GLYCOSIDES OF MARINE INVERTEBRATES.

XV. A NEW TRITERPENE GLYCOSIDE — HOLOTHURIN A₁ —
FROM CARIBBEAN HOLOTHURIANS OF THE FAMILY HOLOTHURIIDAE

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A glycoside, holothurin A₁ has been isolated from the polar glycosidic fractions of the holothurians H. floridana and H. grisea. The complete structure of the glycoside has been established; it is: 3β-[O-(3-0-methyl-B-D-glucopyranosyl)-(1→3)-0-β-D-glucopyranosyl-(1→4)-0-β-D-quinovopyranosyl-(1→2)-(4-sulfato-β-D-xylopyranosyl)oxy]holosta-9(11)-ene-12β,17α,22α-triol. Details of the IR and 1H and 13C 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 (holothurin 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 A₁.

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 A₁ (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 Polykrom-1 and isolated the individual holothurin A₁ (IV) from the glycosidic fraction of H. floridana. The IR spectrum of (IV)

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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 A1 from the Caribbean holothurians H. floridana and H. grisea.

($\nu_{\text{KBr}}^{\text{max}}$ 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 $\beta$ configuration. On the basis of features of the $^1$H and $^{13}$C NMR spectra of the glycoside [7], the structure of the native aglycone of holothurin A1 was established as holost-9(11)-ene-3$\beta$,12$\alpha$,22$\alpha$-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-3$\beta$,17$\alpha$,22$\alpha$-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-$\alpha$- and $\beta$-glucopyranosides, methyl 3-O-acetyl-2,4,6-tri-O-methyl-$\alpha$- and $\beta$-glucopyranosides, methyl 4-O-acetyl-2,3-di-O-methyl-$\alpha$- and $\beta$-quinovopyranosides, and methyl 2-O-acetyl-3,4-di-O-methyl-$\alpha$- and $\beta$-xylopyranosides. The formation of methyl 3-O-acetyl-2,4,6-tri-O-methyl-$\alpha$- and $\beta$-glucopyranosides and of methyl 2,3,4,6-tetra-O-methyl-$\alpha$- and $\beta$-glucopyranosides shows that the terminal monosaccharide residue can only be 3-O-methylglucose, and the