DETERMINATION OF 2-DEOXYALDOSES AND THEIR NATURAL GLYCOSIDES WITH THE AID OF XANTHYDROL

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The majority of photometric methods for the analysis of cardenolide glycosides are based on the reactions of the butenolide ring of the aglycone with various aromatic di- and trinitro compounds (1,3-dinitrobenzene, 3,5-dinitrobenzoic acid, 2,4-dinitrophenyl sulfone, picric acid). However, it is not infrequently necessary to give an estimate of the sugar moiety of a glycoside. For plant pregnanes, the bulk of which are present in plants in the form of glycosides of 2,6-dideoxysugars no methods of quantitative determination whatever have been developed. Arrequine and Pasqualis [1] first turned their attention to the fact that the addition of xanthydrol to a solution of digitoxin leads to the formation of a yellow complex, the color of which changes to red on heating. M. Pesez [2] has shown that the xanthydrol reaction is a general one for deoxysugars and has used it for the quantitative estimation of k-strophanthin. Soon, this reagent was used for the determination of the content of the main glycosides of Digitalis after their separation from a paper chromatogram and the elution of the individual spots [3]. Later, information was published on modifications of the latter method [4-7]. One of them is recommended in pharmaceutical practice as official [8] for the quantitative analysis of celanide (lanatoside C) and its medicinal forms. Vorob'ev and Dzyuba have described a method for determining the amount of erychroside in Erysimum cheiranthoides L. with the aid of xanthydrol [9]. These authors observed that xanthydrol takes part in the reaction in equimolecular ratio, and within the range of concentrations from 3 to 30 mg in 1 ml of solution a linear relationship exists between the optical density and the concentration of the glycoside.

Nevertheless, in spite of the wide use of the xanthydrol reaction in practice, the theoretical principles of its use have not been developed in many respects. In particular, the dependence of this reaction on the structure of the sugar moiety has not been studied.

We have measured the molar extinction coefficients of the individual 2-deoxysugars, of two disaccharides, and of several cardiac glycosides including 2,6-dideoxysugars. The conditions for performing the xanthydrol reaction were similar to those given in the literature.

It can be seen from Table 1 that the highest extinction coefficient is found for the 2,6-dideoxyaldohexoses D-digitoxose and D-cymarose, while within the limits of error of the analysis and taking the relative purities of the samples into account the two sugars have the same index. Of the 2-deoxyaldohexoses, the molar absorption coefficient of 2-deoxy-D-glucose is almost twice and that of 2-deoxy-D-galactose almost half that of the 2,6-dideoxysugars. 2-Deoxy-D-ribose has the same absorption as 2-deoxy-D-glucose. Consequently, the presence of a hydroxy group in the C_5 or the C_6 position opposes reaction with xanthydrol.

For the disaccharides digilanidobiose and strophanthobiose – in which a 2,6-dideoxyaldose is substituted in the C_4 hydroxyl by a D-glucose residue – the absorption index is likewise low. Obviously, under the conditions of analysis generally adopted these disaccharides do not undergo hydrolytic cleavage.

The molar absorption coefficients of the glycosides depend completely on the structures of the sugars composing them. Monoglycosides of the type of erysimin, cymarin, and corchoroside A possess approximately the same extinction as the individual 2,6-dideoxysugars. The extinctions of the cardiac diglycosides – erysimoside, k-strophanthin-β, and olitoriside – although they differ somewhat are completely comparable with the analogous indices of the disaccharides, especially strophanthobiose.

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TABLE 1. Molecular Extinction Coefficients (ε) of the 2-Deoxysugars and Their Glycosides

Compound and its composition	Mol. wt.	c, mg/ml	D	$E^{1\%}_{1cm}$	ε×10³
Monosaccharides					
D-Digitoxose $C_6H_{12}O_4$	148,16	0,0094	0,427	909	135
D-Cymarose $C_7H_{14}O_4$	162,18	0,0075	0,335	893	145
2-Deoxy-D-ribose $C_5H_{10}O_4$	134,14	0,0292	0,498	341	46
2-Deoxy-D-glucose $C_6H_{12}O_5$	164,16	0,0227	0,320	282	46
2-Deoxy-D-galactose $C_6H_{12}O_5$	164,16	0,0191	0,380	398	65
Disaccharides					
Digilanidobiose $C_{12}H_{22}O_9$	310,31	0,0877	0,660	151	47
Strophanthobiose $C_{13}H_{24}O_9$	324,34	0,0958	0,605	126	41
Monoglycosides					
Erysimin $C_{29}H_{42}O_9 \cdot H_2O$	552,66	0,0254	0,330	260	144
Cymarin $C_{30}H_{44}O_9 \cdot CH_3OH$	580,79	0,0275	0,340	247	145
Corchoroside A $C_{29}H_{42}O_9 \cdot 2H_2O$	570,68	0,0453	0,510	225	129
Diglycosides					
Erysimoside $C_{35}H_{52}O_{14} \cdot H_2O$	714,82	0,1968	0,510	52	37
k-Strophanthin-β $C_{36}H_{54}O_{14} \cdot H_2O$	728,84	0,1160	0,307	53	39
Olitoriside $C_{35}H_{52}O_{14} \cdot H_2O$	714,82	0,1426	0,370	52	37
Triglycosides					
Digitoxin $C_{41}H_{64}O_{13}$	764,92	0,0199	0,414	416	318
Gitoxin $C_{41}H_{64}O_{14}$	780,92	0,0251	0,530	422	330
Digoxin $C_{41}H_{64}O_{14}$	780,92	0,0144	0,370	514	401
Tetraglycosides					
Lanatoside C $C_{49}H_{76}O_{20}$	985,10	0,0260	0,361	278	237

Note: The results of experiments agreeing with the mean of three or four determinations are given.

For triglycosides of the type of digitoxin, each containing three molecules of D-digitoxose, an extremely deep color of the product of the reaction with xanthydrol is characteristic. The absorption indices of this type of compounds are extremely high. This can be explained only by the assumption that under the conditions of determination used the glycosides were hydrolyzed to the individual monoses and that the molar absorption coefficient (of digoxin, for example) in an approximate calculation is the sum of the extinctions of the three monosaccharides.

In the case of the tetraglycoside lanatoside C, as was to be expected, the coefficient is considerably lower than in a triglycoside of similar structure, digoxin: at the concentration of hydrochloric acid that is established in the reaction mixture, complete cleavage into four sugar molecules does not take place. The process ceases at the formation of digilanidobiose and two molecules of 2,6-dideoxyaldoses. Consequently, the molar coefficient of lanatoside C is slightly greater than the sum of two D-digitoxoses.

The experimental material presented shows that the xanthydrol reaction is unsuitable for determining mixtures consisting of glycosides with residues of different sugars or with different numbers of residues of the same sugar. The results of a parallel analysis of the amount of cardenolide glycosides in a plant raw material with any reagent for the butenolide ring (for example, picric acid) and with xanthydrol will not coincide, as a rule. Since this type of analysis is based on a comparison of the optical densities of colored complexes of an individual compound and a plant extract, the determination is complicated by the difficulty of selecting a standard sample. For example, in the same sample of Adonis chrysocyathus Hook. f. et Thom., using as standards cymarin and k-strophanthin-β, by the xanthydrol reaction we found 1.80 and 8.40% (!) of glycosides, respectively. On analysis for the butenolide ring (using 2,4-dinitrophenyl sulfone [10]) it was found to contain only 0.58% of glycosides (standard: k-strophanthin-β). It is quite obvious that other substances including 2,6-dideoxysugars take part in this reaction besides the cardenolide glycosides [11, 12]. Objective results in analysis with the aid of xanthydrol can be obtained only in experiments with samples of individual glycosides [8], or, in the case of plant material, only after the preliminary chromatographic separation of the component substances [3-5].

However, if the limitations mentioned above are taken into account, the xanthydrol reaction can be used in various directions. The possibility of a direct analysis of the spots on chromatographic paper without their elution [3] is undoubtedly an advantage of the method under consideration. The reaction with xanthydrol has been used to determine the amount of 2,6-dideoxysugars in cardenolide glycosides of complex

TABLE 2. Information on the Amounts of Glycosides of 2,6-Dideoxysugars in Plants of the Family Asclepiadaceae (wt. % on the air-dried raw material)

Plant	Site of collection	Organ	Amount	
			2,6-dideoxysugars*	pregnane glycosides†
Cynanchum sibiricum Willd., syn. Cynanchum acutum auct. [15]	Syrdar'inskaya oblast of the Uzbek SSR, Bayaut region	Roots Leaves Seeds	0,23 0,04 0,21	0,84 0,12 1,78
Cynanchum pumilum Dcne., syn. Antitoxicum pumilum (Dcne.) Pobed.	TurkmSSR, close to the village of Kara-Kala (Karakum)	Leaves Seeds	0,27 0,83	0,96 3,00
Cynanchum acuminatifolium Hemsl. syn. Antitoxicum acuminatum (Dcne). Pobed.	Maritime Territory, Khanki region, Mount "Sinie Skaly"	Leaves Root	0,14 0,74	0,50 2,65
Cynanchum inamoenum Loes., syn. Antitoxicum (Maxim.) Pobed.	The same	Roots Leaves	0,42 0,04	1,5 0,15
Cynanchum maximoviczii Pobed., syn. Cynanchum caudatum Maxim.	Kuril islands, Kunashir island	Roots	1,03	3,70
Antitoxicum sibiricum (L.) Pobed., syn. Cynanchum sibiricum R. Br.	Mongolia	Epigeal part	0,02	0,08
Cynanchum nigrum C. A. M. syn. Antitoxicum scandens (Somm. et Lev.) Pobed.	Tashkent, Botanical Garden of the Academy of Sciences of the Uzbek SSR	Leaves Seeds	0,03 0,10	0,12 0,37
Metaplexis Japonica (Thunb.) Makino	The same	Seeds	0,09	0,32
Stapelia variegatus	House plant	Epigeal part	0,52	2,17

* Standard: D-digitoxose

† Standard: erysimin

structure [13]. In view of the fact that monoglycosides and diglycosides with terminal D-glucose have different extinctions, this method can be used to develop the optimum conditions for the performance and for determining the completeness of the enzymatic hydrolysis of cardiac glycosides of the corresponding structure. Finally, the same reaction can be recommended as still the only method for testing plant materials for the presence of pregnane compounds.

Our proposal to use xanthydrol for a rough determination of the amount of pregnane glycosides is due to the fact that the 2,6-dideoxysugars are found in plants exclusively in compounds with cardenolides, bufadienolides, and pregnanes [14]. Plants containing cardenolide and bufadienolide glycosides possess cardiotonic activity which can be revealed by well-developed tests on animals. In addition, digitaloid glycosides can be detected and measured quantitatively by means of specific reagents for the butenolide ring. Consequently, if it is reliably known that cardenolide and bufadienolide glycosides are absent from the material being analyzed, it will be possible to judge the amount of pregnane glycosides from the intensity of the spot appearing after treatment with the xanthydrol reagent.

The 2,6-dideoxysugars most widespread in the vegetable kingdom are D-digitoxose and D-cymarose. Consequently, it is desirable to use pure crystalline preparations of these sugars as standards. In the absence of reagent samples, for analytical purposes it is possible to use erysimin (helveticoside) or cymarin. The use of these cardenolide glycosides is all the more justified in that their molecular weights are very close to those of the pregnane monoglycosides. Table 2 gives the results of the analysis of some plants of the family Asclepiadaceae for their content of 2,6-dideoxysugars. It was established by experiments on individual compounds that under the concrete conditions described in the experimental part pregnane glycosides are hydrolyzed completely to monoses. However, in practice it may be found that this requirement is not satisfied in all cases. Because of this, the results obtained must be considered only as indicative.

EXPERIMENTAL

Preparation of the Xanthydrol Reagent. A solution of 10 mg of xanthydrol in 99 ml of glacial acetic acid is treated with 1 ml of concentrated hydrochloric acid.

By increasing the amount of xanthydrol to 50 mg and that of concentrated hydrochloric acid to 2 ml, the sensitivity of the reaction is increased somewhat, but not to such an extent that this has a fundamental influence on the results of the measurements.

Determination of the Molar Extinction Coefficients. A weighed sample of the substance (4-6 mg) was dissolved in 1 ml of 96% ethanol, or, in the case of sugars, 80% ethanol. The solutions were added by means of a microsyringe to 3 ml of freshly prepared xanthydrol reagent: 0.005 ml for 2,6-dideoxyaldohexoses, 0.015 ml for 2-deoxyaldoses, 0.050 ml for disaccharides, 0.02 ml for monoglycosides, 0.10 ml for diglycosides, 0.01 ml for triglycosides, and 0.025 ml for the tetraglycoside.

After the addition of the substances to be analyzed, the solutions were heated in the boiling water bath for 5 min and they were then cooled with water to room temperature (20°C) and were added to cells with a layer thickness of 0.5 cm, and the optical densities were measured in an SF-4 spectrophotometer at 530 nm or on an FÉK-56P photoelectric colorimeter using a green filter (maximum transmission at 540 nm). The standard was the xanthydrol reagent heated and cooled in just the same way as the sample. The measurements were repeated and the mean optical density was calculated. The color is stable and remains for several hours.

Analysis of Plant Raw Material. An accurately weighted sample (2 g) of air-dried carefully comminuted raw material that had been passed through a sieve (with apertures having a diameter of 1.0-1.5 mm) was introduced into a 50-ml measuring flask, 35 ml of 96% ethanol was added, and the mixture was shaken on a vibration shaker for 2 h or was left to stand for 24 h. Then the extract was made up to the mark with the same ethanol, stirred, and filtered. If the solution is strongly colored (chlorophyll), it is desirable to pass it through a layer (1 cm) of previously treated Wofatit SBW [16].

With a microsyringe, 0.1 ml of the extract was added to 3 ml of the xanthydrol reagent, and the subsequent determination was performed as described above. Concentrated solutions the readings for which came off the scale were diluted, while in the case of low optical densities a smaller amont of solvent was used for extraction. A solution of D-digitoxose (in 80% ethanol) in a concentration of 0.3-0.4 mg/ml or of erysimin (in 96% ethanol) in a concentration of 0.8-1.2 mg/ml was used as standard. The solutions of the standards (0.1 ml) were added to the xanthydrol reagent by means of a microsyringe.

The percentage (X) of pregnane glycosides was calculated from the formula

$$X = \frac{D_{exp} \cdot V \cdot c \cdot n \cdot 100}{D_{st} \cdot m \cdot 1000},$$

where D_{exp} and D_{st} are the optical densities of the experimental and standard solutions; c is the concentration of the solution of the standard sample, mg/ml; V is the total volume of extract, ml; m is the weight of the raw material, g; n is the number of dilutions.

In the method described, V = 50 ml, m = 2 g, n = 1.

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SUMMARY

The molecular extinction coefficients in the xanthydrol reaction of 2-deoxyaldoses and their cardenolide glycosides have been measured. The absorption indices vary within wide limits according to the structure of the sugars. The 2,6-dideoxyaldoses have a considerably higher coefficient than the 2-deoxyaldoses. Disaccharides in which 2,6-dideoxyaldohexoses are attached to the D-glucose show a comparatively low optical density. The glycosides reflect the properties of the sugars that they contain.

A rough method of determining the amount of pregnane glycosides in plants has been developed.

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