STRUCTURES OF TWO NEW CYCLOLANOSTANOL XYLOSIDES, CIMIACEROSIDES A AND B[1]

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Abstract — A new cyclolanostanol xyloside, cimiaceroside A, was isolated from *Cimicifuga acerina* (Miyagi) and *Actaea asiatica*, and another, cimiaceroside B, from *C. simplex* and *C. acerina* (Oki) respectively. The absolute stereostructures of cimiacerosides A and B were determined as 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-3β,23,24-trihydroxy-9,19-cyclolanost-7-ene-3-*O*-β-D-xylopyranoside and 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-3β,23, 24-trihydroxy-9,19-cyclolanostane-3-*O*-β-D-xylopyranoside on the basis of chemical and spectral data, and with the aid of a new version of Mosher's method.

We previously reported on the isolation and the structural elucidation of new triterpene glycosides from *Cimicifuga simplex* WORMSK. (Ranunculaceae).[2] During the continuation of our work, we isolated two new cyclolanostanol xylosides, cimiaceroside A (**1**) from *C. acerina* C. TANAKA (Miyagi) and *Actaea asiatica* HARA (Ranunculaceae), and cimiaceroside B (**2**) from *C. simplex* and *C. acerina* (Oki). This paper deals with the isolation and the structural elucidation of these xylosides.

Cimiaceroside A (**1**) was obtained as described in the Experimental section by repeated chromatography of the methanolic extract of the subterranean parts of *C. acerina* (Miyagi) on octadecylsilanized silicic acid (ODS) and silica gel (SiO_2) columns followed by HPLC. Cimiaceroside A (**1**) was also obtained from the subterranean parts of *A. asiatica*.

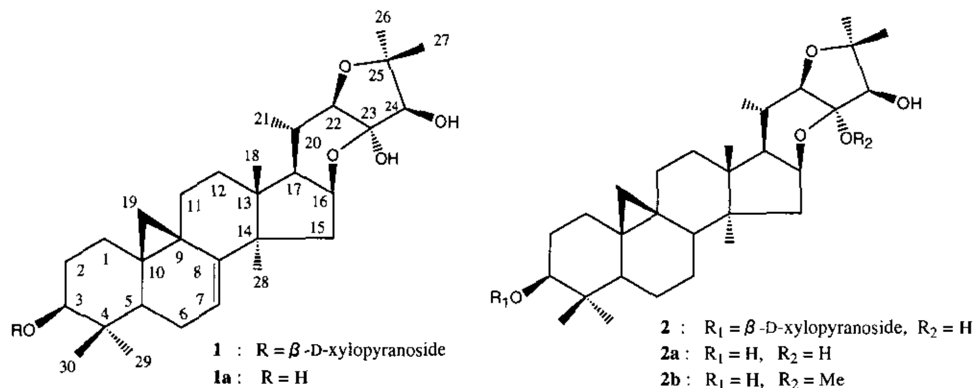

Figure 1. Structures of **1** and **2** and Their Derivatives

Cimiaceroside A (**1**) was obtained as colorless needles, mp 259−260℃, [α]$_D$ -39.3° , and its molecular formula was determined as $C_{35}H_{54}O_9$ on the basis of positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) showing its $(M+H)^+$ ion peak at *m/z*: 619.3850 and the data of the ^{13}C - NMR spectrum. The IR spectrum showed strong hydroxyl bands at 3200−3600 cm^{-1}. ^1H - and ^{13}C - NMR signals were identified by using double quantum filtered correlation spectroscopy (DQF-COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC) and rotating frame nuclear Overhauser effect (ROE) difference spectroscopy spectra. The ^1H - and ^{13}C - NMR spectra showed the presence of partial structures, 3β-hydroxypropanyl (H-3: δ 3.48; C-3: δ 88.19; H-2: 1.96, 2.32; C-2: 29.55; H-1: 1.31, 1.68; C-1: 30.38), a vinyl group (H-7: 5.12; C-7: 113.29; C-8: 149.63), a 9,9,10,10-tetrasubstituted cyclopropane (H-19: 0.45, 0.98; C-19: 28.38; C-9: 21.09; C-10: 28.38), an isolated ethylene (H-11: 1.12, 2.09; C-11: 25.26; H-12: 1.69 (2H); C-12: 33.23), six tertiary methyl groups containing two deshielding methyl groups (H-18: 1.22; C-18: 22.97; H-26: 1.76; C-26: 27.86; H-27: 1.67; C-27: 24.83; H-28: 1.08; C-28: 26.76; H-29: 1.35; C-29: 25.81; H-30: 1.05; C-30: 14.34), a secondary methyl group (H-21: 1.25; C-21: 17.50), 16β:23; 22:25-diepoxy groups (H-16: 5.06; C-16: 72.62; C-23: 106.11; H-22: 3.90; C-22: 86.82; C-25: 83.61), a hydroxy group (H-24: 4.18; C-24: 83.33), a hemiacetal group (C-16 and C-23), and β-xylopyranosyl moiety [3] (H-1': 4.85; C-1': 107.45; other protons: δ 3.73−4.36, carbons: δ 67.12−78.59). Cimiaceroside A (**1**) was hydrolyzed with Cellulase T [Amano] 4 and a genuine aglycone, cimiacerogenin A (**1a**), mp 210 −211℃, pos. HR-EI-MS *m/z*: 486.3342 $(C_{30}H_{46}O_5)^+$, [α]$_D$ -48.0° and an artifact, cimiacerol (**1b**), mp 278-279℃, pos. HR-EI-MS *m/z*: 486.3347 $(C_{30}H_{46}O_5)^+$, [α]$_D$ +47.1° were obtained (Figure 4). ^1H - and ^{13}C - NMR signals of **1a** (Tables 1, 2) were assigned to the structure of the genuine aglycone; they showed similar spectra to those of **1** except for the absence of signals of the xylopyranosyl

group and the glycosylation shift of C-3 from that of **1** by 10.42 ppm. On the other hand, the data of **1b** were different from those of **1**, especially, due to H-3, H-7, H-19, C-3, C-7, C-9, C-10, C-19 (Tables 1 and 2), to assign the structure to suggested that **1b** was an artifact as shown in Figure 4.

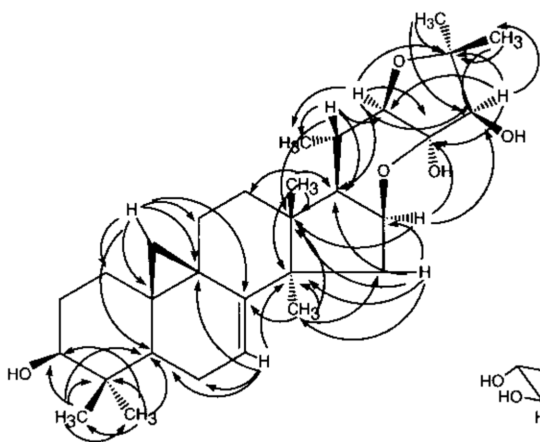

Figure 2. Selected Correlations Observed in HMBC Spectra of **1a**

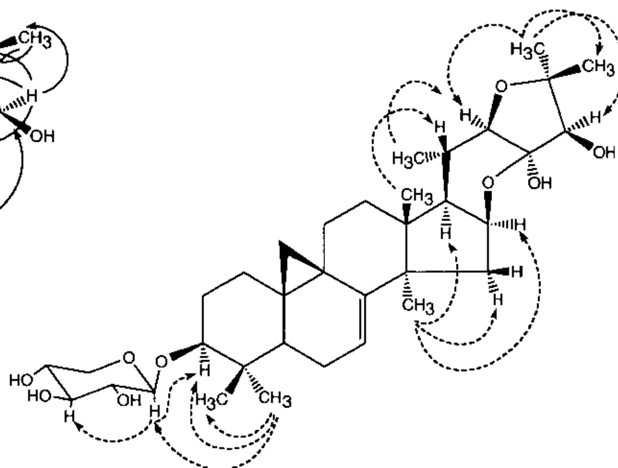

Figure 3. ROEs Observed in ROESY Experiment of **1**

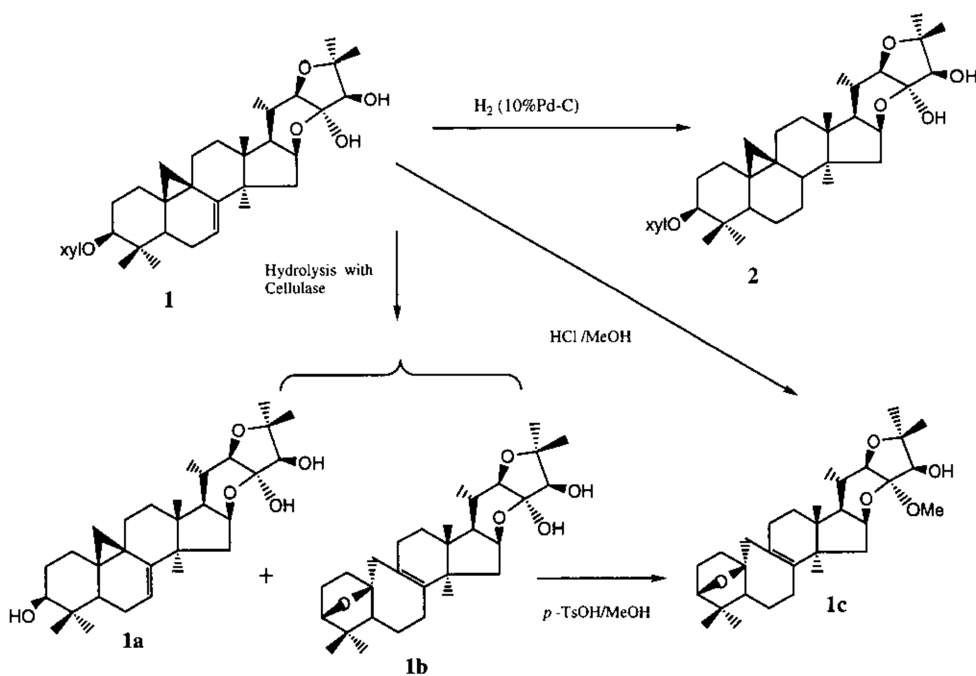

Figure 4. Hydrolysis of **1** and Conversion of **1b** to **1c** and **1** to **2**

The HMBC of **1a** showed long-range correlations between H-28 and C-8, C-13, C-14, C-15; H-18 and C-12, C-13, C-14, C-17; H-29 and C-3, C-4, C-5, C-30; H-30 and C-3, C-4, C-5, C-29; H-19 and C-1, C-5, C-8, C-9, C-10, C-11; H-16 and C-13, C-23, C-24; H-22 and C-17, C-21, C-23, C-25; H-24 and C-22, C-23,C-25,C-27 and other correlations to clarify the relationships between these partial structures of the aglycone moiety (Figure 2).

The relative stereochemistry of **1** was determined on the basis of coupling constants of the protons and ROE difference spectra (Figure 3). Irradiation at H-28 and H-18 increased the signal instensities of H-17(δH 1.60), H-16 and H-15α (δH 2.12), and H-20 (δH 2.28) respectively, and irradiation at H-26 increased those of H-22 (δH 3.90), H-24 and H-27. Similarly, irradiation at H-21 and H-29 increased those of H-22 and H-3α, H-1' and H-30, respectively, and irradiation at H-1' increased those of H-3α, and H-3' (δH 4.15).

The artifact (**1b**) was methylated with methanol containing *p*-toluenesulfonic acid to yield the methyl ether (**1c**) (Figure 4). The methyl ether (**1c**) was identified to *O*-methylcimiacerol, as a result of direct comparison of mp, MS data, TLC and ^{1}H - NMR spctra with those of an authentic specimen. The relative stereostructure of *O*-methylcimiacerol has been established on the basis of the spectral data and X-Ray crystal analysis.[5] The absolute stereostructure of **1c** was determined by a new version of Mosher's method.[6] Esterification of **1c** with (*S*)-(+)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) chloride or (*R*)-(-)-MTPA chloride yielded (*R*)-(+)-MTPA ester and (*S*)-(-)-MTPA ester. The proton signals of (*R*)- and (*S*)-MTPA ester were assigned by DQF- COSY (500 MHz, $CDCl_3$), and $\Delta\delta$ ($=\delta S-\delta R$) values for the protons were obtained. Protons with positive and negative $\Delta\delta$ values are found on the right and left sides of the MTPA planes, respectively. The positive $\Delta\delta$ value of OCH_3 (+15) suggested that the methyl group was located on the right side of the MTPA plates.Thus, the absolute structure of **1c** was fully established as a cyclolanostane type as shown in Figure 5.

The α-orientation of the hemiacetal hydroxy group at C-23 of **1b** was deduced by the solvent effect: the paramagnetic shifts of H-16 (δ 5.01 ppm, Δ 0.41 ppm), H-21 (δ 1.25, Δ0.20) , H-22 (δ 3.90, Δ 0.47) , H-24 (δ 4.22, Δ 0.62) in a pyridine-d_5 solution from those (4.60, 1.05, 3.43, 3.60) in a $CDCl_3$ solution were found, while H-3 (δ 3.75), H_2-19 (δ 1.78, 3.19) in distant positions from the 23-hydroxy group showed almost the same chemical shifts in both solutions.[7]

The methyl ether (**1c**) was also obtained by treatment of **1** with 2% methanolic HCl along with D-xylose. The conversion of **1** to **1c** has often been encountered by dilute acid treatment of 3-*O*-glycosylcycloart-7-ene compounds as shown in Figure 4.[4] D-Xylose was confirmed by TLC, HPLC and $[\alpha]_D$ +20.6° and the connecting position at C-3 was determined by ROEs and the glycosylation shift of C-3 ($\Delta\delta$: 10.42).

Therefore, cimiaceroside A (**1**) was formulated as 20(*S*),22(*R*),23(*S*),24(*R*)-16 β:23;22:25-diepoxy-3 β,23,24-trihydroxy-9,19-cyclolanost-7-ene-3-*O*-β-D-xylopyranoside.

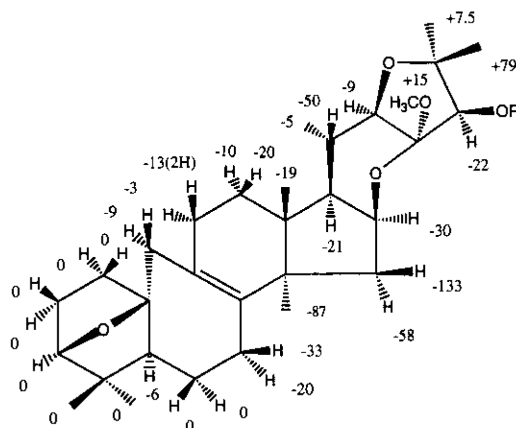

Figure 5. Δ δ Values (Hz) of Protones of **1c** Obtained by a New Version of Mosher's Method

Cimiaceroside B (**2**) was obtained as colorless needles, mp 279−280℃, [α]$_D$ -2.4°, and the molecular formula was determined as $C_{35}H_{56}O_9$ on the basis of pos. HR-SI-MS *m/z*: 603.3898 $(M-OH)^+$, and the data of the ^{13}C - NMR. The IR spectrum showed a strong hydroxyl band at 3200−3650 cm^{-1}. The ^1H - NMR and ^{13}C - NMR signals were assigned as for **1** and similar to those of **1**, except for the absence of the 7(8)-double bond and the signals due to the neighboring moiety of the double bond: (H-5, H-6, H-7, H-8, H-15, H-18, H-19, H-28, C-5, C-7, C-8, C-14, C-15, C-18, C-19, C-28).

Cimiaceroside B (**2**) was hydrolyzed with Cellulase T [Amano] 4 and a genuine aglycone, cimiacerogenin B (**2a**), mp 271−272℃, pos. HR-SI-MS *m/z*: 511.3394 $(C_{30}H_{48}O_5 + Na)^+$, [α]$_D$ +11.4°, was obtained. The ^1H - and ^{13}C - NMR signals of **2a** (Table 1, 2) were assigned to the structure of the genuine aglycone; it showed similar spectra to those of **2** except for the absence of the xylopyranosyl group and the glycosylation shift of C-3 from that of **2** by 10.43 ppm. Hydrolysis of **2** with 2% methanolic HCl yielded 23-*O*-methylcimiacerogenin B (**2b**) and D-xylose, [α]$_D$ +20.8°. The fact that **2** could be produced from **1** by hydrogenation in the presence of 10% Pd-charcoal led to the conclusion that **2** should be 7, 8-dihydrocimiaceroside **A** (Figure 4).[2] Thus, the structure of cimiaceroside B (**2**) was determined as 20(*S*),22(*R*),23(*S*),24(*R*)-16 β:23;22:25-diepoxy-3 β,23,24-trihydroxy-9,19-cyclo-lanostane-3-*O*-β-D-xylopyranoside.

It is interesting from a chemotaxonomical point of view that cimiaceroside A (**1**) was obtained from *C. acerina* (Miyagi), while B (**2**) was obtained from *C. acerina* (Oki).

Table 1. ^{1}H-NMR Data of **1** and **2** and Their Derivatives

	1[a)]	**1a**[a)]	**1b**[a)]	**1c**[b)]	**2**[a)]	**2a**[a)]	**2b**[b)]
1	1.31, 1.68	1.30,1.66	1.42, 1.60	1.42, 1.58	1.23, 1.60	1.20, 1.56	1.24, 1.56
2	1.96, 2.32	1.94 (2H)	1.70, 1.91	1.70, 1.92	1.93, 2.35	1.88, 1.99	1.57, 1.76
3	3.48 dd (4.3, 11.8)	3.52	3.75 d (5.3)	3.73d (5.5)	3.50 dd (4.4, 11.5)	3.53 dd (4.4, 11.5)	3.29dd (4.4, 11.5)
5	1.28	1.32	1.21 d (11.8)	1.18 d (11.8)	1.33	1.32 dd (4.0, 11.9)	1.30
6	1.58, 1.89	1.69, 1.93	1.39, 1.70	1.46, 1.72	0.73, 1.55	0.78, 1.55	0.82, 1.61
7	5.12 dd (2.0, 8.0)	5.15 dd (1.3, 8.1)	1.92, 2.33	2.00, 2.40	1.04, 1.27	1.07, 1.24	1.10, 1.35
8	-	-	-	-	1.58	1.59	1.60
11	1.12, 2.09	1.12, 2.09	2.04 (2H)	2.10 (2H)	1.10, 1.94	1.13, 2.00	1.13, 2.03
12	1.69 (2H)	1.70 (2H)	1.54, 1.73	1.54, 1.76	1.56 (2H)	1.60 (2H)	1.12(2H)
15	1.92, 2.12	1.94, 2.14	1.88 (2H)	1.80 (2H)	1.58, 1.91	1.64, 1.92	1.50, 1.89
16	5.06 ddd (7.8, 7.8, 7.8)	5.08 ddd (7.8, 7.8, 7.8)	5.01 ddd (7.8, 7.8, 7.8)	4.33ddd (7.8, 8.2, 8.0)	4.96 ddd (7.8, 7.8, 7.8)	4.98 ddd (7.8, 7.8, 7.8)	4.29ddd (8.0, 8.0, 8.0)
17	1.60	1.63	1.53	1.41 dd (8.0, 11.0)	1.58	1.57	1.5 dd (8.0, 11.0)
18	1.22 s	1.24 s	1.07 s	0.93 s	1.21 s	1.24 s	1.13s
19	0.45 d (4.0) 0.98 d (4.0)	0.50 d (4.0) 1.04 d (4.0)	1.78 d (13.8) 3.19 d (13.8)	1.66 d (13.7) 3.12 d (13.7)	0.21 d (4.0) 0.51 d (4.0)	0.26 d (4.0) 0.55 d (4.0)	0.36d (4.3) 0.62d (4.3)
20	2.28	2.30	2.29	1.72	2.25	2.25	1.68
21	1.25 d (6.5)	1.25 d (6.5)	1.25 d (6.3)	1.03 d (6.4)	1.22 d (6.5)	1.23 d (6.5)	1.00 d (6.2)
22	3.90 d (10.5)	3.91 d (10.6)	3.90 d (10.6)	3.35 d (10.8)	3.88 d (10.6)	3.90d (10.6)	3.36d (10.8)
24	4.18s	4.18 s	4.22 s	3.80 s	4.16 s	4.17 s	3.78s
26	1.76s	1.77 s	1.76 s	1.33 s	1.75 s	1.76 s	1.33s
27	1.67s	1.68 s	1.67 s	1.31 s	1.67 s	1.68 s	1.31s
28	1.08s	1.09 s	0.89 s	0.90 s	0.87 s	0.89 s	0.91s
29	1.35s	1.23 s	0.98 s	1.02 s	1.33 s	1.22 s	0.96s
30	1.05s	1.11 s	0.99 s	0.95s	1.04 s	1.08 s	0.82s
OCH'				3.26s			3.25s
1'	4.85 d (8.0)				4.86 d (7.5)		
2'	4.03 dd (8.0, 8.0)				4.02 dd (7.5, 8.0)		
3'	4.15 dd (8.0, 8.3)				4.13 dd (8.0, 8.0)		
4'	4.21ddd (5.0, 8.3, 11.0)				4.20 ddd (5.0, 8.0, 10.6)		
5'	3.73 dd (11.0, 11.1) 4.36 dd (5.0, 11.1)				3.73 dd (10.6, 11.3) 4.35 dd (5.0, 11.3)		

a) Obtained on a JEOL α -400, in pyridine-d_5 . b) Obtained on a Varian Unity-INVA-500, in $CDCl_3$.

EXPERIMENTAL

General The instruments used for this study were as follows: a Yanagimoto micromelting apparatus (for melting points, uncorrected); a JASCO DIP 1000 digital polarimeter (for specific rotation, measured at 23 ℃); Perkin-Elmer 1720X-FT IR spectrophotometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); and a Varian Gemini-200, a JEOL α -400 and a Varian Unity-INOVA-500 (for NMR spectra, measured in pyridine-d_5 or $CDCl_3$ containing a drop of D_2O, on the δ scale using tetramethylsilane as an internal standard). Column chromatography was carried out on silica gel (Wakogel C-200) and ODS-A YMC. HPLC was conducted by using a Gilson 305 pump equipped with a JASCO 830-RI as a detector.

Table 2. ^{13}C-NMR Data of **1** and **2** and Their Derivatives

	1$^{a)}$	**1a**$^{a)}$	**1b**$^{a)}$	**1c**$^{b)}$	**2**$^{a)}$	**2a**$^{a)}$	**2b**$^{b)}$
1	30.38	30.64	36.72	36.13	32.16	32.44	31.92
2	29.55	30.73	25.93	25.39	30.04	31.77	30.31
3	88.19	77.77	84.98	85.67	88.42	77.99	78.70
4	40.44	40.24	44.82	44.43	41.31	41.11	40.47
5	42.77	42.53	55.13	54.61	47.51	47.44	47.02
6	21.90	22.18	23.29	22.84	20.94	21.67	20.86
7	113.29	113.45	31.64	31.23	26.28	26.55	26.25
8	149.63	149.57	137.44	136.63	47.47	47.67	47.46
9	21.09	21.10	123.82	123.66	19.65	19.73	19.58
10	28.38	28.69	90.03	89.80	26.65	26.95	26.42
11	25.26	25.33	31.64	31.42	26.35	26.21	26.03
12	33.23	33.24	32.01	31.32	33.47	33.53	33.03
13	44.67	44.68	45.38	45.14	46.88	46.92	46.50
14	50.39	50.40	49.35	48.83	45.27	45.32	44.99
15	42.01	42.02	38.86	37.95	43.34	43.41	42.49
16	72.62	72.63	72.56	72.54	72.41	72.46	72.50
17	52.87	52.88	50.79	49.16	52.37	52.43	50.83
18	22.97	22.97	18.84	18.58	20.59	20.70	20.46
19	28.38	28.49	36.00	35.34	30.15	30.44	30.52
20	34.66	34.67	34.98	34.19	34.74	34.77	33.98
21	17.50	17.49	17.90	17.41	17.48	17.52	17.05
22	86.82	86.82	86.85	84.84	86.91	86.96	85.79
23	106.11	106.11	106.09	108.52	105.99	106.03	108.43
24	83.33	83.33	83.17	76.04	83.35	83.37	76.11
25	83.61	83.61	83.73	83.19	83.55	83.61	83.14
26	27.86	27.84	28.00	26.67	27.75	27.78	26.54
27	24.83	24.82	24.95	23.57	24.76	24.80	23.51
28	26.76	26.79	24.60	24.42	19.64	19.75	19.52
29	25.81	26.19	25.10	25.16	25.76	26.37	25.42
30	14.34	13.65	23.61	23.30	15.41	14.85	13.99
OCH_3				49.70			49.74
1'	107.45				107.48		
2'	75.57				75.55		
3'	78.59				78.59		
4'	71.27				71.25		
5'	67.12				67.08		

a) Measured at 100.4 MHz in pyridine-d_5. b) Measured at125.7MHz in $CDCl_3$.

Silica gel 60 F_{254} (Merck) precoated TLC plates were used, and detection was achieved by spraying with 40% H_2SO_4 followed by heating.

Isolation of 1 The dried subterranean parts (100 g) of *C. acerina* cultivated at the Experimental Station for Medical Plant Studies, Faculty of Pharmaceutical Sciences, Tohoku University, were extracted with methanol (200 mL×3) under reflux for 2 h each. After evaporation *in vacuo*, the extract (12.0 g) was partitioned three times between *n*-BuOH - EtOAc (1: 1) (100 mL) and water (50 mL) . The fraction soluble in the organic solvents was chromatographed on an ODS column (80 g, 3×20 cm) and eluted with MeOH - H_2O (first at 2 : 1, then at 4 : 1). The latter fraction (0.8 g) was rechromatographed on an

SiO_2 column (25 g, 2.2×16 cm). The fractions eluted with $CHCl_3$ - MeOH (19 : 1) were subjected to HPLC [column: Cosmosil 10Ph (i.d. 4.6×250 mm); solvent: MeOH - H_2O - MeCN (10:10:3); column temperature: 40℃; flow rate: 1mL/min]. Recrystallization of the fraction at t_R 10'30" from a mixture of MeOH and MeCN yielded **1** (35 mg) as colorless platelets.

1: mp 259－260℃, [α]$_D$ -39.3 ° (*c*=0.69, MeOH), pos. HR-SI-MS *m/z*: 619.3850 ($C_{35}H_{54}O_9$ + H)$^+$, error 0.7 mmu., pos. SI-MS *m/z*: 619 (M+H)$^+$, *m/z*: 661 (M+ Na)$^+$, *m/z*: 601 (M-OH)$^+$, IR(KBr) cm^{-1}: 3200－3600 (OH). ^{1}H - and ^{13}C - NMR (pyridine-d_5) δ : Tables 1 and 2.

Compound (**1**) (28 mg) was also obtained by the same treatment of the dried subterranean parts (100 g) of *A. asiatica* cultivated at the same Experimental Station as mentioned above.

Hydrolysis of 1 with Cellulase T [Amano] 4 **1** (12.5 mg) was dissolved in MeOH (2 mL) and 0.03% AcOH solution (60 mL) was added with stirring, followed by addition of Cellulase T [Amano] 4 (from *Trichoderuma viride* 100 mg) with stirring. Stirring of the solution was continued for 2 d at rt. The reaction solution was then shaken with EtOAc (60 mL×3), and after washing the EtOAc layer with water followed by drying 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 (1:1) to yield **1a** as colorless powders (4.0 mg) after purification by HPLC and recrystallization from MeOH. When **1** (21.8 mg) was treated with the same enzyme in 1% ethanolic AcOH (20 mL) and water (40 mL) in place of the above methanol - 0.03% AcOH solution, **1b** (7.8 mg) could also be obtained with a small amount of **1a** (1.5 mg).

1a: Colorless powder, mp 210－211℃, [α]$_D$ -48.0° (*c*=0.27, MeOH), pos. HR-EI-MS *m/z*: 486.3342 ($C_{30}H_{46}O_5$)$^+$, error: 0 mmu., pos. EI-MS *m/z*: 486 (M)$^+$, *m/z*: 487 (M+H)$^+$, IR($CHCl_3$) cm^{-1}: 3300 －3600 (OH), ^{1}H - and ^{13}C - NMR (pyridine-d_5) δ : Tables 1 and 2.

1b: mp 278－279℃, [α]$_D$ +47.1° (*c* =0.28, MeOH). pos. HR-EI-MS *m/z*: 486.3347 ($C_{30}H_{46}O_5$)$^+$, error: 0.4 mmu., pos. EI-MS *m/z*: 486 (M)$^+$, 487 (M+H)$^+$. ^{1}H - and ^{13}C - NMR (pyridine-d_5) δ : Tables 1 and 2.

Methylation of 1b **1b** (5.0 mg) was dissolved in 1% methanolic *p*-TsOH (2 mL), and stirred for 1 h at rt. The product was chromatographed on an Al_2O_3 column followed by SiO_2 chromatography. The eluate with *n*-hexane - EtOAc (10:1) was recrystallized from AcOEt to produce **1c** (2 mg) as colorless needles.

1c: mp 235－236℃, [α]$_D$ +20.0° (*c*=0.20, $CHCl_3$), pos. EI-MS *m/z*: 500 ($C_{31}H_{48}O_5$)$^+$, ^{1}H - and ^{13}C - NMR ($CDCl_3$) δ : Tables 1 and 2.

Hydrolysis of 1 with methanolic HCl To the solution of **1** (19.5 mg) in MeOH (1 mL) was

added 3% HCl (2 mL) and the solution was refluxed for 2 h. After usual treatment, the EtOAc soluble fraction was chromatographed on a SiO_2 column to produce **1c** (7.5 mg) as colorless needles by elution with *n*-hexane - EtOAc (10:1) and recrystallization from EtOAc. The water layer was refluxed again for 1 h in order to hydrolyze the methylxylosides, and the reaction solution was chromatographed on an Amberlite IR-35 column. The passed fraction yielded D-xylose (2.8 mg), $[\alpha]_D$ +20.6° [*c*=0.28, MeOH - H_2O (1:1)], which was identified by HPLC [column: LiChrosorb NH_2 (i.d. 4.6×250 mm); solvent: MeOH - H_2O (4:1); column temperature: 40℃, flow rate: 1 mL/min; t_R: 5.5 min], and TLC [*n*-PrOH - H_2O (85:15), R*f*: 0.59] through comparative analysis with an authentic specimen.

Preparation of (*R*)-(+)-MTPA ester and (*S*)-(-)-MTPA ester of 1c To the solution of **1c** (2.9 mg) in pyridine (50 μL) was added (*S*)-(+)-MTPA chloride (5 μL), and the solution was allowed to stand at rt for 17 h. *N*,*N*-Dimethyl-1,3-propanediamine (3 μL) was added, and after having been left standing for 10 min, the product was purified with a TLC preparation to afford **(*R*)-(+)-MTPA ester** (3.4 mg). In a similar manner, **1c** (2.9 mg) and (*R*)-(-)-MTPA chloride (5 μL) were reacted to yield **(*S*)-(-)-MTPA ester** (2.3 mg). ^1H - NMR ($CDCl_3$) of both esters were measured successively on a Varian Unity-INOVA-500, and assigned with the aid of the DQF- COSY, and $\Delta\delta$ (= δS - δR) values were obtained as shown in Figure 5.

(*R*)-(+)-MTPA ester: IR($CHCl_3$)cm^{-1}: 1749 (acetyl), ^1H - NMR (500 MHz, $CDCl_3$) δ: 1.40,* 1.55* (m, H-1), 1.70,* 1.925 (m, H-2), 3.730 (d, *J*=5.5Hz, H-3), 1.175 (m, H-5), 1.50,* 1.72* (m, H-6), 2.000, 2.370 (m, H-7), 2.065 (2H, m, H-11), 1.50,* 1.72* (each, m, H-12), 1.765 (2H, m, H-15), 4.255 (ddd, *J*= 7.8, 8.0, 8.0 Hz, H-16), 1.360 (dd, *J*=8.0, 11.0 Hz, H-17), 0.896 (3H, s, H-18), 1.657, 3.090 (each, d, *J*=13.3 Hz, H-19), 1.650 (m, H-20), 1.008 (3H, d, *J*=6.4 Hz, H-21), 3.379 (d, *J*=10.5 Hz, H-22), 5.301 (s, H-24), 1.424 (3H, s, H-26), 1.119 (3H, s, H-27), 0.802 (3H, s, H-28), 1.020 (3H, s, H-29), 0.953 (3H, s, H-30), 3.357 (3H, s, OCH_3), 3.490 (3H, s, OCH_3-MTPA), 7.39,*(3H, m, H-3,4,5 of Ph), 7.640 (2H, dd, J=7.5, 2.0, H-2,6 of Ph) (*: overlapping).

(*S*)-(-)-MTPA ester: IR($CHCl_3$)cm^{-1}: 1746 (acetyl), ^1H - NMR (500 MHz, $CDCl_3$) δ: 1.40,* 1.55* (m, H-1), 1.70,* 1.925 (m, H-2), 3.731 (d, *J*=5.5Hz, H-3), 1.163 (m, H-5), 1.50,* 1.72* (m, H-6), 1.960, 2.315 (m, H-7), 2.040 (2H, m, H-11), 1.46,*1.70* (each, m, H-12), 1.50,* 1.650 (each, m, H-15), 4.196 (ddd, *J*= 7.8, 8.0, 8.0 Hz, H-16), 1.318 (dd, *J*=8.0, 11.0 Hz, H-17), 0.859 (3H, s, H-18), 1.640, 3.084 (each, d, *J*=13.3 Hz, H-19), 1.550 (m, H-20), 0.998 (3H, d, *J*=6.4 Hz, H-21), 3.361 (d, *J*=11.0 Hz, H-22), 5.257 (3H, s, H-24), 1.439 (3H, s, H-26), 1.277 (3H, s, H-27), 0.629 (3H, s, H-28), 1.019 (3H, s, H-29), 0.953(3H, s, H-30), 3.387 (3H, s, OCH_3), 3.490 (3H, s, OCH_3 - MTPA), 7.40,*(3H, m, H-3,4,5 of Ph), 7.555 (2H, dd, J=7.5, 2.0, H-2,6 of Ph) (*: overlapping).

Isolation of 2 The dried subterranean parts (200 g) of *Cimicifuga simplex* cultivated at the same Experimental Station were treated in the same manner as that used for **1** . The fractions eluted with MeOH - H_2O (4:1) in ODS chromatography were rechromatographed on an SiO_2 column. The fractions eluted with $CHCl_3$ - MeOH (19:1) were subjected to HPLC [column: Deverosil PhA-5 (i.d. 10.0×250 mm); solvent: MeOH - H_2O - MeCN (10:7:3); column temperature: 40℃; flow rate: 2 mL/min]. Recrystallization of the fractions at t_R 20' from MeOH produced **2** (5mg) as colorless needles. **2**: mp 279−280℃, $[\alpha]_D$ -2.4 ° (*c*=0.4, MeOH), pos.HR-SI-MS *m/z*: 603.3898 ($C_{35}H_{56}O_9$ - OH $)^+$, error +0.4 mmu., pos. SI-MS *m/z*: 643 (M+Na $)^+$, *m/z*: 603 (M-OH$)^+$, IR(KBr) cm^{-1}: 3200−3650 (OH). ^1H - NMR and ^{13}C - NMR (pyridine-d_5) δ : Tables 1 and 2.

Compound **2** (65 mg) was also obtained with the same treatment of the dried subterranean parts (150 g) of *C. acerina* collected on Oki Island, Tottori Prefecture, Japan.

Hydrolysis of 2 with Cellulase T [Amano]4 The treatment of **2** (30.4 mg) with Cellulase T [Amano] 4 (300 mg) similar to that used for **1** produced **2a** (9.7 mg) :

2a: Colorless needles, mp 271−272℃, $[\alpha]_D$ +11.4° (*c* =0.25, MeOH), pos.HR-SI-MS *m/z*: 511.3394 ($C_{30}H_{48}O_5$ + Na$)^+$, error: -0.3 mmu., pos. SI-MS *m/z*: 471 (M- OH$)^+$, *m/z*: 511 (M+Na $)^+$, IR($CHCl_3$) cm^{-1}: 3300−3500 (OH), ^1H - and ^{13}C - NMR (pyridine-d_5) δ : Tables 1 and 2.

Hydrolysis of 2 with methanolic HCl The treatment of **2** (17.4 mg) with 2% methanolic HCl [MeOH - H_2O (2: 1) (3 mL)] similarly as that used for **1** produced **2b** (5 mg) and D-xylose (1.6 m g), $[\alpha]_D$ +20.8° [*c* =0.16, MeOH - H_2O (1:1)], which was identified by HPLC [column: LiChrosorb NH_2 (i.d. 4.6×250 mm); solvent: MeOH - H_2O (4:1); column temperature: 40℃,flow rate: 1 mL/min; t_R: 5.5 min], and TLC [*n*-PrOH - H_2O (85:15), R*f*: 0.59] by comparative analysis with an authentic specimen.

2b: Colorless platelets, mp 204−205℃, $[\alpha]_D$ ±0° (*c* =0.32, MeOH), pos.HR-SI-MS *m/z*: 502.3660 ($C_{31}H_{50}O_5$$)^+$, error: 0.5 mmu., IR ($CHCl_3$) cm^{-1}: 3200−3500 (OH), ^1H - and ^{13}C - NMR ($CDCl_3$) δ : Tables 1 and 2.

Hydrogenation of 1 to 2 **1**(9.5mg) was dissolved in EtOH (2mL) and 10% Pd-charcoal (about 20 mg) was added to the solution. The solution was stirred in an H_2 atmosphere for 48 h. After removal of the catalyst by filtering, the products were subjected to HPLC to recover the starting material (**1**) (2.3 mg) and to yield **2** (3.5 mg). **2** was identified as cimiaceroside B by direct comparison of HPLC, ^1H - NMR and MS data with those of an authentic specimen.

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