Cytotoxic Constituents of *Polyalthia longifolia* var. *pendula*

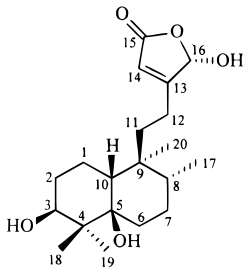
Chung-Yi Chen,[†] Fang-Rong Chang,[†] Yao-Ching Shih,[†] Tian-Jye Hsieh,[†] Yi-Chen Chia,[†,‡] Huang-Yi Tseng,[§] Hua-Chien Chen,[§] Shu-Jen Chen,[§] Ming-Chu Hsu,[§] and Yang-Chang Wu*[,†]

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Tajen Institute of Technology, Ping Tung Hsien 907, Taiwan, and Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Taipei, Taiwan, Republic of China

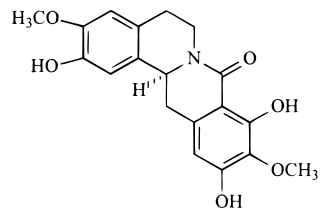
Received April 14, 2000

A new halimane diterpene, 3*β*,5*β*,16α-trihydroxyhalima-13(14)-en-15,16-olide (**1**), and a new oxoprotoberberine alkaloid, (−)-8-oxopolyalthiaine (**2**), along with 20 known compounds, were isolated from a methanolic extract of *Polyalthia longifolia* var. *pendula*. The structures of compounds **1** and **2** were established by spectroscopic analysis. Several of these compounds were evaluated for cytotoxicity toward a small panel of human cell lines.

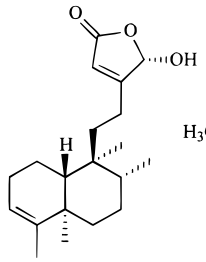
Polyalthia longifolia Benth. & Hook. f. var. *pendula* (Annonaceae) is an evergreen tree cultivated in southern Taiwan and is also widely distributed in the temperate regions of India. Previous phytochemical work on *Polyalthia* species had led to the isolation of clerodane diterpenes,[1−7] halimane diterpenes,[3] labdane diterpenes,[8] triterpenes,[9,10] aporphines,[11−13] indolosesquiterpenes,[14,15] benzylisoquinolines,[16] tetrahydroprotoberberines,[17,18] morphinanedienones,[11] and some azafluorene alkaloids.[19,20] To further understand the chemotaxonomy and to continue searching for novel plant bioactive agents from Annonaceous plants, *P. longifolia* var. *pendula* was chosen for phytochemical investigation. A new halimane diterpene, 3*β*,5*β*,16α-trihydroxyhalima-13(14)-en-15,16-olide (**1**), and a new oxoprotoberberine alkaloid, (−)-8-oxopolyalthiaine (**2**), along with 20 known compounds, 16α-hydroxycleroda-3,13-dien-15,16-olide (**3**),[1−7] 16-hydroxycleroda-3,13-dien-15-oic acid,[1−7] cleroda-3,13*E*-dien-15-oic acid,[1−7] 3,12*E*-kolavadien-15-oic acid-16-al,[1−7] (4→2)-*abeo*-16(*R* and *S*)-2,13*Z*-kolavadien-15,16-olide-3-al,[1−7] labd-13*E*-en-8-ol-15-oic acid,[8] (−)-stepholidine,[21] 1-aza-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione,[22,23] 5-hydroxy-6-methoxyonychine (**4**),[24,25] 6-hydroxy-7-methoxyonychine,[24,25] liriodenine,[26] oxoxylopine,[27] (−)-anonaine (**5**),[26] (−)-asimilobine,[26] (−)-norboldine,[28] (+)-norboldine,[28] (−)-norpallidine,[29] *p*-hydroxybenzoic acid,[30] *β*-sitosterol, and stigmasterol,[26] were isolated and identified from this plant. The structures of these compounds were elucidated by spectroscopic analysis, and several of them were evaluated for their cytotoxicity toward a panel of human cell lines.



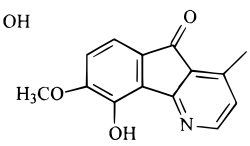
1



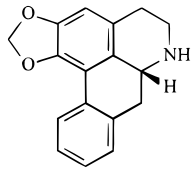
2



3



4



5

Results and Discussion

The combined MeOH extracts of the leaves of *P. longifolia* var. *pendula* were partitioned to yield $CHCl_3$ and aqueous layers. The dried $CHCl_3$ layer was extracted with 3% HCl to remove alkaloids, and then the neutral $CHCl_3$ solution was dried and evaporated to afford a brown viscous residue. Workup of this residue led to the isolation of **1** and **2** along with 20 known compounds.

Compound **1** was obtained as a white amorphous powder. The presence of a hydroxyl group and an α,*β*-unsaturated lactone was indicated by its IR (ν_{max} at 3415 and 1750 cm^{-1}, respectively) and UV (λ_{max} at 210 nm) spectra.[2] The HREIMS gave a molecular ion at *m*/*z* 352.2245 $[M]^+$ (calcd 352.2250), corresponding to the molecular formula $C_{20}H_{32}O_5$. The ^1H NMR spectrum of **1** indicated the presence of four methyl groups at *δ* 0.80, 1.13, 1.19 (each 3H, s, including a pair geminal methyls), and 0.86 (3H, d, $J = 6.4$ Hz), a triplet signal at *δ* 3.48 (1H, $J = 2.8$ Hz), ascribable to an oxymethine, and two downfield singlet signals at *δ* 5.88 and 6.04 (each 1H, s), from a *γ*-hydroxy-α,*β*-unsaturated-*γ*-lactone moiety. These data were consistent with the ^1H NMR features of a halimane-type diterpene.[3] In addition, the ^{13}C NMR and DEPT spectra indicated that **1** possesses a diterpene skeleton based on a total of 20 carbons, comprising four methyl carbons at *δ* 16.4, 17.9, 18.7, and 21.2, six methylene carbons at *δ* 17.6, 22.5, 27.8, 31.0, 33.5, and 36.5, five methine carbons at *δ* 37.4, 41.4, 76.9, 101.4, and 117.4, and five quaternary carbons at *δ* 39.8, 42.6, 77.3, 173.3, and 173.8. COSY NMR correlations enabled three partial structures A−C to be established (Figure 1). In addition, ^1H NMR signals at *δ* 5.88 and 6.04, and typical ^{13}C NMR signals at *δ* 101.4, 117.4, 173.3 and 173.8, demonstrated the presence of a *γ*-hydroxy-α,*β*-unsaturated-*γ*-lactone moiety (partial structure D in Figure 1). The

* To whom correspondence should be addressed. Tel: +886-7-3121101 ext. 2197. Fax: +886-7-3114773. E-mail: yachwu@cc.kmu.edu.tw.
† Kaohsiung Medical University.
‡ Tajen Institute of Technology.
§ National Health Research Institutes.

10.1021/np000176e CCC: $19.00
Published on Web 09/08/2000

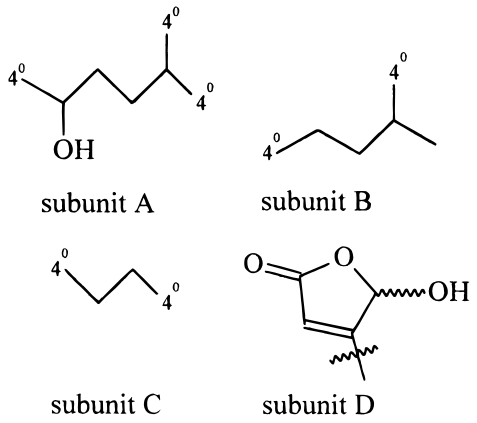


Figure 1. Partial fragments of **1**.

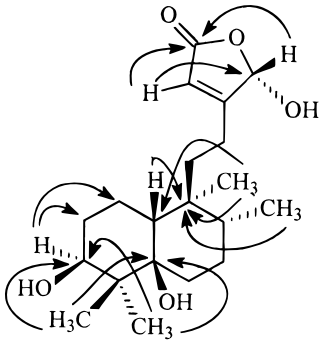


Figure 2. HMBC correlations of **1**.

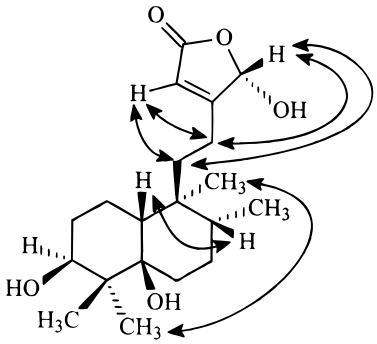


Figure 3. NOESY correlations of **1**.

connections of these subunits were determined by HMBC and NOESY NMR data. In the HMBC spectrum, the hydroxylated methine carbon at δ 76.9 (C-3) showed correlations with Me-18, Me-19, H-1, and H-2, whereas the quaternary carbon at δ 42.6 (C-4) showed $^2J_{C-H}$ connections with Me-18 and Me-19 (Figure 2). The quaternary hydroxylated carbon at δ 77.3 (C-5) revealed $^3J_{C-H}$ interactions with H-3, Me-18, and Me-19 and $^2J_{C-H}$ interactions with H-6 and H-10. Thus, the quaternary carbon C-4, bearing two methyl groups, was linked to C-3 and C-5. On the basis of the HMBC correlations, the locations of two hydroxyl groups at C-3 and C-5 were also confirmed. The quaternary carbon at δ 39.8 (C-9) displayed $^3J_{C-H}$ interactions with H-1, H-7, and Me-17 and $^2J_{C-H}$ interactions with H-10, H-11, and Me-20, which determined the C-11 side-chain connection at C-9. Furthermore, a *cis* A/B ring junction of the bicyclic diterpenoid nucleus was determined by a correlation between Me-19 and Me-20 in the NOESY spectrum (Figure 3). A correlation between H-8 and H-10 suggested that these two protons possess 1,3-diaxial configurations. The β-orientation of H-10 and the hydroxyl groups at C-3 and C-5 was confirmed by a NOESY

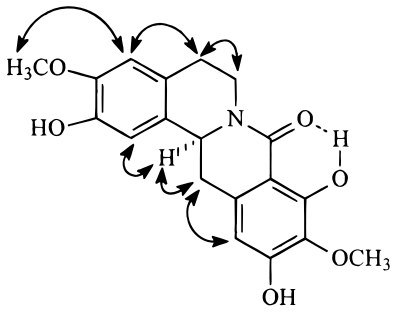


Figure 4. NOESY correlations of **2**.

experiment, in which H-16 and H-14 exhibited significant correlations with the signals of H-11 and H-12. Therefore, the lactone moiety (subunit D) was connected to C-12. To solve the final stereochemistry, the CD spectrum of **1** was measured. On comparison to the Cotton effect data of known butenolides,[31] the absolute configuration at C-16 was correlated to the sign of the Cotton effects of the n$-\pi^*$ (235−250 nm) and $\pi-\pi^*$ (200−220 nm) transitions. The positive n$-\pi^*$ transitions at 246 nm and negative $\pi-\pi^*$ transitions near 200 nm suggested that C-16 has the *S* configuration, and the α-orientation of the hydroxyl group at C-16 could be confirmed. The result was consistent with the literature data described by Ma *et al.*[7] Thus, the structure of **1** was determined as 3β,5β,16α-trihydroxyhalima-13(14)-en-15,16-olide, and its IUPAC name is 4-{2-[(2*S*,3*R*,8*S*,6*R*)-6,8-dihydroxy-2,3,7,7-tetramethylbicyclo-[4.4.0]dec-2-yl]ethyl}-(5*S*)-5-hydroxy-5-hydrofuran-2-one.

Compound **2** was obtained as a yellow amorphous powder from $CHCl_3$. HREIMS revealed a $[M]^+$ ion at *m*/*z* 357.1213 (calcd 357.1212), corresponding to the molecular formula $C_{19}H_{19}O_6N$. The UV spectrum showed absorption maxima at 238, 270, and 353 nm, consistent with the compound being a oxoprotoberberine-type alkaloid.[32,33] The IR spectrum of **2** exhibited absorption bands at ν_{max} 3503 and 1635 cm^{-1}, indicating the presence of hydroxyl and lactam groups.[32,33] The EIMS exhibited characteristic *retro*-Diels−Alder fragments associated with an 8-oxoprotoberberine skeleton possessing two hydroxyl groups and one methoxyl group in the D ring (fragment at *m*/*z* 180), as well as one methoxyl and one hydroxyl group in the A ring (fragment at *m*/*z* 178).[32,33] The negative optical rotation of **2** was consistent with a 14 *S*-configuration, and H-14 was determined to have an α-orientation.[32] The ^{1}H NMR spectrum of **2** displayed seven typical signals for an 8-oxoprotoberberine alkaloid at δ 2.64 (1H, ddd, J = 13.0, 3.0, 2.0 Hz) for H-5α (pseudoequatorial), δ 2.81 (1H, dd, J = 15.6, 13.6 Hz) for H-13β, δ 2.86 (1H, ddd, J = 13.0, 11.0, 2.6 Hz) for H-5β (pseudoaxial), δ 2.90 (1H, ddd, J = 12.5, 11.0, 2.0 Hz) for H-6α (pseudoaxial), δ 3.25 (1H, dd, J = 15.6, 4.0 Hz) for H-13α, δ 4.77 (1H, dd, J = 13.6, 4.0 Hz) for H-14, and δ 4.98 (1H, ddd, J = 12.5, 3.0, 2.6 Hz) for H-6β (pseudoequatorial), respectively.[30] In the ^{13}C NMR spectrum of **2**, a carbonyl carbon at δ 169.1, 12 aromatic carbons between δ 156.9 and 104.9, two methyl carbons at δ 55.1 and 55.9, three methylene carbons at δ 28.9, 37.1, and 38.6, and a methine carbon at δ 60.2 were observed. However, the positions of three aromatic proton signals at δ 7.12 (1H, s), 6.78 (1H, s), and 6.62 (1H, s), two methoxyl signals at δ 3.98 and 3.78, and three hydroxyl groups needed to be determined. The complete assignments of these protons were established from the NOESY NMR spectrum (Figure 4). Significant correlations between OCH_3-3, H-4, H-5, and H-6, as well as between H-1, H-13, H-14, and H-12, were observed. One hydroxyl proton resonated at δ 13.97 (1H, s), indicating that it was

Table 1. ^{1}H NMR (400 MHz, *J* in Hz) and ^{13}C NMR (100 MHz) Spectral Data of **1** in CD_3OD

position	δ_H (mult., *J*, Hz)	δ_C (mult.)
1	1.29 (m), 1.55 (m)	17.6, t
2	1.62 (m), 2.01 (tt, 14.0, 4.0)	31.0, t
3	3.48 (t, 2.8)	76.9, d
4		42.6, s
5		77.3, s
6	1.33 (m), 1.72 (m)	33.5, t
7	1.37 (m), 1.68 (m)	27.8, t
8	1.47 (m)	37.4, d
9		39.8, s
10	1.85 (dd, 12.2, 1.8)	41.4, d
11	1.52 (m), 1.66 (m)	36.5, t
12	2.34 (br s)	22.5, t
13		173.3, s
14	5.88 (s)	117.4, d
15		173.8, s
16	6.04 (s)	101.4, d
17	0.82 (d, 6.4)	16.4, q
18	1.19 (s)	21.2, q
19	1.13 (s)	17.9, q
20	0.80 (s)	18.7, q

Table 2. Cytotoxicity of Compounds **3**−**5**

	IC_{50} (μM)[a]			
compound	AGS[b]	DLD1[c]	HA59T[d]	HepG2[e]
3	26.9	>30	23.6	>30
4	>30	>30	21.7	>30
5	8.6	28.9	16.4	20.8

[a] Mean concentrations for 50% growth inhibition (replicate values). Compounds showing values > 30 μM are considered inactive. [b] AGS (human gastric cancer cell). [c] DLD1 (human colon cancer cell). [d] HA59T (human hepatoma cell). [e] HepG2 (human hepatoma cell).

hydrogen-bonded with a C-8 carbonyl group. No NOE correlation between H-12 and a methoxyl group was observed, which indicated a methoxyl group was attached to C-10, rather than C-11. Therefore, the structure of **2** was determined as 2,8,10-trihydroxy-3,9-dimethoxy-5,6,12,12a, 6a-pentahydroisoquinolino-(2,1-b)-isoquinolin-7-one and assigned the trivial name (−)-8-oxopolyalthiaine.

Besides these two new compounds, 20 known compounds were isolated and identified by means of spectroscopic methods. Among them, 16-hydroxycleroda-3,13-dien-15-oic acid and 1-aza-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione were isolated for the first time as natural products.[2,22] Nine compounds, including 3,12*E*-kolavadien-15-oic acid-16-al,[1−6] (4→2)-*abeo*-16(*R* and *S*)-2,13*Z*-kolavadien-15,16-olide-3-al,[1−6] labd-13*E*-en-8-ol-15-oic acid,[7] 5-hydroxy-6-methoxonychine (**4**),[23,24] 6-hydroxy-7-methoxyonychine,[23,24] (−)-asimilobine,[25] (−)-norboldine,[27] (+)-norboldine,[27] and (−)-norpallidine,[28] were obtained from this plant for the first time.

In a previous study,[7] clerodane-type diterpenoids were reported to possess cytotoxic activity toward various human and murine cancer cell lines by Ma *et al.* In our present investigation, several compounds were evaluated for their cytotoxicity using a panel of human tumor cell lines (Table 2). The new compound **1** showed no cytotoxic effects on any of the cell lines tested. The major secondary metabolite obtained in the present investigation, compound **3** (16α-hydroxycleroda-3,13-dien-15,16-olide), displayed moderate cytotoxicity against two cell lines tested (AGS and HA59T). 5-Hydroxy-6-methoxonychine (**4**) and 6-hydroxy-7-methoxyonychine are two structurally similar compounds. With a methoxy group at C-6 and 5-hydroxy substitution, compound **4** was active against one cell line (HA59T). Unlike the other two aporphinoids tested, stepholidine and norboldine, which showed no cytotoxicity at up to 30 μM, anonaine (**5**) displayed significant cytotoxicity against four cancer cell lines tested, with AGS cells (human gastric cancer, IC_{50} = 8.6 μM) being the most susceptible.

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Devices Mel-Temp II and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 220-20 spectrophotometer in EtOH. CD spectra were determined on a JASCO Model J-720 spectropolarimeter. IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 and 200 MHz), ^{13}C NMR, NOESY, and DEPT spectra were obtained on a Varian NMR spectrometer. LRE-IMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC-MS spectrometer having a direct inlet system. HREIMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography, precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were employed for analytical TLC, and precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. Spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and then heated on a hot plate.

Plant Material. The leaves of *P. longifolia* var. *pendula* were collected from Kaohsiung, Taiwan, in January 1997. A voucher specimen was prepared by Dr. Hsin-Fu Yen and deposited in the Graduate Institute of Natural Products (voucher number: Annona 16), Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The leaves of *P. longifolia* var. *pendula* (9 kg) were extracted with MeOH (10 L × 6) at room temperature for 24−48 h. The combined MeOH extracts were evaporated under reduced pressure to yield a dark-brown syrup (266.7 g). This syrup was partitioned between $CHCl_3$ and water, with the $CHCl_3$ partition extracted with 3% HCl. The remaining $CHCl_3$ solution was evaporated to give a green viscous residue (part A) (260 g). The acidic aqueous layer was alkalinized with NH_4OH and extracted once more with $CHCl_3$. The $CHCl_3$ layer was concentrated to yield part B (2.0 g). Part B gave a positive alkaloidal test with Dragendorff's reagent. The crude alkaloid portion (part B) was chromatographed over Si gel using $CHCl_3$/MeOH as eluent to obtain 11 fractions. Fraction 2 was eluted with *n*-hexane−$CHCl_3$ (1:1 and 1:5) by column chromatography to afford **4** (3.0 mg) (*n*-hexane−$CHCl_3$−MeOH, 5:5:1, R_f 0.32) and **5** (7.0 mg) ($CHCl_3$−MeOH, 10:1, R_f 0.45). 6-Hydroxy-7-methoxyonychine (2.5 mg) ($CHCl_3$−MeOH, 49:1, R_f 0.44), liriodenine (6.6 mg) ($CHCl_3$−MeOH, 49:1, R_f 0.47), and (−)-asimilobine (9.0 mg) ($CHCl_3$−MeOH, 10:1, R_f 0.30) were isolated from fraction 3 by Si gel chromatography using $CHCl_3$−MeOH, 50:1, 20:1, and 5:1, as eluting solvent systems, respectively. Fraction 4 was purified by Si gel chromatography ($CHCl_3$−MeOH, 10:1) and then recrystallized from $CHCl_3$ to obtain oxoxylopine (5.2 mg) ($CHCl_3$−MeOH, 10:0.5, R_f 0.45). Fraction 5 was separated by preparative TLC (*n*-hexane−$CHCl_3$, 4:17) to afford (−)-stepholidine (2.3 mg). (−)-Norpallidine (3.0 mg) ($CHCl_3$−MeOH, 6:1, R_f = 0.21) was isolated from fraction 6, which was eluted with $CHCl_3$−MeOH (10:1) by Si gel column chromatography. 1-Aza-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione (3.0 mg) was isolated from fraction 7 by Si gel column using $CHCl_3$−MeOH (9:1) as solvent system. A racemic mixture of (−)-norboldine and (+)-norboldine (7.0 mg) ($CHCl_3$−MeOH, 5:1, R_f 0.24) was isolated from fraction 9 by passage over a Si gel column using $CHCl_3$−MeOH (5:0.8) for elution.

Part A was concentrated and chromatographed over Si gel using gradient mixtures of *n*-hexane−$CHCl_3$−MeOH as eluents to produce 16 fractions. Fraction 11 was chromatographed over Si gel eluting with a gradient of *n*-hexane−EtOAc (3:1) and recrystallized from MeOH to obtain a mixture of β-sitosterol and stigmasterol (550 mg) (*n*-hexane−EtOAc, 1:1, R_f 0.32). Compound **1** (51.7 mg) (*n*-hexane−EtOAc, 1:1, R_f 0.32)

and cleroda-3,13*E*-dien-15-oic acid (2.8 mg) ($CHCl_3$−MeOH, 99:1, R_f 0.32) were obtained from fraction 11 on elution with *n*-hexane−EtOAc (1:1). Fraction 12, eluted with *n*-hexane−EtOAc (7:1), was further separated and purified by Si gel column chromatography to afford **3** (12.2 g, the major component) (*n*-hexane-acetone, 9:1, R_f 0.25). Further purification from the fraction 12 by chromatography on preparative TLC yielded an alkaloid, **2** (2.0 mg) (*n*-hexane−EtOAc, 3:2, R_f 0.31). 3,12*E*-Kolavadien-15-oic acid-16-al (18.6 mg) (*n*-hexane-acetone, 6:1, R_f 0.56) and (4→2)-*abeo*-16(*R* and *S*)-2,13*Z*-kolavadien-15,16-olide-3-al (36.9 mg) (*n*-hexane−EtOAc, 8:5, R_f 0.38) were isolated from fraction 13 using *n*-hexane−EtOAc (8:1 and 5:1, respectively) as the eluting solvent system. Fraction 14 was eluted with a gradient mixtures of $CHCl_3$−MeOH by Si gel column chromatography to obtain 16-hydroxycleroda-3,13-dien-15-oic acid (15.4 mg) ($CHCl_3$−acetone, 10:1, R_f 0.47) and colorless needles of *p*-hydroxybenzoic acid (10.0 mg) (*n*-hexane−EtOAc, 50:1, R_f 0.58). This fraction was eluted with *n*-hexane−EtOAc (7:1) and was used for the purification of labd-13*E*-en-8-ol-15-oic acid (20.5 mg) (*n*-hexane−EtOAc, 5:1, R_f 0.58).

3*β*,5*β*,16α-Trihydroxyhalima-13(14)-en-16,15-olide (1): white amorphous powder; mp 100−102 °C; $[\alpha]_D$ −26.1° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.3) nm; CD (MeOH, 1×10^{-5} M) ($\Delta\epsilon$) 246 (12.96), 231 (−6.72), 220 (26.39), 200 (−18.60) nm; IR (KBr) ν_{max} 3415, 2957, 1750, 1680, 1656 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* $[M]^+$ 352.2245 ($C_{20}H_{32}O_5$, calcd 352.2250); EIMS *m*/*z* 352 $[M]^+$ (3), 334 (6), 317 (2), 299 (2), 205 (15), 191 (5), 163 (23), 135 (24), 121 (29), 107 (38), 95 (49), 67 (47), 55 (77), 43 (100).

(−)-8-Oxopolyalthiaine (2): yellow amorphous powder; mp 95−97 °C; $[\alpha]_D$ −264.8° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (4.3), 270 (3.9), 353 (3.5) nm; IR (neat) ν_{max} 3503, 1635, 1618, 1580, 1512 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N) δ 2.64 (1H, ddd, J = 13.0, 3.0, 2.0 Hz, H-5α, pseudoeq.), 2.81 (1H, dd, J = 15.6, 13.6 Hz, H-13*β*), 2.86 (1H, ddd, J = 13.0, 11.0, 2.6 Hz, H-5*β*, pseudoax.), 2.90 (1H, ddd, J = 12.5, 11.0, 2.0 Hz, H-6α, pseudoax.), 3.25 (1H, dd, J = 15.6, 4.0 Hz, H-13α), 3.78 (3H, s, OCH_3-3), 3.99 (3H, s, OCH_3-10), 4.77 (1H, dd, J = 13.6, 4.0 Hz, H-14), 4.98 (1H, ddd, J = 12.5, 3.0, 2.6 Hz, H-6*β*, pseudoeq.), 6.62 (1H, s, H-12), 6.78 (1H, s, H-4), 7.12 (1H, s, H-1), 13.97 (OH-9); ^{13}C NMR (100 MHz, C_5D_5N) δ 29.5 (C-5), 37.1 (C-6), 38.6 (C-13), 55.1 (C-14), 55.9 (OCH_3-3), 60.2 (OCH_3-10), 106 (C-12), 112.2 (C-4), 113.1 (C-1), 124.0 (C-8a), 125.4 (C-12a), 128.5 (C-4a), 134.5 (C-14a), 134.9 (C-10), 147.0 (C-2), 147.7 (C-3), 156.3 (C-11), 156.9 (C-9), 169.1 (C-8); HREIMS *m*/*z* $[M]^+$ 357.1213 ($C_{19}H_{19}O_6N$, calcd 357.1212); EIMS *m*/*z* 357 $[M]^+$ (32), 180 (8), 178 (85), 176 (8), 97 (48), 85 (65), 71 (83), 57 (100).

Cytotoxicity Assay. The cytotoxicity of **1**, **3**−**5**, 6-hydroxy-7-methoxyonychine, stepholidine, and norboldine was tested against four human cancer cell lines: AGS (gastric cancer cells), DLD1 (colon cancer cells), HepG2 (hepatoma cells), and HA59T (hepatoma cells). Cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal calf serum (Gibco). Cultures were established in 96-well plates for 24 h. After 72 h exposure to the test compounds, cell viability was determined using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt] colorimetric assay, according to a previously described procedure.[34]

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China.

References and Notes

(1) Zhao, G.; Jung, J. H.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Planta Med.* **1991**, *57*, 380−383.
(2) Phadnis, A. P.; Patwardhan, S. A.; Dhaneshwar, N. N.; Tavale, S. S.; Tayur, N. G. *Phytochemistry* **1988**, *27*, 2899−2901.
(3) Hara, N.; Asaki, H.; Fujimoto, Y.; Gupta, Y. K.; Singh, A. K.; Sahai, M. *Phytochemistry* **1995**, *38*, 189−194.
(4) Kijjoa, A.; Pinto, M. M. M.; Pinho, P. M. M.; Herz, W. *Phytochemistry* **1988**, *32*, 457−460.
(5) Kijjoa, A.; Pinto, M. M. M.; Pinho, P. M. M.; Tantisewie, B.; Herz, W. *Phytochemistry* **1991**, *29*, 653−655.
(6) Hao, X. J.; Yang, X. S.; Zhang, Z.; Shang, L. J. *Phytochemistry* **1985**, *39*, 447−448.
(7) Ma, X.; Lee, I. S.; Chai, H. B.; Zaw, K.; Farnsworth, N. R.; Soejarto, D. D.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn A. D. *Phytochemistry* **1994**, *37*, 1659−1662.
(8) Richomme, P.; Godet, M. C.; Foussard, F.; Toupet, L.; Sevenet, T.; Bruneton, J. *Planta Med.* **1991**, *57*, 552−554.
(9) Goyal, M. M.; Gupta, A. *Indian Drugs* **1985**, *22*, 658.
(10) Li, H. Y.; Sun, N. J.; Kashiwada, Y.; Sun, L.; Snider, J. V.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **1993**, *56*, 1130−1133.
(11) Jossang, A.; Leboeuf, M.; Cavé, A.; Sevenet, T.; Padmawinata, K. *J. Nat. Prod.* **1984**, *47*, 504−513.
(12) Cavé, A.; Guinaudeau, H.; Ramahatra, A.; Razafindrazaka, J. *Planta Med.* **1978**, *33*, 243−250.
(13) Connolly, J. D.; Haque, E.; Kadir, A. A. *Phytochemistry* **1996**, *43*, 295−297.
(14) Hocqemiller, R.; Dubois, G.; Leboeuf, M.; Cavé, A.; Kunesch, N.; Riche, C.; Chiaroni, A. *Terahedron Lett.* **1981**, *22*, 5057−5060.
(15) Kunesch, N.; Cavé, A.; Leboeuf, M.; Hocquemiller, R.; Dubois, G.; Guittet, E.; Lallemand, J. Y. *Terahedron Lett.* **1985**, *26*, 4937−4940.
(16) Jossang, A.; Leboeuf, M.; Cabalion, P.; Cavé, A. *Planta Med.* **1983**, *49*, 20−24.
(17) Chakrabarty, M.; Patra, A. *Indian J. Chem. Sect. B* **1990**, 29B, 394−395.
(18) Lavault, M.; Guinaudeau, H.; Bruneton, J.; Sevenet, T.; Hadi, H. A. *Phytochemistry* **1990**, *29*, 3845−3847.
(19) Wu, Y. C. *Heterocycles* **1989**, *29*, 463−475.
(20) Wu, Y. C.; Duh, C. Y.; Wang, S. K.; Chen, K. S.; Yang, T. H. *J. Nat. Prod.* **1990**, *53*, 1327−1331.
(21) Lange, G. L.; Lee, M. *J. Nat. Prod.* **1986**, *49*, 253−258.
(22) Goulart, M. O. F.; Santana, A. E. G.; Oliveira, A. B. D.; Maia, J. G. S. *Phytochemistry* **1986**, *25*, 1691−1695.
(23) Pérez, J. M.; Vidal, L.; Grande, M. T.; Menéndez, J. C.; Avendaño, C. *Tetrahedron* **1994**, *50*, 7923−7932.
(24) Zhang, J.; El-Shabrawy, A. R. O.; El-Shabrawy, M. A.; Schiff, P. L.; Slatkin, D. J. *J. Nat. Prod.* **1987**, *50*, 800−806.
(25) Bou-Abdallah, É.; Jossang, A.; Tadic, D.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1989**, *52*, 273−278.
(26) Hsieh, T. J.; Chang, F. R.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 607−611.
(27) Talapatra, S. K.; Patra, A.; Talapatra, B. *Chem. Ind.* **1969**, 1056−1057
(28) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Aust. J. Chem.* **1968**, *21*, 1383−1386.
(29) Tokumura, A.; Handa, Y.; Yoshioka, Y.; Higashimoto, M.; Tsukatani, H. *Chem. Pharm. Bull.* **1982**, *30*, 2119−2126.
(30) Sakushima, A.; Coskun, M.; Maoka, T. *Phytochemistry* **1995**, *40*, 257−261.
(31) Gawronski, J. K.; van Oeveren, A.; van der Deen, H.; Leung, C. W.; Feringa, B. L. *J. Org. Chem.* **1982**, *30*, 2119−2126.
(32) Pinho, P. M. M.; Pinto, M. M. M.; Kijjoa, A.; Pharadai, K.; Díaz, J. G.; Herz, W. *Phytochemistry* **1992**, *31*, 1403−1407.
(33) Malhotra, S.; Taneja, S. C.; Dhar, K. L. *Phytochemistry* **1989**, *28*, 1998−1999.
(34) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207−212.

NP000176E