Cytotoxic Hexacyclic Triterpene Acids from *Euscaphis japonica*

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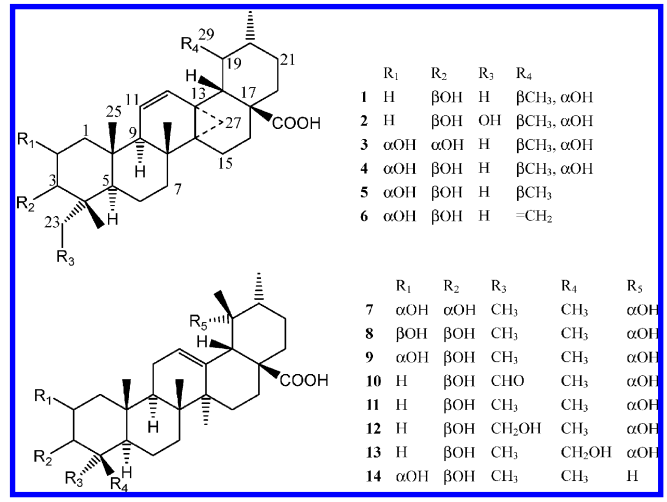
Six hexacyclic triterpene acids (**1**−**6**), named euscaphic acids A−F, and eight known triterpene acid compounds (**7**−**14**) were isolated from an ethanolic extract of twigs of *Euscaphis japonica*. Compounds **8** and **10** were isolated for the first time from a natural source. Triterpenes **1**−**6** possess hexacyclic skeletons with a 13α,27-cyclopropane ring. Structural elucidation of compounds **1**−**6** was established by spectroscopic methods, especially 2D NMR techniques (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY). Compounds **3**, **4**, and **14** showed significant cytotoxicity against different cancer cell lines [IC_{50} = 2.54 (NCI-H460), 3.61 (MCF-7), and 3.27 μM (CEM) for **3**, **4**, and **14**, respectively].

Euscaphis japonica (Thunb.) Kanitz. (Staphyleaceae) is a deciduous shrub or small tree, glabrous, leaves opposite, 5−7-pinnate, generally found in the forests and mountains of southeastern central China, southwestern Japan, and northern Taiwan.[1] Flavonoids,[2] four triterpenes,[3] three amino acid amides,[4] megastigmane and tetraketides,[5,6] and some compounds that showed inhibitory activity toward lipopolysaccaride-induced nitric oxide production in BV2 microglia[7] have been reported from this plant. Although several triterpene components have been reported previously,[3] there is a lack of pharmacological information on this plant. We report herein the isolation of six triterpene acids with 13α,27-cyclours-11-en-28-oic acid skeletons, named euscaphic acids A−F (**1**−**6**), along with eight known triterpene acids, euscaphic acid (**7**),[8] 2α-hydroxypomolic acid (**8**),[9] tormentic acid (**9**),[8] 23-aldehydepomolic acid (**10**),[10] pomolic acid (**11**),[11] rotundic acid (**12**),[11] rotungenic acid (**13**),[10] and 2α-hydroxyursolic acid (**14**)[11] from an EtOH extract of *E. japonica*. Hexacyclic triterpenes with the 13α,27-cyclopropane ring skeleton are rare in nature. The five known compounds of this type are phyllanthol (13α,27-cycloursan-3β-ol),[12] methyl-2α,3α-24-trihydroxy-13α,27-cycloolean-11-en-28-oate,[13] 3β-acetoxy-15α-hydroxy-13α,27-cyclours-11-ene,[14] 3β-acetoxy-13α,27-cyclour-sane,[15] and 2α,3α,19α,23-tetrahydroxy-13α,27-cyclours-11-en-28-oic acid[16] from *Phyllanthus engleri* (Pax), *Prunella vulgaris*, *Ficus microcarpa* L., *Ficus septica*, and *Planchonella duclitan* (Blanco) Bakhuizan, respectively. Semisyntheses of 13α,27-cyclopropane hexacyclic triterpenes were reported by Zürcher[17] and by Beaton.[18] Structural elucidation of compounds **1**−**6** was based on spectroscopic analyses, including 1D and 2D NMR techniques (DEPT, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC). Cytotoxicity of compounds **1**−**14** against the human cancer cell lines (MCF-7, NCI-H460, HT-29, and CEM) was also evaluated.

Results and Discussion

An extract (95% EtOH) of twigs of *E. japonica* was suspended in H_2O and then partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate extract was chromatographed on a silica gel column and then on a Sephadex LH-20 column. The most cytotoxic fractions were then subjected to preparative HPLC, using a reversed-phase (ODS) column, to yield six new (**1**−**6**) and eight known triterpene acids.



	R_1	R_2	R_3	R_4
1	H	βOH	H	βCH_3, αOH
2	H	βOH	OH	βCH_3, αOH
3	αOH	αOH	H	βCH_3, αOH
4	αOH	βOH	H	βCH_3, αOH
5	αOH	βOH	H	βCH_3
6	αOH	βOH	H	$=CH_2$

	R_1	R_2	R_3	R_4	R_5
7	αOH	αOH	CH_3	CH_3	αOH
8	βOH	βOH	CH_3	CH_3	αOH
9	αOH	βOH	CH_3	CH_3	αOH
10	H	βOH	CHO	CH_3	αOH
11	H	βOH	CH_3	CH_3	αOH
12	H	βOH	CH_2OH	CH_3	αOH
13	H	βOH	CH_3	CH_2OH	αOH
14	αOH	βOH	CH_3	CH_3	H

Compound **1** was obtained as a white powder. The HRESIMS of **1** suggested the elemental formula $C_{30}H_{46}O_4$ from a quasi-molecular ion at *m/z* 493.3320 $[M + Na]^+$. The IR spectrum displayed absorption bands for OH (3376 cm^{-1}), carbonyl (1693 cm^{-1}), and olefin (1631 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra revealed 30 carbons containing six methyl, nine methylene, seven methine (including two olefinic and one oxymethine), and eight quaternary (one oxygenated and one carbonyl) carbons. Valence bond calculations revealed that there were eight degrees of unsaturation in compound **1**, accounted for by one carbonyl, a double bond, and six rings. In the ^{1}H NMR spectrum, **1** showed signals for five tertiary methyl groups (δ_H 1.38, 0.97, 0.87, 0.79, and 0.75), one methyl linked to a methine [δ_H 0.91 (d, J = 6.0 Hz)], an oxygenated methine [δ_H 3.12 (dd, J = 11.5 Hz, 5.0 Hz)], and two olefinic protons [δ_H 5.81 (dd, J = 11.5 Hz, 3.0 Hz) and 5.06 (dd, J = 11.5 Hz, 2.5 Hz)]. Detailed analyses of 1D and 2D NMR (^{1}H, ^{13}C NMR spectra, ^{1}H−^{1}H COSY, HMQC, and HMBC) of **1** and comparison with compound **11** (pomolic acid) suggested that the basic skeletons were similar. However, the obvious difference was that the methyl-27 in pomolic acid was absent and appeared as a methylene (δ_C 16.1, δ_H 1.83, 1.26, d, J = 4.0 Hz each 1H) linked to C-13, resulting in the ene-11,12 in **1**. Thus, the 13α,27-cyclours-11-en-28-oic acid skeleton for **1** was deduced. The ^{1}H−^{1}H COSY spectrum of **1** provided the following cross-peaks: H_α-1/H_2-2, H_2-2/H-3; H-5/H_β-6; H-9/H-11/H-12; H_2-15/H_2-16; H-20/H_3-30, H-20/H_2-21, H_2-21/H_2-22. Its HMBC correlations included H-3/C-23 and C-24; H_3-23/C-3, C-4, C-5, and C-24; H_3-25/C-1, C-5, C-9, and C-10; H_3-26/C-7, C-8, C-9, and C-14; H_2-27/C-8, C-12, C-13, C-14, C-15, and C-18; H-18/C-12, C-13, C-14,

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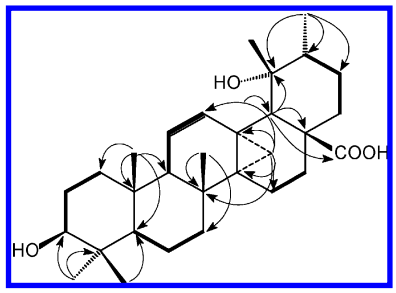

Figure 1. Key HMBC (→) and COSY (—) correlations of **1**.

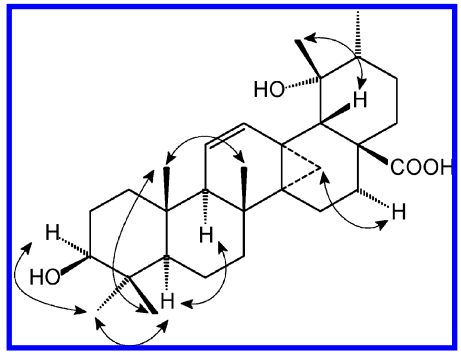

Figure 2. NOE correlations of **1**.

C-16, C-17, C-19, C-20, C-28, and C-29; and H_3-30/C-19, C-20, and C-21 (Figure 1). Thus, the planar structure of **1** was established as shown. The relative configuration of **1** was elucidated from the NOE interactions (Figure 2); the same relative configuration as compound **11** was observed except for the chiral center at C-13 in **1**, indicating that the configuration was *S**. Thus, the structure of **1** was established as 3β,19α-dihydroxy-13α,27-cyclours-11-en-28-oic acid, and it was named euscaphic acid A.

Compound **2** was isolated as a white, amorphous powder, and its HRESIMS gave a quasi-molecular ion at *m/z* 509.3288 $[M + Na]^+$ ($C_{30}H_{46}O_5Na$). The IR and ^1H and ^{13}C NMR data were very similar to those of **1**, except that the signal of a CH_3 was replaced by a CH_2OH at δ_H 3.58 and 3.33 (each 1H, d, $J = 10.0$ Hz) and δ_C 68.55. This CH_2OH group was connected to C-4 by means of the HMQC and HMBC spectra and was determined to be α-oriented due to the NOESY spectrum showing cross-peak of δ_H 3.33 with 3.60 (H-3). Consequently, **2** was deduced to be 3β,19α,23-trihydroxy-13α,27-cyclours-11-en-28-oic acid, and it was named euscaphic acid B.

Compound **3** had the molecular formula $C_{30}H_{46}O_5$, as determined from its pseudomolecular ion peak at *m/z* 509.3281 $[M + Na]^+$ in the HRESIMS spectrum. The ^1H and ^{13}C NMR data of **3** indicated that it had the same hexacyclic triterpene skeleton as compound **1**. The major difference between **1** and **3** was that one of the methylenes of **1** was replaced by a hydroxymethine in **3**. Analysis of HMQC and HMBC experiments placed the hydroxymethine at C-2. NOESY correlations between H-2 and H-3 and H_3-24 indicated that both OH-2 and OH-3 were α-oriented. This result was supported by the NOE of H-2 and H_3-25 and by the small coupling constant of H-3 with H-2, disclosing that H-3 was equatorial (β-orientation), whereas H-2 was axial (β-orientation), due to the coupling constant of H-2 with H-1 ($J = 11.6$ Hz). The similarity of ^{13}C NMR data of C-1 to C-6 and C-10 in compounds **3** and **7**[10] also supported the α-orientation of OH-2 and OH-3 in **3**. Therefore, the structure of compound **3** was deduced to be 2α,3α-dihydroxy-euscaphic acid A (2α,3α,19α-trihydroxy-13α,27-cyclours-11-en-28-oic acid), and it was named euscaphic acid C.

Compound **4** had the molecular formula $C_{30}H_{46}O_5$ [HRESIMS (509.3290 $[M + Na]^+$)]. Comparison of MS and NMR data of **4** with those of **3** and analyses of HMQC and HMBC spectra of **4** suggested that **3** and **4** had the same planar structure. NOESY correlations of H-2 with H_3-24 and H_3-25, and of H-3 with H_3-23 and H-5, indicated the α-orientation of OH-2 and β-orientation of OH-3. The coupling constant ($J = 9.2$ Hz) of H-3 with H-2 indicated that both H-2 and H-3 were axial protons, also supporting the 2α-OH and 3β-OH of **4**. Since the ^{13}C NMR data of C-1 through C-6 and C-10 of **4** were superimposable with those of **14**,[11] the α-orientation of OH-2 and β-orientation of OH-3 in compound **4** were deduced. Accordingly, **4** was elucidated as 3β-hydroxyeuscaphic acid C (2α,3β,19α-trihydroxy-13α,27-cyclours-11-en-28-oic acid), and it was named euscaphic acid D.

Compound **5** [$C_{30}H_{46}O_4$ (*m/z* 493.3337 $[M + Na]^+$)] yielded ^1H and ^{13}C NMR data similar to those of **4** (Tables 1 and 2), except that signals of an oxygenated quaternary carbon and a singlet methyl on the F ring in **4** were replaced by a methine and a doublet methyl group, respectively, in **5**. In compound **5**, Me-29 (δ_H 1.10 d, $J =$ 5.6 Hz) appeared as a doublet instead of a singlet as in **1**—**4**. NOE correlations of H-18 with H_3-29 and H-19 with H_2-27 suggested the β-orientation of Me-29 in compound **5**. Thus, the structure of **5** (2α,3β-dihydroxy-13α,27-cyclours-11-en-28-oic acid) was determined unambiguously, and it was named euscaphic acid E.

The molecular formula $C_{30}H_{44}O_4$, corresponding to 9 unsaturation equivalents, was established for **6** (by HRESIMS). Compared with the NMR spectra of **5** and **6**, signals for a doublet methyl and a methine in **5** were replaced by a vinyl methylene in **6**. The HMBC spectrum of **6** provided the assignment of the methylene group to C-19. On the basis of the above evidence, the structure of compound **6**, named euscaphic acid F, was determined to be 2α,3β-dihydroxy-13α,27-cyclours-11,29-dien-28-oic acid.

Compounds **1**—**14** were evaluated for cytotoxic activity against a panel of human tumor cell lines, including breast cancer (MCF-7), lung cancer (NCI-H460), colon tumor (HT-29), leukemia (CEM), and human normal fibroblast cells. As shown in Table 3, compounds **3**, **4**, and **14** had significant inhibition on some cancer cell lines, while the other triterpenoids (**1**, **2**, and **5**—**13**) were inactive (cancer cell proliferation was >50% at 10 μM). Therefore, only compounds **3**, **4**, and **14** were measured for their IC_{50} values. From the IC_{50} data (Table 4), compounds **3**, **4**, and **14** showed selective cytotoxicity against NCI-H460 cells (IC_{50} 2.54 μM), MCF-7 cells (IC_{50} 3.61 μM), and CEM cells (IC_{50} 3.27 μM), respectively.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 polarimeter. IR spectra were recorded as KBr disks, using an IR-FT Mattson Genesis II spectrometer. NMR spectra were recorded using Bruker UltraShield 400 MHz and Varian Inova-500 MHz spectrometers. High-resolution ESIMS were determined using a Shimadzu IT-TOF HR mass spectrometer. For column chromatography (CC), silica gel 60 (70—230, 230—400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used. Precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. MPLC was performed on a system equipped with a Buchi B-688 pump, a Buchi B-684 fraction collector, and Buchi columns. HPLC separations were performed on a Shimadzu LC-8A series apparatus with a RID-10A RI detector, equipped with a 250 × 20 mm i.d. preparative Cosmosil $5C_{18}$ AR-II column (Nacalai Tesque, Inc.).

Plant Material. Twigs of *E. japonica* (7.6 kg) were collected at Yangming mountain in Taiwan, in April 2008, and identified by Professor Yao-Haur Kuo, National Research Institute of Chinese Medicine, Taipei. A voucher specimen (No. NRICM20080410A) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The twigs of *E. japonica* (7.6 kg) were extracted with 95% EtOH at 55 °C (three times), and the extract was concentrated under reduced pressure. The EtOH extract (600 g) was then suspended in water, and the suspension was extracted successively

Table 1. ^{1}H NMR Spectroscopic Data (δ) of **1**−**6** (J in Hz)[a,b]

position	1	2	3	4	5	6
1	0.90, o	0.89, o	1.70, m	1.23, d (12.0)	1.22, m	0.87, t (11.5)
	1.73, o	1.71, o	2.11, m	2.43, dd (12.8, 4.4)	2.41, dd (12.8, 4.4)	2.04, o
2	1.57, o	1.57, o	4.31, brd (11.6)	4.12, td (11.2, 4.0)	4.11, td (12.0, 4.4)	3.62, td (9.5, 4.5)
	1.64, o	1.64, o				
3	3.12, dd (11.5, 5.0)	3.60, dd (10.0, 5.0)	3.73, brs	3.33, d (9.2)	3.35, d (9.2)	2.89, d (9.5)
5	0.80, dd (10.0, 1.5)	1.11, dd, 11.5, 2.0)	1.68, d (11.2)	0.99, o	0.94, o	0.92, dd (12.0, 2.0)
6	1.46, dd (12.5, 3.5)	1.44, o	1.42, m	1.45, m	1.46, o	1.40, o
	1.62, o	1.52, o	1.55, o	1.60, o	1.64, o	1.63, o
7	1.60, o	1.60, o	1.29, m	1.29, dd (12.0, 3.6)	1.22, o	1.22, m
	1.74, o	1.74, o	1.86, d (12.4)	1.89, m	1.80, o	1.80, d (12.5)
9	1.64, o	1.65, o	2.03, o	1.93, brs	1.83, brs	1.71, t like
11	5.06, dd (10.0, 2.5)	5.06, dd (10.0, 2.0)	5.51, brd (11.6)	5.37, brd (9.6)	5.34, brd (10.0)	5.21, dd (10.5, 2.5)
12	5.81, dd (10.5, 3.0)	5.81, dd (10.5, 2.5)	6.25, brd (10.4)	6.26, brd (10.4)	6.13, brd (10.0)	5.73, dd (10.5, 3.5)
15	1.48, o	1.46, o	1.72, m	1.74, dd (13.2, 5.6)	1.64, o	1.49, m
	2.09, dd (13.0, 5.5)	2.09, o	2.51, td (12.8, 6.4)	2.53, td (13.2, 6.4)	2.49, m	2.06, o
16	1.54, o	1.52, o	1.99, m	2.00, m	1.50, dd (12.8, 5.2)	1.26, m
	2.21, td (12.5, 5.5)	2.20, td (12.5, 5.5)	2.67, td (12.4, 5.6)	2.70, td (12.4, 6.8)	1.81, o	1.59, o
18	2.35, s	2.33, s	2.81, s	2.84, s	2.53, d (10.4)	3.25, s
19					0.97, o	
20	1.37, o	1.35, o	1.43, m	1.45, m	1.09, o	2.02, o
21	1.24, o	1.24, o	1.37, m	1.37, m	1.10, o	1.09, m
	1.61, o	1.61, o	1.92, m	1.93, m	1.36, m	1.58, o
22	1.19, td (13.0, 4.0)	1.20, m	2.02, m	2.00, o	1.80, o	1.15, o
	1.80, o	1.74, o	2.12, m	2.13, d (12.8)	1.95, d (13.2)	1.76, t (12.0)
23	0.97, s	3.33, d (10.0)	1.20, s	1.21, s	1.22, s	0.98, s
		3.58, d (10.0)				
24	0.75, s	0.72, s	0.85, s	1.03, s	1.03, s	0.79, s
25	0.87, s	0.87, s	0.95, s	0.87, s	0.94, s	0.86, s
26	0.79, s	0.76, s	1.11, s	1.11, s	1.03, s	0.97, s
27	1.26, d (4.0)	1.25, o	1.47, d (4.4)	1.58, o	0.47, d (4.4)	0.47, d (5.5)
	1.83, d (4.0)	1.80, d (4.0)	2.31, d (4.4)	2.39, d (4.4)	1.46, o	1.43, d (5.5)
29	1.38, s	1.18, s	1.56, s	1.59, s	1.10, d (5.6)	4.95, 5.20 s each 1H
30	0.91, d (6.0)	0.88, d (6.0)	1.05, d (6.4)	1.06, d (6.4)	0.88, d (5.2)	1.08, d (6.4)

[a] **1**, **2**, and **6** in acetone-d_6, 500 MHz; **3**−**5** in pyridine-d_5, 400 MHz. [b] "o" means "overlapped".

Table 2. ^{13}C NMR Spectroscopic Data (δ_C, mult.) for **1**−**8**, **10**, **11**, and **14**[a]

C	1	2	3	4	5	6	7	8	10	11	14
1	39.4, CH_2	39.1, CH_2	43.1, CH_2	47.9, CH_2	47.9, CH_2	47.5, CH_2	42.6, CH_2	45.0, CH_2	39.4, CH_2	39.4, CH_2	48.0, CH_2
2	28.0, CH_2	27.3, CH_2	65.9, CH	68.6, CH	68.6, CH	68.9, CH	66.2, CH	71.7, CH	26.9, CH_2	28.1, CH_2	68.5, CH
3	78.6, CH	74.1, CH	79.3, CH	83.8, CH	83.8, CH	83.9, CH	79.3, CH	78.5, CH	72.9, CH	78.2, CH	83.8, CH
4	39.5, qC	42.6, qC	38.8, qC	39.8, qC	39.8, qC	39.8, qC	38.9, qC	38.8, qC	56.7, qC	39.4, qC	39.9, qC
5	56.0, CH	49.1, CH	48.4, CH	55.5, CH	55.4, CH	55.9, CH	48.8, CH	56.2, CH	48.7, CH	55.9, CH	55.9, CH
6	19.1, CH_2	18.8, CH_2	18.5, CH_2	18.8, CH_2	18.8, CH_2	19.1, CH_2	18.8, CH_2	19.0, CH_2	21.8, CH_2	18.9, CH_2	18.7, CH_2
7	37.4, CH_2	37.3, CH_2	37.4, CH_2	37.3, CH_2	37.1, CH_2	37.5, CH_2	33.7, CH_2	33.8, CH_2	33.5, CH_2	33.6, CH_2	33.5, CH_2
8	34.0, qC	33.9, qC	34.0, qC	33.8, qC	35.6, qC	35.9, qC	40.8, qC	40.7, qC	41.4, qC	40.3, qC	39.8, qC
9	53.5, CH	53.5, CH	53.1, CH	53.1, CH	53.3, CH	53.8, CH	47.8, CH	48.4, CH	48.5, CH	47.8, CH	48.0, CH
10	37.1, qC	36.9, qC	38.0, qC	37.8, qC	37.8, qC	38.3, qC	38.8, qC	37.5, qC	36.9, qC	37.3, qC	38.4, qC
11	119.4, CH	119.4, CH	119.1, CH	119.0, CH	119.6, CH	120.6, CH	24.3, CH_2	24.4, CH_2	24.6, CH_2	24.0, CH_2	23.7, CH_2
12	141.3, CH	141.2, CH	141.5, CH	141.6, CH	140.7, CH	139.0, CH	128.7, CH	128.9, CH	129.1, CH	128.0, CH	125.5, CH
13	27.8, qC	27.7, qC	27.7, qC	27.8, qC	29.5, qC	27.4, qC	139.6, qC	139.6, qC	140.0, qC	140.0, qC	139.3, qC
14	33.3, qC	33.3, qC	33.1, qC	33.0, qC	33.8, qC	33.7, qC	42.3, qC	42.3, qC	42.7, qC	42.1, qC	42.1, qC
15	22.3, CH_2	22.3, CH_2	22.3, CH_2	22.3, CH_2	22.2, CH_2	21.1, CH_2	29.1, CH_2	29.2, CH_2	29.6, CH_2	29.3, CH_2	28.6, CH_2
16	26.4, CH_2	26.4, CH_2	26.3, CH_2	26.3, CH_2	24.5, CH_2	26.5, CH_2	26.3, CH_2	26.4, CH_2	26.2, CH_2	26.4, CH_2	31.0, CH_2
17	47.3, qC	47.3, qC	47.3, qC	47.3, qC	46.2, qC	47.9, qC	48.3, qC	48.3, qC	48.5, qC	48.4, qC	47.9, qC
18	47.1, CH	47.1, CH	47.0, CH	47.0, CH	46.3, CH	45.9, CH	54.4, CH	54.5, CH	55.1, CH	54.6, CH	53.5, CH
19	76.0, qC	76.0, qC	75.4, qC	75.4, qC	40.6, CH	152.2, qC	73.2, qC	73.2, qC	73.6, qC	72.7, qC	39.4, CH
20	42.6, CH	42.2, CH	42.4, CH	42.4, CH	38.2, CH	37.8, CH	42.5, CH	42.6, CH	43.1, CH	42.4, CH	39.4, CH
21	26.9, CH_2	26.9, CH_2	26.8, CH_2	26.8, CH_2	30.7, CH_2	31.0, CH_2	27.0, CH_2	27.0, CH_2	27.3, CH_2	26.9, CH_2	31.0, CH_2
22	37.8, CH_2	37.4, CH_2	37.5, CH_2	37.5, CH_2	37.9, CH_2	38.1, CH_2	38.4, CH_2	38.5, CH_2	39.0, CH_2	37.3, CH_2	37.4, CH_2
23	28.4, CH_3	68.6, CH_2	29.3, CH_3	28.9, CH_3	28.9, CH_3	28.9, CH_3	29.5, CH_3	29.5, CH_3	208.6, CH	28.8, CH_3	29.3, CH_3
24	15.6, CH_3	12.1, CH_3	21.6, CH_3	17.1, CH_3	17.1, CH_3	16.8, CH_3	22.3, CH_3	17.9, CH_3	9.4, CH_3	16.8, CH_3	17.7, CH_3
25	17.7, CH_3	17.7, CH_3	18.6, CH_3	18.7, CH_3	18.7, CH_3	19.0, CH_3	16.7, CH_3	16.7, CH_3	16.1, CH_3	16.5, CH_3	16.9, CH_3
26	16.8, CH_3	16.5, CH_3	16.4, CH_3	16.7, CH_3	16.2, CH_3	16.8, CH_3	17.4, CH_3	17.3, CH_3	17.4, CH_3	17.4, CH_3	17.5, CH_3
27	16.1, CH_3	16.1, CH_3	15.9, CH_3	16.0, CH_3	16.3, CH_3	20.0, CH_3	24.7, CH_3	24.7, CH_3	24.8, CH_3	24.7, CH_3	23.9, CH_3
28	179.5, qC	179.5, qC	180.7, qC	180.7, qC	179.9, qC	178.6, qC	179.4, qC	179.4, qC	180.3, qC	180.8, qC	180.0, qC
29	27.2, CH_3	27.2, CH_3	26.9, CH_3	26.8, CH_3	17.9, CH_3	109.3, CH_2	27.2, CH_3	27.2, CH_3	27.1, CH_3	27.1, CH_3	21.4, CH_3
30	15.8, CH_3	15.6, CH_3	15.8, CH_3	15.8, CH_3	20.5, CH_3	19.5, CH_3	16.6, CH_3	16.6, CH_3	16.6, CH_3	16.5, CH_3	17.4, CH_3

[a] Compounds **1**, **2**, and **6** in acetone-d_6, **8** in methanol-d_4, 125 MHz; **3**−**5**, **7**, **10**, **11**, and **14** in pyridine-d_5, 100 MHz.

with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc layer provided 160 g of extract and was separated on a MPLC silica gel column, eluted with $CHCl_3$−MeOH (100:0, 20:1, 10:1, 5:1, 2:1, 0:100), to yield six fractions (E1 to E6). Fraction E1 ($CHCl_3$−MeOH, 100:0, 70 g) was submitted to Sephadex LH-20 CC, eluting with MeOH−acetone (2:1), to afford seven fractions (E1.1 to E1.7). Fraction E1.5 (35 g) was subjected to silica gel MPLC, eluted with *n*-hexane−$CHCl_3$−MeOH from 4:1:0 to 0:5:1, to give 15 fractions (E1.5.1 to E1.5.15). Fraction E1.5.13 (7.0 g) was separated on a silica gel MPLC column eluted with $CHCl_3$−MeOH−EtOAc−acetone, 90:1:1:1, to give 15 fractions (E1.5.13.1 to E1.5.13.15). Fraction E1.5.13.2 (1.7 g) was subjected to silica gel CC, eluted with $CHCl_3$−MeOH, 40:1, to afford 18 fractions (E1.5.13.2.1 to E1.5.13.2.18). Fraction E1.5.13.2.5 (42 mg) was purified by preparative HPLC (250 × 20 mm i.d., Cosmosil 5 C_{18} AR-II column, MeOH−H_2O, 83:17, recycle five times), to afford compounds **1** (6.5 mg, 0.00009%) and **11** (12.1 mg, 0.00016%). The other subfractions were also purified by recycle preparative HPLC (250 × 20 mm i.d., Cosmosil 5 C_{18} AR-II column); fraction E1.5.13.2.9 (65 mg) (MeOH−H_2O, 80:20, recycle six times) afforded **7** (30 mg, 0.00039%) and **8** (22 mg, 0.00029%); fraction E1.5.13.2.10 (32 mg) (MeOH−H_2O,

Table 3. Normal and Tumor Cell Proliferation Data of Compounds **1**−**14** (at 10 μM)

	cell proliferation (%)				
compound	MCF-7	NCI-H460	HT-29	CEM	fibroblast
1	85	62	87	83	100
2	84	99	93	80	109
3	10	6	1	19	119
4	41	24	13	55	111
5	90	91	86	87	110
6	76	81	87	86	115
7	85	104	85	89	95
8	70	78	74	73	107
9	85	88	86	93	114
10	93	103	91	88	99
11	79	82	91	92	93
12	78	85	101	106	92
13	98	106	91	97	99
14	75	89	68	33	109

Table 4. Cytotoxicity of **3**, **4**, and **14** against Four Human Tumor Cell Lines

	cell line (IC_{50}, μM)[a]			
compound	MCF-7[b]	NCI-H460[b]	HT-29[b]	CEM[b]
3	15.69	2.54	14.33	65.34
4	3.61	13.34	34.78	8.99
14	[c]	[c]	[c]	3.27
doxorubicin	2.57	6.06	6.4	3.14

[a] IC_{50} = concentration that causes a 50% reduction in absorbance at 570 and 600 nm relative to untreated cells using the SRB assay. [b] The cell lines are described in the Experimental Section. [c] $IC_{50} > 10$ μM.

80:20, recycle four times), afforded **3** (13.2 mg, 0.00017%); fraction E1.5.13.2.14 (113 mg) (MeOH−H_2O, 75:25, recycle 10 times) afforded compounds **6** (6.7 mg, 0.00009%) and **14** (15.4 mg, 0.00020%); fraction E1.5.13.2.15 (MeOH−H_2O, 75:25) afforded fractions E1.5.13.2.15.1 and E1.5.13.2.15.2. Fraction E1.5.13.2.15.2 (25 mg) was purified by HPLC (MeOH−H_2O, 70:30, recycle four times) to afford compound **2** (6.9 mg, 0.00009%). Fraction E1.5.13.5 (1.8 g) was chromatographed on silica gel, using $CHCl_3$−MeOH, 35:1, to afford 10 fractions (E1.5.13.5.1 to E1.5.13.5.10). Subfraction E1.5.13.5.5 (52 mg) was purified by HPLC (MeOH−H_2O, 80:20, recycle four times) to afford **5** (6.4 mg, 0.00008%) and **13** (5.4 mg, 0.00007%); fraction E1.5.13.5.6 (112 mg) (MeOH−H_2O, 75:25, recycle five times) afforded **4** (35.2 mg, 0.00046%), **9** (5.7 mg, 0.00008%), and **12** (22.4 mg, 0.00029%). Fraction E1.5.12 (2.4 g) was subjected to silica gel CC, eluting with $CHCl_3$−EtOAc−acetone−MeOH, 200:1:1:1, to afford 18 fractions; then, the 14th fraction (120 mg) was purified by HPLC (MeOH−H_2O, 75:25, recycle five times) to afford **10** (70.1 mg, 0.00092%).

Euscaphic Acid A (1): white powder, mp 278 °C; $[\alpha]^{25}_D$ +9.23 (*c* 0.65, MeOH); IR ν_{max} (KBr) 3376, 2944, 2836, 1693, 1631, 1514, 1447 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 493.3320 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3294).

Euscaphic Acid B (2): white powder, mp 260 °C; $[\alpha]^{25}_D$ +17.57 (*c* 0.74, MeOH); IR ν_{max} (KBr) 3376, 2944, 2837, 1697, 1632, 1604, 1515, 1448 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 509.3288 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

Euscaphic Acid C (3): white powder, mp 248 °C; $[\alpha]^{25}_D$ +0.87 (*c* 1.15, MeOH); IR ν_{max} (KBr) 3396, 2946, 2837, 1704, 1632, 1604, 1515, 1448 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 509.3281 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

Euscaphic Acid D (4): white powder, mp 272 °C; $[\alpha]^{25}_D$ +7.46 (*c* 0.67, MeOH); IR ν_{max} (KBr) 3365, 2943, 2837, 1697, 1631, 1603, 1515, 1449 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 509.3290 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

Euscaphic Acid E (5): white powder, mp 279 °C; $[\alpha]^{25}_D$ −1.85 (*c* 0.54, MeOH); IR ν_{max} (KBr) 3404, 1700, 1630, 1603, 1515 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 493.3337 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3294).

Euscaphic Acid F (6): white powder, mp 244 °C; $[\alpha]^{25}_D$ +5.88 (*c* 0.85, MeOH).; IR ν_{max} (KBr) 3365, 2931, 2850, 1699, 1631, 1597, 1516, 1455 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 491.3180 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3137).

Pomolic Acid (11): ^{19} ^1H NMR (C_5D_5N, 400 MHz) δ 0.90 (3H, s, H-25), 1.01 (3H, s, H-24), 1.10 (3H, s, H-26), 1.10 (3H, d, J = 6.4 Hz, H-30), 1.22 (3H, s, H-23), 1.44 (3H, s, H-29), 1.72 (3H, s, H-27), 3.06 (1H, s, H-18), 3.12 (1H, td, J = 12.8, 4.4 Hz, H-16), 3.42 (1H, dd, J = 10.8, 5.2 Hz, H-3), 5.09, (1H, s, OH-19), 5.60 (1H, t-like, H-12).

Cytotoxicity Assay. Four cancer cell lines, MCF-7 (breast cancer), NCI-H460 (lung cancer), HT-29 (colon cancer), and CEM (leukemia), were maintained in optimal medium (Life Technologies) supplemented with 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies) under standard culture conditions. After treatment with serial dilutions of test compounds for 48 h, the alamar blue assay (Biosource International, Nivelles, Belgium) was used to evaluate cytotoxic effects on four cancer cell lines, and IC_{50} values were calculated. Doxorubicin was used as a positive control. Plates were incubated at 37 °C for 6 h prior to measuring the absorbance at 570 and 600 nm wavelengths using a spectrophotometric plate reader (DYNEX Technologies, USA). Experimental data were normalized to control values.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**6**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Huang, T. C. *Flora of Taiwan*; Department of Botany, National Taiwan University: Taipei, 1993; Vol. 3, p 661.
(2) Ishikura, N. *Phytochemistry* **1971**, *10*, 3332–3333.
(3) Takahashi, K.; Kawaguchi, S.; Nishimura, K.; Kubota, K.; Tanabe, Y. *Chem. Pharm. Bull.* **1974**, *22*, 650–653.
(4) Konishi, T.; Otani, T.; Kiyosawa.; Fujiwara, Y. *Chem. Pharm. Bull.* **1996**, *44*, 863–864.
(5) Takeda, Y.; Okada, Y.; Masuda, T.; Hirata, E.; Takushi, A.; Otsuka, H. *Pytochemistry* **1998**, *49*, 2565–2568.
(6) Takeda, Y.; Okada, Y.; Masuda, T.; Hirata, E.; Shinzato, T.; Takushi, A.; Yu, Q.; Otsuka, H. *Chem. Pharm. Bull.* **2000**, *48*, 752–754.
(7) Lee, M. K.; Jeon, H. Y.; Lee, K. Y.; Ma, C. J.; Sung, S. H.; Lee, H.-S.; Park, M. J.; Kim, Y. C. *Planta Med.* **2007**, *73*, 782–786.
(8) Numata, A.; Yang, P.-M.; Takahashi, C.; Fujiki, R.; Nabae, M.; Fujita, E. *Chem. Pharm. Bull.* **1989**, *37*, 648–651.
(9) Hattori, M.; Kuo, K. P.; Shu, Y. Z.; Tezuka, Y.; Kikuchi, T.; Namba, T. *Phytochemistry* **1988**, *27*, 3975–3976.
(10) Nakatani, M.; Miyazaki, Y.; Iwashita, T.; Naoki, H.; Hase, T. *Phytochemistry* **1989**, *28*, 1479–1482.
(11) Saimaru, H.; Orihara, Y.; Tansakul, P.; Kang, Y.-H.; Shibuya, M.; Ebizuka, Y. *Chem. Pharm. Bull.* **2007**, *55*, 784–788.
(12) Alberman, K. B.; Kipping, F. B. *J. Chem. Soc.* **1951**, 2296–2297.
(13) Kojima, H.; Tominaga, H.; Shigeki, S.; Takayanagi, H.; Ogura, H. *Phytochemistry* **1988**, *27*, 2921–2925.
(14) Chiang, Y. M.; Su, J. K.; Liu, Y. H.; Kuo, Y. H. *Chem. Pharm. Bull.* **2001**, *49*, 581–583.
(15) Kuo, P. C.; Chiu, C. C.; Shi, L. S.; Li, C. Y.; Wu, S. J.; Damu, A. G.; Wu, P. L.; Kuoh, C. S.; Wu, T. S. *J. Chin. Chem. Soc.* **2002**, *49*, 113–116.
(16) Lee, T. H.; Juang, S. H.; Hsu, F. L.; Wu, C. Y. *J. Chin. Chem. Soc.* **2005**, *52*, 1275–1280.
(17) Zürcher, A.; Jeger, O.; Ruzicka, L. *Helv. Chim. Acta* **1954**, *37*, 2145–2149.
(18) Beaton, J. M.; Easton, J. D.; Macarthur, M. M.; Spring, F. S.; Stevenson, R. *J. Chem. Soc.* **1955**, 3992–3997.
(19) Saimaru, H.; Orihara, Y.; Tansakul, P.; Kang, Y. H.; Shibuya, M.; Ebizuka, Y. *Chem. Pharm. Bull.* **2007**, *55*, 784–788.

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