Antiproliferative Cardenolides of an *Elaeodendron* sp. from the Madagascar Rain Forest[1]

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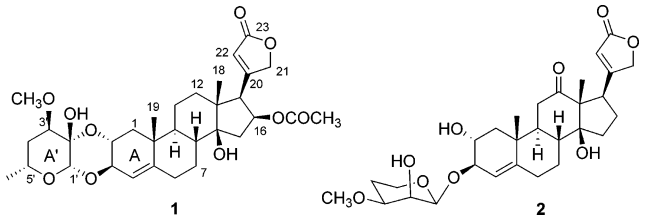
Bioassay-guided fractionation of an ethanol extract obtained from the Madagascar plant *Elaeodendron* sp. led to the isolation of two new cardenolides, elaeodendrosides T and U (**1** and **2**). The structures of the new compounds were elucidated using 1D and 2D NMR experiments and mass spectrometry. Compounds **1**, **3**, **4**, and **5** showed significant antiproliferative activity against A2780 human ovarian cancer cells with IC_{50} values of 0.085, 0.019, 0.19, and 0.10 μM, respectively, while compounds **2** and **6** were less active.

In our continuing search for bioactive molecules from the Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[1] we obtained an EtOH extract of the wood of *Elaeodendron* sp. (Celastraceae). This extract (MG 3232) showed antiproliferative activity against the A2780 ovarian cancer cell line with an IC_{50} value of 7.6 μg/mL. The extract was selected for bioassay-guided fractionation on the basis of its activity.

There are ca. forty species in the genus *Elaeodendron* from the Mexican coast, Bermuda, Africa, Madagascar (including Mascarene), India, Melanesia, and Australia.[2] The plants in this genus are usually glabrous trees or shrubs,[2] and flavonoids,[3] terpenoids,[4] and cardenolides[5] have been isolated from them. Cardenolides are also prominent cardioactive secondary metabolites of many medicinal plants belonging to the Apocynaceae (*Nerium, Strophanthus, Thevetia*), Asclepiadaceae (*Periploca, Calotropis, Xysmalobium*), Scrophulariaceae (*Digitalis*), Ranunculaceae (*Adonis*), and Convallariaceae families (*Convallaria, Speirantha*).[6] The cytotoxicity of cardenolides and their cardiac activity have been widely studied.[7]

An EtOH extract of the wood (MG 3232) of *Elaeodendron* sp. was subjected to liquid−liquid partitioning to give an active CH_2Cl_2 fraction with an IC_{50} value of 0.3 μg/mL in the A2780 assay. Activity-guided separation of this fraction by passage over a C_{18} SPE column yielded three subfractions, and subjection of the active second subfraction to further purification using C_{18} HPLC led to the isolation of the two new compounds **1** and **2** and the four known compounds elaeodendroside B (**3**),[5f] elaeodendroside F (**4**),[5f] elaeodendroside G (**5**),[5f] and (2α,3β,14β)-trihydroxy-3-*O*-(4-deoxy-3-*O*-methyl-α-L-erythropentopyranosyl)card-4,20(22)-dienolide (**6**).[5g] Here we report the structures of the two new compounds elaeodendroside T (**1**) and U (**2**) as well as the antiproliferative activity of all the isolates.

Compound **1** was obtained as a white powder. Its HRFABMS (positive-ion mode) exhibited a quasimolecular ion peak at *m*/*z* 589.2962, consistent with a molecular composition of $C_{32}H_{45}O_{10}$ (calcd 589.3013). The ^{13}C NMR spectrum of compound **1** contained



32 signals, which were assigned to one methoxy, one acetoxy, three methyls, eight methylenes, 11 methines, and eight quaternary carbons on the basis of its ^1H NMR and HSQC spectra. The ^1H and ^{13}C NMR signals (C_6D_6, Table 1) at δ_C 170.3 (C-20), δ_C/δ_H 73.3 (C-21)/4.71 and 4.56 (both as br d, J = 17.9 Hz, H_2-21), δ_C/δ_H 119.0 (C22)/5.85 (br s, H22), and δ_C 173.4 (C23) indicated the presence of an α,β-unsaturated γ-lactone unit. The spin systems in ring A (H_2-1 through H-2 and H-3 to H-4: $CH_2-CH-CH-CH$), rings B and C (H_2-6 through H_2-7, H-8, H-9, and H_2-11 to H_2-12: $CH_2-CH_2-CH-CH-CH_2-CH_2$), and ring D ($H_2$-15 through H-16 to H-17: $CH_2-CH-CH$) of the aglycone were identified from the COSY and TOSCY spectra. The aglycone of **1** was established as a 2,3,14,16-tetraoxygenated card-4,20(22)-dienolide on the basis of its HMBC correlations (Figure 1). The acetoxy group attached to C-16 was detected by HMBC correlations from both H-16 (δ_H 5.15, ddd, J = 11.3, 7.7, 4.1 Hz) and the methyl protons of the acetyl group (δ_H 1.70, s) to the carbonyl carbon at δ_C 169.8. H-3 showed a ROESY correlation (Figure 2) to H_α-1 (axial-like), and H-2 exhibited ROESY correlations to H_3-19 and H_β-1 (equatorial-like), indicating a *trans* relationship between H-3 and H-2. The *trans* and *cis* fusions for the rings B/C and C/D were established by the ROESY correlations from H_α-1 (axial-like) to H-9, from H-15 to H-7, and from both H_3-18 and H_3-19 to H_β-11 (axial) and H-8, separately. The ROESY spectrum of **1** also revealed cross-peaks from H-17 to H-21, H-22, and H-16 and from H_3-18 to H-21 and H-22; the substituents at the 13-, 16-, and 17-positions were therefore designated β. The multiplicities and coupling constants of the protons in the sugar moiety were deduced from the ^1H NMR spectrum as follows: H-1′ showed a singlet at δ 5.04; H-3′ appeared as a broad singlet at δ 3.29; H_2-4′ resonated as two multiplets at δ 1.53 and 1.80; H-5′ appeared as a multiplet at δ 3.90; H_3-6′ resonated as a doublet at δ 1.22 (J = 5.2 Hz), while the 3′-OMe resonated at δ 2.90 as a singlet. The connectivity of protons in

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Table 1. $^{1}H^{a}$ and $^{13}C^{b}$ NMR Data of Compounds **1** and **2**

	^{1}H		^{13}C		
no.	**1**[c]	**2**[c]	**1**[c]	**1**[d]	**2**[d]
1	1.32 t (12.7), 1.75 m	1.28 m, 1.90 m	41.6	40.5	42.9
2	4.54 m	3.90 m	68.9	66.1	67.3
3	4.99 d (8.8)	3.90 m	70.7	69.1	79.7
4	5.45 s	5.25 s	119.3	117.2	119.0
5			145.3	145.4	145.0
6	1.85 m	1.80 br t (10.0), 1.90 m	31.4	30.5	30.6
7	0.60 m, 1.54 m	0.68 m, 1.50, m	28.6	28.0	28.3
8	1.00 m	1.25 m	41.2	40.0	40.0
9	0.70 m	0.90 m	50.4	48.9	46.5
10			40.6	40.0	40.0
11	0.85 m, 1.00 m	1.90 m, 2.15 dd (3.9, 13.7)	21.2	20.4	36.8
12	1.00 m, 1.20 m		39.6	39.0	211.5
13			48.6	48.3	63.7
14			84.2	82.7	84.5
15	1.75 m, 1.82 m	0.80 dd (9.9, 12.0), 0.90 dd (7.7, 12.0)	39.6	39.0	31.7
16	5.15 ddd (11.3, 7.7, 4.1)	1.28 m, 1.50 m	78.7	77.8	26.2
17	2.34 d (3.9)	3.97 dd (8.6, 9.4)	57.9	56.5	40.0
18	0.45 s	0.48 s	15.4	15.0	16.1
19	0.75 s	0.64 s	19.8	19.4	18.8
20			170.3	172.6	173.7
21	4.71 br d (17.9) 4.56 br d (17.9)	4.39 d (17.9) 4.27 d (17.9)	73.3	73.0	73.3
22	5.85 br s	5.79 br s	119.0	116.4	117.1
23			173.4	173.2	175.2
1′	5.04 s	4.65 d (3.3)	96.7	94.0	98.3
2′		3.60 ddd (3.3, 4.4, 9.4)	91.0	90.1	66.7
3′	3.29 br s	3.03 m	80.9	79.3	77.4
4′	1.53 m, 1.80 m	1.15 m, 1.50 m	33.6	33.4	25.5
5′	3.90 m	3.05 m, 3.90 m	66.0	64.8	60.5
6′	1.22 d (5.2)		21.3	20.4	
2-OH		4.54 s			
14-OH		6.13 s			
2′-OH	3.61 s	2.65 d (9.4)			
OCH_3	2.90 s	2.94 s	57.2	57.2	54.8
$OCOCH_3$			169.8	169.8	
$OCOCH_3$	1.70 s		20.5	20.3	

[a] δ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses. [b] δ (ppm) 125 MHz. [c] In C_6D_6. [d] In DMSO-d_6.

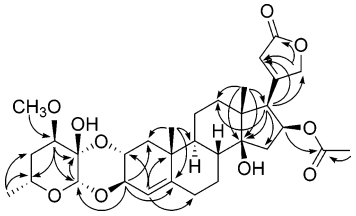


Figure 1. Key HMBC correlations of **1**.

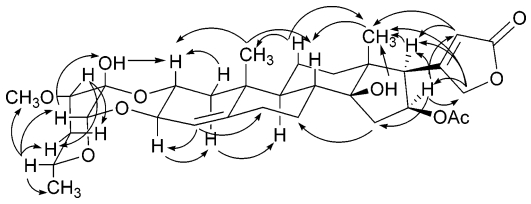


Figure 2. Key ROESY correlations of **1**.

ring A′ (H-3′ through H_2-4′ and H-5′, to H_3-6′: CH−CH_2−CH−CH_3) was confirmed by the COSY and TOCSY spectra. ROESY correlations from H-1′ to 2′-OH (δ 3.61) and from H-5′ to H-1′ and 3′-OMe indicated that the sugar moiety was a 2′-oxygenated 4′,6′-dideoxy-3′-*O*-methylallopyranoside. The connectivity between C-1′ and C-3 through an oxygen bridge was confirmed by the observation of an HMBC correlation from H-1′ to C-3.

In order to determine the orientation of the hydroxyl groups at the 14- and 2′-positions, NMR data were collected in DMSO-d_6 (^{1}H NMR data, Experimental Section; ^{13}C NMR data, Table 1). The 14-OH proton (δ_H 4.59, s) showed ROESY correlations to H-18 (δ_H 0.78, s), H-8 (δ_H 1.57), and H-15 (δ_H 2.06), which confirmed a *cis* fusion of rings C/D. ROESY correlations from 2′-OH (δ_H 6.12, s) to H-1′ (δ_H 4.54, s), H-3′ (δ_H 3.15, br s), and H-2 (δ_H 4.06, ddd, J = 2.8, 8.5, 12.1 Hz) supported the presence of another oxygen bridge from C-2′ to C-2, which formed a 1,4-dioxane ring in the chair conformation between rings A and A′. Furthermore, the ^{13}C NMR chemical shifts of the carbons in rings A′, A, and B of **1** were close to those of affinoside F (**7**),[8] while the carbons in rings C, D, and E had similar chemical shifts to those of cryptostigmin II (**8**).[9] The structure of **1** was thus established as indicated; it was given the trivial name elaeodendroside T.

Compound **2** was also obtained as a white powder. The molecular formula $C_{29}H_{40}O_9$ of **2** was deduced from its HRFABMS. Its ^{1}H NMR spectral data (C_6D_6, Table 1) showed signals for a cardenolide framework, with methylene protons at δ_H 4.27 and 4.39 (H_2-21, d each, J = 17.9 Hz), an olefinic proton at δ_H 5.79 (H-22, br s), and a methine proton at δ_H 3.97 (H-17, dd, J = 8.6, 9.4 Hz). The methyl doublet and acetyl methyl present in **1** were absent in the ^{1}H NMR spectrum of **2**. The ^{13}C NMR (DMSO-d_6, Table 1) spectrum exhibited 29 signals comprised of one methoxy, two methyls, nine methylenes, 10 methines, and seven quaternary carbons, which were assigned from its HSQC spectrum. The HMBC spectrum of **2** (Figure 3) showed key correlations that established the location of the carbonyl group at C-12 and the sugar at C-3. The aglycone of **2** was thus established as a 2,3,14-trioxygenated 12-oxo-card-4,-20(22)-dienolide, which was the same as that of elaeodendroside R.[5h] The orientations of H-2 (β, axial-like) and H-3 (α, axial-like) of **2** were the same as those of **1** because H-2 (δ_H 3.90) and H-3

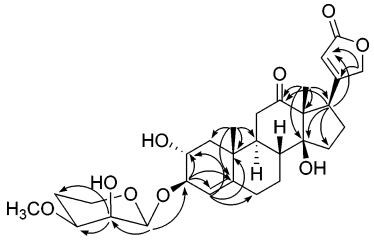


Figure 3. Key HMBC correlations of **2**.

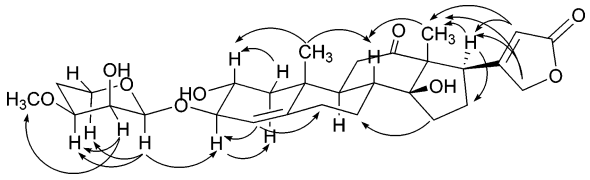
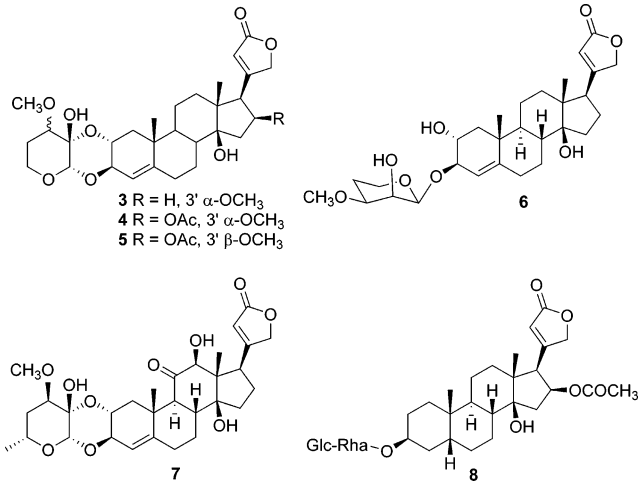


Figure 4. Key ROESY correlations of **2**.

(δ_H 3.90) showed ROESY (Figure 4) correlations to H_3-19 (δ_H 0.64) and 2-OH (δ_H 4.54), respectively (C_6D_6, Table 1), which was also supported by the multiplicity of H-3 (δ_H 3.92, d, $J = 8.0$ Hz) in DMSO-d_6 (Experimental Section). The COSY and TOCSY data for **2** identified a connectivity sequence indicative of a coupling system in the sugar moiety (H-1′ through H-2′, H-3′, and H_2-4′, to H_2-5′: CH−CH−CH−CH_2−CH_2). A small coupling constant of H-1′ at δ_H 4.65 (d, $J = 3.3$ Hz, C_6D_6, Table 1) indicated that H2′ was equatorial. ROESY cross-peaks from H-1′ to H-3′ and from H-2′ to H-3′ and 3′-OMe suggested H-3′ was axial. These considerations established the structure of **2** as indicated, and it was given the trivial name elaeodendroside U.



Compounds **1**−**6** were tested for antiproliferative activity against the A2780 human ovarian cancer cell line, and the two most active compounds, **1** and **3**, were also evaluated against four additional cell lines. The results are shown in Table 2. The assay results demonstrate that the 1,4-dioxane rings between rings A and A′ in compounds **1**, **3**, **4**, and **5** are important for their antiproliferative activity, since compounds **2** and **6**, lacking this structural feature, are significantly less active than the compounds with this feature.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a MIDAC M-series FTIR spectrophotometer. NMR spectra were obtained on a JEOL Eclipse 500 and an Inova 400 spectrometer. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive-ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semipreparative C_8 Varian Dynamax column (5 μm, 250 × 10 mm) and a preparative C_{18}Varian Dynamax column (8 μm, 250 × 21.4 mm).

Antiproliferative Activity. Determinations of antiproliferative activities were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line as previously described.[10] The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[11] Antiproliferative effects of compounds on the four cultured human cancer cell lines MDA-MB-435 breast cancer, HT-29 colon cancer, H522-T1 non-small-cell lung cancer, and U937 histiocytic lymphoma were performed at Eisai Research Institute as previously described,[12] with the exception that luminescence was read on an Envision 2102 multilabel reader.

Plant Material. Wood of the tree *Elaeodendron* sp. (Celastraceae) was collected in the Montagne des Français region, a dry forest on limestone, Antsiranana, Madagascar, at an elevation of 220 m, at 12.24.42 S, 49.22.22 E, on February 14, 2005. The tree was 14 m high with diameter at breast height of 55 cm, growing on a boulder near a stream, and it has yellow petioles, red bark, red wood, gray stem, and immature green fruit. It was determined by R. H. Archer (South African National Biodiversity Institute) in 2007 as a new species; its assigned collection number is Randrianasolo.S (SSR) 520. The species of *Elaeodendron* collected in Madagascar is different from *E. orientale* Jacq., the species known only from the Mascarene Islands. It has similar leaves, but the fruit is much smaller with sharp points at both ends. In addition, juvenile leaves are conspicuously long and narrow. The vernacular of the new species is *tangenala*. Species of *Cerbera* (Apocynaceae) with the same vernacular name were formerly used for poison practice in Madagascar. Nothing is known about the uses of *Elaeodendron* except that the fruit is reported to be toxic to lemurs. Voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); the Missouri Botanical Garden, St. Louis, Missouri (MO); and the Muséum National d'Histoires Naturelles, Paris, France (P).

Extraction and Isolation. Dried wood of *Elaeodendon* sp. (250 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 3232 (15.9 g), of which 5.0 g was shipped to Virginia Polytechnic Institute and State University (VPISU) for fractionation. MG 3232 (1.5 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 150 mL) and extracted with hexanes (3 × 150 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 160 mL portions). The CH_2Cl_2 extract was evaporated *in vacuo* to leave a 128.8 mg of residue (IC_{50}: 0.3 μg/mL). Both the hexane and aqueous MeOH extracts (40.3 mg and 1.3 g) were inactive. The CH_2Cl_2 extract was treated with C_{18} SPE eluting with 50%, then 100% MeOH−H_2O, and 5% CH_2Cl_2−MeOH to furnish three fractions (I−III: 25, 100, and 3 mg, respectively). Only fraction II was active, with an IC_{50} value of

Table 2. Antiproliferative Activity of Compounds **1**−**6**

	antiproliferative activity (IC_{50} against the indicated cell line, μM)				
compound	A2780[a]	MDA-MB-435[b]	HT-29[b]	H522-T1	U937[b]
1	0.085	0.37	0.18	0.18[c]	0.15
2	30	ND	ND	ND	ND
3	0.019	0.15	0.08	0.08[b]	0.05
4	0.19	ND	ND	ND	ND
5	0.10	ND	ND	ND	ND
6	2.5	ND	ND	ND	ND

[a] Average of three determinations. [b] Average of two determinations. [c] Single determination.

0.3 μg/mL, and this fraction was loaded on a C_{18} Varian Dynamax HPLC column [8 μm, 250 × 21.4 mm, 10 mL/min (0 min, 18 min, 40 min; 50%, 56%, 100% MeOH−H_2O)]. Thirteen subfractions (A−M) were collected. Subfractions J, K, and L yielded compounds **5** (3 mg, t_R35.5 min), **6** (1 mg, t_R38.5 min), and **1** (2 mg, t_R49 min), respectively. Purification of subfraction E was carried out by C_8 HPLC with 45% MeOH−H_2O as an eluent to yield **2** (0.5 mg, t_R24 min). Subfraction H yielded compound **3** (1.2 mg, R_f 0.30) and compound **4** (1.1 mg, R_f 0.35) after separation over preparative Si gel TLC developed with CH_2Cl_2−MeOH (20:1).

Elaeodendroside T (1): white powder; $[\alpha]_D^{23}$ +12 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.39) nm; IR (film) ν_{max} 3430, 2930, 1733, 1448, 1373, 1242, 1091, 1027 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 0.78 (3H, s, H_3-18), 0.87 (1H, t, J = 12.1 Hz, H-7a), 1.04 (3H, s, H_3-19), 1.11 (3H, d, J = 5.0 Hz, H_3-6′), 1.19 (1H, t, J = 11.3 Hz, H-9), 1.23 (1H, t, J = 13.0 Hz, H-11a), 1.38 (1H, dd, J = 12.1, 12.4 Hz, H-1a), 1.50 (1H, m, H-11b), 1.55 (1H, m, H-4a′), 1.57 (1H, m, H-8), 1.58 (1H, m, H-12a), 1.61 (1H, dd, J = 2.8, 12.4 Hz, H-1b), 1.75 (1H, dd, J = 2.0, 13.8 Hz, H-4b′), 1.98 (3H, s, 16-OAc), 2.06 (2H, m, H_2-6), 2.06 (1H, m, H-7b), 2.06 (1H, m, H-12b), 2.06 (2H, m, H_2-15), 2.68 (1H, d, J = 3.8 Hz, H-17), 3.15 (1H, br s, H-3′), 3.83 (1H, m, H-5′), 4.06 (1H, ddd, J = 2.8, 8.5, 12.1 Hz, H-2), 4.37 (1H, d, J = 8.5 Hz, H-3), 4.54 (1H, s, H-1′), 4.59 (1H, s, 14-OH), 4.89 (2H, br s, H_2-21), 5.08 (1H, br s, H-4), 5.14 (1H, ddd, J = 3.8, 8.0, 12.1 Hz, H-16), 5.98 (1H, s, H-22), 6.12 (1H, s, 2′-OH); ^1H NMR (500 MHz, C_6D_6) and ^{13}C NMR (125 MHz, C_6D_6 and DMSO-d_6) see Table 1; HRFABMS *m*/*z* 589.2962 (calcd for $C_{32}H_{45}O_{10}$, 589.3013).

Elaeodendroside U (2): white powder; $[\alpha]_D^{23}$ −9 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.01) nm; IR (film) ν_{max} 3434, 2938, 1736, 1445, 1370, 1240, 1067 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 0.98 (3H, s, H_3-18), 1.08 (3H, s, H_3-19), 3.24 (3H, s, 3′-OMe), 3.30 (1H, m, H-3′), 3.35 (1H, m, H-5a′), 3.53 (1H, m, H-2), 3.79 (1H, br s, H-2′), 3.86 (1H, m, H-5b′), 3.92 (1H, d, J = 8.0 Hz, H-3), 3.99 (1H, dd, J = 8.0, 8.3 Hz, H-17), 4.45 (1H, s, H-1′), 4.90 (2H, br s, H_2-21), 5.25 (1H, s, H-4), 5.96 (1H, s, H-22); ^1H NMR (500 MHz, C_6D_6) and ^{13}C NMR (125 MHz, DMSO-d_6) see Table 1; HRFABMS *m*/*z* 555.2528 (calcd for $C_{29}H_{40}O_9Na$, 555.2570).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Biodiversity Conservation and Drug Discovery in Madagascar, Part 28. For Part 27, see: Cao S.; Brodie, P. J.; Miller J. S.; Ratovoson, F.; Birkinshaw, C.; Randrianasolo, S.; Rakotobe, E.; Rasamison, V. E.; Kingston D. G. I. *J. Nat. Prod.* **2007**, *70*, 686−688.

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