II. Cardiac glycosides from *Strophanthus boivinii*

2.1 Introduction

In our continuing search for bioactive natural products from Madagascar rainforests as a part of International Cooperative Biodiversity Group (ICBG) program, we obtained ethanol extract of the plant *Roupellina boivinii*, also known as *Strophanthus boivinii*.[1] This plant extract (MG 2309PE) exhibited antiproliferative activity in the A2780 ovarian cancer cell line (IC_{50} = 11 μg/mL) and hence it was selected for further fractionation to isolate its active components. Bioassay-guided fractionation of this extract yielded three new cardenolide glycosides, boivinides A, B and F, as well as one known cytotoxic cardenolide glycoside. The study and structural characterization of these compounds is reported herein.

2.1.1 Previous studies of *Strophanthus* genus

Strophanthus boivinii (Fig. **2.1**)[2] is a small flowering tree that belongs to the Apocynaceae family. This family is native mainly to Africa, extending to South Africa with a few species in Asia. The family has 424 genera and over 2100 species. The medicinal plants of this family are known to contain lignans,[3] cytotoxic alkaloids and cardenolide glycosides.

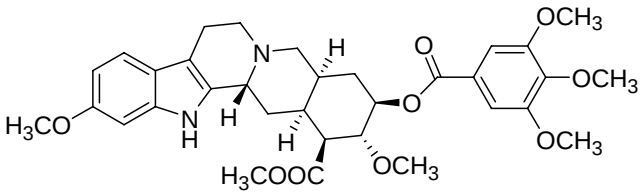
Figure 2.1 *Strophanthus boivinii*
Source: © TopTropicals.com

Reserpine (**2.1**), an indole alkaloid extracted from the roots of *Rauwolfia serpentine*, is an antipsychotic and antihypertensive drug.[4] The vinca alkaloids, vincristine (**2.2**) and vinblastine (**2.3**), isolated from *Catharanthus roseus*, are effective against leukemia.[5] The *Strophanthus* genus within the Apocynaceae family consists of 35-40 species.[6] The name *strophas anthos* (twisted cord flower) is derived from the long twisted threadlike corolla. The *Strophanthus* genus includes small shrubs, trees, and vines which attain an average height of 30 feet.

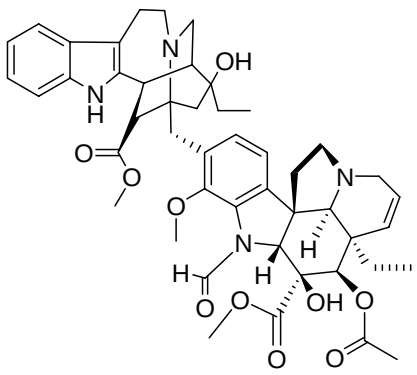
The genus *Strophanthus* is most associated with cardenolide glycosides which are an important class of natural products that can be used as drugs as well as toxins. Since 1500 B. C. the leaves of *Strophanthus* genus have been used to cure skin ulcerations, to reduce fever and the decoction of leaves has been a good remedy for gonorrhea.[7] As an example of their use as toxins, Inee, (also known as onaye), obtained from *Strophanthus hispidus*[8] is used as an arrow poison for hunting in West Africa.

The seeds of *Strophanthus gratus* have anticoagulant properties and have been used on wounds and treatment of snake-bites in Africa.[3] The seeds and the plant extract of *Strophanthus kombé* is beneficial as a cardiac drug or a diuretic.[9] These plants are mainly used in the treatment of congestive heart failure; however, their toxicity limits their extensive use. Ouabain, a more common name for G-strophanthin (**2.6**), a poisonous glycoside obtained from the fruits of *Strophanthus gratus,*[10] is used widely to block the sodium pump for *in vitro* studies.[11] K-strophanthin (**2.7**), isolated from *Strophanthus kombé*, was also used for the treatment of heart diseases until its adverse side effects were observed.[12] As would be expected, the cytotoxicity and structural characterization of various cardenolide glycosides have been extensively studied. However, only two

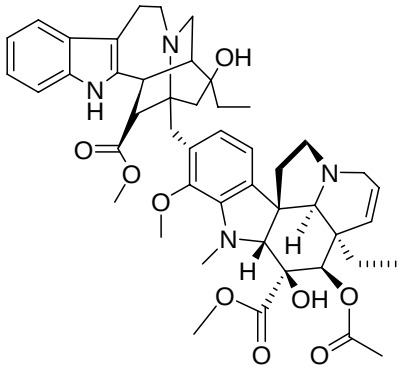
investigations to study the cardenolide glycosides from *Strophanthus boivinii* have been reported.[13, 14]



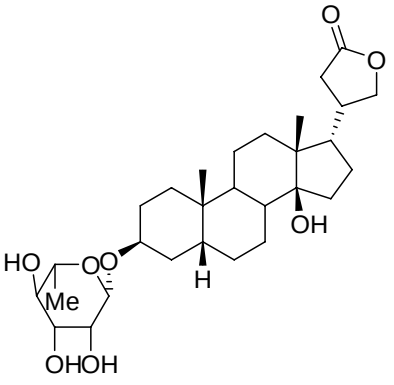
2.1 Reserpine



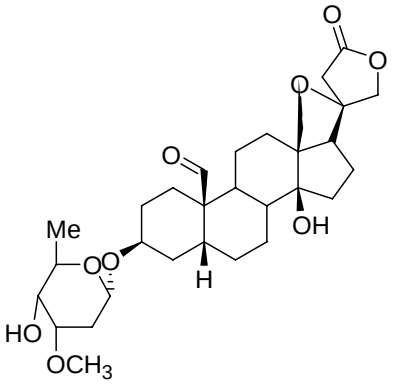
2.2 Vinblastine



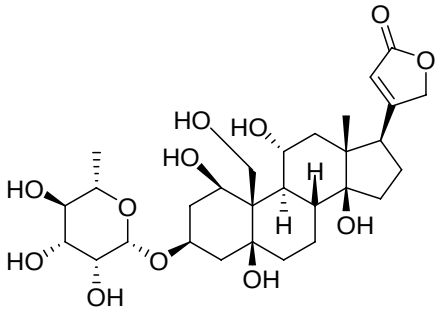
2.3 Vincristine



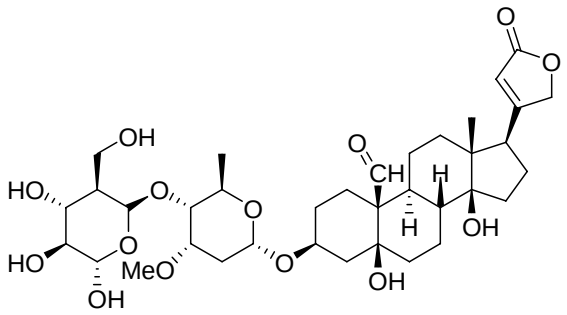
2.4 digitoxigenin α-L-acofrioside



2.5 (*20S*)-18,20-epoxydigitoxigenin α-L-cymarin



2.6 G-strophanthin



2.7 K-strophanthin-β

2.1.2 Structure and Chemistry of Cardiac Glycosides

Cardiac glycosides consist of two parts: the aglycone portion (non-sugar) and the glycone or the sugar portion.

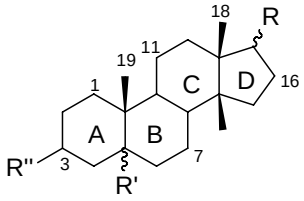


Figure 2.2 Basic skeleton of cardenolide glycosides
R = unsaturated lactone or pyrone ring system
R' = H or OH
R'' = sugar moiety

The R group at position C-17 determines the class of the glycoside. Two classes have been identified in nature.

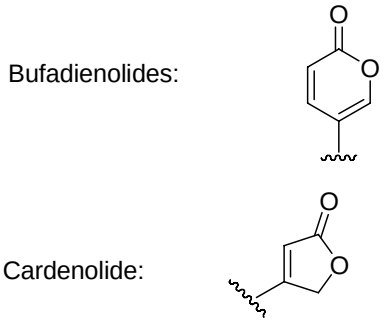
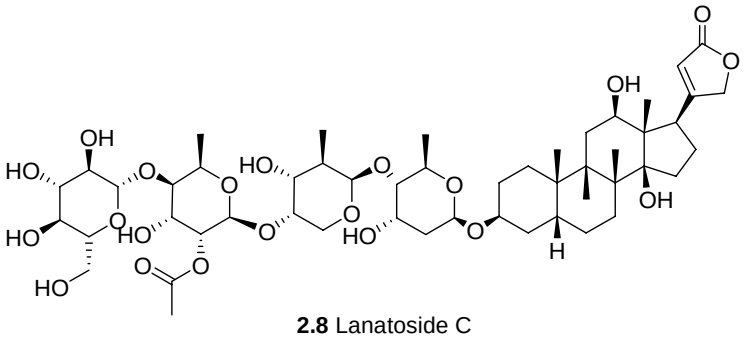
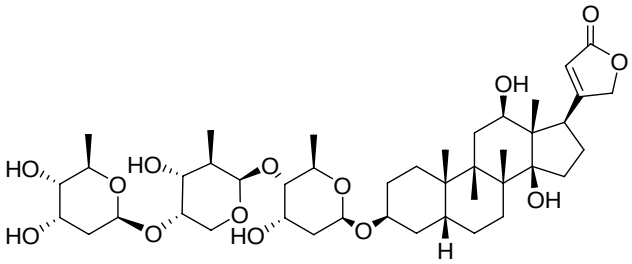


Figure 2.3 Two classes of cardiac glycosides

Cardiac glycosides with a cardenolide substituent are generally found in plants, and cardiac glycosides that contain bufadienolide substituents are obtained from animal sources. The aglycone consists of a four fused ring system, A, B, C and D. The stereochemistry of the rings greatly influences the cytotoxicity of these compounds. Compounds with a *trans* fusion of A/B and *cis* fused C/D rings are less active as compared to the compounds with *cis* fusions of both A/B and C/D rings. Removal of the –OH group at the 14β- position does not affect the activity of the compound. The double bond in the lactone ring plays an important role in the activity of the compound, and saturation of the ring causes a marked decrease in this activity.[15] However, the lactone ring is not absolutely required, and if it is replaced with a nitrile group, the resulting compound shows little or no loss of activity. Also, the lactone ring alone does not exhibit any biological activity. The sugar moiety is not required for the activity of the molecule, but it is essential for the pharmacokinetics, absorption and half-life of the compound, etc.[15] For example, lanatoside C (**2.8**) differs from digoxin (**2.9**) in the sugar residue and is more lipophilic with greater water solubility and a faster absorption rate than digoxin.



2.8 Lanatoside C



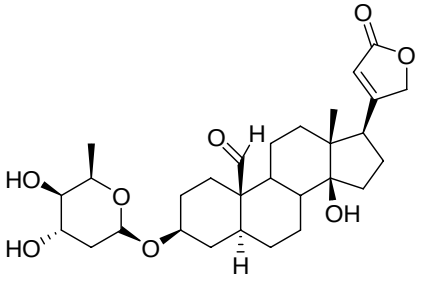
2.9 Digoxin

Cardiac glycosides are common compounds present in plants. These glycosides inhibit Na^+/K^+ ATPase and increase the contraction of the heart muscles. The aglycone portion of these glycosides is very poisonous, and in low concentrations, exhibits antiproliferative activity.[16] Repke et al. in 1988 established the importance of Na^+/K^+ ATPase in cancer cell proliferation. Hence, inhibition of Na^+/K^+ ATPase can play an important role in apoptosis and cancer cell death.[17] However, due to their high levels of toxicity and adverse side effects, their medicinal use has not been much explored. Further studies have to be performed to confirm their use as cancer drugs.

2.2 Results and Discussion

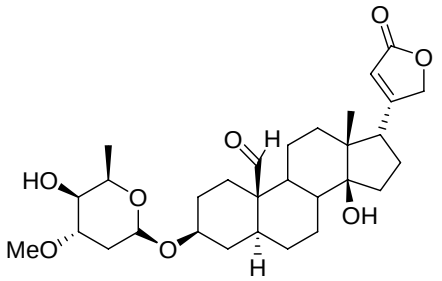
Strophanthus boivinni was studied previously by Mr. Eba Adou, a doctoral student from our research group. The plant extract was subjected to solvent partitioning between hexane and aqueous MeOH, followed by extraction of the methanol fraction with dichloromethane. Both methanol and dichloromethane fractions were active and were filtered through short C_{18} chromatography columns and separated by HPLC-C_{18}

using a MeOH:H_2O solvent system. Mr. Adou isolated three known (**6.1**, **6.2** and **6.4**) and three new cardenolides (**6.3**, **6.5** and **6.6**) from this extract (NO55899). However, the extract yielded many active fractions, and thus the work on this extract was continued to further isolate its additional active compounds.



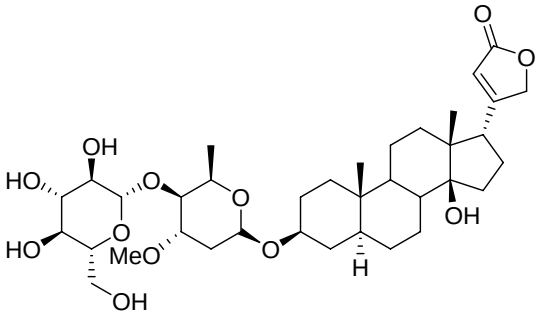
6.1 5α– corotoxigenin-β-D-boivinoside

IC_{50} = 0.08 μg/ml



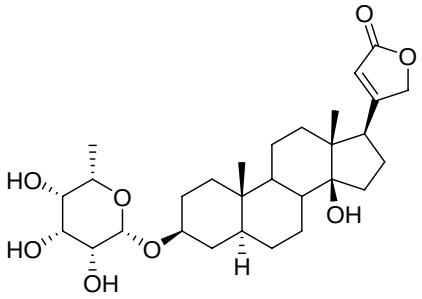
6.2 17α– corotoxigenin-β-D-sarmentoside

IC_{50} = 2.0 μg/ml



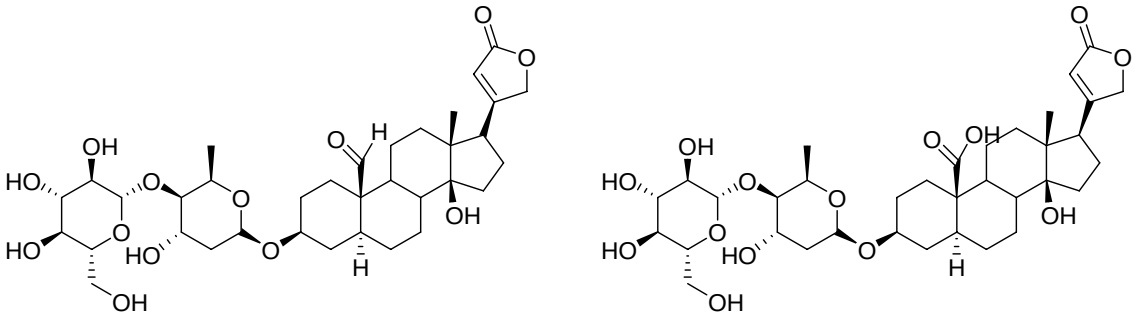
6.3 5α, 17α– uzarigenin-3-O-[β-D-glucopyranosyl-1⟶4)-β-D-sarmentoside

IC_{50} = 2.0 μg/ml



6.4 5α– uzarigenin-3-O-α-L-rhamnoside

IC_{50} = 0.08 μg/ml



6.5 5α– uzarigenin-3-O-[β-D-glucosyl-1⟶4)-β-D-boivinoside

IC_{50} = 0.2 μg/ml

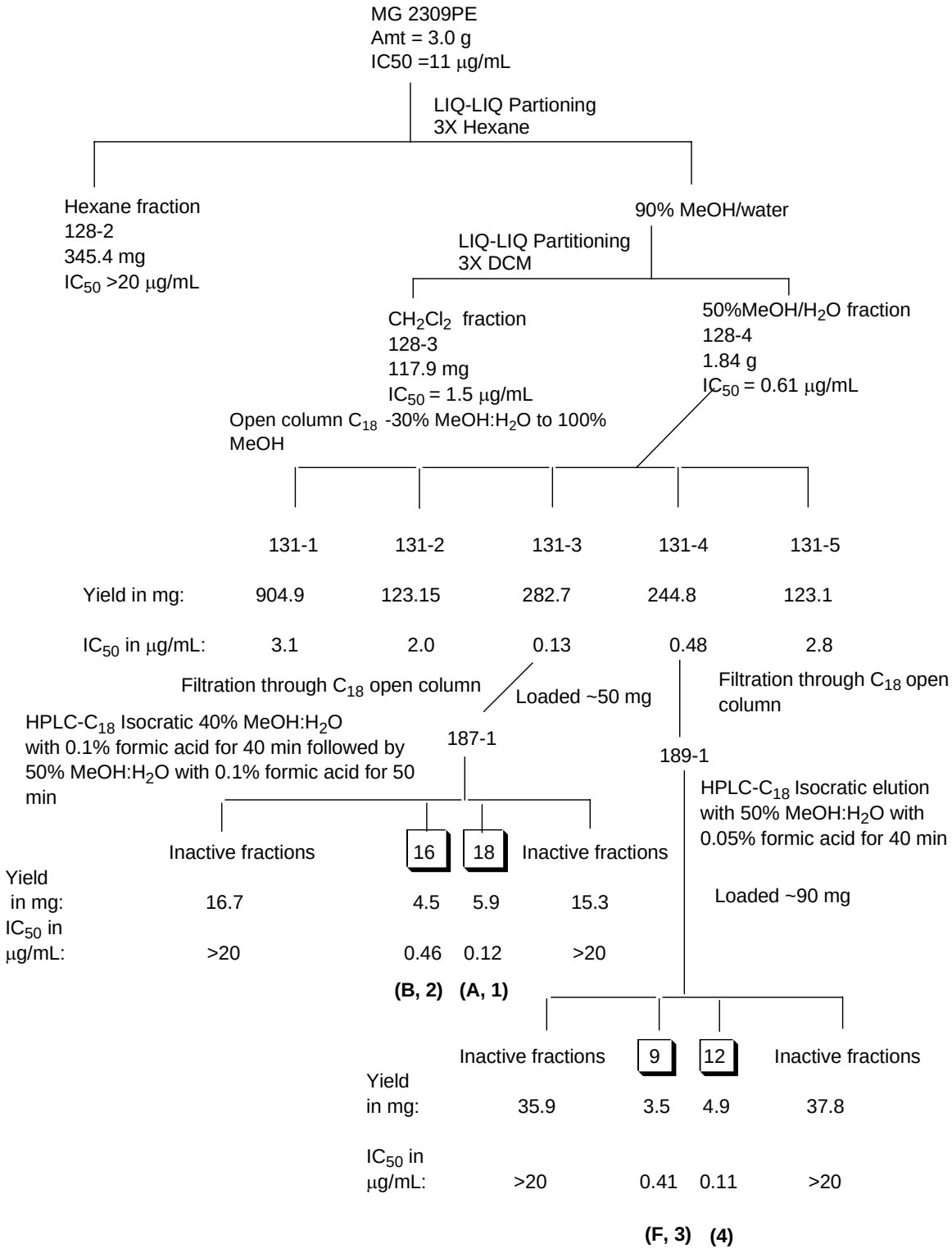
6.6

Figure 2.4 Cardenolide glycosides isolated by Mr. Adou from *Strophanthus boivinii*

2.2.1 Isolation of boivinides A-C and digitoxigenin 3-O-β-D-glucopyranosyl-(1→ 4)-α-L-acofriopyranoside.

The EtOH extract of the plant, designated MG 2309/11 (3.0 g) was suspended in aqueous MeOH (MeOH:H_2O, 9:1, 100 mL) and extracted with hexanes (3 × 100 mL). 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). This fraction was chromatographed over a C_{18} column 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 twenty-five subfractions were collected. Fractions 16 and 18 were pure and new compounds and named boivinide B (**2**) and boivinide A (**1**). Fraction IV was loaded on a C_{18} column and eluted with 50% MeOH:H_2O (0.05% formic acid) for 90 min. Two pure subfractions were collected; one was a new compound, boivinide F (**3**) and the other was a known compound (**4**), digitoxigenin 3-O-β-D-glucopyranosyl-(1→4)-α-L-acofriopyranoside.[18]

Strophanthus boivinii



Scheme 2.1 Fractionation tree for *Strophanthus boivinii*

2.2.2 Structure Elucidation of Boivinide A

Boivinide A (compound **1)** was obtained as a white amorphous solid. Positive ion LC-MS gave molecular ion peak at *m/z* 749.4 [M+K^+], consistent with the molecular composition of $C_{36}H_{54}O_{14}$. Previous studies on this plant indicated that compound **1** belonged to the class of cardenolide glycosides.

The ^1H spectrum of **1** in pyridine-d_5 (Fig. **2.5**) indicated that the compound was pure with several oxygenated protons and methyl groups. A peak at δ_H10.01 (s, H-19) suggested the presence of an aldehyde proton. The spectrum also showed signals for one methoxy group at δ_H 3.62 (s), one methyl at δ_H 0.93 (s, H-18), and a methyl doublet at 1.63 (d, H-6') indicating the presence of a deoxy sugar moiety in the compound. Two anomeric proton signals at δ_H 4.77 (d, H-1') and δ_H 5.20 (d, H-1'') confirmed the presence of two sugar moieties in the compound. The ^{13}C spectrum of compound **1** contained 36 signals: one methoxy, two methyls, eleven methylenes, seventeen methines and five quaternary carbons.

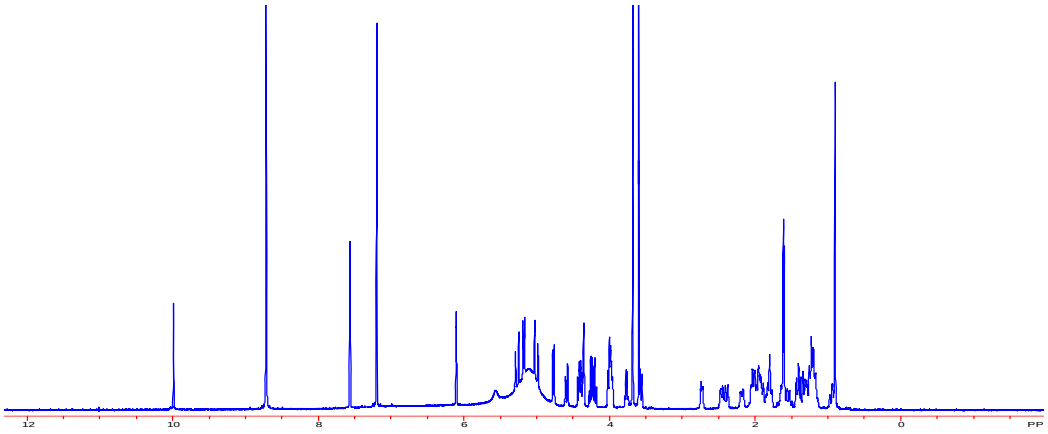


Figure 2.5 ^1H NMR spectrum of compound **1** in pyridine-d_5

The ^{1}H and ^{13}C NMR signals in pyridine-d_5 showed typical signals for an α,β-unsaturated γ- lactone unit with peaks at δ_C 176.0 (C-20), δ_C/δ_H 74.0 (C-21)/5.03 and 5.27 (br d, J = 18.0 Hz, H-21), δ_C/δ_H 118.1 (C-22)/6.10 (br s, H-22), δ_C 174.8 (C-23). Four spin systems, A, B, C and D for the aglycone portion were identified by COSY, 1D and 2D TOCSY spectra. From HSQC and HMBC correlations, the proton-carbon pairs were connected to each other and $^{1}J_{CH}$ correlations were determined (Table **1**) to obtain a cardenolide skeleton (**2.13**).

HMBC correlations from H-17 (δ_H 2.75, s) 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 39.9), C-13 (δ_C 49.9), C-14 (δ_C 84.1) and C-17 (δ_C 50.1). These correlations led to the construction of Fragment 1 and confirmed the C/D ring fusion at C-13 and C-14.

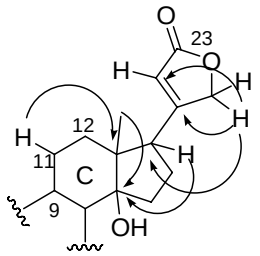


Figure 2.6 Key HMBC correlations of fragment 1

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 43.2), C-9 (δ_C 48.8); H-5 (δ_H 1.19, m) to C-1, C-3 (δ_C 76.9), C-4 (δ_C 36.4), C-6 (δ_C 29.0), C-7 (δ_C 27.4), C-9 and C-10 (δ_C 55.5). 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 22.4), C-

13, and C-14 and of H-9 (1.20, m) to C-1, C-5, C-7, C-8 (δ_C 43.1), C-10, C-11, C-12 and C-19 (δ_C 209.1).

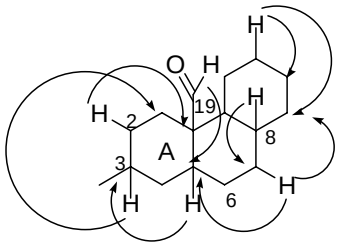


Figure 2.7 Key HMBC correlations of A/B and B/C rings of compound **1**

A strong HMBC correlation from H-3 to C-1' confirmed that the first sugar was connected to the aglycone at position 3 on A ring.

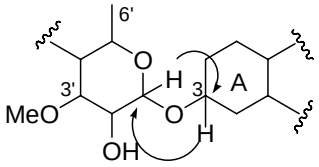


Figure 2.8 Key HMBC correlation for connecting the sugar moiety to the aglycone of compound **1**

In the second sugar, H-1" (δ_H 5.20) correlated to C-4' of the first sugar. Similarly, a correlation of H-4' to C-1" was observed, suggesting a 1→4 attachment of the two sugars.

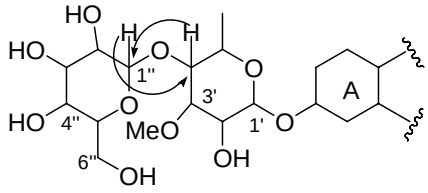


Figure 2.9 Key HMBC correlation for attachment of sugar II to sugar I of compound **1**

The ^{1}H NMR spectrum of compound **1** was very complex, and extensive overlap of peaks especially between 2.0 and 4.0 ppm, made analysis of the spectrum very difficult. However, an HSQC experiment in pyridine-d_5 was extremely useful for detecting single bond C-H correlations, especially for assembling the sugar portion, and made the $^{1}J_{CH}$ correlations very clear. H-5 (δ_H 1.19), H-9 (δ_H 1.20), H-11 (δ_H 1.22) and H-12 (δ_H 1.23) were well resolved and correlated to C-5 (δ_C 43.2), C-9 (δ_C 48.9), C-11 (δ_C 22.3) and C-12 (δ_C 39.5) respectively. Similarly, H-4' (δ_H 4.25), H-3" (δ_H 4.23) and H-4" (δ_H 4.18) correlated to C-4' (δ_C 76.4), C-3" (δ_H 78.5) and C-4" (δ_C 72.2) respectively.

The stereochemistry of compound **1** was determined from 1D and 2D ROESY spectra. 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 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. 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) and H-18 (δ_H 0.93, s) suggested a *trans* fusion of the B/C rings of the aglycone.

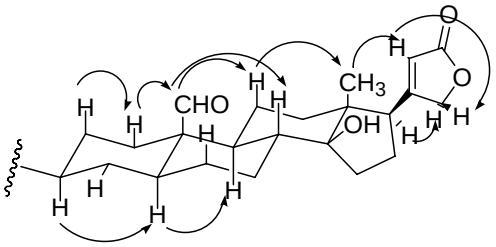


Figure 2.10 Key ROESY correlations of the aglycone portion of compound **1**

The ROESY spectrum also indicated crosspeaks from H-17 (δ_H 2.75, s) to H_2-21 (5.03, m 5.27, d, J = 18.0 Hz), H-22 ((δ_H 6.12, s), and H_2-16 (δ_H 1.96, m 2.06, m), as well

as H-18 (δ_H 0.93, s) to H-21 and H-22, but not H-17, hence the lactone ring was assigned the β configuration.

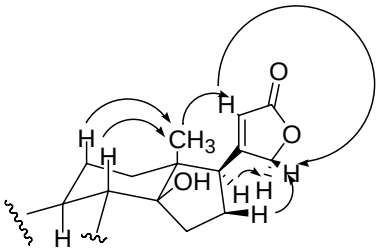


Figure 2.11 ROESY correlations of the D ring and the lactone unit of compound **1**

The ^{1}H and ^{13}C chemical shifts for the sugar moieties were assigned as follows: δ_C/δ_H 102.8 (C-1')/4.77 (br d, J = 7.2 Hz) and δ_C/δ_H 105.7 (C-1")/5.20 (br d, J = 8.0 Hz) indicated the sugars had β-linkages; δ_C/δ_H 85.7 (C-3')/3.58 (dd, J = 10.0, 12.4 Hz), δ_C/δ_H 78.6 (C-3")/4.23 (br s); H-4' appears as a multiplet at δ_H 4.25 (C-4' δ_C 76.4); H-5' is a multiplet at δ_H 3.77 (C-5' δ_C 79.0); H-6' is a prominent broad doublet at δ_H 1.63 (J = 6.8 Hz, C-6' δ_C 18.1), H-6" is a methylene with δ_H 4.38 (m), 4.59 (m) and a 3'-methoxy group appeared as a singlet at δ_H 3.62. The glycone portion of the compound was connected together from COSY, TOCSY and HMBC spectra. The stereochemistry at C-3 position was confirmed by ROESY correlation of H-3 (δ_H 3.97) to H-1' (δ_H 4.77), suggesting a *cis* relationship between H-1' and H-3. (**2.12**)

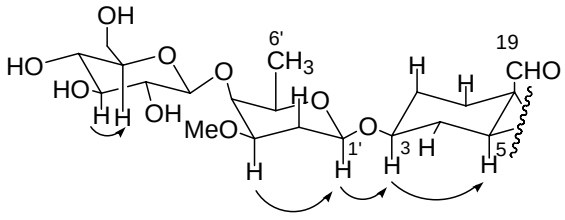


Figure 2.12 Key ROESY correlations of sugar I and the aglycone of compound **1**

The two sugars were attached by a 4'→1" linkage. 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 β-D-glucopyranosyl-β-D-digitaloside[19] very closely. The ^{1}H and ^{13}C NMR shifts of the shifts of the aglycone portion were similar to those of corotoxigenin.[20] This assignment of the sugar and the aglycone moieties in boivinide A led to its structural assignment as 5α-corotoxigenin-β-D-glucopyranosyl-(1→4)-β-D-digitaloside. This compound has been isolated and identified for the first time. The final structure of boivinide A is as shown in Figure 2.13**.**

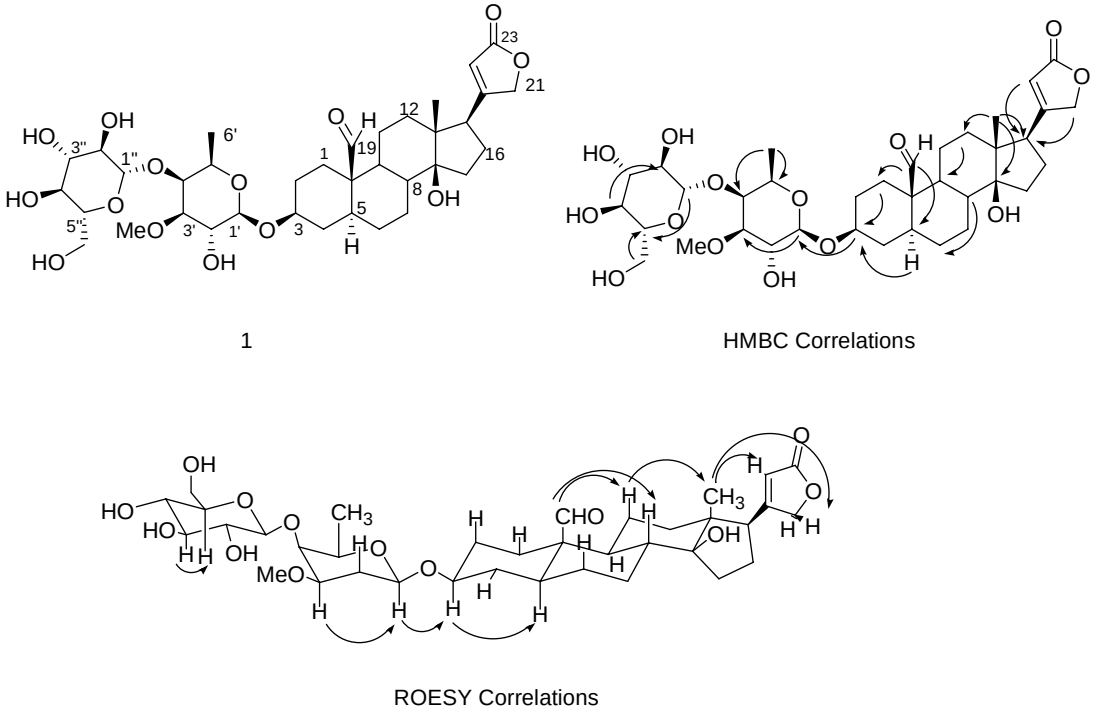


Figure 2.13 Final structure and key HMBC and ROESY correlations of compound **1**

Table 2.1 $^{1}H^{a}$ and $^{13}C^{b}$ NMR data for boivinide A and its comparison to literature values.

	Corotoxigenin		**Boivinide A**	
Position	^{1}H NMR[c]	^{13}C NMR[c]	^{1}H NMR[c]	^{13}C NMR[c]
1		31.5	0.9, 2.39m	31.7
2		31.2	1.56 m, 2.20 m	31.3
3	4.31	76.7	3.97 br s	76.9
4		36.3	2.04 m, 1.42 m	36.5
5		43	1.19 m	43.3
6		28.7	1.89 m, 1.42 m	29.1
7		27.9	2.48 m, 1.24 m	27.4
8		43	1.81 m	43.2
9		48.6	1.20 m	48.9
10		51.7		51.5
11		22.1	1.64 m, 1.22 m	22.5
12		39.3	1.23 m, 1.34 m	39.5
13		49.8		49.9
14		84.1		84.1
15		32.5	1.98 m, 1.81 m	32.8
16		27.1	2.06 m, 1.96 m	28.2
17	2.79 dd	51.2	2.75 s	50.1
18	1.09 s	15.9	0.93 s	16.2
19	9.49 s	208.7	10.01 s	209.1
20		175.6		176.0
21	5.02 d,5.28 d	73.7	5.03 d, 5.27 d (18.0)	74
22	6.12 s	117.7	6.12 s	118.1
23		174.4		174.8
	β-D-glucopyranosyl-3-O-methyl-β-D-digitaloside[c]			
Sugar I				
1'	4.79 d	103.4	4.77 d (7.2)	102.8
2'	4.42	71.2	4.43 d (2.0)	71.6
3'	3.52	85.5	3.58 dd (10.0, 12.4)	85.7
4'	4.33	76.6	4.25[e]	76.4
5'	3.73	70.5	3.77 d (6.8)	70.8
6'	1.56	17.7	1.63 d (6.0)	18.1
OMe	3.62	58.9	3.62 s	59.3
Sugar II				
1''	5.14	105.4	5.20 d (8.0)	105.7
2''	3.96	76	3.96 [e]	76.5
3''	4.23	78.5	4.23 [e]	78.6
4''	4.17	71.9	4.18 [e]	72.2
5''	3.94	78.3	3.95 [e]	79.0
6''	4.56	63.1	4.38 m, 4.59 m	63.4

[a]δ (ppm) 500MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet; [b]δ (ppm) 125MHz; [c] in pyridine-d_5; [e] overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

2.2.3 Structure Elucidation of Boivinide B

Compound **2** (boivinide B) was also obtained as a white amorphous solid. Positive ion LC-MS gave a molecular ion peak at *m/z* 695.4 $[M+H]^-$, consistent with the molecular formula of $C_{36}H_{54}O_{13}$. The ^1H NMR spectrum was characteristic of a cardenolide glycoside.

The ^1H spectrum of compound **2** in pyridine-d_5 (Fig. **2.14**) showed the presence of thirteen oxygenated carbons, one less than boivinide A**.** The ^1H NMR spectrum showed a signal at δ_H10.05 (s, H-19) for an aldehyde proton, and signals for one methoxy 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'). Two anomeric proton signals were seen at δ_H 5.48 (d, H-1') and δ_H 5.04 (d, H-1''), indicating the presence of two sugar moieties in the compound.

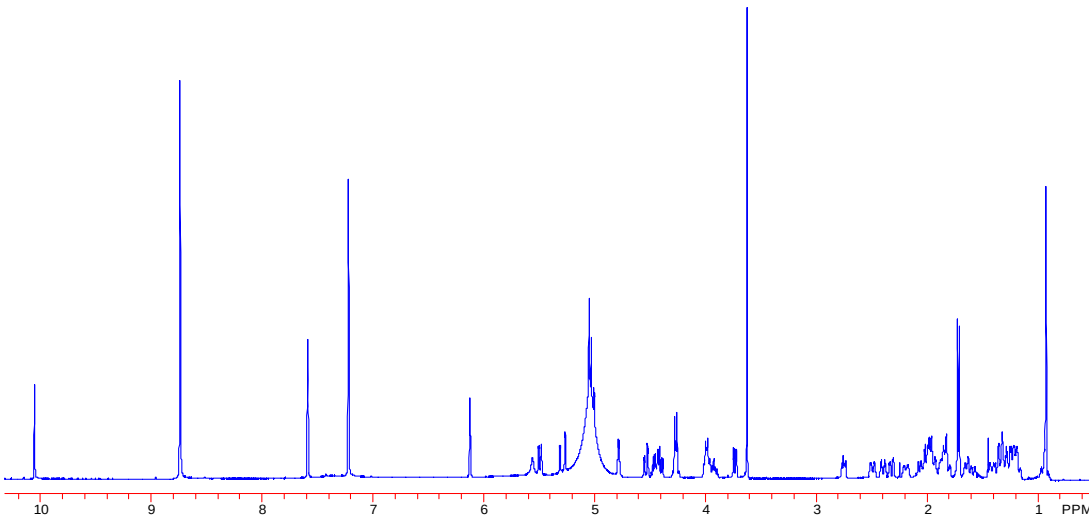


Figure 2.14 ^1H NMR spectrum of compound **2** in pyridine-d_5

The ^{13}C spectrum of compound **2** contained 36 signals: one methoxy, two methyls, twelve methylenes, sixteen methines and five quaternary carbons, consistent with a cardenolide framework. H-18 (δ_H 0.92, s) exhibited correlations to C-12 (δ_C 39.7), C-13 (δ_C 50.2), C-14 (δ_C 84.5), C-17 (δ_C 52.2). H-3 (δ_H 3.92, s) correlated to C-2 (δ_C 31.6), C-4 (δ_C 36.6), and C-1'(δ_C 96.3). The ^{1}H and ^{13}C NMR signals in pyridine-d_5 showed typical signals for an α,β-unsaturated γ-lactone unit. The aldehyde proton at C-19 (δ_C 209.3) position suggested that the aglycone in compound **2** was corotoxigenin,[20] and this was further confirmed by the comparison of the ^{1}H and ^{13}C NMR shifts of the aglycone of compound **2** with those of compound **1**. Further analysis of COSY, 1D and 2D TOCSY confirmed that aglycone portion was the same as that of boivinide A (**1**).

The ROESY correlations of H-19 (δ_H 10.05, s) to H_β-1 (δ_H 2.40, m), H_β-2 (δ_H 2.38, m), H_β-4 (δ_H 1.86, m), H_β-11 (δ_H 1.62, m) and H-5 (δ_H 1.20, m) to H_α-1 (δ_H 0.83, m), H-3 (δ_H 3.92, br s), H_α-6 (δ_H 1.40, m) and H-9 (δ_H 2.75, m) indicated a *trans* orientation of H-5 (δ_H 1.20, m) and H-19 (δ_H 10.05, s) to each other. The stereochemistry at this position was further confirmed by ROESY crosspeaks from H-5 (δ_H 1.20, m) to H-3 (δ_H 3.92, m) and H-3 to H-1' (δ_H 5.48, d, J = 9.6 Hz). *Trans* fusion of the B/C rings and *cis* fusion of the C/D rings further confirmed that the aglycone of compound **2** was corotoxigenin.[20]

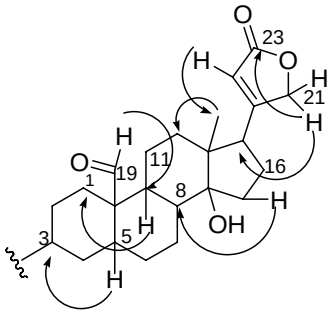


Figure 2.15 Key HMBC correlations of the aglycone of compound **2**

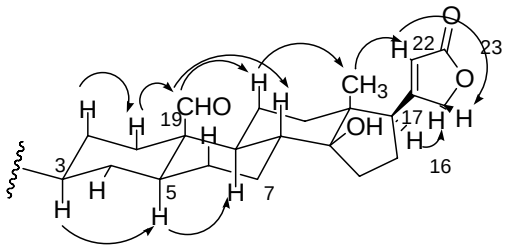


Figure 2.16 Key ROESY correlations of the aglycone of compound **2**

The sugar portion of compound **2** had a 1→4 attachment of the two sugar moieties, as indicated by an HMBC correlation from H-1"→H-4'. Also, a strong correlation from H-1' (δ_H 5.48, d, J = 9.6 Hz) to C-3 (75.8) indicated the attachment of the first sugar to the aglycone portion at C-3, giving a 3→1' connectivity. The two sugars were attached as 4'→1". H-4' and H-1" protons were oriented *cis* to each other as confirmed from ROESY correlations observed from H-1" to H-4'.

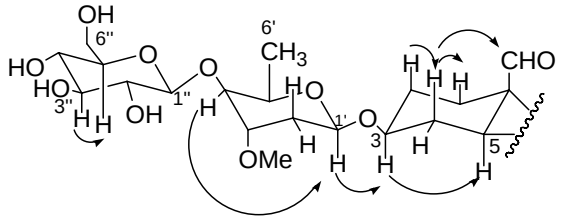


Figure 2.17 Key ROESY correlations of the sugar portion of compound **2**

The HMBC correlations for the sugar portion showed that the sugar moieties of **2** were similar to those of **1**, except for the absence of one oxygenated carbon. HMBC, ROESY and 1D TOCSY spectra suggested that the first sugar of **2** was different from that of **1**. The assignments of the sugars in **2** were made as follows: δ_C/δ_H 96.3 (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 68.2 (C-3')/4.79 (m), δ_C/δ_H 78.8 (C-3")/3.9 (br s); H-4' appeared as a doublet 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 78.8); 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, 4.52 and the 3'-methoxy group appeared as a singlet at δ_H 3.62.

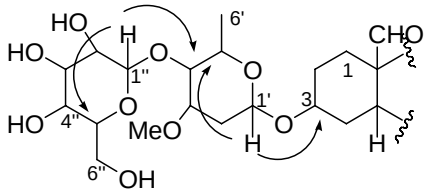


Figure 2.18 HMBC connection of the sugar to the aglycone of compound **2**

The NMR spectra of the sugar portion matched the spectra of β-D-glucopyranosyl-(1→4)-β-D-sarmentoside.[21, 22] The structure of boivinide B (compound **2**)

was thus assigned as 5α-corotoxigenin-β-D-glucopyranosyl-(1→4)-β-D-sarmentoside (**2.19**). This compound has not been previously observed in nature.

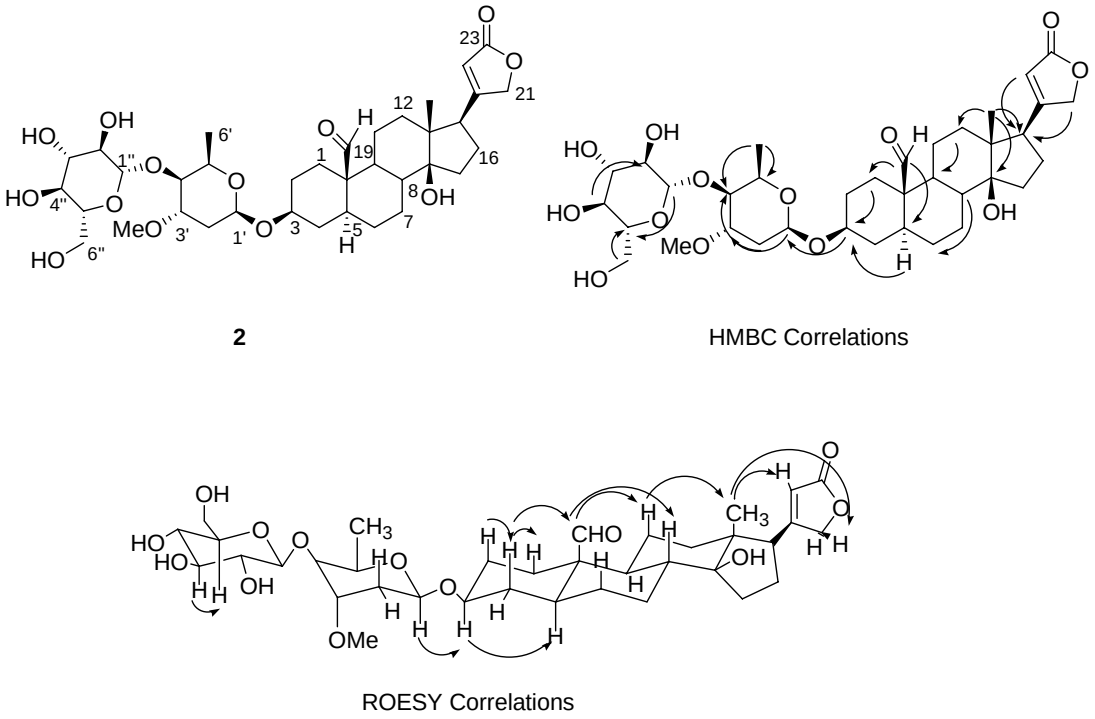


Figure 2.19 Key HMBC and ROESY correlations of compound **2**

Table 2.2 $^{1}H^{a}$ and $^{13}C^{b}$ NMR data for boivinide B and its comparison to literature values.

	Corotoxigenin		**Boivinide B**	
Position	^{1}H NMR[c]	^{13}C NMR[c]	^{1}H NMR[c]	^{13}C NMR[c]
1		31.5	0.83 m, 2.40 m	31.8
2		31.2	1.60 m, 2.38 m	31.6
3	4.28 br s	76.7	3.92 br s	76.2
4		36.3	1.86 m, 1.35 m	36.7
5		43	1.20 m	43.4
6		28.7	1.94 m, 1.40 m	29.2
7		27.9	2.35 m, 1.26 m	28.3
8		43	1.80 m	43.3
9		48.6	2.75 m	51.6
10		51.7		49.0
11		22.1	1.62 m	22.6
12		39.3	1.98 m, 2.31 m	39.7
13		49.8		50.2
14		84.1		84.6
15		32.5	1.98 m, 1.82 m	32.9
16		27.1	2.10 m, 1.98 m	27.6
17		51.2	2.72 m	52.2
18	1.01 s	15.9	0.92 s	16.4
19	10.41 s	208.7	10.05 s	209.3
20		176.7		176.2
21	5.28 dd (18.0 1.0) 5.02 dd (18.0, 2.0)	73.7	5.00 d (1.6), 5.14 d (4.0)	74.1
22	6.13 s	117.8	6.12 s	118.2
23		174.5		174.9
	β-D-glucopyranosyl-3-O-methyl-β-D-sarmentoside[c]			
Sugar I				
1'	5.11 dd (9.0,2.0)	97.6	5.48 d (9.6)	96.4
2'		36.5	1.98 m, 2.31 m	39.7
3'	4.07 q (3.0)	77.9	4.79[e]	68.3
4'	3.65 dd (9.0, 3.0)	82.8	3.73 dd (9.2, 2.8)	84.4
5'	4.20 m	69.5	4.44[e]	69.4
6'	1.61 d (6.0)	18.5	1.71 d (6.4)	19.3
OMe	3.62	58.5	3.62 s	50.1
Sugar II				
1''	4.94 d (8.0)	106.4	5.04 d (7.6)	106.6
2''	3.99 t (9.0)	75.3	3.97[e]	75.6
3''	4.17	78.3	3.99[e]	78.8
4''	4.23	71.9	4.26[e]	71.9
5''	4.57 dd (11.0, 2.0)	78.3	4.26[e]	78.9
6''	4.38 dd (11.0, 5.0)	63.0	4.4[e], 4.52[e]	63.0

[a]δ (ppm) 400 MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet; [b]δ (ppm) 100 MHz; [c] in pyridine-d_5; [e] overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

2.2.4 Structure Elucidation of Boivinide F

Compound **3** (boivinide F) was also obtained as a yellow amorphous solid. Positive ion HRFABMS gave a molecular ion peak at *m/z* 775.3570 $[M+Na]^+$, consistent with the molecular formula of $C_{38}H_{56}O_{15}$ and with the same aglycone portion as boivinides A and B. The presence of a glucose unit was confirmed by 1D TOCSY, 1D and 2D ROESY spectra. However, additional peaks appeared at δ_C 170.0, indicating the presence of an additional carbonyl group, and a singlet at δ_H 2.08 (3H, s). Analysis of the HMBC spectrum led to the conclusion that the first sugar of compound **3** was connected to the aglycone at C-3. An HMBC correlation from H-1' ((δ_H 4.82, d, *J* = 8.4 Hz) to C-3 (δ_C 77.9) confirmed the 1→3 connectivity of the aglycone and the sugar moieties. The proton and carbon chemical shifts of the two sugars were assigned as follows: δ_C/δ_H 100.6 (C-1')/4.82 (d, *J* = 8.4 Hz), and δ_C/δ_H 105.4 (C-1")/5.16 (br d, *J* = 7.6 Hz); δ_C/δ_H 72.3 (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), δ_C/δ_H 78.7 (C-3")/4.26 (br s); H-4' appeared as a multiplet at δ_H 4.46 (C-4' δ_C 75.1); H-5" was a multiplet at δ_H 3.78 (C-5" δ_C 71.2); H-6' was a prominent broad doublet at δ_H 1.60 (*J* = 6.0 Hz, C-6' δ_C 18.0), H-6" was a methylene with δ_H 4.38, 4.60 and the 3'-methoxy group appeared as a singlet at δ_H 3.49.(**2.22**)

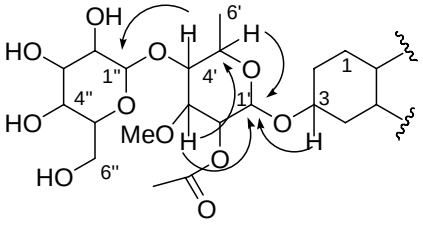


Figure 2.20 Key HMBC correlations of the two the sugar moieties of compound **3**

The acetoxy group (δ_C 170.0, δ_C/δ_H 21.4/2.08) was attached at position C-2' (δ_C/δ_H 72.3/5.83) of the first sugar. After comparing the chemical shifts of the first sugar to literature values, it was concluded that the first sugar is 2-acetyl-3-methyl-β-D-fucopyranoside.[23] The relative stereochemistry of the first sugar was confirmed by ROESY crosspeaks between H-1' ((δ_H 4.82, d, J = 8.4 Hz) and H-3 ((δ_H 3.90, br s); H-3' (δ_H 3.62, d, J = 6.4 Hz) and H-5' (δ_H 3.78, d, J = 2.8 Hz) as well as H-4' (δ_H 4.47, d, J = 2.0 Hz) and H-1" (δ_H 5.16, d, J = 7.6 Hz) (**2.21**).

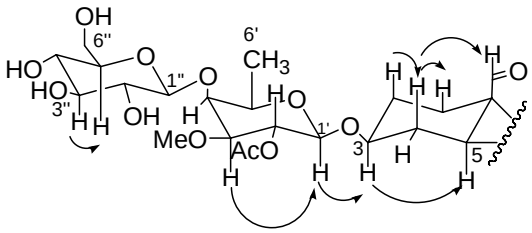


Figure 2.21 Key ROESY correlations of the two sugar moieties of compound **3**

The comparison of the ^{1}H and ^{13}C NMR data of compound **3** to literature values led to the conclusion that the aglycone of **3** was corotoxigenin,[20] and the sugar moiety was β-D-glucoypyranosyl-(1→4)-2-acetyl-3-methyl-β-D-fucopyranoside.[23] The structure of boivinide F (compound **3**) was thus assigned as 5α-corotoxigenin-β-D-glucoypyranosyl-(1→4)-2-acetyl-3-methyl-β-D-fucopyranoside (**2.22**).

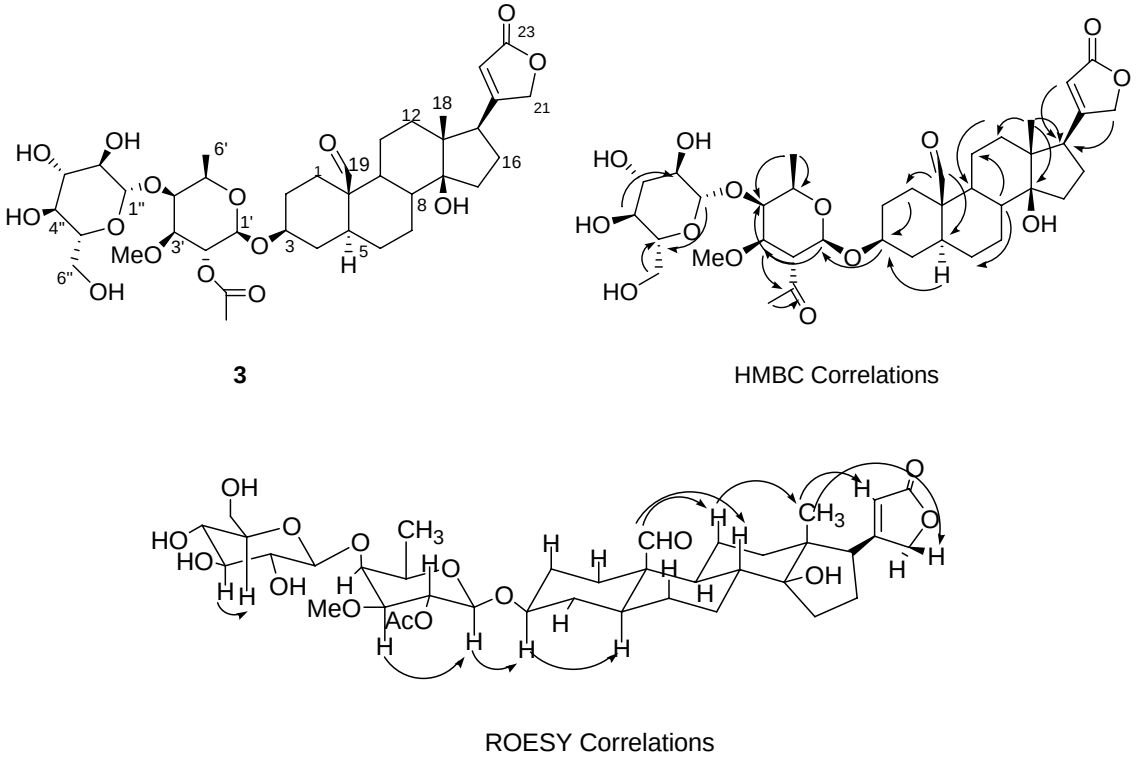


Figure 2.22 Final Structure and key HMBC and ROESY correlations of compound **3**

Table 2.3 $^{1}H^{a}$ and ^{13}C ^{b}NMR data for boivinide C and its comparison to literature values.

	Corotoxigenin		**Boivinide C**	
Position	^{1}H NMR^{c}	^{13}C NMR^{c}	^{1}H NMR^{c}	^{13}C NMR^{c}
1		31.5	0.98, 2.41m	31.8
2		31.2	1.98 m, 1.45 m	31.6
3	4.28 br s	76.7	3.90 br s	76.2
4		36.3	2.03 m, 1.5 m	36.7
5		43	1.24 m	43.4
6		28.7	1.30 m, 1.30 m	29.2
7		27.9	1.83 m, 1.83 m	28.3
8		43	1.22 m	43.3
9		48.6	2.70 m	51.6
10		51.7		49.0
11		22.1	1.63 m, 1.33	22.6
12		39.3	1.21 m, 1.34 m	39.7
13		49.8		50.2
14		84.1		84.6
15		32.5	1.99 m, 1.82 m	32.9
16		27.1	2.15 m, 1.97 m	27.6
17		51.2	2.75 dd	52.2
18	1.01 s	15.9	0.94 s	16.4
19	10.41 s	208.7	10.04 s	209.3
20		176.7		176.2
21	5.28 dd (18.0 1) 5.02 dd (18.0, 2)	73.7	5.29 d (18.4), 5.01 d (4.0)	74.1
22	6.13 s	117.8	6.14 s	118.2
23		174.5		174.9
	β-D-glucopyranosyl-2-O-acteyl-3-O-methyl-β-D-fucopyranoside			
Sugar I				
1'	4.84 d (8.0)	102.1	4.82 d (8.4)	101.1
2'	5.82 dd (8.0, 10.0)	72.7	5.83 d (7.6)	72.9
3'	3.61 dd (10.2, 3.0)	73.5	3.62 d (6.4)	83.7
4'	4.44 br d (3.0)	75.2	4.47 d (2.0)	75.4
5'	3.73 m	71.8	3.78 br d (2.8)	71.7
6'	1.6 d (6.4)	17.2	1.60 d (6.0)	17.4
OMe	3.45 s	58.5	3.49 s	58.5
C=O		172.2		171.9
Me	2.23 s	21.1	2.08 s	21.2
Sugar II				
1''	5.14 d (7.7)	104.6	5.16 d (7.6)	104.4
2''		75.9	4.02 ^{e}	76.0
3''	4.25 dd (8.8, 8.8)	77.8	4.26 ^{e}	77.9
4''	4.19 dd (9.4, 8.8)	71.8	4.19 ^{e}	71.9
5''		78.2	3.98 ^{e}	78.4
6''	4.60 d (11.5), 4.84 d (8.0)	63.0	4.38 m, 4.60 m	63.2

^{a}δ (ppm) 500 MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet; ^{b}δ (ppm) 125 MHz; ^{c} in pyridine-d_5; ^{d} in methanol-d_4; ^{e} overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

2.2.5 The Structure Elucidation of Compound 4

Compound **4** was a yellow solid obtained from the methanol fraction and HRFABMS gave m/z at 697.3523 $[M+H]^+$, giving a molecular composition of $C_{36}H_{55}O_{13}$. The structure was confirmed by examination of ^1H and ^{13}C NMR spectra and comparison of its proton and carbon chemical shifts to literature values.[18] Unlike boivinide A-C, the ^1H NMR spectrum of compound **4** did not show the presence of an aldehyde proton (Fig. **2.23**). Instead, a methyl peak appeared at δ_H 0.96 ppm. In addition to this, a typical methyl doublet at δ_H 1.60 (s, H_3-6', J = 6.0) was observed, indicating the presence of a deoxy sugar in the compound. Two anomeric signals at δ_C/δ_H 100.2 (C-1')/ 5.28 (d, J = 2.0 Hz) and δ_C/δ_H 104.4 (C-1")/5.16 (d, J = 7.6 Hz) suggested two sugars in the compound. The ^{13}C NMR consisted of 36 signals: one methoxy, three methyls, eleven methylenes, sixteen methines and 5 quaternary carbons. The NMR spectra of the aglycone of compound 4 matched the literature values for digitoxigenin. The chemical shifts of compound **4** were similar to that of the known compound, digitoxigenin 3-O-β-D-glucopyranosyl-(1→4)-α-L-acofriopyranoside.[18] However, this compound was isolated from *Strophanthus boivinii* for the first time.

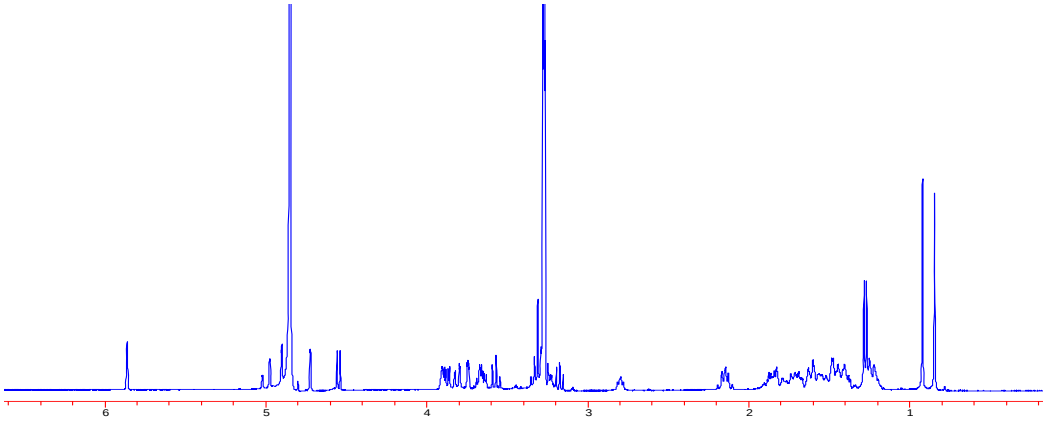


Figure 2.23 ^1H NMR spectrum of compound **4** in methanol-d_4

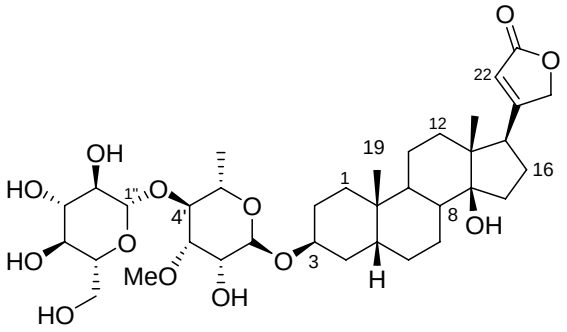


Figure 2.24 Digitoxigenin 3-O-β-D-glucopyranosyl-(1→4)-α-L-acofriopyranoside (compound **4**)

Table 2.4 $^{1}H^{a}$ and $^{13}C^{b}$ NMR data for compound **4** and its comparison to literature values.

	Digitoxigenin		**Compound 4**	
Position	^{1}H NMR[d]	^{13}C NMR[c]	^{1}H NM[d]	^{13}C NM[c]
1		30.1	1.52, 1.42 m	30.6
2		26.9	1.63 m, 1.57 m	27.3
3	4.02 br s	72.4	3.93 br s	72.8
4		31.1	1.46 m, 1.81 m	31.5
5		37.2	1.64 m	37.5
6		27.2	1.28 m, 1.26 m	27.6
7		22.0	1.79 m, 1.27 m	22.4
8		42.0	1.50 m	42.3
9		35.9	1.70 m	36.2
10		35.6		35.9
11		21.6	1.44 m, 1.76	22.0
12		40.0	1.60 m, 1.66 m	40.3
13		50.2		50.1
14		84.7		85.1
15		33.3	1.72 m, 2.16 m	33.6
16		27.4	2.18 m, 1.88 m	27.8
17	2.83 dd (8.5, 6.0)	51.5	2.83 dd	51.9
18	0.88 s	16.3	0.87 s	16.6
19	0.94 s	24.1	0.96 s	24.5
20		175.9		176.5
21	4.92 dd (18.5, 1.5) 5.04 dd (18.5, 1.5)	73.8	4.88 d (18.4, 4.0), 5.05 d (18.4, 4.0)	74.2
22	5.90 br s	117.7	5.85 s	118.1
23		174.5		175.0
	β-D-glucopyranosyl-(1 →4)-β-D-acofriopyranoside			
Sugar I				
1'	5.36 d (2.0)	99.5	5.28 d (2.0)	100.2
2'	4.51 br s	68.5	4.56 br s	72.9
3'	4.04 dd (9.5, 3.0)	82.7	3.92 [e]	73.3
4'	4.47 t (9.5)	79.6	4.46 [e]	85.9
5'		68.3	3.78 [e]	68.8
6'	1.68 d (6.0)	18.7	1.60 d (6.0)	18.9
OMe	3.54 s	56.7	3.34 s	58.5
Sugar II				
1''	5.27 d (8.0)	105.7	5.16 d (7.6)	104.4
2''		76.1	4.02[e]	76.0
3''		78.4	4.26 [e]	77.9
4''		72.0	4.19 [e]	71.9
5''		78.1	3.98 m	78.4
6''	4.34 dd (12.0, 4.5), 4.41 dd (12.0, 2.5)	63.0	4.38 m (12.5, 4.5) 4.60 m (12.0, 2.5)	63.2

[a]δ (ppm) 500MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet; [b]δ (ppm) 125MHz;[c] in pyridine-d_5 [d] in methanol-d_4; [e] overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

2.2.6 Antiproliferative activity of Compounds 1-4

All four compounds were tested for their growth inhibition ability using the A2780 human ovarian cancer cell line. Compounds **1-3** (boivinide A, B and F) are new compounds and they exhibited strong growth inhibition. Since **1-3** are new compounds, no previous activity has been reported for them. However, as they belong to the class of cardenolide glycosides, they exhibited activities similar to those expected for this group of compounds. Compound **4** is a known compound, but no biological activity has been published for it. The activity data for the four compounds, compounds **1-4** is tabulated in Table **2.5**

Table 2.5. Antiproliferative activity of cardenolides against the A2780 human ovarian cancer cell line

Compound	IC_{50} (μg/mL)	IC_{50} (μM)
1	0.12	0.17
2	0.46	0.66
3	0.41	0.52
4	0.11	0.15

2.3 Experimental Section

2.3.1 General Experimental Procedures- Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were collected on UV-1210 series and the IR spectra were measured on a MIDAC M-series FTIR spectrophotometers. 1D and 2D NMR spectra were obtained on a Varian Inova 400 spectrometer and the chemical shifts are given in ppm. Mass spectra were obtained on JEOL JMS-HX-110 instrument in a positive mode and Finnigan LTQ LC/MS^n in positive and negative mode ion with sample elution using MeOH from a C_{18} column. HPLC was carried out using a Shimadzu LC-

10AT 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.

2.3.2 Cytotoxicity Bioassays- The A2780 human ovarian cancer cell line antiproliferative assay was performed at Virginia Polytechnic Institute and State University as previously described.[24]

2.3.3 Plant Material- The dried root (14.1 g), bark (10.9 g) and wood (7.0 g) of *Strophanthus boivinii* were separately ground and extracted with EtOH; the resulting extracts were designated MG 2309 (3.3 g) MG 2310 (2.2 g), and MG 2311 (1.9 g) respectively, of which 3.3 g, 2.2 g, and 1.9 g respectively were made available for this work.

2.3.4 Extraction and Isolation- The dried root, bark and wood parts of the plant *Strophanthus boivinii* was extracted with EtOH and designated MG 2309PE. 3.0 g of the dried plant material, MG 2309PE was suspended in aqueous MeOH (MeOH:H_2O, 9:1, 100mL) and extracted with hexanes (3 × 100mL). The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH_2Cl_2 (3 × 180mL). The aqueous MeOH fraction displayed the highest cytotoxicity (IC_{50} = 0.61 μg/mL). Hence this fraction was selected for further isolation. It was chromatographed using C_{18} open column eluting with 30% MeOH:H_2O to 100% MeOH which yielded five fractions. Only two fractions, III and IV, were active at IC_{50} of 0.13 μg/mL 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 twenty-five subfractions were collected. Fractions 16 and 18 pure and new compounds, boivinide B (**2**) (5.9 mg, t_R 62 min) and boivinide A (**1**)

(4.5 mg, t_R 59 min). Fraction IV was loaded on 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 and one new compound, boivinide F (**3**) (3.5 mg, t_R 39 min) and one known compound (**4**) (4.9 mg, t_R 49 min) were isolated from this fraction.

Boivinide A (1): white amorphous solid; $[\alpha]_D^{25}$ +14.0 (*c* = 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 nm; IR ν_{max} 3409, 2870, 1743, 1616, 1068, 884 cm^{-1}; ^1H NMR (400MHz, pyridine-d_5): H_2-1:0.90/2.39, H_2-2:1.56/2.20, H-3:3.97, H_2-4:2.04/1.42, H-5:1.19, H_2-6:1.89/1.42, H_2-7:2.48/1.24, H-8:1.81, H-9:1.20, H_2-11:1.64/1.22, H_2-12:1.23/1.34, H_2-15:1.98/1.81, H-16:2.06/1.96, H-17:2.75, H_3-18:0.93, H-19:10.01, H_2-21:5.03/5.27, H-22:6.12, H-1':4.77; H-2':4.43; H-3': 3.58; H-4': 4.25; H-5':3.77; H-6':1.63; OMe:3.62; H-1":5.20; H-2":3.96; H-3":4.23; H-4":4.18; H-5":3.95; H-6":4.38/4.59. ^{13}C NMR (100 MHz, pyridine-d_5) see Table **2.1**; 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; $[\alpha]_D^{25}$ +21.0 (*c* = 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215 nm; IR ν_{max} 3400, 2873, 1739, 1616, 1162, 1071, 1024, 679 cm^{-1} ; ^1H NMR and ^{13}C NMR (100 MHz, pyridine-d_5), see Table **2.2**; LC-MS *m/z* 695.4 $[M+H]^-$ (calcd. for $C_{36}H_{55}O_{13}^-$, 695.4)

Boivinide F (3): yellow amorphous solid; $[\alpha]_D^{25}$ +17.0 (*c* = 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 nm; IR ν_{max} 3450, 2871, 1747, 1709, 1372, 1236, 1069, 985, 659 cm^{-1}; ^1H NMR and ^{13}C NMR (100 MHz, pyridine-d_5), see Table **2.3**; HRFABMS *m/z* 775.3570 (calcd. for $C_{38}H_{56}O_{15}Na^+$, 775.3512)

Compound 4: yellow amorphous solid; $[\alpha]_D^{25}$ +28.0 (*c* = 0.1, MeOH); UV (MeOH)

λ_{max} (log ε) 216 nm; IR ν_{max} 3400, 2927, 1737, 1734, 1593, 1447, 1378, 1351, 1063, 885, 659 cm^{-1}; ^{1}H NMR and ^{13}C NMR (100 MHz, pyridine-d_5), see Table **2.4**; HRFABMS *m/z* 697.3523 (calcd. for $C_{36}H_{57}O_{13}^{+}$, 697.3794)

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