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Cytotoxic Hexacyclic Triterpene Acids from *Euscaphis japonica*Jing-Jy Cheng,^{1,†,‡} Li-Jie Zhang,^{1,†} Hui-Ling Cheng,[†] Chun-Tang Chiou,[†] I-Jung Lee,[†] and Yao-Haur Kuo^{*,†,§}*National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, Institute of Biophotonics, National Yang-Ming University, Taipei 112, Taiwan, Republic of China, and Graduate Institute of Integrated Medicine, China Medical University, Taichuang 404, Taiwan, Republic of China*

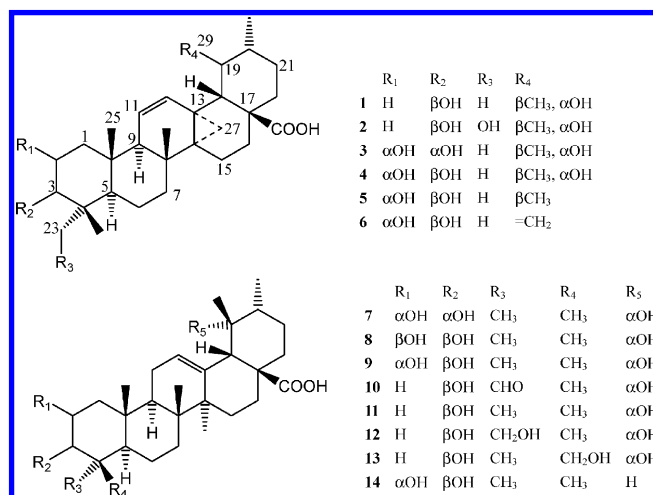
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Six hexacyclic triterpene acids (**1–6**), named euscaphic acids A–F, and eight known triterpene acid compounds (**7–14**) were isolated from an ethanolic extract of twigs of *Euscaphis japonica*. Compounds **8** and **10** were isolated for the first time from a natural source. Triterpenes **1–6** possess hexacyclic skeletons with a 13 α ,27-cyclopropane ring. Structural elucidation of compounds **1–6** was established by spectroscopic methods, especially 2D NMR techniques (¹H–¹H COSY, HMQC, HMBC, and NOESY). Compounds **3**, **4**, and **14** showed significant cytotoxicity against different cancer cell lines [IC₅₀ = 2.54 (NCI-H460), 3.61 (MCF-7), and 3.27 μ M (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 lipopolysaccharide-induced nitric oxide production in BV2 microglia⁷ 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 13 α ,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 2 α -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 2 α -hydroxyursolic acid (**14**)¹¹ from an EtOH extract of *E. japonica*. Hexacyclic triterpenes with the 13 α ,27-cyclopropane ring skeleton are rare in nature. The five known compounds of this type are phyllanthol (13 α ,27-cycloursan-3 β -ol),¹² methyl-2 α ,3 α -24-trihydroxy-13 α ,27-cycloolean-11-en-28-oate,¹³ 3 β -acetoxy-15 α -hydroxy-13 α ,27-cyclours-11-ene,¹⁴ 3 β -acetoxy-13 α ,27-cycloursane,¹⁵ and 2 α ,3 α ,19 α ,23-tetrahydroxy-13 α ,27-cyclours-11-en-28-oic acid¹⁶ from *Phyllanthus engleri* (Pax), *Prunella vulgaris*, *Ficus microcarpa* L., *Ficus septica*, and *Planchonella duclitan* (Blanco) Bakhuizen, respectively. Semisyntheses of 13 α ,27-cyclopropane hexacyclic triterpenes were reported by Zürcher¹⁷ and by Beaton.¹⁸ Structural elucidation of compounds **1–6** was based on spectroscopic analyses, including 1D and 2D NMR techniques (DEPT, ¹H–¹H 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 H₂O 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.



Compound **1** was obtained as a white powder. The HRESIMS of **1** suggested the elemental formula C₃₀H₄₆O₄ 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 ¹³C 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 ¹H 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 (¹H, ¹³C NMR spectra, ¹H–¹H 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 13 α ,27-cyclours-11-en-28-oic acid skeleton for **1** was deduced. The ¹H–¹H COSY spectrum of **1** provided the following cross-peaks: H _{α} -1/H₂-2, H₂-2/H-3; H-5/H _{β} -6; H-9/H-11/H-12; H₂-15/H₂-16; H-20/H₃-30, H-20/H₂-21, H₂-21/H₂-22. Its HMBC correlations included H-3/C-23 and C-24; H₃-23/C-3, C-4, C-5, and C-24; H₃-25/C-1, C-5, C-9, and C-10; H₃-26/C-7, C-8, C-9, and C-14; H₂-27/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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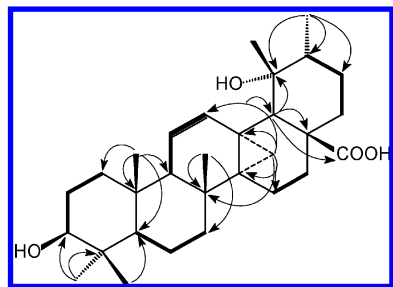


Figure 1. Key HMBC (→) and COSY (---) correlations of **1**.

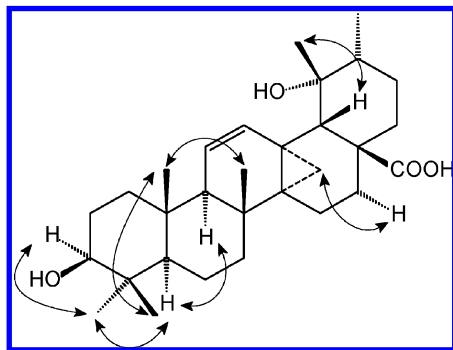


Figure 2. NOE correlations of **1**.

C-16, C-17, C-19, C-20, C-28, and C-29; and H₃-30/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 3 β ,19 α -dihydroxy-13 α ,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]⁺ (C₃₀H₄₆O₅Na). The IR and ¹H and ¹³C NMR data were very similar to those of **1**, except that the signal of a CH₃ was replaced by a CH₂OH at δ_{H} 3.58 and 3.33 (each 1H, d, *J* = 10.0 Hz) and δ_{C} 68.55. This CH₂OH 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 3 β ,19 α ,23-trihydroxy-13 α ,27-cyclours-11-en-28-oic acid, and it was named euscaphic acid B.

Compound **3** had the molecular formula C₃₀H₄₆O₅, as determined from its pseudomolecular ion peak at *m/z* 509.3281 [M + Na]⁺ in the HRESIMS spectrum. The ¹H and ¹³C 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 H₃-24 indicated that both OH-2 and OH-3 were α -oriented. This result was supported by the NOE of H-2 and H₃-25 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 ¹³C 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 2 α ,3 α -dihydroxy-euscaphic acid A (2 α ,3 α ,19 α -trihydroxy-13 α ,27-cyclours-11-en-28-oic acid), and it was named euscaphic acid C.

Compound **4** had the molecular formula C₃₀H₄₆O₅ [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 H₃-24 and H₃-25, and of H-3 with H₃-23 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 2 α -OH and 3 β -OH of **4**. Since the ¹³C 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 3 β -hydroxyeuscaphic acid C (2 α ,3 β ,19 α -trihydroxy-13 α ,27-cyclours-11-en-28-oic acid), and it was named euscaphic acid D.

Compound **5** [C₃₀H₄₆O₄ (*m/z* 493.3337 [M + Na]⁺)] yielded ¹H and ¹³C 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 H₃-29 and H-19 with H₂-27 suggested the β -orientation of Me-29 in compound **5**. Thus, the structure of **5** (2 α ,3 β -dihydroxy-13 α ,27-cyclours-11-en-28-oic acid) was determined unambiguously, and it was named euscaphic acid E.

The molecular formula C₃₀H₄₄O₄, 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 2 α ,3 β -dihydroxy-13 α ,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 μ M). Therefore, only compounds **3**, **4**, and **14** were measured for their IC₅₀ values. From the IC₅₀ data (Table 4), compounds **3**, **4**, and **14** showed selective cytotoxicity against NCI-H460 cells (IC₅₀ 2.54 μ M), MCF-7 cells (IC₅₀ 3.61 μ M), and CEM cells (IC₅₀ 3.27 μ M), 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% H₂SO₄ 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 5C₁₈ 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. ^1H 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 \times 20 mm i.d., Cosmosil 5 C₁₈ AR-II column, MeOH–H₂O, 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 \times 20 mm i.d., Cosmosil 5 C₁₈ AR-II column); fraction E1.5.13.2.9 (65 mg) (MeOH–H₂O, 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–H₂O,

Table 3. Normal and Tumor Cell Proliferation Data of Compounds **1–14** (at 10 μ M)

compound	cell proliferation (%)				
	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

compound	cell line (IC ₅₀ , μ M) ^a			
	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 IC₅₀ = 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 IC₅₀ > 10 μ M.

80:20, recycle four times), afforded **3** (13.2 mg, 0.00017%); fraction E1.5.13.2.14 (113 mg) (MeOH–H₂O, 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–H₂O, 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–H₂O, 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 CHCl₃–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–H₂O, 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–H₂O, 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 CHCl₃–EtOAc–acetone–MeOH, 200:1:1:1, to afford 18 fractions; then, the 14th fraction (120 mg) was purified by HPLC (MeOH–H₂O, 75:25, recycle five times) to afford **10** (70.1 mg, 0.00092%).

Euscaphic Acid A (1): white powder, mp 278 °C; [α]_D²⁵ +9.23 (c 0.65, MeOH); IR ν_{\max} (KBr) 3376, 2944, 2836, 1693, 1631, 1514, 1447 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 493.3320 [M + Na]⁺ (calcd for C₃₀H₄₆O₄Na, 493.3294).

Euscaphic Acid B (2): white powder, mp 260 °C; [α]_D²⁵ +17.57 (c 0.74, MeOH); IR ν_{\max} (KBr) 3376, 2944, 2837, 1697, 1632, 1604, 1515, 1448 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 509.3288 [M + Na]⁺ (calcd for C₃₀H₄₆O₅Na, 509.3243).

Euscaphic Acid C (3): white powder, mp 248 °C; [α]_D²⁵ +0.87 (c 1.15, MeOH); IR ν_{\max} (KBr) 3396, 2946, 2837, 1704, 1632, 1604, 1515, 1448 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 509.3281 [M + Na]⁺ (calcd for C₃₀H₄₆O₅Na, 509.3243).

Euscaphic Acid D (4): white powder, mp 272 °C; [α]_D²⁵ +7.46 (c 0.67, MeOH); IR ν_{\max} (KBr) 3365, 2943, 2837, 1697, 1631, 1603, 1515, 1449 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 509.3290 [M + Na]⁺ (calcd for C₃₀H₄₆O₅Na, 509.3243).

Euscaphic Acid E (5): white powder, mp 279 °C; [α]_D²⁵ –1.85 (c 0.54, MeOH); IR ν_{\max} (KBr) 3404, 1700, 1630, 1603, 1515 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 493.3337 [M + Na]⁺ (calcd for C₃₀H₄₆O₄Na, 493.3294).

Euscaphic Acid F (6): white powder, mp 244 °C; [α]_D²⁵ +5.88 (c 0.85, MeOH); IR ν_{\max} (KBr) 3365, 2931, 2850, 1699, 1631, 1597, 1516, 1455 cm⁻¹; ¹H NMR and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 491.3180 [M + Na]⁺ (calcd for C₃₀H₄₄O₄Na, 491.3137).

Pomolic Acid (11): ¹H NMR (C₅D₅N, 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 IC₅₀ 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 ¹³C NMR spectra of compounds **1–6**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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