TRITERPENE GLYCOSIDES OF THE HOLOTHURIAN Eupentacta pseudoquinquisemita

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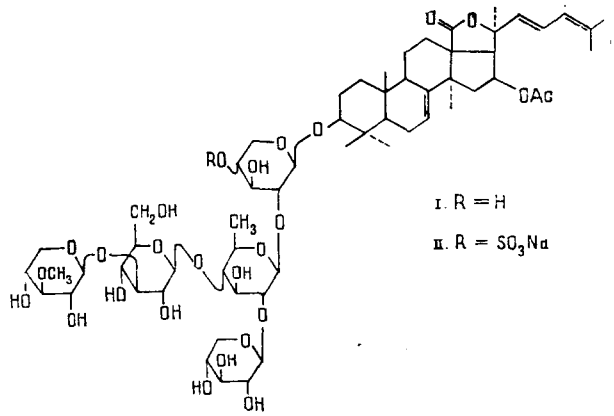
The holothurian Eupentacta pseudoquinquisemita Deichmann collected in Kraternaya Bay, Ushishir Islands has yielded two triterpene pentaosides – the previously known cucumarioside C_2, and cucumarioside H, which is a new glycoside. With the aid of ^{13}C NMR spectroscopy and solvolytic desulfation its structure has been determined as 6β-acetoxy-3β-([3-O-methyl-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)] [β-D-xylopyranosyl-(1 → 4)] [β-D-xylopyranosyl-(1 → 2)]-β-D-quinovopyranosyl-(1 → 2)-(4-O-sulfato-β-D-xylopyranosyloxy)holosta-7,22,24(trans)-triene. Cucumarioside H was also identified in Eupentacta (= Cucumaria) fraudatrix from Posyet Bay, Sea of Japan.

Previously, cucumariosides G_1, C_1 and C_2 were isolated from the holothurian Eupentacta (= Cucumaria) fraudatrix [1, 2] and their structures were established. Continuing chemical investigations of holothurians of this genus we have studied the main components of the glycosidic fraction of the holothurian Eupentacta pseudoquinoquisemita Deichmann (Cucumariidae, Dendrochirota), collected in Kraternaya Bay, Ushishir Islands (Kurile Islands).



From the total glycosidic fraction of E. pseudoquinquisemita, obtained by the usual method [3], we isolated with the aid of reversed-phase chromatography two individual glycosides ((I) and (II)). In the products of the acid hydrolysis of glycoside (I) we identified, in the form of aldononitrile acetates, 3-O-methylxylose, quinovose, xylose, and glucose in a ratio of 1:1:2:1. The ^{13}C and ^{1}H NMR spectra of this glycoside taken in dimethyl sulfoxide and pyridine, respectively, and also the constants were identical with the spectra and constants of cucumarioside C_2 from E. fraudatrix, the structure of which had been established previously [2]. Thus, glycoside (I) was identified as cucumarioside C_2.

On acid hydrolysis, glycoside (II) also gave 3-O-methylxylose, quinovose, xylose, and glucose (1:1:2:1). In its UV spectrum a maximum was observed at 240 nm, which indicated the presence of a conjugated diene grouping in the aglycon. The signals in the aglycon part of the ^{13}C NMR spectrum of glycoside (II) coincided with the corresponding signals in the spectrum of (I). The signals in the carbohydrate part of the spectrum were close to the signals in the spectrum of (I). The differences consisted mainly in a shift of the signal of the C-5 methylene carbon of the first xylose residue from 65.2 ppm in the spectrum of (I) to 63.1 ppm in the spectrum of (II).

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TABLE 1. ^{13}C NMR Spectra of Glycosides (I), (II) (solvent DMSO-d_6, 39.6 ppm)

Atom	I	II	Atom	I	II	Atom	I	II
C-1	35,3	35,2	C-22	133,7	133,7	C_6^2	18,0	18,1
C-2	26,1	26,1	C-23	121,4	121,5	C_1^3	102,7	102,8
C-3	88,1	88,1	C-24	124,4	124,5	C_2^3	72,5	72,6
C-4	*	*	C-25	133,9	133,9	C_3^3	85,8	85,8
C-5	47,0	47,2[a]	C-26	25,5	25,5	C_4^3	68,1	68,2
C-6	22,4	22,5	C-27	17,3	17,3	C_5^3	76,2	76,2
C-7	119,2	119,0	C-30	16,8	16,8	C_6^3	60,7	60,7
C-8	144,8	145,0	C-31	28,1	28,2	C_1^4	104,2	104,2
C-9	46,5	46,6	C-32	31,9	31,9	C_2^4	73,1	73,2
C-10	34,8	34,9	OAc	169,2	169,3	C_3^4	85,8	85,8
C-11	21,6	21,6		21,0	20,9	C_4^4	69,7	68,7
C-12	30,0	30,0	C_1^1	103,7	103,5[d]	C_5^4	65,6[a]	65,6[b]
C-13	58,1	58,1	C_2^1	81,0	79,6	OCH_3	59,6	59,6
C-14	47,0	47,0[a]	C_3^1	76,2	74,5[c]	C_1^5	103,7	103,7[d]
C-15	42,4	42,5	C_4^1	68,9	74,3[c]	C_2^5	73,8	73,5
C-16	72,1	72,2	C_5^1	65,2	63,1	C_3^5	75,3	75,0
C-17	55,2	55,3	C_1^2	101,0	100,2	C_4^5	68,2	69,3
C-18	178,5	178,5	C_2^2	81,0	80,8	C_5^5	65,5[a]	65,1[b]
C-19	23,4	23,5	C_3^2	74,4	75,0[c]			
C-20	82,8	82,8	C_4^2	85,3	85,3			
C-21	29,7	29,8	C_5^2	69,6	69,7			

a,b,c,d – assignment of the signals ambiguous; * – signal masked by the signals of the solvent.

Corresponding shifts were suffered by the C_4^1 and C_3^1 signals (+5.4 and −1.7 ppm, respectively). Such shifts are characteristic of the α- and β-effects of a sulfate group [4]. In the spectrum of (II), the C_2^1 signal (79.6 ppm) was shifted upfield by 1.4 ppm relative to the C_2^1 signal in the spectrum of (I) (81.0 ppm). Boiling glycoside (I) in a mixture of dioxane and pyridine led to solvolytic desulfation. By means of its ^{13}C NMR spectrum and physical constants, we identified the desulfated derivative as cucumarioside C_2 (I). Thus, glycoside (II) is a sulfated derivative of cucumarioside C_2, the sulfate group being attached in position 4 of the first xylose residue. Glycoside (II) was not previously known. We propose to call it cucumarioside H.

After the determination of the structure of cucumarioside H, we made a careful study of the total glycosides from E. fraudatrix. With the aid of reversed-phase chromatography on polikhrom-1 we isolated cucumarioside H and identified it from its ^{13}C spectrum in extracts from this holothurian, also, although it was a minor component in the glycosidic fraction.

Thus, the chemical structures of the components of the glycosidic fractions of E. pseudoquinquisemita and E. fraudatrix coincide. Posyet Bay, where the E. fraudatrix was collected, and Kraternaya Bay, where E. pseudoquinquisemita was collected, are about a 1000 km apart. Moreover, Kraternaya Bay is a seawater-filled crater of the active volcano Ushishir [6]. The ecological system of this bay, which has developed close to submarine outlets of hydrothermal and gaseous sources, is unique in the opinion of marine biologists [5]. The coincidence of the structures of the glycosides from holothurians of the genus

Eupentacta collected, on the one hand, at a considerable distance from one another, and on the other hand, living under greatly differing ecological conditions confirms the conclusion drawn previously that the structure of the triterpene glycosides of holothurians does not depend on ecological factors but is determined genetically and is a reliable chemosystematic characteristic [7].

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. PMR spectra were taken on the same instrument at 250 MHz. UV spectra were recorded on a Specord UV-VIS spectrophotometer. GLC analysis was performed on a Tsvet-110 chromatograph using 0.3 × 150 cm columns with 3% of QF-1 on Chromaton N-HMDS, with argon as the carrier gas (60 ml/min) at temperatures of 150-220°C, 5°C/min. The counter-ion in the sulfate group of (II) was determined as sodium on a AA-780 atomic absorption spectrometer.

Eupentacta pseudoquinquisemita was collected during the second voyage of the Research ship "Akademik Oparin" in Kraternaya Bay, Island of Yankicha, Ushishir Islands, Kurile Islands, at a depth of 10-15 m in September, 1986. The holothurian was determined by A. V. Smirnov. E. fraudatrix was collected in Troitsa Inlet, Pos'et Bay, Sea of Japan, at a depth of 0.5-1 m. The holothurian was determined by V. F. Levin.

Isolation of the Glycosides. The total glycosidic materials were obtained from extracts of the holothurians as described in [3]. Individual glycosides were obtained by chromatography on polikhrom-1 ($H_2O \rightarrow 30\%$ C_2H_5OH). Cucumarioside C_2 (I), mp 198-200°C, $[\alpha]_D^{20}$ −48° (c 0.1; pyridine). Cucumarioside H (II), mp 205-208°C, decomp., $[\alpha]_D^{20}$ −71°C (c 0.1; pyridine), UV spectrum (MeOH): λ_{max} 240 nm.

Acid Hydrolysis of (I) and (II). A solution of 10 mg of one of the glycosides in 2 ml of 12% HCl was heated at 90-100°C for 2 h. Then the reaction mixture was extracted with chloroform, and the aqueous layer was neutralized with Dowex anion-exchange resin (HCO_3^-). The resin was separated off by filtration and was washed with water. The aqueous layer and the wash-waters were combined and 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, after which 1 ml of acetic anhydride was added to the reaction mixture and it was heated at 100°C for another 1 h. Then it was evaporated and the residue was analyzed by GLC. The peracetates of the aldononitriles of 3-O-methylxylose, quinovose, xylose and glucose were identified in a ratio of 1:1:2:1.

Desulfation of Glycoside (II). A solution of 25 mg of glycoside (II) in 10 ml of a mixture of pyridine and dioxane (1:1) was boiled for 1 h. Then it was evaporated to dryness and the residue was purified by column chromatography on silica gel in the chloroform–methanol–water (75:25:1) system. This gave 20 mg of glycoside (1).

SUMMARY

It has been shown that cucumarioside H from holothurians of the genus Eupentacta is 16β-acetoxy-3β-{[3-O-methyl-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)][β-D-xylopyranosyl-(1 → 2)]-β-D-quinovopyranosyl-(1 → 2)-4-O-sulfato-β-D-xylopyranosyloxy}-holosta-7,22,24(trans)-triene.

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