JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Isolation and Synthesis of Antiproliferative Eupolauridine Alkaloids of *Ambavia gerrardii* from the Madagascar Dry Forest[1]

Ende Pan,[†] Shugeng Cao,[†,‡] Peggy J. Brodie,[†] Martin W. Callmander,[§] Richard Randrianaivo,[§] Stephan Rakotonandrasana,[⊥] Etienne Rakotobe,[⊥] Vincent E. Rasamison,[⊥] Karen TenDyke,[∥] Yongchun Shen,[∥] Edward M. Suh,[∥] and David G. I. Kingston*[,†]

[†]Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, United States

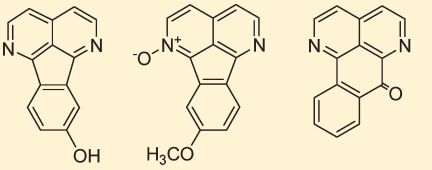
[§]Missouri Botanical Garden, B.P 3391, Antananarivo 101, Madagascar

[⊥]Centre National d'Application des Recherches Pharmaceutiques, B.P 702, Antananarivo 101, Madagascar

[∥]Next Generation Systems, Eisai Inc., 4 Corporate Drive, Andover, Massachusetts 01810, United States

Supporting Information

ABSTRACT: Investigation of the Madagascan endemic plant *Ambavia gerrardii* for antiproliferative activity against the A2780 ovarian cancer cell line led to the isolation of the three new alkaloids 8-hydroxyeupolauridine (**1**), 9-methoxyeupolauridine 1-oxide (**2**), and 11-methoxysampangine (**3**) and the three known alkaloids **4**−**6**. The structures of **1** and **2** were confirmed by synthesis. Compounds **3**, **4**, and **6** showed moderate to good antiproliferative activities, with IC_{50} values of 10.3, 3.5, and 0.60 μM, respectively, against the A2780 human ovarian cancer cell line and with IC_{50} values of 0.57, 1.77, and 0.58 μM, respectively, against the H460 human lung cancer cell line.



1 IC_{50} 12.7μM, IC_{50} >10 μM

2 54.3 μM, >10 μM

6 0.60 μM (A2780), 0.58 μM (H460)

In our continuing search for biologically active natural products from tropical forests, as part of an International Cooperative Biodiversity Group (ICBG) program, we obtained an EtOH extract from the roots of a plant identified as *Ambavia gerrardii* (Baill.) Le Thomas (Annonaceae) from Madagascar. The extract exhibited good antiproliferative activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 8.2 μg/mL. The genus *Ambavia* consists of only two species, *A. capuronii* and *A. gerrardii*, both of which are endemic to Madagascar, and no phytochemical work has been reported on either species. Previous phytochemical studies of plant species in the Annonaceae family have revealed the presence of cytotoxic acetogenins,[2,3] miliusanes,[4] styrylpyrones,[5] polyacetylenes,[6] diterpenoids,[7,8] flavanones,[9] and alkaloids.[6,10] The extract was selected for bioassay-directed fractionation to isolate its active components on the basis of its activity and the absence of previous phytochemical studies.

RESULTS AND DISCUSSION

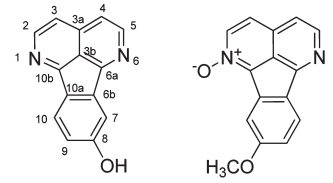
An EtOH extract of the roots of *A. gerrardii* was subjected to liquid−liquid partitioning between hexanes, CH_2Cl_2, and MeOH to give fractions with IC_{50} values of 14, 2.6, and >100 μg/mL, respectively, in the A2780 assay. Fractionation of the active CH_2Cl_2 fraction by C18 open column and high-performance liquid chromatography (HPLC) yielded two new eupolauridine derivatives (**1** and **2**), the new 11-methoxysampangine (**3**), and eupolauridine *N*-oxide (**4**), eupolauridine (**5**), and sampangine (**6**). The structures of compounds **1** and **2** were proposed on the basis of their UV, IR, HRESIMS, and ^{1}H NMR spectral data, but the limited samples available combined with the absence of ^{13}C NMR data and HMBC correlations made it necessary to confirm the structures by synthesis. The synthesis also provided material for the evaluation of the biological activities of **1** and **2**. Herein we report the isolation and structure elucidation of compounds **1**−**3**, the synthesis of compounds **1** and **2**, and the antiproliferative activities of all isolates.

Eupolauridine *N*-oxide (**4**),[11] eupolauridine (**5**),[11] and sampangine (**6**)[12−14] were all obtained as yellow solids. Eupolauridine (**5**) was previously isolated from the annonaceous plant *Cananga odorata* (ylang ylang) collected in Madagascar[15] and from the eupomatiaceous plant *Eupomatia laurina*.[16]

Compound **1** was obtained as a yellow solid. Its UV absorptions in MeOH, with λ_{max} (log ε) 220 (4.15), 240 (3.98), 287 (3.82), 351 (3.28), and 369 (3.35) nm, indicated the presence of an extended aromatic chromophore similar to that of eupolauridine **5**. The IR spectroscopic data of compound **1**, which showed absorptions at 1599, 1580, 1398, 1378, 1202, 842, and 808 cm^{-1}, confirmed the existence of the aromatic and C−O functions. The positive ion HRESIMS of **1** revealed a pseudomolecular ion peak at m/z 221.0713 $[M + H]^+$ corresponding to a molecular formula of $C_{14}H_{10}N_2O$ (calcd for $C_{14}H_9N_2O$: 221.0715). Its ^{1}H NMR signals in CD_3OD exhibited AB, A′B′, and A″B″X″ multiplets at low field [δ_H 8.64 (1H, d, J = 6.1 Hz), 8.55 (1H, d, J = 6.1 Hz),

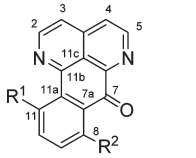
Received: January 29, 2011
Published: April 19, 2011

ACS Publications Copyright © 2011 American Chemical Society and American Society of Pharmacognosy

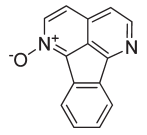


1

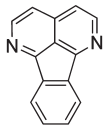
2



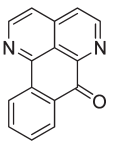
3 $R^1 = OCH_3$, $R^2 = H$
12 $R^1 = H$, $R^2 = OCH_3$



4



5



6

7.78 (1H, d, J = 8.2 Hz), 7.61 (1H, d, J = 6.1 Hz), 7.51 (1H, d, J = 6.1 Hz), 7.42 (1H, d, J = 2.3 Hz), 6.86 (1H, dd, J = 8.2, 2.3 Hz)]. The above data together with the fact that **1** and **5** ($C_{14}H_{10}N_2$) had the same unsaturation number (12) suggested that compound **1** was a hydroxylated eupolauridine. The position of the OH group was assigned at C-8 from the coupling patterns of the protons of the benzene ring of **1** [δ_H 7.78 (1H, d, J = 8.2 Hz), 6.86 (1H, dd, J = 8.2, 2.3 Hz), and 7.42 (1H, d, J = 2.3 Hz)] and comparison of the ^{1}H NMR spectroscopic data of **1** with those of **5** [δ_H 8.71 (2H, d, J = 6.0 Hz), 8.01 (2H, m), 7.67 (2H, d, J = 6.0 Hz), 7.55 (2H, m)]. On the basis of the above data, we proposed the structure of 8-hydroxyeupolauridine for compound **1**.

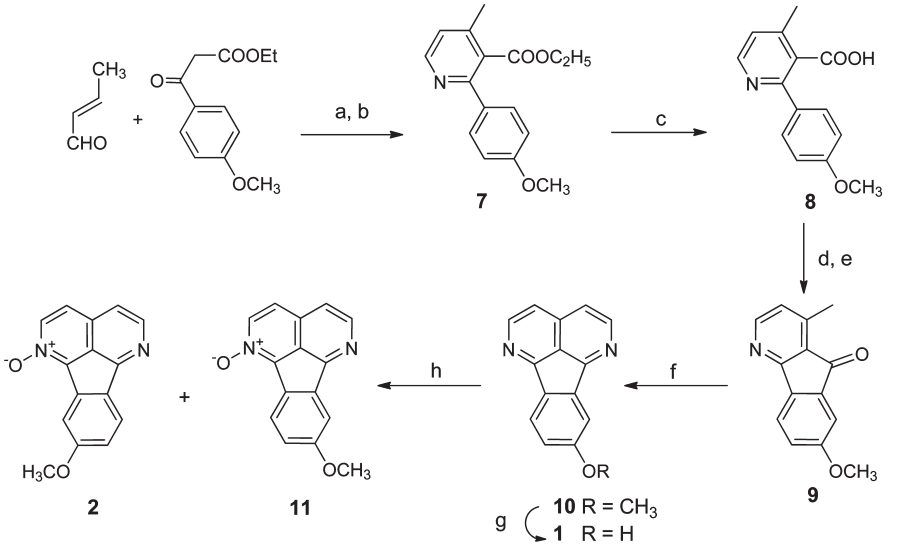
Compound **2** was also obtained as a yellow solid. It showed strong UV absorptions (MeOH) at λ_{max} (log ε) 225 (4.03), 254 (3.99), 292 (3.87), and 384 (3.36) nm. Its IR spectrum displayed aromatic absorptions at 1611, 1593, 1569, 1451, 1423, 846, and 828 cm^{-1}, a C−O stretch absorption at 1022 cm^{-1}, and N−O stretch absorptions at 1487 and 1380 cm^{-1}. The characteristic alkyl C−H stretch absorptions were also observed at 2919 and 2851 cm^{-1}. The positive ion HRESIMS of **2** displayed a pseudomolecular ion peak at m/z 251.0820 $[M + H]^+$, corresponding to a molecular formula of $C_{15}H_{11}N_2O_2$ (calcd for $C_{15}H_{11}N_2O_2$: 251.0821). Its proton spectrum [AB, A′B′, and A″B″X″ aromatic coupling pattern and one OCH_3 group: δ_H 8.58 (1H, d, J = 6.0 Hz), 8.23 (1H, d, J = 7.1 Hz), 7.95 (1H, d, J = 8.2 Hz), 7.92 (1H, d, J = 2.4 Hz), 7.79 (1H, d, J = 7.1 Hz), 7.57 (1H, d, J = 6.0 Hz), 7.09 (1H, dd, J = 8.2, 2.4 Hz)] was very similar to that of **1** except for the presence of a signal for an additional methyl group at δ_H 3.96 (3H, s). The above spectroscopic data thus suggested that **2** was either 8-methoxyeupolauridine N^1-oxide or 9-methoxyeupolauridine N^1-oxide, but the available data did not permit a distinction between these two structures. It was thus necessary to synthesize **1** and **2** to confirm the structure of **1**, to provide additional material for bioassay, and to determine the position of the *N*-oxide in **2**.

The synthesis of 8-hydroxyeupolauridine (**1**) initially followed Wong's method for synthesizing eupolauridine,[17] using 5-methoxy-1,3-indanedione as the starting material instead of the 1,3-indanedione of the published method. The key step to afford 7-methoxyonychin (**9**) involved the thermal rearrangement of an oxime *O*-crotyl ether, and this gave a very low yield of product (<10%) in our hands. We thus changed the synthetic strategy by modifying Bracher's synthesis of eupolauridine (**5**) (Scheme 1).[18] Ethyl 3-(4-methoxyphenyl)-3-oxopropionate was deprotonated by NaH, then underwent Michael addition with crotonaldehyde to afford the intermediate α-(1-methyl-3-oxopropyl)-β-oxo-4-methoxylbenzenepropanoic acetate, which reacted with hydroxylamine hydrochloride to give ethyl 2-(4-methoxy)phenyl-4-methyl-3-pyridinecarboxylate (**7**). Although polyphosphoric acid was reported to catalyze the formation of onychin from ethyl 2-phenyl-4-methyl-3-pyridinecarboxylate in good yield,[18] a very low yield of product was obtained when **7** was used as a substrate in the study. Compound **7** was thus hydrolyzed to the corresponding nicotinic acid **8**, which was then converted to its acyl chloride by treatment with thionyl chloride. A Friedel−Crafts reaction of the acyl chloride in chlorobenzene under reflux gave 7-methoxyonychin (**9**). The acidic methyl group of **9** reacted with dimethylformamide diethyl acetal to afford an enamine intermediate, which yielded 8-methoxyeupolauridine (**10**) through a ring-closing reaction of the intermediate in the presence of ammonium acetate at 140 °C. Cleavage of the methyl ether of **10** in 48% HBr gave 8-hydroxyeupolauridine (**1**) in 1.7% overall yield from **7**. Comparison of the ^{1}H NMR (Table 1) and HRESIMS data of 8-hydroxyeupolauridine with the data of compound **1** confirmed the proposed structure of the isolated natural product.

Mono-oxidation of 8-methoxyeupolauridine (**10**) by one equivalent of *meta*-chloroperoxybenzoic acid yielded the two methoxyeupolauridine *N*-oxides in a 3.7:1 ratio in 2.7% overall yield; the ^{1}H NMR data for the major product matched the corresponding data of the natural product **2** and differed from those of the isomeric product **11**. Comparison of the ^{1}H NMR data of **2** and **11** (Table 1) indicated that the proton signals of H-10 for **2** [δ_H 7.95 (d, J = 8.2 Hz)] were less deshielded than those of **11** [δ_H 8.21 (d, J = 8.4 Hz)]. On the other hand the signals for H-7 of **2** [δ_H 7.92 (d, J = 2.4 Hz)] were more deshielded than those of **11** [δ_H 7.61 (d, J = 2.3 Hz)]. These differences enabled the structures of **2** and **11** to be assigned. The larger deshielding of H-10 of **11** and of H-7 of **2** was consistent with the major resonance structures of each compound. For compound **2** the favored resonance structure is expected to be the one that puts the negative charge on the positively charged oxygenated nitrogen (structure **2a**) rather than a structure such as **2b**, where the charge ends up on the neutral nitrogen atom; this means that H-7 will be preferentially deshielded. The situation is reversed for structure **11**, where the most stable resonance structure **11a** results in deshielding of H-10 and the less favored resonance structure **11b** is the one that deshields H-7 (Figure 1). HMBC correlations of **2** from both downfield shifted H-5 (δ_H 8.23) and H-7 (δ_H 7.92) to C-6a (δ_C 144.0) confirmed it to be 9-methoxyeupolauridine-1-oxide.

11-Methoxysampangine (**3**) was obtained as a yellow solid and had a molecular mass of 263.1, consistent with the composition $C_{16}H_{11}N_2O_2$. Its ^{1}H NMR signals in $CDCl_3$ exhibited AB, A′B′, and A″B″C″ multiplets at low field: δ_H 8.88 (1H, d, J = 5.8 Hz), 7.71 (1H, d, J = 5.8 Hz), 7.86 (1H, d, J = 5.8 Hz), 9.11 (1H, d, J = 5.8 Hz), 7.26 (1H, d, J = 8.0 Hz), 7.77 (1H, t, J = 8.0 Hz), and 8.56 (1H, d, J = 8.0 Hz). A triplet at

Scheme 1. Synthesis of 1, 2, and 11



a) NaH, 1,4-dioxane, 30 min; b) $H_2NOH.HCl$, AcOH, 100-110 °C, 90 min, 32%; c) 40% NaOH/H_2O, reflux, 15h, 66%; d) $SOCl_2$, reflux, 24 h; e) $AlCl_3$, chlorobenzene, reflux, 15 h, 25%; f) (i) $Me_2NCH(OEt)_2$, DMF, 120 °C, 2 h; (ii) NH_4OAc, 140 °C, 1 h, 74%; g) 48% HBr, reflux, 24h, 43%; h) *m*CPBA, DCM, rt, 24 h, 70%.

Table 1. ^1H and ^{13}C NMR Spectra of Compounds 1−3 and ^1H NMR Spectra of Compounds 10 and 11[a]

	1		2		3			10	11
position	^1H (*J*, Hz)	^{13}C[b]	^1H (*J*, Hz)	^{13}C[b]	^1H (*J*, Hz)[c]	^1H (*J*, Hz)	^{13}C	^1H (*J*, Hz)	^1H (*J*, Hz)
2	8.55 d (6.1)	150.3	8.58 d (6.0)	149.6	8.88 d (5.8)	8.87 d (5.7)	148.9	8.57 d (5.7)	8.16 d (7.1)
3	7.61 d (6.1)	117.9	7.57 d (6.0)	117.8	7.71 d (5.8)	7.90 d (5.7)	120.6	7.28 d (6.0)	7.72 d (7.1)
3a		137.0		130.4			140.6		
3b		122.7		125.0					
4	7.51 d (6.1)	119.7	7.79 d (7.1)	124.4	7.86 d (5.8)	8.12 d (5.7)	125.1	7.35 d (5.7)	7.65 d (5.8)
5	8.64 d (6.1)	150.5	8.23 d (7.1)	145.8	9.11 d (5.8)	8.99 d (5.7)	148.9	8.63 d (5.7)	8.65 d (5.8)
6a		163.1		144.0			149.5		
6b		143.0		136.3					
7	7.42 d (2.3)	112.3	7.92 d (2.4)	113.5			182.1	7.49 d (1.4)	7.61 d (2.3)
7a							139.2		
8		164.2		164.5	8.56 d (8.0)	8.52 d (8.0)	119.0		
9	6.86 dd (8.2, 2.3)	118.2	7.09 dd (8.2, 2.4)	117.1	7.77 t (8.0)	7.83 t (8.0)	137.4	6.89 dd (8.2, 1.4)	7.07 dd (8.4, 2.3)
10	7.78 d (8.2)	125.5	7.95 d (8.2)	125.4	7.26 d (8.0)	7.42 d (8.0)	116.7	7.81 d (8.2)	8.21 d (8.4)
10a		130.9		130.7					
10b		163.6		159.6					
11							163.5		
11a							121.7		
11b							152.4		
11c							119.0		
OCH_3			3.96 s	56.5	4.09	4.05	56.8	3.90 s	3.96 s

[a] In CD_3OD unless otherwise stated: δ (ppm); multiplicities; *J* values (Hz) in parentheses. [b] The ^{13}C NMR data on **1** and **2** were obtained on the synthetic samples. [c] In $CDCl_3$, δ (ppm); multiplicities; *J* values (Hz) in parentheses.

δ_H 7.77 with a coupling constant of 8.0 Hz indicated the presence of a contiguous group of three aromatic protons and limited the possible structures to either 8-methoxysampangine (**12**) or 11-methoxysampangine (**3**). The 8-methoxysampangine structure **12** was initially favored, based on the absence of a ^3J HMBC correlation from the H-8 aromatic proton to the C-7 carbonyl carbon, but a comparison of its ^1H NMR spectrum with that of synthetic 8-methoxysampangine prepared by Zjawiony et al.[19]

dx.doi.org/10.1021/np200093n |*J. Nat. Prod.* 2011, 74, 1169–1174

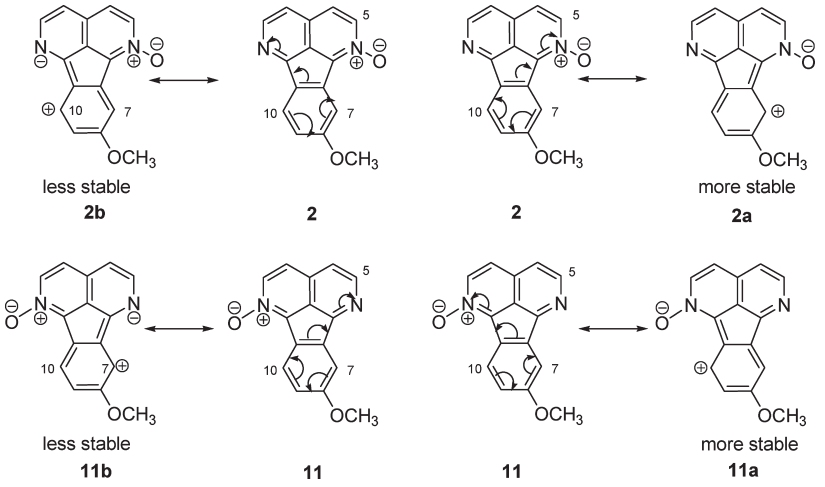


Figure 1. Favored resonance structures of **2** and **11**.

indicated that the two compounds were not identical. Although a sample for direct comparison was not available, Dr. Zjawiony confirmed the structure of his synthetic compound by a comparison of the NMR data of his intermediates with data in the Supporting Information of a recent paper.[20,21] These facts exclude the 8-methoxysampangine structure, and so the structure of compound **3** is assigned as 11-methoxysampangine. The lack of sample regrettably precluded additional experiments to confirm this assignment.

The biosyntheses of these two types of alkaloids have been proposed by Taylor.[22] It was reported that sampangine (**6**) could derive from a dihydroxyoxoaporphine. Then eupolauridine (**5**) could arise from sampangine (**6**) through a ring-contraction pathway with concurrent loss of carbon monoxide.

Previous research has shown eupolauridine to have antifungal activity[23] and sampangine derivatives to have cytotoxic, antimalarial, and antifungal activities.[24] 8-Hydroxyeupolauridine (**1**), 9-methoxyeupolauridine 1-oxide (**2**), 8-methoxyeupolauridine (**10**), and 8-methoxyeupolauridine 1-oxide (**11**), as well as 11-methoxysampangine (**3**), eupolauridine N-oxide (**4**), eupolauridine (**5**), and sampangine (**6**) were tested against the A2780 human ovarian cancer cell line. Among these eight compounds, sampangine (**6**) was the most active against the A2780 cell line, with an IC_{50} value of 0.60 μM, but sampangine derivative **3** showed much weaker activity, with an IC_{50} of 10.3 μM. Among the eupolauridine analogues, compound **4** was the most active, with an IC_{50} value of 3.5 μM.

When tested against the H460 human non-small-cell lung cancer cell line, compounds **3** and **6** both exhibited strong activity, with IC_{50} values of 0.57 and 0.58 μM, respectively. Interestingly, only **4** among the eupolauridine analogues showed antiproliferative activity against this cell line, with an IC_{50} value of 1.77 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. UV was measured on a Shimadzu UV-1201 spectrophotometer. IR spectra of solid samples were obtained directly on a MIDAC M-series FTIR spectrophotometer. Melting points were obtained on a B-540 Büchi melting-point apparatus. NMR spectra were recorded in $CDCl_3$, CD_3OD, or D_2O on either JEOL Eclipse 500 or Bruker 600 spectrometers. The chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. Mass spectra were obtained on an Agilent 6220 TOF mass spectrometer. HPLC was performed on a Shimadzu LC-10AT instrument with a semipreparative C18 or phenyl Varian Dynamax column (5 μm, 250 × 10 mm). Unless otherwise noted, chemicals were obtained from commercial suppliers and used without purification. Solvents were dried if necessary by standard methods. All reactions were performed under air atmosphere unless otherwise noted.

Plant Material. Roots of *Ambavia gerradii* were collected on July 15, 2005, in the Ambohibe dry forest near the village of Betsimiranja, Diana, Antsiranana, Madagascar. The collection coordinates were 13°02′42″ S, 049°09′11″ E, and the elevation was 50 m. The plant sampled was a tree of about 16 m height with a diameter at breast height of 35 cm, and with green fruit; it occurs commonly in this area. The tree was identified by one of the authors (R.R.), and its identity was confirmed by G. E. Schatz (Missouri Botanical Garden). Voucher specimens with colllection number Randrianaivo & al. 1196 have been deposited in herbaria at the Parc Botanique and Zoologique de Tsimbazaza (TAN), at the Centre National d'Application des Recherches Pharmaceutiques in Antananarivo, Madagascar (CNARP), at the Missouri Botanical Garden in St. Louis, Missouri (MO), and at the Muséum National d'Histoire Naturelle in Paris, France (P).

Extraction and Isolation. Dried roots of *A. gerrardii* (250 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at room temperature to give the crude extract MG 3311 (6.4 g), of which 3.0 g was available at Virginia Polytechnic Institute and State University (VPISU) for evaluation. The extract MG 3311 (IC_{50} 8.2 μg/mL, 1.3 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 100 mL) and extracted with hexanes (3 × 100 mL portions). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 150 mL portions). The hexanes extract was evaporated in vacuo to leave 138 mg with an IC_{50} value of 14 μg/mL. The residue from the CH_2Cl_2 extract (354 mg) had an IC_{50} value of 2.6 μg/mL. The aqueous MeOH extract (715 mg) was inactive. The CH_2Cl_2 extract was selected for fractionation, and five fractions of 124, 44, 81, 26, and 38 mg were collected from a C18 open column eluted with MeOH−H_2O (gradient from 60% to 100%). The first four fractions had IC_{50} values of 3.7, 1.9, 3, and 16 μg/mL, respectively, and the last fraction was inactive. Fractions I and II were selected for further work. Separation of fraction I by C-18 preparative HPLC (60% MeOH−H_2O) yielded eight subfractions (IC_{50} 19 μg/mL, inactive, inactive, 3.2 μg/mL, inactive, 2 μg/mL, 4.9 μg/mL, and inactive), and the most active subfractions, I-4 (IC_{50} 3.2 μg/mL) and I-6 (IC_{50} 2 μg/mL), were selected for further separation by phenyl HPLC (60% MeOH−H_2O). Compounds **1** (0.4 mg, t_R 28.1 min) and **4** (2.0 mg t_R 31.4 min) were isolated from subfraction I-4, and

compounds **2** (0.5 mg, t_R 29.8 min) and **3** (1.0 mg, t_R 36.0 min) were isolated from subfraction I-6. Fraction II was applied to a C18 open column. The most active subfraction (IC_{50} 0.14 μg/mL) was subjected to phenyl HPLC (70% MeOH−H_2O) to give compounds **5** (1.9 mg, t_R 25.3 min) and **6** (0.6 mg, t_R 31.4 min).

8-Hydroxyeupolauridine (1): yellow solid; UV (MeOH) λ_{max} nm (log ε) 220 (4.15), 240 (3.98), 287 (3.82), 351 (3.28), 369 (3.35); IR ν_{max} cm^{-1} 1638, 1599, 1580, 1398, 1378, 1289, 1243, 1202, 1091, 1060, 1016, 994, 842, and 808 cm^{-1}; ^1H NMR (500 MHz, CD_3OD), see Table 1; HRESIMS m/z 221.0713 $[M + H]^+$ (calcd for $C_{14}H_{10}N_2O$, 221.0715).

9-Methoxyeupolauridine 1-oxide (2): yellow solid; UV (MeOH) λ_{max} nm (log ε) 225 (4.03), 254 (3.99), 292 (3.87), 384 (3.36); IR ν_{max} cm^{-1} 2919, 2851, 1611, 1593, 1487, 1451, 1423, 1380, 1256, 1233, 1022, 973, 846, and 828 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRESIMS m/z 251.0820 $[M + H]^+$ (calcd for $C_{15}H_{11}N_2O_2$, 251.0821).

11-Methoxysampangine (3): yellow solid; LC-MS m/z 263.1 $[M + H]^+$ (calcd for $C_{16}H_{11}N_2O_2$, 263.1); ^1H NMR (500 MHz, $CDCl_3$), see Table 1.

Eupolauridine *N*-oxide (4): yellow solid; LC-MS m/z 221.1 $[M + H]^+$ (calcd for $C_{14}H_9N_2O$, 221.1); ^1H NMR (500 MHz, $CDCl_3$), see Supporting Information

Eupolauridine (5): yellow solid; LC-MS m/z 205.1 $[M + H]^+$ (calcd for $C_{14}H_9N_2$, 205.1); ^1H NMR (500 MHz, $CDCl_3$), see Supporting Information

Sampangine (6): yellow solid; LC-MS m/z 233.0 $[M + H]^+$ (calcd for $C_{15}H_9N_2O$, 233.1); ^1H NMR (500 MHz, $CDCl_3$), see Supporting Information

Ethyl 2-(4-methoxy)phenyl-4-methyl-3-pyridinecarboxylate (7). To a solution of 3.84 g (17 mmol) of ethyl 3-(4-methoxyphenyl)-3-oxopropionate in 14 mL of dioxane in a 100 mL flask was added 48 mg (2 mmol) of NaH and then 1.68 g (24 mmol) of crotonaldehyde dropwise in 6 mL of dioxane. After the reaction mixture was stirred for another 30 min at room temperature, 4.9 g of $H_2NOH\cdot$HCl (70 mmol) and 20 mL of glacial AcOH were added. The reaction mixture was stirred at 100−110 °C for 90 min and then poured onto 100 g of ice, made basic with K_2CO_3, and extracted with ether (3 × 100 mL). The combined organic phase was extracted with 2 N HCl (3 × 100 mL). The combined acidic aqueous phase was neutralized with K_2CO_3 and extracted with ether (3 × 100 mL), and the ether was dried over K_2CO_3 and evaporated under reduced pressure. The oily crude product was purified by flash silica column chromatography (hexane−EtOAc, 4:1) to afford ethyl 2-(4-methoxy)phenyl-4-methyl-3-pyridinecarboxylate (**7**) (1.47 g, 32%): yellow oil; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.53 (1H, d, J = 5.1 Hz), 7.54 (2H, d, J = 8.8 Hz), 7.08 (1H, d, J = 5.1 Hz), 6.94 (2H, d, J = 8.8 Hz), 4.17 (2H, q, J = 7.2 Hz), 3.82 (3H, s), 2.39 (3H, s), 1.08 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 168.8, 160.0, 156.0, 149.4, 145.4, 132.4, 129.6, 128.9, 123.1, 113.7, 61.3, 55.2, 19.3, 13.7; HRESIMS m/z 272.1268 $[M + H]^+$ (calcd for $C_{16}H_{18}NO_3$, 272.1287).

2-(4-Methoxyphenyl)-4-methyl-3-pyridinecarboxylic acid (8). Compound **7** (670 mg, 2.5 mmol) was refluxed overnight in aqueous NaOH (40%, 10 mL). The solution was extracted with $CHCl_3$ to remove any unreacted starting material. The pH value of the mixture was then adjusted to about 6, the solvent was removed under reduced pressure, and the residue was extracted with hot MeOH (3 × 10 mL). The MeOH extracts were combined and concentrated to a residue, which was purified on a silica column eluted with MeOH−CH_2Cl_2 (3:1) to give 2-(4-methoxy)phenyl-4-methyl-3-pyridinecarboxylic acid (**8**) (403 mg, 66%): off-white solid, mp 198−200 °C; ^1H NMR (500 MHz, D_2O) δ_H 8.43 (1H, d, J = 6.2 Hz), 7.78 (1H, d, J = 6.2 Hz), 7.62 (2H, d, J = 9.0 Hz,), 7.13 (2H, d, J = 9.0 Hz), 3.89 (3H, s), 2.59 (3H, s); ^{13}C NMR (125 MHz, D_2O) δ_C 171.6, 161.8, 155.4, 147.6, 139.1, 130.3, 126.7, 123.6, 115.0, 55.7, 49.0, 20.2; HRESIMS m/z 244.0958 $[M + H]^+$ (calcd for $C_{14}H_{14}NO_3$, 244.0974).

Table 2. Antiproliferative Activities of Compounds 1−6, 10, and 11 against the A2780 and H460 Cancer Cell Lines

	IC_{50} (μM)							
cancer cell line	**1**	**2**	**3**	**4**	**5**	**6**	**10**	**11**
A2780	12.7	54.3	10.3	3.5	35.7	0.60	11.1	50.3
H460	>10	>10	0.57	1.77	>100	0.58	>10	>100

7-Methoxyonychin (9). A solution of **8** (93 mg, 0.38 mmol) in 2 mL of $SOCl_2$ was refluxed 24 h under N_2. After the solvent was removed under vacuum, 51 mg (0.38 mmol) of $AlCl_3$ and 2 mL of chlorobenzene were added to the flask, and the mixture was refluxed overnight under N_2. The reaction was cooled and quenched by pouring into 10 mL of saturated $NaHCO_3$ solution. The resulting mixture was extracted with CH_2Cl_2 (3 × 10 mL) and dried over K_2CO_3, and the CH_2Cl_2 phase was evaporated. The residue was purified by silica gel PTLC (hexanes−EtOAc, 6:4) to afford 7-methoxyonychin (**9**) (21 mg, 25%): yellow solid, mp 134−136 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.30 (1H, d, J = 5.2 Hz), 7.79 (1H, d, J = 8.2 Hz), 7.18 (1H, d, J = 2.3 Hz), 7.04 (1H, dd, J = 8.2, 2.3 Hz), 6.90 (1H, d, J = 5.2 Hz), 3.87 (3H, s), 2.59 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 192.4, 164.8, 162.5, 151.3, 148.2, 136.9, 134.5, 126.1, 125.0, 122.7, 120.4, 109.0, 55.8, 17.3; HRESIMS m/z 226.0865 $[M + H]^+$ (calcd for $C_{14}H_{12}NO_2$, 226.0868).

8-Methoxyeupolauridine (10). A solution of 33 mg (0.15 mmol) of **9** plus *N*,*N*-dimethylformamide diethyl acetal in 1 mL of DMF was stirred 2 h at 120 °C under N_2. NH_4OAc (600 mg) was added to the flask, the reaction mixture was stirred for another 30 min at 140 °C and quenched with 5 mL of water, and the solvent was extracted with EtOAc (3 × 5 mL). The organic phase was combined, dried over K_2CO_3, and concentrated to a residue, which was separated on a silica TLC plate (hexanes−EtOAc, 6:4) to give 8-methoxyeupolauridine (**10**) (25.4 mg, 74%): yellow solid, mp 138−140 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.63 (1H, d, J = 5.7 Hz), 8.57 (1H, d, J = 5.7 Hz), 7.81 (1H, d, J = 8.2 Hz), 7.49 (1H, d, J = 1.4 Hz), 7.35 (1H, d, J = 5.7 Hz), 7.28 (1H, d, J = 5.7 Hz), 6.89 (1H, dd, J = 8.2, 1.4 Hz), 3.89 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 162.8, 162.5, 162.2, 149.7, 149.6, 142.0, 134.9, 131.9, 123.7, 121.3, 117.8, 116.3, 115.5, 109.0, 55.7; HRESIMS m/z 235.0875 $[M + H]^+$ (calcd for $C_{15}H_{11}N_2O$, 235.0871).

8-Hydroxyeupolauridine (1). A solution of **10** (6 mg, 0.026 mmol) in 1 mL of 48% HBr was refluxed for 24 h. The mixture was then cooled and evaporated in vacuo to give a residue, which was purified by PTLC on silica gel (hexanes−EtOAc, 6:4). 8-Hydroxyeupolauridine (**1**) (2.5 mg, 43%) was collected as a yellow solid, mp 278−280 °C. Its ^1H NMR and HRESIMS spectra were identical to those of the isolated material; ^{13}C NMR, see Table 1.

8-Methoxyeupolauridine 1-oxide (11) and 9-methoxyeupolauridine 1-oxide (2). A solution of 8-methoxyeupolauridine (**10**, 10 mg, 0.045 mmol) and *meta*-chloroperoxybenzoic acid (7.4 mg, 0.043 mmol) in 1 mL of CH_2Cl_2 was stirred at room temperature for 24 h. After the solvent was removed, the residue was separated by C-18 HPLC (60% MeOH−H_2O) to afford 9-methoxyeupolauridine 1-oxide (**2**) (1.6 mg, 15%) (mp 198−200 °C) and 8-methoxyeupolauridine 1-oxide (**11**) (5.9 mg, 55%). The NMR and mass spectroscopic data for **2** were identical to those of the natural product; ^{13}C NMR, see Table 1.

8-Methoxyeupolauridine 1-oxide (11): yellow solid, mp 218−220 °C; ^1H NMR (600 MHz, CD_3OD) δ_H 8.65 (1H, d, J = 5.8 Hz), 8.21 (1H, d, J = 8.5 Hz), 8.16 (1H, d, J = 7.1 Hz), 7.72 (1H, d, J = 7.1 Hz), 7.65 (1H, d, J = 5.8 Hz), 7.61 (1H, d, J = 2.3 Hz), 7.07 (1H, dd, J = 8.4, 2.4 Hz), 3.96 (3H, s); ^{13}C NMR (150 MHz, CD_3OD) δ_C 164.6, 159.3,

149.8, 145.6, 144.5, 141.1, 130.7, 128.4, 126.3, 125.3, 123.1, 119.3, 116.5, 111.2, 56.5; HRESIMS m/z 251.0807 $[M + H]^+$ (calcd for $C_{15}H_{11}N_2O_2$, 251.0821).

Antiproliferative Bioassays. The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported,[25] except that the samples were added in 1 μL of 100% DMSO per well instead of 20 μL of 1:1 DMSO−H_2O. The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[26] Assays against the H460 NSCLC lung cancer cell line were carried out at Eisai, Inc., as previously described.[1]

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of **1**, **2**, and **7**−**11** and ^{1}H NMR spectra of **3**−**6**. These materials are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: (540) 231-6570. Fax: (540) 231-3255. E-mail: dkingston@vt.edu.

Present Addresses

‡Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA.

■ ACKNOWLEDGMENT

This International Cooperative Biodiversity Group project was supported by the Fogarty International Center, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart Lung and Blood Institute, the National Center for Complementary and Alternative Medicine, the Office of Dietary Supplements, the National Institute of General Medical Sciences, the Biological Sciences Directorate of the National Science Foundation, and the Office of Biological and Environmental Research of the U.S. Department of Energy under Cooperative Agreement U01 TW00313 with the International Cooperative Biodiversity Groups. This project was also supported by the National Research Initiative of the Cooperative State Research, Education and Extension Service, USDA, Grant #2008-35621-04732. These supports are gratefully acknowledged. Work at Virginia Polytechnic Institute and State University was supported by the National Science Foundation under Grant CHE-0619382 for the purchase of the Bruker Avance 600 NMR spectrometer and Grant CHE-0722638 for the purchase of the Agilent 6220 mass spectrometer. We thank Mr. B. Bebout for obtaining the mass spectra, and Prof. J. K. Zjawiony, University of Misisippi, for consulting with us on the structure of his synthetic compound **12**. Fieldwork essential for this project was conducted under a collaborative agreement between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and a multilateral agreement between the ICBG partners, including the Centre National d'Application des Recherches Pharmaceutiques. We gratefully acknowledge courtesies extended by the Government of Madagascar (Ministère des Eaux et Forêts). We thank S. Randrianasolo, R. Rakotondrajaona, R. L. Andriamiarisoa, V. Benjara, and M. Velonjara for assistance with the plant collection.

■ REFERENCES

(1) Biodiversity Conservation and Drug Discovery in Madagascar, Part 47. For Part 46, see: Pan, E.; Harinantenaina, L.; Brodie, P.; Callmander, M; Rakotonandrasana, S.; Rakotobe, E.; Rasamison, V. E.; TenDyke, K.; Shen, Y.; Suh, E. M.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2011**, *19*, 422–428.

(2) McLaughlin, J. L. *J. Nat. Prod.* **2008**, *71*, 1311–1321.

(3) Nakanishi, Y.; Chang, F. R.; Liaw, C. C.; Wu, Y. C.; Bastow, K. F.; Lee, K. H. *J. Med. Chem.* **2003**, *46*, 3185–3188.

(4) Zhang, H. J.; Ma, C. Y.; Van Hung, N.; Cuong, N. M.; Tan, G. T.; Santarsiero, B. D.; Mesecar, A. D.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. S. *J. Med. Chem.* **2006**, *49*, 693–708.

(5) Wang, S.; Zhang, Y. J.; Chen, R. Y.; Yu, D. Q. *J. Nat. Prod.* **2002**, *65*, 835–841.

(6) Li, C.; Lee, D.; Graf, T. N.; Phifer, S. S.; Nakanishi, Y.; Riswan, S.; Setyowati, F. M.; Saribi, A. M.; Soejarto, D. D.; Farnsworth, N. R.; Falkinham, J. O.; Kroll, D. J.; Kinghorn, A. D.; Wani, M. C.; Oberlies, N. H. *J. Nat. Prod.* **2009**, *72*, 1949–1953.

(7) Li, C.; Lee, D.; Graf, T. N.; Phifer, S. S.; Nakanishi, Y.; Burgess, J. P.; Riswan, S.; Setyowati, F. M.; Saribi, A. M.; Soejarto, D. D.; Farnsworth, N. R.; Falkinham, J. O.; Kroll, D. J.; Kinghorn, A. D.; Wani, M. C.; Oberlies, N. H. *Org. Lett.* **2005**, *7*, 5709–5712.

(8) Chang, F. R.; Hwang, T. L.; Yang, Y. L.; Li, C. E.; Wu, C. C.; Issa, H. H.; Hsieh, W. B.; Wu, Y. C. *Planta Med.* **2006**, *72*, 1344–1347.

(9) Khamis, S.; Bibby, M. C.; Brown, J. E.; Cooper, P. A.; Scowen, I.; Wright, C. W. *Phytother. Res.* **2004**, *18*, 507–510.

(10) Lekphrom, R.; Kanokmedhakul, S.; Kanokmedhakul, K. *J. Ethnopharmacol.* **2009**, *125*, 47–50.

(11) Waterman, P. G.; Muhammad, I. *Phytochemistry* **1985**, *24*, 523–527.

(12) Rao, J. U. M.; Giri, G. S.; Hanumaiah, T.; Rao, K. V. J. *J. Nat. Prod.* **1986**, *49*, 346–347.

(13) Peterson, J. R.; Zjawiony, J. K.; Liu, S.; Hufford, C. D.; Clark, A. M.; Rogers, R. D. *J. Med. Chem.* **1992**, *35*, 4069–4077.

(14) Orabi, K. Y.; Li, E.; Clark, A. M.; Hufford, C. D. *J. Nat. Prod.* **1999**, *62*, 988–992.

(15) Leboeuf, M.; Cave, A. *Lloydia* **1977**, *39*, 459–460.

(16) Bowden, B.; Picker, K.; Ritchie, E.; Taylor, W. *Aust. J. Chem.* **1975**, *28*, 2681–2701.

(17) Tong, T. H.; Wong, H. N. C. *Synth. Commun.* **1992**, *22*, 1773–1782.

(18) Bracher, F. *Arch. Pharm.* **1989**, *322*, 293–294.

(19) Zjawiony, J. K.; Srivastava, A. R.; Hufford, C. D.; Clark, A. M. *Heterocycles* **1994**, *39*, 779–800.

(20) Jiang, C.; Xu, M.; Wang, S.; Wang, H.; Yao, Z.-J. *J. Org. Chem.* **2010**, *75*, 4323–4325.

(21) Zjawiony, J. K. Personal communication. January 13, 2011.

(22) Taylor, W. C. *Aust. J. Chem.* **1984**, *37*, 1095–1104.

(23) Hufford, C. D.; Liu, S.; Clark, A. M.; Oguntimein, B. O. *J. Nat. Prod.* **1987**, *50*, 961–964.

(24) Muhammad, I.; Dunbar, D. C.; Takamatsu, S.; Walker, L. A.; Clark, A. M. *J. Nat. Prod.* **2001**, *64*, 559–562.

(25) Cao, S.; Brodie, P. J.; Miller, J. S.; Randrianaivo, R.; Ratovoson, F.; Birkinshaw, C.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2007**, *70*, 679–681.

(26) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. *Cancer Res.* **1985**, *45*, 2110–2115.