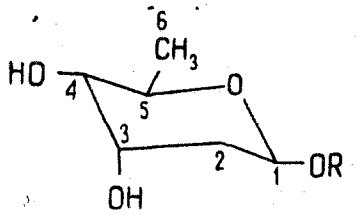
PARTIAL SYNTHESIS OF ERYCHROSIDE

I. F. Makarevich

Khimiya Prirodnykh Soedinenii, Vol. 2, No. 6, pp. 416-420, 1966

We first obtained the cardiac glycoside erychroside in 1960 [1] from Erysimum cheiranthoides L. (treacle erysimum) and characterized it [2] as strophanthidin 3-(β-D-digitoxopyranosido-4'-β-D-xylopyranoside). The presence of xylose in the carbohydrate moiety proved to be unusual for this glycoside. It has not previously been found in the composition of cardiac glycosides.

To confirm the structure of erychroside we have synthesized it by Königs and Knorr's method [3] starting from authentic D-xylose and erysimin (strophanthidin digitoxoside).



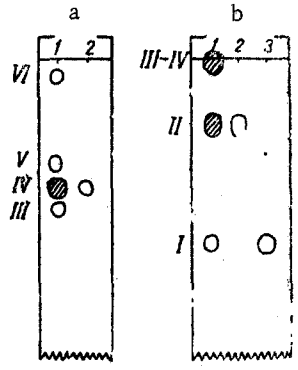
(R= Strophanthidin) Erysimin

The presence in erysimin of two secondary hydroxy groups capable of undergoing condensation with acetobromoxylose gave a basis for assuming that a mixture of glycosides is formed in the synthesis. However, in view of the fact that in the digitoxose residue the hydroxyl at C_4 is in the equatorial position and that at C_3 in the axial position it was expected that the reaction would take place predominantly at the equatorial hydroxyl. The latter assumption has been confirmed by experiment.

The difficulty of the synthesis of glycoside containing 2-deoxy sugars is due to the lability of the glycosidic bond. Even a very small amount of acid hydrolyzes these substances. Consequently, their synthesis requires fairly mild conditions. Attempts to synthesize erychroside under the comparatively severe conditions used in the synthesis of convallatoxin [4, 5] led to the aglycone strophanthidin and some strophanthidin xyloside. When β-bromoacetyl-D-xylose was condensed with erysimin, the hydrogen bromide liberated hydrolyzed the glycoside to the aglycone. Part of this aglycone apparently underwent condensation with the bromoacetylxylose forming the acetate of strophanthidin xyloside, which is more stable to hydrolysis.

Of the various mild synthetic conditions tried, the most successful proved to be synthesis at room temperature in absolute dioxane under the action of silver oxide as condensing agent and potassium oxide as dehydrating agent. Bromoacetylxylose in the dry state was added in small portions over 1.5—2 hr with vigorous stirring. Under such synthetic conditions, the hydrogen bromide liberated was rapidly neutralized and hydrolysis of the glycosides took place to only a slight extent.

The products of synthesis were deacetylated with ammonia in methanolic solution [6]. After deacetylation, chromatography on paper (type B of the Volodarskii Leningrad No. 2 mill) showed (figure, a) that the products of the synthesis included six cardenolides, provisionally denoted by the symbols I–VI. The substances were obtained in the pure crystalline state by absorption chromatography on alumina.



Chromatogram of the products of the synthesis. a) Solvent system: tetrahydrofuran—chloroform—formamide (50:50:6.5), 18 hr. 1) Mixture of cardenolides obtained from the synthesis; 2) erychroside. b) Solvent system: methyl ethyl ketone—m-xylene (1:1)/formamide, 4 hr. 1) Mixture of cardenolides; 2) erysimin 3) strophanthidin.

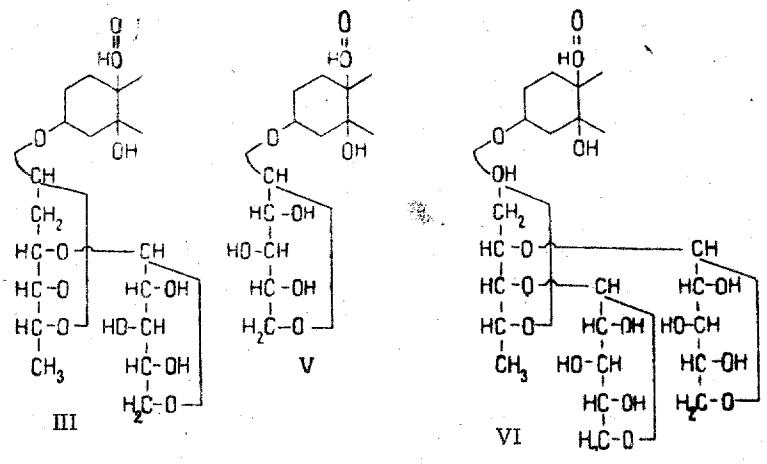
Substances (I) and (II) were identified, respectively, as strophanthidin and erysimin. Substance (IV), $C_{34}H_{50}O_{13}$, was identical in its physicochemical properties, IR spectra, and hydrolysis products with natural erychroside. Substance (V), $C_{28}H_{40}O_{10}$, was a monoglycoside; it gave negative reactions for the presence of a 2-deoxy sugar. Under the action of enzymes, the glycoside was hydrolyzed to form the aglycone strophanthidin and the monosaccharide xylose, which were identified by paper chromatography. Acid hydrolysis of the glycoside took place

under fairly severe conditions, which indicates the pyranose form of the xylose residue. A comparison of the molecular rotations of the glycoside and aglycone in accordance with Klyne's rule [7] showed that the xylose was attached by a β-glycosidic bond.

Substance (III) gave a positive reaction with the Keller-Killiani and Webb-Levy [8] reagents, which shows the presence of a 2-deoxy sugar. This substance evidently consists of a diglycoside, since its polarity is similar to that of erychroside and the action of enzymes decomposed it into D-xylose and the monoglycoside erysimin. The latter was identified by paper chromatography. The specific rotation of the glycoside (+4.0°) gives grounds for assuming that the xylose in it is attached by a β-glycosidic bond, as in erychroside. On acid hydrolysis, the xylose was split off under comparatively severe conditions, which indicates that it is present in the glycoside in the pyranose form. Apparently the xylose in this glycoside is attached at C_3 of the digitoxose residue.

Substance (VI) was formed in very small amount; it possesses a high polarity and gives positive reactions for a 2-deoxy sugar. It is unaffected by the enzymes of the snail Helix pomatia and the fungus Aspergillus oryzae. On acid hydrolysis, the glycoside forms strophanthidin, D-xylose, and D-digitoxose, which were identified by paper chromatography. The xylose is not split off when the glycoside is heated in 0.05 N sulfuric acid at 70° C for 30 min, which shows [9] that it is present in the glycoside in the pyranose form. This cardenolide is probably a triglycoside—a derivative of erysimin in which both secondary hydroxyls are substituted by xylose residues. The glycoside has a considerable negative specific rotation (−35°) which indicates a β-glycosidic linkage of the xylose residues. A molecular model of this substance shows that such a structure is possible.

Quantitative paper chromatography of the products of the synthesis showed that erychroside and substances (III) and (VI) are formed in a ratio of 12:2:1, respectively. This confirms the assumption expressed above that the equatorial hydroxyl of the digitoxose residue is more reactive than the axial hydroxyl; the synthesis forms erychroside predominantly. The synthesis is stereospecific, since it gives β-glycosides exclusively.



Experimental

The substances were analyzed after being dried for 2 hr over P_2O_5 at 80° C under a vacuum of 0.01 mm Hg. To investigate the sugars by paper chromatography, the following systems of solvents were used: butan-1-ol—acetic acid—water (4:1:5), and butan-1-ol—pyridine—water (6:4:3).

Synthesis

A 100-ml reaction flask was charged with 30–40 ml of absolute dioxane, 2 g of freshly-calcined calcium oxide, 0.8 g of erysimin with mp 176°–178° C, and 10 g of silver oxide. Then, at room temperature (20°–25° C) and with vigorous stirring using a magnetic stirrer, 5 g of freshly-prepared crystalline β-bromoacetyl-D-xylose (mp 97°–100° C) was added in 5–7 mg portions over 1.5–2 hr. The reaction mixture was stirred for a further 6 hr. After the end of the reaction, the solution was filtered, the residue was washed with absolute dioxane, and the filtrate was evaporated in vacuum. The residue was dissolved in 30 ml of methanol, and 25 ml of methanol saturated with gaseous ammonia was added to the solution, after which the mixture was left in a closed vessel at room temperature for 17–18 hr. The methanol and the ammonia were driven off in vacuum. The residue was dissolved in 150 ml of a mixture of alcohol and chloroform (1:2) and 10 ml of water. The alcoholic chloroform layer was separated off and was additionally washed free from salts and sugars with water (2 × 5 ml). The wash waters were then treated with 30 ml of a mixture of alcohol and chloroform (1:2). The combined alcoholic chloroform solution was dried with anhydrous sodium sulfate, filtered, and evaporated. As shown by chromatography (Fig. 1), the residue contained a mixture of six cardenolides.

The mixture obtained was chromatographed on 50 g of alumina (activity grade III) in a mixture of chloroform and ethanol, with a gradual increase in the content of ethanol. The substances were eluted in the following order: I, II, V, III, IV, VI.

Substance (I) crystallized from methanol solution: mp 230°–233°C; $[\alpha]_D$ +45.7 ± 3° (chloroform). On paper chromatography, its position was identical with that of strophanthidin. A mixture of substance (I) and strophanthidin melted at 230°–233° C.

Substance (II) crystallized from ethanol: mp 176°–178°C; $[\alpha]_D$ +25.2 ± 2° (methanol). On paper chromatography, it was found at the level of erysimin. A mixture gave no depression of the melting point (176°–178° C).

Substance (III) crystallized from solution in isopropanol: mp 220°–225°C; $[\alpha]_D$ + 4.0 ± 4° (methanol). It dissolved in concentrated sulfuric acid with a gray coloration changing to brown after 5 min. Enzymatic hydrolysis was carried out on the micro scale. For this purpose, 4 mg of the substance was dissolved in 2 ml of water, 5 mg of dry snail enzyme preparation was added, and the solution was left in the thermostat at 37°–40° C for 24 hr. Then 15 ml of hot ethanol was added to the solution to eliminate the enzymes. The precipitate was filtered off and the filtrate was evaporated in vacuum to give an aqueous residue of about 0.5 ml. On cooling, the solution deposited a small amount of crystals with mp 174°–177° C which, on paper chromatography, occupied the same position as erysimin. D-xylose was also found in the aqueous solution by paper chromatography.

Substance (IV) crystallized from solution in isopropanol in the form of small prisms. 219 mg of crystals melting at 243°–246°C was obtained; $[\alpha]_D$ +18.0 ± 2° (methanol).

Found, % C 59.70; H 7.68; mol. wt. 686. Calculatated for $C_{34}H_{50}O_{13}H_2O$, % C 59.63; H 7.65; Mol. wt. 684.79.

With concentrated sulfuric acid the glycoside gave a green coloration changing to orange after 15 min and to yellow after 2 hr. On chromatography on paper, its position was identical with that of erychroside. A mixture with natural erychroside melted at 243°–246° C. With antimony trichloride, substance (IV), like erychroside, gave an orange color (95°C, 2 min). The IR spectra of substance (IV) and erychroside were identical (figure, b). After enzymatic hydrolysis, carried out in a similar manner to that described above, paper chromatography showed the presence of erysimin and D-xylose.

The acid hydrolysis of substance (IV) was carried out in a 0.1 N solution of sulfuric acid at room temperature for 48 hr. The solution was neutralized with barium carbonate, filtered, and evaporated, and the residue was investigated by paper chromatography. The aglycone formed was located at the level of strophanthidin, and the sugar was D-xylose.

Substance (V) crystallized from methanol–ether. It melted at 170°–172° C; $[\alpha]_D$ +12.6 ± 2° (methanol).

Found, %: C 62.48; H 7.66; mol. wt. 532.1. Calculated for $C_{28}H_{40}O_{10}$, %: C 62.67; H 7.51; mol. wt. 536.6.

On enzymatic hydrolysis, the glycoside formed an aglycone and a monosaccharide shown by paper chromatography to be identical with strophanthidin and D-xylose. The glycoside dissolved in concentrated sulfuric acid giving a red coloration which changed to orange after 25 min and to green after 1 hr.

Substance (VI) crystallized from solution in isopropanol; mp 247°–253° C; $[\alpha]_D$ −35.0 ± 5° (methanol). It dissolved in concentrated sulfuric acid with a yellow green coloration, changing to brown after 5 min. The glycoside underwent no change under the action of enzymes. Like erychroside, it readily decomposed in 0.1 N sulfuric acid solution, forming the aglycone strophanthidin. To determine the composition of the sugar component, the glycoside was hydrolyzed by Killiani's method [10] and the hydrolysis products were investigated by paper chromatography. Two monosaccharides were found, one located at the level of D-digitoxose and the other at that of D-xylose.

Summary

The partial synthesis of erychroside from erysimin and D-xylose has been carried out with a yield of 22%. It has been shown that the condensation of erysimin with bromoacetylxylose takes place predominantly at the C_4 equatorial hydroxyl of the digitoxose residue and to a considerably smaller extent at the C_3 axial hydroxyl.

REFERENCES

1. I. F. Makarevich and M. Ya. Tropp, Farmatsevt. zhurnal, **4**, 36, 1960.
2. I. F. Makarevich, M. Ya. Tropp, and D. G. Kolesnikov, DAN SSSR, **136**, 3, 617, 1961.
3. W. Königs and E. Knorr, Ber., **34**, 957, 1901.
4. K. Reyle, K. Meyer and T. Reichstein, Helv. Chim. Acta, **33**, 1541, 1950.
5. V. T. Chernobai, ZhOKh, **34**, 3, 1018, 1964.
6. E. Fischer and M. Bergmann, Ber., **50**, 1047, 1917.
7. W. Klyne, Biochem. J., **47**, N. 4, 1950.
8. J. M. Webb and H. B. Levy, J. Biol. Chem., **213**, 107, 1955.

9. A. Hunger and T. Reichstein, Helv. Chim. Acta., **35**, 1073, 1952.
10. H. Killiani, Arch. d. Pharm., **234**, 438, 1896.

15 March 1966

Khar'kov Chemical and Pharmaceutical Scientific Research Institute