PRESENCE OF GLUCODIGIFUCOSIDE IN ERYSIMUM CHEIRANTHOIDES

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We have obtained from the treacle erysimum (Erysimum cheiranthoides L.), in addition to other cardenolides, a cardiac glycoside which we have provisionally designated glycoside Z [1]. The object of the present communication is to provide a proof of its chemical structure.

The glycoside gives positive Legal, Raimond, and Kedde cardenolide reactions. The empirical formula ($C_{35}H_{54}O_{13} \cdot 3H_2O$) and M 686.3 correspond to a steroid diglycoside, and the UV spectrum shows only one absorption maximum at 218 mμ ($\log \varepsilon = 4.19$), which, together with the qualitative reactions, characterizes the presence of a butenolide ring. The glycoside is readily hydrolyzed by an enzyme preparation obtained from the pancreatic juice of the snail Helix pomatia; an aglycone and a mixture of two monosaccharides are formed and by suitable treatment of the hydrolyzate these can be obtained in the crystalline state. The aglycone has been identified as digitoxigenin, and the monosaccharides as D-fucose* and D-glucose. The acid hydrolysis of glycoside Z carried out by the Mannich-Siewert method [2] gave digitoxigenin, D-fucose and D-glucose as did enzymatic hydrolysis.

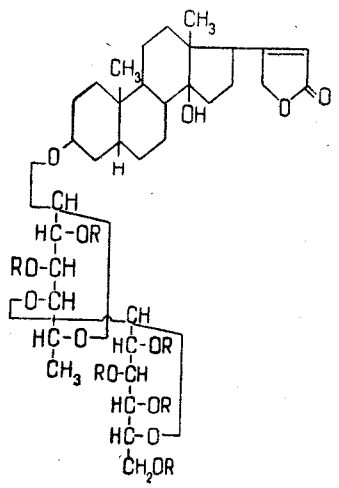
The pyranose form of the monosaccharide residues is indicated by the difficulty of hydrolyzing the glycoside by means of dilute mineral acids. Thus, there was no appreciable hydrolysis of the glycoside after 30 minutes' treatment at 70° with 0.05 N H_2SO_4 solution. This simple method of identifying pyranosides was proposed by A. Hunger and T. Reichstein [3].

In order to determine the linkage arrangement of the monosaccharides in the glycoside and to confirm the dimensions of the oxide ring of the D-glucose, we carried out the exhaustive methylation of the glycoside by Kuhn's method [4]. The methylated glycoside was hydrolyzed, and the hydrolysis products were investigated by means of paper chromatography. The presence of 2, 3, 4, 6-tetramethyl-D-glucose was discovered (see figure). For identification, development with diphenylamine-aniline was used [5]; this differentiates methylated sugars by the color of the spots. On this treatment, the aglycone is destroyed; its degradation products are readily separated, since they are insoluble in water.

The formation of 2, 3, 4, 6-tetramethyl-D-glucose shows that the terminal monosaccharide residue in the diglycoside is a glucose residue and that it is present in the pyranose form. Attempts to obtain a monoglycoside from the diglycoside by partial enzymatic or acid hydrolysis did not give the desired result. Consequently, there was no possibility of establishing the molecular rotation of the monosaccharide residues in order to determine the configuration of the glycosidic linkages. However, judging from the molecular rotation of the disaccharide residue (−14.6°), it may be assumed that the D-fucose and the D-glucose are connected by β-glycosidic bonds. If even a single α-glycosidic bond were present in glycoside Z, one would expect a markedly different molecular rotation of the disaccharide residue.

Thus, the structure of glycoside Z may be represented most probably by formula (I). This is the first case of the detection of glycosides of digitoxigenin in plants of the genus Erysimum.

In addition to Erysimum cheiranthoides L., we have found glycoside Z in E. leptophyllum (M. B.), E. suffruticosum, and E. Marschallianum Andrz. [6]. This glycoside was obtained somewhat before us from foxglove by the Japanese worker A. Okano [7], who called it glucodigifucoside. We shall adopt the same name for glycoside Z.



I. R=H. Glucodigifucoside (glycoside Z); mp 188-192°, $[\alpha]_D$ − 7.9°.

II. R = $CH_3-\overset{\overset{O}{\|}}{C}-$; mp 235-238°; $[\alpha]_D$ −5,2°.

* A sample of D-fucose was kindly provided by Prof. T. Reichstein.

EXPERIMENTAL

The substances were analyzed after drying for 3 hr over P_2O_5 at 110° in a vacuum of 0.1 mm Hg. The following systems of solvents were used to investigate the cardenolides by means of paper chromatography: toluene-butan-1-ol-water (2.5:1.5:1), chloroform-tetrahydrofuran-formamide (50:50:6.5), and m-xylene-methyl ethyl ketone (1:1)/formamide.

Glycoside Z crystallizes from 60% alcohol in the form of thin elongated plates melting at 188-192°; $[\alpha]_D^{21} - 7.9° \pm \pm 5$ (c 0.455; methanol). It dissolves in 84% sulfuric acid with a yellow coloration which changes after 40 minutes to a red coloration lasting for a long time.

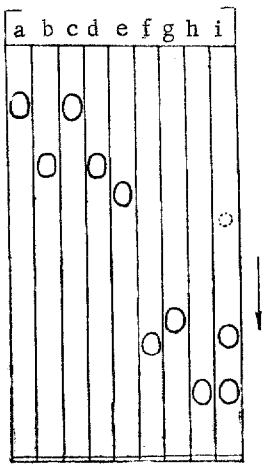
Found %: C 61.38; H 8.05; M 686.3 (lactone titration). $C_{35}H_{54}O_{13}$. Calculated %: C 61.56; H 7.97; M 682.82. The loss in weight of the glycoside on drying amounted to 7.3%.

In order to obtain the acetyl derivative, 50 mg of glycoside Z was dissolved in 0.2 ml of pyridine, 0.4 ml of acetic anhydride was added, and the mixture was left for 24 hr at room temperature. Paper chromatography of a sample showed that acetylation of the glycoside had taken place completely after this time. The solvent was evaporated in vacuum, and the residue was crystallized from alcohol. The crystals obtained melted at 235-238°; $[\alpha]_D^{21} - 5.2 \pm 4°$ (c 0.39; chloroform).

Enzymatic hydrolysis and identification of the hydrolysis products. A solution of 0.6 g of the glycoside in 500 ml of hot water was cooled to room temperature, and 0.5 g of a dry enzyme preparation from the grape snail (Helix pomatia) was added. The solution was left in the thermostat at 37-40° for 48 hr. After this time, as was shown by paper chromatography, hydrolysis of the glycosides had taken place completely. In order to remove the enzymes, the solution was poured into two liters of hot alcohol. The precipitate which deposited was filtered off, and the filtrate was evaporated in vacuum to an aqueous residue. The latter was treated with chloroform (5 × 100 ml). The residue was crystallized from alcohol. The chloroformic extracts were dried with sodium sulfate and evaporated. The resulting crystalline aglycone (0.28 g) melted at 248-252°; $[\alpha]_D^{20} + 18.3 \pm 3°$ (c 0.52; methanol).

The action of acetic anhydride in pyridine on the aglycone gave an acetyl derivative melting at 224-228°; $[\alpha]_D^{21} + + 20.1 \pm 3°$ (c 0.49; chloroform). The aglycone gives positive Legal, Raimond and Kedde reactions; it dissolves in 84% sulfuric acid with a yellow coloration which changes after two hours into a long-lasting blue coloration. According to these properties, the aglycone corresponds to digitoxigenin. A comparison of the aglycone obtained with authentic digitoxigenin by paper chromatography, the mixture test, and IR spectroscopy showed the identity of these substances. The IR spectra were obtained on a IKS-14 spectrometer with LiF and NaCl prisms in the 4000-667 cm^{-1} region.

The aqueous solution freed from the aglycone was evaporated in vacuum. The residue, which consisted of a mixture of two monosaccharides, was chromatographed on a cellulose column by the method described previously [8]. The individual monosaccharides obtained were crystallized from alcohol-ether. One of them melted at 144-146°; $[\alpha]_D^{22} + + 53.0 \pm 4°$ (c 0.483; aqueous solution after 2 hr), and on paper chromatography was located at the same level as D-glucose. A mixture of these substances gave no depression of the melting point (144-146°). The osazone of the sugar was obtained; it melted at 207-208°.



Scheme of the chromatogram. System: butan-1-ol-ethanol-water-ammonia (40:10:49:1). Chromatography for 18 hr. "B" paper of the Volodarskii Leningrad No. 2 mill: a) D-glucose reference sample; b) D-fucose reference sample; c) D-glucose from glycoside Z; d) D-fucose from glycoside Z; e) 3-methyl-D-glucose; f) 2,3,4-trimethyl-D-glucose; g) 2,3,6-trimethyl-D-glucose; h) 2,3,4,6-tetramethyl-D-glucose; i) hydrolyzate of methylated glycoside Z.

The second monosaccharide melted at 139-144°. On paper chromatography it exhibited the same R_f figures as D-fucose. A mixture with the authentic substance gave no depression of the melting point (139-144°).

Methylation of the glycoside by Kuhn's method [4]. A solution of 0.2 g of the glycoside in 5 ml of dimethylformamide was treated with 2 ml of methyl iodide and 4 g of freshly-prepared and vacuum-dried silver oxide. The reaction mixture, evolving heat, was cooled in ice water. After 2 hr, a further 1 ml of methyl iodide and 0.5 g of silver oxide were added. The mixture was left overnight at room temperature. The silver salt was filtered off and washed with chloroform, and the filtrate was evaporated in vacuum. The residue was subjected to a similar methylation process twice more, the completeness of the reaction being followed by thin-layer chromatography on a non-fixed carrier by the method described by N. K. Kochetkov et al. [9].

The methylated glycoside, without further purification, was hydrolyzed for 7 hr at 80° in a sealed tube with 1 N methanolic hydrochloric acid. The hydrolyzate was treated with an equal volume of water and was heated for 3 hr under the same conditions. After hydrolysis, the solution was freed from acid by treatment with silver carbonate and was evap-

orated in vacuum. On paper chromatography, the residue showed the presence of two reducing sugars of low polarity with a small amount of a third sugar (figure). One of the sugars was located at the same level as 2, 3, 4, 6-tetramethyl-D-glucose and showed a coloration similar to this on development with diphenylamine-aniline [5].

SUMMARY

Glycoside Z obtained from Erysimum cheiranthoides L., on the basis of the structure established, is identical with the glucodigifucoside described previously.

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