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Constituents of Cimicifugae Rhizoma. I. Isolation and Characterization of Ten New Cycloartenol Triterpenes from *Cimicifuga heracleifolia* KOMAROV

Jian Xin LI,[a] Shigetoshi KADOTA,*[,a] Masao HATTORI,[a] Sadao YOSHIMACHI,[b] Motoo SHIRO,[b] Naoko OOGAMI,[c] Hiroshi MIZUNO[c] and Tsuneo NAMBA[a]

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University,[a] 2630 Sugitani, Toyama 930–01, Japan, Research and Development Division, Rigaku Industrial Corporation,[b] Akishima, Tokyo 196, Japan, and National Institute of Agrobiological Resources,[c] Tsukuba Science City, Ibaragi 305, Japan. Received September 11, 1992

Ten new highly oxidized cycloartane-type triterpenoids, 24-*epi*-7,8-didehydrocimigenol, 7,8-didehydrocimigenol, 25-*O*-acetyl-7,8-didehydrocimigenol, 3-keto-24-*epi*-7,8-didehydrocimigenol, 2′,4′-*O*-diacetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside, 3′-*O*-acetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside, 24-*epi*-7,8-didehydrocimigenol-3-xyloside, 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside, 24-*epi*-acerinol and heracleifolinol, were isolated from the rhizome of *Cimicifuga heracleifolia* KOMAROV and their structures were determined by the use of two-dimensional nuclear magnetic resonance (2D NMR) techniques (^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C COSY, ^{1}H–^{13}C long-range COSY) and by X-ray diffraction analysis of the 3-keto compound.

Keywords *Cimicifuga heracleifolia*; Ranunculaceae; cycloartane-type triterpene; 24-*epi*-7,8-didehydrocimigenol; 2D NMR

The rhizome of *Cimicifuga heracleifolia* KOMAROV (Ranunculaceae) has been widely used in traditional Chinese medicine as an anti-inflammatory drug to relieve fever.[1)] Chemical constituents of *Cimicifuga* species have been studied by several groups, and isolation and identification of trieterpenes,[2)] sterols,[3)] phenolic acids,[4)] chromones[5)] and yellow pigments[6)] were reported. However, no paper has been published so far on the constituents of *C. heracleifolia*. In the present paper, we wish to present the isolation and structure elucidation of ten new triterpenes: 24-*epi*-7,8-didehydrocimigenol (**1**), 7,8-didehydrocimigenol (**2**), 25-*O*-acetyl-7,8-didehydrocimigenol (**3**), 3-keto-24-*epi*-7,8-didehydrocimigenol (**4**), 2′,4′-*O*-diacetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside (**5**), 3′-*O*-acetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside (**6**), 24-*epi*-7,8-didehydrocimigenol-3-xyloside (**7**), 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside (**8**), 24-*epi*-acerinol (**9**) and heracleifolinol (**10**).

Air-dried rhizome of *C. heracleifolia*, grown in Heilong-

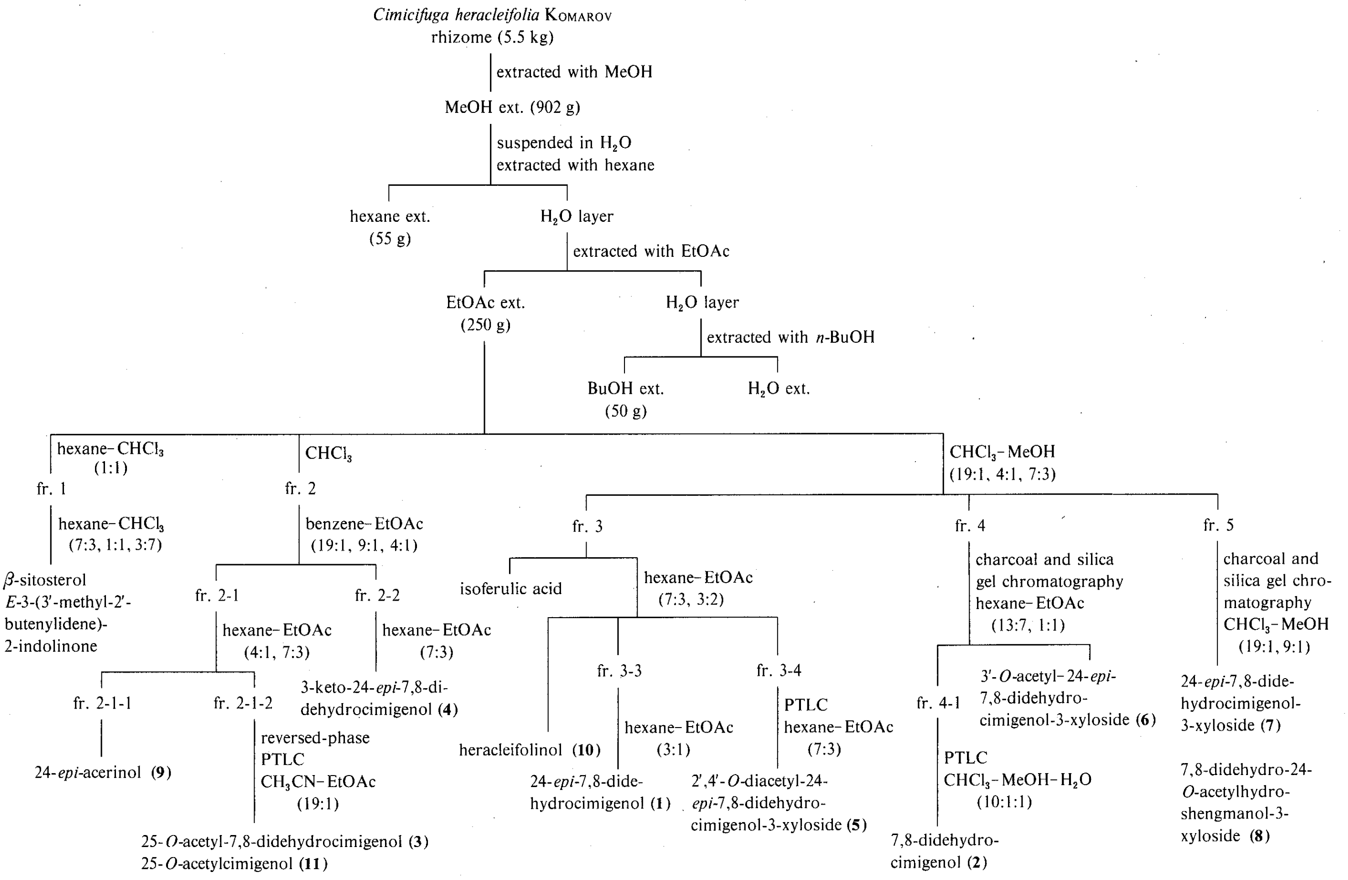

Chart 1

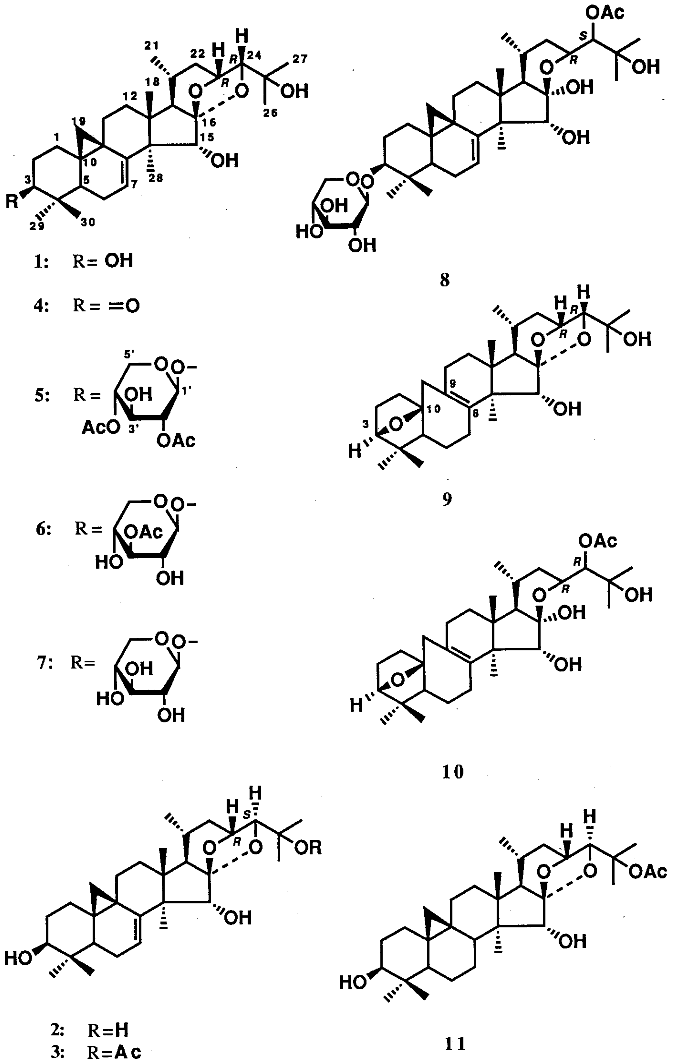

Chart 2

jiang province of China, was pulverized and extracted with hot methanol. The methanol extract was roughly separated into hexane-, EtOAc- and *n*-BuOH-soluble fractions. As shown in Chart 1, from the EtOAc extract, ten new cycloartenol triterpenoids (**1**—**10**) were isolated together with four known compounds: β-sitosterol, isoferulic acid, *E*-3-(3′-methyl-2′-butenylidene)-2-indolinone[6] and 25-*O*-acetylcimigenol (**11**).[7] The structures of **1**—**10** were determined as described below.

Compound **1** was isolated as colorless needles, mp 222—223 °C, and showed $[\alpha]_D$ +6.36° ($CHCl_3$). In the electron impact mass spectrum (EI-MS), it showed a molecular ion peak at *m/z* 486 along with fragment ion peaks at *m/z* 468 ($M^+ - H_2O$) and 453, and its molecular formula was determined to be $C_{30}H_{46}O_5$ by high-resolution EI-MS. The infrared (IR) spectrum of **1** showed absorption bands at 3450 (OH) and 1620 cm^{-1} (C=C). The proton nuclear magnetic resonance (^1H-NMR) spectrum of **1**, analyzed with the aid of ^1H–^1H shift correlation spectroscopy (COSY), showed signals due to a cyclopropane methylene (δ_H 0.55 and 1.09, each d, J=4.0 Hz), one double bond proton (δ_H 5.62), four methine protons on carbon substituted by oxygen (δ_H 4.45, 4.04, 3.57, 3.33), and a secondary methyl group (δ_H 0.9, d, J=6.6 Hz), along with six *tert*-methyl groups (Fig. 1). From these data, we considered that **1** was a highly oxygenated cyclotriterpenoid. Based on a comparison of the ^1H- and ^{13}C-NMR spectral data with those reported in the literature[8,9] for a cycloartane-type triterpene isolated from another plant species, we speculated that the structure of **1** should be very similar to that of cimigenol, but the ^1H- and ^{13}C-NMR spectra of **1** clearly showed characteristic signals due to a double bond, which did not appear in cimigenol. Hence, **1** was suggested to be didehydrocimigenol, a conclusion which was also supported by the ^1H–^{13}C COSY spectrum.

Next, we measured the ^1H–^{13}C long-range COSY spectrum of **1** in order to assign all the signals due to protons and carbons. As shown in Fig. 2, the carbon signals at δ_C 146.88 (C-8) and δ_C 50.41 (C-14) are correlated with proton signals at δ_H 1.06 (28-H_3) and 1.20 (11-H), and at δ_H 5.62 (7-H), respectively. The signal at δ_C 112.09 (C-16) is correlated with proton signals at δ_H 1.30 (17-H), 4.04 (15-H) and 4.45 (23-H), and the carbon signal at δ_C 83.25 (C-24) is correlated with proton signals at δ_H 1.22 (27-H_3) and 1.33 (26-H_3). Some other long-range correlations observed are also shown by arrow. From this experiment, the double bond could be assigned to the 7,8-position.

The relative stereochemistry of **1** was determined on the basis of the coupling constants of each proton (Table I) and nuclear Overhauser effect (NOE) experiments. As shown in Fig. 3, irradiation at 30-H_3 and 18-H_3 increased the signal intensities of the 19-, 6-, and 2-protons and of the 17-, 12-, and 15-protons, respectively, and irradiation at 29-H_3 and 28-H_3 enhanced the signal intensities of the 5-, 6-, and 3-protons and of the 15-OH and 17-protons, respectively. Also, irradiation at 27-H_3 and 26-H_3 gave NOE enhancement of the 22-, 24-, and 23-protons and the 24-protons, respectively. The configuration at the C-24 position of **1** could be assigned as 24*R* by comparing the coupling constant of the C-23 and C-24 proton signals of **1** with those of 24-epimeric triterpenes.[10] The structure of **1** was consequently assigned as 24-*epi*-7,8-didehydrocimigenol.

Compound **2** was obtained as a white powder. It revealed the molecular ion peak at *m/z* 486 (M^+) in the MS and its molecular formula was determined to be $C_{30}H_{46}O_5$ by high-resolution MS. It showed a similar IR spectral pattern to that of **1**, suggesting that the structure of **2** may be similar to that of **1**. The ^1H-NMR spectrum of **2** was also quite similar to that of **1**, but some changes were observed in chemical shifts and splitting patterns of ^1H-signals; a methylene at δ_H 0.97 (m) and 2.33 (ddd, J=13.0, 10.0, 3.0 Hz), carbinol methines at δ_H 4.12 (d, J=7.1 Hz) and 3.46 (d, J=0.5 Hz), and a carbinol methine at δ_H 4.48 (dd, J=10.0, 1.8 Hz), which were assignable to the 22-, 15-, 24-, and 23-protons, respectively (Table I). The ^{13}C-NMR spectrum of **2** was also similar to that of **1** except for the chemical shifts due to C-15, C-22, C-23, C-24, C-25, C-26 and C-27 (Table II). The C-15 hydroxy-bearing methine signal showed NOE enhancement on irradiation of the 18-methyl protons (δ_H 1.03), indicating that the 15-hydroxy group is also in α-configuration. Based on these findings, the structure of **2** was assigned as 7,8-didehydrocimigenol.

Compound **3**, a white powder, is a minor constitutent of the rhizome. It exhibited the molecular ion peak at *m/z* 528 (M^+) in the EI-MS and its molecular formula was deter-

TABLE I. ^{1}H-NMR Spectral Data for Triterpenoids from *Cimicifuga heracleifolia*

Proton	1[a)]	2[a)]	3[a)]	4[b)]	5[a)]	6[b)]	7[b)]	8[b)]	9[a)]	10[a)]	11[a)]
1	1.37 m	1.36 m	1.58 m	1.57 m	1.35 m	1.35 ddd (12.5, 4.1, 3.0)	1.47 m	1.34 m	2.45 m	2.30 m	1.96 m
	1.72 m	1.73 m	1.72 m	1.83 td (13.5, 4.5)	1.69 m	1.63 m	1.68 m	1.69 m		2.49 m	
2	1.68 ddd (13.0, 4.0, 2.2)	1.67 m	1.66 m	2.30 ddd (13.5, 4.5, 2.0)	1.75 m	1.95 m	1.96 m	1.95 m	1.59 m	1.51 m	1.22 m
	1.75 m	1.75 m	1.75 m	2.78 td (13.5, 6.5)	1.93 m	2.29 ddd (12.5, 4.1, 3.0)	2.31 ddd (12.5, 4.3, 3.0)	2.29 ddd (12.0, 4.0, 3.0)	1.70 m	1.70 m	1.58 m
3	3.33 dd (11.4, 4.0)	3.32 dd (11.2, 3.6)	3.33 dd (11.0, 4.0)	—	3.20 dd (11.4, 4.0)	3.45 dd (11.4, 4.1)	3.46 dd (11.6, 4.3)	3.44 dd (11.6, 4.0)	3.73 d (5.5)	3.73 d (5.5)	3.29 dd (11.6, 4.6)
5	1.25 m	1.24 m	1.24 m	1.62 m	1.25 m	1.28 m	1.30 m	1.24 m	1.20 m	1.18 d (12.0)	1.30 m
6	1.63 m	1.63 m	1.63 m	1.74 m	1.65 m	1.59 m	1.60 m	1.61 m	1.73 m	1.72 m	1.32 m
	2.01 ddd (13.0, 7.5, 2.2)	1.99 ddd (17.0, 7.5, 5.5)	1.97 m		1.79 m	1.88 m	1.91 m	1.88 m	1.92 m	1.93 ddd (12.0, 7.5, 4.5)	1.61 m
7	5.62 dd (7.5, 2.2)	5.64 dd (7.5, 2.1)	5.62 dd (7.5, 2.2)	6.05 dd (7.4, 2.0)	5.60 dd (7.5, 2.2)	6.05 dd (7.4, 1.5)	6.04 dd (7.4, 2.0)	6.06 dd (7.5, 1.5)	1.41 td (12.0, 4.5)	1.38 td (12.0, 4.5)	0.80 m
	—	—	—	—	—	—	—	—	1.62 m	1.58 m	1.76 m
8	—	—	—	—	—	—	—	—	—	—	1.63 m
11	1.20 m	1.19 m	1.15 m	1.38 m	1.20 m	1.13 m	1.12 m	1.14 m	2.10 m	2.09 m	1.04 m
	2.13 ddd (13.0, 7.0, 3.5)	2.16 ddd (13.0, 10.0, 4.0)	2.15 m	2.09 m	2.15 m	2.12 ddd (13.0, 8.0, 2.8)	2.12 ddd (13.0, 9.0, 3.0)	2.14 ddd (12.5, 9.0, 4.0)	1.70 m		1.69 m
12	1.70 m	1.69 m	1.67 m	1.70 m	1.71 m	1.67 m	1.67 m	1.66 m	1.54 m	1.47 m	1.67 m
	1.77 ddd (13.0, 8.0, 3.5)	1.79 m	1.79 m	1.78 m	1.77 m		1.71 m	1.77 m	1.86 m	1.85 m	1.79 m
15	4.04 d (8.4)	4.12 d (7.1)	4.12 d (8.4)	4.05 s	4.03 d (7.9)	4.49 d (7.5)	4.48 d (7.5)	4.26 d (7.5)	4.00 d (8.1)	3.89 d (6.0)	3.19 d (8.4)
15-OH	2.78 d (8.4)	2.62 d (7.1)	2.73 d (8.4)	—	2.76 d (7.9)	4.58 d (7.5)	4.52 d (7.5)	4.63 d (7.5)	2.75 d (8.1)	4.09 d (6.0)	2.64 d (8.4)
16-OH	—	—	—	—	—	—	—	8.47 s	—	4.92 s	—
17	1.30 d (6.6)	1.38 d (6.7)	1.37 m	1.74 d (6.8)	1.32 d (6.6)	1.73 m	1.73 m	1.49 m	1.80 d (11.0)	1.58 m	1.38 m
19	0.55 d (4.0)	0.54 d (4.3)	0.54 d (4.0)	0.71 d (4.0)	0.55 d (4.9)	0.51 d (4.0)	0.51 d (4.0)	0.50 d (4.0)	1.68 d (13.4)	1.65 d (13.4)	0.37 d (4.3)
	1.09 d (4.0)	1.11 m	1.07 d (4.0)	1.22 d (4.0)	1.08 d (4.9)	1.06 d (4.0)	1.06 d (4.0)	1.05 d (4.0)	3.14 d (13.4)	3.10 d (13.4)	0.63 d (4.3)
20	1.60 m	1.67 m	1.62 m	1.75 m	1.60 m	1.72 m	1.70 m	1.74 m	1.61 m	1.58 m	1.62 m
22	1.96 ddd (13.0, 6.6, 2.2)	0.97 m	1.01 m	1.98 ddd (13.0, 6.0, 2.5)	1.95 m	1.95 m	1.96 m	1.55 m	1.96 m	1.56 m	1.00 m
	2.15 ddd (13.0, 10.0, 2.2)	2.33 ddd (13.0, 10.0, 3.0)	2.32 ddd (13.0, 6.5, 2.5)	2.65 m	2.13 m	2.64 ddd (13.0, 9.7, 2.1)	2.61 ddd (13.0, 9.7, 2.0)	1.91 m	2.15 ddd (13.0, 9.5, 2.5)	1.70 m	2.34 ddd (13.0, 7.5, 2.5)
23	4.45 ddd (10.0, 4.2, 2.2)	4.48 dd (10.0, 1.8)	4.39 br d (10.0)	4.62 ddd (9.5, 4.2, 2.5)	4.44 ddd (10.0, 4.0, 2.2)	4.60 ddd (9.7, 4.1, 2.1)	4.60 ddd (9.7, 4.1, 2.0)	4.18 t (8.8)	4.45 ddd (9.5, 4.2, 2.5)	4.07 m	4.38 br d (9.2)
24	3.57 d (4.2)	3.46 d (0.5)	3.92 s	3.73 d (4.2)	3.57 d (4.0)	3.72 d (4.1)	3.71 d (4.1)	4.82 d (2.0)	3.57 d (4.2)	5.10 d (9.5)	3.88 s
18[c)]	1.03 s	1.03 s	1.03 s	1.19 s	1.02 s	1.18 s	1.16 s	1.18 s	0.88 s	0.81 s	1.09 s
21[c)]	0.90 d (6.6)	0.91 d (6.7)	0.90 d (7.0)	0.98 d (5.5)	0.89 d (6.6)	0.98 d (5.8)	0.98 d (5.5)	0.96 d (6.7)	0.91 d (6.6)	1.00 d (6.4)	0.88 d (7.2)
26[c)]	1.33 s	1.20 s	1.48 s	1.43 s	1.33 s	1.42 s	1.41 s	1.69 s	1.31 s	1.29 s	1.47 s
27[c)]	1.22 s	1.20 s	1.42 s	1.29 s	1.22 s	1.28 s	1.31 s	1.49 s	1.21 s	1.13 s	1.41 s
28[c)]	1.06 s	1.09 s	1.06 s	1.26 s	1.05 s	1.26 s	1.27 s	1.34 s	0.95 s	0.88 s	0.95 s
29[c)]	1.01 s	1.01 s	1.01 s	1.07 s	0.97 s	1.28 s	1.28 s	1.29 s	1.01 s	1.02 s	1.00 s
30[c)]	0.85 s	0.86 s	0.85 s	1.11 s	0.85 s	1.00 s	1.05 s	1.04 s	0.92 s	0.88 s	0.81 s
1′	—	—	—	—	4.60 d (5.9)	4.81 d (7.3)	4.79 d (7.3)	4.76 d (7.3)	—	—	—
2′	—	—	—	—	4.85 dd (7.0, 5.9)	4.01 dd (9.2, 7.3)	3.96 dd (8.5, 7.3)	3.94 dd (8.6, 7.3)	—	—	—
3′	—	—	—	—	3.78 m	5.68 t (9.2)	4.08 t (8.5)	4.06 t (8.6)	—	—	—
3′-OH	—	—	—	—	2.91 s	—	—	—	—	—	—
4′	—	—	—	—	4.82 td (7.0, 4.0)	4.20 td (9.2, 5.2)	4.17 td (8.5, 5.2)	4.15 td (8.6, 5.2)	—	—	—
5′	—	—	—	—	3.39 dd (12.0, 7.0)	3.96 t (10.7)	3.67 t (10.1)	3.65 t (10.1)	—	—	—
	—	—	—	—	4.15 dd (12.0, 4.0)	4.31 dd (10.7, 5.2)	4.31 dd (10.1, 5.2)	4.29 dd (10.1, 5.2)	—	—	—
$COCH_3$	—	—	1.99 s	—	2.11 s	1.98 s	—	2.02 s	—	2.05 s	1.99 s
$COCH_3$	—	—	—	—	2.13 s	—	—	—	—	—	—

^{1}H–^{1}H shift correlation spectra were measured. δ value in *a)* $CDCl_3$ or *b)* pyridine-d_5 and coupling constants in Hz. *c)* Assignments were confirmed by NOE experiments.

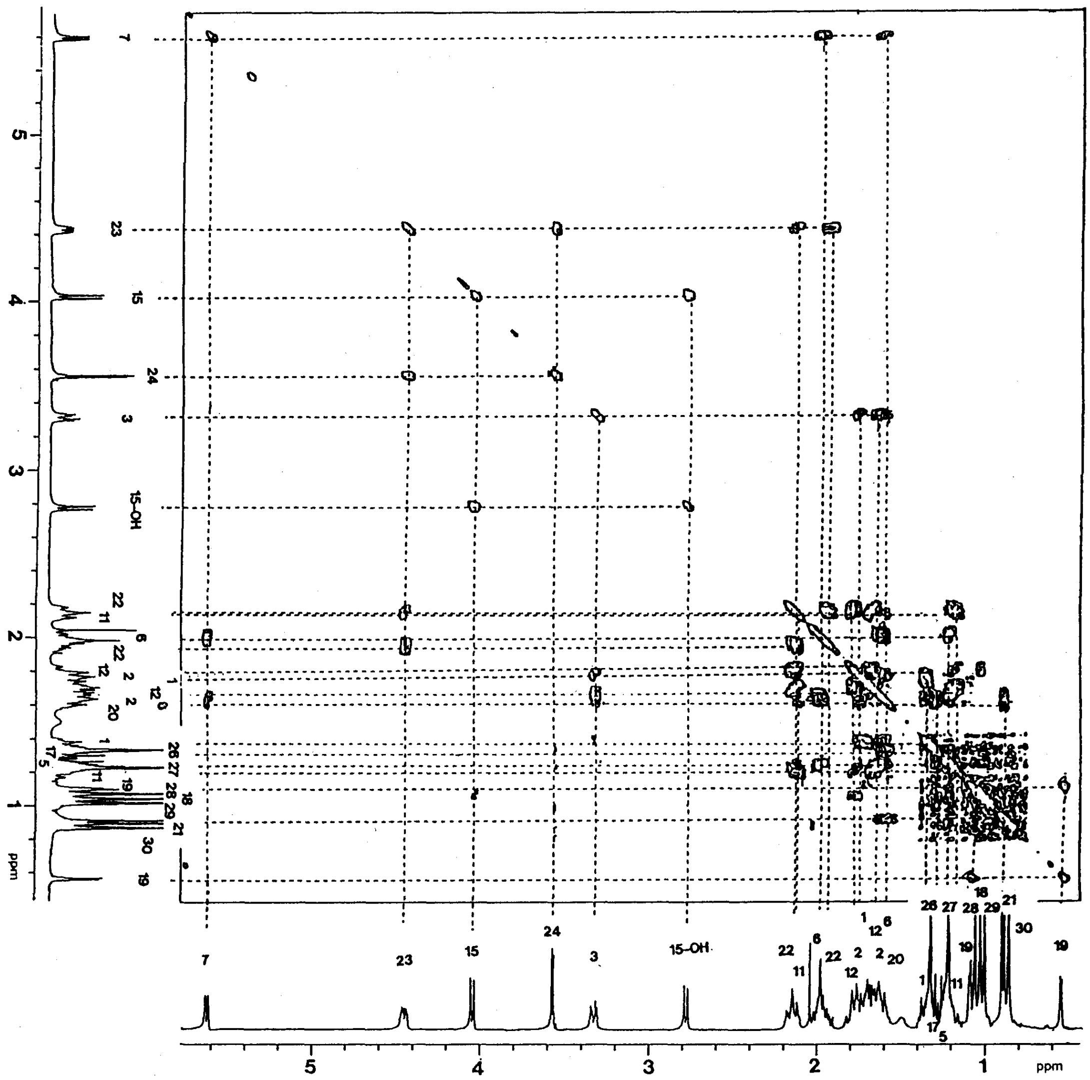

Fig. 1. Contour Map of the ^{1}H–^{1}H COSY Spectrum of 24-*epi*-7,8-Didehydrocimigenol (**1**) in $CDCl_3$

mined to be $C_{32}H_{48}O_6$ by high-resolution MS. The ^{1}H-NMR spectrum resembled that of **2** except for the signals attributable to an acetyl group in **2** (see Table I). When the ^{13}C-NMR spectrum of **3** was compared with those of **1** and **2**, the chemical shifts due to the 24-, 25-, 26-, 27- carbon signals of **3** were distinctly different from those of **1** and **2** (Table II), and the acetyl group was suggested to be attached at the 25-position, in place of a hydroxyl group. Based on these findings, **3** was determined to be 25-*O*-acetyl-7,8-didehydrocimigenol.

Compound **4**, mp 225—226 °C, $[\alpha]_D$ $-12.4°$ ($CHCl_3$), showed a hydroxyl band (3470 cm^{-1}) and a strong carbonyl band (1720 cm^{-1}) in the IR spectrum. The ^{1}H- and ^{13}C-NMR spectral patterns were similar to those of **1**, **2** and **3** (Tables I and II). However the signals due to a methine proton at low field (assigned to 3-H of **1**, **2** and **3**) were not observed in the ^{1}H-NMR spectrum of **4** and the signals of methylene protons (2-H_2) were shifted to low field (Table I). In the ^{13}C-NMR spectrum, the spectral pattern was quite similar to that of **1**, except for an additional carbonyl carbon signal (δ_C 216.34) in place of a signal near δ_C 78 (assigned to carbon bonding with oxygen), suggesting that **4** may be an oxo derivative of **1**. From the NMR spectral data, there was still ambiguity as to whether the carbonyl group should be assigned to the 3- or 15-position, although the ^{1}H–^{13}C long-range COSY experiment was in favor of the 3-position.

The structure was definitively proved by single-crystal X-ray analysis using the direct method. As shown in Fig. 4, the stereochemistry was in agreement with that deduced by NMR spectroscopy. The ORTEP[11] drawing of **4** shows thermal ellipsoids of non-H atoms and the atomic numbering. The final positional parameters are given in Table III. Interatomic bond distances and angles are listed in Tables IV and V. An intermolecular short contact O–H···O is observed [O2···O4 = 2.615 (5) Å], possibly representing a hydrogen bond, although its geometry is distorted [O2–H···O4 = 97°]. Figure 5 shows the molecular packing of **4**. Intermolecular contacts are mainly based on hydrophobic interaction. Short contact is observed only between O3 and O5 of the neighboring molecule (symmetry operation: $x, y, z-1$) [O3···O5 = 2.775 (4) Å]. From these findings, **4** was determined to be 3-keto-24-*epi*-7,8-didehydrocimigenol.

Compound **5** was obtained as a white powder and showed $[\alpha]_D$ $-15.5°$ ($CHCl_3$). It showed IR absorption bands at 3550 (OH), 1740 (C=O) and 1635 cm^{-1} (C=C). The positive ion FAB-MS exhibited an $[M+H]^+$ peak at *m/z*

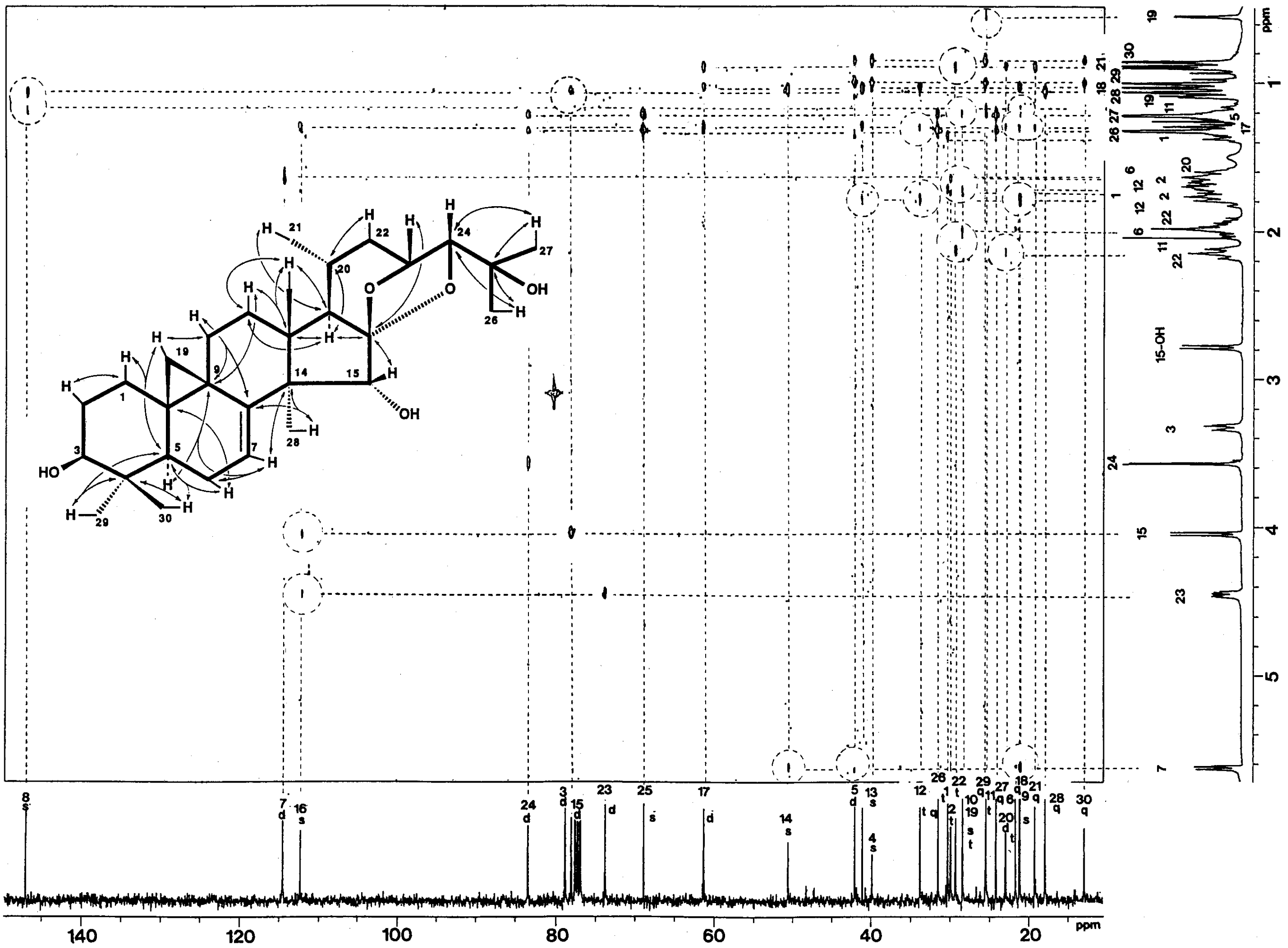

Fig. 2. Contour Map of the ^{1}H–^{13}C Long-Range COSY Spectrum of 24-*epi*-7,8-Didehydrocimigenol (**1**) in $CDCl_3$ ($J_{CH}=10$ Hz)

The multiplicities of carbon signals were determined by means of distortionless enhancement by polarization transfer (DEPT) and are indicated as s, d, t, and q.

703. The ^{1}H- and ^{13}C-NMR spectra of **5** showed very close similarity to those of **1**. However, additional signals due to a five membered sugar and two acetyl groups were observed. The sugar was identified as β-D-xylose by acid hydrolysis followed by GC analysis after trimethylsilylation. Furthermore, by analysis of the ^{1}H- and ^{13}C-NMR spectra of **5** with the aid of ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY, **5** was found to be the diacetyl xyloside of **1**. In the ^{1}H–^{13}C long-range COSY spectrum, the carbon signal due to C-3 (δ_C 89.23) showed long-range correlation with a signal due to an anomeric proton (δ_H 4.60), which suggested that xylose was substituted at the 3-position. In addition, the signals due to 2′- and 4′-H (δ_H 4.85 and 4.82, respectively) were shifted downfield, suggesting the presence of two acetoxy groups at the 2′- and 4′-positions (Table I). On the basis of these findings, the structure of **5** was assigned as 2′,4′-*O*-diacetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside.

Similarly, **6** and **7** were determined as 3′-*O*-acetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside and 24-*epi*-7,8-didehydrocimigenol-3-xyloside, respectively. Assignments of ^{1}H- and ^{13}C-NMR signals of these compounds are shown in Tables I and II.

Compound **8** was obtained as a white powder and showed $[\alpha]_D$ $-27.4°$ ($CHCl_3$: MeOH $=2:3$). The molecular formula for **8** was supposed to be $C_{37}H_{58}O_{11}$ on the basis of the positive ion FAB-MS, which exhibited an $[M+H]^+$ peak at *m/z* 679. The ^{1}H- and ^{13}C-NMR spectra of **8** closely resembled those of **7**, except for the presence of ^{1}H- and ^{13}C-signals due to an acetyl group in **8** and a few signals assignable to a part of the D ring and side chain carbons (C-15, C-16, C-24 and C-25) (Tables I and II). This suggested that **8** might be a 24-acetoxy hemiacetal derivative of **7**. Furthermore, irradiation at 24-H and 18-H_3 enhanced the signal intensities of the 16-OH, 23-, and 27-protons and the 15-, 11β-, and 20β-protons, respectively. The configuration at the C-24 position was deduced to be *S* on the basis of the coupling constant of 24-H (δ_H 4.82, d, $J=2.0$ Hz), which is comparable with that of isodahurinyl diacetate (δ_H 4.75, d, $J=2.5$ Hz).[12] Thus, **8** was determined to be 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside.

Compound **9** was obtained as colorless needles, mp 230—231 °C, and showed $[\alpha]_D$ $+59.23°$ ($CHCl_3$). It exhibited a molecular ion peak at *m/z* 486 (M^+) in the MS and its molecular formula was determined to be $C_{30}H_{46}O_5$ by high-resolution MS. The ^{1}H-NMR spectrum was almost identical with that of acerinol with 24(*S*) configuration.[13] The ^{1}H-NMR data for **9** differed only in the coupling constant of 24-H (δ_H 3.57, d, $J=4.2$ Hz) from those described in the literature.[13] This suggested that **9** has 24(*R*) configuration and is an epimer of acerinol (24*S*). The

TABLE II. ^{13}C-NMR Spectral Data for Triterpenoids from *Cimicifuga heracleifolia*

^{13}C	**1**$^{a,c)}$	**2**$^{a,e)}$	**3**$^{a,e)}$	**4**$^{a,c)}$	**5**$^{a,c)}$	**6**$^{b,d)}$	**7**$^{b,d)}$	**8**$^{b,c)}$	**9**$^{a,d)}$	**10**$^{a,c)}$	**11**$^{a,e)}$
1	30.13 (t)	30.16 (t)	30.37 (t)	31.68 (t)	30.00 (t)	30.31 (t)	30.31 (t)	30.34 (t)	30.67 (t)	30.76 (t)	32.13 (t)
2	29.73 (t)	29.76 (t)	29.75 (t)	36.81 (t)	28.52 (t)	29.43 (t)	29.46 (t)	29.52 (t)	22.82 (t)	22.78 (t)	30.13 (t)
3	78.76 (d)	78.61 (d)	78.67 (d)	216.34 (s)	89.23 (d)	88.54 (d)	88.23 (d)	88.20 (d)	84.96 (d)	84.95 (d)	78.79 (d)
4	39.66 (s)	39.72 (s)	39.71 (s)	48.89 (s)	39.92 (s)	40.33 (s)	40.36 (s)	40.39 (s)	41.08 (s)	45.15 (s)	40.52 (s)
5	41.88 (d)	41.88 (d)	41.86 (d)	43.86 (d)	42.18 (d)	42.70 (d)	42.70 (d)	42.60 (d)	54.67 (d)	54.29 (d)	47.04 (d)
6	21.60 (t)	21.63 (t)	21.61 (t)	21.73 (t)	21.44 (t)	21.69 (t)	21.69 (t)	21.75 (t)	25.47 (t)	25.45 (t)	20.93 (t)
7	114.31 (d)	114.34 (d)	114.20 (d)	114.26 (d)	114.16 (d)	114.28 (d)	114.22 (d)	113.95 (d)	36.25 (t)	36.08 (t)	26.07 (t)
8	146.88 (s)	146.79 (s)	146.90 (s)	146.79 (s)	146.93 (s)	148.04 (s)	148.01 (s)	148.28 (s)	136.64 (s)	137.05 (s)	48.19 (d)
9	21.08 (s)	21.02 (s)	21.02 (s)	26.36 (s)	21.11 (s)	21.23 (s)	21.20 (s)	21.17 (s)	123.80 (s)	123.48 (s)	19.85 (s)
10	28.25 (s)	28.31 (s)	28.29 (s)	28.09 (s)	27.99 (s)	28.18 (s)	28.18 (s)	28.22 (s)	89.77 (s)	89.75 (s)	26.55 (s)
11	25.30 (t)	25.36 (t)	25.33 (t)	25.51 (t)	25.30 (t)	25.51 (t)	25.48 (t)	25.42 (t)	30.94 (t)	30.80 (t)	26.20 (t)
12	33.59 (t)	33.68 (t)	33.71 (t)	33.48 (t)	33.59 (t)	33.89 (t)	33.86 (t)	34.10 (t)	31.87 (t)	31.68 (t)	33.71 (t)
13	40.90 (s)	41.15 (s)	41.86 (s)	40.81 (s)	40.90 (s)	41.06 (s)	41.06 (s)	40.15 (s)	45.19 (s)	41.24 (s)	41.52 (s)
14	50.41 (s)	50.16 (s)	50.24 (s)	50.25 (s)	50.41 (s)	50.77 (s)	50.74 (s)	49.98 (s)	49.53 (s)	48.46 (s)	47.02 (s)
15	77.82 (d)	77.58 (d)	76.84 (d)	77.76 (d)	77.80 (d)	78.58 (d)	78.52 (d)	80.64 (d)	75.37 (d)	77.73 (d)	79.58 (d)
16	112.09 (s)	111.79 (s)	112.20 (s)	111.97 (s)	112.09 (s)	112.40 (s)	112.37 (s)	106.57 (s)	112.25 (s)	103.44 (s)	111.95 (s)
17	60.09 (d)	59.03 (d)	58.82 (d)	61.00 (d)	61.26 (d)	60.61 (d)	60.58 (d)	61.15 (d)	59.39 (d)	57.81 (d)	58.92 (d)
19	28.25 (t)	28.31 (t)	28.29 (t)	27.85 (t)	28.17 (t)	28.30 (t)	28.31 (t)	28.34 (t)	35.71 (t)	35.56 (t)	30.91 (t)
20	22.84 (d)	23.60 (d)	23.54 (d)	22.89 (d)	22.84 (d)	23.36 (d)	23.33 (d)	25.60 (d)	23.25 (d)	26.76 (d)	23.63 (d)
22	29.13 (t)	37.50 (t)	37.63 (t)	29.14 (t)	29.20 (t)	29.55 (t)	29.52 (t)	33.89 (t)	29.26 (t)	32.34 (t)	37.73 (t)
23	73.57 (d)	71.35 (d)	71.88 (d)	73.45 (d)	73.57 (d)	73.81 (d)	73.78 (d)	72.72 (d)	73.63 (d)	74.15 (d)	71.74 (d)
24	83.25 (d)	88.84 (d)	86.26 (d)	83.30 (d)	83.25 (d)	84.01 (d)	83.98 (d)	80.25 (d)	83.33 (d)	78.49 (d)	86.41 (d)
25	68.71 (s)	71.81 (s)	82.51 (s)	68.70 (s)	68.71 (s)	68.56 (s)	68.56 (s)	75.42 (s)	68.70 (s)	73.57 (s)	82.57 (s)
18	21.41 (q)	21.20 (q)	21.19 (q)	21.14 (q)	21.44 (q)	21.60 (q)	21.57 (q)	22.08 (q)	17.13 (q)	18.20 (q)	19.12 (q)
21	19.17 (q)	19.47 (q)	19.43 (q)	19.17 (q)	19.18 (q)	19.65 (q)	19.62 (q)	20.63 (q)	19.73 (q)	21.72 (q)	19.29 (q)
26	31.34 (q)	26.21 (q)	23.16 (q)	31.37 (q)	31.42 (q)	30.67 (q)	30.64 (q)	32.78 (q)	31.32 (q)	26.88 (q)	23.08 (q)
27	24.06 (q)	26.15 (q)	21.79 (q)	24.04 (q)	24.04 (q)	25.66 (q)	25.76 (q)	27.12 (q)	24.17 (q)	24.63 (q)	21.75 (q)
28	17.86 (q)	17.65 (q)	17.65 (q)	17.91 (q)	17.88 (q)	18.50 (q)	18.44 (q)	18.71 (q)	23.25 (q)	16.59 (q)	11.06 (q)
29	25.36 (q)	25.36 (q)	25.38 (q)	22.16 (q)	25.33 (q)	25.94 (q)	25.94 (q)	25.75 (q)	25.20 (q)	25.12 (q)	25.44 (q)
30	12.94 (q)	12.97 (q)	12.94 (q)	20.25 (q)	13.86 (q)	14.19 (q)	14.25 (q)	14.28 (q)	16.85 (q)	23.33 (q)	14.04 (q)
1′	—	—	—	—	102.06 (d)	107.05 (d)	107.36 (d)	107.39 (d)	—	—	—
2′	—	—	—	—	72.51 (d)	73.00 (d)	75.42 (d)	75.18 (d)	—	—	—
3′	—	—	—	—	70.79 (d)	79.19 (d)	78.43 (d)	78.46 (d)	—	—	—
4′	—	—	—	—	71.37 (d)	69.17 (d)	71.11 (d)	71.11 (d)	—	—	—
5′	—	—	—	—	60.94 (t)	66.71 (t)	66.98 (t)	67.01 (t)	—	—	—
$O\underline{C}OCH_3$	—	—	170.31 (s)	—	170.50 (s)	170.69 (s)	—	170.59 (s)	—	169.50 (s)	170.31 (s)
$O\underline{C}OCH_3$	—	—	—	—	170.35 (s)	—	—	—	—	—	—
$OCO\underline{C}H_3$	—	—	22.45 (q)	—	21.00 (q)	21.14 (q)	—	20.93 (q)	—	20.99 (q)	22.45 (q)
$OCO\underline{C}H_3$	—	—	—	—	21.00 (q)	—	—	—	—	—	—

δ value in *a*) $CDCl_3$ or *b*) pyridine-d_5. The multiplicities of carbon signals were determined by the distortionless enhancement by polarization transfer (DEPT) method and are indicated as s, d, t, and q. *c*) ^{1}H–^{13}C and ^{1}H–^{13}C long-range COSY spectra were measured. *d*) ^{1}H–^{13}C spectrum was measured. *e*) Only the complete decoupling spectrum was measured.

structure of **9** was consequently determined to be 24-*epi*-acerinol. The complete assignments of its proton and carbon signals determined by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY are shown in Tables I and II.

Compound **10** was obtained as colorless needles, and showed $[\alpha]_D$ +33.8° ($CHCl_3$). It exhibited a molecular ion peak at m/z 546 (M^+) along with fragment ion peaks at m/z 528 ($M^+ - H_2O$), 311, and its molecular formula was determined to be $C_{32}H_{50}O_7$ by high-resolution MS. The ^{1}H-NMR spectrum of **10** exhibited signals due to an acetoxy-bearing methine proton (δ_H 5.10), a hydroxy-bearing methine (δ_H 3.89), two methines substituted by oxygen (δ_H 3.73 and 4.07), and one *sec*- and six *tert*-methyl groups along with other signals, which were analyzed by ^{1}H–^{1}H COSY. The whole spectral pattern of **10** resembled that of **9**, but it showed a marked downfield shift of an acetoxy-bearing methine signal to δ_H 5.10 (24-H). This spectral behavior resembled that of 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside, whose 24-methine proton resonated at δ_H 4.82. The ^{13}C-NMR spectrum of **10**, which was fully analyzed by a combination of ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation techniques, was found to be parallel with that of 24-*epi*-acerinol. In the ^{1}H–^{13}C long-range COSY spectrum of **10**, the carbon signals at δ_C 89.75 (C-10) and 169.50 ($CH_3\underline{C}=O$) are correlated with proton signals at δ_H 3.73 (3-H), 1.51 (2-H) and 1.65 (19-H) and at δ_H 5.10 (24-H), respectively. Some of the other significant long-range correlations observed are shown by arrows in Fig. 6. The relative stereochemistry of **10** was determined on the basis of the proton coupling constants and NOE experiments. Irradiation at 18-H_3 enhanced the signal intensity of 15-H, indicating that the 15-hydroxy group has α configuration. Furthermore, the configuration at the C-24 position was concluded to be *R* based on the coupling constant of 24-H (δ_H 5.10, d, J=9.5 Hz), which is comparable with that of dahurinyl diacetate (δ_H 4.88, d, J=8.0 Hz).[12] On the basis of the above findings, the structure of **10** was fully established and it was named heracleifolinol.

25-*O*-Acetylcimigenol (**11**) has already been reported by Takemoto and Kusano[7] but complete NMR signal assignments had not been given. All the proton and carbon

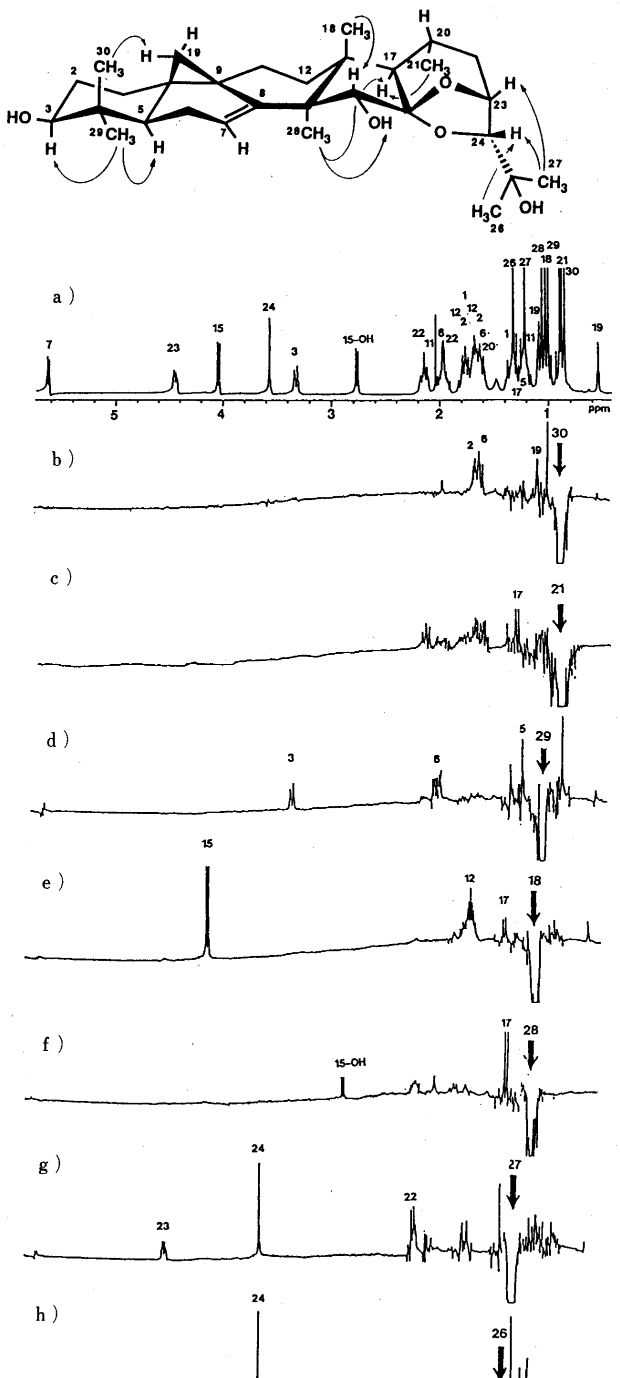

Fig. 3. ^{1}H-NMR (Normal and NOE) Spectra of 24-*epi*-7,8-Didehydrocimigenol (**1**)

a) Normal spectrum. b—h) NOE difference spectra on irradiation at δ 0.85, 0.90, 1.01, 1.03, 1.06, 1.22, and 1.33, respectively.

signals were assigned in this experiment by using distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY (Tables I and II).

In these experiments, 2D NMR methods were effectively applied to the structure elucidation of cycloartenol triterpenoids. The anti-inflammatory effects of these compounds are under investigation in our laboratory.

Table III. Fractional Atomic Coordinates and Isotropic Temperature Factors, B_{eq} for Non-H Atoms with Their Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq}
O(1)	0.0976 (4)	0.5917 (1)	0.6070 (7)	7.2 (2)
O(2)	−0.1427 (2)	0.8718 (1)	0.9130 (6)	4.8 (2)
O(3)	−0.0024 (3)	0.9609 (1)	0.8899 (4)	4.5 (1)
O(4)	−0.0615 (3)	0.9279 (1)	1.1646 (4)	3.8 (1)
O(5)	0.0176 (3)	0.9728 (1)	1.4901 (4)	4.9 (2)
C(1)	0.2012 (4)	0.6901 (2)	0.789 (1)	5.2 (3)
C(2)	0.2159 (5)	0.6512 (2)	0.642 (1)	6.1 (3)
C(3)	0.1155 (4)	0.6315 (2)	0.5894 (8)	4.3 (2)
C(4)	0.0344 (4)	0.6645 (2)	0.5145 (8)	4.3 (2)
C(5)	0.0282 (3)	0.7055 (2)	0.6609 (7)	3.6 (2)
C(6)	−0.0502 (4)	0.7408 (2)	0.603 (1)	5.6 (3)
C(7)	−0.0474 (4)	0.7827 (2)	0.719 (1)	5.9 (3)
C(8)	0.0346 (3)	0.7991 (1)	0.8049 (7)	3.1 (2)
C(9)	0.1335 (3)	0.7739 (1)	0.7954 (7)	3.4 (2)
C(10)	0.1304 (3)	0.7254 (1)	0.7101 (7)	3.4 (2)
C(11)	0.2074 (3)	0.7845 (2)	0.9622 (9)	4.7 (2)
C(12)	0.2162 (3)	0.8340 (2)	1.0258 (8)	4.0 (2)
C(13)	0.1368 (4)	0.8652 (1)	0.9379 (7)	3.3 (2)
C(14)	0.0326 (3)	0.8401 (1)	0.9378 (6)	2.9 (2)
C(15)	−0.0401 (4)	0.8792 (2)	0.8811 (6)	3.5 (2)
C(16)	0.0027 (4)	0.9197 (1)	0.9968 (6)	3.5 (2)
C(17)	0.1110 (4)	0.9094 (1)	1.0567 (7)	3.6 (2)
C(18)	0.1703 (4)	0.8780 (2)	0.7271 (7)	4.2 (2)
C(19)	0.1723 (4)	0.7640 (2)	0.592 (1)	4.9 (3)
C(20)	0.1789 (4)	0.9516 (2)	1.0339 (8)	4.5 (2)
C(21)	0.2798 (4)	0.9461 (2)	1.134 (1)	6.3 (3)
C(22)	0.1219 (4)	0.9931 (2)	1.1124 (7)	4.6 (2)
C(23)	0.0142 (5)	0.9946 (2)	1.0444 (6)	4.8 (2)
C(24)	−0.0696 (4)	0.9768 (2)	1.1848 (7)	3.9 (2)
C(25)	−0.0714 (4)	0.9903 (2)	1.4013 (7)	4.3 (2)
C(26)	−0.1631 (4)	0.9692 (2)	1.4963 (8)	6.2 (3)
C(27)	−0.0706 (5)	1.0422 (2)	1.412 (1)	6.8 (3)
C(28)	0.0025 (4)	0.8231 (2)	1.1449 (7)	3.9 (2)
C(29)	−0.0656 (5)	0.6402 (2)	0.501 (1)	7.7 (4)
C(30)	0.0694 (7)	0.6795 (2)	0.3100 (8)	7.0 (4)

Table IV. Bond Distances (Å) for Non-H Atoms with Their Estimated Standard Deviations in Parentheses

Atom	Atom	Distance	Atom	Atom	Distance
O1	C3	1.197 (6)	C9	C11	1.536 (7)
O2	C15	1.390 (6)	C9	C19	1.514 (8)
O3	C16	1.416 (5)	C10	C19	1.498 (7)
O3	C23	1.466 (6)	C11	C12	1.523 (7)
O4	C16	1.450 (5)	C12	C13	1.519 (6)
O4	C24	1.448 (6)	C13	C14	1.563 (6)
O5	C25	1.422 (6)	C13	C17	1.568 (6)
C1	C2	1.537 (8)	C13	C18	1.558 (6)
C1	C10	1.500 (7)	C14	C15	1.547 (6)
C2	C3	1.492 (8)	C14	C28	1.556 (6)
C3	C4	1.532 (7)	C15	C16	1.539 (6)
C4	C5	1.570 (7)	C16	C17	1.520 (7)
C4	C29	1.503 (8)	C17	C20	1.537 (6)
C4	C30	1.541 (8)	C20	C21	1.508 (8)
C5	C6	1.518 (7)	C20	C22	1.530 (7)
C5	C10	1.511 (6)	C22	C23	1.499 (8)
C6	C7	1.467 (8)	C23	C24	1.559 (8)
C7	C8	1.323 (7)	C24	C25	1.537 (7)
C8	C9	1.504 (6)	C25	C26	1.509 (8)
C8	C14	1.509 (6)	C25	C27	1.526 (8)
C9	C10	1.538 (6)			

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$

TABLE V. Bond Angles (°) for Non-H Atoms with Their Estimated Standard Deviations in Parentheses

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C16	O3	C23	101.3 (3)	C11	C9	C19	120.6 (4)	O3	C16	C17	110.8 (4)
C16	O4	C24	106.4 (3)	C1	C10	C5	111.7 (4)	O4	C16	C15	108.7 (4)
C2	C1	C10	110.8 (5)	C1	C10	C9	119.1 (4)	O4	C16	C17	111.7 (3)
C1	C2	C3	109.5 (5)	C1	C10	C19	119.2 (4)	C15	C16	C17	109.3 (4)
O1	C3	C2	121.9 (5)	C5	C10	C9	117.9 (4)	C13	C17	C16	103.2 (4)
O1	C3	C4	120.8 (5)	C5	C10	C19	120.2 (4)	C13	C17	C20	119.1 (4)
C2	C3	C4	117.3 (4)	C9	C10	C19	59.8 (3)	C16	C17	C20	111.1 (4)
C3	C4	C5	107.9 (4)	C9	C11	C12	117.1 (4)	C9	C19	C10	61.4 (3)
C3	C4	C29	109.6 (4)	C11	C12	C13	114.2 (4)	C17	C20	C21	112.6 (5)
C3	C4	C30	106.0 (5)	C12	C13	C14	108.9 (3)	C17	C20	C22	108.6 (4)
C5	C4	C29	110.9 (5)	C12	C13	C17	116.3 (4)	C21	C20	C22	111.2 (5)
C5	C4	C30	112.2 (4)	C12	C13	C18	108.4 (4)	C20	C22	C23	112.5 (4)
C29	C4	C30	110.2 (6)	C14	C13	C17	101.5 (3)	O3	C23	C22	110.3 (4)
C4	C5	C6	113.2 (4)	C14	C13	C18	111.4 (4)	O3	C23	C24	96.5 (4)
C4	C5	C10	113.2 (4)	C17	C13	C18	110.1 (3)	C22	C23	C24	118.2 (4)
C6	C5	C10	113.8 (4)	C8	C14	C13	111.2 (3)	O4	C24	C23	102.8 (4)
C5	C6	C7	114.5 (4)	C8	C14	C15	116.8 (3)	O4	C24	C25	110.5 (4)
C6	C7	C8	124.5 (5)	C8	C14	C28	107.5 (3)	C23	C24	C25	121.4 (5)
C7	C8	C9	120.9 (4)	C13	C14	C15	101.3 (3)	O5	C25	C24	107.9 (4)
C7	C8	C14	122.9 (4)	C13	C14	C28	112.1 (3)	O5	C25	C26	109.4 (4)
C9	C8	C14	115.7 (4)	C15	C14	C28	107.9 (4)	O5	C25	C27	109.5 (5)
C8	C9	C10	116.7 (4)	O2	C15	C14	116.8 (4)	C24	C25	C26	108.8 (5)
C8	C9	C11	114.9 (4)	O2	C15	C16	113.5 (4)	C24	C25	C27	107.8 (4)
C8	C9	C19	115.4 (4)	C14	C15	C16	102.5 (4)	C26	C25	C27	113.2 (5)
C10	C9	C11	119.2 (4)	O3	C16	O4	104.0 (3)				
C10	C9	C19	58.8 (3)	O3	C16	C15	112.1 (3)				

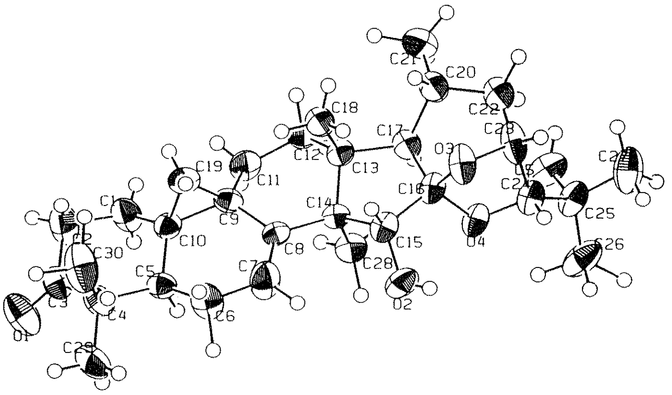

Fig. 4. ORTEP Drawing of 3-Keto-24-*epi*-7,8-didehydrocimigenol (4) with Atomic Numbering

Ellipsoids of 50% probability are drawn for the non-H atoms; the H atoms are represented as spheres.

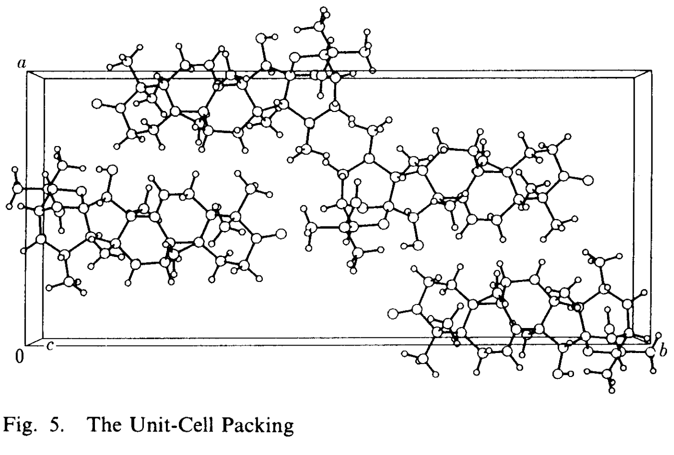

Fig. 5. The Unit-Cell Packing

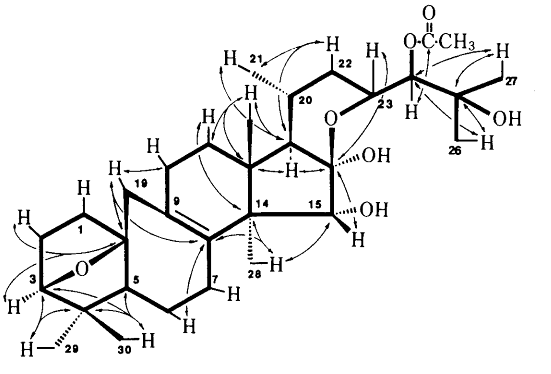

Fig. 6. Significant Long-Range Correlations Observed in the ^1H–^{13}C Long-Range COSY Spectrum of Heracleifolinol (**10**)

or MeOH solutions on a JASCO DIP-360 digital polarimeter at 31 °C. IR spectra were recorded on a Hitachi 260-01 spectrometer in KBr discs. EI-MS (ionization voltage, 70 eV; accelerating voltage, 3 kV) and positive ion FAB-MS were measured with a JEOL JMS DX-300 spectrometer using a direct inlet system and glycerol was used as a matrix in positive ion FAB-MS measurements. ^1H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX 400 spectrometer with tetramethylsilane as an internal standard and chemical shifts were recorded in δ values. Multiplicities of ^{13}C-NMR signals were determined by means of the DEPT method and are indicated as s (singlet), d (doublet), t (triplet), and q (quartet). 2D NMR spectra (^1H–^1H COSY, ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY) were measured by the use of JEOL standard pulse sequences and collected data were treated by JEOL standard software. Difference NOE spectra were obtained by the use of JEOL standard pulse sequences with 5 s irradiation. Column chromatography was performed with Wakogel C-200 (Wako Pure Chemical Co., Japan). TLC and preparative TLC were carried out on precoated Kieselgel F_{254} plates (0.25 or 0.5 mm) or Merck RP-18 F_{254} reversed-phase plates (0.25 mm) with hexane–EtOAc (7:3) and $CHCl_3$–MeOH–H_2O (10:1:1) or acetonitrile–EtOAc (19:1) as a developing solvent, and spots were detected under UV light, or by using $Ce(SO_4)_2$–10% H_2SO_4 (1:99) spray reagent.

Isolation and Properties of Triterpenoids from *Cimicifuga heracleifolia* KOMAROV The dried rhizome (5.5 kg) of *C. heracleifolia*, grown in Heilongjiang province of China, was successively extracted three times with MeOH for 3 h under reflux. The MeOH extract was evaporated to dryness and the residue (902 g) was suspended in water (1000 ml) and fractionated by successive extraction with hexane (1000 ml × 3), EtOAc

(1000 ml × 3) and *n*-BuOH (1000 ml × 3) to give a hexane–soluble fraction (50 g), an EtOAc-soluble fraction (250 g) and a *n*-BuOH-soluble fraction (50 g).

The EtOAc-soluble fraction (235 g) was subjected to column chromatography on silica gel (2.5 kg). Elutions with hexane–CHCl$_3$ (1 : 1), CHCl$_3$, and finally CHCl$_3$–MeOH (19 : 1, 4 : 1 and 7 : 3) gave fractions 1 (15 g), 2 (8 g), 3 (33 g), 4 (16 g) and 5 (85 g).

Fraction 1 (fr. 1) was re-chromatographed on a silica gel (60 g) column with hexane–CHCl$_3$ (7 : 3 and 1 : 1), giving two fractions. The product of the first fraction was crystallized from EtOH to give β-sitosterol (1.12 g), colorless needles, mp 137—138 °C. MS *m/z*: 414 (M$^+$) (base peak), 396, 381, 329, 303, 273, 255. The structure was confirmed by direct comparison of the spectral data with those of an authentic sample. The second fraction was further purified by preparative TLC with hexane–CHCl$_3$ (3 : 7), giving *E*-3-(3′-methyl-2′-butenylidene)-2-indolinone (5 mg), fine yellow needles, mp 213—214 °C. MS *m/z*: 199 (M$^+$). ^{1}H-NMR (CDCl$_3$) δ: 1.99, 2.06 (each 3H, s, 13-, 14-CH$_3$), 6.83 (1H, d, J=7.6 Hz, 7-H), 6.85 (1H, d, J=12.7 Hz, 11-H), 6.96 (1H, t, J=7.6 Hz, 5-H), 7.19 (1H, t, J=7.6 Hz, 6-H), 7.34 (1H, d, J=12.7 Hz, 10-H), 7.75 (1H, d, J=7.6 Hz, 4-H), 10.41 (1H, s, N–H). ^{13}C-NMR (CDCl$_3$) δ: 18.59 (C-13), 26.82 (C-14), 109.55 (C-7), 120.82 (C-11), 121.14 (C-4), 122.22 (C-12), 123.31 (C-3), 123.52 (C-5), 128.51 (C-6), 130.35 (C-9), 141.84 (C-8), 151.64 (C-10), 169.09 (C-2). The compound was identified by comparing the ^{1}H-NMR spectrum with data in the literature.6)

Fraction 2 (fr. 2) was subjected to silica gel column chromatography with benzene–EtOAc (19 : 1, 9 : 1 and 4 : 1), giving two fractions. The first fraction (fr. 2-1) was re-chromatographed on a silica gel (60 g) column with hexane–EtOAc (4 : 1 and 7 : 3) to give two fractions (fr. 2-1-1 and fr. 2-1-2). Fraction (fr. 2-1-1) give 24-*epi*-acerinol (9) (30 mg) on crystallization from EtOAc.

24-*epi*-Acerinol (9) Colorless needles, mp 230—231 °C, $[\alpha]_D$ +59.23° (c=0.27, CHCl$_3$). IR ν_{max} cm^{-1}: 3510 (OH), 1380, 1240. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 486 (M$^+$) (base peak), 468, 453, 435 and 389. High-resolution MS: Found: 486.3330. Calcd for C$_{30}$H$_{46}$O$_5$ (M$^+$): 486.3332. Fraction (fr. 2-1-2) was repeatedly purified by reversed phase preparative TLC with acetonitrile–EtOAc (19 : 1) to give 25-*O*-acetyl-7,8-didehydrocimigenol (3) (2 mg) and 25-*O*-acetylcimigenol (11) (2 mg). Identification of **11** was done by comparison of its ^{1}H-NMR spectrum with data in the literature.7)

25-*O*-Acetyl-7,8-didehydrocimigenol (3) White powder. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 528 (M$^+$), 510, 468, 435, 409, 95. High-resolution MS: Found: 528.3445. Calcd for C$_{32}$H$_{48}$O$_6$ (M$^+$): 528.3449.

25-*O*-Acetylcimigenol (11) White powder. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 530 (M$^+$), 512, 452, 437, 409, 330 (base peak). High-resolution MS: Found: 530.3583. Calcd for C$_{32}$H$_{50}$O$_6$ (M$^+$): 530.3606.

The second fraction (fr. 2-2) was re-chromatographed on silica gel (25 g) with hexane–EtOAc (7 : 3) and the product was crystallized from EtOAc to give 3-keto-24-*epi*-7,8-didehydrocimigenol (4) (40 mg), colorless needles, mp 225—226 °C, $[\alpha]_D$ −12.4° (c=0.37, CHCl$_3$). IR ν_{max} cm^{-1}: 3470 (OH), 1720 (C=O). ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 484 (M$^+$) (base peak), 469, 466, 451, 398, 385. High-resolution MS: Found: 484.3187. Calcd for C$_{30}$H$_{44}$O$_5$ (M$^+$): 484.3187.

Fraction 3 (fr. 3) was crystallized from MeOH to give an additional crop of isoferulic acid (2.5 g): colorless prisms, mp 236—238 °C. MS *m/z*: 194 (M$^+$). ^{1}H-NMR (DMSO-d_6) δ: 3.81 (3H, s, OCH$_3$), 6.24 (1H, d, J=15.6 Hz, 2′-H), 6.95 (1H, d, J=8.3 Hz, 6-H), 7.09 (1H, d, J=8.3 Hz, 5-H), 7.08 (1H, s, 2-H), 7.45 (1H, d, J=15.6 Hz, 1′-H). ^{13}C-NMR (DMSO-d_6) δ: 55.64 (OCH$_3$), 112.04 (C-5), 114.10 (C-2), 116.32 (C-6), 120.92 (C-2′), 127.10 (C-1), 144.11 (C-1′), 146.66 (C-3), 149.80 (C-4), 167.67 (C=O). The structure of this compound was confirmed by comparison of the ^{1}H-NMR spectrum with data in the literature.14) The mother liquor (*ca.* 30 g) was re-chromatographed on a silica gel column with hexane–EtOAc (7 : 3 and 3 : 4), giving three fractions. The product in the first fraction was crystallized from EtOAc to give heracleifolinol (**10**) (32 mg), colorless needles, mp 238—239 °C, $[\alpha]_D$ +33.8° (c=0.45, CHCl$_3$). IR ν_{max} cm^{-1}: 3152 (OH), 1725 (C=O). ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 546 (M$^+$), 528 (M$^+$−18) (base peak), 513, 510, 495, 435, 311. High-resolution MS: Found: 546.3536. Calcd for C$_{32}$H$_{50}$O$_7$ (M$^+$): 546.3538. The second fraction was re-chromatographed on a silica gel column with hexane–EtOAc (3 : 1) to give 24-*epi*-7,8-didehydrocimigenol (1) (46.9 mg), colorless needles, mp 222—223 °C, $[\alpha]_D$ +6.4° (c=0.47, CHCl$_3$). IR ν_{max} cm^{-1}: 3450 (OH), 1620 (C=C). ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 486 (M$^+$) (base peak), 468, 453. High-resolution MS: Found: 486.3360. Calcd for C$_{30}$H$_{46}$O$_5$ (M$^+$): 486.3359. The third fraction was separated by preparative TLC with hexane–EtOAc (7 : 3) to give 2′,4′-*O*-diacetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside (5) (70 mg), white powder, $[\alpha]_D$ −15.5° (c=0.53, CHCl$_3$). IR ν_{max} cm^{-1}: 3550 (OH), 1740 (C=O), 1635 (C=C). ^{1}H- and ^{13}C-NMR: Tables I and II. Positive ion FAB-MS *m/z*: 703 [M+H]$^+$.

Fraction 4 (fr. 4) was chromatographed on a charcoal column with MeOH and then re-chromatographed on a silica gel (60 g) column with hexane–EtOAc (13 : 7 and 1 : 1) to give two fractions. A part of the first fraction was further purified by preparative TLC with CHCl$_3$–MeOH–H$_2$O (10 : 1 : 1) to give 7,8-didehydrocimigenol (2) (6 mg), white powder. IR ν_{max} cm^{-1}: 3425 (OH), 1620 (C=C), and 1260 (C–O). ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 486 (M$^+$) (base peak), 468, 453. High-resolution MS: Found: 486.3379. Calcd for C$_{30}$H$_{46}$O$_5$ (M$^+$): 486.3345. The second fraction was re-chromatographed on a silica gel column with hexane–EtOAc (1 : 1) to give 3′-*O*-acetyl-24-*epi*-7,8-didehydrocimigenol-3-xyloside (6) (15 mg), white powder, $[\alpha]_D$ −13.2° (c=0.53, CHCl$_3$: MeOH=2 : 3). IR ν_{max} cm^{-1}: 3420 (OH), 1725 (C=O), 840 (C=C). ^{1}H- and ^{13}C-NMR: Tables I and II. Positive ion FAB-MS: 661 [M+H]$^+$.

A part of fraction 5 (fr. 5) was passed through a charcoal column with MeOH and then re-chromatographed on a silica gel column with CHCl$_3$–MeOH (19 : 1 and 9 : 1) to give 24-*epi*-7,8-didehydrocimigenol-3-xyloside (7) (80 mg) and 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside (8) (25 mg).

24-*epi*-7,8-Didehydrocimigenol-3-xyloside (7) White powder, $[\alpha]_D$ −9.6° (c=0.35, CHCl$_3$: MeOH=2 : 3). IR ν_{max} cm^{-1}: 3420 (OH), 1630 (C=C). ^{1}H- and ^{13}C-NMR: Tables I and II. Positive ion FAB-MS *m/z*: 619 [M+H]$^+$.

7,8-Didehydro-24-*O*-acetylhydroshengmanol-3-xyloside (8) White powder, $[\alpha]_D$ −27.4° (c=0.62, CHCl$_3$: MeOH=2 : 3). IR ν_{max} cm^{-1}: 3346 (OH), 1740 (C=O), 1240 (C–O). ^{1}H- and ^{13}C-NMR: Tables I and II. Positive ion FAB-MS *m/z*: 679 [M+H]$^+$.

Acid Hydrolysis of 5—8 Compounds **5**—**8** (each 5 mg) were each hydrolyzed in 0.5 N HCl for 3 h at 100 °C. After neutralization with NH$_4$OH, followed by extraction with EtOAc (20 ml × 3), the aqueous layer was lyophilized *in vacuo* to give a residue. The residue was applied to a RP-TLC plate and developed twice with CH$_3$CN–H$_2$O (7 : 3). The *Rf* value of the product was in agreement with that (*Rf*=0.7) of β-D-xylose. The presence of β-D-xylose was further confirmed by GC after trimethylsilylation.

X-Ray Crystallographic Analysis of 3-Keto-24-*epi*-7,8-didehydrocimigenol (4) Prismatic crystals were grown from a mixture of EtOAc and hexane by slow evaporation. The cell dimensions were determined by a least-squares refinement of 20 reflections in the range of $55° < 2\theta < 60°$ measured on a Rigaku AFC5 four-circle diffractometer equipped with a rotating anode (Ni-filtered CuK_α, 40 kV, 200 mA). Crystal data: C$_{30}$H$_{44}$O$_5$, M_r=484.67, orthorhombic, $P2_12_12_1$, a=13.218(3), b=29.363(2), c=6.855(1) Å, V=2661(1) Å^3, Z=4, D_x=1.210 gcm^{-3}, λ(CuK_α)=1.54178 Å, μ=6.04 cm^{-1}, F(000)=1056. Intensity data were collected at 20 °C within $2\theta_{max}$=122° ($0<h<14$, $0<k<23$, $0<l<7$) by the ω-2θ scan technique (scan speed was 4°/min in ω and scan range in ω was $0.9°+0.5\tan\theta$). Background was measured for 3.0 s on either side of the peak. Three standard reflections were monitored every 100 reflections. Of the 2392 reflections, 1896 reflections with $I > 3.0\,\sigma(I)$ were treated as observed. The intensities were corrected for Lorentz and polarization effects, and secondary extinction (coefficient: 0.1833E−05). Decay corrections were applied, but no absorption correction was made.

Structure Determination and Refinement The structure was solved by direct methods.15,16) The non-hydrogen atoms were refined anisotropically. The coordinates of hydrogen atoms were calculated assuming idealized geometries, but were not refined. Full-matrix least-squares refinement was performed to minimize $\Sigma w(|F_O|-|F_C|)^2$ where $w=4F_O^2/\sigma^2(F_O^2)$ and $\sigma^2(F_O^2)=[S^2(C+R^2B)+(pF_O^2)^2]/Lp^2$ (S=scan rate, C=total integrated peak count, R=ratio of scan time to background counting time, B=total background count, Lp=Lorentz and polarization factor, and p=0.06). Final cycles of least-squares refinement yielded R=0.058 and wR=0.071 for 1896 reflections. Maximum and minimum peaks on the final difference Fourier map corresponded to 0.18 and −0.21 eÅ^{-3}, respectively. Neutral atom scattering factors were taken from Cromer and Waber.17) Anomalous dispersion effects were included in F_C; the values for f' and f'' were those of Cromer and Waber.18) All calculations were performed using the TEXSAN software package.19) A complete list of X-ray structure data has been deposited at the Cambridge Crystallographic Data Center.

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