108(50), 107(14), 106(59), 105(10), 104(50). Found, %: C 76.57; H 10.09. $C_{15}H_{24}O_2$. Calculated, %: C 76.22; H 10.23.

Then the same solvent eluted from the column 334 mg (32%) of the keto acid (X), mp 48-49°C, $[\alpha]_D^{20}$ + 15.6° (c 13.3; $CHCl_3$). IR spectrum (cm^{-1}): 1630, 1720, 1270 ($CH_3C{=}O$, CO_2H), 1160 ($CH_3C{=}O$). PMR spectrum (ppm): 0.87 (3H, s), 0.90 (3H, s) [$(CH_3)_2$ at C-5], 1.18 (3H, c, CH_3 at C-1), 2.01 (3H, s, $CH_3{-}C{=}O$), 2.36 (2H, m, $-CH_2-CO-$), 11.55 (1H, s, $-CO_2H$). Found, %: C 70.04; H 10.15. $C_{14}H_{24}O_3$. Calculated, %: C 69.96; H 10.06.

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TWO NEW TRITERPENE GLYCOSIDES FROM THE HOLOTHURIAN *Duasmodactyla kurilensis*

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Two new glycosides have been isolated from the total triterpene glycosides of the holothurian *Duasmodactyla kurilensis*: kurilosides A (III) and C (IV). It has been established that (III) is 16α-acetoxy-3β-{[O-β-D-glucopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)]-[O-(3-O-methyl-β-D-glucopyranosyl)-(1 → 3)-O-(6-O-(sodium sulfato)-β-D-glucopyranosyl)-(1 → 4)]-β-D-xylopyranosyloxy}-4,4,14-trimethylpregen-9(11)-en-20-one, while the minor glycoside (IV) is 16α-acetoxy-3β-{O-β-D-quinovopyranosyl-(1 → 2)-[O-(3-O-methyl-β-D-glucopyranosyl-(1 → 3)-O-(6-O-(sodium sulfato)-β-D-glucopyranosyl)-(1 → 4)]-β-D-xylopyranosyloxy}-4,4,14-trimethylpregn-9(11)-en-20-one.

Previously, having started a study of triterpene glycosides from the Far Eastern holothurian *Duasmodactvla kurilensis* Levin (Phyllophoridae, Dentrochirots), we reported the isolation of two new genins – kurilogenin (I) and nemogenin (II) [1, 2]. Continuing our investigation, we have isolated glycosides which we have called kurilosides A (III) and C (IV) and have determined their structures.

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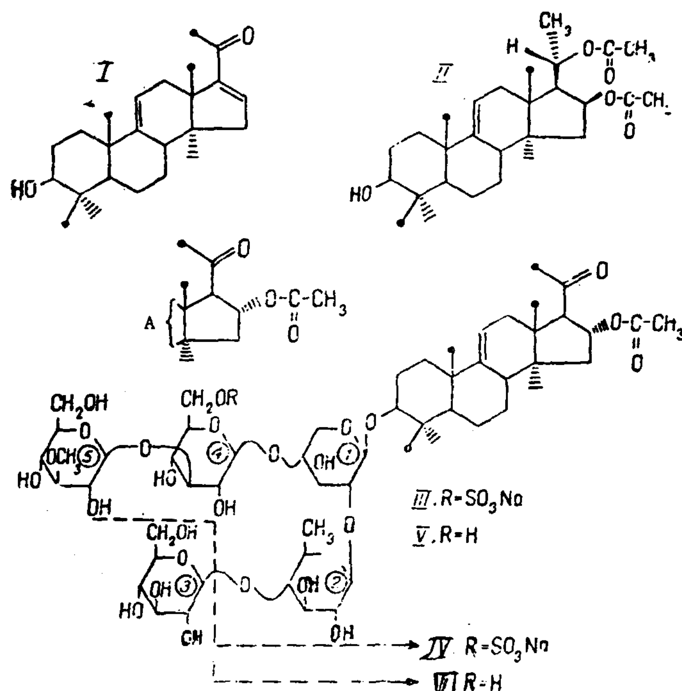

On acid hydrolysis, glycoside (III) gave kurilogenin (I) and a mixture of D-xylose, D-quinovose, D-glucose, and 3-O-methyl-D-glucose in a ratio of 1:1:2:1. A comparison of the ^{13}C NMR spectra of (I) and of the aglycon part of (III) (Table 1) showed a substantial difference between them, which confirmed the assumption that (I) was an artefactual genin. In actual fact, although the signals of the C-1, C-2, C-4—C-13, C-19, and C-32—C-32 atoms in the spectra of these compounds were close or coincided, the spectrum of (III), as compared with that of (I), lacked the signals of a 16-double bond conjugated with a keto group but had the signals of a >CH—O—CO—CH_3 group. These facts permitted the assumption of the presence of fragment A in the native aglycon of kuriloside A. Difference decoupling on the protons of ring D confrmed this assumption and enabled the chemical shifts and spin-spin coupling constants of the H-15α, H-15β, H-16, and H-17 protons to be determined (Table 2).

The calculated spin-spin coupling constants of the protons of ring D for 16α- and 16β-substituted holostane derivatives have been given in the literature [3]. The constants that we observed (Table 2) were close to those calculated for the α-position of an acetate group. Some differences from the calculated values can be explained by a deformation of ring D. In such a deformation caused by a 16α-substituent, the H-16—H-17 angle is obtuse and increases (increase in $J_{16,17}$), the angle H-16—H-15β is acute and decreases (increase in $J_{16,15\beta}$), while $J_{16,15\alpha}$ corresponds to that point on the Karplus curve where the value of the angle has little effect on the constants, which also corresponds to the experimental results. Thus, on the basis of the results of ^{1}H NMR spectroscopy the 16α-configuration of the acetate group in the native aglycon of kuriloside A, which we propose to call neokurilogenin, appears the most probable.

The presence of five monosaccharide residues in glycoside (III) linked with one another and with the aglycon by β-glhcosidic bonds was confirmed by the ^{13}C NMR spectrum (Table 3) in which there were five signals of C-1 atoms of monosaccharides in the 100.0-103.7 ppm region [4]. The assignment of the monosaccharides of the carbohydrate chain of glycoside (III) to the D series as made after a determination of the specific rotation of the total monosaccharides obtained after hydrolysis of the glycoside.

The result of the solvolytic desulfation of kuriloside A indicated the presence of a sulfate group in this glycoside. It led to the desulfated derivative (V).

The Hakomori methylation of (V) followed by methanolysis and acetylation fo the methanolysis products gave the methyl α- and β-glycosides of 2,4-di-O-acetyl-3-O-methylxylopyranose, 4-O-acetyl-2,3-di-O-methylquinovopyranose, 3-O-acetyl-2,4,6-tri-O-methylglucopyranose, and 2,3,4,6-tetra-O-methylglucopyranose, which were identified by GLC-MS. It followed from the

TABLE 1. ^{13}C NMR Spectra of the Aglycon Moiety of Kuriloside A (III) (solvent DMSO-d_6) and Its Derivative (V) (solvent C_5D_5N)

Atom	III	V	Atom	III	V
C-1	35,8	36,5	C-14	46,1	46,8
C-2	26,3	27,2	C-15	42,3	42,7
C-3	87,8	83,6	C-16	74,3	75,9
C-4	*	39,5	C-17	64,8	66,0
C-5	52,3	53,0	C-18	16,2	16,7
C-6	20,9	21,4	C-19	22,1	22,4
C-7	27,7	28,6	C-20	206,6	206,5
C-8	*	41,5	C-21	30,7	30,9
C-9	148,3	149,0	C-30	27,5	28,2
C-10	*	40,0	C-31	16,8	17,4
C-11	113,5	114,0	C-32	19,2	19,6
C-12	34,9	35,8	O-Ac	169,7	170,2
C-13	45,7	46,4		20,6	21,0

*The signals were masked by the signals of the solvent.

TABLE 2. ^1H NMR Spectrum of Derivative (VI) (solvent C_5D_5N, $\delta_{TMS} = 0$)

Positions of the protons	δ	Positions of the protons	δ (J, Hz)
CH_3—	0,65 s	H-11	5,32 m
CH_3—	1,04 s	H-15	1,58 dd (1,5; 14,3)
CH_3—	1,06 s	H-15	2,12 dd (8,5; 14,3)
CH_3—	1,10 s	H-16	5,95 m
CH_3—	1,25 s	H-17	3,41 d (6,1)
CH_3-21	2,25 s	H_1^1	4,72 d (7,5)
CH_3 of quinovose	1 62 d	H_1^2	5,07 d (7,5)
O—CH_3	3,85 s	H_1^4	4,92 d (7,5)
O—Ac	2,05 s	H_1^5	5,17 d (7,5)
H-3	3,20 dd		

methylation results that the carbohydrate chain of kuriloside A had a branched structure. The center of branching was the xylose residue, and glucose and 3-O-methylglucose residues were located at the ends of the chain.

When (III) and (V) were subjected to periodate oxidation, the quinovose and one of the glycose residues decomposed. By Smith cleavage we obtained from (III) a mixture of two progenins having the same carbohydrate chain but stereoisomeric at C-20. Monosaccharide analysis of the mixture obtained showed the presence in the progenins of xylose, glucose, and 3-O-methylglucose in a ratio of 1:1:1, while desulfation followed by Hakomori methylation, methanolysis and acetylation led to the formation of the methyl α- and β-glycosides of 4-O-acetyl-2,3-di-O-methylxylopyranose, of 3-O-acetyl-2,4,6-tri-O-methylglucopyranose, and of 2,3,4,6-tetra-O-methylglucopyranose, which were identified by GLC-MS. It followed from these facts that the carbohydrate chains of the progenins had a linear structure with a terminal 3-O-methylglucose residue.

A comparison of these facts and the results of the methylation of desulfated kuriloside A (V) showed that the biosidic block O-glucosyl-(1 → 4)-quinovose was attached to C-2 of the xylose residue in (III) and was split off on Smith degradation.

A second Smith degradation was accompanied by the breakdown of the xylose. After the chromatography of the product obtained on Polikhrom, a bioside derivative was detected in the eluate the hydrolysis of which gave glucose and 3-O-methylglucose. Subsequent elution of the column with alcohol led to the total genins containing no monosaccharide residue. It followed from this that the xylose was located at the position of attachment of the carbohydrate chain to the aglycon, and the bioside block 3-O-methylglucosyl-(1 → 3)-glucose was attached to C-4

TABLE 3. ^{13}C NMR Spectra of the Carbohydrate Moiety of Kuroloside A and Its Derivatives (δ_{TMS} = 0)

Atom	III DMSO	IV C_5D_5N	V DMSO	V C_5D_5N	VI C_5D_5N
C_1^1	103,4[a]	105.1	103,6	105,1[a]	105,2
C_2^1	81,8	83,2	81,7	83,4	83,2
C_3^1	74,1	75,5	74,3	75,6	75,6
C_4^1	76,0	77,5	75,9	77,5	77,8[a]
C_5^1	62,8	63,9	62,8	64,0	64,0
C_1^2	103,4[a]	105,8	103,6[a]	105,4	105,9
C_2^2	74,9	76,5	74,8	76,3	76,7
C_3^2	74,1	78,7	74,3	75,5	77,8[a]
C_4^2	85,8	76,8	85,9	87,1	77,0
C_5^2	70,0	73,4	70,2[b]	71,7	73,4
C_6^2	17,3	18,5	17,5	18,4	18,6
C_1^3	103,4[a]	—	103,6[a]	105,2[a]	—
C_2^3	73,2[b]	—	73,3[c]	74,9	—
C_3^3	76,6	—	76,5[d]	78,2[b]	—
C_4^3	70,0	—	70,0[b]	71,7	—
C_5^3	76,6	—	76,5[d]	78,1[b]	—
C_6^3	60,9	—	61,0	62,6	—
C_1^4	100,0	103,3	100,8	102,7	102,9
C_2^4	71,5	73,3	71,6	73,1	73,1
C_3^4	86,4	87,1	87,1	88,0	88,2
C_4^4	68,3	69,6	68,4	69,8	69,9
C_5^4	74,6	75,7	76,7[d]	78,2[b]	78,1[a]
C_6^4	65,4	67,3	61,0	62,3[c]	62,3
C_1^5	103,7[a]	105,3	103,7[a]	105,4	105,5
C_2^5	73,3[b]	75,0	73,5[c]	75,0	75,0
C_3^5	85,8	87,7	85,9	87,8	87,8
C_4^5	69,2	70,6	69,3	70,7	70,8
C_5^5	76,6	78,2	76,7[d]	78,2[b]	78,1
C_6^5	60,9	62,2	61,0	62,2[c]	62,3
O—CH_3	59,6	60,5	59,8	60,4	60,5

[a,b,c,d]Ambiguous assignment of the signals.

of the xylose. These facts enabled the structure of the carbohydrate chain of kuriloside A (III) to be determined unambiguously. However, the position of the sulfate group in the glycoside remained undetermined.

A comparison of the ^{13}C NMR spectra of kuriloside A and its derivative (V) showed that the sulfate group was present at the C-6 atom of one of the glucose residues or in the 3-O-methylglucose residue. Thus, the signal of this atom (61.0 ppm) in the spectrum of the desulfated derivative (V) had shifted by 4.5 ppm upfield in comparison with the corresponding signal in the spectrum of (IIi) (65.4 ppm). Correspondingly, the C-5 signal had shifted downfield by 1.8 ppm. These shifts are characteristic for the α- and β-effects of sulfate groups [4].

The establishment of the position of the sulfate group was made on the sulfate-containing product (IV) of the enzymatic cleavage of kuriloside A by cellulase. The acid hydrolysis of the progenin obtained gave a mixture of xylose, quinovose, glucose, and 3-O-methylglucose in

a ratio of 1:1:1:1; thus, on the enzymatic hydrolysis of (III) one terminal unsulfated glucose residue had been split out. The acetylation of (IV) to the peracetate followed by solvolytic desulfation led to a derivative having a free hydroxy group in the position of the O-sulfate group. The methylation of this derivative with diazomethane in the presence of boron trifluoride etherate followed by acid hydrolysis gave a mixture of xylose, quinovose, 6-O-methylglucose, and 3-O-methylglucose, which were identified in the form of aldononitrile peracetates by GLC-MS. It unambiguously followed from this that the sulfate group was attached at C-6 of the glucose residue of progenin (IV).

The minor component (Kuriloside C) isolated from the total glycosides gave, on acid hydrolysis, kuriiogenin and a mixture of xylose, quinovose, glucose, and 3-O-methylglucose in a ratio of 1:1:1:1. In its physical, chemical, and spectral charcateristics it was completely identical with the product of the enzymatic cleavage of kuriloside A. Consequently, its structure is described by formula (IV). Kurilosides A and C are the first nonholostane glycosides of a holothurian, having an aglycon with a 22,23,24,25,26,27-hexanorlanostane (4,4,14-trimethylpregnane) skeleton. Thus, they are the representatives of a new structural type of triterpene glycosides of holothurians. It is most likely that such a sharp difference in the structure of the glycosides indicates an important taxonomic distinction of this species from other representatives of the family Phyllophoridae [5-7] that have been so far studied.

EXPERIMENTAL

The spectral analyses were performed under the conditions described previously [5].

The animals were gathered with an industrial rake-type dredge in the littoral of the island of Onekotan (Kurile Islands, Sea of Okhotsk) in August, 1983 from a depth of 70-120 m.

Isolation of the Glucoside Fraction. The comminuted holothurians were twice extracted with hot ethanol. The ethanolic extracts were combined and evaporated to dryness. The dry residue obtained was dissolved in water, and the solution was filtered and chromatographed on a column of Polikhrom-1. For this purpose, the aqueous solution was deposited on a column of Polikhrom-1 (previously filled in ethanol and washed with water) and the column was washed with water until the reaction for chloride ion with silver nitrate was negative. The glycosididic fraction was eluted from the Polikhrom-1 with 50% ethanol.

Isolation of Individual Glycosides. Kurilosides A and C were isolated by successive column chromatography on silica gel in the chloroform—ethanol—water (100:100:17) system (system A) and on Polikhrom-1 (A, water → 14% ethanol; C, water → 16% acetone). This gave 140 mg of (III), mp 215°C (decomp), $[\alpha]_D^{20}$ − 13° (c 0.1; pyridine) and 35 mg of (IV), mp 210°C (decomp), $[\alpha]_D^{20}$ − 38.0° (c 0.105; pyridine).

The acid hydrolysis of the glycosides and of the progenins, the Smith cleavage of (III), the determination of the position of the sulfate group in (IV), and the methylation of the glycosides and progenins were carried out as described previously [5].

Enzymatic Hydrolysis of (III). A solution of 60 mg of kuriloside A in 35 ml of water was treated with 5 mg of cellulase. The mixture was thermostated at 37°C for 24 h. After this, the solution was evaporated in vacuum, and the residue was chromatographed on a column of silica gel in system A. This gave 42 mg of progenin (IV), mp 211°C (decomp), $[\alpha]_D^{20}$ − 38.0° (c 0.1; pyridine).

The desulfation of (III) and (IV) was achieved by the procedure described in [5]. This gave derivative (V), mp 209-211°C, $[\alpha]_D^{20}$ − 19.2° (c 0.1; pyridine) and derivative (VI), mp 265-266°C, $[\alpha]_D^{20}$ − 11.8° (c 0.15; pyridine).

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