spectrum, m/z(%): M^+ 306 (1), 291 ($M^+ - CH_3$, 100), 273 ($M^+ - CH_3 - H_2O$, 38), 257 ($M^+ - H_2O - CH_2OH$, 0), 243 (8), 208 (35), 205 (28), 177 (61), 135 (34), 121 (34), 109 (49).

The mass spectra of (Ib) and of 19-hydroxy(epimannoyl oxide) [4] were identical. The cyclohexylammonium salt of the acid (I) (Ic) had mp 113-115°C, $[\alpha]_D$ −29.3° (c 1.0; ethanol). ν_{max}^{KBr}, cm^{-1}: 1645, 3045 (C=C), 1327, 1540 (COO^-).

The hydrogenation of (I) in the presence of platinum black in ethanol solution gave 8,13-epoxylabdan-19-oic acid (Id). mp 61-63°C, $[\alpha]_D$ −23.1° (c 1.0; ethanol). M^+ 322. λ_{max}^{KBr}, cm^{-1}: 1465, 1696 (C=O), 943, 1235 (OH), 1275 (C=O).

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GLYCOSIDES OF MARINE INVERTEBRATES. XIV. STRUCTURE OF HOLOTHURIN B_1 FROM THE HOLOTHURIAN *Holothuria floridana*

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UDC 547.925:593.96

The complete structure of a triterpene oligoside — olothurin B_1 — from the holothurian *H. floridana* of the sublittoral of the island of Cuba has been established.

We have previously established the structure of the native aglycone of holothurin B_1 as (I) [1, 2]. The results of acid hydrolysis showed that holothurin B_1 is a sulfated bioside containing D-xylose and D-quinovose as monosaccharide residues. The structure of the carbohydrate chain of the glycoside was not established.

In the present paper we give the complete structure of holothurin B_1. Below are presented the characteristics of the ^{13}C NMR spectra of the carbohydrate chain of holothurin B_1 and its desulfated derivative (2 and 3, respectively):

Atom	2	3	Atom	2	3
C_1^1	105.6	105.79	C_1^2	105.1	105.4
C_2^1	83.05	83.5	C_2^2	76.48	76.8
C_3^1	76.48	77.78	C_3^2	77.52	77.78

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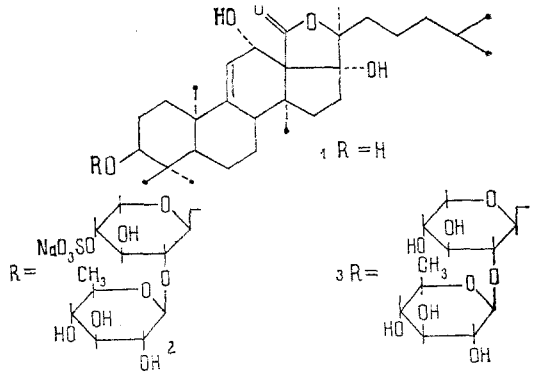
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 482-484, July-August, 1982. Original article submitted August 11, 1981.

0009-3130/82/1804-0449$07.50

C_4^1	75.11	70.83	C_4^2	76.48	76.8
C_5^1	64.2	66.41	C_5^2	73.3	73.3
			C_6^2	18.45	18.45

The C-3 signal in the ^{13}C NMR spectrum of (2) is in a weaker field (88.6 ppm) than the same signal in the spectrum of the aglycone obtained by the acid hydrolysis of the glycoside (78.2 ppm) [1]. This shows the attachment of the carbohydrate chain of the glycoside in position 3 of the aglycone. The values of the C_1^1 and C_1^2 signals (105.6 and 105.1 ppm) in the ^{13}C spectrum of (2) confirm the β configurations of both anomeric carbon atoms of the carbohydrate chain of the glycoside [3, 4]. In the desulfated glycoside (3), the C-4 signal of the cylose residue is located at 70.83 ppm (see above), in a stronger field than the corresponding signal in the ^{13}C NMR spectrum of the initial glycoside (2) (75.11 ppm). Consequently, in compound (2) the sulfate group is attached to the C-4 atom of the xylose residue.



Structure of holothurin B_1 from the holothurian Holothuria floridana.

A comparison of the chemical shifts of the signals of the carbon atoms of the carbohydrate chains of compounds (2) and (3) with those for holothurin B from the holothurians *H. atra* [3] and *H. leucospilota* [4], having O-β-quinovopyranosyl-(1 → 2)-(4-sulfato-β-D-xylopyranosyl) as the carbohydrate chain showed coincidence of the signals. However, the same set of signals of the carbon atoms also corresponds to a O-(4-sulfato-β-D-xylopyranosyl)-(1 → 2)-β-D-quinovopyranosyl structure of the carbohydrate chain of the glycoside. We therefore carried out an additional chemical proof of the structure of the carbohydrate chain in (2). Methanolysis of the fully methylated desulfated holothurin B_1 followed by acetylation of the products formed confirmed the facts that only one carbohydrate chain was present in (3) and that the terminal monosaccharide in this chain was D-quinovose. In the reaction products we identified methyl 2-O-acetyl-3,4-di-O-methyl-α- and -β-xylopyranosides and methyl 2,3,4-tri-O-methyl-α- and -β-quinovopyranosides. The facts given above permit us to put forward structure (2) as that of holothurin B_1.

Holothurin B_1 differs in the structure of its aglycone from holothurin A_1 isolated from the same species of holothurians and characterized by us previously [5]. We are the first to have reported the fact that holothurians contain the glycosidic pairs holothurin A and holothurin B with different aglycones for animals of the family Holothuriidae. It would be logical to suggest that the holothurians use for the construction of the holothurins B and A already-formed aglycones, adding to them first two monosaccharides, xylose and quinovose, and then another two, glucose and 3-O-methylglucose. However, our results indicate that a transformation of the algycone, and in particular, oxidation, may take place even in a synthesized glucoside. The isolation and proof of the structure of the new triterpene glycosides of holothurians will give valuable information for establishing the biosynthetic pathways of these compounds in the animal organism.

There is a report by Japanese authors [6] on the isolation of a similar glycoside from another species of holothurian *Actinopyga echinites*.

EXPERIMENTAL

The ^{13}C NMR spectra were taken on a Bruker HX-90 E instrument in pyridine with tetramethylsilane as internal standard, and the IR spectra on a Specord IR-75 instrument in chloroform. The chromato-mass spectrometry of the products of methanolysis was performed on a LKB-9000S instrument at an ionizing voltage of 70 eV using a 3 m × 3 mm column containing 3% of QF-1 on Chromosorb W. The temperature of analysis was 100-200°C. GLC analysis was performed on Tsvet-100 chromatograph in columns containing 5% of QF-1 on Chromaton N, the temperature of the analysis again being 100-200°C.

The isolation of holothurin B_1 and its constants have been given in a previous paper [1]. The solvolytic cleavage of the pyridinium salt of holothurin B_1 was carried out as described by Kitagawa et al. [7]. The desulfated holothurin B_1 was purified on a column of KSK silica gel in the chloroform–methanol (1:1) system. A chromatographically homogeneous product was obtained.

Methylation and Methanolysis of Glycoside (3). A solution of 20 mg of (3) in 2 ml of absolute dimethyl sulfoxide was treated with 2 ml of the carbanion prepared from 200 mg of sodium hydride and 10 ml of absolute dimethyl sulfoxide in an atmosphere of nitrogen. The reaction mixture was stirred in an atmosphere of nitrogen at 45°C for 2 h. Then 3 ml of methyl iodide was added and the mixture was left for 12 h, after which it was worked up by the usual method. This gave 17 mg of methylation product, which was purified on a column of KSK silica gel in the chloroform–methanol (1:1) system. The yield of fully methylated glycoside (3) with mp 227°C was 4.5 mg. IR spectrum ($CHCl_3$, cm^{-1}): 1747, 1732, 1086. A mixture of 4.5 mg of the methylated glycoside (3) and 2 ml of absolute methanol containing 10% of HCl was boiled for 2 h. The reaction was dried, acetylated in the usual way, and analyzed by GLC.

SUMMARY

The structure of a triterpene oligoside — holothurin B_1 from the holothurian *H. floridana* of the sublittoral of the island of Cuba — has been established.

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