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STRUCTURE OF THE SUGAR COMPONENT OF SECURIDASIDE

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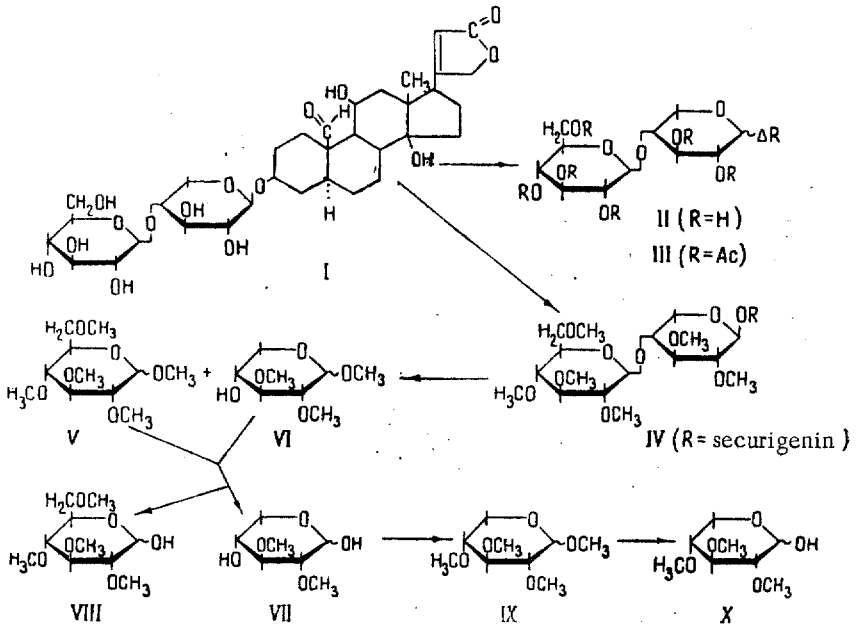
From the seeds of Securigera securidaca (L.) Degen and Derfler we have isolated a new cardiac bioside, securidaside (I) [1]. After its hydrolysis by the Mannich-Sivert method [2] the aglycone securigenin and two monosaccharides, D-xylose and D-glucose, were obtained. In the stepwise enzymatic hydrolysis of securidaside by the enzymes of the fungus Aspergillus oryzae [3] first the monoside securiside and D-glucose were formed and then securigenin and D-xylose, which shows the order of attachment of the monosaccharides in the molecule of the cardenolide under investigation.

On quantitatively determining the content of monosaccharides in the molecule of the glycosides, it was found that the sugar component consists of one molecule of D-xylose and one molecule of D-glucose. By hydrolyzing the diglycoside with a dilute solution of sulfuric acid, a biose (II) was split off; this has been called securidabiose.

The present paper gives information on the determination of the position and nature of the addition of the terminal D-glucose and also on an investigation of securidabiose. We used the method of exhaustive methylation [4]. For this purpose, securidaside was methylated with dimethyl sulfate in an alkaline medium and the methyl glycosides obtained after methanolysis were hydrolyzed with hydrochloric acid solution (scheme):



Chromatographic analysis of the hydrolysis products showed the presence of two methylated monosaccharides having the same R_f values as 2, 3-di-O-methyl-D-xylose and 2, 3, 4, 6-tetra-O-methyl-D-glucose (the samples of 2, 3-di-O-methyl-D-xylose and 2, 3, 4, 6-tetra-O-methyl-D-glucose were provided by N. I. Kuibina and Yu. P. Solov'eva (State Scientific-Research Institute for the Hydrolysis and Sulfite Alcohol Industry).

In chromatographic behavior and also with respect to optical activity, the dimethyl-D-xylose (VII) isolated proved to be identical with a sample of 2, 3-di-O-methyl-D-xylose. The reaction of the dimethyl-D-xylose with dimethylaniline trichloroacetate gave a violet coloration which is characteristic, according to Hough, et al., [6], for methylated aldoses with a free hydroxy group at C-4. The methylation of 2, 3-di-O-methyl-D-xylose with dimethyl sulfate gave a product (X) identified by paper chromatography as 2, 3, 4-tri-O-methyl-D-xylose.

The difficulty of the acid hydrolysis of securidaside permitted the assumption of the presence of pyranose rings of the xylose and glucose residues of the sugar component of this compound. This was confirmed for the xylose by the isolation and identification of 2, 3-di-O-methyl-D-xylose, with a free hydroxy at C-4, and also by the production of 2, 3, 4-tri-O-methyl-D-xylose by the methylation of the dimethylxylose.

A comparison of the specific rotations of the tetramethylglucose isolated from securidaside and fully methylated glucose in the pyranose and furanose forms (Table 1) showed that the glucose in securidaside is present in the pyranose form.

Table 1

Optical Activity of Tetramethyl Glucose in the Pyranose and Furanose Forms

Methylated monosaccharides	Specific rotation	Form of the ring	Reference
2,3,4,6-Tetra-O-methyl-D-glucose	+ 83.3°	pyranose	[7,4]
Tetramethylglucose isolated from securidaside	+ 80.5°	—	—
2,3,5,6-Tetra-O-methyl-D-glucose	− 11.1°	furanose	[7]

The nature of the glycosidic link between the D-glucose and the D-xylose was established by the use of Klyne's rule [8]. From a comparison of the molecular rotation of the glucose moiety of securidaside with the molecular rotation of methyl α- and β-glucosides it may be considered that the glucose and xylose are connected by a β-glucosidic link (Table 2).

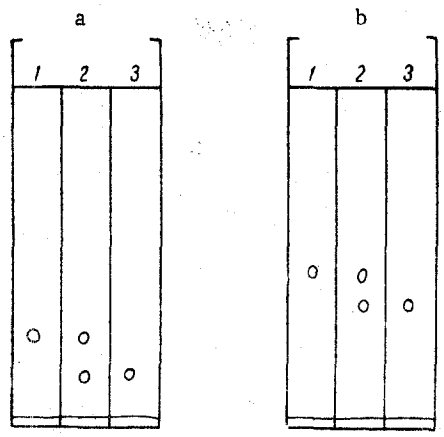


Fig. 1. Chromatograms of the qualitative analysis of a hydrolyzate of methylated securidaside. a) Ethyl acetate – pyridine – water (4:1:1); b) 1-butanol – ethanol – water (5:1:4). 1) 2,3-Di-O-methyl-D-xylose; 2) hydrolyzate of methylated securidaside; 3) 2,3,4-tetra-O-methyl-D-glucose.

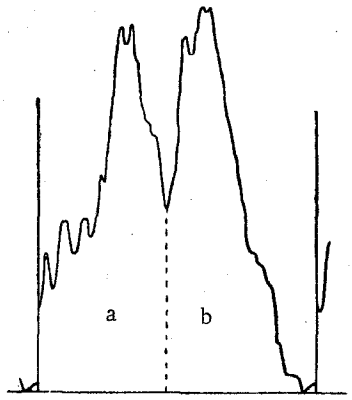


Fig. 2. Graph of the interpretation of the chromatogram of a mixture of the hydrolyzate of methylated securidase. a) Photometry of the spot of 2,3-di-O-methyl-D-xylose; b) photometry of the spot of 2,3,4,6-tetra-O-methyl-D-glucose.

On passing to a chemical study of securidabiose, we had information [10] on only one biose consisting of D-glucose and D-xylose, this being 6-β-(D-xylopyranosyl)-D-glucopyranose (primeverose). The attachment of the glucose to the fourth carbon atom of the xylose was also confirmed by the color reaction proposed by Bailey [11]. On the basis of what has been said, it may be considered that the sugar component of securidaside consists of D-xylose and D-glucose connected by a 1 → 4 β-glycosidic bond and the biose isolated is 4-β-(D-glucopyranosyl)- D-xylopyranose.

Experimental

Methylation of securidaside. To a solution of 10.52 g of securidaside in 50 ml of water were added 20 ml of carbon tetrachloride and 30 ml of dimethyl sulfate, and the mixture was heated on a water bath (35–40° C). Then 120 ml of a 40% solution of caustic soda was added with continuous stirring over 2 hr. The temperature of the reaction mixture rose to 70° C. After the addition of all the alkali, the mixture was stirred for another 1 hr, and then,

with constant stirring, another 30 ml of dimethyl sulfate and 120 ml of 40% caustic soda solution were added simultaneously dropwise over 2 hr, after which the mixture was heated for 30 min at 100° C. A viscous residue of methylated glycoside deposited. The cooled solution was treated with 500 ml of chloroform, in which the precipitate dissolved, and after the separation of the chloroform layer the aqueous phase was repeatedly extracted with chloroform (5 × 50 ml). The combined chloroform extracts were concentrated in vacuum to 200 ml, washed with water (2 × 20 ml), dried over sodium sulfate, and evaporated in vacuum to dryness. This gave 5.45 g of methylated securidaside (IV).

Methanolysis and hydrolysis of methylated securidaside. A solution of 5 g of methylated securidaside in 20 ml of methanol with 20 ml of 27% methanolic hydrogen chloride was treated in a sealed tube at 80°–85° C for 6 hr. After cooling, the contents of the tube were evaporated in vacuum to dryness, the residue was dissolved in 100 ml of 2% hydrochloric acid, and the resulting solution was heated at 100° C for 2 hr and was then left for 14 hr. The hydrolyzate containing the methylated sugars was evaporated in vacuum to dryness and the residue was treated repeatedly with chloroform (15 × 50 ml). The combined chloroform extracts were washed with water, dried with sodium sulfate, and evaporated in vacuum to dryness. This gave 1.7940 g of a resinous residue of methylated sugars. A chromatogram of the methylated saccharides obtained is shown in Fig. 1.

Table 2

Determination of the Configuration of the Glucosidic Link in Securidaside

Substance	$[M]_D$, deg
Securidaside, mol. wt. 720.0	+ 34.6
Securiside, mol. wt. 540.0	+147.9
D-Glucose moiety of securidaside	—113.3
β-Methyl-D-glucopyranoside [9]	— 66.4
α-Methyl-D-glucopyranoside [9]	+308.4

Quantitative determination of the content of monosaccharides in the hydrolyzate. The quantitative content of carbohydrate components in the hydrolyzate of methylated securidaside was determined by interpreting the spots revealed with aniline phthalate on the paper chromatograms by photometry with an EFA-1 recording instrument [12] (Fig. 2). We give the results of determinations of monosaccharides in the hydrolyzate of securidaside expressed as percentages of the total components:

Methylated sugars	Area occupied on the diagram, mm^2	Sugar content hydrolyzate, %	Number of carbohydrate residues
2,3-Di-O-methyl-D-xylose	1900	51.8	1
2,3,4,6-Tetra-O-methyl-D-glucose	1770	48.2	1

Isolation of the methylated sugars. To prepare the cellulose powder, 2 kg of chromatographic paper of type M from the Leningrad Volodarskii Mill was passed through rollers. The compacted pieces of paper so obtained were ground in a ball mill and passed through a sieve with 0.5-mm apertures. The cellulose powder was washed successively with 2% aqueous Trilon B, 3% hydrochloric acid, distilled water to neutrality, and, finally, ethanol. The cellulose was dried in the air and ground in a mortar to a powder.

The column for separating the methylated sugars was prepared from 750 g of cellulose (height 175 cm, diameter 3.5 cm) by the dry method with subsequent packing down of the adsorbent under vacuum. Before the substances were deposited on the column, it was washed with 3 *l* of the organic phase of the solvent mixture 1-butanol–ethanol–water (4:1:5). Afterwards, the column was eluted with the same solvents.

A solution of 1.7 g of the resinous residue of the methylated sugars in the minimum amount of the above-mentioned mixture of solvents was deposited on the column. The first 150 ml of eluate was discarded and then 30-ml fractions were collected. Paper-chromatographic analysis of the fractions obtained showed that fractions 6–7 contained 2, 3, 4, 6-tetra-O-methyl-D-glucose, fractions 8–12 a mixture of tetramethylglucose and dimethylxylose, and fractions 13–18 only 2,3-di-O-methyl-D-xylose. In all, 0.74 g of 2,3,4,6-tetra-O-methyl-D-glucose and 0.1 g of 2,3-di-O-methyl-D-xylose were obtained.

2,3,Di-O-methyl-D-xylose (VII). For identification, the dimethylxylose obtained was compared with a sample of 2,3-di-O-methyl-D-xylose in two systems of solvents (see Fig. 1). As can be seen from the paper chromatograms, the spots were located at the same level. It has not yet been possible to crystallize the 2,3-di-O-methyl-D-xylose. $[\alpha]_D^{22}$ +25.3° (c 0.79; water).

According to the literature, the specific rotation of 2,3-di-O-methyl-D-xylose is +22.6° (in water) [13].

2,3-Di-O-methyl-N-phenylxylosamine. A solution of 40.9 g of 2,3-di-O-methyl-D-xylose in 1 ml of anhydrous ethanol and 3 ml of freshly distilled aniline was heated in a sealed tube at 100° C for 6 hr. The resulting anilide, after crystallization from ethyl acetate, melted at 124°–125° C.

According to the literature, the anilide of 2,3-di-O-methyl-D-xylose melts at 126° C [5, 13].

Methylation of 2,3-di-O-methyl-D-xylose. Under the same conditions as were used for the methylation of securidaside, 30 mg of the dimethylxylose was methylated with dimethyl sulfate in an alkaline medium. The results of the methylation were checked by paper chromatography in the 1-butanol–ethanol–water (4:1:5) system. The spot of the trimethylxylose (X) obtained was located at the same level as a sample of 2,3,4-tri-O-methyl-D-xylose.

2,3,4,6-Tetra-O-methyl-D-glucose (VIII). The tetramethylglucose was crystallized from ethanol in the form of needle-like crystals melting at 90° C. $[\alpha]_D^{22}$ + 80.5° (c 1.0; water). When the methylglucose was chromatographed on paper in two solvent systems, its spot was located at the same level as a sample of 2,3,4,6-tetra-O-methyl-D-glucose (Fig. 1).

Table 3

R_g and R_x Values of Securidabiose in Various Solvent Systems

Solvent systems	R_g	R_x
1-Butanol–acetic acid–water (4:1:5)	0.46	0.31
1-Butanol–pyridine–water (6:4:3)	0.66	0.44
1-Butanol–ethanol–water (5:1:4)	0.32	0.19
1-Butanol–water (2:1)	0.30	0.19

Securidabiose (II). A solution of 10 g of securidaside in 500 ml of water was mixed with 600 ml of 0.05 N sulfuric acid and left in a thermostated vessel at 60° C for hydrolysis. On paper-chromatographic analysis in the benzene–1-butanol (1:1) – water system, on the 10th day only traces of the initial glycoside were detected in the hydrolyzate. The aqueous solution was separated from the precipitate of aglycone and was extracted first with chloroform (5 × 1 *l*) and then with a mixture of chloroform and ethanol (2:1) (3 × 1 *l*). After the aqueous solution had been neutralized with barium carbonate, it was filtered and the filtrate was then evaporated under vacuum to dryness. The residue (3.03 g) was dissolved in ethanol and mixed with an equal volume of ether. The biose which deposited was reprecipitated with ether from methanolic solution and for separation from the monosaccharides it was purified on a column of cellulose, being eluted with the organic phase of the mixture 1-butanol–ethanol–water (4:1:5). The fractions containing the biose were combined and evaporated under vacuum. This gave 0.87 g of amorphous securidabiose.

Securidabiose is an amorphous powder rapidly resinifying in the air. It gives a positive Fehling's reaction. On a paper chromatogram treated with acid aniline phthalate, the spot of securidabiose is colored pink-red $[\vartheta]_D^{22}$ −27.3° (c 1.184; water).

On paper chromatography, the distance travelled by the securidabiose spot was determined as ratios of those for glucose (R_g) and for xylose (R_x) (Table 3).

Reaction of securidabiose with diphenylamine reagents [11]. The following solutions were used to prepare the revealing reagents: a) 2% solution of diphenylamine in acetone, 10% solution of phosphoric acid (by volume); b) 3% solution of urea in butanol saturated with water, 10% solution of phosphoric acid; c) 2% solution of p-anisidine in ethanol, 10% solution of phosphoric acid.

Reagent no. 1, which is used to detect a 1 → 4 bond, was prepared directly before use by mixing equal volumes of solutions a and b. Reagent no. 2, which is used to detect 1 → 2 and 1 → 6 bonds was also prepared before use by mixing equal volumes of solutions a and c.

After the chromatograms had been dried, the spots were sprayed with reagents nos. 1 and 2 and the paper was heated at 95°–100° C for 8–10 min. The spot of the biose, when sprayed with reagent no. 1, acquired a red orange color, which is characteristic for a 1 → 4 bond, and when sprayed with reagent no. 2 it acquired a blue green color.

Securidabiose acetate (III). To a solution of 360 mg of securidabiose in 4 ml of acetic anhydride was added 0.4 ml of acetic acid and 0.7 g of sodium acetate. The mixture was heated at 100° C for 1 hr. After the usual working up, 103 mg of a residue of the acetate was obtained with mp 154°–157° C (from acetone), $[\alpha]_D^{22}$ −18.2° (c 0.98; chloroform).

The degree of acetylation was checked by means of the IR spectroscopy. The spectrum had no band at a wavelength of 3400 cm^{-1} characteristic for hydroxy groups.

Summary

The structure of the sugar component of the new cardiac bioside securidaside, securidabiose, has been established; it is 4-β-(D-glucopyranosyl)-D-xylopyranose.

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