The 1,8-ANS and CTC probes were added in the form of aqueous solutions in a concentration of $5 \cdot 10^{-6}$ M to a suspension of liposomes (0.5 mg/ml). The source of calcium ions was a $5 \cdot 10^{-3}$ M solution of calcium chloride. The final concentrations of the compounds ASB, 17α-ASB, strophanthidin, and ajmaline in the liposome suspensions studied was $2.5 \cdot 10^{-4}$ M. The fluorescence spectra were recorded on a Hitachi-4 spectrophotometer.

SUMMARY

New cardenolide derivatives of the alkaloids ajmaline, hyoscyamine, and scopolamine, and also of 4-aminoantipyrine, have been synthesized. The ajmalinocardenolides combine within themselves antiarrhythmic and cardiotonic actions with low or moderate toxicity. The hyoscyamino- and scopolaminostrophanthidins proved to be highly toxic compounds.

The ajmalinostrophanthidin bromide possessed the high affinity for phospholipid membranes that is characteristic for many antiarrhythmic agents. The presence of the cardenolide fragment in this compound caused an increase in the binding of calcium ions to liposome membranes.

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STRUCTURE OF CUCUMARIOSIDE G_1 — A NEW TRITERPENE GLYCOSIDE FROM THE HOLOTHURIAN *Cucumaria fraudatrix*

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A new triterpene glycoside — cucamarioside G_1 — has been isolated from the Pacific Ocean holothurian *Cucumaria fraudatrix*. On the basis of physicochemical characteristics and the results of chemical transformations, its structure has been established as 16β-acetoxy-3β-[O-(3-O-methyl-β-D-xylopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-(1 → 2)-(4-O-sulfato-β-D-xylopyranosyloxy)]-holosta-7,24-diene.

We have previously established that the glycosidic fraction of the holothurian *Cucumaria fraudatrix* contains cucumarioside G_1 (I) and have determined the structure of the native aglycone of this glycoside as 16β-acetoxyholosta-7,24-dien-3β-ol (II) [1]. The present investigation was devoted to establishing the structure of the carbohydrate chain of cucumarioside G_1.

The acid hydrolysis of glycoside (I) gave, in addition to the aglycone (II), a mixture of D-xylose, D-quinovose, D-glucose, and 3-O-methyl-D-xylose in a ratio of 1:1:1:1, these sugars being identified by GLC in the form of acetates of the corresponding aldononitriles.

This is the first time that one of the monosaccharides, 3-O-methyl-D-xylose has been detected in hydrolysates of holothurian glycosides. We isolated this monosaccharide by prepara-

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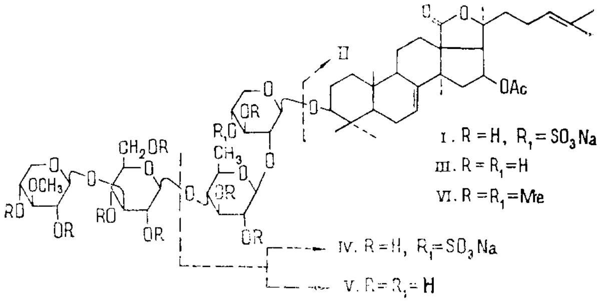
tive paper chromatography of the mixture of sugars and converted it into the following derivatives: the peracetate of the aldononitrile and the peracetate of methyl 3-O-methyl-α-D-xylopyranoside. The presence in the mass spectrum of the aldononitrile peracetate of the peaks of ions with m/z 189, 142, and 129 indicated that the initial monosaccharide was a 3-O-methylpentose [2]. A definitive determination of this monosaccharide as 3-O-methylxylose was made by comparing the mass spectra and GLC behavior of a standard methyl 3-O-methyl-α-D-xylopyranoside peracetate and the sample obtained from the suspected 3-O-methylxylose.

The IR spectrum of (I), ν_{max}^{KBr} (1225 and 825 cm^{-1}), and the result of the solvolytic cleavage of the pyridinium salt showed that glycoside (I) contained a sulfate group.

The desulfated derivative (III) was obtained by the solvolysis of glycoside (I). The Hakomori methylation of (III) [3] followed by methanolysis and acetylation led to the formation of methyl 2-O-acetyl-3,4-di-O-methyl-α- and -β-xylopyranosides, 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-tri-O-methyl-α- and -β-xylopyranosides, which were identified by GLC and mass spectrometry. The results obtained showed that the carbohydrate chain was unbranched and that the terminal position was occupied by the 3-O-methylxylose or the xylose residue.



When the desulfated derivative (III) was subjected to periodate oxidation followed by acid hydrolysis, the xylose and quinovose residues were destroyed, which corresponds to the results of methylation. When glycoside (I) was subjected to periodate oxidation followed by acid hydrolysis, only the quinovose residue was destroyed, which indicated the attachment of the sulfate group to the xylose residue.

To determine the sequence of the monosaccharides in the carbohydrate chain we carried out the enzymatic cleavage of glycoside (I) with cellulase. This gave a progenin (IV), acid hydolysis of which yielded xylose and quinovose. The methylation, methanolysis, and acetylation of the product of the solvolysis of the progenin, (V), led to the formation of methyl 2-O-acetyl-3,4-di-O-methyl-α- and -β-xylopyranosides and of methyl 2,3,4-tri-O-methyl-α- and -β-quinovopyranosides. The results obtained showed that in the progenin (IV) the quinovose was terminal, which means that the xylose was attached to the aglycone. Thus, the terminal monosaccharide in the carbohydrate chain of cucumarioside G_1 is 3-O-methylxylose and between it and the quinovose residue there is a glucose residue.

The position of the sulfate group at C'-4 in the xylose residue was determined by comparing the ^{13}C NMR spectra of the progenin (IV) and that of the desulfated derivative (V) (Table 1). The signal of the C'-5 methylene carbon of the xylose residue at 64.1 ppm in compound (IV) was shifted to 66.6 ppm in compound (V), which is characteristic for the β effect of a sulfate group. Corresponding shifts were undergone by the C'-3 and C'-4 signals (+1.6 and −4.5 ppm, respectively) [4].

The presence in the ^{13}C NMR spectrum of glycoside (I) of the signal of anomeric carbon atoms at 104.6, 103.3, and 104.0 ppm (1 C, 1 C, and 2 C, respectively), showed the β configuration of the glycosidic bonds [5].

The attachment of the carbohydrate chain to the C-3 position of the aglycone followed from a comparison of the ^{13}C NMR spectrum of the aglycone (II) and the spectrum of the glycoside (I) [1].

In a preliminary communication [6], the counter-ion at the sulfate group was erroneously given as the ammonium ion. The cation has now been established by atomic absorption spectroscopy as sodium.

TABLE 1. ^{13}C NMR Spectra of the Carbohydrate Moieties of Glycoside (I) (in $(CD_3)_2SO$) and of Its Derivatives (III-V) (in C_5D_5N) (δ, ppm, TMS = 0)

C	I	III	IV	V	C	I	III
C_1^1	104.0	105,5	105.1	105,5	C_1^3	103.3	104.8
C_2^1	81,9	84,2	83,0	83,8	C_2^3	73,2	73,9
C_3^1	75.0[a]	77,9	76,3	77,9	C_3^3	86.1	87,6
C_4^1	74,7[a]	70,9	75,5	71,0	C_4^3	68.8	70,1
C_5^1	63,3	66.6	64,1	66.6	C_5^3	76,6	77,9
C_1^2	104,0	105.5	105.7	106 0	C_6^3	61.4	62.4
C_2^2	75,3[a]	76,4[a]	76.7	76,7	C_1^4	104.6	106,1
C_3^2	75.0[a]	76.0[a]	77,7	77.9	C_2^4	73,5	74,6
C_4^2	86.0	87,2	76.7	76.9	C_3^4	86.1	87,6
C_5^2	70,9	71,7	73.3	73.4	C_4^4	69,2	69,9
C_6^2	17,8	18,2	18,4	18.4	C_5^4	66 0	67.1
					OCH	60.0	60.4

a — ambiguous assignment of the signals.

The facts given above permitted the complete structure of cucumarioside G_1 to be determined as 16β-acetoxy-3β-[O-(3-O-methyl-β-D-xylopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy] holosta-7,24-diene.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. ^{13}C NMR spectra were recorded on a Bruker HX-90E spectrometer and IR spectra on a Specord IR-75. Sodium was determined on a Hitachi 180/70 atomic absorption spectrophotometer. For chromatography we used type L silica gel (Chemapol, Czechoslovakia, 5/40 μ), silanized silica gel, and the solvent systems: 1) $CHCl_3$–MeOH–H_2O (75:25:1); 2) C_2H_5OH–H_2O (60:40); and 3) $CHCl_3$–MeOH (8:1). Paper chromatography was performed on Whatman-1 paper in the phenol–water (100:40) system. The monosaccharides on the chromatograms were detected with aniline phthalate.

Silanized silca gel was obtained by treating type L silica gel with a mixture of trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) in a ratio of 1:1 (v/v) in dry benzene for 1 h.

GLC analysis was performed on a Tsvet-110 chromatograph using 150 × 0.3 cm glass columns with 3% of QF-1 on Chromaton N-HMDS with argon as the carrier gas (60 ml/min) at temperatures of 110-220°C, 5°C/min. Chromato-mass spectrometric analysis was performed on an LKB-9000 mass spectrometer with a 300 × 0.3 cm column containing 1.5% of QF-1 on Chromaton N-HMDS, with helium as the carrier gas (30 ml/min).

The animals were collected in Posyet Bay, Sea of Japan, in August, 1979, at a depth of 1 m.

Cucumarioside G_1 (I) was isolated as described previously [6]. mp 216-218°C, (MeOH), $[\alpha]_D^{20}$ −21.8° (c 3.4; C_2H_5OH–H_2O (1:1)). IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3420, 1755 (γ-lactone), 1640 (C=C), 1225, 825 (sulfate), 1070 (C–O–C).

Hydrolysis of Cucumarioside G_1. Cucumarioside G_1 (5 mg) was heated with 0.5 ml of 2 N H_2SO_4 at 90-100°C for 3 h. The reaction mixture was diluted with water and extracted with chloroform. The aqueous layer was neutralized with Amberlite AB-17-8 (HCO_3^-). The resin was separated off by filtration and was washed with water (3 × 2 ml). The wash-water and the filtrate was combined and concentrated in vacuum. The residue was dissolved in 1 ml of dry pyridine, 5 mg of $NH_2OH \cdot HCl$ was added, and the mixture was heated at 100°C for 1 h. Then 1 ml of acetic anhydride was added to the reaction mixture and it was heated for another 1 h. The mixture was diluted with water and the aldononitrile peracetates were extracted with chloroform and analyzed by GLC. Xylose, quinovose, glucose, and 3-O-methylxylose were identified in a ratio of 1:1:1:1.

Isolation of 3-O-Methylxylose. Cucumarioside G_1 (300 mg) was hydrolyzed in 50 ml of 2 N H_2SO_4 for 2.5 h. The precipitate of a mixture of aglycones that had deposited was removed by centrifugation and the acid was precipitated with barium oxide. The precipitate was filtered off and was washed with water. The filtrate and the wash-waters were combined and concentrated in vacuum. The resulting combined monosaccharides were separated on paper. This led to the isolation of 8.8 mg of 3-O-methylxylose, $[\alpha]_D^{20}$ +19.1° (c 0.4; H_2O). According to the literature, $[\alpha]_D^{20}$ + 17.0° (c 0.8; H_2O) [7].

To 2 ml of 10% hydrogen chloride in methano was added 5 mg of 3-O-methylxylose and the mixture was heated under reflux for 30 min. Then it was concentrated in vacuum to dryness and the residue was treated with a mixture of pyridine and acetic anhydride (1:1, 1 ml); analysis of the product by GLC led to the identification of methyl 3-O-methyl-α-D-xylopyranoside peracetate.

Methylation of Methyl α-D-Xylopyranoside. A solution of 39.6 mg of methyl α-D-xylopyranoside in 0.5 ml of absolute methanol was treated with 0.2 ml of CH_3I and the mixture was heated at 45°C. Then the mixture was cooled and filtered. The filtrate was concentrated in vacuum to dryness and the residue was treated with a mixture of pyridine and acetic anhydride (1:1) (2 ml). The combined peracetates of methylated methyl α-D-xylopyranoside were analyzed by GLC, and methyl 3-O-methyl-α-D-xylopyranoside peracetate was identified.

Desulfation of Cucumarioside G_1. A solution of 135 mg of the glycoside in water was mixed with KU-2-8 cation-exchange resin (H+) for 2 min. The resin was filtered off, and the solution was neutralized with pyridine and was concentrated in vacuum to dryness. The residue was treated with 50 ml of absolute dioxane containing 1% of pyridine, and the mixture was boiled for 2 h. Then it was concentrated in vacuum to dryness, and the residue was chromatographed on silica gel in system 1. This gave 40 mg of the desulfated derivative (III) with mp 185-187°C, (C_2H_5OH–H_2O, 1:1), $[\alpha]_D^{20}$ – 12.5° (c 1.0; MeOH).

Methylation of the Desulfated Derivative (III). A solution of 30 mg of (III) in 1.2 ml of dry dimethyl sulfoxide was added to a solution of methylsulfinyl anion (prepared from 50 mg of NaH and 1.5 ml of DMSO) and the mixture was stirred at 50°C in an atomosphere of argon for 1 h. Then 1.5 ml of CH_3I was added and the resulting mixture was left at room temperature for 12 h, after which it was diluted with water (10 ml) and extracted with CH_3I (3 × 3 ml). The extract was washed with 4 ml of saturated $Na_2S_2O_3$ solution and with water and was concentrated in vacuum to dryness. The methylation product was purified by column chromatography in silica gel in system 3. This yielded 20 mg of a vitreous product (VI), $[\alpha]_D^{20}$ – 33.2° (c 1.0; $CHCl_3$). Its IR spectrum ($CHCl_3$) lacked absorption in the 3200-3400 cm^{-1} region.

A solution of product (VI) in 10 ml of anhydrous methanol saturated with HCl was boiled under reflux for 2 h. Then it was concentrated in vacuum and the residue was treated with a mixture of pyridine and acetic anhydride and left at room temperature for 12 h. The mixture was concentrated in vacuum and the residue was analyzed by GLC-MS to identify methyl glycosides.

Periodate Oxidation of the Desulfated Derivative (III). A solution of 10 mg of (III) in 1.8 ml of water was treated with 20 mg of sodium periodate and the mixture was left at room temperature for 3 days. The excess of periodate was decomposed by the addition of 50 μl of glycerol (20°C, 1 h). Then the mixture was diluted with water and deposited on a column of polytetrafluoroethylene powder (polikhrom-1). The reaction product was eluted with 50% ethanol and was hydrolyzed and worked up as described above for the ananlysis of the monosaccharides in the form of aldononitrile peracetates. Glucose and 3-O-methylxylose were identified in the hydrolysate obtained.

Periodate Oxidation of Cucumarioside G_1. A solution of 10 mg of (I) in 2 ml of water was treated with 20 mg of sodium periodate and the mixture was left at +5°C for 12 h. The reaction product was isolated and worked up as described above. Xylose, 3-O-methylxylose, and glucose were identified in a ratio of 1:1:1.

Enzymatic Hydrolysis of Cucumarioside G_1. A solution of 200 mg of the glycoside in 50 ml of water was treated with 340 mg of cellulose, and the mixture was left at 37°C for 72 h. Column chromatography of the resulting mixture on silica gel in system 1 gave 80 mg of the progenin (IV) with mp 186-188°C, (C_2H_5OH), $[\alpha]_D^{20}$ – 22.5° (c 0.4; MeOH).

Desulfation of the Progenin (IV). The progenin (IV) (65 mg) was desulfated as described above. This gave 40 mg of a product (V) with mp 222-224°C, (C_2H_5OH), $[\alpha]_D^{20}$ − 20° (c 0.9; MeOH).

Methylation of Product (V). The methylation, methanolysis and acetylation of this substance were performed under the conditions described above.

SUMMARY

The complete structure of cucumarioside G_1, isolated from the holothurian *Cucumaria fraudatrix*, has been established as 16β-acetoxy-3β-[O-(3-O-methyl-β-D-xylopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy]holosta-7,24-diene.

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