Cytotoxic Cardenolide Glycosides of *Roupellina (Strophanthus) boivinii* from the Madagascar Rainforest[1]

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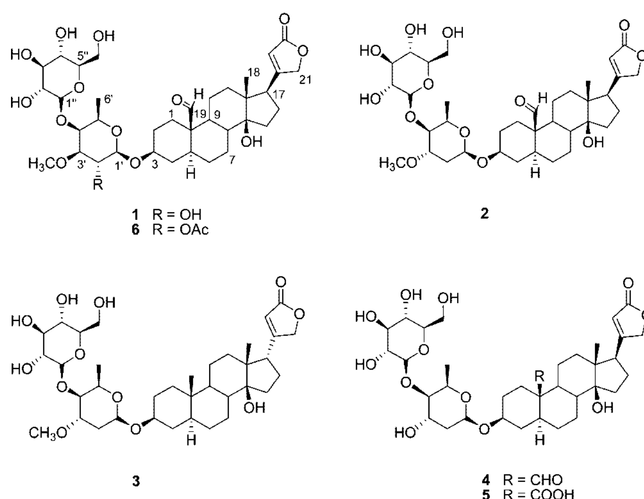
Bioassay-guided fractionation of an ethanol extract of *Roupellina (Strophanthus) boivinii* from the rainforest of Madagascar afforded the six new cardenolide glycosides boivinides **1**–**6**, as well as the four known cardenolide glycosides digitoxigenin 3-*O*-[β-D-glucopyrananosyl-(1→4)-α-L-acofriopyranoside], corotoxigenin 3-*O*-β-D-boivinoside, 17α-corotoxigenin 3-*O*-β-D-sarmentoside, and uzarigenin 3-*O*-α-L-rhamnoside. The structures of these compounds were elucidated by various 1D and 2D NMR techniques. All new compounds showed significant antiproliferative activity against the A2780 human ovarian cancer cell line, with boivinide A being the most active at $IC_{50} = 0.17\ \mu M$.

In our continuing search for bioactive natural products from the Madagascar rainforests as a part of an International Cooperative Biodiversity Group (ICBG) program,[1] we obtained an ethanol extract of the plant *Roupellina boivinii* (Baill.) Pichon (family Apocynaceae); this plant is also known as *Strophanthus boivinii* Baill. The extract, designated MG 2309PE, exhibited good antiproliferative activity against the A2780 ovarian cancer cell line (IC_{50} = 11 μg/mL) and, hence, was selected for fractionation to isolate its active components.

The Apocynaceae family is native mainly to Africa, with a few species in Asia. This family has 424 genera and 2100 species with 35–40 species of flowering plants in the *Roupellina*/*Strophanthus* genus.[2] The medicinal plants of this genus are known to contain lignans,[3] cytotoxic alkaloids,[4] and cardenolide glycosides.[4] Cardiac glycosides have also been isolated from the monarch butterfly (*Danaus plexippus* L.), which feeds on the plants of the milkweed (Apocynaceae) family.[5] Cardenolide glycosides are an important class of natural products that can be used as drugs as well as toxins, and plants of the Apocynaceae family or their extracts have been used as arrow poisons, emetics, or heart tonics since 1500 BC. Cardenolide glycosides are used in the treatment of congestive heart failure, but their toxicity limits their extensive use. The cytotoxicity and structure characterization of various cardenolide glycosides have been extensively studied. However, only two investigations to study the chemistry and the cardenolide glycosides of *Strophanthus boivinii* have been reported,[6,7] and no work has been reported under the name *Roupellina boivinii.*

Results and Discussion

Liquid–liquid partitioning of the ethanol extract of *R. boivinii* yielded an active methanol-soluble fraction with IC_{50} = 0.61 μg/mL and an active dichloromethane-soluble fraction with IC_{50} = 3.7 μg/mL in the A2780 bioassay. Both of the active fractions were separately passed through a short reversed-phase column with $MeOH/H_2O$ as the mobile phase and further purified by HPLC over a C_{18} column to yield six new compounds designated boivinides A–F (**1**–**6**) and the four known cardenolide glycosides digitoxigenin 3-*O*-[β-D-glucopyrananosyl-(1→4)-α-L-acofriopyranoside],[8] 5-α-corotoxigenin-β-D-boivinoside,[6] 17α-corotoxigenin 3-*O*-β-D-sarmentoside,[6] and uzarigenin 3-*O*-α-L-rhamnoside.[9]



Boivinide A (**1**) was obtained as a white, amorphous solid. Positive ion LC-MS gave a molecular ion peak at *m/z* 749.4 [M + K^+], consistent with a molecular composition of $C_{36}H_{54}O_{14}$. The ^{1}H NMR spectrum suggested the presence of an aldehyde group at δ_H10.01 (s, H-19), one methoxy group at δ_H 3.62 (s), and methyl groups at δ_H 0.93 (s, H-18) and 1.63 (d, H-6′, J = 6.0 Hz), suggesting the presence of a deoxy sugar moiety in the compound. Two signals for anomeric protons at δ_H 4.77 (d, H-1′, J = 7.2 Hz) and 5.20 (d, H-1″, J = 8.0 Hz) confirmed the presence of two sugar moieties. The ^{13}C NMR spectrum of **1** contained 36 signals, confirming the composition $C_{36}H_{54}O_{14}$. The signals were assigned as one methoxy, two methyls, 11 methylenes, 17 methines, and five quaternary carbons. The ^{1}H and ^{13}C NMR signals in C_5D_5N showed typical signals for an α,β-unsaturated γ-lactone unit, with peaks at δ_C 176.0 (C-20), δ_C 74.0 (C-21) and δ_H 5.03 and 5.27 (br d, J = 18.0 Hz, H-21), δ_C 118.1 (C-22) and δ_H 6.12 (br s, H-22), and δ_C 174.8 (C-23).

A consideration of the NMR spectra of **1** as discussed below and comparison with literature data indicated that the aglycone portion of **1** was that of corotoxigenin.[10] A detailed analysis of its COSY and 1D and 2D TOCSY spectra and HSQC and HMBC correlations enabled the proton–carbon pairs to be connected to each other and the $^1J_{CH}$ correlations to be determined (Table 1).

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Table 1. $^1H^a$ NMR and $^{13}C^b$ NMR Data of Compounds **1**, **2**, and **3**

	1[c]		**2**[c]		**3**[d]	
position	^1H (J, Hz)	^{13}C	^1H (J, Hz)	^{13}C	^1H (J, Hz)	^{13}C
1	0.90 m, 2.39 m	31.7	0.83 m, 2.40 m	31.8		38.3
2	1.56 m, 2.20 m	31.3	1.60 m, 2.38 m	31.6		31.8
3	3.97 br s	76.9	3.92 br s	76.2	4.03 m	73.3
4	2.04 m, 1.42 m	36.5	1.35 m, 1.86 m	36.7		35.6
5	1.19 m	43.3	1.20 m	43.4	1.18 m	45.6
6	1.89 m, 1.42 m	29	1.94 m, 1.40 m	29.2		28.8
7	2.48 m, 1.24 m	27.4	2.35 m, 1.26 m	28.3	1.05 m	29.9
8	1.80 m	43.2	1.80 m	43.3		42.2
9	1.20 m	48.9	2.75 m	51.6		49.1
10		51.5		49.0		36.9
11	1.64 m, 1.22 m	22.5	1.62 m	22.6	2.02 m	21.4
12	1.23 m, 1.34 m	39.5	1.98 m, 2.31 m	39.7		30.5
13		49.9		50.2		51.2
14		84.1		84.6		87.0
15	1.98 m, 1.81 m	32.8	1.82 m, 1.98 m	32.9		31.7
16	2.06 m, 1.96 m	28.2	2.10 m, 1.98 m	27.6		25.3
17	2.75 m	50.1	2.72 m	52.2	3.17 d (9.6)	49.7
18	0.93 s	16.2	0.92 s	16.4	0.89 s	18.9
19	10.01 s	209.1	10.05 s	209.3	1.03 s	12.6
20		176.0		176.2		176.8
21	5.03 d, 5.27 d (18.0)	74.0	5.00 d (1.6), 5.04 d (4.0)	74.1	4.82, 4.96 dd (2.0, 18.5)	75.2
22	6.12 s	118.1	6.12 s	118.2	5.88 br s	116.9
23		174.8		174.9		175.1
1′	4.77 d (7.2)	102.8	5.48 d (9.6)	96.4	4.85 dd (2.0, 10.0)	97.6
2′	4.47 m	71.6	1.98 m, 2.31 m	39.7		32.2
3′	3.58 dd (10.0, 12.4)	85.7	4.79[e]	68.3	4.20[e]	76.5
4′	4.25[e]	76.4	3.73 dd (9.2, 2.8)	84.4	3.19 br d (3.4)	73.4
5′	3.77 d (6.8)	70.8	4.44[e]	69.4	3.91[e]	70.3
6′	1.63 d (6.0)	18.1	1.71 d (6.4)	19.3	1.26 d (6.6)	17.2
OMe	3.62 s	59.3	3.62 s	50.1	3.24 s	57.2
1″	5.20 d (8.0)	105.7	5.04 d (7.6)	106.6	4.29 d (8.0)	102.2
2″	3.96[e]	76.5	3.97[e]	75.6		74.9
3″	4.23[e]	78.6	3.99[e]	78.8		78.4
4″	4.18[e]	72.2	4.26[e]	71.9		71.9
5″	3.95[e]	79.0	4.26[e]	78.9		78.0
6″	4.38 m, 4.59 m	63.4	4.40 m, 4.52 m	63.0	3.66 dd (5.5, 12.0), 3.86 dd (2.0, 12.0)	63.0

[a] δ(ppm) 500 MHz; s, singlet; br s, broad singlet; d, doublet; m, multiplet. [b] δ (ppm) 125 MHz. [c] In C_5D_5N. [d] In MeOH-d_4. [e] Overlapped and unresolved signals; values obtained from HSQC and 1D TOCSY experiment.

Thus HMBC correlations from H-17 (δ_H 2.75, m) to C-12, C-13, C-14, C-15, C-16, C-20, C-21, and C-22, as well as H-22 (δ_H 6.12, s) to C-17, indicated the point of attachment of the lactone ring system to ring D. H-18 (δ_H 0.93, s) exhibited a very strong correlation to C-12 (δ_C 39.5), C-13 (δ_C 49.9), C-14 (δ_C 84.1), and C-17 (δ_C 50.1). These correlations confirmed the C/D ring fusion at C-13 and C-14. The fusion of the A/B rings was confirmed by the correlation of H-19 (δ_H 10.01, s) to C-1 (δ_C 31.7), C-5 (δ_C 43.3), C-9 (δ_C 48.9) and from H-5 (δ_H 1.19, m) to C-1, C-3 (δ_C 76.9), C-4 (δ_C 36.5), C-6 (δ_C 29.0), C-7 (δ_C 27.4), C-9 and C-10 (δ_C 51.5). Similarly, the B/C ring fusion was confirmed by correlation of H-8 (δ_H 1.81) to C-6, C-7, C-11(δ_C 22.5), C-13, C-14 and H-9 (1.20, m) to C-1, C-5, C-7, C-8 (δ_C 43.2), C-10, C-11, C-12, and C-19 (δ_C 209.1). The ROESY correlations of H-19 (δ_H 10.01, s) to H_β-1 (δ_H 2.39 m), H_β-2 (δ_H 2.20, m), H_β-4 (δ_H 2.04, m), H_β-11 (δ_H 1.22, m) and from H-5 (δ_H 1.19, m) to H_α-1 (δ_H 0.90, m), H-3 (δ_H 3.97, br s), H_α-6 (δ_H 1.42, m), H-9 (δ_H 1.20, m) indicated a *trans* fusion of the A and B rings. The *trans* relationship between H-8 and H-9 and the *cis* fusion of the C/D rings were established by observing correlations of H-8 (δ_H 1.81) to H-19 (δ_H 10.01, s) and H_β-11 (δ_H 1.22, m) as well as correlations of H-9 (δ_H 1.20, m) to H-5 (δ_H 1.19). The ROESY spectrum indicated cross-peaks from H-17 to H-21, H-22, and H-16 as well as from H-18 to H-21 and H-22, but not to H-17, and so the lactone ring was assigned a β-configuration. The ^1H and ^{13}C chemical shifts for the sugar units were assigned as in Table 1 and indicated that the sugars had β-linkages. The aglycone portion of the compound was connected using COSY, TOCSY, and HMBC spectra. The point of attachment of the sugar moieties to the aglycone portion of the molecule was determined from a strong HMBC correlation of H-1′ to H-3 (Figure 1), indicating a 3→1′ connectivity. The stereochemistry at this position was confirmed by a ROESY correlation of H-3 to H-1′ (Figure 1). The two sugars were linked from the 4′-position of the first sugar to the 1″-position of the second, and the H-4′ and H-1″ protons were oriented *cis* to each other. The chemical shifts of the sugar portion matched the literature values for the sugar portion of a β-D-glucopyranosyl-(1→4)-β-D-digitaloside.[11] The structure of boivinide A was thus established as the new compound corotoxigenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-digitalopyranoside] (**1**).[12]

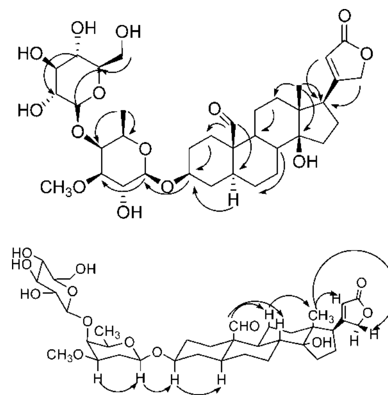

Figure 1. Key HMBC (upper) and ROESY (lower) correlations of compound **1**.

Compound **2** was also obtained as a white, amorphous solid. LC-MS gave a molecular ion peak at m/z 695.4 [M + H^+],

consistent with the molecular formula $C_{36}H_{54}O_{13}$. The ^{13}C NMR spectrum (C_5D_5N, Table 1) showed signals for 13 oxygenated carbons, and the ^1H NMR spectrum showed signals for an aldehyde proton group at δ_H 10.05 (s, H-19), one methoxy group at δ_H 3.62 (s), one methyl group at δ_H 0.92 (s, H-18), and a methyl doublet at 1.71 (d, H-6′), indicating the presence of a deoxy sugar moiety in the compound. Two anomeric proton signals were seen at δ_H 5.48 (d, H-1′) and 5.04 (d, H-1″). The ^{13}C spectrum of compound **2** contained a total of 36 signals: one methoxy, two methyls, 12 methylenes, 16 methines, and five quaternary carbons were consistent with a cardenolide structure. Further analysis of COSY and 1D and 2D TOCSY spectra established that the aglycone portion was the same as that of boivinide A (**1**). ROESY correlations of H-19 to H_β-1, H_β-2, H_β-4, and H_β-11 and of H-5 to H_α-1, H-3, H_α-6, and H-9 indicated a *trans* orientation of H-5 and H-19. HMBC correlations for the sugar portion showed the presence of similar sugar moieties to those in **1**, but with the replacement of one oxygenated carbon with a CH_2 group. HMBC, ROESY, and 1D TOCSY spectra indicated that the aglycone-linked sugar of **2** was different from that of **1**. The assignments of the sugars in **2** were made as follows: δ_C/δ_H 96.4 (C-1′)/5.48 (dd, $J = 9.6$ Hz) and δ_C/δ_H 106.6 (C-1″)/5.04 (br d, $J = 7.6$ Hz); δ_C/δ_H 39.7 (C-2′)/1.98 and 2.31; δ_C/δ_H 68.3 (C-3′)/4.79 (m), δ_C/δ_H 78.8 (C-3″)/3.99 (m); H-4′ appeared as a doublet of doublets at δ_H 3.73 (dd, $J = 9.2$, 2.8 Hz/C-4′ δ_C 84.4); H-5″ was a multiplet at δ_H 4.26 (C-5″ δ_C 78.9); H-6′ was a prominent broad doublet at δ_H 1.71 ($J = 6.4$ Hz, C-6′ δ_C 19.3); H-6″ was a methylene with δ_H 4.40 and 4.52; and a 3′-methoxy group appeared as a singlet at δ_H 3.62. Both sugars were in the β-anomeric form, and these conclusions were confirmed by comparison of the ^1H and ^{13}C NMR spectra of **2** with the corresponding spectra of the carbohydrate portion of oleandrigenin-β-D-glucopyranosyl-(1→4)-β-D-sarmentoside.[10] The structure of boivinide B (**2**) was thus established as corotoxigenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-sarmentopyranoside].

Compound **3** was also isolated as an amorphous substance, and HRFABMS indicated a molecular composition of $C_{36}H_{56}O_{12}$. Its ^1H and ^{13}C NMR spectra showed that this compound also had a cardenolide skeleton. However, a triplet at δ_H 3.17 (t, $J = 9.6$ Hz) indicated that the lactone at C-17 had the α-orientation.[13,14] An additional methyl peak at δ_H 1.03 (s, H-19) and the absence of an aldehyde proton peak in the ^1H NMR spectrum confirmed that the aglycone of **3** was 5α,17α-uzarigenin, and this was confirmed by comparison of the ^{13}C NMR spectrum of the aglycone with that of 5α,17α-uzarigenin.[15] Comparison of chemical shifts of the sugars with those of **2** indicated that **3** had the same sugar moieties as **2**, and its structure was thus established as 5α,17α-uzarigenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-sarmentopyranoside].

Compound **4** was isolated as a white powder, and HRFABMS indicated a molecular formula of $C_{35}H_{52}O_{13}$. Compared to compound **3**, the ^1H NMR spectrum of **4** had a signal for the proton of an aldehyde at δ_H 10.01 (s, H-19). 2D NMR studies confirmed that the aglycone was corotoxigenin.[10] The absence of a prominent methoxy singlet and comparison of its $^3J_{HH}$ coupling constants and carbon NMR data to the sugar portion of digitoxigenin-3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-boivinopyranoside][16] indicated that **4** was the new cardenolide glycoside corotoxigenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-boivinopyranoside].

Compound **5** had the molecular formula $C_{35}H_{52}O_{14}$. ^1H NMR and ^{13}C NMR spectra revealed that the only difference between compounds **4** and **5** was the absence of the peak for the C-19 aldehyde carbonyl group at δ_C 210.4 and the presence of a signal at δ_C 180.9 for a carboxylic acid carbonyl group. These spectroscopic data combined with its elemental composition indicated that compound **5** was the new cardenolide glycoside 5-epicannogeninic acid 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-boivinopyranoside].

Compound **6** had the molecular formula $C_{38}H_{56}O_{15}$, and its aglycone portion was the same as that of compounds **1**, **2**, and **4**, as judged by its NMR spectra. The presence of a glucopyranoside unit was indicated by its ^1H and ^{13}C NMR spectra, and this was confirmed by 1D TOCSY. However, additional peaks appeared at δ_C 170.0 and δ_C/δ_H 21.5/2.08. HMBC correlations connected the first sugar as follows: δ_C/δ_H 100.7 (C-1′)/4.82 (d, $J = 8.4$ Hz), δ_C/δ_H 72.4 (C-2′)/5.83, indicating that it was highly deshielded; δ_C/δ_H 83.4 (C-3′)/3.64 (d, $J = 2.8$ Hz); H-4′ appeared as a multiplet at δ_H 4.46 (C-4′, δ_C 75.1); H-6′ was a prominent broad doublet at δ_H 1.60 ($J = 6.0$ Hz, C-6′ δ_C 18.0); and the 3′-methoxy group appeared as a singlet at δ_H 3.49. The acetoxy group (δ_C 170.0, 21.1, δ_H 2.08, s) was attached at position C-2′ (δ_C/δ_H 72.4/5.83). The relative stereochemistry of the first sugar was confirmed by ROESY cross-peaks from H-1′ to H-3, H-3′, and H-5′ and from H-4′ to H-1″. The sugar moiety was thus assigned as 3-*O*-[β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-β-D-digitalopyranoside].[17]

This extract also yielded the four known cardenolide glycosides digitoxigenin 3-*O*-[β-D-glucopyrananosyl-(1→4)-α-L-acofriopyranoside] (**S7**), corotoxigenin 3-*O*-β-D-boivinoside (**S8**), 17α-corotoxigenin 3-*O*-β-D-sarmentoside (**S9**), and uzarigenin 3-*O*-α-L-rhamnoside (**S10**). The structures of these compounds were confirmed by comparison of their proton and carbon chemical shifts to the literature values.[6,8,9]

All 10 compounds were tested for growth inhibition using the A2780 human ovarian cancer cell line. The six new cardenolides exhibited strong antiproliferative activity with $IC_{50} = 0.17$ μM for boivinide A. The results are listed in Table 3. The major factor in determining bioactivity among this group of compounds is the stereochemistry at C-17; compounds **3** and **S9**, with an α-orientation of the unsaturated lactone ring, were an order of magnitude less active than compounds with a β-orientation of this ring.

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV-1210 series, and IR spectra were measured on a MIDAC M-series FTIR spectrophotometer. NMR spectra were obtained on a Varian INOVA 400 spectrometer at 400 MHz and JEOL Eclipse 500 spectrometer instrument for ^1H NMR spectra and ^{13}C NMR spectra; chemical shifts are given in ppm. High-resolution mass spectra were obtained on a JEOL JMS-HX-110 instrument in the positive ion mode, and low-resolution spectra were obtained on a Finnigan LTQ LC/MS^n instrument in either the positive or negative ion mode after sample elution with MeOH from a C_{18} column. HPLC was carried out using a Shimadzu LC-10AT instrument with analytical (5 μm, 250 × 10 mm) and preparative (8 μm, 250 × 10 mm) C_{18} Varian Dynamax columns coupled with a UV diode array detector.

Antiproliferative Bioassays. Antiproliferative activity against the A2780 human ovarian cancer cell line was determined at Virginia Polytechnic Institute and State University as previously described.[18] The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[19]

Plant Material. Samples of the roots, bark, wood, and leaves of *Roupellina boivinii* (Baill.) Pichon were collected on May 18, 2004, in the vicinity of the Baie des Dunes Orangéa, Antsiranana, Madagascar, at an elevation of 2 m. The collection coordinates were 12°14′26″ S, 49°22′17″ E. Collection was made by one of the authors (N.M.A.) and given collection number ANM 430. The herbarium specimen was from a tree of 5 m, diameter at breast height 12 cm. It had a white latex and green, elongated, spindle-shaped fruit. Its vernacular name is tangeniala. Duplicates of the voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP), the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Muséum National d'Histoires Naturelles, Paris, France (P).

Extraction and Isolation. The dried root (350 g), bark (350 g), and wood (369 g) of *Strophanthus boivinii* were separately ground and extracted with EtOH; the resulting extracts were designated MG2309 (14.1 g) MG2310 (10.9 g), and MG2311 (7.0 g), respectively, of which 3.3, 2.2, and 1.9 g, respectively, were made available for this work. The combined EtOH extracts of the above three parts of the plant, designated MG2309PE (3.0 g), was suspended in aqueous MeOH ($MeOH/H_2O$, 9:1, 100 mL) and extracted with hexanes (3 × 100 mL).

Table 2. ^{1}H[a] and ^{13}C[b] NMR Data of Compounds **4**, **5**, and **6**

	4[d]		**5**[d]		**6**[c]	
position	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1	2.42 m	37.0	2.42 m	37.8	2.41 m, 0.98 m	31.8
2		29.6		30.8	1.98 m, 1.45 m	31.5
3	4.03 m	77.7	4.03 m	77.8	3.90 m	77.9
4		32.1		36.8	2.03 m, 1.50 m	36.8
5	1.30 m	44.1	1.32 m	46.0	1.24 m	43.4
6		28.6		28.9	1.30 m, 1.30 m	28.3
7		31.6		33.2	1.83 m, 1.83 m	29.2
8	1.35 m	43.8	1.30 m	42.6	1.22 m	43.4
9	2.72 m	49.6	2.72 m	49.6	2.70 m	49.1
10		52.8		53.4		52.1
11		22.9		24.6	1.63 m, 1.33 m	22.6
12		40.4		41.3	1.21 m, 1.34 m	39.7
13		50.7		50.7		50.3
14		85.8		85.8		84.5
15		32.8		33.1	1.99 m, 1.82 m	32.9
16		27.9		28.6	1.97 m, 2.15 m	27.6
17	2.81 dd (5.2, 9.1)	51.9	2.81 dd (5.2, 9.1)	51.2	2.75 m	51.7
18	0.81 s	16.1	0.81 s	16.6	0.94 s	16.4
19	10.01 s	210.4		180.9	10.04 s	209.3
20		178.2		178.2		176.2
21	4.92, 5.02 dd (2.0, 18.5)	75.3	4.92 dd, 5.02 dd (2.0, 18.5)	75.9	5.01 d (18.0), 5.29 d (18.4)	75.9
22	5.88 br s	117.9	5.88 br s	117.9	6.14 s	118.3
23		177.2		177.2		174.9
1′	4.87 dd (2.0, 10.0)	97.5	4.87 dd (2.0, 10.0)	97.1	4.82 d (8.4)	100.7
2′		35.0		35.0	5.83 m	72.4
3′		66.5		66.4	3.64 d (2.8)	83.4
4′	3.19 br d (3.4)	75.9	3.19 d (3.4)	75.9	4.46 d (2.0)	75.1
5′		70.1		70.0	3.77 d (6.4)	71.2
6′	1.24 d (6.6)	17.2	1.24 d (6.6)	17.2	1.60 d (6.0)	18.0
OMe					3.49 s	58.5
OCO						170.0
					2.08 s	21.5
1″	4.29 d (8.0)	102.4	4.29 d (8.0)	102.3	5.16 d (7.6)	105.5
2″	4.51[e]	74.9	4.51[e]	74.9	4.02[e]	76.4
3″		77.9		78.1	4.26[e]	78.7
4″		71.8		71.9	4.19[e]	72.2
5″	3.43[e]	78.1	3.45[e]	78.5	3.98[e]	79.2
6″	3.87 dd (5.5, 12.0), 3.67 dd (2.0, 12.0)	63.0	3.87 dd (5.5, 12.0), 3.67 dd (2.0, 12.0)	63.0	4.38 m, 4.6 m	63.0

[a] δ (ppm) 500 MHz; s, singlet; br s, broad singlet; d, doublet; m, multiplet. [b] δ (ppm) 125 MHz. [c] In C_5D_5N. [d] In CD_3OD. [e] Overlapped peaks and unresolved signals; values obtained from HSQC and 1D TOCSY experiment.

Table 3. Antiproliferative Activity of Compounds **1**–**10** against the A2780 Human Ovarian Cancer Cell Line[a]

compound	IC_{50} (μM)
1	0.17
2	0.66
3	2.9
4	0.29
5	0.28
6	0.54
S7	0.15
S8	0.15
S9	3.7
S10	0.15

[a] Concentration of each compound that inhibited 50% (IC_{50}) of the growth of the A2780 human ovarian cell line. Actinomycin D (IC_{50} 0.8−2.4 nM) was the positive control.

The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH_2Cl_2 (3 × 180 mL). The aqueous MeOH fraction displayed the highest activity (IC_{50} = 0.61 μg/mL) followed by the CH_2Cl_2-soluble fraction with IC_{50} = 3.7 μg/mL. About 634 mg of the MeOH fraction was partitioned between *n*-BuOH and H_2O, which yielded 232 mg of an active *n*-BuOH fraction. The BuOH fraction was filtered through a short C_{18} reversed-phase column with MeOH/H_2O (6:4) as the mobile phase. The resulting filtrate was further separated using a C_{18} Varian Dynamax column (8 μm, 250 × 10 mm, 10 mL/min) with MeOH/H_2O (6:4) as the mobile phase to yield two new compounds, 1.5 mg of boivinide D (**4**) and 3.0 mg of boivinide E (**5**). The remaining MeOH fraction was chromatographed over a C_{18} open column, eluting with 30% MeOH/H_2O to 100% MeOH, to yield five fractions. Only fractions III and IV were active, with IC_{50} 0.13 and 0.48 μg/mL respectively. Fraction III was loaded on a C_{18} Varian Dynamax column [5 μm, 250 × 10 mm, 1.8 mL/min, isocratic elution with 40% MeOH/H_2O (0.1% formic acid) for 40 min followed by 50% MeOH/H_2O (0.1% formic acid) for 50 min] and 25 subfractions were collected. Fractions 16 and 18 were pure and new compounds and were named boivinide B (**2**) (4.5 mg, t_R 59 min) and boivinide A (**1**) (5.9 mg, t_R 62 min). Fraction IV was loaded on a C_{18} Varian Dynamax column [8 μm, 250 × 10 mm, 10 mL/min, isocratic elution with 50% MeOH/H_2O (0.05% formic acid) for 90 min]. Two pure subfractions were collected; one was a new compound, boivinide F (**6**) (3.5 mg, t_R 39 min), and the other was the known compound digitoxigenin 3-*O*-[β-D-glucopyrananosyl-(1→4)-α-L-acofriopyranoside] (**S7**) (4.9 mg, t_R 49 min). The CH_2Cl_2 fraction was filtered through a short C_{18} column with elution by 60% MeOH/H_2O, and the resulting filtrate was further purified by C_{18} HPLC using 60% MeOH/H_2O to yield one new compound, boivinide C (**3**) (15.0 mg), and the three known compounds **S8**–**S10**.[6,8,9]

Boivinide A (1): white, amorphous solid; UV (MeOH) λ_{max} (log ε) 217 nm; IR ν_{max} 3409, 2870, 1743, 1616, 1068, 884 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 1; LC-MS *m/z* 749.4 [M + K]$^+$ (calcd for $C_{36}H_{54}O_{14}K^+$, 749.3).

Boivinide B (2): white, amorphous solid; UV (MeOH) λ_{max} (log ε) 215 nm; IR ν_{max} 3400, 2873, 1739, 1616, 1162, 1071, 1024, 679 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 1; LC-MS *m/z* 695.4 [M + H]$^-$ (calcd for $C_{36}H_{55}O_{13}^-$, 695.4).

Boivinide C (3): white, amorphous solid; ^{1}H and ^{13}C NMR spectra, see Table 1; HRFABMS *m/z* 703.3665 [M + Na]$^+$ (calcd for $C_{36}H_{56}O_{12}Na^+$, 703.3664).

Boivinide D (4): white, amorphous powder; ^{1}H and ^{13}C NMR spectra, see Table 2; HRFABMS *m/z* 703.3327 $[M + Na]^+$ (calcd for $C_{35}H_{52}NaO_{13}^+$, 703.3301).

Boivinide E (5): white, amorphous powder; ^{1}H and ^{13}C NMR spectra, see Table 2; HRFABMS *m/z* 719.3251 $[M + Na]^+$ (calcd for $C_{35}H_{52}NaO_{14}^+$, 719.3250).

Boivinide F (6): yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 216 nm; IR ν_{max} 3450, 2871, 1747, 1709, 1372, 1236, 1069, 985, 659 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 2; HRFABMS *m/z* 775.3570 $[M + Na]^+$ (calcd for $C_{38}H_{56}O_{15}Na$, 775.3512).

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Supporting Information Available: Structures of compounds **S7**–**S10**; 1D NMR spectra of compounds **1**–**6**, and 2D NMR spectra of compounds **1**–**4** and **6**. This material is available free of charge on the Internet at http://pubs.acs.org.

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