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Sarasinosides D-G: four new Triterpenoid Saponins from the Sponge *Asteropus sarasinosum*

Alfonso Espada[1], Carlos Jiménez[2], Jaime Rodríguez[1], Phillip Crews[3]* and Ricardo Riguera[1]*

[1]Depart. de Química Orgánica, Fac. de Química, Univ. de Santiago de Compostela, Santiago de Compostela. 15706, Spain

[2]Depart. de Química Fundamental e Industrial, Fac. de Ciencias, Univ. da Coruña, A Coruña. 15071, Spain

[3]Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, USA.

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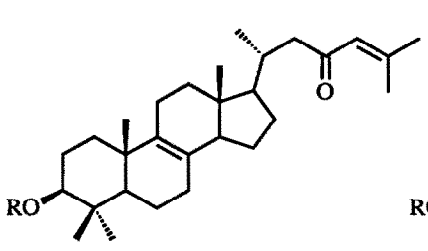
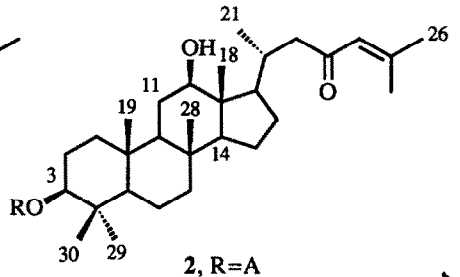
Abstract: Four new triterpenoid oligoglycosides (**2-5**; sarasinosides **D-G**, respectively) and sarasinoside B_1 (**1**) have been isolated from the butanolic extracts of the sponge *Asteropus sarasinosum*, which showed *in vitro* cytotoxic activity against several types of tumour cells and inhibition to protein kinase C. These compounds have identical sugar chains and differ in the aglycon moiety. Structures were established by FAB-MS, COSY, XHCORR, RCOSY, TOCSY, ROESY and HMQC experiments, and confirmed by selective hydrolysis and permethylation followed by methanolysis.

INTRODUCTION

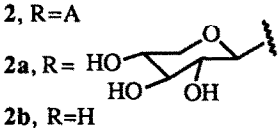
Triterpenoid saponins are a class of secondary metabolites that have very rarely been isolated from marine organisms apart the phylum Echinodermata[1]. Recently, however half a dozen triterpenoid oligoglycosides have been isolated from marine sponges of the genera *Asteropus* [2] and *Erylus* [3]. Previous studies of *Asteropus sp* have yielded five novel triterpene galactosides named pousides **A-E**[4] and several norlanostane triterpenoid oligoglycosides differing in the sugar chain but with the same triterpenoid framework structure. This paper reports the isolation of saponins **1-5** from the cytotoxic[5] butanolic extract of the sponge *Asteropus sarasinosum*, and their identification, by extensive spectroscopic analysis (inverse 2D-NMR experiments and FAB-MS) and chemical transformation, as sarasinoside B_1 (**1**) and the four new related triterpenoid saponins **2-5** (sarasinosides **D-G** respectively).



1, R=A

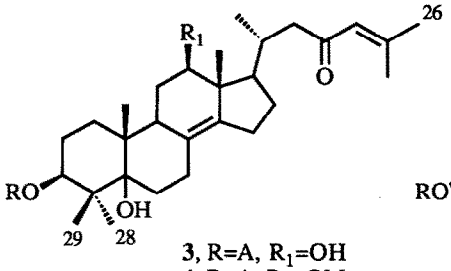


2, R=A

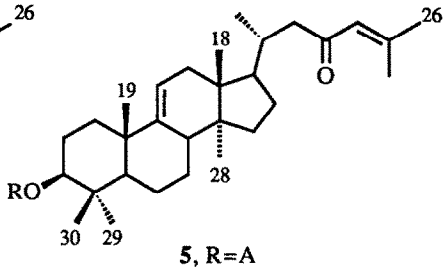


2a, R=

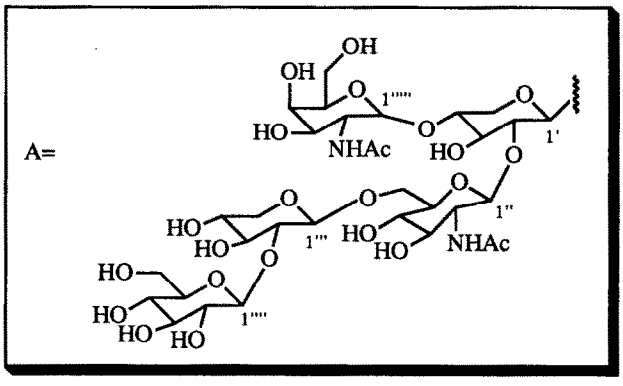
2b, R=H



3, R=A, R_1=OH
4, R=A, R_1=OMe



5, R=A



RESULTS AND DISCUSSION

Specimens of the sponge were collected by hand in the Solomon Islands and immediately inmersed in methanol at room temperature. The fresh methanol extracts were then partitioned into n-hexane, CCl_4, CH_2Cl_2 and n-BuOH. The n-BuOH fraction, shown by pharmacological screening to be the most cytotoxic, was fractionated on a Sephadex LH-20 column to give a mixture of compounds whose ^1H and ^{13}C NMR spectra suggested the presence of triterpenoid saponins. In accordance with our previous experience with saponins,[6] the mixture was subjected to several separation procedures, including DCCC followed by repeated reversed phase HPLC, to give the pure saponins **1**-**5**.

TABLE 1. ^{13}C NMR data of the aglycons of compounds **1-5**.

Carbon	1[a]	2[a]	3[a]	4[a]	5[b]
1	37.4 t	38.9 t	33.2 t	34.2 t	34.4 t
2	27.8 t	26.7 t	26.6 t	28.1 t	25.5 t
3	90.6 d	90.2 d	90.1 d	89.9 d	91.3 d
4	40.1 s	40.8 s	42.2 s	41.5 s	40.3 s
5	51.0 d	55.1 d	75.0 s	74.5 s	50.3 d
6	18.9 t	21.5 t	21.7 t	22.0 t	24.8 t
7	28.6 t	25.1 t	29.1 t	29.8 t	28.1 t
8	127.8 s	42.3 s	128.1 s	137.3 s	44.7 d
9	136.6 s	49.5 d	44.8 d	44.2 d	148.4 s
10	37.2 s	39.8 s	39.3 s	39.7 s	37.3 s
11	22.5 t	29.8 t	31.6 t	33.7 t	115.0 d
12	36.3 t	68.7 d	65.1 t	79.0 d	33.7 t
13	42.6 s	43.2 s	44.2 s	43.5 s	c
14	52.3 d	58.5 d	143.5 s	144.8 s	c
15	24.1 t	34.9 t	26.0 t	28.1 t	30.7 t
16	29.4 t	33.9 t	26.7 t	30.5 t	25.2 t
17	55.4 d	55.0 d	56.9 d	54.2 t	54.1 d
18	11.5 q	17.4 q	17.3 q	18.2 q	17.2 q
19	16.7 q	16.3 q	16.5 q	16.9 q	18.2 q
20	33.9 d	34.1 d	31.2 d	30.9 d	33.2 d
21	20.2 q	19.5 q	19.7 q	20.2 q	18.8 q
22	52.0 t	50.7 t	50.7 t	51.2 t	50.2 t
23	200.9 s	201.0 s	201.6 s	200.4 s	203.9 s
24	124.9 d	124.1 d	124.4 d	124.7 d	125.2 d
25	154.2 s	156.2 s	154.5 s	153.9 s	157.1 s
26	20.8 q	20.1 q	20.2 q	20.4 q	20.9 q
27	27.4 q	26.8 q	26.9 q	27.1 q	27.7 q
28	28.2 q	16.9 q	28.3 q	28.6 q	22.1 q
29	20.2 q	27.9 q	17.4 q	17.9 q	29.0 q
30		17.1 q			20.9 q
$CH_3C(O)$	22.7 (2C)	23.5 (2C)	22.7 (2C)	23.1 (2C)	23.0 (2C)

a. In C_5D_5N.
b. In CD_3OD.
c. Signal under the solvent (CD_3OD).

Compound **1**, the major component of the mixture, was identified by comparison of its spectra with published data as sarasinoside B_1, previously isolated from the same source[2].

Sarasinoside **D** (**2**; $C_{62}H_{102}N_2O_{26}$) was obtained as a yellow amorphous solid of $[\alpha]_D^{20} = -12.7$ °. Its negative ion FAB mass spectrum exhibited a pseudomolecular ion $[M-H]^-$ at m/z 1289, in agreement with the molecular formula, and provided the best information on the sugar sequence of the molecule: fragments at m/z 1127 ([M- 162 -H]$^-$) and 1086 ([M- 203 -H]$^-$) showed terminal glucose and NAc-galactosamine, while fragments at m/z 995 ([M- 162 -132 -H]$^-$; loss of glucose + xylose), 774 ([M- 162- 132 -203 -18 -H]$^-$loss of glucose+xylose+NAc-glucosamine+H_2O) and 589 ([M- 162 -132 -203 -203]$^-$; loss of glucose+xylose+NAc-glucosamine+NAc-galactosamine) indicated the sugar sequence in the oligosaccharide. ^1H and ^{13}C NMR signals, in particular the anomeric peaks at δ_H 5.62 (d, J= 8.4 Hz), 5.50 (d, J= 7.7 Hz), 5.27 (d, J= 8.6 Hz), 5.24 (d, J= 7.6 Hz) and 4.75 (d, J= 8.0 Hz), (δ_C 102.0, 102.4, 103.3, 105.6 and 106.9), confirmed that the oligosaccharide part of **2** comprises 2 xylose unit and 1 unit each of glucose, NAc-glucosamine and NAc-galactosamine. The chemical shifts and J values for the anomeric protons showed that all the sugars are connected through β-glycoside linkages.

Assignment of the anomeric signals of the amino sugars was carried out by inspection of the COSY spectrum, which showed correlation between the NH peak of NAc-galactosamine at δ_H 8.91 (d, J= 8.7 Hz) and a doublet at δ_H 4.62 attributed to H2'''', and this in turn with the anomeric proton at 5.27. A similar correlation was seen among the signals of the NH of the NAc-glucosamine at δ_H 8.73 (d, J= 8.5 Hz), a broad triplet at δ_H 4.39 (H2''), and the anomeric proton at 5.62. [7,8] The arrangement of the sugar chain was corroborated by ROESY experiments and is identical to that of **1**. Exhaustive methylation followed by methanolysis (Hakomori's method)[9] confirmed the sugars and their attachment points.

With regard to the structure of the aglycon, comparison of the ^{13}C NMR data of sarasinoside B_1 (**1**) with those of **2** suggests the presence of additional hydroxyl and methyl groups in **2** and the absence of the Δ^8 double bond that is present in **1**. This agrees with the molecular mass difference of 30 mass units between **1** and **2**.

Tables 1, 2 and 3 show the assignment of ^1H and ^{13}C-NMR data on the basis of HMQC experiment. All but two signals were assigned. A secondary hydroxyl group (peaks at δ_H 4.30 and δ_C 68.7) was easily located at C-12[10] by Relayed-COSY and TOCSY experiments showing the coupling between the deshielded methine proton H12 at δ_H 4.30 (dd, J= 10.3 and 2.0 Hz) and the methylene protons at C-11 (δ_H 2.23 and δ_H 1.54), which in turn are coupled with H9 (δ_H 0.97). The additional methyl group (Me-28) is at C-8 and not at C-14, as normally found. This can be deduced from the coupling between the methylene protons H15 at δ_H 1.76 and the methine proton H14 at δ_H 2.19. TOCSY experiments around ring C from H12 as starting point showed the expected coupling pattern of H9 and H11 and confirmed that C-8 is a quaternary carbon. A precedent for a methyl at this position on a triterpenoid framework is found in 21-O-acetyl toosendantriol, which was isolated from a Chinese plant[11].

The ^{13}C NMR chemical shifts of the methyl groups C-18, C-19 and C-28 are in keeping with their axial orientation and *trans* ABCD ring junctions. The ^1H NMR coupling between H11 and H12 ($J_{H11a\text{-}H12a}$ = 10.3 Hz, $J_{H11e\text{-}H12a}$= 2.0 Hz) is consistent with β orientation for the C-12 hydroxyl group.[10]

Acid hydrolysis of **2** gave the expected sugars, which were identified by GC-MS as alditol acetates. Partial hydrolysis, by refluxing in 0.5 N HCl/toluene gave, after purification by reversed phase HPLC, products identified as the xyloside **2a** and the triterpene **2b**.

Compound **2a**, which was isolated as a white solid, has the molecular formula $C_{35}H_{58}O_7$. The HREIMS exhibits an intense [M^+-H_2O] peak ($C_{35}H_{56}O_6$, m/z 572.4052, Δ 0.025 mmu from calc.). The FAB mass spectrum (positive ion) showed the pseudomolecular ion at m/z 611 ([M-H_2O+K]$^+$) and a fragment at 479 ([M-H_2O+K-132]$^+$), indicating the loss of a xylose unit.

The ^1H NMR data of the aglycon part of xyloside **2a** are in perfect agreement with those of **2**. Slight differences are observed when the NMR spectrum is recorded in $CDCl_3$ instead of C_5D_5N (see experimental part). The J value for the anomeric proton corresponds to a β glycoside. COSY experiments served to establish sequential correlation among aglycon protons at H12 at δ_H 4.19 (dd, J= 10.2 and 2.0 Hz), H11a at δ_H 1.48, H11e at δ_H 2.11 and H9 at δ_H 1.20 and also to correlate the xylose protons. These data confirmed that xylose was bonded directly to the aglycon.

Compound **2b**, also a white solid, has the molecular formula $C_{30}H_{50}O_3$. Its HREIMS shows a [M^+-H_2O] peak (m/z 440.3188 Δ 0.466 mmu from calcd) due to the loss of water. The EI mass spectrum exhibited fragments at m/z 83 (C_5H_7O) and m/z 97 (C_6H_9O) corresponding to characteristic cleavages of the side chain.

The ^{1}H NMR spectrum was very similar to that of **2a** with the obvious exception of the five signals due to the xylose moiety. Together, these findings indicated that **2b** was the aglycon of saponin **2**.

TABLE 2. ^{1}H NMR data of the aglycon moieties of **2-5**.

H	**2**[a] δ, mult. J (Hz)	**3**[a] δ, mult. J (Hz)	**4**[a] δ, mult. J (Hz)	**5**[b] δ, mult. J (Hz)
1	1.50		1.75, 1.48	
2	2.23, 2.07	2.18, 2.07	2.22, 2.06	1.70
3	3.38dd, 12.5, 5.0	3.34dd, 12.0, 4.5	3.40dd, 11.8, 4.0	3.22dd, 13.5, 3.8
5				
6	2.11, 2.03		2.72, 2.50	
7	1.29, 1.14		1.71, 1.42	
9	0.97dd, 12.8, 6.0	2.10m	2.30m	
11	2.23, 1.54	2.25dd, 1.58dd	1.96, 1.52	5.19d, 5.2
12	4.30dd, 10.3, 2.0	4.26dd, 9.8, 2.5	4.11dd, 10.6, 4.8	2.40m
14	2.19dd, 11.4, 6.3			
15	1.76, 1.62		2.46, 2.19	
16	1.86, 1.69	1.74, 1.33	1.73, 1.54	
17	2.08	1.45	1.46	1.75
18	0.94	0.92	0.90	0.98
19	1.30	1.32	1.34	1.17
20	2.32	2.22	2.22	2.19
21	1.02d, 6.5	0.97d, 5.8	0.96d, 6.7	0.93, 5.6
22	2.61, 2.18	2.52, 2.19	2.58, 2.16	2.50, 2.17
24	6.15bs	6.10bs	6.18bs	6.17bs
26	2.26	2.18	2.19	2.14
27	1.67	1.73	1.75	1.93
28	1.10	1.42	1.38	1.17
29	1.34	1.00	1.04	1.11
30	1.05			1.06

a. In C_5D_5N.
b. In CD_3OD.

The above data show that the structure of **2** is 3β-*O*-[β-D-glucopyranosyl (1→2) β-D-xylopyranosyl (1→6) β-D-N-acetyl-2-amino-glucopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-N-acetyl-2-amino-galactopyranosyl]-4,4-dimethyl-8α-methyl-cholesta-24-en-12β-ol-23-one. We named this compound sarasinoside **D**.

Another compound isolated from the butanolic extract of this sponge, sarasinoside **E** (**3**), was crystallized from methanol as a white microcrystalline powder of $[\alpha]_D^{20} = -8.4$ (MeOH). Its molecular formula, $C_{61}H_{98}N_2O_{27}$, was established by HRFAB-MS (negative ion) (m/z 1290.6381, Δ 0.027 mmu from calcd). This formula differs from that of **1** by O_2H_2, suggesting the presence of two extra hydroxyl groups in **3**. Its oligosaccharide moiety was found by negative ion FAB-MS and ^{13}C and ^{1}H NMR spectroscopy to be the same as that of **2**.

The presence of a $\Delta^{8(14)}$ double bond in the aglycon of **3** instead of the $\Delta^{8(9)}$ bond of **1** can be deduced from its ^{13}C NMR and APT spectra which exhibited two tetrasubstituted olefinic carbons at δ_C 128.1 and 143.5. [12,13] The ^1H NMR spectrum and COSY experiments showed correlations between a signal at δ 4.26 (dd, J= 9.8 and 2.5 Hz) assigned to H12 and the signals at δ_H 1.58 (dd, J= 14.6, 9.8 Hz, H-11a) and 2.25 (m, H-11e), indicating the presence of an -OH group at C-12 (δ_C 65.1); the correlation between H11 and the multiplet for H9 at δ_H 2.10 corroborated the location of the double bond at $\Delta^{8(14)}$.

The presence of the quaternary carbon at δ_C 75.0 and the lack of a signal for a tertiary carbon in the range δ_C 55-50 suggested the presence of a hydroxyl group at C-5. Comparison of the chemical shifts of C-4, C-5, C-6, and C-10 in **1** and **3** showed the expected downfield shifts (+2.1, +24.0, +2.8, and +2.1 ppm respectively) due to the α and β -OH effects, and are in good agreement with the values observed for other terpenes, such as vouacapen-5α-ol and suvanine[14,15].

The β stereochemistry of the hydroxyl group at C-12 was deduced from the J values between H11 and H12 ($J_{H11a\text{-}H12a}$=9.8 Hz, $J_{H11e\text{-}H12a}$= 2.4 Hz)[10]. The ^{13}C NMR chemical shifts for the methyl carbons C-18 (at δ_C 16.5) and C-19 (at δ_C 17.3) are typical of axial orientation. Thus compound **3** is 3β-*O* -[β-D-glucopyranosyl (1→6) β-D-xylopyranosyl (1→6) β-D-N-acetyl-2-amino-glucopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-N-acetyl-2-amino-galactopyranosyl]-norlanosta-8(14),24-dien-5α,12β-diol-23-one. We named it sarasinoside **E**.

Sarasinoside **F** (**4**; $[\alpha]_D^{20}$ = -8.4 (MeOH)) was crystallized from MeOH. Its negative ion HRFAB-MS spectrum showed it to have the molecular formula $C_{62}H_{100}N_2O_{27}$ (m/z 1304.6458, Δ 0.006 mmu from calcd), that is 14 mass units (CH_2) more than **3**. The same oligosaccharide moiety as in **1-3** was identified by negative ion FAB-MS, COSY and XHCORR experiments. The ^1H and ^{13}C NMR data for the aglycon of **4** were very similar to those of **3**, with the exception of the presence of an extra signal at δ_H 3.23 (3H, s) which correlated with the signal at δ_C 55.5, indicating the presence of an OMe group. The chemical shifts of C-11, C-12 and C-13 in **4**, and comparison with those of the same carbons in **3**, allocated the methoxy group at C-12. Analysis of the coupling constants and correlations of H12 at δ_H 4.11 (dd, J= 10.6 and 2.8 Hz) with the signals at δ_H 1.96 (H11e) and δ_H 1.52 (H11a), and those of H11a with the peak at δ_H 2.30 (H9), confirmed the position of the double bond as $\Delta^{8(14)}$ and the configuration of the OMe group at C-12 as β. Together, these data show compound **4** to be 3β-O-[β-D-glucopyranosyl (1→2) β-D-xylopyranosyl (1→6) β-D-N-acetyl-2-amino-glucopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-N-acetyl-2-amino-galactopyranosyl]-norlanosta-8(14),24-dien-5α-ol,12β-methoxy-23-one. We named it sarasinoside **F**.

Finally, sarasinoside **G** (**5**; $[\alpha]_D^{20}$ = -29.9 (MeOH)) showed a molecular ion $[M+Na]^+$ at m/z 1295 in the positive ion FAB-MS in keeping with the molecular formula ($C_{62}H_{100}N_2O_{25}$). NMR and FAB-MS data implied the same oligosaccharide moiety as in **1-4**. The molecular formula of **5** is 14 mass units heavier than that of sarasinoside $\mathbf{B_1}$ (**1**). Comparison of their ^{13}C and ^1H NMR spectra suggested the presence of an additional α-Methyl group located at C-14 in the aglycon of **5**, showing a typical lanostane framework with a α,β-unsaturated ketone in the side chain as in **1-4**. The olefinic carbons at δ_C 115.0 (d) and 148.4 (s), along with the olefinic proton at δ_H 5.19 (d, J= 5.2 Hz), indicate the presence of a $\Delta^{9(11)}$ double bond.[16] Thus compound **5** is 3β-*O*-[β-D-glucopyranosyl (1→2) β-D-xylopyranosyl (1→6) β-D-N-acetyl-2-amino-glucopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-N-acetyl-2-amino-galactopyranosyl]-lanosta-9(11),24-dien-23-one. We named it sarasinoside **G**.

TABLE 3. ^{13}C NMR data of the oligosaccharide chain of **2-5**.

Sugar	C	2[a]	3[a]	4[a]	5[b]
Xylose I	1'	105.6	104.9	105.5	105.7
	2'	78.5	78.2	78.3	78.2
	3'	77.4	76.2	77.6	77.5
	4'	79.8	79.2	79.5	79.7
	5'	63.9	63.4	63.8	64.2
Glucose	1"	106.9	105.1	106.9	106.6
	2"	76.6	75.1	76.4	75.8
	3"	77.5	76.8	77.8	77.8
	4"	69.6	68.4	69.5	69.8
	5"	78.0	76.8	78.2	78.1
	6"	62.6	61.9	62.5	63.1
2-NAc-Glc	1"'	102.0	101.0	101.8	102.0
	2"'	58.7	57.2	58.6	57.9
	3"	76.7	76.2	76.9	76.8
	4"'	72.7	71.8	72.6	72.8
	5"'	78.5	77.5	78.3	78.2
	6"'	69.7	69.2	69.6	69.9
Xylose II	1""	103.3	102.5	103.2	103.2
	2""	84.8	82.2	84.8	83.2
	3""	77.7	76.8	77.8	77.8
	4""	71.0	70.2	70.8	71.3
	5""	66.8	66.0	66.6	66.9
2-NAc-Gal	1""'	102.4	101.6	102.2	102.5
	2""'	55.1	53.6	54.9	54.5
	3""'	72.3	71.6	72.1	72.3
	4""'	71.1	70.4	71.0	71.4
	5""'	77.1	76.2	77.3	77.2
	6""'	62.3	61.6	62.1	62.6

a. In C_5D_5N.
b. In CD_3OD.

The sarasinosides are among the few saponins isolated from sponges. In fact, only *Asteropus sp.* from the South Pacific, *Erylus sp.* from New Caledonia and the Red Sea and *Pachastrella sp.* from Japan have been reported as sources of this class of metabolites. The eryloside sugar chain is attached at C-3 and composed exclusively of galactopyranosides. The aglycon of erylosides **C** and **D** is a triterpenoid, while that of eryloside **A** is a 4-methylated steroid.[3,17] Pachastrelloside **A** is the C4-galactopyrano-C-7-xylopyranoside of a 2, 3, 4, 7-tetrahydroxysteroid.[18]

Specimens of *Asteropus sp* from Guam Island, Truk Lagoon and Palauan have produced a great variety of triterpenoid saponins with ichthyotoxic and cytotoxic properties.[2] All the sarasinosides reported from these sources had the same norlanostane-triterpenoid aglycon, which is attached via C-3 to sugar chains that in all cases contain amino sugars. In contrast, the butanolic extract of *Asteropus sarasinosum*, a sponge from the Solomon Islands, yielded new sarasinosides (**D-G**) all with identical sugar chain but different novel

triterpenoid aglycon structures. This butanolic extract was cytotoxic for several types of tumour cells and inhibited protein kinase C.

It is interesting to note that sarasinoside **D** (**2**) has Me-28 located at C-8 instead of at the usual position, C-14. Examples of this kind of arrangement, though $\Delta^{14(15)}$ unsaturated, have been found in certain triterpenoids isolated from plants. Sarasinosides **E** (**3**) and **F** (**4**), which have the same framework as **1**, have a $\Delta^{8(14)}$ double bond and a C-5 hydroxyl group that are rarely found in triterpenes. Finally, the aglycon of sarasinoside **G** (**5**) has been isolated from plants but with a different side chain.

EXPERIMENTAL PART

General Methods. All melting points were determined in a Kofler apparatus and are uncorrected. Optical rotations were measured in a Perkin Elmer model 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded on Varian XL500 and Bruker WM250 spectrometers using CD_3OD, C_5D_5N and Cl_3CD as solvents and internal standards. Mass spectra were recorded on Kratos MS-50 and Hewlett-Packard HP 59970 spectrometers. The fast atom bombardment (FAB) mass spectra were obtained employing Xe atoms at 7-9 keV and a matrix of 2-hydroxyethyldisulphide. The HPLC separations were performed on a Waters Model 6000A equipped with an R401 differential refractometer. DCCC was carried out with an Eyela Model 300-S apparatus equipped with 300 tubes.

Two-dimensional experiments. Two-dimensional COSY spectra (128x1K) were obtained by accumulating 32 scans per t_1; the relaxation delay was 2s. The data were zero-filled to 512 in F_1 and subjected to Fourier transformation and symmetrized.

The two-dimensional ^1H-^{13}C heteronuclear shift correlation (XHCORR) spectrum (256x4K) of **4** for directly bonded protons and carbons was obtained by accumulating 80 scans per t_1; the relaxation delay was 2s and the value of J_{CH} selected was 130 Hz. The data were zero-filled to 512 in F_1 and subjected to Fourier transformation using Gaussian.

The HMQC spectra were obtained using the pulse sequence described by Bax and Subramanian[19]. The refocusing delay was optimized to 150 Hz (3.3 ms). The null delay following the BIRD pulse was 400 ms. 512 x 256 data points were acquired using the TPPI method for phase-sensitive acquisition, zero-filled and subjected to apodization with a Gaussian function prior to both Fourier transforms to afford a 1024 x 1024 point data matriz. The number of transients per t_1 increment was 64. Spectral widths were 5071 Hz in ^1H. The ^1H 90° pulse width was 15.0 μs. The ^{13}C 90° pulse width was 12.6 μs. A 0.9 s interpulse delay was employed.

Biological material. Our voucher collection (Nº 89084) was identified by C. Diaz, UCSC Institute of Marine Sciencies. *Asteropus sarasinosum*, Family Ancorinidae, Order Astrophorida.[20] Massive subspherical, barrel-shaped sponge with a cloacal opening on the top where numerous oscules open. Surface with oval to roundly shaped pores (3-4 mm wide of variable depth) and rounded tubercules (< 1 cm) regularly distributed all over the surface. Sponge just compressible.

Color in life, brown redish externally and tan internally. Spicules: Large oxeas (500-800 x 8-10 μm) and microxeas centrotylote (20-60 x 2 μm). In the ectosome the megascleres are disposed tangentially forming a

clear thick layer (2-3 mm). The choanosomal skeleton is formed by megascleres arranged disorderly or in radiate bundles (100-300 μm in thickness). We must indicate that this specimen lacked asterose microscleres which characterize both the genus and the family. Considering that in all other respects the specimen is very similar to *A. sarasinosum* (Thieli, 1899) described by Bergquist 1965, we consider that the asterose microscleres might have been lost in the small voucher specimen available for identification.

Extraction and Isolation. The sponge (2.6 Kg) was collected in the Solomon Islands in July 1989 and was immediately extracted with MeOH. The MeOH extract was defatted with n-hexano (2 x 200 ml), CCl_4 (200 ml) and CH_2Cl_2 (2 x 200 ml) and partitioned between n-BuOH and H_2O. The n-butanol extract (5.8 g) was concentrated, loaded into a column of Amberlite XAD-2, and eluted with water (3 bed volumes) and methanol (2 bed volumes). The methanol eluates were dried under reduced pressure at room temperature to give (2.5 g) of a glassy material which was separated into five fractions on Sephadex LH-20 using 2:1 methanol-water as eluant. The second fraction (1.2 g) was subjected to ascending DCCC with 7:13:8 $CHCl_3$-MeOH-H_2O (flow rate 12 mL/h). Finally the mixture was purified by reversed phase HPLC with 67:33 MeOH-H_2O on a C_{18} μ-Bondapack column (300 x 7,8 mm i.d), affording 180 mg of compound **1**, 90 mg of compound **2**, 15 mg of compound **3**, 12 mg of compound **4** and 10 mg of compound **5** (retention times 78, 20, 31, 34 and 47 minutes respectively).

Sarasinoside D (2) ($C_{62}H_{102}N_2O_{26}$); m.p. 207-211°C. ^1H NMR (δ_H, C_5D_5N): Aglycon protons: see Table 2. Anomeric protons: 4.75 (d, 1H, J= 8.0 Hz, H-1'); 5.24 (d, 1H, J= 7.6 Hz, H-1''''); 5.27 (d, 1H, J= 8.6 Hz, H-1'''''); 5.50 (d, 1H, J= 7.7 Hz, H-1'''); 5.62 (d, 1H, J= 8.4 Hz, H-1''). FAB-MS (negative ion; m/z, %): 1289 ([M-H]$^-$, 100); 1127 ([M-H-glucose]$^-$, 10); 1086 ([M-H-NAc-galactosamine]$^-$, 18); 995 ([M-H-glucose-xylose]$^-$, 8); 774 ([M-H-glucose-xylose-NAc-glucosamine-H_2O]$^-$, 8); 589 ([M-H-glucose-xylose-NAc-glucosamine-NAc-galactosamine]$^-$, 5).

Sarasinoside E (3) ($C_{61}H_{98}N_2O_{27}$); m.p. 193-197°C. ^1H NMR (δ_H, C_5D_5N): Aglycon protons: see Table 2. Anomeric protons: 4.79 (d, 1H, J= 7.5 Hz, H-1'); 5.24 (d, 1H, J= 7.5 Hz, H-1''''); 5.32 (d, 1H, J= 8.3 Hz, H-1'''''); 5.50 (d, 1H, J= 7.3 Hz, H-1'''); 5.63 (d, 1H, J= 7.6 Hz, H-1''). FAB-MS (negative ion; m/z, %): 1289 ([M-H]$^-$, 100); 1128 ([M-H-glucose]$^-$, 11); 1087 ([M-H-NAc-galactosamine]$^-$, 12); 996 ([M-H-glucose-xylose]$^-$, 7); 793 ([M-H-Glucose-xylose-NAc-glucosamine]$^-$, 5); 571 ([M-H-glucose-xylose-NAc-glucosamine-NAc-galactosamine-H_2O]$^-$, 42).

Sarasinoside F (4) ($C_{62}H_{100}N_2O_{27}$); m.p.192-195°C. ^1H NMR (δ_H, C_5D_5N): Aglycon protons: see Table 2. Anomeric protons: 4.45 (d, 1H, J= 7.6 Hz, H-1'); 5.28 (d, 1H, J= 7.3 Hz, H-1''''); 5.32 (d, 1H, J= 8.0 Hz, H-1'''''); 5.56 (d, 1H, J= 7.4 Hz, H-1'''); 5.65 (d, 1H, J= 7.8 Hz, H-1''). FAB-MS (negative ion; m/z, %): 1303 ([M-H]$^-$, 100); 1272 ([M-H-31]$^-$, 19); 1141 ([M-H-Glucose]$^-$, 14); 1101 ([M-H-NAc-galactosamine]$^-$, 17); 1010 ([M-H-glucose-xylose]$^-$, 11); 806 ([M-H-glucose-xylose-NAc-glucosamine]$^-$, 13); 603 ([M-H-glucose-xylose-NAc-glucosamine-NAc-galactosamine]$^-$, 12); 471 ([M-H-glucose-xylose-NAc-glucosamine-NAc-galactosamine-xylose]$^-$, 61).

Sarasinoside G (5) ($C_{62}H_{100}N_2O_{25}$); m.p. 203-206°C. ^1H NMR (δ_H, CD_3OD): Aglycon protons: see Table 2. Anomeric protons: 4.32 (d, 1H, J= 7.7 Hz, H-1'); 4.51 (d, 1H, J= 8.5 Hz, H-1''''); 4.62 (d, 1H, J= 7.6 Hz, H-1'''''); 4.84 (d, 1H, J= 8.4 Hz, H-1'''); 5.03 (d, 1H, J= 7.6 Hz, H-1''). FAB-MS (positive ion; m/z, %): 1295 ([M+Na]$^+$, 100); 1133 ([M+Na-glucose]$^+$, 10); 1092 ([M+Na-NAc-galactosamine]$^+$, 8); 1001 ([M+Na-glucose-xylose]$^+$, 12); 798 ([M+Na-glucose-xylose-NAc-glucosamine]$^+$, 8); 577 ([M+Na-glucose-

xylose-NAc-glucosamine-NAc-galactosamine-H_2O]$^+$, 3); 463 ([M+Na-glucose-xylose-NAc-glucosamine-NAc-galactosamine-xylose]$^+$, 48).

Hydrolysis of sarasinoside D (2)

Preparation of 2a and 2b. Sarasinoside **D** (**2**, 30 mg) was heated under reflux in 0.5N HCl (6 ml) and toluene (6 ml) for 2h. n-butanol was added to the cooled reaction mixture to facilitate the separation of the emulsion. The n-butanol/toluene phase was concentrated and the concentrate subjected to HPLC with 90:10 MeOH-H_2O as eluant on a C_{18} Nucleosil column (reversed phase), affording compounds **2a** and **2b**. The aqueous phase was dissolved in H_2O (10 ml), $NaBH_4$ (20 mg) was added, and the mixture was stirred at room temperature for 2 h. After addition of AcOH to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and codistilled with MeOH (2 x 5 mL), and the resulting alditols were acetylated by refluxing overnight with 1:1 Ac_2O-pyridine (10 mL). The solution was washed with H_2O and extracted with CH_2Cl_2, and the alditol acetates were then identified as those of xylose, glucose, NAc-glucosamine and NAc-galactosamine (ratio 2:1:1:1)) by GC-MS comparison with authentic samples.

Compound 2a ($C_{35}H_{58}O_7$). ^{1}H NMR (δH, $CDCl_3$): 0.78 (3H, s, Me-18), 0.85 (3H, s, Me-30),1.01 (6H, s, Me-21, Me-29), 1.03 (6H, s, Me-19, Me-28), 1.89 (3H, s, Me-27), 2.15 (3H, s, Me-26), 1.2 (1H, m, H-9), 1.32 (1H, H-1a), 1.48 (1H, H-11a), 1.64 (1H, H-2a), 1.92 (1H, H-2e), 2.11 (1H, H-11e), 2.19 (1H, H-20), 2.45 (1H,H-22), 3.21 (1H, dd, 4.3, 11.7, H-3α), 3.39 (1H, dd, 7, 12, H-5'), 3.52 (1H, m, H-2'), 3.63 (1H, m, H-3'), 3.76 (1H, m, H-4'), 4.09 (1H, dd, 4, 12, H-5'), 4.19 (1H, d, 10.4, H-12β), 4.51 (1H, d, 5.3, H-1'), 6.04 (1H, bs, H-24). FAB-MS (positive ion; m/z, %) 611 ([M+K]$^+$, 100); 479 ([M+K-xylose]$^+$, 18). LREIMS, m/z (relative intensity) 572 (M$^+$ 2); 475 (3); 419 (65); 440 (3); 263 (100); 191 (37); 142 (47); 83 (52). High-resolution EIMS: 440.3688 ($C_{30}H_{48}O_2$, Δ 3.4 from the calcd.); 422.6952 ($C_{30}H_{46}O$, Δ 0.8 from the calcd.); 191.3375 ($C_{14}H_{23}$, Δ 1.8 from the calcd.).

Compound 2b ($C_{30}H_{50}O_3$). ^{1}H NMR (δH, $CDCl_3$): 0.78 (3H, s, Me-18), 0.82 (3H, s, Me-30),1.01 (6H, s, Me-21, Me-29), 1.03 (6H, s, Me-19, Me-28), 1.88 (3H, s, Me-27), 2.15 (3H, s, Me-26), 4.19 (1H, d, 10.4, H-12), 6.04 (1H, s, H-24). LREIMS, m/z (relative intensity) 440 (M$^+$ 3); 422 (5); 356 (2); 342 (4); 287 (100); 97 (4); 83 (42).

Methylation of sarasinoside D (2)

A solution of sarasinoside **D** (45 mg) in dimethyl sulphoxide (DMSO) (2.5 ml) was stirred with a dimsyl carbanion under an Ar atmosphere at 25°C for 1 h. The mixture was treated with CH_3I (3 ml) under ice-cooling and stirred at room temperature for a further 1.5 h in the dark. The reaction mixture was then poured into ice-water and extracted with $CHCl_3$ (3 x 10 ml). The extract was washed with water, dried over $NaSO_4$ and then evaporated under reduced pressure to give an oily residue (50 mg), which was purified by preparative TLC (with 10% MeOH/$CHCl_3$ as eluant) to obtain 40 mg of permethylated compound **2**. $[\alpha]_D^{20}$ = -32.5 ($CHCl_3$).

Methanolysis of permethylated sarasinoside D (2)

A solution of permethylated compound **2** (20 mg) in anhydrous HCl/MeOH (4 ml) was stirred at 70 °C for 3 h. After cooling, the MeOH was evaporated under reduced pressure, the oily residue diluted with 6 ml of water, and the resulting mixture neutralized with 5% NaOH. This aqueous solution was extracted with $CHCl_3$

(3 x 15 ml) to yield 14 mg of oily residue, which was subjected to normal phase HPLC with 99:1 MeOH-$CHCl_3$ on a μ-Porasil column (300 x 7,8 mm i.d.), affording methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 1,3,4-tri-*O*-methylxylopyranoside, methyl 1,3,4-tri-*O*-methyl-N-acetyl-2-amino-glucopyranoside methyl 1,3-di-*O*-methylxylopyranoside and methyl 3,4,6-tri-*O*-methyl-N-acetyl-methyl-2-amino-galactopyranoside, identified by ^1H NMR spectroscopy and LREIMS.

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