Studies on the Chinese Crude Drug "Shoma." IX.[1] Three Novel Cyclolanostanol Xylosides, Cimicifugosides H-1, H-2 and H-5, from Cimicifuga Rhizome

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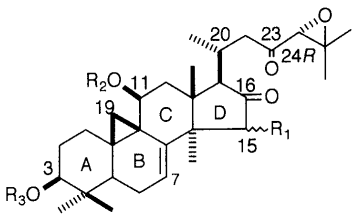
Three new cyclolanostanol xylosides were isolated from a batch of commercial Cimicifuga Rhizome, cimicifugoside H-1 (1), $C_{35}H_{52}O_9$, mp 260—262 °C, $[\alpha]_D$ −43.5°, cimicifugoside H-2 (2), $C_{35}H_{54}O_{10}$, mp 227—229 °C, $[\alpha]_D$ −38.8°, and cimicifugoside H-5 (3), $C_{35}H_{52}O_{10}$, mp 262—264 °C, $[\alpha]_D$ −22.9°, together with known glycosides, actein and 27-deoxyactein. Their structures were determined on the basis of chemical and spectrometric evidence including an X-ray crystallographic analysis. The structure of cimicifugoside H-1 (1) was established as (20*R*,24*R*)-24,25-epoxy-11β-hydroxy-3-β-(β-D-xylopyranosyloxy)-9,19-cyclolanost-7-ene-16,23-dione. Cimicifugoside H-5 (3) is the 15-hydroxylated derivative of 1. Since 1 changed into cimicifugoside H-2 (2) on treatment with *p*-toluenesulfonic acid, 2 has a 24*R*,25-diol structure derived from 1 by opening its epoxy ring.

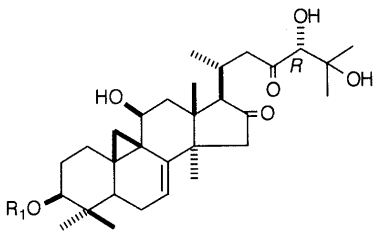
Key words cimicifugoside (H-1, H-2 and H-5); Cimicifuga Rhizome; 9,19-cyclolanostanol; triterpenol glycoside

Cimicifuga Rhizome (The Pharmacopoeia of Japan, 12th ed.) is the rhizome of *Cimicifuga simplex* or other species of the same genus (Ranunculaceae), and has been used as an antipyretic and an analgesic in Chinese traditional medicine. The rhizome of *Cimicifuga* species is known to contain highly oxygenated 9,19-cyclolanostane triterpenol glycosides such as cimigenol xyloside,[2] acetylshengmanol xyloside[3] and 24-*O*-acetylhydroshengmanol xyloside,[4] in addition to cinnamic acid derivatives,[5] chromones[6] and indolinones.[7]

We have preliminarily reported the isolation and the structure determination of cimicifugosides H-1 (**1**), H-3 and H-4.[8] In this paper, we present a full account of the structure elucidation of cimicifugoside H-1 (**1**) and, in addition, two new cimicifugosides H-2 (**2**) and H-5 (**3**). The isolation and purification of these cimicifugosides and two known xylosides, actein and 27-deoxyactein, are described in detail in the experimental section.

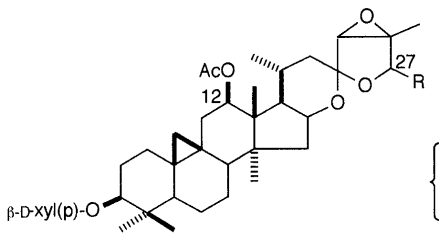
Cimicifugoside H-1 (**1**) was obtained as colorless needles, mp 260—262 °C, $[\alpha]_D$ −43.5°. Its molecular formula was determined as $C_{35}H_{52}O_9$ on the basis of the elemental analysis and the FAB-MS result. The IR spectrum of **1** showed absorptions at 1720 and 1715 cm^{-1} due to two carbonyl groups. The UV spectrum of **1** showed an absorption maximum due to a cyclopropane ring conjugating with a double bond at 205 nm (ε 6275).[9] The ^{13}C-NMR spectrum of **1** showed the signals ascribable to three methine carbons bearing an oxygen atom at δ_C 88.4 (C-3), 63.0 (C-11) and 65.8 (C-24), and oxygen-bearing quaternary carbons including two ketonic carbons at 60.7



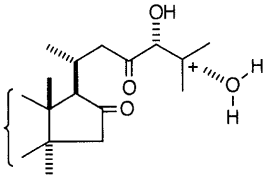


1: R_1=H, R_2=H, R_3=β-D-xylopyranosyl
3: R_1=OH, R_2=H, R_3=β-D-xylopyranosyl
4: R_1=H, R_2=H, R_3=H
5: R_1=H, R_2=Ac, R_3=Ac

2: R_1=β-D-xylopyranosyl
7: R_1=H



10 : R=OH
11 : R=H



intermediate A

Chart 1

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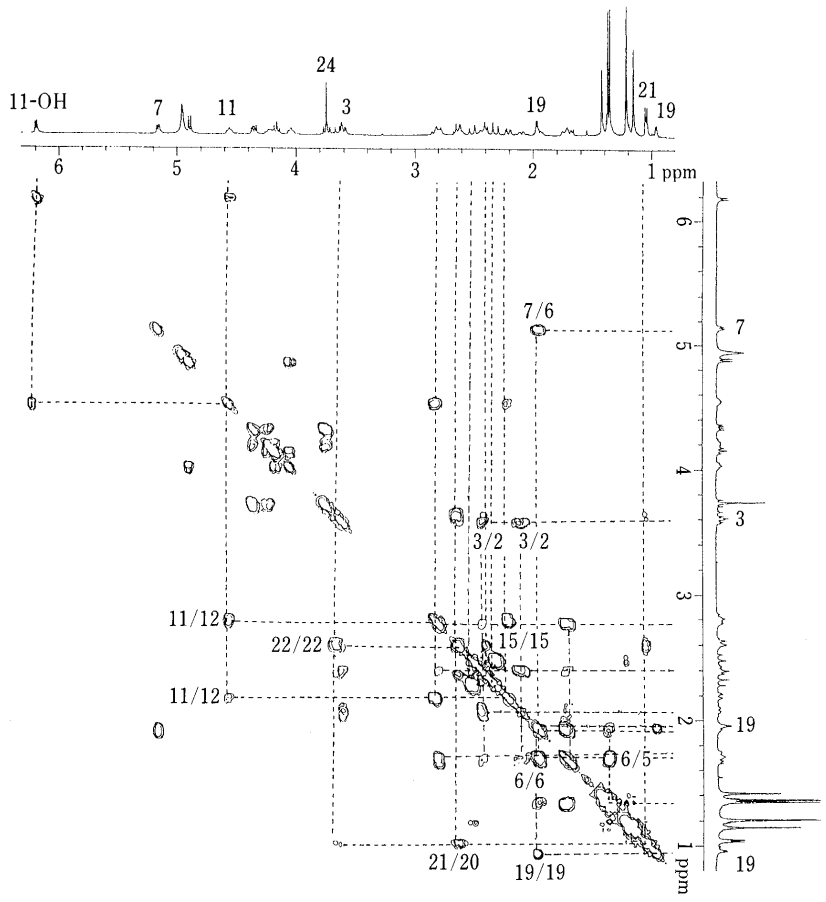

Fig. 1. ^{1}H–^{1}H COSY Spectrum of Compound **1** in Pyridine-d_5

(C-25), 218.4 (C-16) and 205.6 (C-23). The spectrum also gave information about the sugar moiety: five oxygenated carbons assignable to a β-D-xylopyranose moiety were observed [δ_C 107.4 (C-1), 75.5 (C-2), 78.5 (C-3), 71.2 (C-4), 67.1 (C-5)].

The ^{1}H-NMR spectrum of **1** exhibited the presence of a secondary and six tertiary methyl groups (δ_H 1.04—1.41), a trisubstituted double bond (δ 5.15, br d, J=6 Hz), two carbinol methines (δ 3.59, dd, J=8, 4 Hz, and 4.58, m), a methine bearing an oxygen atom (δ 3.74, s) and an anomeric proton (δ 4.88, d, J=7 Hz).

The ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum (Fig. 1) disclosed that the xyloside **1** has partial structures a, b, c, d, e and f (Fig. 2) in the molecule. When the partial structures a to f were applied to a 9,19-cyclolanostanol skeleton, rings A, B, C and D and a part of the side chain of the triterpenic genin were presumable as in the structure **1** in Chart 1, except for the binding site of the hydroxyl in the partial structure d. But the protons assignable to the cyclopropane methylene (19-H_2) in the ^{1}H-NMR spectrum were observed as a pair of AB doublets at markedly low magnetic field (δ_H 0.96 and 1.96 ppm) compared with those of 9,19-cyclolanost-7-enes such as 7,8-didehydrocimigenol (δ_H 0.54 and 1.11 ppm).[10] The downfield shift of the cyclopropane methylene could be explained by the exsistence of 11β-OH on ring C: the spatial arrangement of the cyclopropane methylene and the hydroxyl, similar to that in 1,3-diaxially substituted cyclohexane, reasonably accounts for the marked downfield shift of the methylene protons.[11a]

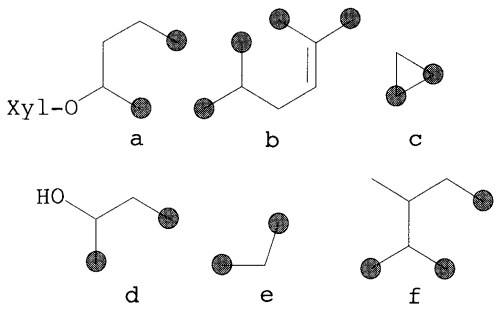

Fig. 2. Partial Structures of Compound **1**

All signals of ^{1}H- and ^{13}C-NMR of **1** were assigned with the assistance of ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY (Tables 1 and 2).

Enzymatic hydrolysis of **1** with molsin afforded a diketonic genin (**4**), $C_{30}H_{44}O_5$, mp 204—206 °C, $[\alpha]_D$ −31.4°, and xylose. Xylose was identified as xylitol acetate by GLC. Application of Klyne's rule[12] to **1** and **4** supported the view that the sugar moiety of **1** is β-D-xylopyranose [molecular rotation difference between **1** and **4**, −116.0°: methyl β-D-xylopyranoside, M_D −108°; methyl α-D-xylopyranoside M_D +253°].[13] On comparison of the ^{13}C-NMR spectra of **1** and **4**, the signal due to C-3 showed a downfield shift ($\Delta\delta$ value; +10.4: glycosylation shift).[14] So, we concluded that the β-D-xylopyranose was bound to C-3 of the genin **4**.

Acetylation of **4** with acetic anhydride in pyridine yielded a diacetate (**5**), $C_{34}H_{48}O_7$, mp 183—184 °C, $[\alpha]_D$

Table 1. ^{13}C-NMR Chemical Shifts of **1**, **2**, **3**, **4** and **11** in Pyridine-d_5

	1	2	3	4	11
1	27.5	27.7	27.5	27.6	31.9
2	29.8	29.8	29.8	30.9	29.8
3	88.4	88.4	88.5	78.0	88.1
4	40.7	40.7	40.7	40.5	41.2
5	43.9	43.8	43.9	43.5	47.0
6	22.1	22.0	22.0	22.3	20.3
7	115.5	115.3	115.9	115.6	25.7
8	147.2	147.3	146.3	147.1	45.6
9	27.6	27.4	28.0	27.6	20.2
10	29.4	29.3	29.2	29.7	26.8
11	63.0	63.0	63.1	63.1	36.6
12	47.4	47.2	46.8	47.3	77.0
13	44.4	44.5	40.9	44.4	48.8
14	46.2	46.1	49.7	46.1	47.9
15	49.8	49.8	80.7	49.8	44.2
16	218.4	218.3	220.5	218.4	74.5
17	61.2	61.4	58.7	61.1	56.2
18	20.1	20.1	19.5	20.1	14.3
19	18.6	18.5	18.5	18.7	29.4
20	27.7	27.5	27.8	27.7	23.3
21	20.4	20.4	20.9	20.4	21.3
22	47.4	47.6	48.9	47.3	37.6
23	205.6	213.7	205.3	205.6	105.9
24	65.8	84.0	65.8	65.8	62.3
25	60.7	72.5	60.7	60.6	62.5
26	18.4	27.4	18.3	18.3	13.4
27	24.6	25.7	24.6	24.6	68.2
28	27.7	28.0	21.0	27.7	19.6
29	26.0	25.9	25.9	26.3	25.6
30	14.5	14.5	14.5	13.8	15.3
CH_3COO–					170.6
					21.6
Xyl-1	107.4	107.4	107.3		107.4
Xyl-2	75.5	75.4	75.4		75.5
Xyl-3	78.5	78.5	78.4		78.5
Xyl-4	71.2	71.2	71.1		71.2
Xyl-5	67.1	67.0	67.0		67.0

Xyl, β-D-xylopyranosyl.

+10.1°. The ^{1}H-NMR of **5** showed a signal due to two acetyl methyl groups at δ 2.06 ppm (6H, s), and the signals of cyclopropane methylene observed at δ 1.01 and 1.99 ppm (each d, J=4 Hz) in **4** showed an upfield shift to 1.00 and 1.60 ppm (each d, J=5 Hz) in **5**.[11b]

Finally, in order to confirm the structure of cimicifugoside H-1 (**1**), an X-ray crystallographic analysis of the diacetate (**5**) was carried out. The ORTEP[15] drawing is shown in Fig. 3.[16] The 20*R* and 24*R* configurations and location of the β-hydroxy group at C-11 were determined unambiguously.

From this evidence, cimicifugoside H-1 (**1**) was determined to be (20*R*,24*R*)-24,25-epoxy-11β-hydroxy-3β-(β-D-xylopyranosyloxy)-9,19-cyclolanost-7-ene-16,23-dione.

A second new xyloside **2**, $C_{35}H_{54}O_{10}$, mp 227—229 °C, $[\alpha]_D$ −38.8°, was isolated as colorless needles, and named cimicifugoside H-2. In the ^{13}C- and ^{1}H-NMR spectra of **2**, the chemical shifts were very similar to those of **1** (Tables 1 and 2). The differences observed in the ^{13}C-NMR spectra were in the chemical shifts ascribable to C-23, C-24 and C-25 (Table 1). In the ^{1}H-NMR spectra, there were some differences between **1** and **2**: the signals assignable to 22-H_2 [δ 3.62 and 2.61 ppm for **1**; 3.79 (dd, J=14, 3 Hz) and 3.44 (dd, J=14, 9 Hz) for **2**] and 24-H [δ 3.74 (s) ppm for **1**; 4.50 (s) for **2**]. The reducing nature of **2** was demonstrated by a positive coloration (blue) with alkaline blue tetrazolium reagent on TLC: this is consistent with an α-ketol system at C-23 and C-24 in the proposed structure for **2** (below).

Acetylation of **2** with acetic anhydride afforded a hexaacetate (**6**), mp 189—192 °C. The ^{1}H-NMR spectrum of **6** showed six singlets due to acetyl methyl groups (δ_H 1.97—2.15 ppm). The IR spectrum of **6** showed no absorption due to hydroxy group(s). These results indicate that **2** has six hydroxy groups (including three hydroxyls of the sugar moiety) in its molecule.

Enzymatic hydrolysis of **2** with Cellulase T [Amano] 4 in diluted acetic acid (pH 5) afforded a diketonic genin (**7**), mp 190—192 °C, $C_{30}H_{46}O_6$ and xylose. The ^{1}H-NMR spectrum of **7** showed the cyclopropane methylene signals at δ 0.96 and 2.00 ppm (each d, J=4 Hz), and one secondary and six tertiary methyl groups at δ 1.09—1.65 ppm. The ^{13}C-NMR spectrum showed thirty signals including two carbonyl signals at δ 218.5 (C-16) and 213.6 (C-23) ppm, trisubstituted double bond signals at δ 147.1 (C-8) and 115.3 (C-7) ppm, and four oxygenated carbon signals at δ 83.8 (C-24), 77.9 (C-3), 72.4 (C-25) and 62.9 (C-11) ppm.

From these results, the structure of **2** was presumed to be **2** (Chart 1), except for the absolute configuration at C-24.

To confirm the structure of cimicifugoside H-2 (**2**), the following reaction was employed. On treatment with *p*-toluenesulfonic acid in H_2O–MeOH (20 : 1), cimicifugoside H-1 (**1**) changed into a product, mp 226—228 °C, $[\alpha]_D$ −34.3°, which was identical with cimicifugoside H-2 (**2**). This transformation of **1** into **2** under acidic conditions can be explained as follows. The acid-catalyzed opening of the 24,25-epoxide ring of **1** proceeds through intermediate A in Chart 1, with retention of the configuration at C-24, and addition of a water molecule to the cationic center at C-25 followed by elimination of a proton affords **2**. Thus, the structure of cimicifugoside H-2 (**2**) including the configuration at C-24 was established as (20*R*,24*R*)-11β,24,25-trihydroxy-3β-(β-D-xylopyranosyloxy)-9,19-cyclolanost-7-ene-16,23-dione.

Cimicifugoside H-5 (**3**), $C_{35}H_{52}O_{10}$, mp 262—264 °C, $[\alpha]_D$ −22.9°, was isolated as colorless needles from aq. MeOH. The ^{1}H-NMR spectrum of **3** revealed the presence of a cyclopropane methylene, and one secondary and six tertiary methyl groups. The ^{1}H-NMR spectrum of **3** was very similar to that of **1**, but with two different signals [δ 6.17 (br d, J=6 Hz, 7-H) and 4.56 ppm (s, 15-H)].

The ^{13}C-NMR spectrum of **3** showed two ketonic signals at δ 220.5 (C-16) and 205.3 (C-23) ppm, a trisubstituted double bond at δ 146.3 (C-8) and 115.9 (C-7) ppm, and ten oxygenated carbon signals including those due to β-D-xylopyranose at δ 88.5 (C-3), 63.1 (C-11), 80.7 (C-15), 65.8 (C-24), 60.7 (C-25), 107.3 (xyl-1), 75.4 (xyl-2), 78.4 (xyl-3), 71.1 (xyl-4) and 67.0 (xyl-5). The ^{13}C-NMR spectrum was very similar to that of **1**, except for the signal assignable to C-15 (Table 1).

The ^{1}H–^{1}H COSY spectrum of **3** showed the same partial structures as those of **1**, except for partial structure e in Fig. 2. The reducing nature of **3** was also demonstrated by a positive coloration (blue) with alkaline blue

Table 2. ^{1}H-NMR Chemical Shifts of **1**, **2**, **3**, **4**, **10** and **11** in Pyridine-d_5

	1[a]	**2**[a]	**3**[a]	**4**[a]	**10**[a]	**11**[a]
1	1.72, 2.79	1.69, 2.80	1.72, 2.80	1.68, 2.78	1.15, 1.56	1.13, 1.52
2	2.08, 2.40	2.05, 2.40	2.05, 2.32	2.04, 2.04	1.80, 2.23	1.84, 2.23
3	3.59 dd (8, 4)	3.59 dd (8, 4)	3.58 m	3.62 m	3.42 dd (11, 4)	3.44 dd (11, 4)
5	1.34	1.35 dd (9, 4)	1.39	1.37	1.22	1.25
6	1.70, 1.92	1.70, 1.93	1.72, 1.98	1.78, 2.00	0.64, 1.43	0.64, 1.40
7	5.15 br d (6)	5.13 br d (6)	6.17 br d (6)	5.18 dd (7, 2)	0.90, 1.23	0.91, 1.22
8					1.59	1.61
11	4.58 m	4.53 m	4.59 m	4.58 m	1.20, 2.69 dd (16, 9)	1.18 dd (16, 3), 2.71 dd (16, 9)
12	2.18 dd (10, 4), 2.78	2.21, 2.78	2.22, 2.82	2.22 dd (14, 4), 2.84	5.05 dd (9, 4)	5.10 dd (9, 3)
15	2.37 d (18), 2.50 d (18)	2.26 d (18), 2.48 d (18)	4.56 s	2.31 d (18), 2.53 d (18)	1.53, 1.93	1.77, 1.89
16					4.57 dd (14, 7)	4.25 m
17	2.39	2.43	2.20	2.40 d (9)	1.72	1.79
18	1.21 s	1.22 s	1.30	1.24 s	1.34 s	1.46 s
19	0.96 d (4), 1.96 d (4)	0.94 d (4), 1.95 d (4)	1.02 d (4), 1.95 d (4)	1.01 d (4), 1.99 d (4)	0.25 d (4), 0.59 d (4)	0.27 d (4), 0.54 d (4)
20	2.61	2.40	2.65	2.63	1.80	2.21 m
21	1.04 d (6)	1.13 d (7)	1.05 d (6)	1.06 d (7)	0.97 d (6)	1.02 d (7)
22	2.61, 3.62	3.44 dd (14, 9), 3.79 dd (14, 3)	2.65, 3.62	2.63, 3.68 m	1.63, 2.20	1.43, 1.56
24	3.74 s	4.50 s	3.72 s	3.75 s	3.86 s	3.61 s
26	1.35 s	1.55 s	1.31 s	1.36 s	1.75 s	1.41 s
27	1.36 s	1.67 s	1.36 s	1.37 s	5.66 s	3.62 d (10), 4.05 d (10)
28	1.21 s	1.16 s	1.37 s	1.23 s	0.79 s	0 84 s
29	1.41 s	1.42 s	1.36 s	1.30 s	1.27 s	1.29 s
30	1.15 s	1.14 s	1.12 s	1.20 s	0.96 s	0.98 s
CH_3COO–					2.11 s	2.12 s
Xyl-1	4.88 d (7)	4.89 d (7)	4.82 d (7)		4.78 d (7)	4.82 d (7)
Xyl-2	4.02	4.04	3.98		3.94	4.00
Xyl-3	4.15	4.17	4.09		4.10	4.14
Xyl-4	4.22	4.24	4.15		4.18	4.21
Xyl-5	3.74 dd (10, 7), 4.34 dd (10, 4)	3.73 t (10), 4.35 dd (10, 4)	3.66, 4.33 dd (11, 5)		3.67 t (10), 4.30 dd (10, 5)	3.72 t (10), 4.35 dd (10, 5)

a) Signal assignments were based on ^{1}H–^{1}H COSY spectra.

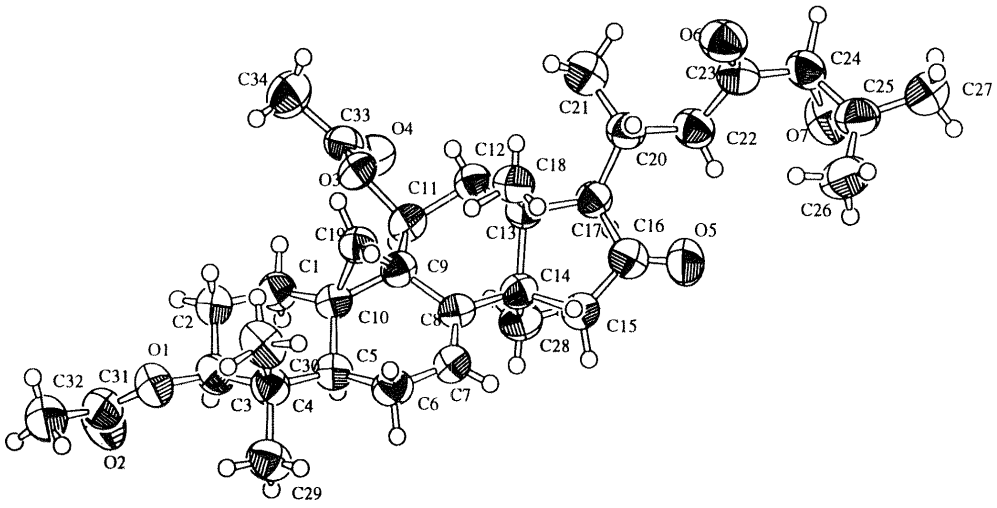

Fig. 3. ORTEP Drawing of the Diacetate of **4** (**5**) with Atomic Numbering

tetrazolium reagent on TLC. These findings suggested the location of a hydroxyl at C-15: an α-ketol structure at C-15 and C-16 would be consistent with the reducing nature of **3**. Thus, the structure was elucidated as **3** in Chart 1. Namely, **3**, designated cimicifugoside H-5, was determined to be (20*R*,24*R*)-24,25-epoxy-11β,15ξ-dihydroxy-3β-(β-D-xylopyranosyloxy)-9,19-cyclolanost-7-ene-16,23-dione.

Cimicifugosides H-1 (**1**), H-2 (**2**) and H-5 (**3**) are particular components, having a hydroxy group at C-11, of known *Cimicifuga* triterpenol glycosides. Cimicifugosides H-3 (**8**) and H-4 (**9**), whose genin is a trinor-triterpenol, also have a hydroxy group at C-11. The details of the structures of **8** and **9** will be presented elsewhere.

Actein (**10**)[17] and 27-deoxyactein (**11**)[18] have been isolated from *C. racemosa*, but no physical or ^{1}H- or ^{13}C-NMR spectral data for **11** have been reported. The proton signals of **10** have not been assigned, either. The data for **10** and **11** are given in the experimental section: all the proton and carbon signals were assigned on the basis of two-dimensional (2D) NMR spectra (Tables 1 and 2).

The plants of origin of the commercial Cimicifuga Rhizome on the Japanese market have been investigated on the basis of morphological study.[19] We were unable to detect cimicifugosides H-1 to H-5 in authentic specimens of the rhizomes of *C. dahurica* and *C. heracleifolia*.[20]

Experimental

General Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter at 25 °C. NMR spectra were recorded with JEOL JMN GX-270 and JEOL JMN GX-400 spectrometers. Tetramethylsilane was used as the internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants (J values) are given in Hz. Mass spectra (MS) were recorded with a JEOL JMS D-300 spectrometer, and FAB-MS with a JEOL JMS SX-102 spctrometer. Gas-liquid partition chromatography (GLC) was run on a Shimadzu GC-4A chromatograph with a hydrogen flame ionization detector. IR spectra were recorded with a Hitachi IR 260-10 spectrometer. UV spectra were recorded with a Shimadzu UV 250 spectrometer. Silica gel 60 F_{254} (Merck) precoated TLC plates were used, and detection was carried out by spraying 10% H_2SO_4 followed by heating. Preparative HPLC was performed using an ODS column (YMC-pack ODS-AQ, Nishio, 4.6 × 150 mm, detector: reflective index). The crude drug was purchased from Uchida Wakanyaku Co., Ltd. in 1991.

Isolation of Compounds 1, 2, 3, 10 and 11 An EtOAc-soluble fraction (160 g) of the MeOH extract (500 g) from Cimicifuga Rhizome (10 kg)[20] was applied to a column of silica gel with EtOAc–MeOH (98 : 2—0 : 1) to give fr. A_1—A_3. Fraction A_3, eluted with MeOH from the column, was rechromatographed on silica gel with $CHCl_3$–MeOH (9 : 1—0 : 1) to give fr. B_1—B_3. Fraction B_3, eluted with MeOH from the column, was rechromatographed on silica gel with EtOAc–acetone–MeOH–H_2O (20 : 3 : 1 : 1) to give fr. C_1—C_3. Fraction C_2 was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give fr. D_1—D_5. Fraction D_1 afforded **11** (0.8 g) and fr. D_2 afforded **10** (1 g). Fraction D_4 was rechromatographed on ODS (RP-18) with MeOH–H_2O (2 : 1) to afford fr. E_1—E_3. Fraction E_1 and E_2 gave **1** (0.8 g) and **2** (50 mg), respectively. On purification by preparative HPLC using octadecyl silica (ODS) column with aq. 50% MeOH, fr. E_3 (30 mg) gave **3** (24 mg).

Properties of Cimicifugoside H-1 (1) Colorless needles (MeOH), mp 260—262 °C. Optical rotatory dispersion (ORD) (c = 0.50, MeOH) [α] (nm): −43.5° (589), −47.2° (577), −56.1° (546), −134.2° (435), −357.1° (365). *Anal.* Calcd for $C_{35}H_{52}O_9$: C, 68.15; H, 8.50. Found: C, 67.87; H, 8.70. Alkaline blue tetrazolium reaction (on TLC): negative (no coloration). IR ν_{max}^{KBr} cm^{-1}: 3500—3300, 1040 (br OH), 1720, 1715 (C=O). UV λ_{max}^{MeOH} nm (ε): 205 (6275). Positive FAB-MS m/z: 639 $[M+Na]^+$. Positive HRFAB-MS Calcd for $C_{35}H_{52}O_9Na$ m/z: 639.3509. Found: 639.3508. EI-MS m/z: 598 $(M^+ - H_2O)$, 484 $(M^+ - C_5H_8O_4)$. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Enzymatic Hydrolysis of 1 A solution of **1** (200 mg) in a mixture of EtOH (10 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (40 ml) was treated with molsin (*Aspergillus saitoi*) (300 mg) in H_2O (10 ml), and the mixture was kept for 7 d with gentle stirring at 37 °C. The EtOH was removed *in vacuo* and the residue was extracted with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed over silica gel with benzene–EtOAc (1 : 1) to afford the diketonic genin (**4**) (115 mg). The water-soluble part was treated with Amberlite MB-3, and concentrated under reduced pressure. The residue was treated with $NaBH_4$ (*ca.* 3 mg) at room temperature under N_2 for 1 h. The reaction mixture was passed through an Amberlite MB-3 column and concentrated to dryness. Boric acid was removed by co-distillation with MeOH. The residue was acetylated with acetic anhydride (10 ml) and dry pyridine (2 ml) at room temperature overnight. The reagents were evaporated *in vacuo*. Alditol acetate was detected by GLC. GLC conditions: column, 2% OV-17 (support Gas-chrom Q) 3 mm × 2 m; column temperature, 200 °C; carrier gas, N_2. t_R: 7.4 min (xylitol acetate).

Properties of 4 Colorless needles (MeOH), mp 204—206 °C. ORD (c = 0.7, MeOH) [α] (nm): −31.4° (589), −57.3° (577), −66.8° (545), −148.6° (435), −397.9° (365). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (br OH), 1740, 1705 (C=O). UV λ_{max}^{MeOH} nm (ε): 205 (6214). EI-MS m/z: 484 (M^+), 466 $(M^+ - H_2O)$, 448 $(M^+ - 2H_2O)$. HREI-MS Calcd for $C_{30}H_{44}O_5$: 484.3187. Found 484.3192. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Acetylation of 4 A solution of **4** (20 mg) in acetic anhydride (5 ml) and dry pyridine (1 ml) was left standing at room temperature overnight. Usual work-up afforded **5** (5 mg), colorless needles, mp 183—184 °C (from MeOH). ORD (c = 0.51, $CHCl_3$) [α] (nm): +10.1° (589), −0.3° (577), −3.4° (546), −50.6° (435), −266.2° (365). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1720, 1705, 1240. UV λ_{max}^{MeOH} nm (ε): 203 (6638). EI-MS m/z: 568 (M^+), 508 $(M^+ - AcOH)$, 448 $(M^+ - 2AcOH)$. HREI-MS Calcd for $C_{32}H_{44}O_5$ m/z: 508.3189. Found: 508.3192. ^{1}H-NMR (pyridine-d_5) δ: 1.00, 1.60 (each d, J = 5 Hz, 19-H_2), 2.06 (6H, s, 2 × CH_3COO), 4.75 (dd, J = 11, 4 Hz, 3-H), 5.48 (dd, J = 10, 4 Hz, 11-H).

X-Ray Crystallographic Analysis[21] of 5 Prismatic crystals were grown from MeOH as orthorhombic crystals (mp 183—184 °C, $C_{34}H_{48}O_7$). Space group $P2_12_12_1$ with a = 13.582(3), b = 22.856(3), c = 10.297(3) Å, Z = 4, D_c = 1.182 g cm^{-3}. Intensity measurements were made with Cu$K\alpha$ radiation (λ = 1.542 Å: graphite monochromator) on a Rigaku AFC-7R diffractometer in the ω-2θ mode within $2\theta < 120.1°$. The structure was solved using MITHRIL 90[22] and refined by the full-matrix least-squares method using 2395 unique reflections with $I > 3\sigma(I)$. All hydrogen atoms were found on the difference electron-density map at the calculated locations. Anisotropic thermal parameters were used for all non-hydrogen atoms and parameters for all hydrogen atoms were fixed. The R-value was 0.056. Rw 0.049.

Properties of Cimicifugoside H-2 (2) Colorless needles, mp 227—229 °C (from MeOH). $[\alpha]_D$ −38.8° (c = 1.1, MeOH). Alkaline blue tetrazolium reaction (on TLC): positive (blue). Positive FAB-MS m/z: 635 $[M+H]^+$. Positive HR-FABMS Calcd for $C_{35}H_{55}O_{10}$ m/z: 635.3795. Found: 635.3788. IR ν_{max}^{KBr} cm^{-1}: 3400—3300 (OH), 1720, 1700 (C=O), 1650 (C=C). ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Acetylation of 2 A solution of **2** (10 mg) in acetic anhydride (7 ml) and dry pyridine (3 ml) was left standing at room temperature overnight. Usual work-up afforded the hexaacetate (**6**) (6 mg), colorless needles (from MeOH), mp 189—192 °C. IR ν_{max}^{KBr} cm^{-1}: 1740—1710, 1240 ($OCOCH_3$), 1595 (C=C). ^{1}H-NMR (pyridine-d_5) δ_H: 0.97, 1.82 (each 1H, d, J = 4 Hz, 19-H_2), 1.97, 1.98, 2.03, 2.05, 2.11, 2.15 (each 3H, 6 × CH_3COO), 5.12 (1H, br d, J = 6 Hz, 7-H).

Enzymatic Hydrolysis of 2 Compound **2** (20 mg) was dissolved in MeOH (2 ml) and 0.003% AcOH (about 40 ml) was added to the solution under stirring to adjust the pH to 5. Cellulase T [Amano] 4 (from *Trichoderma viride*) (50 mg) was added, and the mixture was stirred for 3 d at 30 °C, then shaken with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed over silica gel with benzene–EtOAc (5 : 2) to afford the diketonic genin (**7**) (7 mg), colorless needles, mp 190—192 °C (MeOH). Positive FAB-MS m/z: 503 $[M+H]^+$. Positive HRFAB-MS Calcd for $C_{30}H_{46}O_6Na$ m/z: 525.3192. Found 525.3162 $[M+Na]^+$. IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1725, 1700 (C=O). ^{1}H-NMR (pyridine-d_5) δ: 1.09 (3H, d, J = 8 Hz, 21-CH_3), 1.15, 1.16, 1.23, 1.27, 1.53, 1.65 (each 3H, s, 6 × CH_3), 0.96, 2.00 (each 1H, d, J = 4 Hz, 19-CH_2), 3.40 (1H, dd, J = 18, 8 Hz, 22-H), 3.60 (1H, m, 3-H), 3.81 (1H, dd, J = 18, 3 Hz, 22-H), 4.48 (1H, s, 24-H), 4.54 (1H, m, 11-H). ^{13}C-NMR (pyridine-d_5) δ: 27.6 (C-1), 30.6 (C-2), 77.9 (C-3), 40.3 (C-4), 43.4 (C-5), 22.1 (C-6), 115.3 (C-7), 147.1 (C-8), 27.4 (C-9), 29.5 (C-10), 62.9 (C-11), 47.0 (C-12), 44.3 (C-13), 45.9 (C-14), 49.6 (C-15), 218.5 (C-16), 61.2 (C-17), 20.0 (C-18), 18.5 (C-19), 27.2 (C-20), 20.2 (C-21), 47.4 (C-22), 213.6 (C-23), 83.8 (C-24), 72.4 (C-25), 27.5 (C-26), 25.5 (C-27), 27.7 (C-28), 26.1 (C-29), 13.6 (C-30). The water-soluble part was treated with Amberlite MB-3, and concentrated under reduced pressure. The residue was subjected to TLC with BuOH–AcOH–H_2O (6 : 1 : 2), and identified as xylose, Rf value: 0.37.

Treatment of 1 with *p*-Toluenesulfonic Acid Xyloside **1** (44 mg) in MeOH (2 ml) was treated with aqueous *p*-toluenesulfonic acid (pH 5) (about 40 ml) at room temperature for 5 d. Usual work-up afforded a mixture of **1** and **2**. The mixture was chromatographed on ODS (RP-18) with

MeOH–H_2O (2 : 1) to afford **2** (23 mg), colorless needles (MeOH), mp 226—228 °C, $[\alpha]_D$ −34.3° (c=0.62, MeOH) and unchanged **1** (15 mg). The product was identical with authentic **2** by TLC and ^{1}H- and ^{13}C-NMR spectral comparisons.

Properties of Cimicifugoside H-5 (3) Colorless needles, mp 262—264 °C (from MeOH–H_2O, 2 : 1). $[\alpha]_D$ −22.9° (c=1.9, MeOH). Alkaline blue tetrazolium reaction (on TLC): positive (blue). Positive FAB-MS m/z: 655 $[M+Na]^+$. Positive HRFAB-MS Calcd for $C_{35}H_{52}O_{10}Na$ m/z: 655.3458. Found: 655.3467. IR ν_{max}^{KBr} cm^{-1}: 3450—3350 (OH), 1740, 1725 (C=O). ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Properties of Actein (10) Colorless prisms (MeOH), mp 232—233 °C. $[\alpha]_D$ −66.0° (c=1.6, $CHCl_3$–MeOH, 1 : 1). IR ν_{max}^{KBr} cm^{-1}: 3500—3350 (OH), 1715 (C=O). ^{1}H-NMR: Table 2.

Properties of 27-Deoxyactein (11) Colorless prisms (from MeOH), mp 252—254 °C. ORD (c=1.4, $CHCl_3$–MeOH (1 : 1)) [α] (nm): −44.3° (589), −48.8° (577), −55.7° (546), −92.6° (435), −142.5° (365). Positive FAB-MS m/z: 661 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3550—3400 (OH), 1715, 1250 (CH_3COO). ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Enzymatic Hydrolysis of 11 A solution of **11** (300 mg) in a mixture of EtOH (100 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (250 ml) was treated with molsin (*Aspergillus saitoi*) (300 mg) in H_2O (100 mg), and the mixture was kept for 3 d with gentle stirring at 37 °C. Usual work-up afforded an aglycone **12** (150 mg), $C_{32}H_{48}O_6$, colorless prisms (from MeOH), mp 289—291 °C. ORD (c=1.9, $CHCl_3$) [α] (nm): −82.1° (589), −85.8° (577), −97.1° (546), −162.8° (435), −251.4° (365). ^{1}H-NMR (pyridine-d_5) δ: 2.14 (3H, s, CH_3COO), 2.76 (1H, dd, J=16, 9 Hz, 11-H), 3.47 (1H, dd, J=12, 4 Hz, 3-H), 3.62, 4.06 (each 1H, d, J=10 Hz, 27-H_2), 4.24 (1H, dd, J=14, 8 Hz, 16-H), 5.12 (1H, dd, J=9, 4 Hz, 12-H). The aglycone **12** was identified as 27-deoxyacetylacteol by comparison of the data with those given in the literature.[23]

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