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NOVEL CYTOTOXIC RING-A *SECO*-CYCLOARTANE TRITERPENES FROM *GARDENIA CORONARIA* AND *G. SOOTEPENSIS*

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Abstract: Coronalolide methyl ester (**1**), coronalolide (**2**), and coronalolic acid (**3**) were isolated from the leaves and/or stems of *Gardenia coronaria.* A further compound, methyl coronalolate acetate (**4**), was purified from the stems after methylation. The novel compounds **1-4** have the rare ring-A *seco*-cycloartane carbon skeleton and their structures were assigned on the basis of spectral data and molecular modeling, as well as X-ray crystallography performed on **1**. Compounds **1** and **2** were also isolated from the leaves of *Gardenia sootepensis* and showed broad cytotoxic activity when evaluated against a panel of human cancer cell lines.Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The genus *Gardenia* (Rubiaceae) comprises more than 80 species with several being used ethnomedically in various countries primarily for abortifacient (*G. griffithii*[1)]) and contraceptive (*G. jasminoides*,[1)] *G. jovis-tonantis*,[2)] *G. turgida*[3)]) purposes. Other uses are as a febrifuge (*G. laeta*),[4)] for the treatment of headaches (*G. taitensis*[5)]), and as a larvicide (*G. campanulata*).[6)] Extracts of various *Gardenia* species have shown anti-implantation and abortifacient effects,[7)] as well as antibacterial,[8)] antiulcer,[9)] analgesic,[10)] diuretic,[10)] hypotensive,[10)] and larvicidal activity.[11)] In our ongoing project on the discovery of new anticancer agents from plants,[12)] we have studied the chloroform extracts of both the stems and leaves of *G. coronaria* (Buch.-Ham.) and the leaves of *G. sootepensis* Hutch., both species for which there appears to be no previous chemical literature. In the present work, we report the isolation and structure elucidation of four novel *seco*-cycloartanes (**1-4**), of which compounds **1** and **2** showed significant cytotoxic effects against a panel of human cancer cell lines, along with two known compounds, 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone and benzoic acid. This is the first report of ring-A 3,4-*seco*-cycloartanes in the genus

Gardenia; to our knowledge only a small group of such compounds have been isolated from natural sources, including nigranoic acid,[13,14] schisanlactone B,[15] 24-methylene-3,4-*seco*-cycloart-4(28)-en-3-oic acid,[16] kadsulactone A,[17] kadsudilactone,[18] pseudolarolide C,[19] and several constituents from *Tillandsia usneoides*.[20] Compounds **1-4** and compounds **1-2** were isolated from *G. coronaria* and *G. sootepensis*, respectively.

RESULTS AND DISCUSSION

Compound **1** was shown to possess a molecular formula of $C_{31}H_{44}O_5$ by HREIMS. Its IR spectrum showed three carbonyl group absorbances at 1768, 1739 and 1684 cm^{-1}, indicating the presence of two ester or lactone groups and one conjugated carbonyl group. The ^{1}H-NMR spectrum of **1** exhibited a characteristic pair of doublets at δ 0.43 (5.5 Hz) and δ 0.19 (5.5 Hz), corresponding to the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene.[13-20] The signal at δ 9.40 (1H, s) confirmed the presence of an aldehyde group, which, together with a triplet of quartets at δ 6.49 (*J*=6.5, 1.5 Hz, H-24), a three-proton doublet at 0.94 (H_3-21), and a broad three-proton singlet at δ 1.76 (H_3-27), indicated the presence of a side-chain attached to C-17. This was confirmed by the EIMS fragment ion at *m/z* 371, consistent with a cleavage between C-17 and C-20.

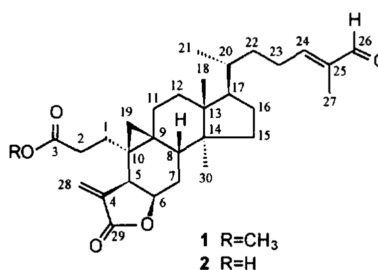
The above data suggested initially that compound **1** might be a normal cycloartane-type triterpene; however, the absence of signals corresponding to *gem*-dimethyl groups at C-4, the presence of two more carbonyl peaks (either ester or lactone) in the IR spectrum, and the analysis of a combination of the ^{1}H- (Table 1), ^{13}C- (Table 2) (broad-band and DEPT), DQF-COSY, HETCOR, HOHAHA, ROESY, and selective INEPT (Table 3) NMR spectra, led to the proposal of structure **1** for this compound. This structure is substantially different from those ring-A *seco*-cycloartanes so far isolated,[13-20] since C-4 was lactonized to C-6, showing a similar structural characteristic to the sesquiterpene lactones.[21] Two doublets at δ 6.34 (2.5 Hz) and δ 5.75 (2.3 Hz) in the ^{1}H-NMR spectrum of **1** were assigned to H-28a and H-28b of the exomethylene γ-lactone ring. Using these signals as a starting point in the DQF-COSY spectrum, signals were assigned to H-5 (δ 3.23), H-6 (δ 4.74), H-7α (δ 1.77), H-7β (δ 1.55), and H-8 (δ 2.12). Three methyl groups appeared as singlets (H_3-18, H_3-27, H_3-30), and another as a doublet at δ 0.94 (6.5 Hz, H_3-21). From the latter signal, the DQF-COSY spectrum was used to assign the resonance corresponding to H-20 at δ 1.48, which did not overlap any other signal. Following the cross-peaks from H-20, the resonances of H-22a and H-22b could be found at δ 1.63 and δ 1.24, respectively. An additional cross-peak was found at *ca.* δ 1.65, and assigned to H-17. This signal showed cross-peaks with the resonances at δ 1.95 (m) and δ 1.37 (m), attributable to H-16a and H-16b, respectively. No further correlations were observed between these signals and those corresponding to H-15a,b. Both H-15 proton resonances were found at the same chemical shift, δ 1.37. Most previous structure elucidation arguments for cycloartane derivatives have been based on data derived from ^{13}C-NMR

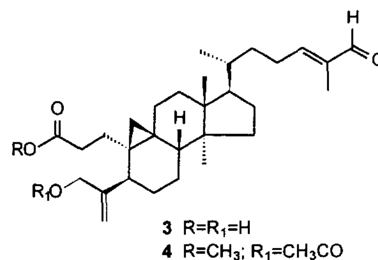
spectroscopy and there are only a few reports on the use of high-field ^{1}H-NMR studies (500-600 MHz).[22] There was another group of correlations from the signals corresponding to H-22a and H-22b with the resonances of H-23a and H-23b (δ 2.43 and δ 2.32), the resonance of H-24 at δ 6.49, and the broad singlet of H_3-27 at δ 1.76 (Table 1). The signal of H-24 showed a nOe cross-correlation peak in the ROESY experiment with the resonance of the aldehyde group at δ 9.40 (H-26), indicating an *E*-configuration of the double bond between C-24 and C-25. The HOHAHA experiment exhibited a correlation peak between H-20 and both the H-16a and H-16b signals, due to a relay through H-17. A double-relay cross-peak was observed between H-22a and H-16b.

Table 1. ^{1}H-NMR data of compounds **1-4** ($CDCl_3$).*

H#	**1**[a]	**2**[b]	**3**[b]	**4**[a]
1a	2.25 obsc.	--	--	--
1b	1.61 obsc.	--	--	--
2a	2.55 obsc.	--	--	--
2b	2.45 obsc.	--	--	--
5	3.23 (bd, 8.0)	3.25 (bd, 8.4)	--	--
6	4.74 (td, 8.0, 6.5)	4.77 (td, 8.4, 6.7)	--	--
7α	1.77 obsc.	--	--	--
7β	1.55 obsc.	--	--	--
8	2.12 (t, 5.5)	--	--	--
11 } 12 }	1.6-1.7	--	--	--
15a	1.37 obsc.	--	--	--
15b	1.37 obsc.	--	--	--
16a	1.95 obsc.	--	--	--
16b	1.37 obsc.	--	--	--
17	~1.65 obsc.	--	--	--
18	0.95 (s)	0.95 (s)	0.98 (s)	0.97 (s)
19β	0.43 (d, 5.5)	0.44 (d, 5.4)	0.74 (d, 4.2)	0.72 (d, 5)
19α	0.19 (d, 5.5)	0.19 (d, 5.4)	0.50 (d, 4.2)	0.49 (d, 5)
20	1.48 (m)	--	--	--
21	0.94 (d, 6.5)	0.94 (d, 6.5)	0.93 (d, 6.5)	0.93 (d, 6.5)
22a	1.63 obsc.	--	--	--
22b	1.24 obsc.	--	--	--
23a	2.43 obsc.	--	--	--
23b	2.32 obsc.	--	--	--
24	6.49 (tq, 6.5, 1.5)	6.51 (bt, 6.2)	6.51 (bt, 6.8)	6.49 (bt, 6.5)
26	9.40 (s)	9.40 (s)	9.40 (s)	9.40 (s)
27	1.76 (bs)	1.76 (bs)	1.78 (bs)	1.76 (bs)
28a	6.34 (d, 2.5)	6.35 (d, 2.2)	5.11 (s)	5.14 (s)
28b	5.75 (d, 2.3)	5.75 (d, 2.0)	5.09 (s)	5.09 (s)
29	--	--	4.12 (bs)	4.57 (bs)
30	0.92 (s)	0.92 (s)	0.94 (s)	0.94 (s)
	3.69 (s, OMe)	--	--	2.10 (s, Ac)
		--	--	3.65 (s, OMe)

* Chemical shifts given in ppm using TMS as internal reference. Multiplicities and coupling constants (Hz) are given in parentheses.
[a] Obtained at 500 MHz. [b] Obtained at 300 MHz. Obsc.=Obscured signals.





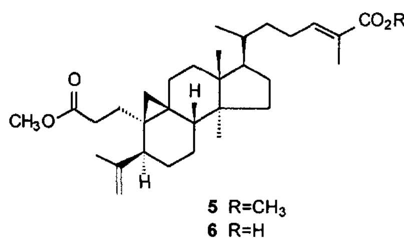


Figure 1. Structures of compounds **1-6**.

We assumed initially that the stereochemistry at C-5, C-8, C-9, C-10, C-13, C-14, C-17, and C-20 in **1** was the same as that long-established for the cycloartanes. The stereochemistry was determined using molecular modeling and a ROESY experiment. The structures of both epimers at C-6 (*cis* and *trans* fusion of the γ-lactone ring) were generated by the molecular modeling software PCMODEL V 5.1 for Windows using the MMX force field. From the calculated dihedral angles involving H-5, H-6 and H_2-7, we have estimated vicinal coupling constant using the Karplus generalized equation developed by Haasnoot *et al.*[23] The observed vicinal coupling constants involving H-5, H-6 and H_2-7 were very similar to those calculated for the model of the *cis*-isomer (Table 4). In the ROESY experiment H-19a (δ 0.43) showed NOE cross-correlation peaks with the signals corresponding to H-7β (δ 1.55) and H-8 (δ 2.12); and the signals of H-5 and H-6 showed nOe

Table 2. ^{13}C-NMR data of compounds **1-3**, and **5**.*

C#	**1**	**2**	**3**	**5**†
1	30.8	30.7	28.1	29.1
2	31.1	31.2	30.3	31.5
3	173.3	178.1	179.3	174.4
4	139.0	139.1	152.1	149.5
5	38.9	39.0	41.9	45.9
6	74.3	74.4	28.7	27.8
7	27.1	27.2	25.2	25.1
8	38.2	38.2	47.9	47.8
9	25.0	25.1	21.8	21.4
10	28.1	28.1	27.4	27.2
11	26.4	26.5	26.9	27.1
12	32.9	33.0	33.0	33.1
13	45.7	45.7	45.1	45.3
14	48.6	48.6	48.9	49.0
15	34.7	34.8	35.6	35.7
16	27.7	27.8	28.1	28.1
17	51.2	51.4	52.1	52.2
18	15.9	16.0	18.1[a]	18.1[a]
19	23.1	23.2	30.3	30.0
20	35.9	36.0	36.0	36.0
21	18.2	18.2	18.2[a]	18.2[a]
22	34.7	34.8	34.7	35.2
23	25.9	26.0	26.1	25.7
24	155.2	155.4	155.7	143.2
25	139.0	139.1	139.2	127.2
26	195.2	195.5	196.0	168.8
27	9.1	9.2	9.2	12.4
28	123.0	123.1	110.6	111.6
29	170.6	170.7	64.7	19.4
30	20.0	20.1	19.4	19.8
OMe	51.7			51.5, 51.7

* Chemical shifts given in ppm using TMS as internal reference in $CDCl_3$ (90.8 MHz).
[a] Interchangeable assignments.
† ^{13}C-NMR data from literature.[18]

Table 3. Selective INEPT NMR data for **1** ($CDCl_3$).

Proton (s) irradiated	Carbon (s) enhanced
H-5	23.1 (C-19), 27.1 (C-7), 28.1 (C-10) 30.8 (C-1), 74.3 (C-6), 123.0 (C-28) 139.02 (C-4), 170.6 (C-29)
H-6	28.1 (C-10), 38.2 (C-8), 139.02 (C-4) 170.6 (C-29)
H-8	20.0 (C-30), 23.1 (C-19), 25.0 (C-9) 27.1 (C-7), 28.1 (C-10), 34.66 (C-15) 45.7 (C-13), 48.6 (C-14), 74.3 (C-6)
H_3-18, 21, 30	32.9 (C-12), 34.66 (C-15, C-22) 35.9 (C-20), 38.2 (C-8), 45.7 (C-13) 48.6 (C-14), 51.2 (C-17)
H-19α	25.0 (C-9), 28.1 (C-10), 30.8 (C-1) 38.2 (C-8), 38.9 (C-5)
H-19β	25.0 (C-9), 28.1 (C-10), 30.8 (C-1) 38.2 (C-8), 38.9 (C-5). 26.4 (C-11)
H-24	9.1 (C-27), 34.66 (C-22), 25.9 (C-23) 195.2 (C-26)
H-26	138.97 (C-25)
H-28a	38.9 (C-5), 139.02 (C-4), 170. 6 (C-29)
H-28b	38.9 (C-5), 170.6 (C-29)

Table 4. Observed vs. calculated vicinal *J* values (Hz) involving H-5, H-6 and H_2-7 in compound **1**.

	Calculated		Observed
J (Hz)	*cis*-isomer	*trans*-isomer	**1**
H-5, H-6	8.99	10.85	8.0
H-6, H-7α	7.34	10.11	6.5
H-6, H-7β	8.48	5.67	8.0

cross-correlation peaks with the three-proton singlet at δ 0.92, which was consequently assigned to H_3-30. These results again agreed very well with the spatial relationship observed for these protons in the model of the *cis*-isomer. The signal of H-8 also showed a cross-peak with the resonance of H_3-18 (δ 0.95, s), and the signal of H-7α showed a cross-peak correlation with the singlet corresponding to H_3-30. The relative stereochemistry of this compound was confirmed by single-crystal X-ray diffraction analysis, and a three-dimensional view of the structure is given in Figure 2.

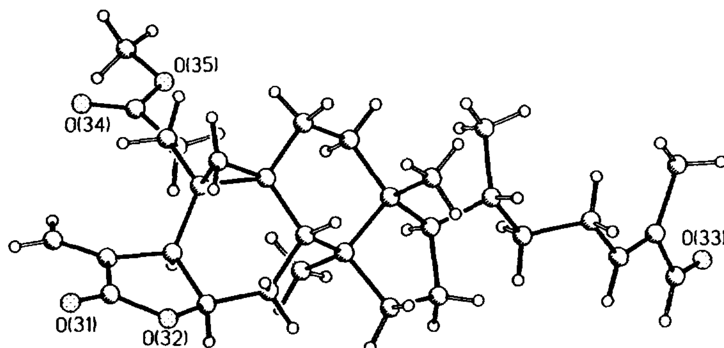


Figure 2. Perspective view of the molecular structure of **1** as determined by X-ray crystallography.

The ^{13}C-NMR (Table 2) signals of **1** were fully assigned using the DEPT, HETCOR and selective INEPT experiments, and also by comparison with the published ^{13}C-NMR data of cycloartanes. The selective INEPT experiment results (Table 3) were helpful in the assignment of the quaternary carbons and also allowed the differentiation of the resonances of C-4 and C-25, which varied by only 0.05 ppm. Irradiation of the aldehyde signal at δ 9.40 (H-26) produced an enhancement of the signal at 138.97 (C-25), while selective polarization transfer from the resonances of H-5, H-6 and H-28a enhanced the signal at δ 139.02 (C-4). The remaining irradiations presented in Table 3 substantiated the proposed structure for coronalolide methyl ester (**1**). Assignments for the ^{1}H-NMR signals corresponding to protons H_2-1 and H_2-2 are in agreement with analogous data reported for dimethyl 3,4-*seco*-mangiferonate from *Tillandsia usneoides*.[24)]

Compound **2** revealed a molecular formula of $C_{30}H_{42}O_5$ by HREIMS, representing one carbon and two hydrogen atoms less than compound **1**. An EIMS fragment peak also showed the loss of 125 Da from the molecular ion at *m/z* 482, indicating the presence of the same side-chain attached to C-17 as in **1**. The IR spectrum of **2** lacked the band at 1739 cm-1 corresponding to the saturated ester at C-3 and exhibited new bands at 1707 cm^{-1} and 3221 cm^{-1} (broad shoulder), suggesting the presence of a free carboxylic acid group. The ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) of **1** and **2** were almost superimposable, except that the signals corresponding to the ester OMe group were lacking, and the signal due to the carbonyl group at C-3 in the ^{13}C-NMR spectrum of coronalolide (**2**) was shifted downfield *ca.* 5 ppm with respect to compound **1**. Hydrolysis

of compound **1** with LiOH in refluxing THF afforded **2** after purification by prep. TLC using 40% CH_2Cl_2-MeOH as solvent system.

Compound **3** demonstrated a molecular formula of $C_{30}H_{46}O_4$ by HREIMS, representing one oxygen atom and two degrees of unsaturation less than compound **2**. The IR spectrum exhibited aldehyde (1688 cm^{-1}) and free carboxylic acid (1707 cm^{-1}) carbonyl groups, however, as well as hydroxyl group absorption (3431 cm^{-1}). The ^1H- and ^{13}C-NMR spectra of **3** (Tables **1** and **2**) revealed the presence of the same side-chain as in compounds **1** and **2**. The signal corresponding to the carbonyl of the γ-lactone ring in **1** and **2** was absent, as was the ^1H-NMR signal corresponding to the γ-lactone carbinol proton H-6 of **1** and **2**. Two one-proton, broad singlets at δ 5.09 and δ 5.11 suggested the presence of a terminal double bond. This signal exhibited allylic coupling with the two-proton, broad singlet at δ 4.12. The pair of doublets corresponding to H-19a (δ 0.50) and H-19b (δ 0.74) appeared shifted downfield with respect to analogous signals in compounds **1** and **2**. These data suggested a change in the substitution pattern of ring B, leading to the proposal for coronalolic acid (**3**) of a 3,4-*seco*-cycloartane structure with C-29 oxidized to a primary alcohol.

The ^{13}C-NMR DEPT spectrum of **3** (Table 2) revealed the presence of a carbonyl doublet (aldehyde), a carbonyl singlet, four sp^2 carbons (two C, one CH and one CH_2), and twenty-four sp^3 carbons (four C, four CH, twelve CH_2 and four CH_3). One of these methylenes resonated at δ 64.7, confirming the presence of a primary alcohol group. The ^{13}C-NMR data correlated closely with those reported for the 3,4-*seco*-cycloartanes **5**[24] and **6**[20] isolated from *Tillandsia usneoides*, which differ from compound **3** in the side-chain (methyl ester or free acid instead of aldehyde), at C-3 (methyl ester instead of free acid), and at C-29 (alcohol instead of methyl group). It can be seen in Table 2 that oxidation of the methyl group C-29 to an alcohol produced a γ-effect at C-5 (δ 45.9 in **5** and **6**, δ 41.9 in **3**). Also, a δ-effect was observed in the resonance of C-6, which was shifted slightly downfield in coronalolic acid (**3**) with respect to **5** and **6** (Table 2).

Finally, a very small amount of a fourth 3,4-*seco*-cycloartane, **4**, was purified from a fraction treated with diazomethane. Its CIMS exhibited a quasimolecular ion peak ($[M+1]^+$) at *m/z* 527, corresponding to a molecular formula of $C_{33}H_{50}O_5$. The base peak was observed at *m/z* 467, produced by the loss of acetic acid from the quasimolecular ion. Due to the paucity of **4**, we were only able to obtain a ^1H-NMR spectrum (Table 1). Analysis of the data, however, enabled compound **4** to be proposed as the C-29-acetyl, C-3-methyl ester derivative of **3**, presumably derived from the corresponding acid, **3**.

Compounds **1-3** were evaluated for their cytotoxic effects against a panel of human cancer cell lines.[25] While coronalolic acid (**3**) was inactive in all cell lines tested, coronalolide methyl ester (**1**) and coronalolide (**2**) were broadly cytotoxic with ED_{50} values in the range of 0.1-1.0 μg/ml [most potent activity for **1**, ED_{50} 0.6 μg/ml against a hormone-dependent breast cancer cell line (ZR-75-1); most potent activity for **2**, ED_{50} 0.5 μg/ml against a glioma cell line (U373)]. Based on the inactivity of **3**, the cytotoxicity of **1** and **2** appears to be facilitated by the exomethylene lactone ring portion of each molecule.

EXPERIMENTAL

General procedures: Mps: uncorr.; UV: MeOH; IR: film; ^{1}H- and ^{13}C-NMR spectra were recorded on 300, 360 or 500 MHz instruments with TMS as int. standard; low- and high-resolution mass spectra were obtained on a Finnigan MAT-90 instrument.

Plant material: The leaves and stems of *Gardenia coronaria* Buch.-Ham. (Rubiaceae) and the leaves of *G. sootepensis* Hutch. were collected at Doi Suthep, Chiangmai, Thailand in September, 1993 and in February, 1990, respectively, and identified by one of us (T.S.). The voucher specimens for *G. coronaria* (BKF 93742) and *G. sootepensis* (BKF 101411) have been deposited at the Herbarium, Royal Forestry Department, Bangkok, Thailand.

Extraction and isolation: The dried, powdered leaves of *G. coronaria* (35 g) were extracted by maceration with $CHCl_3$ three times and the residue was concentrated under vacuum to afford a gum (3.74 g). The dried $CHCl_3$ extract was absorbed on Si gel (8 g) and fractionated by flash CC (column i.d 2.5 cm packed with Si gel, 15 cm deep) and eluted with hexanes, hexanes-toluene (1:1), toluene, toluene-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-EtOAc mixtures of increasing polarity, EtOAc, EtOAc-MeOH (1:1), and MeOH. Fraction 7 (293 mg, $CHCl_3$-EtOAc 4%) showed a main UV-active spot, that appeared pink when sprayed with H_2SO_4/vanillin, and was purified by flash CC with silica H, eluting with $CHCl_3$, EtOAc, and MeOH in gradients. Subfractions 3 and 4 were combined (59.8 mg) and coronalolide methyl ester (**1**, 9.0 mg, 0.025%) was purified by prep. TLC with $CHCl_3$-EtOAc (9:1) as solvent. From fraction 8 (185 mg), coronalolide (**2**, 12.5 mg, 0.036%) was separated by a combination of normal and C_{18}-reversed phase CC. Fractions 14-16 (207.8 mg, $CHCl_3$-EtOAc 70%) were fractionated by flash CC eluted with $CHCl_3$ and $CHCl_3$-MeOH (1, 2, 4, 8 and 12%) and washed with MeOH. Subfraction 13 (62.4 mg) showed a main UV active spot with R_f 0.75 using $CHCl_3$-MeOH (85:15), and coronalolic acid (**3**, 9.5 mg, 0.027%) was purified by prep. TLC using this solvent system.

The powdered stems of *G. coronaria* (1.5 kg) were extracted with $CHCl_3$ (3 x 5 L) at room temperature, filtered, and concentrated under vacuum, giving a dried extract (75.17 g) which, in turn, was fractionated by VLC, eluting with the same solvent system (1.5 L) as for the leaf extract. Fraction 5 (7.01 g, $CHCl_3$-EtOAc 5%) was subfractioned by gravity CC (Si gel, 450 g) using as solvent systems hexane, hexane-EtOAc mixtures of increasing polarity, EtOAc-MeOH (19:1 and 9:1), and MeOH. Subfraction 14 (1.31 g) was purified by CC with $CHCl_3$ to afford 550 mg of **1**. Fraction 6 (15.08 g, $CHCl_3$-EtOAc 10%) was fractionated by VLC, and subfraction 6 (2.35 g, hexane-EtOAc 25%) after being purified by CC with $CHCl_3$, afforded a further 1.2 g of compound **1**. Fraction 7 (3.67 g, $CHCl_3$-EtOAc 15%) was purified by CC (180 g Si gel) using $CHCl_3$, and $CHCl_3$-MeOH mixture of increasing polarity, and MeOH for elution. Subfractions 5-16 were combined affording a yellow precipitate (126.5 mg, 0.008%) identified as 5,7,3′-trihydroxy-6,4′,5′-

trimethoxyflavone.[26] Fraction 8 (2.69 g, $CHCl_3$-EtOAc 20%) was purified by VLC, subfraction 4 affording crystals that purified by sublimation that were identified as benzoic acid[27] (2 mg). Fraction 10 (4.13 g) was fractionated by VLC using petroleum ether-$CHCl_3$ mixtures (20 and 50%) as solvent system. Subfraction 7 after treatment with CH_2N_2 afforded a complex mixture (535 mg) that was purified further by flash CC eluted with $CHCl_3$-MeOH mixtures (2, 5, 10, 20 and 50%), and prep. TLC [hexanes-MeOH (8:2)] gave methyl coronalolate acetate (**4**, 1.2 mg, 0.00008%).

The dried, powdered leaves of *G. sootepensis* (8.67 kg) were extracted with hexanes (3 x 20 L), $CHCl_3$ (3 x 20 L), EtOAc (3 x 20 L), and MeOH (4 x 20 L) at room temperature. Removal of solvents from each extract under vacuum afforded greenish residues (369.7, 535.8, 212.6 and 191.3 g, respectively). The active chloroform extract (348 g, P-388 murine lymphocytic leukemia cell line, ED_{50} 1.6 μg/ml) was subjected to flash CC (Si gel H) using hexanes and hexanes-EtOAc mixtures of increasing polarity, and MeOH for elution. Fraction 8 (74 g) was purified by flash CC using hexanes, CH_2Cl_2 and MeOH mixtures of increasing polarity to afford a gum (17.6 g) which was crystallized to yield **1** (3.8 g) as colorless plates upon the addition of hexanes-CH_2Cl_2 mixture. Fraction 9 (129 g) was repeatedly chromatographed on Si gel 60 using hexanes, CH_2Cl_2 and MeOH mixtures of increasing polarity to afford **2** (2.3 g).

*Coronalolide methyl ester [3,4-*seco*-cycloart-4(28),24-diene-6-hydroxy-26-ol-3,29-dioic acid-29-α,γ-lactone-3-methyl ester] (1).* Colorless needles from MeOH: mp 91-92.5°; $[\alpha]^{25}_D$ +121.6° (*c* 0.86, $CHCl_3$); UV λ^{MeOH}_{max} nm: 213 and 229; IR ν_{max} (film) cm^{-1}: 2943, 1768 (γ-lactone), 1739 (COOR), 1684 (CHO), 1641 ($C=CH_2$), 1265 and 1167; ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 2; EIMS *m/z* (rel. int. %): 496 (14) $[M]^+$, 481 (9) $[M-CH_3]^+$, 371 (46) $[M-C_8H_{13}O]^+$, 231 (18), 217 (23), 157 (45), 145 (49), 129 (68), 125 (24), 105 (100); HR-EIMS: *calcd* for $C_{31}H_{44}O_5$ 496.3189, found 496.3182.

Coronalolide (2). White powder from hexanes-CH_2Cl_2: mp 82.5-83°; $[\alpha]^{25}_D$ +119.1° (*c* 0.69, $CHCl_3$); UV λ^{MeOH}_{max} nm: 241 and 268; IR ν_{max} (film) cm^{-1}: 3221 (broad shoulder, COOH), 2946, 2880, 1761 (γ-lactone), 1707 (COOH), 1686 (CHO), 1641 ($C=CH_2$), 1458, 1268, 1148, 1028, 988 and 757; ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 2; EIMS *m/z* (rel. int. %): 482 (25) M^+, 464 (15) $[M-H_2O]^+$, 384 (22), 357 (100) $[M-C_8H_{13}O]^+$, 245 (13), 219 (18), 147 (37), 121 (41), 107 (49); HR-EIMS: *calcd* for $C_{30}H_{42}O_5$ 482.3032, found 482.3027.

*Coronalolic acid [3,4-*seco*-cycloart-4(28),24-dien-26-ol-29-hydroxy-3-oic acid] (3).* Amorphous gum: $[\alpha]^{20}_D$ -36.4° (*c* 0.21, $CHCl_3$); UV λ^{MeOH}_{max} nm: 232; IR ν_{max} (film) cm^{-1}: 3431 (OH), 2930, 1707 (COOH), 1688 (CHO), 1456, 1377, 1177, 1059, 988 and 901; ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 2; EIMS *m/z* (rel. int. %): 470 (36) M^+, 455 (100), 452 (39) $[M-H_2O]^+$, 437 (55), 424 (38), 345 (20) $[M-C_8H_{13}O]^+$, 327 (30), 285 (18), 233 (54), 147 (84), 109 (86), 105 (90); HR-EIMS: *calcd* for $C_{30}H_{46}O_4$ 470.3396, found 470.3405.

Methyl coronalolate acetate (4). Amorphous gum: ^{1}H-NMR (500 MHz, $CDCl_3$): Table 1; CIMS *m/z* (rel. int. %): 527 (42) $[M+1]^+$, 485 (14) $[M+1\text{-}CH_2{=}C{=}O]^+$, 467 (100) $[M+1\text{-}AcOH]^+$.

X-ray experimental data and structure analysis of 1: *Crystal data.* $C_{31}H_{44}O_5$, M_r=496.66, Monoclinic, $P2_1$, a=7.413(4), b=27.85(2), c=7.486(5) Å, β=116.44(5)°, V=1384(2) Å^3 (by least squares refinement on diffractometer angles for 12 automatically centered reflections), λ=1.54178 Å, Z=2, D_c=1.192 Mg/m^{-3}, F(000) = 540, μ(Cu-Kα)=0.627 mm^{-1}. *Data Collection and Processing.* Three-dimensional, room temperature (293°K) X-ray data were collected on a Rigaku AFC6S diffractometer with monochromatized Cu-Kα X-radiation. 2Θ/ω mode with scan range (ω) 6.36-57.46° plus Kα separation and a variable scan speed (4.88-14.65 min^{-1}). A total of 2110 reflections was measured ($3<2\Theta<115°$, min. hkl -8 0 0, max. hkl 7 30 8); 1945 independent reflections were obtained [R(σ)=0.0319, Friedel opposites merged]. No absorption correction was applied. One control reflection monitored every 99 reflections showed no appreciable decay during 13.6 h of exposure of the crystal to X-rays. *Structure Analysis and Refinement.* Direct methods resulted in the location of all of the non-hydrogen atoms. Full matrix least-squares refinement with anisotropic thermal parameters was used for all non-hydrogen atoms. Hydrogen atoms were refined in riding mode. Refinement converged at R=0.0463, R_w=0.1228. Maximum and mean shift/error in the final cycle of refinement were 0.006 and 0.003, respectively. The final electron-density difference synthesis showed no peaks >0.16 or <-0.14 eÅ^{-3}. All computations were carried out using the SHELXTL for IRIS V5.03 system of programs.[28)]

Bioassay evaluations: Compounds **1-3** were screened for cytotoxicity against a panel of human cancer cell lines, according to established protocols.[25)] ED_{50} values of >4 μg/ml were regarded as inactive.

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