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GLYCOSIDES OF MARINE INVERTEBRATES.

XV. A NEW TRITERPENE GLYCOSIDE — HOLOTHURIN A_1 — FROM CARIBBEAN HOLOTHURIANS OF THE FAMILY HOLOTHURIIDAE*

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A glycoside, holothurin A_1 has been isolated from the polar glyosidic fractions of the holothurians *H. floridana* and *H. grisea*. The complete structure of the glycoside has been established; it is: 3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)-(4-sulfato-β-D-xylopyranosyl)oxy]holosta-9(11)-ene-12α,17α,22ξ-triol. Details of the IR and ^1H and ^{13}C NMR spectra of the compounds obtained are given.

It has been established that holothurians of the family of Holothuriidae synthesize, in addition to triterpene biosides (holothurins B), a series of polar glycosides — tetraosides (holothurins A) [1, 2] and hexaosides (bivittosides [3]). The results of a study of the products of acid hydrolysis of these metabolites has permitted the conclusion to be drawn that they are not individual compounds but difficulty separable mixtures of substances close in structure. The sets of components in these mixtures are different for different species of holothurians [1]. Recently, in connection with the study of the mechanism of the physiological action of the holothurian triterpene glycosides and the biosynthesis of these compounds, great value has been placed upon work on the isolation of individual substances from the glycosidic fractions and the determination of their complete chemical structures. For glycosides of the holothurin A type, the chemical structures of only a few oligosides having the aglycones (I) and (II) have been established [4, 5].

We have shown that in the polar glycosidic fractions of holothurians of the sublittoral of the island of Cuba, *Holothuria floridana* and *Holothuria grisea* (the species of holothurian were determined by V. V. Kiselev), include a new triterpene glycoside — holothurin A_1.

The compositions of the holothurin A fractions of the holothurians studied were different. Thus, the glycosidic fraction of the holothurian *H. floridana* contained, in addition to holothurin A_1 (65%), a glycoside with the aglycone (I), and the same fraction of *H. grisea* also contained oligosides with the genins (I), (II), and (IIa). To separate compounds so close in structure we used reversed-phase chromatography on Polykhrom-1 and isolated the individual holothurin A_1 (IV) from the glycosidic fraction of *H. floridana*. The IR spectrum of (IV)

*For Communication XIV, see. p. 449.

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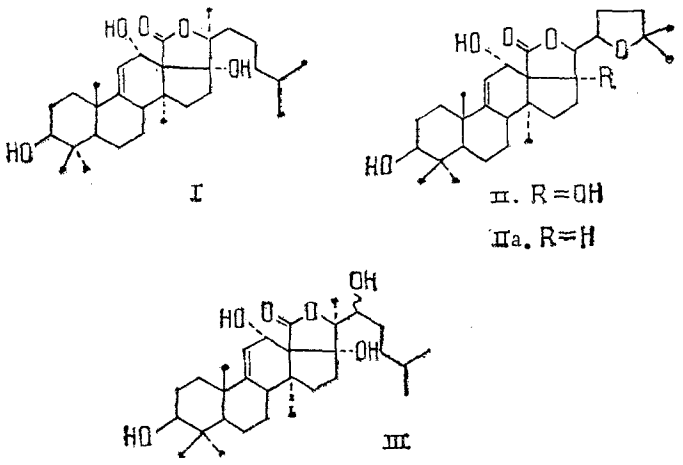

Fig. 1. Structures of the native aglycones of glycosides of the holothurin A type isolated from holothurians of the family Holothuriidae.

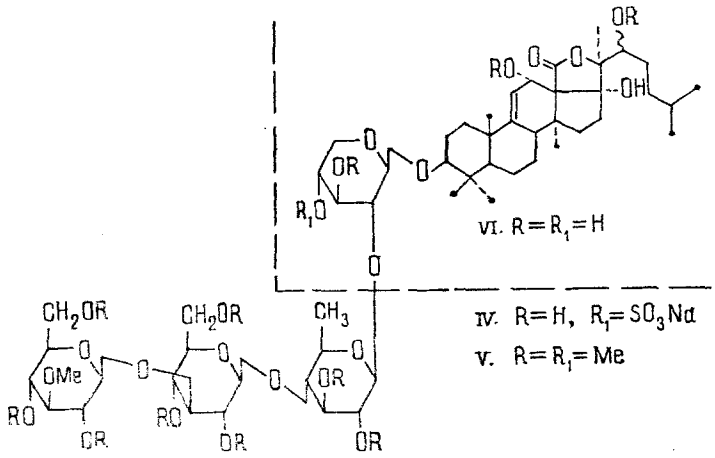

Fig. 2. Structure of holothurin A_1 from the Caribbean holothurians *H. floridana* and *H. grisea*.

(ν_{max}^{KBr}: 1325 and 825 cm^{-1}) and the result of solvolytic cleavage of the pyridinium salt [6] showed that the glycoside (IV) includes a sulfate group. We have previously reported [7] that D-glucose, 3-O-methyl-D-glucose, D-quinovose, and D-xylose residues are present in the glycoside in a ratio of 1:1:1:1, and the glycosidic bonds have the β configuration. On the basis of features of the ^1H and ^{13}C NMR spectra of the glycoside [7], the structure of the native aglycone of holothurin A_1 was established as holost-9(11)-ene-3β,12α,22ξ-tetraol (III). The structure of the native aglycone was confirmed by the result of the acid hydrolysis of compound (IV), which led to the formation of a single aglycone — holosta-7,9(11)-diene-3β, 17α,22ξ-triol (griseogenin) [7, 8].

The structure of the carbohydrate chain of the glycoside was established by the usual methods of carbohydrate chemistry — methylation with subsequent methanolysis of the completely methylated product (V), periodate oxidation of the glycoside, and Smith degradation [9].

The methylation of glycoside (IV) under the conditions of the Hakomori reaction [10] led to the formation of the methylated product (V). The methanolysis of compound (V) with subsequent acetylation confirmed the presence in compound (IV) of a single unbranched carbohydrate chain. In the reaction products we identified methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides, methyl 4-O-acetyl-2,3-di-O-methyl-α- and -β-quinovopyranosides, and methyl 2-O-acetyl-3,4-di-O-methyl-α- and -β-xylopyranosides. The formation of methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides and of methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides shows that the terminal monosaccharide residue can only be 3-O-methylglucose, and the

TABLE 1. Characteristics of the ^{13}C NMR Spectrum of Holothurin A_1

C atom	δ, ppm	C atom	δ, ppm	C atom	δ, ppm	C atom	δ, ppm	C atom	δ, ppm
C_1	36,6[b]	C_{12}	71,7	C_{23}	30,8	C_4^1	75,4	C_4^3	69,8
C_2	27,4[a]	C_{13}	59,5	C_{24}	36,5	C_5^1	64,1	C_5^3	77,6
C_3	88,9	C_{14}	46,1	C_{25}	28,4	C_1^2	105,1	C_6^3	62,1
C_4	40,1	C_{15}	37,0[c]	C_{26}	22,9	C_2^2	76,2	C_1^4	105,4[c]
C_5	53,0	C_{16}	36,0[b]	C_{27}	22,7	C_3^2	75,4	C_2^4	75,1
C_6	21,7	C_{17}	90,2	C_{30}	16,8	C_4^2	86,7	C_3^4	87,7
C_7	28,0[a]	C_{18}	174,5	C_{31}	28,4	C_5^2	71,7	C_4^4	71,0
C_8	41,2	C_{19}	22,7	C_{32}	20,3	C_6^2	18,1	C_5^4	78,1
C_9	153,9	C_{20}	87,1	C_1^1	105,1[c]	C_1^3	104,6	C_6^4	62,3
C_{10}	39,8	C_{21}	19,2	C_2^1	83,2	C_2^3	73,9	OCH_3	60,6
C_{11}	115,5	C_{22}	75,3	C_3^1	76,2	C_3^3	88,2		

a, b, c — assignment of the signals ambiguous.

glucose is linked to the monosaccharides by a (1 → 3) bond. This was confirmed by the periodate oxidation of compound (IV), in which only one monosaccharide residue — the quinovose residue — was destroyed. According to the results of methanolysis and periodate oxidation, the quinovose residue is bound in the carbohydrate chain by 1 → 4 bonds. When the desulfated glycoside (IV) was subjected to periodate oxidation, two monosaccharide residues — those of quinovose and xylose — were oxidized. It follows from this and from the methanolysis results that the xylose residue contains the sulfate group in position 3 or 4. The choice between these carbon atoms was made on the basis of an analysis of the ^{13}C NMR spectrum of holothurin A_1, in which the signals of the C-4 and C-5 atoms of the xylose residue are located at (δ, ppm) 75.06 and 64.1 ppm (Table 1), respectively. Such a spectrum is characteristic for a monosaccharide sulfated at position 4 [4, 11]. The sequence of linkage of the monosaccharides in the carbohydrate chain of the glycoside was determined by the Smith cleavage of glycoside (IV). This gave a progenin (VI) having only one monosaccharide — xylose. Consequently, the quinovose residue cleaved on periodate oxidation is attached to the xylose. On the basis of all the results obtained, the complete structure of holothurin A_1 can be represented by formula (IV).

The holothurin A_1 isolated from the holothurian *H. grisea* was, according to ^1H and ^{13}C NMR spectra and the results of acid hydrolysis and of methylation followed by methanolysis, identical with the holothurin A_1 from *H. floridana*. We ascribed some noncorrespondence of the analytical results to the fact that the glycoside from *H. grisea* contained as impurity another glycoside with the aglycone (II), as was shown by the formation after acid hydrolysis of the aglycone 22,25-epoxyholosta-7,9(11)-diene-3β,17-diol. This aglycone was identical in all its analytical and spectral characteristics with an authentic sample [12].

EXPERIMENTAL

The melting points of the substances were determined on a Boëtius stage and the optical rotations of the substances on a Perkin-Elmer 141 polarimeter. For chromatography we used type L silica gel (Chemapol, Czechoslovakia, 5/40 μm) and the solvent systems: 1) $CHCl_3$—MeOH—H_2O (75:25:1), and 2) EtOAc—C_6H_{14} (2:1). The spots on the chromatogram were revealed with concentrated sulfuric acid on heating.

The ^{13}C and ^1H NMR spectra were determined on a Bruker HX-90E spectrometer in chloroform and pyridine solutions with tetramethylsilane as internal standard. IR spectra were recorded on a Specord IR-75 instrument in KBr tablets or in chloroform solutions.

The GLC analysis of the sugars was performed on a Tsvet-110 chromatograph using glass columns 0.3 × 150 cm containing 3% of QF-1 on Chromaton N-HMDS, the carrier gas being argon

(60 ml/min, temperature 125-230°C, 5°C/min). Chromato-mass-spectrometric analysis was performed on a LKB 9000S mass spectrometer with a 0.3 × 300 cm column containing 1.5% of QF-1 on Chromaton N-HMDS, the carrier gas being helium (30 ml/min).

Holothurin A_1 (IV) from the holothurian *H. floridana* — mp 263-264°C (from ethanol), $[\alpha]_D^{20}$ −9.22° (c 0.03; ethanol).*

Holothurin A_1 from the holothurian *H. Grisea* — mp 251-253°, $[\alpha]_D^{18}$ −17° (c 0.23; pyridine). IR spectrum of holothurin A_1, (KBr, cm^{-1}): 1762 (C=O in a γ-lactone), 1225, 825 (sulfate group).

Methylation of the Glycoside (IV) followed by Methanolysis. Glycoside (IV) (30 mg) was dissolved in 1.5 ml of dry DMSO and, in a current of argon with heating to 45°C and continuous stirring, 3 ml of methylsulfinyl anion was added. After 1 h, the green gel that had formed was treated with 6 ml of CH_3I, and the mixture was left in the dark at room temperature for 12 h. Then it was diluted with water and extracted with $CHCl_3$ (3 × 3 ml), and the extract was washed with saturated $Na_2S_2O_3$ solution and with water and was concentrated in vacuum. This yielded 35 mg of the methylated product (V), amorphous after purification on silica gel, $[\alpha]_D^{20}$ −5.36° (c 0.28; chloroform). IR spectrum ($\nu_{max}^{CHCl_3}$, cm^{-1}): 3537 (tert-OH).

A solution of compound (V) in 10 ml of MeOH containing 10% of HCl was boiled for 2 h and was concentrated in vacuum. The residue was treated with a mixture of pyridine and acetic anhydride (1:1) and was left at room temperature for 12 h. Then the mixture was concentrated in vacuum and analyzed by GLC-MS to identify the methyl glycosides.

Periodate Oxidation of the Holothurin (IV). A solution of 4 mg of glycoside (IV) in water was treated with 20 mg of $NaIO_4$ and the mixture was left at 5°C for 12 h. Then it was extracted with butan-1-ol (3 × 1 ml), and the extract was washed with water and concentrated in vacuum. The product was hydrolyzed with 12% HCl (100°C, 2 h), and then the peracetates of the aldononitriles were obtained by a known method [13] and were analyzed by GLC.

Desulfation of Holothurin (IV) followed by Periodate Oxidation. A solution of 5 mg of glycoside (IV) in water was mixed with Dowex (in the H^+ form), the residue was filtered off, and the filtrate was neutralized with pyridine and concentrated in vacuum. The residue was boiled with 3 ml of 1% pyridine in dioxane for 1 h. According to TLC, the reaction took place completely. The desulfated glycoside that had been dried in vacuum was dissolved in a mixture of butan-1-ol and water, and 30 mg of $NaIO_4$ was added. The mixture was left for 7 days at room temperature and it was then worked up and the reaction products were analyzed as described above.

Smith Cleavage of Holothurin (IV). A solution of 33 mg of glycoside (IV) in water was treated with 50 mg of $NaIO_4$, and the mixture was left at 5°C for 12 h. Then it was desalted on a column of Polikhrom-1 and was concentrated in vacuum. The residue was dissolved in 50% ethanol, 50 mg of KBH_4 was added, and the mixture was left at room temperature for 1.5 h. The course of the reaction was monitored by TLC. The solution was acidified with glacial acetic acid to pH 5 and was concentrated in vacuum, and 0.5% HCl was added until the solid matter had dissolved completely. The new precipitate that had formed after 15 min was filtered off, washed with water, and dissolved in 20 ml of $CHCl_3$—MeOH (1:1). The extract was concentrated in vacuum and purified on a column of silica gel. In this way, 4 mg of the progenin (VI) was isolated, with mp 267-269°C, $[\alpha]_D^{20}$ −18.75° (c 0.16; ethanol).

Hydrolysis of the Progenin (VI). Compound (VI) (2 mg) was treated with 12% HCl (100°C, 2 h), the mixture was diluted with water and was neutralized with Dowex (HCO_3^- form), the resin was filtered off, and the filtrate and wash-waters were concentrated in vacuum. The peracetates of the corresponding aldononitriles were obtained by a known method and analyzed by GLC.

Acid Hydrolysis of Holothurin A_1 from *H. grisea*. The severe acid hydrolysis of the glycoside was carried out by the method described above. The combined aglycones obtained were separated on a column of silica gel in system 2. From 24 mg of the mixture of aglycones were obtained 14 mg (58%) of holosta-7,9(11)-diene-3β,17α,22ξ-triol, $C_{30}H_{46}O_5$, mp

*The constants of holothurin A_1 are given here again, since those given in the first communication [7] were wrong.

282-285°C, $[\alpha]_D^{20}$ −22.5° (c 0.3; chloroform); mass spectrum, m/z: 486 (M^+), 468 ($M^+ - H_2O$), 453 ($M^+ - H_2O - 15$); and 10 mg of 22,25-epoxyholosta-7,9(11)-diene-3β,17α-diol, $C_{30}H_{44}O_5$, mp 298-300°C, $[\alpha]_D^{20}$ −26.8° (c 0.14; chloroform); ^1H NMR spectrum (δ, ppm): 0.89, 1.01, 1.09, 1.10, 1.12, 1.34, 3.25 (3α-H), 4.2 (22-H), 5.3 and 5.51 (11-H, 7-H). Mass spectrum, m/z: 484 (M^+), 466 ($M^+ - H_2O$), 451 ($M^+ - H_2O - 15$), 99.

SUMMARY

We have detected a triterpene tetraoside not described previously — holothurin A_1 — in two species of holothurians of the sublittoral of the island of Cuba. It has been shown that holothurin A_1 is 3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)-(4-sulfato-β-D-xylopyranosyl)oxy]holosta-9(11)-ene-12α,17α,22ξ-triol. The determination of the complete structure of the glycoside has confirmed the common nature of the biosynthesis of the carbohydrate chain of oligosides of the holothurin A series produced by animals of the family Holothuriidae.

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