Bioactive Constituents of the Stem Bark of *Mitrephora glabra*

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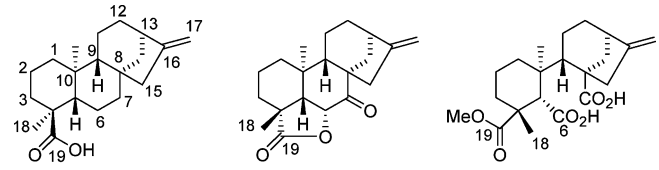
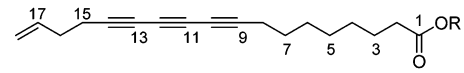
Bioactivity-guided fractionation of the stem bark of *Mitrephora glabra* yielded nine compounds, comprising three *ent*-kaurenoids (**1**−**3**), five polyacetylenic acids/esters (**4**−**8**), and one aporphine alkaloid, liriodenine (**9**). The structures of the six new compounds (**1**−**3**, **5**, **7**, and **8**) were determined by spectroscopic data interpretation. All compounds were evaluated for their inhibitory activities against a panel of cancer cell lines and a battery of microorganisms.

Over the last two decades, plants of the Annonaceae have been investigated rather extensively, largely for the presence of cytotoxic and/or insecticidal acetogenins.[1] Of these natural product-based investigations, however, the genus *Mitrephora* is relatively underexplored, probably because Annonaceous acetogenins have not been discovered therein, at least to date. There are nearly 50 described species of *Mitrephora*, and although a few species are relatively widespread in its native range, which extends from mainland China in the north to India in the west to Australia in the southeast, most of these shrubs and small to large trees have very narrow to localized distributions.[2−4] Representatives of such ecological niches may yield secondary metabolites of unique structures; yet, to the best of our knowledge, only five species of *Mitrephora* have been investigated for bioactive secondary metabolites, specifically, *M. celebica*,[5,6] *M. maingayi*,[7−9] *M. thorelii*,[10,11] *M. tomentosa*,[12] and *M. zippeliana*.[13]

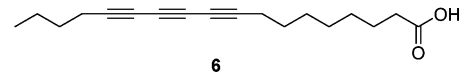
In an ongoing study of potential anticancer agents from the plant kingdom,[14,15] an investigation of the constituents of the stem bark of *Mitrephora glabra* Scheff. (Annonaceae), collected in Indonesia, has been conducted. In an earlier communication, we reported from this plant three new *ent*-trachylobane diterpenoids, mitrephorenones A−C, of which one was shown to possess an unusual oxetane ring, making it the first hexacyclic representative of this compound class.[16] A more comprehensive investigation of this species, with the aim of isolating more potent cytotoxic constituents, led to the isolation and elucidation of six additional new compounds (**1**−**3**, **5**, **7**, and **8**) and three known substances (**4**, **6**, **9**). These can be subdivided into three *ent*-kaurane diterpenoids (**1**−**3**) and five polyacetylenic acids/esters (**4**−**8**). The known alkaloid liriodenine (**9**), for which the structural data were in good agreement with those in the literature,[17,18] was isolated as well. This alkaloid was reported recently from *M. maingayi*[9] and may be common to species in the *Mitrephora* genus; it has been found in at least two other genera of the Annonaceae as well.[17] Along with testing in cytotoxicity assays, all compounds were evaluated for antimicrobial activity, largely due to the literature precedence for antimicrobial constituents from this genus.[5,6]



1 **2** **3**



4 R=H
5 R=Me



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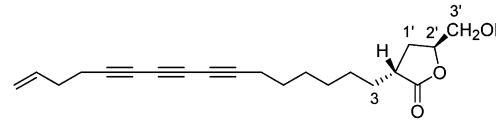

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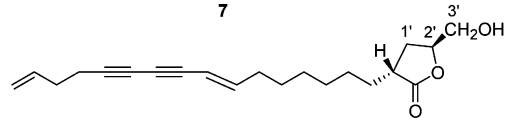

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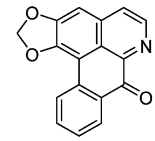

liriodenine (**9**)

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Table 1. NMR Data for Compounds **1**−**3** in $CDCl_3$

	4-*epi*-kaurenic acid (**1**)		mitrekaurenone (**2**)			methylmitrekaurenate (**3**)		
position	δ_C	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	HMBC	δ_C	δ_H (mult.; *J* in Hz)	HMBC
1	39.5	1.84 (brd; 13); 0.87 (ddd;13, 13, 4)	34.7	1.58 (m) 1.04 (m)	2, 3, 5, 10, 20 2, 3, 9, 10, 20	34.9	1.73 (m)	2, 3, 4
2	18.0	1.58 (m)	18.0	1.56 (m)	1, 3, 4	19.7	1.83(m); 1.73 (m)	3, 4, 10
3	39.8	1.97 (dd; 12, 3); 1.10 (dd; 12, 5)	29.0	2.22 (ddd; 14, 6, 6) 1.42 (m)	1, 2, 4, 5, 19 1, 2, 4, 18, 19	33.6	2.21 (m); 1.26 (m)	4, 5, 18, 19
4	47.6		41.7			44.5		
5	50.1	1.68 (m)	52.8	2.25 (d; 7.0)	4, 9, 10, 18, 19, 20	59.3	2.65 (s)	4, 6, 9, 10, 18, 19, 20
6	23.2	1.19 (m)	76.6	4.86 (d; 7.0)	7, 8, 10	177.0		
7	33.2	1.66 (m); 1.51 (m)	209.1			183.8		
8	43.9		53.9			52.9		
9	56.0	1.18 (m)	57.3	1.66 (m)	5, 10, 11, 12, 15, 20	47.6	2.45 (brd; 7)	1, 5, 8, 10, 12, 14
10	38.6		35.5			42.8		
11	40.6	1.58 (m)	31.9	2.11 (m) 1.40 (m)	9, 12, 13 8, 12, 13, 14	32.1	1.81 (m); 1.50 (m)	8, 9
12	17.7	1.62 (m); 1.46 (m)	17.1	1.40 (m) 1.25 (m)	9, 16 9, 11, 13, 14	18.3	1.82 (m); 1.61 (m)	9, 16
13	44.3	2.64 (brs)	37.5	2.71 (brdd; 8.5, 5.5)	8, 11, 12, 15, 16, 17	43.8	2.77 (brs)	
14	36.9	1.76 (m); 1.62(m)	32.1	1.98 (dd; 12.0, 5.5) 1.63 (dd; 12.0, 4.0)	7, 8, 9, 11, 12, 13, 15 7, 8, 10, 11, 12, 15, 16	34.2	1.94 (dd; 11, 5); 1.81 (brd; 11)	7, 8, 9, 13
15	49.0	2.09 (d; 15); 2.06 (d; 15)	48.2	2.36 (d; 15) 2.27 (d; 15)	9, 13, 14, 16 9, 16	46.8	2.62 (d; 18); 2.49 (d; 18)	8, 9, 14, 16
16	155.8		156.9			152.4		
17	103.0	4.80 (s); 4.74(s)	108.3	5.06(s); 4.90 (s)	13, 15	104.1	4.87 (s); 4.78 (s)	13, 15, 16
18	16.1	1.17 (s)	26.6	1.32 (s)	3, 4, 5, 19	29.1	1.37 (s)	3, 4, 5, 19
19	185.4		180.0			178.5		
20	17.8	1.06 (s)	15.8	0.71 (s)	1, 5, 9, 10	19.1	1.20 (s)	1, 5, 9, 10
OMe						52.3	3.71 (s)	19

Results and Discussion

Compound **1** exhibited a molecular formula of $C_{20}H_{30}O_2$, as deduced from HREIMS and 1D NMR data, suggesting an index of hydrogen deficiency of six. An examination of the literature quickly led to the known compound kaurenic acid,[19] which has both the same molecular formula as and similar NMR data to those of **1**[19](Table 1). COSY, HMBC, and ^{13}C NMR analyses confirmed that compound **1** shares the same planar structure as kaurenic acid. In particular, key resonances were evident at δ_C 44.3, 36.9, and 103.0, corresponding to positions C-13, C-14, and C-17, respectively. However, the chemical shifts for C-4 (δ_C 47.6) and C-18 (δ_C 16.1) were distinctly different from those reported for kaurenic acid,[19] suggesting that compound **1** possesses an opposite configuration at C-4. Indeed, this was supported by comparing the chemical shifts of related *ent*-kaurane and *ent*-trachylobane diterpenoids, where a methyl in the β configuration results in resonances of approximately δ_C 44 and 29 for positions C-4 and C-18, respectively, as was observed for kaurenic and grandiflorenic acids,[19] *ent*-trachyloban-19-oic acid,[20] and 15-oxo-(−)-*ent*-trachyloban-19-oic acid,[21] as opposed to resonances of approximately δ_C 47 and 16 when the methyl was in the α configuration for the same positions in *ent*-trachyloban-18-oic acid.[20] In further support for the C-18 methyl group of **1** being in the α configuration, H_3-18 and H_3-20 were found to be *syn* to one another, as measured via a strong ROESY correlation between these resonances. This compound was ascribed the trivial name 4-*epi*-kaurenic acid, demonstrating its close similarity to kaurenic acid.

The spectroscopic and spectrometric data for compound **2** ($C_{20}H_{26}O_3$; index of hydrogen deficiency of eight) were similar to those of **1**, suggesting a structural relationship. For example, the bicyclic C-ring portion of the molecule was intact, as evident from the COSY and HMBC data. However, comparison of the DEPT and ^{13}C NMR data between these compounds (Table 1) revealed some key differences, including the replacement of the C-7 and C-6 methylene units in **1** with a ketone moiety (δ_C 209.1) and a downfield shifted methine (δ_C 76.6), respectively, in **2**. Both methylene protons on C-14 displayed HMBC correlations with the ketone carbonyl (C-7). The H-6 resonance had HMBC correlations with C-7, C-8, and C-10, suggesting its position adjacent to the ketone. Moreover, the downfield chemical shift of this signal (δ_H/δ_C 4.86/76.6) indicated further oxygenation, which was satisfied by an ester linkage between C-6 and C-19. This was supported by the upfield chemical shift of C-19, from δ_C 185.4 as the free acid in **1** to δ_C 180.0 as the cyclic ester in **2**; this new lactone moiety added another degree of unsaturation, satisfying the index of hydrogen deficiency of eight. The complete HMBC data set (Table 1) was supportive of the structure of this compound being assigned as **2**. This compound was described previously as an intermediate in a synthetic degradation experiment;[22] however, its physical properties were not reported. To the best of our knowledge, it has not been described previously as a natural product. Therefore, we ascribed the trivial name mitrekaurenone to **2** as a new natural product.

Analysis of ^1H and ^{13}C NMR data for compound **3** revealed resonances that were characteristic for portions of the *ent*-kaurane ring system (C-8 through C-17), consistent with compounds **1** and **2**. Three carbonyl moieties were evident from the ^{13}C and DEPT NMR data (Table 1), and one of these was assigned as a methyl ester due to the HMBC correlation from δ_H 3.71 to δ_C 178.5 (C-19). Examination of these data in light of the molecular formula information from the HRMALDITOFMS ($C_{21}H_{30}O_6$; index of hydrogen deficiency of seven) revealed at least two exchangeable protons, supportive of the other two carbonyls being carboxylic acids (C-6 and C-7). COSY and HMBC data enabled the assignment of an aliphatic chain comprised of methylene units C-1, C-2, and C-3; HMBC correlations were used to position C-4, C-18, and C-19 to this chain (Table 1). The H_3-20 signal displayed HMBC correlations with C-1, C-5, C-9, and C-10, while H_3-18 correlated with C-3, C-4, C-5, and C-19. Using these data in conjunction with the HMBC correlations of a methine singlet proton (δ_H 2.65; H-5) to C-4, C-9, C-10 C-18, C-19, and C-20 permitted the assignment of the A-ring. The H-5 resonance also displayed a HMBC correlation with one of the carboxylic acid moieties, which was placed at C-6 (δ_C 177.0). The remaining carboxylic acid was positioned at C-7, largely due to a key HMBC correlation from H_2-14, finalizing the structure of **3** as a ring-B C-6, C-7-*seco ent*-kaurane diterpenoid. ROESY data revealed correlations of H-5 with both H-9 and H_3-18, but not with H_3-20, suggesting the relative

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **5**, **7**, and **8** in $CDCl_3$

	methyloropheate (**5**)		oropheolide (**7**)		9,10-dihydrooropheolide (**8**)	
position	δ_C	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)
1	174.2		179.8		179.4	
2	34.1	2.25 (m)	32.1	2.25 (m)	32.7	2.25 (m)
3−6[a]	28.0−	1.35−	27.8−	1.35−	27.3−	1.35−
	29.7	1.55 (m)	29.5	2.03 (m)	28.8	2.03 (m)
7[a]	24.9	1.53 (m)	27.0	1.53 (m)	29.6	1.53 (m)
8	19.4	2.30 (t; 7)	19.3	2.28 (t; 7)	31.2	2.70 (m)
9	78.3		78.4		147.1	6.27 (dt; 15, 6)
10	65.8		65.7		109.2	5.51 (d; 15)
11	60.7		60.2		83.6	
12	60.3		60.5		73.1	
13	66.2		66.0		65.4	
14	79.4		79.3		74.0	
15	19.3	2.38 (t; 7)	19.2	2.38 (t; 7)	19.1	2.38 (t; 7)
16	32.2	2.28 (dd; 14, 7)	31.1	2.30 (m)	32.5	2.30 (m)
17	136.1	5.84 (m)	136.0	5.84 (m)	137.3	5.84 (m)
18	116.2	5.10 (dd; 16, 2)	116.2	5.12 (dd; 16, 2)	115.4	5.12 (dd; 16, 2)
		5.04 (dd; 11, 2)		5.04 (dd; 11, 2)		5.04 (dd; 11, 2)
1′			39.5	2.70 (m)	39.5	2.70 (m)
2′			78.6	4.60 (m)	78.3	4.60 (m)
3′			64.4	3.87 (dd; 12, 3)	64.5	3.87 (dd; 12, 3)
				3.65 (dd; 12, 5)		3.65 (dd; 12, 5)
OMe	51.5	3.67 (s)				

[a] Signals may be interchangeable.

configurations of positions 4, 5, 9, and 10. Although no direct evidence was obtained from ROESY data for the stereochemical assignments for C-8 and C-13 due to overlapping signals, these two chiral centers were postulated to carry the same configuration as all other known *ent*-kaurane-type diterpenoids. Therefore, the structure of compound **3** was established as shown, and this substance was ascribed the trivial name methylmitrekaurenate.

The remaining five compounds (**4**−**8**) represented a different structural type than the diterpenoids discussed above. Two of these were known compounds. NMR data for compound **4** revealed the presence of a monosubstituted olefinic unit, a carboxylic acid unit, an aliphatic chain, and three triple bonds, as evidenced from the characteristic signals in the ^{13}C NMR spectrum. These data matched those of a polyacetylenic acid, oropheic acid,[23] which was isolated initially from *Orophea enneandra* and later obtained from *Mitrephora celebica*.[5] The ^{1}H and ^{13}C NMR data indicated that **6** has the same number of carbons as **4**, but the terminal double bond was absent, apparent in the chemical shift data for positions 17 and 18 (data not shown). This was in agreement with the HREIMS and molecular formula data, which showed that **6** has two more protons and one less degree of unsaturation than **4**. Compound **6** was reported in 2007 from the roots of *Polyalthia cerasoides* (also a member of the Annonaceae) and named octadeca-9,11,13-triynoic acid;[24] our structural data were in agreement with spectroscopic values reported previously.[24]

Compound **5** gave a molecular formula of $C_{19}H_{24}O_2$, as deduced from HRCIMS data. Its spectroscopic data were very similar to those of **4**, and the major difference was the presence of a methyl ester unit (δ_H/δ_C 3.67/51.5) in **5**, which had an HMBC correlation to C-1. Due to the structural relationship to **4**, this new compound was ascribed the trivial name methyloropheate (**5**).

NMR data for compound **7** indicated a structural relationship with compounds **4** and **5**. However, several extra signals were observed in the ^{13}C NMR spectrum, and the molecular formula ($C_{21}H_{26}O_3$ from HRCIMS) suggested the presence of an extra ring or double bond, relative to **4** or **5**, to satisfy the index of hydrogen deficiency of nine. Studies of the COSY and coupling constant data revealed a proton spin system comprising H_2-1′, H-2′, and H_2-3′, and this partial structure was incorporated into the oropheic acid backbone, on the basis of HMBC correlations. For example, H-2′ displayed correlations with C-1 and C-2, while H_2-1′ showed correlations with C-1, C-2, and C-3, suggesting a γ-lactone ring; the IR data (1770 cm^{-1}) were supportive of a lactone moiety.[25] ROESY experiments were conducted to establish the relative stereochemistry, and H-2 correlated with both H_2-3′ protons, indicating their *syn* configuration. This compound was assigned the trivial name oropheolide.

The NMR data of compound **8** were found to be similar to those of **7** (Table 2). On the basis of the molecular formula ($C_{21}H_{28}O_3$ from HRCIMS), compound **8** gave two more protons and one less degree of hydrogen deficiency than **7**. These data were consistent with replacement of one of the triple-bond moieties in **7** with a *trans* double bond in **8** (*J* value of 15 Hz; Table 2). COSY and HMBC data were examined to verify the position of this double bond. One of the olefinic protons displayed correlations with C-7 and C-8, while the other displayed correlations with C-11 and C-12, supportive of an olefinic unit between positions 9 and 10. The relative configurations at C-2 and C-2′ were presumed to be similar to those observed in compound **7**, and **8** was given the trivial name 9,10-dihydrooropheolide.

The compounds isolated from *M. glabra* in the present investigation were examined against a panel of cancer cell lines. Among the *ent*-kaurane diterpenoids (**1**−**3**), all were inactive (IC_{50} values >10 μM); the diterpenoids of the *ent*-trachylobane class, reported previously from the same plant, were more potent in this regard.[16] The polyacetylenes (**4** and **6**−**8**) gave IC_{50} values ranging from 10 to 40 μM. However, compound **5** was completely inactive, suggesting that the methyl ester diminishes cytotoxicity. The known alkaloid liriodenine (**9**) was the most cytotoxic against all four of the cell lines in which it was evaluated (IC_{50} value ~5 μM).

Diterpene and polyacetylene compounds have been reported from a related species (*M. celebica*) to have antibacterial activity.[5,6] Modest antimicrobial activity was detected for all the isolated compounds when tested against a microbial panel consisting of bacteria (*Micrococcus luteus* and *Mycobacterium smegmatis*), yeast (*Saccharomyces cerevisiae*), and fungus (*Aspergillus niger*) (Table 3). Compound **6** was reported previously to have activity against *Mycobacterium tuberculosis* (MIC of 6.25 μg/mL);[24] against a similar organism (*M. smegmatis*) it displayed a modest MIC value of 25 μg/mL. Antifungal activity in the absence of cytotoxicity, such as was observed for compounds **5** and **8**, suggests possible utility as antimicrobial agents for the treatment of human fungal infections.

Table 3. Antimicrobial Assay Data for Compounds Isolated from *M. glabra*

		antimicrobial activity (MIC in μg/mL)[a]			
compound	solution turbidity[b]	*M. luteus*	*M. smegmatis*	*S. cerevisiae*	*A. niger*
1	clear	50	50	50	100
2	clear	150	38	38	38
3	clear	150	75	150	150
4	clear	94	94	47	188
5	clear	50	12	25	25
6	clear	25	25	25	50
7	turbid	125	31	63	250
8	clear	50	12	12	12
9	clear	6.3	12	12	25
positive controls[c]	clear	0.1	0.2	25	25

[a] Minimal inhibitory concentration (MIC) is the lowest concentration of compound completely inhibiting growth (as turbidity) expressed in μg/mL.[27] [b] Presence of turbidity or color in compound dissolved in 1 mL of EtOH. Presence of turbidity could lead to an overestimation of MIC, because turbidity due to compound would have obscured end point (no turbidity due to microbial growth). [c] Average values. Ampicillin was the positive control for *M. luteus*, kanamycin was the positive control for *M. smegmatis*, and amphotericin B was the positive control for *S. cervisiae* and *A. niger*.

Experimental Section

General Experimental Procedures. Optical rotation, UV, and IR data were acquired on a Rudolph Autopol III polarimeter, a Varian Cary 3 UV−vis spectrophotometer, and a Nicolet Avatar 360 FT-IR, respectively. All NMR experiments were performed in $CDCl_3$ with TMS as an internal standard; gs-COSY, ROESY, gs-HSQC, gs-HMBC, and ^{1}H NMR spectra were acquired on a Varian Unity INOVA-500 instrument using a 5 mm broadband inverse probe with z-gradient, while a Bruker DPX-300 instrument was utilized for some of the ^{1}H NMR, ^{13}C NMR, and DEPT-135 NMR spectra using a Bruker 5 mm QNP probe. LRESIMS data were acquired using an API 150EX mass spectrometer (Applied Biosystems; Foster City, CA). HREIMS were performed with a Kratos MS-25 using 70 eV ionization conditions, or HREIMS and HRCIMS were performed on a Finnigan MAT 95Q hybrid-sector instrument (ThermoFinnigan; San Jose, CA). HRMALDITOFMS data were acquired with an Applied Biosystems TOF/TOF mass spectrometer (Framingham, MA), equipped with a Nd:YAG laser operating at 355 nm and 200 Hz. This instrument was operated in the reflectron mode, and the matrix employed was 2,5-dihydroxybenzoic acid prepared at a concentration of 9 mg/mL in 70:30 (v/v) acetonitrile−0.1% trifluoroacetic acid. Column chromatography was carried out on silica gel 60 (70−230 mesh, Merck, Darmstadt, Germany), and fractions were monitored via TLC (silica gel 60 F254 plates, 0.25 mm thickness) visualized with vanillin−sulfuric acid in EtOH. Preparative HPLC was carried out on a Varian Prostar HPLC system (Walnut Creek, CA) equipped with Prostar 210 pumps and a 330 photodiode array detector (PDA), with data collected and analyzed using Star Chromatography Workstation software (version 5.52) using an ODS-A column (250 × 25 mm, i.d., 5 μm; YMC, Wilmington, NC). The percent yields of isolated compounds were calculated on the basis of the dry weight of the plant material (w/w).

Plant Material. The bark of *Mitrephora glabra* Scheff. (Annonaceae) was collected in Kasintu village, Tewah district, Kapuas regency, Central Kalimantan province, Indonesia, in October 1999. A voucher herbarium specimen (Riswan et al. TWH077) has been deposited at the John G. Searle Herbarium of the Field Museum in Chicago under accession number F-2221275.

Extraction and Isolation. The dried bark (984 g) was extracted in a Soxhlet with hot MeOH (3 L × 2) for 24 h. The extracts were combined, concentrated in vacuo, and diluted with H_2O to give a MeOH−H_2O (9:1) solution (0.6 L), which was defatted with hexane (0.6 L × 2). The aqueous MeOH fraction was concentrated in vacuo and partitioned between $CHCl_3$−MeOH (4:1, 0.3 L) and H_2O (0.3 L × 2). The organic fraction was then washed with 1% saline until there was no evidence of tannins[26] and concentrated to afford a crude organic extract (22.8 g). This sample was separated on a flash silica gel column that was developed using a gradient of 100% hexane to 100% $CHCl_3$ to 100% MeOH. The fractions were combined into 13 pools based on TLC properties and examined against the KB assay, and further studies were conducted on the bioactive fractions. An aliquot (170 mg) of pool 5 (ca. 4.4 g), which eluted using 2.8% MeOH in $CHCl_3$, was purified via RP-HPLC (flow rate of 10 mL/min using a gradient of 70% to 90% CH_3CN in H_2O over 40 min) to yield compounds **1** (16 mg), **2** (8 mg), and **4** (11 mg). Pools 6 and 7 were combined and then subjected consecutively to another silica gel column using a hexane−ethyl acetate gradient to give 10 subfractions (A-1 to A-10). Fraction A-1 (1.8 g) was then subjected to a diol column followed by preparative RP-HPLC (same conditions as above) to yield compounds **3** (10 mg) and **5** (3 mg), while fraction A-5 (1.3 g) was subjected directly to preparative RP-HPLC (same conditions as above) to yield compounds **6** (4 mg), **7** (35 mg), **8** (2 mg), and liriodenine (11 mg).

4-*epi*-Kaurenic acid (1): colorless oil (16 mg, yield 0.0016% w/w); $[\alpha]_D$ −72.1 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 206 (ε 4434) nm; IR (CH_2Cl_2) ν_{max} 3063, 2984, 2926, 2864, 1692 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; ESIMS *m/z* 303 $[M + 1]^+$ (100%); HREIMS *m/z* 302.2248 $[M]^+$, calcd for $[C_{20}H_{30}O_2]^+$ 302.2246.

Mitrekaurenone (2): colorless oil (8 mg, yield 0.0008% w/w); $[\alpha]_D$ −2.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 206 (ε 5420), 264 (268), 298 (166) nm; IR (CH_2Cl_2) ν_{max} 3069, 2948, 2877, 1780, 1717 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; ESIMS *m/z* 315 $[M + 1]^+$ (100%); HREIMS *m/z* 314.1884 $[M]^+$, calcd for $[C_{20}H_{26}O_3]^+$ 314.1881.

Methylmitrekaurenate (3): colorless oil (10 mg, yield 0.0010% w/w); $[\alpha]_D$ −66.8 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} 204 (ε 4647), 278 (511) nm; IR (CH_2Cl_2) ν_{max} 3061, 2947, 2867, 1736, 1718, 1698 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; ESIMS *m/z* 379 $[M + 1]^+$ (100%); HRMALDITOFMS *m/z* 379.2115 $[M + H]^+$ and 401.1935 $[M + Na]^+$ (calcd for $[C_{21}H_{30}O_6 + H]^+$ 379.2121 and $[C_{21}H_{30}O_6 + Na]^+$ 401.1940, respectively).

Methyloropheate (5): colorless oil (3 mg, yield 0.0003% w/w); UV (MeOH) λ_{max} 217 (ε 6678), 240 (673), 253 (633), 266 (666), 281 (625) nm; IR (CH_2Cl_2) ν_{max} 2935, 2856, 2214, 1736 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 307 $[M + Na]^+$ (100%); HRCIMS *m/z* 285.1841 $[M + H]^+$, calcd for $[C_{19}H_{24}O_2 + H]^+$ 285.1856.

Octadeca-9,11,13-triynoic acid (6): colorless oil (4 mg, yield 0.0004% w/w); UV (MeOH) λ_{max} 216 (ε 6317), 241 (660), 253 (786), 267 (900), 282 (720) nm; IR (CH_2Cl_2) ν_{max} 2933, 2858, 2211, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data were in good agreement with the literature;[24] ESIMS *m/z* 273 $[M + H]^+$ (100%); HREIMS *m/z* 272.1780 $[M]^+$, calcd for $[C_{18}H_{24}O_2]^+$ 272.1771.

Oropheolide (7): colorless oil (35 mg, yield 0.0036% w/w); $[\alpha]_D$ −12.3 (*c* 1.0, CH_2Cl_2); UV (MeOH) λ_{max} 217 (ε 1755), 240 (242), 253 (471), 267 (699), 282 (575) nm; IR (CH_2Cl_2) ν_{max} 3466, 2938, 2856, 2216, 1770 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; selected HMBC data, H-2′ → C-1, C-2; H_2-1′ → C-1, 2, 3; H_2-7 → C-8, 9; H_2-8 → C-9, 10; H_2-15 → C-14, 16; H_2-16 → C-15, 17, 18; H-17 → C-15, 16, 18; H-18 → C-16, 17; key ROESY data, H-2 → H_2-3′; ESIMS *m/z* 349 $[M + Na]^+$ (100%); HRCIMS *m/z* 327.1953 $[M + H]^+$, calcd for $[C_{21}H_{26}O_3 + H]^+$ 327.1960.

9,10-Dihydrooropheolide (8): colorless oil (2 mg, yield 0.0002% w/w); $[\alpha]_D$ −3.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 213 (ε 13 890), 240 (1793), 253 (3340), 267 (4845), 281 (625) nm; IR (CH_2Cl_2) ν_{max} 2933, 2856, 2214, 1773 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; key ROESY data, H-1 → H_2-3′; ESIMS *m/z* 351 $[M + Na]^+$ (100%); HRCIMS *m/z* 329.2101 $[M + H]^+$, calcd for $[C_{21}H_{28}O_3 + H]^+$ 329.2117.

Liriodenine (9): yellow, amorphous solid (11 mg, yield 0.0011% w/w); ^{1}H and ^{13}C NMR data were in good agreement with the literature.[17,18]

Cytotoxicity Assays. The cytotoxicity measurements against the KB human oral epidermoid carcinoma (American Type Culture Collection, Manassas, VA), MCF-7 human breast carcinoma (Barbara A. Karmanos Cancer Center, Detroit, MI), NCI-H460 human large cell lung carcinoma (American Type Culture Collection, Manassas, VA), and SF-268 human astrocytoma (NCI Developmental Therapeutics Program, Frederick, MD) cell lines were performed as described previously with one modification.[27] The assay was monitored via absorbance at the optimal UV maximum of 562 nm.

Antimicrobial Assays. Strains of *Micrococcus luteus, Mycobacterium smegmatis, Saccharomyces cerevisiae*, and *Aspergillus niger* were grown and suspensions prepared as described previously.[28] Minimal inhibitory concentrations (MICs) for complete inhibition by pure

compounds were measured by broth microdilution in 96-well microtiter dishes as described previously.[27]

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Supporting Information Available: NMR spectra for new compounds (**1**−**3**, **5**, **7**, **8**). This information is available free of charge via the Internet at http://pubs.acs.org.

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