A STUDY OF A CULTURE OF STEM TISSUE OF *Rauwolfia serpentina*

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In a culture of the tissue of *Rauwolfia serpentina* Benth. et Kurz. (Java devilpepper) originating from the stems, products of secondary biosynthesis are synthesized — serpentine, ajmaline, and several unidentified alkaloids [1]. A combined preparation with a raunatin-like action containing reserpine, ajmaline, and serpentine is known [2].

The strain of *R. serpentina* stem tissue culture that we are investigating was obtained by A. G. Vollosovich.

The tissue consists of an undifferentiated white biomass of dense consistency. The yield of biomass in a three-month period amounts to 1.64 ± 0.03 g per 60 ml of nutrient medium.

The air-dry biomass was extracted with an organic solvent, the latter was distilled off, and the residue was dissolved in methanol. The methanol-insoluble residue was dissolved in chloroform. The chloroform-insoluble fraction dissolved readily in water and crystallized from aqueous ethanol in the form of colorless crystals with mp 324-327°C. The substance is inorganic and was identified by qualitative reactions as KNO_3. The detection of KNO_3 in a tissue culture shows that the isolated tissue accumulates individual components from the nutrient medium *in vitro*.

The chloroform-soluble part of the extract was separated by chromatography in a column of Al_2O_3 (activity grade II). Chloroform elution yielded three fractions. The first, oily, fraction gave in a thin layer of Al_2O_3 (activity grade II, chloroform) a single spot with R_f 0.9, but it could not be obtained in the crystalline state. The IR spectrum of this fraction had an absorption band at 1740 cm^{-1}, which shows the presence of an ester grouping. Saponification of this fraction gave fine acicular crystals with mp 135-136°C (from ethanol).

From the following fractions II and III a substance was isolated with mp 135-137°C (from ethanol) identical in terms of melting point with the substance isolated previously.

By means of their R_f values (Al_2O_3, activity grade II, chloroform) in comparison with an authentic sample and the absence of a depression of mixed melting points, the substances isolated were identified as β-sitosterol. This sitosterol has been found in the roots of the intact plant [3].

From the mother liquors of fractions II and III after the isolation of the β-sitosterol small colorless crystals were obtained from aqueous ethanol with mp 120-126°C with R_f values and qualitative reactions close to those of β-sitosterol but possibly consisting of a mixture of other sterols.

We were unable to detect in the strains studied the α_2- and γ-sitosterols and serposterol [3-5] that are present in the intact *Rauwolfia serpentina* plant.

Thus, from a strain of stem tissue culture of *R. serpentina* we have isolated and identified for the first time β-sitosterol and an ester of β-sitosterol, and we have also obtained an unidentified substance or mixture of substances with mp 120-126°C, probably phytosterols.

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STEROID GLYCOSIDES.

XVIII. THE STRUCTURE OF FUNKIOSIDES C AND D FROM THE LEAVES OF *Funkia ovata (Hosta caerulea)*

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We have previously reported the isolation from *Funkia (Hosta)* of nine steroid glycosides and have described the structure of funkiosides A and B [1]. In the present paper we give information to show the structures of another two glycosides of this plant — funkiosides C and D.

The individual compounds — funkioside C with mp 258-262°C, $[\alpha]_D^{20}$ −78° (c 0.58; CH_3OH), and funkioside B with mp 188-202°C, $[\alpha]_D^{20}$ −60° (c 0.35; CH_3OH) — were obtained by repeated chromatography of the combined material on a column of SiO_2 in the chloroform–methanol–water (65:25:10) system. Each of the glycosides mentioned was hydrolyzed with 2.5% H_2SO_4 at 120°C for 15 h in sealed tubes in order to determine the monosaccharide composition. In the carbohydrate moiety of the first of them, glucose and galactose (1:1) were detected by gas–liquid and paper chromatography, and the same monosaccharides but in a ratio of 2:1 in the second compound. In both cases the aglycone was identified as diosgenin (from its melting point, specific rotation, chromatographic mobility in a thin layer, and IR spectrum).

The sequence of attachment of the carbohydrates was determined by partial hydrolysis (1% H_2SO_4, 100°C, 5 h), as a result of which diosgenin galactoside (mp 230-235°C, $[\alpha]_D$ −91° (c 1.1; CH_3OH) was obtained as a progenin of funkioside C, and for funkioside D hydrolyzed under the same conditions we detected, in addition to the galactoside, a galactoglucoside of diosgenin (mp 258-261°C, $[\alpha]_D$ −75° (c 0.58; CH_3OH).

Methylation by Kuhn's method [2] followed by methanolysis showed the position of the bonds of the monosaccharides in the carbohydrate chain. From funkioside C we obtained methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,6-tri-O-methyl-D-galactopyranoside, and from funkioside D, in addition, methyl 3,4,6-tri-O-methyl-D-glucopyranoside.

When the funkiosides under study were subjected to periodate oxidation, not one of the monosaccharides remained unattacked, which also agrees with the results of methylation concerning the absence of branching in the carbohydrate chain and 1→3 bonds.

The configurations of the glycosidic centers were determined by means of Klyne's rule [3].

The facts given, and also the results of a study of the progenins obtained on partial hydrolysis have permitted the following structures to be assigned to funkiosides C and D:

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