STEROID SAPONINS.

XIII. THE STRUCTURE OF ALLIUMOSIDES D AND E FROM *Allium narcissiflorum*

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We previously reported a proof of the structures of alliumosides A and B isolated from a methanolic extract of the onion *Allium narcissiflorum* Wills. — a plant of the family Liliaceae [1]. In the present paper we give information on the structure of alliumosides D and E as the compounds available in greatest amount.

After acid hydrolysis, alliumoside D had decomposed into diosgenin, rhamnose, and glucose. By the GLC analysis of the acetates of the aldonitriles of the sugars [2], it was established that their ratio was 2:4. The spirostanol analog obtained by the enzymatic hydrolysis with β-glucosidase of the glycoside D [3] contained rhamnose and glucose in a ratio of 2:3.

In order to prove the type of bonds between the carbohydrate residues in the chain, glycoside D was subjected to methylation by Kuhn's method [4] and methylation was brought to completion by Purdie's method [5], followed by methanolysis with perchloric acid in methanol.

By means of TLC and GLC in the presence of markers, the following methyl glycosides were identified among the methanolysis products: methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3,4-tri-O-methyl-D-glucoside, methyl 2,3-di-O-methyl-L-rhamnoside, and methyl 4,6-di-O-methyl-D-glucoside in a ratio of 2:1:1:1.

To determine the sequence of the carbohydrate residues in the chain, we performed partial hydrolysis with oxalic acid until the compound had decomposed into a series of progenins: trillin, a bioside, a trioside, and a pentaoside. The products of partial hydrolysis were also methylated by Kuhn's method. By the TLC and GLC methods in comparison with markers, the following glycosides were identified among the products of the methanolysis of the permethylated progenins: for trillin only methyl 2,3,4,6-tetra-O-methyl-D-glucoside; for the bioside methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 3,4,6-tri-O-methyl-D-glucoside; and for the trioside methyl 2,3,4-tri-O-methyl-D-glucoside, methyl 3,4,6-tri-O-methyl-D-glucoside, and methyl 2,3,4-tri-O-methyl-L-rhamnoside. The pentaoside yielded the same methyl glycosides as the initial alliumoside D but it contained one molecule less of completely methylated glucose. In its quantitative set of sugars, the pentaoside was identical with the spirostanol analog obtained by the enzymatic hydrolysis of glycoside D.

The fact that glycoside D belonged to the furostanol series was also shown by the oxidative cleavage with CrO_3 of the peracetylated initial compound [3], with the formation of a glycoside of pregnenolone and the tetraacetate of a glucoside of methyl γ-methyl-δ-hydroxyvalerate, having characteristic peaks in the mass spectrum. Acid hydrolysis of the derivative of the pregnenolone glycoside and subsequent acetylation gave 3β-acetoxypregna-5,16-dien-20-one. The monosaccharide composition of the glycoside derivative of pregnenolone was the same as that of the pentaoside of glycoside D.

The reduction of glycoside D with sodium tetrahydroborate in aqueous solution followed by hydrolysis of the reduction product led to dihydrodiosgenin, which confirms the presence of two carbohydrate chains [3].

The configurations of the glycosidic centers of the sugars are given in accordance with Klyne's rule [6].

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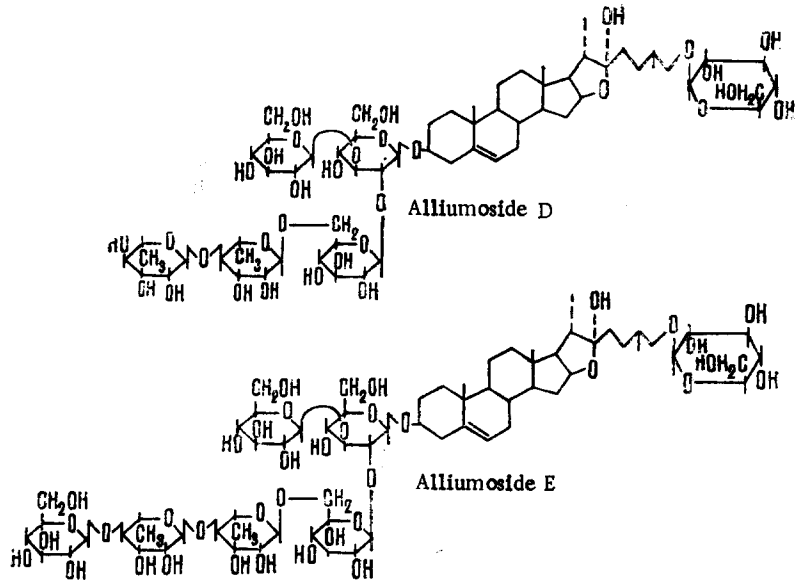

Alliumoside D

Alliumoside E

Structure of the Glycoside E. From its chromatographic mobility on TLC, glycoside E is more polar than alliumoside D. After acid hydrolysis, diosgenin was again identified as the aglycone, and the carbohydrate component consisted of rhamnose and glucose in a ratio of 2:5. With Ehrlich's reagent, glycoside E gave a positive reaction [7], and the action of β-glucosidase formed the spirostanol analog. When glycoside E was methylated, as described for alliumoside D, the following methyl glycosides were identified by GLC: methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-D-glucoside, methyl 2,3-di-O-methyl-L-rhamnoside, and methyl 4,6-di-O-methyl-D-glucoside in a ratio of 3:1:2:1.

To determine the position of branching in the carbohydrate chain, we performed the partial hydrolysis of glycoside E. It was found that the progenins were structurally identical with the products of the partial hydrolysis of glycoside D, with the exception of a tetraoside and a hexaoside, which gave the same methyl glycosides as the initial compound. When the permethylated tetraoside was subjected to methanolysis, the following glycosides were identified by TLC and GLC with markers: methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3-di-O-methyl-L-rhamnoside, methyl 2,3,4-tri-O-methyl-D-glucoside, and methyl 3,4,6-tri-O-methyl-D-glucoside.

Thus, the difference of glycoside E from glycoside D consists in the presence of an additional glucose molecule attached to C_4 of rhamnose. This conclusion is confirmed by the methylation of the products of partial hydrolysis. In the determination of the structure of alliumoside E we again used $NaBH_4$ reduction and oxidative cleavage with CrO_3.

On the basis of the results obtained, the structures of alliumosides D and E can be regarded as demonstrated.

EXPERIMENTAL METHOD

FN-3 and FN-13 papers, types KSK and LS 50/100 (Czechoslovakia) silica gels, neutral alumina, and the following solvent systems were used: 1) butanol—ethanol—water (10:2:5); 2) chloroform—methanol—water (65:35:10); 3) butanol—benzene—pyridine—water (5:1:3:3); 4) chloroform—methanol (9:1); 5) benzene—ethanol (9:1); and 6) benzene—acetone (2:1). Gas—liquid chromatography was performed on a "Khrom-4" instrument with FID; the sugars were investigated in the form of the acetates of the aldononitrile derivative with a dual system on glass columns (220 × 0.35 cm) filled with 5% of XE-60 on Chromaton N-AW HMDS (0.200-0.250 mm), programmed temperature from 180 to 225°C, rate of the program 3 deg/min, feed of carrier gas 45 ml/min. The analysis of the methyl glycosides was performed on the same instrument with programmed temperatures from 100 to 150°C at the rate of 2 deg/min with a dual system of glass columns, one of which was filled with 3% of NPGS on Chromaton N-AW HMDS (0.160-0.200 mm) and the second with 3% of XE-60; the carrier gas was helium at a rate of 30 ml/min. The methyl glycosides were identified by comparing the results of analysis obtained on the two columns. Column partition chromatography was performed with a fraction collector of the KhKOV-1 type, the fractions collected having a volume of 4-15 ml and the separation being monitored by TLC in systems, 1, 2, and 4. The organic substances on the TLC plates were re-

vealed by spraying with concentrated H_2SO_4. The methyl glycosides and the aglycones were separated into the individual substances by column chromatography on SiO_2, with elution by systems 4, 5, and 6.

Hydrolysis of Glycosides D and E. As described previously [1], 30 mg each of glycosides D with mp 268-269°C, $[\alpha]_D^{18}$ −88° (c 0.85; methanol) and E with mp 286-288°C, $[\alpha]_D^{18}$ −83° (c 0.6; methanol) was dissolved in 5% H_2SO_4 and heated in a glass tube at 100°C for 8 h. The precipitate of aglycone that deposited was filtered off, decolorized with activated carbon, and recrystallized three times from hot methanol. The aglycone, with mp 204-206°C, $[\alpha]_D^{18}$ −119° (c 2.0; chloroform) was identified by its IR spectra, retention time on GLC [8], and chromatographic mobility on TLC in system 4 as diosgenin. The mixture of sugars was neutralized with anion-exchange resin (HCO_3^- form) and, after evaporation, was analyzed by paper chromatography in system 3. The remainder of the hydrolyzate was converted into the aldononitrile acetates [2] and used for analysis by the GLC method. Paper chromatography in system 3 showed the presence of rhamnose and glucose in the hydrolyzates of the glycosides D and E. It was shown by the GLC method that for glycoside D their ratio was 2.1:3.9 and for glycoside E 2.1:4.8.

Methylation of Glycosides D and E. In each case, 1.5 g of the glycoside was methylated by Kuhn's method [0.8 g of $Ba(OH)_2$ + 10 g of BaO + 70 ml of dimethylformamide + 70 ml of CH_2I] three times. The products obtained were methylated to completion by Purdie's method, with the addition of 20 ml of CH_3I + 1 g of Ag_2O. The completeness of methylation was checked by IR spectroscopy from the absence of an absorption band at 3400 cm^{-1}. The product obtained was purified on a column of silica gel in system 5. The yield of permethylated products was the same for glycoside D [1.8 g with mp 158-160°C, $[\alpha]_D^{18}$ −20° (c 2.0; methanol)] and for glycoside E [1.9 g with mp 166-167°C, $[\alpha]_D^{18}$ −34° (c 1.65; methanol)].

The compounds synthesized were subjected to methanolysis with 72% $HClO_4$ in methanol (1:10) at 110°C for 5 h. After cooling, the reaction mixture was diluted with water and filtered, and the filtrate was neutralized with anion-exchange resin (HCO_3^- form) and concentrated. The filtrates were shown by TLC and GLC in the presence of markers to contain the methyl glycosides described in the discussion of the results for glycosides D and E.

Enzymatic Hydrolysis. In each case, 0.2 g of the glycoside D or E was dissolved in 50 ml of H_2O, 20 mg of the enzyme complex from *Helix pomatia* was added, and the mixture was left at room temperature for 12 h. Then 20 ml of methanol was added and it was boiled for 5 min. After extraction three times with n-butanol, the organic layer was evaporated and the products of enzymatic hydrolysis were purified on a column of silica gel in system 2. After chromatography, 0.16 g of a glycoside of the spirostanol series was obtained with mp 264-266°C, $[\alpha]_D^{18}$ −86° (c 0.7; methanol) in the case of glycoside D and with mp 282-284°C, $[\alpha]_D^{18}$ −93° (c 1.2; methanol) in the case of glycoside E.

Partial Hydrolysis. In each case, 1.0 g of glycoside D or E was dissolved in 15 ml of 5% $H_2C_2O_4$ and the solution was heated at 80°C for 3.5 h. Then the reaction mixture was diluted with water, and the reaction products were extracted with n-butanol (3 × 50 ml). The organic extracts were combined and evaporated. The resulting precipitate was chromatographed on a column of silica gel, with elution by system 2. After repeated chromatography, the hydrolyzates from glycosides D and E yielded a number of progenins: trillin with mp 260-262°C, $[\alpha]_D^{18}$ −103, (c 0.7; methanol) and a bioside with mp 258-260°C, $[\alpha]_D^{18}$ −180°, (c 0.63; methanol) in which glucose was found on acid hydrolysis. The trioside from the glycosides D and E, with mp 254-256°C, $[\alpha]_D^{18}$ −71° (c 0.7; methanol), on acid hydrolysis, gave rhamnose and glucose in a ratio of 1:2, and a pentaoside with mp 264-266°C, $[\alpha]_D^{18}$ −86° (c 0.8; methanol) gave one molecule of glucose less than glycoside D and two molecules of glucose less than glycoside E. The set of methyl glycosides derived from the progenins obtained has been described in the discussion of the results. In addition, from the products of the partial hydrolysis of glycoside E we isolated a tetraoside with mp 276-278°C, $[\alpha]_D^{18}$ −63° (c 0.9; methanol) and a hexaoside with mp 282-284°C, $[\alpha]_D^{18}$ −93° (c 1.2; methanol). In the permethylate of the tetraoside after hydrolysis with perchloric acid in methanol (1:10), by TLC in system 6, and also by GLC in comparison with markers, we identified methyl 3,4,6-tri-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-D-glucoside, methyl 2,3-di-O-methyl-L-rhamnoside, and methyl 2,3,4-tri-O-methyl-L-rhamnoside, and when the hexaoside was subjected to methanolysis we found the same methyl glycosides as in the case of glycoside E but with one completely methylated glucose molecule less. The acid hydrolysis of the tetraoside and of the hexaoside led to rhamnose and glucose in ratios of 2:2 and 2:4, respectively.

Oxidative Cleavage of Glycosides D and E. In each case, 1.0 g of the peracetylated glycoside D or E was oxidized with CrO_3 by the method described by Tschesche [3]. The reaction products were extracted three times with chloroform and, after evaporation, were hydrolyzed with potassium tert-butoxide [3] in a current of nitrogen at 30°C for 3.5 h. Then the reaction mixture was diluted with water, and the solvent was distilled off in vacuum. The residue was dissolved in 200 ml of H_2O, and the reaction products were extracted three times with n-butanol. The aqueous phase was acidified to pH 3 and extracted with n-butanol. The product obtained was acetylated by the usual method and was methylated with diazomethane. This gave 320 mg of the tetraacetate of the glucoside of methyl γ-methyl-δ-hydroxyvalerate. The mass spectra obtained for each of the glycosides of the compounds showed ions with m/e 331, 243, 242, 200, 169, 157, 145, 141, 140, 115, 109, 103, 98, which are characteristic for completely methylated glucose, and also ions with m/e 129, 97, 89, and 81, which are characteristic for the acid residue. The butanol phases, containing 170 and 160 mg of material, respectively, were evaporated to dryness and subjected to acid hydrolysis with 4 N HCl at 80°C for 3 h. Pregnenolone derivatives were obtained which, in both cases, were purified on a column of silica gel and were acetylated. This gave 3β-acetoxypregna-5,16-dien-20-one with mp 170-172°C, $[\alpha]_D^{18}$ −40° (c 0.9; methanol).

SUMMARY

Two new steroid glycosides of the furostanol series have been isolated from *Allium narcissiflorum* Wills., and their structures have been shown.

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