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DETERMINATION OF THE POSITION OF THE GLUCOSE BOND IN SOME CARDIAC GLYCOSIDES

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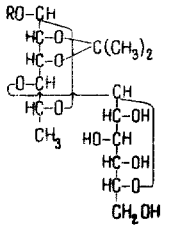
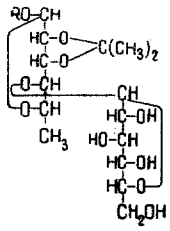
In some cardiac oligosides known at the present time, the monosaccharide residues are attached to one another successively in a chain. In determining the position of attachment of each monosaccharide to the preceding one, the same methods are used as for the oligosaccharides: methylation and oxidation with periodic acid. In this paper we show the possibility of determining the position of the glucose bond in individual diglycosides by the formation of isopropylidene derivatives (ISPD).

Isopropylidene derivatives, the products of the condensation of polyhydric alcohols with acetone, are formed when two neighboring hydroxyl groups in the cis position are present. In the free monosaccharides, it is true, a rearrangement may take place even when a cis-α-glycol group is absent, especially through the conversion of the pyranose form into the furanose form, with the formation of ISPD [1]. However, when the reaction is carried out under mild conditions with sugars connected in the form of glycosides no such rearrangements are observed. We have also taken into account the fact that ISPD may sometimes be formed in oligosides by the hydroxyls of adjacent monosaccharide residues and in glucose, under severe conditions, by the hydroxyls attached to C_4 and C_6.

We have investigated cardiac glycosides of two groups—derivatives of D-gulomethylose (erychordin [2]) and of L-rhamnose (convalloside [3]): convallatoxoloside [4], and hellebrin [5, 6], in which the site of attachment of glucose has not yet been elucidated. The sugar components of these glycosides, as shown previously, are present in the pyranose forms.





I. R-Cannogenol.
Isopropylideneerychordin

II. R-Strophanthidin.
Isopropylideneconvalloside

III. R-Strophanthidol.
Isopropylideneconvallatoxoloside

IV. R-Hellebrigenin.
Isopropylidenehellebrin

D-Gulomethylose, L-rhamnose, and their monoglycosides have a cis-α-glycol grouping at the second and third carbon atoms. It was therefore necessary to determine whether these groupings are free in the diglycosides studied. We first selected conditions under which monorhamnosides and monogulomethylosides form ISPD while monoglucopyranosides and monoxylopyranosides do not form such derivatives.

Glycosides of erychordin, convalloside, convallatoxoloside, and hellebrin readily form the ISPDs **I-IV** when their acetone solutions were kept over anhydrous copper sulfate. Isopropylideneerychordin **(I)** was obtained in the crystalline state. It has the composition $C_{38}H_{58}O_{14}$. The other substances, **II-IV**, because of the limited amount of starting material, were obtained in small quantities in the amorphous state. The formation of the ISPDs was checked by paper chromatography and also by the capacity of the reaction products for being converted into the initial glycosides on acid hydrolysis under mild conditions.

For an additional proof of the fact that the glucose residues do not take part in condensation with acetone, the isopropylidenediglycosides **I-IV** obtained were hydrolyzed with the enzymes of the grape snail Helix pomatia. They all hydrolyzed with the formation of free glucose and isopropylidenemonoglycosides of low polarity. The latter, in their turn, were converted by mild acid hydrolysis into monoglycosides: desglucoerychordin, convallatoxin, convallatoxol, and desglucohellebrin. The substances were identified by paper chromatography.

The capacity of the diglycosides studied for giving isopropylidene derivatives shows that in the D-gulomethylose and L-rhamnose residues, the cis-α-glycol groupings are free. On this basis it may be concluded unambiguously that in erychordin, convalloside, convallotoxoloside, and hellebrin the glucose is attached to C_4 of the D-gulomethylose and L-rhamnose.

Experimental

For the synthesis of the ISPDs, the glycosides were dried in vacuum (10^{-2} mm Hg) at 100° C for 2 hr. Paper chromatography was carried out in the following systems of solvents: tetrahydrofuran—chloroform (1 : 1)/formamide and methyl ethyl ketone—m-xylene (1 : 1)/formamide.

Isopropylideneerychordin. (I). A reaction flask was charged with 0.4 g of erychordin ground to a fine powder, and 5 ml of acetone and 2 g of anhydrous copper sulfate were added. The mixture was left in a thermostat at 40° C for 18 hr. To eliminate the salt and the residues of erychordin that had not reacted, the solution was filtered through a column of 4 g of alumina (activity grade III). The adsorbent was eluted with 100 ml of chloroform—ethanol (85 : 15). The combined filtrate was evaporated in vacuum. The residue was dissolved in 2 ml of acetone and was precipitated by the addition of 20 ml of benzene. This operation was repeated three times. Then the substance was dissolved in 15 ml of acetone and the solution was treated with 10 ml of benzene, concentrated on the hot water bath to about 12 ml and left at room temperature. Pure isopropylideneerychordin (I) crystallized out. Substance I melted at 180–184° C; $[\alpha]_D^{21} -33.3 \pm 3°$ (c 0.91; methanol); it was soluble in conc H_2SO_4 giving a coloration changing with time: 0 min) brown; 1 min) yellow; 170 min) orange; 190 min) red.

Found, %: C 61.92; H 8.01; mol. wt. 743. Calculated for $C_{38}H_{58}O_{14}$, %: C 61.77; H 7.91; mol. wt. 738.9.

The ISPD of convallatoxoloside (III) was obtained similarly. So far as concerns the derivatives of convalloside (II) and of hellebrin (IV), the conditions of synthesis given were unsuitable for them, since the prolonged standing of these glycosides in acetone over $CuSO_4$ leads to a considerable degree of oxidation of the aldehyde group by atmospheric oxygen. Consequently substances II and IV were synthesized by heating acetone solutions of the glycosides over $CuSO_4$ in sealed glass tubes at 80° C for 5 min. Then the solutions were filtered through a small layer of alumina and the filtrates were treated as described above.

For the selective splitting off of the isopropylidene groups, the compounds obtained were heated in 0.05 N HCl in 50% ethanol (100° C, 10 min). The enzymatic hydrolysis was carried out by the usual method (see, for example [7]).

Conclusions

1. The possibility of determining the position of attachment of glucose in some diglycosides by their capacity for forming isopropylidene derivatives has been shown.

2. It has been established that in the cardiac glycosides erychordin, convalloside, convallatoxoloside, and hellebrin, the glucose is attached to C_4 of the D-gulomethylose and L-rhamnose.

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