ERYCORDINOBIOSE AND ERYCHROBIOSE

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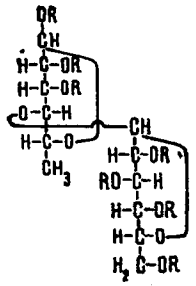
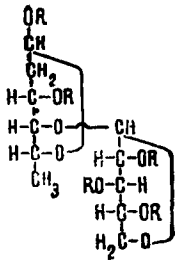
Erycordinobiose, $C_{12}H_{22}O_{10}$, has been obtained by the partial acid hydrolysis of the cardiac glycoside erycordin, which is cannogenol 3β-O-[O-β-D-glucopyranosyl-(1→4)-β-D-gulomethylopyranoside] [1]. The structure of the sugar component in the glycoside was established [1] from the results of stepwise enzymatic and acid hydrolysis, from the formation of 2', 3'-O-isopropylideneerycordin, and from an analysis of molecular rotations. On this basis, erycordinobiose may be characterized as 4-O-β-D-glucopyranosyl-D-gulomethylose (I). The structure of (I) is also confirmed by the formation of a phenylosazone, which excludes the possibility of the attachment of the D-glucose at C-2. When the disaccharide was methylated by Kuhn's method and the methylated product was hydrolyzed, 2,3,4,6-tetra-O-methyl-D-glucose was obtained (identified by chromatography). Erycordinobiose is not hydrolyzed in any appreciable amounts whatever by 0.05 N sulfuric acid at 80°C in 30 min. These results indicate [2] that the D-glucose residue is in the pyranose form.

Erychrobiose, $C_{11}H_{20}O_8$, has been isolated from the cardiac diglycoside erychroside in the amorphous state, and its structure was established as long ago as 1962 [3]. The disaccharide has now been obtained in the crystalline state. We give the properties of erychrobioside and information showing that it is 4-O-β-D-xylopyranosyl-D-digitoxose (III). A sugar of precisely this structure has been described under the name of gypsobiose [9].



I. R = H
II. R = CH_3CO



III. R = H
IV. R = CH_3CO

Erychrobiose is readily hydrolyzed by an enzyme preparation from the grape snail with the formation of D-digitoxose and D-xylose. The sequence of attachment of the monosaccharide residues in the form D-xylose→D-digitoxose is shown by the formation in the enzymatic hydrolysis of erychroside [4] of D-xylose and the monoglycoside erysimin, which is strophanthidin 3β-O-β-D-digitoxoside. An analysis of the molecular rotations of erychroside and the monoglycoside obtained from it (erysimin) by Klyne's rule [5] shows that the D-xylose is attached by a β-glycosidic bond. The pyranose form of the D-xylose residue is shown by two facts: in the first place, 0.05 N sulfuric acid does not hydrolyze erychrobiose appreciably under the conditions given above; in the second place, the methylation of the disaccharide and subsequent hydrolysis gave 2,3,4-tri-O-methyl-D-xylose and D-cymarose (3-O-methyl-D-digitoxose). The formation of D-cymarose, in its turn, shows that the OH group at C_3 of digitoxose is free in erychrobiose. This excludes a 1→3 bond.

The hydrolysis of 1,2',3,3',4'-penta-O-methylerychrobiose under mild conditions gave 2',3,3',4'-tetra-O-methylerychrobiose. The latter was oxidized with bromine and the oxidation product was distilled

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in vacuum in order to convert it completely into the lactone. The IR spectrum of the distillate showed an absorption band at 1749 cm^{-1} which is characteristic for the carbonyl group of a δ-lactone. The formation of a δ-lactone (the lactone of 2',3,3',4'-tetra-O-methylerychrobionic acid) excludes the furanose form of the digitoxose in the disaccharide and a 1→5 bond with it. Consequently, the only possible linkage of the monosaccharide residues is a 1→4 linkage.

Thus, erychrobiose can be characterized as 4-O-β-D-xylopyranosyl-D-digitoxose, and its structure may be represented by formula (III).

EXPERIMENTAL

Preparation of Erycordinobiose. A solution of 3 g of erycordin in 300 ml of methanol was treated with 300 ml of a 0.15 N aqueous solution of sulfuric acid and the mixture was boiled under reflux for 7 h. Then the methanol was distilled off in vacuum at 45°C. The aglycone fraction was extracted from the aqueous residue with a mixture of chloroform and isopropanol (2:1; 5 × 250 ml). The aqueous solution was neutralized with barium carbonate, filtered through a layer of kieselguhr, and evaporated in vacuum. As was shown by the results of chromatographic analysis, the residue consisted of three sugars: a disaccharide (the main component), D-glucose, and D-gulomethylose. The amounts of the last two substances were relatively small. To separate them from the disaccharide, the mixture was chromatographed in a column of cellulose using the butan-1-ol–acetic acid (4:1)/water solvent system. A 250-fold amount of cellulose on the total amount of substances to be separated was used. The fractions containing the pure disaccharide were evaporated in vacuum (0.01 mm Hg) at 7-10°C. The residue was crystallized from methanol–butan-1-ol. This gave 0.48 g of crystalline erycordinobiose (35% of the theoretically possible yield) with mp 133-136/160-163°C, $[\alpha]_D^{19} -22.4 \pm 2°$ (c 1.20; aqueous solution after the establishment of equilibrium). The results of elementary analysis corresponded to the figures calculated for the composition $C_{12}H_{22}O_{10}$. In the butan-1-ol–acetic acid (4:1)/water system, $R_{Glu} = 0.61$.

A solution of 15 mg of erycordinobiose and 10 mg of an enzyme preparation from the grape snail in 1 ml of water was left in the thermostat at 41°C for 17 h. The enzymes were precipitated with ethanol, the precipitate was separated off, and the solution was evaporated in vacuum. Paper-chromatographic analysis of the residue showed that it consisted of D-glucose and D-gulomethylose.

Erycordinobiose (60 mg) was methylated with methyl iodide in dimethylformamide by Kuhn's method [6]. The methylated disaccharide was hydrolyzed with a mixture of hydrochloric acid, acetic acid, and water (1:3.5:5.5; Kiliani's mixture [7]) in a sealed glass tube at 100°C for 1 h. The methylated monosaccharides were extracted from the hydrolyzate with chloroform–ethanol (2:1). The ethanolic chloroform extract was treated with 2 N sodium carbonate solution and was evaporated. As was shown by paper chromatography, the residue consisted of two monosaccharides, one of which was identified as 2,3,4,6-tetra-O-methyl-D-glucose.

1,2,2',3,3',4',6'-Hepta-O-acetylerycordinobiose. A solution of 150 mg of erycordinobiose in 10 ml of absolute pyridine was treated with 6 ml of acetic anhydride and the mixture was left at room temperature for 46 h. Then it was stirred with ice for 2 h and the acetate was transferred into chloroform solution. The latter was treated with 1 N sulfuric acid and with water to neutrality and was evaporated. The residue, which consisted of hepta-O-acetylerycordinobiose (II), after crystallization from acetone–water, had mp 65-70°C. Substance (II) was recrystallized from a mixture of diethyl ether and petroleum ether, and then melted at 71-73°C, $[\alpha]_D^{18} -1.8 \pm 2°$ (c 1.67; chloroform).

The melting point of erycordinobiose phenylosazone was 214.5-216°C (from water); $[\alpha]_D^{21} -34.7 \pm 5°$ (c 0.35; pyridine). UV spectrum: $\lambda_{max}^{ethanol}$ 256.5, 311, 394.5 nm (log ε, respectively, 4.49, 4.26, 4.48) and two shoulders in the ~230- and 327-nm regions (log ε 4.39 and 4.25).

Preparation of Erychrobiose. A solution of 2.5 g of erychroside in 100 ml of ethanol was treated with 100 ml of a 0.1 N aqueous solution of sulfuric acid. The mixture was heated at 80°C under reflux for 40 min. The aglycone was extracted from the cooled hydrolyzate with chloroform (300 ml) and with a mixture of chloroform and ethanol (5:1; 8 × 100 ml). The aqueous solution was neutralized with barium carbonate, filtered, and evaporated. The residue was dissolved in methanol and the resulting solution was filtered. The filtrate was treated with butan-1-ol (15 ml) and was then concentrated in vacuum to a volume of about 7 ml. Slowly, over 2 h, 3 ml of diethyl ether was added and the resulting mixture was kept in a closed vessel at room temperature for 17 h. Small colorless crystals formed. An additional 5 ml of ether was added dropwise over 6 h, and the mixture was left for another 12 h. The crystals were separated off by ordinary

vaccum filtration and were washed first with butanol–ether (1 : 1) and then with pure ether. This gave 0.72 g of erychrobiose (III) (yield about 70% of that theoretically possible) with mp 88-90°C, $[\alpha]_D^{20}$ +2.7 ± 2° (c 1.2; pyridine solution after the establishment of equilibrium). The results of elementary analysis corresponded to the figures calculated for the composition $C_{11}H_{20}O_8$. Enzymatic hydrolysis (see above) of the disaccharide gave D-xylose and D-digitoxose (identified by paper chromatography).

1,2',3,3',4'-Penta-O-acetylerychrobiose (IV). The substance was obtained in the same way as penta-O-acetylerycordinobiose (see above). It crystallized from 50% ethanol in the form of elongated needles; mp 184-186°C, $[\alpha]_D^{20}$ −19.1 ± 3° (c 0.82; chloroform).

1,2',3,3',4'-Penta-O-methylerychrobiose. With heating, 0.5 g of erychrobiose was dissolved in 4 ml of dimethylformamide in a three-necked flask. It was methylated (Haworth's method) with the careful elimination of atmospheric oxygen from the reaction flask (continuous passage of nitrogen) and ice cooling. To the sugar solution was added 1.5 ml of dimethyl sulfate and then, over 40 min, 3 ml of 30% aqueous caustic soda from a dropping funnel. The reaction mixture was stirred with a magnetic stirrer and its temperature was allowed to rise gradually to 20°C after which another 1 ml of dimethyl sulfate and 2.5 ml of the solution of alkali were added over 1 h. Stirring of the mixture at room temperature was continued for 18 h. Then 3 ml of ethanol was added to it, and it was stirred for another 2 h. The mixture was cooled with ice and the alkali was neutralized with 1 N sulfuric acid. The methylation product was extracted with chloroform–isopropanol (3 : 1). The extract was washed four times with water and was dried over anhydrous sodium sulfate and evaporated. The residue (0.60 g) was further methylated by Kuhn's method [6] in the following way: it was dissolved in 4 ml of dimethylformamide and 5 ml of methyl iodide and 5 g of freshly prepared silver oxide were added. The mixture was heated under reflux with continuous stirring (4 h) and was then diluted with 100 ml of chloroform and filtered through a layer of kieselguhr. The chloroform extract was washed with water (5 × 20 ml), dried over anhydrous sodium sulfate, and evaporated. This gave 0.62 g of penta-O-methylerychrobiose in the form of a light-brownish viscous mass. The IR spectrum of the substance did not contain absorption bands characteristic for OH groups.

Hydrolysis of 1,2',3,3',4'-Penta-O-methylerychrobiose; Isolation and Identification of the Hydrolysis Products. The penta-O-methylerychrobiose (200 mg) was hydrolyzed by Kiliani's method [7] for 1 h, and the methylated monosaccharides were separated from the acids as described above. The results of paper chromatography showed that the hydrolyzate consisted of two substances the spots of which were located at the levels of D-cymarose and of 2,3,4-tri-O-methyl-D-xylose. The substances were separated by chromatography on neutral alumina (activity grade III). The trimethylxylose was desorbed with chloroform–methanol (99 : 1 to 97 : 3) and the cymarose with chloroform–methanol (80 : 20 to 70 : 30). The D-cymarose had mp 90-94°C (ethanol–ether). A mixture with a sample of the monosaccharide gave no depression of the melting point (90-94°C).

The 2,3,4-tri-O-methyl-D-xylose had mp 78-86°C, $[\alpha]_D^{21}$ +23.2 ± 4° (c 0.65; in ethanol after the establishment of equilibrium). In the methyl ethyl ketone–m-xylene (1 : 1)/formamide system, R_{TMG} = 0.91 (TMG = 2,3,4,6-tetra-O-methyl-D-glucose). The results of a direct comparison of the monosaccharide obtained with a sample of 2,3,4-tri-O-methyl-D-xylose shows their identity.

2',3,3',4'-Tetra-O-methylerychrobiose. A solution of 400 mg of 1,2',3,3',4'-penta-O-methylerychrobiose in 30 ml of methanol was mixed with 15 ml of 0.15 N aqueous sulfuric acid and heated at 64°C under reflux for 40 min. After the acid had been neutralized with barium carbonate, the solution was filtered and evaporated. The residue consisted of a slightly yellowish vitreous mass which vigorously reduced Fehling's reagent, formed a green coloration with the Keller–Kiliani reagent, and gave a positive Webb–Levy reaction [8]. Paper chromatography revealed only one spot of a reducing sugar. This substance was used for subsequent reactions without further purification.

Lactone of 2',3,3',4'-Tetra-O-methylerychrobionic Acid. A solution of 300 mg of 2',3,3',4'-tetra-O-methylerychrobiose in 20 ml of 50% dioxane was mixed with three drops of bromine, and the mixture was kept at 3°C for 74 h. Then ethylene was passed into the solution to bind the bromine. After this, it was neutralized with silver carbonate and filtered. The filtrate was saturated with hydrogen sulfide and it was then filtered again and evaporated. The light brown syrupy residue obtained was distilled in vacuum (0.01 mm Hg) at 140-155°C. The distillate, which set to a colorless vitreous mass, did not reduce Fehling's reagent and its IR spectrum showed an absorption band at 1749 cm^{-1} (solution in CCl_4) due to the carbonyl group of a δ-lactone; $[\alpha]_D^{20}$ −15.4 ± 5° (c 1.12; ethanol).

SUMMARY

The partial acid hydrolysis of the cardiac glycosides erycordin and erychroside has given two new disaccharides which have been called erycordinobiose and erychrobiose; their structures have been established. Erycordinobiose is 4-O-β-D-glucopyranosyl-D-gulomethylose, and erychrobiose is 4-O-β-D-xylopyranosyl-D-digitoxose.

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