Constituents of *Polyalthia longifolia* var. *pendula*

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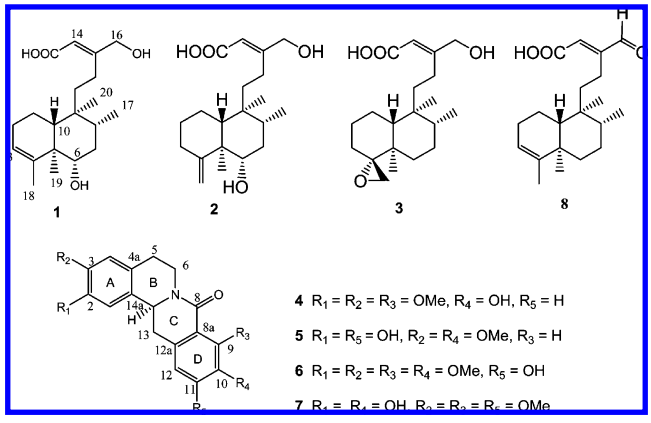
Three new clerodane-type diterpenes, 6α,16-dihydroxycleroda-3,13-dien-15-oic acid (**1**), 6α,16-dihydroxycleroda-4(18),13-dien-15-oic acid (**2**), and 4α,18β-epoxy-16-hydroxyclerod-13-en-15-oic acid (**3**), and four new protoberberine alkaloids, (−)-8-oxo-10-hydroxy-2,3,9-trimethoxyberberine (**4**), (−)-8-oxo-2,11-dihydroxy-3,10-dimethoxyberberine (**5**), (−)-8-oxo-11-hydroxy-2,3,9,10-tetramethoxyberberine (**6**), and (−)-8-oxo-2,10-dihydroxy-3,9,11-trimethoxyberberine (**7**), together with 11 known substances, were isolated from a methanol extract of the stems of *Polyalthia longifolia* var. *pendula*. The structures of **1**−**7** were elucidated on the basis of spectroscopic data analysis. Compounds were evaluated for their antiproliferative activities against A549 and MCF-7 cancer cells, and among the substances tested, only 16-oxo-cleroda-3,13-dien-15-oic acid (**8**) exhibited cytotoxicity.

Polyalthia longifolia Benth. & Hook. f. var. *pendula* (Annonaceae) is cultivated widely throughout tropical and subtropical Asia as an ornamental and has had use as a folk medicine for the treatment of pyrexia and as an anthelmintic and germicide.[1] Previous studies on its leaves,[2] bark,[3] roots,[4] root bark,[5] and seeds[6] have revealed various types of diterpenoids and alkaloids with numerous biological activities such as anti-inflammatory, antihypertensive, antimicrobial, and cytotoxic effects. In Taiwan, *P. longifolia* var. *pendula* is an alien species, but is cultivated commonly as a landscape tree, rather than for any medicinal uses. In an attempt to survey possible novel bioactive agents from this plant, its stems were thus chosen for a phytochemical investigation, which afforded three new clerodane-type diterpenes (**1**−**3**) and four new protoberberine alkaloids (**4**−**7**), accompanied by 11 known compounds. This paper deals with structural characterizations of these new compounds and their cytotoxicity toward two cancer cell lines.



Results and Discussion

A methanol extract of the stems of *P. longifolia* var. *pendula* was partitioned in a preliminary manner to give a medium-polarity layer. Gravity column separation of this layer over silica gel followed by HPLC purification afforded three new diterpenes (**1**−**3**) and four new alkaloids (**4**−**7**) along with 11 known compounds. The known compounds were classified as two clerodane-type diterpenes, 16-oxocleroda-3,13-dien-15-oic acid (**8**)[7] and 2-oxo-3,13*E*-clerodien-15-oic acid;[8] three protoberberine alkaloids, (−)-8-oxotetrahydroplamatine,[9] (−)-8-oxo-9,10-dihydroxy-2,3-dimethoxyberberine,[10] and (−)-8-oxo-11-hydroxy-2,3,9-trimethoxyberberine;[11] an azafluorene alkaloid, darienine;[12] an amide, *N-trans*-feruloyldopamine;[13] two fatty acids, 1-*O*-(9*Z*-octadecadienoyl)glycerol[14] and 1-*O*-(9*Z*,12*Z*-octadecadienoyl)-glycerol;[14] and a phytosterol glycoside mixture containing β-sitosterol 3β-D-glucopyranoside and stigmasterol 3β-D-glucopyranoside (2:1).[15]

Compound **1** was obtained as a yellow oil, and its IR absorptions at 3414, 1689, and 1653 cm^{-1} indicated the presence of a hydroxy group, an α,β-unsaturated carboxylic acid functionality, and a double bond, respectively. Twenty carbon resonances, attributed to five quaternary carbons, five methines, six methylenes, and four methyls, were observed in the ^{13}C NMR spectrum coupled with the DEPT spectrum of **1** (Table 1). On account of the molecular formula, $C_{20}H_{32}O_4$, as assigned by HREIMS, the index of hydrogen deficiency (IHD) of **1** was determined as five, including one α,β-unsaturated carboxylic acid unit and one olefinic functionality, as supported by two low-field pairs of carbon signals at δ_C 122.9 (C-3)/145.3 (C-4) and δ_C 164.2 (C-13)/114.3 (C-14), and a carbonyl signal at δ_C 169.8 (C-15). Thus, a two-ring moiety remained. The above assignments were characteristic for a clerodane-type diterpene skeleton.[16] The planar structure of **1** was deduced by COSY (H-1/H-2; H-2/H-3; H-6/H_2-7; H_2-7/H-8; H-8/H_3-17; H-10/H-1; H_2-11/H_2-12) and key HMBC (H_3-20/C-8, -9, -10, and -11; H_3-19/C-4, -5, and -6; H_3-18/C-3, -4, and -5; H-14/C-12, -13, -15, and -16) correlations. The relative configuration of the decalin system of rings A and B was determined to be *trans*, as evidenced from the ^{13}C NMR chemical shifts of C-19 (δ_C 15.7) and C-20 (δ_C 18.2), when compared with literature values.[3] NOESY correlations, including H_3-19/H_3-20, H_3-20/H_3-17, H-14/H_2-16, H-6/H-10, H-10/H_2-12, and H-8/H_2-12, were used to establish the relative configuration of **1**, as shown in Figure 1. Accordingly, the structure of **1** was assigned as 6α,16-dihydroxycleroda-3,13-dien-15-oic acid.

The molecular formula ($C_{20}H_{32}O_4$) and UV spectrum (λ_{max} 216 nm) of **2**, along with its IR absorption bands at 3451, 1691, and 1641 cm^{-1}, were similar to those of its analogue, **1**. When the ^1H and ^{13}C NMR spectra of **2** were compared with those of **1** (Table 1), major differences involved a methylene group and an exomethylene functionality [H_2-3 at δ_H 2.01 (m) and 2.24 (td, J = 13.2, 4.6 Hz)] in **2**, instead of a signal at δ_H 5.17 (s) in **1**, and signals for

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **1**−**3** [δ in ppm, mult. (J in Hz)]

	1[a]		**2**[b]		**3**[c]	
position	^{13}C[d]	^{1}H	^{13}C[d]	^{1}H	^{13}C[d]	^{1}H
1	18.8 t	1.76 m	37.6 t	1.33 m 1.46 m	25.1 t	1.92 m
2	27.7 t	2.01 m	21.9 t	1.83 m	26.8 t	1.40 m
3	122.9 d	5.17 s	34.2 t	2.01 m 2.24 td (13.2, 4.6)	31.8 t	1.02 m
4	145.3 s		157.8 s		66.9 s	
5	45.1 s		47.0 s		38.0 s	
6	76.4 d	3.44 dd (7.1, 8.8)	74.2 d	3.81 dd (7.9, 9.6)	36.7t	1.50 m
7	38.8 t	1.54 m	37.4 t	1.57 m	23.4 t	2.30 m 2.50 m
8	35.7 d	1.68 m	35.2 d	1.69 m	36.9 d	1.37 m
9	39.8 s		40.1 s		39.5 s	
10	46.9 d	1.29 m	49.1 d	1.15 dd (2.6, 7.5)	47.8 d	1.27 m
11	38.2 t	1.35 m	37.6 t	1.32 m 1.47 m	32.3 t	1.38 m
12	23.9 t	2.33 m	23.4 t	2.33 m	21.0 t	2.13 m
13	164.2 s		164.7 s		165.3 s	
14	114.3 d	5.89 brs	113.3 d	5.96 brs	112.4 t	6.00 brs
15	169.8 s		167.6 s		170.5 s	
16	65.8 t	4.06 brs	65.4 t	4.10 brs	65.8 t	4.20 brs
17	16.1 q	0.86 d (6.7)	16.1 q	0.85 d (6.6)	15.9 q	0.83 d (6.4)
18	22.8 q	1.78 s	105.0 t	4.58 d (1.2) 4.89 d (1.2)	52.9 t	2.40 d (4.4) 3.08 dd (4.4, 2.0)
19	15.7 q	1.00 s	15.3 q	1.05 s	17.9 q	1.13 s
20	18.2 q	0.69 s	18.0 q	0.70 s	18.0 q	0.72 s

[a] Measured in methanol-d_4. [b] Measured in acetone-d_6. [c] Measured in $CDCl_3$. [d] Multiplicities were obtained from DEPT experiments.

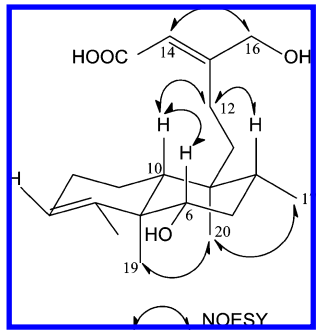

Figure 1. Key NOESY correlations of **1**.

H_2-18 at δ_H 4.58 (d, J = 1.2 Hz) and 4.89 (d, J = 1.2 Hz) in **2** instead of δ_H 1.78 (s) in **1**. These data revealed that the double bond at C-3 in **1** was translocated to C-4 in **2**. The relative configurations of the asymmetric carbons C-5, -6, -8, -9, and -10 in **2** were deduced to be the same as those of **1** from the assignments of the cross-peaks in the NOESY spectrum. After considering all the spectroscopic data, the structure of **2** was thus determined to be 6α,16-dihydroxycleroda-4(18),13-dien-15-oic acid.

Compound **3** was also assigned as a clerodane-type diterpene from its spectroscopic data. Its ^{1}H NMR data were comparable with those of **2** except that H_2-18 was incorporated in an epoxy group [δ_H 2.40 (d, J = 4.4 Hz) and 3.08 (dd, J = 4.4, 2.0 Hz)], and the nearby methylene group at H_2-3 shifted to δ_H 1.02 (m). This was reflected in its ^{13}C NMR spectrum, in which chemical shifts of C-3, C-4, and C-18 resonated at δ_C 31.8, 66.9, and 52.9, respectively. The orientation of the epoxy methylene H_2-18 borne at C-4 was deduced to be β, when the chemical shifts and coupling constants of **3** were compared with those of similar structures in the literature.[17] Accordingly, **3** was assigned as 4α,18β-epoxy-16-hydroxyclerod-13-en-15-oic acid.

Compound **4** was isolated as a brown, amorphous powder, and the UV absorption maxima at 227 and 255 nm and IR absorptions at 3431 (hydroxy), 1639 (lactam), 1591 (aromatic), and 1513 (aromatic) cm^{-1} indicated this compound to be an oxoprotoberberine-type alkaloid.[18] An important feature for this oxoprotoberberine alkaloid in the ^{1}H NMR spectrum was a downfield-shifted proton at δ_H 5.02 (H-6$_{quasi-eq}$), caused by the deshielding effect of the amide and the anisotropic effect of the C-8 carbonyl group, whereas H-6$_{quasi-ax}$ appeared at δ_H 2.94. The negative optical rotation ($[\alpha]^{27}_D$ −26.9) as well as a typical ^{1}H NMR signal at δ_H 4.77 (1H, dd, J = 13.3, 3.1 Hz, H-14) revealed that **4** adopts a 14*S*-configuration (α-orientation).[19] An AB coupling system [δ_H 6.90 (d, J = 8.1 Hz, H-12) and 7.08 (d, J = 8.1 Hz, H-11)] and two singlet protons at δ_H 6.69 (s, H-1 and -4) indicated four aromatic substitutions on rings A and D of the oxoprotoberberine nucleus of **4**. These substitutions were determined to be three methoxy groups at δ_H 3.90 (OMe-2), 3.90 (OMe-3), and 4.01 (OMe-9) and a hydroxy group, from the ^{1}H NMR spectrum in combination with the ^{13}C NMR data. The detailed NMR assignments for **4** (Table 2) were established by further 2D NMR experiments. Since the relative configuration of H-14 was confirmed, NOESY correlations assisted in the determination of the orientations of H_2-5, H_2-6, and H_2-13 as well as the positions of all the substituents (Figure 2). Therefore, **4** was characterized as (−)-8-oxo-10-hydroxy-2,3,9-trimethoxyberberine.

The physical and spectroscopic characteristics of **5**−**7** were closely comparable to those of **4**. On comparing the ^{1}H and ^{13}C NMR spectra of **5**−**7** with those of **4**, the differences involved were only the number of methoxy groups attached to the A and D rings. Compound **5** has two methoxy groups (δ_H 3.80, 3.78) attached to C-3 and C-10, which were deduced by HMBC and NOESY experiments. Compound **6** has one additional methoxy (δ_H 3.97) attached at C-10 as compared with **4**, which was also confirmed by 2D NMR assignments. The three methoxy functionalities at δ_H 3.89, 4.00, and 3.96 evident from the ^{1}H NMR spectrum of **7** were determined to be located at C-3, C-9, and C-11, respectively, as interpreted from the HMBC experiment. The elemental formulas of **5**−**7** were determined by their HRMS data. Accordingly, **5**−**7** were assigned as (−)-8-oxo-2,11-dihydroxy-3,10-dimethoxyberberine, (−)-8-oxo-11-hydroxy-2,3,9,10-tetramethoxyberberine, and (−)-8-oxo-2,10-dihydroxy-3,9,11-trimethoxyberberine, respectively.

Altogether, 15 of the compounds isolated were evaluated for cytotoxicity against MCF-7 (human breast carcinoma) and A549 (non-small cell lung cancer) cells with cell viabilities assessed using a MTT assay. Among the compounds tested, only 16-oxo-cleroda-3,13-dien-15-oic acid (**8**) was cytotoxic against both MCF-7 and

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **4**−**7** [δ in ppm, mult. (J in Hz)]

	4[a]		**5**[b]		**6**[a]		**7**[a]	
position	^{13}C[c]	^{1}H	^{13}C[c]	^{1}H	^{13}C[c]	^{1}H	^{13}C[c]	^{1}H
1	111.4 d	6.69 s	114.2 d	7.17 s	111.5 d	6.66 s	108.9 d	6.66 s
2	148.0 s		147.1 s		148.1 s		144.5 s	
3	148.1 s		147.7 s		148.2 s		145.5 s	
4	109.1 d	6.69 s	112.4 d	6.78 s	108.8 d	6.69 s	111.8 d	6.61 s
4a	127.6 s		129.5 s		127.5 s		126.8 s	
5α	29.4 t	2.76 ddd (13.0, 3.0, 2.0)	30.0 t	2.68 brd (14.9)	29.4 t	2.76 m	29.5 t	2.74 m
5β		2.92 m		2.90 ddd (12.3, 2.4, 2.0)		2.92 m		2.90 m
6α	38.3 t	2.94 m	39.5 t	2.97 ddd (12.3, 2.4, 2.0)	38.1 t	2.93 m	38.1 t	2.92 m
6β		5.02 m		5.20 m		5.00 m		4.96 m
8	162.5 s		165.3 s		162.7 s		162.7 s	
8a	121.5 s		121.2 s		111.5 s		111.8 s	
9	147.4 s		112.2 d	8.07 s	154.3 s		154.3 d	
10	149.0 s		151.9 s		139.8 s		139.8 s	
11	118.1 d	7.08 d (8.1)	147.7 s		152.2 s		152.0 s	
12	122.8 d	6.90 d (8.1)	115.0 d	7.06 s	109.1 d	6.64 s	110.7 d	6.76 s
12a	130.6 s		132.7 s		135.6 s		135.7 s	
13α	39.1 t	3.05 dd (15.4, 3.1)	37.5 t	3.23 dd (15.3, 3.4)	39.5 t	3.01 brd (13.4)	39.4 t	2.98 dd (15.5, 2.9)
13β		2.84 dd (15.4, 13.3)		2.86 dd (15.3, 14.3)		2.84 dd (13.4, 13.4)		
14	55.1 d	4.77 dd (13.3, 3.1)	55.6 d	4.80 dd (14.3, 3.4)	54.7 d	4.73 brd (13.4)	54.4 d	4.70 dd (13.4, 2.4)
14a	127.4 s		126.0 s		127.5 s		128.4 s	
2-OMe	56.2 q	3.90 s			56.0 q	3.89 s		
3-OMe	55.9 q	3.90 s	56.1 q	3.80 s	56.2 q	3.89 s	56.0 q	3.89 s
9-OMe	62.4 q	4.01 s			61.6 q	4.10 s	61.2 q	4.00 s
10-OMe			56.1q	3.78	61.2 q	3.97 s		
11-OMe							61.6 q	3.96 s

[a] Measured in $CDCl_3$. [b] Measured in pyridine-d_5. [c] Multiplicities were obtained from DEPT experiments.

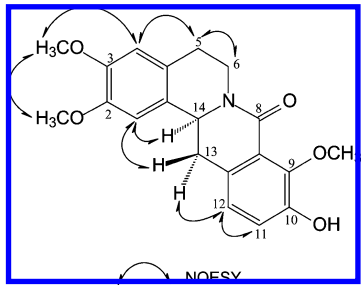

Figure 2. Key NOESY correlations of **4**.

A549 cell lines, with IC_{50} values of 3.7 ± 0.2 and 3.1 ± 0.3 μM, respectively. Under the same conditions, the IC_{50} values of the corresponding positive controls, paclitaxel and doxorubicin, were 0.0020 ± 0.0001 and 0.837 ± 0.034 μM, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 polarimeter. UV spectra were recorded with a Thermo UV−visible Helios α spectrometer. IR spectra were taken on a JASCO FT/IR 4100 IR spectrometer. NMR spectra were acquired on a Bruker DRX-500 NMR spectrometer. LREIMS were recorded on a Finnigan TSQ-46C spectrometer, with HREIMS and HRESIMS measured on Finnigan TSQ-95S and Shimadzu LCMS-IT-TOF mass spectrometers, respectively. Silica gel 60 (70-230 mesh, Merck) was used for open column chromatography. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). HPLC was performed using a silica gel column (Luna 5 μm silica gel, 10 mm i.d. × 250 mm, Phenomenex; detector, RI).

Plant Material. The fresh stems of *Polyalthia longifolia* var. *pendula* were collected in Niaosong Township, Kaohsiung County, Taiwan, in January 2007, and were identified by Dr. Fang-Rong Chang of the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan. A voucher specimen (LCK9602) is deposited at the College of Pharmacy, Taipei Medical University, Taipei, Taiwan.

Extraction and Isolation. The air-dried stems of *P. longifolia* var. *pendula* (2.0 kg) were extracted with MeOH (15 L × 2) at room temperature. Evaporation of the organic solvent under reduced pressure gave a crude extract (108.3 g). The crude MeOH extract was dissolved in MeOH−H_2O (9:1), then partitioned with *n*-hexane to afford an *n*-hexane layer (16.6 g) and an aqueous MeOH layer (45.2 g). The latter layer was absorbed on 68 g of silica gel and then subjected to column chromatography on silica gel 60 (230−400 mesh, 600 g), using mixtures of *n*-hexane, EtOAc, acetone, and MeOH as eluents, in a stepwise gradient mode, to afford four fractions, Fr. I [eluted with *n*-hexane−EtOAc (2:3)], Fr. II (eluted with EtOAc), Fr. III (eluted with acetone), and Fr. IV (eluted with MeOH). Repeated chromatography of Fr. I with *n*-hexane−EtOAc−acetone (12:5:1) as eluent and purification of each compound was carried out by using HPLC on a silica gel column (*n*-hexane−EtOAc−acetone, 12:2.5:1) to afford 16-oxo-cleroda-3,13-dien-15-oic acid (153.9 mg, t_R = 12.7 min), 2-oxo-3,13*E*-clerodien-15-oic acid (21.0 mg, t_R = 5.5 min), darienine (35.6 mg, t_R = 10.4 min), 1-*O*-(9*Z*-octadecadienoyl)glycerol (71.2 mg, t_R = 8.1 min), and 1-*O*-(9*Z*,12*Z*-octadecadienoyl)glycerol (50.3 mg, t_R = 9.2 min). Fr. II was chromatographed on a silica gel 60 column (230−400 mesh) with *n*-hexane−EtOAc−acetone (2:1:0.2), followed by HPLC on a silica gel column (*n*-hexane−EtOAc−acetone, 2:0.3:0.2) to obtain **1** (62.0 mg, t_R = 8.1 min), **2** (2.8 mg, t_R = 9.0 min), and **3** (3.4 mg, t_R = 6.3 min). Fr. III was subjected to a silica gel 60 column chromatography (230−400 mesh) with *n*-hexane−EtOAc−$CHCl_3$ (1:2:0.3), followed by HPLC on a silica gel column (*n*-hexane−EtOAc−CH_2Cl_2, 1:1:0.4, and *n*-hexane−EtOAc−$CHCl_3$, 1:1:0.4), to give **4** (7.6 mg, t_R = 9.3 min), **5** (6.5 mg, t_R = 18.5 min), **6** (2.3 mg, t_R = 7.4 min), **7** (4.9 mg, t_R = 14.1 min), (−)-8-oxo-tetrahydroplamatine (4.0 mg, t_R = 4.5 min), (−)-8-oxo-9,10-dihydroxy-2,3-dimethoxyberberine (10.0 mg, t_R = 13.0 min), (−)-8-oxo-11-hydroxy-2,3,9-trimethoxyberberine (2.9 mg, t_R = 5.9 min), and *N-trans*-feruloyldopamine (2.8 mg, t_R = 3.2 min). Fr. IV was recrystallized using acetone to obtain a cocrystal of β-sitosterol 3β-D-glucopyranoside and stigmasterol 3β-D-glucopyranoside (65.0 mg).

6α,16-Dihydroxycleroda-3,13-dien-15-oic acid (1): yellow oil; $[\alpha]^{26}_D$ +1.6 (*c* 1.0, MeOH); IR (neat) λ_{max} 3414, 1689, 1653, 1451 cm^{-1}; UV λ_{max} (MeOH) (log ε) 219 (4.5) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS $[M - H_2O]^+$ *m/z* 318, 303; HREIMS $[M]^+$ *m/z* 336.2309 (calcd for $C_{20}H_{32}O_4$, 336.2301).

6α,16-Dihydroxycleroda-4(18),13-dien-15-oic acid (2): yellow oil; $[\alpha]^{27}_D$ +7.7 (*c* 1.0, MeOH); IR (neat) λ_{max} 3451, 1691, 1641 cm^{-1}; UV λ_{max} (MeOH) (log ε) 216 (4.7) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS $[M - H_2O]^+$ *m/z* 318, 303, 285; HREIMS $[M]^+$ *m/z* 336.2305 (calcd for $C_{20}H_{32}O_4$, 336.2301).

4α,18β-Epoxy-16-hydroxyclerod-13-en-15-oic acid (3): yellow oil; $[\alpha]^{28}_D$ −2.6 (*c* 1.0, $CHCl_3$); IR (neat) λ_{max} 3415, 1689, 1451, 1262 cm^{-1}; UV λ_{max} (MeOH) (log ε) 216 (4.6) nm; ^{1}H and ^{13}C NMR data,

see Table 1; EIMS $[M - H_2O]^+$ *m/z* 318; HREIMS $[M]^+$ *m/z* 336.2302 (calcd for $C_{20}H_{32}O_4$, 336.2301).

(−)-8-Oxo-10-hydroxy-2,3,9-trimethoxyberberine (4): brown powder; $[\alpha]^{27}_D$ −26.9 (*c* 1.0, MeOH); IR (neat) λ_{max} 3431, 1639, 1591, 1513 cm^{-1}; UV λ_{max} (MeOH) (log ε) 227 (4.9), 255 (4.5) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS $[M]^+$ *m/z* 355, 340, 324; HREIMS $[M]^+$ *m/z* 355.1426 (calcd for $C_{20}H_{21}NO_5$, 355.1420).

(−)-8-Oxo-2,11-dihydroxy-3,10-dimethoxyberberine (5): yellow powder; $[\alpha]^{27}_D$ −12.8 (*c* 1.0, MeOH); IR (neat) λ_{max} 3427, 1635, 1512 cm^{-1}; UV λ_{max} (MeOH) (log ε) 222 (4.9), 266 (4.6) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS $[M]^+$ *m/z* 341; HREIMS $[M]^+$ *m/z* 341.1256 (calcd for $C_{18}H_{17}NO_5$, 341.1263).

(−)-8-Oxo-11-hydroxy-2,3,9,10-tetramethoxyberberine (6): brown powder; $[\alpha]^{28}_D$ −5.1 (*c* 1.0, MeOH); IR (neat) λ_{max} 3448, 1635, 1606, 1512 cm^{-1}; UV λ_{max} (MeOH) (log ε) 220 (4.9), 274 (3.1) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS $[M]^+$ *m/z* 385; HREIMS $[M]^+$ *m/z* 385.1520 (calcd for $C_{20}H_{21}NO_5$, 385.1525).

(−)-8-Oxo-2,10-dihydroxy-3,9,11-trimethoxyberberine (7): brown powder; $[\alpha]^{27}_D$ −10.7 (*c* 1.0, MeOH); IR (neat) λ_{max} 3395, 1627, 1603, 1515 cm^{-1}; UV λ_{max} (MeOH) (log ε) 224 (4.8), 268 (4.1) nm; ^1H and ^{13}C NMR data, see Table 2; ESIMS $[M + H]^+$ *m/z* 372; HRESIMS $[M + H]^+$ *m/z* 372.1440 (calcd for $C_{20}H_{21}NO_5$, 372.1431).

Cytotoxicity Assay. Human breast carcinoma (MCF-7) cells and non-small cell lung cancer (A549) cells were maintained in RPMI-1640 medium supplied with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cell growth in the presence or absence of experimental agents was determined using a MTT-microculture tetrazolium assay. Briefly, 100 μL of cell suspension in logarithmic growth phase was seeded into a 96-well plate (MCF-7, 1.0×10^4/well; A549, 4.5×10^3/well). After 24 h, the cells were exposed to various concentrations of the test compound in a volume of 50 μL for 72 h. Two hours prior to the end of incubation, 15 μL of MTT solution (5 mg/mL) was added into the culture medium. Cells were lysed with 75 μL of MTT lysis buffer (20% SDS−50% DMF), and cell lysis solution was incubated at 37 °C for another 12 h to dissolve the dark blue crystals. The absorption of formazan solution at 570 nm was measured using a microplate reader.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of the new compounds (**1**−**7**). This material is available free of charge via the Internet at http://pubs.acs.org.

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