CARDIAC GLYCOSIDES OF DIGITALIS CILIATA TRAUTV.

II. Acetyldigitoxin

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Khimiya Prirodnykh Soedinenii, Vol. 1, No. 6, pp. 379-382, 1965.

As was shown in a previous communication [1], the paper-chromatographic investigation of the leave of Digitalis ciliata (ciliated foxglove) reveals 25 substances of the cardenolide series, including acetyldigitoxin.

Acetyldigitoxin, or deglucolanatoside A, like other deglucolanatosides, has two isomeric, α and β, forms differing only in the position of the acetyl groups. In acetyldigitoxin-α the acetyl group is attached to the terminal molecule of digitoxose at C_3, and in acetyldigitoxin-β at C_4 [2-9].

In 1961 a report [10] appeared of a third type of isomerism of the secondary acetyl glycosides of the foxglove--acetyldigitoxin-γ. In contrast to the glycosides obtained previously, the acetyl group in these compounds is located on the first molecule of the digitoxose; as in the case of the α-isomer, it is attached at C_3.

Acetyldigitoxin was first obtained by Stoll and Kreis [2] from the leaves of D. lanata by the enzymatic cleavage of lanatoside A. Then Stoll and Renz [11] isolated acetyldigitoxin-β from fresh leaves of D. ferruginea as a genuine glycoside of this plant. Subsequently, another α isomer was isolated from this plant [12]. The presence of acetyldigitoxin-α in the leaves of D. grandiflora, D. lutea, D. sibirica, and D. amandiana has also been established [13-15].

In 1954, D. G. Kolesnikov [16] obtained acetyldigitoxin-β from the leaves of D. purpurea, and he first showed that the purple foxglove contains acetyl derivatives of glycosides not previously considered characteristic for glycosides of this type.

Taking the considerable therapeutic value of acetyldigitoxin into account, we have made an attempt to isolate it from the ciliated foxglove.

In a paper-chromatographic investigation of the leaves of D. ciliata, a fairly large spot of acetyldigitoxin appeared on the chromatogram. At the same time, the paper-chromatographic analysis showed the presence of a comparatively small amount of digitoxin in the leaves. However, we have isolated a considerable amount of this glycoside from the ciliated foxglove. In our opinion, this can be explained by the fact that when the raw material used for obtaining digitoxin is treated, the acetyl group splits off from the acetyldigitoxin and the corresponding deacetylglycoside, digitoxin, is produced. The deacetylation of acetyldigitoxin while preparing digitoxin [1] can take place during the washing of an ethereal extract with a solution of sodium carbonate or during the precipitation of srabilizing substances from an aqueous alcoholic solution with a solution of lead acetate in the presence of ammonium hydroxide.

In order to isolate acetyldigitoxin, the alcohol was distilled off from an aqueous alcoholic extract of the seeds of D. ciliata and the remaining extract was treated with ethyl ether, into which the acetyldigitoxin passed together with other secondary glycosides. The acetyldigitoxin, which proved to be the α-isomer, was isolated from the total glycosides obtained from the ethereal extract by adsorption chromatography on alumina.

Whether the acetyldigitoxin-α is present in the ciliated foxglove in the natural state or is obtained only after the enzymatic cleavage of lanatoside A can be shown only after an investigation of the freshly gathered leaves.

The chemical study of Digitalis ciliata is proceeding.

Experimental

Isolation of the total glycosides. 10 kg of the comminuted air-dried leaves of D. ciliata was extracted with 80% methanol. The extract was concentrated until the alcohol had been completely eliminated. The aqueous liquid was treated successively with ethyl, chloroform, mixtures of chloroform and alcohol (4 : 1, 2 : 1), and butan-1-ol.

Treatment of the ethereal extract. The ethereal extract was concentrated and the residue was dissolved in a small amount of chloroform. A yellow crystalling powder giving a reaction for flavonoids was isolated*. The powder was separated from the liquid. The chloroform solution was poured into petroleum ether. The precipitate which was deposited was dissolved in ethanol, the solution was diluted with water, and the accompanying substances were eliminated by reprecipitation with lead hydroxide and washing to neutrality. The purified aqueous alcoholic liquid was concentrated and the residue was treated with chloroform. Removal of the solvent from the chloroform extract gave the total glycosides in

*The results of an investigation of this compound will be reported separately.

the form of a yellow mass weighing 27.7 g.

It was shown by paper chromatography in system 1 according to Kaiser [17] [methylethylketone and xylene (1 : 1 or 1 : 2), saturated with formamide] that the total glycosides contained nine substances of the cardenolide series. One substance with R_f 0.82 appears in the form of a large spot at the level of a standard sample of acetyldigitoxin.

Isolation of acetyldigitoxin. 10 g of the total glycosides was dissolved in 150 ml of chloroform and passed through a column containing 300 g of alumina. Elution was carried out first with pure chloroform and then with a mixture of chloroform and alcohol with the concentration of alcohol being increased gradually to 10%.

Fractions 45-69, eluted by chloroform containing 1% of alcohol, gave, after two recrystallizations from aqueous methanol, 0.79 g of a white crystalline powder. On parallel chromatography with authentic acetyldigitoxin-α, the substance isolated had the same mobility (R_f 0.83). After the chromatogram was sprayed with the Svendsen-Jensen reagent [18] and then heated to 120°C, a golden yellow fluorescence characteristic for derivatives of digitoxin was shown in UV light. The Legal, Kedde, Raymond, and Keller-Kiliani tests were positive.

The presence of an acetyl group in the molecule of the glycoside was established by Frèrejacque's method [19]: 0.5 mg of the substance under investigation was dissolved in three drops of pyridine, and the liquid was transferred to a sheet of paper for chromatography, dried at 60°C, and sprayed with reagent A (2 g of $NH_2OH \cdot HCl$, 25 ml of water, and 25 ml of ethanol). The sheet of paper was dried again, treated with a normal solution of caustic potash, and after 10 minutes sprayed with solution B (0.5 g of $FeCl_3$, 25 ml of water, and 6 g of CCl_3COOH). A purple spot appeared on the paper, which showed the presence of an acetyl group in the substance under investigation. To determine the acetyl group, we used a different composition of the reagents given, as proposed by Zaffaroni and used for the qualitative and quantitative determination of acetyl groups in cardiac glycosides [20, 21]. The reaction was also positive in this case. 1 g of the substance that we isolated dissolved in 18 ml of methanol, 65 ml of acetone, 1 *l* of ethyl acetate, and 20 *l* of water. Acetyldigitoxin-α has approximately the same solubilities, which sharply distinguish it from the β-isomer [7].

The substance obtained crystallized from aqueous methanol in the form of rectangular plates. Coarser crystals of the same shape were formed from a mixture of chloroform and ether.

To determine its optical activity and elementary composition, the substance was dried at 80°C under high vacuum; the values obtained were $[\alpha]_D^{20}$ + 25.2° (c 0.405; methanol), $[\alpha]_D^{20}$ + 5.2° (c 1.3205: pyridine). Literature data for the α-isomer: +4.8°, for the β-isomer: +16.2° [7].

Found, %: C 62.53; 62.30; H 8.36, 8.49; equiv. 420.83 (by alkalimetry). Calculated for $C_{43}H_{66}O_{14} \cdot H_2O$, %: C 62. 60; H 8.32; equiv. 412.49.

Hydrolysis of α-acetyldigitoxin to digitoxin. A solution of 0.35 g of the substance in 15 ml of methanol was cooled to 0°C, and an equal volume of previously cooled 0.2 N caustic potash solution was added. The liquid was neutralized with 0.1 N hydrochloric acid. The hydrolysis product was recrystallized first from aqueous methanol and then from a mixture of chloroform and ether. The digitoxin obtained melted at 246°C, $[\alpha]_D^{20}$ + 17.6° (c 1.25; chloroform + 1% alcohol).

The biological activity of 1 g of glycoside, as determined by the Biological Investigations Division of the Institute of Pharmaceutical Chemistry AS Georgian SSR was 8000 frog units.

Summary

Acetyldigitoxin-α has been isolated from the leaves of Digitalis ciliata Trautv.

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7 July 1965

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