A PARTIAL SYNTHESIS OF k-STROPHANTHIN-β

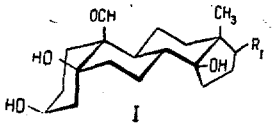
V. T. Chernobai

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 3, pp. 162-168, 1965

Extensive investigations are being carried out in the Soviet Union on the possibility of obtaining the valuable medicinal substance k-strophanthin from plates of the native flora. These investigations were crowned with success when N. K. Abubakirov and R. Sh. Yamotova [1-3], and also N. A. Bugrim and D. G. Kolesnikov [4], isolated k-strophanthin-β from dogbane and adonis. Simultaneously, N. K. Kochetkov, A. Ya. Khorlin, and A. F. Bochkov [5] effected for the first time a partial synthesis of this substance from cymarin (III) and acetobromo-D-glucose (II). The authors mentioned obtained a complex mixture of cardenolides from which k-strophanthin-β was isolated with a yield of 17.3% [6]. A considerable amount of an unknown substance possessing more pronounced polar properties on paper chromatography was present in the reaction mixture.

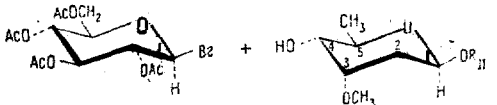
We have also been engaged in an investigation of the partial synthesis of the bioside of k-strophanthin-β, attempting primarily to elucidate the mechanism of this process.

The partial synthesis of cardiac monosides is generally carried out by condensing any aglycone and an acetohalogenose. On considering the conformation of strophanthidin (I) (R_1 represents an α, β-unsaturated five-membered lactone ring) it can be seen that the hydroxyl group at C_3 is relatively free from steric influences from the direction of the other functional groups. It is obviously due to this that the condensation stage, for example in the synthesis of convallatoxin [7], proceeds fairly readily with a yield of 55-60%.



I

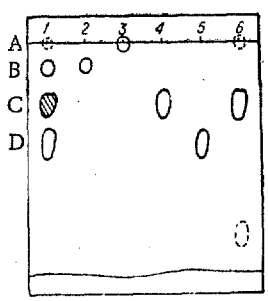
A somewhat different spatial arrangement of the functional groups at the position of the formation of the glycosidic bond is found in the synthesis of k-strophanthidin-β from cymarin (III) and acetobromo-D-glucose (II).



II III (R_{II}=1)

The hydroxyl group at C_4 of the carbohydrate part of the molecule of cymarin (III), in contrast to the preceding case, is probably subject to considerable steric hindrance from two voluminous substituents: the axial methoxy group at C_3 and the methyl group at C_5. Because of this, the approach of the acetobromo-D-glucose (II) to the hydroxyl group at C_4 is obstructed and synthesis under conditions analogous to those of [7] takes place with a relatively low yield (7%). It is possible that in this case the steric factor has a predominating influence on the process of synthesis.

Moreover, it must be borne in mind that cymarin (III), the molecule of which contains a 2-deoxysugar — cymarose is a very labile compound (in an acid medium) and is capable of hydrolyzing even in dilute organic acids.



Paper chromatogram (benzene-formamide system, temperature 18°, 3 hr): 1) reaction mixture obtained by the condensation of cymarin and acetobromo-D-glucose; 2) cymarin; 3) strophanthidin; 4) tetra-O-acetylstrophanthidin-(3)-β-D-glucoside; 5) tetra-O-acetyl-k-strophanthin-β; 6) reaction mixture obtained by the condensation of strophanthidin and acetobromo-D-glucose.

To elucidate the mechanism of the synthesis of k-strophanthin-β, we have isolated and studied all the main products formed in the condensation of cymarin (III) with acetobromo-D-glucose (II). From the results of paper chromatography (Figure, sample 1), the resulting reaction mixture contained four substances of a cardenolide nature: A, B, C, and D. After the separation of these substances on an alumina column, compounds A, B, and C were isolated in the individual crystalline state; it was impossible to crystallize substance D. From their main properties, substance A proved to be identical with strophanthidin, and substance B with cymarin.

On studying substance C, it was found that it contained four acetyl residues. After saponification of the latter with $KHCO_3$, the crystalline des-acetate of substance C was obtained; this did not contain the 2-deoxysugar cymarose (negative Keller-Kiliani reaction). In order to elucidate the chemical nature of the deacetate of substance B, it was hydrolyzed by Mannich's method, after which paper chromatography showed only a single spot of D-glucose. The neutral part contained a series of five cardenolides, one of which was identical with strophanthidin. The deacetate of substance C, the molecule of which contained a D-glucose residue, is a glucoside of the third group in Reichstein's classification [8], those which are difficult to hydrolyze in an acid medium. In order to obtain the aglycone in unchanged form, hydrolysis of the deacetate of substance C was carried out by enzymes from the mold Aspergillus oryzae [9, 10], which made it possible to obtain the aglycone strophanthidin, while paper chromatography showed the presence of D-glucose.

The investigations carried out indicate that the des-acetate of substance C is strophanthidin-(3)-β-D-glucoside, while substance C is its tetraacetyl derivative.

For a final proof of the structure of the des-acetate of substance C, the directed synthesis of strophanthidin-(3)-β-D-glucoside was carried out by the condensation of strophanthidin and acetobromo-D-glucose. After saponification of the reaction mixture, strophanthidin-(3)-β-D-glucoside was isolated with a yield of more than 50%. Its acetyl derivative was also obtained. According to the data of paper chromatography, mixed melting points, and IR spectra, the two compounds were respectively identical with the des-acetate of substance C and substance C isolated in the synthesis of k-strophanthin-β.

Without preliminary crystallization, the fraction containing substance D was deacetylated with $KHCO_3$ to give a crystalline bioside which, from the results of acid and enzymatic hydrolysis and paper chromatography, was identical with k-strophanthin-β.

It has therefore been established that in the condensation of cymarin (III) and acetobromo-D-glucose (II) with subsequent saponification of the reaction products, a mixture of strophanthidin, cymarin, strophanthidin-(3)-β-D-glucoside, and k-strophanthin-β is formed.

On the basis of the investigations carried out, the synthesis of k-strophanthin-β can be explained as follows: the condensation of cymarin (III) and acetobromo-D-glucose (II) takes place incompletely, evidently because of the steric hindrance mentioned previously. The hydrobromic acid liberated in the synthesis cannot be bound rapidly and completely by the silver carbonate present in the solid phase and therefore an acid medium is formed in the reaction mixture in which the cymarin (which has a 2-deoxysugar in its molecule) readily hydrolyzes to D-cymarose and strophanthidin [11]. The hydroxyl group at C_3 of the latter, being freer from steric influences, reacts comparatively readily with the acetobromo-D-glucose continuously entering the reaction medium. This gives rise to tetra-O-acetylstrophanthidin-(3)-β-D-glucoside, which is the main product of this synthesis. Consequently, an interesting process of interglycosidation takes place in which the readily eliminated 2-deoxysugar of cymarose is replaced by the normal sugar D-glucose with the formation of a new glucoside more resistant to acid hydrolysis.

Depending on the conditions and, particularly, the duration of the synthesis, the hydrobromic acid may also lend to the degradation of the aglycone, particularly with the production of its anhydro form [12-14].

Experimental

For analysis, the substances were dried over P_2O_5 in a high vacuum at 110° for 3 hr. The melting points were determined on a Kofler block. The following systems of solvents were used for paper chromatography: I) benzene-formamide; II) chloroform-formamide; III) n-butanol–acetic acid–water (4: 1: 2); IV) benzene–n-butanol (2: 1)-water 35%; V) tetrahydrofuran-chloroform-formamide (50: 50: 6.5). Absolutely dry solvents were used for the synthesis, and the substances were dried in a vacuum desiccator over P_2O_5.

Condensation of cymarin (III) and acetobromo-D-glucose (II). A flask was charged with 1 g of cymarin, 5 g of silver carbonate, 2.5 g of calcium oxide, 50 ml of toluene, and 25 ml of dichloroethane and was immersed in an oil bath heated to 140-150°. The boiling mixture of solvents was distilled off to 50 ml, after which, with continuous stirring, 2.5 g of acetobromo-D-glucose in 60 ml of dichloroethane was added to the reaction mixture in small portions over 25 min. The volume of the solvent was kept at 50 ml by the periodic addition of toluene. After 30 min from the start of the addition of acetobromo-D-glucose, the reaction mixture was cooled, and the precipitate was filtered off and washed with dichloroethane. The filtrate was evaporated in vacuum, and the resinous residue was dissolved in 30 ml of benzene and chromatographed on a column of alumina (h = 20 cm, d = 3.5 cm). The column was eluted with benzene; with a mixture of benzene and chloroform, the concentration of the latter being increased gradually; then with chloroform; and finally with a mixture of chloroform and alcohol. The 50-ml fractions that were collected were evaporated and the residues were analyzed by paper chromatography. Only those fractions containing individual substances of a cardenolide nature were subjected to further investigation:

Chloroform-benzene (1: 1)	– 275 mg of resinous residue of substance D
Chloroform	– 530 mg of resinous residue of substance C
Chloroform containing 3% alcohol	– 650 mg of resinous residue of substance B
Chloroform containing from 5 to 30% of alcohol	– 140 mg of dry residue of substance A.

Substance A (strophanthidin). A solution of 140 mg of substance A from the last fraction in 20 ml of methanol was treated with 30 ml of water, and the mixture was evaporated in vacuum to eliminate the methanol. For purification, the aqueous solution was treated with benzene three times and then the substance A was extracted with chloroform until Raymond's test was negative. The chloroform extracts were combined, washed with water, dried with sodium sulfate, and filtered. After concentration of the filtrate in vacuum, the residue was crystallized from methanol-water. Weight – 60 mg, mp 142-143°/224°, $[\alpha]_D^{18}$ + 42.9 ± 2° (c 1.62; methanol).

A mixture of substance A and strophanthidin showed no depression of the melting point. On paper chromatography in system II, substance A was identical with a sample of strophanthidin, and after spraying with antimony trichloride it gave an identical fluorescence in UV light.

Substance B (cymarin). The 650 mg of the chloroformic-alcoholic (97: 3) residue was dissolved in 50 ml of methanol, and 50 ml of water was added. The methanol was distilled off in vacuum, and the aqueous solution was extracted three times with a mixture of benzene and ether (3: 1) to eliminate the acylated glucose and other byproducts of the synthesis. Then the substance B was extracted with chloroform until Raymond's reaction was negative, and the chloroform extract was washed with distilled water, dried with sodium sulfate, filtered, and evaporated to dryness in vacuum. The residue was crystallized from dilute alcohol. Weight 260 mg, mp 145-147°, $[\alpha]_D^{18}$ + 37.3 ± 2° (c 1.21; methanol).

A mixture of substance B and cymarin gave no depression of the melting point. On paper chromatography in system I, substance B proved to be identical with a sample of cymarin.

Substance C [tetra-O-acetyl-strophanthidin-(3)-β-D-glucoside]. The 530 mg of residue from the chloroform fraction was dissolved in 15 ml of methanol and mixed with 50 ml of water. On standing, the solution deposited crystals. After two recrystallizations from dilute methanol, 285 mg of substance C was obtained with mp 166-167/240-242°, $[\alpha]_D^{19}$ + 11.6 ± 2° (c 1.02; chloroform).

Found %: C 59.80; H 7.09.

Calculated for $C_{37}H_{50}O_{15}$: C 60.48; H 6.86%.

Des-acetate of substance C [strophanthidin-(3)-β-D-glucoside]. A solution of 0.15 g of potassium bicarbonate in 15 ml of water was added to a solution of 150 mg of substance C in 15 ml of methanol, and the mixture was left at room temperature. Paper chromatography in system I showed that the saponification of substance C was complete after 10 days. Then 15 ml of water was added to the solution, the methanol was distilled off in vacuum, the aqueous solution was purified by extraction with three 30-ml portions of chloroform, and the des-acetate of substance C was extracted 6-7 times with a mixture of chloroform and alcohol (4: 1). Evaporation of the chloroformic-alcoholic extract gave 100 mg of an amorphous white powder readily soluble in water and alcohol. The reaction with tetranitromethane and the Keller-Kiliani reaction were negative. On paper chromatography in system IV, this substance gave one spot and possessed more highly polar properties than a sample of k-strophanthin β.

Mannich hydrolysis of the deacetate of substance C. A solution of 20 mg of the substance in 2.5 ml of anhydrous acetone containing 0.025 ml of concentrated hydrochloric acid was left at room temperature for 10 days. Then 3 ml of water was added to the hydrolyzate and the acetone was distilled off in vacuum. The turbid solution was treated with 3 ml of methanol and the mixture was heated for 30 min in a flask with a reflux condenser in the boiling water bath, after which the methanol was distilled off. The aqueous solution was extracted with chloroform until Raymond's test was negative. The residue obtained after the distillation of the chloroform gave several cardenolide spots on paper chromatography in system II, one of which coincided with the spot of strophanthidin.

The acidic aqueous solution was neutralized with freshly-precipitated silver carbonate and the precipitate was filtered off. The filtrate was evaporated in vacuum to a syrupy residue which, on paper chromatography in system III, gave only one spot, identical with that of D-glucose.

Enzymatic hydrolysis of the des-acetate of substance C. A solution of 50 mg of the amorphous substance in 15 ml of water was mixed with 70 mg of a purified enzymatic preparation from the mold Aspergillus oryzae, and the solution was acidified with acetic acid to pH 5.5, treated with two drops of toluene, and left in the thermostat at 38° for a day. Then the hydrolyzate was extracted with chloroform until Raymond's reaction was negative. The chloroformic extracts were washed with water, dried with sodium sulfate, filtered, and evaporated to dryness, and the residue was crystallized from dilute alcohol. On paper chromatography in system II, the crystals gave only one spot, identical with that of a sample of strophanthidin.

The aqueous solution was evaporated in vacuum, the residue was dissolved in a small amount of alcohol, and the solution was chromatographed on paper in system III, giving a single spot identical with that of D-glucose.

Synthesis of strophanthidin-(3)-β-D-glucoside. A flask was charged with 1 g of strophanthidin, 4 g of silver carbonate, 2 g of calcium oxide, 40 ml of toluene, and 25 ml of dichloroethane, and was placed in an oil bath heated to 135-140°. The boiling mixture was stirred for 2-3 min and then a solution of 2.5 g of acetobromo-D-glucose in 60 ml of dichloroethane was added in small portions over 1 hr. The remainder of the process was carried out as in the synthesis of k-strophanthin-β.

The filtered reaction mixture was evaporated in vacuum to give 3.6 g of a resinous residue. This was dissolved in 300 ml of methanol and was treated with a solution of 3.6 g of potassium bicarbonate in 225 ml of water, and the mixture was left at room temperature for 10 days. The completion of deacetylation was determined by paper chromatography in system I. Then the methanol was distilled off and the aqueous solution was extracted:

With chloroform,	3 × 200 ml – 135 mg of residue
With a mixture of chloroform and alcohol (9: 1)	5 × 200 ml – 220 mg of residue
With a mixture of chloroform and alcohol (4: 1)	8 × 200 ml – 880 mg of residue

From the results of paper chromatography in system IV, the chloroformic-alcoholic extracts (4: 1) contained almost pure glucoside. For purification, the 880 mg of residue was dissolved in 20 ml of water, the solution was filtered through a small layer of alumina, which was washed with water, and the eluate was extracted with eight 60-ml portions of a mixture of chloroform and alcohol (4: 1). Evaporation of the chloroformic-alcoholic extracts gave 780 mg of pure amorphous strophanthidin-(3)-β-D-glucoside, which was crystallized from moist isopropyl alcohol-ether in the form of compact nodules with mp 238-244°, $[\alpha]_D^{19}$ + 19.8 ± 2° (c 1.08; water).

Found %: C 60.89; H 7.38.

Calculated for $C_{29}H_{42}O_{11}$: C 61.47; H 7.47%.

Enzymatic hydrolysis of strophanthidin-(3)-β-D-glucoside. 100 mg of the glucoside was hydrolyzed in the same way as the des-acetate of substance C. The chloroform extracts gave crystalline strophanthidin and by paper chromatography in system III the aqueous solution was found to contain D-glucose.

Tetra-O-acetate of strophanthidin-(3)-β-D-glucoside. A solution of 200 mg of strophanthidin-(3)-β-D-glucoside in 3 ml of absolute pyridine was treated with 3 ml of acetic anhydride and left at room temperature for one day. Then the mixture was poured into 8 ml of salt water and left for crystallization. After recrystallization from methanol-ether, a substance was obtained with mp 166-169/241-243°, $[\alpha]_D^{19}$ + 12.0 ± 2° (c 1.02; chloroform).

Found %: C 59.83; H 7.19.

Calculated for $C_{37}H_{50}O_{15}$: C 60.48; H 6.86%.

Substance D [tetra-O-acetate of k-strophanthin-β]. Deacetylation. A solution of 275 mg of the resinous residue obtained from the chloroformic-benzene fraction (1: 1) in 25 ml of methanol was treated with a solution of 0.3 g of potassium bicarbonate in 20 ml of water and the mixture was left for nine days. Then 20 ml of water was added to the solution and the methanol was distilled off in vacuum. For purification, the aqueous solution was extracted with chloroform, and the substance D was extracted with a mixture of chloroform and alcohol (2: 1). After concentration of the chloroformic-alcoholic extracts, the residue was dissolved in 30 ml of water and was again extracted, with six 25-ml portions of a mixture of chloroform and alcohol (6: 1). The extracts were evaporated in vacuum to give a dry white residue (90 mg) of the des-acetate of substance D giving, on paper chromatography in systems IV and V, a single spot identical with that of a sample of k-strophanthin-β. The aqueous acetone yielded 45 mg of crystals with mp 192-195°.

Hydrolysis of the des-acetate of substance D (k-strophanthin-β). A solution of 20 mg of the des-acetate of substance D in 2 ml of methanol was treated with 2 ml of 0.1 N sulfuric acid, and the mixture was heated for 30 min in a flask with a reflux condenser in a boiling water bath. Then 2 ml of water was added to the solution and the methanol was distilled off in vacuum. The aqueous solution was extracted with chloroform until Raymond's reaction was negative. The chloroformic extracts were washed with water, dried with sodium sulfate, filtered, and evaporated to dryness in vacuum. On paper chromatography in system II, the residue gave a single spot identical with that of strophanthidin.

The aqueous solution was neutralized with barium carbonate, and the precipitate was filtered off and washed with water. The filtrate was evaporated in vacuum to give a syrupy residue, which was dissolved in a small amount of alcohol. On paper chromatography in system III, the alcoholic solution gave a spot corresponding to that of strophanthobiose.

Enzymatic hydrolysis of the des-acetate of substance D. 20 mg of the substance was hydrolyzed in a similar manner to the des-acetate of substance C. The residue obtained from the chloroformic extracts gave, on paper chromatography in systems I and II, a single spot identical with that of a sample of cymarin, and the residue obtained after the

evaporation of the aqueous solution gave, in system III, a spot identical with that of a sample of D-glucose.

Summary

1. The condensation of cymarin (III) and acetobromo-D-glucose (II) and subsequent saponification gives a mixture containing strophanthidin, k-strophanthin-β, strophanthidin-(3)-β-D-glucoside, and the initial cymarin.

2. The main product of the synthesis is strophanthidin-(3)-β-D-glucoside formed as a result of the transglycosidation of the cymaroside.

3. The directed synthesis of strophanthidin-(3)-β-D-glucoside from strophanthidin and acetobromo-D-glucose has been carried out with a yield of 50%.

REFERENCES

1. N. K. Abubakirov and R. Sh. Yamatova, Med. prom., SSSR, no. 1, 15, 1960.
2. N. K. Abubakirov and R. Sh. Yamatova, ZhOKh, 31, 2424, 1961.
3. N. K. Abubakirov and R. Sh. Yamatova, ZhOKh, 30, 2082, 1960.
4. D. K. Kolesnikov and N. A. Burgim, Med. prom., SSSR, no. 7, 27, 1960.
5. N. K. Kochetkov, A. Ya. Khorlin, and A. F. Bochkov, DAN SSSR, 136, 613, 1961.
6. A. Ya. Khorlin and A. F. Bochkov, Author's Certificate No. 786080/31-16, 1962.
7. V. T. Chernobai, ZhOKh, 34, 1018, 1964.
8. T. Reichstein, Angew. Chem., 63, 412, 1951.
9. P. I. Gvozdyak and D. G. Kolesnikov, Ukr. farm. zh., no. 4, 70, 1963.
10. P. I. Gvozdyak and D. G. Kolesnikov, DAN UkrSSR, no. 3, 352, 1964.
11. H. P. Sigg, Ch. Tamm, and T. Reichstein, Helv. Chim. Acta, 38, 166, 1955.
12. K. Reyle, K. Meyer, and T. Reichstein, Helv. Chim. Acta, 33, 1541, 1950.
13. K. Reyle and T. Reichstein, Helv. Chim. Acta, 35, 195, 1952.
14. Ch. Tamm and J. P. Rosselet, Helv. Chim. Acta, 36, 1309, 1953.

18 February 1965

Kharkov Scientific Research Chemical-Pharmaceutical Institute