STEROID GLYCOSIDES FROM THE STARFISH Crossaster papposus

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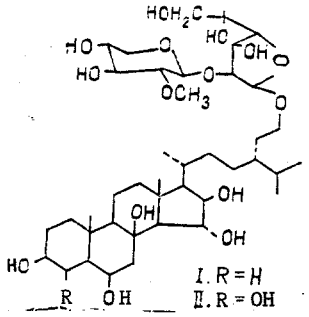
Two new steroid glycosides have been isolated from an ethanolic extract of the starfish Crossaster papposus – crossasterosides P_1 and P_2. On the basis of chemical transformations and spectral characteristics, the structure of crossasteroside P_1 has been established as (24R)-24-ethyl-5α-cholestane-3β,6β,8,15α,16β,29-hexaol 29-O-[2-O-methyl-β-D-xylopyranosyl-(1 → 2)-β-D-galactofuranoside]. Crossasteroside P_2 is its 4β-hydroxy analogue.

In studying the composition of the glycosidic fraction of the starfish Crossaster papposus, we have isolated two new steroid glycosides – crossasterosides P_1 (I) and P_2 (II).



The acid hydrolysis of glycoside (I) and (II) gave one and the same mixture of two monosaccharides in a ratio of 1:1. The monosaccharides were identified as galactose and 2-O-methylxylose (TLC, GLC, GLC-MS). From the specific rotation of the total monosaccharides it was established that they belonged to the D-series [1].

The dimensions of the rings of the monosaccharide residues and the configurations of the anomeric carbon atoms in the glycosides (I) and (II) were determined by a comparison of the ^{13}C NMR and PMR spectra of (I) and (II) (Tables 1 and 2) with information in the literature. The signals of the carbon atoms and of the protons of the 2-O-methyl-D-xylose unit coincided with the corresponding values for the 2-O-methyl-β-D-xylopyranose residue of echinasteroside B_2 (III) from the starfish Echinaster sepositus [2]. The CSs of C-1'–C-4' and of H-1'–H-4' and the H-1'–H-4' SSCC of the D-galactose unit were close to the corresponding values for the 2-O-substituted α-L-arabinofuranose residue of culcitoside C_1 (IV) from the starfish Culcita novaeguineae [3]. The C-4'–C-6' signals of the D-galactose residue agreed well with the same signals in the spectrum of methyl β-D-galactofuranoside (V) [4]. On the basis of these facts, the D-galactose residue was assigned the furanose form, the 2-O-methyl-D-xylose residue the pyranose form, and the glycosidic bond the β-configuration.

To establish the sequence of the monosaccharide residues in the carbohydrate chain, we acetylated glycoside (I) and obtained crossasteroside P_1 acetate (VI). As can be seen from Table 2, the O-acetyl groups in the carbohydrate moiety of (VI) were present at C-3', C-5', C-6', C-3", and C-4". It follows from this that the β-2-O-methyl-D-xylose residue was attached to C-2' of the β-D-galactofuranose residue of glycoside (I). The coincidence of the spectral characteristics of the carbohydrate chains of glycosides (I) and (II) (Tables 1 and 2) permitted the conclusion that these chains were completely identical.

The arrangement of the hydroxy groups in the aglycon of glycoside (I) was determined by spin-decoupling experiments. Starting from the characteristic H-3 multiplet (4.00 ppm) the sequence of H-4–H-7 protons was established, and starting from the H-15 signal (5.03

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TABLE 1. ^{13}C NMR Spectra of Glycosides (I) and (II) (C_5D_5N, δ, TMS = 0

Atom	I	II	Atom	I	II
C 1	40,9	40,6	C 21	19.0[a]	19,0
C 2	32.0	26,9	C 22	34,2	34,2
C 3	71,4	72,2	C 23	28,4	28.3
C 4	37,0	77.1	C 24	41 8	41.8
C 5	48,6	50.3	C 25	30,5	30,5
C 6	73,3	75,5	C 26	18,6[a]	18,6[b]
C 7	45.5	44,9[a]	C 27	19 8	19,8
C 8	76,1	75,7	C 28	31 2	31,5
C 9	56,8	57.1	C 29	67,4	67,3
C 10	36,2	36,2	C 1′	107,4	107,4
C 11	19,3	18,7	C 2′	91,5	91,4
C 12	42,8	42,6	C 3′	77,5b	77.4
C 13	44,7	44.7a	C 4′	83.8	83,8
C 14	63,9	63,8	C 5′	72,6	72,5
C 15	80,7	80,5	C 6′	64,8	64.7
C 16	82,5	82,4	C 1″	104,3	104,3
C 17	60,2	60,2	C 2″	84,7	84,7
C 18	17,0	17,0	C 3″	77 6[b]	77,5
C 19	15.9	18,7[b]	C 4″	70 9	70,9
C 20	29.9	29,9	C 5″	67.0	66 9
			OMe	60 6	60,5

[a,b]Assignment of the signals ambiguous.

TABLE 2. PMR Spectra of the Carbohydrate Moieties of Glycosides (I) and (II) and of the Acetate (VI) (C_5D_5N: TMS = 0; δ, ppm; J*, Hz)

Proton	I	II	VI
H-1′	5,32d (1,8)	5,60d (1,7)	5,57s
H-2′	4, 1 dd (1,8; 4,0)	4,87dd (1,7; 4,0)	4,72d
H-3′	5.11 dd (4,0; 7,8)	5,07dd (4,0; 7,9)	5,55dd
H-4′	4,77 dd (7,8; 4,0)	4,75dd (7,8; 4,0)	4,76t
H-5′	4 58td (3,8; 6,0; 6,0)	4,53td (3,8; 6,0; 6,0)	5,86m
2H-6′	4,49 *AB*dd	4,36 *AB* dd	4,65m 4,75m
H-1″	5,08 d (7,5)	5,03 d (7,5)	5,15d
H-2″	3,45 dd	3,42dd	3,53dd
H-3″	4,50 t	3,95t	5,60t
H-4″	4,20 m	4,17m	5,27td
2H-5″	4,29 dd 3,57 t	4,26dd ; 3,57 t	4,25dd ; 3,57 dd
OMe	3,76 s	3,75s	3,70s

*Values of the splittings in multiplets. The spectra were recorded with an accuracy of 0.23 Hz/point.

ppm), the sequence of H-14, H-16, and H-17 protons (Table 3). The configurations of the substituents were determined from the SSCCs of the protons.

The structure of the aglycon of (I) was also confirmed by a comparison of the spectral characteristics of (I) (Table 3) with the spectra of (24R)-24-ethyl-5α-cholestane-3β,6α,8,-15α,16β,29-hexaol 29-O-(α-L-arabinofuranoside) (VII) from the starfish Patiria pectinifera [5]. Thus, the CSs of the H-3, H-15, and H-16 protons and the corresponding SSCCs in the PMR spectrum of (I) were close to the corresponding signals in the spectrum of glycoside (VII). The H-6 signal (4.20 ppm) of glycoside (I) was present in the form of a narrow quartet, in contrast to the broad triplet of doublets (4.39 ppm) for H-6 of compound (VII). The H-4a signal (2.43 ppm) had shifted downfield, while the H-4e signal (2.00 ppm) has shifted upfield in comparison with the same signals (1.86 and 3.15 ppm, respectively) for (VII). These facts indicated that the substituent at C-6 had the β-configuration. Thus, the following arrangement of hydroxy groups in the steroid nucleus has been found in glycoside (I): 3β,6β,8,15α,16β.

TABLE 3. PMR Spectra of the Aglycon Moieties of Glycosides (I) and (II) (C_5D_5N; TMS = 0; δ, ppm; J, Hz)

Proton	I	II
H-3	4,00 m	3,84 dt
H-4a	2,43 q (11,2)	
H-4e	2,00 dm	4,41 m ($\Delta W_{1/2}$ =7,5)
H-5	1,40 dt	1,31 t
H-6	4,20 q (2,2)	4,40 m ($\Delta W_{1/2}$ =7,5)
H-7a	2,10 dd (14,8; 2,7)	2,02 dd (14,5; 3,0)
H-7e	3,25 dd (15,0; 3,0)	3,19 dd (14,5; 2,8)
H-14	1,57 d (10,5)	1,51 d (10,5)
H-15	5,03 dd (10,5; 2,5)	4,99 dd (10,5; 2,7)
H-16	4,73 dd (7,5; 2,3)	4,70 dd (7,5; 2,5)
CH_3-18	1,75 s	1,70 s
CH_3-19	1,60 s	1,86 s
CH_3-21	1,15 d (6,5)	1,12 d (6,5)
CH_3-26	0,80 d (6,7)	0,80 d (6,6)
CH_3-27	0,82 d (6,7)	0,82 d (6,7)
H-29	4,00 m	3,96 m
H-29'	3,58 m	3,57 m

According to its ^{13}C NMR spectrum, the aglycon of compound (I) contained 29 carbon atoms (Table 1). The signals in the ^{13}C NMR spectrum of (I) at (ppm) 29.9 (C-20); 19.0 (C-21); 34.2 (C-22); 28.3 (C-23); 41.8 (C-24); 30.5 (C-25); 18.6 (C-26); 19.8 (C-27); 31.5 (C-28); 67.3 (C-29) practically coincided with the signals of the side chain of glycoside (VII). From this, we concluded that glycoside (I) had a stigmastane skeleton, and the carbohydrate chain was attached to C-29 of the aglycon.

The R-configuration of C-20 in (I) was determined from the CS of the CH_3-21 protons (0.94 ppm) in the PMR spectrum (CD_3OD) [6].

According to information in the literature [7], for 29-hydroxyclionasterol (24R) (VII) and 29-hydroxysitosterol (24S) (IX), in the case of the (24R)-configuration the difference in the CSs of the CH_3-26 and CH_3-27 protons amounts to 0.3 ppm, while in the case of the (24S)-configuration the doublets of these methyl groups are superposed. Furthermore, it has been shown for (24R)- and (24S)-24-ethyl-5α-cholest-7-ene-3β,29-diols (Xa and Xb) that in the case of the (24R)-configuration the C-26 and C-27 signals in the ^{13}C NMR spectrum differ by 1 ppm, while for the (24S)-isomer they coincide [7]. For glycoside (I), the difference between the signal of the CH_3-26 and CH_3-27 protons amounted to 0.02 ppm (Table 3), and that between the signals of the carbon atoms to 1.2 ppm (Table 1). On this basis we assumed that the C-24 asymmetric center in (I) had the R-configuration.

By using expedients analogous to those described above, we also determined the structure of the aglycon of glycoside (II). Spectral information for it is given in Tables 1 and 3. It was established that, in comparison with glycoside (I), compound (II) had an additional hydroxy group at C-4. In actual fact, a comparison of the sections of the ^{13}C NMR and PMR spectra of (II) relating to the A/B rings with the corresponding sections of the spectra of echinasteroside B_2 (III) [2] revealed the presence in (II) and (III) of an identical 3β,4β,6β-trihydroxy fragment.

Thus, the structure of crossasteroside P_1 has been established as (24R)-24-ethyl-5α-cholestane-3β,6β,8,15α,16β,29-hexaol 29-O-[O-(2-O-methyl-β-D-xylopyranosyl)-(1 → 2)-β-D-galactofuranoside]. Crossasteroside P_2 is its 4β-hydroxy analogue. This is the first time that galactose has been found in polyhydroxysteroid glycosides.

EXPERIMENTAL

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

Isolation of Crossasterosides P_1 and P_2. A mixture of crossasterosides P_1 and P_2 not separable by column chromatography was obtained from the total fraction of polyhydroxysteroids of C. papposus by a method described previously [9]. Acetylation of the mixture with acetic anhydride in pyridine (1:1) led to the combined acetates. The combined acetates of

the glycosides (150 mg) were chromatographed on columns of silica gel in the hexane–ethyl acetate (1:1) system and of Florisil in the hexane–ethyl acetate (18:10) system. This gave 78 mg of crossasteroside P_1 acetate and 51 mg of crossasteroside P_2 acetate.

By saponifying the acetates with a 3% solution of sodium methanolate and separating the products by column chromatography on Polychrome-1 in the ethanol–water (from 0:100 to 50:50) system and on silica gel in the chloroform–ethanol–water (300:100:to saturation) system we obtained 39 mg of crossasteroside P_1 (0.0017% of the lyophilizate of the ethanolic extract and 24 mg of crossasteroside P_2 (0.0011% of the lyophilizate of the ethanolic extract).

Crossasteroside P_1 (I), $C_{41}H_{72}O_{15}$, amorphous, $[\alpha]_{Hg}$ −12.9° (c 1.13; methanol).

Crossasteroside P_2 (II), $C_{41}H_{72}O_{16}$, amorphous, $[\alpha]_{Hg}$ −14.0° (c 1.00; methanol).

Hydrolysis of Crossasterosides P_1 and P_2. The acid hydrolysis of (I) and (II) was carried out with 2N HCl at 100°C for 2 h. D-Galactose and 2-O-methyl-D-xylose were identified by the methods of TLC on silica gel and Silufol in the butanol–acetone–water (4:5:1) system and by GLC and GLC-MS of the corresponding aldononitrile acetates. The total monosaccharides from (I) had $[\alpha]_{Hg}$ +22.0° (c 0.05; water), and those from (II) $[\alpha]_{Hg}$ +24.0° (c 0.05; water). The values calculated from the literature [1] for the sum of β-D-galactose and 2-O-methyl-D-xylose (1:1) is $[\alpha]_D$ +30.3° (water), and for the sum of α-L-galactose and 2-O-methyl-D-xylose (1:1) $[\alpha]_D$ −143.9° (water).

SUMMARY

Two new steroid glycosides have been isolated from Crossaster papposus and characterized: (24R)-24-ethyl-5α-cholestane-3β,6β,8,15α,16β,19-hexaol 29-O-[O-(2-O-methyl-β-D-xylopyranosyl)-(1 → 2)-β-D-galactofuranoside] – crossasteroside P_1 – and its 4β-hydroxy analogue – crossasteroside P_2.

LITERATURE CITED

1. F. Micheel and A. Klemer, Chemie der zucker und Polysaccharide, Academische Verlagsgesellschaft, Geest und Portig, Leipzig (1956), pp. 400, 435.
2. É. V. Levina, A. I. Kalinovskii, P. V. Andriyashchenko, and A. A. Kicha, Khim. Prir. Soedin., No. 2, 246 (1987).
3. A. A. Kicha, A. I. Kalinovskii, E. V. Levina, and P. V. Andriyashchenko, Khim. Prir. Soedin., No. 6, 801 (1985).
4. R. G. S. Ritchie, N. Cyr. B. Korsch, H. J. Koch, and A. S. Perlin, Can. J. Chem., 53, 1424 (1975).
5. A. A. Kicha, A. I. Kalinovskii, É. V. Levina, Ya. V. Rashkes, V. A. Stonik, and G. B. Elyakov, Khim. Prir. Soedin., No. 3, 356 (1985).
6. D. J. Vanderah and C. Djerassi, J. Org. Chem., 43, No. 7, 1442 (1978).
7. R. Riccio, M. V. D'Auria, M. Iorizzi, L. Minale, D. Laurent, and D. Duhet, Gazz. Chim. Ital., 115, No. 8, 405 (1985).
8. A. A. Kicha, A. I. Kalinovskii, and É. V. Levina, Khim. Prir. Soedin., No. 6, 738 (1984).
9. A. A. Kicha, A. I. Kalinovsky [Kalinovskii], E. V. Levina, V. A. Stonik, and G. B. Elyakov, Tetrahedron Lett., 24, No. 36, 3893 (1983).