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Antiproliferative cardenolide glycosides of *Elaeodendron alluaudianum* from the Madagascar Rainforest ☆

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ABSTRACT

Bioassay-guided fractionation of an ethanol extract of a Madagascar collection of *Elaeodendron alluaudianum* led to the isolation of two new cardenolide glycosides (**1** and **2**). The ^{1}H and ^{13}C NMR spectra of both compounds were fully assigned using a combination of 2D NMR experiments, including ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY sequences. Both compounds **1** and **2** were tested against the A2780 human ovarian cancer cell line and the U937 human histiocytic lymphoma cell line assays, and showed significant antiproliferative activity with IC_{50} values of 0.12 and 0.07 μM against the A2780 human ovarian cancer cell line, and 0.15 and 0.08 μM against the U937 human histiocytic lymphoma cell line, respectively.

1. Introduction

In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Groups (ICBG) program,[2] we obtained an extract of the stems of a plant initially identified as a *Hippocratea* sp. from Madagascar. The extract had significant antiproliferative activity against the A2780 ovarian cancer cell line, and it was thus selected for bioassay-guided fractionation. While the work was in progress the plant was reidentified as *Elaeodendron alluaudianum* H. Perrier (Celastraceae). As noted previously,[3] there are about 40 species in the genus *Elaeodendron* from the Mexican coast, Bermuda, Africa, Madagascar (incl. the Mascarenes), India, Melanesia, and Australia.[4] The plants in this genus are usually glabrous trees or shrubs,[4] and flavonoids,[5] terpenoids,[6] and cardenolides[7] have been isolated from them. The cytotoxicities and cardiac activities of cardenolides have been widely studied.[8]

The extract of *E. alluaudianum* had an IC_{50} value of 3.3 μg/mL against the A2780 human ovarian cancer cell line. The crude extract afforded two new cardenolide glycosides, designated elaeodendroside V (**1**) and W (**2**), after solvent partitioning and reversed-phase C18 HPLC. Herein, we report the structural elucidation of the two new cardenolide glycosides and their bioactivities against the A2780 human ovarian cancer cell line and the U937 human histiocytic lymphoma cell line.

2. Results and discussion

Elaeodendroside V (**1**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{35}H_{54}O_{16}$ on the basis of a protonated molecular ion peak at *m*/*z* 731.3496 in its HRFAB mass spectrum. Its ^{1}H NMR spectrum in CD_3OD showed characteristic signals of an α,β-unsaturated γ-lactone (δ_H 5.01, dd, J = 18.4, 1.6 Hz, H-21a; δ_H 4.92, dd, J = 18.4, 1.6 Hz, H-21b; and δ_H 5.91, s, H-22) (Table 1). Its ^{13}C NMR spectrum contained

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35 signals (Table 2), which were assigned as two methyls, 11 methylenes (including three oxymethylenes), 16 methines (including 12 oxymethines and one olefinic carbon), and six quaternary carbons (including two oxyquaternary carbons, one olefinic carbon and one carbonyl carbon) based on ^{13}C NMR (Table 2) and HSQC spectra.

The complete ^{1}H and ^{13}C NMR signal assignments and connectivity were determined from a combination of COSY, TOCSY, HSQC, and HMBC data and comparison with the spectra of known cardenolides.[9]

COSY and TOCSY correlations established three spin systems, which were H_2-1–H_2-2–H-3–H_2-4 in ring A, H_2-6–H_2-7–H-8–H–9–H-11–H_2-12 in rings B and C, and H_2-15–H_2-16–H-17 in ring D (Fig. 1). Further assembly of rings A–D and the α,β-unsaturated γ-lactone of the aglycone was determined on the basis of HMBC correlations. HMBC correlations of H_2-19 to C-1, C-5, and C-9, H_2-1 to C-9, and H_2-6 to C-5 established the connectivity of rings A and B. Correlations of H_2-12, H_2-16, H-17, and H_3-18 to C-13 and of H_2-16 and H_3-18 to C-14 indicated the connectivity of rings C and D. In the meantime, an HMBC correlation of H_2-16 to C-20 suggested the location of the lactone ring at C-17 (Fig. 1).

These correlations established the flat structure of the aglycone of **1**. The structures of the sugar moieties of **1** were determined by 1D TOCSY, COSY, and HMBC data. Two sugar units were clearly shown by COSY and TOCSY correlations of two spin systems, H-1′–H-2′–H-3′–H-4′–H-5′–H_3-6′ and H-1″–H-2″–H-3″–H-4″–H-5″–H_2-6″ (Fig. 1). An HMBC correlation of H-3 to C-1′ indicated the sugars were connected to the aglycone at C-3. In the meantime, HMBC correlations of H-1″ to C-4′ and of H-4′ to C-1″ established that the two sugars were connected from C-1″ to C-4′ (Fig. 1).

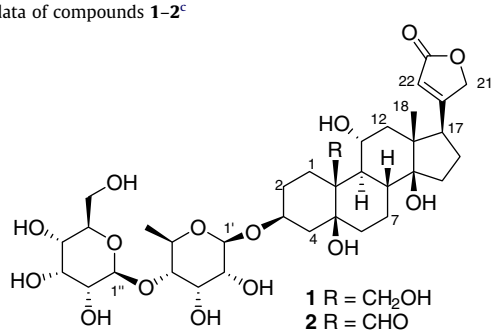
The relative configuration of the aglycone of **1** was established by analysis of its ROESY correlations and calculation of coupling constants (Fig. 2 and Table 1). The ROESY correlations of H_3-18 to H-22, H_2-21, H-8, and H-11, and of H_2-19 to H-8 and H-11 indicated that the C-17 side chain, H-8, H-11, and the oxymethylene at C-10 were all β-oriented. Calculation of coupling constants of H-11 (td, J = 9.8, 4.2 Hz) suggested that the adjacent H-9 occupied

Table 1
^{1}H NMR data of compounds **1** and **2**[c]

No.	**1**[a]	**1**[b]	**2**[b]
1	3.03 m	2.24 m	2.47 m
	2.85 td (13.8, 2.5)	2.1 9m	2.24 m
2	2.45 m	1.94 m	1.92 m
	2.25 m	1.82 m	1.85 m
3	4.48 m	4.15 m	4.19 br s
4	2.21 m	2.10 dd (15.4, 3.0),	2.09 m
	1.84 m	1.64 m	1.72 m
6	2.34 m	1.87 m	1.89 m
	1.67 br d (12.9)	1.47 m	1.68 m
7	2.44 m	1.99 m	2.06 m
	1.49 m	1.25 m	1.28 m
8	2.40 m	1.81 m	2.00 m
9	2.19 m	1.78 m	1.73 m
11	4.52 m	3.93 td (9.8, 4.2)	3.94 dd (9.8, 4.7)
12	1.97 m	1.66 m	1.67 m
	1.89 m	1.54 dd (13.2, 9.8)	1.49 dd (13.2, 10.8)
15	2.31 m	2.17 m	2.15 m
	1.97 m	1.72 m	1.68 m
16	2.09 m	2.17 m	2.14 m
	2.00 m	1.91 m	1.89 m
17	3.00 m	2.93 t (7.2)	2.94 t (7.2)
18	1.13 s	0.91 s	0.90 s
19	4.66 d (10.7)	4.18 d (11.2)	9.97 s
	4.38 m	3.80 m	
21	5.28 br d (18.2)	5.01 dd (18.4, 1.6),	5.00 br d (18.4)
	5.03 m	4.92 dd (18.4, 1.6)	4.92 br d (18.4)
22	6.11 s	5.91 s	5.91 s
1′	5.37 d (8.0)	4.72 d (8.0)	4.73 d (8.0)
2′	3.90–4.60[d]	3.35 m	3.35 m
3′	3.90–4.60[d]	4.33 t (3.0)	4.33 t (2.8)
4′	3.90–4.60[d]	3.28 dd (9.6, 3.0)	3.28 m
5′	3.90–4.60[d]	3.85 m	3.85 m
6′	1.60 d (6.0)	1.30 d (6.0)	1.30 d (6.0)
1″	5.49 d (7.7)	4.73 d (7.6)	4.73 d (8.0)
2″	3.90–4.60[d]	3.33 m	3.33 m
3″	3.90~4.60[d]	4.05 t (2.9)	4.05 t (2.8)
4″	3.90~4.60[d]	3.53 dd (9.2, 2.9)	3.52 dd (9.2, 2.9)
5″	3.90~4.60[d]	3.67 m	3.67 m
6″	4.37 m	3.84 m	3.84 m
	4.30 m	3.68 m	3.69 m
5-OH	5.88 s		
11-OH	5.77 d (4.7)		
14-OH	5.53 s		

[a] In pyridine-d_5.
[b] In CD_3OD.
[c] δ (ppm) 500 MHz.
[d] Overlapped resonances not assigned.

Table 2
^{13}C NMR data of compounds **1**–**2**[c]



No.	**1**[a]	**1**[b]	**2**[b]
1	22.7	22.4	21.3
2	27.6	27.7	27.1
3	74.8	76.2	75.5
4	35.3	35.9	35.7
5	76.3	77.5	75.5
6	37.3	37.0	38.7
7	25.0	25.1	25.5
8	40.8	41.1	42.2
9	45.1	45.2	46.1
10	45.4	45.7	56.7
11	68.7	69.1	68.5
12	50.7	50.6	50.1
13	51.0	51.8	51.0
14	85.0	85.9	85.4
15	33.5	33.5	33.1
16	28.0	28.0	28.0
17	51.4	51.1	51.5
18	18.2	17.8	17.6
19	65.6	65.9	211.1
20	174.8	177.2	177.2
21	74.0	75.5	75.5
22	118.1	118.1	118.1
23	175.8	177.7	177.5
1′	99.3	99.5	99.5
2′	65–80[d]	72.3	72.4
3′	65–80[d]	72.2	72.2
4′	83.8	84.0	84.0
5′	65–80[d]	69.9	69.9
6′	18.7	18.3	18.3
1″	104.3	103.7	103.7
2″	65–80[d]	72.4	72.4
3″	65–80[d]	73.3	73.3
4″	65~80[d]	68.6	68.6
5″	65–80[d]	75.4	75.4
6″	63.0	62.8	62.8

[a] In pyridine-d_5.
[b] In CD_3OD.
[c] δ (ppm) 100 MHz.
[d] Resonances not assigned because of overlapping ^{1}H NMR resonances in the HMBC spectrum.

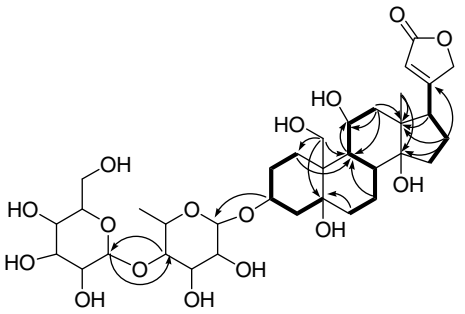


Figure 1. Key COSY (bold) and HMBC (arrows) correlations of **1**.

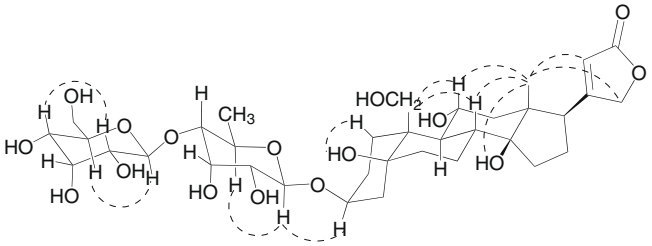


Figure 2. Key ROESY correlations of 1.

the α-orientation. The above assignments indicated that the B/C ring junction was *trans*-fused. ROESY spectra of **1** obtained in pyridine-d_5 showed a correlation of C-14-OH to H_3-18 that suggested a *cis*-fused ring junction of rings C and D. Determination of the relative configuration of the remaining portions of **1** was carried out by comparison with literature data.[9,8l] These comparisons indicated that rings A and B were connected by *cis*-fused ring junctions and that the C-3 side chain (sugar moiety) existed in the β-orientation. The relative configuration of the sugar moiety was established mainly by calculation of coupling constants and was further proved by ROESY correlations (Table 1 and Fig. 2). Coupling constants of H-1′ (d, J = 8.0 Hz), H-3′ (t, J = 3.0 Hz) and H-4′ (dd, J = 9.6, 3.0 Hz) indicated that H-1′, H-2′, H-4′, and H-5′ were in the axial orientation, and that H-3′ was in the equatorial orientation. Coupling constants of H-1″ (d, J = 7.6 Hz), H-3″ (t, J = 2.9 Hz) and H-4″ (dd, J = 9.2, 2.9 Hz) suggested that H-1″, H-2″, H-4″, and H-5″ were in the axial orientation, and that H-3″ was in the equatorial orientation. Those assignments were proved by ROESY correlations of H-1′ to H-5′, H-1″ to H-5″ and H-2″ to H-4″. In addition, the structure of the sugar moiety of **1** was confirmed by comparison with the known compounds sarmentogenin-3β-*O*-[β-allosyl-(1 → 4)-β-6-deoxyalloside] and securigenin-3β-*O*-[β-allosyl-(1 → 4)-β-6-deoxyalloside].[8l] The sugar moieties of **1** had identical ^{1}H and ^{13}C NMR data to those of the reported compounds. Therefore, the structure and configuration of **1** was determined as sarmentologenin-3β-*O*-[β-allosyl-(1 → 4)-β-6-deoxyalloside].

Elaeodendroside W (**2**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{35}H_{52}O_{16}$, which was two units less than that of **1**, on the basis of a sodiated molecular ion peak at m/z 751.311 in its MALDI-TOF/TOF mass spectrum. The ^{1}H NMR spectrum of **2** showed characteristic signals of a α,β-unsaturated γ-lactone (δ_H 5.00, br d, J = 18.4 Hz, H-21a, δ_H 4.92, br d, J = 18.4 Hz, H-21b, and δ_H 5.91, s, H-22) (Table 1). Comparison of the ^{1}H NMR and ^{13}C NMR spectra of compounds **1** and **2** showed that they were very similar, but that the oxymethylene resonances (δ_H 4.18, d, J = 11.2 Hz, H-19a, δ_H 3.80, m, H-19b and δ_C 65.9, C-19) that appeared in the spectra of **1** were absent in the spectra of **2**, and that the aldehyde resonances (δ_H 9.97, s, H-19 and δ_C 211.1, C-19) that appeared in the spectra of **2** were absent in the spectra of **1** (Tables 1 and 2). Those data indicated that **2** had a similar structure to **1** except that **2** has an aldehyde instead of a hydroxymethyl group at C-19. Further comparison of the ^{1}H and ^{13}C NMR spectra of **2** and the known cardenolide glycoside, sarmentosigenin-3β-*O*-β-6-deoxyguloside,[8l] confirmed the assignments, since the ^{1}H NMR and ^{13}C NMR data of the aglycone of **2** were identical to the literature data for sarmentosigenin. The structure of **2** was further confirmed by analysis of 2D NMR spectra including COSY, HMQC, HMBC, and ROESY spectra. Therefore, the structure and configuration of **2** was determined as sarmentosigenin-3β-*O*-[β-allosyl-(1 → 4)-β-6-deoxyalloside].

All of the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cancer cell line and the U937 human histiocytic lymphoma cell line. It was found that both **1** and **2** showed significant antiproliferative activity, with IC_{50} values of 0.12 and 0.07 μM against the A2780 human ovarian cancer cell line and 0.15 and 0.08 μM against the U937 human histiocytic lymphoma cell line, respectively. The antiproliferative activities of **1** and **2** do not appear to be correlated with the oxidation status of C-19 because their IC_{50} values were very close to each other. The known cardenolide glycoside, sarmentosigenin-3β-*O*-β-6-deoxyguloside, which possesses the same aglycone as **2** but contains a 6-deoxygulose, also showed significant cytotoxicity with an IC_{50} value of 0.074 μM against a KB cell line,[8l] while another cardenolide with an identical aglycone to **2** but glycosylated with rhamnose instead of allose also showed significant cytotoxicity with an IC_{50} value of 0.049 μM (0.028 μg/mL) against the HSG cell line.[10] Those data suggested that compounds with the same skeletons as **1** and **2** might show significant activities against cultured cancer cells. The cytotoxicity and antiproliferative activities of many structurally diverse cardenolide glycosides against cultured tumor cells have been widely investigated. Some recent reports are cited.[3,8m,8n,11] This class of compounds has not however found any clinical applications as anticancer agents, in part because of unfavorable toxicity profiles.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were performed on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. Mass spectra were obtained on a JEOL-JMS-HX-110 and an Applied Biosystems 4800 MALDI-TOF/TOF instruments. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. HPLC was performed with Shimadzu LC-10A pumps coupled with a Varian Dynamax semi-preparative C18 column (250 × 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector.

3.2. Antiproliferative bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[12] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[13]

The U937 human histiocytic lymphoma cell line assay was performed at Eisai Research Institute. The cells were cultured in 96-well plates in the absence or continuous presence of 0.005 to 10 μg/mL extract for 96 h. Cell growth was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturer's recommendations. Luminescence was read on the EnVision 2102 Multilabel Reader (Perkin-Elmer). IC_{50} values were determined as the concentration of an extract at which

cell growth was inhibited by 50% compared to untreated cell population. Two independent repeating experiments were performed.

3.3. Plant material

Root, stem, and leaf samples of *Elaeodendron alluaudianum* H. Perrier (Celastraceae) were collected in the forest of Bemosa, a dense humid forest, in northern Madagascar, at an elevation 200 m, at 13.14.17S, 49.37.50E, on November 2, 2005. The tree was 10 m high with diameter at breast height of 12 cm and white flowers. It was identified by R. H. Archer (South African National Biodiversity Institute); its assigned collector number is Randrianaivo et al. 1281.

3.4. Extraction and isolation

The stems of the dried plant sample described above (270 g) were extracted with EtOH to give 5.78 g of extract designated MG 3593. A total of 1.63 g of extract was supplied to VPISU, and this had an IC_{50} value of 3.3 μg/mL against A2780 cells. A portion of this extract (96 mg) was suspended in 20 mL of 30% MeOH/CH_2Cl_2 and filtered. The filtrate was evaporated to afford 67 mg residue (IC_{50} 0.14 μg/mL). The residue was suspended in aqueous MeOH (90% MeOH/H_2O, 10 mL), and extracted with *n*-hexane (3 × 10 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 15 mL). The aqueous MeOH extract (44 mg) was found to be the most active (IC_{50} 0.50 μg/mL) and a portion of this (39 mg) was loaded on a C18 SPE cartridge and eluted with solvent systems of 30% MeOH/H_2O, 70% MeOH/H_2O and MeOH to obtain 3 fractions (I–III). The most active fraction was Fraction I (IC_{50} 0.14 μg/mL), and this was separated via semi-preparative HPLC over a C18 column using MeOH/H_2O (75:25) to afford 12 fractions (IV–XV). Fraction XII afforded elaeodendroside V (**1**, 5.3 mg, t_R 35.7 min), and fraction XIV afforded elaeodendroside W (**2**, 2.4 mg, t_R 44.1 min).

3.5. Elaeodendroside V (1)

white amorphous solid; $[\alpha]_D^{23}$ +1.6 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.31) nm; IR: ν_{max} 3382, 2944, 2828, 1732, 1028 cm^{-1}; ^1H and ^{13}C NMR spectra, see Tables 1 and 2; HRFABMS *m*/*z* 731.3496 $[M+H]^+$ (calcd for $C_{35}H_{55}O_{16}$, 731.3490).

3.6. Elaeodendroside W (2)

white amorphous solid; $[\alpha]_D^{23}$ +1.6 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.34) nm; IR: ν_{max} 3382, 2945, 2833, 1731, 1026 cm^{-1}; ^1H and ^{13}C NMR spectra, see Tables 1 and 2; MALDI-TOF/TOF-MS *m*/*z* 751.311 $[M+Na]^+$ (calcd for $C_{35}H_{52}O_{16}Na$, 751.315).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.092.

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Cytotoxic Prenylated Stilbenes and Flavonoids from *Macaranga alnifolia* from the Madagascar Rainforest[#]

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Abstract

Bioassay-guided fractionation of an extract of the fruit of *Macaranga alnifolia* from Madagascar led to the isolation of four new prenylated stilbenes, schweinfurthins E–H (**1–4**), and one new geranylated dihydroflavonol, alnifoliol (**5**). The known prenylated stilbene, vedelianin (**6**), and the known geranylated flavonoids, bonanniol A (**7**), diplacol (**8**), bonannione A (**9**) and diplacone (**10**), were also isolated. All ten compounds were tested for antiproliferative activity in the A2780 human ovarian cancer cell line assay. Vedelianin (IC_{50} = 0.13 μM) exhibited the greatest activity among all isolates, while schweinfurthin E (IC_{50} = 0.26 μM) was the most potent of the new compounds.

The genus *Macaranga* is a large genus of the Euphorbiaceae family. Observation of *Macaranga* plants in their natural environment has revealed that they produce thread-like wax crystals on their ste ms, which make the slippery surfaces impassable for all insects except a species of ants known as “wax runners”. Chemical analysis has indicated that terpenoids make up a majority of the wax bloom content that helps maintain this symbiotic relationship between plant and insect.[2] One of the more commonly studied species of this genus is *M. tanarius*, noted for its diterpenoid[3,4] and flavonoid[5–7] content. Work has also been performed on the isolation and characterization of terpenes from *M. carolinensis*,[8] flavonoids from *M. conifera*[9] and *M. denticulate*,[10] chromenoflavones from *M. indica*,[11] clerodane diterpenes from *M. monandra*,[12] bergenin derivatives and polyphenols from *M. peltata*,[13,14] prenylflavones from *M. pleiostemona*,[15] a geranyl flavanone from *M. schweinfurthii*,[16] tannins from *M. sinensis*,[17] a rotenoid and other compounds from *M. triloba*,[18] and a geranylflavonol from *M. vedeliana*.[19] No phytochemical studies have been previously reported for *M. alnifolia*.

[#]Dedicated to the late Dr. Kenneth L. Rinehart, of the University of Illinois at Urbana-Champaign, for his pioneering work on bioactive natural products.

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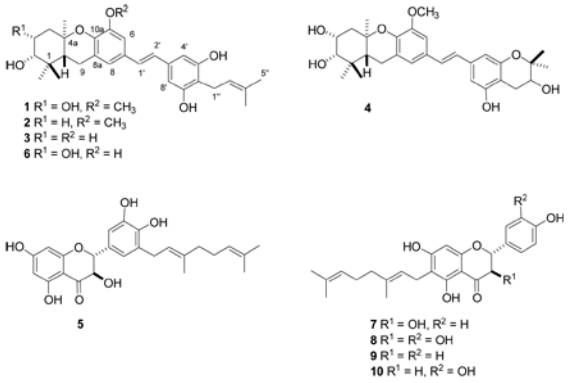
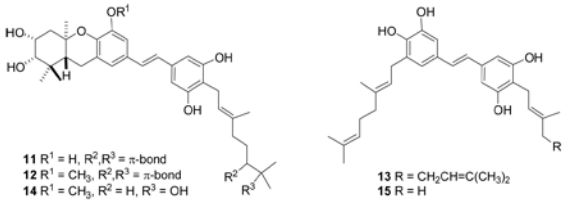
Supporting Information Available: Characterization data for compounds **6** – **10**, NCI 60-cell line data for **1**, ^{1}H and ^{13}C NMR spectra for compounds **1–10**, and gCOSY, HMQC, HMBC, and ROESY spectra of compound **1**. (20 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

Results and Discussion

As part of an ongoing search for cytotoxic natural products from tropical rainforests in Madagascar through the International Cooperative Biodiversity Group (ICBG) program, we obtained an ethanolic extract of the fruit of *Macaranga alnifolia* Baker (Euphorbiaceae) for phytochemical investigation. This extract was found to be active in the A2780 ovarian cancer cytotoxicity assay, with an IC_{50} value of 3.5 μg/mL. Bioassay-guided fractionation led to the isolation of the five new compounds; the four new prenylated stilbenes schweinfurthins E–H (**1–4**), and the new geranylated dihydroflavonol, alnifoliol (**5**). Five known compounds were also isolated: the prenylated stilbene, vedelianin (**6**), the two geranylated dihydroflavonols, bonanniol A (**7**) and diplacol (**8**), and the two geranylated flavanones, bonannione A (**9**) and diplacone (or nymphaeol A) (**10**).



Schweinfurthins E–H (**1–4**) are closely related to schweinfurthins A, B, and D (**11, 12**, and **14**) [20,21] and vedelianin (**6**),[22] and are also more distantly related to the prenylated stilbenes schweinfurthin C (**13**)[20] and mappain (**15**) isolated from *M. mappa*.[23]



Schweinfurthin E (**1**) was isolated as a pale yellow solid with a molecular formula of $C_{30}H_{38}O_6$, based on its HRFABMS. Its UV spectrum, with λ_{max} 331 and 224 nm, correlated well with literature values for compounds of the schweinfurthin class. Its ^{1}H NMR spectrum indicated the presence of an asymmetrical stilbene core (δ 6.87 ppm, 1H, d, *J* = 16 Hz, H-1′; δ 6.77 ppm, 1H, d, *J* = 16.5 Hz, H-2′) with both an AA′ benzene ring system (δ 6.46 ppm, 2H, s, H-4′ and -8′) and an AB benzene ring system (δ 6.91 ppm, 1H, d, H-6; δ 6.84 ppm, 1H, d, H-8). Proton signals at δ 5.23 (1H, tq, *J* = 7, 1.5 Hz, H-2″), 3.27 (H-1″, partially obscured by solvent), 1.76 (3H, s, H-4″), and 1.65 ppm (3H, s, H-5″) indicated the presence of an isoprenyl group. Also present in this spectrum were signals for the protons of three other methyl groups at δ 1.40 (3H, s, H-13), 1.10 (3H, s, H-12) and 1.09 (3H, s, H-11) ppm; protons of a methoxy group at δ 3.84 ppm (3H, s, CH_3O-5); and two methine protons bonded to oxygenated carbons at δ 4.14 (1H, q, *J* = 3.5, H-3) and 3.27 ppm (H-2, partially obscured by solvent).

The presence of an isoprenyl group was indicated by ^{13}C NMR signals at δ 131.1 (C-3″), 124.6 (C-2″), 26.0 (C-5″), 23.3 (C-1″), and 17.9 ppm (C-4″). The three other methyl carbons resonated at δ 29.4 (C-12), 22.0 (C-13) and 16.5 ppm (C-11), and the methoxy carbon resonated

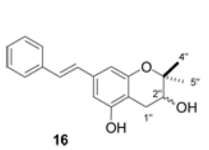
at δ 56.5 ppm. Signals for three oxygenated sp^3 carbons (C-2, C-4a, and C-3) were present in the spectrum at δ 78.8, 78.1, and 71.8 ppm, respectively, and the carbons of the AA′ benzene ring of the stilbene were observed at δ 157.3 ppm for the hydroxylated carbons (C-5′ and C-7′) and δ 105.8 ppm for the hydrogenated carbons (C-4′ and C-8′).

The NMR spectra of **1** corresponded closely with those of vedelianin (**6**)[22] and schweinfurthin B (**12**).[20] In particular, the observation of a "quartet" with J = 3.5 Hz for H-3 was in complete agreement with the "quartet" observed for H-3 of schweinfurthin B with J = 3.4 Hz,[20] and confirmed the cis stereochemistry of the C-2 and C-3 hydroxyl groups. The gCOSY, HMBC, and ROESY spectra of **1** (Figure 1 and supporting information) and the observed spectroscopic differences between it and the reference compounds were also in complete agreement with this assignment and with the assignment of its structure as 5-*O*-methylvedelianin (or 4″-desisoprenyl-schweinfurthin B).

Schweinfurthin F (**2**) was isolated as a pale yellow solid with a molecular formula of $C_{30}H_{38}O_5$, based on HRFABMS. Its ^1H and ^{13}C NMR spectra were very similar to those of **1**, with the major differences that the NMR signals for H-3 and C-3 were shifted significantly upfield (from δ 4.14 to 2.03 ppm and from δ 71.7 to 39.4 ppm, respectively) when compared to those of **1**. These observations, coupled with the fact that the molecule of **2** has five oxygen atoms in place of the six oxygens of **1**, suggested that **2** is a 3-deoxy derivative of **1**. This was confirmed by the upfield shifts for neighboring hydrogens on the α-side of the molecule (H-4, H-11, H-13) and also for adjacent carbons (C-4, C-11, C-12, C-13). The NMR spectra of **2** were essentially identical with those of a recently prepared synthetic sample,[24] thus confirming its structure unambiguously.

Schweinfurthin G (**3**) was isolated as a pale yellow solid. Its ^1H and ^{13}C NMR spectra were very similar to those of **2**, differing significantly only in the lack of signals at δ ~3.8 and ~56 ppm, respectively, corresponding to the methoxy group in **2**. The structure of **3** was thus assigned as 3-deoxyvedelianin.

Schweinfurthin H (**4**) was isolated as a pale yellow solid. It gave a molecular formula of $C_{30}H_{38}O_7$, based on HRFABMS, differing from that of **1** by a single oxygen. The ^1H NMR spectrum of **4** indicated the presence of a different asymmetrical stilbene group with a second, alternate AB benzene ring system rather than an AA′ benzene ring system; the signals for H-4′ (δ 6.52 ppm) and H-8′ (δ 6.44 ppm) appeared as two separate peaks. The absence of a ^1H NMR signal for the isoprenyl double bond and upfield shifts of H-2″ (δ 3.73 ppm), H-4″ (δ 1.33 ppm) and H-5″ (δ 1.23 ppm), and the appearance of H-1″ as doublet of doublets at δ 2.90 and 2.53 ppm, all indicated that the isoprenyl group was cyclized with one of the phenolic oxygens. The hydroxylation of C-2″ was also apparent from its ^{13}C NMR chemical shift of δ 76.4 ppm. The final structure was confirmed through NMR comparison with the literature values reported for chiricanine B (**16**) a tricyclic prenylated stilbene from *Lonchocarpus chiricanus*.[25] The stereochemistry of the 2″-hydroxyl group of **4** was not determined.



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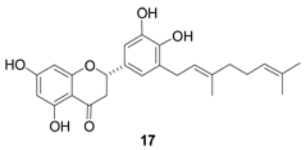

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The data presented to this point demonstrate the relative stereochemistry of compounds **1 – 4**, but do not establish their absolute stereochemistry. Fortunately this can be established by a comparison of CD spectra and of optical rotations between these compounds and the enantiomers of schweinfurthin F (**2**). The CD spectra of compounds **1 – 4** were essentially identical, with strong positive differential dichroic absorptions at 196 nm and strong negative

absorptions at 210 nm. Their specific optical rotations at 589 nm were all also similarly positive, with values of +49.2, +50.8, +33.3, and +32.4 for schweinfurthins E–H (**1 – 4**). These comparisons establish that compounds **1 – 4** all belong to the same stereochemical series.

Both the *R*,*R*,*R*– and the *S*,*S*,*S*–enantiomers of schweinfurthin F (**2**) have recently been synthesized by the Wiemer group.[24] They obtained optical rotations of +53.4 for the 1*R*, 4a*R*,9a*R* isomer and −55.8 for the 1*S*,4a*S*,9a*S* isomer in CH_3OH; the value for the 1*R*,4a*R*, 9a*R* isomer matches well with the value for the natural product (+50.8 in CH_3OH). We conclude that schweinfurthin F (**2**) has the 1*R*,4a*R*,9a*R* stereochemistry, and thus that compounds **1, 3**, and **4** also have the same 1*R*,4a*R*,9a*R* stereochemistry.

The flavonoid alnifoliol (**5**) was isolated as a yellow-brown solid with a molecular formula of $C_{25}H_{28}O_7$, based on HRFABMS. The ^{1}H NMR spectrum of **5** showed signals for four aromatic protons (δ 6.81, d, H-2′; δ 6.74, d, H-6′; δ 5.91, s, H-8; δ 5.87, s, H-6), one oxymethine (δ 4.88, d, H-2), and one methine α to a carbonyl (δ 4.47, d, H-3). These data suggested that **5** possesses a dihydroflavanol skeleton. Signals for a geranyl substituent (δ 5.33, m, H-2″; δ 5.10, m, H-7″; δ 3.33, d, H-1″; δ 2.09, td, H-6″; δ 2.02, t, H-5″; δ 1.70, s, H-4″; δ 1.61, s, H-9″; δ 1.56, s, H-10″) were also observed. The fact that proton signals for both H-6 and H-8 were present indicated that the geranyl group was on the B-ring. The splitting patterns for H-2′ and H-6′ confirmed the location of the geranyl group at C-5. Compound **5** is identical to a known component of propolis, isonymphaeol-B (**17**), except for the presence of the HO-3 group. Comparison of the NMR spectra of **5** with the literature spectra of **17**[26] fully supported the structural assignment of **5**. The coupling constant of the C-2 and C-3 protons (11 Hz) was consistent with their anticoplanar orientation, indicating a *trans* stereochemistry for the C-2 aryl and C-3 hydroxyl substituents.

The known compounds vedelianin (**6**),[22] bonanniol A (**7**),[27] diplacol (**8**),[28 , 29] bonannione A (**9**),[27] and diplacone (**10**, also known as nymphaeol A)[30,31] were also isolated, and their structures were determined based upon comparison of their ^{1}H NMR, ^{13}C NMR, and HRFABMS spectra to literature values.[22,26–31]

All ten compounds isolated from the fruits of *M. alnifolia* were tested for antiproliferative activity against the A2780 ovarian cancer cell line, and the results are provided in Table 1.

Schweinfurthin E (**1**) was also tested in the 60-cell human tumor cancer screen at the National Cancer Institute (NCI). The assay measure used by NCI that most closely corresponds to the IC_{50} values for antiproliferative activity is the GI_{50} value, and schweinfurthin E exhibited a mean panel GI_{50} of 0.19 μM. All lines of the leukemia subpanel were found to be highly sensitive to **1**, while all lines of the ovarian cancer subpanel (OVCAR-3, -4, and -8 and SK-OV-3) were somewhat resistant, with GI_{50} values averaging 2.2 μM. This result is somewhat surprising in view of the sensitivity of the A2780 ovarian cancer cell line to **1**, but can be explained in part by the fact that the A2780 cell line is a drug-sensitive line.[32] The most sensitive lines included leukemia (MOLT-4) and CNS (SF-295) and renal (A498 and CAKI-1) cancers, which all gave GI_{50} and TGI values of < 10 nM. The complete mean graph for **1** is proved as Supporting Information to this manuscript. These differential cytotoxicity results suggest that schweinfurthin E (**1**), similar to the other schweinfurthins, may share a similar mechanism of action with the stelletins.

It is instructive to compare the data reported above with the previously reported data for the schweinfurthins A–D[20,21] and for 3-deoxyschweinfurthin and its synthetic analogs.[24,33] The literature data were reported primarily as mean GI_{50} values from the NCI 60-cell line screen, and the data above are only for one cell line, so comparisons are only possible between compounds determined in the same bioassay.

The first comparisons are between the cytotoxicities of schweinfurthin E (**1**, IC_{50} 0.26 μM) and vedelianin (**6**, IC_{50} 0.13 μM) and those of schweinfurthin G (**3**, IC_{50} 0.39 μM) and vedelianin. In the first case, vedelianin is twice as potent as schweinfurthin E, suggesting that the replacement of the C-5 hydroxyl group with a methoxy group is deleterious to activity. In the second case, vedelianin (**6**) is about three times as potent as schweinfurthin G (**3**), indicating that the C-3 hydroxyl group enhances activity in this series. However, the issue is not as simple as this, because 3-deoxyschweinfurthin B is slightly more active than schweinfurthin B,[34] so clearly the length of the side chain has an influence on how the C-3 hydroxyl group affects activity.

Another direct comparison is possible between the mean GI_{50} values of schweinfurthin E (**1**, 0.19 μM) and schweinfurthin B (**12**, 0.79 μM); this indicates that the shorter geranyl side chain of **1** enhances its activity. A final comparison between schweinfurthin F (**2**, IC_{50} 5.0 μM) and schweinfurthin G (**3**, IC_{50} 0.39 μM) indicates that the combination of the loss of the C-3 hydroxyl group with methylation of the C-5 hydroxyl group results in a greater loss of activity than would have been predicted by either modification alone. The trend in all these comparisons is for the more polar compound to be more active, so it is possible that the observed activity is limited in some way by aqueous solubility, but further experiments are required to confirm this suggestion. Schweinfurthin C (**13**) was found to be much less active than schweinfurthins A, B, and D, so cyclization of the geranyl group must play an important role in mediating the biological activity of these compounds.

Experimental Section

General Experimental Procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. CD spectra were recorded on a JASCO J-700 spectrometer. NMR spectra were obtained on a JEOL Eclipse 500 or a Varian INOVA 400 MHz spectrometer. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. FAB mass spectra were obtained on a JEOL JMS-HX-110 instrument. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C_8 Varian Dynamax column (5 μm, 250 × 10 mm) and a preparative phenyl Varian Dynamax column (8 μm, 250 × 21.4 mm). Finnigan LTQ LC/MS with a C_{18} Hypersil column (5 μm, 100 × 2.1 mm) was also used for crude sample analysis.

Antiproliferative Bioassays

Antiproliferative activity measurements were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line as previously described. The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[32]

Plant Material

Immature and mature fruits of *Macaranga alnifolia* Baker (Euphorbiaceae) (vernacular name “Mokaranana”) were collected in November 2001. The specimens were collected around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar, at coordinates 17.41.01S and 48.38.28E, at an elevation of 900 m. Duplicate voucher specimens have been deposited at the Centre National d’Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d’Histoire Naturelle in Paris, France (P).

Extraction and Isolation

Dried fruits of *M. alnifolia* (275 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 1021 (12.8 g), of which 2.84 g was

made available to Virginia Polytechnic Institute and State University. The crude bioactive extract MG 1021 (IC_{50} = 3.5 μg/mL, 2.32 g) was partitioned between hexanes (200 mL) and MeOH-H_2O (4:1, 200 mL). The aqueous fraction was dried and subsequently partitioned between 1-BuOH and H_2O. The evaporated 1-BuOH fraction (1.96 g) displayed cytotoxicity (IC_{50} = 1.0 μg/mL) and was further separated by repeated RP-C_{18} column chromatography. The fractions eluted with 70% and 80% MeOH-H_2O showed the most improved activity and were separated by solid-phase extraction into fractions eluting with MeOH-H_2O (3:2) and MeOH. Preparative RP-C_{18} HPLC using MeOH-H_2O (4:1, 1 mL/min) on these bioactive eluates and combination of similar fractions yielded a total of 16 new fractions (A–K and L–P). Fraction D was identified as schweinfurthin E (**1**, t_R 21.5 min, 25.4 mg), while fractions A–C yielded vedelianin (**6**, t_R 17.1 min, 4.1 mg), schweinfurthin G (**3**, t_R 18.2 min, 0.9 mg) and schweinfurthin H (**4**, t_R 19.5 min, 1.5 mg), respectively, upon additional purification by semipreparative RP-C_{18} and RP-phenyl HPLC, eluting with MeOH-H_2O, 4:1. Fraction F was also identified as schweinfurthin F (**2**, t_R 25.9 min, 10.6 mg). Fractions G (t_R 32.6 min) and H (t_R 30–45 min) were combined and purified by semipreparative RP-phenyl HPLC to obtain both alnifoliol (**5**, 24.9 mg) and diplacone (**10**, 34.1 mg). Additionally, fractions M, N and P yielded diplacol (**8**, t_R 19 min, 6.7 mg), bonanniol A (**7**, t_R 21 min, 27.1 mg), and bonannione A (**9**, t_R 35 min, 3.0 mg). The structures of the known compounds were identified by comparison of their spectroscopic data with literature values.[22,27–31]

Schweinfurthin E (1)

pale yellow solid; $[\alpha]^{22}_D$ +49.2 (*c* 0.13, CH_3OH); UV (MeOH) λ_{max} (log ε) 223 (4.5), 331 (4.5) nm; CD (MeOH) λ_{max} (Δε, $dm^3\ mol^{-1}\ cm^{-1}$) 196 (+173), 210 (−139), 222 (−135), 250 (+11); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.91 (1H, d, *J* = 1.5 Hz, H-6), 6.87 (1H, d, *J* = 16 Hz, H-1′), 6.84 (1H, d, H-8), 6.77 (1H, d, *J* = 16.5 Hz, H-2′), 6.46 (2H, s, H-4′, 8′), 5.23 (1H, tq, *J* = 7, 1.5 Hz, H-2″), 4.14 (1H, q, *J* = 3.5 Hz, H-3), 3.84 (3H, s, CH_3O-5), 3.30 (partially obscured by solvent, H-2, 1″), 2.76 (2H, m, H-9), 2.34 (1H, dd, *J* = 14, 3 Hz, H-4), 1.93 (1H, dd, *J* = 13.5, 3.5 Hz, H-4), 1.76 (3H, s, H-4″), 1.74 (1H, dd, *J* = 12.5, 6 Hz, H-9a), 1.65 (3H, s, H-5″), 1.40 (3H, s, H-13), 1.10 (3H, s, H-12), 1.09 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5′, 7′), 150.2 (C-5), 143.4 (C-10a), 137.6 (C-3′), 131.1 (C-3″), 130.8 (C-7), 128.6 (C-1′), 127.7 (C-2′), 124.6 (C-2″), 124.4 (C-8a), 121.7 (C-8), 116.0 (C-6′), 108.3 (C-6), 105.8 (C-4′, 8′), 78.8 (C-2), 78.1 (C-4a), 71.8 (C-3), 56.5 (CH_3O-5), 48.5 (C-9a) 44.8 (C-4), 39.2 (C-1), 29.4 (C-12), 26.0 (C-5″), 24.0 (C-9), 23.3 (C-1″), 22.0 (C-13), 17.9 (C-4″), 16.5 (C-11); HRFABMS *m/z* 494.2646 $[M]^+$ (calcd for $C_{30}H_{38}O_6$, 494.2668).

Schweinfurthin F (2)

pale yellow solid; $[\alpha]^{22}_D$ +50.8 (*c* 0.06, CH_3OH); UV (MeOH) λ_{max} (log ε) 224 (4.4), 331 (4.4) nm; CD (MeOH) λ_{max} (Δε, $dm^3\ mol^{-1}\ cm^{-1}$) 196 (+176), 210 (−134), 220inf (−130) 247 (+9.5), 258 (+16); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.91 (1H, d, *J* = 1.5 Hz, H-6), 6.86 (1H, d, *J* = 16.5 Hz, H-1′), 6.83 (1H, d, *J* = 1.5 Hz, H-8), 6.77 (1H, d, *J* = 16.5 Hz, H-2′), 6.46 (2H, s, H-4′, 8′), 5.23 (1H, tq, *J* = 7, 1.5 Hz, H-2″), 3.83 (3H, s, CH_3O-5), 3.30 (partially obscured by solvent, H-2, 1″), 2.72 (2H, m, H-9), 2.03 (2H, m, H-3), 1.79 (1H, m, H-4), 1.76 (3H, s, H-4″), 1.75 (1H, m, H-9a), 1.65 (1H, m, H-4), 1.65 (3H, s, H-5″), 1.21 (3H, s, H-13), 1.09 (3H, s, H-12), 0.87 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5′, 7′), 150.2 (C-5), 143.7 (C-10a), 137.6 (C-3′), 131.2 (C-3″), 130.9 (C-7), 128.6 (C-1′), 127.8 (C-2′), 124.6 (C-2″), 124.1 (C-8a), 121.8 (C-8), 116.0 (C-6′), 108.3 (C-6), 105.8 (C-4′, 8′), 78.8 (C-2), 78.2 (C-4a), 56.5 (CH_3O-5), 39.5 (C-3), 39.0 (C-1), 29.0 (C-4), 27.9 (C-12), 26.0 (C-5″), 24.1 (C-9), 23.3 (C-1″), 20.2 (C-13), 17.9 (C-4″), 14.9 (C-11); HRFABMS *m/z* 478.2737 $[M]^+$ (calcd for $C_{30}H_{38}O_5$, 478.2719).

Schweinfurthin G (3)

pale yellow solid; $[\alpha]^{22}_{D}$ +33.3 (*c* 0.03, CH_3OH); UV (MeOH) λ_{max} (log ε) 228 (4.2) 329 (3.9) nm; CD (MeOH) λ_{max} (Δε, $dm^3\ mol^{-1}\ cm^{-1}$) 196 (+177), 210 (−133), 220 (−123), 247 (+10); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.80 (1H, d, *J* = 17 Hz, H-1′), 6.79 (1H, d, H-6), 6.72 (1H, d, *J* = 1.5 Hz, H-8), 6.70 (1H, *J* = 16 Hz, H-2′), 6.44 (2H, s, H-4′, 8′), 5.23 (1H, tq, *J* = 7, 1.5 Hz, H-2″), 3.30 (partially obscured by solvent, H-2, 1″), 2.71 (2H, m, H-9), 2.06 (2H, m, H-3), 1.80 (1H, m, H-4), 1.76 (3H, s, H-4″), 1.75 (1H, m, H-9a), 1.68 (1H, m, H-4), 1.65 (3H, s, H-5″), 1.23 (3H, s, H-13), 1.10 (3H, s, H-12), 0.88 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5′, 7′), 147.0 (C-5), 142.2 (C-10a), 141.3 (C-3″), 137.6 (C-3′), 131.0 (C-7), 128.6 (C-1′), 127.5 (C-2′), 124.6 (C-2″), 124.0 (C-8a), 120.4 (C-8), 115.9 (C-6′), 111.1 (C-6), 105.7 (C-4′, 8′), 78.8 (C-2), 78.2 (C-4a), 39.5 (C-3), 38.9 (C-1), 29.0 (C-4), 27.9 (C-12), 26.0 (C-5″), 24.0 (C-9), 23.3 (C-1″), 20.3 (C-13), 17.9 (C-4″), 14.8 (C-11). HRFABMS *m/z* 464.2595 $[M]^+$ (calcd for $C_{29}H_{36}O_5$, 464.2563).

Schweinfurthin H (4)

pale yellow solid; $[\alpha]^{22}_{D}$ +32.4 (*c* 0.04, CH_3OH); UV (MeOH) λ_{max} (log ε) 224 (4.5), 329 (4.4) nm; CD (MeOH) λ_{max} (Δε, $dm^3\ mol^{-1}\ cm^{-1}$) 196 (+232), 210 (−145), 220inf (−135), 250 (+12); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.93 (1H, d, *J* = 1.5 Hz, H-6), 6.90 (1H, d, *J* = 16 Hz, H-1′), 6.85 (1H, d, *J* = 1 Hz, H-8), 6.80 (1H, d, *J* = 16 Hz, H-2′), 6.52 (1H, d, *J* = 1.5 Hz, H-4′), 6.44 (1H, d, *J* = 1 Hz, H-8′), 4.14 (1H, q, *J* = 3.5 Hz, H-3), 3.84 (3H, s, CH_3O-5), 3.73 (1H, dd, *J* = 7.5, 5.5 Hz, H-2″), 3.30 (1H, m, H-2), 2.90 (1H, dd, *J* = 17, 5.5 Hz, H-1″), 2.76 (2H, m, H-9), 2.53 (1H, dd, *J* = 17, 7.5 Hz, H-1″), 2.34 (1H, dd, *J* = 14, 3 Hz, H-4), 1.92 (1H, dd, *J* = 14.5 Hz, H-4), 1.74 (1H, dd, *J* = 12, 5.5 Hz, H-9a), 1.40 (3H, s, H-13), 1.33 (3H, s, H-4″), 1.23 (3H, s, H-5″), 1.10 (3H, s, H-12), 1.09 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.1 (C-5′), 155.3 (C-7′), 150.2 (C-5), 143.5 (C-10a), 138.5 (C-3′), 130.6 (C-7), 129.1 (C-1′), 127.5 (C-2′), 124.4 (C-8a), 121.9 (C-8), 108.4 (C-4′), 108.4 (C-6), 107.6 (C-6′), 105.0 (C-8′), 78.8 (C-2), 78.1 (C-4a), 77.7 (C-3″), 71.8 (C-3), 70.6 (C-2″), 56.5 (CH_3O-5), 44.8 (C-4), 39.2 (C-1), 29.4 (C-12), 27.4 (C-1″), 25.8 (C-5″), 24.0 (C-9), 22.0 (C-13), 20.8 (C-4″), 16.6 (C-11); HRFABMS *m/z* 510.2579 $[M]^+$ (calcd for $C_{30}H_{38}O_7$, 510.2618).

Alnifoliol (5)

yellowish-brown solid; $[\alpha]^{23}_{D}$ +15.3 (*c* 0.25, CH_3OH); UV (MeOH) λ_{max} (log ε) 213 (4.7), 290 (4.4) nm; CD (MeOH) λ_{max} (Δε, $dm^3\ mol^{-1}\ cm^{-1}$) 196 (+150), 210 (−123), 220inf (−116), 262 (+16), 295 (−19); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.81 (1H, d, H-2′), 6.74 (1H, d, *J* = 2 Hz, H-6′), 5.91 (1H, d, *J* = 2.5 Hz, H-8), 5.87 (1H, d, H-6), 5.34 (2H, t, H-2″), 5.10 (2H, t, H-7″), 4.88 (1H, d, H-2), 4.47 (1H, d, *J* = 11 Hz, H-3), 3.31 (2H, d, *J* = 7.5 Hz, H-1″), 2.09 (2H, q, *J* = 7.5 Hz, H-6″), 2.02 (2H, t, *J* = 8 Hz, H-5″), 1.70 (3H, s, H-4″), 1.61 (3H, s, H-9″), 1.56 (3H, s, H-10″); ^{13}C NMR (CD_3OD, 125 MHz) δ 197.0 (C-4), 167.4 (C-7), 164.0 (C-5), 163.2 (C-9), 144.5 (C-3′), 143.6 (C-4′), 135.5 (C-3″), 130.9 (C-8″), 128.1 (C-1′), 127.6 (C-5′), 124.1 (C-7″), 122.5 (C-2″), 120.0 (C-6′), 111.8 (C-2′), 100.5 (C-10), 96.0 (C-8), 95.0 (C-6), 84.1 (C-2), 72.4 (C-3), 39.6 (C-5″), 27.8 (C-1″), 26.4 (C-6″), 24.6 (C-9″), 16.4 (C-10″), 14.9 (C-4″); HRFABMS *m/z* 440.1831 $[M]^+$ (calcd for $C_{25}H_{28}O_7$, 440.1835).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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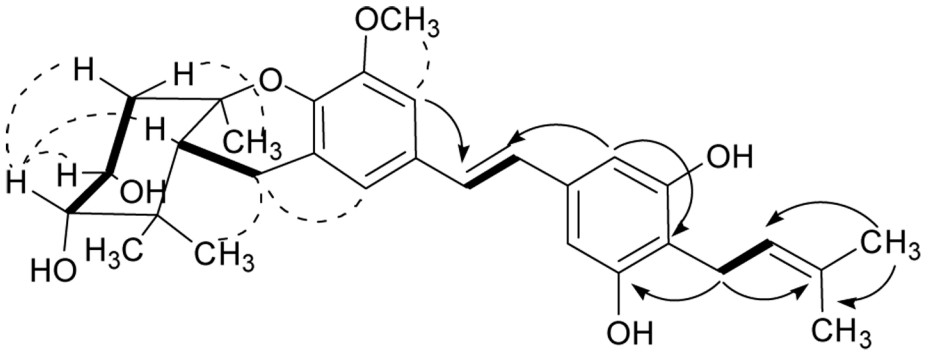

Figure 1.
Key COSY (bold), HMBC (arrows) and ROESY (dashed) correlations of **1**

Table 1

Cytotoxicity Data of *Macaranga alnifolia* Compounds to A2780 Cells.

compound	IC_{50} (μM)
schweinfurthin E (**1**)	0.26
schweinfurthin F (**2**)	5.0
schweinfurthin G (**3**)	0.39
schweinfurthin H (**4**)	4.5
alnifoliol (**5**)	27.3
vedelianin (**6**)	0.13
bonanniol A (**7**)	23.5
diplacol (**8**)	11.5
bonannione A (**9**)	24.5
diplacone (**10**)	10.5