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SYNTHETIC GLYCOSIDES OF STROPHANTHIDIN

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The natural cardiac drugs, with the exception of the toad venoms and a few members of the group which appear to be true alkaloids, occur as glycosides. The question of the role of the carbohydrate component of the glycosides remains unanswered. Until comparatively recently the view was fairly widely held that the function of the carbohydrate moiety was to increase the solubility of the aglycon in the aqueous body fluids, and, in the case of the polysaccharides of the aglycons, to provide a larger molecule, the speed of absorption of which into the body tissues would be slower and hence tend to produce a cardiotonic effect of longer duration. Recently, however, some evidence has been secured to the effect that, while the aglycon portion of the glycoside molecule undoubtedly plays a dominant role in determining the qualitative actions of the glycosides, the carbohydrate portion is not without influence on the quantitative action of the drugs. As illustrative of this effect, the cases of cymarin and convallatoxin may be cited. Cymarin has been shown to be the cymaroside of strophanthidin (1), whereas convallatoxin has been more recently shown to be the *l*-rhamnoside of strophanthidin (2). No information is available as to whether these two substances are α- or β-glycosides, or as to whether they possess the furanoside or pyranoside ring structure. However, it is of interest to note that, whereas convallatoxin is the most potent of the known cardiac glycosides, cymarin is significantly less active. A similar, though less striking, variation has been observed in α- and β-antiarin, which are glycosides of a common aglycon with antiarose and *l*-rhamnose respectively (3). The actual values for the minimum systolic doses for these glycosides are shown in Table I. It thus becomes apparent that the carbohydrate constituent of these pairs of glycosides, at least, plays a decidedly positive role in determining the quantitative action of the drugs. It is, therefore, of great interest to determine the effect on activity of varying the carbohydrate component of the cardiac glycosides.

In the present study the synthesis of four strophanthidin glycosides is described and quantitative data on the activity of both the glycosides and their acetylated precursors are presented. The pharmacological data were secured through the kind cooperation of Dr. K. K. Chen of the Lilly Research Laboratories, Indianapolis, Indiana; and a detailed report of the methods used has appeared elsewhere (6). It should be pointed out that, in commencing this study, we have chosen to keep constant both the aglycon component and the configuration of the glycosides prepared. The mode of synthesis, on the basis of past experience, can lead only to β-glycosides. It has thus been possible to restrict the study to the effect of variation of the carbohydrate constituent on the activity of strophanthidin β-glycosides and their acetyl derivatives. The glycosides investi-

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gated are the β-glycosides of strophanthidin with *l*-arabinose, *d*-xylose, *d*-glucose and *d*-galactose. It is planned in the future to extend the study to α-glycosides, as well as to glycosides of aglycons other than strophanthidin, in order to ascertain the effect of these variables.

The method used for the synthesis of the glycosides was a variation of the classical procedure of Koenigs and Knorr (7) embodying the condensation of an alcohol and an acetobromo sugar. The reactions were carried out in anhydrous dioxane solution in the presence of silver carbonate and anhydrous magnesium sulfate. The use of dehydrating agents more active than magnesium sulfate, such as anhydrous calcium sulfate (Drierite), led to some decomposition of the strophanthidin. It is important that the procedures detailed in the experimental part be followed closely if a crystalline acetyl glycoside is to be obtained. Numerous variations of these methods led only to complex oily mixtures from which no crystalline products could be isolated.

The acetyl glycosides thus obtained are β-glycosides by the mode of synthesis (7). Furthermore, the secondary hydroxyl group on carbon atom 3 of strophanthidin, rather than one of the tertiary hydroxyl groups, is involved in the

TABLE I

MINIMUM SYSTOLIC DOSES OF GLYCOSIDES

GLYCOSIDE	CAT UNITS (MG./KG.)	FROG UNITS (MG./G.)
Cymarin (4)	0.11	0.00060
Convallatoxin (4)	.08	.00021
α-Antiarin (5)	.13	.00050
β-Antiarin (4)	.10	.00039

glycosidic linkage. This was demonstrated by acetylation of strophanthidin *d*-glucoside with acetic anhydride in pyridine solution. The strophanthidin acetyl glucoside thus obtained was identical with the strophanthidin tetraacetylglucoside obtained from strophanthidin and acetobromoglucose. If one of the tertiary hydroxyl groups of strophanthidin had been involved in the glucoside formation, the secondary C-3 hydroxyl group would have been left unsubstituted and, under the conditions of the re-acetylation of the glucoside, a pentaacetyl derivative of the latter should result, with the four hydroxyl groups of the glucose component and the secondary C-3 hydroxyl group undergoing acetylation (8). On the basis of this proof of structure of the strophanthidin acetylglucoside, it is logical to assume that the other glycosides possess an analogous structure.

Deacetylation of the acetylglycosides was carried out catalytically with barium methoxide in absolute methanol solution (9). The resulting crystalline glycosides gave a strong positive nitroprusside (Legal) color test, thus showing that the unsaturated lactone side chain of the strophanthidin had not been altered during the treatment. Deacetylation of strophanthidin triacetyl β-*l*-arabinoside with methyl alcoholic ammonia also did not affect the lactone. However, we feel that the milder barium methoxide method is preferable.

The synthetic strophanthidin glycosides were extremely difficult to obtain in crystalline form originally. However, once they had been obtained in the crystalline state, recrystallization was readily accomplished. Strophanthidin β-*d*-galactoside has not been obtained in crystalline form as this is written. The other three glycosides all contain water of crystallization, the presence of which apparently is necessary in order to secure them in crystalline form.

TABLE II

ASSAY IN CATS (AVERAGE OF SEVERAL ANIMALS)

COMPOUND	MEAN LETHAL DOSE ● STANDARD ERROR (MICROGM./KG. CAT)
Strophanthidin	306.2 ± 38.7
Strophanthidin acetate	186.6 ± 24.6
Strophanthidin-β-tetraacetyl-*d*-glucoside	1166 ± 125
Strophanthidin-β-*d*-glucoside	91.3 ± 2.46
Strophanthidin-β-triacetyl-*d*-xyloside	591.6 ± 70.4
Strophanthidin-β-*d*-xyloside	109.5 ± 4.39
Strophanthidin-β-triacetyl-*l*-arabinoside	1230 ± 136.6
Strophanthidin-β-*l*-arabinoside	94.5 ± 2.95
Strophanthidin-β-tetraacetyl-*d*-galactoside	1692 ± 168
Cymarin	110.1 ± 3.75 (11)

TABLE III

ASSAY IN FROGS (AVERAGE OF SEVERAL ANIMALS)

COMPOUND	MEAN SYSTOLIC DOSE ± STANDARD ERROR (MICROGM./G. FROG)
Strophanthidin	2.71 ± 0.49
Strophanthidin acetate	2.19 ± 0.13
Strophanthidin-β-tetraacetyl-*d*-glucoside	18.77 ± 3.07
Strophanthidin-β-*d*-glucoside	0.583 ± 0.04
Strophanthidin-β-triacetyl-*d*-xyloside	8.07 ± 1.35
Strophanthidin-β-*d*-xyloside	0.64 ± 0.04
Strophanthidin-β-triacetyl-*l*-arabinoside	6.33 ± 0.38
Strophanthidin-β-*l*-arabinoside	0.308 ± 0.03
Strophanthidin-β-tetraacetyl-*d*-galactoside	11.29 ± 1.85
Cymarin	0.60 ± 0.006

The substances prepared have been assayed in cats and frogs, and the data thus obtained are shown in Tables II and III. The observed mean lethal doses are given (6), and the standard error is also indicated. Data obtained previously (4, 5) on the four glycosides given in Table I are presented for comparison, together with previous data on strophanthidin (10) and new data on strophanthidin acetate. It is not possible to draw any definite conclusions as to the influence of the configuration of the sugar component on the activity of the glycosides from the number of cases studied at present. However, certain

definite trends can be pointed out. The acetylglycosides are notably less potent than the glycosides, which, in turn, are more potent than the aglycon. The latter fact has previously been noted in several cases (10). However, it is interesting to note that introduction of an acetyl group directly onto the aglycon (strophanthidin acetate) results in greatly increased activity, whereas acetylation on the sugar component of the glycosides lowers activity in most cases. It is significant that, whereas the activity of the glycosides falls within the same general range, that of the acetylglycosides varies over a much wider range.

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EXPERIMENTAL

All melting points are corrected for stem exposure.

Preparation of the acetylglycosides. The preparation of strophanthidin tetraacetyl-β-*d*-glucoside is given as typical of the general procedure used. The other glycosides were prepared by this general method, with the variations noted below for the individual cases.

A mixture of 4 g. (0.0097 mole) of strophanthidin, 3.5 g. (0.0127 mole) of dry silver carbonate, 6 g. of anhydrous magnesium sulfate, and 40 cc. of dioxane (which had been refluxed over and distilled from sodium) was stirred for one hour in a three-necked flask equipped with a dropping-funnel. All solutions, as well as the reaction mixture, were carefully protected from moisture by calcium chloride tubes. One-half gram of iodine was then added and a solution of 8 g. (0.0193 mole) of acetobromoglucose in 20 cc. of dry dioxane was added dropwise over a period of an hour. After the mixture had been allowed to react at room temperature for 20 hrs., the silver salts and magnesium sulfate were filtered off and the filtrate was concentrated under reduced pressure to a viscous straw-colored oil. The oil was exhaustively stirred with several portions of anhydrous ether until it had completely solidified. Three grams of solid material was thus obtained. When petroleum ether (Skellysolve B) was added to the combined ether washings, an additional 0.7 g. of solid material was obtained which was combined with the main crop. The crude acetylglucoside was crystallized from a mixture of alcohol and water. After two recrystallizations, 2 g. of product corresponding to a yield of 28%, based on the strophanthidin used, was obtained. The acetylglucoside forms long needles which tend to felt when dry. The melting point varies with the rate of heating, but the compound ordinarily begins to soften at about 165° and melts with decomposition between 240° and 250°. For analysis the substance was dried over calcium chloride at 75° and 10 mm. pressure; $[\alpha]_D^{27}$ + 24° (c = 0.978 in chloroform).

Anal. Calc'd for $C_{37}H_{50}O_{15}$: C, 60.5; H, 6.9.
Found: C, 60.3; H, 6.7.

*Strophanthidin tetraacetyl-β-*d*-galactoside* was prepared exactly as was the glucoside. The yield was 31%, and the substance crystallized from dilute alcohol as prismatic needles which sintered at 230° and melted with decomposition at 236–237°. It contains 0.5 mole of water of crystallization. For analysis it was dried over calcium chloride at 75° and 10 mm. $[\alpha]_D^{28}$ + 16° (c = 1.756 in chloroform).

Anal. Calc'd for $C_{37}H_{50}O_{15}\cdot 0.5\ H_2O$: C, 59.7; H, 6.9.
Found: C, 59.7; H, 6.7.

*Strophanthidin triacetyl-β-*d*-xyloside* was prepared in 7.5% yield, as in above cases. It crystallizes from dilute alcohol as long needles which contain two moles of water of crystallization, and melt with decomposition at 240–250° after preliminary sintering. For analysis the substance was dried at 75° and 10 mm. over calcium chloride; $[\alpha]_D^{28}$ − 10° (c = 0.676 in chloroform).

Anal. Calc'd for $C_{34}H_{46}O_{13}\cdot 2H_2O$: C, 58.4; H, 7.2.
Found: C, 58.4; H, 7.5.

Strophanthidin triacetyl-β-l-arabinoside was prepared as above, except that no iodine was used to catalyze the reaction. The yield was 14%. The glycoside crystallizes from dilute alcohol as needles, the melting point of which varies greatly with the rate of heating. Ordinarily it begins to sinter at about 155° and melts with effervescence and decomposition at about 200°. Attempts to improve the yield by the use of iodine resulted in the formation of brown oily products which could not be obtained in crystalline form. For analysis it was dried at 75° and 10 mm. over calcium chloride; $[\alpha]_D^{28}$ + 20° (c = 1.600 in chloroform).

Anal. Calc'd for $C_{34}H_{46}O_{13}$: C, 61.6; H, 7.0.
Found: C, 61.3; H, 7.2.

Strophanthidin β-d-glucoside. To a solution of 0.5 g. of strophanthidin tetraacetyl-β-*d*-glucoside in 75 cc. of absolute methanol was added 1 cc. of approximately 0.5 *N* barium methoxide solution in absolute methanol. After the solution had been allowed to stand for 8 hrs. in the refrigerator, the barium was quantitatively precipitated with dilute sulfuric acid. The filtrate from the barium sulfate was concentrated under reduced pressure, leaving an oily residue which was very soluble in water and sparingly soluble in absolute alcohol and ethyl acetate. Repeated attempts to crystallize the glucoside from the ordinary solvents yielded it only in the amorphous state. It was finally obtained crystalline from ethyl acetate which had been previously saturated with water. Once crystalline, the glucoside may be conveniently recrystallized by dissolving it in 95% alcohol and adding ether to the solution until a slight turbidity appears. The glucoside separates as fine needles which contain 0.5 mole of water of crystallization and melt with decomposition at 234–236° after sintering at 228°. For analysis it was dried at 60° and 10 mm. over calcium chloride for 8 hrs.; $[\alpha]_D^{30}$ + 21° (c = 0.620 in water).

Anal. Calc'd for $C_{29}H_{42}O_{11}\cdot 0.5\ H_2O$: C, 60.5; H, 7.5.
Found: C, 60.3; H, 7.8.

Strophanthidin β-d-xyloside was prepared and purified exactly as was the glucoside. It crystallizes as needles which contain 2.5 moles of water of crystallization and melts with decomposition at 152–154°. For analysis it was dried at 75° and 10 mm. over calcium chloride; $[\alpha]_D^{29}$ + 7° (c = 0.366 in 95% alcohol).

Anal. Calc'd for $C_{28}H_{40}O_{10}\cdot 2.5\ H_2O$: C, 57.8; H, 7.8.
Found: C, 58.0; H, 7.8.

Strophanthidin β-l-arabinoside was prepared as in the above examples, except that the substance was crystallized directly without treatment with wet ethyl acetate, by dissolving it in alcohol, adding water to the solution and then evaporating most of the alcohol. The arabinoside crystallizes in long needles containing 0.5 mole of water of crystallization. The melting point varies with the rate of heating, but ordinarily the substance melts with decomposition and effervescence at about 210° after preliminary sintering. For analysis it was dried at 75° and 10 mm. over calcium chloride; $[\alpha]_D^{30}$ 31° (c = 1.100 in 95% alcohol).

Anal. Calc'd for $C_{28}H_{40}O_{10}\cdot 0.5\ H_2O$: C, 61.6; H, 7.6.
Found: C, 61.6; H, 7.8.

The acetylarabinoside was also deacetylated with methyl alcoholic ammonia as follows: To a solution of 100 mg. of the acetylarabinoside in 20 cc. of absolute methanol was added 100 cc. of an approximately 15% solution of dry ammonia in absolute methanol. After the solution had been allowed to stand in the refrigerator for 24 hrs., it was concentrated under reduced pressure and the residue extracted with dry ethyl acetate. The product remaining from the extraction crystallized from dilute alcohol when seeded with the arabinoside obtained by the use of barium methoxide.

Reacetylation of strophanthidin β-d-glucoside. One hundred twenty milligrams of strophanthidin β-*d*-glucoside was dissolved in 5 cc. of dry pyridine, 0.25 cc. of acetic anhydride was added, and the mixture was allowed to stand twelve hours. The solution was then diluted with ice-water and concentrated under diminished pressure to one-half its volume; a crystalline precipitate separated. This was shown to be identical with strophanthidin tetraacetyl-β-*d*-glucoside by saponification equivalent. Equivalent weight: Calc'd 147; Found 152. As a control, strophanthidin tetraacetyl-β-*d*-glucoside prepared from aceto-

bromoglucose and strophanthidin was similarly saponified. Equivalent weight: Calc'd 147; Found 152.

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