*-BuOH (300 ml) three times and the combined *n*-BuOH layer was washed with water (200 ml) twice. The solvent was evaporated *in vacuo* to provide the residual gum (270 g), which was dissolved in MeOH (500 ml) containing SiO_2 (600 g). This mixture was concentrated to dryness *in vacuo*.

One-third of a homogenous mixture of SiO_2 and the extract was applied to a SiO_2 (100 g) column and chromatographed (SiO_2: i.d. 5.5 cm × 34 cm) first with *n*-hexane and then with increasing amounts of EtOAc in *n*-hexane to give six fractions, followed by five fractions eluted with EtOAc and with increasing amounts of MeOH in EtOAc. This chromatography was repeated twice more with the remaining material.

The fractions (30 g) eluted with *n*-hexane–EtOAc (1 : 1, 3 × 600 ml) and EtOAc (3 × 500 ml) were combined and homogenized with SiO_2 (100 g). The dried homogenous mixture was applied to a SiO_2 column (200 g, i.d. 5.5 cm × 20 cm). After elution with EtOAc (900 ml), elution with EtOAc (100 ml), EtOAc : MeOH (9 : 1, 600 ml) and EtOAc : MeOH (4 : 1, 600 ml) gave mixtures of crude glycosides (11.0 g). The last mixture was chromatographed on SiO_2 (100 g, i.d. 4.0 cm × 22.5 cm). After elution with $CHCl_3$ (40 ml × 16) and $CHCl_3$: MeOH (20 : 1, 40 ml × 9), elution with $CHCl_3$: MeOH (20 : 1, 40 ml × 12) provided a glycoside fraction (3.6 g).

This fraction was chromatographed on ODS-A (100 g, i.d. 3.8 cm × 18.5 cm). After elution with MeOH : water (4 : 1, 30 ml × 6), elution with the same solvent (30 ml × 8) provided the crude xyloside (1.4 g). This was chromatographed on SiO_2 (30 g, i.d. 2.2 cm × 16.0 cm) with $CHCl_3$ (40 ml × 3) and $CHCl_3$: MeOH (20 : 1, 40 ml × 6) to afford a product as a colorless crystalline powder (0.9 g) after recrystallization from MeOH.

The powder (0.9 g) showed one major spot (*Rf* 0.6, $CHCl_3$: MeOH = 10 : 1) with traces of other components on TLC. Repeated recrystallization of the powder from a mixture of EtOH and isopropyl ether provided colorless needles (I, 223 mg), mp 252—253 °C. *Anal.* Calcd for $C_{37}H_{58}O_{11}$: C, 65.47; H, 8.61. Found: C, 64.76; H, 8.52. $[\alpha]_D$ −51.4° (c=0.14, MeOH). ORD: $[M]_{290}$ + 10111.9°, $[M]_{334}$ −9530.7°. FAB-MS m/z: 679.3625 (M^++1). ^{1}H- and ^{13}C-NMR: Fig. 1.

Acetylation of I I (6.1 mg) was dissolved in 0.5 ml of pyridine and acetic anhydride (0.1 ml) was added. The mixture was kept at room temperature overnight, then warmed at 50 °C for 2 h, poured into water (10 ml) and extracted with EtOAc (20 ml × 3). The residue after evaporation of the solvent was recrystallized from EtOAc to afford the pentaacetate of I (II) as colorless needles, mp 120—121 °C, $C_{47}H_{68}O_{16}$ MS m/z: 889 (M^++1). ^{1}H-NMR spectrum: Table I.

Hydrolysis of I with Cellulase I (20.0 mg) was dissolved in MeOH (10 ml) and water (20 ml) was added with stirring. A solution of cellulase (50 mg; Wako, from *Trichoderma viride*) was added dropwise and the mixture was kept at 25 °C in an incubator for a week. The reaction mixture was shaken with EtOAc (20 ml × 3) and the EtOAc layer was washed with water (10 ml × 3). After evaporation of the solvent *in vacuo*, the residue was chromatographed on SiO_2 (15 g, 2.7 × 7.5 cm). Elution with *n*-hexane : EtOAc (1 : 1) provided an aglycone (III, 7.0 mg), mp 194—196 °C. *Anal.* Calcd for $C_{32}H_{50}O_7 \cdot H_2O$: C, 68.05; H, 9.28. Found: C, 68.38; H, 8.90. HR-MS m/z: 546.3546 (M^+); Calcd for $C_{32}H_{50}O_7$: 546.3553. EI-MS m/z: 546 (M^+), 528 (M^+−18), 486 (M^+−AcOH). $[\alpha]_D$ −27.0° (c=0.83, $CHCl_3$).

Hydrolysis of I with 0.5 N HCl I (22.0 mg) was dissolved in 0.5 N HCl in MeOH : water (1 : 1) (10 ml) and refluxed for 2 h. The reaction solution was shaken with EtOAc (20 ml × 3) and the EtOAc layer was washed with water (10 ml × 3) and dried over Na_2SO_4. The residue (16.4 mg) after removal of Na_2SO_4 and evaporation of the solvent was chromatographed on SiO_2 (12 g). Elution with *n*-hexane : EtOAc (3 : 2, 40 ml × 3) and evaporation of the eluate provided colorless needles (IV, 4.0 mg), mp 213—214 °C, $C_{31}H_{50}O_6$, MS m/z: 518. The EtOAc (40 ml × 4) eluate afforded colorless needles (V, 4.7 mg), mp 255—256 °C, $C_{30}H_{48}O_6$, MS m/z: 504.

The water layer after EtOAc extraction was applied to an Amberlite IR-35 (10 ml) column and the passed fraction was concentrated *in vacuo*

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to yield D-xylose (4.3 mg), $[\alpha]_D$ +17.0° (c=0.41, H_2O), of which the TLC behavior (*Rf* 0.45, *n*-BuOH : AcOH : water = 4 : 1 : 1) and ^{1}H-NMR spectrum were identical with those of an authentic specimen.

Hydrolysis of Crude Glycoside Mixture with Cellulase Crude glycoside mixture (200 mg), eluted with $CHCl_3$: MeOH (5 : 1) after I in the chromatography, was dissolved in MeOH (30 ml) and water (50 ml) was added to form a white emulsion. Cellulase (100 mg) was added and the mixture was stirred at room temperature for 4 weeks. The reaction solution was extracted with EtOAc (50 ml × 3) and the EtOAc layer was washed with water (20 ml × 3). The residue (180 mg) after evaporation of the solvent was chromatographed on SiO_2 (10 g). Compound III (37 mg) was isolated from the *n*-hexane–EtOAc (1 : 1) eluate, and the *n*-hexane–EtOAc (1 : 2) eluate afforded colorless needles (V, 25 mg), mp 255—256 °C, $C_{30}H_{48}O_6$, after recrystallization from EtOAc. MS *m/z*: 504 (M^+, 15%), 489 ($M^+ - 15$, 25%), 486 ($M^+ - 18$, 95%). The NMR spectrum in $CDCl_3$ was identical to that of 7β-hydroxycimigenol prepared by acidic hydrolysis of I (Table I).

Acetylation of V A solution of V (9 mg) in acetic anhydride (a few drops) and pyridine (1 ml) was stirred at room temperature overnight. Water was added and the mixture was shaken with EtOAc (10 ml × 3). The organic layer was washed with 1% Na_2CO_3, 1% HCl and water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on SiO_2 (2.5 g). Elution with *n*-hexane–EtOAc (5 : 1) and (3 : 1) provided a diacetate (Vb), mp 283—285 °C, $C_{34}H_{52}O_8$, MS *m/z*: 588. ^{1}H-NMR: Table I. Elution with *n*-hexane–EtOAc (2 : 1) provided a monoacetate (Va), colorless needles, mp 227—228 °C, $C_{32}H_{50}O_7$, after recrystallization from EtOAc. MS *m/z*: 546 (M^+). ^{1}H-NMR: Table I.

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7) The instruments used in this work were as follows: Yanagimoto micro melting point apparatus (melting points), JASCO DIP digital polarimeter (specific rotation), JASCO ORD/UV-5 spectrometer (ORD), Perkin-Elmer 1720X-FT IR spectrometer (IR spectra), Varian Gemini-200, Varian XL-300, General Electric GN-300 and GN-500 spectrometers (NMR spectra), Hitachi M-80 and JEOL JMS-DX-300 spectrometers (mass spectra). Melting points are uncorrected. NMR spectra were measured in pyridine-d_5 and $CDCl_3$ solution, and chemical shifts are expressed on the δ scale using tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Wakogel C-200) and ODS-A YMC.

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