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STEROID SAPONINS

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

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UDC 547.917+547.918

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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By means of the GLC of the acetates of the aldononitrile derivatives of the sugars in a hydrolyzate of glycoside G we found galactose, glucose, xylose, and rhamnose in a ratio of 1:3:1:1.

The type of bond between the monosaccharides was shown by the methylation of the initial saponin using Kuhn's method [7]. The permethylated product was subjected to methanolysis with perchloric acid, and the methyl glycosides obtained were identified by thin-layer and gas–liquid chromatography in the presence of markers. The sequence of the monosaccharides in agavoside G was shown by partial hydrolysis, which gave five progenins (II-VI). On hydrolysis, progenin (II) decomposed into galactose and hecogenin, and (III) proved to be a bioside consisting of galactose, glucose, and hecogenin; progenin (IV) is a trioside with a carbohydrate chain consisting of galactose and glucose in a ratio of 1:2 and having physical constants coinciding with those of the agavoside C, obtained previously [1].

The fourth progenin (V), after hydrolysis and methylation followed by methanolysis, proved to be identical with agavoside C' [2].

In a hydrolyzate of progenin (VI) the same sugars were identified, by gas–liquid chromatography, as in the initial saponin – galactose, glucose, xylose, and rhamnose – but in a ratio of 1:2:1:1. The methanolysis of the permethylated progenin (VI) gave the same methylated glycosides as agavoside G with the exception of methyl 2,3,4,6-tetra-O-methyl-D-glucoside.

The PMR spectrum of this progenin lacked the signal at 3.24 ppm characteristic for a 22-O-methoxy group of a furostanol aglycone.

For a definitive proof that the saponin belonged to the furostanol series, the paracetylated $\Delta^{20(22)}$-agavoside G (VII) was oxidized [4] with CrO_3 followed by the hydrolysis of the oxidation products with KOH in tert-butanol, as a result of which two compounds (VIII and IX) were obtained.

Compound (VIII) proved to be δ-hydroxy-γ-methylvaleric acid glucoside, and after acetylation followed by methylation with diazomethane it gave methyl δ-hydroxy-γ-methylvalerate tetraacetylglucoside. The mass spectrum of the latter showed characteristic peaks for acetylated glucose, and also peaks of fragments with m/e 129 ($C_7H_{13}O_2$) and 97. The acid hydrolysis of compound (IX) led to 3β-hydroxy-5α-pregn-16-ene-12,20-dione which was acetylated to give a compound identified by IR and UV spectroscopy as 3β-acetoxy-5α-pregn-16-ene-12,20-dione. The monosaccharide composition of (IX) was the same as that of agavoside D [2].

The configurations of the glycosidic centers determined from the differences in the angles of rotation of the progenins at the saponin correspond to Klyne's rule [8].

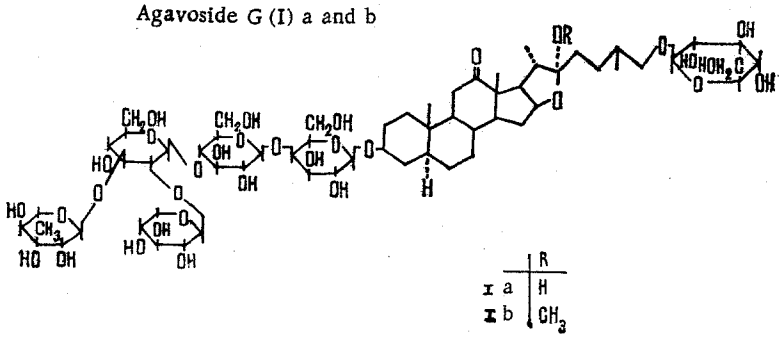
Monosaccharide methyl glycoside	α, degrees	β, degrees	Glycoside	$[M]^{20}$, degrees	Δ, degrees	Bond form
Methyl D-glucopyranoside [9]	+309	−266	Agavoside C' Agavoside D (Progenin VI)	−1415 −716	−699	β
Methyl L-rhamnopyranoside [10]	−111	+170	Agavoside D Progenin V	−716 −619	−97	α
Methyl D-xylopyranoside [11]	+253	−108	Progenin V Progenin IV	−619 −503	−116	β
Methyl D-glucopyranoside	+309	−66	Progenin IV Progenin III	−503 −527	+24	β
	+309	−66	Progenin III Progenin II	−527 −473	−54	β
Methyl D-galactopyranoside [12]	+380	0	Progenin II Hecogenin	−473 +430	−903	β

Thus, agavoside G is 3β,22α,26-trihydroxy-(25R)-5α-furostan-12-one 26-O-β-D-glucopyranosides 3-O-{[O-α-L-rhamnopyranosyl-(1→3)]-[O-β-D-xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}: (see scheme at top of following page).

EXPERIMENTAL

For chromatography we used L 40/100 μ, 5/40 Merck silica gel, Sephadex G-25, FN-3 chromatographic paper, and the following solvent systems: 1) chloroform–methanol–water (65:35:7); 2) butanol–ethanol–water (10:2:5); 3) benzene–acetone (2:1); 4) chloroform–methanol

Agavoside G (I) a and b



	R
I a	H
I b	CH_3

(9:1); 5) butanol—benzene—pyridine—water (5:1:3:3); 6) benzene—ethanol (9:1); 7) chloroform—methanol (96:4).

On the silica gel plates, the glycosides were revealed with the Sannié reagent [13] and concentrated H_2SO_4, and on paper chromatograms the sugars were revealed with aniline phthalate.

The GLC of the acetates of the aldononitrile derivatives of the sugars was performed on a "Chrom-4" instrument with a glass column 2 m long filled with 5% of Xe-60 on Chromaton N-AW-HMDS with helium as the carrier gas, V_{He} = 45 ml/min; the PMR spectra were taken on an RS-60 instrument; the mass spectra on an MKh-1303 instrument; and the IR spectra on an OR-20 instrument.

Enzymatic hydrolysis was performed with the aid of the complex enzyme from *Helix pomatia*.

Preparation of Pure Glycoside G. A solution of 7 g of the total furostanol glycosides in 40 ml of pyridine was added to a solution of 40 ml of acetic anhydride and the mixture was left at room temperature for 10 h. Then 100 ml of ice water was added and it was extracted with chloroform (3 × 50 ml). The chloroform extract was evaporated and chromatographed on a column of Merck silica gel gradientwise starting with system 7 and increasing the concentration of methanol to 20%. The process was monitored on thin-layer plates in system 7.

This gave 2.3 g of the acetate of glycoside G, mp 128-130°C, $[\alpha]_D^{20}$ − 90° (c 1.0; $CHCl_3$).

The product obtained (2.2 g) was heated with 30 ml of 5% KOH in methanol at 100°C for 5 h. After dilution with 50 ml of H_2O, the reaction mixture was extracted with butanol (3 × 30 ml). The butanolic extract was evaporated and chromatographed on a column of silica gel. The process was monitored on thin-layer plates. In system 1, two spots were obtained, and in system 2 one spot, these being colored red by Ehrlich's reagent. The yield of agavoside G with mp 240-245°C, $[\alpha]_D^{20}$ − 130° (c 1.0; MeOH), was 1.5 g. The IR spectrum had a broad absorption band at ~900 cm^{-1} and a band at 1700 cm^{-1}.

The agavoside G (50 mg) was boiled with methanol for 5 h. This gave compound (Ib) with mp 236-239°C and $[\alpha]_D^{20}$ − 117° (c 1.0; MeOH). PMR spectrum (pyridine, δ, HMDS): 0.71 ppm (3 H, s, 18-CH_3); 1.01 ppm (3 H, d, 27-CH_3); 1.04 ppm (3 H, s, 19-CH_3); 1.18 ppm (3 H, d, 21-CH_3), and 3.24 ppm (3 H, s, $-OCH_3$).

After the 22-O-methyl derivative of agavoside G had been boiled in water for 10 h, the signal with the chemical shift of 3.24 ppm in the PMR signal [6] had disappeared, and the substance had been converted into the 22-hydroxy compound, agavoside G.

Enzymatic Hydrolysis of Agavoside G. A solution of 100 mg of the saponin in 20 ml of H_2O was treated with 10 mg of the complex enzyme from *Helix pomatia* and the mixture was left at room temperature overnight. The process was monitored by TLC in system 1. Then the reaction mixture was extracted with butanol (3 × 10 ml) and chromatographed on a column of silica gel in system 1. This gave 70 mg of agavoside D, mp 297-299°C, $[\alpha]_D^{20}$ − 60° (c 1.33; CH_3OH).

Acid hydrolysis of the glycoside obtained permitted the identification of hecogenin, galactose, glucose, xylose, and rhamnose. The GLC of the acetates of the aldononitrile derivatives of the sugars showed that they were present in a ratio of 1:1.9:1:0.9.

Agavoside G (50 mg) was hydrolyzed with 5 ml of 5% H_2SO_4 at 100°C for 10 h. Then the mixture was diluted with water and the precipitate was filtered off and recrystallized from

methanol. This gave hecogenin with mp 266-268°C, $[\alpha]_D^{20}$ + 12° (c 0.83; $CHCl_3$). Its chromatographic mobility was identical with that of an authentic sample in system 4. The IR spectrum showed absorption bands at 862, 900, 912, 962, and 1700 cm^{-1}. The filtrate was shown by paper chromatography in system 5 to contain galactose, glucose, xylose, and rhamnose, and the GLC of the acetates of the aldononitrile derivatives showed that these sugars were present in a ratio of 1:2.9:0.9:1.

Methylation of Agavoside G and Methanolysis of the Permethylate. Agavoside G (400 mg) was methylated by Kuhn's method. The completeness of methylation was checked by TLC in system 6 and by IR spectroscopy. The product obtained was purified on a column of silica gel in system 6. mp 115-117°C, $[\alpha]_D^{20}$ − 82° (c 1.0; $CHCl_3$).

The permethylated agavoside G was subjected to methanolysis with 72% perchloric acid in methanol (1:10) at 105°C for 5 h. After cooling, the reaction mixture was diluted with water and filtered, the filtrate was neutralized with the anion-exchange resin Dowex-8 and was then shown by TLC on silica gel in system 5 to contain five substances which were identified with the aid of authentic samples of such compounds as fully methylated methyl glucoside, xyloside, and rhamnoside and also methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 2,3,6-tri-O-methyl-D-glucoside, and methyl 4,6-di-O-methyl-D-glucoside. The same results were obtained by GLC. After deuteration with CD_3I by Hakomori's method [14], the last-mentioned compound was subjected to PMR and mass-spectrometric analysis. The spectra obtained coincided with literature information for methyl 4,6-di-O-methyl-D-glucoside: m/e 91, 94, 104, 107, 114, 152, 182, 190, 211, 185 [12]; PMR spectrum: 3.21 ppm (3 H, s, C_1—OCH_3); 3.45 ppm (3 H, s, C_4—OCH_3); 3.23 ppm (3 H, s, C_6—OCH_3); and 4.48 ppm (1 H, d, J = 3.8 Hz, C_1—H) [15].

Partial Hydrolysis of Agavoside G. A mixture of 300 mg of agavoside G and 20 ml of 2% H_2SO_4 was heated in the boiling water bath for 6 h. The resulting precipitate was filtered off, dissolved in system 1, and chromatographed on a column of silica gel in the same system giving, in addition to hecogenin (traces) five progenins.

Progenin (II) (the least polar), with mp 220-223°C, $[\alpha]_D^{20}$ − 80° (c 1.0; dimethylformamide), on hydrolysis gave hecogenin and galactose.

Progenin (III), with mp 260-264°C, $[\alpha]_D^{20}$ − 70° (c 1.0; dimethylformamide), on hydrolysis gave galactose and glucose in a ratio of 1:1 (GLC).

Progenin (IV), with mp 275-279°C, $[\alpha]_D^{20}$ − 54° (c 11; CH_3OH), on hydrolysis gave galactose, glucose, and xylose (1:1.9:0.9; GLC).

The methylation of this progenin followed by methanolysis gave methyl 2,3,4-tri-O-methyl-D-xylopyranoside, methyl 3,4,6-tri-O-methyl-D-glucopyranoside, methyl 2,3,6-tri-O-methyl-D-glucopyranoside, and methyl 2,3,6-tri-O-methyl-D-galactopyranoside.

Progenin (VI), with mp 296-300°C, $[\alpha]_D^{20}$ − 60° (c 1.33; CH_3OH), on hydrolysis gave galactose, glucose, xylose, and rhamnose in a ratio of 1:1.9:1:0.9 (GLC). The methanolysis of the methylated progenin (VI) gave the following products identified by TLC in system 3 and by GLC: methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 2,3,6-tri-O-methyl-D-glucoside, methyl 4,6-di-O-methyl-D-glucoside, and the completely methylated xyloside and rhamnoside.

The PMR spectrum showed, in addition to the signals of the CH_3 groups of the aglycone, a doublet at 1.71 ppm (SSCC 6 Hz) from the CH_3 group of rhamnose.

Oxidation of Agavoside G. A solution of 1 g of the acetylated glycoside in 10 ml of acetic acid was treated with 200 mg of NaOAc and oxidation was performed by Tschesche's method [4]. A solution of 500 mg of CrO_3 in 15 ml of 80% acetic acid and 3 ml of water was added dropwise to the solution obtained at 15°C over 15 min with stirring by a magnetic stirrer. Then the mixture was extracted with chloroform and the extract was dried. The oxidized product was hydrolyzed with 30 ml of tert-butanol and 1 g of KOH in 2 ml of water in a current of nitrogen at 30°C with sitrring for 3.5 h and it was then kept for another 30 min at room temperature, 20 ml of water was added, and it was evaporated. The residue was extracted with n-butanol, as a result of which a butanolic phase A and an aqueous B were obtained.

Phase B was acidified to Ph 3 and was extracted twice with n-butanol and twice with chloroform, the extracts were evaporated, and the residue was acetylated with acetic anhydride and pyridine (1:1). The acetylated product was methylated with diazomethane.

The mass spectrum showed characteristic peaks for completely acetylated glucose (m/e: 331, 243, 242, 200, 168, 157, 145, 141, 115, 100), and also for valeric acid (m/e: 129, 97, 89, 81).

The butanolic phase A was washed several times with water and evaporated. The residue was purified on a column of silica gel in system 1, and 50 mg of this substance was hydrolyzed with 3 ml of 4 N HCl in the presence of 2 ml of benzene at 80°C for 3 h. The substance that passed into benzene, after purification on silica gel and acetylation, proved to be 3β-acetoxy-5α-pregn-16-ene-12,20-dione with mp 179-180°C; $[\alpha]_D^{20}$ + 125° (c 1.0; $CHCl_3$)[16]. The monosaccharide composition was identical with that of the progenin (VI) and of glycoside D.

Reduction of Agavoside G with $NaBH_4$. A solution of 200 mg of the glycoside in 90 ml of water was treated with 20 mg of $NaBH_4$ and the mixture was left overnight at room temperature. Then sulfuric acid was added to a concentration of 2.5% and the mixture was boiled on the water bath for 7 h, diluted with water, and extracted with chloroform. The chloroform extract was evaporated and the residue was purified preparatively on silica gel plates. This gave 20 mg of dihydrorockogenin [17], mp 212-215°C.

Its IR spectrum lacked the absorption band characteristic for the oxo group as shown by hecogenin. When the progenin (VI) was reduced under the same conditions, rockogenin was obtained.

SUMMARY

The complete structure of agavoside G, a new steroid glycoside of the furostanol series, has been shown.

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