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A NEW STEROID SAPONIN FROM THE LEAVES OF *Yucca aloifolia*

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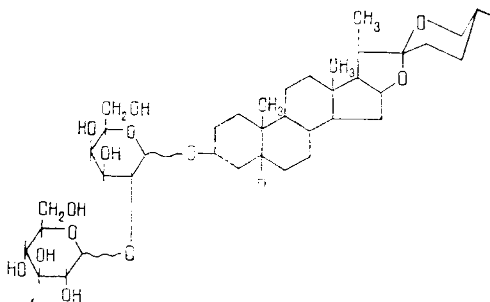
A new steroid saponin has been isolated from the air-dry leaves of *Yucca aloifolia* L., and it has been shown to be (25R)-5β-spirostan-3β-ol O-D-glucopyranosyl-(1 → 2)-D-galactopyranoside. The substance melts at 302-303°C $[\alpha]_D^{20}$ −27.2, (c 1.0; $CHCl_3$).

The genus *Yucca* is known for its content of steroid saponins [1]. From the leaves of *Y. aloifolia* L. (aloe yucca) we have isolated the steroid saponins smilagenin, tigogenin, hecogenin, chlorogenin, and gitogenin [2].

In the present paper we give the results of a study of the steroid saponins of the leaves of the aloe yucca collected in the Sukhumi Botanical Garden in the summers of 1980-1982.

In an ethanolic extract of the leaves by TLC analysis we established the presence of five glycosides, which we called, in order of increasing polarity, glycosides A, B, C, D, and E. A methanolic extract of the leaves was fractionated into less polar and more polar saponins. From the less polar materials, by chromatography on a column of silica gel, we isolated glycoside A. According to its physicochemical properties, the aglycone of the compound was smilogenin [3, 4]. The carbohydrate fraction from complete acid hydrolysis was found to contain glucose and galactose. After the reduction of the hydrolysate followed by acetylation, sorbitol and dulcitol acetates were identified in a ratio of 1:1. The nature of the bond between the glucose and galactose residues was established with the aid of Hakomori methylation [5] of the compound under investigation. As a result of the methanolysis of the methylated saponin followed by GLC, completely methylated methyl glucopyranoside, which obviously represents the terminal monosaccharide residue, and methyl 3,4,6-tri-O-methylgalactopyranoside, representing the monosaccharide residue attached to the aglycone, were identified in a ratio of 1:1. The methylated saponin was subjected to acid hydrolysis followed by reduction and acetylation. 1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyldulcitol [6] were identified by chromato-mass spectrometry, which confirmed the presence of a (1 → 2) glycosidic bond between the glucose and galactose residues in the initial glycoside.



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On the basis of the results obtained, substance A may be assigned the following structure: (25R)-5β-spirostan-3β-ol O-D-glucopyranosyl-(1 → 2)-D-galactopyranoside.

EXPERIMENTAL

For chromatography we used type KSK silica gel; GLC analysis was performed on a Chrom-5 instrument in a copper column (1.5 m × 4 mm) filled with the sorbent Chromaton N-AW HMDS impregnated with 5% of the silicone phase XE-60 at temperatures of the column, the evaporator, and the detector of 110°C, 160°C, and 200°C, respectively, or 170°C, 200°C, and 200°C for the acetates of partially methylated polyols and 210°C, 250°C, and 270°C for the polyol acetates. The rate of flow of the carrier gas, helium, was 40 ml/min. Chromato-mass-spectrometric analysis was performed on a Varian MAT-111 instrument using a column containing 3% of ECNSS (inlet temperature 120°C, 4°C/min).

Isolation of the Combined Saponins. The air-dry comminuted leaves (3 kg) of the aloe yucca were exhuastively extracted with 70% aqueous methanol. The extract was evaporated to dryness at 60°C, and the residue, amounted to 400 g, was dissolved in 2 liters of water and was extracted successively with ether, chloroform, and butanol. The solvents were distilled off from the extracts obtained and the residues were subjected to TLC analysis. Free genins (tigogenin, gitogenin, and chlorogenin) were found in the ethereal extract. The main components of the chloroform extract were the less polar glycosides A, B, and C. The more polar saponins, D and E were present in the butanolic extract.

The chloroform-methanol (20 g) was deposited on a column containing 20 g of type KSK silica gel 50/90. Elution of the column with chloroform-methanol (9:2) yielded the least polar glycoside A. This substance was recrystallized from methanol, to form 1.1 g of a white crystalline powder. Glycoside A had mp 302-306°C, $[\alpha]_D^{20}$ −27.2° (c 1.0; $CHCl_3$). The substance gave a positive Matthews reaction and a negative reaction with the Ehrlich reagent [7, 8].

Hydrolysis of Glycoside A. This was carried out on 150 mg of the substance in 50 ml of 8% H_2SO_4 solution at 100°C for 5 h. The precipitate that deposited was filtered off, washed with water, dried, and twice recrystallized from methanol, to give 50 mg of an aglycone with mp 184-187°C; $[\alpha]_D^{20}$ −73.0° (c 1.0; $CHCl_3$). IR spectrum, cm^{-1}: λ_{max}^{KBr} 3400 (OH), 990, 920, 898, 850 (intensity 898 > 920; 25R-spiroketal). In the carbohydrate fraction (after neutralization of the hydrolysate with $BaCO_3$, filtration, and evaporation), galactose and glucose were detected by TLC (butanol—methanol—water (5:3:1) system; revealing agent: o-toluidine salicylate). A solution of 20 mg of the hydrolysate in 4 ml of 50% aqueous MeOH was reduced with sodium tetrahydroborate at room temperature (12 h), after which the mixture was neutralized with KU-2 resin (H^+ form), filtered and evaporated to dryness with the addition of methanol to eliminate the last traces of boric acid. The carefully dried residue was acetylated in a mixture of acetic anhydride (2 ml) and pyridine (2 ml) at room temperature (12 h), after which the reaction mixture was evaporated to dryness with the addition of methanol and toluene to eliminate the last traces of acetic anhydride and pyridine, respectively. Sorbitol and dulcitol acetates in a ratio of 1:1 were identified by the GLC method in comparison with authentic samples.

Methylation of Saponin A. The Hakomori [5] methylation of 50 mg of glycoside A gave 35 mg of methylated product. Of this, 15 mg was subjected to methanolysis with 5% HCl/MeOH solution (5 ml, 100°C, 4 h), and then the methanolysate was neutralized with saturated aqueous ammonia and evaporated to dryness, and the residue was extracted with chloroform. By the GLC method, methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 3,4,6-tri-O-methylgalactopyranoside in a ratio of 1:1 were identified in the methanolysate.

By the hydrolysis of the methylated saponin A (20 mg) (2 N HCl, 100°C, 4 h) followed by reduction with sodium tetrahydroborate and acetylation in a Ac_2O/Py mixture (in a similar manner to that described above) a product was obtained in which the acetates of partially methylated polyols — 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyldulcitol — were identified by the chromato-mass-spectrometric method.

SUMMARY

The structure of a new glycoside from the leaves of the aloe yucca has been established; it is (25R)-5β-spirostan-3β-ol O-D-glucopyranosyl-(1 → 2)-D-galactopyranoside.

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TRITERPENE GLYCOSIDES OF *Hedera colchica*. STRUCTURE OF HEDERACOLCHISIDES E AND F

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The leaves of Colchis ivy (family Araliaceae) have yielded polar glycosides — hederacolchisides E and F — and their structures have been established. It has been shown on the basis of the results of methylation and of acid and alkaline hydrolysis that these glycosides are hexaosides of oleanolic acid and of hederagenin.

In the leaves of *Hedera colchica* C. Koch (Colchis ivy), family Araliaceae, we have detected six glycosides — hederacolchisides A, B, C, D, E, and F [1]. Hederacolchiside D has been identified as kalopanax saponin B [2], and a preliminary structure has been proposed for hederacolchiside E [3, 4]. In the present paper we give information enabling the structure of hederacolchiside E to be refined and the structure of the most polar glycoside F to be established.

The complete acid hydrolysis of the glycosides gave the aglycones: oleanolic acid for hederacolchiside E and hederagenin for hederacolchiside F. In the carbohydrate moieties of both glycosides rhamnose, arabinose, and glucose in a ratio of 2:1:3 were identified by the GLC of the acetates of the corresponding polyols. The nature of the substitution of the monosaccharide residues was established by the Hakomori methylation [5] of the glycosides followed by methanolysis and identification of the methyl glycosides obtained. In both cases, methyl 2,3,4,6-tetra-O-methylglycopyranoside (1), methyl 2,3,4-tri-O-methylglucopyranoside (2), methyl 2,3,6-tri-O-methylpyranoside (3), methyl 2,3,4-tri-O-methylrhamnopyranoside (4), and methyl 3-O-methylarabinopyranoside (5) were identified by the GLC method. These results were confirmed by the identification of the acetates of the partially methylated polyols obtained as the result of the acid hydrolysis of the metholated glycosides followed by reduction and acetylation. The following polyols were identified by chromato-mass spectrometry (CMS): 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol (6), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylsorbitol (7), 1,4,5-tri-O-acetyl-2,3,6-O-methylsorbitol (8), 1,5-di-O-acetyl-2,3,4-tri-O-methylrhamnitol (9), and 1,2,4,5-tetra-O-acetyl-3-O-methylarabitol (10) [6]. Thus, hederacolchisides E and F contained rhamnose and glucose as terminal monosaccharide residues. The arabinose residue formed a point of branching and was substituted in positions 2 and 4, while the glucopyranose residue in the chain was substituted in positions 4 and 6.

In order to determine the localization of the carbohydrate chains we performed the alkaline hydrolysis of the compounds under investigation. In the oligosaccharide fractions

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