17. Organic Syntheses [Russian translation], Moscow, Vol. 2 (1949), p. 195.
18. F. C. Chang and R. T. Blickenstaff, J. Am. Chem. Soc., 80, 2906 (1958).
19. E. Muller and W. Rundell, Angew. Chem., 70, 105 (1958).

STEROID GLYCOSIDES OF THE ROOTS OF *Capsicum annuum*

I. THE STRUCTURE OF CAPSICOSIDES A_1, B_1, AND C_1

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UDC 547.918+547.917

Three new steroid glycosides of the spirostan series — capsicosides A_1, B_1, and C_1 — have been isolated from a methanolic extract of the roots of red pepper. In an investigation of the products of complete acid hydrolysis of these glycosides, a single aglycon — gitogenin — was identified. The complete chemical structure of each of the capsicosides has been shown with the aid of complete and partial acid hydrolysis, methylation and methanolysis, and periodate oxidation, and also by physicochemical methods of investigation.

Pepper seeds contain steroid glycosides of the furostan series. The structure of one of them has been established [1].

In the roots of red pepper of the variety Podarok Moldovy we have detected glycosides belonging to the spirostan and series [2, 3].

In the present paper we give the results of the isolation and a proof of the chemical structures of three new glycosides of gitogenin which we have called capsicosides A_1 (I), B_1 (II), and C_1 (III).

By chromatography on a silica gel column of the total substances of a methanolic extract of the roots of the plant collected in the flowering and fruit-bearing phase we isolated three (1, 2, 3) chromatographically individual glycoside fractions which were numbered in order of increasing polarity in a thin layer of silica gel and gave positive reactions with the Sannie reagent [4] and negative reactions with Ehrlich's reagent [5], which showed their spirostanol nature.

For each fraction of the glycosides the IR spectrum showed characteristic absorption bands with λ_{max} in KBr (cm^{-1}) of 3500-3400 (OH), 987, 920, 900, 850 (900 > 920) of a spiroketal chain of the (25R) series [6].

To determine the nature of the aglycon, each of the fractions was subjected to complete acid hydrolysis with 2.5% sulfuric acid. Three aglycons were detected in each fraction. After their separation on silica gel impregnated with 2% silver nitrate, three compounds were isolated which were identified as gitogenin, tigogenin, and diosgenin.

These results permitted the assumption that each fraction of glycosides consisted of a three-component mixture of glycosides of close structures and had as the aglycons gitogenin, diosgenin, and tigogenin.

We did not succeed in separating and isolating individual glycosides from the fractions obtained. We first acetylated fractions 1-3, after which the acetylated glycosides were separated on a column of silica gel.

By the acetylation and chromatographic separation of the peracetates followed by their saponification we obtained only capsicosides A_1, B_1, and C_1 in the individual state.

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0009-3130/86/2206-0661$12.50

The complete acid hydrolysis of these glycosides yielded a single aglycon, identical with gitogenin. Paper chromatography of the aqueous hydrolysates showed the presence of galactose for (I), galactose and glucose for (II), and galactose, glucose, and xylose for (III). The following molar ratios of the monosaccharides were established with the aid of gas-liquid chromatography of the acetates of the aldononitrile derivatives of the sugars [7]: galactose and glucose (1:1) for capcisocide B_1 and galactose, glucose, and xylose (1:1:1) for capsicoside C_1.

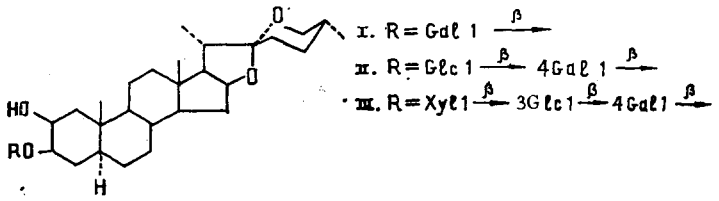
Information on the structure of the carbohydrate chains and the positions of their attachment was obtained for each glycoside by analysis of the products of complete Hakomori methylation [8] followed by methanolysis. By GLC in the presence of authentic markers, methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside was identified for A_1, methyl 2,3,6-tri-O-methyl-D-galactopyranoside and methyl 2,3,4,6-tera-O-methyl-D-glucopyranoside for B_1, and methyl 2,3,4-tri-O-methyl-D-xylanopyranoside, methyl 2,4,6-tri-O-methyl-D-glucopyranoside, and methyl 2,3,6-tri-O-methyl-D-galactopyranoside for C_1. In the case of capsicosides A_1-C_1 the aglycon derivative isolated from the reaction mixture by chromatography was the 2-monomethyl ether of gitogenin, which shows that the carbohydrate chains in these glycosides were attached to C_3 of the aglycon.

The sequence of attachment of the monosaccharide residues was determined for capsicosides B_1 and C_1 with the aid of partial acid hydrolysis.

In the case of capsicoside B_1 a monoside was obtained which decomposed on hydrolysis into galactose and gitogenin. Capsicoside C_1 gave a monoside, a bioside, and gitogenin. On hydrolysis, the monoside gave galactose and gitogenin, and the bioside gave galactose, glucose, and gitogenin.

In order to confirm conclusions concerning the structure of glycoside (III), it was subjected to periodate oxidation followed by acid hydrolysis. Glucose was detected in the hydrolysate. This indicates that it was attached to the monosaccharides through the C_3-OH group and confirmed the results of methylation. The configurations of the glycosidic centers were shown on the basis of Klyne's rule [9] in the light of the molecular rotations of the glycosides and their progenins. A calculation of the molecular rotation differences for each capsicoside and their progenins showed that all the glycosidic bonds had the β-configuration.

On the basis of the results obtained, the following structures are proposed for capsicosides A_1 (I), B_1 (II), and C_1 (III):



EXPERIMENTAL

The combined steroid glycosides were separated and the individual glycosides, their aglycones, and their methylated and acetylated derivatives were separated on columns filled with silica gel of types L 40/100 and L 100/160 μ. The following solvent systems were used: 1) chloroform–methanol (9:1); 2) chloroform–methanol (4:1); 3) chloroform–methanol–water (65:35:10, lower layer); and 4) chloroform–acetone (45:5); 5) acetone–methylene chloride (1:49); and 6) butanol–benzene–pyridine–water (5:1:3:3, upper layer).

For paper chromatography we used FN-3 paper, and for thin-layer chromatography silica gel L 5/40 μ + 13% of gypsum, and also Silufol plates. The thin-layer chromatograms were sprayed with concentrated sulfuric acid and with the Sannie reagent (a 5% solution of vanillin in ethanol) followed by treatment with sulfuric acid. A solution of aniline phthalate was used to reveal sugars on the paper chromatograms.

Melting points were determined on a Boëtius stage. Specific rotations were measured on a Zeiss polarimeter. IR spectra were obtained on a Specord spectrophotometer with the samples in the form of tablets with KBr or in paraffin oil.

Mass spectra were taken on a MKh-1320 instrument. The gas–liquid chromatography of the acetates of the aldononitrile derivatives of the sugars and of the methylated methyl glycosides was performed on a Chrom-5 chromatograph with a flame-ionization detector. For the sugar derivatives we used a glass column 2.4 m long filled with 5% of XE-60 on Chromaton N-AW-HMDS, and for the aglycons a glass column 1.2 m long filled with 3% of QF-1 on Chromaton Super.

Isolation of the Steroid Glycosides. The dry roots of red pepper of the Podarok Moldovy variety in the flowering and fruit-bearing stage (1 kg) were comminuted and extracted twice by steeping with 70% methanol solution for a day. After this, the further extraction of the glycosides was carried out another three times with heating to 65°C for 5 h. The combined methanolic extract obtained was evaporated to dryness. Yield 42 g. The combined glycosides isolated were chromatographed repeatedly on silica gel in solvent systems 1, 2, and 3. The process was monitored with the aid of TLC. Three chromatographically individual fractions were obtained: 1 — 530 mg; 2 — 620 mg; 3 — 600 mg.

Acetylation of the Individual Fractions. For each of fractions 1, 2, and 3, 800 mg was acetylated with acetic anhydride (15 ml) in the presence of pyridine (10 ml) at room temperature for 24 h. Each reaction mixture was diluted with water and the extraction product was extracted with chloroform (3 × 20 ml). Then each total acetate product was chromatographed on silica gel with elution by system 4. The completeness of acetylation was determined with the aid of IR spectroscopy. After column chromatography for each total glycoside material we obtained two individual fractions of acetates differing in polarity.

Saponification of the Capsicoside Peracetates. The peracetates of capsicosides A_1, B_1 and C_1 were saponified with 5% KOH in methanol at 100°C for 5 h. After the saponification of capsicoside A_1 peracetate, 175 mg of pure capsicoside A_1 (I) was obtained with mp 279-282°C, $[\alpha]_D^{20}$ — 29° (s 1.4; CH_3OH). The peracetate of glycoside B_1 gave 182 mg of capsicoside B_1 (II), mp 288-289°C, $[\alpha]_D^{20}$ — 41° (s 2.0; CH_3OH), and the peracetate of glycoside C_1 gave 187 mg of capsicoside C_1 (III), mp 247-248°C, $[\alpha]_D^{20}$ — 22° (s 3.7; CH_3OH).

Acid Hydrolysis of Capsicosides A_1, B_1, and C_1. In each case, 50 mg of the glycoside was hydrolyzed with 2.5% sulfuric acid in sealed tubes at 110°C for 18 h, after which the reaction mixture was diluted with water. The solution of the hydrolysate was extracted with diethyl ether (4 × 20 ml), and gitogenin with mp 264-265°C was isolated from the ethereal extract.

Paper chromatography in system 6 and also gas-liquid chromatography of the acetates of the aldononitrile derivatives of the sugars showed the presence of galactose in the hydrolysates of capsicoside A_1, of galactose and glucose (1.0:0.95) in those of capsicoside B_1, and of galactose, glucose, and xylose (0.98:1:0.95) in those of capsicoside C_1.

Methylation of the Capsicosides and of Their Progenins. To 10 ml of dimethyl sulfoxide were added 300 mg of sodium hydride and then 50 mg of one of the glycosides. The resulting mixture was stirred for one hour at 50°C in an atmosphere of argon. Then 20 ml of methyl iodide was added to the reaction mixture and it was left at room temperature in the dark for 12 h. After this, it was diluted with water and extracted with chloroform. The chloroform extract obtained was washed with saturated sodium thiosulfate solution and with water and was concentrated in vacuum. The methylation product was purified chromatographically on a column of silica gel in system 1.

The methanolysis of the permethylated glycosides obtained in the preceding experiment was performed in each case in 5 ml of anhydrous methanol and 0.5 ml of perchloric acid. The reaction mixture was heated in the boiling water bath for 6 h. Then the precipitate that had deposited was filtered off, and the solution was neutralized with ion-exchange resin evaporated, and analyzed by TLC and GLC in the presence of markers, as a result of which the methyl glycosides referred to in the discussion were identified.

The precipitate obtained was purified by chromatography on a column of silica gel L 40/100 μ in an amount of 20 g (1 × 30 cm) in system 1 and was recrystallized from acetone. As a result, the 2-monomethyl ether of gitogenin was identified, with mp 220-223°C, $[\alpha]_D^{20}$ — 122° (s 0.42; $CHCl_3$). According to the literature: mp 221-224°C, $[\alpha]_D$ — 111° [10].

The Partial Hydrolysis of Capsicosides B_1 and C_1. Capsicosides B_1 and C_1 were subjected to partial hydrolysis. A solution of 100 mg of each glycoside in 20 ml of 2.5% sulfuric acid solution was heated in the boiling water bath for 2 h. The course of the reaction was monitored every 20 minutes by TLC in systems 2 and 3. After cooling, the hydrolysate was diluted with water. The acid solution was neutralized with ion-exchange resin and was extracted with

butanol (3 × 50 ml). The butanolic extract was evaporated and the residue was separated preparatively in a thin layer of silica gel in system 3. The partial hydrolysis of capsicoside B_1 gave capsicoside A_1, and that of capsicoside C_1 gave glycosides (I) and (II).

Periodate Oxidation of Capsicoside C_1. A solution of 20 mg of capsicoside C_1 in 10 ml of methanol was treated with 10 ml of 2% sodium periodate solution. The resulting mixture was left at room temperature in the dark for 3 days. After this, a few drops of ethylene glycol were added and after an hour the mixture was extracted with butanol (2 × 10 ml). The butanolic extract was evaporated and the residue was hydrolyzed with 3 ml of 4% sulfuric acid at 110°C for 12 h. The hydrolysate was neutralized with an anion-exchange resin. On a paper chromatogram in the presence of known samples of sugars in systems 6, glucose was identified.

SUMMARY

Three steroid glycosides of the spirostan series have been isolated for the first time from the roots of the red pepper *Capsicum annuum*.

It has been shown that capsicoside A_1 is (25R)-5α-spirostan-2α,3β-diol-3-O-β-D-galactopyranoside; capsicoside B_1 is (25R)-5α-spirostan-2α,3β-diol-3-O-[O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside]; and capsicoside C_1 is (25R)-5α-spirostan-2α,3β-diol-3-O-[O-β-D-xylopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

LITERATURE CITED

1. R. Tschesche and H. Gutwinski, Chem. Ber., 108, 265 (1975).
2. P. K. Kintia [Kintya], E. V. Gutsu, G. V. Lazurievsky [Lazur'evskii], and N. N. Balachova and L. P. Kovalchuk, in: Second International Conference on the Chemistry and Biotechnology of Biologically Active Natural Products. Abstracts, Budapest (1983), p. 156.
3. E. V. Gutsu, P. K. Kintya, and G. V. Lazur'evskii, in: Abstracts of Lectures at Conferences of the VIIth Indosoviet Symposium on the Chemistry of Natural Compounds [in Russian], Tbilisi (1983), p. 48.
4. C. Sannié and H. Japin, Bull. Soc. Chem. Fr., 1237 (1957).
5. S. Kiyosawa and M. Hutoh, Chem. Pharm. Bull., 16, 1162 (1968).
6. M. Wall, C. S. Fenske, and H. S. Gentry, J. Am. Pharm. Assoc., 44, 4381 (1955).
7. V. V. Krokhmalyuk, P. K. Kintya, and V. Ya. Chirva, Izv. Akad. Nauk MSSR, Ser. Biol. Khim. Nauk, No. 1, 85 (1975).
8. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
9. W. Klyne, Biochem. J., 47, xli (1950).
10. T. Kawasaki, I. Nishioka, T. Komori, T. Yamauchi, and K. Miyahara, Tetrahedron, 21, 299 (1965).