2,3,4-Tri-O-methyl-L-rhamnose was isolated from fraction 1 in the form of a syrupy substance, $[\alpha]_D^{25}$ +22.5 → +23.8° (c 1.08; water) [9]. The $R_f$ values of TLC (systems 1c and 2a) of the substance isolated and of an authentic sample were identical. The retention time of the methyl tri-O-methylrhamnoside, $T_{rel}$ = 0.43, on analysis by the GLC method (phases 1 and 2) [8] coincided with the retention time of an authentic sample.

4,6-Di-O-methyl-α-D-glucopyranose. Fraction 2 yielded 12 mg of a substance with mp 161-164°C (methanol); $[\alpha]_D^{25}$ +91.6 → +72.1° (c 0.64; water) [10]. The GLC of the methyl di-O-methylglucoside (phase 2) gave two peaks with $T_{rel}$ = 2.54 and 2.71, which correspond to the indices of an authentic sample [8].

## SUMMARY

An ethanolic extract of the roots of *Tacca cheancer* (family Taccaceae) has yielded a new steroid glycoside of the spirostan series — taccaoside, which is (25R)-spirost-5-en-3β-ol 3-O-{[O-α-L-rhamnopyranosyl-(1 → 2)][O-α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranoside}.

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# STEROID SAPONINS AND SAPOGENINS OF *Allium*.

# XVII. THE STRUCTURE OF KARATAVIOSIDE C

Yu. S. Vollerner, M. B. Gorovits,
T. T. Gorovits, and N. K. Abubakirov

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A new furostanol glycoside — karatavioside C (I) has been isolated from a methanolic extract of the inflorescences of *Allium karataviense* Rgl. (family Liliaceae). By the complete acid hydrolysis, enzymatic hydrolysis, methylation, and reduction of compound (I), and also by the reduction of yuccagenin (II), the structure of the glycoside (I) has been established as 25(R)-furost-5-ene-2α,3β,22α,26-tetraol 26-O-β-D-glucopyranoside 3-O-{[O-β-D-glucopyranosyl-(1→2)][O-β-D-xylopyranoside-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

We have previously reported on a new spirostan tetraoside haratavioside A — isolated from the inflorescences of *Allium karataviense* Rgl. (family Liliaceae) [1] and of the presence of the combined extractive substances of more highly polar glycosides. In the present paper we give a proof of the structure of one of them, a new pentaoside of the furostan series haratavioside C.

After the preliminary working up of a methanolic extract by chromatography and repeated rechromatography of enriched fractions, a mixture of two compounds with close $R_f$ values (Ia/

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Ib) was obtained. When the chromatogram was sprayed with the Sannié reagent [2], both glycosides were revealed in the form of green-yellow spots, while the use of the Ehrlich reagent [3] gave pink-red spots. After two-dimensional TLC [4], a mixture (Ia/Ib) was found in the form of four spots. The IR spectrum of product (Ia/Ib) has a weak broadened band at 895 $cm^{-1}$ [5]. There were no bands corresponding to compounds of the spirostan series [6, 7]. When the combined glycosides Ia/Ib were heated in water and methanol, the individual compounds (Ia) and (Ib), respectively, were obtained. The PMR spectrum of glycoside (Ib) had a three-proton singlet at 3.12 ppm (methoxy group) [5]. This signal was absent from the PMR spectrum of glycoside (Ia). All this is characteristic for glycosides of the furostan series.

Glycosides of the 22-hydroxyfurostan series are always accompanied by the somewhat less polar 22-O-methyl ethers. It is obvious that the 22-methoxyfurostans are artefacts [4]. They are formed in the process of isolation and purification of the glycosides under the action of methanol. On this basis, it must be assumed that karatavioside C (Ia) is the native furostanol glycoside and compound (Ib) is its 22-O-methyl ether. In view of the ease of interconversion of glycosides (Ia) and (Ib), in demonstrating the structure of karatavioside C (Ia) we used the product (Ia/Ib).

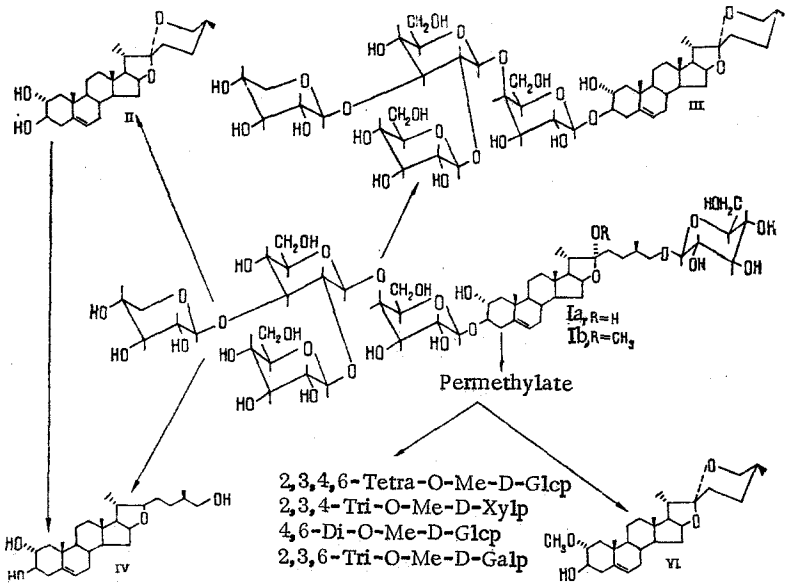
Complete acid hydrolysis of the mixture of glycosides (Ia/Ib) led to yuccagenin (II) [1, 8] and a mixture of monosaccharides. The presence of D-glucose, D-galactose, and D-xylose in a ratio of 3:1:1 was shown by TLC and GLC.

The enzymatic hydrolysis of product (Ia/Ib) with the complex enzyme of the snail *Helix plectotropis* gave compound (III), identical with karatavioside A [1], the carbohydrate chain of which consists of D-glucose, D-galactose, and D-xylose in a ratio of 2:1:1 (lycotetraose).

It is known [9, 10] that the complete acid hydrolysis of the furostanol glycosides forms the corresponding steroid sapogenins, while enzymatic hydrolysis forms glycosides of the spirostan series with the loss of one D-glucose molecule. As a rule, the latter is attached to the hydroxy group at C-26 of the aglycone.

By analogy with other glycosides of the furostan series, it was to be expected that in the karatavioside C molecule (Ia) one D-glucose molecule would be attached to the hydroxy group at C-26 of 25(R)-furost-5-ene-2α,3β,22α,26-tetraol and the tetrasaccharide lycotetraose to the hydroxy group at C-3, as follows from the results of the enzymatic hydrolysis of the product (Ia/Ib).

To prove that karatavioside C(Ia) was a glycoside of the furostan series, product (Ia/Ib) was reduced with sodium tetrahydroborate [4]. After complete acid hydrolysis of the reaction mixture, compound (IV), identical with the product of the reduction of yuccagenin (II) with lithium tetrahydroaluminate (in the presence of aluminum chloride) [11] — dihydroyuccagenin (IV) — was isolated.




To determine the structures and positions of attachment of the carbohydrate chains and to establish the configurations of the glycosidic bonds and the conformations of the carbo-

hydrate rings in the karatavioside C molecule (Ia) the permethylate (V) was obtained by the Hakomori methylation [12] of the mixture of glycosides (Ia/Ib). Completeness of methylation was confirmed by the fact that the IR spectrum of this substance contained no absorption characteristic for hydroxy groups. The PMR spectrum of compound (V) has five doublets in the 4.12-4.95 ppm region corresponding to the anomeric protons of sugars. The spin–spin coupling constants of these signals (J = 7-8 Hz) show the β-configurations of the glycosidic bonds [13] and the C1 conformations of the carbohydrate rings [14]. A comparative analysis of the PMR spectra of the permethylate (V) and of the permethylate of karatavioside A (III) [1] showed that the anomeric proton of the D-glucose attached to the hydroxy group at C-26 of karatavioside C (Ia) has a chemical shift of 4.12 ppm.

After the complete acid hydrolysis of the permethylate (V), compound (VI), identical with the 2-O-methyl ether of yuccagenin described previously, and a set of methylated sugars was obtained. The latter were identified in the presence of authentic samples by TLC and GLC as 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,4-tri-O-methyl-D-xylopyranose, 4,6-di-O-methyl-D-glucopyranose, and 2,3,6-tri-O-methyl-D-galactopyranose.

Thus, the products of the complete acid hydrolysis of the permethylates of karatavioside C (Ia) and karatavioside A (III) [1] are identical. Consequently, we arrive at the conclusion that the hydroxy group at $C_2$ of the aglycone in the molecule of karatavioside C (Ia) is free. The additional glucose molecule by which glycosides (Ia) and (III) differ from one another is terminal and can be attached only to the hydroxy groups at C-26. The remaining four sugar molecules forming the tetrasaccharide lycotetraose are attached to the hydroxy group at C-3. Consequently, the structure of karatavoiside C corresponds to formula (Ia).

## EXPERIMENTAL

General Observations. For thin-layer chromatography (TLC), preparative thin-layer chromatography (PTLC), and two-dimensional thin-layer chromatography (TTLC) we used KSK silica gel (<63 mμ) containing 15% of gypsum, and for column chromatography (CC) KSK silica gel (63-100 mμ). Chromatography was performed in the following solvent systems: 1) chloroform–methanol–water (65:35:8), 2) butanol–ethanol–water (5:3:2), 3) chloroform–methanol (10:1), 4) chloroform–methanol (20:1), and 5) benzene–methanol (10:1). Other information is given in our previous paper [1].

Isolation of the Combined Glycosides (Ia/Ib). The extraction of the plant raw material and the preliminary treatment of the total extractive substances have been described previously [1]. By column chromatography and repeated rechromatography of the enriched fractions, 5 g of a mixture of the glycosides (Ia/Ib) ($R_f$ 0.35 and 0.47, respectively, system 1) was obtained. The yield was 0.1% on the weight of the air-dry raw material. $\nu_{max}^{KBr}$, $cm^{-1}$: 895 (weak broadened band), 3300-3500 (OH).

Karatavioside C (Ia). A solution of 150 mg of product (Ia/Ib) in 50 ml of water was heated at 100°C for 16 h. Part of the water was evaporated off, 200 ml of acetone was added, and the precipitate was filtered off and dried. This gave 120 mg of glycoside (Ia), $C_{56}H_{92}O_{29}$, mp 241-245°C (decomp.), $[\alpha]_D^{20}$ −54.4 ± 2° (c 1.14; pyridine), $\nu_{max}^{KBr}$, $cm^{-1}$: 895 (weak broadened band), 3300-3500 (OH). The PMR spectrum contained no signals of the protons of methoxy groups. (OH). The PMR spectrum contained no signals of the protons of methoxy groups.

22-O-Methyl Ether of Karatavioside C (Ib). A solution of 165 mg of the combined glycosides (Ia/Ib) in 50 ml of absolute methanol was heated at the boil for 16 h. Part of the methanol was evaporated off, 200 ml of anhydrous acetone was added, and the precipitate was filtered off and dried. This gave 90 mg of glycoside (Ib), $C_{57}H_{94}O_{29}$, mp 232-236°C (decomp.), $[\alpha]_D^{20}$ −55.6 ± 2° (c 1.19; pyridine), $\nu_{max}^{KBr}$, $cm^{-1}$: 895 (weak broadened band), 3300-3500 (OH). PMR spectrum ($C_5D_5N$, δ, ppm): 3.12 (22-O-$CH_3$, s).

Complete Acid Hydrolysis of Product (Ia/Ib). A solution of 300 mg of the substance in a mixture of 95 ml of water and 5 ml of concentrated sulfuric acid was heated at 100°C for 6 h. The precipitate that deposited was filtered off, washed with water, and dried. After purification by the CC method in system 4, 35 mg of yuccagenin (II), $C_{27}H_{42}O_4$, was obtained with mp 242-243°C (methanol), $[\alpha]_D^{20}$ −118.5 ± 2° (c 1.19) chloroform), $\nu_{max}^{KBr}$, $cm^{-1}$: 870, 905 > 925 (spiroketal chain of the 25(R) series), 3420 (OH); $M^+$ 430. The compound was identical with an authentic sample [1].

In the filtrate, D-glucose, D-galactose, and D-xylose were detected by TLC (system 2) and GLC (phase 1 [1]) in a ratio of 1.00:0.30:0.33).

Enzymatic Hydrolysis of the Combined Glycosides (Ia/Ib). A solution of 170 mg of product (Ia/Ib) in 20 ml of water was treated with 1 ml of the complex enzyme of the snail *Helix plectotropis* and the mixture was left at 20°C for 16 h. The precipitate that had deposited was filtered off, washed with water, and dried. After recrystallization from methanol, 110 mg of karatavioside A (III), $C_{50}H_{80}O_{23}$, was obtained with mp 281-285°C (decomp.), $[\alpha]_D^{20}$ −69.8 ± 2° (c 1.29; pyridine); $\nu_{max}^{KBr}$, $cm^{-1}$: 905 > 920 (spiroketal chain of the 25(R) series), 3300-3500 (OH); identical with an authentic sample [1].

Methanolysis of the product of enzymatic hydrolysis of (III) gave yuccagenin and the sugars D-glucose, D-galactose, and D-xylose in a ratio of 200:0.94:1.12 as found by TLC (systems 2 and 3) and GLC (phase 1 [1]).

Dihydroyuccagenin (IV) from Product (Ia/Ib). A solution of 1.0 g of the combined glycosides (Ia/Ib) in 150 ml of water was treated with 0.6 g of sodium tetrahydroborate and the mixture was left at 20°C for 16 h. Then a solution of 10 ml of concentrated sulfuric acid in 40 ml of water was added to the reaction mixture and it was heated at 100°C for 6 h. The precipitate that deposited was filtered off, washed with water, and dried. After separation by the CC method in system 4, 18 mg of compound (IV), $C_{27}H_{44}O_4$, was isolated, with mp 179-181°C (acetone), $[\alpha]_D^{20}$ −58.0 ± 2° (c 0.96; chloroform); $\nu_{max}^{KBr}$, $cm^{-1}$: 3360-3380 (OH); $M^+$ 418.

Dihydroyuccagenin (IV) from (II). In portions, 0.45 g of lithium tetrahydroaluminate was added to a solution of 5 g of aluminum chloride in 50 ml of absolute ether in an ice bath, and then a solution of 0.5 g of yuccagenin (II) in 200 ml of absolute ether was added over 15 min. After 45 min, the reaction mixture was boiled for 2 h. All the operations were carried out with stirring. Then 250 ml of dilute hydrochloric acid was added, the ethereal layer was separated off, and the aqueous layer was extracted with ether. The combined ethereal solution was washed with $NaHCO_3$ solution and with water, dried over anhydrous sodium sulfate, and evaporated to dryness. By the CC method in system 4 were isolated 300 mg of yuccagenin (II) and 130 mg of dihydroyuccagenin (IV), $C_{27}H_{44}O_4$, mp 180-182°C (acetone), $[\alpha]_D^{20}$ −59.4 ± 2° (c 0.98; chloroform); $\nu_{max}^{KBr}$, $cm^{-1}$: 3360-3380 (OH); PMR spectrum ($CDCl_3$, δ, ppm): 0.78 (3 H at C-18, s), 0.88 (3 H at C-27, d, J = 6 Hz), 1.02 (6 H at C-19 and C-21, broadened singlet), 3.39 (5 H at C-2, C-3, C-22, and C-26, m), 4.30 (H at C-16, m), 5.31 (H at C-6, m); $M^+$ 418.

Permethylate of Karatavioside C (V). In small portions, 0.9 g of sodium hydride was added to a solution of 1.0 g of the combined glycosides (Ia/Ib) in 100 ml of dimethyl sulfoxide. After 1 h, 13 ml of methyl iodide was added over 15 min, and then the reaction mixture was left for three hours. All the operations were carried out with stirring at 20°C. The reaction products were poured into 250 ml of water and exhaustively extracted with chloroform. The chloroform extracts were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. By the CC method in system 5, 0.66 g of the permethylate (V), $C_{74}H_{128}O_{29}$, was obtained with $[\alpha]_D^{20}$ −54.2 ± 2° (c 1.00; chloroform). The IR spectrum lacked the absorption characteristics of hydroxy groups. PMR spectrum ($CDCl_3$, δ, ppm): 0.77 (3 H at C-18, s), 0.90 (3 H at C-27, d, J = 6 Hz), 1.04 (6 H at C-19 and C-21, broadened singlet), 3.30-3.50 (signals of 18 methoxy groups), 4.12 (anomeric proton of glucose at C-26, d, J = 7 Hz), 4.30, 4.65, 4.85, 4.95 (4 H, anomeric protons of the sugars of the carbohydrate chain at C-3, d, J = 7-8 Hz), 5.24 (H at C-6, m); $M^+$ 1480.

Products of the Complete Acid Hydrolysis of the Permethylate (V). A solution of 350 mg of compound (V) in 100 ml of 50% aqueous methanol containing 5 vol. % of concentrated sulfuric acid was heated at the boil for 10 h. Then 50 ml of water was added to the cooled solution, the methanol was distilled off, and the resulting precipitate was filtered off and dried. Recrystallization from methanol gave 25 mg of the 2-O-methyl ether of yuccagenin (VI), $C_{28}H_{44}O_4$, mp 229-231°C, $[\alpha]_D^{20}$ −147.6 ± 2° (c 0.88; chloroform), $\nu_{max}^{KBr}$, $cm^{-1}$: 868, 905 > 925 (spiroketal chain of the 25(R) series), 3420 (OH); $M^+$ 444; identical with an authentic sample [1].

The filtrate was heated at 100°C for 4 h, after which it was cooled, neutralized with ÉDÉ-10P anion-exchange resin, and evaporated to dryness, and the mixture of methylated sugars was separated by the PTLC method in system 3. The following methylated sugars were found by TLC (system 3) and GLC (phases 2 and 3 [1]) in the presence of authentic samples:

| | $T_{rel}$ | |
|---|---|---|
| | Phase 2 | Phase 3 |
| 2,3,4,6-Tetra-O-methyl-D-glucopyranose | 1.00, 1.45 | 1.00, 1.37 |
| 2,3,4-Tri-O-methyl-D-xylopyranose | 0.44, 0.57 | 0.42, 0.50 |
| 4,6-Di-O-methyl-D-glucopyranose | | 2.62, 2.77 |
| | 3.31, 4.11 | 1.66, 2.22 |
| 2,3,6,Tri-O-methyl-D-galactopyranose | 4.56, 4.91 | 2.32. 2.66 |


## SUMMARY

The inflorescences of *Allium karataviense* Rgl. have yielded a new steroid glycoside of the furostan series, karatavioside C, which is 25R-furost-5-ene-2α,3β,22α,26-tetraol 26-O-β-D-glucopyranoside 3-O-{[O-β-D-glucopyranosyl-(1→2)][O-β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-glactopyranoside}.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