STEROID GLYCOSIDES FROM THE STARFISH

Crossaster papposus

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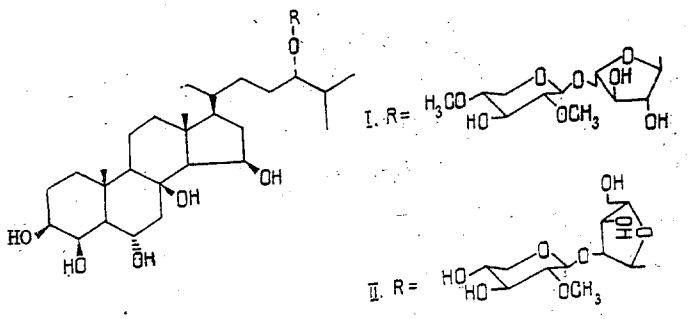
The following have been isolated from the starfish Crossaster papposus and characterized: the new glycoside (24S)-5α-cholestane-3β,4β,6α,8,15β,24-hexaol 24-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 5)-α-L-arabinofuranoside] (crossasteroside P_4) and the previously known attenuatoside B-1.

Continuing a study of the total polyhydroxysteroids and their glycosides from the starfish Crossaster papposus [1, 2], we have isolated two compounds: a new glycoside, which has been called crossasteroside P_4 (I), and the previously known attenuatoside B-1 (II). The structures of (I) and (II) were established mainly by NMR spectroscopy. Their spectral characteristics are given in Tables 1 and 2.

The acid hydrolysis of glycoside (I) gave a mixture of arabinose and 2,4-di-O-methylxylose in a ratio of 1:1. The monosaccharides were identified by TLC and by GLC in the form of aldononitrile acetates. It was established from the specific rotation of the mixture of monosaccharides that the arabinose belonged to the L-series and the 2,4-di-O-methylxylose to the D-series.



The positions of the hydroxy groups in the aglycon of glycoside (I) were determined by difference spin-decoupling experiments and by a comparison of the NMR spectra of (I) with the spectra of model compounds [3, 4]. The configurations of the substituents were established from the SSCCs of the protons (Table 1). On the basis of these results we established that in the steroid nucleus of compound (I) hydroxy groups were present in the 3β,4β,6α,8, 15β positions. The position of attachment (C-24) of the carbohydrate chain to the aglycon and the order (1-5) of the bond between the monosaccharide residues were found by recording nuclear Overhauser effects. Thus, when H-1' (5.57 ppm) was irradiated, enhancements of the signals of H-24 (3.65 ppm) and H-2' (4.85 ppm) were observed. The irradiation of one H-5' (4.24 ppm) gave enhancements of the signals of the other H-5' (4.46 ppm) and of H-1" (4.70 ppm). In its turn, on the irradiation of H-1" (4.70 ppm) enhancements were observed of the signals of one H-5' (4.24 ppm), H-2" (3.38 ppm), H-3" (3.96 ppm), and one H-5" (3.30 ppm).

The assignment of the signals of the carbon atoms of glycoside (I) was made by comparing its ^{13}C NMR spectrum (Table 2) with the spectrum of culcitoside C_1 (III) from the starfish Cucita novaeguineae and of asterosaponin P_2 (IV) from the starfish Patiria pectinifera [3, 4]. The CSs of the carbon atoms of the aglycon moiety and of the 2,4-di-O-methylxylopyranose unit in the spectrum of (I) were identical with the analogous values for glycoside (III). The signals of C-1'–C-5' in the spectrum of compound (I) corresponded to the signals

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TABLE 1. Chemical Shifts and Multiplicities of the Signals of the Protons of Compounds (I) and (II) (C_5D_5N; TMS = 0; δ, ppm; J, Hz)

Protons	I	II
H-2	2,38 m	2,40 qd
H-3	3,95 m	3,97 m
H-4	5,23 m	5,26 t
H-5	1,47 dd (10,5; 2,2)	1,48 dd (10,7; 2,2)
H-6	5,05 td (10,5; 4,0)	5,08 td (10,6; 4,2)
H-7e	3,15 dd (12,0; 4,2)	3,14 dd (12,2; 4,3)
H-7a	1,85 dd (12,0; 10,7)	
H-14	1,15 d (5,6)	1,09 d (5,7)
H-15	4,77 m	4,75 m
H-16	2,62 td (14,2; 7,7)	2,63 dt (14,0; 7,5)
H-16′	1,80 m	
H-17	1,13 m	
H-24	3,65 m	3,65 m
H-25	1,95 m	2,06 m
CH_3-18	1,63 s	1,63 s
CH_3-19	1,85 s	1,86 s
CH_3-21	1,07 d (6,5)	1,05 d (6,5)
CH_3-26	0,95 d (6,7)	1,02 d (6,7)
CH_3-27	0,95 d (6,7)	0,99 d (6,7)
H-1′	5,57 d (2,2)	5,73 d (1,0)
H-2′	4,85 dd (2,2; 4,2)	4,84 dd (1,0; 4,0)
H-3′	4,75 m	4,90 m
H-4′	4,80 m	4,76 m
H-5′	4,24 dd (2,5; 11,2)	4,43 dd (3,0; 12,0)
H-5′	4,46 dd (5,0; 11,2)	4,29 dd
H-1″	4,70 d (7,5)	4,99 d (7,5)
H-2″	3,38 dd (7,5; 9,0)	3,46 dd (7,5; 9,0)
H-3″	3,96 t (8,7)	4,01 t (8,5)
H-4″	3,52 m	4,22 m
H-5″	3,30 dd (10,0; 11,2)	4,29 dd (5,0; 11,2)
H-5″	4,17 dd (5,0; 11,2)	3,56 t
OMe	3,51 s	3,78 s
OMe	3,84 s	

of the unsubstituted arabinofuranose residue of glycoside (IV) with the exception of the fact that the C-4' signal in the spectrum of (I) was shifted upfield by 2.3 ppm as compared with compound (IV), and that of C-5' downfield by 6.9 ppm. These values agree well with the β- and α-effects of glycosylation given in the literature for the C-5 and C-6 atoms of the glucopyranose residue of β-gentiobiose [5], which additionally confirmed the attachment of the terminal monosaccharide residue to C-5' of the arabinofuranose residue in glycoside (I).

The CSs of the carbon atoms of the side chain of compound (I) proved to be close to the corresponding values in the ^{13}C NMR spectrum of nodososide from the starfish Protoreaster nodosus [6]. By analogy with nodososide, the C-24 configuration in glycoside (I) was determined as 24S.

Thus, the structure of compound (I) was established as (24S)-5α-cholestane-3β,4β,6α,8,15β,24-hexaol 24-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1→5)-α-L-arabinofuranoside]. Biosides of starfish polyhydroxysteroids usually have (1→2)-glycosidic bonds between the monosaccharide residues [1, 2, 6]. This is the first time that a (1→5)-glycosidic bond has been detected in this group of compounds.

The acid hydrolysis of glycoside (II) yielded a mixture of two monosaccharides identified as L-arabinose and 2-O-methyl-D-xylose (TLC, GLC, specific rotation) in a ratio of 1:1.

The structure of the aglycon of (II) was determined by the same methods as described above. The agreement of the spectral characteristics for the aglycon moieties of compounds (I) and (II) confirmed their identity (Tables 1 and 2). The CSs of the protons and of the carbon atoms, and also the SSCCs of the protons of the carbohydrate chain of glycoside (II) were close to those of the corresponding values for the carbohydrate chains of crossasterosides P_1 and P_2 and of culcitoside C_1 [1, 3].

As a result, we determined the structure of (II) as (24S)-5α-cholestane-3β,4β,6α,8,15β,24-hexaol 24-O-[O-(2-O-methyl-β-D-xylopyranosyl)-(1→2)-α-L-arabinofuranoside]. As shown by a comparison of its ^{13}C NMR spectrum with that given in the literature, this compound was identical with attenuatoside B_1, obtained previously from the starfish Hacelia attenuata. In the present paper we give for the first time the PMR spectrum of (II) taken in C_5D_5N.

TABLE 2. ^{13}C NMR Spectra of Compounds (I) and (II) (C_5D_5N; TMS = 0; δ, ppm)

Atom	I	II	Atom	I	II
C-1	39,5	39,5	C-21	18,9	19,0
C-2	26,8	26,8	C-22	32,2	32,3
C-3	72,9	72,8	C-23	28,4	28,3
C-4	68,8	68,7	C-24	83,0	83,2
C-5	57,3	57,1[b]	C-25	30,9	30,8
C-6	63,7	63,7	C-26	18,2	18,2
C-7	50,4	50,4	C-27	18,0	18,2
C-8	76,5	76,4	C-1′	109,1	107,2
C-9	58,0	57,8	C-2′	83,8	92,5
C-10	37,6	37,7	C-3′	79 0	77,6[b]
C-11	18,8	18,8	C-4′	83,3	84,2
C-12	42,6	42,6	C-5′	69,8	62,5
C-13	43,7	43,8	C-1″	104,5	104,7
C-14	62,0	61,9	C-2″	84,6	84,7
C-15	70,1	70,1	C-3″	76,0	77,4[b]
C-16	42,2	42,2	C-4″	80,5	70,9
C-17	57,1	57,3[b]	C-5″	63,9	66,9
C-18	16,5	16,6	OMe	60,5	60,5
C-19	17,2	17,2	OMe	58,6	
C-20	35,6	35,6			

[a], [b] Assignments of the signals interchangeable.

EXPERIMENTAL

For general information on the methods used, see [8]. The animals were collected in August, 1983, in the Sea of Okhotsk, from the littoral of the island of Onekotan (Kurile Islands) at a depth of 100 m.

Isolation of Compounds (I) and (II). The comminuted animals were extracted with ethanol at room temperature. The extract was concentrated to an aqueous residue in vacuum, and was lyophilized. The lyophilisate (2.240 kg) was extracted with ethyl acetate–methanol (1:5), and the residue was separated on a KA-70 centrifuge at 3000 rpm and was processed. The supernatant was evaporated, dissolved in water, and chromatographed on Polikhrom-1, with elution by water and 50% aqueous ethanol. The ethanolic eluate was concentrated. The total polyhydroxysteroid fraction obtained was chromatographed on columns of silica gel in the chloroform–ethanol–water (3:1:to saturation; and 30:7:to saturation) systems. Further purification was achieved by high-performance liquid chromatography (Du Pont chromatograph; refractometer detector) on Altex Ultrasfere-Si (5 μm, 10.0 × 250 mm) and Zorbax ODS (5 μm, 4.6 × 250 mm) columns with the eluents chloroform–methanol–water (150:40:1) and methanol–water (3:1), respectively. This gave 14 mg of glycoside (I) (0.00062% on the lyophilisate of the ethanolic extract) and 13 mg (0.00058%) of glycoside (II).

Crossasteroside P_4 (I), $C_{39}H_{68}O_{14}$, amorphous, $[\alpha]_{Hg}$ −40.6° (c 0.7, methanol).

Glycoside (II), $C_{38}H_{66}O_{14}$, mp 295-298°C, $[\alpha]_{Hg}$ −24.6° (c 1.2; methanol).

Hydrolysis of Glycosides (I) and (II). The acid hydrolysis of (I) and (II) was carried out with 2 N HCl at 100°C for 2 h. L-Arabinose and 2,4-di-O-methyl-D-xylose were identified in the hydrolysate from (I), and L-arabinose and 2-O-methyl-D-xylose in that from (II), by TLC on silica gel and Silufol in the butanol–acetone–water (4:5:1) system and by the GLC of the corresponding aldononitrile acetates.

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TRITERPENE GLYCOSIDES FROM THE HOLOTHURIAN Cucumaria frondosa

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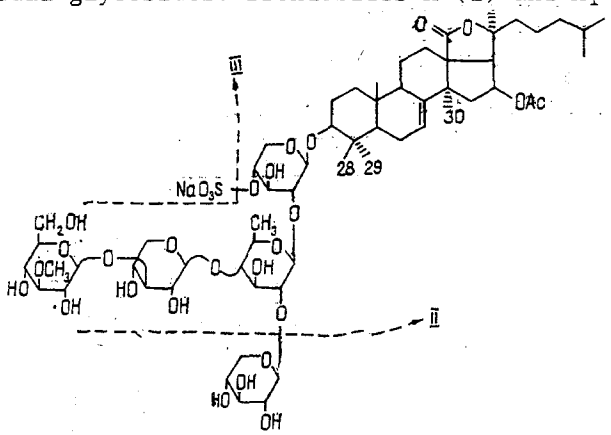
Two triterpene glycosides have been isolated from an alcoholic extract of the holothurian Cucumaria frondosa collected in the Kola littoral, Barents Sea: the main component of the glycoside fraction – frondoside A (I) – and a minor component – frondoside A_1 (II).

From the total glycoside fraction of the holothurian C. frondosa, obtained by the usual procedure, we have isolated by column chromatography on silica gel and reversed-phase chromatography two individual glycosides: frondosides A (I) and A_1 (II).



3-O-Methylglucose, xylose, and quinovose in a ratio of 1:3:1 were identified, in the form of aldononitrile peracetates, in the products of the acid hydrolysis of (I). The solvolytic desulfation of (I) with a mixture of pyridine and dioxane gave the desulfated derivative (III), which showed the presence of a sulfate group in (I). The results of acid hydrolysis, ^{13}C NMR spectra, and the values of the physical constants permitted (I) to be identified as frondoside A, isolated previously by Canadian authors [1] in a study of the same holothurian but collected in the Atlantic littoral of Canada.

Frondoside A_1 was a minor component of the glycoside fraction. Its amount was about 0.6% of that of frondoside A. A comparison of the ^{13}C NMR spectra of (I) and (II) showed that the aglycon of both glycosides was 16β-acetoxyholost-7-en-3β-ol, and the difference consisted in the structures of the carbohydrate chains. In fact, 3-O-methylglucose, xylose, and quinovose in a ratio of 1:2:1 were identified in the products of the acid hydrolysis of (II), and in its ^{13}C NMR spectra the signals of four anomeric carbon atoms were observed (Table 1).

A comparative study of the spectra of (I) and (II) showed that the spectrum of (II), as compared with that of (I), lacked the signals corresponding to a terminal xylose residue, and therefore (II) possessed an unbranched chain consisting of four carbohydrate residues. In fact, the signals of the carbohydrate part of the spectrum of (II) coincided with the sig-

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