Chem. Pharm. Bull. **43**(10) 1640—1642 (1995) Vol. 43, No. 10

Composite Constituents: Three New Triterpene Triols Isolated from Fresh Roots of *Picris hieracioides* subsp. *japonica*

Kenji SHIOJIMA, Hideki SUZUKI, and Hiroyuki AGETA*

Shôwa College of Pharmaceutical Sciences, Higashi-Tamagawagakuen, Machida, Tokyo 194, Japan.

Received March 6, 1995; accepted June 10, 1995

Three new triterpenoids, olean-12-ene-2β,3β,22α-triol (1), urs-12-ene-2β,3β,22α-triol (2), urs-12-ene-2β,3β,28-triol (3), and a known one, olean-12-ene-2β,3β,28-triol (4), were isolated from the fresh roots of *Picris hieracioides* subsp. *japonica*, and their structures were elucidated on the basis of spectral data.

Key words *Picris hieracioides* subsp. *japonica*; Compositae; olean-12-ene-2β,3β,22α-triol; urs-12-ene-2β,3β,22α-triol; urs-12-ene-2β,3β,28-triol; triterpenoid

In the preceding paper of this series,[1] we reported the structure elucidation of forty-two triterpenoids including eight novel compounds isolated from the fresh roots of *Picris hieracioides* LINNÉ subsp. *japonica* (THUNB.) KRYLOV. (kôzorina in Japanese, Compositae). Further investigation of the same extract has resulted in the isolation of three new triterpenoids, olean-12-ene-2β,3β,22α-triol (**1**), urs-12-ene-2β,3β,22α-triol (**2**) and urs-12-ene-2β,3β,28-triol (**3**) (Chart 1), together with five known compounds **4**—**8**. This paper deals with the isolation and structure elucidation of the new compounds.

Results and Discussion

More polar fractions of the hexane extract of the fresh roots[1] were separated by various kinds of chromatography (see Experimental) to give compounds **1**—**8**, which are shown in Table 1 with their physical constants and yields. The mixture of **5**—**8** was esterified and acetylated, and the products were identified as the corresponding acetates or acetate-methyl esters.

Compound **1** was obtained as colorless needles, and the IR spectrum of **1** indicated the presence of hydroxyl groups in the molecule. The high-resolution MS (HR-MS) of **1** showed the molecular ion at *m/z* 458.3773 ($C_{30}H_{50}O_3$), and many significant fragment ions at *m/z* (relative intensity): 443 (2, M^+-CH_3), 440 (3, M^+-H_2O), 425 (3, $M^+-CH_3-H_2O$), 422 (2, M^+-2H_2O), 407 (4, $M^+-CH_3-2H_2O$), 234 (100, a), 223 (19, b), 216 (85, a$-H_2O$), 201 (40, a$-CH_3-H_2O$), and 187 (14, b$-2H_2O$) (Chart 2). These fragment ions suggested that **1** is an oleanane or ursane derivative with functional groups in the A and E rings of the molecule.[2] The ^{1}H-NMR spectrum of **1** indicated the presence of eight tertiary methyl groups and three secondary hydroxyl groups, and therefore, **1** was suggested to be an oleanane derivative. The methyl protons of H-23, 24, 25, 26 and 27 of **1** were observed at almost the same positions as those of olean-12-ene-2β,3β,28-triol (**4**),[3] the structure of which was confirmed by detailed analysis of the two dimensional (2D) NMR spectra, as shown in Table 2. The chemical shifts and splitting patterns of an olefinic proton, and the H-2α and H-3α hydroxy methine protons of **1** were very similar to those of **4** (Table 2). The orientations of the 2β and 3β hydroxyl groups of **1** were indicated by the nuclear Overhauser effect spectroscopy (NOESY) of **1**, because cross peaks were observed between H-2α and H-3α, H-3α

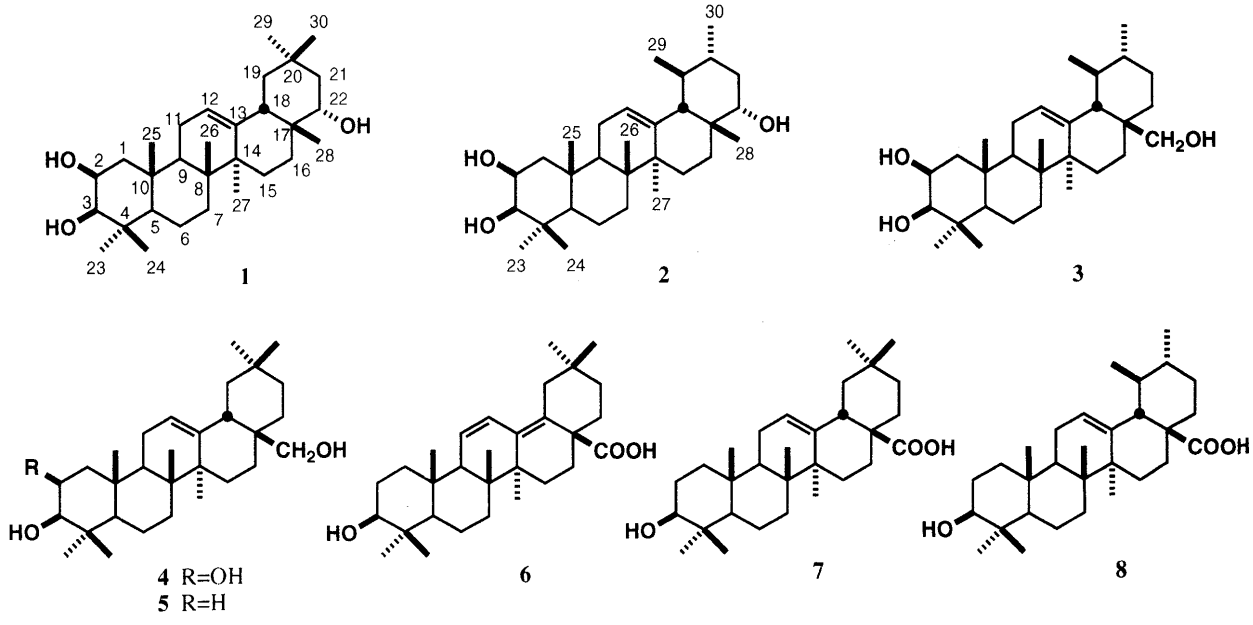

Chart 1

* To whom correspondence should be addressed.

Table 1. Triterpenoids Isolated from *Picris hieracioides* subsp. *japonica*

	mp (°C)	$[\alpha]_D$ (°)	Yield[a] (%) Roots	Ref.
Olean-12-ene-2β,3β,22α-triol (**1**)	220—220.5	+91.2	0.0014	
Urs-12-ene-2β,3β,22α-triol (**2**)	256—257	+87.3	0.0011	
Urs-12-ene-2β,3β,28-triol (**3**)	127—128	+62.5	0.00005	
Olean-12-ene-2β,3β,28-triol (**4**)	110—112	+69.8	0.0003	3
Erythrodiol (**5**)			0.00005	5
3β-Hydroxyoleana-11,13(18)-dien-28-oic acid (**6**)			0.00004	6
Oleanolic acid (**7**)			0.0067	7
Ursolic acid (**8**)			0.0049	7

a) Yield from the dried materials after removal of water by azeotropic distillation.

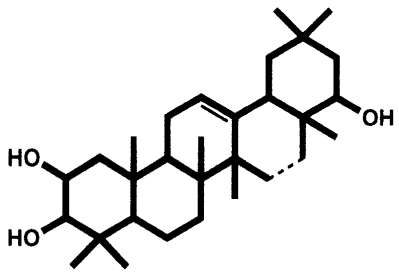

Fig. 1. Partial Structure of **1**, Based on the HMBC Spectrum

and H-23, and H-23 and H-5α. Thus, the structure of rings A, B, C, and D of **1** is presumed to be the same as in **4**. The third hydroxyl group of **1** was determined to be at C-22α from the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) and NOESY spectra. The partial structure of **1**, shown by heavy lines in Fig. 1, was established by the HMBC spectrum. The cross peaks in the NOESY spectrum of **1** were observed between H-22β (δ 3.546) and H-28, H-28 and H-18 (δ 1.99), and H-18 and H-30. In the ^{13}C-NMR spectrum (Table 3), signals of **1** were coincident with those of **4** except for C-22, C-28, and neighbors. Thus, the structure of **1** was established as olean-12-ene-2β,3β,22α-triol (**1**). Assignments of the ^{1}H- and ^{13}C-NMR spectra of the compounds shown in Tables 2 and 3 were confirmed by proton-proton and ^{13}C-proton correlated spectroscopy (^{1}H–^{1}H and ^{13}C–^{1}H COSY), heteronuclear single quantum coherence spectroscopy (HSQC), HMBC spectrum, and distortionless enhancement by polarization transfer (DEPT) spectrum methods.[4]

Compound **2** was obtained as colorless needles, and the IR spectrum of **2** indicated the presence of hydroxyl groups. The HR-MS of **2** showed the molecular ion at *m/z* 458.3778 ($C_{30}H_{50}O_3$), and many significant fragment ions at *m/z* (relative intensity): 443 (1, M^+-CH_3), 440 (8, M^+-H_2O), 425 (5, $M^+-CH_3-H_2O$), 422 (9, M^+-2H_2O), 407 (9, $M^+-CH_3-2H_2O$), 234 (100, a′), 223 (18, b), 216 (61, a′$-H_2O$), 201 (38, a′$-CH_3-H_2O$), and 187 (14, b$-2H_2O$) (Chart 2). These fragment patterns were very similar to those of **1**. The ^{1}H-NMR spectrum of **2** indicated the presence of six tertiary and two secondary methyl groups and three secondary hydroxyl groups, and therefore, **2** was suggested to be an ursane derivative. The chemical shifts and splitting patterns of an olefinic proton and H-2α, H-3α and H-22β hydroxy methine protons were very similar to those of **1** (Table 2). In the ^{13}C-NMR spectrum (Table 3), signals of **2** were coincident with those of **1** except for C-19, 20, 29, and 30.

Table 2. ^{1}H-NMR Spectral Data (500 MHz, $CDCl_3$, δ)

	1	**2**	**3**	**4**
H-23	1.027	1.026	1.024	1.023
H-24	1.018	1.020	1.017	1.014
H-25	1.264	1.281	1.274	1.256
H-26	0.995	1.034	1.009	0.962
H-27	1.149	1.078	1.096	1.159
H-28	0.991	0.967	3.195 (d, 11.0)	3.218 (d, 10.9)
			3.533 (d, 11.0)	3.557 (d, 10.9)
H-29	0.917	0.784 (d, 5.8)	0.806 (d, 5.8)	0.888
H-30	0.932	0.966 (d, 6.1)	0.936 (d, 5.8)	0.876
H-2α	4.095 (ddd, 3.4, 3.4, 3.5)	4.103 (ddd, 3.4, 3.4, 3.6)	4.104 (ddd, 3.2, 3.2, 3.4)	4.094 (ddd, 3.4, 3.4, 3.6)
H-3α	3.226 (d, 4.0)	3.229 (d, 3.9)	3.225 (d, 3.7)	3.224 (d, 4.3)
H-5α	0.84 (m)	0.83 (m)	0.83 (m)	0.83 (m)
H-18β	1.99 (m)	1.31 (m)	1.38 (m)	1.98 (m)
H-22β	3.546 (dd, 5.2, 11.6)	3.353 (dd, 4.5, 12.1)		
C=C<u>H</u>–	5.228 (dd, 3.7, 3.7)	5.161 (dd, 3.7, 3.7)	5.156 (dd, 3.7, 3.7)	5.212 (dd, 3.5, 3.5)

Signals, unless otherwise stated, are 3H, singlet. Multiplicity and coupling constants (*J*) are shown in parentheses.

Table 3. ^{13}C-NMR Spectral Data (125 MHz, $CDCl_3$, δ)

	1	**2**	**3**	**4**
C-1	44.24	44.41	44.42	44.24
C-2	71.11	71.09	71.13	71.11
C-3	78.45	78.47	78.47	78.45
C-4	38.11	38.10	38.10	38.11
C-5	55.14	55.14	55.14	55.15
C-6	18.11	18.09	18.09	18.11
C-7	32.52	32.80	32.80	32.55
C-8	40.03	40.22	40.15	39.91
C-9	48.02	48.09	48.08	48.01
C-10	36.62	36.59	36.58	36.62
C-11	23.63	23.53	23.48	23.61
C-12	122.54	125.21	125.11	122.41
C-13	143.78	138.75	138.73	144.23
C-14	42.30	42.86	42.19	41.86
C-15	25.49	25.88	25.89	25.43
C-16	19.07	20.71	23.29	21.99
C-17	38.24	39.07	38.02	36.95
C-18	47.34	58.60	54.03	42.35
C-19	46.07	39.20	39.34	46.46
C-20	31.59	37.26	39.42	30.96
C-21	42.79	39.12	30.62	34.09
C-22	76.35	79.10	35.18	31.04
C-23	29.71	29.74	29.74	29.70
C-24	17.32	17.35	17.35	17.31
C-25	16.45	16.64	16.66	16.47
C-26	16.72	16.76	16.74	16.71
C-27	26.26	23.39	23.35	25.99
C-28	24.64	24.66	69.93	69.70
C-29	33.39	17.39	17.35	33.20
C-30	24.89	21.06	21.33	23.58

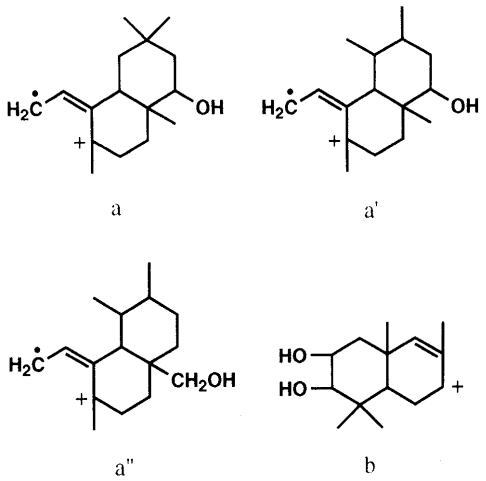

Chart 2

Furthermore, the locations of hydroxyl groups in **2** were 2β, 3β and 22α, based on the HMBC and NOESY spectra. Thus, the structure of **2** was established as urs-12-ene-2β, 3β, 22α-triol (**2**).

Compound **3** was obtained as colorless needles, and the IR spectrum of **3** indicated the presence of hydroxyl groups. The HR-MS of **3** showed the molecular ion at *m/z* 458.3790 ($C_{30}H_{50}O_3$), and many significant ions at *m/z* (relative intensity): 427 (2, $M^+ - CH_2OH$), 234 (18, a''), 223 (12, b), 205 (10, $b - H_2O$), 203 (100, $a'' - CH_2OH$), 187 (5, $b - 2H_2O$) (Chart 2). The fragment ions of the left-hand part of the molecule were very similar to those of **1** and **2**. Thus, the left-hand part of **3** is suggested to be the same as in **1** and **2**. The ^{1}H-NMR spectrum of **3** indicated the presence of five tertiary methyl, two secondary methyl, one hydroxymethyl, and two secondary hydroxyl groups, and therefore, **3** was also suggested to be an ursane derivative (Table 2). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **2** and **4** clearly showed that **3** has a hydroxymethyl group at C-28 (Tables 2 and 3). Furthermore, the locations of hydroxyl groups in **3** were 2β, 3β, and 28, based on the HMBC and NOESY spectra. Thus, the structure of **3** was established as urs-12-ene-2β,3β,28-triol (**3**).

It is noteworthy that four triterpenoid triols **1**—**4** were isolated only from the roots of *P. hieracioides* subsp. *japonica*, and not from the aerial parts. These compounds may be components of the latex in the perennials.

Experimental

Melting points were measured on a Yanagimoto micro apparatus without correction. Specific rotations were observed in $CHCl_3$ solution ($c = 0.1$—0.6) at 22—24 °C. ^{1}H- and ^{13}C-NMR spectra were taken at 500 and 100/125 MHz, respectively, by the Fourier-transform (FT) method in $CDCl_3$ solution with tetramethylsilane as an internal standard. MS was recorded (direct inlet) at 30 eV and the relative intensities of peaks were reported with reference to the most intense peak higher than *m/z* 100. HPLC was performed on a C-18 reverse-phase column (with a refraction index detector), with MeOH–H_2O (9 : 1) or CH_3CN–$CHCl_3$ (9 : 1) as the eluent. Silica gel 60, 230—400 mesh (Merck), and 20% $AgNO_3$-impregnated silica gel were used for column chromatography (CC).

Plant Material Refer to the preceding paper.[1]

Erythrodiol (5), 3β-Hydroxyoleana-11,13(18)-dien-28-oic Acid (6), Oleanolic Acid (7), Ursolic Acid (8) Fraction L (see the preceding paper[1]) was methylated with diazomethane. The products were chromatographed repeatedly on silica gel with benzene–ether (9 : 1) to give two fractions (frs. L-I, L-II). Fraction L-I was acetylated with acetic anhydride–pyridine. The products were separated by HPLC with CH_3CN–$CHCl_3$ (9 : 1) to give the following crystalline solids (recrystallized from MeOH to obtain pure specimens). Erythrodiol diacetate (**5a**, 1 mg), mp 189—190 °C. ^{1}H-NMR δ: 0.857 (H-23), 0.857 (H-24), 0.943 (H-25), 0.759 (H-26), 1.144 (H-27), 3.984, 4.126 (d, $J = 7.1$ Hz, H-28), 0.894 (H-29), 0.943 (H-30), 4.498 (dd, $J = 7.6$, 8.3 Hz, H-3), 5.242 (dd, $J = 3.8$, 3.8 Hz, H-12), 2.046 (–OCOC$\underline{H}_3$). Methyl 3β-acetoxyoleana-11,13(18)-dien-28-oate (**6'a**, 1 mg), mp 230—231 °C, UV λ_{max}^{EtOH} nm (ε): 242 (21000), 250 (21500), 259 (16200). ^{1}H-NMR δ: 0.865 (H-23), 0.865 (H-24), 0.928 (H-25), 0.791 (H-26), 0.958 (H-27), 0.791 (H-29), 0.943 (H-30), 4.524 (dd, $J = 6.8$, 9.0 Hz, H-3), 6.435 (dd, $J = 3.2$, 10.5 Hz, H-11), 5.618 (d, $J = 11.2$ Hz, H-12), 2.061 (–OCOC$\underline{H}_3$), 3.662 (–COOC$\underline{H}_3$). Methyl *O*-acetyl oleanolate (**7'a**, 137 mg), mp 225—226 °C. ^{1}H-NMR δ: 0.857 (H-23), 0.857 (H-24), 0.928 (H-25), 0.723 (H-26), 1.125 (H-27), 0.899 (H-29), 0.928 (H-30), 4.490 (dd, $J = 6.9$, 9.0 Hz, H-3), 5.278 (dd, $J = 3.4$, 3.4 Hz, H-12), 2.046 (–OCOC$\underline{H}_3$), 3.622 (–COOC$\underline{H}_3$). Methyl *O*-acetyl ursolate (**8'a**, 100 mg), mp 240.5—241.5 °C, ^{1}H-NMR δ: 0.860 (H-23), 0.860 (H-24), 0.946 (H-25), 0.745 (H-26), 1.073 (H-27), 0.789 (d, $J = 8.8$ Hz, H-29), 0.919 (d, $J = 5.4$ Hz, H-30), 4.498 (dd, $J = 6.6$, 9.0 Hz, H-3), 5.245 (dd, $J = 3.4$, 3.4 Hz, H-12), 2.046 (–OCOC$\underline{H}_3$), 3.603 (–COOC$\underline{H}_3$). Compounds **5a**, **6'a**, **7'a** and **8'a** were identified by comparison of their melting point and ^{1}H-NMR data with published values.[5-7]

Olean-12-ene-2β,3β,22α-triol (1), Urs-12-ene-2β,3β,22α-triol (2), Urs-12-ene-2β,3β,28-triol (3), Olean-12-ene-2β,3β,28-triol (4) Fraction L-II was separated by HPLC with MeOH–H_2O (9 : 1) to give the following crystalline solids (recrystallized from MeOH–H_2O to obtain pure specimens). **1** (37 mg). IR ν_{max}^{KBr} cm^{-1}: 3448, 1050, 1030. **2** (28 mg). IR ν_{max}^{KBr} cm^{-1}: 3420, 1045, 1030. **3** (1.5 mg). IR ν_{max}^{KBr} cm^{-1}: 3422, 1049, 1017. **4** (8 mg). IR ν_{max}^{KBr} cm^{-1}: 3422, 1051, 1033. Compound **4** was identified by comparison of its ^{1}H-NMR data with published values.[3]

Acknowledgement The authors are indebted to Mr. Yôichi Takase of this College for MS measurements.

References and Notes

1) Shiojima K., Masuda K., Suzuki H., Lin T., Ooishi Y., Ageta H., *Chem. Pharm. Bull.*, **43**, 1634 (1995).
2) Shiojima K., Arai Y., Masuda K., Takase Y., Ageta T., Ageta H., *Chem. Pharm. Bull.*, **40**, 1683 (1992).
3) Bohlmann F., Wallmeyer M., Jakupovic J., *Phytochemistry*, **21**, 1806 (1982).
4) Ageta H., Shiojima K., Arai Y., Suzuki H., Kiyotani T., *Chem. Pharm. Bull.*, **42**, 39 (1994).
5) Unpublished data from our laboratory.
6) Asada M., Amagaya S., Takai M., Ogihara Y., *J. Chem. Soc., Perkin Trans. 1*, **1980**, 325.
7) Arai Y., Kusumoto Y., Nagao M., Shiojima K., Ageta H., *Yakugaku Zasshi*, **103**, 356 (1983).