

2. H. Beierbeck, J. K. Saunders, and J. W. ApSimon, Can. J. Chem., **55**, 2818 (1977).
3. Sh. Sh. Afiyatullo, V. A. Stonik, A. I. Kalinovskii, and G. B. Elyakov, Khim. Prir. Soedin., No. 1, 59 (1983).
4. V. F. Sharypov, A. I. Kalinovskii, V. A. Stonik, S. A. Avilov, and G. B. Elyakov, Khim. Prir. Soedin., No. 1, 55 (1985).

STRUCTURE OF PSOLUSOSIDE B - A NONHOLOSTANE TRITERPENE GLYCOSIDE
OF THE HOLOTHURIAN GENUS *Psolus*

V. I. Kalinin, A. I. Kalinovskii, V. A. Stonik,
P. S. Dmitrenok, and Yu. N. El'kin

UDC 547.996:593.96

The structure of psolusoside B - a minor triterpene oligoglycoside from the holothurian *Psolus fabricii* and the main glycoside from *Psolus sp.* has been determined by the methods of partial acid hydrolysis, methylation, ^{13}C NMR, and FAB mass spectrometry as 20S-acetoxy-3 β -{2'-O-[O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-4'-O-(6"-O-sulfato- β -D-glucopyranosyl)- β -D-xylopyranosyloxy}holosta-7,25-diene-18,16-carbolactone. 3 β -[O-(3'''-O-Methyl-6'''-O-sulfato- β -D-glucopyranosyl)-(1 \rightarrow 3)-O-(6"-O-sulfato- β -D-glucopyranosyl)-(1 \rightarrow 4)-O- β -D-quinovopyranosyl-(1 \rightarrow 2)- β -D-xylopyranosyloxy]holosta-9(11),25-dien-16-one (psolusoside A), known previously for *Psolus fabricii*, has been identified in a holothurian - *Psolus sp.* - from Kraternaya Bay (island of Ushishir).

We have previously reported on the determination of the structure of psolusoside A (I) - the main triterpene glycoside of the Far Eastern holothurian *Psolus fabricii* Düben et Koren [1, 2]. Continuing a study of the glycoside fraction of this holothurian, we have isolated a minor component which has been called psolusoside B (II). After the acid hydrolysis of its hydrated desulfated derivative we obtained an artefactual genin, 20S-acetoxy-3 β -hydroxylanost-7-ene-18,16-carbolactone (III) (onekotanogenin), and the structure of the native aglycon was determined as 20S-acetoxy-3 β -hydroxylanosta-7,25-diene-18,16-carbolactone (IV) [3].

In the present paper we describe the determination of the constitution of the carbohydrate chain of psolusoside B and give its complete structure.

The acid hydrolysis of (II) led to the total monosaccharides, which were identified by the GLC-MS method in the form of aldonitrile peracetates as xylose and glucose (1:3). The FAB mass spectrum of (II) contained intense $\text{M}^+ - \text{Na} - \text{H}$ ion at 1209 m/z, which confirmed the results of monosaccharide analysis.

After solvolysis by heating in a mixture of pyridine and dioxane, (II) gave the desulfated derivative (V) the FAB mass spectrum of which contained the $\text{M}^+ - \text{H}$ peak at 1129 m/z showing the presence of only one sulfate group in psolusoside B.

After (V) had been methylated by Hakomori's method followed by the methanolysis and acetylation of the products, the methyl α - and β -glycopyranosides of 2,4-di-O-acetyl-3-O-methylxylose, 4-O-acetyl-2,3,6-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylglucose, which were identified by the GLC-MS method, were obtained. It followed from this that the carbohydrate chain in glycoside (II) was branched at the xylose residue and had glucose residues at its ends. On the periodate oxidation and hydrolysis of (II), all the glucose residues were decomposed.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 361-368, May-June, 1989. Original article submitted July 25, 1988.

TABLE 1. ^{13}C NMR Spectra of Compounds (II) and (V) (DMSO- d_6) and (V) and (VIII-XI) ($\text{C}_5\text{D}_5\text{N}$)**

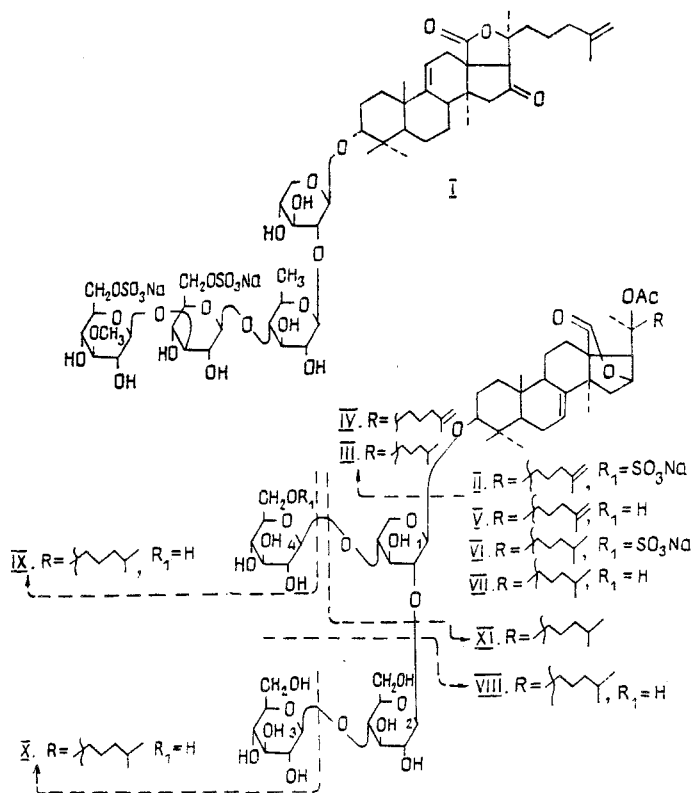
Atom	II	V*	V	Atom	II	V*	V	V	IX	X	VIII	XI
C-1	35,0	34,8	36,0	C-31	28,1	28,0	28,8		—	—	—	—
C-2	26,1	26,0	27,1	C-32	33,9	33,7	34,4		—	—	—	—
C-3	87,8	87,7	88,7	OAc	169,0	169,1	167,0		—	—	—	—
C-4	38,4	38,4	39,4		21,5	21,4	21,3		—	—	—	—
C-5	46,9	46,8	47,9	C ₁ ¹	103,2 ^a	103,2 ^a	105,0 ^b		105,2 ^a	105,5 ^a	106,7	107,5
C-6	22,4	22,4	23,4	C ₂ ¹	89,8	80,7	83,0		82,6	83,5	75,0	75,4
C-7	146,5	146,4	147,0	C ₃ ¹	74,2	74,1	75,7		75,8	77,6	76,2	78,4
C-8	121,7	121,5	122,0	C ₄ ¹	76,4	76,5	78,3		78,1 ^c	71,0	78,2 ^a	71,1
C-9	45,2	45,1	46,1	C ₅ ¹	62,8	62,5	64,2		64,2	66,4	64,5	67,0
C-10	34,8	34,7	35,7	C ₁ ²	103,6 ^a	103,5 ^a	105,9 ^b		105,8 ^a	105,2 ^a	—	—
C-11	20,9	20,9	22,0	C ₂ ²	74,4	74,6	76,4		76,9	76,5	—	—
C-12	19,3	19,2	20,4	C ₃ ²	74,7	74,4 ^c	76,3		77,9 ^b	76,2	—	—
C-13	53,9	53,8	54,9	C ₄ ²	80,6	80,5	81,7		71,7	81,8	—	—
C-14	45,0	44,9	45,8	C ₅ ²	74,7	74,3 ^c	76,0		77,7 ^b	76,2	—	—
C-15	43,7	43,6	44,6	C ₆ ²	67,7	60,6 ^d	62,6 ^c		62,6	62,7	—	—
C-16	78,3	78,3	79,3	C ₁ ³	102,9 ^a	102,8	104,8		—	104,8	—	—
C-17	59,4	59,4	60,5	C ₂ ³	73,2	73,2	74,7		—	74,7	—	—
C-18	180,2	180,0	180,5	C ₃ ³	75,7	76,2	78,1		—	78,2	—	—
C-19	23,5	23,4	24,1	C ₄ ³	69,7	70,1 ^b	71,7 ^d		—	71,9	—	—
C-20	82,9	83,0	83,9	C ₅ ³	75,7	76,2	78,1		—	78,2	—	—
C-21	23,0	23,0	23,6	C ₆ ³	60,5	60,8 ^d	62,5 ^c		—	62,7	—	—
C-22	38,7	38,7	38,1 ^a	C ₁ ⁴	99,8	101,3	103,3		103,4	—	103,5	—
C-23	20,7	20,7	22,0	C ₂ ⁴	74,2	72,6	74,2		74,3	—	74,2	—
C-24	37,1	37,0	38,0 ^a	C ₃ ⁴	75,0	76,7	78,6		78,7 ^c	—	78,6 ^a	—
C-25	144,5	114,6	145,1	C ₄ ⁴	69,7	70,0 ^b	71,5 ^d		71,7	—	72,0	—
C-26	110,1	110,0	110,6	C ₅ ⁴	74,7	76,0	78,2		78,2	—	78,2	—
C-27	21,7	21,8	22,1	C ₆ ⁴	65,5	61,0 ^d	62,3 ^c		62,8	—	63,0	—
C-30	16,6	16,5	17,4									

*The spectrum of (V) was taken in dimethyl sulfoxide.

**For the progenins, only the signals of the carbon atoms of the carbohydrate chains are given; a, b, c, d - assignment of the signals uncertain.

To determine the sequence of monosaccharide residues in the chain, partial acid hydrolysis (2N sulfuric acid-butanol 100°C, 1 h) of the hydrogenated desulfated derivative (VI) was carried out. This gave: the aglycon (III) and the progenins (VIII), (IX), and (X), containing glucose and xylose in ratios of 1:1, 2:1, and 2:1, respectively.

The Smith degradation of the derivative (VI) gave the monoxyloside (XI). Its formation showed that xylose was attached directly to the aglycon in psolusoside B. The Smith degradation of (XI) led, in its turn, to the aglycon (III).



The methylation of (VIII) followed by methanolysis and acetylation led to the formation of the methyl α - and β -glycopyranosides of 4-O-acetyl-2,3-di-O-methylxylose and 2,3,4,6-tetra-O-methylglucose. Consequently, glucose was attached in position 4 of the xylose.

The methylation, methanolysis, and acetylation of the progenin (IX) gave the corresponding methyl glycopyranosides of 2-O-acetyl-3,4-di-O-methylxylose, 4-O-acetyl-2,3,6-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylglucose. Consequently, the 1 \rightarrow 4-glucobiose was attached in position 2 of the xylose residue.

The methylation of the progenin (X) with the same sequence of treatment of the products permitted the identification of the methyl glycopyranosides of 2,4-di-O-acetyl-3-O-methylxylose and 2,3,4,6-tetra-O-methylglucose, which also completed the determination of the sequence of monosaccharides in the carbohydrate chain of the glycoside. The structures of all the progenins obtained were, in addition, confirmed by the results of ^{13}C NMR spectroscopy (Table 1).

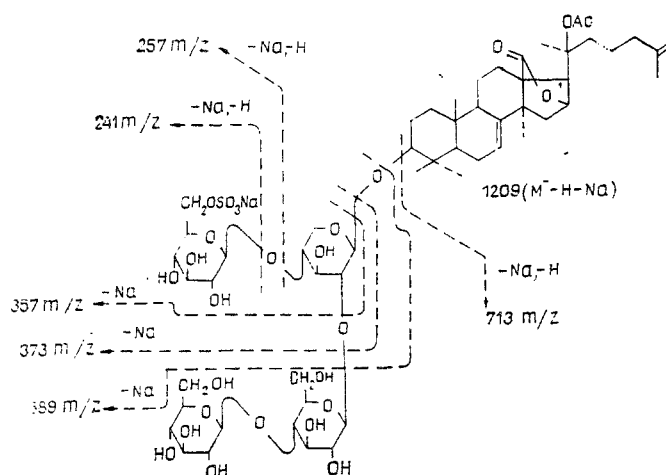
The positions of the signals of the anomeric carbon atoms in the ^{13}C NMR spectra of (V) and of the progenins showed the β -configurations of all the glycosidic bonds [4].

In the spectrum of (II), the signal of one of the C-6 atoms of the glucose residues was shifted to 65.5 ppm as compared with 62.3 ppm in the spectrum of the desulfated derivative (V). The C-5 signal was shifted correspondingly upfield from 78.2 to 74.7 ppm. These shifts are due to the α - and β -effects of a sulfate group [4] and indicate that this group is attached at C-6 in one of the glucose residues.

In actual fact, the acetylation of (II) followed by solvolytic desulfation, methylation by diazomethane in the presence of boron trifluoride etherate, and acid hydrolysis led

to a mixture of monosaccharides in which 6-O-methylglucose was identified in the form of the aldonitrile peracetate.

The counterion at the sulfate group of psolusoside A was sodium (according to atomic absorption spectroscopy). The position of the sulfate group in the half-chain of psolusoside B was determined by mass spectroscopy. The FAB mass spectrum of the glycoside had a series of intense peaks formed through the cleavage of the glycosidic bonds with retention of the negative charge on the fragments bearing the sulfate group. These included peaks at 241 and 257 m/z, corresponding to the splitting out of the terminal sulfated glucose, and at 357, 373, and 389 m/z, connected with the splitting out of a sulfated glucose-xylose fragment. The corresponding fragmentation from which the position of the sulfated glucose in the carbohydrate chain followed is shown in the scheme given below.



Thus, the complete structure of psolusoside B has been established as (II). In studying the glycosidic fraction from Psolus sp. collected in Kraternaya Bay in the Ushishir Islands (Kurile Islands), we detected in it psolusosides B (II) (the main component) and A (I), which were identified by their physical constants, ^{13}C NMR spectra, and monosaccharide compositions: the presence of glycosides (I) and (II) is obviously a taxonomic characteristic of the genus Psolus.

The majority of triterpene glycosides of holothurians known hitherto belong to the so-called holostane series, i.e., they have been aglycons with 18 \rightarrow 20-carbolactone groupings. Psolusoside B is a representative of a new structural series of holothurian glycoside containing an 18 \rightarrow 16-carbolactone.

The presence in animals of the genus Psolus of both holostane and nonholostane derivatives permits the assumption that the aglycons with 18 \rightarrow 16-carbolactone groups are phylogenetic precursors of holotoxinogenin - the aglycon of psolusoside A and a number of other glycosides from the holothurians Stichopus japonicus, Parastichopus californicus (order Aspidochirota) [5], Paracaudina ransonetii (order Molpadonia) [6] and Neothyonidium magnum (order Dendrochirota) [7].

We consider that the holotoxinogenins in representatives of various orders have parallel and independent origins on a similar genetic basis. Consequently, the taxonomic distribution of holothurian glycosides is subject to N. I. Vavilov's law of homologous series in hereditary variability [8]. If this is the case, then the glycosides with 18 \rightarrow 16-carbolactone groupings may be detected in other taxonomic groups of holothurians, as well.

EXPERIMENTAL

Melting points were determined on a Boetius stage. Specific rotations were measured on a Perkin-Elmer 141 polarimeter at room temperature. ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer. GLC analysis was performed on a Tsvet 110 chromatograph with 0.3 \times 150 cm glass columns containing 0.3% of QF-1 on Chromaton N-HMDS with argon as the carrier gas at a column temperature of 110-225°C (5°C/min), and chromatographic analysis on a LKB 9000S instrument using a 0.3 \times 300 cm column containing 1.5% of QF-1 on Chromaton N-HMDS with helium as the carrier gas. Analysis was carried out under the

following conditions: temperature of the evaporator 275°C and of the column 110-220°C (5°C/min), molecular separator at 265°C, ion source at 255°C, ionizing voltage 70 eV. The mass spectra of glycoside (II) and of the desulfated derivative (V) were obtained on LKB-2091 instrument with a primary source for ionization by fast atoms using the FAB method in the regime of negative-ion recording. Xe atoms with an energy of 7 keV were used as the bombarding particles. Atom-absorption spectral analysis for metals was carried out on AA-780 instrument.

Psolus fabricii was collected in the Sea of Okhotsk in the littoral of the island of Onkotan (Kurile Islands) from a depth of 100 m with a comb-type dredge during the expedition of the research vessels Mekhanik Zhukov and Dalarik in August-September, 1982, and Psolus sp. in Kraternaya Bay, island of Ushishir (Kurile Islands) from a depth of 15 m in August, 1986, during the second voyage of the scientific research ship Akademik Oparin. The holothurians were determined by V. S. Levina and A. V. Smirnov.

The isolation of psolusoside B (II) was carried out by the procedure described previously [1], mp 217-220°C (decomp.), $[\alpha]_D^{20}$ -67° (c 0.1; pyridine).

Monosaccharide Analysis of Psolusoside B. A mixture of 5 mg of (II) and 2 ml of 12% HCl was heated 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 separated by filtration and washed with water. The aqueous layer and the wash-waters were combined and evaporated to dryness in vacuum. The residue was dissolved in 1 ml of dry pyridine, 5 mg of hydroxylamine hydrochloride was added and the mixture was heated at 100°C for 1 h. Then 1 ml of acetic anhydride was added to it and it was heated at 100°C for another 1 h, after which it was evaporated and analyzed by GLC. The aldonitrile peracetates derived from xylose and glucose were identified (1:3).

Desulfation of (II). A solution of 90 mg of psolusoside B in 10 ml of pyridine-dioxane (1:1) was boiled for 1 h. The reaction mixture was evaporated to dryness and the derivative obtained was purified by column chromatography on silica gel in the chloroform-methanol (3:1) system. This gave 85 mg of the desulfated derivative (V). mp 208-211°C. $[\alpha]_D^{20}$ -61.3° (c 0.1; pyridine).

Hydrogenation of Psolusoside B. A solution of 100 mg of (II) in 25 ml of water was added to a suspension of 50 mg of Adams catalyst in 10 ml in 50 ml of water and hydrogenation was carried out for 1.5 days. The catalyst was separated off by filtration and the filtrate was evaporated, giving 95 mg of (VI). mp 212-215°C, $[\alpha]_D^{20}$ -56° (c 0.1; pyridine).

The desulfation of the hydrogenated glycoside (VI) was carried out by the method described above. From 90 mg of (IV) was obtained 80 mg of (VII). mp 216-219°C (decomp.) $[\alpha]_D^{20}$ -30° (c 0.2; pyridine).

Partial Acid Hydrolysis of the Hydrogenated Desulfated Derivative (VII). A mixture of 240 mg of (VII), 20 ml of 2 N H_2SO_4 , and 10 ml of butanol was heated in the boiling water bath with vigorous stirring for 30 min. The butanol layer was separated off and the aqueous layer was washed with butanol (2 × 5 ml). Then the combined butanol layers were washed with water (5 ml), with 1% NaHCO_3 solution (2 × 5 ml), and again with water (2 × 5 ml) and were evaporated to dryness. The dry residue was chromatographed on silica gel in the chloroform-methanol (6:1) system. This gave 2.5 mg of total progenins and 14 mg of crude total aglycons.

Chromatography of the total aglycons on silica gel in the benzene-ether acetate system gave 7 mg of the aglycon (III) [3]. The combined progenins were separated by chromatography on silica gel in the chloroform-methanol (6:1) system. This gave 15 mg of progenin (VIII), mp 305-308°C, $[\alpha]_D^{20}$ -70.6° (c 0.3; pyridine), 20 mg of progenin (IX), mp 167-170°C (decomp.); $[\alpha]_D^{20}$ -40° (c 0.2; pyridine), and 17 mg of progenin (X), mp 180-183°C (decomp.) $[\alpha]_D^{20}$ -37° (c 0.2; pyridine).

Determination of the Monosaccharide Compositions of Progenins (VIII), (IX), and (X). After the hydrolysis of 4 mg of a progenin with 2 N HCl (100°C, 2 h) the monosaccharides obtained were analyzed as described above in the form of aldonitrile peracetates. On hydrolysis, progenin (VIII) gave xylose and glucose (1:1), while (IX) and (X) gave xylose and glucose in a ratio of 1:2.

Hakomori Methylation of the Desulfated Derivative (V). A solution of 10 mg of (V) in 2 ml of dry dimethyl sulfoxide (DMSO) was added to a solution of the methyl sulfinyl

anion (prepared from 100 mg of NaH and 2 ml DMSO) and the mixture was stirred at 50°C for 1 h in an atmosphere of argon. Then 1 ml of CH₃I was added to it and the resulting reaction mixture was left at room temperature for 2 h. It was then 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 residue obtained was boiled with 1 ml of anhydrous methanol saturated with HCl for 2 h. The solution was evaporated and the residue was covered with a mixture of pyridine and acetic anhydride, and this mixture was heated at 100°C for 1 h and was evaporated, and the residue was analyzed by GLC-MS to identify the methyl glycosides that it contained.

Methylation of Progenins (VIII), (IX), and (X). In the same way as described above, 5 mg of each of the progenins was methylated and methanolysis and acetylation were carried out. The methyl glycosides obtained were analyzed by GLC-MS.

Periodate Oxidation by Psolusoside B. 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 for 2 days at +5°C. The reaction product was extracted with butanol (3 × 2 ml), and the butanol layer was washed with water. The residue was heated with 0.5 ml of 12% HCl at 90-100°C for 2 h. The monosaccharides obtained were analyzed in the form of aldonitrile peracetates as described above. A xylose residue was identified.

Double Smith Degradation of Psolusoside B. A solution of 100 mg of glycoside (II) in water was treated with 200 mg of NaIO₄ and the reaction mixture was left at 5°C for 3 days. The product was separated from salts on a column with the tetrafluoroethylene powder Polikhrom-1 (water → ethanol) and the aqueous ethanolic eluate (200 ml) was reduced with 100 mg of NaBH₄. The solution was acidified with CH₃COOH to pH 5 and was evaporated to dryness with the addition of butanol and methanol (successively), and the residue was dissolved in 30 ml of water. This solution was treated with 6 ml of concentrated HCl and was then left at room temperature for 3 days. The precipitate that had deposited was filtered off on a folded paper filter, washed with water, and removed from the filter with chloroform-ethanol (1:1). After concentration of the mixture, 20 mg of progenin (XI) was obtained with mp 182-185°C, $[\alpha]_D^{20}$ -29° (c 0.1; pyridine), giving on acid hydrolysis xylose, which was identified in the way described above.

A solution of 15 mg of (XI) in 10 ml of butanol was treated with 10 ml of water and 200 mg of NaIO₄, and the reaction mixture was stirred at room temperature for a week; then the butanol layer was separated off and the aqueous layer was washed with butanol (2 × 5 ml). The combined butanol layers were washed with water (2 × 5 ml). The butanol solution was treated with 10 ml of ethanol and 100 mg of NaBH₄ and the mixture was stirred for 3 h, after which it was acidified with CH₃COOH until the evolution of hydrogen ceased and was then evaporated to dryness with the addition of methanol, and the residue was dissolved in 30 ml of water. To this solution was added 4 ml of concentrated HCl, and after 1.5 h the resulting precipitate was filtered off and washed with water and was removed from the filter by the chloroform-ethanol system (1:1). This gave 10 mg of crude aglycon, which was purified by column chromatography on silica gel in the benzene-ethyl acetate (6:1) system, giving 5 mg of (III).

Determination of the Position of the Sulfate Group in one of the Glucose Residues.

A 1:1 mixture of pyridine and acetic anhydride was added to 50 mg of the glycoside (II) and the mixture was left for two days. The resulting solution was evaporated to dryness and the residue was boiled in a mixture of pyridine and acetic anhydride for 1 h. The reaction mixture was evaporated and was filtered through silica gel in the chloroform-ethyl acetate (1:1) system. The product was dissolved in 15 ml of dry methylene chloride and, after the addition of 0.1 ml of boron trifluoride etherate, the mixture was cooled to 0°C. Then 100 ml of a solution of diazomethane in methylene chloride was added and the reaction mixture was left at 0°C for 90 min. The precipitate that had deposited was filtered off and was washed with methylene chloride, and the filtrates were combined and evaporated to dryness. The residue was chromatographed on a column of silica gel in the chloroform-ethyl acetate (1:1) system and then the methylation product was hydrolyzed with 12% HCl at 100°C. The monosaccharides obtained were identified in the form of aldonitrile peracetates as described above. Xylose, glucose, and 6-O-methyl glucose (1:2:1) were detected.

SUMMARY

It has been shown that psolusoside B from holothurians of the genus Psolus is 20S-acetoxy-3 β -{2'-O-[O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-4'-O-(6"-O-sulfato- β -D-glucopyranosyl)- β -D-xylopyranosyloxy}-lanosta-7,25-diene-18,16-carbolactone.

LITERATURE CITED

1. V. I. Kalinin, B. R. Stepanov, and V. A. Stonik, *Khim. Prir. Soedin.*, 789 (1983).
2. V. I. Kalinin, A. I. Kalinovskii, and V. A. Stonik, *Khim. Prir. Soedin.*, 212 (1985).
3. V. I. Kalinin, A. I. Kalinovskii, and V. A. Stonik, *Khim. Prir. Soedin.*, 674 (1987).
4. A. S. Shaskov and O. S. Chizhov, *Bioorg. Khim.*, **2**, 437 (1976).
5. V. S. Levin, V. I. Kalinin, S. N. Fedorov, and S. Smaili, *Biol. Morya*, No. 4, 72 (1985).
6. V. I. Kalinin, A. N. Malyutin, and V. A. Stonik, *Khim. Prir. Soedin.*, 378 (1986).
7. M. B. Zurita, A. Ahond, C. Poupat, and P. Potier, *J. Nat. Products*, **49**, 809 (1986).
8. N. I. Vavilov, *The Law of Homologous Series in Hereditary Variability* [in Russian], Moscow (1987), p. 11.

STRUCTURE OF CODONOSIDE B

N. G. Alad'ina, Yu. N. El'kin,
and E. A. Chezhina

UDC 547.918:547.914.4

On the basis of chemical transformations and physicochemical characteristics of the compounds obtained it has been established that codonoside B — the main triterpene glycoside of Codonopsis lanceolata (Sieb. et Zucc.) Benth. et Hook., has the structure of echinocystic acid 3-O- β -D-glucopyranuronoside 28-O-[O- β -D-xylopyranosyl-(1 \rightarrow 4)-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside].

The structure of codonoside B (I), a triterpene glycoside from Codonopsis lanceolata (Sieb. et Zucc.) Benth. et Hook, isolated previously, has been established; its aglycon is echinocystic acid (II) and the carbohydrate moiety consists of D-glucose, D-xylose, L-rhamnose, L-arabinose, and D-glucuronic acid residues [1].

In the ^{13}C NMR spectrum of (I) the number of signals from sp^3 -hybridized carbon atoms in the 60-110 ppm region corresponds to a pentaoside. Consequently, in codonoside B the sugars are present in equimolar ratio.

The alkaline hydrolysis of (I) led to the progenin (III). On the acid hydrolysis of (III), glucuronic acid was identified, and its position at C-3 was established by comparing the ^{13}C NMR spectra of the acetate of the methyl ester of codonoside B (V) and the acetate of the methyl ester of its aglycon (IV). The C-3 signal at 80.9 ppm in the spectrum of (IV) had undergone a downfield shift to 90.3 ppm in the spectrum of compound (V). These characteristics are in harmony with the well-known fact that the glycosylation of an aglycon at C-3 causes a shift of the signal of the carbon atom downfield by approximately 9 ppm in comparison with its signal in the acetate [2]. At the same time, the C-16 signal underwent practically no shift — 75.9 ppm in (IV) and 76.3 ppm in (V) (see scheme on following page).

After (I) had been treated with diazomethane and methylated by Kuhn's method [3], the permethylate (VI) was obtained, and this was subjected to methanolysis and hydrolysis. 2,3,4-Tri-O-methyl-D-xylose was identified by TLC. Analysis by the method of chromatomass spectrometry of the mixtures of methylated monosaccharide derivatives that had been obtained

Far Eastern State University, Vladivostok. Pacific Ocean Institute of Organic Chemistry, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok. Translated from *Khimiya Prirodnikh Soedinenii*, No. 3, pp. 368-372, May-June, 1989. Original article submitted July 25, 1988.