seco-Cycloartane Triterpenes from *Gardenia aubryi*

Raphaël Grougnet,[†] Prokopios Magiatis,[†] Sofia Mitaku,*[,†] Stella Loizou,[‡] Paraskevi Moutsatsou,[‡] Aris Terzis,[§] Pierre Cabalion,[⊥] François Tillequin,[▽] and Sylvie Michel[▽]

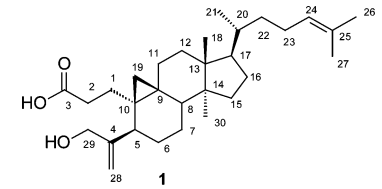
Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens GR-15771, Greece, Laboratory of Biological Chemistry, Medical School, University of Athens, Athens GR-11527, Greece, X-ray Laboratory, NCSR Demokritos, Athens 15310, Greece, Laboratoire des Substances Naturelles Terrestres et Savoirs Traditionnels, Centre IRD de Nouméa, 98800 Nouméa, New Caledonia, and Laboratoire de Pharmacognosie de l'Université René Descartes, UMR/CNRS 8638, Faculté de Pharmacie, F-75006 Paris, France

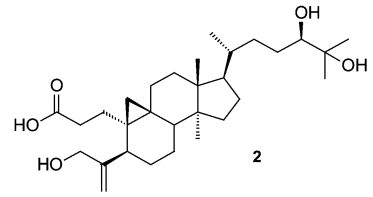
Received June 9, 2006

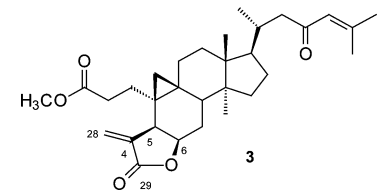
Three new 3,4-*seco*-cycloartanes, secaubryenol (**1**), secaubrytriol (**2**), and secaubryolide (**3**), were isolated from an exudate collected on the aerial parts of *Gardenia aubryi*, in addition to the known (24*S*)-cycloartane-24,25-diol-3-one, coccinetane A, herbacetin 3,8-dimethyl ether, hibiscetin 3,8,3′,4′-tetramethyl ether, and conyzatin. The structures of **1** and **2** were established by mass spectrometry and NMR experiments, while the relative configuration of **2** was defined unequivocally using X-ray crystallography. The in vitro cytotoxic activity of compounds **1**−**3** was evaluated against four human solid tumor cell lines.

The genus *Gardenia* (Rubiaceae) includes some two hundred species of trees and shrubs widely distributed in the warm and tropical regions of the Old World. Six endemic species occur in New Caledonia.[1,2] The leaves and flower buds of two of these, *Gardenia aubryi* Vieill. and *Gardenia urvillei* Montrouz., are covered with an exudate used locally for chewing, especially by children. This fact led us to investigate its chemical composition, in order to establish the potential for local or systemic toxicity by diffusion of the compounds during chewing. In a continuation of our chemical studies on the plants from New Caledonia,[3,4] we report herein the isolation and structure elucidation of the secondary metabolites of *G. aubryi* gum.

Eight compounds were isolated by fractionation of the dichloromethane extract of the exudate from the aerial parts of *G. aubryi*. Three known 3-methoxyflavones were identified: herbacetin 3,8-dimethyl ether,[5] hibiscetin 3,8,3′,4′-tetramethyl ether,[6] and conyzatin.[7] Known terpenoids included (24*S*)-cycloartane-24,25-diol-3-one[8,9] and the *seco*-cycloartane, coccinetane A.[10] We report herein the structure elucidation of three 3,4-*seco*-cycloartanes, secaubryenol (**1**), secaubrytriol (**2**), and secaubryolide (**3**), the crystal structure of **2**, and the cytotoxic activity of these compounds against four human solid tumor cell lines.







Results and Discussion

Secaubryenol (**1**) was obtained as a colorless, amorphous solid, for which the empirical formula was determined as $C_{30}H_{48}O_3$ by HREIMS. The ^{1}H NMR spectrum recorded in $CDCl_3$ exhibited a characteristic pair of doublets (J = 4.5 Hz), at δ 0.49 and 0.73, associated with the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene.[11,12] A three-proton multiplet centered at δ 5.10 was resolved into a one-proton triplet (J = 6.5 Hz) corresponding to a trisubstituted alkene and two one-proton doublets (J = 1 Hz) attributable to an exomethylene group, when the spectum was recorded in C_6D_6. These latter signals exhibited allylic coupling with a two-proton broad singlet at δ 4.07, suggesting a 3,4-*seco*-cycloartane, with C-29 oxidized to a primary alcohol.[13] Inspection of the ^{13}C NMR spectrum revealed the presence of a carboxyl singlet, four sp^2 carbons (two quaternary C, one CH, and one CH_2), and 25 sp^3 carbons (four quaternary C, four CH, 12 CH_2, and five CH_3). Methylene signals at δ 110.6 and 64.6 confirmed the presence of a terminal alkene and a primary alcoholic group, respectively. These data were closely related to those previously reported for coronalolic acid,[13] but clearly differed in the signals corresponding to the side chain at C-17. Indeed, a typical set of resonances associated with a 2-methylhept-2-ene system was noticed at δ 35.8 (C-20), 18.2 (C-21), 36.2 (C-22), 25.2 (C-23), 125.2 (C-24), 130.9 (C-25), 17.6 (C-27), and 25.7 (C-26), similar to analogous signals in the ^{13}C NMR spectrum of coccinetane A.[10] These data permitted the structure of secaubryenol to be depicted as **1**. The relative stereochemistry was deduced from NOESY correlations (Figure 1). Of particular interest were NOESY cross-peaks observed between H-8 and the signals of H-19b and CH_3-18 on one hand, and CH_3-30 and the signals of H-17 and H-5 on the other hand. These were in good agreement with the relative stereochemistry at C-5, C-8, C-9, C-10, C-13, C-14, and C-17 long-established for the cycloartane tricyclic core.[13]

Secaubrytriol (**2**) was found to possess a molecular formula of $C_{30}H_{50}O_5$ by HRCIMS. In the ^{1}H NMR spectrum, typical signals for a cyclopropane methylene proton appeared as two doublets (J

* Corresponding author. Tel: +30 210 727 4290. Fax: +30 210 727 4594. E-mail: mitakou@pharm.uoa.gr.
[†] Department of Pharmacy, University of Athens.
[‡] Medical School, University of Athens.
[§] NCSR Demokritos, Athens.
[⊥] Centre IRD de Nouméa.
[▽] Université René Descartes.

10.1021/np060273t CCC: $33.50
Published on Web 12/01/2006

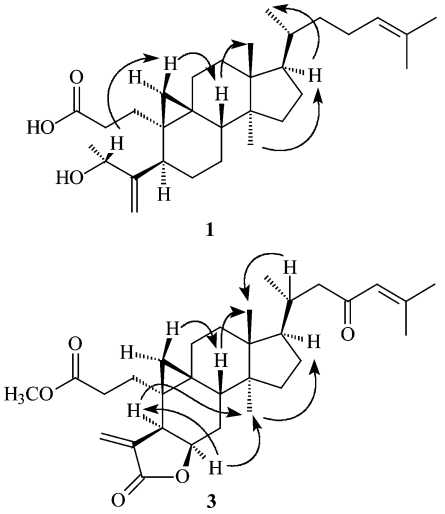


Figure 1. Important NOE correlations of **1** and **3**.

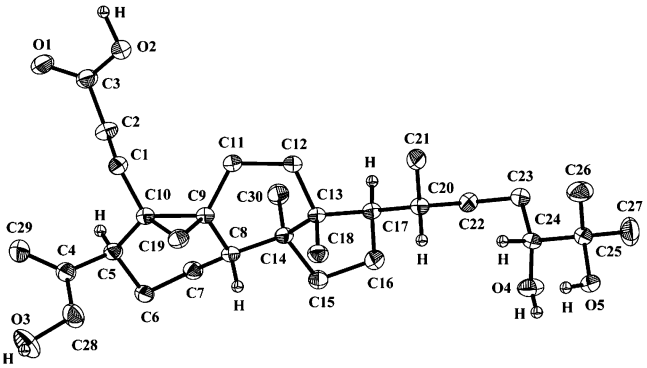


Figure 2. Labeled ORTEP plot of **2** with ellipsoids drawn at the 40% probability level. Only the critical hydrogen atoms are shown.

= 4.5 Hz) at δ 0.48 and 0.74. Allylic coupling observed in the COSY spectrum between a two-proton broad singlet at δ 4.09 accounting for a primary alcoholic group and the two doublets (J = 1 Hz) of a terminal alkene at δ 5.08 and 5.12 was suggestive of the structure of a 29-hydroxy-*seco*-cycloartane closely related to that of **1**. The ^{1}H and ^{13}C NMR data of compounds **1** and **2** were closely comparable. Significant differences appeared only in the resonances associated with the side chain at C-17. Indeed, the replacement in **2** of the olefinic triplet of H-24 in **1** by a double doublet (J = 6.5, 1 Hz) at δ 3.23 and the shielding of the two three-proton singlets associated with the methyl groups at C-26 and C-27 at 1.13 and 1.16 ppm indicated the 24,25-diol structure of that chain. This was confirmed by ^{13}C NMR signals of C-24, C-25, C-26, and C-27, observed at δ 79.8, 73.9, 25.7, and 24.9, respectively, in agreement with analogous data reported for related cycloartane-24,25-diols.[14] Consequently, the structure of this new *seco*-cycloartane derivative was established as **2**. The same cross-peak correlations as for compound **1** observed in the NOESY spectrum gave evidence for the relative stereochemistry of **2** at C-5, C-8, C-9, C-10, C-13, C-14, C-17, and C-20. Finally, the relative stereochemistry at C-24 was established unequivocally by X-ray diffraction analysis, with **2** represented as 5*R**, 8*R**, 9*S**, 10*R**, 13*R**, 14*S**, 17*R**, 20*R**, 24*R** (Figure 2).

The empirical formula of secaubryolide (**3**) was deduced as $C_{31}H_{44}O_5$ from the HRCIMS. The ^{1}H NMR spectrum exhibited the typical signals associated with a 3,4-*seco*-cycloartane triterpene, including two tertiary methyl singlets (δ 0.90 and 0.97), one secondary methyl doublet (δ 0.88, J = 7 Hz), and a characteristic pair of doublets at δ 0.17 (J = 5 Hz) and 0.42 (J = 5 Hz), corresponding to the C-19 methylene protons of the cyclopropane ring. Both the ^{1}H and ^{13}C NMR signals attributable to the D-ring and side chain of **3** closely resembled those previously described for 5α-cycloart-24-ene-3,23-dione and methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate,[15] giving evidence for a 2-methylhept-2-en-4-on-6-yl unit attached at C-17. Additional features comprised two doublets (J = 1.5 Hz) at δ 5.74 and 6.33 in the ^{1}H NMR spectrum, along with signals accounting for a methylene at δ 123.1, a quaternary olefinic carbon at δ 139.1, and a conjugated lactone carbonyl at δ 170.7 in the ^{13}C NMR spectrum, typical of the exomethylene γ-lactone ring system commonly encountered in natural sesquiterpene lactones.[16] Therefore, C-4 was lactonized onto C-6 and the structure of secaubryolide was established as **3**. Observation of a strong NOESY cross-peak between the signals of H-5 and H-6 permitted the assignment of a relative 5,6-*cis*-configuration (Figure 1). Additional correlations between CH_3-30 and H-5, H-6, and H-17, between H-8 and H-19b, H-7b, and H-18, and between H-18 and H-20 allowed the relative stereochemistry of **3** to be depicted as shown.

The cytotoxicity of the three new compounds was evaluated against four solid tumor cell lines: MCF-7 (breast cancer cells, ER positive), MDA (breast cancer cells, ER negative), PC-3 (prostate cancer cells), and HeLa (cervical cancer cells). Only compound **3**, which possesses an exomethylene γ-lactone ring[13] system, was found to be very weakly active and exhibited IC_{50} values of 52, 21, 35, and 40 μM against these cell lines, respectively. Compounds **1** and **2** exhibited IC_{50} values over 100 μM.

From a chemotaxonomic point of view, the isolation of 3,4-*seco*-cycloartane triterpenes from *G. aubryi* is in agreement with previous chemical data on the genus. Indeed, other *seco*-cycloartanes were isolated from *G. coronaria* Buch.-Ham.,[13] *G. sootepnensis* Hutchinson,[13] and *G. obtusifolia* Roxb.,[15] as well as from the related *Antirhea acutata* (DC.) Urb.[17] Methoxylated flavones are represented by herbacetin 3,8-dimethyl ether and 3,8,3′,4′-tetramethyl ether, and conyzatin in *G. aubryi* exudate. Related lipophilic highly oxygenated and polymethoxylated flavonoids recorded from the bud exudate of several *Gardenia* species endemic to Fiji Islands have been claimed to act as antiherbivory digestibility-reducing secondary metabolites.[18]

The occurrence of *seco*-cycloartanes possessing an exomethylene γ-lactone substituent, such as **3**, could lead to the risk of toxicity in the case of ingestion of the bud exudate of this *Gardenia* species, which could occur during chewing.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu-160A spectrophotometer. The IR spectra were obtained on a Perkin-Elmer Paragon 500 instrument. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in ppm downfield from TMS. The 2D NMR experiments (HMQC-DEPT, HMQC-TOCSY, HMBC, NOESY) were performed using standard Bruker microprograms. Electron impact (EIMS) and desorption chemical ionization (DCIMS) mass spectra were determined on a Nermag R-10-10-C spectrometer. HREIMS and HRCIMS were obtained on a AEI MS-902 mass spectrometer. Flash liquid chromatography was performed on columns containing Si gel 60, Merck (40−63 μm).

Plant Material. Aerial parts of *Gardenia aubryi* were collected in July 2003 at Les Bois du Sud, New Caledonia. A voucher sample (JWHJ-75) is kept in the Herbarium of the Centre IRD of Nouméa, New Caledonia. The plant material was collected by J. Waikedre and identified by one of the authors, P.C.

Extraction and Isolation. The exudate (2.35 g), collected manually from fresh *G. aubryi* aerial parts (1.4 kg), was dissolved in CH_2Cl_2 (35 mL). Crude secaubrytriol (**2**) (122 mg) crystallized upon cooling. It was collected by vacuum filtration and further purified by recrystallization from CH_2Cl_2 (94 mg). The solvent was evaporated to dryness under reduced pressure. An aliquot of the solid residue (2.2 g) was subjected to silica gel (60H) liquid vacuum chromatography, using a

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3**

	^{1}H NMR			^{13}C NMR		
position	**1**	**2**	**3**	**1**	**2**	**3**
1	2.12	2.10	2.25	28.7	30.3	30.9
	1.36	1.36	1.58			
2	2.51	2.51	2.48	31.5	32.7	31.2
	2.30	2.23	2.41			
3				179.1	177.9	173.4
4				152.1	154.1	139.1
5	2.51	2.54	3.21, d, *J*=8	41.9	43.6	39.0
6	1.69	1.67	4.74, td, *J*=8; 7	28.9	30.2	74.4
	0.99	1.07				
7	1.28	1.30	1.75	25.2	26.5	27.2
	1.07	1.10	1.51			
8	1.55	1.59	2.12	47.9	49.6	38.3
9				21.8	23.1	25.0
10				27.3	28.7	28.2
11	2.10	2.18	1.70	26.9	28.1	26.5
	1.22	1.26	1.65			
12	1.65	1.72	1.60	32.9	34.3	32.8
13				45.0	46.3	45.8
14				48.8	50.1	48.7
15	1.29	1.34	1.32	35.6	36.9	34.7
16	1.91	1.98	1.89	28.1	29.2	27.9
	1.30	1.37	1.33			
17	1.60	1.64	1.62	52.2	53.8	51.5
18	0.97, s	1.05, s	0.97, s	18.1	18.7	15.9
19	0.73, d, *J*=4.5	0.74, d, *J*=4.5	0.42, d, *J*=5	30.2	31.4	23.0
	0.49, d, *J*=4.5	0.48, d, *J*=4.5	0.17, d, *J*=5			
20	1.40	1.45	2.02	35.8	37.1	33.3
21	0.88, s	0.93, d, *J*=7	0.88, d, *J*=7	18.2	18.8	19.5
22	1.40	1.52	2.51	36.2	34.4	51.6
	1.05	1.28	2.09			
23	2.03	1.52		24.9	28.8	201.3
	1.86	1.34				
24	5.10	3.23, dd, *J*=6.5; 1	6.06, br s	125.2	79.8	124.2
25				130.9	73.9	154.9
26	1.70, s	1.13, s	2.12, s	25.7	25.7	20.6
27	1.62, s	1.16, s	1.86, s	17.6	24.9	27.6
28	5.10, m	5.12, d, *J*=1	6.33, d, *J*=1.5	110.6	110.2	123.1
	5.10, m	5.08, d, *J*=1	5.74, d, *J*=1.5			
29	4.07, br s	4.09, br s		64.6	64.6	170.7
30	0.93, s	1.02, s	0.90, s	19.3	19.9	20.1
OCH_3			3.70, s			51.8

stepwise gradient elution of EtOAc in cyclohexane, collecting 10 mL fractions. Fractions of similar composition were pooled on the basis of TLC analysis (cyclohexane/EtOAc, 80/20, 50/50, 0/100). Further purifications by column chromatography on silica gel performed with cyclohexane/CH_2Cl_2 (gradient from 70/30 to 0/100, 100 fractions of 10 mL) and CH_2Cl_2/MeOH (gradient from 100/0 to 85/15, 150 fractions of 10 mL) yielded successively herbacetin 3,8-dimethyl ether (32 mg), hibiscetin 3,8,3′,4′-tetramethyl ether (27 mg), conyzatin (48 mg), (24*S*)-cycloartane-24,25-diol-3-one (6 mg), coccinetane A (5 mg), secaubryolide (**3**) (15 mg), secaubryenol (**1**) (34 mg), and a second crop of secaubrytriol (37 mg) (**2**).

Secaubryenol (3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-3-oic acid) (1): white, amorphous solid; $[\alpha]_D$ +49 (*c* 0.2, MeOH); IR (MeOH, CaF_2) ν_{max} 3600−2500 (br), 2937, 1707, 1653, 1611, 1457, 1378 cm^{-1}; ^{1}H NMR ($CDCl_3$) data, see Table 1; ^{1}H NMR (C_6D_6) δ 5.11 (1H, d, *J* = 1 Hz, H-28a), 5.20 (1H, d, *J* = 1 Hz, H-28b), 5.38 (1H, t, *J* = 6.5 Hz, H-24); ^{13}C NMR ($CDCl_3$) data, see Table 1; EIMS *m*/*z* 456 $[M]^+$ (20), 441 (100), 423 (25), 410 (20), 205 (40); HREIMS *m*/*z* 456.3609 (calcd for $C_{30}H_{48}O_3$ 456.3603).

Secaubrytriol (3,4-*seco*-cycloart-4(28)-ene-24,25,29-trihydroxy-3-oic acid) (2): whitish needles; mp 164 °C ($CHCl_3$); $[\alpha]_D$ +63 (*c* 0.2, MeOH); IR (MeOH, CaF_2) ν_{max} 2678 (br), 1708, 1654, 1490 cm^{-1}; ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRCIMS *m*/*z* 508.4011 (calcd for $[C_{30}H_{50}O_5 + NH_4]^+$ 508.4002).

X-ray Crystal Structure of Secaubrytriol (2). M_r = 490.70; μ = 0.593 mm^{-1}, d_x = 1.137 g cm^{-3}, $P2_1$, Z = 2, a = 12.911(8) Å, b = 7.138(5) Å, c = 15.565(9) Å, β = 92.70(2)°, V = 1432.9(16) Å^3. Slow crystallization from CH_2Cl_2/MeOH, 1:1 v/v, yielded colorless prismatic crystals. A crystal with approximate dimensions 0.10 × 0.30 × 0.70 mm was mounted in a capillary. Diffraction measurements were made on a $P2_1$ Nicolet diffractometer upgraded by Crystal Logic using graphite-monochromated Cu Kα radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections in the range 22° < 2θ < 54°. Intensity data were recorded using a θ−2θ scan to $2\theta_{max}$ = 118°, with scan speed 3.0°/min and scan range 2.45 plus $\alpha_1\alpha_2$ separation. Three standard reflections monitored every 97 reflections showed less than 3% variation and 24.9% decay, which was corrected. Lorentz, polarization corrections were applied using Crystal Logic software. Symmetry equivalent data were averaged with R_{int} = 0.0391 to give 3526 independent reflections from a total of 3671 collected. The structure was solved by direct methods using SHELXS-86[19] and refined by full-matrix least-squares techniques on F^2 with SHELXL-97[20] using 3526 reflections and refining 377 parameters. Some hydrogen atoms were located by difference maps, and the rest were placed at calculated positions as riding on their respective carbon or oxygen atoms. All were refined isotropically. All non-hydrogen atoms were refined anisotropically. The final values for R_1, wR_2, and GOF for all data are 0.0686, 0.1531, and 1.034, respectively, and R_1 = 0.0534 for observed data. The maximum and minimum residual peaks in the final difference map were 0.150 and −0.16 e/Å. The largest shift/esd in the final cycle was 0.000. There are four intermolecular hydrogen bonds between the donor O2, O3, O4, O5 atoms and the acceptor O5, O4, O3, O1 atoms, respectively, through which an extended two-dimensional network is formed. They appear in the CIF file. Crystallographic data, excluding stucture factors, have been deposited at the Cambridge Crystallographic Data Centre under the deposition number CCDC 609806. Copies can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-0-1223-226033, e-mail: deposit@ccdc.cam.ac.uk).

Secaubryolide (3,4-*seco*-cycloart-4(28),24-dien-6-hydroxy-23-oxo-3,29-dioic acid-29-α,γ-lactone-3-methyl ester) (3): white, amorphous solid; $[\alpha]_D$ +120 (MeOH, *c* 0.15); UV (MeOH) λ_{max} (log ϵ) 235 (4.01) nm; IR ($CHCl_3$) ν_{max} 3024, 2952, 1755, 1737, 1707, 1686, 1615, 1438, 1379 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Table 1; HRCIMS *m*/*z* 497.3259 (calcd for $[C_{31}H_{44}O_5 + H]^+$ 497.3267).

Cell Culture and Assessment of Cytotoxicity. Compounds **1**−**3** were tested for their cytotoxic activity on the following human solid tumor cell lines: MCF-7, derived from a mammary adenocarcinoma of a 69-year-old Caucasian (ATCC); MDA-MB-231, derived from a mammary adenocarcinoma of a 51-year-old Caucasian (ATCC); PC-3, derived from a prostate adenocarcinoma of a 62-year-old Caucasian (ATCC); HeLa, derived from a cervical carcinoma from a 31-year-old African-American woman.

All cell lines were routinely cultured in Dulbecco's minimal essential medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum (media and antibiotics from Biochrom KG, Berlin, Germany), in an environment of 5% CO_2, 85% humidity, and 37 °C. Adherent cells were subcultured using a trypsin 0.25%−EDTA 0.02% solution. Cytotoxicity was estimated by a modification of the MTT assay.[21] Briefly, cells were plated in 96-well flat-bottomed microplates at a density of 5000 cells/well. Then 24 h after the plating, the test compounds were added, appropriately diluted in DMSO. After a 48 h incubation, the medium was replaced with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, St. Louis, MO), dissolved at a final concentration of 1 mg/mL in serum-free, phenol-red-free RPMI (Biochrom KG), for a further 4 h incubation. Then, the MTT-formazan was solubilized in isopropanol and the optical density was measured at a wavelength of 550 nm and a reference wavelength of 690 nm. Daunorubicin HCl was included as a positive control (IC_{50}: 0.44, 0.22, 0.25, and 0.32 μM for PC-3, MCF-7, MDA, and HeLa, respectively).

Supporting Information Available: Crystal data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Guillaumin, A. *Flore Analytique et Synoptique de la Nouvelle-Calédonie, Phanérogames*; Office de la Recherche Scientifique Coloniale: Paris, 1948.

(2) Jaffré, T.; Morat, P.; Veillon, J.-M.; Rigault, F.; Dagostini, G. *Composition et Caractérisation de la Flore Indigène de Nouvelle-Calédonie*; Centre IRD de Nouméa: Nouméa, 2001.

(3) Graikou, K.; Aligiannis, N.; Skaltsounis, A.-L.; Chinou, I.; Michel, S.; Tillequin, F.; Litaudon, M. *J. Nat. Prod.* **2004**, *67*, 685−688.

(4) Grougnet, R.; Magiatis, P.; Fokialakis, N.; Mitaku, S.; Skaltsounis, A.-L.; Tillequin, F.; Sévenet, T.; Litaudon, M. *J. Nat. Prod.* **2005**, *68*, 1083−1086.

(5) Ghisalberti, E. L.; Jeffries, P. R.; Stacey, C. I. *Aust. J. Chem.* **1967**, *20*, 1049−1053.

(6) Chow, P. W.; Jefferies, P. R. *Aust. J. Chem.* **1968**, *21*, 2529−2542.

(7) Horie, T.; Tsukayama, M.; Kawamura, Y.; Yamamoto, S. *Phytochemistry* **1988**, *27*, 1491−1495.

(8) Barik, B. R.; Bhaumik, T.; Dey, A. K.; Kundu, A. B. *Phytochemistry* **1994**, *35*, 1001−1004.

(9) Inada, A.; Murayta, H.; Inatomi, Y.; Nakanishi, T.; Darnaedi, D. *J. Nat. Prod.* **1995**, *58*, 1143−1146.

(10) Sy, L.-K.; Brown, G. D. *Tetrahedron* **1999**, *55*, 119−132.

(11) Sun, H.; Qiu, S.; Lin, L.; Wang, Z.; Lin, Z.; Pengsuparp, T.; Pezzuto, J. M.; Fong, H. H. S.; Cordell, G. A.; Farnsworth, N. R. *J. Nat. Prod.* **1996**, *59*, 525−527.

(12) Cantillo-Ciau, Z.; Brito-Loeza, W.; Quijano, L. *J. Nat. Prod.* **2001**, *64*, 953−955.

(13) Silva, G. L.; Gil, R. R.; Cui, B.; Chai, H.; Santisuk, T.; Srisook, E.; Reutrakul, V.; Tuchinda, P.; Sophasan, S.; Sujarit, S.; Upatham, S.; Lynn, S. M.; Farthing, J. E.; Yang, S.-L.; Lewis, J. A.; O'Neill, M. J.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **1997**, *53*, 529−538.

(14) Della Greca, M.; Fiorentino, A.; Monaco, P.; Previtera, L. *Phytochemistry* **1993**, *35*, 1017−1022.

(15) Tuchinda, P.; Pompimon, W.; Reutrakul, V.; Pohmakotr, M.; Yoosook, C.; Kongyai, N.; Sophasan, S.; Sujarit, K.; Upathum, S. E.; Santisuk, T. *Tetrahedron* **2002**, *58*, 8073−8086.

(16) Fischer N. H.; Olivier, E. J.; Fischer, H. D. In *Fortschritte der Chemie Organischer Naturstoffe*; Herz, W., Kirby, G. W., Steglich, W., Tamm, C., Eds.; Springer-Verlag: Wien, 1979; Vol. 38, pp 47−390.

(17) Lee, D.; Cuendet, M.; Axelrod, F.; Chavez, P. I.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **2001**, *57*, 7107−7112.

(18) Miller, J. M.; Sotheeswaran, S. *Biotropica* **1993**, *25*, 117−122.

(19) Sheldrick, G. M. *SHELXS-86*: Structure Solving Program; University of Göttingen: Germany, 1986.

(20) Sheldrick, G. M. *SHELXL-97*: Crystal Structure Refinement Program; University of Göttingen: Germany, 1997.

(21) Kasiotis, K.; Magiatis, P.; Pratsinis, H.; Skaltsounis, A.-L.; Abadji, V.; Charalambous, A.; Moutsatsou, P.; Haroutounian, S. *Steroids* **2001**, *66*, 785−791.

NP060273T