STEROID GLYCOSIDES.

XIX. A CHEMICAL STUDY OF FUNKIOSIDES E AND G FROM THE LEAVES OF *Funkia ovata*

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Steroid glycosides — funkiosides A, B, C, and D — have been isolated from the leaves of *Funkia ovata* Spr. (*Hosta caerulea*; blue plantain lily) and their structures have been established [1, 2]. In addition to the combined saponins, a methanolic extract of this plant yielded a set of free steroids which were separated by thin-layer chromatography into individual compounds. Their mobility on TLC in the presence of markers and the determination of their physicochemical constants permitted the identification of gitogenin, diosgenin, kogagenin, ruscogenin, and tokorogenin.

Further chromatography of the extract on a column of SiO_2 has enabled another two compounds — funkiosides E and G — to be isolated in the idividual state. From their spectral characteristics, both substances have been identified as glycosides of the isospirostanol series.

Their qualitative compositions were determined by acid hydrolysis. The aglycone in both cases was diosgenin, which was confirmed by its IR and mass spectra and also its melting point, specific rotation, and mobility in a thin layer in the presence of a marker. The monosaccharide composition of the hydrolyzates was determined by paper chromatography and gas—liquid chromatography (GLC). The GLC of the acetates of the aldononitrile derivatives of the sugars show the quantitative ratio of the monosaccharides obtained.

Funkioside E proved to be a tetraoside of diosgenin containing in the monosaccharide chain galactose, glucose, and rhamnose in a ratio of 1:2:1, and funkioside G proved to be a pentaoside with galactose, glucose, rhamnose, and xylose in a ratio of 1:2:1:1.

The glycosides investigated were subjected to methylation by Kuhn's method [3] and to methanolysis in order to determine their partial structures. As a result, for funkioside E we obtained methyl 2,3,6-tri-O-methyl-D-galactopyranoside, methyl 3,4,6-tri-O-methyl-D-glucopyranoside, methyl 2,3,6-tri-O-methyl-D-glucopyranoside, and methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, and for funkioside G methyl 2,3,6-tri-O-methyl-D-galactopyranoside, methyl 2,3,6-tri-O-methyl-D-glucopyranoside, methyl 4,6-di-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, and methyl 2,3,4-tri-O-methyl-D-xylopyranoside.

Partial hydrolysis of the glycosides gave progenins, the analysis of which showed the sequence of connection of the monosaccharides in the chain and, thus, the complete structure of the funkiosides. In the case of funkioside E, three progenins (I-III) were isolated, the least polar of which (I) contained diosgenin and galactose, while (II) contained diosgenin, galactose, and glucose and (III) contained diosgenin, galactose, and two molecules of glucose.

The three least polar progenins of funkioside G coincided in composition with the progenins of funkioside E, while IV contained in its carbohydrate chain galactose, glucose, and rhamnose in a ratio of 1:2:1 and V contained galactose, glucose, and xylose (1:2:1).

The physicochemical constants of progenins (I)-(IV) coincided with those of diosgenin galactoside and of funkiosides C, D, and E, respectively. The fact that it was actually

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galactose that was attached to the aglycone in both cases was established by the acid hydrolysis of the progenin (I).

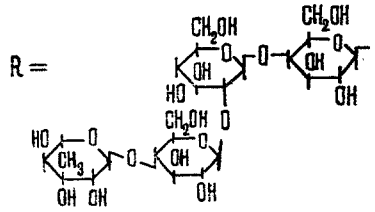
When the permethylated progenin V was subjected to methanolysis methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 4,6-di-O-methyl-D-glucoside, methyl 2,3,4,6-tetra-O-methyl-glucoside, and methyl 2,3,4-tri-O-methyl-D-xyloside were identified, and on this basis it may be concluded that one molecule of glucose is a center of branching. The physicochemical constants of progenin V coincided with those of a known glycoside isolated from *Aspidistra elatior* by Kawasaki et al. [4].

The results of methylation were confirmed by periodate oxidation. For funkioside G and progenin V, after oxidation a glucose molecule remained undestroyed, which confirms the presence of branching in just this link of the carbohydrate chain. The methylation of (III) gave methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 3,4,6-tri-O-methyl-D-glucoside, and methyl-2,3,6-tri-O-methyl-D-galactoside. These results show that in funkioside G the glucose that is the center of branching is attached to the galactose by a 1→4 bond. In this glucose, another molecule of glucose is attached at position 2 and xylose at C-3.

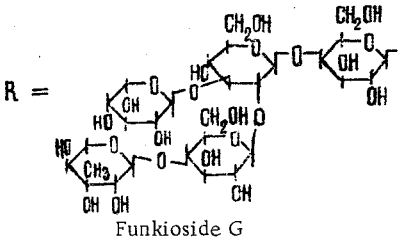
When funkioside E was subjected to periodate oxidation, no monosaccharide remained uncleaved, which, taking the results of methylation into account shows the linear structure of its saccharide moiety.

As is well known [5], in steroid glycosides containing galactose in the carbohydrate chain together with other monosaccharides, it is just at this link that attachment to the aglycone takes place. Funkiosides E and G confirm this rule.

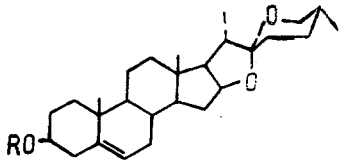
The configurations of the glycosidic centers established by molecular rotation differences between the glycosides the progenins, and the aglycone, are in harmony with Klyne's rule [6]. Consequently, funkiosides E and G have the following structures:



Funkioside E



Funkioside G



EXPERIMENTAL

The absorbents used for chromatography were silica gel of types L 40/100, L 100/160, and L 5/40 + 13% of gypsum, Al_2O_3, and FN-3 chromatographic paper. Gas–liquid chromatography was performed on a "Chrom-4" instrument. The following solvent systems were used: 1) chloroform–methanol–water (65:30:10), lower layer; 2) chloroform–methanol–water (65:35:10) lower layer; 3) benzene–acetone (2:1); 4) benzene–pyridine–butanol–water (1:3:5:3), upper layer; and 5) chloroform–methanol (9:1). The revealing agents used were H_2SO_4 and aniline phthalate.

Isolation of Individual Compounds. A methanolic extract from *Funkia ovata* (450 g) was chromatographed on a column of Al_2O_3 in system 5. This gave 0.5 g of combined free steroid aglycones, which were separated into individual compounds by TLC on SiO_2 in system 5, water being used as the revealing agent (the zones not wetted by water were taken). Aglycones, the characteristics of which are given below,were isolated:

Compound	mp, °C; $[\alpha]_D^{20}$, deg. (experimental)		mp, °C; $[\alpha]_D^{20}$, deg. (lit. data)		Amount, mg
Diosgenin	206	−112	208	−129	81
Gitogenin	265-266	−69	264-267	−78	85
Tokorogenin	266	−46	266-268	−50	41
Ruscogenin	204-208	−118	205-210	−127	52
Kogagenin	317-320	−30	318-322	−27	32

The purified combined saponins were chromatographed on a column of SiO_2 in systems 1 and 2. Monitoring was performed in a thin layer of silica gel in the same solvent systems. After the issuance of funkiosides A, B, C, and D, the eluates yielded glycosides E (0.4 g, mp 222-229°C, $[\alpha]_D^{20}$ −101° (c 0.5; CH_3OH)) and G (0.6 g, mp 258-266° $[\alpha]_D^{20}$ −57° (c 0.47; CH_3OH)).

Hydrolysis of the Funkiosides. In amounts of 50 mg each, the funkiosides were hydrolyzed in sealed tubes with 2.5% H_2SO_4 at 100°C for 10 h. Then the contents of the tubes were diluted with water and extracted with ether. After recrystallization from methanol, the aglycone obtained in each case had mp 204-206°C, $[\alpha]_D^{20}$ −112° (c 1; $CHCl_3$) R_f 0.76 in system 5 in the presence of a marker, and their IR and mass spectra were also identical with those of diosgenin.

The aqueous layer of this hydrolyzate was neutralized with ion-exchange resins (Amberlite IR and KW-2) and was analyzed by paper chromatography in system 4. The carbohydrate chain in funkioside E consisted of galactose, glucose, and rhamnose in a ratio of 1:1.9:0.9 (GLC), and in the case of funkioside G it also included xylose — 0.9:1.9:0.9:0.9 (GLC).

Methylation. Samples of funkiosides E and G (100 mg each) were methylated by Kuhn's method at 40-48°C with constant stirring for 12 h. The completeness of methylation was checked in a thin layer of silica gel in the benzene—ethanol (9:1) system. The methylation products were separated on a column of SiO_2 with gradually increasing proportions of methanol (up to 5%). The substances obtained were treated with a mixture of methanol and 72% $HClO_4$ in a ratio of 10:1 at 100°C for 5 h. The hydrolyzates were neutralized with IR-45 anion-exchange resin and evaporated. Gas—liquid chromatography and thin-layer chromatography in system 3 in the presence of authentic samples showed the presence of the methyl glycosides mentioned in the discussion.

Partial Hydrolysis. Funkiosides E and G (400 mg each) were heated in the water bath with 50 ml of 1% H_2SO_4 for 6 h. This time is the optimum as was confirmed by following the course of the reaction by analyzing samples every hour in a thin layer of silica gel in system 1. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was evaporated and chromatographed on a column of SiO_2 successively in systems 5 and 1, the separation being monitored with aid of TLC in the same systems. A number of progenins as obtained with the following characteristics:

Glycoside	Progenin	Yield, mg	mp, °C	$[\alpha]_D^{20}$, deg.
Funkioside E	I	120	230—235	−91
	II	103	256—262	−75
	III	105	188 - 202	−60
Funkioside G	I	40	230—235	−91
	II	52	256—262	−75
	III	45	188—202	−60
	IV	100	222—227	−57
	V	110	256—264	−68

A 50-mg sample of each of the progenins (III), (IV), and (V) was methylated and was then subjected to methanolysis. By means of GLC in the presence of markers all the methyl glycosides described above were identified.

Periodate Oxidation. To a solution of 50 mg of each of the funkiosides in 20 ml of methanol was added 20 ml of 2% $NaIO_4$ solution. The mixture was kept at room temperature for 48 h, and then a few drops of ethylene glycol were added and after an hour the mixture was extracted with n-butanol. The butanolic extracts were evaporated and the residues were hydrolyzed with 2.5% H_2SO_4 at 100°C for 10 h. Only glucose was identified in a hydrolyzate of the diosgenin pentaoside by paper chromatography. In the case of funkioside E, no sugars could be detected.

SUMMARY

1. Gitogenin, diosgenin, kogagenin, ruscogenin, and tokorogenin have been isolated for the first time from *Funkia ovata* Spr.

2. Structures of two new steroid glycosides — funkiosides E and G — have been determined.

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GLYCOSYLATION OF CARDENOLIDES.

VI. PERIPLOGENIN MONO- AND DIGLUCOSIDURONIC ACIDS

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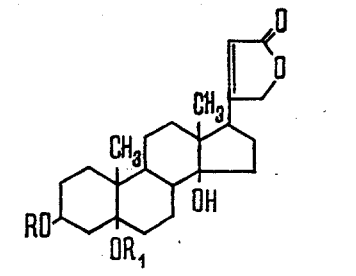
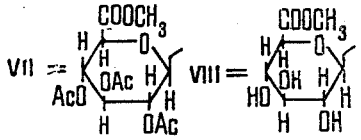
Work on the synthesis of cardiac 3,5-bisglycosides [1] has been continued. The condensation of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucuronate with periplogenin (I) under the conditions of the Koenigs–Knorr method [2] has led to the acetylated glycoside (III) and the less polar compound (V).

The NMR spectrum of the acetate (III) (see Fig. 1) contains the signals of the protons of three acetyl groups in the 2.00-2.05 ppm region, the three-proton singlet of a methoxycarbonyl group $COOCH_3$ at 3.69 ppm, and the doublet of the proton of a glucuronic acid residue at C-5' at 4.00 ppm (J = 10 Hz). This means that product (III) is the triacetate of the methyl ester of a periplogenin monoglucosiduronic acid. It is obvious that the sugar residue is attached to the OH group at carbon atom 3. The NMR spectrum of compound (III) clearly exhibits the doublet of the anomeric proton at 4.64 ppm with J = 7.5 Hz, which shows the β configuration of the glycoside bond [3]. Thus, substance (III) is periplogenin 3α-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucosiduronate).



I. R = R_1 = H
II. R = Ac; R_1 = H
III. R = VII; R_1 = H
IV. R = VIII; R_1 = H
V. R = R_1 = VII
VI. R = R_1 = VIII



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