STEROID SAPONINS AND SAPOGENINS OF *Allium*.

XVIII. THE STRUCTURE OF KARATAVIOSIDE B

Yu. S. Bollerner, N. D. Abdullaev,
M. B. Gorovits, and N. K. Abubakirov

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A new glycoside of the spirostan series — karatavioside B (I) — has been isolated from an ethanolic extract of the inflorescences of *Allium karataviense* Rgl. (family Liliaceae). On the basis of chemical transformations and spectral characteristics the structure of (I) has been established as 25(R)-spirost-5-ene-2α,3β-diol S-O-<{O-[4-O(β-hydroxy-β-methylglutaryl)-β-D-xylopyranosyl]-(1 → 3)}-[O-β-D-glucopyranosyl-(1 → 2)]-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside>.

We have previously reported the isolation of karataviosides A and C — new neutral steroid saponins of the spirostan and furostan series, respectively, from inflorescences of *Allium karataviense* Rgl. (family Liliaceae) [1]. In the present paper we give a proof of the structure of karatavioside B (I) isolated from the same material.

As was found, karatavioside B (I) is a more labile compound than glycosides A and C. Under the conditions of column chromatography on silica gel in the chloroform—ethanol—water system it is partially converted into karatavioside A (III). In order to avoid such a transition, we used as eluent an acidic solvent system: chloroform—methanol—water—acetic acid (65:27:5:1).

The fact that karatavioside B (I) is a compound of the spirostan series was confirmed by a positive reaction with a Sannie reagent [2] and by the presence in its IR spectrum of a series of bands characteristic for a spiroketal grouping of the 25(R) series [3, 4]. Furthermore, in the IR spectrum of glycoside (I) there was a strong band at 1735 cm^{-1} which, on the basis of the facts given below, was assigned to the carbonyl absorption of an ester group and of a free carbonyl group.

Karatavioside B (I) was readily saponified by an aqueous methanolic solution of caustic soda. The reaction mixture yielded karatavioside A (III), which has been described previously [1], and a compound (IV) identified as β-hydroxy-β-methylglutaric acid [5-9].

Thus, karatavioside B (I) is an ester of karatavioside A (III) and β-hydroxy-β-methylglutaric acid (IV). Additional proofs were obtained by subjecting the glycoside (I) to complete acid cleavage. After this, yuccagenin (V) [10] was isolated from the reaction mixture, and D-glucose, D-galactose, D-xylose, and β-hydroxy-β-methylglutaric acid (IV) were detected in the hydrolysate by the TLC method. It was shown with the aid of GLC that the sugars mentioned were present in a ratio of 2:1:1.

Characteristic for the PMR spectrum of karatavioside A (III) is complex mutual overlapping of the signals, particularly in the 3.3-5.3 ppm region where the resonance multiplets of the carbohydrate chain and some of those of the aglycone are present. In the strong field, the signals of the protons of the methyl groups appear clearly at 0.58 ppm (3 H at C-27, d with broadened components), 0.68 ppm (3 H at C-18, s), 0.81 ppm (3 H at C-19, s), and 0.99 ppm (3 H at C-21, d, ^3J = 6 Hz). The signals of the methyl groups in the PMR spectrum of karatavioside B (I) are characterized by practically the same chemical shifts: 0.57 ppm (3 H at C-27, d, with broadened components), 0.68 (3 H at C-18, s), 0.80 ppm (3 H at C-19, s), and 0.99 ppm (3 H at C-21, d, ^3J = 6 Hz). The signals of the methyl group in β-hydroxy-β-methylglutaric acid (IV) are located at 1.43 ppm and fall into the region of the methylene hump.

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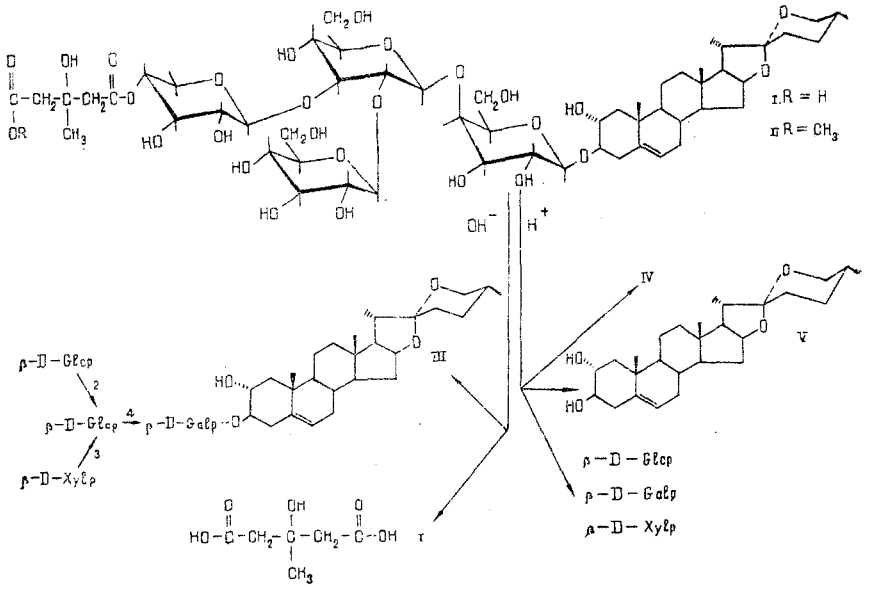

It must be mentioned that the nature of the manifestation of the methylene protons of the acyl moiety (IV) depends substantially on the conditions of recording the spectrum. Thus, in the spectrum of compound (I) (C = 0.05 M) at +25°C the multiplet with its center at 2.70 ppm corresponding to the resonance of the methylene protons is greatly broadened. This signal underwent appreciable narrowing when the temperature of the solution under investigation was raised. Only in the presence of very small amounts of trifluoroacetic acid do the methylene protons under consideration become more equivalent and appear at 2.95 ppm in the form of a broadened four-proton singlet.

The presence of a β-hydroxy-β-methylgutaric acid acid (IV) residue in the molecule of karatavioside B (I) can also be easily detected by comparing the ^{13}C NMR spectra of compounds (I) and (III). In the spectrum of glycoside (I), the carbonyl carbon atoms of the acid residue (IV) resonate at 170.9 ppm, its methylene carbons at 46.7 ppm, its methyl carbon at 28.0 ppm, and its quaternary carbon atom at 70.0 ppm. The values of the chemical shifts given are in good agreement with those for the residue of the same acid present in deltofolin [11, 12] — the only ester of a neutral steroid saponin (dioscin) and a dibasic carboxylic acid known at the present time. The signals mentioned are absent from the spectrum of the glycoside (III).

The methylation of karatavioside B (I) with diazomethane gave the methyl ester (II). The PMR spectrum of compound (II) contained, in addition to the signals given in the discussion of the PMR spectrum of glycoside (I), a three-proton singlet at 3.44 ppm corresponding to the resonance of the protons of a methoxy group. It follows from the facts given that the karatavioside B (I) molecule contains only one residue of β-hydroxy-β-methylglutaric acid (IV) and that one of the carboxy groups of the dibasic acid (IV) esterifies a molecule of karatavioside A (III) and the other remains free.

It must be mentioned that the Kuhn methylation [13] of deltofolin [11, 12] gave its full methyl derivative. In our case, methylation by this method was accompanied by the saponification of the glycoside (I). The position of attachment of the acyl residue (IV) in the molecule of karatavioside B (I) was established on the basis of a comparative analysis of the ^{13}C NMR spectra of yuccagenin (V), karatavioside A (III), and karatavioside B (I).

In the ^{13}C NMR spectrum of karatavioside A (III) taken under conditions of partial suppression of spin—spin coupling with protons, signals at 60.4 ppm (one CH_2 group), 62.7 ppm (two CH_2 groups), and 66.7 ppm (two CH_2 groups) are characterized by triplet splittings and correspond to the resonance of five methylene carbon atoms. In the ^{13}C NMR spectrum of yuccagenin (V), likewise, at 66.8 ppm there is a signal with a triplet-splitting structure which, from the value of its chemical shift, can belong only to the resonance of C-26 of this compound [14, 15]. Thus, in the spectrum of karatavioside A (III) one of the two signals superposed upon one another at 66.7 ppm corresponds to the resonance of C-26 of the aglycone and the second to the resonance of the C-5' atom of the D-xylopyranose molecule.

The C-6' atoms of the two D-glucopyranose molecules resonate at 62.7 ppm, and C-6' of the D-galactopyranose at 60.4 ppm [16, 17].

In the ^{13} NMR spectrum of karatavioside B (I), the signals of the atoms under consideration are located at 60.6 ppm (one CH_2 group), 62.8 ppm (three CH_2 groups), and 66.8 ppm (one CH_2 group). It follows from a comparison of these facts in the spectrum of karatavioside A (III) and its esterified analog karatavioside B (I) that the signals corresponding to the resonance of the C-26 atom of the aglycone, the C-6' atoms of two D-glucopyranose residues, and the C-6' atom of the D-galactopyranose residue have practically coincident positions. On passing from compound (III) to (I), only one of the five carbon-atom signals under consideration — namely that at 66.7 ppm and corresponding in its chemical shift to the C-5' atom of D-galactopyranose [16, 17] — underwent a diamagnetic shift by —3.9 ppm.

This fact permitted us to assume that the acyl group (IV) in glycoside (I) was attached to the D-xylopyranose molecule. From the sign of the shift mentioned, it must be located at C-4' of the pentose molecule [18, 19]. If this is the case, we should detect definite changes as the consequence of esterification also for the C-3' and C-4' atoms of the D-xylopyranose residue. In the ^{13}C NMR spectrum of karatavioside A (III), the C-3' and C-4' atoms of this monosaccharide residue resonate at 78.2 and 70.4 ppm, respectively [16, 17]. A comparative analysis of the spectra of the tetrasides (III) and (I) unambiguously showed that on passing to karatavioside B (I) the positions of precisely these two signals underwent changes. The first of them (C-3') shifted upfield by —3.4 ppm (74.8 ppm), and the second (C-4') downfield by +2.1 ppm (72.5 ppm). Consequently, the magnitude of the α and β contributions of esterification to the chemical shifts of the C-3', C-4', and C-5' atoms ($\Delta\delta_{C\text{-}3'} = -3.4$ ppm; $\Delta\delta_{C\text{-}4'} = +2.1$ ppm and $\Delta\delta_{C\text{-}5'} = -3.9$ ppm) permit us to state that the residue of β-hydroxy-β-methylglutaric acid (IV) in the karatavioside B (I) molecule is located at C-4' of the pentose residue. Thus, the structure of karatavioside B corresponds to formula (I).

EXPERIMENTAL

General Observations. For thin-layer chromatography (TLC) we used silica gel of types KSK (<63 μm) and L (5/40) with the addition of 50% of gypsum, and also Silufol plates. For column chromatography (CC) we used silica gel of types KSK and L (63-100 μm). Craig countercurrent distribution was carried out in the solvent system chloroform—methanol—water (50:50:25). The following solvents systems were used for chromatography: 1a) chloroform—methanol—water (65:30:6); 1b) chloroform—methanol—water (65:22:4); 2) chloroform—methanol—water—acetic acid (65:27:5:1); 3) butanol—ethanol—water (5:3:2); 4) benzene—acetic acid—methanol (45:8:4); 5) ethyl acetate—formic acid—water (100:2:2); and 6) chloroform—methanol (10:1).

PMR spectra were recorded on a JNM 4H-100 instrument (C_5D_5N, 0 — HMDS) and ^{13}C NMR spectra on a CFT-20 (Varian) instrument (C_5D_5N, 0 — TMS). For other details, see [1].

Isolation of Karatavioside B (I). The total methanol-extracted substances (700 g) of the inflorescences of the Turkestan onion, *Allium karataviense*, were treated as described earlier [1]. This gave 98.4 g of mixture 1 (karataviosides A, B, and C) and 66.5 g of mixture 2 (karataviosides A and B). Mixture 1 was subjected to Craig countercurrent distribution in three separatory funnels with unilateral discarding of the lower phase. After ten transfers, 34.8 g of mixture 3 (karataviosides A and B) was obtained. Mixtures 2 and 3, separately, were subjected to CC in portions in system 1a. This gave 18.7 g of mixture 4 (karataviosides A and B), the rechromatography of which in system 3 gave 5.6 g of karatavioside B (I). Yield: 0.08 g calculated on the weight of air-dry raw material.

Karatavioside B (I). Empirical formula $C_{56}H_{88}O_{27}$; after recrystallization from aqueous methanol it had mp 224-227°C (decomp.), $[\alpha]_D^{25} - 75.0 \pm 2^\circ$ (*c* 1.36; dimethyl sulfoxide). ν_{max}^{KBr} (cm^{-1}): 870, 905 > 925 (spiroketal chain of the 25(R) series); 1735 (C=O); 3200-3600 (OH). PMR spectrum (C_5D_5N, δ, ppm): 0.57 (3 H at C-27, d with broadened components); 0.68 (3 H at C-18, s); 0.80 (3 H at C-19, s); 0.99 (3 H at C-21, d, ^3J = 6 Hz); 1.43 (CH_3 of the acyl moiety); 2.70 (methylene protons of the acyl moiety); 4.74 (H at C-16, m); 5.12 (4 H, anomeric protons of sugar residues, m); 5.37 (H at C-6, m).

The Methyl Ether (II) from (I). With cooling in an ice bath, 50 ml of a solution of diazomethane in methylene chloride was added to a solution of 250 mg of karatavioside B in

200 ml of chloroform and methanol (1:1). After 15 min, the reaction mixture was washed with dilute hydrochloric acid and with water, evaporated to dryness, and subjected to CC in system 1b. Recrystallization of the product from methanol yielded 51 mg of the methyl ester (II), $C_{57}H_{90}O_{27}$, mp 201-203°C, $[\alpha]_D^{25} - 54.0 \pm 2^\circ$ (*c* 1.11; pyridine); ν_{max}^{KBr} (cm^{-1}): 870, 905 > 925 (spiroketal chain of the 25(R) series); 1740 (C=O); 3300-3500 (OH). PMR spectrum (C_5D_5N, δ, ppm): 0.57 (3 H at C-27, d with broadened components); 0.68 (3 H at C-18, s); 0.81 (3 H at C-19, s); 0.99 (3 H at C-21, d, ^3J = 6 Hz); 2.86 (4 H, methylene protons of the acyl moiety, broadened singlet); 3.44 (3 H, protons of a methoxy group, s); 4.74 (H at C-16, m); 5.12 (4 H, anomeric protons of the sugar residues, m); 5.37 (H at C-6, m).

Alkaline Hydrolysis of Karatavioside B (I). A solution of 1.0 g of glycoside (I) in 200 ml of 0.5% KOH in 50% aqueous methanol was left at room temperature for 16 h. The precipitate that deposited after the addition of 100 ml of water and the elimination of the methanol by distillation was filtered off and washed with water. After recrystallization from methanol, 640 mg of karatavioside A (III) [1] with mp 280-284°C (decomp.) $[\alpha]_D^{25} - 71.3 \pm 2^\circ$ (c 1.12; pyridine), was obtained. This glycoside had the same R_f value as an authentic sample on TLC in system 1a and showed no depression of the melting point in a mixture with it. The IR spectra of the sample obtained and of the native glycoside were identical.

The filtrate was extracted with ethyl acetate, acidified to pH 1, and again extracted with ethyl acetate (5 × 50 ml). The ethyl acetate extracts were washed with water. After evaporation of the solvent and recrystallization of the residue from a mixture of benzene and methanol, 42 mg of β-hydroxy-β-methylglutaric acid (IV) with mp 109°C was obtained. Mass spectrum of the dimethyl ester (sample obtained for mass spectrometry by methylation of the acid (IV) with diazomethane), m/z (%): 175 ($M^+ - 15$) (15), 117 (100), 101 (30), 85 (70). After a thin-layer chromatograph had been sprayed with 50% aqueous sulfuric acid and had been heated at 120-130°C for 15 min, on observation in UV light a sample of the acid (IV) was revealed in the form of a spot with a bright blue fluorescence having R_f 0.28 and 0.76 in systems 4 and 5, respectively [5-9].

Complete Acid Hydrolysis of Karatavioside B (I). A solution of 400 mg of glycoside (I) in 100 ml of 50% aqueous ethanol containing 5 ml of concentrated sulfuric acid was boiled for 5 h. The precipitate that deposited after the addition of 50 ml of water and the elimination of the methanol by distillation was filtered off and washed with water. Recrystallization from methanol yielded 56 mg of yuccagenin (V) [1, 10] with mp 241-243°C, $[\alpha]_D^{25} - 117.2 \pm 2^\circ$ (*c* 1.40; chloroform). The sample obtained had the same R_f value (TLC) in system 6 as an authentic sample of yuccagenin and gave no depression of the melting point in admixture with it. IR spectra of the sample obtained and of authentic yuccagenin were identical.

The filtrate was extracted with ethyl acetate (3 × 20 ml) and the extracts were washed with water and concentrated, after which β-hydroxy-β-methylglutaric acid (IV) was detected in systems 4 and 5. Then the filtrate was neutralized with EDE-10P anion-exchange resin and was concentrated, and glucose, galactose, and xylose were detected by TLC in system 3. The methanolysis of karatavioside B (I) was performed as described previously [1]. It was shown by GLC (phase 1 [1]) that the ratio of the β-D-glucopyranose, β-D-galactopyranose, and β-D-xylopyranose was 1.00:0.44:0.41.

SUMMARY

The influorescences of *Allium karataviense* Rgl. (family Liliaceae) have yielded a new steroid glycoside of the spirostan series — karatavioside B, which is 25(R)-spirost-5-ene-2α,3β-diol 3-O-<{O-[4-O-(β-hydroxy-β-methylglutaryl)-β-D-xylopyranosyl]-(1 → 3)}-[O-β-D-glucopyranosyl-(1 → 2)]-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside>.

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TRITERPENE GLYCOSIDES OF *Climacoptera transoxana*. I.

Ch. Annaev, M. Isamukhamedova, and N. K. Abubakirov

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Two tripene glycosides — copterosides B and C — have been isolated from the epigeal part of *Climacoptera transoxana* (Iljin) Botsch. On the basis of chemical transformations and physicochemical characteristics, copteroside B has been identified as hedaragenin 3-O-β-D-glucopyranosiduronic acid while copteroside C has the structure of hederagenin 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside].

Plants of the family Chenopodiaceae have been little studied for their saponin content. There is information on the presence of glycosides of oleanolic acid and of hederagenin in the common beet (*Beta vulgaris*) [1, 2] and pricklyseed spinach (*Spinacia oleracea*) [3].

We have investigated the triterpene glycosides of the annual plant *Climacoptera transoxana* (Iljin) Botsch. The material has been studied previously for its flavonoid content [4] and in the same paper the presence of triterpene glycosides in the plant was mentioned. The raw material was collected at the end of the vegetation period in the steppe salt marches in southern-eastern Turkmenia (Kerkinskii district).

When a methanolic extract of the plant was chromatographed in a thin layer (TLC) in various solvent systems, we detected no fewer than eight substances of glycosidic nature. They were called, in order of increasing polarity, copterosides A, B, C, D, F, G, and H. In addition to glycosides, the presence of compounds of low polarity (free aglycones — hederagenin and oleanolic acid) and also of more polar substances of genin nature was detected. The same aglycones were formed on the acid hydrolysis of the combined glycosides. The carbohydrates in the hydrolysate consisted of D-glucose, D-glucuronic acid, and D-xylose.

The results of alkaline hydrolysis of the combined material showed that glycosides D, F, G, and H each had an acyloside chain.

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