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

Akiko KUSANO, Makio SHIBANO, and Genjiro KUSANO*

Osaka University of Pharmaceutical Sciences, 2–10–65 Kawai, Matsubara, Osaka 580, Japan.
Received July 19, 1995; accepted September 1, 1995

Four new xylosides (1—4) were isolated from the aerial parts of *Cimicifuga simplex* (Ranunculaceae), and their structures were determined to be 24-*epi*-7β-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-7β-hydroxy-24-*O*-acetylhydroshengmanol-3-*O*-β-D-xylopyranoside (3) and 25-*O*-methyl-1α-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 (SiO_2), 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°, and the molecular formula was determined as $C_{37}H_{60}O_{12}$ 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 ^{13}C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3500—3200 and an acetyl band at 1732 cm^{-1}. The ^{1}H- and ^{13}C-NMR signals were assigned using the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and the ^{13}C–^{1}H 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 7α-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*-acetylhydroshengemanol (**5a**) has been recorded in a $CDCl_3$ 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 D_2O. A xyloside (**5**) was obtained from the same herb and identified by direct comparison of TLC and ^{1}H-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*)-3β, 15α,16ζ,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 ^{13}C-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-diepoxy 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°, $C_{32}H_{52}O_8$. 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}. ^{1}H- and ^{13}C-NMR signals of **1a** were similar to those of 7β-hydroxycimigenol[1,4] and 24-*O*-acetylhydroshengmanol[2] as summarized in Tables 1 and 2.

Compound **1a** was treated with 1% Na_2CO_3, followed by acidification with 1 N HCl to afford a desacetyl dehydrate **1b**, mp 251—252 °C, $[\alpha]_D$ +44.0°, $C_{30}H_{48}O_6$. 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 ^{1}H-NMR spectra of **1a** and **1b** indicated a conversion of a 16-hydroxy-24-acetoxy-16,23-epoxy moiety [δ 2.18 (3H, s, CH_3CO), 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-diepoxy 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-diepoxy as described in the structural elucidation of cimigol.[5]

Cimigenol derivatives having (23*R*,24*S*)-16,23;16,24-diepoxy 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-diepoxy moieties have not been known, but model consideration indicates that ^{1}H-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

* To whom correspondence should be addressed.

Table 1. ^{1}H-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 br s	3.85 br s	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	3.56 dd	3.50 dd	3.58 dd	3.49 dd	3.55 dd	4.32 dd	4.41 dd	3.48 dd
	(11.0, 4.0)	(11.3, 4.4)	(11.5, 4.5)	(11.0, 4.2)	(12.0, 4.4)	(11.5, 6.5)	(12.0, 8.2)	(12.0, 8.2)	(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	3.71 ddd	1.12, 2.05	1.15, 2.05	3.68 ddd	3.70 ddd	1.35, 1.52	1.35, 1.50	1.12, 2.00
	(12.0, 10.0, 3.0)	(12.0, 10.0, 3.0)			(12.0, 10.0, 3.0)	(12.0, 10.0, 3.0)			
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)	0.41 d (4.2)	0.28 d (3.8)	0.34 d (3.9)	0.35 d (4.1)	0.38 d (4.1)	0.40 d (4.0)	0.47 d (4.2)	0.23 d (4.1)
	0.63 d (3.9)	0.70 d (4.2)	0.50 d (3.8)	0.57 d (3.9)	0.62 d (4.1)	0.65 d (4.1)	0.65 d (4.0)	0.72 d (4.2)	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	4.21	4.25 ddd	4.25	4.23 ddd	4.20	4.23 ddd	4.28 ddd
		(11.9, 8.4, 6.4)		(10.7, 8.4, 6.4)		(11.3, 8.4, 6.3)		(10.8, 8.5, 6.7)	(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		4.05 dd		4.03 dd		4.02 dd		
	(8.3, 8.2)		(8.3, 7.8)		(8.3, 7.8)		(8.5, 7.7)		
3′	4.20 dd		4.18 dd		4.18 dd		4.12 dd		
	(8.6, 8.2)		(8.6, 8.3)		(8.6, 8.3)		(8.6, 8.3)		
4′	4.26 ddd		4.30 ddd		4.22 ddd		4.23 ddd		
	(10.6, 8.6, 4.8)		(10.1, 8.6, 4.8)		(10.1, 8.6, 5.0)		(10.1, 8.6, 5.1)		
5′	3.77 dd		3.77 dd		3.68 dd		3.59 dd		
	(10.8, 10.6)		(10.8, 10.1)		(10.9, 10.1)		(10.6, 10.1)		
5′	4.38 dd		4.38 dd		4.37 dd		4.34 dd		
	(10.8, 4.8)		(10.8, 4.8)		(10.9, 5.0)		(10.6, 5.1)		

a) Obtained on a General Electric GN-500; others on a Varian XL-300 in pyridine-d_5 containing D_2O. Chemical shift were found from ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY.

cimigol type, namely, 24-epicimigenol derivative. The ^{1}H-NMR signals due to cyclopropane methylene, 3α-H and 7α-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 15β-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 ^{1}H-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, $[\alpha]_D$ −34.4°, $C_7H_{14}O_5$, $(M)^+$, 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.

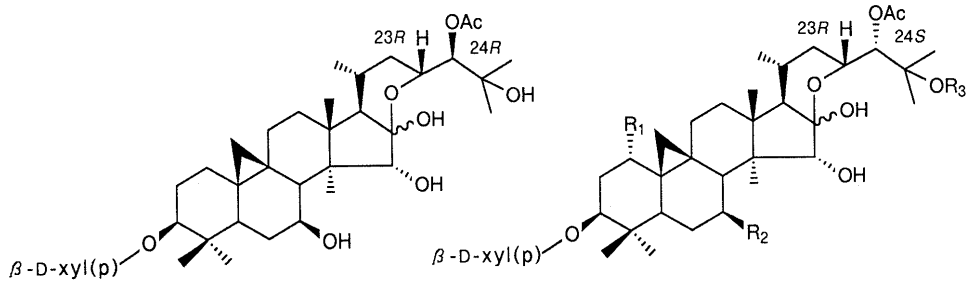
Compound **2** was obtained as colorless needles, mp 205—206 °C, $[\alpha]_D$ −1.5°, and the molecular formula was determined as $C_{38}H_{62}O_{11}$ on the basis of pos. HR-SI-MS $[(M-OH)^+, m/z$ 677.4269], pos. SI-MS $[(M-OH)^+, m/z$ 677; $(M+H)^+, m/z$ 695] and the data of the ^{13}C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3550—3300 and an acetyl band at 1715 cm^{-1}. The ^{1}H-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–CH_3 (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, $[\alpha]_D$ +6.8°, $C_{33}H_{54}O_7$. 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
$\underline{C}OCH_3$	171.37	170.45	171.18	171.25	171.38	171.38	171.20	171.17	171.34
$CO\underline{C}H_3$	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.



1

2 : $R_1 = R_2 = H$, $R_3 = Me$

3 : $R_1 = H$, $R_2 = OH$, $R_3 = Me$

4 : $R_1 = OH$, $R_2 = H$, $R_3 = Me$

5 : $R_1 = R_2 = R_3 = H$

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 ^{1}H-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

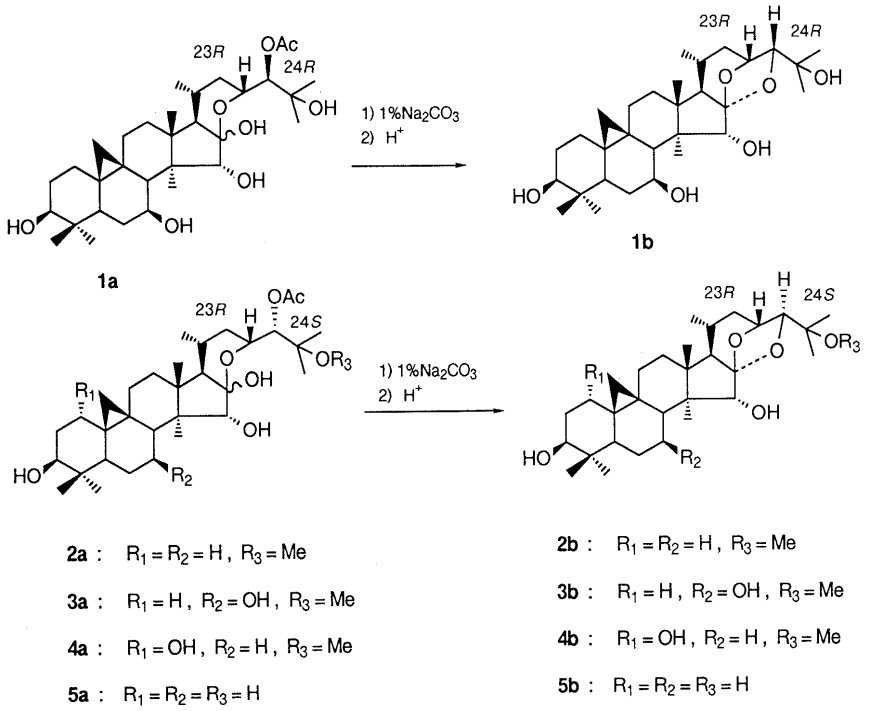


Fig. 2. Conversion of **1a**—**5a** into **1b**—**5b**

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, $[\alpha]_D -40.4°$ as in **1**. The ^{1}H- and ^{13}C-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, $[\alpha]_D +4.6°$, and the molecular formula was determined to be $C_{38}H_{62}O_{12}$ 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 ^{13}C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3500—3200 and an acetyl band at 1725 cm^{-1}. The ^{1}H-NMR (Table 1) and the ^{13}C-NMR (Table 2) signals were similar to those of **2**, except the signals of 7α-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, $[\alpha]_D +13.5°$, $C_{33}H_{54}O_8$. 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% Na_2CO_3, 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-7β-hydroxycimigenol after direct comparison of ^{1}H-NMR spectra with an authentic specimen.[4] These results suggested that **3a** should be 25-*O*-methyl-7β-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, $[\alpha]_D -35.4°$ as in **1**. The ^{1}H- and ^{13}C-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-7β-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, $[\alpha]_D +5.0°$, and the molecular formula was determined as $C_{38}H_{62}O_{12}$ 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 ^{13}C-NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3600—3300 and an acetyl band at 1729 cm^{-1}. The ^{1}H-NMR (Table 1) and ^{13}C-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 (1β-H, 19-2H, C-1, C-19) were similar to those of 1α-hydroxycimigenol, 1α-hydroxyshengmanol and their xylosides.[8]

Compound **4** was hydrolyzed with Cellulase T[Amano]4 to afford **4a**, mp 110—111 °C, $[\alpha]_D +24.0°$, $C_{33}H_{54}O_8$. 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% Na_2CO_3 as in **1a**, **2a** and **3a**, followed by acidification with 5% AcOH to afford a desacetyl dehydrate **4b**, mp 190—191 °C, $[\alpha]_D +41.9°$, $C_{31}H_{50}O_6$. 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 ^{1}H-NMR spectrum was similar to that of 1α-hydroxycimigenol,[8] with an additional *O*-methyl signal (δ 3.26, s). Those data suggested that **4b** should be 25-*O*-methyl-1α-hydroxycimigenol and **4a** 25-*O*-methyl-1α-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° as in **1**. The ^{1}H- 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-1α-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 7β-hydroxy-24*R* congener (**1**), 7β-hydroxy-24*S* one (**3**), and 1α-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 D_2O, 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 F_{254} (Merck) precoated TLC plates were used and detection was carried out by I_2 or 40% H_2SO_4 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 SiO_2 [1.0 kg, i.d. 6.0 × 40.0 cm, eluted with $CHCl_3$–MeOH (10 : 1—0 : 1)] as in the previous report.[1] Fractions eluted with $CHCl_3$–MeOH (9 : 1) were rechromatographed on ODS (100 g, i.d. 3.5 × 24 cm). Fractions eluted with MeOH–H_2O (3 : 1) were subjected to HPLC [column: CrestPak C18T-5 (5 μm, i.d. 4.6 mm × 250 mm); solvent, MeOH–H_2O–CH_3CN (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 $CHCl_3$–MeOH (10 : 1) in the first SiO_2 chromatography were rechromatographed on ODS as above. Fractions eluted with MeOH–H_2O (3 : 1) were subjected to HPLC [the same conditions as in **1** and **4** except the solvent: MeOH–H_2O–CH_3CN (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 CH_3CN and MeOH.

1: Colorless needles, mp 276—277 °C, $[\alpha]_D$ +10.3° (c = 0.6, MeOH), $C_{37}H_{60}O_{12}$. 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 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2. CD ($c = 6.6 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm) = -0.84×10^3 (310) (negative maximum).

2: Colorless needles, mp 205—206 °C, $[\alpha]_D$ −1.5° (c = 0.55, MeOH). $C_{38}H_{62}O_{11}$. 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 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2. CD ($c = 5.6 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm) = -1.87×10^3 (312) (negative maximum).

3: Colorless powder, mp 199—200 °C, $[\alpha]_D$ +4.6° (c = 0.52, MeOH). $C_{38}H_{62}O_{12}$. 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 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2. CD ($c = 5.3 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm) = -1.21×10^3 (310) (negative maximum).

4: Colorless powder, mp 149—150 °C, $[\alpha]_D$ +5.0° (c = 1.15, MeOH), $C_{38}H_{62}O_{12}$. 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 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2. CD ($c = 2.1 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm) = -5.4×10^3 (314) (negative maximum).

Hydrolysis of 1—4 with Cellulase T[Amano]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[Amano]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 Na_2SO_4, the solvent was evaporated *in vacuo*. The residue was chromatographed on SiO_2 (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° (c = 0.35, MeOH), $C_{32}H_{52}O_8$. 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 ($CHCl_3$) cm^{-1}: 3500—3250 (OH), 1743 (CH_3CO). ^{1}H- 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° (c = 0.41, MeOH). $C_{33}H_{54}O_7$. Pos. HR-SI-MS: m/z 545.3846 [(M−OH)$^+$], error: −0.7 (m mass). Pos. SI-MS: m/z 545 [(M−OH)$^+$]. IR ($CHCl_3$) cm^{-1}: 3500—3250 (OH), 1721 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2.

3a: mp 116—117 °C, $[\alpha]_D$ +13.5° (c = 0.31, MeOH). $C_{33}H_{54}O_8$. 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 ($CHCl_3$) cm^{-1}: 3500—3250 (OH), 1718 (CH_3CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: Tables 1 and 2.

4a: mp 110—111 °C, $[\alpha]_D$ +24.0° (c = 0.99, MeOH). $C_{33}H_{54}O_8$. 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 ($CHCl_3$) cm^{-1}: 3550—3300 (OH), 1718 (CH_3CO). ^{1}H- 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[Amano]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 SiO_2. Elution with $CHCl_3$–MeOH (9 : 1) gave ethyl-β-D-xyloside each time. $C_7H_{14}O_5$. 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° has been reported.[6] ^{1}H-NMR (pyridine-d_5) δ: 1.20 (3H, t, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 4.20 (2H, q, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 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, ddd, 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% Na_2CO_3 [MeOH (2 ml) and 2% Na_2CO_3 (2 ml)] for 24 h at 25 °C. After neutralization with 1 N 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 1 N 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 SiO_2 (12 g) and eluates with *n*-hexane–EtOAc (1 : 2) were purified by HPLC to give **1b**, mp 251—252 °C, $[\alpha]_D$ +44° (c = 0.05, MeOH). IR ($CHCl_3$) 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)$^+$]. ^{1}H-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. ^{1}H-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, *Rf* 0.30) and the ^{1}H-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 ^{1}H-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 1 N HCl to give **4b**, colorless needles, mp 190—191 °C. $[\alpha]_D$ +41.9° (c=0.21, MeOH). IR ($CHCl_3$) cm^{-1}: 3580—3300 (OH). $C_{31}H_{50}O_6$. Pos. HR-SI-MS: *m/z* 541.3495 $[(M+Na)^+]$, error: −0.7 (m mass). Pos. SI-MS: 519 $[(M+H)^+]$, 541 $[(M+Na)^+]$. ^{1}H-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, OCH_3), 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).

Acknowledgements The authors are grateful to Amano Pharmaceutical Company, Nagoya, for the generous gift of Cellulase T[Amano]4. They are also grateful to Mr. K. Minoura at their university and Mrs.K. Mushiake of the Faculty of Pharmaceutical Sciences, Tohoku University for NMR spectral measurements, to Mrs. M. Fujitake for mass spectral measurements at thier university, and to Mr. H. Hayasaka and Mr. K. Ohba of the Faculty of Pharmaceutical Sciences, Tohoku University, for collecting the aerial parts of *Cimicifuga simplex*.

References and Notes

1) Part XVII: Kusano A., Shibano M., Kusano G., *Chem. Pharm. Bull.*, **43**, 1167—1170 (1995).
2) Sakurai N., Kimura O., Inoue T., Nagai M., *Chem. Pharm. Bull.*, **29**, 955—960 (1981).
3) Sakurai N., Koeda M., Inoue T., Nagai M., *Chem. Pharm. Bull.*, **42**, 48—51 (1994).
4) Kusano G., Idoji M., Sogoh Y., Shibano M., Kusano A., Iwashita T., *Chem. Pharm. Bull.*, **42**, 1106—1110 (1994).
5) Kusano G., Takemoto T., *Yakugaku Zasshi*, **95**, 1133—1137 (1975).
6) de Bruyne C. K., Loontiens F.G., *Nature* (London), **209**, 396—397 (1966).
7) Takemoto T., Kusano G., *Yakugaku Zasshi*, **88**, 623—626 (1968).
8) Kusano A., Shimizu K., Idoji M., Shibano M., Minoura K., Kusano G., *Chem. Pharm. Bull.*, **43**, 279—283 (1995).
9) Li, J. X., Kadota S., Hattori M., Yoshimachi S., Shiro M., Oogami N., Mizuno H., Namba T., *Chem. Pharm. Bull.*, **41**, 832—841 (1993).