Studies on the Constituents of *Cimicifuga* Species. XXVII.[1] Malonyl Cyclolanostanol Glycosides from the Underground Parts of *Cimicifuga simplex* Wormsk.

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Five malonyl cyclolanostanol glycosides, 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-(2′-*O*-malonyl)-β-D-xylopyranoside, 23-*O*-acetylshengmanol 3-*O*-(2′-*O*-malonyl)-β-D-xylopyranoside, 2′-*O*-malonylcimicifugoside, 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-(2′-*O*-malonyl)-β-D-xylopyranoside, and 2′-*O*-malonylcimiaceroside B, were isolated from the underground parts of *Cimicifuga simplex* Wormsk. (Ranunculaceae), together with two related cyclolanostanol glycosides, 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-β-D-xylopyranoside, 24-*O*-acetyl-25-*O*-methyl-7,8-didehydrohydroshengmanol 3-*O*-β-D-xylopyranoside. Their structures were elucidated on the basis of spectral and chemical evidence.

Key words *Cimicifuga simplex*; Ranunculaceae; malonate; cyclolanostanol; glycoside

Cimicifugae Rhizoma, rhizomes of *Cimicifuga* (*C.*) *simplex* Wormskjord, *C. dahurica* (Turcz) Maximowicz, *C. foetida* Linne and *C. heracleifolia* Komarov (Ranunculaceae) (The Pharmacopoeia of Japan, 13th ed. supplementary), have been used as anti-inflammatory, analgesic, and antipyretic agents in traditional Chinese medicine. During a series of chemical investigations of *C.* species, we isolated cyclolanostanol glycosides, fukiic acid esters, piscidic acid esters, caffeic acid derivatives, phenolic acid derivatives and chromones.[1] In continuing our work, we have now isolated five malonyl cyclolanostanol glycosides (**1**—**5**), and two related cyclolanostanol glycosides (**6**, **7**) from the underground parts of *C. simplex*. These malonyl cyclolanostanol glycosides were isolated for the first time from *C.* species and are the first examples of 2'-malonylxyloside, which are unstable and convert to their corresponding glycosides. This paper deals with the isolation and structural elucidation of these malonyl glycosides and the related glycosides .

The underground parts of *C. simplex* were extracted with MeOH at room temperature and the extract was subjected on octadecyl silica (ODS) chromatography and preparative (p) TLC. Malonyl compounds (**1**—**5**) were obtained from the lower zone and the related glycosides (**6**, **7**) from the higher zone of p-TLC followed by p-HPLC as described in the Experimental. The ^{1}H- and ^{13}C-NMR signals were assigned by using ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser enhancement and exchange spectroscopy (NOESY).

Compound **1** showed a broad hydroxyl band (3200—3650 cm^{-1}), a carbonyl band (1735 cm^{-1}) and carboxyl bands (2400—3000, 1735 cm^{-1}) in the IR spectrum. In the secondary ion mass spectrometry (SI-MS), it showed a $(M+Na)^+$ peak at *m/z*: 769.3762 ($C_{40}H_{58}O_{13}+Na)^+$ [positive high resolution (pos. HR) SI-MS], a malonic acid (MA) ion peak at *m/z*: 105 ($C_3H_4O_4+H)^+$ (pos. SI-MS) and at *m/z*: 103 $(C_3H_3O_4)^-$ [negative (neg.) SI-MS] , and a $(M-87)^-$ peak at *m/z*: 659 (neg. SI-MS) due to the loss of malonate. The ^{13}C-NMR of **1** showed the signals of a malonyl group at δ 167.23, 169.53, and 42.40 (COOR, COOH, $-CH_2-$). Hydrolysis of **1** by 1% Na_2CO_3 gave the corresponding glycoside (**6**), and treatment of **1** with diazomethane gave a methyl ester (**1a**) and a glycoside (**6**). Compound **1** was also converted into **6** by dissolving in MeOH and allowing it to stand at room temperature. Thus, compound **1** was suggested to be a malonate of **6**.

Compound **6**, a colorless powder, showed a $(M+H)^+$ peak at *m/z*: 661.3947 ($C_{37}H_{56}O_{10}+H)^+$ (pos. HR-SI-MS). The ^{1}H- and ^{13}C-NMR spectra were similar to those of 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-α-L-arabinopyranoside,[2] except for the sugar moiety. On enzymatic hydrolysis, the genuine aglycone was obtained, and was identified as 23-*O*-acetyl-7,8-didehydroshengmanol by direct comparison with an authentic specimen.[2] On acid hydrolysis, D-xylose was detected as the sugar. The coupling constant (J=7.5 Hz) of 1′-H, the NOE effect between 1′-H and 3-H, and the HMBC correlations between 1′-H and 3-C, and 1′-C and 3-H, showed the presence of a 3-*O*-β-D-xylopyranosyl group. Thus, the structure of **6** was determined to be 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-β-D-xylopyranoside.

Acylation shifts[3—6] of 2′-H (Δ 1.55 ppm), 1′-C (Δ −3.17 ppm), 2′-C (Δ 1.22 ppm), and 3′-C (Δ −2.08 ppm) between **1** and **6**, and the HMBC correlation between 2′-H and 1″-C of **1a**, suggested that the malonyl group was attached to the 2′-*O* of the D-xylopyranosyl group. Thus, the structure of **1** was determined to be 23-*O*-acetyl-7,8-didehydroshengmanol 3-*O*-(2′-*O*-malonanyl)-β-D-xylopyranoside.

Compound **2** showed a similar IR spectrum as that of **1**, a broad hydroxyl band (3200—3650 cm^{-1}), a carbonyl band (1738 cm^{-1}) and carboxyl bands (2400—3000, 1738 cm^{-1}), and a $(M+H)^+$ peak at *m/z*: 749.4123 ($C_{40}H_{60}O_{13}+H)^+$ (pos. HR-SI-MS), which was 2 mass units greater than that of **1**, and an MA ion peak at *m/z*: 105 ($C_3H_4O_4+H)^+$ (pos. HR-SI-MS), at *m/z*: 103 $(C_3H_3O_4)^-$ (neg. SI-MS), and a $(M-87)^-$ peak at *m/z*: 661 (neg. SI-MS) due to the loss of malonate. The ^{13}C-NMR of **2** showed the signals of a malonyl group at δ 168.11, 169.50, and 42.40 (COOR, COOH, $-CH_2-$) as in **1**. Treatment of **2** with diazomethane gave a methyl ester (**2a**) and 23-*O*-acetylshengmanol 3-*O*-β-D-xylopyranoside (**2b**).[7] Compound **2** was also converted into **2b** by dissolving in MeOH and allowing it to stand at room temperature. Thus, compound **2** was suggested to be a malonate of **2b**. Acylation

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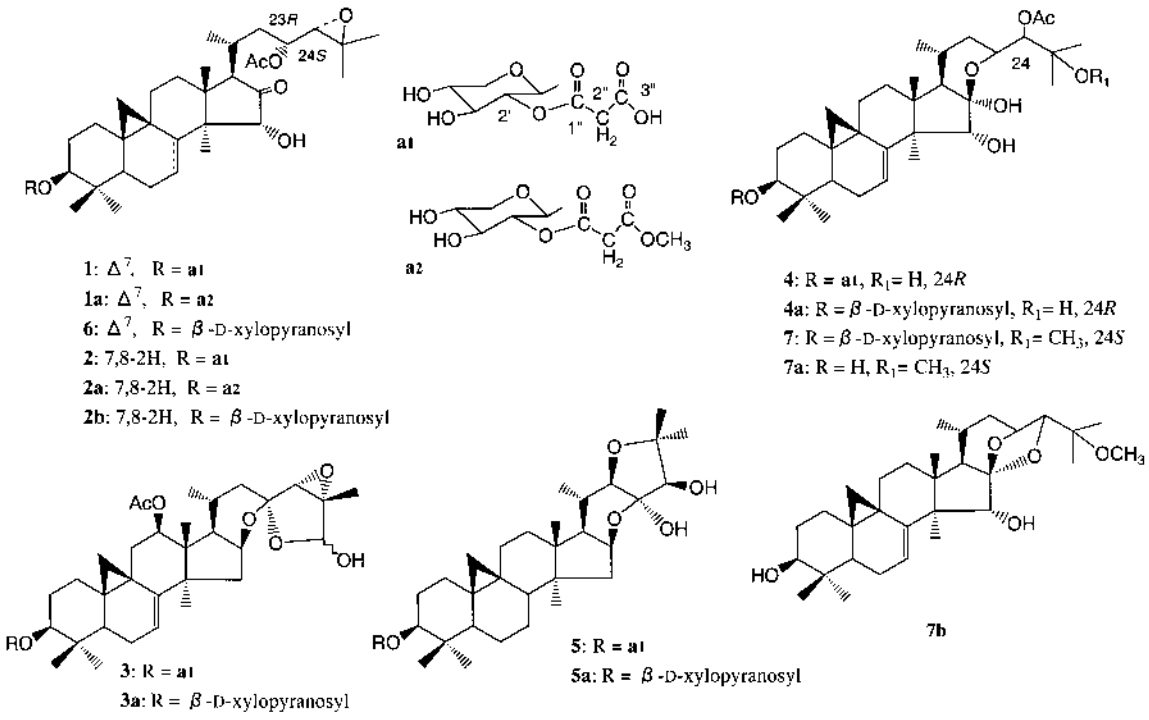

Fig. 1. Structures of Compounds **1**—**7** and Their Derivatives

shifts of 2′-H, 1′-C, 2′-C, 3′-C between **2** and **2b** and the HMBC correlation between 2′-H and 1″-C of **2a**, suggested that the malonyl group was attached to the 2′-*O* of the D-xylopyranosyl group. Thus, the structure of **2** was determined to be 23-*O*-acetylshengmanol 3-*O*-(2′-*O*-malonyl)-*β*-D-xylopyranoside.

Compounds **3**, **4**, and **5** showed carboxyl bands (2400—3000, 1737/1742 cm^{-1}) in their IR spectra, respectively, and their $(M+Na)^+$ peaks at *m/z*: 783.3558 ($C_{40}H_{56}O_{14}+Na)^+$, *m/z*: 787.3876 ($C_{40}H_{60}O_{14}+Na)^+$, *m/z*: 729.3811 ($C_{38}H_{58}O_{12}+Na)^+$ along with the common MA ion peak at *m/z*: 105 ($C_3H_4O_4+H)^+$, in pos. SI-MS, respectively. Their ^1H- and ^{13}C-NMR spectra suggested that they were malonates of cimicifugoside (**3a**),[8,9] 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-*β*-D-xylopyranoside (**4a**),[2] cimiaceroside B (**5a**),[10] respectively. (Tables 1 and 2). Treatment of **3**, **4**, and **5** with diazomethane gave **3a**, **4a**, and **5a** along with traces of the corresponding methyl esters. They were also converted into **3a**, **4a**, and **5a** by dissolving in MeOH and allowing them to stand at room temperature. Acylation shifts of 2′-H, 1′-C, 2′-C, 3′-C between **3** and **3a**, **4** and **4a**, and **5** and **5a**, suggested that the malonyl groups were attached to the 2′-*O* of the D-xylopyranosyl groups. Thus, the structures of **3**, **4**, and **5** were determined to be 2′-*O*-malonylcimicifugoside, 24-*epi*-24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-(2′-*O*-malonyl)-*β*-D-xylopyranoside, and 2′-*O*-malonylcimiaceroside B.

Compound **7**, a colorless powder, showed a $(M+H)^+$ peak at *m/z*: 693.4214 ($C_{38}H_{60}O_{11}+H)^+$ (pos. HR-SI-MS). The ^1H- and ^{13}C-NMR spectra were similar to those of 24-*O*-acetyl-7,8-didehydrohydroshengmanol 3-*O*-*β*-D-xylopyranoside[1] except for the 25-*O*-methyl moiety (OCH_3, 26-H, 27-H, 24-C, 25-C, 26-C, 27-C). On enzymatic hydrolysis, the genuine aglycone (**7a**) was obtained as a colorless powder, and showed a $(M)^+$ peak at *m/z*: 560.3708 ($C_{33}H_{52}O_7)^+$ (pos. HR-EI-MS). Treatment of **7a** with 1% Na_2CO_3 followed by acidification with 2.5% CH_3COOH gave 25-*O*-methyl-7,8-didehydrocimigenol (**7b**), as colorless needles. The spectral data were reasonably assigned to the structure (**7b**). The conversion of **7a** to **7b**, suggested that **7a** was 24-*O*-acetyl-25-*O*-methyl-7,8-didehydrohydroshengmanol.[1] On acid hydrolysis, D-xylose was detected as the sugar. The coupling constant (J=7.8 Hz) of 1′-H, the NOE effect between 1′-H and 3-H, and the HMBC correlations between 1′-H and 3-C, and 1′-C and 3-H, showed the presence of a 3-*O*-*β*-D-xylopyranosyl group. Thus, the structure of **7** was determined to be 24-*O*-acetyl-25-*O*-methyl-7,8-didehydrohydroshengmanol 3-*O*-*β*-D-xylopyranoside.

Compounds **6** and **7** were also obtained by dissolving **1** in MeOH and allowing it to stand at room temperature. We propose that the malonyl group of **1** is sufficiently acidic to hydrolyze the malonylate and produce **6**, followed by cleavage of the 24,25-epoxy ring with the introduction of a methoxy group to 25-C, rearrangement of the acetyl group of 23-*O*, and formation of a hemiacetal to produce **7**.

Recently several 6′-malonylglucosides of ginsenosides, isoflavones, anthocyanins, flavonols, flavones, and chalcones, which were hydrolyzed easily to the demalonyl glucosides, have been reported from ginseng radix,[11] red clover[12] and soybeen seeds,[6] *Jasonia*,[13] leaves and fruits of pears,[3] barren sprouts,[5] *Dahlia*,[4] and so on.[14,15] The isolation of these unstable compounds was carried out by improved isolation techniques, especially HPLC using reversed phase columns. At this time, five new 2′-malonylxylosides have been isolated from *C. simplex* and they are supposed to be transportation forms of glycosides in the plant bodies similarly to other 6′-malonylglycosides.

Table 1. ^{1}H-NMR Data of Compounds **1**—**7**, **7a**, and **7b**

	1	**2**	**3**[a)]	**4**	**5**	**6**	**7**	**7a**	**7b**
1	1.25, 1.70	1.22, 1.58	1.14, 1.54	1.34, 1.64	1.20, 1.50	1.39, 1.70	1.35, 1.70	1.35, 1.70	1.25, 1.65
2	1.90, 2.25	1.90, 2.25	1.82, 2.14	1.91, 2.22	1.86, 2.22	2.00, 2.35	1.93, 2.33	1.93, 2.00	1.95, 199
3	3.41 dd (4.0, 11.5)	3.42 dd (4.5, 11.5)	3.35 dd (4.0, 11.5)	3.39 dd (4.0, 11.5)	3.39 dd (4.3, 11.7)	3.60 dd (4.0, 11.5)	3.49 dd (4.0, 11.5)	3.54 dd (4.2, 11.5)	3.53 dd (4.3, 11.2)
5	1.30	1.35	1.16	1.26	1.28	1.32	1.28	1.27	1.25
6	1.60, 1.93	0.75, 1.57	1.54, 1.80	1.55, 1.88	0.68, 1.50	1.59, 1.95	1.55, 1.85	1.60, 1.88	1.65, 1.93
7	6.08 dd (1.5, 7.0)	1.25, 2.08	5.04 dd (1.5, 7.5)	5.98 dd (1.5, 7.0)	1.00, 1.27	6.10 dd (1.8, 7.5)	6.00 dd (1.5, 7.5)	6.03 dd (1.5, 7.5)	6.12 dd (1.8, 7.5)
8	—	1.83	—	—	1.50	—	—	—	—
11	1.20, 2.20	1.18, 2.10	1.22 2.91 dd (8.5, 16.0)	1.16, 2.15	1.02, 1.90	1.23, 2.22	1.18, 2.20	1.20, 2.20	1.20 2.20 ddd (6.5, 12.0, 14.0)
12	1.95 (2H)	1.80 (2H)	5.19 d (8.5)	1.66, 1.82	1.58 (2H)	1.95 (2H)	1.68, 1.84	1.70, 1.85	1.68, 1.80
15	4.56 s	4.36 s	1.90, 2.00	4.44 s	1.62. 1.88	4.58 s	4.43 s	4.45 s	4.54 s
16	—	—	4.70	—	4.97 ddd (8.0, 8.0, 8.0)	—	—	—	—
17	2.30 d (8.0)	2.34 d (6.5)	1.78	1.80	1.60	2.31 d (8.0)	1.79	1.80	1.46 d (11.0)
18	1.29 s	1.36 s	1.38 s	1.24 s	1.20 s	1.30 s	1.26 s	1.29 s	1.17 s
19	0.50 d (4.0) 1.01 d (4.0)	0.28 d (4.0) 0.53 d (4.0)	0.51 d (4.0) 1.10 d (4.0)	0.46 d (4.0) 1.02 d (4.0)	0.16 d (4.0) 0.42 d (4.0)	0.56 d (4.0) 1.07 d (4.0)	0.51 d (4.0) 1.07 d (4.0)	0.55 d (4.0) 1.07 d (4.0)	0.55 d (4.0) 1.09 d (4.0)
20	2.14	2.12	1.86	1.78	2.26	2.15	1.78	1.78	1.68
21	1.24 d (6.5)	1.26 d (6.5)	0.96 d (6.5)	1.01 d (6.5)	1.22 d (6.4)	1.24 d (6.6)	1.05 d (6.3)	1.06 d (6.6)	0.90 d (6.6)
22	1.72, 2.87	1.75, 2.65	1.68, 2.20	1.84, 2.06	3.90 d (10.7)	1.75 2.88 ddd (1.8, 10.5, 13.5)	1.96, 2.04	1.93, 2.05	1.20 ddd (1.5, 11.0, 13.0) 2.28 ddd (6.5, 9.2, 13.0)
23	5.41 ddd (2.5, 8.5, 11.0)	5.38 ddd (2.5, 8.0, 11.0)		4.45 ddd (6.0, 8.0, 11.0)	—	5.42 ddd (2.5, 8.5, 10.5)	4.22	4.23 ddd (6.0, 7.0, 11.5)	4.46 br d (9.2)
24	3.06 d (8.5)	3.03d (8.5)	3.94 s	5.75 d (8.1)	4.20 s	3.07 d (8.5)	5.66 d (8.0)	5.64 d (8.0)	3.69 s
26	1.28 s	1.27 s	5.74 s	1.48 s	1.76 s	1.30 s	1.27 s	1.27 s	1.28 s
27	1.42 s	1.41 s	1.79 s	1.52 s	1.67 s	1.43 s	1.21 s	1.22 s	1.27 s
28	1.43 s	1.20 s	1.00 s	1.42 s	0.85 s	1.45 s	1.46 s	1.47 s	1.44 s
29	1.19 s	1.17 s	1.16 s	1.15 s	1.18 s	1.36 s	1.31 s	1.18 s	1.19 s
30	1.06 s	1.03 s	0.99 s	1.02 s	1.00 s	1.09 s	1.04 s	1.09 s	1.11 s
$COCH_3$	2.05 s	2.07 s	2.21 s	2.15 s		2.06 s	2.02 s	2.09 s	—
OCH_3							3.24 s	3.25 s	3.22 s
Sugar									
1′	4.87 d (8.0)	4.87 d (8.0)	4.84 d (8.0)	4.86 d (8.0)	4.86 d (7.8)	4.87 d (7.5)	4.84 d (7.8)		
2′	5.61 dd (8.0, 8.5)	5.61 dd (8.0, 8.5)	5.59 dd (8.0, 8.5)	5.59 dd (8.0, 8.5)	5.59 dd (7.8, 8.5)	4.06 dd (7.5, 8.8)	4.03 dd (7.8, 8.0)		
3′	4.21	4.20	4.19	4.20	4.20	4.20 dd (8.8, 8.8)	4.17 dd (8.0, 8.0)		
4′	4.22	4.22	4.20	4.22	4.21	4.26 ddd (5.0, 8.8, 10.0)	4.22 ddd (5.0, 8.0, 10.0)		
5′	3.73 dd (10.0, 11.0)	3.72 dd (10.0, 11.0)	3.68 dd (10.0, 11.0)	3.72	3.70	3.78 dd (10.0, 11.0)	3.75 dd (10.0, 11.0)		
	4.34 dd (5.0, 11.0)	4.32 dd (5.0, 11.0)	4.31 dd (5.0, 11.0)	4.33 dd (5.0, 11.5)	4.31 dd (5.0, 11.0)	4.39 dd (5.0, 11.0)	4.37 dd (5.0,11.0)		
Malonyl									
2″	3.89 (2H)	3.89 (2H)	3.86 (2H)	3.90 (2H)	3.89 (2H)				

Obtained on a Varian Unity-INOVA-500 in pyridine-d_5. *a*) signals due to the major compound (26*S*) of the 26-isomers of **3**.

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 Mercury-300, 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—7 *Cimicifuga simplex* was collected in Miyagi prefecture in Japan. The underground parts were dried at 60 °C in the drying room for several days. The powdered material (520 g) was extracted three times with MeOH (2000 ml) at room temperature. The extract was partitioned between EtOAc–*n*-BuOH and water (1 : 1 : 1). After evaporation of the former phase, the residue was chromatographed on ODS column using by MeOH–H_2O (2 : 1—5 : 0) to afford fractions (fr.) 1 to 9. Frs. 2 and 3 [eluted with MeOH–H_2O (2 : 1)], and fr. 4 [eluted with MeOH–H_2O (3 : 1)] were subjected to p-TLC [Whatman, PLK5F Silica Gel 150A; developing solvent: $CHCl_3$–MeOH (2 : 1)] , respectively. Compounds **1** (30.7 mg), **2** (34.7 mg), and **3** (16.4 mg) were obtained from the lower zone (*Rf* 0.05—0.1) of p-TLC of fr. 2, and compounds **4** (28.6 mg) and **5** (16.4 mg) from the the lower zone (*Rf* 0.05—0.1) of frs. 3 and 4 followed by p-HPLC [column: Cosmosil 5Ph, i.d. 10×250 mm; mobile phase CH_3CN–1% CH_3COOH (40 : 60/35 : 65); flow rate: 2.0 ml/min; column temperature; 40 °C], respectively. Compounds **6** (39.0 mg) and **7** (23.0 mg) were obtained from the higher zone (*Rf* 0.5—0.7) of p-TLC of Fr. 2 followed by p-HPLC [same conditions as above except for mobile phase CH_3CN–H_2O (40 : 60/35 : 65)].

Table 2. ^{13}C-NMR Data of Compounds **1**—**7**, **7a**, and **7b**

	1	**2**	**3**[a)]	**4**	**5**	**6**	**7**	**7a**	**7b**
1	29.95	32.06	30.02	30.08	32.06	30.30	30.33	30.37	30.51
2	29.22	29.93	29.20	29.19	29.84	29.34	29.53	30.30	30.51
3	88.23	88.60	87.96	88.25	88.73	88.01	88.20	77.45	77.60
4	40.07	40.98	40.07	40.01	41.05	40.23	40.36	39.92	40.11
5	42.40	47.31	42.25	42.41	47.42	42.42	42.67	42.21	42.32
6	21.77	20.98	21.72	21.65	20.97	21.66	21.76	21.82	21.95
7	114.94	26.65	113.87	113.31	26.42	114.87	113.38	113.34	114.29
8	147.14	48.24	147.65	150.02	47.58	147.04	149.22	148.77	147.87
9	21.42	20.07	21.20	21.10	19.78	21.29	21.22	21.04	21.22
10	28.41	26.65	28.18	28.20	26.53	28.34	28.32	28.42	28.63
11	25.13	25.90	36.57	25.29	26.26	25.02	25.49	25.33	25.51
12	33.45	32.98	76.44	33.71	33.49	33.30	33.93	33.74	33.98
13	40.77	41.50	47.99	41.46	46.87	40.66	41.35	41.17	41.18
14	49.38	46.02	50.52	49.84	45.29	49.24	49.89	49.67	50.41
15	80.70	82.80	42.34	79.84	43.37	80.52	80.34	80.12	77.93
16	220.37	220.78	73.14	102.97	72.44	220.31	103.23	103.04	112.18
17	60.04	59.92	56.79	60.56	52.39	59.91	60.20	59.98	59.16
18	21.77	19.80	14.77	22.45	20.72	21.66	21.38	21.18	21.60
19	27.77	30.41	28.66	28.20	30.21	27.75	28.35	28.29	28.28
20	28.41	27.90	25.77	26.92	34.74	28.28	27.49	27.28	23.86
21	19.79	20.33	20.96	21.52	17.53	19.64	21.63	21.42	19.67
22	37.24	36.91	37.31	32.66	86.90	37.10	33.50	33.29	37.93
23	71.96	72.08	105.86	74.02	105.95	71.87	74.38	74.16	71.74
24	65.21	65.14	63.36	81.26	83.10	65.10	79.54	79.32	88.11
25	58.58	58.58	65.60	72.00	83.65	58.53	76.26	76.08	76.26
26	24.72	24.69	98.29	27.12	27.80	24.59	22.58	22.39	19.35
27	19.35	19.33	13.07	27.06	24.86	19.22	23.27	23.06	21.99
28	18.80	11.95	26.75	18.06	19.53	18.69	18.23	18.06	18.39
29	25.59	25.54	25.56	25.53	25.69	25.59	25.75	25.94	26.08
30	14.08	15.26	14.01	14.04	15.31	14.15	14.30	13.45	13.58
$\underline{C}OCH_3$	170.68	170.67	170.80	170.47		170.66	171.25	171.22	
$CO\underline{C}H_3$	20.99	20.98	21.64	21.05		20.88	21.22	21.02	
OCH_3							49.39	49.20	49.17
Sugar									
1′	104.08	104.20	104.05	104.01	104.25	107.25	107.39		
2′	76.43	76.12	76.76	76.37	76.53	75.21	75.36		
3′	76.09	75.37	76.04	76.06	76.21	78.17	78.34		
4′	71.00	70.98	70.98	70.86	71.00	70.90	71.07		
5′	67.02	67.00	66.99	66.96	67.09	66.86	67.01		
Malonyl									
1″	167.23	168.11	167.34	167.48	167.56				
2″	42.40	42.40	42.34	41.46	42.80				
3″	169.53	169.50	169.58	169.72	169.76				

Measured at 125.7 MHz, in pyridine-d_5. *a*) Signals due to the major compund (26*S*) of the 26-isomers of **3**.

1: A colorless powder, $[\alpha]_D$ −16.7° (*c*=0.39, MeOH). Pos. SI-MS *m/z*: 105 $(C_3H_4O_4+H)^+$, 747 $(M+H)^+$, 769 $(M+Na)^+$. Neg. SI-MS *m/z*: 103 $(C_3H_3O_4)^-$, at *m/z*: 659 $(M-87)^-$. Pos. HR-SI-MS *m/z*: 769.3762 $(C_{40}H_{58}O_{13}+Na)^+$, error: −1.0 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1735 (AcO, cyclopentanone, COOR, COOH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

2: A colorless powder, $[\alpha]_D$ −19.6° (*c*=1.59, MeOH). Pos. SI-MS *m/z*: 105 $(C_3H_4O_4+H)^+$, 749 $(M+H)^+$. Neg. SI-MS *m/z*: 103 $(C_3H_3O_4)^-$, at *m/z*: 661 $(M-87)^-$. Pos. HR-SI-MS *m/z*: 749.4123 $(C_{40}H_{60}O_{13}+H)^+$, error: 1.4 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1738 (AcO, cyclopentanone, COOR, COOH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

3: A colorless powder, $[\alpha]_D$ −55.9° (*c*=0.88, MeOH). Pos. SI-MS *m/z*: 105 $(C_3H_4O_4+H)^+$, 783 $(M+Na)^+$. Pos. HR-SI-MS *m/z*: 783.3558 $(C_{40}H_{56}O_{14}+Na)^+$, error: −0.6 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1737 (AcO, COOR, COOH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

4: A colorless powder, $[\alpha]_D$ −8.0° (*c*=0.52, MeOH). Pos. SI-MS *m/z*: 105 $(C_3H_4O_4+H)^+$, 787 $(M+Na)^+$. Pos. HR-SI-MS *m/z*: 787.3867 $(C_{40}H_{60}O_{14}+Na)^+$, error: −1.0 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1742 (AcO, COOR, COOH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

5: A colorless powder, $[\alpha]_D$ −0.72° (*c*=0.33, MeOH). Pos. SI-MS *m/z*: 105 $(C_3H_4O_4+H)^+$, 729 $(M+Na)^+$. Pos. HR-SI-MS *m/z*: 729.3811 $(C_{38}H_{58}O_{12}+Na)^+$, error: −1.2 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1742 (COOR, COOH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

6: Recrystallization from a mixture of MeOH , EtOAc and isopropyl ether. A colorless powder, mp 268—269 °C, $[\alpha]_D$ −49.6° (*c*=1.27, MeOH). Pos. SI-MS *m/z*: 661 $(M+H)^+$. Pos. HR-SI-MS *m/z*: 661.3947 $(C_{37}H_{56}O_{10}+H)^+$, error: −0.1 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 1735 (AcO, cyclopentanone). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

7: Recrystallization from MeOH. Colorless needles, mp 216—217 °C, $[\alpha]_D$ −14.7° (*c*=0.51, MeOH). Pos. SI-MS *m/z*: 693 $(M+H)^+$, *m/z*: 675 $(M-OH)^+$. Pos. HR-SI-MS *m/z*: 693.4214 $(C_{38}H_{60}O_{11}+H)^+$, error: 0.3 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 1700 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Methylation of 1 and 2 **1** (9.0 mg) was dissolved in MeOH (1 ml) and an ether solution of CH_2N_2 was added in the usual manner. The products after removal of the solvent were subjected to HPLC [column: Develosil PH5, i.d. 10×250 mm; mobile phase CH_3CN–H_2O (40 : 60); flow rate: 2.0 ml/min; column temperature; 40 °C] to give methyl ester of **1** (**1a**) (5.1 mg) and **6** (2.3 mg). Similar treatment of **2** (7.4 mg) gave the methyl ester of **2** (**2a**) (1.7 mg), and 23-*O*-acetyl-shengmanol 3-*O*-*β*-D-xylopyranoside (**2b**) (3.5 mg) which was identified by direct comparison with an authentic specimen.[7)]

1a: A colorless powder, $[\alpha]_D$ −20.3° (c=0.51, MeOH). Pos. SI-MS m/z: 105 $(C_3H_4O_4+H)^+$, 119 $(C_4H_6O_4+H)^+$, 783 $(M+Na)^+$. Pos. HR-SI-MS m/z: 783.3921 $(C_{41}H_{60}O_{13}+Na)^+$, error: −0.7 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1735 (AcO, cyclopentanone, COOR). ^{1}H- and ^{13}C-NMR (pyridine-d_5): δ_H 3.68 (3H, s, OCH_3), 3.79 (2H, 2″-H) δ_C 41.49 (2″-C), 52.22 (OCH_3), 166.41(1″-C), 167.13 (3″-C).

2a: A colorless powder, $[\alpha]_D$ −24.5° (c=0.17, MeOH). Pos. SI-MS m/z: 105 $(C_3H_4O_4+H)^+$, 119 $(C_4H_6O_4+H)^+$, 785 $(M+Na)^+$. Pos. HR-SI-MS m/z: 785.4089 $(C_{41}H_{62}O_{13}+Na)^+$, error: 0.4 m.m.u. IR (KBr) cm^{-1}: 3200—3650 (OH), 2400—3000 (COOH), 1735 (AcO, cyclopentanone, COOR). ^{1}H- and ^{13}C-NMR (pyridine-d_5): δ_H 3.57 (3H, s, OCH_3), 3.79 (2H, 2″-H), δ_C 42.07 (2″-C), 52.22 (OCH_3), 166.44 (1″-C), 166.65 (3″-C).

Alkaline Hydrolysis of 1 **1** (9.6 mg) was dissolved in MeOH (2 ml), and after 2% $NaHCO_3$ (2 ml) was added, the solution was stirred for 16 h at room temperature. After evaporation of MeOH from the reaction solution, it was extracted with EtOAc (20 ml×3). The extract was purified by HPLC to give **6** (2.0 mg).

Enzymic Hydrolysis of 6 and 7 **6** (21.0 mg) was dissolved in MeOH (1 ml), and then 0.03% AcOH (80 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. Then, the reaction solution was extracted with EtOAc. The extract was subjected to SiO_2 chromatography followed by HPLC to afford the genuine aglycone (9.0 mg), which was identified as 23-*O*-acetyl-7,8-didehydroshengmanol by direct comparison with an authentic specimen.[2] Similar treatment of **7** (16.5 mg) with Cellulase T [Amano] 4 as in the case of **6** gave **7a** (5.4 mg) after recrystallization from a mixture of MeOH and isopropyl ether.

7a: A colorless powder, mp 122—123 °C, $[\alpha]_D$ −20.3° (c=0.35, MeOH). Pos. HR-EI-MS m/z: 560.3708 $(C_{33}H_{52}O_7)^+$, error: −0.2 m.m.u. IR (KBr) cm^{-1}: 3200—3600 (OH), 1723 (AcO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Conversion of 7a to 7b **7a** (4.7 mg) was dissolved in MeOH (1 ml), and after 2% Na_2CO_3 (1 ml) was added, the solution was stirred for 16 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 2 h at 60 °C. After evaporation of the solvent *in vacuo*, the products were purified by SiO_2 chromatography, followed by recrystallization from MeOH to give **7b** (3.4 mg).

7b: Colorless needles, mp 249—250 °C, $[\alpha]_D$ +4.1 (c=0.34, MeOH). Pos. HR-EI-MS m/z: 500.3497 $(C_{31}H_{48}O_5)^+$, error: −0.2 m.m.u. IR (KBr) cm^{-1}: 3200—3500 (OH). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Sugar Analysis of 6 and 7 **6** (13.4 mg), or **7** (5.2 mg) was dissolved in dioxane (1 ml), and after 3% HCl (2 ml) was added, 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] and HPLC with a chiral detector OR-I; [column, Shodex NH_2P-50 (i.d. 4.6×250 mm); solvent, MeCN–H_2O (80 : 20); effluent rate, 1 ml/min; column temperature, 45 °C, D-(+)-xylose: t_R 5.00′]. D-(+)-Xylose was detected from **6** and **7**, respectively.

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