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Abstract

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SYNTHESIS OF K-STROPHANTHIN-β

Among the large group of cardiac glycosides, the glycosides of various species of *Strophanthus* are of great importance for practical medicine. The principal place among them is occupied by the bioside known as K-strophanthin-β, first isolated in pure form by Jacobs ([1]), for which the structure of strophanthidin 4-β-*D*-glucosyl-*D*-cymaroside (II) has been established. In view of the difficulties associated with the isolation of K-strophanthin-β from natural material, which moreover is not always available, the partial synthesis of this practically important glycoside from a more accessible natural raw material is of interest, in particular from the accessible monoside cymarin (*D*-cymaroside of strophanthidin). Preliminary data on this synthesis are presented in the present paper.

In the literature several syntheses of monosides containing cardiac aglycones have been described ([2–7]). By contrast, we have been unable to find mention in the literature of attempts to synthesize biosides, and in particular the most important of them, K-strophanthin-β. Since in cymarin (I) the carbohydrate residue of cymarose contains the only free hydroxyl, the tertiary hydroxyl groups of the aglycone are only slightly reactive in hydrogen-substitution reactions, and the linkage of glucose with cymarose in K-strophanthin-β has the β-configuration, it seemed possible to carry out the synthesis of this bioside by the Koenigs–Knorr method with subsequent removal of the acetyl groups by some sufficiently mild method.

In view of the high lability of the glycosidic bond of cymarin both as the glycoside of a 2-deoxy sugar and of its aglycone moiety, the entire synthesis required very careful selection of the reaction conditions and monitoring of its course. Preliminary experiments on the condensation of tetraacetylglucosyl bromide with cymarin under the usual conditions of the Koenigs–Knorr synthesis, i.e., in the presence of silver carbonate in benzene–dioxane solution, gave negative results or led only to the formation of traces of bioside. Examination of the condensation conditions showed that the failures were apparently associated with the presence of water in the reaction mixture, which is formed as a result of the interaction of HBr with Ag_2CO_3. It therefore became necessary to select an effective method for removing water from the reaction mixture. The use of the usual methods for this purpose—water-removing agents such as anhydrous magnesium sulfate, calcium chloride, aluminum oxide, molecular sieves, or azeotropic distillation—was impossible or ineffective under the conditions of the reaction.

Fig. 1. Apparatus for carrying out the reaction: 1 –metallic sodium; 2 – porous glass plate; 3 –reflux condenser; 4 –nitrogen inlet

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Fig. 2. IR absorption spectra in Vaseline oil. 1 –synthetic K-strophanthin-β; 2,3 –first and second samples of natural K-strophanthin-β. 1710 and 1714 cm^{-1} –stretching vibrations of the C_{19} C=O group; 1744, 1742, and 1759 cm^{-1} –stretching vibrations of the C=O group of the butenolide ring; 1630 and 1624 cm^{-1} –stretching vibrations of the C=C group of the butenolide ring

Figure 2: Fig. 2. IR absorption spectra in Vaseline oil. 1 –synthetic K-strophanthin-β; 2,3 –first and second samples of natural K-strophanthin-β. 1710 and 1714 cm^{-1} –stretching vibrations of the C_{19} C=O group; 1744, 1742, and 1759 cm^{-1} –stretching vibrations of the C=O group of the butenolide ring; 1630 and 1624 cm^{-1} –stretching vibrations of the C=C group of the butenolide ring

Königs-Knorr synthesis, with drying agents ($MgSO_4$, etc.), as well as an attempt at continuous distillation of water in vacuo in a stream of nitrogen, did not lead to the desired result. Use of the known method of azeotropic distillation required considerable dilution of the reaction mixture, which led to an almost complete cessation of the reaction. To eliminate this difficulty, the reaction was carried out by us in a special apparatus (Fig. 1), in which water was continuously removed by distillation with benzene and was completely absorbed by metallic sodium. This made it possible to conduct the process in a fairly concentrated solution at a constant volume of the reaction mixture.

Fig. 1. Apparatus for carrying out the reaction: 1 –metallic sodium; 2 –porous glass plate; 3 –reflux condenser; 4 –nitrogen inlet

Investigation of the reaction mixture by paper chromatography showed that under these conditions a considerable amount of a substance is formed corresponding in R_f to K-strophanthin-β. Along with this, a considerable amount of the starting cymarin remains in the reaction mixture, which persists even when an excess of acetobromoglucose is used and the duration of the reaction is increased. In addition, some new substance is formed which, judging from the paper-chromatography data, has greater polarity.

The reaction product obtained by the method described above, without isolation in pure form, was subjected to deacetylation by the action of $KHCO_3$ in aqueous methanol solution, followed by purification by extraction, chromatography, repeated extraction, and recrystallization.

The pure preparation did not differ from a sample of natural K-strophanthin-β in melting point, color reactions, or biological action.

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It proved to be homogeneous and identical with an authentic natural sample* in three systems. The presence of a glucose residue in the compound was demonstrated–

* The authors consider it a pleasant duty to express their sincere gratitude to Prof. Bauer (Slovak Academy of Sciences) for the sample of pure K-strophanthin-β provided.

underwent hydrolysis, after which glucose was detected chromatographically in the hydrolysate, as well as by the color reaction with anthrone (8). The absence of changes in the aglycone molecule, which might have been expected in view of the lability of strophanthidin, was shown by the positive Legal reaction, indicating preservation of the butenolide ring, and also by the UV spectrum, which has maxima characteristic of the butenolide ring (λ_{max}223 mμ, $\lg \varepsilon = 4.119$) and of the C_{19} aldehyde group (λ_{max}306 mμ, $\lg \varepsilon = 1.715$). In addition, the identity of the preparation obtained with K-strophanthin-β was proved by comparison of the IR spectra (Fig. 2). At our disposal

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Fig. 3. Chromatogram of the reaction mixture after separation. System I. 1–cymarin; 2–$CHCl_3$ extract; 3–$CHCl_3$–alcohol (9:1) extract; 4–$CHCl_3$–alcohol (4:1) extract; 5–$CHCl_3$–alcohol (3:2) extract; 6–K-strophanthin-β. The intensity of spot coloration was assessed by a three-point system (numbers inside the spots).

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we had two samples of natural K-strophanthin-β, obtained from different sources. The IR spectrum of our preparation proved completely identical with the IR spectrum of one of them, and showed a shift of the maximum corresponding to the valence vibrations of the C=O group of the butenolide ring by 15 cm^{-1} toward lower frequencies in comparison with the corresponding maximum of the other sample of K-strophanthidin-β (1744 and 1759 cm^{-1}, respectively).

Fig. 4. Chromatograms of pure synthetic K-strophanthin-β: No. 1–system I; No. 2–system II; No. 3–system III. 1, 3–first and second samples of natural K-strophanthin-β; 2–synthetic preparation.

Experimental Part

All solvents used, except in specially indicated cases, were distilled. Evaporation of solutions in all cases was carried out in vacuo in a stream of nitrogen at a temperature not above 40°.

Paper chromatography. Descending, Schleicher & Schüll paper. System I: *n*-butanol–toluene–water (1:1:1), upper layer. The paper was impregnated with a 25% solution of water in methanol. System II: chloroform–*n*-butanol–ethanol (100:4:1). The paper was impregnated with a 15% solution of formamide in methanol. System III: *n*-butanol saturated with water. Spots were revealed with an aqueous-alcoholic alkaline solution of sodium picrate.

Starting materials. Cymarin: a commercial preparation was twice recrystallized from methanol, dried in vacuo at 100°,

m.p. 147-150°. **Acetobromoglucose:** m.p. 88-89° $[\alpha]_D$ + 205° ($CHCl_3$). **Silver carbonate.** The freshly precipitated salt is thoroughly washed with water and ethanol. It is dried in vacuo over P_2O_5 and immediately introduced into the reaction.

Synthesis of K-strophanthin-β. In a round-bottom flask fitted with an apparatus (Fig. 1) are placed 721 mg (1.3 mM) of cymarin and 6 ml of abs. benzene. The mixture is heated in a stream of dry, oxygen-free nitrogen at a bath temperature of 105 ± 2°. To the mixture are added 4.5 g (16.3 mM) of silver carbonate and a solution of 2.5 g (6.1 mM) of acetobromoglucose in 3.75 ml of abs. dioxane in the following sequence (the times are counted from the beginning of heating): 2 hours–2.0 g Ag_2CO_3; 2 hours 30 min.–3 ml of the acetobromoglucose solution; 2 hours 50 min.–1.5 g Ag_2CO_3 + 0.75 ml of the acetobromoglucose solution; 4 hours 50 min.–1.0 g Ag_2CO_3; 7 hours 10 min.–1.0 g Ag_2CO_3; 8 hours 40 min.–the mixture is cooled in a stream of nitrogen, filtered, the precipitate is washed with dioxane, chloroform, and methanol, and the filtrate is evaporated to dryness. The residue is dissolved in 200 ml of methanol and a solution of 7.0 g of potassium bicarbonate in 150 ml of water is added. The mixture is left at room temperature for 7 days, filtered, and the filtrate is evaporated to one half of its volume. The aqueous solution obtained is extracted successively with chloroform (400 ml), with chloroform-alcohol mixtures 9:1 (400 ml), 4:1 (400 ml), and 3:2 (700 ml) (compare, for example, ([5])). The extracts are evaporated to dryness and dried in vacuo at 60-80°. This gives: from the chloroform extract–a yellow powder, weight 255 mg (recovered cymarin); from the chloroform-alcohol extracts: 9:1–a yellow powder, weight 43 mg; 4:1–a yellow powder, weight 97 mg; 3:2–a yellow powder, weight 230 mg.

The products obtained are examined by paper chromatography (Fig. 3).

85 mg of the chloroform-alcohol (4:1) extract is subjected to partition chromatography on cellulose in the system $CHCl_3$–alcohol/H_2O with gradient elution. Column cross section 5.6 cm^2, height of the adsorbent bed 34 cm. The

cellulose is saturated with water vapor at 25°, and the column is packed by the wet method in a 5% solution of alcohol in chloroform. The volume of solvents in the mixer is 400 ml, the alcohol concentration 5%. A 20% solution of alcohol in chloroform is added. After 250 ml of blank fractions, 150 ml of a solution containing 50 mg of K-strophanthin-β with an insignificant admixture of cymarin is obtained from the column. Fractions containing a more polar component of the mixture are then collected. The K-strophanthin-β thus obtained is dissolved in 25 ml of water, the solution is filtered and extracted successively with $CHCl_3$ 100 ml, $CHCl_3$—alcohol (20:1) 70 ml, and $CHCl_3$—alcohol (2:1) 100 ml. The last extract is evaporated to dryness and dried in vacuo. Snow-white powder, weight 25 mg. The substance is reprecipitated with ether from alcohol and dried at 100° (10^{-5} mm). M.p. 190–196° (decomp.).

The substance is chromatographically homogeneous and identical with authentic samples (Fig. 4). It gives a positive reaction with anthrone, a positive Legal reaction, and a green coloration with H_2SO_4.

Found, %: C 58.80; 58.98; H 7.54; 7.36
$C_{36}H_{54}O_{14} \cdot H_2O$. Calculated, %: C 59.32; H 7.74;

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Note: Figure translations are in progress. See original paper for figures.