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STEROID SAPONINS AND SAPOGENINS OF *Allium*

XII. TUROSIDE A FROM *Allium turcomanicum*

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Continuing a study of the steroid saponins and sapogenins of *Allium turcomanicum* Rgl. [1, 2], we have investigated a methanolic extract of the bulbs of this plant. From the total extractive substances we isolated a new steroid glycoside which we have call turoside A (I).

A hydrolyzate of glycoside (I) was shown by the GLC method [3, 4] to contain D-xylose, D-glucose, and D-galactose in a ratio of 1:2:1. Neoagigenin (II) was isolated as the aglycone of (I).

The chemical shifts of the C-27 and C-21 methyl groups in the PMR spectrum of (I) (0.94, d, J = 6 Hz, and 1.03, d, J = 6 Hz, respectively), and also the resonance lines of the C-26 proton at 3.24 ppm, from their positions and multiplicities, confirm that glycoside (I) belongs to the 25S series [5].

The partial acid hydrolysis of turoside A yielded three progenins (VI-VIII). Progenin (VI) contained one molecule of galactose in the sugar chain, (VII) contained galactose and glucose in a ratio of 1:1, and (VIII) one molecule of galactose and two molecules of glucose. Consequently, the galactose is bound directly to the aglycone, and, in its turn, the glucose is attached to the galactose.

To determined the positions of the xylose and the second molecule of glucose, we methylated the glycoside (I) [6] and then subjected the resulting permethylate (III) to acid hydrolysis. This yielded dimethoxyneoagigenin (IV) and a mixture of methylated sugars. By separating this mixture on a column of SiO_2 we isolated methylated carbohydrates which were identified by their physicochemical constants, GLC, and TLC as 2,3,4-tri-O-methyl-D-xylopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,6-tri-O-methyl-D-galactopyranose, and 4,6-di-O-methyl-D-glucopyranose.

The products obtained showed that the carbohydrate chain of the new glycoside contains branching the center of which is one of the glucose molecules.

When the mixture of methylated sugars was separated, in addition to those we have mentioned, a pentamethylated disaccharide was also isolated which, when subjected to further acid hydrolysis, decomposed into 2,3,6-tri-O-methyl-D-galactopyranose and 4,6-di-O-methyl-glucopyranose. In view of the fact that in turoside A the galactose is attached directly to the aglycone, it must be assumed that the pentamethylated biose is based on 4-O-D-glucopyranosyl-D-galactose. The PMR spectrum of the biose pentamethylate contained at 4.41 ppm a 1-

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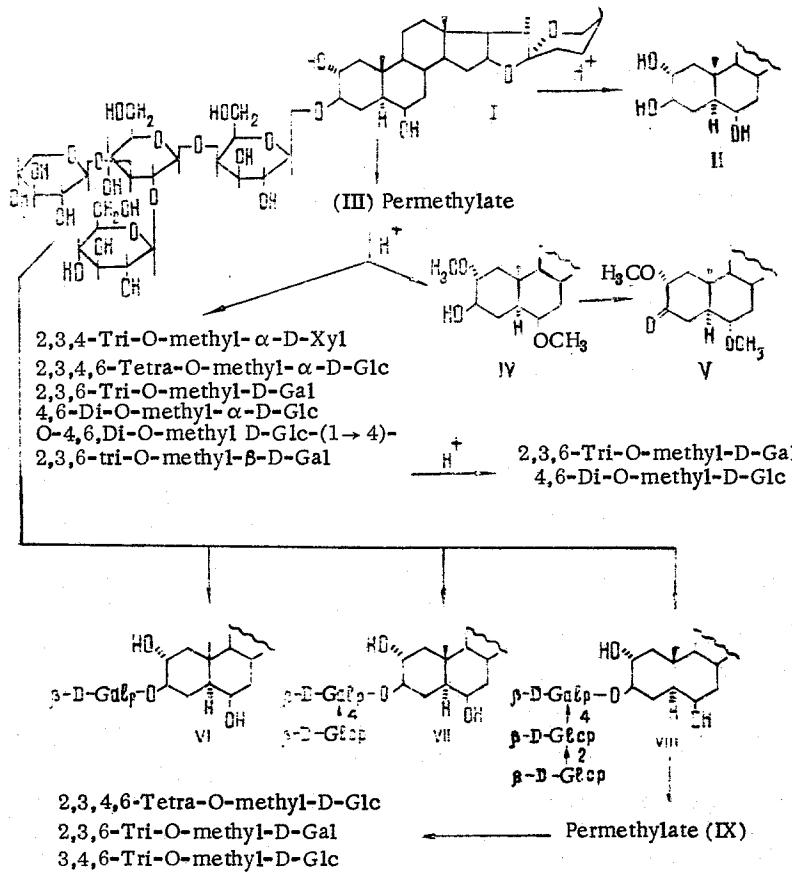
proton doublet with J = 7 Hz, which shows the β configuration of the glycoside bond [7, 8]. The complete structure of the methylated biose is therefore O-4,6-di-O-methyl-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-β-D-glactose and it is the penta-O-methyl derivative of a known disaccharide — lycobiose [9].

To determine the position of attachment of the terminal sugars, the trioside (VIII) was methylated to give the permethylate (IX). 2,3,4,6-Tetra-O-methyl-D-glucopyranose, 3,4,6-tri-O-methyl-D-glucopyranose, and 2,3,6-tri-O-methyl-D-galactopyranose were identified in the products of the acid hydrolysis of (IX). Consequently, to the glucose molecule forming the center of branching terminal molecules of glucose and xylose are attached at C-2 and C-3, respectively, and the disubstituted glucose itself, as we have already shown, is attached to the C-4 hydroxyl of the galactose.

In the PMR spectrum of the permethylate (III), four doublets of anomeric sugar protons appeared in the 4.28-5.01 ppm region. The coupling constants (J = 7 Hz) showed the β configurations of all the glycosidic bonds [7, 8]. The presumed configurations coincide with those calculated by the method of molecular rotation differences [10].



Analysis of the experimental material led to the conclusion that the carbohydrate chain of turoside A is identical in structure with that of the known tetrasaccharide lycotetraose [11]. A sugar chain of this structure is found fairly frequently in steroid saponins [11, 12, 13], including the spirostan glycosides of plants of the genus *Allium* [14].

The position of attachment of the carbohydrate residue to the aglycone was determined in the following manner. Acid hydrolysis of the permethylate (III) led to dimethoxyneoagigenin (IV), which was oxidized with chromium trioxide to the ketone (V). The optical rotatory dispersion curve of the dimethoxy ketone (V), taken in methanol, with a positive Cotton effect, had the form characteristic for 2-oxo- or 3-oxo-5α-steroids [15]. When hydrochloric acid was added to the solution, a marked decrease in amplitude was observed. This shows that compound (V) is a 3-oxo-5α-steroid [15, 16], and, consequently, the carbohydrate residue can be attached only to the hydroxyl at C-3. Turoside A has the structure corresponding to formula (I).

EXPERIMENTAL

For general observation, see [14].

Isolation of Turoside A (I). The comminuted and dried bulbs of *Allium turcomanicum* (1.4 kg) collected in the phenophase of budding in May, 1976 (Turkmen SSSR, environs of the village of Babadurmez, Kopet Dagh range) were extracted with methanol at 60°C (6 times). The extract was concentrated to 0.7 liter, and the residue was treated with 3 liters of acetone. The precipitate that deposited was separated off and was redissolved in methanol and reprecipitated with acetone. This operation was repeated once more. As a result, 200 g of total extractive substances was obtained in the form of a powder. Part of this total (50 g) was chromatographed on a column of silica gel, being eluted first with chloroform–methanol (5:1) and then with choroform–methanol–water (65:35:8). A fraction was isolated which, after repeated recrystallization from methanol, gave 3.4 g (0.97% of the wieght of the air-dry raw material) of compound (I), $C_{50}H_{82}O_{24}$, mp 279-284°C, $[\alpha]_D^{20}$ −60.9±3° [c 0.92; chloroform–methanol (10:1)]. ν_{max}^{KBr}, cm^{-1}: 3200-3500 (OH), 855, 900, 925, 990 (spiroketal chain). PMR spectrum (HMDS, C_5D_5N, δ, ppm): 0.72 (3H at C-18, s); 0.94 (3H at C-27, d, J = 6 Hz), 1.03 (3H at C-21, d, J = 6 Hz), 1.12 (3H at C-19, s); 3.24 (H at C-26, doublet with broadened components, J_{gem} = 11 Hz); the resonance lines of the second proton of the C-26 methylene group are masked by the broad signal (3.4-4.6 ppm) of the protons of the carbohydrate part of the molecule.

Hydrolysis of Turoside A (I). A solution of 100 mg of glycoside (I) on 50 ml of aqueous methanol containing 6% HCl was boiled on the water bath for 7 h, after which the reaction mixture was diluted with water (200 ml) and the methanol was evaporated off as completely as possible. Recrystallization of the resulting precipitate from methanol gave 15 mg of neoagigenin (II), $C_{20}H_{44}O_5$, mp 267-269°C, $[\alpha]_D^{20}$ −72.3±2° [c 0.84; chloroform–methanol (10:1)]; ν_{max}^{KBr}: 3300-3500 (OH), 855, 900 < 930, 990 (spiroketal chain of the 25S series); M^+ 448 [17].

In the hydrolyzate D-xylose, D-glucose, and D-galactose were found by TLC and GLC. GLC showed that these sugars were present in a ratio of 1.10:2.00:1.06.

Permethylate (III) of Turoside A (I). A solution of 2 g of glycoside (I) in 150 ml of dimethyl sulfoxide was treated with 1.7 g of sodium hydride and the mixture was stirred at room temperature for 1 h. Then 20 ml of methyl iodine was added and stirring was continued for another 4 h, after which the reaction products were poured into water and extracted with chloroform. The extract was treated with a solution of sodium hyposulfite washed with water, and dried over anhydrous sodium sulfate. The residue from the distillation of the solvent was methylated in the same way twice more. The methylation products so obtained were chromatographed on a column of silica gel. By elution with benzene–methanol (50:1), 1.3 g of the amorphous permethylate (III) was obtained with $[\alpha]_D^{20}$ −82.2 ±3° (c 1.12; chloroform), the IR spectrum of which lacked the absorption in the region of hydroxy groups. The PMR spectrum of the permethylate (III) in the part relating to the protons of the carbohydrate chain coincided with the spectrum of the permethylate of aginoside [14].

2,6-Dimethyl Ether of (25S)-5α-spriostan-2α,3β,6β-triol (IV) from (III). A solution of 1.15 g of the permethylate (III) in 80 ml of 60% aqueous methanol containing 5% of H_2SO_4 was heated on a boiling-water bath for 6 h. The hydrolyzate was diluted with water (200 ml) and the methanol was evaporated off. Recrystallization of the resulting precipitate from acetone yielded 80 mg of compound (IV), $C_{29}H_{48}O_5$, mp 180-182°C, $[\alpha]_D^{20}$ −107.4±3° (c 1.1; chloroform); ν_{max}^{KBr}, cm^{-1}: 3400-3500 (OH), 855, 900 < 925, 990 cm^{-1} spiroketal chain of the 25S series); M^+ 476.

Separation of the Methylated Sugars. The hydrolyzate of the permethylate (III) (from the preceding experiment) was treated with 7 ml of concentrated H_2SO_4 and boiled for 4 h after which it was neutralized with $BaCO_3$, and the precipitate was removed. The filtrate was evaporated to dryness, and the residue obtained (920 mg) was deposited on a column of SiO_2. Elution was carried out with chloroform–methanol (12:1). Fractions were isolated which contained individual compounds: 1 — 140 mg; 2 — 160 mg; 3 — 60 mg; 4 — 30 mg. Further washing of the column with chloroform–methanol (7:1) gave fraction 5 (100 mg).

2,3,4-Tri-O-methyl-α-D-xylopyranose. Recrystallization of fraction 1 gave 120 mg of a substance with mp 87-89°C (chloroform–methanol), $[\alpha]_D^{20}$ +59.4 → 21.8° (c 1.01; water). According to the literature [11]: mp 91-92°; $[\alpha]_D^{20}$ +64.5 → 17.7° (water). The R_f value in TLC (system 2) coincided with the R_f value of an authentic sample. Gas–liquid chromatography of

methyl 2,3,4-tri-O-methylxyloside (phase 1) [14] gave two peaks, the retention times T_{rel} (0.45 and 0.57) and intensities of which coincided with the corresponding indices of an authentic sample.

2,3,4,6-Tetra-O-methyl-α-D-glucopyrose. The recrystallization of fraction 2 from ethyl acetate gave 130 mg of a substance with mp 89-92°C, $[\alpha]_D^{22}$ +88.7 → +79.6° (c 0.98; water). According to the literature [12], mp 88-94°C, $[\alpha]_D^{13}$ +90 → +85° (water). The R_f values on TLC (system 2) of the substance isolated and of an authentic sample were identical. Gas–liquid chromatography of the methyl tetra-O-methylglucoside (phase 1) [14] gave two peaks, the intensities and T_{rel} values (1.00 and 1.48) of which also coincided with the corresponding indices of the authentic sample.

2,3,6-Tri-O-methyl-D-galactopyranose was isolated in the form of a syrupy substance with $[\alpha]_D^{22}$ +74.6 (c 1.34; water). According to the literature [12]: $[\alpha]_D^{9}$ +79° (water). The R_f value on TLC (system 2) was identical with that of an authentic sample. Gas–liquid chromatography of methyl tri-O-methylgalactoside (phase 1) [14] gave four peaks which coincided in intensities and T_{rel} values (3.28, 4.07, 4.47, and 4.88) with the corresponding indices of an authentic sample.

O-4,6-Di-O-methyl-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-β-D-galactopyrose was obtained by recrystallizing fraction 4 from ethyl acetate (25 mg); $C_{17}H_{32}O_{11}$, mp 88-92°C, $[\alpha]_D^{20}$ +60.2 → +50.6° (c 0.83; water). The disaccharide (5 mg) was hydrolyzed with 7% H_2SO_4 for 14 h. 4,6-Di-O-methyl-D-glucopyranoside and 2,3,6-tri-O-methyl-D-galactopyranoside were identified in the hydrolyzate by TLC (system 2) [14] in the presence of markers.

4,6-Di-O-methyl-α-D-glucopyranose. Fraction 5 yielded a substance with mp 162-164°C (ethyl acetate), $[\alpha]_D^{22}$ +105.8 → 70.5° (c 0.85; water). According to the literature [11]: mp 163-164°C; $[\alpha]_D^{23}$ +118 → 67.5° (water). Gas–liquid chromatography of themethyl di-O-methylglucoside (phase 2) gave one peak with T_{rel} 2.86, which corresponds to the literature figure [14]. The value of R_f on TLC (system 2) of the 4,6-di-O-methyl-α-D-glucopyranose isolated coincided with the index for an authentic sample.

2α,6β-Dimethoxy-(25S)-5α-spirostan-3-one (V) from (IV). A solution of 60 mg of the dimethoxygenin (IV) in 2 ml of glacial acetic acid was treated with 160 mg of CrO_3 in 13 ml of acetic acid, and the mixture was left at room temperature for 74 h. The chromium trioxide that had not reacted was decomposed with sodium sulfite. The reaction mixture was diluted with twice its volume of water and was extracted with chloroform. The extract, after being washed with 5% Na_2CO_3 solution and with water, was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (50 mg) was deposited on a column of SiO_2 and was eluted with chloroform. This gave 15 mg of compound (V), $C_{29}H_{46}O_5$ with mp 144-146°C (acetone), $[\alpha]_D^{21}$ −41.2± 2° (c 0.51; methanol). Optical rotatory dispersion (c 0.102; $[M]_{309}$ + 1768°, $[M]_{269}$ −5256°, α = +70°; 1 h after the addition of one drop of concentrated HCl: $[M]_{309}$ +465°, $[M]_{269}$ −4791°, α = +53°; ν_{max}^{KBr}: 1720 (>C = O); 855, 900 < 925, 990 (spiroketal chain of the 25S series); M^+ 474.

Partial Hydrolysis of Turoside A (I). A solution of 1 g of glycoside (I) in 100 ml of 50% aqueous methanol was treated with 5 ml of concentrated HCl and the mixture was boiled on the water bath for 5 h. Then the hydrolyzate was diluted with water (250 ml) and the methanol was distilled off as completely as possible. The aqueous solution was extracted with butanol (6 × 50 ml). The extract was washed with water to neutrality and evaporated to dryness. The residue (580 mg) was deposited on a column of SiO_2 and was eluted with chloroform–methanol–water (80:35:7). Fractions containing individual substances were isolated: 1 — 25 mg; 2 — 120 mg; 3 — 50 mg.

3-O-[β-D-Galactopyranosyl]neoagigenin (VI). The recrystallizationof fraction 1 from acetone gave 18 mg of compound (VI) $C_{33}H_{54}O_{10}$, with mp 295-297°C, $[\alpha]_D^{23}$ −51.2±2° [c 0.73; chloroform–methanol (10:1)]. A solution of 5 mg of glycoside (VI) in 1 ml of 50% aqueous ethanol containing 5% of H_2SO_4 was boiled in a sealed tube for 7 h. GLC and TLC (system 1) showed the presence of D-galactose in the hydrolyzates.

3-O-[O-β-D-Glucopyranosyl-(1 → 4)-β-D-galactopyranosyl]neoagigenin (VII). Fraction 2 yielded 100 mg of the glycoside (VII), $C_{39}H_{64}O_{15}$, mp 270-272°C (methanol), $[\alpha]_D^{23}$ −45.7±2° [c 0.92; chloroform–methanol (10:1)]. The hydrolysis conditions were the same as in the preceding experiment. TLC (system 1) showed the presence of glucose and galactose. The ratio of these sugars was 1.00:1.03 (GLC).

3-O-[O-β-D-Glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl]neoagigenin (VIII). The recrystallization of fraction 3 from methanol yielded 45 mg of substance (VIII), $C_{45}H_{74}O_{20}$ with mp 278-282°C, $[\alpha]_D^{23}$ −43.5±2° [c 0.92; chloroform–methanol (10:1)]. The hydrolysis conditions were similar to those described above. According to GLC, glucose and galactose were present in a ratio of 2.00:1.04.

Neoagigenin Trioside Permethylate (IX) from (VIII). The neoagigenin trioside (VIII) (35 mg) was methylated three times as described for glycoside (I). The methylation product obtained (20 mg) was chromatographed on a column of SiO_2. The column was washed with a mixture of benzene and methanol (50:1). This gave 4 mg of substance (IX), the IR spectrum of which showed no absorption in the region of hydroxy groups. The hydrolysis of compound (IX) was carried out in the same way as described for the permethylate (III). 2,3,4,6-Tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose, and 3,4,6-tri-O-methyl-D-glucose were in detected in the hydrolysate by the TLC method (system 2) with markers. The reaction of the 3,4,6-tri-O-methyl-D-glucose for an α-diol grouping [18] was positive, and that of its methyl glucoside was negative

SUMMARY

A methanolic extract of the bulbs of *Allium turcomanicum* Rgl. has yielded a new steroid glycoside — turoside A — which is 3β-[O-β-D-xylopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyloxy-(25S)-5α-spirostan-2α,6β-diol.

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