



Published in final edited form as:

J Nat Prod. 2007 June ; 70(6): 1064–1067.

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

Shugeng Cao[†], Peggy J. Brodie[†], James S. Miller[‡], Fidy Ratovoson[§], Martin W. Callmänder[§], Sennen Randrianasolo[⊥], Etienne Rakotobe[⊥], Vincent E. Rasamison[⊥], Edward M. Suh^{||}, Karen TenDyke^{||}, and David G. I. Kingston^{†*}

Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166-0299, and B.P. 3391, Antananarivo, Madagascar, and Centre National d'Application des Recherches Pharmaceutiques, B. P. 702, Antananarivo 101, Madagascar, and Eisai Research Institute, 4 Corporate Drive, Andover, MA 01810

Abstract

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 IC₅₀ 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 IC₅₀ value of 7.6 μg/mL. The extract was selected for bioassay-guided fractionation based on its activity, and also on the absence of any previous chemical investigation of the species.

There are ca. forty species in the genus *Elaeodendron* from the Mexican coast, Bermuda, Africa, Madagascar (incl. Mascarene), India, Melanesia, and Australia.² The plants in this genus are usually glabrous trees or shrubs,² and flavonoids,³ terpenoids,⁴ and cardenolides⁵ 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 as well as their cardiac activity have been widely studied.⁷

An EtOH extract of the woods (MG 3232) of *Elaeodendron sp.* was subjected to liquid-liquid partitioning to give an active CH₂Cl₂ fraction with an IC₅₀ value of 0.3 μg/mL in the A2780

*To whom correspondence should be addressed. Tel: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu.

[†]Virginia Polytechnic Institute and State University.

[‡]Missouri Botanical Garden, St. Louis; current address: The New York Botanical Garden, 200th St. & Kazimiroff Blvd., Bronx, NY 10458-5126.

[§]Missouri Botanical Garden, Antananarivo.

[⊥]Centre National d'Application des Recherches Pharmaceutiques.

^{||}Eisai Research Institute.

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

assay. Activity-guided separation of this fraction by passage over a C₁₈ SPE column yielded three subfractions, and subjection of the active second subfraction to further purification using C₁₈ 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 (2 α ,3 β ,14 β)-trihydroxy-3-O-(4-deoxy-3-O-methyl- α -L-erythro-pentopyranosyl)-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 C₃₂H₄₅O₁₀ (calcd 589.3013). The ¹³C NMR spectrum of compound **1** contained 32 signals, which were assigned to one methoxy, one acetoxy, three methyls, eight methylenes, eleven methines, and eight quaternary carbons based on its ¹H NMR and HSQC spectra. The ¹H and ¹³C NMR signals (C₆D₆, 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, H₂-21), 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 (H₂-1 through H-2 and H-3 to H-4: CH₂—CH—CH—CH), rings B and C (H₂-6 through H₂-7, H-8, H-9, and H₂-11 to H₂-12: CH₂—CH₂—CH—CH—CH₂—CH₂), and ring D (H₂-15 through H-16 to H-17: CH₂—CH—CH) of the aglycone were identified from the COSY and TOCSY spectra. The aglycone of **1** was established as a 2,3,14,16-tetraoxygenated card-4,20(22)-dienolide based on its HMBC correlations (Fig. 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 (Fig. 2) to H $_{\alpha}$ -1 (axial-like), and H-2 exhibited ROESY correlations to H₃-19 and H $_{\beta}$ -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 $_{\alpha}$ -1 (axial-like) to H-9, from H-15 to H-7, and from both H₃-18 and H₃-19 to H $_{\beta}$ -11 (axial) and H-8, separately. The ROESY spectrum of **1** also revealed crosspeaks from H-17 to H-21, H-22, and H-16, and from H₃-18 to H-21 and H-22; the substituents at 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; H₂-4' resonated as two multiplets at δ 1.53 and 1.80; H-5' appeared as a multiplet at δ 3.90; H₃-6' 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 ring A' (H-3' through H₂-4' and H-5', to H₃-6': CH—CH₂—CH—CH₃) 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-methyl-allopyranoside. 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 (¹H NMR data, Experimental Section; ¹³C 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 ¹³C 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 1H 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 (H₂-21, 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 1H 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, ten methines, and seven quaternary carbons, which were assigned from its HSQC spectrum. The HMBC spectrum of **2** (Fig. 3) showed key correlations which 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 orientation 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 (δ_H 3.90) showed ROESY (Fig. 4) correlations to H₃-19 (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 H₂-4', to H₂-5': CH—CH—CH—CH₂—CH₂). 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 crosspeaks 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 semi-preparative C₈ Varian Dynamax column (5 μ m, 250 \times 10 mm) and a preparative C₁₈ Varian Dynamax column (8 μ m, 250 \times 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 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 elevation: 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 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 collector number is Randrianasolo.S (SSR) 520. The species of *Elaeodendron* collected in Madagascar is different from *E. orientale* Jacq., the type of the genus, 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 the ordeal by 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); at the Missouri Botanical Garden, St. Louis, Missouri (MO); and at the Muséum National d'Histoires Naturelles, Paris, France (P).

Extraction and Isolation

Dried wood of *Elaeodendron* 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-H₂O, 9:1, 150 mL) and extracted with hexanes (3 × 150 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H₂O and extracted with CH₂Cl₂ (3 × 160 mL portions). The CH₂Cl₂ extract was evaporated *in vacuo* to leave a 128.8 mg of residue (IC₅₀: 0.3 µg/mL). Both the hexane and aqueous MeOH extracts (40.3 mg and 1.3 g) were inactive. The CH₂Cl₂ extract was treated with C₁₈ SPE eluting with 50%, then 100% MeOH-H₂O, and 5% CH₂Cl₂-MeOH to furnish three fractions (I–III: 25, 100, and 3 mg, respectively). Only fraction II was active with an IC₅₀ value of 0.3 µg/mL, and this fraction was loaded on a C₁₈ Varian Dynamax HPLC column [8 µm, 250 × 21.4 mm, 10 mL/min (0 min, 18 min, 40 min; 50%, 56%, 100% MeOH-H₂O)], and thirteen subfractions (A–M) were collected. Subfractions J, K and L yielded compounds **5** (3 mg, *t*_R 35.5 min), **6** (1 mg, *t*_R 38.5 min) and **1** (2 mg, *t*_R 49 min), respectively. Purification of subfraction E was carried out by C₈ HPLC with 45% MeOH-H₂O as an eluent to yield **2** (0.5 mg, *t*_R 24 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 CH₂Cl₂-MeOH (20:1).

Elaeodendroside T. (**1**)

white powder; $[\alpha]_D^{23} +12$ (c 0.05, 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, H₃18), 0.87 (1H, t, *J* = 12.1 Hz, H7a), 1.04 (3H, s, H₃19), 1.11 (3H, d, *J* = 5.0 Hz, H₃6'), 1.19 (1H, t, *J* = 11.3 Hz, H9), 1.23 (1H, t, *J* = 13.0 Hz, H11a), 1.38 (1H, dd, *J* = 12.1, 12.4 Hz, H1a), 1.50 (1H, m, H11b), 1.55 (1H, m, H4a'), 1.57 (1H, m, H8), 1.58 (1H, m, H12a), 1.61 (1H, dd, *J* = 2.8, 12.4 Hz, H1b), 1.75 (1H, dd, *J* = 2.0, 13.8 Hz, H4b'), 1.98 (3H, s, 16-OAc), 2.06 (2H, m, H₂6), 2.06 (1H, m, H7b), 2.06 (1H, m, H12b), 2.06 (2H, m, H₂15), 2.68 (1H, d, *J* = 3.8 Hz, H17), 3.15 (1H, br s, H3'), 3.83 (1H, m, H5'), 4.06 (1H, ddd, *J* = 2.8, 8.5, 12.1 Hz, H2), 4.37 (1H, d, *J* = 8.5 Hz, H3), 4.54 (1H, s, H1'), 4.59 (1H, s, 14-OH), 4.89 (2H, br s, H₂21), 5.08 (1H, br s, H4), 5.14 (1H, ddd, *J* = 3.8, 8.0, 12.1 Hz, H16), 5.98 (1H, s, H22), 6.12 (1H, s, 2'-OH); ¹H NMR (500 MHz, C₆D₆) and ¹³C NMR (125 MHz, C₆D₆ and DMSO-*d*₆) see Table 1; HRFABMS *m/z* 589.2962 (calcd for C₃₂H₄₅O₁₀, 589.3013).

Elaeodendroside U. (2)

white powder; $[\alpha]_D^{23}$ -9 (c 0.11, 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, H₃18), 1.08 (3H, s, H₃19), 3.24 (3H, s, 3'-OMe), 3.30 (1H, m, H3'), 3.35 (1H, m, H5a'), 3.53 (1H, m, H2), 3.79 (1H, br s, H2'), 3.86 (1H, m, H5b'), 3.92 (1H, d, J = 8.0 Hz, H3), 3.99 (1H, dd, J = 8.0, 8.3 Hz, H17), 4.45 (1H, s, H1'), 4.90 (2H, br s, H₂21), 5.25 (1H, s, H4), 5.96 (1H, s, H22); ^{13}C NMR (125 MHz, DMSO- d_6) see Table 1; HRFABMS m/z 555.2528 (calcd for C₂₉H₄₀O₉Na, 555.2570).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This project was supported by the Fogarty International Center, the National Cancer Institute, the National Science Foundation, the National Heart, Lung and Blood Institute, the National Institute of Mental Health, the Office of Dietary Supplements, and the Office of the Director of NIH, under Cooperative Agreement U01 TW 00313 with the International Cooperative Biodiversity Groups, and this support is gratefully acknowledged. We thank Mr. B. Bebout for obtaining the mass spectra, Mr. T. Glass for assistance with the NMR spectra and Dr. R. H. Archer at the National Herbarium, South African National Biodiversity Institute, Pretoria for data on the genus *Elaeodendron*. 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).

References and Notes

- Biodiversity Conservation and Drug Discovery in Madagascar, Part 28. For Part 27, see: Cao S, Brodie PJ, Miller JS, Ratovoson F, Birkinshaw C, Randrianasolo S, Rakotobe E, Rasamison VE, Kingston DGI. *J. Nat. Prod.* 2007
- (a) Archer RH, Van Wyk AE. *S. Afr. J. Bot* 1998;64:93–109. (b) Islam MB, Simmons MP, Archer RH. *Syst. Bot* 2006;31:512–524.
- (a) Weeratunga G, Bohlin L, Sandberg F, Kumar V. *Acta Pharm. Suecica* 1984;21:73–76. (b) Weeratunga G, Bohlin L, Verpoorte R, Kumar V. *Phytochemistry* 1985;24:2093–2095.
- (a) Anjaneyulu ASR, Narayana Rao M. *Phytochemistry* 1980;19:1163–1169. (b) Weeratunga G, Kumar V, Sultanbawa MUS, Balasubramaniam S. *J. Chem. Soc., Perkin Trans. 1: Org. Bioorg. Chem* 1982:2457–2459. (c) Weeratunga G, Kumar V, Sultanbawa MUS. *Tetrahedron Lett* 1982;23:2031–2032. (d) Weeratunga G, Kumar V, Sultanbawa MUS. *Aust. J. Chem* 1983;36:1067–1072. (e) Weeratunga G, Kumar V. *Phytochemistry* 1985;24:2369–2372. (f) Kubo I, Fukuhara K. *J. Nat. Prod* 1990;53:968–971. (g) Tsanuo MK, Hassanali A, Jondiko IJO, Torto B. *Phytochemistry* 1993;34:665–667.
- (a) Kupchan SM, Uchida I, Shimada K, Fei BY, Stevens DM, Sneden AT, Miller RW, Bryan RF. *J. Chem. Soc. Chem. Comm* 1977:255–256. (b) Shimada K, Nambara T, Uchida I, Kupchan SM. *Heterocycles* 1979;12:1445–1448. (c) Anjaneyulu ASR, Rao MN. *Indian J. Chem., Sect B* 1980;19B:944–949. (d) Shimada K, Kyuno T, Nambara T, Uchida I. *Heterocycles* 1981;15:355–360. (e) Shimada K, Kyuno T, Nambara T, Uchida I. *Chem. Pharm. Bull* 1982;30:4075–4081. [PubMed: 7165878] (f) Shimada K, Kyuno T, Nambara T, Uchida I. *Phytochemistry* 1985;24:1345–1350. (g) Shimada K, Ishii N, Ohishi K, Ro JS, Nambara T. *J. Pharmacobio-Dyn* 1986;9:755–759. [PubMed: 3025406] (h) Shimada K, Masuda H, Ohtaki H, Kobayashi N, Nambara T. *Heterocycles* 1990;30:441–450. (i) Tsujino Y, Ogoche JIJ, Tazaki H, Fujimori T, Mori K. *Phytochemistry* 1995;40:753–756. (j) Kasai HF, Kawai K, Shimada K. *Heterocycles* 2000;53:2689–2700.
- Pauli GF, Matthiesen U, Fronczek FR. *Phytochemistry* 1999;52:1075–1084.
- (a) Hoffmann JJ, Cole JR. *J. Pharm. Sci* 1977;66:1336–1338. [PubMed: 903878] (b) Hembree JA, Chang CJ, McLaughlin JL, Peck G, Cassady JM. *J. Nat. Prod* 1979;42:293–298. (c) Koike K, Bevelle C, Talapatra SK, Cordell GA, Farnsworth NR. *Chem. Pharm. Bull* 1980;28:401–405. [PubMed: 7389015] (d) Wagner H, Habermeier H, Schulten HR. *Helv. Chim. Acta* 1984;67:54–64. (e) Kamano

- Y, Sato N, Nakayoshi H, Pettit GR, Smith CR. *Chem. Pharm. Bull* 1988;36:326–332. [PubMed: 2837339] (f) Kaneda N, Chai H, Pezzuto JM, Kinghorn AD, Farnsworth NR, Tuchinda P, Udchachon J, Santisuk T, Reutrakul V. *Planta Med* 1992;58:429–431. [PubMed: 1470666] (g) Baek NI, Lee YH, Park JD, Kim SI, Ahn BZ. *Planta Med* 1994;60:26–29. [PubMed: 8134412] (h) Decosterd L, Gustafson KR, Cardellina JH II, Cragg GM, Boyd MR. *Phytotherapy Res* 1994;8:74–77. (i) Gil RR, Lin L-Z, Chai H-B, Pezzuto JM, Cordell GA. *J. Nat. Prod* 1995;58:848–856. [PubMed: 7673928] (j) Kitanaka S, Takido M, Mizoue K, Nakaike S. *Chem. Pharm. Bull* 1996;44:615–617. [PubMed: 8882460] (k) Kiuchi F, Fukao Y, Maruyama T, Obata T, Tanaka M, Sasaki T, Mikage M, Haque ME, Tsuda Y. *Chem. Pharm. Bull* 1998;46:528–530. [PubMed: 9549894] (l) Ankli A, Heilmann J, Heinrich M, Sticher O. *Phytochemistry* 2000;54:531–537. [PubMed: 10939358] (m) Laphookhieo S, Cheenpracha S, Karalai C, Chantrapromma S, Rat-a-pa Y, Ponglimanont C, Chantrapromma K. *Phytochemistry* 2004;65:507–510. [PubMed: 14759549] (n) Cheenpracha S, Karalai C, Rat-a-Pa Y, Ponglimanont C, Chantrapromma K. *Chem. Pharm. Bull* 2004;52:1023–1025. [PubMed: 15305009] (o) Roy MC, Chang F-R, Huang H-C, Chiang MY-N, Wu Y-C. *J. Nat. Prod* 2005;68:1494–1499. [PubMed: 16252914] (p) Lhinhatrakool T, Sutthivaiyakit S. *J. Nat. Prod* 2006;69:1249–1251. [PubMed: 16933890]
8. Hanada R, Abe F, Mori Y, Yamauchi T. *Phytochemistry* 1992;31:3547–3551.
9. Kamel MS, Assaf MH, Abe Y, Ohtani K, Kasai R, Yamasaki K. *Phytochemistry* 2001;58:537–542. [PubMed: 11576593]
10. Cao S, Brodie PJ, Randrianaivo R, Ratovoson F, Callmander M, Andriantsiferana R, Rasamison VE, Kingston DGI. *J. Nat. Prod.*
11. Louie KG, Behrens BC, Kinsella TJ, Hamilton TC, Grotzinger KR, McKoy WM, Winker MA, Ozols RF. *Cancer Res* 1985;45:2110–2115. [PubMed: 3986765]
12. Cao S, Norris A, Miller JS, Ratovoson F, Razafitsalama J, Andriantsiferana R, Rasamison VE, TenDyke K, Suh T, Kingston DGI. *J. Nat. Prod* 2007;70:361–366. [PubMed: 17263578]

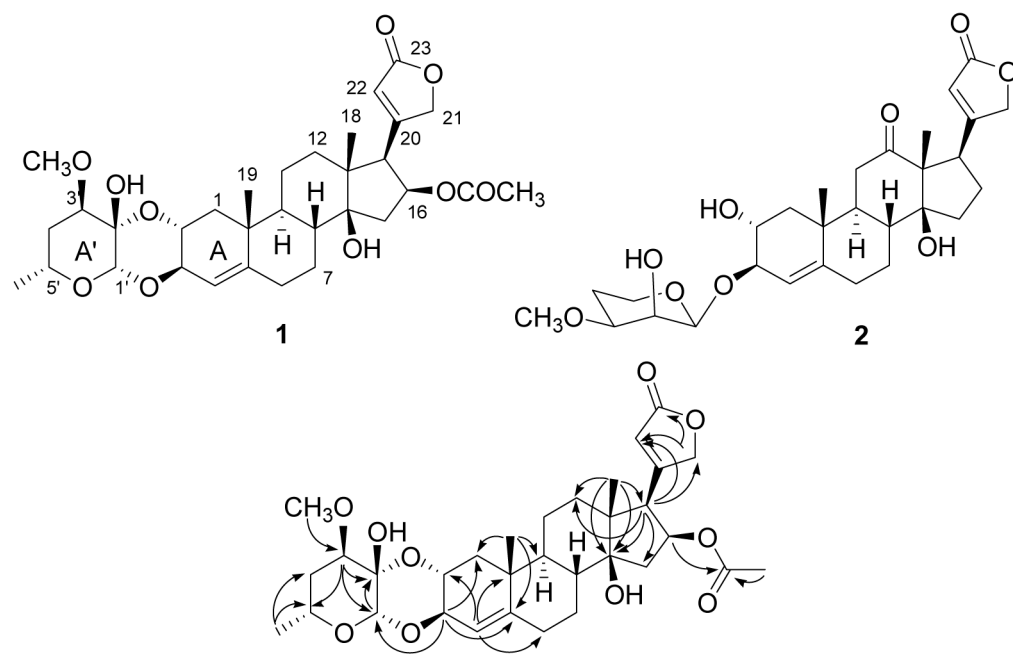


Figure 1.
Key HMBC correlations of **1**

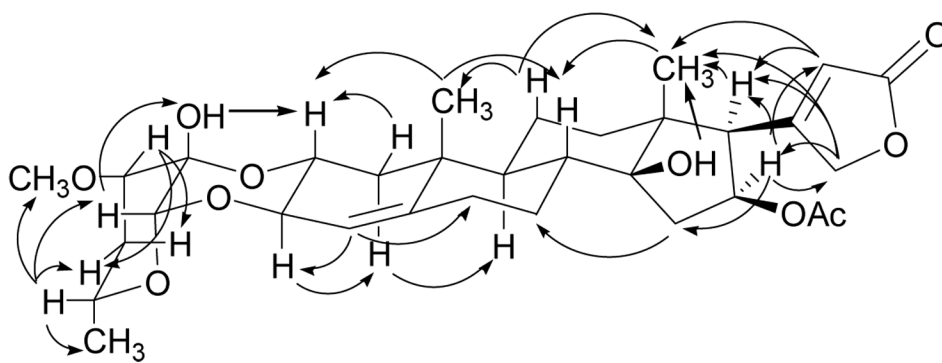


Figure 2.
Key ROESY correlations of **1**

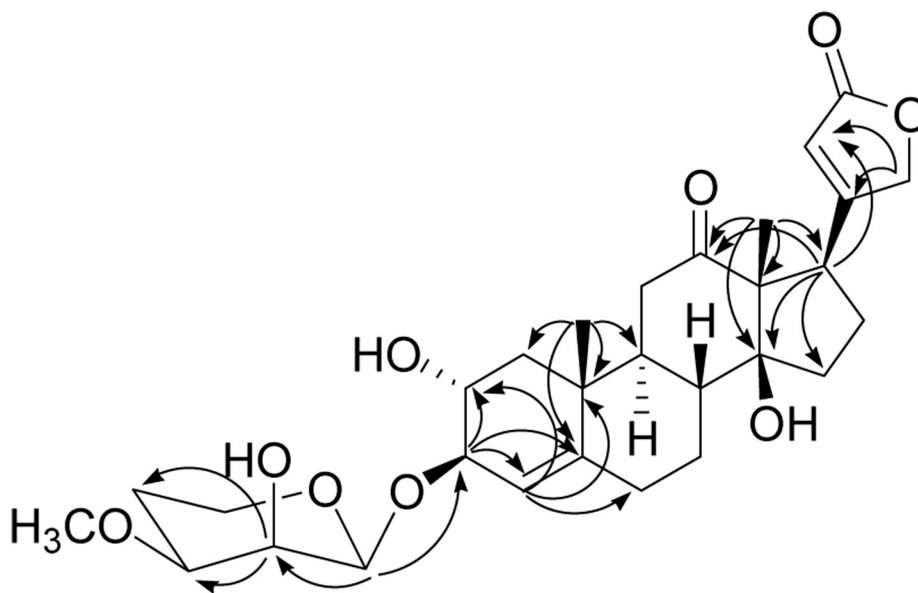


Figure 3.
Key HMBC correlations of 2

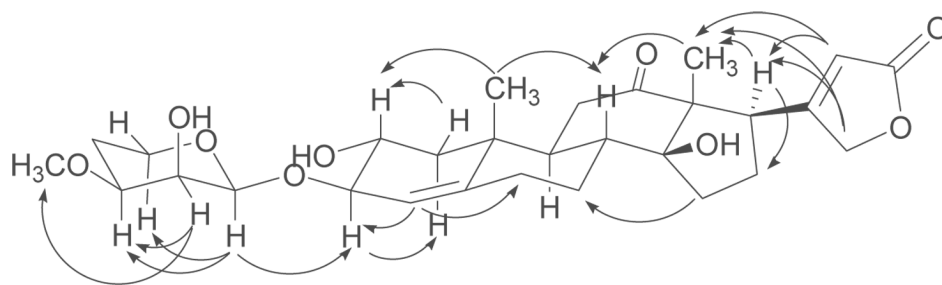


Figure 4.
Key ROESY correlations of **2**

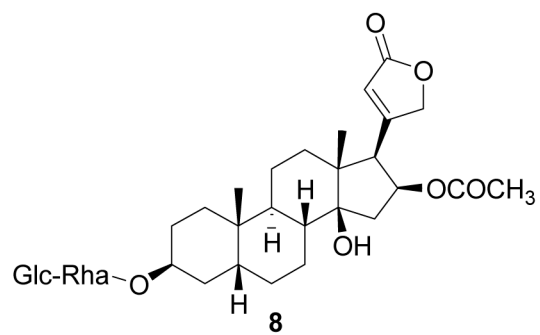
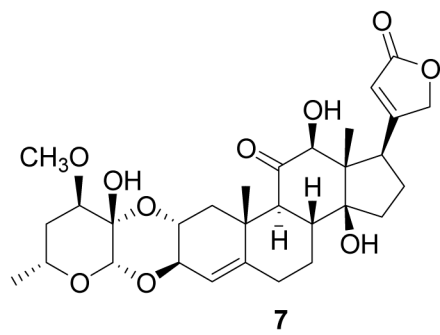
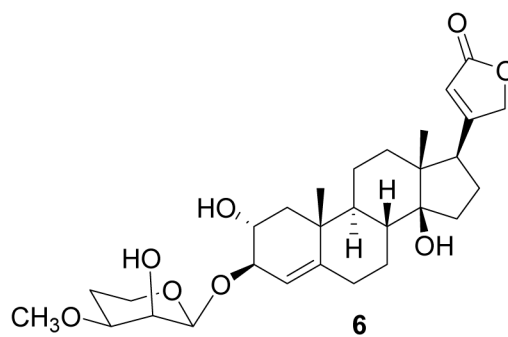
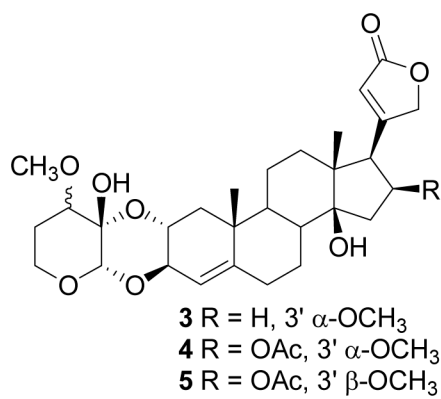


Table 1

 $^1\text{H}^a$ and $^{13}\text{C}^b$ NMR Data of Compounds 1 and 2

no.	^1H		^{13}C		
	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		145.0
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)	4.39 d (17.9)	73.3	73.0	73.3
22	4.56 br d (17.9)	4.27 d (17.9)			
23	5.85 br s	5.79 br s			
1'	5.04 s	4.65 d (3.3)	119.0	116.4	117.1
2'		3.60 ddd (3.3, 4.4, 9.4)	173.4	173.2	175.2
3'	3.29 br s	3.03 m	96.7	94.0	98.3
4'	1.53 m, 1.80 m	1.15 m, 1.50 m	91.0	90.1	66.7
5'	3.90 m	3.05 m, 3.90 m	80.9	79.3	77.4
6'	1.22 d (5.2)	3.05 m, 3.90 m	33.6	33.4	25.5
2-OH		4.54 s	66.0	64.8	60.5
14-OH		6.13 s			
2'-OH	3.61 s	2.65 d (9.4)	57.2	57.2	54.8
OCH_3	2.90 s	2.94 s	169.8	169.8	
OCOCH_3			20.5	20.3	
OCOCH_3	1.70 s				

 a δ (ppm) 500 MHz; multiplicities; J values (Hz) in parentheses. b δ (ppm) 125 MHz. c in C_6D_6 . d in $\text{DMSO}-d_6$.

Table 2

Antiproliferative Activity of Compounds **1** – **6**

compound	A2780 ^a	MDA-MB-435 ^b	antiproliferative activity (IC ₅₀ against the indicated cell line, μ M)	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