Tetrahedron Vol. 47, No. 26, pp. 4753-4762, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00
© 1991 Pergamon Press plc

HOLOTHURINOSIDES: NEW ANTITUMOUR NON SULPHATED TRITERPENOID GLYCOSIDES FROM THE SEA CUCUMBER *HOLOTHURIA FORSKALII.*

JAIME RODRIGUEZ, RITA CASTRO and RICARDO RIGUERA*

Departamento de Química Orgánica, Facultad de Química, Universidad de Santiago de Compostela, Spain.

(*Received in UK* 7 *March* 1991)

ABSTRACT

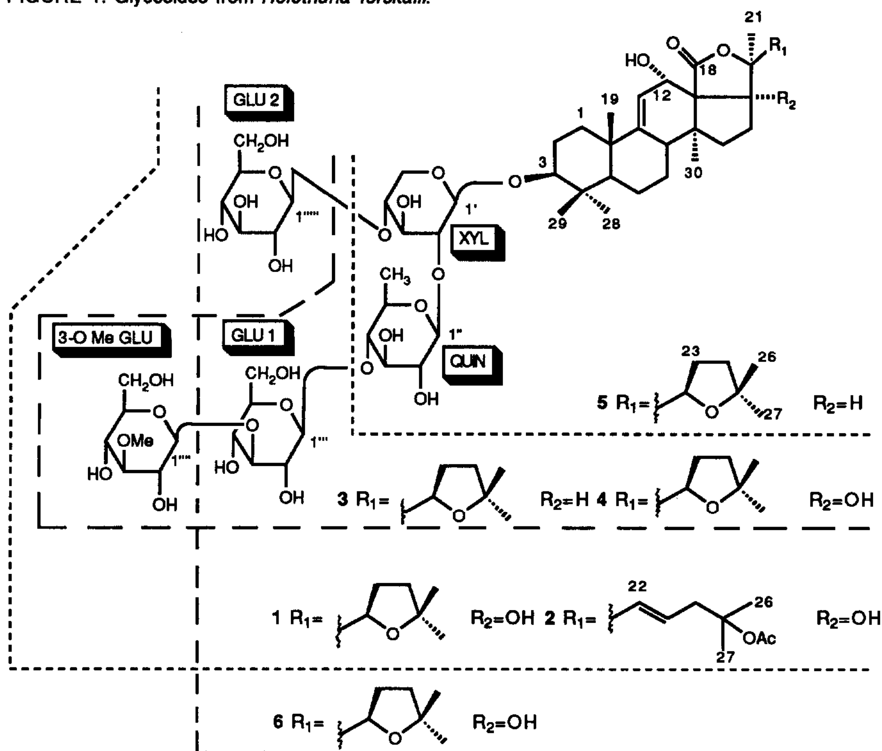
Five triterpene glycosides (holothurinosides A, B, C and D; **1**, **2**, **3** and **5** respectively) and desholothurin A (**4**) have been isolated from the sea cucumber *Holothuria forskalii*. Compounds **1** and **2** are the first non-sulphated pentasaccharide saponins isolated from marine echinoderms. **3** and **5** are di and tetrasaccharides, and **4** is a tetrasaccharide that has been described as a synthetic intermediate. The structures were established by FAB-MS, COSY, NOESY and long range and direct XHCORR experiments, and selective hydrolysis. **1-5** and the related saponin **6** have antitumour and antiviral activity, **1** and **4** being the most active with IC_{50} values of 0.46 and 0.38 mg/ml respectively for P388 cells.

INTRODUCTION

In the search for new pharmacologically active substances from marine organisms, attention has been paid to echinoderms, and among them, to sea cucumbers (class Holothuridae). These invertebrates have been shown to contain a variety of triterpene glycosides of lanosterol type with a distinctive γ-lactone skeleton named holostane, and a sugar chain composed of up to six monosaccharide units (principally D-xylose, D-glucose, D-quinovose, D-3-O-methylglucose and D-3-O-methylxylose). In addition, sulphate groups can be found at certain positions of the aglycone and sugars [1]. The structures of these compounds have usually been determined by selective hydrolysis, chemical transformations, FAB-MS and extensive ^{1}H and ^{13}C nmr characterization of the intact saponin. Due to the complexity of the spectra, direct comparison of the nmr data of the glycoside with those obtained for related terpenes and monosaccharides has been the principal basis for nmr signal assignment, which explains the erroneous identification of some signals in the aglycone. As for the sugar chain, NT_1 data and NOESY experiments have been successfully applied to deduce, in the former case, the sequence of sugars and the interglycosidic linkages [2], and in the second case to obtain general information about the relative position of certain protons [3]; however, the use of the latter methodology is limited by the complexity of the ^{1}H nmr spectra in the 3-5 ppm region, which usually precludes the measurement of nOe for a large number of protons.

In the work described here we used COSY, NOESY and direct and long-range ^{13}C-^{1}H correlation (XHCORR) nmr spectroscopy for complete signal assignment and structural analysis of the non-sulphated pentasaccharide triterpene saponins **1-5**, which were isolated from the polar extracts of *Holothuria forskalii*. Antitumour and antiviral activity of **1-5** is also described.

FIGURE 1. Glycosides from *Holothuria forskalii.*



RESULTS AND DISCUSSION

Glycosides **1-5** were isolated from the separate methanolic-aqueous extracts of the body walls and Cuverian tubules of fresh sea cucumbers collected by divers on the Galician coast (NW Spain). The extracts were purified by recovery of the polar material on a column of Amberlite XAD-2 and subsequent chromatography of the methanol eluates on a Sephadex-LH20 column. Final isolation was accomplished by DCCC and reverse phase HPLC.

Holothurinoside A (1) is a glassy material (m.p. 232-233°C; $[\alpha_D]$=-0.9 (MeOH), c=0.0135) which upon treatment with 10% HCl gave D-quinovose, D-xylose, 3-O-methyl-D-glucose and D-glucose in the ratios 1:1:1:2 together with a mixture of triterpene aglycones. The monosaccharides were identified by GC-MS in the form of the peracetates of the corresponding alditols.

The FAB mass spectrum of 1 (positive-ion mode) showed molecular species at m/z 1303 $[(M+Na)]^+$ and 1325 $[(M-H+2Na)]^+$, where M is the molecular weight of 1280 corresponding to the molecular formula $C_{60}H_{96}O_{29}$, and fragments indicative of the cleavage of the sugar chain at m/z 801 $[($pentasaccharide portion$+Na)]^+$, 639 $[(801$-glucose$)]^+$, 625 $[(801$-3*O*-Me-glucose$)]^+$ and 477 $[(801$-glucose-3*O*-Me-glucose$)]^+$, showing that glucose and 3-O-Me-glucose must be terminal. In addition, the fragments corresponding to the loss of sugars from the $[(M+Na)]^+$ peak were also observed at m/z 1141 $[(M+Na$-glucose$)]^+$, 1127 $[(M+Na$-3*O*-Me-glucose$)]^+$, 965 $[(M+Na$-3*O*-Me-glucose-glucose$)]^+$, 803 $[(M+Na$-3*O*-Me-glucose-2glucose$)]^+$ and 657 $[(M+Na$-3*O*-Me-glucose-2glucose-quinovose$)]^+$.

TABLE 1. ^1H and ^{13}C nmr chemical shifts of holothurinoside A (1) in C_5D_5N (298 K).

CARBON	δ_C	DEPT	δ_H J (Hz)	CARBON	δ_C	DEPT	δ_H J (Hz)
1	36.2	CH_2		1'	103.5	CH	4.68 d 7.1
2	26.8	CH_2	1.89, 2.06 m	2'	83.3	CH	3.89 m
3	88.6	CH	3.12 dd 13.4, 3.8	3'	75.7	CH	4.16 m
4	39.8	C	-	4'	77.9	CH	3.93 m
5	52.6	CH	0.90, m	5'	64.1	CH_2	4.35 3.59 m
6	21.1	CH_2	1.61, 0.93 m	1''	105.5	CH	5.12 d 7.3
7	28.2	CH_2	1.50, 1.71 m	2''	75.8	CH	3.95 m
8	40.7	CH	3.34 dd 12.8, 4.0	3''	76.3	CH	4.20 m
9	153.6	C	-	4''	87.2	CH	3.56 m
10	39.5	C	-	5''	71.7	CH	3.69 m
11	115.5	CH	5.63 d 4.8	6''	18.0	CH_3	1.71 d 6.0
12	71.6	CH	4.96 d 4.8	1'''	104.9	CH	4.94 d 7.8
13	58.7	C	-	2'''	73.7	CH	3.98 m
14	45.7	C	-	3'''	87.8	CH	3.63 m
15	36.7	CH_2	-	4'''	69.8	CH	3.92 m
16	35.3	CH_2	-	5'''	77.3	CH	3.91 m
17	89.7	C	-	6'''	62.1	CH_2	4.38 m
18	174.5	C	-	1''''	105.6	CH	5.26 d 7.7
19	22.3	CH_3	1.38 s	2''''	74.9	CH	3.96 m
20	86.5	C	-	3''''	87.7	CH	3.93 m
21	18.7	CH_3	1.74 s	4''''	70.5	CH	4.02 m
22	80.5	CH	4.18 t	5''''	78.3	CH	4.17 m
23	28.0	CH_2	1.95 m	6''''	62.5	CH_2	4.40 m
24	38.3	CH_2	1.67 m	OMe	60.7	CH_3	3.81 s
25	81.3	C	-	1'''''	105.3	CH	5.01 d 7.8
26	28.6	CH_3	1.19 s	2'''''	74.3	CH	3.95 m
27	27.9	CH_3	1.26 s	3'''''	78.7	CH	3.92 m
28	27.3	CH_3	1.18 s	4'''''	71.7	CH	4.14 m
29	16.5	CH_3	1.04 s	5'''''	78.1	CH	4.15 m
30	20.2	CH_3	1.67 s	6'''''	62.2	CH_2	4.37 m

These data lead directly to the sugar sequence indicated in the Figure 1. The peak at m/z 501 corresponds to the aglycone part of the molecule.

The presence of five sugar units was confirmed by the ^{1}H nmr spectrum (Table I), where peaks for the five β-anomeric protons appeared as doublets (J = 7-8 Hz) in the 4.6-5.3 ppm region. Other significant signals were the singlet at 3.83 ppm ascribable to the 3-O-Me-glucose unit, and the doublet at 1.71 (J=6Hz) due to the methyl group of quinovose. The aglycone contributes seven singlets in the 0.9-1.9 ppm region that are due to the methyl groups of a triterpene skeleton, one olefinic proton at 5.63 ppm, and a doublet at 4.96 ppm due to a proton that is geminal to a hydroxyl group. The rest of the spectrum is too complex to be reliably interpreted without further nmr experiments.

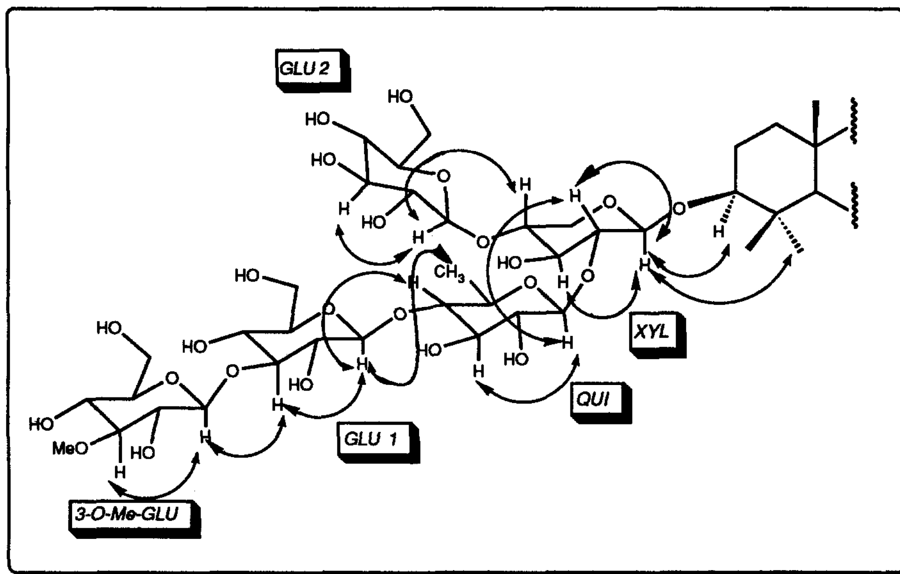
The ^{13}C nmr spectrum recorded in C_5D_5N showed peaks for 60 carbons. Among them, five signals in the 106-103 ppm region evidently corresponded to the anomeric carbons. As for the aglycone, a carbonyl group at 174.5 confirms the presence of a γ-lactone ring (I.R.=1750 cm^{-1}), and signals at δ_C 115.5 and 153.6 indicate a $\Delta^{9(11)}$ double bond with a hydroxyl group at the allylic position. The quaternary carbons at δ_C 86.5 and 81.3 suggest that the lateral chain is in furane form, and the signal at 89.7 reflects a hydroxyl group at C17. On the basis of these data we conclude that the aglycone of **1** is 22,25-epoxy-holost-9-(11)-ene-12α,17α-diol. This aglycone was reported by Kitagawa as that of holothurins A and B [4-7], although certain discrepancies in nmr assignment have to be explained (*vide infra*).

To check the sequence of sugars indicated by FAB-MS and determine the points of interglycosidic attachment, we used a combination of COSY, NOESY and direct and long range ^{13}C-^{1}H (XHCORR) correlation spectroscopy.

These experiments showed that xylose is bound both to C3 of the aglycone and to C2' of quinovose since there was correlation between C2' (δ_C 83.3) and the proton at 3.89 identified as H2' on account of its coupling with the anomeric xylose proton (δ_H 4.68, δ_C 103.5). Furthermore, that C4' of xylose is bound to the anomeric carbon of glucose 2 via C4 may be deduced from the observed high-field shift (Δ= 2 ppm) of C5' (δ_C 64.1) with respect to the free sugar. The ^{1}H-^{1}H and ^{13}C-^{1}H correlations of the C1'-C4' fragment of quinovose show that this sugar is bound via C4' to glucose 2. Finally, the extensive use of NOESY on compound 1 confirmed the general arrangement of the molecule (Figure 2).

With respect to the triterpene part of the molecule, the direct and long-range ^{1}H-^{13}C correlation spectra corroborated the presence of the holostane skeleton but there werealso certain discrepancies with the published data for this structure, principally as regards the side chain R_1 and the methyl groups. The correct assignment for R_1 (Table I) are based the fact that the triplet for H22 at δ_H 4.18 correlates with the multiplet at 1.95 for the methylene protons H23, and that these correlate with the multiplet at 1.68 for the protons H24 (which are hidden under the methyl group of quinovose but are detected by NOESY); furthermore, the ^{13}C-^{1}H direct XHCORR shows that H22, H23 and H24 correlate with the carbon peaks at δ_C 80.5 ppm, 28.0 and 38.3 ppm respectively, so that published data for C23 and C24 should be interchanged. The exact location of the methyl groups was deduced from the following long range ^{13}C-^{1}H correlations: Me21/C20 (two bonds), Me21/C17, Me21/C22 (three bonds); Me19/C10 (two bonds), Me19/C9 (three bonds); Me30/C13 (three bonds); Me28/C4, Me29/C4 (two bonds) and Me27/C25 (two bonds).

FIGURE 2. ^1H-^1H NOESY of Holothurioside A (1).



On the basis of the above results, the structure of 1 is established as 3β-*O*-[β-D-3-O-methylglucopyranosyl (1→3) β-D-glucopyranosyl (1→4) β-D-quinovopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-glucopyranosyl]-22,25-epoxy-holost-9-(11)-ene-12α,17α-diol. It is the first example of a naturally occurring nonsulphated holothurin containing a five-sugar chain.

Compound **2**, named holothurinoside B, had a FAB-MS peak for $[M+Na]^+$ at m/z 1345, indicating a molecular weight of 1322 (molecular formula $C_{62}H_{98}O_{30}$) and a sugar chain identical to that of **1**. The nmr data show that the aglycone is also that of **1** except for the side chain, which in **2** is acyclic and has an acetate group and a *trans* double bond (J=16.0 Hz) whose positions (C25 and Δ^{22}) are implied by the fact that the proton and carbon nmr data show the chemical shifts that are to be expected for methyl and methylene groups at C21 and C24 respectively. Thus **2** is 3β-*O*-[β-D-3-O-methylglucopyranosyl (1→3) β-D-glucopyranosyl (1→4) β-D-quinovopyranosyl (1→2) β-D-xylopyranosyl (4→1) β-D-glucopyranosyl]-25-acetoxy-holost-9-(11)-22-diene-12α,17α-diol. The aglycone appears to have been found previously in *Actinopyga flammea* [8] though the exact position of the double bond.was not reported.

FAB-MS of compound **3** yields a molecular weight of 1112 (for a molecular formula $C_{41}H_{64}O_{12}$) and fragments indicative of a tetrasaccharide tree formed by a xylose-quinovose-glucose chain and 3-O-methyl-glucose, i. e. the same as for **1** and **2** except that the terminal glucose 2 is now absent (Fig. 1). The aglycone of **3** has nmr spectra like those of **1**, the main difference being the replacement of the quaternary oxygen-substituted (δ_C 89.7) by a methine (δ_C 47.6), in agreement with the chemical shifts for C13, C16 and C20.

Table 2. ^{13}C data of the aglycones of compounds **2-5** at 298 K in C_5D_5N.

CARBON	2	3	4	5
1	35.6	36.4	36.4	36.3
2	27.6	27.3	27.3	27.2
3	90.4	88.8	88.9	88.8
4	41.0	40.1	40.1	39.5
5	53.9	52.8	52.7	52.8
6	22.0	21.2	21.2	21.1
7	29.3	28.7	28.1	28.0
8	40.7	40.8	39.6	40.0
9	154.3	153.3	153.9	153.3
10	40.5	40.1	39.7	39.5
11	115.6	116.1	115.6	116.1
12	71.2	71.5	71.2	68.1
13	65.6	63.7	58.8	57.3
14	46.7	46.2	45.9	46.2
15	37.3	23.6	36.8	30.0
16	35.2	38.4	38.6	37.3
17	87.6	47.6	89.7	47.6
18	178.3	177.5	174.6	177.6
19	22.7	22.0	22.5	22.3
20	90.4	83.6	86.6	86.3
21	21.4	18.7	18.0	18.6
22	140.1	80.2	80.6	81.2
23	136.0	28.7	28.8	29.0
24	28.3	36.7	36.5	36.2
25	82.1	81.2	81.3	81.6
26	24.9	28.7	28.0	28.5
27	24.7	28.0	28.1	27.7
28	17.1	27.2	27.2	27.3
29	28.6	16.7	16.7	16.6
30	23.5	20.1	21.2	20.7
	$CO\underline{C}H_3$ 22.8			
	$\underline{C}OCH_3$ 172.0			

Compound **4** has the aglycone of **1** and the tetrasaccharide chain of **3**. Compound **5** is **3** without its terminal 3-O-methylglucose and glucose units.

Complementary data confirming the structures of glycosides **1**-**5** were obtained by controlled hydrolysis of the terminal sugar units of **1**. Treatment with 0.1 N HCl in methanol at r.t. for 2 h gave a mixture of two tetrasaccharides which were separated by hplc and whose spectroscopic data showed them to be **4** and **6**.

ANTITUMOUR ACTIVITY

The holothurinosides A (**1**), C (**3**), D(**5**), desholothurin A (**4**), the related glycoside **6** and the aglycones [6] were tested for their antitumour activity against P388, A549, HeLa and B-16 cells *in vitro*. The IC_{50} values in the antitumour assays of compounds **1**, **3**, **5** and **6** are listed in Table **4**. These compounds also showed some antiviral activity (20% inhibition of VSV cells in BHK at dosage of 20 μg/ml).

TABLE 3. ^{13}C data of the sugar moieties of holothurinosides A, B and C in C_5D_5N.

POSITION	1	2	3
1'	103.5	104.9	105.6
2'	82.9	84.2	83.6
3'	76.3	77.9	77.8
4'	78.1	70.8	70.8
5'	64.2	66.7	66.6
1"	105.3	105.6	106.1
2"	75.4	76.4	76.8
3"	76.0	75.9	77.5
4"	86.9	87.4	77.0
5"	71.7	71.6	73.4
6"	18.1	18.2	18.5
1'''	104.8	105.6	
2'''	74.7	73.7	
3'''	87.6	88.0	
4'''	69.9	70.5	
5'''	77.7	78.1	
6'''	62.5	62.1	
1''''	105.8	105.6	
2''''	75.4	75.0	
3''''	87.6	88.0	
4''''	69.4	69.8	
5''''	78.1	78.2	
6''''	62.5	62.1	
OMe	61.0	60.7	
1'''''	105.3		
2'''''	74.5		
3'''''	78.7		
4'''''	71.7		
5'''''	77.9		
6'''''	62.5		

Table 4. IC_{50} values againnst tumour cell growth *in vitro* (μg/ml)

	1	3	5	6
P388	0.46	0.34	2.00	2.00
A549	0.33	0.16	2.50	5.00
HeLa	0.86	0.47		
B-16	0.71	0.93		

EXPERIMENTAL PART

General

All melting points were determined in a Kofler-Thermogenerăte apparatus. ^1H and ^{13}C nmr spectra were recorded on a Bruker WM-250 spectrometer at 250 and 60.13 MHz. ^1H, ^{13}C and DEPT nmr spectra were obtained in C_5D_5N and CD_3OD at 298K. HPLC was performed with 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. Conventional and FAB mass spectra were obtained on a Kratos MS50 mass spectrometer equipped with a Kratos FAB source. The FAB mass spectra were obtained by dissolving the samples in a 1:1 glycerol:thioglycerol matrix with NaCl as additive and bombarding with Xe atoms of 2-8 kV energy. GCMS analyses were carried out on a Carlo Erba MFC 500 HRGC/MS chromatograph using a 0.25 mm x 10 m SP-2330 capillary column and the following experimental conditions: column temp 240°, injection temp 50° for 1 min and 12°/min up to 240° maintained for 2 min.

Two-dimensional experiments

The two-dimensional ^1H-^1H COSY spectra (128x1K) of the holothurinosides 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 ^1H-^1H NOESY spectra of holothurinoside A (**1**) (256x1K) was obtained by accumulating 16 scans per t_1; the relaxation delay was 2s. The mixing time (t_m) was 0.2 s and the random variation of t_m was 10%. 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) spectra (256x1K) of **1**, **3** and **5** for directly bonded protons and carbons were obtained by accumulating 80 scans per t_1; the relaxation delay was 2s and the value of J_{CH} selected was 130 Hz. The long-range correlation XHCORR (256x2K) spectrum of holothurinoside A (**1**) was obtained by accumulating 94 scans per t_1; the relaxation delay was 2s and the value of J_{CH} selected was 9Hz. Both types of XHCORR data (long-range and direct correlation) were zero-filled to 512 in F_1 and subjected to Fourier transformation using Gaussian data manipulation in F_2 (LB_2=-4, GB_2=0.3).

Isolation of the glycosides of *Holothuria forskalii*

Body walls and Cuverian tubules of 19 specimens (approx 300 g each) were collected by diving off Villagarcía de Arosa (Pontevedra, Spain) in July 1989 and were immediately extracted with methanol. The

extracts were concentrated to dryness under reduced pressure. The dry extract of the body walls (79 g) was partitioned between water and hexane and the water layer further partitioned between water and n-butanol. The n-butanol extract (9.7 g) was concentrated and passed through a column of Amberlite XAD-2 which was then washed with water (3 bed volumes) and methanol (2 bed volumes). The methanol eluates were dried under reduced pressure at room temperature to give 2.2 g of a glassy material which was chromatographed on Sephadex LH-20 using methanol-water (2:1) as the eluent. The first fractions contained 0.93 g of glycosides, which was rechromatographed into six fractions by DCCC (ascending mode, 7:13:8 $CHCl_3$:MeOH:H_2O). Fractions 2 and 3 were purified with 7:3 methanol-water on a 30 cm x 19 mm i.d. C_{18} μ-Bondapack column, affording 40 mg of holothurinoside A (1), 9 mg of holothurinoside B (2), 15 mg of holothurinoside C (3) , 10 mg of holothurinoside D (5) and 20 mg of des-holothurin A (4).

Holothurinoside B (2) ($C_{62}H_{98}O_{30}$); M.p. 230-232°C. Nmr ^1H (δ_H, C_5D_5N): 1.06 (s, 3H, Me-29); 1.22 (s, 3H, Me-28); 1.35 (s, 3H, Me-19); 1.49 (s, 3H, Me-26); 1.50 (s, 3H, Me-27); 1.70 (s, 3H, Me30); 1.71 (d, 3H, J=5.5 Hz, H-6"); 1.96 (s, 3H Me-21); 2.08 (s, 3H, OAc); 2.79 (dd, 1H, J=13.0 and 6.0 Hz, H-24_a); 2.81 (dd, 1H, J=13.0 and 6.0 Hz, H-24_b); 3.12 (dd, 1H, J=13.1 and 4.0 Hz, H-3); 3.32 (dd, 1H, J=12.6 and 3.9 Hz, H-8); 3.83 (s, 3H, OMe); 4.67 (d, 1H, J=7.1 Hz, H-1'); 4.93 (d, 1H, J=7.6 Hz, H-1'"); 4.99 (d, 1H, J=7.8 Hz, H-1'""); 5.27 (d, 1H, J=7.7 Hz, H-1""); 5.66 (bd, 1H, H-11); 5.95 (dd, 1H, J=15.0 and 6.0 Hz, H-23); 6.02 (d, 1H, J=15.0 Hz, H-23). FAB-MS (positive ion mode; m/z, %): 1367, ([M-H+2Na]$^+$, 15); 1345, ([M+Na]$^+$, 100); 801 ([pentasaccharide portion+Na]$^+$, 20); 639 ([801-glucose]$^+$, 23); 625 ([801-3*O*-Me-glucose]$^+$, 18); 477 ([801-glucose-3*O*-Me-glucose]$^+$, 17).

Holothurinoside C (3) ($C_{54}H_{86}O_{23}$); m.p. 223-225°C. Nmr ^1H (δ_H, C_5D_5N): 1.14 (s, 3H, Me-29); 1.19 (s, 3H, Me-26); 1.24 (s, 3H, Me-28); 1.25 (s, 3H, Me-27); 1.28 (s, 3H, Me-30); 1.41 (s, 3H, Me19); 1.60 (s, 3H, Me-21); 1.73 (d, 3H, J= 6.0 Hz, H-6"); 3.12 (dd, 1H, J=11.1 and 3.6 Hz, H-3); 3.83 (s, 3H, OMe); 4.77 (d, 1H, J=7.2 Hz, H-1'); 4.94 (d, 1H, J=7.8 Hz, H-1'"); 4.97 (d, 1H, J= 5 Hz, H-12); 5.27 (d, 1H, J=7.8 Hz, H-1""); 5.64 (d, 1H, J=5.0 Hz, H-11). FAB-MS (positive ion mode; m/z, %): 1125, ([M+Na]$^+$, 100), 639 ([801-glucose]$^+$, 23); 625 ([801-3*O*-Me-glucose]$^+$, 18); 477 ([801-glucose-3*O*-Me-glucose]$^+$, 17).

Holothurinoside D (5) ($C_{41}H_{64}O_{13}$); m.p. 219-221°C. Nmr ^1H (δ_H, C_5D_5N): 0.80 (m, 1H, H-5); 1.146(s, 3H, Me-29); 1.18 (s, 3H, Me-26); 1.22 (s, 3H, Me-28); 1.26 (s, 3H, Me-27); 1.31 (s, 3H, Me-30); 1.40 (s, 3H, Me-19); 1.58 (s, 3H, Me-21); 1.64 (d, 3H, J= 5.5 Hz, H-6"); 2.50 (t, J= 7.3 Hz, H-17); 3.21 (dd, 1H, J=11.1 and 3.2 Hz, H-3); 4.54 (d, 1H, J=5.0 Hz, H-12); 4.78 (d, 1H, J=7.1 Hz, H-1'); 4.96 (d, 1H, J= 7.4 Hz, H-1"); 5.73 (dd, 1H, J=5.0 and 1.0 Hz, H-11). FAB-MS (positive ion mode; m/z, %): 787, ([M+Na]$^+$, 100).

Compound 6. FAB-MS (positive ion mode; m/z, %): 1127, ([M+Na]$^+$, 100).

Acid hydrolysis of 1

To a solution of holothurinoside A (1) (20 mg in 10 ml of MeOH) was added 1 ml of 0.1N HCl, and the mixture was refluxed for 2 hr. The solution was neutralized with Ag_2CO_3 and filtered, and the mixture of aglycones was extracted with *n*-BuOH (3x20 ml), concentrated under reduced pressure and subjected to hplc on a 30 cm x 7.8 mm i. d. μ-Bondapack C-18 column with 7:3 methanol:H_2O as eluent, affording compounds **4** and **6**.

Hydrolysis of holothurinosides

Treatment of 2 mg of each holothurinoside with 2N HCl gave sugars and a mixture of triterpenes. The sugar fraction was dissolved in H_2O (10 ml), $NaBH_4$ (20 mg) was added, and the mixture was stirred at r.t. for 2 hr. After addition of AcOH to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and co-distilled with MeOH (2x5 ml), and the resulting alditols were acetylated with 1:1 Ac_2O-pyridine (10 ml) by refluxing overnight. The soln. was washed with H_2O and extracted with Cl_2CH_2, and the alditol acetates were then identified by GC-MS.

Antitumour activity

Antitumour activity was determined in the cell lines P-388 (lymphoid neoplasm from DBA/2 mice), A-549 (human lung carcinoma), HeLa (epitheloid carcinoma from human cervix) and B-16-F1 (mouse melamona). Antiviral activity against HSV-1 (herpes simplex virus type 1) and VSV (vesicular stomatitis virus) was assayed in the cell lines CV-1 (mouse kidney) and BHK (baby hamster kidney). The assays were carried out by Dr. Dolores García Gravalos of Pharmamar.

ACKNOWLEDGEMENTS

This work was supported by the Plan Nacional de Investigación (FAR 88-0512). J. R. and R. C. acknowledge fellowships from the Xunta de Galicia. We are also grateful to Pharmamar (Madrid)

REFERENCES

1. For a general review see V. A. Stonik and G. B. Elyakov. Bioorganic Marine Chemistry, 1988. Springer-Verlag, Berlin, Heidelberg, Germany.
2. T. Miyamoto, K. Togawa, R. Miguchi, T. Komori, and S. Takamura. *Liebigs Ann. Chem.* 1990 453.
3. M. Girard, J. Bélanger, J. W. Apsimon, F. X. Garneau, C. Harvey and J. R. Brisson *Can. J. Chem.* 1990, **68**, 11.
4. I. Kitagawa, T. Nishino, T. Matsuno, H. Akutsu and Y. Kyogoku *Tetrahedron Lett.* 1978, 985.
5. I. Kitagawa, T. Nishino, M. Kobayashi, T. Matsuno, H. Akutsu and K. Yoshimasa *Chem. Pharm. Bull.* 1981, **29**, 1942.
6. I. Kitagawa, T. Nishino and Y. Kyogoku *Tetrahedron Lett.* 1979, 1419.
7. I. Kitagawa, T. Nishino, M. Kobayashi and Y. Kyogoku *Chem. Pharm. Bull.* 1981, **29**, 1951.
8. S. Bhatnager, B. Dudonet, A. Ahond, C. Poupat, O. Thoison, A. Clastres, D. Laurent and P. Potier, *Bull. Soc. Chim. Fr.* , 1985, 124.