469(3), 445(7), 443(5), 442(5), 427(15), 331(100), 327(4), 326(3), 325(7), 169(67), 152(30), 139(16), 127(16).

CONCLUSION

A new phytoecdysteroid has been isolated from the roots of *Silene brahuica* Boiss. and has been shown to be integristerone A 22-O-α-D-galactoside.

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GLYCOSIDES OF MARINE INVERTEBRATES.

STRUCTURE OF HOLOTHURIN A_2 FROM THE HOLOTHURIAN *Holothuria edulis*

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UDC 547.995:593.96

The structure of a new glycoside from *Holothuria edulis*, holothurin A_2, has been established with the aid of periodate oxidation, methylation, Smith degradation, and ^{13}C NMR spectroscopy. The structure of the glycoside has been determined as holost-9(11)-ene-3β,12α,17α-triol 3-O-{2-O-[3-O-methyl-β-D-glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-quinovopyranosyl]-4-O-sulfate-β-D-xylopyranoside}.

We have previously reported the identification in the holothurian *Jolothuria edulis* of the previously known holothurin B and a new variant of holothurin A, the so-called holothurin A_2 [1]. We have shown that this glycoside differs from the holothurins A and A_1 [2, 3] by the structure of the aglycone and is a sulfated tetraoside of holost-9(11)-ene-3β,12α,17α-triol. Its monosaccharides were determined as D-xylose, D-quinovose, 3-O-methyl-D-glucose, and D-glucose. We have now established that the structure of the carbohydrate chain of holothurin A_2 (I), thereby completing the interpretation of its entire structure.

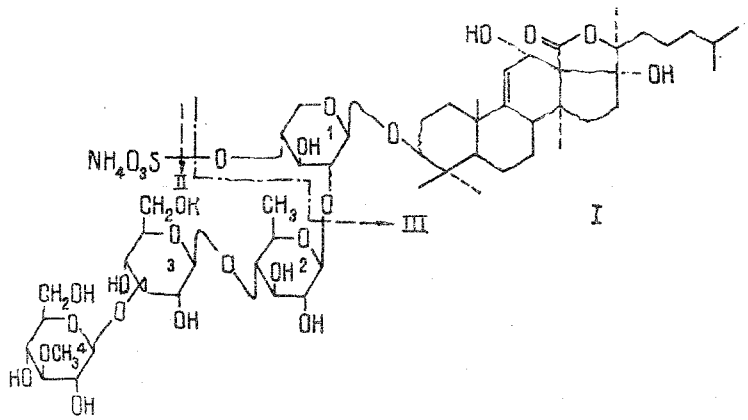

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TABLE 1. ^{13}C NMR Spectra of the Carbohydrate Moieties of Glycoside (I), and Desulfated Derivative (II) (in C_5D_5N), and the Progenin (III) (in $(CD_3)_2SO$)

I				II				III	
C_1^1	105.4	C_1^3	104.6	C_1^1	105,4	C_1^3	104.6	C_1	106,2
C_2^1	83.0	C_2^3	73,8	C_2^1	83,9	C_2^3	73,8	C_2	74,0
C_3^1	76,2	C_3^3	88,2	C_3^1	77,8	C_3^3	88,3	C_3	76,8
C_4^1	75,3	C_4^3	69,9	C_4^1	70,8	C_4^3	69,9	C_4	69,8
C_5^1	64,1	C_5^4	77,7	C_5^1	66,5	C_5^3	77,8	C_5	65,7
C_1^2	105.0[a]	C_6^3	62,4	C_1^2	105,4	C_6^3	62,4		
C_2^2	76,2[b]	C_1^4	105,2	C_2^2	76,3[a]	C_1^4	105,4		
C_3^2	75,6[b]	C_2^4	74,9	C_3^2	75,9[a]	C_2^4	74,9		
C_4^2	87,1	C_3^4	87,7	C_4^2	87,1	C_3^4	87,8		
C_5^2	71,1	C_4^4	70,8	C_5^2	71,7	C_4^4	70,8		
C_6^2	18,1	C_5^4	78,1	C_6^2	18,2	C_5^4	78,2		
		C_6^4	62,4			C_6^4	62,4		
		OCH_3	60,5			OCH_3	60,5		

Note. a and b denote that the assignment of the signals is ambiguous.

The solvolysis of glycoside (I) gave the desulfated derivative (II). The Hakomori methylation [4] of (II) followed by methanolysis and acetylation led to the formation of methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside, methyl 4-O-acetyl-2,3-di-O-methyl-α- and -β-quinovopyranosides, methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides, and methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, which were identified by GLC and chromato-mass spectrometry.

The results obtained showed that the carbohydrate chain is unbranched, and the 3-O-methyl glucose residue occupies the terminal position.

When the desulfated derivative (II) was subjected to periodate oxidation followed by acid hydrolysis, the xylose and quinovose residues underwent decomposition, which corresponds to the results of methylation.

When the glycoside (I) was subjected to periodate oxidation and subsequent acid hydrolysis, only the quinovose residue decomposed which showed the attachment of the sulfate group to the xylose residue. The position of the sulfate group at C-4 in the xylose residue was established by comparing the ^{13}C NMR spectra of (I) and the desulfated derivative (II) (Table 1).

The signal of the C-5 methylene carbon of the xylose residue at 64.1 ppm is shifted to 66.5 ppm, which is characteristic for the β effect of a sulfate group [5]. Corresponding shifts are undergone by the C-3 and C-4 signals (+1.6 ppm and −4.5 ppm, respectively).

The signals at 105.4, 105.2, 105.0, and 104.6 ppm of the anomeric carbon atoms showed the β configurations of the glycosidic bonds.

To determine the sequence of the xylose, quinovose and glucose residues, we carried out the Smith degradation of glycoside (I) [6], followed by desulfation. This led to a progenin (III), the acid hydrolysis of which gave xylose. Since on the periodate oxidation of glycoside (I) the quinovose residue was destroyed, and on Smith degradation a progenin containing only xylose was formed, it was clear that the xylose residue is attached to the aglycone and to this, in its turn, is attached the quinovose residue. According to the results of methylation, the terminal monosaccharide residue is that of 3-O-methylglucose, and therefore it was concluded that the glucose residue was located between it and the quinovose residue in the carbohydrate chain.

The next structural question was that of the cation associated with the sulfate group. During storage at room temperature, the glycoside (I) underwent spontaneous desulfation, which

we have not observed in the case of other sulfated glycosides. It is known that ammonium or pyridinium salts readily undergo desulfation [10]. A test of the glycoside with the Nessler reagent was positive, from which it follows that the counter-ion for the sulfate group is the ammonium ion.

The attachment of the carbohydrate chain at position 3 of the aglycone followed from a comparison of the ^{13}C NMR spectra of a model genin, holosta-7,9(11)-diene-3β,17α-diol, with the spectrum of the glycoside (I). The C-3 signal in the glycoside (89.4 ppm) is shifted in comparison with a signal of the genin C-3 atom (78.2 ppm) as the result of glycosylation [8].

When our work was already in the stage of being completed, a report of Japanese authors on the structure of echinoside A from the holothurian *Actinopuga echinites* appeared [9]. Unfortunately, this paper does not give details of the ^{13}C NMR spectrum of echinoside A and it was impossible to compare the glycoside with glycoside (I) on the basis of their ^{13}C NMR spectra. But the structures established in this and in our investigations coincide with the exception of the cation associated with the sulfate group (in echinoside A it is sodium).

The agreement of our results on the structure of holothurin A_2 from *Holothuria edulis* with those of the Japanese authors on the structure of echinoside A from *Actinopuga echinites* confirms the hypothesis put forward previously [10] that there are no fundamental structural differences between the glycosides of the holothurians of the genera *Holothuria* and *Actinopuga*.

In a study of the glycosidic fraction of *Holothuria edulis* collected in the Gilbert Islands in 1972, by acid hydrolysis the artefactual genin 22,25-epoxyholosta-7,9(11)-diene-3β-17α-diol was obtained together with trace amounts of the 17-epoxy derivative of this genin [10]. An aglycone with an open side chain was not then detected in *Holothuria edulis*. It may be assumed that in the holothurians of some species but of different catches there may be differences in the details of the structure of the aglycones.

As is well known, the glycosides of holothurians of the family *Holothuridae* (with the exception of the glycosides of holothurians of the genus *Bochadshia*) have aglycones of similar structure which differ, as a rule, only by details of the structure of the side chain and which are biosides and tetraosides the sugar moieties of which are composed of D-xylose, D-quinovose, 3-O-methyl-D-glucose, and D-glucose residues [10, 11].

The agreement of the structures of the carbohydrate moieties of holothurins A from the holothurians *Holothuria leucospilota* [2], *Holothuria edulis* [1], and *Actinopuga echinites* [9] collected in the south of Japan in the Miyazaki prefecture, in the Maldive Islands, and on the island of Okinawa, respectively and of the holothurin B from the same holothurians and also from *Holothuria atra* [12] is evidence in favor of the assumption that in the biosynthesis of the carbohydrate chains the glycosides of these holothurians the main role is played by genetic, and not ecological, factors.

EXPERIMENTAL

Melting points were determined on a Boëtius block. Specific rotations were measured on a Perkin-Elmer 141 polarimeter in pyridine solutions at room temperature. ^{13}C NMR spectra were obtained on a Bruker HX-90E spectrometer in pyridine solutions. The signals in the NMR spectra are given on the δ scale, relative to TMS. GLC analysis was performed on a Tsvet-110 chromatograph with glass columns 0.3 × 150 cm containing 3% of QF-1 on Chromaton N-HMDS with argon as the carrier gas (60 ml/min) at temperatures of 110-220°C, 5 deg/min. Chromato-mass spectrometric analysis was carried out on a LKB-9000s mass spectrometer using 0.3 × 300 cm columns containing 1.5% of QF-1 on Chromaton N-HMDS with helium as the carrier gas (30 ml/min). Analysis was performed under the following conditions: temperature of the evaporator 275°C, of the columns 105-220°C (5 deg/min), of the molecular separator 265°C, and of the ion source 225°C; ionizing voltage 70 V.

The animals were collected in the Maldive Islands in February, 1980.

The isolation of holothurin A_2 (I) was carried out by the method described previously [1]. mp 244-246°C, $[\alpha]_D^{20}$ −10.8° (c 0.073; pyridine).

Desulfination of Holothurin A_2. A solution of 100 mg of glycoside I in 50 ml of water was stirred with Dowex cation-exchange resin (H^+) for 2 min. The resin was filtered off, and the solution was dried with pyridine and evaporated to dryness. The residue was boiled in 50 ml of absolute dioxane containing 1% of pyridine for 1 h. The reaction mixture was evaporate to dryness and the residue was purified by column chromatography on silica gel in the chloro-

form–methanol–water (75:25:1) system. This gave 60 mg of desulfated derivative (II) with mp 228-231°C, $[\alpha]_D^{20}$ −39° (c 0.1; pyridine).

Methylation of the Desulfated Derivative (II). A solution of 10 mg of (II) in 2 ml of dry dimethyl sulfoxide (DMSO) was added to a solution of the methylsulfinyl anion (prepared from 100 mg of NaH and 2 ml of DMSO), and the mixture was stirred at 50°C in an atmosphere of argon for 1 h. Then 1 ml of CH_3I was added to it and it was left at room temperature for 12 h. The reaction mixture was diluted with water (10 ml) and extracted with chloroform (3 × 2 ml). The extract was washed with 2 ml of saturated sodium thiosulfate solution and with 2 ml of water and was evaporated.

The resulting residue was boiled with 1 ml of anhydrous methanol saturated with HCl for 2 h. The solution was evaporated, the residue was covered with a mixture of pyridine and acetic anhydride (1:1), the mixture was left for 12 h and was evaporated, and the residue was analyzed by GLC-MS in order to identify the methyl glycosides present.

Periodate Oxidation of the Desulfated Derivative (II). A solution of 4 mg of (II) in 2 ml of water was treated with 30 mg of sodium periodate and the mixture was left at +5°C for two weeks. The reaction product was extracted with butanol (3 × 2 ml), and the butanolic layer was washed with water and evaporated. The residue was heated under reflux with 0.5 ml of 12% HCl 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 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 to the solution 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 to 100°C for another 1 h, after which it was evaporated to dryness. The residue was analyzed by GLC, the peracetates of the aldononitriles derived from 3-O-methylglucose and from glucose being identified in a ratio of 1:1.

Periodate Oxidation of Glycoside (I). A solution of 4 mg of glycoside (I) in 2 ml of water was treated with 30 mg of sodium periodate and the mixture was left at +5° for three days. The reaction product was isolated and hydrolyzed as described above. Xylose, 3-O-methylglucose, and glucose were identified in a ratio of 1:1:1.

Smith Degradation of Glycoside (I). A solution of 100 mg of glycoside (I) in 20 ml of water was treated with 200 mg of sodium periodate and the mixture was left at +5°C for 2 days. Then it was transferred in water to a columm of Teflon powder (Polikhrom-1) and was washed free from salts with water, after which the reaction products was eluted with ethanol. The aqueous ethanolic solution obtained in this way (50 ml) was treated with 200 mg of potassium tetrahydroborate and the mixture was stirred for 3 h. Then it was acidified with acetic acid to pH 4.5 and was concentrated in vacuum, and the boric acid was eliminated in the form of methyl borates by the addition of portions of methanol to the residue and their evaporation off in vacuum. The residue was dissolved in 20 ml of water and the solution was treated with 2 ml of concentrated HCl. After 20 min, the precipitate that deposited was filtered off and was washed with water to neutrality. Then it was removed from the filter with a mixture of chloroform and methanol (1:1), and the solution was evaporated to dryness.

The dry residue was covered with 10 ml of absolute dioxane, 1 ml of pyridine was added, and the mixture was boiled under reflux for 1 h. Then it was evaporated and the residue was chromatographed on silica gel in the chloroform–methanol (10:1) system. This gave 12.5 mg of the progenin (III) with mp 298-301°C, $[\alpha]_D^{20}$ −9° (c 0.1; pyridine). After acid hydrolysis of the progenin by the method described above, xylose was identified.

CONCLUSION

It has been shown that holothurin A_2 from the holothurian *Holothuria edulis* is holost-9(11)-ene-3β,12α,17α-triol 3-O-{2-O-[3-O-methyl-β-D-glucopyranosyl-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl]-4-O-sulfato-β-D-xylopyranoside}.

A. I. Kalinovskii took part in the treatment of the ^{13}C data and the compilation of the Table.

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MASS SPECTRA OF 18,19-EPOXYCARDENOLIDES

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The mass spectra of six 18,19-epoxycardenolides (ECs) with various substituents in the 3β position — OH, OAc, rhamnosyloxy have been studied. In all the spectra the contribution of the fragments characteristic for cardenolides formed by the cleavage of the bonds of rings C and D were lowered. The strongest peaks were those of ions with m/z 259, 272, and 285 arising on the splitting out of the elements of rings A and B. It was established by the metastable defocusing (MD) method that in the case of the 3β-ols these fragments are formed from M^+ and in the case of acetyl derivatives, in addition, they may arise from the $(M - AcOH)^+$ ions. The spectra of the epoxycardenolides were compared with the structure of 3β,5β,14β,19-tetrahydroxycardenolide (strophanthidol) and its 3β-monoacetate and 3β,19-diacetate. The MD spectra and elementary compositions of the ions showed that other mass-spectrometric conditions strophanthidol monoacetate decomposes partially in the form of the 8,19-epoxycardenolide.

The task of the fragmentary mass spectrometry of organic compounds is, in addition to establishing the general directions of the fragmentation of certain groups of substances, the analysis of spectra revealing unusual properties with relatively small structural changes. Having studied over a number of years the mass spectra of the aglycones of cardenolides and their derivatives [1-3], we have established that the intensities of the peaks of the molecular and key fragmentary ions depend not only on the number and positions of the oxygen-containing functional groups but also on the nature of the substituent at C-10 of the steroid nucleus. The role of this factor is due to the fact that the first act of the fragmentation of cardenolides, as of other steroid systems, is the cleavage of one of the C-C bonds adjacent to a quaternary carbon atom, most frequently the C-1—C-10 bond. In all the spectra studied previously, the changes on passing from substance to substance have borne a mainly quantitative nature.

We have achieved the possibility of observing qualitative changes, and these sharp ones, on analyzing the spectra of the 8,19-epoxycardenolides: 3β,5-dihydroxy-5,10-epoxy-5β,14α,17β-card-20(22)-enolide (I), its 14β,17α- isomer (II), their 3β-acetates (III) and (IV), the 3β-rhamnoside of the 14β,17α-epoxycardenolide (V), and its triacetate (VI). These substances were obtained as by-products in the synthesis of the rhamnosides of strophanthidol by the orthoester method [4].

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