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Note

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Antiproliferative Acetogenins from a *Uvaria* sp. from the Madagascar Dry Forest[1]

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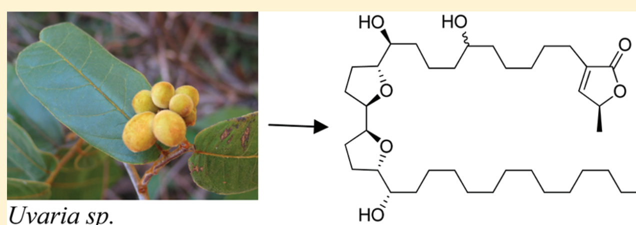
S *Supporting Information*

ABSTRACT: Investigation of the endemic Madagascan plant *Uvaria* sp. for antiproliferative activity against the A2780 ovarian cancer cell line led to the isolation of two new acetogenins. The structures of these two compounds were elucidated on the basis of analysis of their 1D and 2D NMR spectra, circular dichroism, and mass spectrometric data, together with chemical modification. The two acetogenins display weak antiproliferative activity against the A2780 ovarian cancer, the A2058 melanoma, and the H522 lung cancer cell lines.



Uvaria sp.

As part of our engagement in an International Cooperative Biodiversity Group (ICBG) program, we are focusing on searching for antiproliferative natural products from both tropical dry forests and rain forests in Madagascar. As a part of this research, an EtOH extract from the aerial parts of a *Uvaria* sp. (Annonaceae) from the dry forest of northern Madagascar exhibited weak antiproliferative activity against the A2780 human ovarian cancer cell line, with an IC_{50} value of 20 μg/mL. Compounds of the Annonaceae family are well known for their broad range of bioactivity, including immunosuppression, antimalarial, insecticidal, antifeedant, and antitumor activities.[2−6] The genus *Uvaria* has been investigated extensively, with over 300 references in the chemical and biological literature, and the chemical constituents of *Uvaria* species have been summarized.[7,8] The genus *Uvaria* is also one of only seven of the 120 genera of the Annonaceae family known to produce acetogenins,[9] and uvaricin, the first example of these compounds, was isolated from the roots of *Uvaria accuminata* Oliv. by Jolad et al. in 1982.[10]

Although the genus *Uvaria* has been well investigated, no work has been done on this new species. A total of 17 *Uvaria* species are currently known in Madagascar, and 11 remain to be described.[11] This extract was thus selected for bioassay-guided fractionation to isolate its active components.

The EtOH extract of the aerial parts of the *Uvaria* sp. was subjected to liquid−liquid partitioning to give an active CH_2Cl_2 fraction with an IC_{50} value of 20 μg/mL. Bioassay-guided separation, including LH-20 size-exclusion, silica gel normal-phase, and C_{18} reverse-phase chromatography, was used to obtain the two new acetogenins uvaricin A (**1**) and uvaricin B (**2**) with modest antiproliferative activity against A2780 ovarian cancer cells, with IC_{50} values of 6.4 and 8.8 μM, respectively. Herein, we report the structural elucidation and antiproliferative properties of the two isolates.

Uvaricin A (**1**), $[\alpha]^{21}_{D}$ +23 (*c* 1.2, MeOH), was isolated as a white, wax-like solid. The positive ion HRESIMS of uvaricin A revealed a quasi-molecular ion peak at *m/z* 623.4908 $[M + H]^+$ and adduct ions at *m/z* 640.5147 $[M + NH_4]^+$ and 645.4726 $[M + Na]^+$, corresponding to a molecular formula of $C_{37}H_{66}O_7$. A carbonyl absorption at 1747 cm^{-1} in its IR spectrum, UV absorption at 210 nm (MeOH), ^{1}H NMR signals at δ_H 6.98 (H-35), 4.98 (H-36), and 0.88 (H-34), and ^{13}C NMR resonances at δ_C 174.2 (C-1), 149.1 (C-35), 134.6 (C-2), 77.6 (C-36), and 19.4 (C-37) were indicative of a methylated α,β-unsaturated γ-lactone ring (Table 1).[12,13] This was confirmed by three-bond HMBC correlations observed between H-35 and C-1 and between H-35 and C-37, as well as two-bond HMBC correlations between H-35 and C-2 and between H-36 and C-37 (Figure S1a). Three OH functionalities in **1** were evident from the IR absorption at 3376 cm^{-1} and the ^{13}C NMR resonances at δ_C 74.3 (C-21), 72.1 (C-8), and 71.6 (C-12). The presence of a bis-THF ring system with two flanking OH groups was suggested by the four ^{13}C NMR

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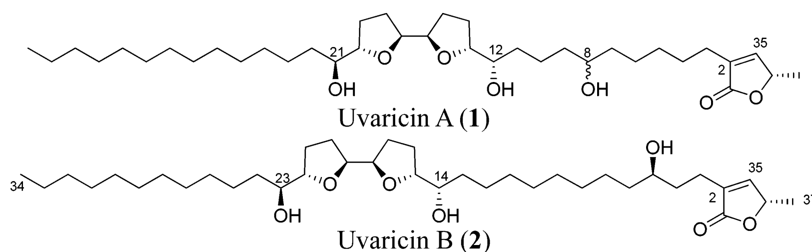

Table 1. NMR Spectroscopic Data for 1 and 2 in $CDCl_3$ (500 MHz)

	1		2	
position	δ_H (*J*, Hz)	δ_C, type	δ_H (*J*, Hz)	δ_C, type
1		174.2, C		174.0, C
2		134.6, C		133.9, C
3a	2.26 tdd (7.2, 1.6, 1.6)	25.1, CH_2	2.37 dtd (15.2, 7.6, 1.6)	21.6, CH_2
3b	2.26 tdd (7.2, 1.6, 1.6)		2.51 dtd (15.2, 7.6, 1.6)	
4	1.54 m	27.6, CH_2	1.65 m	35.4, CH_2
5	1.25 m	29.6–29.9, CH_2	3.60 m	70.9, CH
6	1.25 m	25.4, CH_2	1.39 m	37.6, CH_2
7	1.39–1.42 m	37.7, CH_2	1.48 m	26.1, CH_2
8	3.60 m	72.1, CH	1.26 m	29.6–29.8, CH_2
9	1.39–1.42 m	37.4, CH_2	1.26 m	29.6–29.8, CH_2
10	1.25 m	22.2, CH_2	1.26 m	29.6–29.8, CH_2
11	1.39–1.42 m	32.7, CH_2	1.26 m	29.6–29.8, CH_2
12	3.87 m	71.6, CH	1.48 m	25.6, CH_2
13	3.83 m	83.0, CH	1.41 m	32.5, CH_2
14a	1.62 m	28.6, CH_2	3.86 m	71.4, CH
14b	1.87 m			
15a	1.62 m	29.1, CH_2	3.83 m	82.9, CH
15b	1.87 m			
16a	3.93 m	82.5, CH	1.67 m	28.4, CH_2
16b			1.82 m	
17a	3.91 m	82.8, CH	1.67 m	29.0, CH_2
17b			1.82 m	
18a	1.60 m	29.1, CH_2	3.93 m	82.4, CH
18b	1.97 m			
19a	1.60 m	28.6, CH_2	3.91 m	82.6, CH
19b	1.97 m			
20a	3.84 m	83.5, CH	1.61 m	29.1, CH_2
20b			1.95 m	
21a	3.39 m	74.4, CH	1.61 m	29.4, CH_2
21b			1.95 m	
22	1.39–1.42 m	33.5, CH_2	3.84 m	83.3, CH
23	1.25 m	25.9, CH_2	3.37 m	74.2, CH
24	1.25 m	29.6–29.9, CH_2	1.41 m	33.5, CH_2
25	1.25 m	29.6–29.9, CH_2	1.48 m	25.7, CH_2
26–31	1.25 m	29.6–29.9, CH_2	1.26 m	29.6–29.8, CH_2
32	1.25 m	32.1, CH_2	1.26 m	32.00, CH_2
33	1.25 m	22.8, CH_2	1.26 m	22.8, CH_2
34	0.88 t (6.9)	14.3, CH_3	0.86 t (6.9)	14.2, CH_3
35	6.98 td (1.5, 1.5)	149.1, CH	7.05 td (1.5, 1.5)	149.3, CH
36	4.98 qdd (6.8, 1.5, 1.5)	77.6, CH	5.01 qd (6.8, 1.5)	77.7, CH
37	1.41, d (6.8)	19.4, CH_3	1.40 d (6.9)	19.3, CH_3

resonances at δ_C 83.5, 83.0, 82.8, and 82.5, which were correlated to the proton signals at δ_H 3.79–3.93 (5H) in the HMQC spectrum, as described for similar structures.[14] The existence of the THF ring was confirmed by the HMBC correlations between C-13 and H-16. The one-bond $^1H-^{13}C$ correlations detected in the HMQC spectrum, along with the observed mutiple-bond $^1H-^{13}C$ correlations in the HMBC spectrum, permitted the assignment of the carbon signals at δ_C 74.4 and 71.6 to the carbons adjacent to the THF rings at C-12 and C-21, respectively, with the proton signals at δ_H 3.39 and 3.87. A third OH group in the hydrocarbon chain was suggested by the resonances at δ_H 3.60 and δ_C 72.1.

The locations of the THF rings and the third OH group on the hydrocarbon chain were determined by analysis of 1D NMR, HMBC, HMQC, and COSY spectroscopic data and confirmed by analysis of the EIMS fragmentation shown in

Figure S2a. In the vicinity of the THF moiety, the chemical shift at δ 22.2 in the ^{13}C NMR spectrum was assigned to C-10 by the observation of an HMBC long-range correlation between H-12 (δ_H 3.87) and C-10 (δ_C 22.2). The third OH was assigned to C-8 by the presence of HMBC correlations between H-9 and C-10, and H-8 and C-9, as well as the COSY coupling of H-9 and H-8. The presence of an oxygen-bearing methine at C-8 was confirmed by the HMBC correlations between H-9 and C-7, H-8 and C-7, H-7 and C-6, H-4 and C-6, H-4 and C-3, H-3 and C-5, and H-3 and C-35, as well as a COSY cross-peak between H-4 and H-5. The number of carbons between the THF rings and the lactone ring was therefore restricted to 10.

The planar structure was further supported by the analysis of EIMS fragmentation. Intense fragment ions were observed at m/z 399, 347, 295, and 207. As shown in Figure S2a, these fragmentations are consistent with those observed for similar acetogenins, and they fully support the assigned structure.[15] In particular, the unusual loss of H_2O accompanied by H_2 has been observed before.[16]

The relative configurations around the bis-THF rings were determined by analysis of the ^1H NMR spectrum and comparison with literature data. In Figure S3a, the chemical shifts at δ_H 3.39 (H-21) and 3.84 (H-20) indicated the S^* and S^* relative configurations, while the S^* and R^* relative configurations were deduced for C-12 and C-13 and for C-16 and C-17, due to the similarity of the chemical shift of H-12 (δ_H 3.87) and H-13 (δ_H 3.83) and that of H-16 (δ_H 3.93) and H-17 (δ_H 3.91).[17,18] The methylene protons H-18 and H-19 were assigned to signals at δ_H 1.60 and 1.97 on the basis of COSY correlations between H-20 and H-21, H-19 and H-20, and H-18 and H-17, combined with HMBC coupling between H-19 and C-17. The difference between these chemical shifts indicated a *trans* configuration for the C-17/C-20 THF ring.[19] In the same manner, a *trans* configuration was assigned to the C-13/C-16 THF ring based on the divergent chemical shifts at δ_H 1.62 and 1.87 for H-14 and H-15.[19] Thus, the relative configuration around the bis-THF rings was concluded to be S^*, R^*, R^*, S^*, S^*, S^* for C-12, C-13, C-16, C-17, C-20, and C-21. The absolute configuration of C-36 was determined by CD spectroscopic analysis. The negative n$-\pi^*$ Cotton effect ($\Delta\varepsilon$ = -1.13) at 238 nm and a positive $\pi-\pi^*$ Cotton effect ($\Delta\varepsilon$ = 8.79) at 208 nm clearly indicated the *S* configuration at C-36 in the lactone ring.[16] The absolute configurations of the OH groups adjacent to the THF rings were determined by Mosher ester methodology[20] using the (*R*)-MPA and (*S*)-MPA derivatives[21] and the "in NMR tube" method.[22] As shown in Figure S4a, the $\Delta\delta_H$ ($=\delta_S - \delta_R$) was negative on both chain sides and positive along the THF rings, indicating *S* configurations at both C-12 and C-21.[23] The configuration of C-8 could not be determined due to the negative $\Delta\delta_H$ values displayed by both H-7 and H-9 on the hydrocarbon chain.

Uvaricin B (**2**), $[\alpha]^{21}_D$ +18 (c 1.2, MeOH), was also isolated as a white, wax-like solid. The positive ion HRESIMS of **2** revealed a quasi-molecular ion peak at m/z 623.4908 $[M + H]^+$ and adduct ions at m/z 640.5147 $[M + NH_4]^+$ and 645.4726 $[M + Na]^+$, corresponding to a molecular formula of $C_{37}H_{66}O_7$. The NMR, IR, and UV data of compound **2** were very similar to those of compound **1**. As with compound **1**, absorptions typical of a methylated α,β-unsaturated γ-lactone ring in its IR and UV spectra and of a bis-THF ring system with two flanking OH groups in the ^1H and ^{13}C NMR spectra were observed (Table 1). These structures were supported by the long-range HMBC correlations shown in Figure S1b. A third OH group was also present in the hydrocarbon chain, as indicated by NMR signals at δ_H 3.60 and δ_C 70.9.

The locations of the THF rings and of the third OH group on the hydrocarbon chain were determined by analysis of the 1D NMR, HMBC, HMQC, and COSY spectra and confirmed by analysis of EIMS fragmentations. The two diastereotopic protons (δ_H 2.37/2.51) were assigned to H_2-3 on the basis of HMBC correlations between H-3 and C-1, H-3 and C-2, and H-35 and C-3. The presence of an HMBC long-range coupling between a methylene carbon at δ_C 21.5 and H-3, as well as its connected proton (δ_H 1.65) and C-2, confirmed the assignment of this methylene carbon at C-4. The adjacent oxygen-bearing carbon was located at C-5 by the presence of HMBC correlations between H-4 and C-5, and H-3 and C-5. The C-5 OH was confirmed by comparing the chemical shift of H-35 with those of other acetogenins of the same molecular formula and with OH groups at C-4, C-5, C-6, C-12, or C-15. As previously reported, the chemical shift of H-35 in C-5 hydroxylated acetogenins such as narumicin I and calamistrin F[24,25] is always more shielded (δ_H 7.05) than with C-4 hydroxylated acetogenins, such as bullatacin ($\delta_{H\text{-}35}$ 7.16) and squamotacin ($\delta_{H\text{-}35}$ 7.17).[26] Its chemical shift is however deshielded compared with the corresponding protons of acetogenins without an OH group near the lactone ring, such as the C-12 hydroxylated acetogenin, folianin B ($\delta_{H\text{-}35}$ 6.98),[27] and the C-15 hydroxylated acetogenin, guanaconetin-1 ($\delta_{H\text{-}35}$ 6.98).[28] Acetogenins with an OH at C-5 could be distinguished from C-6 hydroxylated acetogenins by their divergent chemical shift at H-3, albeit sharing similar $\delta_{H\text{-}35}$ values.[29]

Analysis of the EIMS fragmentation supported the above structure. The fragment ions at m/z 155 and 135 are consistent with the conclusion that the third OH is connected to C-5, while the fragment ions at m/z 437, 367, 355, and 267 and their subsequent daughter ions indicated that the bis-THF rings and the two flanking OH groups are located between C-14 and C-23 (Figure S2b).

The relative configurations around the bis-THF rings were determined by examination of the data from proton NMR spectra and comparison to those in the literature. As shown in Figure S3b, the ^1H NMR chemical shifts around the bis-THF rings for compound **2** were similar to those of compound **1**, and the relative configurations were therefore assigned as S^*, R^*, R^*, S^*, S^*, S^* for C-14, C-15, C-17, C-18, C-22, and C-23. The CD spectrum of **2** revealed a negative n$-\pi^*$ Cotton effect ($\Delta\varepsilon$ = -1.15) at 238 nm and a positive $\pi-\pi^*$ Cotton effect ($\Delta\varepsilon$ = 9.64) at 208 nm, indicating an *S* configuration at the stereogenic center (C-36) in the lactone ring.[16] The absolute configurations of the three secondary alcohols were determined by Mosher ester methodology in the same way as for **1**. As depicted in Figure S4b, the $\Delta\delta_H$ ($=\delta_S - \delta_R$) is negative on the chain side and positive toward the THF rings, indicating the *S* configuration of both C-14 and C-23.[23] The positive values for H-3 and H-4 and negative values for H-6 to H-8 indicated an *S* configuration for C-5.

Uvaricin A (**1**) and B (**2**) were evaluated for antiproliferative activity against the A2780 ovarian cancer, the A2058 melanoma, and the H522 lung cancer cell lines (Table 2). Both compounds showed weak inhibition of all three cell lines, with IC_{50} values in the low micromolar range. Several hundred acetogenins have been identified from these plants, but not all of them have antiproliferative activity. According to previous studies of the structure–activity relationships of acetogenins,

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Table 2. Antiproliferative Activities of Compounds 1 and 2

compound	IC_{50} (μM)		
	A2780	A2058	H522
1	6.4	6.6	<10
2	8.8	7.2	<10
paclitaxel	0.02	ND	ND

the relative configuration of the THF rings and the positions of the OH groups on the hydrocarbon chains have a significant influence on their activities. The *threo-cis-threo-cis-erythro* configuration was described as the most potent subgroup of acetogenins with bis-THF rings;[30] as an example, rolliniastatin-1 has an IC_{50} value of 0.7×10^{-7} μM against SW480 human colon cancer cells.[31] In addition, the presence of two OH groups adjacent to the THF rings is necessary for potent inhibitory effects.[32] The moderate cytotoxicity of uvaricins A and B can thus be explained by the stereochemistry of the bis-THF rings flanked by the two OH groups, whose configuration is different from that of rolliniastatin-1. The weak antiproliferative activity may also be due to the number and the location of the OH groups.[32,33]

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO P-2000 polarimeter. UV and IR spectroscopic data were measured on a Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively. CD spectra were obtained on a JASCO J-815 circular dichroism spectrometer. NMR spectra were recorded in CD_3OD or $CDCl_3$ on either JEOL Eclipse 500 or Bruker Avance 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 LC-TOF-MS in the positive ion mode; NH_4^+ adducts are commonly observed with this instrument. HPLC was performed on a Shimadzu LC-10AT instrument with a semipreparative C18 Varian Dynamax column (5 μm, 250 × 10 mm).

Plant Material. The above-ground parts of *Uvaria* sp. were collected in July 2005. The plant was a liana with immature yellowish-green fruit and probably represents a new species still to be described.[34] The collection was made in dry degraded evergreen broadleaf forest near Betsimiranjana, in the Ambilobe region of Madagascar, at coordinates 13°02′35″ S, 049°09′15″ E. Voucher specimens have been deposited at the Parc Botanique and Zoologique de Tsimbazaza (TAN), the Centre National d'Application des Recherches Pharmaceutiques in Antananarivo, Madagascar (CNARP), the Missouri Botanical Garden in St. Louis, Missouri (MO), and the Muséum National d'Histoire Naturelle in Paris, France (P), voucher Stéphan Rakotonandrasana et al. 923.

Antiproliferative Bioassays. Antiproliferative activities were obtained at Virginia Polytechnic Institute and State University against the drug-sensitive A2780 human ovarian cancer cell line as previously described.[35] Antiproliferative activities against the A2058 melanoma and the H522 lung cancer cell lines were determined at Eisai Inc. by similar procedures to those used for the H460 cell line.[36]

Extraction and Isolation. Dried aerial parts of *Uvaria* sp. (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 3352 (24.9 g), of which 6.9 g was shipped to Virginia Tech for bioassay-guided isolation against the A2780 cell line. A 1.0 g sample of MG 3352 (IC_{50} 20.0 μg/mL) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 100 mL) and extracted with hexane (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 hexane fraction was evaporated in vacuo to leave 567 mg of material with IC_{50} >20 μg/mL. The residue from the CH_2Cl_2 fraction (200 mg) had an IC_{50} of 20 μg/mL, and the remaining aqueous MeOH fraction had an IC_{50} >20 μg/mL. LH-20 size exclusion open column chromatography of the CH_2Cl_2 fraction was used to obtain four fractions, of which the most active fraction (106 mg) had an IC_{50} of 6.9 μg/mL. This fraction was applied to a silica gel column eluted with a gradient of hexane−EtOAc, 9:1, 4:1, and 0:1, to give four fractions, of which the 100% EtOAc fraction (71.0 mg) was the most active (IC_{50} 5.5 μg/mL). A C-18 open column was employed for further separation, eluted by 90% MeOH to give eight fractions, and the most active one (IC_{50} 4.3 μg/mL) was separated by C-18 HPLC with the solvent system MeOH−H_2O, 85:15, and a flow rate of 2 mL/min. This yielded two active compounds, with IC_{50} values of 4.0 and 5.5 μg/mL and with elution times of 28.3 and 35 min, respectively.

Uvaricin A (1): white, wax-like solid; $[\alpha]^{21}_D$ +23 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (ε) 210 (2.60); IR ν_{max} cm^{-1} 3376, 2920, 1748, 1466, 1027 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 645.4726 [M + Na]$^+$ (calcd for $C_{37}H_{66}NaO_7$, 645.4726).

Uvaricin B (2): white, wax-like solid; $[\alpha]^{21}_D$ +18 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (ε) 210 (2.20); IR ν_{max} cm^{-1} 3449, 2923, 1753, 1460, 1024 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 645.4726 [M + Na]$^+$ (calcd for $C_{37}H_{66}NaO_7$, 645.4726).

Preparation of the (*R*)- and (*S*)-MPA Ester Derivatives of 1 and 2. In a 25 mL round-bottom flask, 5.00 mg of (*R*)-(−)-α-methoxyphenylacetic acid ((*R*)-MPA) was dissolved in 5 mL of CH_2Cl_2 and 15 mg of oxalyl chloride. Several drops of dimethylformamide were added to the reaction system as a catalyst. The reactants were mixed with a magnetic stir bar in an ice bath for 1 h to obtain 4.98 mg of (*R*)-(−)-α-methoxyphenylacetyl chloride (91% yield). The in-NMR-tube reaction was carried out to prepare the MPA ester derivatives.[22] The selected acetogenin (0.2 mg) dissolved in $CDCl_3$ was transferred to a clean NMR tube, and the solvent was completely evaporated. (*R*)-(−)-α-Methoxyphenylacetyl chloride (0.5 mg) was dissolved in $CDCl_3$ (0.5 mL) and added to the NMR tube immediately under an N_2 gas flow, and the NMR tube was shaken carefully to mix the acetogenin and MPA chloride. The reaction NMR tube was kept at room temperature under N_2 for 6 h to form the (*R*)-MPA ester and was then analyzed by ^{1}H NMR spectroscopy. The (*S*)-MPA ester of the acetogenin was prepared from (*S*)-MPA by the same method.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1−S4 and ^{1}H and ^{13}C NMR spectra of **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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spectra and Dr. H. Azurmendi for assistance with the NMR spectra. We also thank Dr. T. Deroin, a specialist on the Annonaceae family, for confirming the determination of the collection. Field work 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'Applications des Recherches Pharmaceutiques. We gratefully acknowledge courtesies extended by the Government of Madagascar (Ministère des Eaux et Forêts).

■ DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

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