STEROID SAPONINS AND SAPOGENINS OF *Allium*

IX. THE STRUCTURE OF AGINOSIDE

A. N. Kel'ginbaev, M. B. Gorovits, T. T. Gorovits, and N. K. Abubakirov

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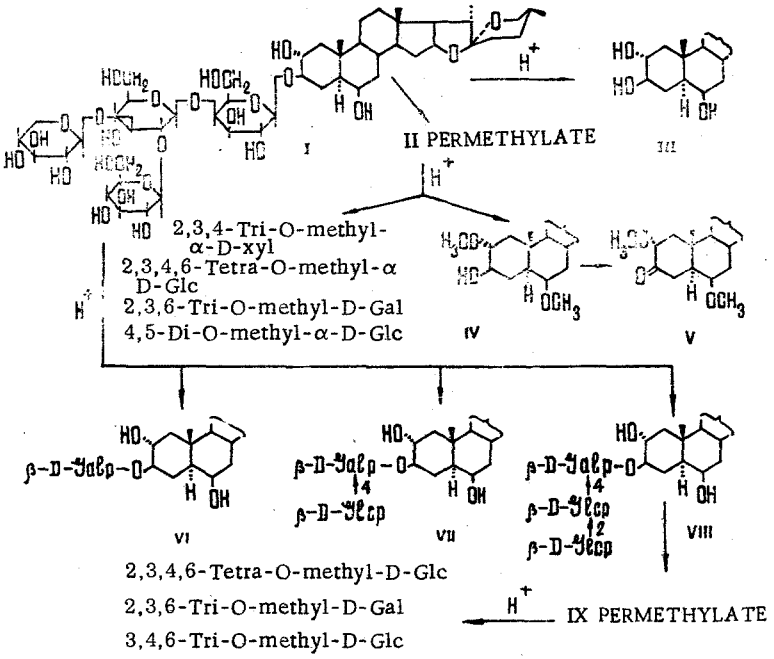
Continuing a study of the more polar fractions of a methanolic extract of the skins of the bulbs of *Allium giganteum* Rgl. (family Alliaceae) [1, 2] we have isolated a new steroid glycoside which we have called aginoside (I). According to its IR spectrum, glycoside (I) belongs to the spirostan compounds of the 25R series.

When the glycoside was hydrolyzed with weak hydrochloric acid, agigenin (III) [2] was identified as the aglycone. By GLC [3, 4], D-xylose, D-glucose, and D-galactose in a ratio of 1:2:1 were found in the hydrolyzate.



In a study of the products of the Smith degradation of the glycoside (I) [5], we identified D-glucose and agigenin (III). Consequently, one of the two molecules of the glucose either is a center of branching of the carbohydrate chains or has a 1→3 bond.

After acid hydrolysis, the permethylate (II) of the glycoside (I), obtained by Hakomori's method [6], gave dimethoxyagigenin (IV) and a mixture of various methylated monosaccharides. The mixture was separated by chromatography on silica gel. On the basis of the combination of the physicochemical constants, some chemical reactions, and GLC and TLC, the methylated carbohydrates were identified 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. These facts and the results of the Smith degradation of the glycoside (I) permit the conclusion that the center of branching is glucose, and galactose is attached directly to the aglycone.

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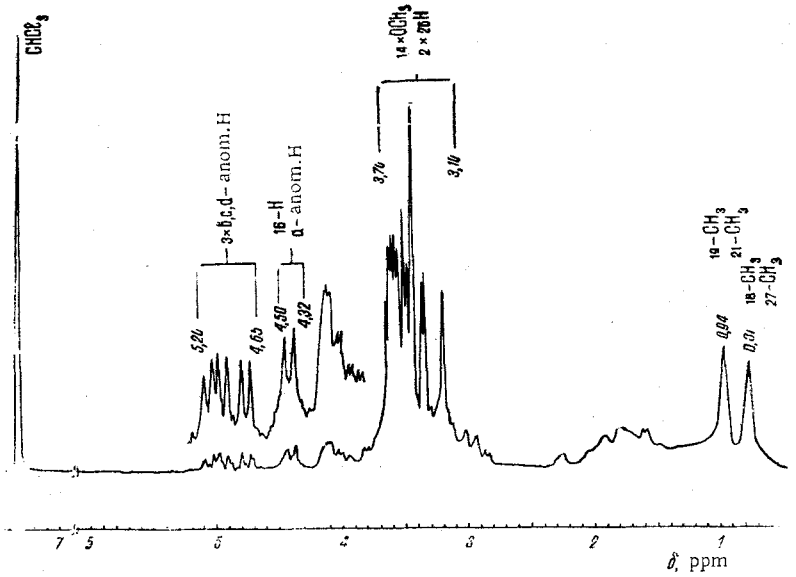

Fig. 1. NMR spectrum of the permethylate of aginoside (II).

Further information on the structure of the carbohydrate chain of aginoside (I) was obtained by studying the products of its partial acid hydrolysis. From these it was possible to isolate three glycosides (VI-VIII). According to GLC, glycoside (VI) is a monoside and contains only galactose. Compound (VII) is a bioside containing galactose and glucose (1:1). Substance (VIII) is a trioside, the carbohydrate chain of which consists of one molecule of galactose and two molecules of glucose.

To determine the position of attachment of the terminal sugars glucose and xylose in (I), the trioside (VIII) was also subjected to exhaustive methylation by Hakomori's method. In the products of the hydrolysis of 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. Consequently, in the carbohydrate chain of aginoside (I) the terminal glucose and xylose molecules substitute the hydroxyls at C_2 and C_3, respectively, of the second glucose molecule, which, in its turn, is attached to the hydroxyl at C_4 of galactose.

The configuration of the glycosidic centers is given on the basis of a study of the NMR spectrum (Fig. 1) of the permethylate (II), in which four doublets (a, b, c, and d) of the anomeric protons of the sugars clearly appear in the 4.3-5.2 ppm region. The coupling constants (J = 7-8 Hz) of the signals given show the β configurations of all four glycosidic bonds [7, 8]. The proposed configurations agree with those calculated by the method of molecular rotation differences [9].

A similar carbohydrate chain, called lycotetraose [10] has previously been found in the steroid glycoalkaloids tomatine and dimessine [10], and also in the steroid saponins F-gitonin [11] and purpureagitoside [12].

It was necessary to determine the position of attachment of the carbohydrate chains to the steroid nucleus. Although the most probable position was the hydroxyl at C_3, it was not excluded that the sugar moiety was attached to one of the hydroxyls at C_2 and C_6 of the steroid nucleus. For an unambiguous answer to the question, the dimethoxyagigenin (IV) obtained by the hydrolysis of the permethylate (II) was oxidized with chromium trioxide. The optical rotatory dispersion (ORD) curve of the dimethoxy ketone (V), with a positive Cotton effect (see Experimental), has the form characteristic for 2-oxo- or 3-oxo-5α-steroids [13]. In view of the fact that in the determination of the ORD in methanol with the addition of hydrochloric acid a sharp decrease in amplitude is observed, compound (V) can be considered to be a 3-oxo-5α-steroid [14]. Thus, the carbohydrate residue is attached to the hydroxyl at C_3. Consequently, aginoside has the structure corresponding to formula (I).

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed on a fixed layer of KSK silica gel containing 7% of gypsum. The following solvent systems were used: 1) butanol—ethanol—water

(5:3:2) and 2) chloroform–methanol (10:1). The glycosides were detected with Sannié's reagent [15] and the sugars with o-toluidine salicylate on plates impregnated with a 0.3 M solution of NaH_2PO_4.

Gas–liquid chromatography (GLC) was performed on a "Tsvet-4" chromatograph. The glycosides were hydrolyzed with 5% H_2SO_4 in sealed tubes at 100°C for 6 h. The monosaccharides were analyzed in the form of the trimethylsilyl ethers of the methyl glycosides [3, 4], using a column (2 m × 4 mm) filled with 5% of the silicone phase SE-30 on Chromaton N-AW; the temperature of the thermostat was 170°C, and the carrier gas was He at a rate of 40 ml/min. The methyl glycosides of the methylated sugars were obtained by boiling the methyl ethers in 5% methanolic HCl for 4 h and were chromatographed on a column (1 m × 4 mm) containing 20% of tetramethylene succinate on Celite (phase 1) or 10% of poly(phenyl ether) on Chromaton N-AW (phase 2); the temperature of the thermostat was 180°C, and the carrier gas was He at a rate of 50 ml/min. The retention times T_{rel} for the methylated methyl glycosides were calculated in relation to the retention time of methyl 2,3,4,6-tetra-O-methyl-β-D-glycopyranoside [16].

The mass spectra were obtained on an 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. The molecular weights were determined by mass spectrometry, the IR spectra were taken on a UR-20 spectrometer in KBr, the NMR spectra on a JNM-4H-100 instrument in $CDCl_3$ with HMDS as internal standard (δ scale), and the optical rotatory dispersion spectra on a J-20 spectropolarimeter.

Isolation of Aginoside (I). The air-dry skins of the bulbs of *A. giganteum* (5 kg) were extracted with methanol at 60°C (five times). The yield of extractive substances was 500 g. Of this material, 60 g was chromatographed on a column of silica gel (1200 g) in chloroform–methanol with gradientwise increasing proportions of methanol (0 → 50%). When the column was eluted with the system having a 3:1 ratio of the components, a fraction was obtained from which, after precipitation with acetone and with repeated recrystallization from methanol, 4.3 g of compound (I) was obtained: $C_{50}H_{82}O_{24}$ with mp 272–276°C (decomp.), $[\alpha]_D^{29}$ − 66.7 ± 2° (c 1.21; chloroform–methanol, 10:1); γ_{max}^{KBr}: 3250–3550 (OH), 872, 910 > 930 cm^{-1} (spiroketal chain of the 25R series).

Acid Hydrolysis of Aginoside (I). A solution of 200 mg of the glycoside (I) in 100 ml of 50% aqueous methanol containing 6% of HCl was heated in the boiling-water bath for 7 h. The reaction mixture was diluted with water (100 ml), evaporated to its initial volume, and heated for another 2 h. The precipitate that deposited was recrystallized from methanol. This gave 20 mg of agigenin (III), $C_{27}H_{44}O_5$, with mp 265–267°C, $[\alpha]_D^{29}$ − 74.2 ± 2° (c 1.27; chloroform); γ_{max}^{KBr}: 3300–3500 (OH), 872, 905 > 927, 965 cm^{-1} (spiroketal chain of the 25R series): M^+ 448.

In the hydrolyzate, D-xylose, D-glucose, and D-galactose were found by TLC in system 1. GLC showed the presence of the same sugars in a ratio of 1.08:2.00:0.98.

Smith Degradation of Aginoside (I). A solution of 100 mg of the glycoside (I) in 100 ml of 50% methanol was treated with 0.8 g of sodium periodate and 1 ml of acetic acid, and the mixture was kept at room temperature for six days. The unchanged periodate was decomposed with ethylene glycol. The methanol was evaporated off from the reaction mixture, and the residue was diluted with 25 ml of water and was extracted with butanol (5 × 20 ml). The butanolic extracts were washed with water and concentrated. The concentrate was treated with 5 ml of water and 0.7 g of sodium tetrahydroborate, and the mixture was heated at 70–80°C for 8 h. Then it was neutralized with KU-2 cation-exchange resin and evaporated with the addition of methanol (3 × 10 ml).

The product obtained was hydrolyzed in 20 ml of 2% H_2SO_4 at room temperature for 20 h. After recrystallization, the precipitate that had deposited (24 mg) was identified as agigenin (III). In the neutralized and concentrated filtrate, D-glucose was detected by TLC (system 1) and GLC.

Permethylate of Aginoside (II) from (I). The glycoside (I) (1.70 g) in 120 ml of dimethyl sulfoxide was treated with 1.46 g of sodium hydride, and the mixture was shaken at room temperature for 40 min. Then 15 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 treated with a solution of hyposulfite, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was methylated

by the same method twice more. The mixture of products obtained after the third methylation was chromatographed on a column of silica gel. Elution with benzene–methanol (50:1) yielded 1.03 g of the amorphous permethylate (II) with $[\alpha]_D^{29}$ – 76.1 ± 3° (c 1.05; chloroform). The IR spectrum of compound (II) showed no absorption in the hydroxy-group region.

(25R)-5α-Spirostan-2α,3β,6β-triol 2,6-Dimethyl Ether (IV) from (II). A solution of 0.91 g of the permethylate (II) in 62 ml of 60% aqueous methanol containing 5% of H_2SO_4 was hydrolyzed at 100°C for 6 h. The hydrolyzate was diluted with water (200 ml) and evaporated to its initial volume. The precipitate that had deposited was recrystallized from acetone, giving 65 mg of compound (IV), $C_{29}H_{48}O_5$, mp 176-178°C; $[\alpha]_D^{29}$ – 100 ± 2° (c 0.7; chloroform); ν_{max}^{KBr}: 3350-3550 (OH), 875, 910 > 930 cm^{-1} (spiroketal chain of the 25R series); M^+ 476.

Separation of the Methylated Sugars. The aqueous solution from the preceding experiment, after the extraction of the aglycone, was treated with 5 ml of concentrated H_2SO_4 and boiled for 4 h. The hydrolyzate was neutralized with $BaCO_3$ and filtered. The filtrate was evaporated to dryness and the residue (720 mg) was transferred to a column of silica gel (260 g). The contents of the column were eluted first with benzene and then with benzene–acetone with gradually increasing proportions of acetone. The process was monitored by TLC in system 2. On elution with benzene–acetone at a ratio of 8:1, fraction 1 was isolated – 120 mg (R_f 0.86); 7:1 – fraction 2, 20 mg (0.86, 0.9); 6:1 – fraction 3, 135 mg (0.9); 5:1 – fraction 4, 15 mg (0.9, 0.77, 0.73); 4.5:1 – fraction 5, 10 mg (0.9; 0.73); 4:1 – fraction 6, 17 mg (0.9, 0.77, 0.73); 4:1 – fraction 7, 20 mg (0.77, 0.73, 4:1 – fraction 8, 25 mg (0.73); 4:1 – fraction 9, 40 mg (0.77, 0.73; 0.68); 3:1 – fraction 10, 15 mg (0.73, 0.68); 2:1 – fraction 11, 24 mg (0.68, 0.5); 1:1 – fraction 12, 30 mg (0.68, 0.5, 0.31); 1:1.5 – fraction 13, 40 mg (0.5, 0.31); 1:1.5 – fraction 14, 15 mg (0.5, 0.31).

2,3,4-Tri-O-methyl-α-D-xylopyranose. Recrystallization of fraction 1 gave a substance with mp 84-85°C (chloroform–methanol); $[\alpha]_D^{29}$ + 59.3 → 19.3° (c 0.31; water). Literature information [10]: mp 91-92°C; $[\alpha]_D^{20}$ + 64.5 → +17.7° (water). The R_f value in TLC (system 2) coincided with that of an authentic sample. The GLC of the methyl ether of 2,3,4-tri-O-methylxylose (phase 1) gave two peaks with T_{rel} (0.44, 0.56) the intensities of which coincided with the analogous indices of an authentic sample.

2,3,4,6-Tetra-O-methyl-α-D-glucopyranose. Fraction 3 yielded a substance with mp 92-94°C (ethyl acetate); $[\alpha]_D^{29}$ + 103 → +81° (c 1.13; water). Literature data [11]: mp 88-94°C; $[\alpha]_D^{13}$ + 90° → +85° (water). On comparison by TLC (system 2), the R_f values of the product obtained and of an authentic sample were identical. The GLC of the methyl tetra-O-methyl glucoside (phase 1) gave two peaks the intensities and T_{rel} values (1.00, 0.45) of which coincided with the analogous values for the authentic sample.

2,3,6-Tri-O-methyl-D-galactopyranose. Fraction 8 consisted of a syrupy mass, $[\alpha]_D^{29}$ + 67.0° (c 0.7; water). Literature data [11]: $[\alpha]_D^{9}$ + 79.0° (water). When the methyl tri-O-methylgalactoside was subjected to GLC (phase 1), four peaks were obtained coinciding in intensity and T_{rel} (3.25, 4.00, 4.38, 4.76) with literature data [16] for this compound. The product of demethylation with BCl_3 [17] was identified as galactose by TLC (system 1) and GLC.

4,6-Di-O-methyl-α-D-glucopyranose. The recrystallization of fraction 13 from ethyl acetate gave a product with mp 164-165°C; $[\alpha]_D^{29}$ + 91.3 → +65.2° (c 0.46; water). Literature data [10]: mp 163-164°C; $[\alpha]_D^{23}$ + 118 → 67.5° (water). After the demethylation of the dimethoxyglucopyranose obtained with BCl_3, TLC (system 1) and GLC showed the presence of glucose. The GLC of the methyl di-O-methylglucoside on phase 2 gave a peak with T_{rel} 3.00.

The other fractions consisted of mixtures of the compounds described above obtained in the separation of the mixture of methylated sugars.

Partial Hydrolysis of Aginoside (I). A solution of 700 mg of the glycoside (I) in 100 ml of 50% aqueous methanol containing 5 ml of HCl was heated at the boil for 3 h. The hydrolyzate was diluted with water (200 ml) and the methanol was distilled off as completely as possible. The aqueous residue was extracted with butanol (5 × 40 ml), and the butanolic extract was washed with water to neutrality and evaporated in vacuum. The dry residue (570 mg) was mixed with a fivefold amount of silica gel and deposited on a column containing 260 g of silica gel. The contents of the column were washed with chloroform–methanol–water (80:35:7); 100-ml fractions being collected and examined by TLC in the same system. The following fractions were obtained: 1, 40 mg (R_f 0.97, 0.92); 2, 36 mg (0.97, 0.92); 3, 45 mg (0.65); 4, 105 mg (0.65, 0.46); 5, 73 mg (0.46); 6, 31 mg (0.46, 0.38); 7, 22 mg (0.46, 0.38,

0.33); 8, 30 mg (0.38; 0.33); 9, 57 mg (0.33); 10, 64 mg (0.33, 0.3); 11, 20 mg (0.3); 12, 15 mg (0.3).

Agigenin 3-O-β-D-Galactopyranoside (VI). Recrystallization of fraction 3 from acetone gave compound (VI), $C_{33}H_{54}O_{10}$, with mp 300-302°C (decomp.); $[\alpha]_D^{29}$ − 42.0 ± 2° [c 0.6; chloroform–methanol (10:1)]. A solution of 5 mg of glycoside (VI) in 1 ml of 50% aqueous methanol containing 5% H_2SO_4 was boiled in a sealed tube for 6 h. The hydrolyzate was shown to contain D-galactose by GLC and TLC in system 1.

Agigenin 3-O-[O-β-D-Glucopyranosyl-(1→4)-β-D-galactopyranoside] (VII). Recrystallization of fraction 5 from methanol gave the glycoside (VII), $C_{39}H_{64}O_{15}$ with mp 308-310°C (decomp.), $[\alpha]_D^{29}$ − 60.9 ± 2° [c 1.14; chloroform–methanol (10:1)]. The conditions for the hydrolysis of the bioside (VII) were similar to those described in the preceding experiment. TLC in system 1 showed the presence of glucose and galactose. According to GLC, the ratio of these sugars was 1.00:1.07.

Agigenin 3-O-[O-β-D-glucopyranosyl-(1→2)]-[O-β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside (VIII). Fraction 9 was recrystallized from methanol and yielded substance (VIII), $C_{45}H_{74}O_{20}$ with mp 290-292°C (decomp.); $[\alpha]_D^{29}$ − 52.0 ± 2° [c 1.34; chloroform–methanol (10:1)]. The conditions for performing hydrolysis were similar to those described above. GLC showed the presence of glucose and galactose in a ratio of 2.00:1.10.

Permethylate of the Agigenin Trioside (IX) from (VIII). The trioside (VIII) (28 mg) was methylated and treated as described above for aginoside (I). This gave 25 mg of crude product, which was mixed with a fivefold amount of silica gel and transferred to a column containing 18 g of silica gel. The contents of the column were washed with benzene–methanol with gradually increasing concentrations of methanol. On elution with the 50:1 mixture, 4 g of product (IX) was obtained, the IR spectrum of which showed no absorption in the region of hydroxy groups. The compound (IX) was hydrolyzed under conditions similar to those described above for the permethylate (II). In the aqueous part of the hydrolyzate by TLC in system 2 and comparison with authentic samples, 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 identified. The reaction for an α-diol grouping with the Bonner reagent [18] was positive for the 3,4,6-tri-O-methyl-D-glucose and negative for its methyl glucoside.

2α,6β-Dihydroxy-(25R)-5α-spirostan-3-one 2,6-Dimethyl Ether (V) from (IV). A solution of 160 mg of CrO_3 in 13 ml of acetic acid was treated with 60 mg of compound (IV) in 2 ml of acetic acid and the mixture was left at room temperature for 48 h. Then it was diluted with 30 ml of methanol, and the unchanged CrO_3 was decomposed with Na_2SO_3. The residue was treated with two volumes of water, the methanol was distilled off as completely as possible, and the residual mixture was extracted with chloroform. The chloroform extract was washed with water, with 5% Na_2CO_3 solution, and with water again, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (reaction product) (45 mg) was mixed with a fivefold amount of silica gel and deposited on a column containing 18 g of silica gel. The contents of the column were eluted first with benzene and then with benzene–methanol with gradually increasing proportions of methanol. On elution with the 250:1 mixture, 7 mg was obtained of compound (V), $C_{29}H_{46}O_5$, with mp 142-144°C (acetone); $[\alpha]_D^{23}$ − 36.0° (c 0.08; methanol). Optical rotatory dispersion (c 0.08; methanol): $[M]_{309}$ + 1310°, $[M]_{263}$ − 5580°, a = +69°; an hour after the addition of one drop of concentrated HCl: $[M]_{309}$ − 455°, $[M]_{263}$ − 5010°; a = +45.5°; γ_{max}^{KBr}: 1727 (>C = O), 875, 910 > 930 cm^{-1} (spiroketal chain of the 25R series); M^+ 474.

SUMMARY

From a methanolic extract of the skins of the bulbs of *Allium giganteum* Rgl, a new steroid glycoside has been isolated – aginoside, which is (25R)-5α-spirostan-2α,3β,6β-triol 3-O-{[O-β-D-xylopyranosyl-(1→3)-]-[O-β-D-glucopyranosyl-(1→2)]-O-β-D-glucopyranosyl-(1→4)-O-β-D-galactopyranoside}.

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STEROID SAPONINS

XIV. THE STRUCTURE OF AGAVOSIDE G FROM THE LEAVES OF *Agava americana*

P. K. Kintya, V. A. Bobeiko, and A. P. Gulya

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In the preceding papers we have reported the detection in the leaves of *Agava americana* (century plant agave) of steroid glycosides and have given a proof of the chemical structures of the spirostanol glycosides agavosides C, C', and D [1, 2]. In the present paper we discuss the structure of a new glycoside of the furostanol series — agavoside G (Ia).

Chromatography of the total agavosides on a column of silica gel gave the total furostanol glycosides, which were subjected to acetylation. The acetates were separated on silica gel.

After the saponification of the acetate of agavoside G and purification on a column of silica gel, the pure glycoside G was obtained, giving a positive reaction with Ehrlich's reagent [3] in TLC. In methanol systems, the glycoside gave two spots, (Ia) and (Ib), on a thin-layer chromatogram, which is characteristic for the furostanol glycosides [4, 5].

Under the action of the complex enzyme from *Helix pomatia* in aqueous solution at room temperature for a day, agavoside G was converted into agavoside D [2], forming only one spot on TLC.

The acid hydrolysis of agavoside G gave an aglycone which was identified by its melting point, specific rotation, IR spectrum, and chromatographic mobility as hecogenin. In view of the fact that ring F closes on enzymatic hydrolysis, the native genin must be considered to be 3β,22α,26-trihydroxy-(25R)-5α-furostan-12-one. This was confirmed by the IR spectrum of glycoside G — there are no absorption bands characteristic for spirostanol glycosides while the broad band at 900 cm^{-1} that is characteristic for the furostanol glycosides [5] is present.

The presence of a signal in the PMR spectrum with a value of chemical shift δ of 3.24 ppm, which is characteristic for a methoxy group at C_{22} [6] also confirms that 3β,22α,26-trihydroxy-(25R)-5α-furostan-12-one is the aglycone of glycoside G.

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