Constituents of the Root Bark of *Severinia buxifolia* Collected in Hainan

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Two new tetranortriterpenoids, 7-isovaleroylcycloseverinolide (**1**) and 7-isovaleroylcycloepiatalantin (**2**), together with 28 known compounds, were isolated and characterized from the root bark of *Severinia buxifolia* collected in Hainan. The structures of **1** and **2** were elucidated on the basis of spectral evidence including 2D NMR and X-ray techniques. The cytotoxicity of several acridone alkaloid isolates (**3**−**8**) was evaluated against a small tumor cell panel.

Severinia buxifolia Tenore [*Atalantia buxifolia* (Poir.) Oliv.] (Rutaceae) has been used in Chinese folk medicine for the treatment of chronic rheumatism, malaria, paralysis, and snakebite.[1,2] We have isolated several compounds from the root bark of *S. buxifolia* collected in Taiwan.[3−6] However, Gu and Qin have only isolated simple acridone alkaloids from the root bark of this plant collected from Hainan Province, People's Republic of China.[7,8] To better understand the relationship between bioactive constituents and the collection location of the plant, we reinvestigated the root of *S. buxifolia*, collected from Hainan Province, and have also reported several acridone alkaloids.[9] In the present paper, we describe the isolation and structural elucidation of two new tetranortriterpenoids, namely, 7-isovaleroylcycloseverinolide (**1**) and 7-isovaleroylcycloepiatalantin (**2**), from the root bark of *S. buxifolia*. In addition, 28 known compounds were obtained. The cytotoxicity of six acridone alkaloid isolates (**3**−**8**) against a small cancer panel was also investigated.

Results and Discussion

7-Isovaleroylcycloseverinolide (**1**) was isolated as optically active colorless plates and exhibited the molecular formula $C_{31}H_{38}O_9$ by HRFABMS. Compound **1** showed UV maxima at 216 and 313 nm, an IR absorption band at 1684 cm^{-1}, a pair of olefinic protons at δ 6.19 and 7.72 (each 1H, d, $J = 5.6$ Hz) in the ^1H NMR spectrum, and signals at δ 131.3, 170.6, and 213.9 in the ^{13}C NMR spectrum, characteristic of a cyclopentenone system.[5] A β-substituted furan ring in the molecule was inferred by proton signals at δ 6.28 (1H, d, $J = 1.8$ Hz) and 7.39 (2H, d, $J = 1.8$ Hz) and the carbon signals at δ 109.7 (d), 120.2 (s), 141.2 (d), and 143.1 (d). The ^1H NMR spectrum of **1** also revealed the presence of typical H-15 and H-17 signals of a limonoid ring D epoxylactone at δ 4.01 and 5.56, respectively. The appearance of four tertiary methyl signals at δ 1.05, 1.20, 1.21, and 1.50 (each 3H, s) and AB quartet signals at δ 3.84 and 3.95 (each 1H, d, $J = 10.0$ Hz) suggested a limonin carbon skeleton with an ether bridge from C-19 to C-4. On the basis of the above data, we assumed that **1** was similar to cycloseverinolide.[5] Comparison of the spectral data of **1** with those of the latter compound indicated that two hydroxyl protons (δ 2.50 and 6.29) were replaced in **1** by a hydroxyl group (δ 5.79, exchangeable with D_2O) and an isovaleroyl group [δ 2.17 (1H, m), 2.11 (1H, m), 2.04 (1H, m), 0.95 (3H, d, $J = 6.4$ Hz), and 0.93 (3H, d, $J = 6.4$ Hz)] in **1**. The H-7 signal at δ 3.42 in cycloseverinolide was shifted downfield to δ 4.98 in **1**. The cross-peaks between H-6 (δ 4.40) with C-3 (δ 213.9) and H-7 (δ 4.98) with C-1′ (δ 171.6) in the HMBC experiment of **1** (Figure 2), together with the coupling constant of H-6 and H-7 ($J = 2.4$ Hz), confirmed that the isovaleroyl and hydroxyl groups were located at C-7α and C-6α, respectively. The complete structure and relative stereochemistry of 7-isovaleroylcycloseverinolide was determined by a single-crystal X-ray diffraction analysis (Figure 3). Thus, compound **1** was assigned the structure 7-isovaleroylcycloseverinolide.

7-Isovaleroylcycloepiatalantin (**2**) was obtained as optically active colorless plates. It displayed a protonated molecular ion peak at *m/z* 533.2435 $[M + H]^+$ in its HRFABMS, corresponding to the molecular formula $C_{31}H_{36}O_9$. The UV, IR, ^1H NMR, and ^{13}C NMR spectral data were similar to those of **1**, indicating the presence of a tetranortriterpenoid skeleton. The presence of a δ-lactone group, an α,β-unsaturated carbonyl system, and a β-substituted furan ring in the molecule were characterized spectroscopically in a manner similar to **1**. On the basis of the comparison of its spectral data, we found **2** to be similar to cycloepiatalantin.[5] The differences between **2** and the latter compound were that the H-7 signal at δ 3.43 and the C-7 hydroxyl proton at δ 3.60 were substituted in **2**, in turn, by a C-7 proton at δ 4.64 and an isovaleroyl group at δ 0.91 (6H, d, $J = 6.4$ Hz), 2.00 (1H, m), and 2.02 (2H, m). On the other hand, the spectral data of **2** were also similar to those of **1**. The difference between **1** and **2** was only a hydroxyl substituent on C-6 (δ_C 71.1) in **1** instead of a carbonyl group (δc 195.1) in **2**, which was also confirmed by an IR absorption band for the carbonyl group at 1755 cm^{-1} in **2**. To confirm the substitution of the isovaleroyl group, a HMBC experiment was carried out. The results showed ^2J and ^3J correlations of H-7 (δ 4.64) with C-1′ (δ 169.9), C-6 (δ 195.1), and C-9 (δ 34.5) (Figure 2) and indicated that the isovaleroyl group was located at C-7. The complete structure and relative stereochemistry of 7-isovaleroylcycloepiatalantin was also determined by a single-crystal X-ray analysis (Figure 4). The structure of 7-isovaleroylcycloepiatalantin was assigned to **2**.

In addition to these new terpenoids, 28 known compounds were also isolated and identical, namely, atalantolide,[5] α-santalene,[3] α-photosantalol,[3] (*E*)-5-(2,3-dimethyl-3- nortricyclyl)pent-3-en-2-one,[3] dihydro-α-santalen-12-one,[3] buxifoliadine-A,[9] -C,[9] -E,[9] -F,[9] -G,[9] buxifoliadine-B (**3**),[9]

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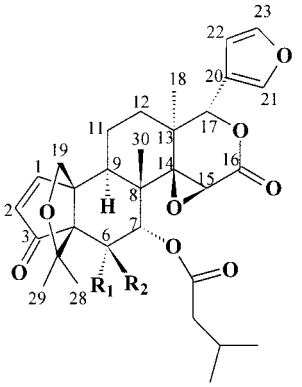
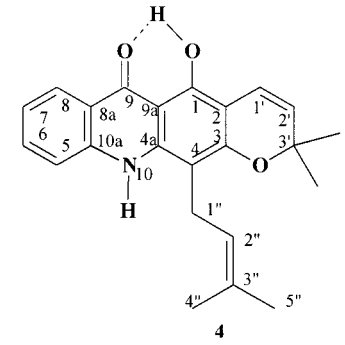
Chart 1



1 R_1=OH, R_2=H
2 R_1=R_2=O



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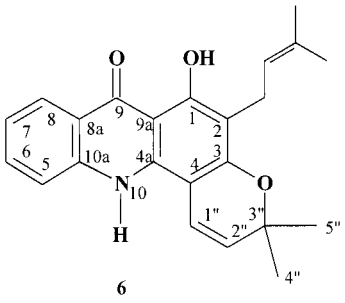
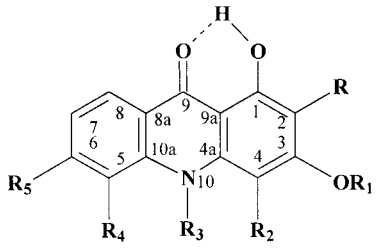
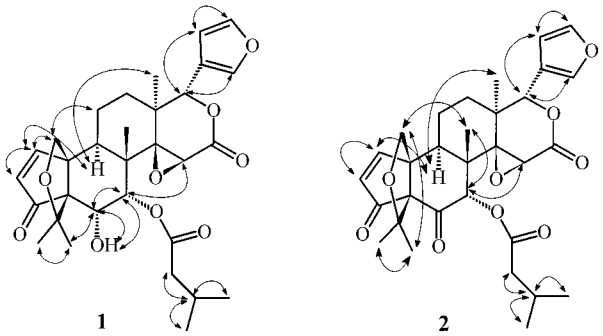


6

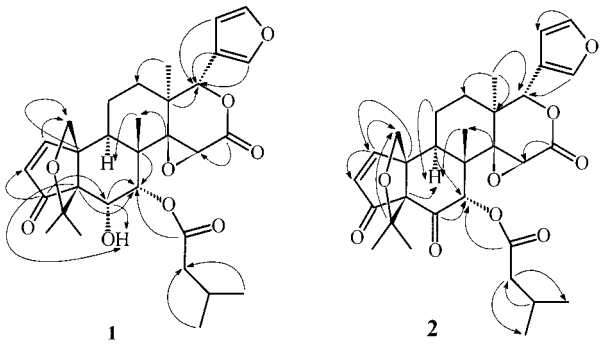


3 R=R_2=prenyl, R_1=CH_3, R_3=R_5=H, R_4=OH
5 R=R_1=H, R_2=R_4=OCH_3, R_3=CH_3, R_5=OH
7 R=R_5=H, R_1=R_3=CH_3, R_2=OCH_3, R_4=OH
8 R=R_1=R_5=H, R_3=CH_3, R_2=OCH_3, R_4=OH



1 **2**

Figure 1. NOESY correlations for compounds **1** and **2**.



1 **2**

Figure 2. HMBC correlations for compounds **1** and **2**.

buxifoliadine-D (**4**),[9] buxifoliadine-H (**5**),[9] severifoline (**6**),[4] atalaphyllinine,[4] atalaphyllidine,[9] citrusinine-I (**7**)[9] and -II (**8**),[9] *N*-methylatalaphylline,[9] 1,2,3-trihydroxyacridone,[9] 5-hydroxy-*N*-methylseverifoline,[6] glycocitrine-I,[9] *N*-methylswietenidine-B,[10] auraptene,[6] 8-geranyl-7-hydroxycoumarin,[6] β-sitosterol,[6] stigmasterol,[6] and methyl ferulate.[11]

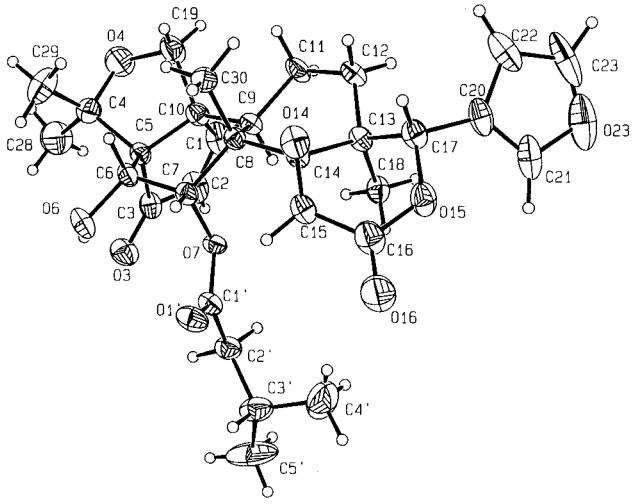


Figure 3. Single-crystal X-ray crystallographic diagram of **1**.

The acridone alkaloids buxifoliadine-H (**5**), severifoline (**6**), citrusinine-I (**7**), and citrusinine-II (**8**) showed significant cytotoxic activities against KB cells (nasopharyngeal carcinoma), with ED_{50} values of 0.22, 0.09, 0.09, and 0.82 μg/mL, respectively. Compounds **5** and **7** also exhibited marginal cytotoxic activities against Hepa-3B (hepatoma) cells with ED_{50} values of 5.2 and 6.6 μg/mL, respectively. Buxifoliadine-B (**3**), buxifoliadine-D (**4**), and citrusinine-I (**7**) exhibited significant cytotoxic activities against colo-205 cell (colon carcinoma) (ED_{50} values of 1.2, 0.58, and 6.3 μg/mL, respectively) (Table 1).

Our present and previous results[9] showed that the constituents of the roots of *S. buxifolia* vary according to the area from where they are collected. Hence, plant-based traditional Chinese medicines must be obtained from

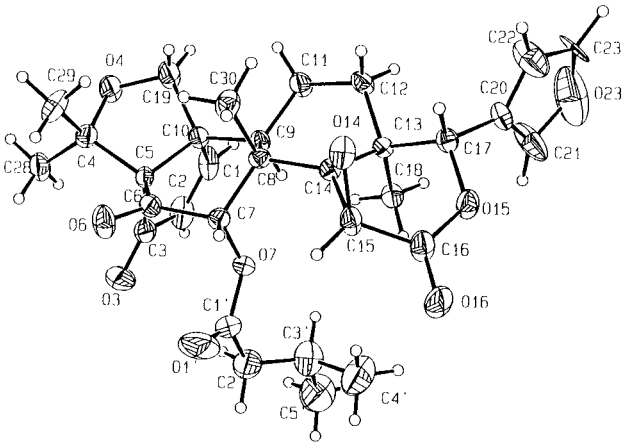


Figure 4. Single-crystal X-ray crystallographic diagram of **2**.

Table 1. Cytotoxicity of Acridone Alkaloids Isolated from Root Bark of *Severinia buxifolia*

compound	cell line (ED_{50}, μg/mL)[a]		
	colon-205	hep-3B	KB
buxifoliadine-B (**3**)	1.2	NA[b]	NA
buxifoliadine-D (**4**)	0.58	NA	NA
buxifoliadine-H (**5**)	NA	5.3	0.22
severifoline (**6**)	NA	NA	0.09
citrusinine-I (**7**)	6.3	6.6	0.09
citrusinine-II (**8**)	NA	NA	0.82

[a] Cell lines used: Colo-205 (colon carcinoma); Hep-3B (hepatoma); KB (nasopharyngeal carcinoma). [b] NA= no activity (ED_{50} > 25 μg/mL).

specific collection areas to maintain defined pharmacological activities.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto MP-S3 micromelting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO Dip-370 polarimeter. The IR spectra were recorded on a Shimadzu FT IR-8501 spectrophotometer as KBr disks, and the UV spectra were recorded on a Hitachi U-3210 spectrophotometer. The ^{1}H and ^{13}C NMR spectra (400 MHz) were recorded with Varian Unity plus 400 MHz and Bruker AMX-400 spectrophotometers ($CDCl_3$ and acetone-d_6 as solvent). Chemical shift values are shown in ppm (δ) with TMS as an internal standard. Mass spectra were recorded on a VG 70-250S mass spectrometer. X-ray crystallography was conducted on an Enraf-Nonius CAD4 instrument.

Plant Material. The plant material (980 g) used for this study (root bark of *Severinia buxifolia* Tenore) was collected in February 1997 from Hainan Province, People's Republic of China, and identified by Prof. C. S. Kuoh. A voucher specimen (TSWu 970001) was deposited in the Herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried root bark of *S. buxifolia* (980 g) was extracted with hot methanol (40 L) and concentrated under reduced pressure to give a dark brown syrup (180 g). The syrup was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer (120 g) was concentrated under reduced pressure to give a further brown syrup, which was directly subjected to Si gel column chromatography with successive elution with *n*-hexane, C_6H_6, EtOAc, and MeOH, to afford 20 fractions. Fraction 1 was chromatographed over a Si gel coating with 5% silver nitrate and eluted with *n*-hexane to give α-santalene (3.15 g), α-photosantalol (2 mg), (*E*)-5-(2,3-dimethyl-3-nortricyclyl)pent-3-en-2-one (2 mg), and dihydro-α-santalen-12-one (1.5 mg). Fraction 3 was chromatographed over Si gel and eluted with a gradient of *n*-hexane and EtOAc to give buxifoliadine D (**4**, 15 mg). Fraction 9 was rechromatographed over a Si gel column with *n*-hexane−*i*-Pr_2O (3:1) as eluent to afford buxifoliadine A (450 mg), severifoline (**6**, 23 mg), and auraptene (1.2 g). Fraction 10 was rechromatographed over a Si gel column and eluted with *n*-hexane−*i*-Pr_2O (4:1) to obtain *N*-methylatalaphylline (3 mg), 1,2,3-trihydroxyacridone (3 mg), 8-geranyl-7- hydroxycoumarin (3 mg), a mixture of β-sitosterol and stigmasterol (520 mg), and methyl ferulate (0.9 mg). Fraction 11 was separated by Si gel column chromatography by elution with *n*-hexane−EtOAc (3:1) to give atalantolide (40 mg), buxifoliadine B (**3**, 238 mg), 5-hydroxy-*N*-methylseverifoline (1 mg), and glycocitrine-I (32 mg). Fraction 13 was purified over a Si gel column and eluted with *n*-hexane−EtOAc (6:1) to afford 7-isovaleroylcycloepiatalantin (**2**, 250 mg) and α-santalane-11,12,13-triol (6 mg). Fraction 14 was chromatographed over a Si gel column by elution with $CHCl_3$−Me_2CO (14:1) to give 7-isovaleroylcycloseverinolide (**1**, 207 mg), buxifoliadine C (5 mg), buxifoliadine E (2 mg), buxifoliadine F (6 mg), buxifoliadine G (15 mg), buxifoliadine H (**5**, 232 mg), atalaphyllinine (0.8 mg), atalaphyllidine (0.5 mg), citrusinine-I (**7**, 32 mg), citrusinine-II (**8**, 40 mg), and *N*-methylswietenidine B (2 mg).

7-Isovaleroylcycloseverinolide (1): colorless plates; mp 242−244 °C; $[\alpha]_D$ +73° (*c* 0.63, $CHCl_3$); IR ν_{max} 3350, 2955, 2878, 1742, 1684, 1595, 1506, 1472, 1057, 1026, 895 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 313 (1.87), 216 (4.05) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.94 (3H, d, J = 6.4 Hz, H-4′), 0.96 (3H, d, J = 6.4 Hz, H-5′), 1.05 (3H, s, H-18), 1.20 (3H, s, H-28), 1.21 (3H, s, H-30), 1.50 (3H, s, H-29), 1.71 (2H, m, H-11β, H-12α), 1.94 (1H, m, H-11α), 2.04 (1H, m, H-2′), 2.11 (1H, m, H-3′), 2.14 (1H, m, H-12β), 2.17 (1H, m, H-2′), 2.57 (1H, m, H-9), 3.84 (1H, d, J = 10.0 Hz, H-19), 3.95 (1H, d, J = 10.0 Hz, H-19), 4.01 (1H, s, H-15), 4.40 (1H, dd, J = 8.4, 2.4 Hz, H-6), 4.98 (1H, d, J = 2.4 Hz, H-7), 5.56 (1H, s, H-17), 5.79 (1H, d, J = 8.4 Hz, OH-6), 6.19 (1H, d, J = 5.6 Hz, H-2), 6.28 (1H, d, J = 1.8 Hz, H-22), 7.39 (2H, d, J = 1.8 Hz, H-21, H-23), 7.72 (1H, d, J = 5.6 Hz, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.0 (C-11), 18.0 (C-18), 18.4 (C-30), 22.4 (C-4′, C-5′), 24.7 (C-3′), 24.9 (C-29), 25.3 (C-12), 28.1 (C-28), 35.8 (C-9), 38.5 (C-13), 43.0 (C-2′), 43.4 (C-14), 56.7 (C-15), 59.7 (C-10), 62.4 (C-8), 69.2 (C-19), 71.1 (C-6), 74.7 (C-7), 77.3 (C-5), 77.6 (C-17), 85.5 (C-4), 109.7 (C-22), 120.2 (C-20), 131.3 (C-2), 141.2 (C-23), 143.1 (C-21), 166.7 (C-16), 170.6 (C-1), 171.6 (C-1′), 213.9 (C-3); FABMS *m*/*z* 593 ([M + K]$^+$, 5), 577 ([M + Na]$^+$, 31), 555 ([M + H]$^+$, 100), 497 (30), 395 (42); HRFABMS *m*/*z* 555.2595 ([M + 1]$^+$) (calcd for $C_{31}H_{39}O_9$, 555.2594).

X-ray Crystallography of 1. *Crystal data*: colorless crystal (0.31 × 0.44 × 0.19 mm) grown from acetone; $C_{31}H_{38}O_9$, mol wt = 554.64, monoclinic, space group $P2_12_12_1$, a = 8.4454(18) Å, b = 14.755(6) Å, c = 11.7790(20) Å, V = 1413.4(7) Å^3, Z = 2, D_c = 1.303 g/cm^3, F(000) = 591.94, μ = 0.09 mm^{-1}, λ(Mo Kα) = 0.70930 Å), 2764 measured intensities (−10 ≤ h ≤ 10, k = −17 → 0, l = −13 → 13), 2580 unique (R_{int} = 0.019), of which 1796 observed with $I \geq 2.5\sigma(I)$. *Data collection and structure refinement:* The intensity data were collected on a Nonius diffractometer, using graphite-monochromated Mo Kα radiation and the θ−2θ scan technique up to 49.8°. Cell parameters were refined from 24 well-centered reflections with 7.42° ≤ θ ≤ 18.91°. The structure was solved by direct methods using the NRCVAX System[12] and refined by full-matrix least-squares refinement. The last least-squares cycle was calculated with 78 atoms, 360 paramenters, and 1796 out of 2580 reflections. Weights based on counting statistics were used. The weight modifier K in KF_o^2 is 0.000100. Thus, for significant reflections, R_F = 0.034, R_w = 0.032, GoF = 1.29, and for all reflections, R_F = 0.051, R_w = 0.044.[13]

7-Isovaleroylcycloepiatalantin (2): colorless plates; mp 208−210 °C; $[\alpha]_D$ +22.3° (*c* 0.85, $CHCl_3$); IR ν_{max} 3400, 1755, 1742, 1684, 1595, 1506, 1393, 1367, 1028, 820 cm^{-1}, UV (MeOH) λ_{max} (log ϵ) 316 (1.94), 215 (4.28) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91 (6H, d, J = 6.4 Hz, H-4′, H-5′), 1.14 (3H, s, H-18), 1.17 (3H, s, H-30), 1.22 (3H, s, H-28), 1.38 (3H, s, H-29), 1.60 (1H, m, H-12β), 1.80 (1H, m, H-12α), 1.85 (1H, m, H-11β), 2.00 (1H, m, H-3′), 2.02 (3H, m, H-11α, H-2′), 2.91 (1H, dd, J = 12.6, 6.4 Hz, H-9), 3.67 (1H, s, H-15), 3.87 (1H, d, J = 10.0 Hz, H-19), 3.98 (1H, d, J = 10.0 Hz, H-19), 4.64 (1H, s, H-7),

5.57 (1H, s, H-17), 6.26 (1H, d, $J = 5.4$ Hz, H-2), 6.31 (1H, d, $J = 1.2$ Hz, H-22), 7.41 (2H, d, $J = 1.2$ Hz, H-21, H-23), 7.74 (1H, d, $J = 5.4$ Hz, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 15.5 (C-30), 16.7 (C-11), 17.9 (C-18), 22.4 (C-4′, C-5′), 24.9 (C-29), 25.1 (C-3′), 25.5 (C-12), 28.4 (C-28), 34.5 (C-9), 38.5 (C-13), 42.9 (C-2′), 43.0 (C-8), 56.4 (C-15), 61.1 (C-10), 68.4 (C-14), 69.9 (C-19), 72.9 (C-7), 75.5 (C-17), 77.6(C-5), 86.0 (C-4), 109.7 (C-22), 120.0 (C-20), 130.2 (C-2), 141.3 (C-23), 143.2 (C-21), 166.2 (C-16), 169.0 (C-1), 169.9 (C-1′), 195.1 (C-6), 200.2 (C-3); FABMS *m/z* 591 ($[M + K]^+$, 4), 575 ($[M + Na]^+$, 6), 553 ($[M + H]^+$, 74), 538 (12), 495 (100), 411 (45), 393 (23), 379 (26), 365 (32), 327 (27), 269 (30), 187 (44); HRFABMS *m/z* 552.2435 ($[M + 1]^+$) (calcd for $C_{31}H_{37}O_9$, 553.2438).

X-ray Crystallography of 2. *Crystal data*: colorless crystal (0.44 × 0.38 × 0.31 mm) grown from acetone; $C_{31}H_{36}O_9$, mol wt = 552.62, orthorhombic, space group $P2_12_12_1$, $a = 10.7327(16)$ Å, $b = 14.1631(17)$ Å, $c = 18.6762(13)$ Å, $V = 2838.9(6)$ Å^3, $Z = 4$, $D_c = 1.293$ g/cm^3, $F(000) = 1175.87$, $\mu = 0.09$ mm^{-1}, λ(Mo Kα) = 0.70930 Å, 5473 measured intensities ($-11 \le h \le 12$), $k = -15 \rightarrow 16$, $l = 0 \rightarrow 22$), 4893 unique ($R_{int} = 0.008$), of which 3800 observed with $I \ge 2.5\sigma(I)$. *Data collection and structure refinement:* conducted in the same manner as described for **1**. The last least-squares cycle was calculated with 76 atoms, 362 parameters, and 3800 out of 4983 reflections. Weights based on counting statistics were used. The weight modifier K in KF_o^2 is 0.000100. Thus, for significant reflections, $R_F = 0.041$, $R_w = 0.042$, GoF = 1.57, and for all reflections, $R_F = 0.051$, $R_w = 0.048$.[13]

Biological Assays The in vitro cytotoxicity assay was carried out according to the method reported by Elliott and Auersperg.[14]

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