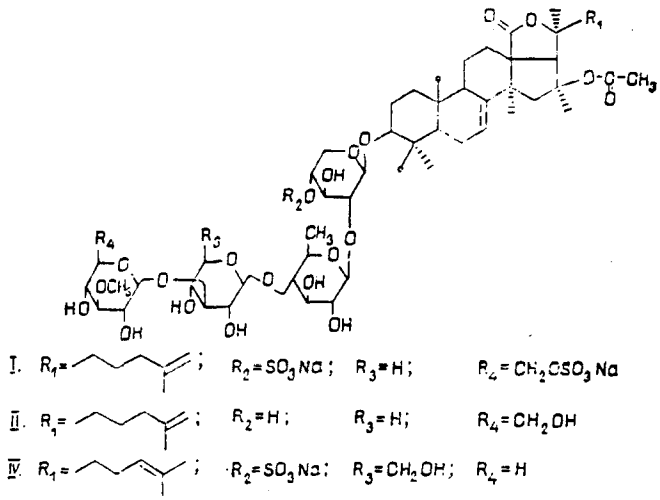
NEW TRITERPENE GLYCOSIDE FROM THE HOLOTHURIAN *Neothyonidium magnum*

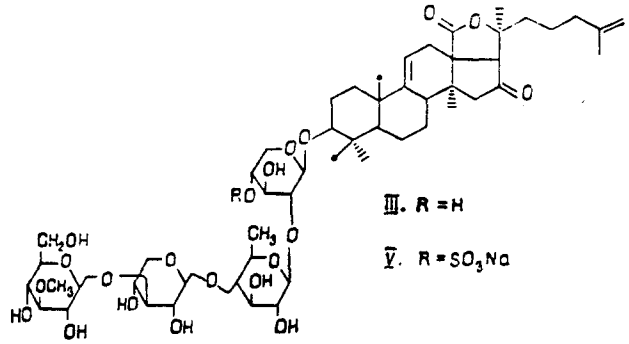
S. A. Avilov, A. I. Kalinovskii, and V. A. Stonik

UDC 547.996.593.96

The main component of the triterpene glycosides from the holothurian Neothyonidium magnum *— neothyonidioside C — has been isolated and its structure has been determined by physical and chemical methods as 16β-acetoxy-3β-[O-(3-O-methyl-6-O-(sodium sulfato)-β-D-glucopyranosyl-(1 → 3)-O-β-D-xylopyranosyl-(1 → 4)-O-β-D-quinovopyranosyl-(1 → 2)-(4-O-(sodium sulfato)-β-D-xylopyranosyloxy)]holosta-7,25-diene.*

Continuing an investigation of glycosides of holothurians of the family Phyllophoridae [1], we have isolated the total triterpene glycosides from an alcoholic extract of the holothurian *Neothyonidium magnum* collected in Vietnam and have established the structure of the main component — neothyonidioside C (I).





The acid hydrolysis of (I) gave D-xylose, D-quinovose, and 3-O-methyl-D-glucose in a ratio of 2:1:1. A comparison was made of the ^{13}C NMR spectra of (I), its desulfated derivative (II), and the model compound (III) obtained in a structural study of the glycosides of the Far Eastern trepang *Stichopus japonicus* [2].

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 53-57, January-February, 1990. Original article submitted February 20, 1989; revision submitted May 6, 1989.

0009-3130/90/2601-0042$12.50

TABLE 1. ^{13}C NMR Spectrum of Glycoside (I) and of the Desulfated Derivative (II)

Atom	I	II	Atom	I	II
C-1	36,2	36,2	C-31	28,8	28,8
C-2	27,3	27,3	C-32	32,3	32,3
C-3	89,2	89,2	O—C—CH_3	169,7	169,7
			‖		
C-4	39,7	39,7	O	21,3	21,3
C-5	48,1	48,1			
C-6	23,4	23,4	C_1^1	105,1	105,6
C-7	120,3	120,3	C_2^1	83,0	84,2
C-8	145,2	145,2	C_3^1	76,2	78,1
C-9	47,2	47,2	C_4^1	75,3	70,6
C-10	35,7	35,7	C_5^1	64,1	66,7
C-11	22,7	22,7	C_1^2	104,9	105,3
C-12	31,5	31,5	C_2^2	76,2	76,5
C-13	59,4	59,3	C_3^2	75 6	75,5
C-14	47,5	47,4	C_4^2	85,5	85,9
C-15	43,3	43,7	C_5^2	71,6	71,7
C-16	75,1	75,0	C_6^2	18,1	18,1
C-17	54,8	54,8	C_1^3	104,4	105,1
C-18	179,4	179,4	C_2^3	73,5	74,4
C-19	24,0	24,0	C_3^3	87,2	87,9
C-20	84,8	84,8	C_4^3	68,9	69,0
C-21	28,2	28,2	C_5^3	66,8	66,5
C-22	38,5	38,5	C_1^4	104,9	105,4
C-23	22,3	22,3	C_2^4	74,9	75,0
C-24	38,5	38,4	C_3^4	86,9	87,5
C-25	145,6	145,6	C_4^4	70,2	70,6
C-26	110,7	110,7	C_5^4	76,2	78,2
C-27	23,3	23,3	C_6^4	67,3	62,2
C-30	17,4	17,4	O—CH_3	60,6	60,7

On the basis of ideas developed recently, according to which the chemical shifts of the signals of the carbon atoms of monosaccharide residues in ^{13}C NMR spectra depend not only on the structure of the monosaccharides but also on the sequence of their linkage in the oligosaccharide chain [3], and also from the accumulated results on the ^{13}C NMR spectra of the carbohydrate chains of holothurian glycosides [4], we came to the conclusion that the carbohydrate chains in (II) and (III), the spectra of which practically coincided, were identical. To confirm this conclusion, we carried out the Smith degradation of glycoside (I). In actual fact, as the result of the cleavage of its carbohydrate chain with oxidation of the quinovose, a progenin containing sulfated xylose was obtained, as was confirmed by desulfation and acid hydrolysis. Furthermore, as the result of this cleavage, a bioside derivative was isolated which gave, after acid hydrolysis, 3-O-methyl-D-glucose and xylose. The results unambiguously determined the sequence of monosaccharide residues in the carbohyrate chain of glycoside (I).

In contrast to the desulfated derivative (II), glycoside (I) had two sulfated hydroxy groups in the carbohydrated moiety of the molecule. Thus, the C-4 signal of xylose (70.6 ppm) in the desulfated derivative (II) was located in a stronger field by 4.7 ppm as compared with the spectrum of (I), while the C-3 and C-5 signals were in weaker fields by 1.9 and 2.6 ppm, respectively. The C-6 signal of the 3-O-methyl-D-glucose residue in (II) (62.2 ppm) was shifted upfield by 5.1 ppm in comparison with the corresponding signal in (I), and the C-5 signal downfield by 2.0 ppm. These shifts, which are characteristic for the α- and β-effects of sulfate groups [5] showed that one sulfate group was attached at C-4 of the first xylose residue and the second at C-6 of the 3-O-methyl-D-glucose residue. We confirmed these positions of the sulfate groups by an independent method.

The acetylation of (I) to form a peracetate followed by solvolytic desulfation led to a derivative having free hydroxy groups at the positions of the O-sulfate groups. The methylation of this derivative with diazomethane in the presence of boron trifluoride etherate followed by acid hydrolysis gave not only xylose and quinovose, but also 4-O-methylxylose and 3,6-di-O-methylglucose, which were identified in the form of the peracetates of the corresponding aldenonitriles.

The positions of the signals of the anomeric carbon atoms in the ^{13}C NMR spectra showed the β-configuration of all the glycosidic bonds [5].

The structure of the aglycon of neothyonidioside C was established by comparing the ^{13}C NMR spectra of the desulfated derivative (II) and of cucumarioside G_1 (IV), the aglycon of which is 16β-acetoxyholosta-7,24-dien-3β-ol [4, 6]. A study of the spectra of (II) and (IV) showed their considerable structural similarity. The only difference consisted in the signals of the carbon atoms of the side chain of the aglycon. In (II) the signals of the double bond in the side chain were located at 145.6 and 110.7 ppm, which is characteristic for a 25(26)-double bond [7]. Furthermore, the presence of a 25(26)-double bond was confirmed by signals at 4.82 ppm (2H-26) and 1.50 ppm (CH_3-27), and the presence of a 16β-acetoxy group by the values of the chemical shifts and spin-spin coupling constants of the $H_{15\alpha}$, $H_{15\beta}$, $H_{16\alpha}$, and H_{17} protons in the ^1H NMR spectra of the aglycon of cucumariosides (I) and (II).

In this way it was established that (I) was the disulfated tetraoside of 16β-acetoxyholosta-7,24-dien-3β-ol.

French workers who have studied the composition of the triterpene glycosides of the holothurian *Neothyonidium magnum* gathered on the shores of New Caledonia have recently isolated as the main glycosidic component another compound – "neothyonidioside" (V) [8]. We assume, however, that these results do not contradict ours. In actual fact, *Neothyonidium magnum* contains a complex mixture of triterpene glycosides. As preceding studies [9-11] have shown, in holothurians belonging to one and the same or related species the structures of the individual glycosidic components do not, as a rule, undergo changes for different catches, although the amounts of these compounds may vary considerably. Thus, psolusoside A as the main component of the glycosidic fraction from *Psolus fabricii* proved to be a minor component in *Psolus* sp. [7, 12].

It must be mentioned that neothyonidioside C (I) is the second, after psolusoside A, of the known tripene glycosides having two sulfate groups in the carbohydrate chain.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. ^{13}C and ^1H NMR spectra were recorded on a Bruker WM-250 spectrometer with pyridine as the solvent ($\delta_{TMS} = 0$). Specific rotations were measured on a Perkin–Elmer 141 polarimeter. The GLC analysis of mixtures of monosaccharide derivatives was performed on a Tsvet-110 chromatograph with 0.3 × 150 cm glass columns containing 3% of QF-1 on Chromaton N-HMDS using argon as the carrier gas (60 ml/min) at temperatures of 150-220°C, 5°C/min. Chromato-mass spectrometric analysis (GLC-MS) was carried out on an LKB-9000 S instrument with a 0.3 × 300 cm column containing 1.5% of QF-1 on Chromaton N-HMDS using helium as the carrier gas (30 ml/min). The analysis was performed under the following conditions: temperature of the evaporator 275°C, of the column 140-220°C (5°C/min), of the molecular separator 265°C, and of the ion source 255°C; ionizing energy 70 eV.

The animals were collected in the winter of 1988 on the shores of South Vietnam and were determined by V. S. Levin (Institute of Marine Biology, Far Eastern Branch, Academy of Sciences of the USSR).

Isolation of Neothyonidioside C. An alcoholic extract of the holothurian was evaporated to dryness, and the residue was dissolved in water and chromatographed on a column of Polikhrom-1 (water → 50% ethanol), and then on a column containing silica gel in the chloroform–ethanol–water (100:100:17) system. The fraction containing the (I) (300 mg) was separated by the HPLC method on a Du Pont 8800 chromatograph with a Zorbax C-8 column (4.6 × 250 mm) using as the mobile phase 30% ethanol at a rate of elution of 1 ml/min. This gave 65 mg of (I) with mp 186°C (decomp.), $[\alpha]_D^{20}$ –15° (c 1.0; pyridine).

Desulfation of (I). A solution of 30 mg of neothyonidioside C in 8 ml of a mixture of pyridine and dioxane (1:1) was boiled for 1 h and was then concentrated in vacuum, and the residue was chromatographed on a column of silica gel in the chloroform–ethanol–water (100:50:4) system. This gave 18 mg of (II) with mp 247-249°C, $[\alpha]_D^{20}$ –23.6° (c 1.0; pyridine). ^1H NMR: 1.17 s (3H), 1.21 s (3H), 1.26 s (3H), 1.37 s (3H) – (CH_3-19, 30, 31, 32), 1.50 s (3H, CH_3-21), 1.71 s (3H, CH_3-27), 1.77 d (3H, CH_3 group in quinovose), 3.37 dd (1H, H-3α), 5.70 m (1H, H-7), 3.50 m (1H, H-9), 4.82 m (2H, H-26), 2.62 dd (1H, $J_{16,15_\alpha}$ = 7.6 Hz, H-15α), 1.79 dd (1H, $J_{16,15_\beta}$ = 8.5 Hz, H-15β), 5.95 m (1H, $J_{16,17}$ = 9 Hz, H-16), 2.65 d (1H, H-17).

Acid Hydrolysis. A solution of 5 mg of the glycoside in 2 ml of 2 N HCl was heated in the boiling water bath for 2 h and was then cooled and diluted with water (5 ml). The precipitate was extracted with chloroform and the aqueous layer was neutralized with Dowex anion-exchange resin (HCO_3^-). The resin was filtered off and the aqueous layer was evaporated to dryness. The residue was dissolved in 1 ml of pyridine, and then 5 mg of hydroxylamine hydrochloride was added and the mixture was heated at 100°C for 1 h, after which 1 ml of acetic acid was added to it and it was heated at 100°C for another 1 h. It was then evaporated, and the aldonitrile peracetates obtained were analyzed by the GLC-MS method.

Smith Cleavage of Glycoside (I). A solution of 15 mg of glycoside (I) in 10 ml of water was treated with 30 mg of sodium periodate and the mixture was left for 3 days. Then it was deposited on a column of Polikhrom-1 that had been filled into it in water and the column was washed free from salts with water. The oxidized glycoside was eluted with 50% ethanol. The solution obtained was treated with 50 mg of sodium tetrahydroborate, the reaction mixture was stirred at 20°C for 2 h and was then acidified with acetic acid to pH 5.0 and was concentrated in vacuum with the addition to the residue of methanol in order to eliminate boric acid in the form of methyl borates. The residue so obtained was dissolved in 20 ml of 0.5 N HCl and, after 2 h, the solution was passed through a column of Polikhrom-1 as described above. The progenin was eluted with 50% ethanol and this fraction was evaporated to dryness and desulfated as described above. The desulfate obtained was hydrolyzed with 1 ml of 2 N HCl, and xylose was identified. The aqueous eluate from the column contained a disaccharide fragment. The solution was evaporated to dryness, and the residue was dissolved in 2 ml of 2 N HCl and hydrolyzed under the same conditions. Xylose and 3-O-methyl-D-glucose were identified.

Determination of the Positions of the Sulfate Groups in (I). A solution of 8 mg of the glycoside in a mixture of pyridine and acetic anhydride was left for 24 h. Then the reaction mixture was concentrated in vacuum, the residue was dissolved in 6 ml of pyridine–dioxane (1:1), and this solution was boiled for 1 h. Then it was evaporated and the residue obtained was chromatographed on a column of silica gel in the chloroform–ethyl acetate (1:1) system. The desulfate derivative so obtained was dissolved in 2 ml of dry methylene chloride, and 0.2 ml of boron trifluoride etherate was added to the solution, which was then cooled to 0°C, and a solution of diazomethane in methylene chloride was added until a permanent yellow coloration appeared. After this, the reaction mixture was left at 0°C for 2 h. The volatile substances were driven off in vacuum and the residue was chromatographed on a column of silica gel in a chloroform–ethyl acetate (1:1) system. The product obtained (6 mg) was hydrolyzed with 2 N HCl (95°C, 4 h). The monosaccharides formed were identified as described above.

LITERATURE CITED

1. S. A. Avilov and V. A. Stonik, *Khim. Prir. Soedin.*, 764 (1988).
2. I. I. Maltsev, V. A. Stonik, A. I. Kalinovsky [Kalinovskii], and G. B. Elyakov, *Comp. Biochem. Physiol.* **78B**, 421 (1984).
3. O. S. Chizhov and A. S. Shashkov, *Progress in Carbohydrate Chemistry* [in Russian], Nauka, Moscow (1985), p. 39.
4. A. I. Kalinovskii, I. I. Mal'tsev, A. S. Antonov, and V. A. Stonik, *Biooorg. Khim.*, 1655 (1984).
5. A. S. Shashkov and O. S. Chizhov, *Bioorg. Khim.* **2**, 437 (1976).
6. Sh. Sh. Afiyatullov, V. A. Stonik, A. I. Kalinovskii, and G. B. Elyakov, *Khim. Prir. Soedin.*, 59 (1983).
7. V. I. Kalinin, A. I. Kalinovskii, and V. A. Stonik, *Khim. Prir. Soedin.*, 212 (1985).
8. M. B. Zurita, A. Ahond, C. Poupat, and P. Potier, *J. Nat. Prod.*, 809 (1986).
9. V. I. Kalinin and V. A. Stonik, *Khim. Prir. Soedin.*, 215 (1982).
10. V. A. Stonik, I. I. Mal'tsev, A. I. Kalinovskii, K. Konde, and G. B. Elyakov, *Khim. Prir. Soedin.*, 194 (1982).
11. B. S. Levin, V. I. Kalinin, I. I. Mal'tsev, and V. A. Stonik, *Biol. Morya*, 3 (1985).
12. V. I. Kalinin, A. I. Kalinovskii, V. A. Stonik, P. S. Dmitrenok, and Yu. N. El'kin, *Khim. Prir. Soedin.*, No. 3, 361 (1989).