Studies on the Constituents of *Cimicifuga* Species. XXVI.[1] Twelve New Cyclolanostanol Glycosides from the Underground Parts of *Cimicifuga simplex* Wormsk.

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Twelve new cyclolanostanol glycosides (1—12) were isolated from the underground parts of *Cimicifuga simplex* Wormsk. (Ranunculaceae) together with five known cyclolanostanol glycosides (13—17). On the basis of spectral and chemical evidence, the structures of 1—12 were determined to be 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-xylopyranoside and 3-*O*-α-L-arabinopyranoside, 24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-xylopyranoside and 3-*O*-α-L-arabinopyranoside, 7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside and 3-*O*-α-L-arabinopyranoside, 25-*O*-acetyl-7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside and 3-*O*-α-L-arabinopyranoside, 1α-hydroxycimigenol 3-*O*-α-L-arabinopyranoside and 3-*O*-β-D-galactopyranoside, 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-β-D-galactopyranoside, and 26-deoxycimicifugoside, respectively. The last compound (12) was also obtained from the underground parts of *Actaea asiatica* Hara (Ranunculaceae).

Key words *Cimicifuga simplex*; *Actaea asiatica*; Ranunculaceae; cyclolanostanol; glycoside

Cimicifugae Rhizoma, rhizomes of *Cimicifuga* (*C.*) *simplex* Wormskjord, *C. dahurica* (Turcz) Maximmowicz, *C. foetida* Linne and *C. heracleifolia* Komarov (Ranunculaceae) (The Pharmacopoeia of Japan, 13th ed. supplementary), have been used as an anti-inflammatory, analgesic, and antipyretic agents in traditional Chinese medicine. During a series of chemical investigations of *Cimicifuga* species, we reported twenty cyclolanostanol glycosides from the aerial parts of *C. simplex*,[2—7] and 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-α-L-arabinopyranoside,[7] cimicifugoside,[8,9] bugbanosides A and B,[10] and cimiaceroside B[11] from the underground parts of *C. simplex*. Cimiacerosides A and B have also been isolated from the underground parts of *C. acerina*, and cimiaceroside A from *Actaea* (A.) *asiatica* Hara.[11] Fukiic acid esters,[12] piscidic acid esters,[13] caffeic acid derivatives, phenolic acid derivatives and chromones have also been isolated from *C. species*.[1] In continuing work, we have now isolated twelve new cyclolanostanol glycosides (**1**—**12**), and five known cyclolanostanol glycosides (**13**—**17**) from the underground parts of *C. simplex*. Compound **12** was also obtained from the underground parts of *A. asiatica* at this time. This paper deals with the isolation and structural elucidation of these glycosides.

Compounds **1**—**17** were obtained by repeated chromatography of the methanol extract of the underground parts of the plants on octadecyl silanized silicic acid (ODS) and silica-gel (SiO_2) columns, followed by HPLC separation (Fig. 1). Compound **13** was identified by comparison of ^{1}H- and ^{13}C-NMR spectral data with reported data as 24-*epi*-7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside, which has been isolated from *C. heracleifolia*.[14] Compound **14** was identified as 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-galactopyranoside, **15** as 24-*epi*-24-*O*-acetylhydroshengmanol 3-*O*-β-D-galactopyranoside, **16** as 7,8-didehydrocimigenol 3-*O*-β-D-galactopyranoside, and **17** as cimigenol 3-*O*-β-D-galactopyranoside, respectively, by direct comparison with authentic specimens.[7] The ^{1}H- and ^{13}C-NMR signals were attributed by using ^{1}H–^{1}H correlated spectroscopy, heteronuclear signal quantum coherence, heteronuclear multiple bond connectivity (HMBC), and rotating frame nuclear Overhauser effect (ROE) difference spectroscopy spectra.

Compound **1** was obtained as colorless needles. The molecular formula was determined to be $C_{37}H_{58}O_{11}$ by positive high resolution secondary ion mass spectrometry (pos. HR-SI-MS) and ^{13}C-NMR. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **14**, except for the sugar moiety (Tables 1, 2). On enzymatic hydrolysis, a genuine aglycone (**1a**) and an artifact (**1b**) were obtained, and **1a** was identified as 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol by direct comparison with an authentic specimen, and **1b** as heracleifolinol by comparison with the reported data.[14] On acid hydrolysis, D-xylose was detected as the sugar. The ROE experiment involving irradiation of the anomeric proton signal at δ 4.87 (1H, d, J=7.5 Hz, 1′-H) enhanced the protons at δ 4.16 (1H, dd, J=8.1, 8.1 Hz, 3′-H), δ 3.76 (1H, dd, J=10.0, 11.0 Hz, 5′-H), and δ 3.51 (1H, dd, J=4.4, 11.9 Hz, 3-H), suggesting the presence of a 3-*O*-β-D-xylopyranosyl group. In the HMBC spectrum, a correlation was observed between 1′-H (δ 4.87) and 3-C (δ 88.18). A glycosylation shift for 3-C ($\Delta\delta$, 10.33 ppm) between **1** and **1a** was also observed. Thus, the structure of **1** was determined to be 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-xylopyranoside.

Compound **2**, colorless needles, $C_{37}H_{58}O_{11}$, gave L-arabinose on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **1** and **14**, except for the sugar moiety (Tables 1, 2). The ROE experiment and the HMBC spectrum showed the presence of a 3-*O*-α-L-arabinopyranosyl group. Thus, the structure of **2** was determined to be 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-α-L-arabinopyranoside.

Compound **3**, colorless needles, $C_{37}H_{58}O_{11}$, and compound **4**, colorless powder, $C_{37}H_{58}O_{11}$, gave D-xylose and L-arabinose on acid hydrolysis, respectively. Their ^{1}H- and ^{13}C-NMR spectra were similar to those of **1** and **2**, respectively, except for the side chain moiety (indicated by underlined signals in Tables 1 and 2). Enzymatic hydrolysis of **3** with cellu-

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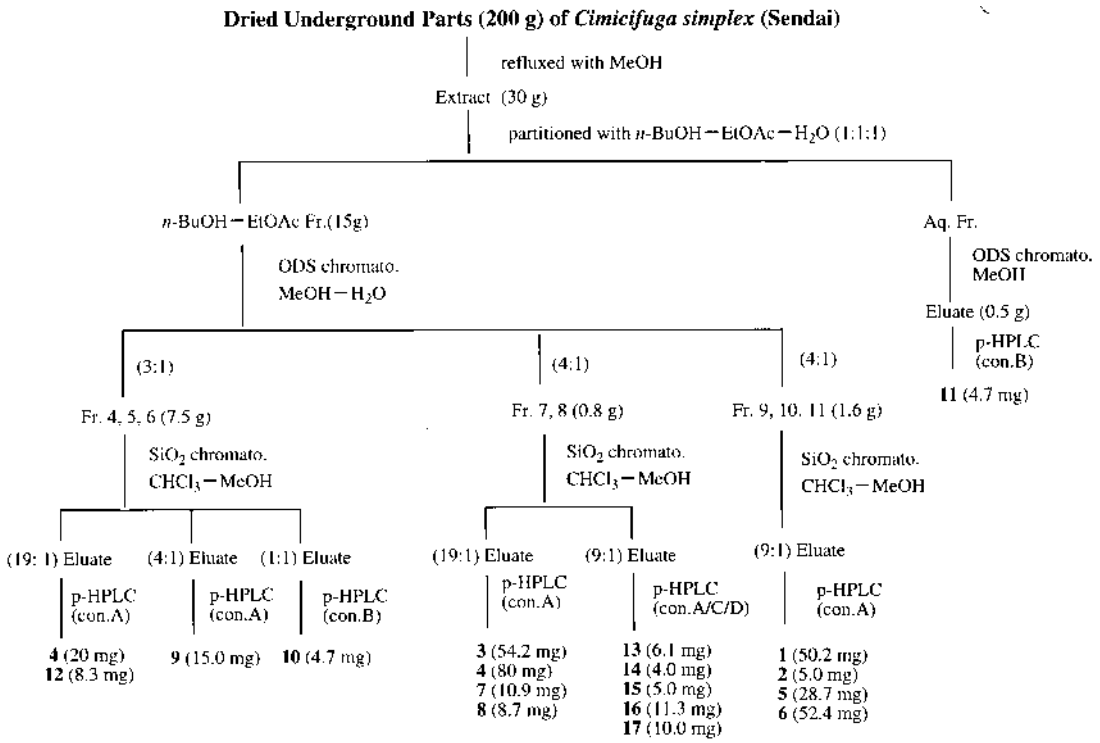

Fig. 1. Isolation Procedure of Compounds **1**—**17**

See con. A, B, C and D in the Experimental section.

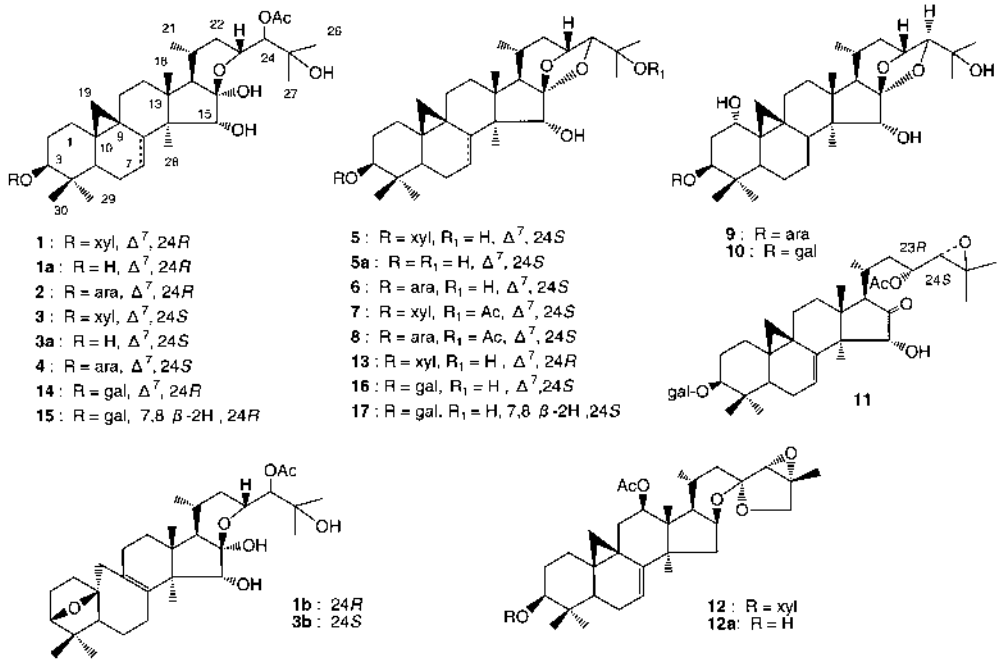

Fig. 2. Structures of Compounds **1**—**7** and Their Derivatives

lase and **4** with lactase gave the same genuine aglycone (**3a**) and an artifact (**3b**). Treatment of **3a** with 1% Na_2CO_3 followed by 2.5% CH_3COOH gave 7,8-didehydrocimigenol (**5a**), suggesting that **3a** was 24-*O*-acetyl-7,8-didehydrohydroshengmanol.[7] The ^{1}H- and ^{13}C-NMR spectra of **3b** were similar to those of **1b**, except for the side chain moiety, and the structure was formulated to be **3b** (proacerinol). The ROE experiment, and the HMBC spectra of **3** and **4**, showed the presence of a 3-*O*-β-D-xylopyranosyl group and a 3-*O*-α-L-arabinopyranosyl group, respectively. Thus, the structures of **3** and **4** were determined to be 24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-xylopyranoside and 24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-α-L-arabinopyranoside. Comparison of ^{1}H- and ^{13}C-NMR spectra between 24(*S*) compounds (**3**, **4**) and 24(*R*) compounds (**1**, **2**) spported the configuration of 24*S* (24-H: δ 5.63, d, J=7.5 Hz; acetyl: δ 2.00, s in pyridine-d_5) and 24*R* (24-H: δ 5.74, d, J=8.1 Hz; acetyl: δ 2.14, s in pyridine-d_5), before chemical conversions

Table 1. ^{1}H-NMR Data of Compounds **1**—**12** and Their Aglycones

	1[a)]	**2**[a)]	**3**[a)]	**3a**[a)]	**3b**[a)]	**4**[a)]	**5**[a)]	**6**[a)]
1	1.33, 1.73	1.33, 1.68	1.34, 1.70	1.33, 1.69	1.48, 1.68	1.34, 1.68	1.35, 1.71	1.36, 1.70
2	1.93, 2.35	1.98, 2.35	1.95, 2.33	1.96 (2H)	1.68, 1.90	1.93, 2.34	1.97, 2.34	1.97, 2.36
3	3.51 dd (4.4, 11.9)	3.48 dd (4.0, 11.3)	3.49 dd (4.0, 11.3)	3.54 dd (4.4, 11.3)	3.73 d (5.3)	3.48 dd (4.4, 11.3)	3.49 dd (4.4, 11.9)	3.47 dd (4.4, 11.5)
5	1.33	1.35	1.35	1.31	1.30	1.31	1.30	1.26
6	1.65, 1.95	1.63, 1.95	1.62, 1.92	1.68, 1.98	1.48, 1.76	1.62, 1.93	1.59, 1.84	1.59, 1.88
7	6.01 dd (1.5, 6.9)	5.99 dd (1.5, 6.9)	6.00 dd (1.3, 6.8)	6.04 dd (1.3, 7.5)	2.60, 2.94	6.01 dd (1.3, 6.8)	6.06 dd (1.5, 7.5)	6.06 dd (1.5, 7.5)
8	—	—	—	—	—	—	—	—
11	1.16, 2.18	1.18, 2.17	1.16, 2.18	1.20, 2.21	1.98, 2.13	1.17, 2.18	1.17, 2.19	1.15, 2.18
12	1.68, 1.83	1.66, 1.80	1.68, 1.80	1.68, 1.82	1.50 (2H)	1.66, 1.83	1.67, 1.83	1.67, 1.80
15	4.45 s	4.44 s	4.44 s	4.47 s	4.34 s	4.43 s	4.52 s	4.52 s
17	1.82	1.81	1.80	1.86	1.80	1.83 d (8.8)	1.54	1.52
18	1.27 s	1.25 s	1.29 s	1.31 s	1.10 s	1.28 s	1.17 s	1.17 s
19	0.54 d (4.0) 1.09 d (4.0)	0.52 d (4.0) 1.09 d (4.0)	0.52 d (4.0) 1.08 d (4.0)	0.57 d (4.0) 1.14 d (4.0)	1.84 d (13.5) 3.27 d (13.5)	0.53 d (4.0) 1.09 d (4.0)	0.51 d (4.0) 1.05 d (4.0)	0.51 d (4.0) 1.07 d (4.0)
20	1.83	1.80	1.80	1.83	1.79	1.80	1.71	1.70
21	1.03 d (6.3)	1.01 d (6.3)	1.03 d (6.3)	1.05 d (6.3)	1.03 d (6.0)	1.04 d (6.3)	0.91 d (6.3)	0.91 d (6.6)
22	1.84, 2.10	1.83, 2.08	2.08, 2.22	2.11, 2.21	2.06, 2.22	2.09, 2.23	1.02, 2.28	1.06, 2.31
23	4.46	4.44	4.41	4.41 ddd (6.3, 7.5, 11.3)	4.42 ddd (6.3, 7.0, 12.0)	4.41	4.73 d (9.0)	4.75 d (9.4)
24	5.74 d (8.1)	5.73 d (8.1)	5.63 d (7.5)	5.64 d (7.5)	5.59 d (7.3)	5.63 d (7.6)	3.79 s	3.79 s
26	1.50 s	1.49 s	1.47 s	1.48 s	1.48 s	1.48 s	1.49 s	1.49 s
27	1.47 s	1.45 s	1.47 s	1.48 s	1.46 s	1.47 s	1.47 s	1.47 s
28	1.44 s	1.43 s	1.46 s	1.46 s	1.30 s	1.47 s	1.41 s	1.42 s
29	1.34 s	1.29 s	1.31 s	1.20 s	0.90 s	1.29 s	1.30 s	1.27 s
30	1.06 s	1.02 s	1.04 s	1.10 s	0.96 s	1.09 s	1.05 s	1.03 s
$COCH_3$	2.14 s	2.12 s	2.00 s	2.00 s	1.98 s	2.00 s		
1′	4.87 d (7.5)	4.79 d (7.3)	4.85 d (7.5)			4.79 d (7.5)	4.84 d (7.5)	4.78d (7.5)
2′	4.04 dd (7.5, 8.1)	4.43	4.02 dd (7.5, 8.2)			4.43 dd (7.5, 8.1)	4.02 dd (7.5, 8.1)	4.43 dd (7.5, 7.5)
3′	4.16 dd (8.1, 8.1)	4.16 dd (3.1, 8.1)	4.15 dd (8.2, 8.2)			4.17 dd (3.1, 8.1)	4.14 dd (8.1, 8.1)	4.15 dd (3.1, 7.5)
4′	4.23 ddd (5.0, 8.1, 10.0)	4.32	4.21 ddd (5.0, 8.2, 10.0)			4.33	4.21 ddd (5.0, 8.1, 10.0)	4.30
5′	3.76 dd (10.0, 11.0) 4.39 dd (5.0,11.0)	3.81 dd (2.5, 11.2) 4.31	3.74 dd (10.0, 11.0) 4.36 dd (5.0,11.0)			3.81 dd (2.5, 11.2) 4.31 dd (2.5, 11.2)	3.73 dd (10.0, 11.3) 4.39 dd (5.0, 11.3)	3.79 dd (2.5, 11.0) 4.30 dd (2.5, 13.0)

to cimigenol and cimigol type structures. The structure of **3** is the same as that of 7,8-didehydro-24-*O*-acetylhydroshengmanol 3-xyloside, reported from *C. heracleifolia*,[14] but the reported data were very different from those of our compound **3**. Therefore, we regard **3** as a new compound and confirmed the structure by chemical conversion of the aglycone (**3a**) to the known compound (**5a**) as mentioned above.

Compound **5**, colorless needles, $C_{35}H_{54}O_9$, and compound **6**, colorless needles, $C_{35}H_{54}O_9$, gave D-xylose and L-arabinose on acid hydrolysis, respectively. Enzymatic hydrolysis of **5** with cellulase and **6** with lactase gave the same genuine aglycone (**5a**), which was identified as 7,8-didehydrocimigenol by direct comparison with an authentic specimen.[7] The ROE experiment, and the HMBC spectra of **5** and **6**, showed the presence of a 3-*O*-β-D-xylopyranosyl group and a 3-*O*-α-L-arabinopyranosyl gruop, respectively. Thus, the structures of **5** and **6** were respectively determined to be 7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside and 7,8-didehydrocimigenol 3-*O*-α-L-arabinopyranoside.

Compound **7**, colorless needles, $C_{37}H_{56}O_{10}$, and compound **8**, colorless powder, $C_{37}H_{56}O_{10}$, gave D-xylose and L-arabinose on acid hydrolysis, respectively. The aglycone was identified as 25-*O*-acetyl-7,8-didehydrocimigenol by comparison of the ^{1}H- and ^{13}C-NMR spectra with the reported data.[14] The ROE experiment, and the HMBC spectra of **7** and **8**, showed the presence of a 3-*O*-β-D-xylopyranosyl group and a 3-*O*-α-L-arabinopyranosyl group, respectively. Thus, the structures of **7** and **8** were respectively determined to be 25-*O*-acetyl-7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside, and 25-*O*-acetyl-7,8-didehydro cimigenol 3-*O*-α-L-arabinopyranoside.

Compound **9**, colorless powder, $C_{35}H_{56}O_{10}$, and compound **10**, colorless powder, $C_{36}H_{58}O_{11}$, gave L-arabinose and D-galactose on acid hydrolysis, respectively. The aglycone was identified as 1α-hydroxycimigenol by comparison of the ^{1}H- and ^{13}C-NMR spectra with the reported data.[4] The ROE experiment, and the HMBC spectra of **9** and **10**, showed the presence of a 3-*O*-α-L-arabinopyranosyl group and a 3-*O*-β-D-galactopyranosyl group respectively. Thus, the structures of **9** and **10** were respectively determined to be 1α-hydroxycimigenol 3-*O*-α-L-arabinopyranoside and 1α-hydroxycimigenol 3-*O*-β-D-galacto pyranoside.

Compound **11**, colorless powder, $C_{38}H_{58}O_{11}$, gave D-galactose on acid hydrolysis. The aglycone was identified as 23-*O*-acetyl-7,8-didehydroshengmanol by comparison of the ^{1}H- and ^{13}C-NMR spectra with the reported data.[7] The ROE ex-

Table 1. (Continued)

	7[a]	8[a]	9[a]	10[b]	11[b]	12[a]	12a[a]
1	1.36, 1.71	1.30, 1.76	3.80 brs	3.76 brs	1.28, 1.64	1.15, 1.60	1.14, 1.56
2	1.97, 2.34	1.95, 2.33	2.22, 2.70	2.20, 2.85	1.95, 2.44	1.88, 2.25	1.84 (2H)
3	3.49 dd (4.4, 11.9)	3.47 dd (4.4, 11.9)	4.30 dd (4.3, 11.8)	4.37	3.53 dd (4.0, 11.5)	3.43 dd (4.2, 11.5)	3.45 dd (4.4, 11.3)
5	1.28	1.25	2.43 dd (4.3, 12.5)	2.42 dd (4.3, 12.5)	1.28	1.18	1.16
6	1.59, 1.90	1.59, 1.90	0.86, 1.65	0.85, 1.65	1.60, 1.92	1.48, 1.80	1.58, 1.84
7	6.10 dd (1.3, 7.5)	6.10 dd (1.3, 7.5)	1.38, 2.13	1.39, 2.12	6.09 dd (1.5, 7.8)	5.11 dd (1.2, 7.5)	5.14 dd (1.8, 7.5)
8	—	—	1.75	1.74	—	—	—
11	1.15, 2.18	1.15, 2.18	1.40, 2.85	1.38, 2.83	1.20, 2.20	1.25 d (16.3) 2.94 dd (8.7,16.3)	1.25 d (15.6) 2.95 dd (8.7,15.6)
12	1.68, 1.82	1.63, 1.82	1.62, 1.78	1.60, 1.75	1.93 (2H)	5.23 d (8.7)	5.23 d (8.7)
15	4.53 s	4.55 s	4.28 s	4.29 s	4.56 s	2.08, 2.13	2.09, 2.15
16	—	—	—	—	-	4.33	4.34
17	1.49 d (10.0)	1.47 d (10.6)	1.50	1.46	2.35	1.78	1.82
18	1.15 s	1.16 s	1.20 s	1.19 s	1.29 s	1.49 s	1.49 s
19	0.51 d (4.0) 1.05 d (4.0)	0.55 d (4.0) 1.05 d (4.0)	0.43 d (4.0) 0.71 d (4.0)	0.38 d (4.0) 0.68 d (4.0)	0.52 d (4.0) 1.04 d (4.0)	0.52 d (4.0) 1.06 d (4.0)	0.54 d (4.0) 1.04 d (4.0)
20	1.68	1.65	1.70	1.68	2.14	2.25	2.25
21	0.89 d (6.5)	0.90 d (6.5)	0.86 d (6.5)	0.85 d (6.5)	1.24 d (6.5)	1.02 d (6.6)	1.02 d (6.3)
22	1.01, 2.29	1.00, 2.30	1.05, 2.25	1.02, 2.28	1.70 ddd (2.5, 10.5, 13.5) 2.87 ddd (2.0, 10.5, 13.5)	1.46, 1.60	1.50, 1.62
23	4.60 d (9.3)	4.63 d (9.0)	4.73 d (8.8)	4.74 d (8.8)	5.41 ddd (2.5, 8.5,10.5)	—	—
24	4.13 s	4.16 s	3.75 s	3.79 s	3.07 d (8.5)	3.65 s	3.68 s
26	1.67 s	1.69 s	1.45 s	1.47 s	1.29 s	3.62 d (10.2) 4.05 d (10.2)	3.63 d (10.2) 4.05 d (10.2)
27	1.66 s	1.68 s	1.48 s	1.50 s	1.43 s	1.47 s	1.47 s
28	1.42 s	1.44 s	1.28 s	1.29 s	1.45 s	1.05 s	1.06 s
29	1.30 s	1.28 s	1.34 s	1.38 s	1.35 s	1.30 s	1.18 s
30	1.06 s	1.03 s	1.08 s	1.08 s	1.05 s	0.99 s	1.05 s
$COCH_3$	1.96 s	1.99 s			2.05 s	2.17 s	2.18 s
1′	4.84 d (7.5)	4.80 d (7.5)	4.81 d (6.9)	4.89 d (7.8)	4.89 d (8.0)	4.83 d (7.6)	
2′	4.02 dd (7.5, 8.1)	4.46 dd (7.5, 8.8)	4.42 dd (6.9, 8.1)	4.45 dd (7.8, 9.5)	4.47	4.01 dd (7.6, 8.3)	
3′	4.13 dd (8.1, 8.1)	4.18 dd (3.1, 8.8)	4.11 dd (3.1, 8.1)	4.09 dd (3.5, 9.5)	4.18 dd (3.5, 9.5)	4.13 dd (8.3, 8.5)	
4′	4.21 ddd (5.0, 8.1, 10.0)	4.33	4.27	4.50 d (3.5)	4.60 d (3.5)	4.20 ddd (5.0, 8.5, 10.0)	
5′	3.72 dd (10.0, 11.0) 4.35 dd (5.0,11.0)	3.81 dd (2.5, 11.3) 4.32 dd (2.5, 11.3)	3.66 dd (2.5, 11.8) 4.20 dd (2.5, 11.8)	4.00 dd (6.0, 7.5)	4.12 dd (6.0, 7.0)	3.71 dd (10.0, 11.0) 4.33 dd (5.0,11.0)	
6’				4.38 4.38	4.47 4.47		

a) Obtained on a JEOL α-400, *b*) on a Varian Unity-INOVA-500 in pyridine-d_5. Underlined numbers show distinct signals due to the 24*S* and 24*R* isomers.

periment, and the HMBC spectrum of **11**, showed the presence of a 3-*O*-β-D-galactopyranosyl group. The structure of **11** was thus determined to be 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-β-D-galacto pyranoside.

Compound **12** was obtained as colorless needles, $C_{37}H_{54}O_{10}$. The ^{1}H- and ^{13}C-NMR spectra were similar to those of cimicifugoside,[9] except for a pair of doublets due to 26-2H (δ 3.63, d, J=10.2; δ 4.05, d, J=10.2) and 26-C (δ 68.18), instead of the signals due to the hemiacetal structure. Reduction of cimicifugoside with sodium borohydrate provided 26-deoxycimicifugoside, which was identified by direct comparison with the isolated compound. The genuine aglycone (**12a**), 26-deoxycimicifugenin, was obtained by enzymatic hydrolysis of **12** with cellulase.

Experimental

General The instruments used in this investigation were as follows: a Yanagimoto micromelting apparatus (for melting points, uncorrected); a JASCO DIP-1000 digital polarimeter (for specific rotation, measured at 25 °C); a Perkin–Elmer 1720X-FT IR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); and a Varian Gemini-200, a varian Mercury-300, a JEOLα-400 and a Varian Unity-INOVA-500 instrument (for NMR spectra, measured in pyridine-d_5 solution containing a few drops of D_2O, on the δ scale using tetramethylsilane as an internal standard). Column chromatography was carried out on silica-gel (Wakogel C-200, 75—150 μm) and ODS-A (YMC, 60—400/230 mesh) columns. HPLC was carried out using a Gilson 305 pump equipped with a JASCO 830-RI detector. Silica-gel 60 F_{254} (Merck) precoated TLC plates were used and detection was carried out by spraying with 40% H_2SO_4 followed by heating.

Isolation of 1—17 *Cimicifuga simplex* was grown at the Experimental Station for Medicinal Plant Studies, Faculty of Pharmaceutical Sciences, Tohoku University for seven years and the underground parts were dried at

Table 2. ^{13}C-NMR Data of Compound **1**—**12** and Their Aglycones

	1[a]	**2**[a]	**3**[a]	**3a**[a]	**3b**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]	**10**[b]	**11**[b]	**12**[a]	**12a**[a]
1	30.40	30.38	30.41	30.78	36.69	30.41	30.39	30.30	30.38	30.46	72.50	72.32	30.11	30.29	30.58
2	29.55	29.48	29.57	30.68	25.54	29.41	29.57	29.48	29.56	29.59	37.77	37.32	29.33	29.47	30.62
3	88.18	88.22	88.24	77.78	84.93	88.28	88.29	88.31	88.26	88.42	84.68	84.56	88.19	87.90	77.58
4	40.43	40.41	40.42	40.23	45.28	40.42	40.43	40.41	40.42	40.52	41.57	41.18	40.23	40.39	40.21
5	42.76	42.75	42.79	42.56	55.04	42.79	42.72	42.70	42.68	42.76	40.11	39.82	42.49	42.49	42.30
6	21.63	21.63	21.85	22.14	23.47	21.86	21.77	21.32	21.78	21.88	21.01	20.70	21.72	21.81	22.14
7	113.48	113.46	113.43	113.60	30.92	113.42	114.31	114.29	114.31	114.36	26.38	26.06	114.94	114.08	114.31
8	149.10	149.10	149.24	149.22	138.41	149.26	148.03	148.00	147.96	148.10	48.85	48.60	147.11	147.70	147.73
9	21.86	21.86	21.55	21.33	123.56	21.26	21.65	21.78	21.64	21.78	21.04	20.70	21.30	21.28	21.35
10	28.48	28.43	28.46	28.75	89.85	28.45	28.45	28.44	28.46	28.54	31.10	30.58	28.40	28.30	28.67
11	25.46	25.44	25.55	25.62	31.31	25.48	25.58	25.56	25.57	25.64	25.86	25.49	25.08	36.63	36.79
12	33.87	33.86	33.97	34.19	32.18	33.97	34.09	34.09	34.38	34.15	34.21	33.84	33.37	76.80	76.96
13	41.60	41.60	41.40	41.43	41.44	41.41	41.32	41.32	41.27	41.36	41.96	41.58	40.73	48.13	48.19
14	50.04	50.04	50.03	50.04	49.18	50.05	50.66	50.66	50.52	50.57	47.46	47.07	49.31	50.53	50.61
15	80.10	80.08	80.74	80.77	78.00	80.75	78.16	78.15	78.12	78.15	80.30	80.00	80.58	43.06	43.10
16	103.19	103.19	103.41	103.43	103.28	103.42	112.30	112.29	112.75	112.88	112.05	111.72	220.33	74.53	74.60
17	60.76	60.76	60.32	60.34	58.53	60.33	59.43	59.43	59.23	59.34	59.66	59.32	59.99	56.62	56.66
18	22.56	22.55	22.63	22.66	18.49	22.64	21.34	21.64	21.33	21.69	19.61	19.33	21.72	14.79	14.86
19	28.44	28.43	28.41	28.62	36.26	28.41	28.25	28.25	28.25	28.34	30.85	30.70	27.81	28.86	29.07
20	27.07	27.07	27.64	27.66	27.81	27.82	24.01	24.01	23.86	23.98	24.16	23.82	28.34	23.11	23.17
21	21.26	21.26	21.79	21.33	22.45	21.79	19.75	19.75	19.65	19.79	19.66	19.36	19.73	21.38	21.44
22	32.80	32.79	34.17	34.20	34.14	34.17	38.08	38.08	37.82	37.93	38.27	37.93	37.17	37.29	37.32
23	74.26	74.26	74.82	74.81	74.79	74.82	71.25	70.96	71.95	72.05	70.98	71.63	71.92	105.95	106.03
24	81.37	81.36	82.56	82.58	82.36	82.57	90.28	90.26	86.80	86.92	90.25	89.94	65.15	62.39	62.45
25	72.18	72.98	71.29	71.29	71.35	71.30	72.11	72.10	83.13	83.38	71.94	70.82	58.56	62.44	62.52
26	27.38	27.37	29.02	29.04	29.03	29.03	27.08	27.08	23.86	23.48	27.03	25.07	24.66	68.18	68.24
27	27.14	27.13	25.47	25.47	25.83	25.83	25.78	25.78	21.60	21.42	25.47	26.48	19.29	14.22	14.31
28	18.14	18.13	18.27	18.33	17.47	18.27	18.44	18.43	18.41	18.58	11.74	11.47	18.75	26.88	27.00
29	25.81	25.81	25.81	26.20	24.98	25.48	25.46	25.45	25.78	25.90	25.86	25.55	25.72	25.71	26.18
30	14.35	14.31	14.35	13.68	23.54	14.32	14.38	14.28	14.32	14.40	14.70	14.44	14.19	14.26	13.61
C<u>O</u>CH$_3$	170.38	170.37	171.32	171.32	171.17	171.33			170.13	170.47			170.67	170.69	170.85
CO<u>C</u>H$_3$	21.07	21.07	21.30	21.26	21.21	21.26			22.25	22.44			20.93	21.57	21.64
1′	107.46	107.31	107.44			107.31	107.46	107.31	107.46	107.40	107.39	107.20	107.25	107.42	
2′	75.56	72.17	75.57			72.98	75.56	72.96	75.56	72.88	72.81	72.74	72.89	75.58	
3′	78.59	74.63	78.56			74.62	78.56	74.60	78.57	74.56	74.49	74.96	75.18	78.59	
4′	71.27	69.50	71.26			69.50	71.00	69.48	71.25	69.48	69.29	69.89	69.95	71.24	
5′	67.12	66.73	67.10			66.70	67.10	66.69	67.10	66.78	66.45	76.26	76.57	67.10	
6′												62.13	62.12		

a) Measured at 100.4 MHz, *b*) at 125.7 MHz, in pyridine-d_5. Underlined numbers show distinct signals due to the 24*S* and 24*R* isomers.

60 °C in a drying room for several days. The powdered materials (200 g) were extracted three times with boiling MeOH (200 ml). After evaporation of the solvent, the extracts were dissolved in water (50 ml) and the mixture was extracted five times with EtOAc–*n*-BuOH (1 : 1, 100 ml). Fractions from the upper layer and water layer were treated as described in Fig. 1 and the compounds **1**—**17** were obtained. Preparative HPLC was carried out as follows: condition A (con. A): [column, Develosil PhA-5 (10.0 i.d.×250 mm); solvent, MeOH–H_2O–MeCN (10 : 7 : 3); elution rate, 2 ml/min; column temperature, 40 °C], condition B (con. B): [column, Cosmosil 5Ph (10.0 i.d.×250 mm); solvent, MeCN–H_2O (35 : 65); elution rate, 2 ml/min; column temperature, 40 °C], condition C (con. C): [column, Cosmosil 5Ph (4.6 i.d.×250 mm); solvent, MeOH–H_2O–MeCN (10 : 12 : 3); elution rate, 1 ml/min; column temperature, 40 °C], and condition D (con. D): [column, Develosil PhA-5 (10.0 i.d.×250 mm); solvent, MeOH–H_2O–MeCN (10 : 13 : 3); elution rate, 2 ml/min; column temperature, 40 °C].

1: (t_R 30′ in con. A, recrystallization from MeOH, colorless needles, 50.2 mg), mp 224—225 °C, $[\alpha]_D$ −25.5° (*c*=1.00, MeOH). Pos. SI-MS *m/z*: 661 $(M-OH)^+$. Pos. HR-SI-MS *m/z*: 661.3951 $(C_{37}H_{58}O_{11}-OH)^+$ which is specific to hydroshengmanol type compounds, error: 0.3 m.m.u. IR (KBr) cm^{-1}: 3200—3600 (OH), 1720 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

2: (t_R 28′ in con. A, recrystallization from MeOH, colorless needles, 5.0 mg), mp 221—222 °C, $[\alpha]_D$ −9.2° (*c*=0.50, MeOH). Pos. SI-MS *m/z*: 701 $(M+Na)^+$, 661 $(M-OH)^+$. Pos. HR-SI-MS *m/z*: 701.3846 $(C_{37}H_{58}O_{11}+Na)^+$, error: −2.8 m.m.u. IR (KBr) cm^{-1}: 3200—3600 (OH), 1720 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

3: (t_R 22′ in con. A, recrystallization from MeOH, colorless needles, 54.2 mg), mp 227—228 °C, $[\alpha]_D$ −29.7° (*c*=0.77, MeOH). Pos. SI-MS *m/z*: 678 $(M)^+$, 679 $(M+H)^+$, 661 $(M-OH)^+$. Pos. HR-SI-MS *m/z*: 678.3985 $(C_{37}H_{58}O_{11})^+$, error: 1.0 m.m.u. IR (KBr) cm^{-1}: 3250—3760 (OH), 1720 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

4: (t_R 19′ in con. A, recrystallization from MeOH, colorless powder, 100 mg), mp 172—173 °C, $[\alpha]_D$ −14.1° (*c*=0.51, MeOH). Pos. SI-MS *m/z*: 701 $(M+Na)^+$. Pos. HR-SI-MS *m/z*: 701.3876 $(C_{37}H_{58}O_{11}+Na)^+$, error: 0.3 m.m.u. IR (KBr) cm^{-1}: 3200—3700 (OH), 1723 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

5: (t_R 27′ in con. A, recrystallization from MeOH, colorless needles, 28.7 mg), mp 291—292 °C, $[\alpha]_D$ −14.8° (*c*=0.88, MeOH). Pos. SI-MS *m/z*: 619 $(M+H)^+$. Pos. HR-SI-MS *m/z*: 619.3837 $(C_{35}H_{54}O_9+H)^+$, error: −0.6 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

6: (t_R 24′ in con. A, recrystallization from a mixture of MeOH and $CHCl_3$, colorless needles, 52.4 mg), mp 272—273 °C, $[\alpha]_D$ −3.64° (*c*=1.17, MeOH). Pos. SI-MS *m/z*: 619 $(M+H)^+$. Pos. HR-SI-MS *m/z*: 619.3842 $(C_{35}H_{54}O_9+H)^+$, error: −0.1 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

7: (t_R 48′ in con. A, recrystallization from MeOH, colorless needles, 10.9 mg), mp 255—256 °C, $[\alpha]_D$ −14.9° (*c*=0.74, MeOH). Pos. SI-MS *m/z*: 661 $(M+H)^+$. Pos. HR-SI-MS *m/z*: 661.3976 $(C_{37}H_{56}O_{10}+H)^+$, error: 2.8 m.m.u. IR (KBr) cm^{-1}: 3250—3650 (OH), 1738 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

8: (t_R 46′ in con. A, recrystallization from MeOH, colorless powder, 8.7 mg), mp 167—168 °C, $[\alpha]_D$ −4.7° (*c*=0.70, MeOH). Pos. SI-MS *m/z*: 661 $(M+H)^+$. Pos. HR-SI-MS *m/z*: 661.3942 $(C_{37}H_{56}O_{10}+H)^+$, error: −0.7 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 1738 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

9: (t_R 15′40″ in con. A, recrystallization from a mixture of MeOH and isopropylether, colorless powder, 15.0 mg), mp 186—187 °C, $[\alpha]_D$ +33.6° (c= 0.98, MeOH). Pos. SI-MS m/z: 659 $(M+Na)^+$, 619 $(M-OH)^+$. Pos. HR-SI-MS m/z: 659.3749 ($C_{35}H_{56}O_{10}$+Na)$^+$, error: −1.9 m.m.u. IR (KBr) cm^{-1}: 3250—3650 (OH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

10: (t_R 13′ in con. B, recrystallization from a mixture of MeOH and isopropylether, colorless powder, 4.7 mg), mp 185—186 °C, $[\alpha]_D$ +25.7° (c= 0.47, MeOH). Pos. SI-MS m/z: 689 $(M+Na)^+$. Pos. HR-SI-MS m/z: 689.3885 ($C_{36}H_{58}O_{11}$+Na)$^+$, error: 1.1 m.m.u. IR (KBr) cm^{-1}: 3250—3650 (OH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

11: (t_R 21′ in con. B, recrystallization from a mixture of MeOH and MeCN, colorless powder, 4.7 mg), mp 170—171 °C, $[\alpha]_D$ −44.1° (c=0.47, MeOH). Pos. SI-MS m/z: 691 $(M+H)^+$. Pos. HR-SI-MS m/z: 691.4043 ($C_{38}H_{58}O_{11}$+H)$^+$, error: −1.1 m.m.u. IR (KBr) cm^{-1}: 3250—3650 (OH), 1735 (AcO, cyclopentanone). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

12: (t_R 18′ in con. A, recrystallization from MeOH, colorless needles, 8.3 mg), mp 285—286 °C, $[\alpha]_D$ −121.0° (c=0.57, MeOH). Pos. SI-MS m/z: 659 $(M+H)^+$. Pos. HR-SI-MS m/z: 659.3796 ($C_{37}H_{54}O_{10}$+H)$^+$, error: 0.4 m.m.u. IR (KBr) cm^{-1}: 3250—3600 (OH), 1732 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2. **12** (30.2 mg) was also isolated by similar treatment of the underground parts of *A. asiatica* (60 g).

13, (t_R 24′30″ in con. A, 6.1 mg), was identified by comparison with the reported values.[14] **14** (t_R 82′ in con. D, 4.0 mg), **15** (t_R 86′ in con. D, 5.0 mg), **16** (t_R 45′ in con. C, 11.3 mg), and **17** (t_R 50′ in con. C, 10.0 mg) were identified by direct comparison with authentic specimens.[7]

Hydrolysis of 1, 3, 4, 5, 6 and 12 with Enzymes **1** (25.0 mg) was dissolved in MeOH (2 ml), and 0.03% AcOH (100 ml) was added with stirring. Cellulase T [Amano] 4 (from *Trichoderma viride*, 300 mg) was added to the solution with stirring for 1 d at room temperature. The reaction solution was then shaken with EtOAc (100 ml×3) and, after washing the combined 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 $CHCl_3$–MeOH (19 : 1) to afford **1a** (9.0 mg) as a colorless powder and **1b** (1.2 mg) as a colorless powder after purification by HPLC [column, Develosil PHA-5 (10.0 i.d.×250 mm); solvent, MeOH–H_2O–MeCN (10 : 9 : 3); elution rate, 2 ml/min; column temperature, 40 °C] and recrystallization from MeOH. **1a** was identified as 24-*epi*-24-*O*-acetyl-7,8-didehydrohydro shengmanol by direct comparison with an authentic specimen[7] and **1b** as heracleifolinol by comparison with the reported data.[14]

Similar treatment of **3** (33.2 mg) with Cellulase T [Amano] 4 as in the case of **1**, and **4** (41.5 mg) with Lactase F [Amano] (from *Aspergillus orizae)*, both gave **3a** (6.0, and 10.7 mg, respectively) as a colorless powder and **3b** (1.3 and 2.3 mg, respectively) as a colorless powder after purification by HPLC and recrystallization from MeOH. **3a**: mp 125—126 °C, $[\alpha]_D$ −23.7° (c=0.56, MeOH). Pos. HR-SI-MS m/z: 546.3554 $[C_{32}H_{50}O_7]^+$, error: 0.0 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 1720 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2. **3b**: mp 122—123 °C, $[\alpha]_D$ +22.2° (c=0.36, MeOH). Pos. HR-EI-MS m/z: 546.3543 $[C_{32}H_{50}O_7]^+$, error: −1.1 m.m.u., IR (KBr) cm^{-1}: 3200—3600 (OH), 1720 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Similar treatment of **5** (15.3 mg) with Cellulase T [Amano] 4, and **6** (20.9 mg) with Lactase F [Amano], both gave the same aglycone, **5a** (3.2, and 3.3 mg), which was identified as 7,8-didehydrocimigenol by direct comparison with an authentic specimen.[7]

Similar treatment of **12** (22.5 mg) with Cellulase T [Amano] 4 gave **12a** (13.1 mg) as a colorless powder by recrystallization from MeOH. **12a**: mp >300 °C, $[\alpha]_D$ −135.5° (c=0.73, MeOH). Pos. SI-MS m/z: 527 $(M+H)^+$. Pos. HR-SI-MS m/z: 527.3352 ($C_{32}H_{46}O_6$+H)$^+$, error: −1.8 m.m.u. IR (KBr) cm^{-1}: 3503 (OH), 1732 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Conversion of 3a to 5a **3a** (5.3 mg) was dissolved in MeOH (1 ml), and 2% Na_2CO_3 (1 ml) was added and the solution stirred for 2 h at room temperature. The solution was neutralized with 5% AcOH, and shaken with EtOAc (20 ml×3). The residue after removal of the solvent was dissolved in dioxane (1 ml) and 5% AcOH (1 ml), and stirred for 16 h at room temperature. After evaporation of the solvent *in vacuo*, the products were purified by recrystallization from MeOH to give **5a** (4.3 mg), which was identified as 7,8-didehydrocimigenol by direct comparison with an authentic specimen.[7]

Sugar Analysis of 1—11 **1** (7.0 mg), **2** (1.5 mg), **3** (5.3 mg), **4** (5.0 mg), **5** (4.0 mg), **6** (11.6 mg), **7** (3.6 mg), **8** (3.4 mg), **9** (3.0 mg), **10** (1.5 mg), or **11** (1.5 mg) was dissolved in dioxane (0.5 ml), 3% HCl (1 ml) was added, and the solution was refluxed for 2 h. The reaction solution was diluted with water and extracted with EtOAc (20 ml×3). The water layer was passed through an Amberlite IR-35 column. The eluate was concentrated *in vacuo* and analyzed by TLC [*n*-PrOH–H_2O (85 : 15); D-xylose, *Rf* 0.59; L-arabinose, *Rf* 0.51; D-galactose, *Rf* 0.39], and HPLC with a chiral detector OR-I; [column, Shodex NH2P-50 (4.6 i.d.×250 mm); solvent, MeCN–H_2O (80 : 20); elution rate, 1 ml/min; column temperature, 45 °C; L-(+)-arabinose, t_R 4.60′; D-(+)-xylose, t_R 5.00′; D-(+)-galactose, t_R 7.20′]. D-(+)-Xylose was detected from **1**, **3**, **5**, **7**, L-(+)-arabinose from **2**, **4**, **6**, **8**, **9**, and D-(+)-galactose from **10**, **11**.

Conversion of Cimicifugoside to 26-Deoxycimicifugoside (12) Cimicifugoside (54 mg) was dissolved in MeOH (50 ml), and $NaBH_4$ (80 mg) was added and the solution was stirred for 13 h at room temperature. The solvent was evaporated *in vacuo* and the residue was shaken with EtOAc–*n*-BuOH–H_2O (50 : 10 : 50) (110 ml×3).The residue from the upper layer was chromatographed on a SiO_2 column (18 g), and the eluate with $CHCl_3$–MeOH (9 : 1) was subjected to HPLC [column, Cosmosil 10Ph (4.6 i.d.×250 mm); solvent, MeOH–H_2O–MeCN (10 : 10 : 3); elution rate, 1 ml/min; column temperature, 40 °C] to provide 26-deoxycimicifugoside, which was identified by direct comparison with the isolated compound (**12**).

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