Preparation and Cytotoxic Effect of Ceanothic Acid Derivatives

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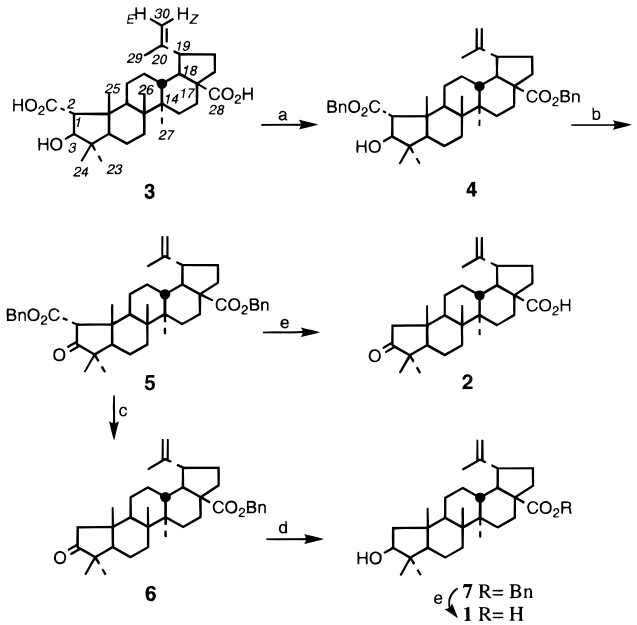
Six ceanothane and 1-norceanothane derivatives (**1**, **2**, **8**−**11**) were prepared from ceanothic acid dibenzyl ester. These ring-A homologues of betulinic acid exhibited cytotoxic effects. Among these, 1-decarboxy-3-oxo-ceanothic acid (**2**) was found to be the most cytotoxic against OVCAR-3 and HeLa cancer cell lines, with an IC_{50} of 2.8 and 6.6 μg/mL, respectively, and an IC_{50} of 11.3 μg/mL against normal cell line FS-5.

The lupane-type triterpene, betulinic acid, is widely distributed in plants. This pentacyclic compound has proved to be selectively cytotoxic to human melanoma[1] and is active against the HIV virus.[2] Structure−activity relationship studies of the latter activity showed that the 3-hydroxyl, 17-carboxylic acid, and 19-isopropyl (or isopropylidene) moieties are essential pharmacophores.[2] A recent study revealed that *Paliurus ramossisimus* (Rhamnaceae) is rich in ceanothic acid (**3**), a ring-A homologue of betulinic acid.[3] As a result, we decided to investigate the biological activity of analogues of this acid. Results of this work are described in this report.

Results and Discussion

1-Decarboxy-ceanothic acid (**1**) and 1-decarboxy-3-oxo-ceanothic acid (**2**) were prepared as shown in Scheme 1. Benzylation of ceanothic acid (**3**), by reacting with benzyl chloride in alkaline conditions, gave ceanothic acid dibenzyl ester (**4**), whose ^{1}H NMR spectrum showed two AB systems for benzylic proton signals, one at δ 4.95 (d) and 5.08 (d) (J = 11.9 Hz) and the other at δ 5.07 (d) and 5.12 (d) (J_{AB} = 12.3 Hz), in addition to the signals of **3**.[4] Oxidation of **4** with pyridinium chlorochromate (PCC)[5] yielded 3-oxo-ceanothic acid dibenzyl ester (**5**), whose ^{1}H NMR spectrum showed the absence of an H-3 signal and a downfield shift of H-1 (δ 3.01, s) relative to that in **4** (δ 2.60, s). Treatment of **5** under alkaline conditions yielded **6**, which had a molecular formula of $C_{36}H_{50}O_3$ based on HREIMS. The ^{1}H NMR spectrum of **6** exhibited only one AB system for benzylic protons (δ 5.08 and 5.14, J_{AB} = 12.3 Hz) and one additional AB system at δ 1.83 and 2.13, J_{AB} = 15.9 Hz), as well as the absence of the corresponding H-1 singlet (δ 3.01) of **5**. These data established **6** as 1-decarboxy-2-oxo-ceanothic acid benzyl ester. This reaction apparently saponified the C-2 ester selectively, accompanied by a six-membered decarboxylation mechanism for β,γ-unsaturated carboxylic acid. Reduction of **6** with sodium borohydride yielded exclusively 1-decarboxy-ceanothic acid benzyl ester (**7**), with an IR absorption at 3450 cm^{-1} (OH) and a molecular formula of $C_{36}H_{52}O_3$. The orientation of the hydroxyl group of **7** was confirmed to be the same as **3** by an NOE experiment in which enhancement of H-3 (δ 3.95, d, J = 7.7 Hz) and H-24 (δ 0.88) was observed upon irradiation of H-23 (δ 0.98). The hindered benzyl group at C-28 was removed by treating **7** with lithium iodide/γ-collidine under reflux[6] to give 1-decarboxy-ceanothic acid (**1**). The IR spectrum of **1** showed absorption at 2500−3100 cm^{-1} and 1698 cm^{-1} for the carboxylic acid function. Its ^{1}H NMR spectrum lacked signals of benzylic protons, and the molecular formula $C_{29}H_{46}O_3$, as deduced from the HREIMS, supported the assigned structure. Treating **5** directly with lithium iodide/γ-collidine under reflux yielded 1-decarboxy-3-oxo-ceanothic acid (**2**). The IR spectrum displayed the absorption at 2600−3200 cm^{-1} and 1690 cm^{-1} for a carboxylic function and 1738 cm^{-1} for a five-membered ketone function. Its ^{1}H NMR spectrum is similar to that of **6** except for lack of benzyl proton signals. These data and the molecular formula $C_{29}H_{44}O_3$, deduced from the HREIMS, established the structure of **2** as shown.

Scheme 1[a]



[a] Key: (a) BnCl, K_2CO_3, MeOH, reflux; (b) PCC, CH_2Cl_2, room temperature; (c) KOH, EtOH, reflux; (d) $NaBH_4$, EtOH−dioxane (1:1), room temperature; (e) LiI, γ-collidine, reflux.

2,3,28-Trihydroxy-ceanoth-20(30)-ene (**8**), ceanotha-1(3),-20(30)-diene-2,28-dioic acid (**9**), 2-norceanotha-1(3),20(30)-dien-28-oic acid (**10**, 1-deformyl-zizyberenalic acid), and 2-norceanothan-28-oic acid (**11**) were prepared as shown in Scheme 2. Reduction of **4** with lithium aluminum hydride gave **8**, with a molecular formula $C_{30}H_{50}O_3$ as deduced from the HREIMS. The ^{1}H NMR spectrum of **8** (in C_5D_5N + D_2O) exhibited signals for H-28 as an AX system at δ 4.09 and 3.65, J_{AX} = 10.6 Hz, for H-2 as multiplets (δ 4.14 and 3.81), and for H-3 as a doublet (δ

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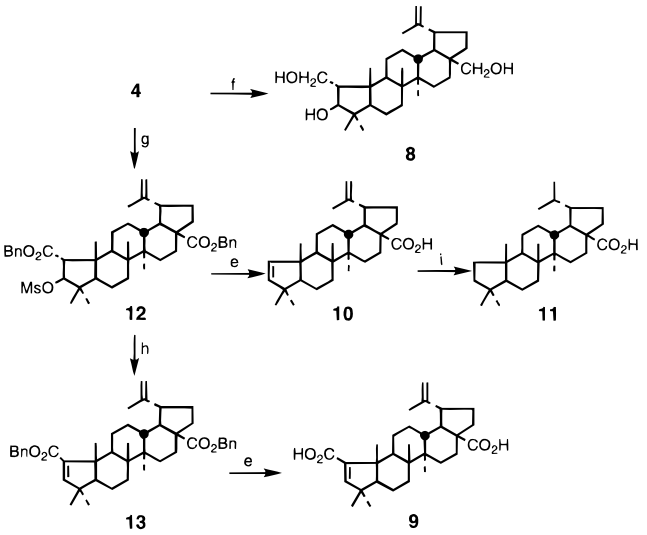
Scheme 2[a]



[a] Key: (f) $LiAlH_4$, THF, reflux; (g) MsCl, pyridine, room temperature; (h) K_2CO_3, *i*-PrOH, reflux; (i) H_2, 10% Pd−C, EtOAc, room temperature.

4.69). *O*-Mesylation of **4** (MsCl−pyridine, 0 °C)[7] gave 3-*O*-mesylceanothanic acid dibenzyl ester (**12**), whose ^{1}H NMR spectrum showed the characteristic signal, δ 2.92 (s, 3H), for the mesyl group, and a downfield-shifted H-3 singlet (δ 4.92) relative to that in the reactant (δ 4.16). Treatment of **12** under alkaline conditions gave the elimination product ceanotha-1(3),20(30)-diene-2,28-dioic acid dibenzyl ester (**13**). The ^{1}H NMR spectrum of **13** revealed an additional olefinic proton at δ 6.20 (s) for H-3, but lacked a signal for 3-OMs. *O*-Debenzylation of the ester **13** using lithium iodide yielded ceanotha-1(3),20(30)-diene-2,28-dioic acid (**9**). The HREIMS showed the molecular ion at *m/z* 468.3238, consistent with that calculated for the expected formula $C_{30}H_{44}O_4$. The ^{1}H NMR spectrum of **9** was similar to that of **13** except for the absence of signals for two benzyl groups.

Treatment of **12** with lithium iodide/γ-collidine under reflux yielded 2-norceanotha-1(3),20(30)-dien-28-oic acid (**10**). The ^{1}H NMR spectrum of **10** displayed two coupled olefinic proton signals at δ 5.90 and 5.42, J_{AX} = 5.7 Hz, assignable to H-1 and H-3, which were confirmed by an NOE experiment, which enhanced H-3 (δ 5.42, d) upon irradiation at the frequency of H-23 (δ 1.00, s) or H-24 (δ 0.89, s). Another irradiation at the signal of H-25 (δ 0.92) enhancing H-1 (δ 5.90, d) and H-24 also confirmed the assignment of H-1 and H-24. HREIMS showed the molecular ion at *m/z* 424.3351, consistent with that calculated for $C_{29}H_{44}O_2$. Catalytic hydrogenation of **10** (Pd−H_2) yielded 2-norceanothan-28-oic acid (**11**). The ^{1}H NMR spectrum of **11** lacked the AX system for H-1 and H-3 and two broad singlets for H-30's in the ^{1}H NMR spectrum of **10**. Instead, two doublet methyls at δ 0.84 (J = 7.0 Hz) and 0.74 (J = 6.8 Hz), assignable for both H-29 and H-30, appeared in the spectrum of **11**.

Compounds **8** and **9** are reduction and dehydration derivatives of **3**, respectively. Their ^{1}H and ^{13}C NMR assignments, obtained by analysis of NOE, COSYDQF, HSQC, and HMBC spectra, are shown in Tables 1 and 2.

The cytotoxicity assay of these prepared compounds was undertaken using the tetrazolium salt (MTT) method.[8] The results indicate that 1-norceanothane derivatives such as **1**, **2**, and **10** are more potent than the other ceanothane analogues tested. It appears that the exo-ring C-1 substitution decreases cytotoxicity, especially polar groups such as **3** and **9** (Table 3). Further study on the IC_{50} of the most cytotoxic compounds (Table 4) revealed that 1-norceanthane derivatives **2** and **10** were about equally potent, but **10** was about two times less toxic to normal cells than was **2**. This observation could be critical to design a lead compound having selective toxicity. This result indicates that the double bond between C-20 and C-30 and the 3-OH group are not essential to the cytotoxic activity of 1-norceanothane derivatives. However, the carboxylic group at C-17 is required for this activity, as was reported in the betulinic acid study.[2]

Experimental Section

General Experimental Procedures. The physical data of the prepared compounds were obtained from the following instruments: Fisher−Johns melting point apparatus (uncorrected); Perkin−Elmer 1760-X infrared FT spectrometer; Hitachi 150-20 UV (MeOH); JEOL JMX-HX110 (HREIMS) and Finnigan TSQ-700 (EIMS) mass spectrometers; Bruker AMX-400 spectrometers using solvent peak as reference standard.

Ceanothic Acid Dibenzyl Ester (4). The ceanothic-acid (**3**) -rich fraction was obtained from the partitioning of the

Table 1. ^{1}H NMR and HMBC (J = 8 Hz) Data for Compounds **8** and **9** (δ/ppm, J in Hz) in C_5D_5N[a]

proton	**8**[b]	HMBC of **8** correlations (C#)	**9**	HMBC of **9** correlations (C#)
1	2.26 dd (9.6, 4.3)	2, 3, 4, 5, 10		
2	4.14 m/3.81 m			
3	4.69 d (3.2)	2, 10	6.52 s	1, 2, 4, 5, 10
5	1.55 m	4, 10, 23, 24	1.57 m	4, 6, 10
9	1.87 dd (12.3, 2.5)	8, 11, 25	2.17 dd (12.7, 3.1)	8, 10, 11, 25, 26
13	1.80 dt (3.2, 12.2)		2.73 dt (3.5, 12.2)	12, 18, 27
18	1.69 t (11.6)	13, 16, 17, 28	1.65 t (11.3)	13, 16, 17, 28
19	2.60 dt (5.7, 11.0)	13, 18, 20, 21, 29, 30	3.46 dt (5.5, 10.8)	18, 20, 21, 29, 30
23	1.21 s	3, 4, 5, 24	1.08 s	3, 4, 5, 24
24	1.26 s	3, 4, 5, 23	0.93 s	3, 4, 5, 23
25	1.34 s	1, 5, 9, 10	1.44 s	1, 5, 9, 10
26	1.06 s	7, 8, 9, 14	1.14 s	7, 8, 9, 14
27	1.09 s	8, 13, 14, 15	0.97 s	8, 13, 14, 15
28	4.09 dd (4.2, 10.6)	22		
	3.65 dd (4.6, 10.6)	16		
29	1.75 br s	19, 20, 30	1.71 br s	19, 20, 30
30	4.87 br s (*Z*)	19, 29	4.83 br s (*Z*)	19, 20, 29
	4.73 br s (*E*)		4.65 br s (*E*)	

[a] Data overlapped or poorly resolved signals were assigned from COSY-45, HSQC, and HMBC spectra: **8** δ 1.49/1.37 (H-7), 1.40−1.46 (H-11), 1.68/1.22 (H-12), 1.93/1.05 (H-15), 2.42/1.30 (H-16), 2.14/1.49 (H-21), 2.40/1.15 (H-22); **9** δ 1.58 (H-6), 1.40 (H-7), 2.49/1.83 (H-11), 1.91/1.21 (H-12), 1.90/1.19 (H-15), 2.61/1.54 (H-16), 2.23/1.50 (H-21), 2.23/1.52 (H-22). [b] δ_{2-OH} 5.93 (br s), δ_{3-OH} 6.21 (d, J = 3.2 Hz), δ_{28-OH} 5.80 (dd, J = 4.6, 4.2 Hz).

Table 2. ^{13}C NMR Data for Compounds **8** and **9** (δ/ppm)[a] in C_5D_5N

carbon	**8**	**9**	carbon	**8**	**9**
1	62.07 d	149.03 s	16	30.11 t	33.07 t
2	61.77 d	171.08 s	17	48.51 s	56.51 s
3	85.33 d	149.57 d	18	49.22 d	49.84 d
4	43.27 s	43.46 s[b]	19	48.31 d	47.82 d
5	58.59 d	63.17 d	20	151.31 s	151.10 s
6	19.21 t	17.28 t	21	30.44 t	31.27 t
7	34.55 t	35.75 t	22	34.85 t	37.66 t
8	41.95 s	42.91 s	23	33.07 q	28.64 q
9	42.64 d	48.58 d	24	20.44 q	20.91 q
10	46.40 s	53.27 s	25	19.38 q	20.31 q
11	23.99 t	24.02 t	26	17.12 q	18.12 q
12	25.50 t	25.83 t	27	15.13 q	14.96 q
13	38.05 t	38.43 d	28	59.50 t	178.90 s
14	43.57 s	43.39 s[b]	29	19.38 q	19.45 q
15	27.87 t	30.49 t	30	109.92 q	110.04 t

[a] Multiplicities were obtained from DEPT experiments. [b] Both close signals were not resolved in HMBC spectrum and might be interchangeable.

Table 3. Cytotoxicity of Betulinic Acid and Ceanothic Acid Derivatives (**1**, **2**, **8**−**11**) on OVCAR-3, HeLa, and FS-5 Cells using Doxorubicin (10 μg/mL) as Positive Control

	cell survival (%)[a] ($n = 2$)		
compound	OVCAR-3	HeLa	FS-5
betulinic acid	0	25	39
ceanothic acid (**3**)	68	65	81
1	0	18	67
2	0	4	16
8	25	22	43
9	51	50	87
10	0	17	33
11	55	48	85
doxorubicin	21	19	56
2% FCS	100	100	100

[a] The cell survival (%) is equal to the OD_{50} value of cells cultured in the presence of tested compounds divided by the OD_{50} value of cells cultured in the presence of 2% FCS.

Table 4. Inhibitory Effect of Betulinic Acid and Norceanothic Acid Derivatives (**1**, **2**, and **10**) on OVCAR-3, HeLa, and FS-5 Cells

	IC_{50} (μg/mL) ($n = 4$)		
compound	OVCAR-3	HeLa	FS-5
betulinic acid	0.9	0.8	20.7
1	4.7	9.1	29.6
2	2.8	6.6	11.3
10	3.5	7.1	22.7

EtOH extract of roots of *P. ramosissimus*, which had been described earlier.[4] Part of this fraction (57 g) was mixed with MeOH (250 mL), benzyl chloride (13.8 mL, 0.36 mol), and K_2CO_3 (16.60 g, 0.12 mol). The suspension was refluxed for 24 h, and the cooled mixture was partitioned between H_2O (350 mL) and toluene (350 mL × 3). The toluene layer was dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure to give a viscous residue (11 g). This reaction was repeated for the remaining insoluble residue and worked up similarly to give an additional 32 g of the crude ceanothic acid dibenzyl ester. These two fractions were combined and subjected to column chromatography over Si gel (500 g, 230−400 mesh) eluted with 0−1% MeOH in $CHCl_3$ to give **4** (19.00 g): amorphous solid; R_f 0.62 [MeOH−$CHCl_3$ (1:9)]; mp 143−145 °C; UV λ_{max} (log ε) 257.0 (2.70); IR (KBr) ν_{max} 3500 (br s), 2950 (s), 1720 (s), 1700 (m), 1640 (w), 1179 (s), 1159 (s), 1125 (s), 900 (m), 740 (s), and 700 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60 (1H, s, H-1), 4.16 (1H, s, H-3), 2.13 (1H, ddd, $J = 3.3$, 12.2, 12.2 Hz, H-13), 2.97 (1H, ddd, $J = 4.4$, 11.0, 11.0 Hz, H-19), 1.09 (3H, s, H-23), 0.89 (3H, s, H-24), 1.01 (3H, s, H-25), 0.84 (3H, s, H-26), 0.72 (3H, s, H-27), 1.64 (3H, s, H-29), 4.70 (1H, br s, H-30_Z), 4.58 (1H, br s, H-30_E), 4.95 (d) and 5.08 (d) ($J =$ 11.9 Hz) (2-OCH_2Ph), 5.07 (d) and 5.12 (d) ($J = 12.3$ Hz) (28-OCH_2Ph); EIMS m/z $[M]^+$ 666 (2), 575 (6), 91 (100), 338 (2), 203 (2), 327 (3), 311 (1), 175 (6); HREIMS (20 eV) m/z $[M]^+$ 666.4313 (calcd for $C_{44}H_{58}O_5$, 666.4286).

3-Oxo-ceanothic Acid Dibenzyl Ester (5). PCC (1.00 g, 4.64 mmol) was added to compound **4** (4.00 g, 6.01 mmol) dissolved in CH_2Cl_2 (80 mL). The resultant solution was stirred at room temperature for 2 h, and then Et_2O (30 mL) was added. Removal of the brown solid residue via filtering through a Celite pad and evaporation of the filtrate gave a viscous residue, which was chromatographed over Si gel (160 g, 230−400 mesh) and eluted with toluene to give **5** (3.20 g, 80.2% yield): R_f 0.50 ($CHCl_3$); mp 123−126 °C; UV λ_{max} (log ε) 257.5 (2.58); IR (KBr) ν_{max} 2950 (s), 1745 (s), 1723 (s), 1710 (s), 1640 (m), 1378 (s), 1459 (s), 1320 (s), 1220 (s), 1159 (s), 880 (m), 738 (s), 700 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.01 (1H, s, H-1), 2.13 (1H, ddd, $J = 3.2$, 11.8, 11.8 Hz, H-13), 2.96 (1H, ddd, $J = 4.5$, 10.8, 10.8 Hz, H-19), 1.64 (3H, s, H-29), 4.70 (1H, br s, H-30_Z), 4.60 (1H, br s, H-30_E), 5.03 (d) and 5.08 (d) ($J =$ 12.0 Hz) (2-OCH_2Ph), 5.07 (d) and 5.12 (d) ($J = 12.3$ Hz) (28-OCH_2Ph), 7.33 (10H, m, CH_2Ph); EIMS m/z $[M]^+$ 664 (1), 574 (13), 91 (10), 338 (2), 203 (2), 325 (1), 311 (1), 175 (5); HREIMS (20 eV) m/z $[M]^+$ 664.4077 (calcd for $C_{44}H_{56}O_5$, 664.4130).

1-Decarboxy-3-oxo-ceanothic Acid Benzyl Ester (6). Powdered KOH (1.00 g, 17.86 mmol) was added to compound **5** (3.20 g, 4.82 mmol) dissolved in EtOH (60 mL). The resultant suspension was stirred under reflux for 3 h. After removal of the organic solvent, the residue was triturated with H_2O (100 mL), and the suspension was adjusted to pH 3 with concentrated HCl, and partitioned against $CHCl_3$ (100 mL × 3). The combined $CHCl_3$ layer, after drying over anhydrous Na_2SO_4, was evaporated to give a residue that was purified on a Si gel column (120 g, 230−400 mesh) eluted with toluene to give **6** (1.80 g, 70.5% yield): R_f 0.35 [Me_2CO−toluene (1:99)]; mp 143−144 °C; UV λ_{max} (log ε) 257.5 (2.14); IR (KBr) ν_{max} 3060 (w), 2950 (s), 1738 (s), 1640 (m), 1475 (s), 1150 (s), 1129 (s), 885 (m), 755 (s), 700 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.83 and 2.12 (each 1H, d, $J = 15.9$ Hz, H-1), 2.17 (1H, ddd, $J = 3.3$, 12.1, 12.1 Hz, H-13), 2.99 (1H, ddd, $J = 4.7$, 10.9, 10.9 Hz, H-19), 1.66 (3H, s, H-29), 4.70 (1H, br s, H-30_Z), 4.57 (1H, br s, H-30_E), 5.08 (d) and 5.14 (d) ($J = 12.3$ Hz) (28-OCH_2Ph), 7.34 (5H, m, CH_2Ph); EIMS m/z $[M]^+$ 530 (11), 441 (12), 91 (100), 338 (11), 203 (12), 193 (27), 311 (1), 175 (20); HREIMS (20 eV) m/z $[M]^+$ 530.3764 (calcd for $C_{36}H_{50}O_3$, 530.3762).

1-Decarboxy-ceanothic Acid Benzyl Ester (7). $NaBH_4$ (42.3 mg, 1.12 mmol) was added portionwise at room temperature to compound **6** (150 mg, 0.28 mmol) dissolved in EtOH (10 mL) and dioxane (10 mL) and then was stirred for 1 h. After removal of the organic solvent, the residue was partitioned between H_2O (50 mL) and $CHCl_3$ (50 mL × 3). The combined $CHCl_3$ layer, after drying over anhydrous Na_2SO_4, was evaporated to give a residue that was purified on a Si gel column (5 g, 230−400 mesh) eluted with $CHCl_3$ to give **7** (130 mg, 86.3% yield): R_f 0.50 [MeOH−$CHCl_3$ (3:97)]; mp 75−78 °C; UV λ_{max} (log ε) 257.5 (2.15); IR (KBr) ν_{max} 3450 (br s), 2948 (s), 1722 (s), 1640 (m), 1155 (s), 1123 (s), 1060 (m), 880 (s), 745 (s), 700 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95 (1H, d, $J = 7.7$ Hz, H-3), 2.14 (1H, ddd, $J = 3.4$, 12.3, 12.3 Hz, H-13), 2.99 (1H, ddd, $J = 4.6$, 11.1, 11.1 Hz, H-19), 1.66 (3H, s, H-29), 4.70 (1H, br s, H-30_Z), 4.57 (1H, br s, H-30_E), 5.07 (d) and 5.13 (d) ($J = 12.1$ Hz) (28-OCH_2Ph), 7.33 (5H, m, CH_2Ph); EIMS m/z $[M]^+$ 532 (3), 441 (12), 91 (100), 338 (11), 203 (12), 193 (27), 311 (1), 175 (20); HREIMS (20 eV) m/z $[M]^+$ 532.3865 (calcd for $C_{36}H_{52}O_3$, 532.3919).

1-Decarboxy-ceanothic Acid (1). The mixture of compound **7** (110 mg, 0.21 mmol), γ-collidine (0.7 mL), and LiI (85 mg, 0.64 mmol) was heated under reflux (180 °C) for 1.5 h. The viscous reaction mixture was partitioned between $CHCl_3$ (50 mL) and H_2O (pH 1, 50 mL × 3). The $CHCl_3$ layer, after drying over anhydrous Na_2SO_4, was evaporated in vacuo to give a residue that was purified on a Si gel column (8 g, 230−400 mesh) eluted with 0.25% trifluoroacetic acid in $CHCl_3$ to give **1** (80 mg, 87.5% yield): R_f 0.33 [MeOH−$CHCl_3$ (3:97)]; mp 240−241 °C; IR (KBr) ν_{max} 3450 (br s), 2950 (s), 1698 (s), 1640 (m), 1450 (s), 1377 (s), 1180 (s), 880 (m) cm^{-1}; ^1H NMR

($CDCl_3$) δ 3.96 (1H, d, J = 7.9 Hz, H-3), 2.10 (1H, ddd, J = 3.4, 12.1, 12.1 Hz, H-13), 2.90 (1H, ddd, J = 4.0, 10.4, 10.4 Hz, H-19), 1.67 (3H, br s, H-29), 4.71 (1H, br s, H-30$_Z$), 4.58 (1H, br s, H-30$_E$); EIMS m/z [M]$^+$ 442 (24), 424 (25), 248 (40), 203 (23), 193 (100), 220 (10), 175 (52); HREIMS (20 eV) m/z [M]$^+$ 442.3450 (calcd for $C_{29}H_{46}O_3$, 442.3449).

1-Decarboxy-3-oxo-ceanothic Acid (2). Under similar reaction conditions and workup process for the preparation of **1**, **5** (300 mg, 0.45 mmol) gave **2** (170 mg, 85.5%): R_f 0.25 [MeOH−$CHCl_3$ (3:97)]; mp 235−236 °C; IR (KBr) ν_{max} 3300 (br s), 2950 (s), 1738 (s), 1690 (s), 1640 (m), 1450 (s), 1375 (s), 880 (s), 755 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.85 and 2.13 (each 1H, d, J = 15.8 Hz, H-1), 2.19 (1H, ddd, J = 3.2, 12.3, 12.3 Hz, H-13), 2.98 (1H, ddd, J = 4.8, 10.8, 10.8 Hz, H-19), 1.67 (3H, br s, H-29), 4.72 (1H, br s, H-30$_Z$), 4.59 (1H, br s, H-30$_E$); EIMS m/z [M]$^+$ 440 (42), 425 (100), 248 (62), 203 (50), 191 (62), 220 (9), 175 (49); HREIMS (20 eV) m/z [M]$^+$ 440.3292 (calcd for $C_{29}H_{44}O_3$, 440.3292).

2,3,28-Trihydroxy-ceanoth-20(30)-ene (8). Anhydrous THF (4 mL) was injected to $LiAlH_4$ (40 mg, 0.53 mmol) under nitrogen. The suspension was stirred at room temperature for 10 min, then **4** (40 mg, 0.06 mmol), dissolved in anhydrous THF (1 mL), was added dropwise. The mixture was heated under reflux for 2.5 h. After cooling, hydrated Na_2SO_4 was added to the suspension to destroy excess $LiAlH_4$. The suspension was then filtered through a Celite pad and the filtered residue washed with MeOH. The filtrate and washings were combined and evaporated to give a residue that was purified on a Si gel column (3 g, 230−400 mesh) eluted with 2% MeOH in $CHCl_3$ to give **8** (20 mg, 72.7% yield): R_f 0.30 [MeOH−$CHCl_3$ (1:19)]; mp 219−220 °C; IR (KBr) ν_{max} 3380 (br s), 2947 (s), 1640 (m), 1020 (s), 1458 (s), 1377 (s), 883 (s) cm^{-1}; ^{1}H and ^{13}C NMR (C_5D_5N) data, see Tables 1 and 2, respectively; NOE data (C_5D_5N), H-23 (δ 1.21) to H-3 (δ 4.69) (12.1%), H-5 (δ 1.55) (5.6%), and H-24 (δ 1.26) (5.9%); H-24 to H-23 (3.7%) and H-25 (δ 1.34) (4.0%); H-25 to H-1 (δ 2.26) (9.6%), H-24 (8.4%), and H-26 (δ 1.06) (10.8%); H-26 to H-13 (δ 1.80) (7.6%) and H-25 (8.1%); H-27 (δ 1.09) to H-9 (δ 1.87) (9.4%) and H-18 (δ 1.69) (8.3%); H-29 (δ 1.75) to H-19 (δ 2.60) (1.4%), H-30$_E$ (δ 4.73) (6.2%), and H-30$_Z$ (δ 4.87) (−1.0%); EIMS m/z [M]$^+$ 458 (41), 440 (42), 427 (49), 234 (17), 203 (45), 223 (45), 206 (16), 175 (45); HREIMS (20 eV) m/z [M]$^+$ 458.3751 (calcd for $C_{30}H_{50}O_3$, 458.3762).

3-*O*-Mesylceanothic Acid Dibenzyl Ester (12). Methanesulfonyl chloride (MsCl, 0.5 mL) was added slowly to compound **4** (200 mg, 0.30 mmol) dissolved in anhydrous pyridine (1 mL) in an ice bath. The reaction was carried at 0 °C for 0.5 h and at room temperature for 1 h. Then pyridine−H_2O (2:1, 0.15 mL) was added and stirred for 15 min to destroy excess MsCl. The resultant mixture was partitioned between H_2O (50 mL) and $CHCl_3$ (50 mL × 3). The $CHCl_3$ layer, after drying over anhydrous Na_2SO_4, was evaporated to give a residue that was purified on a Si gel column (10 g, 230−400 mesh) eluted with toluene to give **12** (160 mg, 71.6% yield): R_f 0.68 [MeOH−$CHCl_3$ (3:97)]; mp 163−164 °C; UV λ_{max} (log ϵ) 257.5 (2.44); IR (KBr) ν_{max} 2948 (s), 1724 (s), 1715 (s), 1641 (m), 1340 (s), 1180 (s), 1152 (s), 1130 (s), 1030 (m), 870 (s), 760 (s), 737 (s), 700 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.58 (1H, s, H-1), 4.92 (1H, s, H-3), 2.92 (3H, s, 3-OMs), 2.13 (1H, ddd, J = 3.2, 12.2, 12.2 Hz, H-13), 2.96 (1H, m, H-19), 1.63 (3H, s, H-29), 4.70 (1H, br s, H-30$_Z$), 4.58 (1H, br s, H-30$_E$), 4.96 (d) and 5.15 (d) (J = 12.1 Hz) (2-OCH_2Ph), 5.06 (d) and 5.12 (d) (J = 12.4 Hz) (28-OCH_2Ph), 7.34 (5H, m, CH_2Ph); EIMS m/z [M]$^+$ 744 (3), 653 (25), 91 (100), 338 (1), 203 (6), 405 (7), 311 (1), 175 (4); HREIMS (20 eV) m/z [M]$^+$ 744.4072 (calcd for $C_{45}H_{60}O_7S$, 744.4079).

Ceanotha-1(3),20(30)-diene-2,28-dioic Acid Dibenzyl Ester (13). The mixture of compound **12** (400 mg, 0.54 mmol), *i*-PrOH (40 mL), and K_2CO_3 (10.00 g) were heated under reflux for 48 h. After cooling, the precipitate was filtered and the filtrate was evaporated to give a residue that was purified on a Si gel column (20 g, 230−400 mesh) eluted with *n*-hexane−$CHCl_3$ (1:1) to give **13** (210 mg, 60.3% yield): R_f 0.45 [Me_2-CO−toluene (1:99)]; mp 129−131 °C; UV λ_{max} (log ϵ) 258.0 (2.22); IR (KBr) ν_{max} 2950 (s), 1725 (s), 1717 (s), 1640 (m), 1278 (s), 1156 (s), 1122 (s), 1023 (s), 885 (s), 743 (s), 700 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.20 (1H, s, H-3), 2.10 (1H, ddd, J = 2.8, 12.3, 12.3 Hz, H-13), 2.97 (1H, ddd, J = 4.8, 11.0, 11.0 Hz, H-19), 1.65 (3H, s, H-29), 4.69 (1H, br s, H-30$_Z$), 4.59 (1H, br s, H-30$_E$), 5.09 (d) and 5.12 (d) (J = 12.3 Hz) (2-OCH_2Ph), 5.08 (d) and 5.17 (d) (J = 12.4 Hz) (28-OCH_2Ph), 7.34 (10H, m, CH_2Ph); EIMS m/z [M]$^+$ 648 (1), 557 (5), 91 (100), 338 (1), 203 (2), 309 (2), 311 (2), 175 (4); HREIMS (20 eV) m/z [M]$^+$ 648.4370 (calcd for $C_{44}H_{56}O_4$, 648.4181).

Ceanotha-1(3),20(30)-diene-2,28-dioic Acid (9). When reaction conditions and a workup process similar to those for the preparation of **1** were used, **13** (180 mg, 0.28 mmol) gave **9** (120 mg, 92.3%): R_f 0.42 [MeOH−$CHCl_3$ (1:19)]; mp 270−271 °C; IR (KBr) ν_{max} 3300 (br s), 2950 (s), 1960 (s), 1640 (m), 1450 (s), 1378 (s), 1230 (s), 1210 (s), 1192 (s), 880 (s) cm^{-1}; ^{1}H and ^{13}C NMR (C_5D_5N) data, see Tables 1 and 2, respectively; NOE data (C_5D_5N), H-23 (δ 1.08) to H-3 (δ 6.52) (10.6%) and H-24 (δ 0.93) (7.5%); H-24 to H-3 (6.7%), H-23 (5.8%), and H-25 (δ 1.44) (10.4%); H-25 to H-24 (7.8%) and H-26 (δ 1.14) (7.8%); H-26 to H-13 (δ 2.73) (10.5%) and H-25 (13.6%); H-27 (δ 0.97) to H-9 (δ 2.17) (9.9%) and H-18 (δ 1.65) (8.0%); H-29 (δ 1.71) to H-19 (δ 3.46) (2.0%), H-30$_E$ (δ 4.65) (6.1%), and H-30$_Z$ (δ 4.83) (−2.3%); EIMS m/z [M]$^+$ 468 (57), 248 (62), 220 (32), 219 (100), 203 (62), 175 (95); HREIMS (20 eV) m/z [M]$^+$ 468.3238 (calcd for $C_{30}H_{44}O_4$, 468.3241).

1-Norceanotha-1(3),20(30)-dien-28-oic Acid (10). When reaction conditions and a workup process similar to those for the preparation of **1** were used, **12** (350 mg, 0.47 mmol) gave **10** (86 mg, 43.1%): R_f 0.55 [MeOH−$CHCl_3$ (3:97)]; mp 203−204 °C; IR (KBr) ν_{max} 3300 (br s), 2950 (s), 1690 (s), 1640 (m), and 880 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.90 (1H, d, J = 5.7 Hz, H-1), 5.42 (1H, d, J = 5.7 Hz, H-3), 4.72 (1H, br s, H-30$_Z$−), 4.59 (1H, br s, H-30$_E$−), 2.98 (1H, dt, J = 4.8, 12.5 Hz, H-19), 2.15 (1H, dt, J = 3.6, 12.5 Hz, H-13), 1.72 (1H, dd, J = 3.2, 12.5 Hz, H-9), 1.67 (3H, s, H-29), 1.58 (1H, dt, J = 3.1, 13.7 Hz, H-15β), 1.58 (1H, t, J = 11.3 Hz, H-18), 1.51 (1H, dq, J = 4.7, 12.5 Hz, H-11β), 1.39 (1H, m, H-6β), 1.42 (1H, dt, J = 3.6, 13.2 Hz, H-7α or H-12α), 1.23 (1H, br d, J = 11.8 Hz, H-5), 1.15 (1H, br d, J = 13.0 Hz, H-15), 1.00 (3H, s, H-23), 0.98 (3H, s, H-27), 0.93 (3H, s, H-26), 0.92 (3H, s, H-25), 0.89 (3H, s, H-24); NOE data, H-23 to H-24 (6.3%), H-3 (5.2%), and H-5 (7.2%); H-24 to H-3 (3.5%), H-23 (3.9%), and H-25 (2.4%); H-25 (δ 0.92) to H-1 (1.1%) and H-11β (5.5%); H-26 to H-11β (4.0%), H-13 (8.9%), H-6β (9.1%), and H-15β (7.9%); H-27 (δ 0.98) to H-9 (9.3%), H-18 (9.4%), H-15 (5.2%), and H-7α (or H-12α) (10.2%); EIMS m/z [M]$^+$ 424 (14), 409 (100), 248 (1), 220 (1), 203 (5), 175 (41); HREIMS (20 eV) m/z [M]$^+$ 424.3351 (calcd for $C_{29}H_{44}O_2$, 424.3343).

1-Norceanothan-28-oic Acid (11). Compound **10** (60 mg) in EtOAc (5 mL) was catalytically hydrogenated (H_2, 1 atm; 10% Pd−C, 30 mg) in the usual manner at room temperature overnight. The resultant suspension was filtered through a Celite pad and the residue washed with $CHCl_3$. The combined filtrate and washings were evaporated to give a residue that was purified on a Si gel column (4 g, 230−400 mesh) eluted with 1% MeOH in $CHCl_3$ to give **11** (40 mg, 66.0% yield): R_f 0.38 [MeOH−$CHCl_3$ (1:19)]; mp 200−202 °C; IR (KBr) ν_{max} 3300 (br s), 2950 (s), 1680 (m), 1640 (m), 1460 (w), 1380 (w), and 1240 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.18 (1H, dt, J = 3.4, 11.7 Hz, H-13), 0.95 (3H, s, H-27), 0.94 (3H, s, H-26), 0.88 (3H, s), 0.85 (3H, s) and 0.76 (3H, s) (H-23, H-24, and H-25), 0.84 (3H, d, J = 7.0 Hz) and 0.74 (3H, d, J = 6.8 Hz) (H-29 and H-30); EIMS m/z [M]$^+$ 428 (9), 409 (100), 250 (1), 222 (1), 205 (3), 177 (100); HREIMS (20 eV) m/z [M]$^+$ 428.3638 (calcd for $C_{29}H_{48}O_2$, 428.3656).

Cytotoxicity Assay.[8] Human ovarian adenocarcinoma (OVCAR-3) cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 μg/mL streptomycin, 100 IU/mL penicillin, and 2 mM glutamine. Human foreskin fibroblast (FS-5) and cervical carcinoma (HeLa) cells were grown in DMEM supplemented with similar components. All cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. The cytotoxic activity against OVCAR-3, HeLa, and FS-5 cells was determined after 48 h of incubation in the presence and absence of

the test compounds. Briefly, cells were seeded at a density of 2×10^4 cells/well in 96-well microtiter plates and incubated at 37 °C for 24 h before treatment. The growth medium was then replaced with one that contained the tested compounds and 2% FCS, and cells were incubated for 48 h. After incubation with the compounds, the medium was withdrawn, the cells were washed, 0.1 mL of fresh serum-free medium and 25 μL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] were added to each well and incubated at 37 °C for 4 h. Lysis buffer (100 μL, 20% SDS in 50% dimethylformamide) was then added, and, after overnight incubation, the absorbance of each culture was read at 570 nm using a microplate reader (Labsystems, Finland).

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References and Notes

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