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Leucospilotaside C, a new sulfated triterpene glycoside from sea cucumber *Holothuria leucospilota*

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Abstract

A new triterpene glycoside, leucospilotaside C, along with two known saponin, was isolated from sea cucumber *Holothuria leucospilota* collected from the South China Sea, and its structure was elucidated as 3-*O*-{4′-*O*-sodiumsulfate-*β*-D-xylopyranosyl}-holosta-22,25-epoxy-9-ene-3*β*,12*α*,17*α*-triol (**1**) by extensive spectroscopic analysis and chemical methods. The glycosides have the same triterpene aglycone, but differ in the oligosaccharide moieties.

Keywords: *Holothuria leucospilota*; Triterpene glycoside; Leucospilotaside C

Sea cucumber saponins known as holothurins are a rich source of triterpene glycosides. The triterpene is usually of the lanosterol-type with a 18(20)-lactone and a sugar chain of up to six monosaccharide linked to the C-3 of the aglycon. These compounds exhibit wide spectrum of biological activity, such as antifungal, cytotoxic, hemolytic, cytostatic and immunomodulatory effects [1–3]. Here we report the isolation and structure elucidation of a new monosulfated glycoside, leucospilotaside C (**1**), along with the known holothurin B (**2**) and A (**3**).

The sea cucumber, identified as *Holothuria leucospilota* [4] by Prof. Yu Lin Liao in the Institute of Oceanology, Chinese Academy of Science, Qingdao, were collected from the South China Sea, along the coast of Hainan Province, in spring 2005. A voucher specimen (reg. no. HYSC-2005-03) is preserved at Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai. The 80% ethanolic extract of *H. leucospilota* was sequentially submitted to column chromatography on DA-101 resin (Nankai University, Tianjin), silica gel, and reversed-phase silica (lichroprep RP-18, 40–63 μm). Finally, purification of these compounds was achieved by reversed-phase HPLC on zobax SB C-18 to give the pure leucospilotaside C (**1**), along with the main component of the glycosidic fraction.

Leucospilotaside C (**1**), colorless amorphous powder, $[\alpha]_D^{20} + 7$ (c 0.4, pyridine), mp 214–216 °C, was positive to Liebemann-Burchard and Molish test. The molecular formula was established as $C_{35}H_{53}O_{13}NaS$ from the $[M+Na]^+$ ion at *m/z* 759.2267 (calcd. 759.2251) in the positive ion mode HRESIMS and $[M-Na]^-$ ion at *m/z* 713 in the negative ion mode ESIMS. The fragment ion peak at *m/z* 639 $[M+Na\text{-}NaHSO_4]^+$ in the positive ion mode ESIMS indicated the presence of a sulfate group in the glycoside. This was supported by extensive analysis of 1D NMR and 2D NMR

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(^{1}H–^{1}H COSY, NOESY, HMQC, HMBC) and MS. IR spectrum showed the presence of hydroxyl (3464 cm^{-1}), carbonyl (1736 cm^{-1}), olefinic (1653 cm^{-1}), and sulfate (1060 cm^{-1}) groups.

The data of the ^{1}H, ^{13}C NMR and DEPT suggested the presence of a triterpenoid aglycon with a 9(11)-double bond [δ_C 153.9 (s,C-9) and 115.8 (d,C-11), δ_H 5.98 (br. d,H-11)], one oxomethine [δ_C 88.6 (d,C-3), δ_H 3.30 (dd,H-3)] and one epoxy group (δ_C 80.8, d,C-22 and 81.5, s,C-25). NMR signal characteristic for an oxygenated methane [δ_C 71.7 (C-12), δ_H 5.09 (H-12)] in the holostane nucleus indicated α-configuration of the allylic OH group at C(12) [5]. Therefore, a 12-hydroxylated $\Delta^{9(11)}$ terpenoid aglycone was identified, together with the analysis of the TOCSY and HMBC experiments. The NMR spectra of **1** showed resonances for a lactone group at δ_C 174.7 (C-18) and an oxygenated quaternary C-atom at δ_C 86.8 (C-20) in accordance with a 18,20-lactone. Correlation from H-21 to C-22, H-23 to C-22 and H-24 to C-25, H-26 to C-25 in the HMBC spectrum determined epoxy group at C-22/C-25 of the aglycon. The assignments of the NMR signal associated with the aglycon moiety (Table 1) were derived and confirmed from the ^{1}H–^{1}H COSY, TOCSY, HMBC, and NOESY experiments, and comparison with those of the aglycons of the known related compounds from *H. leucospilota* [6,7], which had been identified as holothurigenol. The NOSEY spectrum of **1** allowed us to establish the relative configuration of all stereogenic centers of the aglycon.

The presence of one β-sugar unit in the compound **1** was deduced from the ^{13}C- and ^{1}H NMR spectra, which showed one anomeric C-atom and one anomeric H-atom resonances, both with a coupling constant doublets (J = 7.2–7.8 Hz). The sugar moiety was determined as xylose (Xyl), as supported by acidic hydrolysis (aq. 2 mol/L CF_3COOH) and chemical derivatization to the corresponding aldononitrile peracetate [8], which were analyzed by GC/MS and by comparing the GC retention time of the corresponding trimethylsilylated hydrolysate with those of the authentic samples prepared in the same manner [9]. The ^{1}H- and ^{13}C NMR signals attributable to the sugar unit were assigned by

Table 1
^{13}C and ^{1}H NMR data for the aglycon moieties of leucospilotaside C (**1**).

Position	δ_C mult[a]	δ_H mult[b] (J in Hz)	HMBC	NOESY
1	36.5 t	1.48–1.90 m		
2	27.1 t	1.96–2.18 m		Me(19)
3	88.6 d	3.30 dd (4.0, 12.0)		H–C(26), Me(29), Hα–C(1)
4	40.0 s			
5	52.8 d	1.11 m		Hα–C(3), Me(29), H–C(7)
6	21.3 t	1.57–1.81 m		
7	28.4 t	1.61–1.79 m		H–C(17), Me(29), Me(30), H–C(5)
8	41.0 d	3.43 m		
9	153.9 s			
10	39.8 s			
11	115.8 d	5.98 d (4.4)	C-8, -10, -12	
12	71.7 d	5.09 m	C-9, -11, -14, -18	
13	58.9 s			
14	46.0 s			
15	37.0 t	1.50–1.91 m		
16	35.7 t	2.49–3.05 m		Hα–C(17), Me(30)
17	89.9 s			Hα–C(16), H–C(7), Me(30), Me(21)
18	174.7 s			
19	22. 6 q	1.41 s	C-1, -5, -9, -10	Hβ–C(2), Me(30)
20	86.8 s			
21	19.0 q	1.84 s	C-17, -20, -22	H–C(17)
22	80.8 s	4.44 m		
23	28.3 t	1.83 m	C-20, -22, -24, -25	
24	38.6 t	1.73 m	C-22, -23, -26, -27	
25	81.5 s			
26	27.1 q	1.27 s	C-24, -25, -27	
27	28.8 q	1.29 s	C-24, -25, -26	
30	16.9 q	1.07 s	C-3, -4, -5, -31	Me(19)
31	28.3 q	1.34 s	C-3, -4, -5, -30	H–C(3), H–C(5), H–C(7)
32	20.5 q	1.76 s	C-8, -13, -14, -15	H–C(16), H–C(17), H–C(7)

[a] Recorded at 150 MHz in C_5D_5N–D_2O (4:1); multiplicity by DEPT.
[b] Recorded at 600 MHz in C_5D_5N–D_2O (4:1).

Table 2
^{1}H and ^{13}C NMR data for the sugar moieties of leucospilotaside C (**1**).

Position	δ_C mult[a]	δ_H mult[b] (J in Hz)	HMBC
Xyl(1 → C-3)			
1	107.3	4.79 d (7.2)	C-3, Xyl C-2
2	77.0	4.06 m	Xyl C-1, -3
3	76.8	4.32 m	Xyl C-2, -4
4	76.4	5.28 m	Xyl C-2, -3
5	65.0	3.86 m	Xyl C-1, -4
		4.88 m	

[a] Recorded at 150 MHz in C_5D_5N–D_2O (4:1); multiplicity by DEPT.
[b] Recorded at 600 MHz in C_5D_5N–D_2O (4:1).

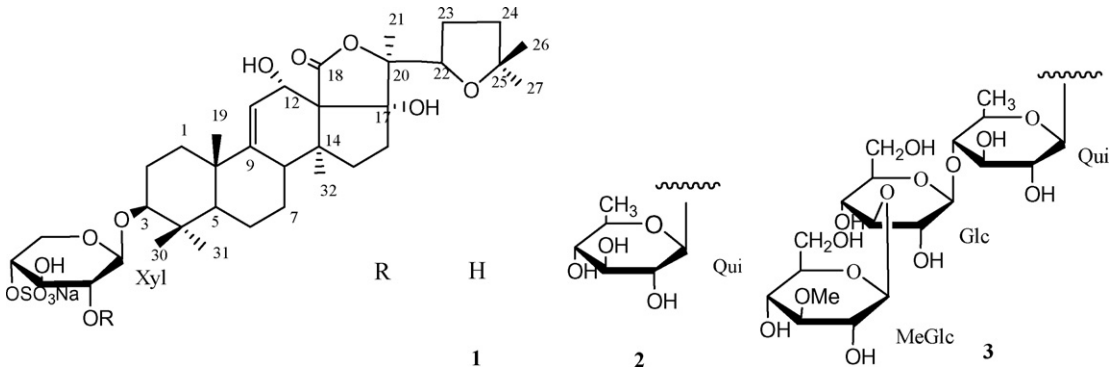

Fig. 1. Structure of leucospilotaside C (**1**), holothurin B (**2**) and holothurin A (**3**).

the 2D NMR experiment, and the data (Table 2) indicated that sugar residue was in its pyranose form. The monosaccharide sequence was determined by analysis of HMBC correlations and the chemical shifts for C-3 of the aglycone (δ 88.6), which was shifted downfield relative to the resonances expected for the corresponding methyl glycopyranosides. Cross-peaks at δ 4.79/88.6 (Xyl H-1/C-3) indicated the sugar residues: Xyl-(1 → 3)-aglycon. The position of the sulfate group was determined by comparing the ^{13}C NMR data of **1** with those of the desulfated derivative, and esterification shift was observed for the signal of C-4′ (Xyl, from δ 69.4 to 76.4) [10]. These data were also confirmed by the NOESY spectrum. Therefore, structure of leucospilotaside C (**1**) was deduced as 3-*O*-{4′-*O*-sodiumsulfate-β-D-xylopyranosyl}-holosta-22,25-epoxy-9-ene-3β,12α,17α-triol (**1**) (Fig. 1).

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