

Studies on the Constituents of *Cimicifuga* Species. XVIII.¹⁾ Four New Xylosides from the Aerial Parts of *Cimicifuga simplex* WORMSK.

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Four new xylosides (1–4) were isolated from the aerial parts of *Cimicifuga simplex* (Ranunculaceae), and their structures were determined to be 24-*epi*-7 β -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside (1), 25-*O*-methyl-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside (2), 25-*O*-methyl-7 β -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside (3) and 25-*O*-methyl-1 α -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside (4).

Key words *Cimicifuga simplex*; β -D-xyloside; cycloartane; hemiketal; 24*R*-stereostructure; 24*S*-stereostructure

We recently reported on the isolation of four new triterpenic glycosides from the aerial parts of *Cimicifuga simplex*.¹⁾ In our continuing work, we isolated four new xylosides (1–4) from the same herb. This paper deals with the isolation and the structural elucidation of these xylosides.

Compounds 1–4 were obtained as described in the experimental section by repeated chromatographies on silica gel (SiO₂), octadecylsilanized silicic acid (ODS) columns, and HPLC, preparative TLC (pTLC) and recrystallization of the aqueous fraction after removal of the *n*-BuOH fraction from MeOH extracts of the aerial parts.

Compound 1 was obtained as colorless needles, mp 276–277 °C, $[\alpha]_D + 10.3^\circ$, and the molecular formula was determined as C₃₇H₆₀O₁₂ on the basis of positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) [(M–OH)⁺, *m/z* 679.4062], pos. FAB-MS [(M–OH)⁺, *m/z* 679, (M+Na)⁺, *m/z* 719] and the data of the ¹³C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3500–3200 and an acetyl band at 1732 cm^{–1}. The ¹H- and ¹³C-NMR signals were assigned using the ¹H–¹H shift correlation spectroscopy (¹H–¹H COSY) and the ¹³C–¹H COSY, and similar assignments were carried out in other compounds (2–4, 1a–5a) and summarized in Tables 1 and 2. The spectra of 1 were similar to those of 24-*O*-acetylhydroshengmanol xyloside except signals of 7 α -H (δ 3.69, ddd) and C-7 (δ 70.55).

The reported value (δ 4.73, d, *J* = 5.0 Hz) of 24-H due to 24-*O*-acetylhydroshengmanol (5a) has been recorded in a CDCl₃ solution.²⁾ The values in Table 1 [(especially, δ 5.61, d, *J* = 8.1 Hz due to 24-H of the same compound (5a)] were obtained in a pyridine-*d*₅ solution containing a few drops of D₂O. A xyloside (5) was obtained from the same herb and identified by direct comparison of TLC and ¹H-NMR spectra (pyridine-*d*₅) with an authentic specimen kindly given by Dr. Sakurai. The aglycone (5a) was also obtained by hydrolysis of 5 with Cellulase T [Amano]⁴ and converted into cimigenol (5b) by the same treatments as described later in 1–4 and 1a–4a. Experimental details about 5, 5a and 5b are omitted from this report.

The circular dichroism (CD) of 1 showed a weak negative Cotton effect curve superimposable on that of 24-

O-acetylhydroshengmanol xyloside, namely (23*R*,24*S*)-3 β , 15 α ,16 ζ ,25-tetrahydroxy-24-acetoxy-16,23-epoxy-9,19-cyclolanostane-3-*O*- β -D-xylopyranoside; this effect has been attributed to a 16-keto-23-ol form (minor) equilibrated with its hemiketal form (16,23-epoxy-16-ol, major).²⁾ A ¹³C-NMR signal (δ 103.61) was characteristic of a hemiketal carbon at C-16 of 1 instead of ketal carbons (*ca.* 111 ppm) of 16,23;16,24-diepoxo compounds as reported in 24-*O*-acetylhydroshengmanol glycosides.³⁾

Compound 1 was hydrolyzed with Cellulase T [Amano]⁴ to afford 1a, mp 189–190 °C, $[\alpha]_D + 48.0^\circ$, C₃₂H₅₂O₈. The pos. HR-SI-MS showed a (M–OH)⁺ ion at *m/z* 547.3628, clarifying the molecular formula. The IR spectrum showed broad hydroxyl bands at 3500–3250 cm^{–1} and an acetyl band at 1743 cm^{–1}. ¹H- and ¹³C-NMR signals of 1a were similar to those of 7 β -hydroxycimigenol^{1,4)} and 24-*O*-acetylhydroshengmanol²⁾ as summarized in Tables 1 and 2.

Compound 1a was treated with 1% Na₂CO₃, followed by acidification with 1*N* HCl to afford a desacetyl dehydrate 1b, mp 251–252 °C, $[\alpha]_D + 44.0^\circ$, C₃₀H₄₈O₆. The pos. HR-SI-MS showed a (M+H)⁺ ion at *m/z* 505.3557, clarifying the molecular formula. The IR spectrum showed broad hydroxyl bands at 3400–3250 cm^{–1}.

Comparison of ¹H-NMR spectra of 1a and 1b indicated a conversion of a 16-hydroxy-24-acetoxy-16,23-epoxy moiety [δ 2.18 (3H, s, CH₃CO), 5.79 (1H, d, *J* = 8.4 Hz, 24-H), 4.46 (1H, ddd, *J* = 11.9, 8.4, 6.4 Hz, 23-H)] in 1a to a 16,23;16,24-diepoxo moiety [δ 3.78 (1H, d, *J* = 4.0 Hz, 24-H), 4.62 (1H, ddd, *J* = 9.0, 4.0, 2.0 Hz, 23-H)] in 1b. The splitting pattern of the latter signals (24-H and 23-H in 1b) suggested the stereostructure of the moiety to be (23*R*,24*R*)-16,23;16,24-diepoxo as described in the structural elucidation of cimigol.⁵⁾

Cimigenol derivatives having (23*R*,24*S*)-16,23;16,24-diepoxo moieties show a singlet due to 24-H and a double doublet (*J* = 9, 2 Hz) due to 23-H. Compounds having (23*S*,24*R*)- or (23*S*,24*S*)-16,23;16,24-diepoxo moieties have not been known, but model consideration indicates that ¹H-NMR spectra should show a doublet (*J* = 4 Hz) to 24-H and a ddd signal (*J* = 4, 4, 4 Hz) to 23-H in the former type and a singlet to 24-H and a double doublet (*J* = 4, 4 Hz) to 23-H in the latter type.

These considerations indicated that 1b should be a

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Table 1. ^1H -NMR Data of **1**–**4** and **1a**–**5a**

	1	1a	2	2a	3	3a	4	4a	5a ^{a)}
1	1.28, 1.60	1.30, 1.60	1.38, 1.56	1.40, 1.55	1.30, 1.55	1.32, 1.60	3.82 brs	3.85 brs	1.15, 1.50
2	2.05, 2.35	2.09, 2.00	1.95, 2.36	1.98, 2.10	1.90, 2.45	1.92, 2.45	2.22, 2.70	2.24, 2.43	1.87, 2.02
3	3.50 dd (11.0, 4.0)	3.56 dd (11.3, 4.4)	3.50 dd (11.5, 4.5)	3.58 dd (11.0, 4.2)	3.49 dd (12.0, 4.4)	3.55 dd (11.5, 6.5)	4.32 dd (12.0, 8.2)	4.41 dd (12.0, 8.2)	3.48 dd (11.4, 4.4)
5	1.65	1.73	1.32	1.25	1.50	1.55	2.40	2.45	1.20
6	1.28, 2.02	1.27, 2.02	0.68, 1.30	0.75, 1.40	1.18, 1.70	1.18, 1.68	0.80, 1.65	0.88, 1.67	0.65, 1.45
7	3.69 ddd (12.0, 10.0, 3.0)	3.71 ddd (12.0, 10.0, 3.0)	1.12, 2.05	1.15, 2.05	3.68 ddd (12.0, 10.0, 3.0)	3.70 ddd (12.0, 10.0, 3.0)	1.35, 1.52	1.35, 1.50	1.12, 2.00
8	1.80	1.80	1.73	1.75	1.78	1.80	1.75	1.80	1.68
11	1.08, 1.98	1.01, 1.96	1.05, 2.03	1.05, 2.05	1.05, 2.00	1.01, 2.00	1.60, 2.85	1.65, 2.90	1.05, 2.02
12	1.55, 1.65	1.56, 1.65	1.59, 1.65	1.55, 1.60	1.55, 1.65	1.55, 1.65	1.45, 1.75	1.40, 1.80	1.53, 1.68
15	4.16 s	4.20 s	4.15 s	4.19 s	4.17 s	4.19 s	4.16 s	4.19 s	4.11 s
17	1.78	1.81	1.80 d (8.0)	1.83 d (8.4)	1.80	1.78	1.78 d (8.5)	1.81 d (9.2)	1.75
18	1.27 s	1.30 s	1.30 s	1.30 s	1.29 s	1.31 s	1.29 s	1.31 s	1.20 s
19	0.36 d (3.9) 0.63 d (3.9)	0.41 d (4.2) 0.70 d (4.2)	0.28 d (3.8) 0.50 d (3.8)	0.34 d (3.9) 0.57 d (3.9)	0.35 d (4.1) 0.62 d (4.1)	0.38 d (4.1) 0.65 d (4.1)	0.40 d (4.0) 0.65 d (4.0)	0.47 d (4.2) 0.72 d (4.2)	0.23 d (4.1) 0.46 d (4.1)
20	1.85	1.88	1.75	1.75	1.80	1.80	1.75	1.80	1.75
21	0.98 d (5.5)	0.99 d (5.8)	1.02 d (5.7)	1.06 d (5.7)	1.01 d (5.6)	1.03 d (5.8)	0.98 d (5.4)	1.00 d (5.5)	0.93 d (5.5)
22	1.80, 2.00	1.80, 2.00	1.95, 2.00	1.95, 2.00	1.95, 2.05	1.90, 2.05	1.96, 2.10	2.00, 2.10	2.06, 2.15
23	4.43	4.46 ddd (11.9, 8.4, 6.4)	4.21	4.25 ddd (10.7, 8.4, 6.4)	4.25	4.23 ddd (11.3, 8.4, 6.3)	4.20	4.23 ddd (10.8, 8.5, 6.7)	4.28 ddd (11.5, 8.1, 6.0)
24	5.76 d (8.3)	5.79 d (8.4)	5.70 d (8.6)	5.72 d (8.4)	5.65 d (8.6)	5.65 d (8.4)	5.66 d (8.5)	5.69 d (8.5)	5.61 d (8.1)
26	1.57 s	1.58 s	1.25 s	1.31 s	1.28 s	1.29 s	1.37 s	1.38 s	1.43 s
27	1.53 s	1.53 s	1.25 s	1.28 s	1.26 s	1.27 s	1.28 s	1.30 s	1.42 s
28	1.21 s	1.24 s	1.23 s	1.25 s	1.22 s	1.19 s	1.22 s	1.22 s	1.18 s
29	1.32 s	1.21 s	1.28 s	1.21 s	1.31 s	1.24 s	1.35 s	1.28 s	1.11 s
30	1.05 s	1.10 s	1.04 s	1.09 s	1.04 s	1.07 s	1.07 s	1.12 s	0.99 s
COCH ₃	2.19 s	2.18 s	2.12 s	2.15 s	2.11 s	2.14 s	2.12 s	2.14 s	2.09 s
OCH ₃			3.27 s	3.29 s	3.25 s	3.27 s	3.27 s	3.25 s	
1'	4.86 d (8.3)		4.86 d (7.8)		4.84 d (7.8)		4.86 d (7.7)		
2'	4.05 dd (8.3, 8.2)		4.05 dd (8.3, 7.8)		4.03 dd (8.3, 7.8)		4.02 dd (8.5, 7.7)		
3'	4.20 dd (8.6, 8.2)		4.18 dd (8.6, 8.3)		4.18 dd (8.6, 8.3)		4.12 dd (8.6, 8.3)		
4'	4.26 ddd (10.6, 8.6, 4.8)		4.30 ddd (10.1, 8.6, 4.8)		4.22 ddd (10.1, 8.6, 5.0)		4.23 ddd (10.1, 8.6, 5.1)		
5'	3.77 dd (10.8, 10.6)		3.77 dd (10.8, 10.1)		3.68 dd (10.9, 10.1)		3.59 dd (10.6, 10.1)		
5'	4.38 dd (10.8, 4.8)		4.38 dd (10.8, 4.8)		4.37 dd (10.9, 5.0)		4.34 dd (10.6, 5.1)		

a) Obtained on a General Electric GN-500; others on a Varian XL-300 in pyridine-*d*₅ containing D₂O. Chemical shift were found from ^1H - ^1H COSY and ^{13}C - ^1H COSY.

cimigol type, namely, 24-epicimigenol derivative. The ^1H -NMR signals due to cyclopropane methylene, $3\alpha\text{-H}$ and $7\alpha\text{-H}$ of **1b** were similar to those of an authentic specimen of 7β -hydroxycimigenol.¹⁾

The nuclear Overhauser effect (NOE) difference spectrum of **1a** suggested a 15α -hydroxy group, due to the NOE between 18–3H and $15\beta\text{-H}$. **1b** should then be 24-*epi*- 7β -hydroxycimigenol and **1a** 24-*epi*- 7β -hydroxy-24-*O*-acetyl hydroshengmanol as shown in Fig 2.

The ^1H -NMR spectrum of **1** showed 1'-H (δ 4.86, d, $J=8.3$ Hz), 2'-H (δ 4.05, dd, $J=8.3, 8.2$ Hz), 3'-H (4.20, dd, $J=8.6, 8.2$ Hz), 4'-H (δ 4.26, ddd, $J=10.6, 8.6, 4.8$ Hz), 5'-H (δ 4.38, dd, $J=10.8, 4.8$ Hz), 5'-H (δ 3.77, dd, $J=10.8, 10.6$ Hz), suggesting the presence of a β -xylopyranosyl group. On hydrolysis of **1** with Cellulase T[Amano]⁴ in EtOH–water (1:2), followed by dialysis with a Spectrapor membrane tube, ethyl- β -D-xylopyranoside, $[\alpha]_{\text{D}} -34.4^\circ$, $\text{C}_7\text{H}_{14}\text{O}_5$, (M)⁺, m/z 178 in pos. SI-MS, was obtained and identified by direct comparison with an authentic specimen.⁶⁾

The ^{13}C -NMR spectrum of **1** showed that C-3 appeared

at δ 88.77 by a glycosylation shift of 11.37 ppm from that of **1a**. Thus, **1** should be formulated as 24-*epi*- 7β -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside as shown in Fig. 1.

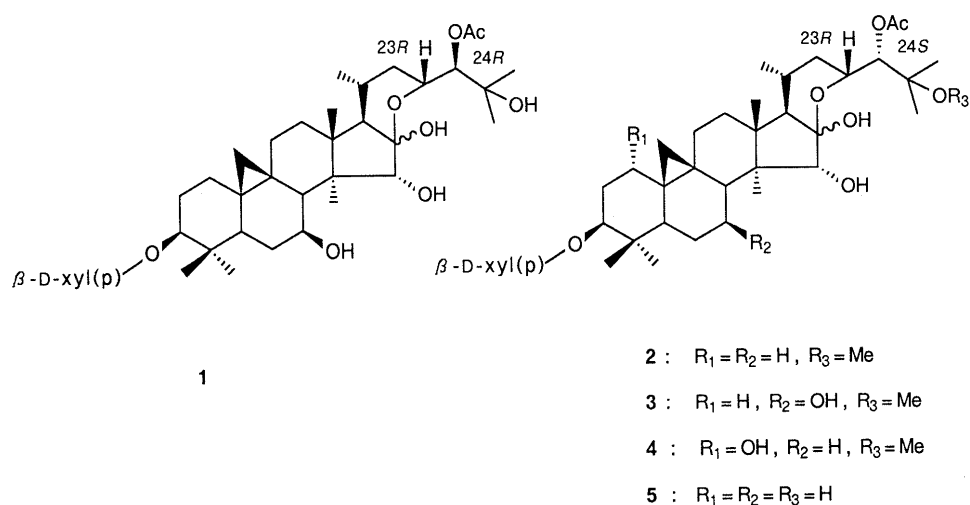
Compound **2** was obtained as colorless needles, mp 205–206 °C, $[\alpha]_{\text{D}} -1.5^\circ$, and the molecular formula was determined as $\text{C}_{38}\text{H}_{62}\text{O}_{11}$ on the basis of pos. HR-SI-MS [(M–OH)⁺, m/z 677.4269], pos. SI-MS [(M–OH)⁺, m/z 677; (M+H)⁺, m/z 695] and the data of the ^{13}C -NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3550–3300 and an acetyl band at 1715 cm^{-1} . The ^1H -NMR (Table 1) and the ^{13}C -NMR (Table 2) signals were similar to those of 24-*O*-acetylhydroshengmanol xyloside, except for additional signals of O–CH₃ (δ_{H} 3.27, s; δ_{C} 49.18).

Compound **2** was hydrolyzed with Cellulase T[Amano]⁴ to afford **2a**, mp 185–186 °C, $[\alpha]_{\text{D}} +6.8^\circ$, $\text{C}_{33}\text{H}_{54}\text{O}_7$. The pos. HR-SI-MS showed a (M–OH)⁺ ion at m/z 545.3846, clarifying the molecular formula. The IR spectrum showed strong hydroxyl bands at 3500–3250 and an acetyl band at 1715 cm^{-1} .

Table 2. ^{13}C -NMR Data of **1**–**4** and **1a**–**5a** in Pyridine- d_5

	1	1a	2	2a	3	3a	4	4a	5a ^{a)}
1	31.37	30.88	32.09	32.29	30.60	30.52	72.10	72.41	32.09
2	31.37	30.88	30.56	30.75	30.60	30.52	37.41	38.58	30.52
3	88.77	77.40	88.26	77.60	87.98	77.25	84.25	73.03	77.43
4	41.59	40.65	41.03	40.73	40.80	40.37	41.15	41.03	40.52
5	47.05	46.22	47.23	47.08	46.27	45.96	39.69	39.72	46.87
6	34.38	33.68	21.04	21.20	32.11	32.16	20.62	20.99	20.77
7	70.55	69.93	26.10	26.26	69.66	69.62	25.58	25.74	26.05
8	56.79	56.15	48.83	48.90	56.14	55.94	49.15	49.20	48.66
9	19.56	18.82	19.72	19.63	18.82	18.62	20.62	20.70	19.43
10	27.57	26.85	26.38	26.59	26.83	26.90	30.54	31.05	26.40
11	26.81	26.17	26.25	26.26	26.18	26.05	26.01	26.27	26.05
12	32.88	32.39	33.33	32.29	33.30	33.15	33.29	33.41	33.56
13	43.42	42.73	41.70	41.69	42.42	42.28	41.65	41.73	41.49
14	47.61	46.94	46.34	46.33	46.90	46.73	46.38	46.53	46.11
15	82.35	81.66	82.15	82.12	81.96	81.80	82.27	82.47	82.07
16	103.61	102.92	102.74	102.72	102.98	102.85	102.70	102.88	102.58
17	61.72	61.05	60.14	60.11	60.43	60.28	60.09	60.29	59.85
18	21.03	20.35	20.29	20.22	20.31	20.17	20.23	20.35	20.00
19	30.54	31.01	29.82	30.75	29.75	29.55	30.62	30.75	30.52
20	27.46	26.98	27.27	27.30	27.26	27.10	27.28	27.40	27.16
21	21.92	21.20	21.10	21.03	21.12	20.98	21.15	21.20	21.06
22	33.43	32.74	33.81	33.80	33.72	33.55	33.80	33.92	33.68
23	74.31	73.63	74.02	73.97	73.81	73.68	73.96	74.07	74.06
24	81.93	81.15	79.33	79.26	79.37	79.24	79.27	79.36	82.13
25	72.73	71.97	76.00	75.99	76.04	75.95	75.96	76.04	70.73
26	27.57	27.15	23.02	23.00	23.06	22.91	22.99	23.07	28.29
27	27.57	26.97	21.05	21.03	21.12	20.98	21.15	21.27	24.79
28	12.23	11.54	11.64	11.65	11.57	11.42	11.49	11.61	11.46
29	26.15	25.91	25.42	25.84	25.38	25.66	25.44	25.97	25.64
30	15.91	14.65	15.19	14.60	15.10	14.39	14.40	13.91	14.37
COCH ₃	171.37	170.45	171.18	171.25	171.38	171.38	171.20	171.17	171.34
COCH ₃	21.75	20.99	20.74	20.97	21.12	20.98	21.02	21.08	20.89
OCH ₃			49.18	49.19	49.20	49.06	49.03	49.20	
1'	107.98		107.19		102.21		107.19		
2'	75.88		75.11		75.08		75.08		
3'	78.83		78.06		78.05		77.94		
4'	71.58		70.81		70.78		70.69		
5'	67.52		66.92		66.76		66.53		

a) Measured at 125 MHz; others: at 75.4 MHz.

Fig. 1. Structures of **1**–**5**

Compound **2a** was treated with 1% Na_2CO_3 , followed by acidification with 1 N HCl as in **1a** to afford a desacetyl dehydrate **2b**, which was identified as 25-*O*-methylcimigenol after direct comparison of mp, TLC, and ^1H -NMR spectra with an authentic specimen.⁷⁾ These results

suggested that **2b** should be 25-*O*-methylcimigenol and **2a** 25-*O*-methyl-24-*O*-acetylhydroshengmanol as shown in Fig. 2.

The CD of **2** showed a weak negative Cotton effect curve superimposable on that of **1**, and was attributable

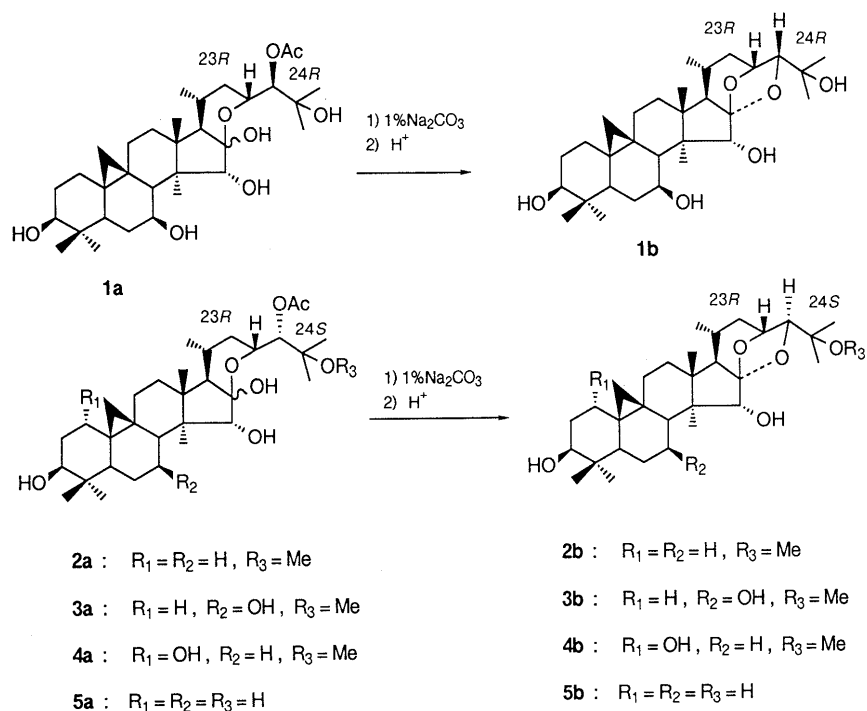


Fig. 2. Conversion of **1a**—**5a** into **1b**—**5b**

to a 16-keto-23-ol form as mentioned above. Enzymatic hydrolysis of **2** and dialysis of the sugar fraction afforded ethyl- β -D-xylopyranoside, $[\alpha]_D -40.4^\circ$ as in **1**. The ¹H- and ¹³C-NMR spectra of **2** suggested the presence of a 3-*O*- β -xylopyranosyl group as in **1**. Thus, **2** should be formulated as 25-*O*-methyl-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside as shown in Fig. 1.

Compound **3** was obtained as colorless needles, mp 199—200 °C, $[\alpha]_D +4.6^\circ$, and the molecular formula was determined to be C₃₈H₆₂O₁₂ on the basis of pos. HR-SI-MS [(M—OH)⁺, m/z 693.4214], pos. SI-MS [(M+Na)⁺, m/z 733] and the data of the ¹³C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3500—3200 and an acetyl band at 1725 cm⁻¹. The ¹H-NMR (Table 1) and the ¹³C-NMR (Table 2) signals were similar to those of **2**, except the signals of 7 α -H and C-7 (δ_H 3.68, ddd; δ_C 69.66) and small paramagnetic shifts of 19-2H (Δ : 0.07, 0.12 ppm).

Compound **3** was hydrolyzed with Cellulase T[Amano]⁴ to afford **3a**, mp 116—117 °C, $[\alpha]_D +13.5^\circ$, C₃₃H₅₄O₈. The pos. HR-SI-MS showed a (M—OH)⁺ ion at m/z 561.3783, clarifying the molecular formula.

Compound **3a** was treated with 1% Na₂CO₃, followed by acidification with 1 N HCl as in **1a** and **2a** to afford a desacetyl dehydrate **3b**, which was identified as 25-*O*-methyl-7 β -hydroxycimigenol after direct comparison of ¹H-NMR spectra with an authentic specimen.⁴⁾ These results suggested that **3a** should be 25-*O*-methyl-7 β -hydroxy-24-*O*-acetylhydroshengmanol as shown in Fig. 2.

The CD of **3** showed a weak negative Cotton effect curve superimposable on those of **1** and **2**, attributable to the 16-keto-23-ol form as mentioned above. Enzymatic hydrolysis of **3** and dialysis of the sugar fraction afforded ethyl- β -D-xylopyranoside, $[\alpha]_D -35.4^\circ$ as in **1**. The ¹H- and ¹³C-NMR spectra of **3** suggested the presence of a 3-*O*- β -xylopyranosyl group as in **1**. Thus, **3** should be

formulated as 25-*O*-methyl-7 β -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside as shown in Fig. 1.

Compound **4** was obtained as colorless needles, mp 149—150 °C, $[\alpha]_D +5.0^\circ$, and the molecular formula was determined as C₃₈H₆₂O₁₂ on the basis of pos. HR-SI-MS [(M—OH)⁺, m/z 693.4193], pos. SI-MS [(M—OH)⁺, m/z 693, (M+Na)⁺, m/z 733] and the data of the ¹³C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3600—3300 and an acetyl band at 1729 cm⁻¹. The ¹H-NMR (Table 1) and ¹³C-NMR (Table 2) signals (15-, 23-, 24-H and C-15, 16, 23, 24) were partially similar to those of **2** and **3**. The signals (1 β -H, 19-2H, C-1, C-19) were similar to those of 1 α -hydroxycimigenol, 1 α -hydroxyshengmanol and their xylosides.⁸⁾

Compound **4** was hydrolyzed with Cellulase T[Amano]⁴ to afford **4a**, mp 110—111 °C, $[\alpha]_D +24.0^\circ$, C₃₃H₅₄O₈. The pos. HR-SI-MS showed a (M—OH)⁺ ion at m/z 561.3784, clarifying the molecular formula. The IR spectrum showed broad hydroxyl bands at 3550—3300 and an acetyl band at 1718 cm⁻¹.

Compound **4a** was treated with 1% Na₂CO₃ as in **1a**, **2a** and **3a**, followed by acidification with 5% AcOH to afford a desacetyl dehydrate **4b**, mp 190—191 °C, $[\alpha]_D +41.9^\circ$, C₃₁H₅₀O₆. The pos. HR-SI-MS showed a (M+H)⁺ ion at m/z 519.3724 and (M+Na)⁺ ion at m/z 541.3495, clarifying the molecular formula. The IR spectrum showed broad hydroxyl bands at 3580—3300 cm⁻¹. The ¹H-NMR spectrum was similar to that of 1 α -hydroxycimigenol,⁸⁾ with an additional *O*-methyl signal (δ 3.26, s). Those data suggested that **4b** should be 25-*O*-methyl-1 α -hydroxycimigenol and **4a** 25-*O*-methyl-1 α -hydroxy-24-*O*-acetylhydroshengmanol as shown in Fig. 2.

The CD of **4** showed a weak negative Cotton effect curve superimposable on those of **1**, **2** and **3**, attributable

to a 16-keto-23-ol form as mentioned above. Enzymatic hydrolysis of **4** and dialysis of the sugar fraction afforded ethyl- β -D-xylopyranoside, $[\alpha]_D -37.8^\circ$ as in **1**. The ^1H - and ^{13}C -NMR spectra of **4** suggested the presence of a 3-*O*- β -xylopyranosyl group as in **1**. Thus, **4** should be formulated as 25-*O*-methyl-1 α -hydroxy-24-*O*-acetylhydroshengmanol-3-*O*- β -D-xylopyranoside as shown in Fig. 1.

It is interesting that aglycones **1a**–**4a** of the new xylosides **1**–**4** were 24-*epi*-24-*O*-acetylhydroshengmanol and 24-*O*-acetylhydroshengmanol derivatives, which are assumed to be biosynthetic intermediates between 24,25-epoxide type and 16,23;16,24-diepoxide type of genins of *Cimicifuga* glycosides. Such related hemiketal glycosides have also been reported from *Cimicifuga japonica*,²⁾ *C. dahurica*,³⁾ *C. heracleifolia*,⁹⁾ and now 7 β -hydroxy-24*R* congener (**1**), 7 β -hydroxy-24*S* one (**3**), and 1 α -hydroxy-24*S* one (**4**) were obtained from *C. simplex*. These results might advance future studies on the constituents of *Cimicifuga* species.

Experimental

General The instruments used in this work were as follows: Yanagimoto micromelting apparatus (for melting points, uncorrected); JASCO digital polarimeter (for specific rotation, measured at 20°C); JASCO ORD/UV-5 spectrometer (for CD, measured at 20°C); Perkin-Elmer 1720X-FT IR spectrometer (for IR spectra); Hitachi M-80 and JEOL JMS-DX-300 spectrometer (for MS spectra); and Varian Gemini-200, Varian XL-300 and General Electric GN-500 (for NMR spectra, measured in pyridine-*d*₅ solution containing a few drops of D₂O, on the δ scale using tetramethyl silane as an internal standard). Column chromatography was carried out on silica gel (Wakogel C-200) and ODS-A YMC. HPLC was conducted on a Gilson 305 pump equipped with a JASCO 830-RI as a detector. Silica gel 60 F₂₅₄ (Merck) precoated TLC plates were used and detection was carried out by I₂ or 40% H₂SO₄ followed by heating.

Extraction of 1–4 The residue (185 g) of the aqueous fraction after removal of the BuOH fraction from MeOH extracts of the aerial parts of *Cimicifuga simplex* (6.1 kg) was chromatographed on SiO₂ [1.0 kg, i.d. 6.0×40.0 cm, eluted with CHCl₃–MeOH (10:1–0:1)] as in the previous report.¹⁾ Fractions eluted with CHCl₃–MeOH (9:1) were rechromatographed on ODS (100 g, i.d. 3.5×24 cm). Fractions eluted with MeOH–H₂O (3:1) were subjected to HPLC [column: CrestPak C18T-5 (5 μm , i.d. 4.6 mm×250 mm); solvent, MeOH–H₂O–CH₃CN (10:7:3); effluent speed: 1 ml/min; column temperature, 40°C] to afford **1** and **4**. **1** was obtained as colorless needles (17 mg) after recrystallization from MeOH and **4** as colorless needles (53 mg) from a mixture of MeOH, EtOAc and isopropylether. Fractions eluted with CHCl₃–MeOH (10:1) in the first SiO₂ chromatography were rechromatographed on ODS as above. Fractions eluted with MeOH–H₂O (3:1) were subjected to HPLC [the same conditions as in **1** and **4** except the solvent: MeOH–H₂O–CH₃CN (10:7:4.5)] to afford **2** (18 mg) after recrystallization from MeOH and **3** (35 mg) after pTLC and recrystallization from a mixture of CH₃CN and MeOH.

1: Colorless needles, mp 276–277°C, $[\alpha]_D +10.3^\circ$ ($c=0.6$, MeOH), C₃₇H₆₀O₁₂. Pos. HR-SI-MS: m/z 679.4062 [(M–OH)⁺], error: 0.8 (m mass). Pos. FAB-MS: m/z 679 [(M–OH)⁺], m/z 719 [(M+Na)⁺]. IR (KBr) cm^{−1}: 3500–3200 (OH), 1732 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2. CD ($c=6.6\times 10^{-4}$, MeOH) $[\theta]^{20}(\text{nm})=-0.84\times 10^3$ (310) (negative maximum).

2: Colorless needles, mp 205–206°C, $[\alpha]_D -1.5^\circ$ ($c=0.55$, MeOH), C₃₈H₆₂O₁₁. Pos. HR-SI-MS: m/z 677.4269 [(M–OH)⁺], error: 0.8 (m mass), pos. SI-MS: m/z 677 [(M–OH)⁺], m/z 695 [(M+H)⁺]. IR (KBr) cm^{−1}: 3550–3300 (OH), 1715 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2. CD ($c=5.6\times 10^{-4}$, MeOH) $[\theta]^{20}(\text{nm})=-1.87\times 10^3$ (312) (negative maximum).

3: Colorless powder, mp 199–200°C, $[\alpha]_D +4.6^\circ$ ($c=0.52$, MeOH), C₃₈H₆₂O₁₂. Pos. HR-SI-MS: m/z 693.42147 [(M–OH)⁺], error: 0.4 (m mass). Pos. SI-MS: m/z 694 [(M–OH)⁺], 733 [(M+Na)⁺]. IR (KBr)

cm^{−1}: 3500–3200 (OH), 1725 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2. CD ($c=5.3\times 10^{-4}$, MeOH) $[\theta]^{20}(\text{nm})=-1.21\times 10^3$ (310) (negative maximum).

4: Colorless powder, mp 149–150°C, $[\alpha]_D +5.0^\circ$ ($c=1.15$, MeOH), C₃₈H₆₂O₁₂. Pos. HR-SI-MS: m/z 693.4193 [(M–OH)⁺], error: −1.8 (m mass). Pos. SI-MS: m/z 693 [(M–OH)⁺], 733 [(M+Na)⁺]. IR (KBr) cm^{−1}: 3600–3300 (OH), 1729 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2. CD ($c=2.1\times 10^{-4}$, MeOH) $[\theta]^{20}(\text{nm})=-5.4\times 10^3$ (314) (negative maximum).

Hydrolysis of 1–4 with Cellulase T[Amamo]4 **1** (10.2 mg) was dissolved in 1% ethanolic AcOH (20 ml), water (40 ml) was then added with stirring and the solution was adjusted to pH 4.5 by a dropwise addition of AcOH. Cellulase T[Amamo]4 (from *Trichoderma viride*, 200 mg) was added. The solution was stirred for 2 d at 25°C. Then, the reaction solution was shaken with EtOAc (30 ml×3), and after washing the joined EtOAc layer with water and drying it over Na₂SO₄, the solvent was evaporated *in vacuo*. The residue was chromatographed on SiO₂ (12 g) and eluted with *n*-hexane–EtOAc (1:2) to afford **1a** as colorless needles (4.0 mg) by recrystallization from MeOH. Similar treatments of **2** (10.0 mg), **3** (22.5 mg) and **4** (33.0 mg) provided **2a** (3.5 mg), **3a** (10.3 mg) and **4a** (12.3 mg) as aglycones.

1a: mp 189–190°C, $[\alpha]_D +48.0^\circ$ ($c=0.35$, MeOH), C₃₂H₅₂O₈. Pos. HR-SI-MS: m/z 547.3628 [(M–OH)⁺], error: −0.4 (m mass). Pos. SI-MS: m/z 547 [(M–OH)⁺], 587 [(M+Na)⁺]. IR (CHCl₃) cm^{−1}: 3500–3250 (OH), 1743 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2. Irradiation at δ 1.30 ppm (18–3H) enhanced the signal intensity of a singlet (15-H) at 4.20 ppm, and irradiation at δ 1.88 ppm (20-H) enhanced the signal intensities of 23-H at δ 4.46 ppm and 15-H.

2a: mp 185–186°C, $[\alpha]_D +6.8^\circ$ ($c=0.41$, MeOH), C₃₃H₅₄O₇. Pos. HR-SI-MS: m/z 545.3846 [(M–OH)⁺], error: −0.7 (m mass). Pos. SI-MS: m/z 545 [(M–OH)⁺]. IR (CHCl₃) cm^{−1}: 3500–3250 (OH), 1721 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2.

3a: mp 116–117°C, $[\alpha]_D +13.5^\circ$ ($c=0.31$, MeOH), C₃₃H₅₄O₈. Pos. HR-SI-MS: m/z 561.3783 [(M–OH)⁺], error: −0.5 (m mass). Pos. SI-MS: m/z 561 [(M–OH)⁺], 601 [(M+Na)⁺]. IR (CHCl₃) cm^{−1}: 3500–3250 (OH), 1718 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2.

4a: mp 110–111°C, $[\alpha]_D +24.0^\circ$ ($c=0.99$, MeOH), C₃₃H₅₄O₈. Pos. HR-SI-MS: m/z 561.3784 [(M–OH)⁺], error: −0.5 (m mass). Pos. SI-MS: m/z 561 [(M–OH)⁺], 601 [(M+Na)⁺]. IR (CHCl₃) cm^{−1}: 3550–3300 (OH), 1718 (CH₃CO). ^1H - and ^{13}C -NMR (pyridine-*d*₅) δ : Tables 1 and 2.

Identification of Ethyl- β -D-xyloside After EtOAc extraction of **1a**–**4a** as aglycones in hydrolysis with Cellulase T[Amamo]4, the aqueous fractions were each dialyzed with Spectrapor membrane tubes (6000–8000 MW cut off, Spectrum Medical Industries Inc.) overnight. The dialysates were concentrated *in vacuo* and chromatographed on SiO₂. Elution with CHCl₃–MeOH (9:1) gave ethyl- β -D-xyloside each time. C₇H₁₄O₅. Pos. SI-MS: m/z 178 [(M)⁺], 177 [(M–H)⁺]. $[\alpha]_D -34.4$ – -40.0° . An authentic specimen was prepared from methyl- β -D-xyloside by the same treatment with cellulase. $[\alpha]_D -37.0^\circ$ has been reported.⁶⁾ ^1H -NMR (pyridine-*d*₅) δ : 1.20 (3H, t, $J=7.0$ Hz, CH₃CH₂O), 4.20 (2H, q, $J=7.0$ Hz, CH₃CH₂O), 3.65 (1H, dd, $J=9.7$, 7.8 Hz, 2-H), 3.72 (1H, dd, $J=11.0$, 7.8 Hz, 5-H), 4.00 (1H, dd, $J=9.7$, 7.8 Hz, 3-H), 4.15 (1H, dd, $J=9.7$, 7.8, 3.9 Hz, 4-H), 4.35 (1H, dd, $J=11.0$, 3.9 Hz, 5-H), 4.67 (1H, d, $J=7.8$ Hz, 1-H).

Conversion of 1a–4a into 1b–4b **1a** (1.5 mg) was stirred in 1% Na₂CO₃ [MeOH (2 ml) and 2% Na₂CO₃ (2 ml)] for 24 h at 25°C. After neutralization with 1N AcOH, the mixture was shaken with EtOAc (20 ml×3) and washed with water. The residue after evaporation of the solvent was dissolved in dioxane (2 ml) and a few drops of 1N HCl were added. After standing for 30 min. at r.t., the reaction solution was diluted with water and extracted with EtOAc (20 ml×3). The product was chromatographed on SiO₂ (12 g) and eluates with *n*-hexane–EtOAc (1:2) were purified by HPLC to give **1b**, mp 251–252°C, $[\alpha]_D +44^\circ$ ($c=0.05$, MeOH). IR (CHCl₃) cm^{−1}: 3400–3250 (OH). Pos. HR-SI-MS: m/z 505.3551 [(M+H)⁺], error: 3.0 (m mass). Pos. SI-MS: m/z 505 [(M+H)⁺], 527 [(M+Na)⁺]. ^1H -NMR (pyridine-*d*₅) δ : 0.40 (1H, d, $J=4.0$ Hz, 19-H), 0.72 (1H, d, $J=4.0$ Hz, 19-H), 1.00 (3H, d, $J=6.0$ Hz, 21-3H), 3.56 (1H, dd, $J=12.0$, 4.0 Hz, 3-H), 3.68 (1H, ddd, $J=12.0$, 10.0, 3.0 Hz, 7-H), 3.78 (1H, d, $J=4.0$ Hz, 24-H), 4.44 (1H, s, 15-H), 4.62 (1H, ddd, $J=9.0$, 4.0, 2.0 Hz, 23-H). Ref. ^1H -NMR (pyridine-*d*₅) δ of cimigol: 0.31 (1H, d, $J=4.0$ Hz, 19-H), 0.55 (1H, d, $J=4.0$ Hz, 19-H), 0.97 (3H, d, $J=6.0$ Hz, 21–3H), 3.55 (1H, dd, $J=12.0$, 4.0 Hz, 3-H), 3.75

(1H, $J=4.0$ Hz, 24-H), 4.23 (1H, s, 15-H), 4.62 (1H, dd, $J=9.0, 4.0, 2.0$ Hz, 23-H). **2a** (2.7 mg) was treated as in **1a** to give **2b**, mp 241–242 °C. TLC (*n*-hexane–EtOAc=3:1, *R_f* 0.30) and the ¹H-NMR were identical with those of an authentic specimen of 25-*O*-methylcimigenol.⁷⁾ **3a** (3.4 mg) was also treated as in **1a** to give **3b**, Pos. SI-MS: *m/z* 519 [(M+H)⁺]. The ¹H-NMR spectrum was identical to that of 25-*O*-methyl-7β-hydroxycimigenol.⁴⁾ **4a** (5.0 mg) was treated as in **1a** except for acidification of 5% AcOH instead of 1N HCl to give **4b**, colorless needles, mp 190–191 °C. $[\alpha]_D +41.9^\circ$ ($c=0.21$, MeOH). IR (CHCl₃) cm^{-1} : 3580–3300 (OH). C₃₁H₅₀O₆. Pos. HR-SI-MS: *m/z* 541.3495 [(M+Na)⁺], error: –0.7 (m mass). Pos. SI-MS: 519 [(M+H)⁺], 541 [(M+Na)⁺]. ¹H-NMR (pyridine-*d*₅) δ : 0.45 (1H, d, $J=4.0$ Hz, 19-H), 0.72 (1H, d, $J=4.0$ Hz, 19-H), 0.85 (3H, d, $J=7.0$ Hz, 21-3H), 3.26 (3H, s, OCH₃), 3.68 (1H, s, 24-H), 3.83 (1H, br s, 1-H), 4.20 (1H, s, 15-H), 4.40 (1H, dd, $J=12.0, 4.0$ Hz, 3-H), 4.60 (1H, d, $J=9.0$ Hz, 23-H).

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