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CONFORMATIONS OF THE SUGARS FOUND IN CARDIAC GLYCOSIDES

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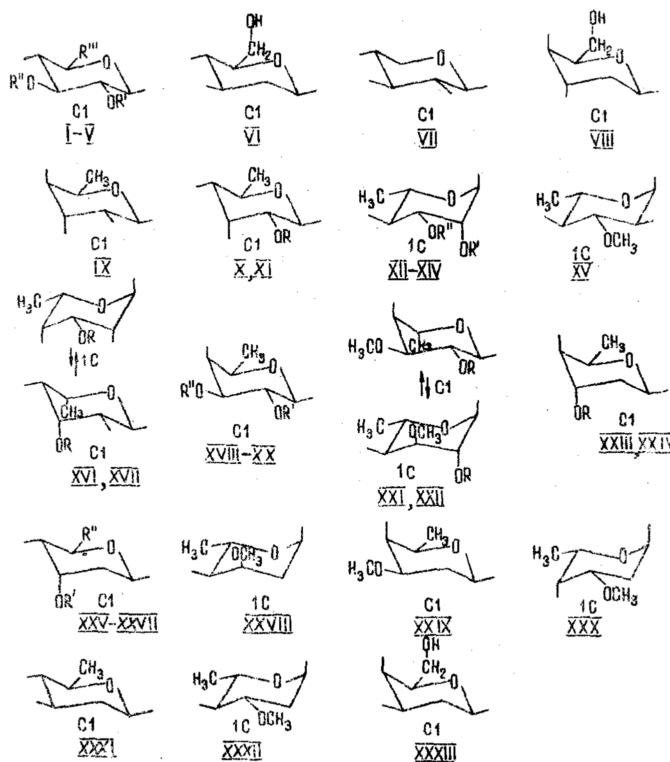
At present, 33 monosaccharides—I–XXXIII—are known which have been detected (mainly by T. Reichstein and his co-workers) in cardiac glycosides. The majority of them are "specific," i.e., found so far only in this particular group of natural compounds. Krasso and Weiss [1], Klyne [2], Gvozdyak and Kolesnikov [3, 4], Tschesche et al. [5], and Chernobai [6] give the conformations of individual monosaccharides. Unfortunately, some workers [2–5] have erroneous opinions relating above all to the sugars of the L-series.

In a preceding paper [7], we showed that equatorial hydroxyls are acetylated 3–5 times faster than axial hydroxyls. On the basis of these differences in the rate of the reaction a method was proposed for determining the conformations of OH groups in steroid compounds. Subsequent investigations have made it possible to use this method to determine the conformations of OH groups in carbohydrates. Although the rate of acetylation of hydroxyls of the same conformations in sugars and steroids are different, in the sugars, just as in the steroids, e-hydroxyls are acetylated three and more times faster than axial hydroxyls. This situation exists only when voluminous substituents are absent from the cis position with respect to the OH group undergoing acetylation. For example, the presence of a methoxyl group in D-cymarose with a cis position with respect to the e-hydroxyl at C_4 considerably slows down the acetylation of the latter.

The conformations of I–XXXIII were constructed mainly according to Collie and Tollens' formulas with account taken of Reeves' instability factors [8]. The calculated figures show that the sugars of the L-series are mainly in the 1-C form and the sugars of the D-series in the C_1 form. Experimental investigations were then carried out, although on only some of the monosaccharides, the results being in good agreement with the conformations shown.



In the conformational formulas, apart from the ring bonds, only those bonds going to hydroxyl and methyl groups and the six carbon atoms are shown:

I) R' = R" = H; R"' = CH_2OH. β-D-Glucose; II) R' = H; R" = CH_3; R"' = CH_2OH. 3-O-Methyl-β-D-glucose. III) R' = R" = CH_3; R"' = CH_2OH. 2,3-Di-O-methyl-β-D-glucose. IV) R' = R" = R"' = CH_3. 2,3,-Di-O-methyl-6-deoxy-β-D-glucose. V) R' = H; R" = R"' = CH_3. β-D-Thevetose (-quinovose). VI) 2-Deoxy-β-D-glucose. VII) β-D-Xylose. VIII) 2-Deoxy-β-D-gulose. IX) β-D-Gulomethylose. X) R = H. β-D-Allomethylose. XI) R = CH_3. 2-O-Methyl-β-D-allomethylose. XII) R' = R" = H. α-L-Rhamnose. XIII) R' = H; R" = CH_3. α-L-Acofriose. XIV) R' = CH_3CO; R" = CH_3. 2-O-Acetyl-α-L-acofriose. XV) α-L-Thevetose. XVI) R = H. α-L-Talomethylose. XVII) R = CH_3. α-L-Acovenose. XVIII) R' = R" = H. β-D-Fucose. XIX) R' = R" = CH_3. 2,3-Di-O-methyl-β-D-fucose. XX) R' = H; R" = CH_3. β-D-Digitalose. XXI) R = H. α-L-Vallorose. XXII) R = CH_3CO. 2-O-Acetyl-α-L-vallorose. XXIII) R = H. β-D-Bovinose. XXIV) R = CH_3. β-D-Sarmentose. XXV) R' = H; R" = CH_3. β-D-Digitoxose. XXVI) R' = R" = CH_3. β-D-Cymarose. XXVII) R' = H; R" = COOH. β-D-Digicuronic acid. XXVIII) α-L-Cymarose. XXIX) β-D-Diginose. XXX) α-L-Diginose. XXXI) β-D-Canarose. XXXII) α-L-Oleandrose. XXXIII) 2-Deoxy-β-galactose.

β-D-Gulomethylose (IX), 2-deoxy-β-D-gulose (VIII), α-L-rhamnose (XII), α-L-acofriose, (XIII), and 2-O-acetyl-α-L-acofriose (XIV). D-Gulomethylose (IX) and L-rhamnose (XII) are an extremely suitable pair for comparative investigation. It follows from calculated figures that the conformations of their hydroxyls at C_4 are opposite. If the conformations of the OH groups at C_4 have been shown, it is easy to determine the spatial position of the remaining ones, bearing in mind the fact that in the chair-shaped forms with the substituents on neighboring carbon atoms in the cis position one of them is axial and the other is equatorial; in the trans position they are both axial or both equatorial. We may assume that the hydroxyl at C_4 of D-gulomethylose is axial. Then, as follows from the Collie-Tollens formula, the hydroxyl at C_3 will also be axial (occupying the trans position with respect to the OH group at C_4). The hydroxyl at C_2 is in the cis position with respect to the OH group at C_3 and, consequently, it will be equatorial. Continuing these considerations, it is easy to see that in β-D-gulomethylose (chair form) the semiacetal hydroxyl is also equatorial.

In order to determine the conformation of the hydroxyls at C_4 from the rate of their acetylation, it was necessary to protect the OH groups at C_2 and C_3 in the corresponding glycosides of L-rhamnose and D-gulomethylose. The situation is facilitated by the fact that these hydroxyls form a cis-α-glycol grouping and are capable under suitable conditions of interacting with acetone to form isopropylidene derivatives.

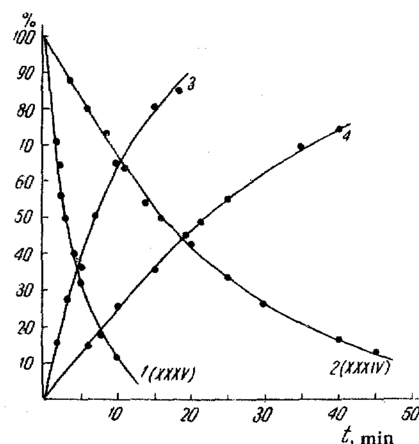
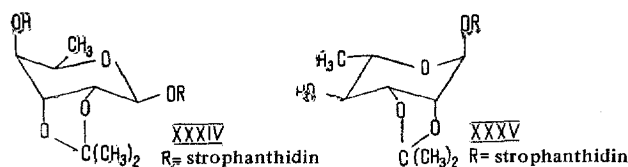


Fig. 1. Kinetic curves of the change in the amount of convallatoxin isopropylidene derivative (XXXV) (1), desglucocheirotoxin isopropylidene derivative (XXXIV) (2), bovoside A diacetate (3) and corchoroside A diacetate (4) in the acetylation reaction.

From convallatoxin (strophanthidin α-L-rhamnopyranoside) and desglucocheirotoxin (strophanthidin β-D-gulomethylopyranoside) we synthesized the isopropylidene derivatives XXXIVaand XXXV. The five-membered ring so formed was not apparently coplanar and did not appreciably change the degree of the chair form of the initial six-membered ring with which it was condensed. Definite indications of this are the comparatively small changes in the specific rotations of the isopropylidene derivatives as compared with the initial glycosides and the marked difference in the conformations of the hydroxyls at C_4 in the compounds obtained XXXIV and XXXV. It is not without interest that the deviation of the specific rotation for compound XXXIV as compared with desglucocheirotoxin (a derivative of a monosaccharide of the D-series) is negative, −8.1°, while for compound XXXV, in contrast to convallatoxin, it is positive and of almost the same value, +7.6° (derivative of a monosaccharide of the L-series).



The course of the acetylation reaction was analyzed by quantitative paper chromatography as described previously [7, 9]. The kinetic curves 1 and 2 (Fig. 1), constructed from the measured change in the amount of starting materials, show that the period of half-conversion of the desglucocheirotoxin isopropylidene compound XXXIV is 16 min and for the convallatoxin isopropylidene compound XXXV it is 3 min, i.e., substance XXXV acetylates 5.3 times faster than XXXIV. This difference in the rates of reaction undoubtedly shows different conformations of the OH group. Consequently, ly, the hydroxyl at C_4 in the L-rhamnose residue is equatorial and in the D-gulomethylose residue it is axial. If it is assumed that the L-rhamnose and D-gulomethylose are present in the chair form, the conformations of the other hydroxyls are consequently (for the course of the determination, see above) as shown by formulas IX and XII.

To confirm the structure XII of L-rhamnose, the rate of the acetylation of the semiacetal hydroxyl was determined. A comparison of the rates of the reactions was carried out with D-glucose (D-gulomethylose could not be used because of the inadequate amount available to us). In view of the fact that the semiacetal hydroxyls differ in their specific reactivity, in order to slow down the reaction acetylation was carried out at 0° C. It proved to be possible to determine the rates of acetylation of the OH groups at C_1 by interrupting the reaction after definite intervals of time and measuring the reducing power of the reaction products or the intensity of the coloration with a suitable reagent. As such a reagent we used aniline hydrogen phthalate [10]. Since the monosaccharides form colors of different depths and shades with it, calibration curves were constructed for each sugar separately.

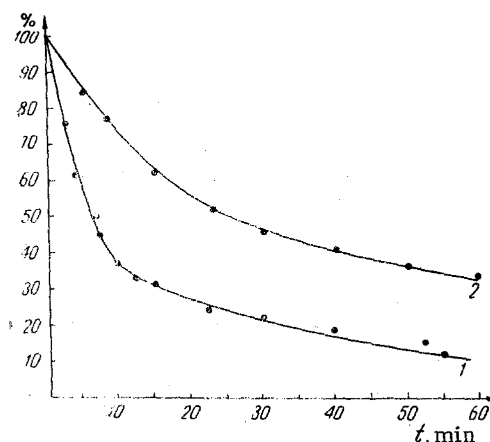


Fig. 2. Kinetic curves of the acetylation (at 0° C) of the semiacetal hydroxyls in D-glucose (1) and L-rhamnose (2).

Account was taken of the fact that in solution an equilibrium exists between the α- and β-forms with a predominance of that anomer which is conformationally most stable. Thus, it is known that in a solution of D-glucose after the establishment of equilibrium there is about 65% of the β-form with an equatorial semiacetal hydroxyl and about 35% of the α-form with this hydroxyl in the axial position. Consequently, before acetylation the monosaccharides were kept for a definite time in water and pyridine solutions. The shape of the kinetic curves of the acetylation of the D-glucose clearly confirms this assumption (see Fig. 2, curve 1). In 10 min, 63% of D-glucose reacts and then the reaction slows down markedly. This shows that the β-form of D-glucose does in fact predominate; its acetylation takes place rapidly. Subsequently the α-form with the axial semiacetal hydroxyl acetylates, but more slowly. The time of half-conversion of the D-glucose (at the semiacetal hydroxyl) is 7 min. L-Rhamnose acetylates slowly and relatively uniformly (see Fig. 2, curve 2). Its half-conversion period is 25 min, i.e., the semiacetal hydroxyl of L-rhamnose acetylates 3.6 times more slowly than that in glucose in the β-form. This shows that for L-rhamnose that form is stable which contains axial semiacetal hydroxyls.

Thus, the experimental results unambiguously show for L-rhamnose the stability of the 1-C form represented by formula XII. D-Gulomethylose and the 2-deoxy-β-D-gulose related to it, exist in the C-1 form with the conformational formulas IX and VIII, L-Acofriose (XIII) and 2-O-acetyl-L-acofriose (XIV), which are derivatives of L-rhamnose, obviously have conformations similar to it.

α-L-Thevetose (XV). The investigation was carried out with the cardiac glycoside bovoside A, which is bovogenin α-L-thevetosopyranoside. The rate of formation of bovoside A diacetate (see Fig. 1, curve 3) shows that both acetylatable hydroxyls at C_2 and C_4 in the L-thevetose residue are equatorial. α-L-Thevetosides are distinguished by a number of features: in the first place, their acid hydrolysis is hindered, and in the second place of all the monosaccharides I–XXXIII shown, only α-L-thevetose has the cis position of the hydroxyl at C_2 with respect to the semiacetal hydroxyls or the glycosidic oxygen atom. The difficulty of the hydrolysis of thevetosides is apparently due to the fact that the hydroxyl at C_2 considerably opposes the approach of the hydroxonium ion (H_3O^+) to the glycosidic bond. If this idea is correct, then, conversely, the observed ease of hydrolysis of glycosides containing a 2-deoxysugar can be explained by the spatial accessibility of the glycosidic center on reaction with the hydroxonium ion.

β-D-Bovinose (XXIII) and β-D-sarmentose (XXIV). Corchoroside A (strophanthidin β-D-bovinosopyranoside) contains two OH groups capable of acetylation in the sugar moiety, at C_3 and C_4. The half-conversion period of the glycoside (see Fig. 1, curve 4) is 22 min, i.e., its acetylation takes place 3.1 times more slowly than that of bovoside A and 7.3 times more slowly than that of substance XXXV. This shows that both free hydroxyls in the D-bovinose residue are axial. β-D-Sarmentose is 3-O-methyl-β-D-bovinose and, obviously has the same configuration.

β-D-Digitoxose (XXV), β-D-cymarose (XXVI), and β-D-digicuronic acid (XXVII). In preceding papers [7, 11, 12] we have shown that in the digitoxosides the hydroxyl at C_4 is equatorial and that at C_3 is axial. Since β-D-cymarose and β-D-digicuronic acid may be regarded as derivatives of D-digitoxose, the stable conformation for them will possibly be common (see XXV-XXVII). As already mentioned, the presence of a methoxyl group in D-cymarose in the cis position with respect to the equatorial hydroxyl at C_4 markedly slows down the acetylation of the latter. The half-period of acetylation of cymarin is 38 min [7].

The conformations of D-glucose (I) and its derivatives (II-VI), and also that of D-xylose (VII) dc not require discussion. Reeves' investigations [8, 13] have shown, and it is today generally accepted, that they have the C-1 form and

all the voluminous substituents in them occupy the equatorial positions. The results of acetylation of the glycosides and xylosides obtained by us also agree with these ideas.

A special study was devoted to L-vallorose (XXI) and L-talomethylose (XVI) and their derivatives. From the number of axial and equatorial voluminous substituents and their mutual position the existence of the 1-C ⇄ C-1 transformation in these compounds may be assumed. This relates above all to L-vallorose. It is possible that for α-L-talomethylose the 1-C conformation will be preferred in spite of 3α-hydroxyl groups in it. The C-1 conformation must be less stable: voluminous substituents at C_3 and C_5 experience strong torsional strain.

The conformations of the α-D- and α-L- and the β-D- and β-L-monosaccharides, respectively, must be regarded as mirror isomers. Let us consider, for example, in respect of the features of their conformation (in this case without taking the configuration at C_1 into account, since the examples given in pairs below are not monotypical, i.e., α-L- and β-D- monosaccharides are compared), D-thevetose (V) and L-thevetose (XV), D-cymarose (XXVI) and L-cymarose (XXVIII), and D-diginose (XXIX), and L-diginose (XXX).

In order to determine more readily from the conformational formulas in which form (α or β) a monosaccharide is present in hexapyranoses for the C-1 and 1-C conformations it is desirable to compare the mutual positions of the semiacetal hydroxyl and the sixth carbon atom. In the α-form of a monosaccharide one of the substituents has the equatorial and the other the axial position. In the β-form, both the substituents mentioned are equatorial or both axial.

It follows from what has been said that in the cardenolides the glycosidic centers exhibit all forms of conformational associations: ee, ea, ae, aa (for example, horfuside, bovoside A, erysimin, convallatoxin, etc.).

Experimental

Convallatoxin and desglucocheirotoxin isopropylidene derivatives XXXIV and XXXV. A solution of 0.2 g of the glycoside in 1.5 ml of acetone was treated with 1 g of anhydrous copper sulfate, and the mixture was heated at 80° C in a sealed glass tube with frequent shaking for 5 min. The solution was vacuum-filtered through a filter packed with several layers of kieselguhr and activated alumina. The filter was washed with 10 ml of acetone. The filtrate was evaporated and the residue, consisting of the isopropylidene derivative contaminated with the initial glycoside, was dissolved with heating in 50 ml of benzene. The solution was concentrated to a volume of about 10 ml, 50 mg of activated carbon was added, and it was filtered under vacuum. The isopropylidene cardenolide crystallized from the filtrate in the cold.

Isopropylidenedesglucocheirotoxin (XXXIV) melts at 174–178° C; $[\alpha]_D^{19}$ −17.7 ± 3° (c 1.19; methanol). It dissolves in concentrated H_2SO_4 forming a coloration changing with time: 0 min) red-brown; 1 min) yellow-brown; 100 min) green.

Isopropylideneconvallatoxin (XXXV), mp 156–160° C, $[\alpha]_D^{20}$ +7.6 ± 3° (c 0.77; methanol). Under the action of conc H_2SO_4 it gives a coloration changing with time; 0 min) red; 1 min) light brown; 20 min) yellow; 180 min) green.

Method for the quantitative determination of substances during acetylation. The method is similar to that described previously [7, 9]. The quantitative determination of the diacetate of bovoside A formed in the reaction differs only by the fact that the substance eluted from the corresponding part of the chromatogram and dried was treated with a mixture of acetic anhydride and conc H_2SO_4 (100 : 0.5; volume ratio). The extinctions of the colored soltuion formed were measured in a photoelectric colorimeter—an FEK-56 nephelometer (filter with maximum transmission at a wavelength of 540 mμ.

The method for the quantitative determination of the rate of acetylation of the semiacetal hydroxyls of the sugars was as follows: a calibration graph was first constructed. For this purpose we used a 2% solution of a monosaccharide in pyridine; 1 ml of the solution contained 20 mg of sugar. An accurate volume of the solution (between 0.1 and 1 ml) was evaporated in vacuum with heating. For a blank experiment, the same volume of pyridine was taken and it was evaporated in a separate flask. Then 2 ml of the reagent—aniline hydrogen phthalate [10] dried with anhydrous sodium sulfate—was added to each flask and they were heated in the boiling water bath for 1 hr. The solutions were transferred to 100-ml measuring flasks and were made up with ethanol to the marks. The extinctions were measured in cells 1-cm thick. The calibration graph was constructed in the form of the dependence of the extinction on the amount of monosaccharide (mg).

Acetylation was carried out with 1 ml of the same 2% solution of the sugar in pyridine cooled to 0° C, to which 0.5 ml of acetic anhydride was added (the reaction likewise being carried out at 0° C). After a definite time, 1 ml of water was added to stop the reaction. The solution was kept at 0° C for 15 min and at room temperature for 20 min (in the presence of pyridine the acetic anhydride reacted with the water extremely vigorously). The solvents were eliminated

in vacuum with heating. Chloroform was added to facilitate their elimination. To the dry residue in the same flask was added 2 ml of the reagent (aniline hydrogen phthalate) and the mixture was heated in the boiling water bath for 1 hr. A blank experiment was carried out in parallel using initially 1 ml of pyridine and then the same reagents. The extinctions of the colored solutions were measured (filter with a maximum transmission at a wavelength of 400 mμ) and the amount of monosaccharide that had not reacted was determined with the aid of the calibration graphs. The kinetic curve was constructed in the form of the change in the content of initial monosaccharide as a function of the time of the reaction.

Conclusions

On the basis of calculated and, for some of the compounds, experimentally determined figures, the most stable conformational formulas of all the monosaccharides present in cardiac glycosides have been determined. It has been shown that the majority of monosaccharides of the L-series are present in the 1-C form while the monosaccharides of the D-series are present in the C-1 form. It has also been established that the glycosidic centers in the cardenolides exhibit all types of conformational linkages: ee, ea, ae, and aa.

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