SUMMARY

It has been shown that psolusoside B from holothurians of the genus Psolus is 20S-acetoxy-3β-{2'-O-[O-β-D-glucopyranosyl-(1 → 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

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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 → 4)-O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside].

The structure of codonoside B (1), 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 chromato-mass spectrometry of the mixtures of methylated monosaccharide derivatives that had been obtained

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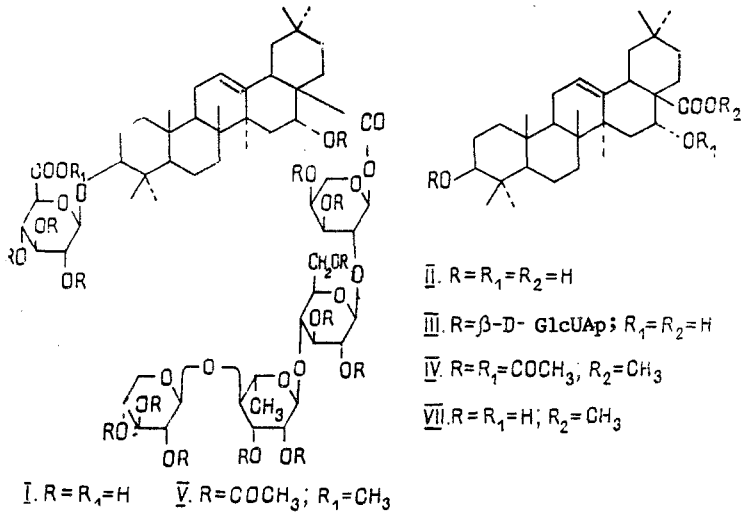


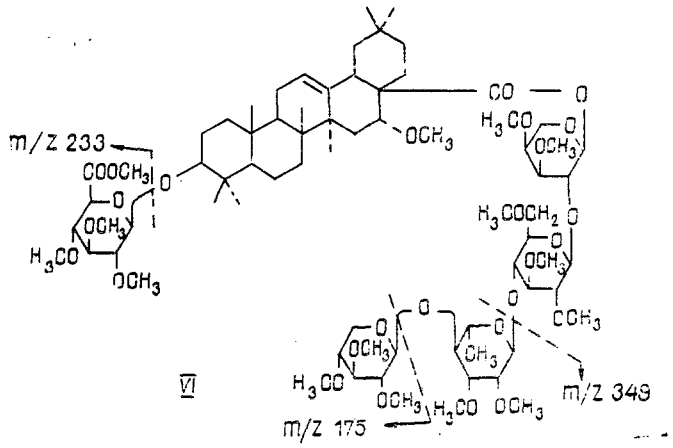
I. $R = R_1 = H$ V. $R = COCH_3$; $R_1 = CH_3$

II. $R = R_1 = R_2 = H$

III. $R = \beta$-D- GlcUAp; $R_1 = R_2 = H$

IV. $R = R_1 = COCH_3$; $R_2 = CH_3$

VII. $R = R_1 = H$; $R_2 = CH_3$



VI

in the form of acetates of methyl glycosides and acetates of aldononitriles showed the presence in the permethylate (VI) of 2,3-di-O-methyl-L-rhamnopyranose, 3,4-di-O-methyl-L-arabinopyranose, 2,3,4-tri-O-methyl-D-xylopyranose, 2,3,6-tri-O-methyl-D-glucopyranose, and methyl 2,3,4-tri-O-methyl-D-glucopyranosiduronate.

The completely methylated xylose that was detected showed that this monosaccharide was terminal in the acylosidic tetrasaccharide. In the alkaline hydrolysis of (I), the arabinose underwent degradation, which showed that it was attached to the carboxy group of the aglycon. The results obtained permitted the assumption for the tetrasaccharide of two variants of the structure differing by the mutual positions of the rhamnose and glucose residues. The choice was made on the basis of an analysis of the mass spectrum of the permethylated (VI), in which the characteristic peaks of ions corresponding to fragments of the carbohydrate chains in the aglycon were detected. This showed that the rhamnose glycosylated a glucose residue.

The presence in the ^{13}C NMR spectrum of glycoside (I) of the signals of anomeric carbon atoms at (ppm) 105.1 (1 C), 104.4 (1 C), and 104.1 (1 C) showed that the D-glucose, D-glucuronic acid, and D-xylose residues were linked by β-glycosidic bonds [4]. The chemical shifts of (ppm) 99.1 (1 C) and 91.8 (1 C) agree well with literature figures for C-1 of a (1 → 4)-α-L-rhamnopyranoside and of a (1 → 2)-α-L-arabinopyranoside attached by a O-acylglycosidic bond, respectively [5].

Calculation by Klyne's rule [6] of the molecular rotation for glycoside (I) (−702°) agreed with the experimentally found value (−664°), which confirmed the configuration of the glycosidic centers.

Thus, codonoside B has the structure of echinocystic acid 3-O-β-D-glucopyranuronoside 28-O-[O-β-D-xylopyranosyl-(1 → 4)-O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside].

EXPERIMENTAL

Type KSK silica gel impregnated with a 0.2 M solution of NaH_2PO_4 and fixed with gypsum was used for TLC. The glycoside and its derivatives were detected with a 50% solution of H_2SO_4.

The following solvent systems were used:

1) chloroform–ethanol–water (15:15:2); 2) chloroform; 3) butan-1-ol–acetic acid–water (4:1:1); 4) butan-1-ol–acetone–water (4:5:1); 5) benzene–ethyl acetate (10:1 → 1:1); 6) chloroform–ethanol (100:0 → 50:50); 7) petroleum ether–chloroform (8:1 → 1:10); 8) petroleum ether–ethyl acetate (10:1 → 4:1); 9) hexane–ethyl acetate (25:1 → 1:1); and 10) chloroform–ethanol (40:1).

Melting points were determined on an instrument of the Boetius type. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. ^{13}C NMR spectra were recorded on Bruker WM-250 instrument (δ, ppm, 0 – TMS; (I) in DMSO-d_6 and (IV) and (V) in $CDCl_3$). Mass spectra were obtained on a LKB-9000S mass spectrometer with direct introduction of the substance into the ion source. Chromato-mass spectrometric analysis was performed on the LKB-9000S mass spectrometer using a 0.3 × 300-cm column with 1.5% of QF-1 on Chromaton N-HMDS with helium as the carrier gas (30 ml/min) at an ionizing voltage of 70 eV.

Isolation of Codonoside B (I). The air-dry comminuted roots (150 g) were boiled with water (1.5-liter portions, 3 times) for 0.5 h. The combined aqueous extract was passed through a column of Polikhrom-1. The substance was eluted with 40% ethanol. The fractions were analyzed in system 1. This gave a glycosidic fraction (1.58 g) which was subjected to column chromatography on silica gel in system 1. The main component of the fraction, codonoside B (I) was isolated: $C_{58}H_{92}O_{27}$, mp 250-256°C (from aqueous n-butanol); $[\alpha]_D^{20}$ −54.4° (c 0.57; aqueous pyridine). IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1614 (COO^-), 1730 (C=O), 2600-2800 (O–H).

Acid Hydrolysis of Codonoside B (I). Codonoside B was hydrolyzed with 8% H_2SO_4 in the water bath for 5 h. The precipitate that had deposited was separated off, washed with water until the wash-waters had a neutral reaction, and chromatographed on silica gel. The column was eluted with system 6 and the fractions were analyzed in system 2. This gave echinocystic acid (II), $C_{30}H_{48}O_4$, mp 306-308.5°C (aqueous ethanol): $[\alpha]_D^{28}$ +35.4° (c 0.78; ethanol). IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1634 (C=C), 1691 and 2653 (COOH), 3430 (O–H). Mass spectrum, m/z (%): 472 (M^+, 9.6), 457 (1.0), 454 (8.0), 439 (1.0), 426 (4.8), 410 (4.8), 264 (72.3), 246 (100), 231 (15.5), 219 (14.4), 207 (44.7), 201 (44.7), 190 (28.8).

In the aqueous part of the hydrolysate after the usual working up, D-glucose, D-xylose, L-rhamnose, and L-arabinose (in system 4) and D-glucuronic acid (in system 3) were identified by TLC.

Alkaline Hydrolysis of Codonoside B (I). A mixture of 40 mg of codonoside B and 2 ml of 1% aqueous ethanolic KOH solution was heated for 3 h. The reaction mixture was neutralized with Amberlite IR-120 (H^+). The precipitate of the progenin (III) that had deposited and the Amberlite were filtered off and were washed with water and ethanol. The ethanolic solution was evaporated. The residue was hydrolyzed with 8% H_2SO_4 at 100°C for 4 h. The reaction mixture was extracted with $CHCl_3$, and echinocystic acid was identified by TLC in system 2. D-Glucuronic acid was detected in the aqueous layer, after its neutralization with $BaCO_3$, by TLC in system 3.

The aqueous solution obtained after the separation of the Amberlite was treated with AB-16 GS anion-exchange resin (OH^-). The resin was filtered off and the filtrate was evaporated to dryness. The residue was hydrolyzed with 8% H_2SO_4 for 4 h. After neutralization, D-xylose, L-rhamnose, and D-glucose were identified by TLC in system 4.

Acetate of the Methyl Ester of Echinocystic Acid (IV). Compound (II) (90 mg) was treated with an ethereal solution of diazomethane. The reaction product was chromatographed on silica gel with elution by system 7. This gave methyl echinocystate (VII), $C_{31}H_{50}O_4$, mp 213-215°C (aqueous ethanol), $[\alpha]_D^{28}$ +30.2° (c 0.53; ethanol). IR spectrum $\nu_{max}^{CCl_4}$, cm^{-1}:

1720 (C=O), 3637 (OH). Mass spectrum, m/z (%): 486 (M^+, 14.5), 468 (14.5) 425 (12.8), 278 (24.2), 260 (87.0), 245 (24.0), 207 (41.2), 201 (100), 200 (32.6).

The ester (VII) obtained (90 mg) was heated in 4 ml of pyridine–acetic acid anhydride (1:1) at 100°C for 3 h, and then the mixture was evaporated. The reaction product was chromatographed on silica gel. Elution with system 8 yielded 70 mg of the amorphous acetate of the methyl echinocystate (IV), $C_{35}H_{54}O_6$. IR spectrum: $\nu_{max}^{CCl_4}$, cm^{-1}: 1249 (C–O), 1738 (C=O), no absorption of hydroxy groups. Mass spectrum, m/z (%): 570 (M^+, 0.9), 510 (3.0), 495 (2.9), 451 (9.6), 450 (15.4), 391 (2.9), 260 (80.0), 249 (8.3), 245 (1.5), 201 (100), 190 (14.5).

Acetate of the Methyl Ester of Codonoside B (V). Glycoside (I) (160 mg) was acetylated in 6 ml of mixture [sic] at room temperature for 12 h and at 100°C for another 3 h. After the end of the reaction the mixture was evaporated. The residue, 260 mg, was treated with diazomethane. The reaction product was chromatographed on silica gel in system 5. This gave 100 mg of the amorphous compound (V). IR spectrum: $\nu_{max}^{CHCl_3}$, cm^{-1}: 1744 (C=O), no absorption bands of carboxy and hydroxy groups.

Permethylate of Codonoside B (VI). Codonoside B (I) (500 mg) was treated with an ethereal solution of diazomethane and was then methylated by Kuhn's method [3]. The reaction product was chromatographed on silica gel. Elution with system 9 gave 230 mg of the amorphous permethylate (VI) $C_{73}H_{122}O_{27}$, mp 121-128°; $[\alpha]_D^{20}$ −63.6° (c 0.67; $CHCl_3$). IR spectrum: $\nu_{max}^{CHCl_3}$, cm^{-1}: 1740 (C=O); no absorption of carboxy and hydroxy groups.

Hydrolysis of the Permethylate (VI). The permethylate (VI) (25 mg) was hydrolyzed with 7% methanolic H_2SO_4 solution (8 ml) at the boiling point of the reaction mixture for 7 h. Then the reaction mixture was diluted with 16 ml of cold H_2O, poured into a saturated solution of $NaHCO_3$, and extracted with $CHCl_3$, and the extract was evaporated to dryness. The residue was heated in a mixture of pyridine and acetic anhydride (1:1) at 100°C for 1 h and the reaction mixture was cooled, diluted with cold H_2O, and extracted with $CHCl_3$, and the extract was washed with H_2O, dried with Na_2SO_4, and concentrated. The sum of the methyl glycoside acetates of methylated monosaccharides so obtained was analyzed by GLC-MS.

The permethylate (VI) (200 mg) was hydrolyzed with methanolic H_2SO_4 as described above. Then the reaction mixture was diluted with H_2O and was heated for another 7 h. After cooling, it was neutralized with $BaCO_3$. The precipitate was separated off, and 2,3,4-tri-O-methyl-D-xylose was identified in the filtrate by TLC in system 10 in the presence of an authentic sample. The filtrate was evaporated to dryness, 60 mg of the residue obtained was dissolved in 3 ml of dry C_5H_5N, and 150 mg of $NH_2OH \cdot HCl$ was added and the mixture was heated at 100°C for 1 h. Then 3 ml of acetic anhydride was added and the resulting mixture was heated for another 1 h. The reaction mixture was worked up as described above. The combined aldononitrile acetates of methylated monosaccharides were analyzed by GLC-MS.

SUMMARY

It has been shown that codonoside B from Codonopsis lanceolata (Sieb. et Zucc.) Benth. et Hook. has the structure of echinosystic acid 3-O-β-D-glucopyranuronoside 28-O-[O-β-D-xylopyranosyl-(1 → 4)-O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside].

LITERATURE CITED

1. N. G. Alad'ina, P. G. Goroboi, and G. B. Elyakov, Khim. Prir. Soedin., 137 (1988).
2. H. Ishii, K. Tori, T. Tozyo, and Y. Yoshimura, Chem. Pharm. Bull., 26, 674 (1978).
3. R. Kuhn, H. Trishmann, and I. Löw, Angew. Chem., 67, 32 (1955).
4. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
5. T. Konishi, A. Tada, J. Shoji, R. Kasai, and O. Tanaka, Chem. Pharm. Bull., 26, 668 (1978).
6. W. Klyne, Biochem. J., 47, xli (1950).