Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 2987–2992
Tetrahedron

Thyonosides A and B, two new saponins isolated from the holothurian *Thyone aurea*

Isabelle Bonnard* and Kenneth L. Rinehart

Roger Adam Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Received 15 December 2003; revised 30 January 2004; accepted 3 February 2004

Abstract—The structures of two saponins, thyonosides A and B, isolated from the holothurian *Thyone aurea* collected in Namibia, were elucidated by 1D and 2D NMR (^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{1}H J-resolved, TOCSY, HMQC, HMBC and NOESY). The two compounds have the same aglycon but different oligosaccharidic chains. Thyonoside A has a 3-*O*-methyl-β-D-xylopyranosyl-(1→3)-6-*O*-sodium sulphate-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulphate-β-D-xylopyranosyl chain, and thyonoside B a 3-*O*-methyl-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulphate-β-D-xylopyranosyl chain. The holostane-type aglycon features an endocyclic double bond at position 7–8, a double bond at position 25–26 and a β-acetoxy group at C16.

1. Introduction

More than 100 saponins found in holothurians have been described to date. Most of them present a sugar chain of up to six monosaccharide units [principally D-glucose (Glu), D-xylose (Xyl), D-fucose (Fuc), D-quinovose (Qui), D-3-*O*-methylglucose (3-*O*-Me-Glu), and D-3-*O*-methylxylose (3-*O*-Me-Xyl)] linked to C-3 of the aglycon, which is usually represented by a triterpene 18(20)-lactone with a lanostane skeleton (holostane). Another notable feature of many of the glycosides from marine organisms is the sulphatation of the aglycon or the sugar moiety. Traditionally, structural studies of saponins have involved hydrolytic removal of the sugars, which usually results in the decomposition of the aglycon to give complex mixtures including many artifacts. The development of high-resolution NMR techniques in the last two decades, and more specifically ^{13}C NMR spectroscopy, now permits the structural studies on intact saponins and avoids the need for large quantities of material. The advent of modern high-field two-dimensional (2D) NMR techniques provides an extremely useful tool for the characterization of complex molecules since these techniques often allow unambiguous assignment of most signals observed in conventional unidimensional ^{1}H and ^{13}C NMR spectra. This paper illustrates the use of these 2D-NMR experiments to determine the structures of the two saponins isolated from the holothurian *Thyone aurea*.

2. Results and discussion

Thyonosides A and B were isolated from an alcoholic extract of the sea cucumber *Thyone aurea* collected in Namibia in December 1995. The extract showed interesting activity against murine tumor cell line, L1210, and herpes simplex virus type 1, HSV-1. After subsequent chromatography, the final isolation was accomplished by reverse phase HPLC and Counter Current Chromatography (CCC). The structures were elucidated mainly by 500 MHz NMR analyses including 1D and 2D (^{1}H–^{1}H COSY, TOCSY, ^{1}H–^{1}H J-resolved, HMQC, HMBC and NOESY) spectroscopy.

2.1. Aglycon portion

The assignments for the NMR signals associated with the aglycon moiety (Table 1) show the same aglycon for both thyonosides A and B. Since the ^{13}C NMR spectra gave inadequate results, most of the ^{13}C assignment were established by HMQC, HMBC, and by comparison with those of other saponins (frondoside A,[1] frondogenin,[2] eximisoside A[3]…).

Signals at 145.5 and 119.9 ppm for thyonoside A (145.5 and 120.0 for thyonoside B) in the downfield region, are indicative of the presence of an endocyclic double bond at

Keywords: *Thyone aurea*; Holothurian; Triterpene saponin; Holostane aglycon.
* Corresponding author. Address: Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Universite de la Reunion, 15 Avenue Rene Cassin, BP 7151, Saint Denis de la Reunion 97715, France. Tel.: +262-262-93-81-98; fax: +262-262-93-81-83; e-mail address: ibonnard@univ-reunion.fr

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.02.004

Table 1. ^{13}C and ^{1}H NMR data and assignments for the aglycon moieties of thyonosides A (**1**) and B (**2**) in $C_5D_5N–D_2O$ 9:1

	^{13}C NMR in ppm			^{1}H NMR in ppm, mult. (*J* in Hz)	
C	(**1**)	(**2**)	H	(**1**)	(**2**)
1	35.5	35.6	1α;1β	1.33 m	1.40 m
2		26.4	2α;2β	1.83 m; 2.05 m	1.86 m; 2.07 m
3	88.5	89.0	3α	3.18 dd (3.7, 11.5)	3.22 dd (3.5, 11.7)
4	39.0	39.6			
5	47.3	48.0	5α	0.92 m	0.99 t (7.8)
6	22.7 or 20.8[a]	23.3	6α;6β	1.95 m	2.00 m
7	119.9	120.0	7	5.63 m	5.54 m
8	145.5	145.5			
9	46.5	47.0	9β	3.41 bd (14.5)	3.43 bd (14.4)
10		36.1			
11		22.6	11α;11β	1.47 m; 1.72 m	1.51 m; 1.76 m
12		31.6	12α;12β	2.07 m; 1.96 m	1.94 m; 2.15 m
13	59.0	59.3			
14	47.0	47.5			
15	43.0	43.2	15α	2.56 dd (7.3, 12.5)	2.60 dd (7.3, 12.0)
			15β	1.67 m	1.74 m
16	74.8	74.8	16α	5.88 ddd (7.3, 8.9, 9.0)	5.91 ddd (7.3, 8.7, 9.3)
17	54.2	54.5	17α	2.63 d (9.0)	2.61 d (9.3)
18	180.0	179.6			
19	23.6	24.1	19	1.12 s	1.19 s
20	85.0	85.0			
21	27.8	28.2	21	1.47 s	1.46 s
22	38.0	38.4	22; 22′	2.32 m; 1.82 m	2.36 m; 1.88 m
23			23; 23′	1.45 m; 1.35 m	1.50 m
24	37.8	38.4	24; 24′	1.93 m	1.96 m
25	145.6	145.8			
26	110.6	110.0	26; 26′	4.74 b	4.77 b
27	21.7	22.2	27	1.64 s	1.66 s
28	31.8	32.2	28	1.15 s	1.09 s
29	16.7	17.3	29	0.97 s	1.12 s
30	28.2	28.7	30	1.16 s	1.25 s
31	170.0	170.4	31		
32	22.7 or 20.8[a]	21.5	32	1.99 s	2.01 s

[a] Assignments may be reversed.

the 7, 8-position (9, 8-double bond feature chemical shifts around 140 and 130 ppm, whereas 9, 11-double bond resonate around 150 and 110 ppm). Signals at 145.6 and 110.6 ppm for thyonoside A (145.8 and 110.0 for thyonoside B) show an another double bond consistent with a terminal isoprenyl function.[4,5] Two low field resonances at 180.0 and 170.0 ppm for thyonoside A (179.6 and 170.4 ppm for thyonoside B) are assigned to the γ-lactone and acetoxy carbonyl carbons, respectively.

The high field region of the ^{1}H NMR spectrum shows a pattern characteristic of most holothurins, featuring many overlapping signals. In this instance, the use of the COSY experiment allowed the establishment of the connectivities within the framework and thus permitted the assignment of the chemical shifts for the overlapping proton signals. Assignments were confirmed by TOCSY and completed using 2D-NOESY experiments. Of particular interest, the presence of two double bonds in the aglycon was confirmed by: (1) the 1H multiplet at 5.63 ppm (5.54 ppm for thyonoside B), correlated to H-6 at 1.95 ppm (2.00 ppm for thyonoside B), which can be attributed to the H-7 vinylic proton, (2) the strong broad 2H singlet at 4.74 ppm (4.77 ppm for thyonoside B) correlated to H_3-27 and H-24, which can be attributed to *exo* H-26 methylenic proton. The presence of an acetoxy group at C-16 was deduced from the chemical shift of the H-16 signal which shows coupling signals with H-17, Hα-15 and Hβ-15 in the 2D-COSY spectrum.

The relative stereochemistry of the aglycon was deduced using 2D-NOESY experiments (mixing time of 250 ms) for

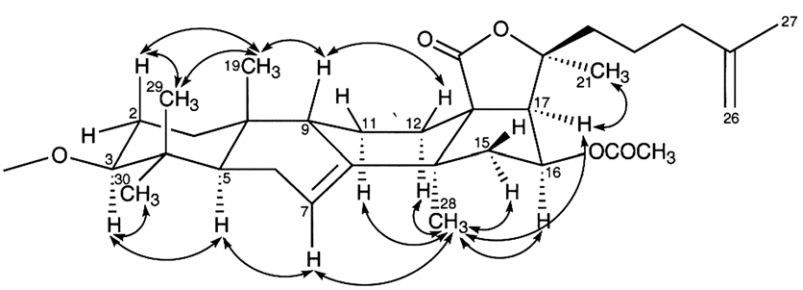


Figure 1. NOE correlations for the aglycon of thyonoside A (**1**).

Table 2. Experimental and calculated vicinal proton coupling constants for the D-ring protons of thyonosides A and B

	Thyonoside A	Thyonoside B	Frondoside A[a]		
H–H	$J_{exp.}$	$J_{exp.}$	$J_{exp.}$	$J_{calc.}$[b]	Dihedral angle (deg.)
15α 16α	7.32	7.33	7.4	8.0	32
15β 16α	8.92	8.75	8.6	9.0	155
16α 17α	9.03	9.28	9.0	9.2	345
16β 17α				1.5	110
15β 16β				6.0	32
15α 16β				1.2	270

[a] Ref. 1.
[b] Calculated assuming a distorted envelope conformation.

NOEs and 2D-^{1}H–^{1}H J-resolved experiments for coupling constants (Fig. 1 and Table 2). The orientation of the hydroxyl function at C-3 proved to be β-equatorial from the large coupling constant (J=11.5 Hz for thyonoside A and J=11.7 Hz for thyonoside B) due to H-3α (axial) and H-2β (axial). Strong NOEs between H-3α and H_3-30, and between H-3α and H-5α (even between H_3-30 and H-5α for thyonoside B) indicate the α position of the C-30 methyl group and H-5. Strong NOEs between H-2β and H_3-29, H-2β and H_3-19, and between H_3-29 and H_3-19, indicate the 1,3-axial orientation of these methyl groups. These suggest that the A/B ring has a *trans* junction. The H-9β configuration was confirmed by: (1) the presence of a broad doublet at 3.41 ppm for thyonoside A and 3.43 ppm for thyonoside B, the downfield shift of which have been previously attributed to the anisotropic effect of the γ-lactone carbonyl[6] and (2) a strong NOE between H-9β and H_3-19. NOEs observed between H-9β and H-12β and between H-12α and H_3-28 indicate the α-position of the C-28 methyl group. This methyl group is also correlated via NOEs with H-7, H-15α, H-16α, H-17α. These data show that the C/D-ring has a *trans* junction and the D/E-ring a *cis* junction. The α configuration of the H_3-21 group was determined by the observation of a NOE between the H_3-21 singlet and H-17α. The β configuration of the C-16 acetoxy group was deduced from the NOE mentioned between H_3-28 and H-16α, and from coupling constants for the four-proton system 2H-15/H-16/H-17. Comparisons between our values and the calculated coupling constants for the D-ring protons of frondoside A[1], support the β configuration for the C-16 acetoxy group (Table 2).

The aglycon appears to be the same as that in neothyonidioside C[7] from *Neothyone magnum*, in cucumarioside A_0-2[8] from *Cucumaria japonica*, in lefevreioside D[9] from *Cucumaria lefevrei* and in cucumarioside G_1[10] from *Eupenctata fraudatrix*.

2.2. Oligosaccharide chain

The nature of the sugar units, the oligosaccharide sequence, and the position of interglycosidic linkages were determined using a combination of ^{1}H–^{1}H COSY, TOCSY, ^{1}H–^{1}H J-resolved, ^{13}C–^{1}H correlations and NOESY experiments. The carbon and proton chemical shift assignments, and coupling constant values are summarized in Table 3.

Firstly, the proton and carbon resonances corresponding to the sugar part of the molecule suggested the presence of four monosaccharide units for both saponins. This conclusion is clearly indicated by signals for four anomeric carbons at

Table 3. ^{13}C and ^{1}H NMR data and assignments for the oligosaccharide subunits of thyonosides A (**1**) and B (**2**) in C_5D_5N–D_2O 9:1

Sugars		^{13}C NMR in ppm		^{1}H NMR in ppm, mult. (J in Hz)	
		(**1**)	(**2**)	(**1**)	(**2**)
Xyl-4-OSO_3Na	1	104.6	105.0	4.65 d (7.1)	4.69 d (7.3)
	2	82.0	84.0	3.83 dd (7.1, 8.7)	3.98 dd (7.3, 9.0)
	3	74.3	75.5	4.23 dd (8.7, 8.7)	4.32 dd (9.0, 9.0)
	4	75.3	75.0	5.07 ddd (5.5, 8.7, 9.6)	5.12 m
	5	64.0	64.2	4.68 m	4.82 dd (5.1, 11.5)
				3.68 dd (9.6, 11.8)	3.72 dd (9.7, 11.5)
Qui	1	104.3	105.5	4.79 d (7.8)	5.09 d (7.6)
	2	75.3	73.8	3.89 dd (7.8, 9.9)	3.99 dd (7.6, 9.5)
	3	74.3	68.8	3.98 dd (8.4, 9.9)	4.02 dd (9.5, 8.9)
	4	87.0	86.1	3.37 dd (8.4, 9.5)	3.63 dd (8.9, 8.9)
	5	71.0	71.8	3.55 dd (5.9, 9.5)	3.74 dd (5.9, 8.9)
	6	17.4	17.9	1.57 d (5.9)	1.71 d (5.9)
Glu-6-OSO_3Na or Xyl	1	104.2	105.5	4.73 d (7.8)	4.85 d (7.6)
	2	74.6[a]	75.5	3.86 dd (7.8, 10.4)	3.98 dd (7.6, 9.0)
	3	85.7	75.5	4.13 dd (10.4, 10.4)	4.12 dd (9.0, 9.0)
	4	69.2	86.6	3.73 dd (8.7, 10.4)	4.04 ddd (5.6, 9.0, 10.6)
	5	74.6	66.5	4.15 m	4.19 dd (5.6, 11.5)
					3.65 dd (10.6, 11.5)
	6	67.3		5.10 m	
				4.70 m	
3-*O*-Me-Xyl	1	105.4	106.0	5.19 d (7.8)	5.25 d (8.0)
	2	73.6[a]	74.5	3.85 dd (7.8, 9.2)	3.94 dd (8.0, 8.9)
	3	87.0	87.8	3.55 dd (8.8, 9.2)	3.60 dd (8.9, 8.9)
	4	69.3	70.2	4.02 ddd (5.6, 8.8, 10.6)	4.08 ddd (5.7, 8.9, 10.4)
	5	66.4	67.1	4.15 dd (5.6, 11.2)	4.20 dd (5.7, 11.6)
				3.55 dd (10.6, 11.2)	3.61 dd (10.4, 11.6)
	OMe	60.3	60.7	3.81 s	3.84 s

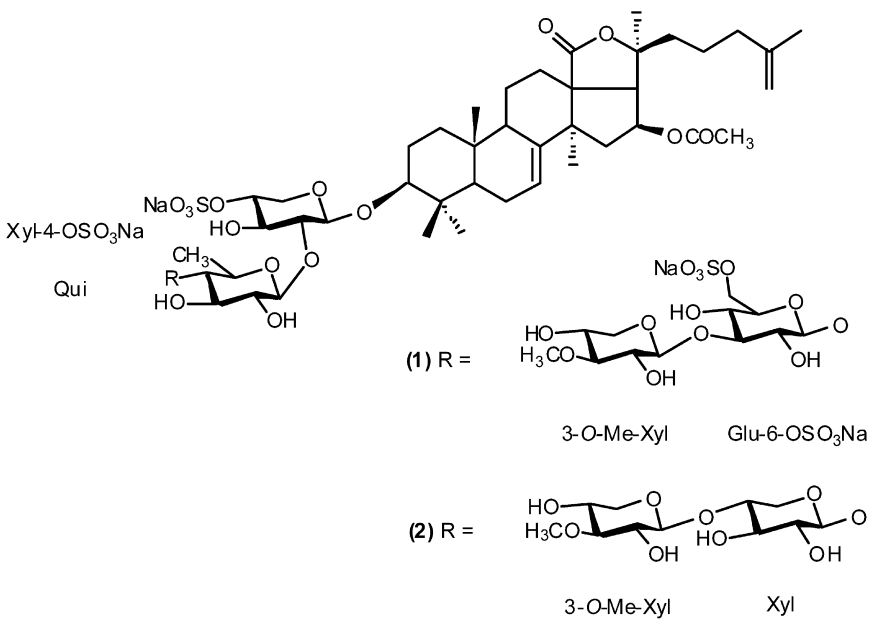
[a] Assignment may be reversed in the vertical column.

105.4, 104.6, 104.3, 104.2 ppm for thyonoside A (106.0, 105.5, 105.5, 105.0 ppm for thyonoside B) and four anomeric protons at 5.19, 4.79, 4.73, 4.65 ppm for thyonoside A (5.25, 5.09, 4.85, 4.69 ppm for thyonoside B). All anomeric protons resonate as doublets with coupling constants between 7 and 8 Hz, indicating a β stereochemistry of the glycoside bond. Furthermore, the 104–106 ppm range of the anomeric carbon signals is indicative of a β configuration.[11,12] A doublet at 1.57 ppm (J=5.9 Hz, 3H) for thyonoside A (1.71 ppm, J=5.9 Hz, 3H for thyonoside B), and a singlet at 3.81 ppm (s, 3H) for thyonoside A (3.84 ppm, s, 3H for thyonoside B) in both ^{1}H spectra are ascribable to a 6-deoxy sugar and an *O*-methyl sugar, respectively. The ^{1}H–^{1}H COSY and TOCSY-NMR spectra allowed the identification of the separate carbohydrate ring spin systems. NOESY-NMR spectra (t_{mix}=250 ms) were used to define completely the relative stereochemistry of each carbohydrate unit with ^{1}H–^{1}H J-resolved for coupling constants. Coupling constants in the 8–9 Hz range are indicative of axial–axial coupling[13,14] for proton 2–3, 3–4, 4–5. For xylose units, a coupling constant of 5–6 Hz can be observed between H-4 axial and H-5 equatorial protons.[13,14] The four sugars for thyonoside A appear to be xylose-4-sulphate, quinovose, glucose-6-sulphate and 3-*O*-methylxylose; the sugars in thyonoside B are xylose-4-sulphate, quinovose, xylose and 3-*O*-methylxylose.

The sequence of the oligosaccharide chains was deduced from the NOESY and HMBC experiments (Fig. 2). The position of the interglycosidic linkages was determined by comparing the carbon chemical shifts observed with those of the corresponding methyl glycopyranoside[15,16] and taking into account the downfield shift resulting from glycosidation. The Xyl-4-OSO_3Na subunit of thyonoside A must be linked to the aglycon at C-3 due to the strong NOE and the HMBC correlation observed between H-1 of Xyl-4-OSO_3Na and the H-3α proton or C-3 carbon of the aglycon. The interglycosidic position of attachment of Xyl-4-OSO_3Na is assigned to C-2 based on the significant downfield shift ($\Delta\delta$=82.0−74.6=7.4 ppm difference from the corresponding methyl glycopyranoside).[16] This residue appears to be linked to Qui, according to the NOE observed between H-1 of Qui and H-2 of Xyl-4-OSO_3Na. Another important downfield shift ($\Delta\delta$=75.3−70.9=4.4 ppm), observed at C-4 of Xyl-4-OSO_3Na is attributed to the presence of a sulphate group at that position. This shift cannot be the consequence of a glycosidic linkage since no inter-residue NOE involving H-4 of Xyl-4-OSO_3Na is observed. The presence of an inter-residue NOE between H-1 of Glu-6-OSO_3Na and H-4 of Qui, as well as a large glycosidation shift on C-4 of Qui ($\Delta\delta$=87.0−73.8=13.2 ppm) suggest that Glu-6-OSO_3Na is β1-4 linked to Qui. A distinctive downfield shift for the C-6 of Glu-6-OSO_3Na (67.0 instead of 60–62 ppm), indicates the C-6 attachment of the sulphate group.[15,17] The absence of any ^{13}C glycosidation shift for 3-*O*-Me-Xyl (except the one due to the methylation on C-3 hydroxyl function and identified by HMBC and NOESY), suggests that this residue must be the terminal unit. A NOE between H-1 of 3-*O*-Me-Xyl and H-3 of Glu-6-OSO_3Na, and the downfield shift of C-3 of Glu-6-OSO_3Na ($\Delta\delta$=85.7−78.3=7.4 ppm) confirmed that 3-*O*-Me-Xyl is β1-3 linked to Glu-6-OSO_3Na. The glycoside sequence is supported by the following HMBC correlations: H-1 of Qui with C-2 of Xyl-4-OSO_3Na, H-1 of Glu-6-OSO_3Na with C-4 of Qui, H-1 of 3-*O*-Me-Xyl with C-3 of Glu-6-OSO_3Na.

Therefore, based on the above results and the HRFAB data (see Section 3), thyonoside A possesses structure **1**; that is, 16β-acetoxy-3β-*O*-{3-*O*-methyl-β-D-xylopyranosyl-(1→3)-6-*O*-sodium sulphate-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulphate-β-D-xylopyranosyl}-holosta-7(8),25(26)-diene, assuming that each monosaccharide belongs to the D series.

The difference between thyonosides A and B resides not only in one glycosidic residue, Xyl instead of Glu-6-OSO_3Na, but also in the linkage between 3-*O*-Me-Xyl and Xyl which is β1-4 according to the downfield shift of C-4 of Xyl. The absence of a strong NOE between these two



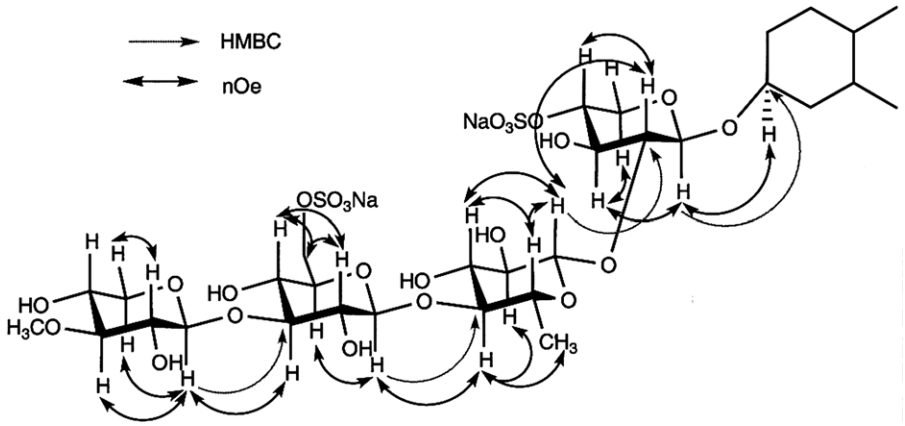


Figure 2. NOE and HMBC correlations for the oligosaccharide chain of thyonoside A (**1**).

residues and a HMBC correlation between H-1 of 3-*O*-Me-Xyl and C-4 of Xyl establishes the linkage position. Otherwise, similar correlations and glycosidation shifts are observed for this saponin. Hence, thyonoside B possesses structure **2**; that is, 16β-acetoxy-3β-*O*-{3-*O*-methyl-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulphate-β-D-xylopyranosyl}-holosta-7(8), 25(26)-diene.

The extensive use of ^{1}H and ^{13}C NMR techniques led us to the structure determination of two new saponins, thyonosides A and B, isolated from *Thyone aurea*. They possess a classical holostane-type aglycon which has been found previously in neothyonidioside C,[7] cucumarioside A_0-2,[8] lefevreioside D,[9] and in cucumarioside G_1.[10] Differences between thyonosides A and B and these known saponins appear in the oligosaccharide chain, principally due to the combination of the two final sugars: glucose-6-sulphate and 3-*O*-methylxylose, or xylose and 3-*O*-methylxylose.

3. Experimental

3.1. General methods

High resolution FAB mass experiments were recorded on a VG 70-4SE mass spectrometer. NMR spectra were obtained on a Varian 500NB spectrometer at 300 K using 5 mg of thyonoside A and 7 mg of thyonoside B in 0.75 mL of pyridine-d_5 plus one drop of D_2O. ^{13}C Experiments were run on a Varian 500 MHz. HPLC was carried out on a Econosil C_{18} (10 μm) column (10×250 mm) at 1 mL/min, UV detection at 210 nm. CCC separations were performed under 150 psi at 12 mL/min.

3.2. Extraction and purification

The specimen of the sea cucumber *Thyone aurea* was collected in Namibia. Extraction of 70 g of material with a mixture of methanol–acetone 5:5 gave 3.43 g of dry extract. This crude extract was partitioned between hexane, then chloroform, and a mixture of methanol–water, then between butanol and water. The alcoholic fraction (590 mg) showed interesting activities against L1210 cells and HSV-1. This part of the extract was submitted to silica gel chromatographies (eluent CH_2Cl_2 to MeOH) to give 115 mg of a glycoside mixture. After a first purification on HPLC reversed-phase (eluent CH_3CN-H_2O 5:5), a final purification was performed using CCC with $CHCl_3$–MeOH–H_2O–*i*PrOH–EtOH (9:6:8:1:8) as solvent, upper phase as mobile phase. Pure thyonoside A (6.2 mg) and pure thyonoside B (8.0 mg) were collected as white amorphous powders. The molecular formula of thyonoside A was determined as $C_{55}H_{84}O_{28}S_2Na_2$ by pseudo-molecular ion at *m/z* 1325.4260 $[M+Na]^+$ in the HRFABMS (positive ion mode), calcd for $C_{55}H_{84}O_{28}S_2Na_3$ 1325.428366. Fragment ion peaks at *m/z* 1223.5 $[M-SO_3Na+Na+H]^+$, and 1121.6 $[M-2SO_3Na+Na+2H]^+$ indicate the presence of two sulphate groups in the glycoside. The molecular formula of thyonoside B was determined as $C_{54}H_{83}O_{24}SNa$ by pseudo-molecular ion at *m/z* 1193.4766 $[M+Na]^+$ in the HRFABMS (positive ion mode), calcd for $C_{54}H_{83}O_{24}SNa_2$ 1193.479041. Fragment ion peaks at *m/z* 1091.6 $[M-SO_3Na+Na+H]^+$ indicate the presence of one sulphate group in the glycoside.

Acknowledgements

We thank Professor R. M. Coates (Department of Chemistry, University of Illinois at Urbana-Champaign) for helpful comments. This work was supported by a grant from the National Institute of Health, USA (GM 54063) and a research agreement with PharmaMar, S.A., Madrid, Spain.

References and notes

1. Girard, M.; Belanger, J.; Apsimon, J. N.; Garneau, F.-X.; Harvey, C.; Brisson, J.-R. *Can. J. Chem.* **1990**, *68*, 11–18.
2. Findlay, J. A.; Daljeet, A. *J. Nat. Prod.* **1984**, *47*, 320–324.
3. Kalinin, V. I.; Avilov, S. A.; Kalinina, E. Y.; Korolkova, O. G.; Kalinovsky, A. I.; Stonik, V. A.; Riguera, R.; Jiménez, C. *J. Nat. Prod.* **1997**, *60*, 817–819.
4. Miyamoto, T.; Togawa, K.; Higuchi, R.; Komori, T.; Sasaki, T. *J. Nat. Prod.* **1992**, *55*, 940–946.
5. Bedoya Zurita, M.; Ahond, A.; Poupat, C.; Potier, P.; Menou, J.-L. *J. Nat. Prod.* **1986**, *49*, 809–813.
6. Kitagawa, I.; Kobayashi, M.; Inamoto, T.; Yasuzawa, T.;

Kyogoku, Y.; Kido, M. *Chem. Pharm. Bull.* **1981**, *29*, 1189–1192.
7. Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A. *Khim. Prir. Soedin.* **1990**, 53–57.
8. Drozdova, O. A.; Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. W. *Khim. Prir. Soedin.* **1993**, 242–248.
9. Rodriguez, J.; Riguera, R. *J. Chem. Res. Synop.* **1989**, 342–343.
10. Kalinin, V. I.; Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A. *Khim. Prir. Soedin.* **1992**, 729–730.
11. Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933–1944.
12. Beier, R. C.; Mundy, B. P.; Strobel, G. A. *Can. J. Chem.* **1980**, *58*, 2800–2804.
13. Findlay, J. A.; Jaseja, M.; Brisson, J.-R. *Can. J. Chem.* **1987**, *65*, 2605–2611.
14. Findlay, J. A.; Jaseja, M.; Burnell, D. J.; Brisson, J.-R. *Can. J. Chem.* **1987**, *65*, 1384–1391.
15. Archibald, P. J.; Fenn, M. D.; Roy, A. R. *Carbohydrate Res.* **1981**, *93*, 177–190.
16. Kitagawa, I.; Nishino, M.; Kobayashi, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1951–1956.
17. Kitagawa, I.; Nishino, M.; Kyogoku, Y. *Tetrahedron Lett.* **1979**, *16*, 1419–1422.