Cytotoxic Triterpenes from a Chinese Medicine, Goreishi[1)]

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Bioactivity-guided fractionation of the methanol extract of Goreishi (the feces of *Trogopterus xanthipes* MILNE-EDWARDS) afforded one new and three known cytotoxic triterpenes, namely, 3-*O*-*cis*-*p*-coumaroyltormentic acid, pomolic acid, 2α-hydroxyursolic acid, and jacoumaric acid. In the course of this investigation, six additional compounds having no cytotoxic activity were isolated, namely, maslinic acid, 3-*O*-*trans*-*p*-coumaroylmaslinic acid, ursolic acid, tormentic acid, euscaphic acid, and a new triterpene, 3-*O*-*trans*-*p*-coumaroyltormentic acid. The structures of the new compounds were established on the basis of X-nucleus-proton correlation with fixed evolution time (XCORFE) and other spectroscopic evidence.

Keywords Goreishi; *Trogopterus xanthipes*; triterpene; 3-*O*-*p*-coumaroyltormentic acid; 2α-hydroxyursolic acid; pomolic acid; jacoumaric acid; cytotoxicity; P-388 cell; XCORFE

Previously it was reported by Kosuge *et al.* that a Chinese traditional medicine, Goreishi (the feces of *Trogopterus xanthipes* MILNE-EDWARDS) exhibited tumor-inhibitory activity in the Ehrlich ascites carcinoma *in vivo* bioassay.[2)] This result led us to investigate the constituents of this drug manifesting the activity, by using the P-388 lymphocytic leukemia cell culture test system.[3)]

The 80% MeOH extract of Goreishi was dissolved in 90% MeOH and the solution was extracted successively with hexane, carbon tetrachloride, and chloroform. The chloroform extract (ED_{50} 21 μg/ml) was purified by Sephadex LH-20 and silica gel column chromatographies and high-performance liquid chromatography (HPLC) to afford two new triterpenes (**1**, **2**) as well as eight known ones (**3**—**10**). Among them, four compounds (**2**, **5**—**7**) showed significant cytotoxic activities against P-388 culture cells (Table I).

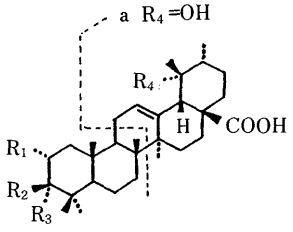
TABLE I. Evaluation of the Cytotoxic Activity of Triterpenes

Compound	Test system P388 (ED_{50} μg/ml)[a)]
1	5.6
2	3.9
3	5.4
4	17.5
5	4.0
6	3.2
7	2.9
8	9.0
9	6.9
10	13.0
5-Fluorouracil (standard)	8.0×10^{-2}

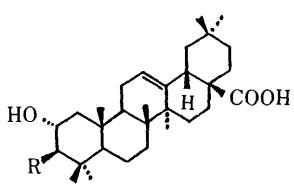
a) For significant activity, an ED_{50} = 4.0 μg/ml is required.[4)]

The known compounds (**3**, **4**, **6**—**10**) were identified, by comparison of spectral data with published values for the compounds themselves or their derivatives, as tormentic acid,[5)] euscaphic acid,[6)] 2α-hydroxyursolic acid,[7)] pomolic acid,[8)] ursolic acid,[7)] 3-*O*-*trans*-*p*-coumaroylmaslinic acid,[9)] and maslinic acid,[9)] respectively (Chart 1). Compound **5**, mp 236—238 °C (MeOH), $[\alpha]_D^{25}$ +18° (*c* = 0.4, MeOH), afforded 2α-hydroxyursolic acid (**6**) and *trans*-*p*-coumaric acid on alkaline hydrolysis. This result and spectroscopic evidence showed **5** to be jacoumaric acid, which had been isolated as the methyl ester derivative from *Jacaranda caucana*.[10)] This is the first isolation of the original acid. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **3**, **4** and **5** were assigned by the application of distortionless enhancement by polarization transfer (DEPT) and ^{1}H–^{13}C heteronuclear shift-correlated (HETCOR) 2D-NMR spectra and comparison with the spectra of other related triterpenes since they had not previously been assigned (Table II).

The molecular formula of compound **1**, mp 245—247 °C (MeOH), $[\alpha]_D^{25}$ +25° (*c* = 0.3, MeOH), was determined as $C_{39}H_{54}O_7$ on the basis of the fast atom bombardment mass spectrum (FAB-MS) [*m*/*z*: 657 $(M+Na)^+$], and proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR



1 : $R_1=R_4=OH$, $R_2=OCC(OH)=C(H)$-(1′ 2′ 3″ 5″ 6″ ring)-OH, $R_3=H$
2 : $R_1=R_4=OH$, $R_2=OCC(OH)=C(H)$-(1′ 2′ 3″ 5″ 6″ ring)-OH, $R_3=H$
3 : $R_1=R_2=R_4=OH$, $R_3=H$
4 : $R_1=R_3=R_4=OH$, $R_2=H$
5 : $R_1=OH$, $R_2=OCC(OH)=C(H)$-(ring)-OH, $R_3=R_4=H$
6 : $R_1=R_2=OH$, $R_3=R_4=H$
7 : $R_1=R_3=H$, $R_2=R_4=OH$
8 : $R_1=R_3=R_4=H$, $R_2=OH$



9 : $R=OCC(OH)=C(H)$-(ring)-OH
10 : R=OH

Chart 1

TABLE II. ^{13}C-NMR Chemical Shifts (δ ppm) of **3**, **4**, and **5** in Pyridine-d_5

Position	3	4	5	Position	3	4	5
1	47.93	42.91	48.61	21	26.96	26.98	31.11
2	68.62	66.12	66.40	22	38.53	38.53	37.47
3	83.88	79.38	85.04	23	29.36	29.48	29.04
4	39.87	42.21	39.51	24	17.68	22.32	18.29
5	55.98	48.78	55.55	25	16.89	16.67	17.43
6	19.02	18.63	18.68	26	17.28	17.32	17.54
7	33.55	33.55	33.35	27	24.72	24.64	23.97
8	40.45	40.60	39.84	28	180.74	180.74	179.94
9	47.85	47.66	47.99	29	27.12	27.08	21.44
10	38.53	38.69	38.30	30	16.81	16.80	16.92
11	24.14	24.12	23.72	1′			167.90
12	127.97	127.98	125.41	2′			116.11
13	140.00	139.99	139.38	3′			144.79
14	42.18	42.21	40.02	1″			126.29
15	29.36	29.29	28.67	2″			130.61
16	26.41	26.42	24.92	3″			116.81
17	48.31	48.30	42.56	4″			161.35
18	54.63	54.63	53.53	5″			116.81
19	72.72	72.73	39.51	6″			130.61
20	42.18	42.37	39.44				

TABLE III. ^{1}H- and ^{13}C-NMR Chemical Shifts (δ ppm) and Coupling Constants (J_{H-H} Hz, in Parenthesis) of **1** and **2** in Pyridine-d_5

Position	Proton		Carbon	
	1	2	1	2
1	1.41 t (9.5)	1.38 t (9.9, 4.0)	48.49	48.59
	2.33 dd (9.5, 3.4)	2.30 dd (9.9, 4.0)		
2	4.32 td (9.5, 3.4)	4.27 td (9.9, 4.0)	66.40	66.32
3	5.26 d (9.5)	5.20 d (9.9)	85.09	84.98
4			39.85	39.68
5			55.58	55.58
6			18.79	18.79
7			33.36	33.33
8			40.38	40.38
9			47.71	47.68
10			38.36	38.36
11			24.09	24.04
12	5.59 t (3.5)	5.58 t (3.5)	127.74	127.74
13			140.08	140.08
14			42.15	42.12
15			29.29	29.24
16 (β)	3.16 td (12.0, 5.5)	3.15 td (12.0, 5.0)	26.39	26.35
17			48.29	48.27
18	3.07 s	3.06 s	54.64	54.60
19			72.69	72.70
20			42.41	42.37
21			26.99	26.94
22			38.52	38.53
23	1.06 s	1.05 s	29.00	29.00
24	1.05 s	0.99 s	18.24	18.15
25	1.03 s	1.00 s	16.81	16.80
26	1.11 s	1.09 s	17.20	17.21
27	1.73 s	1.69 s	24.72	24.71
28			180.88	180.74
29	1.46 s	1.43 s	27.12	27.12
30	1.15 d (6.6)	1.13 d (6.6)	16.81	16.69
1′			167.95	167.19
2′	6.68 d (15.8)	6.09 d (13.3)	116.05	111.07
3′	8.01 d (15.8)	6.29 d (13.3)	144.80	143.60
1″			126.25	126.71
2″	7.19 d (8.6)	7.14 d (9.1)	130.58	133.75
3″	7.57 d (8.6)	8.14 d (9.1)	116.77	115.88
4″			161.35	160.44
5″	7.57 d (8.6)	8.14 d (9.1)	116.77	115.88
6″	7.19 d (8.6)	7.14 d (9.1)	130.58	133.75

spectra. In the electron ionization (EI) MS, **1** exhibited a characteristic fragment peak at *m/z* 264 (ion a), resulting from *retro*-Diels–Alder (RDA) cleavage of ring C.[11] In addition to this evidence, the observation of one *sec*-methyl and six *tert*-methyl signals in the ^{1}H-NMR spectrum (Table III) of this compound suggested that **1** was a Δ^{12}-unsaturated triterpene. The infrared (IR), ^{1}H- and ^{13}C-NMR spectra (Table III) indicated that **1** possessed four functionalities, namely, a carboxylic acid unit, a *sec*-hydroxyl, a *tert*-hydroxyl and a *trans-p*-coumaroyloxyl groups. Assignments of ^{1}H- and ^{13}C-NMR spectra were based on the application of DEPT, and ^{1}H–^{13}C HETCOR (Fig. 1), X-nucleus-proton correlation with fixed evolution time (XCORFE) (Fig. 2) and ^{1}H–^{1}H homonuclear shift correlation (COSY) 2D-NMR spectra, and comparison with published data for related triterpenes.[6-8] In the ^{1}H-NMR spectrum, the coupling constant of 9.5 Hz between the *sec*-hydroxyl-bearing methine and *trans-p*-coumaroyloxyl-bearing methine protons suggested that these groups, located at the vicinal position, should be affixed in an equatorial manner. In addition, the XCORFE 2D-NMR spectrum[12] (XCORFE is a newly modified technique of long-range ^{1}H–^{13}C HETCOR) showed that the carbon signal of the *p*-coumaroyloxyl-bearing methine correlated with the 23-methyl protons (Fig. 2). These data indicate that the *sec*-hydroxyl and *p*-coumaroyloxyl groups are located at the 2- and 3-positions, respectively.

In the ^{1}H-NMR spectrum of **1** the allylic 18-proton signal appears as a singlet at δ 3.07 ppm. Furthermore, in the XCORFE spectrum the *tert*-hydroxyl-bearing carbon signal has cross peaks with the *sec*-methyl proton signal as well as the *tert*-methyl proton signal observed at lower field (δ 1.46 ppm) due to the linkage with the hydroxyl group. The *tert*-methyl proton signal at δ 1.46 ppm also has cross peaks with the 18- and 20-carbon signals, and the carbon signal of the carboxylic acid moiety has a cross peak with the signal at δ 3.16 ppm (dt, J=5.5, 12 Hz) assignable to one of the 16-protons. These data show that the *sec*- and *tert*-methyl groups, and the carboxylic acid moiety are located at the 20-, 19-, and 17-positions, respectively. On the other hand, alkaline hydrolysis of **1** afforded tormentic acid (**3**) and *trans-p*-coumaric acid. Based on this evidence, compound **1** was assigned as 3-*O*-*trans-p*-coumaroyltormentic acid.

The general spectral features of compound **2**, mp 238—240 °C (MeOH), $C_{39}H_{54}O_7$ [FAB-MS *m/z*: 657 $(M+Na)^+$], $[\alpha]_D^{25}$ +2.1° (c=0.19, MeOH), closely resembled those of **1** except for the signals of the coumaroyl group in the ^{1}H- and ^{13}C-NMR spctra (Table III). In the ^{1}H-NMR spectrum of **2**, *cis*-conjugated olefinic protons (δ 6.09 and 6.29 ppm, each d, J=13.3 Hz) were apparent, in contrast to the analogous *trans*-conjugated signals (δ 6.68 and 8.01, each d, J=15.8 Hz) observed in the ^{1}H-NMR spectrum of **1**. Therefore, compound **2** was assigned the structure 3-*O*-*cis*-*p*-coumaroyltormentic acid.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The ultraviolet (UV) spectra were recorded with a Hitachi 323 spectrophotometer and the IR spectra with a Hitachi EPI-G2 spectrometer. Optical rotations were measured on a

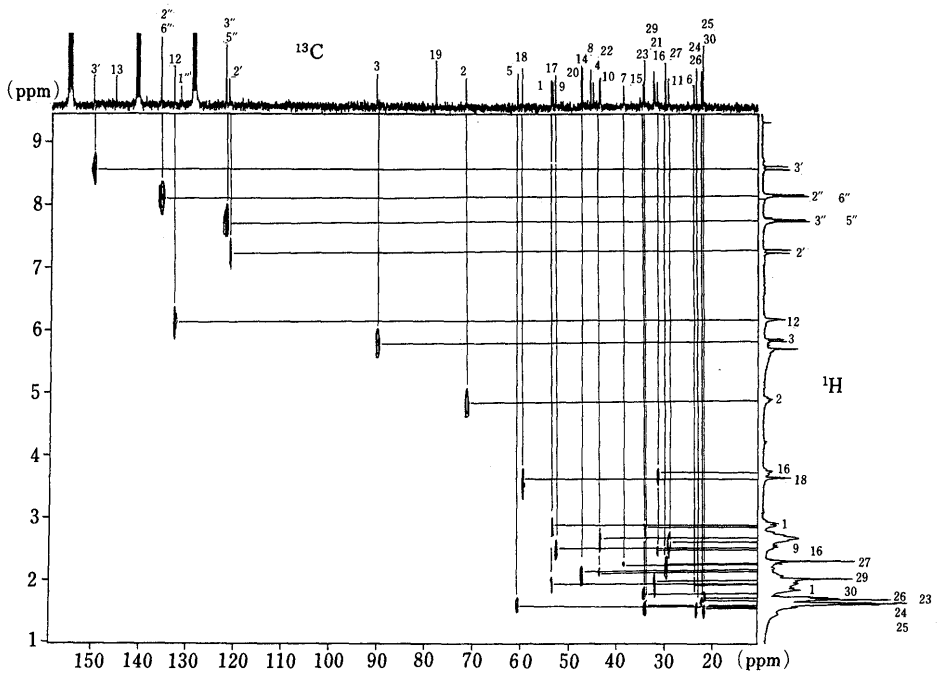


Fig. 1. The HETCOR Spectrum of **1**

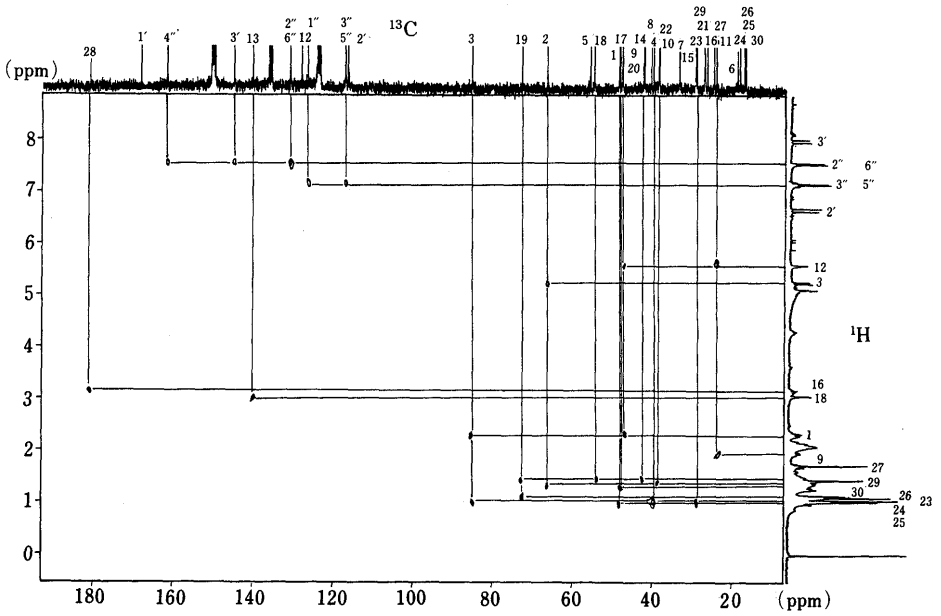


Fig. 2. The XCORFE Spectrum of **1**

JASCO ORD/UV-5 spectropolarimeter. NMR spectra were recorded at 27 °C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^{1}H and ^{13}C, respectively, in pyridine-d_5 with tetramethylsilane (TMS) as an internal reference. The parameters used for 2D experiments were as follows. (i) The ^{1}H–^{13}C HETCOR experiment: 128 experiments of 256 scans each; relaxation delay, 0.5 s; D3, 3.7 ms; D4, 2.5 ms; spectral width, 5399.6 Hz (F1), 17361.1 Hz (F2); number of data points in F2, 2 K; sine bell multiplication in both dimensions, zero-filling to 256 W in t_1. (ii) The XCORFE experiment: 128 experiments of 2048 scans, relaxation delay, 0.5 s; T, 22.7 ms; Δ, 48 ms; τ, 3.7 ms; spectral width, 2820.9 (F1), 14025.2 (F2); number of data points in F2, 2 K; sine bell multiplication in both dimensions, zero-filling to 256 W in t_1. FAB-MS and EI-MS were take on ZAB-SE and Hitachi M 80 spectrometers, respectively. HPLC was run on a Waters ALC-200 instrument modified with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.).

Extraction and Separation Goreishi (3 kg), purchased from Tochimototenkaido Co., Ltd., was extracted three times with boiling 80% MeOH (5 l each) for 3 h. The combined extracts were evaporated *in vacuo*. The residue (232.5 g) was dissolved in MeOH–H_2O (9 : 1, 2 l) and the solution was extracted with hexane (2 l × 3). The water layer was diluted with water (200 ml) and extracted with CCl_4 (2 l × 3). The water layer was further diluted with water (1.4 l) and extracted with $CHCl_3$ (2 l × 3). The four fractions obtained were separately evaporated *in vacuo* to give the hexane (16.5 g), CCl_4 (42.2 g), $CHCl_3$ (53.5 g) and water (128.3 g) extracts, the cytotoxic activities of which were ED_{50} 40, 33, 21, and 80 μg/ml, respectively. The $CHCl_3$ extract (37.9 g) was passed through Sephadex LH-20, using $CHCl_3$–MeOH (1 : 1) as the eluent. The third fraction (14.2 g) was repeatedly chromatographed on a silica gel column with an MeOH–CH_2Cl_2 gradient as the eluent. Elution with 1% MeOH in CH_2Cl_2 gave **8** (337 mg). Further elution with 1% MeOH in CH_2Cl_2 gave a crystalline substance which was then subjected to HPLC with 90% MeOH, giving **1** (71.5 mg), **2** (23.7 mg), **3** (10.2 mg), **4** (7 mg), **5** (13.8 mg), **6** (15.3 mg), **7** (34.2 mg), **9** (6.6 mg) and **10** (10.7 mg).

3-*O*-*trans*-*p*-Coumaroyltormentic Acid (1) Colorless needles, mp 245—247 °C (from MeOH), $[\alpha]_D^{25}$ +25° (c=0.3, MeOH). FAB-MS m/z: 657 $(M+Na)^+$. EI-MS m/z: 634 (M^+), 573, 453, 408, 264 (RDA ion). UV

λ_{max}^{MeOH} nm (log ε): 225 (4.22), 303 (4.38), 314 (4.42). IR ν_{max}^{KBr} cm^{-1}: 3550—2400, 1680 (COOH), 1700 (α,β-unsaturated ester), 1630 (C=C), 1600, 1590, 1510 (ar. C–C).

Hydrolysis of 1 Compound **1** (20 mg) was saponified with 2% KOH–EtOH (10 ml) for 1 h. The reaction mixture was partitioned between EtOAc and H_2O. The EtOAc layer was evaporated to dryness and the residue was recrystallized from MeOH–H_2O to give **3**, which was identified by direct comparison. The H_2O layer was neutralized with diluted HCl and extracted with EtOAc. After evaporation of the solvent, the residue was recrystallized from H_2O to give *trans-p*-coumaric acid, identical with an authentic sample.

3-*O*-*cis*-*p*-Coumaroyltormentic Acid (2) Colorless needles, mp 238—240 °C (from MeOH), $[\alpha]_D^{25}$ +2.1° (c=0.19, MeOH). FAB-MS m/z: 657 $(M+Na)^+$. EI-MS m/z: 572, 452, 408, 264 (RDA ion). UV λ_{max}^{MeOH} nm (log ε): 225 (4.14), 302 (4.25), 313 (4.27). IR ν_{max}^{KBr} cm^{-1}: 3550—2400, 1670 (COOH), 1700 (α,β-unsaturated ester), 1630 (C=C), 1598, 1580, 1505 (ar. C–C).

Tormentic Acid (3) Colorless crystalline powder, mp 260—262 °C (lit.[6] mp 264—266 °C) (from MeOH–H_2O). High resolution (HR)-MS m/z: 488.3490 (M^+) (Calcd for $C_{30}H_{48}O_5$: 488.3489), 264.1730 (RDA ion) (Calcd for $C_{16}H_{24}O_3$: 264.1719). ^{1}H-NMR (pyridine-d_5) δ ppm: 1.02 (3H, s, 25-H), 1.10 (3H, s, 24-H), 1.13 (3H, s, 26-H), 1.13 (3H, d, J=6.1 Hz, 30-H), 1.28 (3H, s, 23-H), 1.45 (3H, s, 29-H), 1.73 (3H, s, 27-H), 3.07 (1H, s, 18-H), 3.40 (1H, d, J=9.3 Hz, 3α-H), 4.12 (1H, dt, J=3.8, 9.3 Hz, 2β-H), 5.60 (1H, t, J=3.5 Hz, 12-H).

Methyl tormentate diacetate, prepared in a usual manner, was identified by comparison of the ^{1}H-NMR data with published values.[5]

Euscaphic Acid (4) Colorless crystalline powder, mp 266—268 °C (lit.[6] mp 270 °C) (from MeOH). HR-MS m/z: 488.3521 (M^+) (Calcd for $C_{30}H_{48}O_5$: 488.3489), 264.1732 (RDA ion) (Calcd for $C_{16}H_{24}O_3$: 264.1719). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.91 (3H, s, 25-H), 0.99 (3H, s, 24-H), 1.12 (3H, s, 26-H), 1.13 (3H, d, J=6.3 Hz, 30-H), 1.27 (3H, s, 23-H), 1.43 (3H, s, 29-H), 1.65 (3H, s, 27-H), 3.05 (1H, s, 18-H), 3.62 (1H, d, J=2.6 Hz, 3β-H), 4.31 (1H, dt, J=6.8, 2.6 Hz, 2β-H), 5.59 (1H, t, J=3.5 Hz, 12-H).

Methyl euscaphate prepared in a usual manner was identified by comparison of the ^{1}H-NMR data with reported values.[6]

Jacoumaric Acid (5) Colorlss crystalline powder, mp 236—238 °C (from MeOH), $[\alpha]_D^{25}$ +18° (c=0.4, MeOH). FAB-MS m/z: 641 $(M+Na)^+$. EI-MS m/z: 454, 408, 248 (RDA ion). IR ν_{max}^{KBr} cm^{-1}: 3550—2400, 1690 (COOH), 1710 (α,β-unsaturated ester), 1630 (C=C), 1603, 1590, 1514 (ar. C–C). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.98 (3H, d, J=7.2 Hz, 29-H), 0.99 (3H, d, J=7.0 Hz, 30-H), 1.01 (3H, s, 25-H), 1.05 (3H, s, 24-H), 1.05 (3H, s, 23-H), 1.08 (3H, s, 26-H), 1.24 (3H, s, 27-H), 2.65 (1H, d, J=9.2 Hz, 18-H), 4.11 (1H, dt, J=4.4, 9.8 Hz, 2β-H), 5.27 (1H, d, J=9.8 Hz, 3α-H), 5.48 (1H, t, J=3.5 Hz, 12-H).

Hydrolysis of 5 **5** (10 mg) was saponified with 2% KOH–EtOH (5 ml) for 1 h. The reaction mixture was subjected to the same procedure as described for hydrolysis of **1** to afford **6** and *trans-p*-coumaric acid, which were identified by direct comparison with authentic samples.

2α-Hydroxyursolic Acid (6) Colorless needles, mp 239—241 °C (lit.[13] 243—245 °C) (from MeOH). HR-MS m/z: 472.3557 (M^+) (Calcd for $C_{30}H_{48}O_4$: 472.3540), 248.1767 (RDA ion) (Calcd for $C_{16}H_{24}O_2$: 248.1770). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.98 (3H, d, J=6.6 Hz, 29-H), 0.99 (3H, s, 25-H), 1.00 (3H, d, J=6.2 Hz, 30-H), 1.01 (3H, s, 24-H), 1.09 (3H, s, 26-H), 1.22 (3H, s, 27-H), 1.29 (3H, s, 23-H), 2.64 (1H, d, J=10.8 Hz, 18-H), 3.42 (1H, d, J=9.7 Hz, 3α-H), 4.11 (1H, dt, J=3.9, 9.7 Hz, 2β-H), 5.47 (1H, t, J=3.5 Hz, 12-H).

The methyl ester of **6** was prepared in a usual manner and identified by comparison of the ^{13}C-NMR data with published values.[7]

Pomolic Acid (7) Colorless crystalline powder, mp >300 °C (lit.[6] mp >300 °C) (from MeOH). HR-MS m/z: 472.3562 (M^+) (Calcd for $C_{30}H_{48}O_4$: 472.3540), 264.1715 (RDA ion) (Calcd for $C_{16}H_{24}O_3$: 264.1719). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.93 (3H, s, 25-H), 1.05 (3H, s, 24-H), 1.14 (3H, s, 26-H), 1.14 (3H, d, J=6.1 Hz, 30-H), 1.25 (3H, s, 23-H), 1.47 (3H, s, 29-H), 1.75 (3H, s, 27-H), 3.08 (1H, s, 18-H), 3.45 (1H, dd, J=10.2, 4.0 Hz, 3α-H), 5.63 (1H, t, J=3.5 Hz, 12-H).

The methyl ester of **7** was prepared in a usual manner and identified by comparison of the ^{13}C-NMR data with reported values.[8]

Ursolic Acid (8) Colorless needles, mp 279—281 °C (lit.[14] mp 278—280 °C) (from MeOH). HR-MS m/z: 456.3605 (M^+) (Calcd for $C_{30}H_{48}O_3$: 456.3591), 248.1756 (RDA ion) (Calcd for $C_{16}H_{24}O_2$: 248.1770). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.91 (3H, s, 25-H), 0.97 (3H, d, J=6.3 Hz, 29-H), 1.02 (3H, d, J=6.6 Hz, 30-H), 1.05 (3H, s, 24-H), 1.08 (3H, s, 26-H), 1.25 (3H, s, 27-H), 1.27 (3H, s, 23-H), 2.66 (1H, d, J=3.5 Hz, 18-H), 3.48 (1H, dd, J=10.3, 4.1 Hz, 3α-H), 5.52 (1H, t, J=3.5 Hz, 12-H).

The methyl ester of **8** was prepared in a usual manner and identified by comparison of the ^{13}C-NMR data with reported values.[7]

3-*O*-*trans*-*p*-Coumaroylmaslinic Acid (9) Colorless needles, mp 277—279 °C (lit.[9] mp 278—282 °C) (from benzene–acetone). FAB-MS m/z: 641 $(M+Na)^+$. EI-MS m/z: 454, 408, 371, 248 (RDA ion), 203. The ^{1}H-NMR data were in accord with the published ones.[9]

Maslinic Acid (10) Colorless crystalline powder, mp 257—259 °C (lit.[9] mp 258—260 °C) (from MeOH). HR-MS m/z: 472.3563 (M^+) (Calcd for $C_{30}H_{48}O_4$: 472.3540), 248.1770 (RDA ion) (Calcd for $C_{16}H_{24}O_2$: 248.1770). The ^{1}H-NMR data were in accord with the published ones.[9]

Cytotoxic Activity P-388 lymphocytic leukemia cells were cultured at 37 °C in suspension in Eagle's minimum essential medium (Gibco) supplemented with 10% fetal bovine serum (Whittaker M. A. Bioproducts). The test material was dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 10 mg/ml and the solution was diluted with the medium to give concentrations of 200, 20, and 2 μg/ml. Each solution was combined in the ratio of one to one with the cell suspension (2×10^5/ml) in the medium. After incubation at 37 °C for 3 d, the cell number in the test cell suspension was determined and expressed as a percentage, relative to the control cell suspension which was prepared without the test substance by the same procedure as described above. All assays were performed three times, semilogarithmic plots were constructed from the averaged data, and the effective dose of the substance required to inhibit cell growth by 50% (ED_{50}) was determined.

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References and Notes

1) This work was presented at the 37th Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Kobe, Nov. 1987, Abstract of Papers, p. 37.
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