New Cytotoxic Oleanane-Type Triterpenoids from the Cones of *Liquidamber styraciflua*

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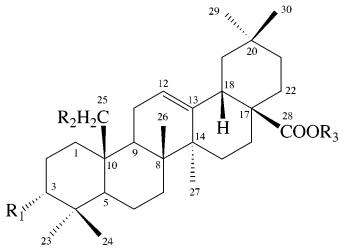
Two new oleanane-type triterpenoids (**1** and **2**), together with two known compounds, 6*β*-hydroxy-3-oxo-lup-20(29)-en-28-oic acid (**3**) and 3,11-dioxoolean-12-en-28-oic acid (**4**), were isolated from the stem bark of *Liquidamber styraciflua*. The structures of **1** and **2** were determined to be 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid (**1**) and 3α,25-dihydroxyolean-12-en-28-oic acid (**2**) on the basis of spectroscopic methods and chemical conversion. Compound **1** showed strong cytotoxicity against a disease-oriented panel of 39 human cancer cell lines, although compounds **2**, **3**, and **4** showed weaker activity compared to **1**.

In the course of a search for biologically active constituents from the cones, bark, and leaves of common trees that have been treated as wastes in the forestry industry, we found that several triterpenoids and diterpenoids isolated from the stem bark of *Picea jezoensis* Carr. var. *jezoensis* (Pinaceae)[1−3] and *Thuja standishii* (Gord.) Carr (Cupressaceae)[4,5] exhibit potent anti-tumor-promoting activity against two stages of mouse skin carcinogenesis. In our search for active compounds from natural sources, we examined the constituents from *Liquidamber styraciflua* (Japanese name: Momijibafuu) (Hamamelidaceae), which is planted as a roadside tree and grows taller than *L. formosana* (Japanese name: Fuu). Researchers had reported 36 monoterpenes and sesquiterpenes from the leaf oil of this plant by GC and GC−MS.[6] Triterpene constituents of this tree have not been previously reported.

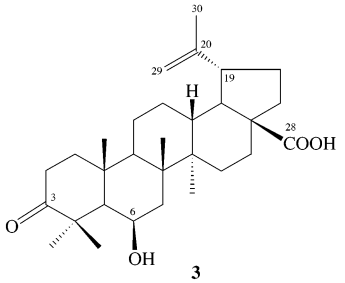
Triterpene carboxylic acids, such as oleanolic acid, ursolic acid, and betulinic acid, are among the most widely distributed triterpene series and are reported to exhibit several types of biological activities. For example, ursolic acid showed significant cytotoxicity in the lymphocytic leukemia cells P-388 and L-1210, the human lung carcinoma cell A-549, KB, and human colon (HCT-8) and mammary (MCF-7) tumor cells.[7] Ursolic acid inhibited lipoxygenase activity and HL60 leukemic cell proliferation.[8] Oleanolic acid showed anti-inflammatory action.[9] Ursolic acid, 2α-hydroxyursolic acid, and 3-*O*-caffeoyloleanolic acid were responsible for the cytotoxicity against A549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system), and HCT-15 (colon) human tumor cell lines.[10] Pomolic acid (3*β*,19α-hydroxyurs-12-en-28-oic acid) showed significant cytotoxic activity against M-14 melanoma and ME180 cervical carcinoma.[11] Natural 3-*O*-coumaroylalphitolic acid showed high cytotoxic activities against K562, B16(F-10), SK-MEL-2, PC-3, LOX-IMVI, and A549 tumor cell lines.[12] Hederagenin (3*β*,23-dihydroxyurs-12-en-28-oic acid) was found to be cytotoxic against P-388, L-1210, U-937, HL-60, SNU-5, and Hep G2 tumor cell lines.[13] The synthetic oleanane triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid showed potent antiproliferative and anti-inflammatory activity.[14] Oleanolic acid reduced azoxymethane (AOM)-induced ACF, colonic mucosal ODC activity, and AgNOR number in the colonic epithelium.[15] Topical application of ursolic acid together with TPA twice weekly for 20 weeks to DMBA-initiated mice inhibited the number of tumors per mouse.[16]

Results and Discussion

Stem bark of *L. styraciflua* was extracted with $CHCl_3$, and the extract was separated with silica gel column



1: R_1 = OH, R_2 = $OCOCH_3$, R_3 = H
1a: R_1 = OH, R_2 = $OCOCH_3$, R_3 = Me
1b: R_1 = R_2 = $OCOCH_3$, R_3 = Me
1c: R_1 = R_2 = OH, R_3 = H
1d: R_1 = R_2 = $OCOCH_3$, R_3 = H
2: R_1 = R_2 = OH, R_3 = H
2a: R_1 = R_2 = $OCOCH_3$, R_3 = H
2b: R_1 = R_2 = $OCOCH_3$, R_3 = Me



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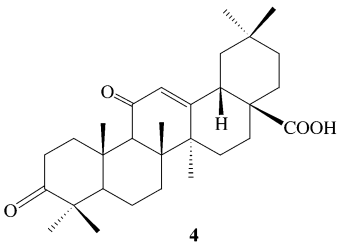

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Table 1. NMR Data for Compounds **1**[a] and **1a**[b] (125 and 500 MHz)[c]

	1				**1a**	
position	δ_C	δ_H	HMBC (C → H)	$^1H-^1H$ COSY	δ_C	δ_H
1α	29.6 t	1.36 m	2α, 2*β*, 3*β*	1*β*, 2α, 2*β*	28.4 t	1.10 m
1*β*		2.42 m		1α, 2α, 2*β*		1.98 m
2α	24.8 t	1.80 m	1α, 1*β*, 3*β*	1α, 1*β*, 2*β*	24.0 t	1.62 m
2*β*		2.44 m		1α, 1*β*, 2α		2.00 m
3*β*	78.5 d	4.99 t (2.5)	1α, 1*β*, 2α, 2*β*, 5α	2α, 2*β*	78.1 d	4.70 t (2.5)
4	36.7 s		2α, 2*β*, 3*β*, 5α, 6α, 6*β*		36.3 s	
5α	50.8 d	1.59 dd (11.0, 2.2)	3*β*, 6α, 6*β*	6α, 6*β*	50.8 d	1.29 dd (11.0 2.2)
6α	18.3 t	1.44 m	5α, 6α, 6*β*	5α, 6*β*	17.8 t	1.42 m
6*β*		1.44 m		5α, 6α		1.28 m
7α	33.3 t	1.40 m	5α, 6α, 6*β*	6α, 6*β*, 7*β*	32.5 t	1.30 m
7*β*		1.62 m		6α, 6*β*, 7α		1.50 m
8	40.1 s		7α,7*β*, 9α		39.5 t	
9α	49.1 d	2.02 dd (11.3, 4.1)	11α, 11*β*	11α, 11*β*	48.0 t	1.74 dd (11.3, 4.1)
10	41.8 s		1α, 1*β*, 2α, 2*β*, 5α, 9α		41.2 s	
11α	25.3 t	2.32 ddd (19.0, 4.1, 3.6)	9α, 12	9α, 11*β*, 12	24.8 t	2.04 ddd (19.0, 4.1, 3.6)
11*β*		2.90 ddd (19.0, 11.3, 3.6)		9α, 11α, 12		2.28 ddd (19.0, 11.3, 3.6)
12	123.8 d	5.54 t (3.6)	11α, 11*β*, 18α	11α, 11*β*	122.6 d	5.28 t (3.6)
13	144.6 s		18α		143.7 s	
14	42.4 s		15α, 15*β*, 16α, 16*β*, 18α		41.8 s	
15α	28.4 t	1.22 m	16α, 16*β*, 18α	15*β*, 16α, 16*β*	27.7 t	1.06 m
15*β*		2.24 ddd (13.2, 13.2, 3.5)		15α, 16α, 16*β*		1.64 m
16α	23.7 t	1.98 m	15α, 15*β*, 18α	15α, 15*β*, 16*β*	23.0 t	1.63 m
16*β*		2.12 m		15α, 15*β*, 16α		1.98 m
17	46.6 s		15α, 15*β*, 16α, 16*β*, 18α		46.7 s	
18*β*	41.9 d	3.32 dd (13.7, 4.1)	12,16α, 16*β*, 19α, 19*β*	19α, 19*β*	41.2 d	2.90 dd (13.7, 4.1)
19α	46.7 t	1.81 m	18α, 21α, 21*β*	18α, 19*β*	45.9 t	1.64 m
19*β*		1.32 m		18α, 19α		1.16 m
20	31.0 s		19α, 19*β*, 21α, 21*β*		30.7 s	
21α	34.2 t	1.45 m	19α, 19*β*, 22α, 22*β*	21*β*, 22α, 22*β*	33.8 t	1.34 m
21*β*		1.21 m		21α, 22α, 22*β*		1.20 m
22α	33.2 t	1.82 m	21α, 21*β*	21*β*, 21α, 22*β*	32.4 t	1.54 m
22*β*		2.05 m		21α, 21α, 22*β*		1.70 m
23	28.5 q	1.02 s	3α, 5α, 24		28.2 q	0.88 s
24	21.8 q	1.06 s	3α, 5α, 23		21.7 q	0.97 s
25A	60.3 t	4.17 d (12.1)	1α, 1*β*, 5α, 9*β*	25B	61.3 t	3.97 d (12.1)
25B		4.29 d (12.1)		25A		4.08 d (12.1)
26	17.9 q	1.24 s	7α, 7*β*, 9α		17.1 q	0.81 s
27	26.6 q	1.26 s	15α, 15*β*		26.4 q	1.18 s
28	180.2 s		16α, 16*β*, 18α, 22α, 22*β*		178.3 s	
29	33.2 q	0.94 s	19α, 19*β*, 21α, 21*β*, 30		33.1 q	0.90 s
30	23.8 q	1.00 s	19α, 19*β*, 21α, 21*β*, 29		23.6 q	0.93 s
OCO*CH*$_3$	21.2 q	2.08 s	3b		21.3 q	2.07 s
O*C*OCH$_3$	170.4 s		3b		170.7 s	
COO*CH*$_3$					51.5 q	3.63 s

[a] Pyridine-d_5. [b] $CDCl_3$. [c] Assignments confirmed by decoupling, H/H COSY, NOESY, HMQC, and HMBC spectra. *J* values are given in Hz.

chromatography, Sephadex LH-20, and medium-pressure liquid chromatography (MPLC). Two new (**1**, **2**) and two known (**3**, **4**) triterpenoids were obtained. Compounds **3** and **4** were confirmed as 6*β*-hydroxy-3-oxolup-20(29)-en-28 oic acid[17] and 3,11-dioxoolean-12-en-28-oic acid[18] because their physical and spectral data showed good agreement with those already published.

The molecular formula of compound **1** was assigned as $C_{32}H_{50}O_5$ (M^+; *m*/*z* 514.3654) by HREIMS. The IR spectrum showed a hydroxyl group (ν_{max} 3463 cm^{-1}), a carboxyl group (ν_{max} 3100−2700, 1703 cm^{-1}), and an acetoxyl group (ν_{max} 1721, 1265 cm^{-1}). The ^1H and ^{13}C NMR spectra (pyridine-d_5) of **1** (Table 1) exhibited six tertiary methyls, a primary acetoxyl group [δ_H 2.08 (3H, s), 4.17(1H, d), 4.29 (1H, d); δ_C 21.2 (q), 60.3 (t), 170.4 (s)], 10 methylenes, three methines, a secondary axial hydroxyl group [δ_H 4.99 (1H, t); δ_C 78.5 (d)], a trisubstituted double bond [δ_H 5.54 (1H, t); δ_C 123.8 (d), 144.6 (s)], five quaternary carbons, and a carboxylic acid [δ_C 180.2 (s)]. The HMBC spectrum of **1** indicated a long-range correlation between CH_2OCOCH_3 (δ_C 60.3) and H-1α, 1*β*, 5α, 9α and between CHOH (δ_C 78.5) and H-1α, 1*β*, 2α, 2*β*, 5α; therefore the primary acetoxyl group must be attached at C-25. The secondary hydroxyl group was assigned as C-3 axial, as its coupling constant with H_2-2 is 2.5 Hz. Correlations were also observed between C-12 (δ_C 123.8) and H-11α, 11*β*,18*β*; between C-23 (δ_C 28.5) and H-3α, 5α, Me-24; between C-24 (δ_C 21.8) and H-3α, 5α, Me-23; between C-26 (δ_C 17.9) and H-7α, 7*β*, 9α; between C-27 (δ_C 26.6) and H-15α, 15*β*; between C-29 (δ_C 33.2) and H-19α, 19*β*, 21α, 21*β*, Me-30; and between C-30 (δ_C 23.8) and H-19α, 19*β*, 21α, 21*β*, Me-29. In addition, a long-range correlation with C-28 (δ_C 180.2) was shown for H-16α, 16*β*, 18*β*, 22α, and 22*β* . In the $^1H-^1H$ COSY spectra of **1**, H-3*β* (δ_H 4.99) correlated with H-2α and 2*β*, and H-12 (δ_H 5.54) correlated with H-11α and 11*β*, respectively. In the NOESY spectra of **1**, significant NOEs (Figure 1) were observed between H-3*β* and H-6*β*, Me-23, Me-24; between Me-24 and H-2*β*; and between H-12 and H-18*β* and H-19*β*. The EIMS of **1** showed two predominant ion peaks characteristic for cleavage of the C ring at *m*/*z* 248 and a loss of COOH at 203, which indicates the presence of a carboxyl group at C-17.[19] Methylation with trimethylsilyl-diazomethane gave a methyl ester (**1a**), $C_{33}H_{52}O_5$ (M^+; *m*/*z* 528), δ_H 3.63 (3H, s, COOMe), and subsequent acetylation of **1a** with Ac_2O−pyridine gave a methyl acetyl derivative (**1b**), $C_{35}H_{54}O_6$ (M^+; *m*/*z* 570), in which the

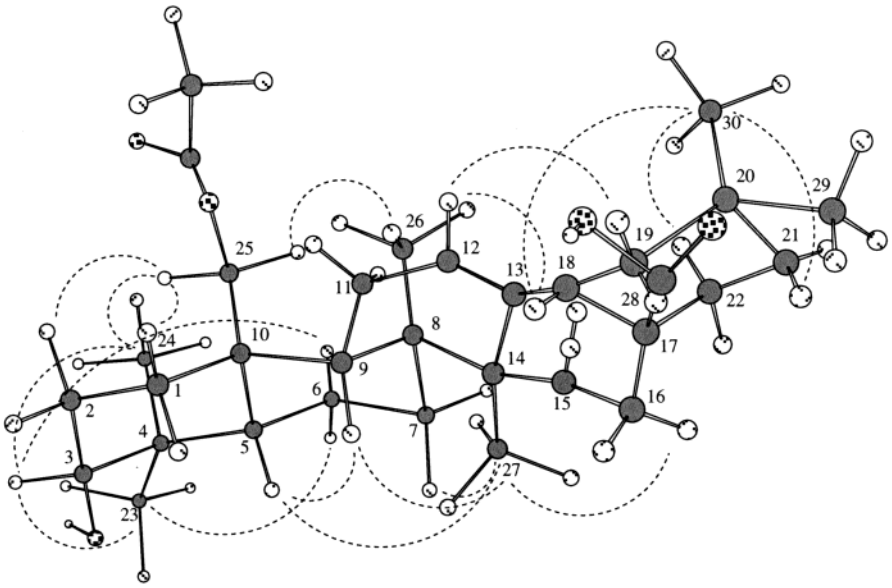

Figure 1. Selected NOESY correlations of **1**.

acetoxymethine proton signal appeared at δ_H 5.27 (t). On the other hand, alkaline hydrolysis of **1** with KOH−MeOH afforded a corresponding diol (**1c**), $C_{30}H_{48}O_4$ (M^+; *m/z* 470), δ_H 3.97 (t, H-3β), 3.97 and 4.05 (each 1H, d, H_2-25), and acetylation of **1** gave a diacetate (**1d**), $C_{34}H_{52}O_6$ (M^+; *m/z* 556), δ_H 4.68 (t, H-3β), 4.41 and 4.49 (each 1H, d, H_2-25). These data established the structure of **1** as 25-acethoxy-3α-hydroxyolean-12-en-28-oic acid.

Compound **2** was assigned as $C_{30}H_{48}O_4$ (M^+; *m/z* 472.3541) by HREIMS. The IR spectrum of **2** showed a hydroxyl (ν_{max} 3446 cm^{-1}) and a carboxyl group (ν_{max} 3100−2700, 1703 cm^{-1}). The ^1H and ^{13}C NMR spectra of **2** (Table 2) exhibited six tertiary methyls, a primary hydroxyl group [δ_H 3.97 (1H, d), 4.07 (1H, d); δ_C 61.5 (t)], 10 methylenes, three methines, a hydroxymethine group [δ_H 3.47 (1H, t); δ_C 75.9 (d)], a trisubstituted double bond [δ_H 5.28 (1H, t); δ_C 122.8 (d), 143.6 (s)], and five quaternary carbons and a carboxylic acid [δ_C 181.5 (s)]. Acetylation of **2** furnished a diacetyl derivative (**2a**), $C_{34}H_{52}O_6$ (M^+; *m/z* 556), and subsequent methylation of **2a** gave a diacetyl-methyl derivative (**2b**), $C_{35}H_{54}O_5$ (M^+; *m/z* 570), in which the primary hydroxymethyl group shifted to δ 4.44 (2H, s) and the secondary hydroxymethine proton shifted to δ 4.68 (t). Compounds **2**, **2a**, and **2b** were identical with synthetic **1c**, **1b**, and **1d** in all respects. Therefore the structure of **2** was established as 3α,25-dihydroxyolean-12-en-28-oic acid. Although a lot of oleanane-type triterpenes are found in nature, **1** and **2** are regarded as new compounds because the two hydroxyl groups at C-3 and C-25 adopt α axial and β axial configurations, respectively, and condensation between C-3 and C-25 is impossible. The availability of compounds **1** and **2** in quantity from readily available natural sources should be of considerable interest.

Growth Inhibition against a Panel of 39 Human Cancer Cell Lines. To evaluate drugs for their cell growth inhibition profile, a human cancer cell line panel combined with a database was established.[20] The system as a whole was developed according to the method of the National Cancer Institute, with modification.[21] The cell line panel consisted of 37 human cancer cell lines and 2 xPg. With this system, more than 200 standard compounds including various anticancer drugs have been examined, and a new database has been established.

The cancer growth inhibitory properties of compounds **1**−**4** were examined using a disease-oriented panel of 39 human cancer cell lines including 2 xPg cell lines (HCC panel) at the Japanese Foundation for Cancer Research.[20] Compound **1** showed significant cytotoxic activity against the colon HT-29 (log GI_{50} −5.47), HCT-116 (−5.34), and lung A549 (−5.37) cell lines (Table 3), and the average logarithm of the GI_{50} (MG-MID) across all cell lines tested was −5.00. Compounds **2** (MG-MID: −4.44), **3** (MG-MID: −4.34), and **4** (MG-MID: −4.38) were weaker in activity compared to **1** (MG-MID: −5.00). A known triterpenoid, betulinic acid, showed strong cytotoxic activity against breast BSY-1 (log GI_{50} −5.53) and CNS SF-539 (−5.39) cell lines, and the MG-MID was −4.84. The fingerprints of compound **1** and betulinic acid showed differences in their mechanisms of action (positive correlation $r = 0.225$). As shown in Table 3, the delta and range values of **1** were 0.47 and 0.92, respectively (effective value: delta >0.5 as well as range >1.0), indicating that compound **1** has selective cytotoxic activity.

Furthermore, evaluation of the pattern of differential cytotoxicity using the COMPARE program[21] suggested the possibility that the mode of action for **1** might be different from that shown by any other anticancer drug developed to date. Compounds **2**, **3**, and **4** resembled E7010 (r = 0.547), W80 (r = 0.585); E7010 (r = 0.571), vinblastine (r = 0.565); and E7010 (r = 0.6), W80 (r = 0.549), vinblastine (r = 0.505), respectively, suggesting that their molecular target is tubulin. Compounds **1** and **2** belong to the Δ_{12} oleanane series of triterpenes, and the only structural difference between **1** and **2** is the presence of a C-25 acetyl group instead of a C-25 hydroxyl group. It is noteworthy that the C-25 acetyl group in Δ_{12} oleanane is important to enhance the cytotoxic activity.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micro-melting point apparatus without correction. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. IR spectra were recorded using a Perkin-Elmer 1720X FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ and pyridine-d_5 were used as the solvent and Me_4Si (TMS) as the internal standard. EIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out

Table 2. NMR Data for Compounds **2**[a] and **2b**[a] (125 and 500 MHz)[b]

	2			**2b**	
position	δ_C	δ_H	HMBC (C → H)	δ_C	δ_H
1α	27.8 t	1.24 m	2α, 2*β*, 3*β*, 25	28.8 t	1.15 m
1*β*		1.92 m			1.84 m
2α	26.6 t	1.58 m	1α, 1*β*, 3*β*	23.6 t	1.59 m
2*β*		2.05 m			1.87 m
3*β*	75.9 d	3.47 t (2.7)	1α, 1*β*, 2α, 2*β*, 23, 24	77.6 d	4.68 t (2.7)
4	37.2 s		3α, 23, 24	36.2 s	
5α	48.8 d	1.34 m	23, 24, 25	49.9 d	1.34 m
6α	17.9 t	1.40 m	5α,7*β*	17.9 t	1.47 m
6*β*		1.27 m			1.32 m
7α	32.6 t	1.48 m	5α, 26	32.4 t	1.53 m
7*β*		1.30 m			1.32 m
8	39.6 s		7α, 7*β*, 26, 27	39.4 s	
9α	48.0 d		12, 25, 26	48.0 d	
10	41.4 s		1α, 1*β*, 25	39.9 s	
11α	24.8 t	2.08 m	9α, 12	24.4 t	2.02 m
11*β*		2.27 ddd (18.5, 11.4, 3.4)			2.16 ddd (18.5, 11.4, 3.4)
12	122.8 d	5.28 t (3.4)	9α, 11α, 11*β*	122.4 d	5.27 t (3.7)
13	143.6 s		12, 27	143.7 s	
14	41.8 s		15α, 15*β*, 26, 27	41.8 s	
15α	27.6 t	1.07 m	16α, 16*β*, 27	27.6 t	1.07 m
15*β*		1.74 m			1.63 m
16α	22.9 t	1.99 m	15α, 15*β*	23.0 t	1.98 m
16*β*		1.60 m			1.66 m
17	46.4 s		18*β*, 22α, 22*β*	46.6 s	
18α	41.1 d	2.82 dd (13.5, 3.7)	12, 19α, 19*β*	41.1 d	
19α	45.8 t	1.62 m		45.9 t	1.63 m
19*β*		1.17 m	18*β*, 29, 30		1.14 m
20	30.7 s		29, 30	30.7 s	
21α	33.8 t	1.33 m	22α, 22*β*, 29, 30	33.8 t	1.35 m
21*β*		1.21 m			1.21 m
22α	32.4 t	1.58 m	21α, 21*β*	32.3 t	1.53 m
22*β*		1.78 m			1.69 m
23	28.6 q	0.98 s	24	28.2 q	0.88 s
24	33.01 q	0.92 s	23	21.6 q	0.95 s
25A	61.5 t	3.97 d (12.1)		63.5 t	4.44 s
25B		4.07 d (12.1)			4.44 s
26	17.2 q	0.83 s	9α	17.1 q	0.79 s
27	26.5 q	1.15 s		26.4 q	1.19 s
28	181.5 s		18*β*	178.2 s	
29	33.1 q	0.91 s	30	33.1 q	0.90 s
30	23.6 q	0.94 s	29	23.6 q	0.93 s
OCO*CH*$_3$				21.2 q	2.05 s
OCO*CH*$_3$				21.3 q	2.07 s
O*C*OCH$_3$				170.6 s	
O*C*OCH$_3$				170.9 s	
COOC*H*$_3$					3.63 s
*C*OOCH$_3$				51.6 q	

[a] $CDCl_3$. [b] Assignments confirmed by decoupling, H/H COSY, NOESY, HMQC, and HMBC spectra. *J* values are given in Hz.

over silica gel (70−230 mesh, Merck), and medium-pressure liquid chromatography (MPLC) was carried out with silica gel (230−400 mesh, Merck) and LH-20. Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}, Merck). Preparative TLC was carried out on Merck silica gel F_{254} plates (20 × 20 cm, 0.5 mm thick).

Plant Material. Cuticles of *L. styraciflua* were collected at a neglected garden around the Senri Cetral Park, Suita City, Osaka Prefecture, Japan, in Novemver 2002. A voucher specimen (LSC-01) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and Isolation. The freshly chopped cones of *L. styraciflua* (6.8 kg) were extracted with $CHCl_3$ (20 L) employing an automatic percolator for 7 days at 50 °C. The $CHCl_3$ solution was evaporated under reduced pressure, and the resulting dark brown residue (98.3 g) was subjected to silica gel (2.0 kg) column chromatography. Elution of the column with $CHCl_3$ afforded residues A (22.9 g), B (35.0 g), and C (1.4 g), from fractions 1−22, 23−28, and 29−32 (each 1 L). Elution was continued with $CHCl_3$−EtOAc (10:1) to give residue D (5.5 g) from fractions 33−50 and subsequent CC with $CHCl_3$−EtOAc (5:1) to give residues E (3.3 g), F (2.1 g), and G (4.9 g) from fractions 51−59, 60−68, and 69−78. Further elution with $CHCl_3$−EtOAc (2:1) and EtOAc gave residues H (7.9 g) and I (6.5 g) from fractions 79−98 and 99−120, respectively. Recrystallization from *n*-hexane−$CHCl_3$ of residue E gave 6*β*-hydroxy-3-oxolup-20(29)-en-28-oic acid (**3**) (2.55 g). Repeated column chromatography of the filtrate of **3** (0.8 g) on silica gel (50 g) eluting with $CHCl_3$−EtOAc (10:1) afforded a crystalline solid (fractions 18−40, 0.5 g), which was recrystallized from MeOH−$CHCl_3$ to give compound **4** (415 mg). Repeated column chromatography of residue G on silica gel (200 g) eluting with $CHCl_3$−EtOAc (5:1) afforded a crystalline solid (fractions 51−81, 3.8 g), which was subjected to LH-20 using $CHCl_3$−MeOH (1:1) and recrystallized from MeOH−$CHCl_3$ to give compound **1** (3.1 g). Repeated column chromatography of residue H on MPLC (300 g) eluting with $CHCl_3$−EtOAc (5:1) afforded a crystalline solid (fractions 74−76, 26 mg), which was recrystallized from MeOH−$CHCl_3$ to give compound **2** (21 mg).

Compound 1: colorless prisms; mp 282−284 °C (from MeOH−$CHCl_3$); $[\alpha]^{15}_D$ +42.9° (*c* 0.22, $CHCl_3$); HREIMS *m*/*z* 514.3654 $[M]^+$ ($C_{32}H_{50}O_5$, calcd for 514.3656); IR (KBr) ν_{max} cm^{-1} 3463 (OH), 3100−2700 and 1703 (COOH), 2968, 2920, 2860, 1721, and 1265 (OAc), 1461, 1379, 1363, 1211, 1172,

Table 3. Cytotoxicity of Compound **1** against a Panel of 39 Human Cancer Cell Lines

origin of cancer	cell line	log GI_{50} (M)[a]
breast	HBC-4	−5.10
	BSY-1	−4.97
	HBC-5	−4.96
	MCF-7	−5.08
	MDA-MB-231	−4.71
central nervous system	U251	−5.22
	SF-268	−4.81
	SF-295	−5.06
	SF-539	−5.20
	SNB-75	−4.80
	SNB-78	−4.80
colon	HCC2998	−4.55
	KM-12	−5.29
	HT-29	−5.47
	HCT-15	−5.19
	HCT-116	−5.34
lung	NCI-H23	−5.07
	NCI-H226	−4.73
	NCI-H522	−4.86
	NCI-H460	−4.86
	A549	−5.37
	DMS273	−5.20
	DMS114	−4.87
melanoma	LOX-IMVI	−4.95
ovary	OVCAR-3	−4.96
	OVCAR-4	−5.00
	OVCAR-5	−4.74
	OVCAR-8	−4.95
	SK-OV-3	−4.69
kidney	RXF-631L	−4.93
	ACHN	−5.27
stomach	St-4	−5.04
	MKN1	−4.96
	MKN7	−5.18
	MKN28	−5.25
	MKN45	−4.75
	MKN74	−5.11
prostate	DU-145	−4.88
	PC-3	−4.94
MG-MID[b]		−5.00
delta[c]		0.47
range[d]		0.92

[a] Log concentration of compound for inhibition of cell growth at 50% compared to control. [b] Mean value of log GI_{50} over all cell lines tested. [c] The difference in log GI_{50} value of the most sensitive cell and MG-MID value. [d] The didifference in log GI_{50} value of the most sensitive cell and the least sensitive cell.

1060, 1014, 983; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* (rel int) 514 (0.3) $[M]^+$, 454 (7) $[M - HOAc]^+$, 439 (1) $[M - HOAc - Me]^+$, 436 (2) $[M - HOAc - H_2O]^+$, 424 (10), 397 (22), 279 (11), 248 (44), 234 (14), 203 (100), 189 (31), 175 (28), 173 (11), 133 (23), 119 (21), 105 (18), 95 (15).

Methyl 25-Acetoxy-3α-hydroxyolean-12-en-28-oate (1a). To a MeOH (7 mL) and C_6H_6 (7 mL) solution of compound **1** (92.8 mg) was added a trimethylsilyldiazomethane 2.0 M solution in *n*-hexane ($TMSCHN_2$) (1.6 mL) and the mixture stirred for 5 h at room temperature. Evaporation of the solvent under reduced pressure afforded a residue, which was purified by PTLC ($CHCl_3$−MeOH, 25:1) to afford compound **1a** (92.4 mg): colorless prisms; mp 110−113 °C (from MeOH−$CHCl_3$); $[\alpha]^{21}_D$ +22.9° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3546 (OH), 2953, 2878, 1737 (COOMe), 1727 and 1248 (OAc), 1457, 1375, 1165, 1031, 979; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* (rel int) 528 (0.5) $[M]^+$, 510 (0.6) $[M - H_2O]^+$, 468 (28) $[M - HOAc]^+$, 450 (7) $[M - HOAc - H_2O]^+$, 437 (30), 279 (19), 262 (36), 248 (19), 203 (100), 189 (59), 175 (21).

Methyl 3α,25-Diacetoxyolean-12-en-28-oate (1b). A mixture of compound **1a** (60.5 mg) and Ac_2O (1.2 mL) in pyridine (1.2 mL) was kept at room temperature overnight. Usual workup gave a residue (63.5 mg), which was recrystallized from MeOH−$CHCl_3$ to a corresponding methyl 3α,25-diacetoxyolean-12-en-28-oate (**1b**) (58.3 mg): colorless prisms; mp 234−236 °C (from MeOH−$CHCl_3$); $[\alpha]^{21}_D$ +121.4° (*c* 0.10, $CHCl_3$); EIMS *m/z* (rel int) 570 (2) $[M]^+$, 510 (72) $[M - HOAc]^+$, 450 (60) $[M - 2HOAc]^+$, 437 (27), 391 (22), 262 (79), 248 (8), 203 (100), 189 (49), 175 (29).

3α,25-Dihydroxyolean-12-en-28-oic acid (1c). Compound **1** (20.0 mg) was refluxed with a solution of 0.02 N KOH−MeOH on a steam bath for 6 h. Evaporation of the solvent under reduced pressure afforded a residue, which was recrystallized with MeOH−$CHCl_3$ to afford compound **1c** (18.3 mg): colorless prisms; IR (KBr) ν_{max} cm^{-1} 3446 (OH); ^{1}H NMR δ 3.97 (1H, d, J = 12.1 Hz, H-25A), 4.07 (1H, d, J = 12.1 Hz, H-25B); ^{13}C NMR δ 61.5 (t, C-25); EIMS *m/z* (rel int) 472 (0.3) $[M]^+$, 454 (5) $[M - H_2O]^+$, 436 (3) $[M - 2H_2O]^+$, 423 (14), 248 (80), 237 (37), 234 (19), 203 (100), 189 (55), 175 (46), 173 (7), 133 (15), 119 (13), 105 (10), 95 (10).

3α,25-Diacetoxyolean-12-en-28-oic acid (1d). A mixture of compound **1** (20.0 mg) and Ac_2O (1 mL) in pyridine (1 mL) was kept at room temperature overnight. Usual workup gave a residue (21.2 mg), which was recrystallized from MeOH−$CHCl_3$ to a corresponding 3α,25-diacetoxyolean-12-en-28-oic acid (**1d**) (19.8 mg): colorless prisms; $[\alpha]^{21}_D$ +31.8° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3100−2800 and 1712 (COOH), 2907, 1728 and 1282 (OAc), 1473, 1375, 1047, 1003, 960; ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, s, Me-26), 0.89 (3H, s, Me-23), 0.92 (3H, s, Me-29), 0.93 (3H, s, Me-30), 0.96 (3H, s, Me-24), 1.21 (3H, s, Me-27), 2.04 and 2.07 (each 3H, s, OAc), 4.36 (1H, d, J = 12.1 Hz, H-25A), 4.50 (1H, d, J = 12.1 Hz, H-25B), 4.66 (1H, t, J = 2.5 Hz, H-3β), 5.31 (1H, t, J = 3.4 Hz, H-12); ^{13}C NMR δ 17.4 (C-26), 17.9 (C-6), 21.2 (OAc), 21.3 (OAc), 21.7 (C-24), 23.0 (C-16), 23.5 (C-2), 23.6 (C-30), 24.5 (C-11), 26.3 (C-27), 27.5 (C-15), 28.2 (C-23), 28.8 (C-1), 30.7 (C-20), 31.4 (C-22), 32.5 (C-7), 33.0 (C-29), 33.6 (C-21), 36.2 (C-4), 39.6 (C-8), 39.9 (C-10), 42.1 (C-14), 45.8 (C-19), 48.0 (C-9), 48.3 (C-17), 50.0 (C-5), 63.4 (C-25), 123.2 (C-12), 143.1 (C-13), 170.6 (OAc), 170.8 (OAc), 173.0 (C-17); EIMS *m/z* (rel int) 570 (2) $[M]^+$, 510 (72) $[M - HOAc]^+$, 450 (60) $[M - 2HOAc]^+$, 437 (27), 391 (22), 262 (79), 248 (8), 203 (100), 189 (49), 175 (29).

Compound 2: colorless prisms; mp 167−169 °C (from MeOH−$CHCl_3$); $[\alpha]^{21}_D$ +77.6° (*c* 0.10, $CHCl_3$); HREIMS *m/z* 472.3541 $[M]^+$ ($C_{30}H_{48}O_4$, calcd for 472.3550); IR (KBr) ν_{max} cm^{-1} 3100−2700 and 1703 (COOH), 3630, 3468 (OH), 2935, 2784, 1457, 1387, and 1364 (*gem*-dimethyl), 1030, 980; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* (rel int) 472 (0.2) $[M]^+$, 454 (3) $[M - H_2O]^+$, 436 (2) $[M - 2H_2O]^+$, 423 (11), 248 (75), 237 (32), 234 (13), 203 (100), 189 (46), 175 (34), 173 (6), 133 (12), 119 (11), 105 (9), 95 (9). Compound **2** was identified by direct comparison with synthetic **1c**.

3α,25-Diacetoxyolean-12-en-28-oic acid (2a). A mixture of compound **2** (10.0 mg) and Ac_2O (1 mL) in pyridine (1 mL) was kept at room temperature overnight. Usual workup gave a residue (21.2 mg), which was recrystallized from MeOH−$CHCl_3$ to a corresponding 3α,25-diacetoxyolean-12-en-28-oic acid (**2b**) (9.5 mg). This material was identified by direct comparison with **1d**.

Methyl 3α,25-Diacetoxyolean-12-en-28-oate (2b). To a MeOH (1 mL) and C_6H_6 (1 mL) solution of compound **2a** (10.0 mg) was added a trimethylsilyldiazomethane 2.0 M solution in *n*-hexane ($TMSCHN_2$) (0.5 mL) and the mixture stirred for 5 h at room temperature. Evaporation of the solvent under reduced pressure afforded a residue, which was purified by PTLC ($CHCl_3$−MeOH, 20:1) to afford compound **2b** (10.1 mg). This material was identified by direct comparison with **1b**.

Cell Lines. The following human cancer cell lines were generously donated by the National Cancer Institute (Fredrick, MD): lung cancer NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; colon cancer HCC-2998, KM-12, HT-29, HCT-15, and HCT-116; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; breast cancer MCF-7, BSY-1, HBC-4, HBC-5, and MDA-MB-231; renal cancer RFX-631L and ACHN; melanoma LOX-IMVI; brain tumor U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; human stomach cancer MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, and St-4; xPg, DU-145, and PC-3.

Human Cancer Cell Line Panel and the Database. To evaluate drugs for the cell growth inhibition profile, Yamori

et al. established a human cancer cell line panel combined with a database. The system as a whole was developed according to the method of the National Cancer Institute,[22] with modification. The cells were plated at proper density in 96-well plates in RPMI 1640 medium with 5% fetal bovine serum and allowed to attach overnight. The cells were exposed to drugs for 48 h. Then, the cell growth was determined according to the sulforhodamine B assay, described by Skehan et al.[23] Data calculations were made according to the methods described previously.[20] By using the computer to process percent growth values, the 50% growth inhibition parameter (GI_{50}) was determined. The mean graph, which shows the differential growth inhibition of the drug in the cell line panel, was drawn on the basis of a calculation using a set of GI_{50}.[21] To analyze the correlation between the mean graphs of drugs A and B, the COMPARE computer algorithm was developed according to the method described by Paull et al.[21]

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