STEROID SAPONINS AND SAPOGENINS OF *Allium*.

XV. ERUBOSIDE B FROM *Allium erubescens*

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A new steroid glycoside of the spirostan series — eruboside B (I) — has been isolated from an ethanolic extract of the bulbs of *Allium erubescens* C. Koh. In an acid hydrolysate, the aglycone β-chlorogenin (II) and the sugars D-glucose and D-galactose in a ratio of 3:1 have been found. By methylation, partial hydrolysis, and oxidation the structure of the spirostanol (I) has been established as (25R)-5α-spirostan-3β,6β-diol 3-O-{[O-β-D-glucopyranosyl-(1→3)]-[O-β-D-glucopyranosyl-(1→2)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

Continuing a search for plants of the genus *Allium* rich in saponins [1], we have investigated *Allium erubescens* C. Koh., family Liliaceae. A preliminary study with the aid of TLC (system 2a) of an ethanolic extract of the inflorescences of this plant showed the presence in them of four substances of glycosidic nature which we have called in order of increasing polarity erubosides A, B, C, and D. In this paper we give the results of a proof of the structure of the glycoside present in greatest amount — eruboside B (I) (Scheme).

Glycoside (I), according to its IR spectrum (972, 903>929 cm^{-1}), belongs to the spirostan compounds of the 25R series [2]. The carbohydrate chain of eruboside B, which was analyzed with the aid of GLC, consists of D-glucose and D-galactose in a ratio of 3:1. β-Chlorogenin (II) [3, 4] was identified as the aglycone. The partial hydrolysis of (I) yielded two progenins — (III) and (IV). It was shown by the GLC method that glycoside (III) contained one galactose molecule, and (IV) contained galactose and glucose in a ratio of 1:1. The analytical results given show that D-galactose is bound directly to the aglycone.

Further information on the structure of the carbohydrate chain, the position of its attachment to the aglycone, the nature of the bonds, and the size of the oxide rings was obtained in a study of the products of the methylation of eruboside B (I). As the result of three methylations of glycoside (I) by Hakomori's method [5] followed by chromatographic separation of the reaction mixture on a column containing SiO_2, products (V) and (VI) were isolated with yields of 5.7 and 66.4%, respectively.

Compound (V) had a molecular weight of 1276. The IR spectrum of (V) lacked absorption in the region of hydroxy groups, which permits it to be considered as the completely methyl ether of glycoside (I).

The IR spectrum of the more polar product (VI) had absorption at 3480-3520 cm^{-1} (OH). The presence in the mass spectrum of compound (VI) of the peak of the molecular ion (M^+ 1262) 14 m. u. less than for (V) showed the presence of one free hydroxy group in the molecule of (VI). Since in the aglycone of eruboside B, β-chlorogenin, there is a spatially hindered 6β-hydroxy group, it may be assumed that this is the one that remained unmethylated. In actual fact, as the result of the oxidation of the hydroxy methyl ether (VI) with N-bromosuccinimide a keto derivative (VII) was obtained which, after hydrolysis, gave the known sapogenin laxogenin (VIII) [6]. The formation of laxogenin quite obviously shows that the addition of the carbohydrate chain to the hydroxyl at C-6 of β-chlorogenin in aruboside B (I) is impossible.

The structure of the sugar chain was determined in the following way. The hydroxy methyl ether (VI) was subjected to acid hydrolysis, which gave β-chlorogenin (II) and a mixture of

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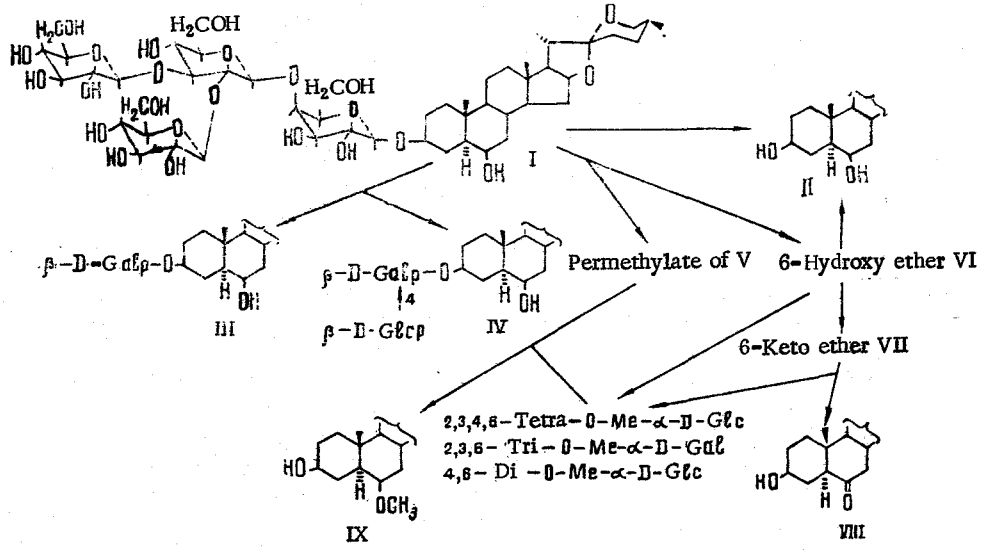

methylated sugars. The latter were separated by chromatography on silica gel. The individual methylated sugars were identified on the basis of the agreement of their physicochemical constants and GLC and TLC characteristics as 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose, and 4,6-di-O-methyl-D-glucose.

The acid cleavage of the permethylate (V) led to the 6-monomethyl ether of β-chlorogenin (IX). The methylated carbohydrates were identical with those that were obtained by the hydrolysis of product (VI).

These facts and the results of the partial hydrolysis of the glycoside (I) permit the conclusion that in the carbohydrate chain of eruboside B the two terminal glucose residues are bound to the hydroxyls at C-2 and C-3 of the third glucose molecule, which, in its turn, is attached to the hydroxyl at C-4 of galactose.

The configuration of the glycosidic centers and the conformations of the carbohydrate components are given on the basis of a study of the PMR spectra of the permethylate (V) and of compound (VI). In both compounds there are four doublets in the 4.18-5.05 ppm region which are due to the resonance lines of the anomeric protons of the sugar residues. The spin–spin coupling constants (J = 7-8 Hz) of these signals show the β configuration of all four glycosidic bonds [4, 7] and the fact that the rings of the carbohydrate components of eruboside B (I) are present in the Cl conformation [9]. This is the first time that such a carbohydrate chain has been found in plants of the genus *Allium*. The structure corresponding to formula (I) is suggested for eruboside B.

EXPERIMENTAL

Thin-layer chromatography was performed in a fixed layer of KSK silica gel containing 7% of gypsum. The following solvent systems were used for chromatography: 1) benzene–methanol [a) 50:1, b) 20:1, c) 10:1]; 2) chloroform–methanol–water [a) 65:35:7; b) 80:35:7; c) 80:32:6]; 3) butanol–ethanol–water (5:3:2); and 4) chloroform–methanol [a) 20:1; b) 10:1]. The glycosides were detected with the Sannie reagent [10] and the sugars with o-toluidine salicylate; the free carbohydrates were chromatographed on plates impregnated with a 0.3 M solution of NaH_2PO_4. The gas–liquid chromatography of the free sugars in the form of the trimethylsilyl ethers of the methyl glycosides and of the methyl glycosides of the methylated carbohydrates was performed as described previously [1].

The mass spectra were obtained on a MKh-1303 instrument fitted with a system for the direct introduction of the substance into the ion source at an ionizing voltage of 40 V and a temperature of 110-160°C, and also on a MKh-1310 instrument at an ionizing voltage of 70 V and a temperature of 110°C. The molecular weights were determined mass-spectrometrically; the IR spectra were taken on a UR-10 spectrometer in KBr or in paraffin oil, and the PMR spectra on a JNM-4H-100 instrument (HMDS as internal standard (δ scale)).

Isolation of Eruboside B (I). The air-dry inflorescences of *A. erubescens* collected in the environs of Tbilisi (village of Kodzhori) in the flowering phenophase (0.5 kg) were ex-

tracted five times with 80% ethanol. The yield of extractive substances was 100 g. Part of this total (50 g) was chromatographed on a column of silica gel with elution by chloroform–methanol with a gradient of increasing amounts of methanol (0 → 50%). This yielded a fraction (5.2 g; chloroform–methanol (4:1) system) enriched with glycoside B, which was rechromatographed in system 2a. As a result, 4.2 g of compound (I) was obtained with the composition $C_{51}H_{84}O_{24}$, mp 274-280°C (decomp.); $[\alpha]_D^{25}$ −71.8 ± 2° (c 0.91; chloroform–methanol (10:1)), ν_{max}^{KBr}, cm^{-1}: 3300-3500 (OH), 972, 903>929 (spiroketal chain of the 25R series).

Acid Hydrolysis of Eruboside B (I). Glycoside (I) (200 mg) was dissolved in 100 ml of 50% aqueous methanol containing 6% of HCl, and the solution was heated in the boiling water bath for 6 h, after which it was diluted with water (100 ml) and evaporated to its original volume. The precipitate that deposited was filtered off and recrystallized from benzene. This gave 42 mg of compound (II), $C_{27}H_{44}O_4$, mp 231-233°C, $[\alpha]_D^{25}$ −74.2 ± 2° (c 1.42; chloroform), M^+ 432. The distances of migration of TLC (systems 1c and 4a) and the characteristic frequencies in the IR spectrum of compound (II) corresponded to those for an authentic sample of β-chlorogenin [3, 4]. The evaporated filtrate was heated in the boiling water for another 2 h. D-glucose and D-galactose were found in the filtrate by the TLC method (system 3). GLC showed the presence of the same sugars in a ratio of 3.00:1.14.

Partial Hydrolysis of Eruboside B (I). A solution of 1.0 g of glycoside (I) in 150 ml of 50% aqueous methanol containing 5 ml of concentrated HCl was heated at the boil for 2.5 h. The hydrolysate was diluted with water (250 ml), and the methanol was distilled off to the greatest possible degree. The aqueous residue was extracted with butanol (6 × 40 ml), and the butanolic extract was washed with water to neutrality and evaporated to dryness. The dry residue (720 mg) was deposited on a column of SiO_2 and was eluted by systems 2a, 2b, and 2c. Fractions containing individual substances were isolated: fraction 1 amounted to 200 g, fraction 2 to 20 g, fraction 3 to 35 g, and fraction 4 to 200 mg. Fraction 1 contained β-chlorogenin (II), and fraction 4 contained eruboside B (I).

β-Chlorogenin 3-O-β-D-Galactopyranoside (III). The recrystallization of fraction 2 from acetone yielded 14 mg of compound (III), $C_{33}H_{54}O_9$, with mp 283-285°C (decomp.); $[\alpha]_D^{25}$ −46.3 ± 2° (c 0.69; chloroform–methanol (10:1)). A solution of 5 mg of glycoside (III) in 1 ml of 50% aqueous methanol containing 5% of H_2SO_4 was boiled in a sealed tube for 6 h. D-Galactose was found in the hydrolyzate by GLC and TLC (system 3).

β-Chlorogenin 3-O-[O-β-D-Glucopyranosyl-(1→4)-O-β-galactopyranoside] (IV). The recrystallization of fraction 3 from methanol yielded 21 mg of substance (IV), $C_{39}H_{64}O_{14}$, with mp 290-293°C (decomp.), $[\alpha]_D^{25}$ −64.2 ± 2° (c 0.79; chloroform–methanol (10:1)). The hydrolysis conditions were the same as in a preceding experiment. TLC in system 3 showed the presence of glucose and galactose. According to GLC, the ratio of these sugars was 1.00:0.82.

Methylation of Eruboside B (I). A solution of 1.0 g of glycoside (I) in 70 ml of dimethyl sulfoxide was treated with 0.86 g of sodium hydride and the reaction mixture was stirred at room temperature for 45 min. Then 9 ml of methyl iodide was added and the mixture was stirred for another 3 h. The reaction product was poured into water and extracted with chloroform. The chloroform extract was washed with sodium hyposulfite solution and with water and was dried over anhydrous sodium sulfate. The dry residue obtained after the distillation of the solvent was methylated in the same way twice more. The methylation products were chromatographed on a column of SiO_2. Elution with system 1a gave 57 mg of the permethylate (V), and elution with system 1b gave 664 mg of compound (VI).

Permethylate of Eruboside B (V) was isolated in the form of an amorphous powder with $[\alpha]_D^{25}$ −71.4 ± 2° (c 1.21; chloroform). The IR spectrum of compound (V) lacks absorption in the region of hydroxy groups. PMR spectrum ($CDCl_3$, δ, ppm): 0.74 (6 H at C-18 and C-27, broadened singlet); 0.91 (6 H at C-19 and C-21, broadened singlet); 3.00-3.57 (14 × OCH_3 and 2 H at C-26, m); 4.28, 4.68, 4.83, and 4.93 (4 H, anomeric protons of the carbohydrate moiety, d, J ≈ 7-8 Hz; H at C-16, m; the multiplet from the proton at C-16 is superimposed on the signal of the anomeric proton at 4.28). M^+ 1276.

The 6-Hydroxy Methyl Ether (VI) was isolated in the form of an amorphous powder with $[\alpha]_D^{25}$ −57.4 ± 2° (c 1.34; chloroform); ν_{max}^{KBr}: 3480-3520 cm^{-1} (OH). PMR spectrum ($CDCl_3$, δ, ppm): 0.74 (6 H at C-18 and C-27, broadened singlet); 0.93 (3 H at C-21, d, J ≈ 5-6 Hz); 1.01 (3 H at C-19, s); 3.04-3.70 (13 × OCH_3; 2 H at C-26, m); 4.28, 4.68, 4.85, and 4.95

(4 H, anomeric protons of the carbohydrate moiety, d, J ≈ 7-8 Hz, H at C-16, m; the multiplet of the proton at C-16 is superimposed on the signal of the anomeric proton at 4.28). M^+ 1262.

Acid Hydrolysis of the 6-Hydroxy Methyl Ether (VI). A solution of 300 mg of compound (VI) in 35 ml of 60% aqueous methanol containing 5% of H_2SO_4 was heated in the boiling water bath for 6 h. The hydrolysate was diluted with water (50 ml), and the methanol was evaporated off. Recrystallization of the precipitate that deposited gave 18 g of a compound $C_{27}H_{44}O_{41}$, mp 232-235°C; $[\alpha]_D^{25}$ −69.8 ± 2° (c 1.08; chloroform), identical with the β-chlorogenin (II) obtained in the hydrolysis of eruboside B (I).

After the aglycone had been extracted, 1 ml of concentrated H_2SO_4 was added to the aqueous solution and the mixture was boiled for 4 h. The hydrolysate was neutralized with $BaCO_3$ and the precipitate was separated off. The filtrate was evaporated to dryness, and the residue obtained (210 mg) was deposited on a column of SiO_2 and was eluted with systems 4a and 4b. Fractions containing individual compounds were isolated: fraction 1) 85 mg; fraction 2) 27 mg; and fraction 3) 46 mg.

2,3,4,6-Tetra-O-methyl-α-D-glucopyranose. The recrystallization of fraction 1 from ethyl acetate gave 57 mg of a substance with mp 90-93°C; $[\alpha]_D^{25}$ +94° → +82.3° (c 1.07; water) [11, 12]. The R_f values in TLC (system 4a) of the substance isolated and of the authentic sample were identical. The GLC of the methyl tetra-O-methylglucoside (phase 2) [1] showed two peaks. The intensities and relative retention times T_{rel} (1.00 and 1.45) of the two peaks also coincided with the corresponding indices of an authentic sample.

2,3,6-Tri-O-methyl-D-galactopyranose was isolated from fraction 2 in the form of a syrupy substance with $[\alpha]_D^{25}$ +72.8 ± 2° (c 1.07; water) [11, 12]. On being compared in TLC (system 4a), the R_f values of the compound obtained and of an authentic sample were identical. The GLC of the methyl tri-O-methylgalactoside (phase 2) gave four peaks the intensities and T_{rel} values of which (3.27, 4.04, 4.41, and 4.82) coincided with the corresponding indices for an authentic sample [1].

4,6-Di-O-Methyl-α-D-glucopyranose. Fraction 3 yielded 99 mg of a substance with mp 163-165°C (methanol): $[\alpha]_D^{25}$ +97.4° → +69.7° (c 0.74; water) [11]. GLC of the methyl dimethylglycoside (phase 3) gave two peaks with T_{rel} 2.55 and 2.72, which corresponds to literature figures [1].

(25R)-5α-Spirostan-3β,6β-diol 6-Methyl Ether (IX) from (V). A solution of 40 mg of the permethylate (V) in 7 ml of 60% aqueous methanol containing 5% of H_2SO_4 was heated at 110°C for 6 h. The hydrolysate was then diluted with water (15 ml), and the methanol was evaporated off. Recrystallization from acetone of the precipitate that had deposited gave 4 mg of compound (IX), $C_{28}H_{46}O_4$, mp 162-166°C. M^+ 446.

The hydrolysate after the extraction of the aglycone was boiled for 4 h after the addition of 1 ml of concentrated H_2SO_4, and it was then neutralized with $BaCO_3$. In the filtrate, 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 were identified by TLC in comparison with authentic samples.

The 6-Keto Methyl Ether (VII) from (VI). A solution of 300 mg of compound (VI) in 40 ml of 72% aqueous dioxane was treated with 85 mg of N-bromosuccinimide. The reaction mixture was stirred in the dark at room temperature for 3 h and was then left to stand for 17 h. After this, 85 mg of N-bromosuccinimide was added and the reaction was continued for another 24 h. The reaction product was poured into water (150 ml), and the precipitate that had deposited was filtered off. The dry residue obtained (240 mg) was deposited on a column of SiO_2 and was eluted with systems 1a and 1b. This gave 165 mg of amorphous compound (VII), $[\alpha]_D^{25}$ −61.3 ± 2° (c 1.12; chloroform); ν_{max}^{KBr}: 1708 cm^{-1} (>C=O). There was no absorption in the region of hydroxy groups.

3β-Hydroxy-(25R)-5α-spirostan-6-one (Laxogenin) (VIII) from (VII). A solution of 140 mg of the keto derivative (VII) in 20 ml of 60% aqueous methanol containing 5% of H_2SO_4 was hydrolyzed in the boiling water bath for 6 h. The hydrolysate was diluted with water (30 ml) and the methanol was evaporated off. Recrystallization from methanol of the precipitate that had deposited gave 18 mg of laxogenin (VIII) with mp 209-213°C, $[\alpha]_D^{25}$ −78.9 ± 2° (c 1.07; chloroform). The characteristics of the IR spectrum and the R_f value in TLC (system 4a) of substance (VIII) coincided with those of an authentic sample of laxogenin [6, 13].

The hydrolysate after the extraction of the aglycone was treated with 1.5 ml of concentrated H_2SO_4 and boiled for 4 h and was then neutralized with $BaCO_3$. 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 were identified in the aqueous fraction by TLC (system 4b) in comparison with authentic samples.

SUMMARY

A new steroid glycoside of the spirostan series — eruboside B — has been isolated from a methanolic extract of the influorescences of *Allium erubescens*; erboside B is (25R)-5α-spirostan-3β,6β-diol 3-O-{[O-β-D-glucopyranosyl-(1→3)]-[O-β-D-glucopyranosyl-(1→2)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

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STEROID SAPONINS AND SAPOGENINS OF *Allium*.

XVI. TUROSIDE C FROM *Allium turcomanicum*

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From a methanolic extract of the bulbs of *Allium turcomanicum* Rgl. we have isolated a new furostanol glycoside, turoside C (I). An acid hydrolysate was found to contain the aglycone — neoagigenin (II) — and the sugars D-xylose, D-glucose, and D-galactose in a ratio of 1:4:1. The structure of the furostanol (I) has been established by methylation, enzymatic hydrolysis, and oxidative cleavage, and also by the oxidative cleavage of (II), as (25S)-5α-furostan-2α,3β,6β,22α,26-pentaol 26-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→3)]-[O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

We have previously reported the isolation from the bulbs of *Allium turcomanicum* Rgl. (family Liliaceae) of a glycoside of the spirostanol series — turoside A — and of turoside A 6-O-benzoate [1, 2]. Continuing the study of the steroid components of this plant, we have isolated from a methanolic extract of the bulbs a new furostanol glycoside, turoside C (Ia).

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