A NEW STEROID GLYCOSIDE, TACCAOSIDE, FROM *Tacca cheancer*

Fam Khoang Ngok, A. N. Kel'ginbaev,
M. B. Gorvits, and N. K. Abubakirov

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A new steroid glycoside of the spirostan series — taccaoside (I) — has been isolated from an ethanolic extract of the roots of *Tacca cheancer* (family *Taccaceae*). An acid hydrolysate was found to contain the aglycone diosgenin (II) and the sugars D-glucose and L-rhamnose in a ratio of 1:2. By methylation and hydrolysis of the permethylate (IV) it has been established that the two terminal L-rhamnose residues are attached at C-2 and C-3 of the D-glucose molecule which, in its turn, substitutes the hydroxy group at C-3 of diosgenin. Glycoside (I) has the structure of (25R)-spirost-5-en-3β-ol 3-O-{[O-α-L-rhamnopyranosyl-(1→2)][O-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside}.

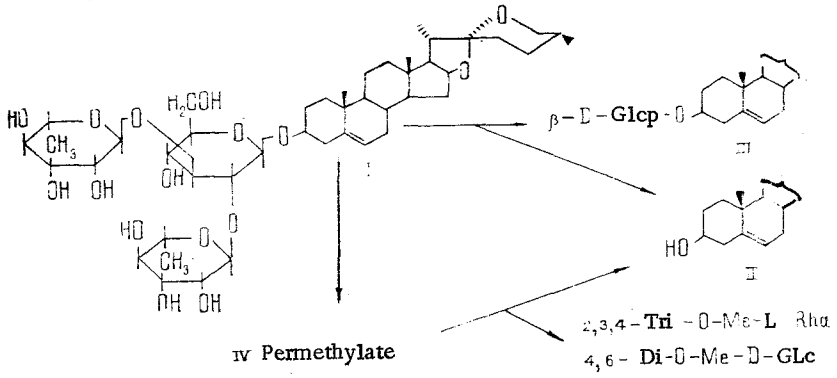
From the roots of the plant *Tacca cheancer* (family Taccaceae) growing in the Socialist Republic of Vietnam we have isolated a new steroid glycoside which has been called taccaoside (I).

According to its IR spectrum (975, 928 < 905 cm^{-1}), glycoside (I) belongs to the spirostan compounds of the 25R series [1]. According to GLC, the carbohydrate chain of taccaoside consists of D-glucose and L-rhamnose in a ratio of 1:2. Among the products of the acid hydrolysis of (I) were found diosgenin (II) and trillin (III) [2]. The formation of the monoside (III) shows that in taccaoside (I) the D-glucose is attached directly to the aglycone.

To determine the positions of attachment of the two L-rhamnose molecules, taccaoside (I) was exhaustively methylated by Hakomori's method [3], and the permethylate (IV) obtained was subjected to acid hydrolysis. This yielded diosgenin (II) and a mixture of methylated sugars which were separated by chromatography on silica gel. The individual methylated carbohydrates were identified on the basis of their physicochemical constants and GLC and TLC behavior as 2,3,4-tri-O-methyl-L-rhamnose and 4,6-di-O-methyl-D-glucose.

The facts given show that in taccaoside the two L-rhamnose molecules are terminal and are attached through the hydroxy groups to C-2 and C-3 of the D-glucose residue, which, in its turn, substitutes the hydroxy group at C-3 of diosgenin.

A calculation by the method of molecular rotation differences [4] ($[M]_D$ for (I), 810.7°; $[M]_D$ for (III), 513.3°; $\Delta[M]_D$, 297.4°) showed the α-configuration of the glycosidic bonds of both L-rhamnose molecules with the D-glucose. In the PMR spectrum of the permethylate (IV) a one-proton doublet appeared at 4.26-4.39 ppm (J ≈ 7-8 Hz) due to the resonance lines of the anomeric proton of the D-glucose [5, 6] and confirming the β-configuration of the gly-



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cosidic bond of the D-glucose with the aglycone. Consequently, taccaoside has a structure corresponding to formula (I).

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed in a fixed layer of KSK silica gel containing 7% of gypsum. Column chromatography was performed on silica gel of the same type (100 > SiO_2 > 56 μm). The following solvent systems were used: 1) benzene–methanol (a, 100:1; b, 50:1; c, 25:2); 2) chloroform–methanol (a, 20:1; b, 5:1; c, 1:1); and 3) chloroform–methanol–water (80:28:5). The glycosides were revealed with the Sannié reagent [7], and the sugars with o-toluidine salicylate. The gas–liquid chromatography (GLC) of the free sugars in the form of trimethylsilyl ethers of methyl glycosides and the methyl glycosides of methylated carbohydrates was carried out as described previously [8].

Mass spectra were obtained on a MKh-1310 instrument at an ionizing voltage of 70 V and a temperature of 110°C. Molecular weights were determined mass-spectrometrically, IR spectra were taken on a UR-20 spectrometer in KBr or paraffin oil, and PMR spectra on a JNM-4H-100 instrument with HMDS as internal standard (δ scale).

Isolation of Taccaoside (I). The ground and comminuted roots of *Tacca cheancer* (1 kg) were extracted with 90% ethanol (8 × 2 liters) at room temperature. The ethanolic extract was concentrated to one liter, diluted with two volumes of water, and extracted with benzene–ethyl ether (1:1), and then with butanol. This gave 4.1% of a benzene-ether extract and 8.6% of a butanol fraction, calculated on the air-dry raw material. Part of the combined butanol-soluble material was chromatographed on a column of Al_2O_3 with elution successively by benzene, chloroform, ethyl acetate, and methanol. The ethyl acetate fraction was rechromatographed on a column of SiO_2 (system 3). Recrystallization from methanol–chloroform then yielded taccaoside (I), $C_{45}H_{72}O_{16}$, mp 249-251°C (decomp.); $[\alpha]_D^{25}$ − 93.4 ± 2° (c 2.14; dimethylformamide); ν_{max}^{KBr}, cm^{-1}: 3550-3250 (OH), 975, 928 < 905 (spiroketal chain of the 25R series); M^+ 868.

Acid Hydrolysis of Taccaoside (I). A solution of 730 mg of the glycoside (I) in 100 ml of 50% aqueous methanol containing 10% of H_2SO_4 was heated at 100°C for 10 h. The reaction mixture was diluted with water (100 ml) and evaporated to small volume. The precipitate that deposited (420 mg) was chromatographed on a column of SiO_2. Elution with chloroform gave a fraction (143 mg), which after recrystallization from acetone, yielded 60 mg of sapogenin (II), $C_{27}H_{42}O_3$, mp 205-206°C, $[\alpha]_D^{25}$ − 119.7 ± 2° (c 1.18; chloroform). In its mobility on TLC (systems 1c and 2a) and characteristic IR frequencies, compound (II) corresponded to an authentic sample of diosgenin. Elution with system 2c gave a fraction (196 mg) the recrystallization of which, from chloroform–methanol yielded 75 mg of a monoside (III), $C_{33}H_{52}O_8$, mp 254-256°C, $[\alpha]_D^{25}$ −88.2 ± 2° (c 1.7; dimethylformamide), $[\alpha]_D^{25}$ −104.3 ± 2° (c 1.13; dioxane), which was identified by the constants given as trillin [2]. The GLC of taccaoside (I) showed the presence of glucose and rhamnose in a ratio of 1.00:2.09.

Permethylate of Taccaoside (IV) from (I). Over 20 min, 460 mg of sodium hydride was added to 490 mg of glycoside (I) in 35.3 ml of dimethyl sulfoxide, and the mixture was stirred at room temperature for 40 min. Then 4.4 ml of methyl iodide was added and the mixture was stirred for another 3 h. The reaction product was poured into water (200 ml) and extracted with chloroform (5 × 30 ml). The chloroform extract was treated with a solution of sodium hyposulfite, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (590 mg) was chromatographed on a column of Al_2O_3 (systems Ia and Ib). Elution with system Ib gave 320 mg of the amorphous permethylate (IV): $[\alpha]_D^{25}$ −94.2 ± 2° (c 1.34; chloroform). The IR spectrum of compound (IV) lacked absorption in the region of hydroxy groups M^+ 980.

Acid Hydrolysis of the Permethylate (IV). A solution of 180 mg of compound (IV) in 90 ml of 50% aqueous methanol, containing 5% of H_2SO_4 was heated at 100°C for 10 h. The hydrolysate was diluted with 50 ml of water and evaporated to small volume. Recrystallization of the precipitate that had deposited gave 16 mg of a compound $C_{27}H_{42}O_3$ with mp 203-205°C (methanol), $[\alpha]_D^{25}$ −118.1 ± 2° (c 0.91; chloroform), identical with diosgenin.

Separation of the Methylated Sugars. The aqueous solution after the extraction of the aglycone was boiled in the water bath for 4 h. The hydrolysate was neutralized with $BaCO_3$ and the precipitate was filtered off. The filtrate was evaporated to dryness, and the resulting residue was chromatographed on a column of SiO_2. Chloroform elution yielded fraction 1 (56 mg), and system 2b eluted fraction 2 (21 mg).

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}.

LITERATURE CITED

1. M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klump, Anal. Chem., 24, 1337 (1952).
2. R. E. Marker and J. Kreuger, J. Am. Chem. Soc., 62, 2548 (1940).
3. S. Hakomori. J. Biochem., 55, 205 (1964).
4. W. Klyne, Biochem. J., 47, xli (1950).
5. J. M. van der Veen, J. Org. Chem., 28, 564 (1963).
6. V. V. Isakov, A. K. Dzizenko, G. I. Oshitok, N. I. Uvarova, and G. B. Elyakov, Khim. Prir. Soedin., 78 (1972).
7. C. Sannié, S. Heitz, and H. Lapin, C. R., 233, 1670 (1951).
8. G. V. Pirtskhalava, M. B. Gorovits, T. T. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 514 (1979).
9. I. Heilbron, Dictionary of Organic Compounds, 4th ed., Eyre and Spottiswoode, London (1965), Vol. V, p. 2863.
10. R. Kuhn, L. Low, and H. Trischman, Chem. Ber., 90, 203 (1957).

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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