CUCUMARIOSIDE G_4 – A NEW TRITERPENGLYCOSIDE FROM THE HOLOTHURIAN Eupentacta fraudatrix

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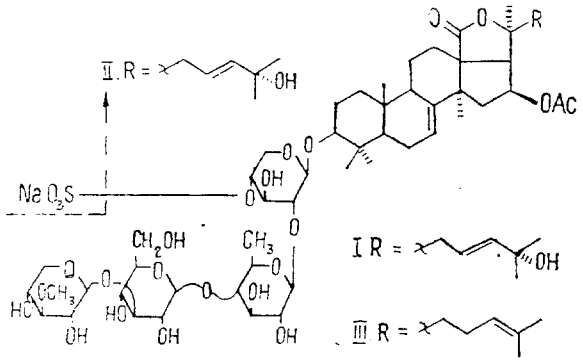
A minor glycoside – cucumarioside G_4 (I) – has been isolated from the total triterpeneglycosides of the holothurian Eupentacta fraudatrix, and its structure has been determined by physical and chemical methods as 16β-acetoxyholosta-7,23E-diene-3β, 25-diol 3-O-(3-O-methyl-β-D-xylopyranosyl)-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-quinovopyransoyl-(1→2)-(4-O-(sodium sulfato)-β-D-xylopyranoside)].

Continuing an investigation of the triterpeneglycosides of the holothurian Eupentacta fraudatrix [1, 2], we have isolated a minor glycoside – cucumarioside G_4 (I) in a proportion of about 0.01% of the weight of the total glycosides, and have established its structure



In the molecular region of the LSIMS mass spectrum of (I) obtained in a glycerol matrix, we observed the peaks of ions with m/z 1233 $(M_K + H)^+$, 1239 $(M_{Na} + Na)^+$, and 1255 $(M_{Na} + K)^+$. After the addition of NaCl to the matrix, only the peak of the ion with m/z 1239 appeared in this region. A comparison of the mass of this ion with the mass of the $(M_{Na} + Na)^+$ with m/z 1223 of cucumarioside G_1 (III) in the spectrum of a mixture in the high-resolution regime showed that the composition of these ions differed by an oxygen atom.

The structure of the aglycon of cumcumarioside G_4 was determined by comparing the ^{13}C NMR spectra of (I), that of its desulfated derivative (II) and that of cucumarioside G_1 (III) [3, 4]. The C-1–C-21 and C-30–C-32 signals were close or coincided in the spectra of these compounds, which indicated an identity of the structures of the polycyclic fractions of the aglycons of these glycosides (Table 1). This conclusion was confirmed by a comparison of the corresponding signals in the ^{13}H NMR spectra of (I) and (III) (Table 2).

In the ^{13}C NMR spectrum of (I) there were also six signals of carbon atoms belonging to the side chain of the aglycon: two of them corresponded to carbon atoms linked by a double bond (143.5 (d) and 120 (d) ppm), one belonged to a carbon atom bearing a hydroxy group

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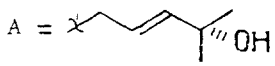
TABLE 1. ^{13}C NMR Spectrum of Cucumarioside G_4 (I) and of its Desulfated Derivative (II); Solvent C_5D_5N ($\delta_{TMS} = 0$)

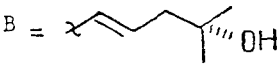
Atom	I	II	Atom	I	II
C-1	36,0	36,2	C-31	28,8	28,9
C-2	27,0	27,3	C-32	32,2	32,2
C-3	89,1	89,2	O-Ac	170,3	169,5
C-4	39,7	39,7		21,3	21,1
C-5	48,2	48,4	—	—	—
C-6	23,3	23,4	C_1^I	104,8	105,5[b]
C-7	120,6	120,5	C_2^1	82,4	84,1
C-8	145,6	145,0	C_3^1	75,2	77,9
C-9	47,2	47,2	C_4^1	75,0	70,3
C-10	35,6	35,7	C_5^1	63,9	66,6
C-11	22,5	22,7	C_1^2	104,8	105,4[b]
C-12	31,4	31,5	C_2^2	76,1[b]	76,3[a]
C-13	59,3	59,3	C_3^2	75,8	75,9
C-14	47,4	47,5	C_4^2	86,5	87,2
C-15	43,7	43,7	C_5^2	71,6	71,7
C-16	75,0	74,8	C_6^2	18,0	18,2
C-17	54,6	54,8	C_1^3	104,2	104,7
C-18	179,7	179,0	C_2^3	73,6	73,8
C-19	24,0	24,0	C_3^3	87,0[a]	87,6[d]
C-20	84,8	84,3	C_4^3	69,8	70,0
C-21	28,3	28,3	C_5^3	77,4	77,9
C-22	41,6	41,7	C_6^3	61,9	62,4
C-23	143,5	144,1	C_1^4	105,4	106,0
C-24	120,2	120,4	C_2^4	74,2	74,5
C-25	69,9	69,7	C_3^4	86,9[a]	87,5[d]
C-26	30,0	30,5	C_4^4	69,3	69,7
C-27	30,1	30,6	C_5^4	66,6	67,1
C-30	17,3	17,4	O-CH_3	60,4	60,4

a, b, c, d Assignments of the signals uncertain.

(69.9 (s) ppm) and two signals corresponded to methyl groups (30.0 (q) and 30.1 (q), ppm) and one signal to the carbon atom of a CH_2 group (41.6 (t), ppm).

In the ^1H NMR spectra, in addition to a singlet at 1.52 ppm corresponding to two methyl groups, an isolated four-spin system of the ABXZ type was observed in which two protons belonged to a methylene group (XZ) and the other two to protons at a double bond, with a spin-spin coupling constant of 15.5 Hz, which indicates their trans-configuration (Table 2). Two variants of the structure of the side-chain of the aglycon correspond to these facts: A and B. The LSIMS mass spectrum of (I) which contained peaks with m/z 551 $(AglOH + Na)^+$ and 533 $(551 - H_2O)^+$ confirmed that the aglycon of cucumarioside G_4 was an acetoxyholostadienediol.

A = 

B = 

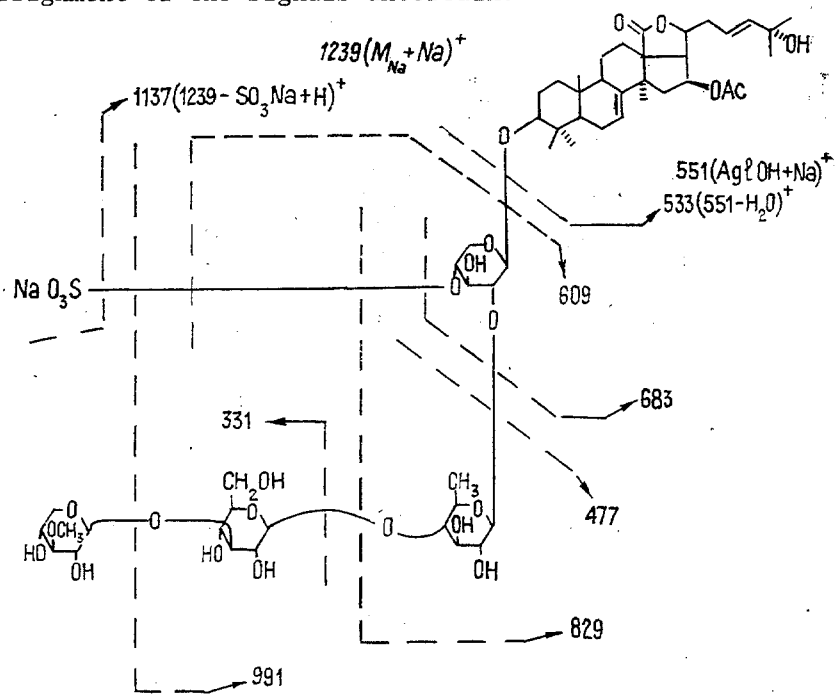
The ^{13}C NMR spectrum of holesta-7,22E-dien-3β-ol (IV) has been given in [5], and in it the C-24 signal was observed at 22.0 ppm. If compound (IV) were hydroxylated at C-25 (variant B for the side-chain), the C-24 signal would have shifted downfield by 4-5 ppm [6]. At the same time, in the spectrum of cucumarioside G_4 the value of 41.6 ppm for the signal of the methylene carbon atom in the side chain shows that this signal must relate to C-22 and, consequently, the structure of the aglycon corresponds to formula (I) (variant A for the side-chain).

The acid hydrolysis of (I) gave a mixture of xylose, quinovose, glucose, and 3-O-methylglucose in a ratio of 1:1:1:1, these being identified by GLC in the form of aldononitrile peracetates. A comparison of the ^{13}C NMR spectra of the carbohydrate moieties of cucumariosides G_4 and G_1 and also of their desulfated derivatives permitted the conclusion that they were completely identical. This conclusion was confirmed by mass spectrometry. Thus, in the LSIMS mass spectrum of glycoside (I) there were the peaks of the ion $(1239 - SO_3Na + H)^+$ with m/z 1137, breaking down by the alternative, or successive, ejection of the carbohydrate units beginning with the terminal ones (m/z 991, 829, 683, 551), and also peaks corresponding to the fragments of the carbohydrate chain (m/z 609, 477, 331). The fragmentation of glycoside (I) is shown in the scheme below (in the mass numbers of the fragmentary ions, the presence of a sodium atom in place of a hydrogen atom has been taken into account).

TABLE 2. ^{1}H NMR Spectrum of Cucumarioside G_4 (I); Solvent C_5D_5N (δ_{TMS} = 0)

Positions of the protons	δ (J, Hz)	Positions of the protons	δ (J, Hz)
CH_3-19	1,24[a] s	H-15a	2,60 dd (7,8; 12.0)
CH_3-21	1,52 s	H-15β	1,77 dd (9,2; 12,0)
CH_3-26	1,53 s	H-16	5.96 m
CH_3-27	1,53 s	H-17	2,62 d (9,4)
CH_3-30	1,30[a] s	H-22	2,70 dd (7,7; 13,0)
CH_3-31	1,14[a] s	H-22′	3,34 dd (5 5; 13,0)
CH_3-32	1,08[a] s	H-23	5,90 ddd (5,7; 7,5; 15, 5)
O-Ac	1,98 s	H-24	6,24 d (15,5)
CH_3- of quinovose	1,77 d (6,3)	H_1^1	
H-3	3,27 dd (5,0; 12,0)	H_1^2	4,74; 5,01:
H-7	5,67 m	H_1^3	5,10; 5,26
H-9	3,47 m	H_1^4	(7,5)

[a]Assignment of the signals uncertain.



On the basis of the results obtained, cucumarioside G_4 has been assigned the structure described by formula (I).

EXPERIMENTAL

NMR spectra were obtained under the conditions described in [6]. LSIMS mass spectra were obtained on a MKh 1310 instrument fitted with a LSIMS ion source (manufactured by IAP RAN [Institute of Agrochemistry and Soil Science of the Russian Academy of Sciences, St. Petersburg]. The target was bombarded with a beam of Cs^+ ions with an energy of 7 keV. The accelerating voltage was 5 kV. The resolving capacity of the instrument in the recording of the general spectra was ~1000 and in the determination of the accurate masses of the ions it was >10,000. The liquid matrices were glycerol and glycerol with added NaCl.

The animals were collected in May, 1990 in the Marine Experimental Station (MÉS) of TIBOKh DVO RAN in Troitsa Bay, Peter the Great Bay, Sea of Japan, from a depth of 1-1.5 m.

The total triterpeneglycosides were isolated as described in [4], and acid hydrolysis and monosaccharide analysis were carried out under the conditions described in the same paper.

Cucumarioside G_4. The total glycosides (9.8 g) were chromatographed repeatedly on silica gel in the chloroform–methanol–water (65:24:4) system, and then the fraction containing the (I) was separated by the HPLC method on a Zorbax-ODS column, 4.8 × 250 mm, at a rate of elution of 1 ml/min with the eluent water–acetone (73:27). This gave 98 mg of (I) with mp 211-213°C, $[\alpha]_{578}^{20}$-11° (c 0.1; pyridine).

The desulfation of (I) by solvolysis in a mixture of pyridine and dioxane (1:1) was carried out by the procedure described in [4]. The derivative obtained was finally purified by chromatography on silica gel in the chloroform–methanol (8:1) system. This gave (II) with mp 265-267°C, $[\alpha]_{578}^{20}$-12° (c 0.1; pyridine).

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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XLIV. STRUCTURE OF CYCLOCARPOSIDES A AND C

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The structures of two new cycloartane glycosides – cyclocarposides A and C, isolated from the herb Astragalus coluteocarpus Boiss. – have been established on the basis of spectral characteristics and chemical transformations. Cyclocarposides A and C are: 20R,24S-epoxycycloartane-3β,6α,17β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside)6-O-(2-O-acetyl-α-L-rhamnopyranoside) and 20R,24S-epoxycyloartane-3β,6α,16β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside) 6-O-α-L-rhamnopyranoside, respectively.

Continuing investigations of cycloartane methylsteroids and their glycosides from plants of the genus Astragalus (Leguminosae), we have determined the structures of two new glycosides isolated from Astragalus coluteocarpus Boiss. [1] which we have called cyclocarposide A (III, substance 3) and C (IV), substance 5).

The presence in the PMR signals of the new glycosides (III) and (IV) of signals characteristic for the methylene protons of a tetrasubstituted cyclopropane and also of seven methyl groups showed that the glycosides under consideration were cycloartane derivatives [2, 3]. The IR spectra of cyclocarposides A and C each contained an absorption band at 3055 cm^{-1} assigned to the methylene of a three-membered ring [4].

The correctness of the assignment of the glycosides under discussion to the cycloartane series was also confirmed by the fact that on acid hydrolysis both glycosides formed cyclosieversigenin (I) [3]. It was shown by the GLC method [5] that cyclocarposides A and C each

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