STEROID GLYCOSIDES FROM THE STARFISH *Echinaster sepositus*

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Two new steroid glycosides, which have been called echinasterosides B_1 and B_2 have been isolated from the starfish *Echinaster sepositus*. Using chemical transformations (methylation, hydrolysis) and also spectral methods (^1H and ^{13}C NMR spectroscopy and GLC-MS) the complete chemical structure of B_1 has been established as 15α-acetoxy-5α-cholestane-3β,4β,6β,8,24ξ-pentaol 24-O[O-(2)O÷ methyl-β-D-xylopyranosyl)-(1→3)-α-L-arabinofuranoside] (I) and that of glycoside B_2 as 5α-cholestane-3β,4β,6β,8,15α,24ξ-hexaol 24-O-[O÷(2-O-methyl-β-D-xylopyranosyl)-(1→3)-α-L-arabinofuranoside] (II).

Continuing investigations of glycosylated steroid polyols, we have studied the composition of the glycoside fraction from the starfish *Echinaster sepositus*. Two new glycosides have been obtained — echinasterosides B_1 and B_2. The acid hydrolysis of glycosides B_1 and B_2 led to the same mixture of two monosaccharides in a ratio of 1:1, and these were identified as L-arabinose and 2-O-methyl-D-xylose (PC, GLC, $[\alpha]_D$). After glycoside B_2 had been methylated, the permethyl derivative had been subjected to methanolysis, and the methyl glycosides so formed had been acetylated, we identified the following monosaccharide derivatives with the aid of GLC and GLC–MS: methyl 2,3,4-tri-O-methyl-α- and -β-D-xylopyranosides and ethyl 3-O-acetyl÷2,5-di-O-methyl-α- and -β-L-arabinofuranosides [1].

The methylation results showed that the carbohydrate chain of echinasteroside B_2 consisted of L-arabinose attached to the aglycon and linked to a terminal 2-O-methyl-D-xylose residue by a 1→3 bond.

The assignment of the signals of the atoms of the carbohydrate moieties in the ^{13}C and ^1H NMR spectra of glycosides B_1 and B_2 (Tables 1 and 2) showed their complete identity. The configuration of the 2-O-methyl-D-xylopyranose residue was determined from its spin–spin coupling constant ($J_{1'',2''}$ = 7.5 Hz) as β and that of the L-arabinofuranose residue ($J_{1',2'}$ = 2 Hz, δC-1' = 109.7 ppm) as α. The chemical shifts of the other carbon atoms (Table 2) agreed with the s structure of the carbohydrate chain given above [2] apart from an unusually low value for C-1" (101.5 ppm).

A comparison of the ^{13}C NMR spectra for the standard compounds methyl-β-D-xylopyranoside and its 2-O-methyl derivative showed that the influence of the substitutent at C-2 on the chemical shift of C-1 was insignificant, amounting to 0.1 ppm. As has been shown for a series of synthetic oligosaccharides [3], the C-1 signal in the ^{13}C NMR spectrum of a terminal β-xylopyranosyl residue may be shifted upfield to 102.0 ppm, depending on the position of the glycosidic bond. Since in glycosides B_1 and B_2 the C-1"H bond deviated somewhat from the syn position with respect to C-3'H and interacted with C-2'H (β-effect — 4.8 ppm), it may be considered that the low value of the C-1 doublet chemical shift was due to conformational effects [2, 4].

According to the ^{13}C NMR spectra, the aglycons of glycosides B_1 and B_2 (Table 2) each had 27 carbon atoms and five hydroxy groups in the nucleus. The sequence of the substituents was established for the glycosides by high-resolution ^1H NMR spectroscopy in just the same way as was done previously for glycoside P_1 [6]. The stereochemistry of the hydroxy functions was determined from their spin–spin coupling constants, which were approximately equal to the values for the splittings of the multiplets of the protons as a consequence of the substantial difference in their chemical shifts. Thus, the equatorial position of the substituent at C-3 and the axial position of that at C-4 followed from the splittings of the H-3 signal and the small widths of the H-4 and H-5 multiplets. The equatorial position of the OH group at C-15 followed from the SSCC of the corresponding geminal proton with H-14 (10 Hz) (Table1).

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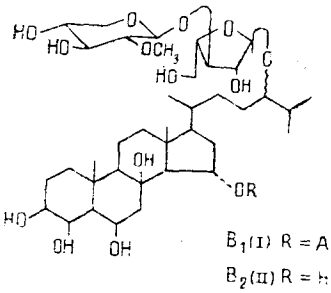
TABLE 1. ^{1}H NMR Spectra of Glycosides B_1 and B_2 (C_5D_5N, δ, TMS = 0). The spectra were recorded with an accuracy of 0.23 Hz/point)

Proton	B_2	B_1
	δ, ppm (J, Hz)	
H-3	3,92 dt (4,4; 4,4; 11,2)	4,00 dt (4,2; 4,2; 11,5)
H-4	4,51 m	4,60 m
H-5	1,26 m	1,28 m (2,4; 1,5)
H-6	4,52 m	4,45 m (3,0; 1,5)
H-7e	3,16 dd (2,9; 14,8)	2,40 dd (2,9; 14,8)
H-7a	2,03 dd (2,9; 15,0)	1,88 dd (3,0; 14,8)
H-14	1,53 d (10,0)	1,53 d (10,0)
H-15	4,85 td	5,62 td
H-16	2,27 m	
H-16	2,13 m	
H-24	3,57 m	3,60 m
H-25	1,93 m	1,90 m
3H-18	1,25 s	1,16 s
3H-19	1,82 s	1,79 s
3H-21	1,02 d (6,6)	0,99 d (6,7)
6H-26,27	0,95 d (6,6)	0,97 d (6,6)
OAc		2,0 s
H-1′	5,57 d (2,0)	5,58 d (2,0)
H-2′	4,85 m	4,90 m
H-3′	4,80 m	4,90 m
H-4′	4,80 m	4,77 m
H-5′	4,40 dd	4,40 dd
H-5′	4,26 dd	4,30 dd
H-1″	4,90 d (7,5)	4,95 d (7,5)
H-2″	3,45 t	3,48 t
H-3″	4,08 t	4,10 t
H-4″	4,20 m	4,23 m
H-5″	3,68 t	3,70 t
H-5″	4,36 m	4,38 m
OMe	3,83 s	3,85 s

It is difficult to deduce the corresponding constants of glycoside B_2 because of the pronounced overlapping of the H-4 and H-6 multiplets. On the basis of the results obtained, we assigned to glycoside B_2 the 3β,4β,6β,8,15α sequence of the hydroxy groups in the nucleus.

The presence of an oxygen function at C-24 (H-24, 3.57 ppm) was revealed by difference spin decoupling. Starting both from the 3.57 ppm multiplet and also from the CH_3-26,27 signals we obtained a characteristic multiplet at 1.93 ppm (H-25) which was converted into a doublet on double resonance with irradiation of the CH_3-26,27 methyl groups and into a septet on irradiation of H-24. The fact that the carbohydrate chain was attached to the aglycon at C-24 followed from the shifts of the glycosylation signal of the C-23, C-24, and C-25 carbon atoms of the side chain [6, 7].

On the basis of the results obtained, we suggested for echinasteroside B_2 the structure of 5α-cholestane-3β,4β,6β,8,15α,24ξ-hexaol 24-O-[O-(2-O-methyl-β-D-xylopyranosyl)-(1→3)-α-arabinofuranoside] (II).



B_1 (I) R = Ac

B_2 (II) R = H

A comparison of the spectral characteristics of B_1 and B_2 showed that they differed by the presence of an acetoxy function in B_1. In the ^{1}H NMR spectrum of glycoside B_1 there was a three-proton singlet at 2.06 ppm, and the H-15 signal was shifted downfield by 0.77 ppm in comparison with the corresponding signal in the spectrum of B_2 (Table 1). In the ^{13}C spectrum of this glycoside the acetoxy group was shown by signals at 170.1 (s) and 21.2 (q) ppm, and the acetylation effects observed for the neighboring atoms were −3.7 (C-14), +4.1 (C-15), and −3.0 (C-16) ppm. These values were close to those that we obtained previously for glycoside B_1 and its acetates [6] and confirmed the presence of a 15α-OAc group in B_1.

On the basis of what has been said above, as ascribed to glycoside B_1 the structure of 15α-acetoxy-5α-cholestane-3β,4β,6β,8,24ξ-pentaol 24-O-[O-(2-O-methyl-β-D-xylopyranosyl)-(1→3)-α-L-arabinofuranoside] (I).

TABLE 2. ^{13}C NMR Spectra of Glycosides B_1 and B_2 (C_5D_5N, δ, TMS = 0)

Atom	B_1	B_2	Atom	B_1	B_2
C-1	40,3	40,2	C-23	28,9	28,4
C-2	24,7	24,6	C-24	83,4	83,4
C-3	73,6	73,6	C-25	31,2	31,0
C-4	78,8	78,8	C-26	18,2	17,9
C-5	49,9	49,8	C-27	17,9	18,3
C-6	75,0	75,2	OMe	60,4	60,3
C-7	44,1	44,9	OAc	170,1; 21,2	
C-8	75,0	75,6	C-1′	109,7	109,6
C-9	56,7	57,0	C-2′	78,8	78,8
C-10	36,1	36,1	C-3′	85,6*	85,5*
C-11	18,7	18,7	C-4′	83,7	83,7
C-12	41,6	41,9	C-5′	62,8	62,8
C-13	44,3	44,7	C-1″	101,6	101,5
C-14	62,5	66,2	C-2″	84,5*	84,4*
C-15	73,0	68,9	C-3″	77,7	77,6
C-16	38,4	41,4	C-4″	71,0	70,9
C-17	55,4	55,0	C-5″	66,9	66,7
C-18	15,0	15,2			
C-19	18,3	18,3			
C-20	35,2	35,2			
C-21	18,7	18,7			
C-22	32,1	31,9			

*Assignment of the signals ambiguous.

EXPERIMENTAL

All the spectral characteristics and physical constants were determined under the conditions described in [5]. ^1H and ^{13}C NMR spectra were taken on a Bruker WM-250 spectrometer. Mass spectra were obtained on a LKB-9000S spectrometer at an ionization energy of 70 eV. The starfish *Echinaster sepositus* were collected on the north-western littoral of the island of Madagascar in February-March 1983. The starfish was determined by A. V. Smirnov. Samples of methyl β-Dβxylopyranoside and 2-O-methyl-β-D-xylopyranoside were kindly supplied by E. V. Evtushenko.

Echinasteroside B_1 (I), $C_{40}H_{68}O_{15}$, mp 266-269°C; $[\alpha]_D^{20}$ −12,36° (c 0.6; ethanol) was isolated with a yield of 0.002% from an ethanolic extract of the starfish *E. sepositus* by a method described previously [6].

Echinasteroside B_2 (II), $C_{38}H_{66}O_{13}$, mp 262-265°C; $[\alpha]_D^{20}$ −24,22° (c 2.2; ethanol) was isolated in a similar manner to (I) with a yield of 0.05%.

The methylation of echinasteroside B_2 was carried out by Hakomori's method [8]. Methanolysis of the methylation products, and the acetylation and identification of the methyl 2,3,4-tri-O-methyl-α- and -β-D-xylopyranosides and methyl 3-O-acetyl-2,5-di-O-methyl-α- and -β-L-arabinofuranosides were carried out as described in [5].

The acid hydrolysis of echinasterosides B_1 and B_2 was performed by heating 20 mg of each glycoside with 2 N HCl at 85-90°C for 2 h. The monosaccharides were separated preparatively on Whatman 3 MM paper in the butanol—pyridine—water (6:4:43) system and were analyzed by TLC on silica gel impregnated with 0.02 M sodium dihydrogen phosphate in the butanol—acetone—water (4:1:5) system and by GLC-chromato-mass spectrometry in the form of aldononitrile peracetates. L-Arabinose and 2-O-methyl-D-xylose were identified.

SUMMARY

Two new steroid glycosides have been isolated from the starfish *Echinaster sepositus* and have been characterized: 15α-acetoxy-5α-cholestane-3β,4β,6β,8,24ξ-pentaol 24-O-[O-(2-O-methyl-β-D-xylopyranosyl-(1→3)-α-L-arabinofuranoside and 5α-cholestane-3β,4β,6β,8,15α,24ξ-hexaol 24-O-[O-(2-O-methyl-β-D-xylopyranosyl-(1→3)-α-L-arabinofuranoside], which have been called echinasterosides B_1 and B_2, respectively.

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NEW ASTEROSAPONINS FROM THE STARFISH *Dismolasterias nipon*

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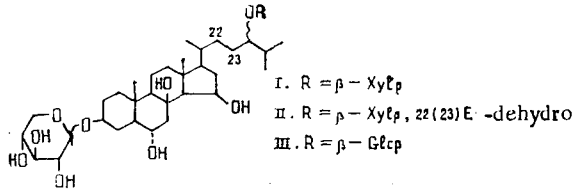
Three new glycosides, D_1, D_2, and D_3, have been isolated from the Far Eastern starfish *Distolasterias nipon*. They have been identified by chemical and physicochemical methods as 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside, tα-cholest-22-ene-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside (II), and 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 24-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside (III).

Asterosaponins are a group of physiologically active steroid saponins present in extracts of starfish [1-3]. Recently, Italian chemists have reported the isolation of two asterosaponins of a new structural type having monosaccharide residues attached to C-3 and C-24 of the aglycon [4].

In studying physiologically active substances from mass species of Far Eastern marine invertebrates, we have isolated three new glycosides belonging to this group from extracts of the starfish *Distolasterias nipon*.

The structures of asterosaponins D_1 (I), D_2 (II), and D_3 (III) were determined by chemical and physicochemical methods.



The structures of the aglycons of glycoside (I) and (III) were established with the aid of spin-decoupling experiments (high-resolution ^{1}H NMR), and also by comparing the ^{1}H and ^{13}C NMR spectra of glycosides D_1 and D_3 (Tables 1 and 2) with the spectra of model compounds: 5α-cholestane-3β,6α,8β,15α,24ξ-pentaol [3] and 5α-cholestane-3β,4β,6α,8β,15β,24ξ-hexaol [25].

The glycon of glycoside (II) was characterized by the presence of a 22(23)-double bond. Its position was confirmed with the aid of double resonance and difference ^{1}H NMR spectroscopy. The double bond had the E-configuration. In actual fact, signals at 39.9, 139.5, and 127.6 ppm for C-20, C-22, and C-23 (Table 1) were close to the corresponding signals in the spectra of 22E-cholestenes, while in the 22Z-isomers the C-20 signal resonates in a stronger field [6].

To confirm that (II) was a 22(23)-dehydro derivative of glycoside (I) we hydrogenated asterosaponin D_2 over Adams catalyst, and obtained D_1.

The carbohydrate chains of glycosides (I) and (II) included only residues of D-xylose, which was identified in hydrolysates with the aid of TLC, GLC, and chromato-mass spectrometry and by a determination of specific rotation. At the same time, the presence of ^{13}C NMR spectra of (I) and (II) (Table 1) of two signals of anomeric carbon atoms showed that each of

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