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TRITERPENE GLYCOSIDES FROM THE HOLOTHURIAN Cucumaria frondosa

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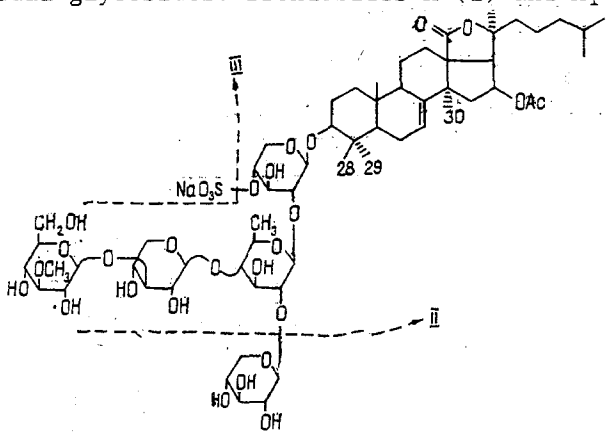
Two triterpene glycosides have been isolated from an alcoholic extract of the holothurian Cucumaria frondosa collected in the Kola littoral, Barents Sea: the main component of the glycoside fraction – frondoside A (I) – and a minor component – frondoside A_1 (II).

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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TABLE 1. ^{13}C NMR Spectra of Glycosides (I), (II), and (III) in C_5D_5N ($\delta_{TMS} = 0$)

Atom	I	III	II	Atom	I	III	II
C-1	36,1	36,2	36,2	C_1^1	104,9	105,0	105,3
C-2	27,0	27,1	27,0	C_2^1	81,6	82.9	83,2
C-3	89,1	89,1	89,1	C_3^1	75,8	77.7	76,1
C-4	39,6	39,6	39,6	C_4^1	76,1	70.3	75,5
C-5	48,2	48,2	48,2	C_5^1	64.4	66,9	64,2
C-6	23.4	23,4	23,4	C_1^2	102,3	103,0	105.3
C-7	120,3	120.2	120,3	C_2^2	82,3	83,1	76,3
C-8	145.6	145,6	145,7	C_3^2	75,0	75,5	75,0
C-9	47,2	47,2	47,2	C_4^2	85,5	85 3	85,3
C-10	35,6	35,7	35,7	C_5^2	71,1	71,2	71,7
C-11	22,6	22,7	22,7	C_6^2	18,0	18,0	18,0
C-12	31.5	31,5	31,6	C_1^3	104.9	104,8	105,0
C-13	59,3	59.4	59,4	C_2^3	73,5	73,2	73,5
C-14	47,5	47,5	47,5	C_3^3	87,4	87,5	87,4
C-15	43.7	43,7	43,7	C_4^3	69,0	69,0	69.0
C-16	75,4	75.0	75.3	C_5^3	66,4	66,4	66,5
C-17	54,8	54,8	54,9	C_1^4	105,3	105,3	104,9
C-18	179,2	179.1	179,2	C_2^4	74,9	74,9	74,9
C-19	24,0	24,0	24,0	C_3^4	87,8	87,8	87,8
C-20	84,9	84,9	85,0	C_4^4	70,7	70,7	70.7
C-21	28,4	28,3	28,4	C_5^4	78,1	78,1	78,1
C-22	39,2	39,2	39,1	C_6^4	62,2	62,2	62,2
C-23	22,4	22,4	22,4	OMe	60,6	60,6	60.6
C-24	39.7	39,7	39,7	C_1^5	105,3	105,8	—
C-25	28,2	28,2	28,2	C_2^5	74,9	75,5	—
C-27	22,8	22,8	22,8	C_3^5	76,4	77,1	—
C-28	17,5	17,5	17,4	C_4^5	70,5	70,4	—
C-29	28,8	28,9	28,8	C_5^5	66,4	66,4	—
C-30	32,2	32,2	32,3				
OAc	169,5 21,2	169,4 21,3	169,5 21,3				

nals of the known pseudostichoposide A from the holothurian Pseudostichopus trachus [2], which has an analogous carbohydrate chain. To prove the structure of glycoside (II), we subjected frondoside A (I) to Smith degradation, in which the terminal xylose residue was split off, giving a progenin identical in its physical constants and ^{13}C NMR spectrum with frondoside A_1.

From these results it followed that frondoside A_1 (I) is 16β-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl-(1→2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy]holost-7-ene.

Thus, the main component of the glycoside fractions from the Atlantic holothurian Cucumaria frondosa collected in the Kola littoral, Barents Sea, and that from the same species collected in the Atlantic littoral of Canada are one and the same glycoside – frondoside A. At the same time, neither frondoside A nor frondoside A_1 is identical with any of the glycosides known at the present time from the far-eastern holotype, Cucumaria japonica [3], which is morphologically so close to Cucumaria frondosa that they were assigned to a single species comparatively recently [4].

EXPERIMENTAL

Melting points were determined on a Boëtius stage. Specific rotations were measured on a Perkin–Elmer 141 polarimeter at room temperature. ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer. GLC analysis was conducted on a Tsvet-110 chromatograph with 0.3 × 150 cm steel columns containing 3% of QF-1 on Chromaton N-HMDS, using argon as the carrier gas (60 ml/min) at column temperatures of 150-220°C, 5°C/min.

The Cucumaria frondosa was collected in Dal'ne-Zelenetskaya Bay (Kola littoral, Barents Sea) from a depth of 10-15 m in August, 1988.

Isolation of the Glycosides. The comminuted animals (40 kg) were extracted three times with hot ethanol, and the combined extracts were evaporated in vacuum to dryness. The residue was chromatographed on a column with the polytetrafluoroethylene powder Polikhrom-1 ($H_2O \rightarrow$ 50% C_2H_5OH), and then on silica gel in the chloroform–ethanol–water (100:100:17) system. The total glycosides obtained were separated on silica gel in the chloroform–methanol–water (100:75:9) system. The fractions containing frondosides A and A_1 were separated into their individual components by HPLC on a 10 × 150 mm Silasorb C-18 column with the eluent water–ethanol (55:45) at 3 ml/min. This gave frondoside A (I), mp 234-236°C, $[\alpha]_{578}$ −31° (c 0.1; pyridine), and frondoside A_1 (II), mp 242-244°C, $[\alpha]_{578}$ −14° (c 0.1; pyridine).

Acid Hydrolysis of (I) and (II). A solution of 5 mg of one of the glycosides in 2 ml of 2 N HCl was heated at 90-100°C for 2 h. The reaction mixture was extracted with chloroform, and the aqueous layer was neutralized with Dowex anion-exchange resin (HCO_3^-). The resin was filtered off and was washed with water. The aqueous layer and the wash-waters were combined and were concentrated in vacuum to dryness. The residue was dissolved in 1 ml of dry pyridine, 5 mg of hydroxylamine hydrochloride was added, and the mixture was heated at 100°C for 1 h; then 1 ml of acetic anhydride was added and the reaction mixture was heated at 100°C for another 1 h. After this it was evaporated and was analyzed by GLC.

Desulfation of Frondoside A (I). A solution of 25 mg of glycoside (I) in 10 ml of pyridine–dioxane (1:1) was boiled for 1 h. The reaction mixture was evaporated to dryness, and the residue was purified by column chromatography on silica gel in the chloroform–methanol–water (65:15:2) system. This gave 20 mg of derivative (III), mp 242-246°C, $[\alpha]_{578}$ −23° (c 0.1; pyridine).

Smith Degradation of Frondoside A. A solution of 100 mg of glycoside (I) in 50 ml of water was treated with 200 mg of $NaIO_4$, and the mixture was left at room temperature for three days. The product was freed from salts on a column with the polytetrafluoroethylene powder Polikhrom-1 (water→alcohol) and the aqueous-alcoholic eluate (200 ml) was reduced with 10 mg of $NaBH_4$. The solution was acidified with CH_3COOH to pH 5 and was evaporated to dryness with the addition of butanol and methanol (successively), the residue was dissolved in 30 ml of 0.5 N HCl, and the resulting solution was left for 3 h. Then the acid was eliminated on a column of polytetrafluoroethylene powder (water→alcohol). The reaction product was chromatographed on silica gel in the chloroform–methanol–water (65:25:4) system, giving 25 mg of glycoside (II).

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