STEROID GLYCOSIDES OF THE ROOTS OF *Capsicum annuum*.

II. THE STRUCTURE OF THE CAPSICOSIDES

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Two tigogenin glycosides and two diosgenin glycosides have been isolated from a methanolic extract of the roots of bush red pepper. An attempt to isolate the individual spirostanol glycosides directly was unsuccessful. These compounds have very similar structures. For their separation, acetylation and epoxidation of the double bond of the aglycon, diosgenin, in the corresponding glycosides was performed. The derivatives obtained were purified by chromatography. To establish the complete chemical structure of each capsicoside we used both chemical and physical methods: complete and partial acid hydrolysis, methylation followed by methanolysis, IR spectroscopy, mass spectroscopy, etc. It was shown that capsicoside A_2 is (25R)-5α-spirostan-3β-ol 3-O-β-D-galactopyranoside, capsicoside B_2 is (25R)-5α-spirostan-3β-ol 3-O-[O-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside], capsicoside A_3 is (25R)-spirost-5-en-3β-ol 3-O-β-D-galactopyranoside, and capsicoside B_3 is identical with funkioside C.

We have previously reported the isolation from the roots of bush red pepper of several chromatographically individual fractions containing steroid glycosides of gitogenin, diosgenin, and tigogenin similar in structure and difficult to separate. The gitogenin glycosides were isolated via their acetylation [1, 2].

Continuing invesigations of chromatographically individual fractions we have isolated another four glycosides. In the present paper we give proofs of their structures.

After the separation of the gitogenin glycosides, each individual fraction containing tigogenin and diosgenin glycosides was subjected to the action of m-chloroperbenzoic acid, and the double bond of the diosgenin aglycon of the corresponding glycoside was epoxidized [3]. As a result of this process, new derivatives of the diosgenin glycosides were obtained — peracetates of the tigogenin glycosides (II) by chromatography on a column of silica gel. The completeness of epoxidation was determined with the aid of TLC and IR spectroscopy.

The individual peracetates (II) were saponified and the glycosides were isolated and have been named by us in order of increasing polarity capsicosides A_2 (III) and B_2 (IV). The complete hydrolysis of (III) and (IV) gave one and the same aglycon — tigogenin (V).

To eliminate the epoxide group, the epoxide peracetates (I) were treated with chloromethylsilane and sodium iodide in acetonitrile [4]. The diosgenin glycoside periodates obtained in this way were purified by chromatography on silica gel. Their saponification led to glycosides which we have called capsicosides A_3 (VI) and B_3 (VII). After the acid hydrolysis of these substances, the aglycon was isolated; it was identical in its physicochemical constants with diosgenin (VIII).

All the glycosides isolated, (III), (IV), (VI), and (VII), gave a positive reaction with the Sannié reagent and a negative one with the Ehrlich reagent, which showed their spirostanol nature. The IR spectrum of these glycosides contained absorption bands at 900 > 920 cm^{-1} which are characteristic for a spiroketal chain of the (25R)-series.

The qualitative and quantitative compositions of the monosaccharides in the oligosaccharide moieties of the glycosides isolated were identified with the aid of the PC and

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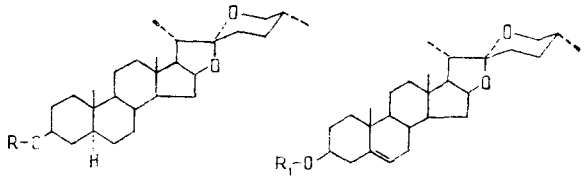
GLC of the acetates and aldononitrile derivative of the sugars [5]. For (III) and (IV) only galactose was detected, and for (VI) and (VII) galactose and glucose in a ratio of 1:1.

To determine the type of bond between the monosaccharide residues, each capsicoside was methylated by Hakomori's method [6], and the permethylates obtained were subjected to methanolysis. By GLC in the presence of markers, methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside was identified in the cases of permethylated (III) and (VI), and methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,6-tri-O-methyl-D-galactopyranoside in the cases of (IV) and (VII).

The sequence of attachment of the monosaccharide residues in glycosides (IV) and (VII) was determined with the aid of partial acid hydrolysis. In the case of glycoside (IV), progenin (IX) was obtained which broke down into tigogenin and galactose, while glycoside (VII) yielded progenin (X), giving diosgenin and galactose on hydrolysis. In its physicochemical constants, progenin (IX) was identical with capsicoside A_2 and progenin (X) with capsicoside A_3. The methylation of (IX) and (X) followed by methanolysis gave methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside. Consequently, in capsicosides B_2 and B_3 the galactose residue was attached directly to the aglycon.

The configurations of the glycosidic centers were shown by means of Klyne's rule [7] on the basis of the molecular rotations of the glycosides and their progenins.

Thus, the facts given above determine the following structures for the capsicosides:



where R for III – Gal_p^{α}, IV – Glc_p^{β} – $4Gal_p^{\beta}$

and R_1 for VI – Gal_p^{β}, VII – Glc_p^{β} – $4Gal_p^{\beta}$

Capsicoside B_3 was identical with funkioside C isolated previously from *Funkia* leaves [8].

EXPERIMENTAL

The compounds obtained and their derivatives were identified on the basis of a comparison of physicochemical properties and spectral characteristics, and also by direct comparison with authentic samples. Column chromatography was carried out with silica gel L 100/160 μ and the following solvent systems: 1) chloroform–methanol (9:1); 2) chloroform–methanol (4:1); 3) chloroform–methanol–water (65:35:10, lower layer); 4) chloroform–acetone (9:1); 5) chloroform–acetone–methanol (45:5:1); and 6) butanol–benzene–pyridine–water (5:1:3:3, upper layer).

Type FN-3 paper was used for paper chromatography and silica gel L 5/40 μ + 13% of gypsum for thin-layer chromatography. Melting points were determined on a Boëtius stage. IR spectra were taken on a Specord IR spectrophotometer. Mass spectra were recorded on a MKh-1302 instrument. GLC analysis was performed on a Chrom-5 chromatograph. Monosaccharides were analyzed in the form of acetates of the aldononitrile derivatives of the sugars and of methylated methyl glycosides. A glass column 2.4 m long filled with 5% of XE on Chromaton N-AW-HMDS was used for the sugar derivatives and a glass column 1.2 m long filled with 3% of QF-1 on Chromaton Super for the aglycons.

Acetylation was performed with acetic anhydride in pyridine [2].

Epoxidation of the Diosgenin Glycosides. Over 15 min, a solution of 160 mg of m-chloroperbenzoic acid in 4 ml of chloroform was added dropwise to 600 mg of a mixture of the peracetates of glycosides A_2 and A_3 dissolved in 10 ml of freshly distilled chloroform, and the mixture was stirred at room temperature for 5 h. The reaction was monitored by TLC in solvent system 5. After the end of the reaction, the excess of peracid was decomposed with a 5% solution of sodium sulfite. The resulting solution was treated with water (3 × 50 ml) with 5% sodium carbonate solution (3 × 100 ml), and again with water (2 × 100 ml), and was

then dried over anhydrous sodium sulfate and concentrated in vacuum. The completeness of the epoxidation was determined by IR spectroscopy (absence of the absorption band at 1640 cm^{-1} that is characteristic for a C=C bond). The mixture of acetates of capsicoside A_2 and of the epoxidized diosgenin glycoside A_3 was separated by preparative thin layer chromatography in solvent system 5.

This led to the isolation of 165 mg of the peracetate of capsicoside A_2 (XI), mp 114-116°C, $[\alpha]_D^{20}$ −38° (c 1.0; chloroform) and 292 mg of the peracetate of epoxidized capsicoside A_3 (XII) with mp 122°C, $[\alpha]_D^{20}$ −76° (c 1.6; chloroform).

In a similar manner, 160 mg of the peracetate of capsicoside B_2 (XIII) was isolated with mp 112°C, $[\alpha]_D^{20}$ −53° (c 1.9; chloroform) and 326 mg of the peracetate of epoxidized capsicoside B_3 (XIV), with mp 131-132°C, $[\alpha]_D^{20}$ −70° (c 0.9; chloroform).

De-epoxidation of the Epoxy Derivatives of the Glycosides. With stirring by means of a magnetic stirrer, 0.1 g of chlorotrimethylsilane was added to a solution of sodium iodide (0.3 g) in 2 ml of acetonitrile. Then 280 mg of the epoxy derivatives of capsicoside A_3 in acetonitrile was added to the resulting light yellow suspension. The solution acquired a red-brown color. Stirring was continued for an hour. Then the reaction mixture was treated with a saturated solution of sodium thiosulfate until it had become decolorized, and the reaction product was extracted with diethyl ether (3 × 20 ml). The extract obtained was washed with water, with sodium hydrogen carbonate solution, and again with water and was dried over anhydrous sodium sulfate, after which the solvent was distilled off. The epoxy derivative of capsicoside B_3 was de-epoxidized by a similar procedure. The peracetates of the diosgenin glycosides were purified chromatographically on silica gel in solvent system 4. The de-epoxidation of compound (XII) yielded 235 mg of the peracetate of capsicoside A_3 (XV) with mp 109°C, $[\alpha]_D^{20}$ −41° (c 3.2; chloroform), and compound (XIV) yielded 284 mg of the peracetate of capsicoside B_3 (XVI), mp 113°C, $[\alpha]_D^{20}$ −56° (c 2.1; chloroform).

De-acetylation of the Acetates of the Steroid Glycosides. A flask fitted with a reflux condensor was charged with 500 mg of the peracetate of one of the glycosides in 18 ml of a 5% solution of potassium hydroxide in methanol. The mixture so obtained was boiled in the water bath for 5 h, after which the contents of the flask were diluted with water (30 ml). The reaction product was extracted with butanol (3 × 15 ml). The butanolic extracts were washed with water to neutrality, and the solvent was evaporated. The glycosides so obtained were purified chromatographically in solvent system 3.

After the saponification of the peracetate (XI), 100 mg of capsicoside A_2 was isolated with mp 290-291°C, $[\alpha]_D^{20}$ −28° (c 0.8; CH_3OH); (XIII) similarly gave 95 mg of capsicoside B_2 with mp 293°C, $[\alpha]_D^{20}$ −37° (c 1.0; CH_3OH); (XV) gave 180 mg of capsicoside A_3 with mp 295-297°C, $[\alpha]_D^{20}$ −63° (c 1.0; CH_3OH); and (XVI) gave 203 mg of capsicoside B_3, mp 290°C, $[\alpha]_D^{20}$ −64° (c 1.3; CH_3OH).

Acid Hydrolysis of the Capsicosides. To 20 mg of each capsicoside in a glass ampul was added 3 ml of 2.5% sulfuric acid. The ampul was sealed and was heated in a thermostat at 105°C for 12 h. After this, the ampul was opened and 15 ml of water was added to the contents. Then the hydrolysate was treated with diethyl ether.

From the ethereal extracts of capsicoside A_2 and B_2 tigogenin was isolated as the aglycon, with mp 202-203°C, $[\alpha]_D^{20}$ −65° (c 1.0; chloroform), $[M]^+$ 416, while capsicosides A_3 and B_3 gave diosgenin, with mp 208°C, $[\alpha]_D^{20}$ −120° (c 1.0; chloroform), $[M]^+$ 414.

Methylation of the Steroid Glycosides and their Progenins. To 20 ml of dimethyl sulfoxide was added 500 mg of sodium hydride and then 100 mg of one of the capsicosides. The resulting mixture was stirred for about an hour at 50°C in an atmosphere of argon.

The reaction mixture was treated with 20 ml of methyl iodide and was left at room temperature in the dark for 12 h. Then this reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with saturated sodium thiosulfate solution and with water and was concentrated in vacuum. The methylation product was purified on a column of silica gel in solvent system 4.

Methanolsis of the Permethylated Glycoside. A flask fitted with a reflux condenser was charged with 50 mg of the methylated glycoside obtained in the preceding experiment, 5 ml of absolute methanol, and 0.5 ml of perchloric acid. The mixture was heated in the water

bath for 6 h. Then the precipitate was filtered off and the solution was neutralized with an ion-exchange resin and evaporated, and the residue was analyzed by TLC and GLC in the presence of markers.

Partial Acid Hydrolysis of Capsicosides B_2 and B_3. A solution of 50 mg of each glycoside in 5 ml of 1% sulfuric acid was heated in the water bath under reflux for an hour. The course of the reaction was monitored every 30 min by TLC in solvent systems 1, 2, and 3.

After the hydrolysate had been cooled, the acid solution was neutralized with ion-exchange resin and was extracted with butanol (3 × 20 ml). The butanolic extract was evaporated and separated preparatively in a thin layer of silica gel. As a result of the partial acid hydrolysis of capsicoside B_2, tigogenin and a progenin identical in its physicochemical constants with capsicoside A_2 were identified, while in the case of capsicoside B_3 diosgenin and a progenin identical with capsicoside A_3 were identified.

SUMMARY

Two tigogenin glycosides and two diosgenin glycosides have been isolated from the roots of bush red pepper for the first time.

It has been shown that capsicoside A_2 is (25R)-5α-spirostan-3β-ol 3-O-β-D-galactopyranoside, capsicoside B_2 is (25R)-5α-spirostan-3β-ol 3-O-[O-β-D-glucopyranosyl-(1→4)-4-D-galactopyranoside, capsicoside A_3 is (25R-spirost-5-en-3β-ol 3-O-β-D-galactopyranoside, and capsicoside B_3 is funkioside C [8].

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