PARTIAL SYNTHESIS OF COROTOXYGENIN (3)-β-D-GLUCOSIDE

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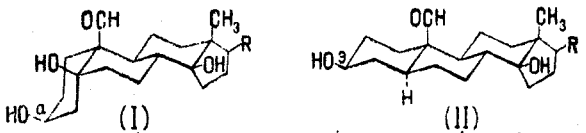
Up to the present time, only the aglycones of the coprostane series (cis-A/B series) have been used in the partial synthesis of the cardiac glycosides [1].



(I) cis-A/B series (II) trans-A/B series

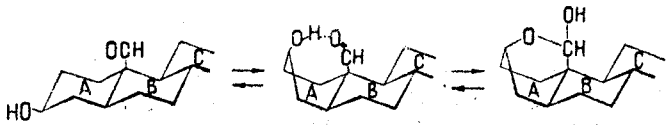
(R – α, β-unsaturated five-membered lactone ring).

In the present communication we consider the synthesis of corotoxygenin (3)-β-D-glucoside, the aglycone of which belongs to the trans-A/B series (II). The conformation of the molecule of corotoxygenin (II) permits the assumption that the equatorial hydroxyl group at C_3 should take part in condensation with acetobromo-D-glucose more actively than the axial hydroxyl group of strophanthidin at C_3 (I). However, contrary results were obtained in an experiment: the synthesis of strophanthidin (3)-β-D-glucoside [1] took place with a yield of not less than 50%, while the yield of corotoxygenin (3)-β-D-glucoside was only 5-6%.

We have carried out an investigation to elucidate the factors hindering the synthesis of corotoxygenin (3)-β-D-glucoside. According to the results of paper chromatography, at the stage of the condensation of corotoxygenin with acetobromo-D-glucose the reaction mixture contained a large amount of the initial aglycone and only a very small amount of the synthesized tetra-O-acetate of corotoxygenin (3)-β-D-glucoside. In contrast to the situation in the synthesis of strophanthidin (3)-β-D-glucoside [1] (cis-A/B series), an increase in the duration of the state of condensation of corotoxygenin with acetobromo-D-glucose had no appreciable influence on the yield of the final product, in spite of the presence of unchanged aglycone in the reaction mixture. The possibility arises that the hydroxyl group of corotoxygenin at C_3 may have lost its reactivity in the process of synthesis. The difficulty in carrying out this synthesis may be due to features of the conformation of the structure of corotoxygenin (II) which, as we have established, substantially affect its reactivity during the partial synthesis of the glucoside and in the production of a series of derivatives (acylation, reduction of the carbonyl group at C_{10}, oximation).

It is known [2] that in cholestane (trans-A/B series), the methyl group at C_{10} and the hydrogen atom at C_3 prevent the conversion of ring A into the boat form; thus, it has the chair form. In cholestanol, however, ring A may also partially assume the boat conformation because of the presence in its molecule of a polar hydroxyl group at C_3.

In contrast to cholestane and cholestanol, corotoxygenin contains two polar functional groups (the hydroxyl group at C_3 and the carbonyl group at C_{10}), because of which the chair-boat equilibrium of ring A (III, IV) may be displaced towards predominance of the boat form (IV) through the formation of an intramolecular hydrogen bond between the hydroxyl group at C_3 and the carbonyl group at C_{10}. The hydrogen bromide arising from the condensation stage is not removed from the reaction medium completely by the silver carbonate (which is present in the solid phase), as a result of which the boat form of ring A (IV) of corotoxygenin is capable of forming a $C_3 \rightarrow C_{19}$ semiacetal (V), which prevents the further synthesis of corotoxygenin (3)-β-D-glucoside.



(III) (IV) (V)

To conform the results obtained, the reactivity of the carbonyl group at C_{10} was investigated. It was found that on reduction with sodium borohydride in dioxane-acetic acid, corotoxygenin (trans-A/B series) remains unchanged. This confirms the assumption that the carbonyl group at C_{10} is converted into the semiacetal form (V) in an acid medium. However, in methanolic solution corotoxygenin is very readily reduced to coroglaucigenin: the addition of sodium borohydride imparts a basic character to the medium, in which corotoxygenin is present in the aldehyde form (III) and is easily reduced.

In dioxane-acetic acid, strophanthidin (cis-A/B series) is reduced to strophanthidol with a yield of 70-80%.

To prove the formation of the semiacetal form (V) in an acid medium, the crystalline methylal of corotoxygenin was obtained. In contrast to the conditions described previously [3], we carried out the reaction in methanol-acetic acid solution, which excluded the possibility of the formation of anhydro products. As was to be expected, the methylal of corotoxygenin was not reduced by sodium borohydride, did not undergo acetylation, and did not react with acetobromo-D-glucose.

Under the same conditions, in accordance with the features of its conformation (cis-A/B series) strophanthidin (I) does not form a methylal.

As is well known [4, 5], the oximation of corotoxygenin takes place with a relatively good yield but with considerably greater difficulty than that of strophanthidin (I). Since oximation is carried out in the presence of such agents as $NH_2OH \cdot HCl$, CH_3COONa, and H_2O, corotoxygenin is obviously present in both the aldehyde (III) and the semiacetal (V) forms. This situation explains the negative effect on the rate of oximation of corotoxygenin as compared with strophanthidin, which is present only in the aldehyde form (I).

The acylation of corotoxygenin generally [5] takes place readily and with high yields, since the process is carried out in a basic medium (pyridine).

On the basis of the investigations carried out, it may be assumed that in anhydrous media of an acidic nature corotoxygenin is present completely or predominantly in the $C_3 \rightarrow C_{19}$ semiacetal form (V); however, in solutions of a basic nature it exists wholly or predominantly in the aldehyde form (III).

Experimental

The substances for analysis were dried in a high vacuum over P_2O_5 at 110°C for 3 hr. The melting points were determined on a Kofler block. The following systems of solvents were used for paper chromatography: 1) benzene-formamide, 2) chloroform-formamide, 3) butan-1-ol-acetic acid-water (4 : 1 : 2), 4) tetrahydrofuran-chloroform-formamide (50 : 50 : 6.5). Absolutely dry solvents were used for the synthesis, and the solid reagents were dried in a vacuum desiccator over P_2O_5.

Condensation of corotoxygenin and acetobromo-D-glucose. A mixture of 0.5 g of corotoxygenin, 3 g of silver carbonate, 1.5 g of calcium oxide, and 150 ml of dichloroethane was heated in an oil bath at 140°; the boiling dichloroethane was distilled off with stirring to a level of 100 ml and this level was maintained in the process of synthesis by the addition of dichloroethane. Then a solution of 1.5 g of acetobromo-D-glucose in 100 ml of dichloroethane was added in small portions over 60 min, after which the reaction mixture was cooled to room temperature, and the precipitate was filtered off and washed with dichloroethane. The filtrate was concentrated in vacuum to give a resinous residue, which was dissolved in 165 ml of methanol; to this solution was added 2 g of potassium bicarbonate in 125 ml of water, and the mixture was left for 12 days.

After the saponification, the methanol was distilled off in vacuum and the aqueous fraction was filtered through a small layer of alumina which was subsequently washed with water. For purification, the filtrate was treated with benzene three times, and then the unchanged corotoxygenin was extracted with chloroform. The chloroform extracts were washed twice with small amounts of water, dried with sodium sulfate and evaporated to dryness in vacuum. The residue was crystallized from acetone-water. This gave 180 mg of pure corotoxygenin with mp 204-208°C and, after crystallization from methanol-ether, 218-224°C.

The aqueous fraction was extracted with a mixture of chloroform and alcohol (2 : 1) until the aqueous phase gave a negative Raymond test. The extract was evaporated in vacuum, the residue was dissolved in 30 ml of methanol, 30 ml of water was added, and the methanol was distilled off. The aqueous fraction (20 ml) was re-extracted five times with a mixture of chloroform and alcohol, the solvent was distilled off in vacuum, and the residue was crystallized from aqueous alcohol. Weight of the crystals, 45 mg, mp 263-265°C $[\alpha]_D^{18}$ + 12.3 ± 2° (c 0.80; methanol).

Found, %: C 62.98; H 7.53. Calculated for $C_{29}H_{42}O_{10}$, %: C 63.25; H 7.67.

On paper chromatography in system 4, the glucoside synthesized and a sample of glucocorotoxygenin [6] had the same R_f value (0.11). A mixture of the two substances gave no depression of the melting point.

Enzymatic hydrolysis of corotoxygenin (3)-β-D-glucoside. A solution of 10 mg of the glucoside in 3 ml of water was mixed with 10 mg of an enzyme preparation from the fungus Aspergillus oryzae, and the solution was acidified with acetic acid to pH 5.5 and left in a thermostat at 45°C for 30 min. Then the hydrolyzate was extracted with chloroform. The extracts were washed with a small amount of water, dried with sodium sulfate and evaporated to dryness. On paper chromatography in system 2, the residue gave only one spot, identical with that of a sample of corotoxygenin.

The aqueous solution and the washing liquid were evaporated in vacuum, the residue was dissolved in a small amount of alcohol, and the solution was chromatographed on paper in system 3, a single spot of D-glucose being obtained.

Corotoxygenin methylal. With heating, 200 mg of corotoxygenin was dissolved in 30 ml of dry methanol, 12 ml of glacial acetic acid was added, and the mixture was left at room temperature. Paper chromatography in system 1 showed that the formation of the methylal was complete at the end of two days. A considerably more rapid reaction takes place if the reaction mixture is heated in a water bath periodically at 40-50°C. Then 50 ml of water was added and the methanol was distilled off in vacuum. The aqueous fraction was extracted three times with chloroform, and the extracts were washed with water, dried with sodium sulfate, and evaporated to dryness in vacuum. The residue was crystallized from dilute methanol. This gave 160 mg of crystals with mp 187-191°C; after recrystallization from methanol-ether, mp 204-206°C. In system 1, R_f 0.78; $[\alpha]_D^{18}$ +30.0 ± 2° (c 1.00; chloroform).

Found, %: C 71.38; H 8.36. Calculated for $C_{24}H_{34}O_5$, %: C 71.61; H 8.51.

As was to be expected, strophanthidin does not form a methylal under analogous conditions.

Reduction of corotoxygenin methylal. A solution of 10 mg of the methylal in 2 ml of methanol was treated with 5 mg of sodium borohydride and the mixture was left in the refrigerator for 2 hr. When the reaction mixture was chromatographed on paper in system 1, it gave only the spot of the unchanged starting material.

Acetylation of corotoxygenin methylal. A solution of 10 mg of the methylal in 0.2 ml of absolute pyridine was treated with 0.2 ml of acetic anhydride, and the mixture was left for a day. Paper chromatography of the reaction mixture in system 1 showed that it contained the unchanged starting material.

Reduction of corotoxygenin in an acid medium. A solution of 100 mg of corotoxygenin in 3 ml of dioxane was treated with 1.2 ml of glacial acetic acid, and the mixture was cooled to 0°C. Then 50 mg of sodium borohydride was added in small portions over 40 min, and the solution was left in the refrigerator for 2 hr. The reaction mixture was treated with 20 ml of water, and the solution was extracted three times with chloroform. The extracts were washed 2-3 times with water, dried with sodium sulfate, and evaporated to dryness in vacuum. When the resulting residue was subjected to paper chromatography in system 2, only the spot of the initial corotoxygenin was found. The residue was dissolved in 10 ml of methanol, 5 ml of water was added, and the methanol was distilled off, whereupon corotoxygenin crystallized with mp 204-208°C. Weight, 90 mg. After recrystallization from methanol-ether, mp 220-224°C.

Reduction of strophanthidin in an acid medium. Strophanthidin (500 mg) was reduced in a similar manner to corotoxygenin. According to the results of paper chromatography in system 2, the dry residue obtained after the evaporation of the chloroform extract contained mainly strophanthidol and a very small amount of the starting material. Crystallization of the residue from acetone-ether gave 400 mg of strophanthidol with mp 218-224°C, $[\alpha]_D^{18}$ +36.0 ± 2° (c 1.52; methanol).

Reduction of corotoxygenin in an alkaline medium. A solution of 50 mg of corotoxygenin in 4 ml of methanol was cooled to 0°C, and 10 mg of sodium borohydride was added (creating an alkaline medium). The mixture was stirred until this had dissolved and was then left in the refrigerator. After 30 min, the reaction mixture was chromatographed on paper in system 2. It was found that the corotoxygenin had been reduced completely to coroglaucigenin. The reaction mixture was acidified with acetic acid to litmus, 15-20 ml of water was added, and the methanol was distilled off in vacuum, when the coroglaucigenin readily crystallized. Weight 43 mg, mp 246-249°C.

The sample of glucocorotoxygenin was provided by Yu. N. Beletskii.

Summary

The partial synthesis of corotoxygenin (3)-β-D-glucoside with a yield of about 6% has been effected. The low yield of the product of the synthesis is due to the conformational features of the corotoxygenin molecule. It is assumed that at the stage of the condensation of the aglycone with acetobromo-D-glucose an acid medium is formed, under the influence of which the semiacetal form of corotoxygenin is produced, which excludes the possibility of the further progress of the synthetic reaction.

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