DETERMINATION OF 2,6-DIDEOXYSUGARS IN CARDENOLIDES BY GAS–LIQUID CHROMATOGRAPHY

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At the present time, gas–liquid chromatography (GLC) is being used successfully for the analysis of carbohydrates of various classes [1-4]. However, this method has still not acquired wide employment for the determination of the 2,6-dideoxysugars that are common components of cardiac glycosides and some antibiotics.

In the present paper we show the possibility of using GLC for the qualitative and quantitative analysis of the carbohydrate chains of a number of cardiac glycosides containing the dideoxysugars frequently found in cardenolides: D-boivinose, D-cymarose, and D-digitoxose [5]. We chromatographed the sugars in the form of the trimethylsilyl (TMS) ethers obtained as described by Sweeley et al. [1]. As the stationary phase we used SE-30 silicone rubber.

To determine the sugars in the glycosides, the latter were hydrolyzed in aqueous solutions of hydrochloric acid. After the aglycone had been separated off, the residual sugar fraction was silylated and analyzed by GLC.

It is known that the glycosides of 2-deoxysugars hydrolyze very easily [6]. The hydrolysis conditions for our investigations were selected for digitoxin, which contains three molecules of D-digitoxose. Hydrolysis was performed with 1.0%, 0.5%, and 0.1% solutions of hydrochloric acid for 1 h and with a 0.1% solution of HCl for 3 h in the boiling water bath. The completeness of hydrolysis was checked by thin-layer chromatography on silica gel in the chloroform–methanol (9 : 1) system.

Table 1 gives the relative retention times of the products formed in the hydrolysis of digitoxin under various conditions and their percentages. As can be seen from Table 1, the treatment of digitoxin with a 1.0% solution of acid gives a complex mixture of products forming a large number of peaks on the chromatogram. When the concentration of acid was lowered to 0.5% the number of peaks fell and the two main peaks (relative retention times 0.71 and 1.00) appeared clearly. The chromatogram of the products of the hydrolysis of digitoxin by 0.1% acid for 1 h contained only these two peaks. When digitoxin was hydrolyzed with 0.1% HCl for 3 h, a small number of additional peaks appeared on the chromatogram.

In a series of parallel experiments, the percentage compositions of the peaks obtained after the chromatography of the products of the hydrolysis of digitoxin with 0.1% HCl for 1 h remained unchanged,

TABLE 1. Percentage Composition of the Peaks in the Chromatography of the Silyl Derivatives of Digitoxose after the Hydrolysis of Digitoxin with Hydrochloric Acid

Hydrolysis conditions		Relative retention time							
concn. of HCl, %	time, h	1,33	1,00	0,71	0,32	0.24	0,16	0.08	0,06
1,0	1	34,3	38,3	5,7	5,6	3,0	5,7	4,5	2,0
0,5	1	4,3	81,7	6,6	3,0	—	1,3	1,5	1,6
0,1	1	—	92,5	7,5	—	—	—	—	—
0,1	3	3,6	85,0	8,5	—	—	—	1,5	1,4

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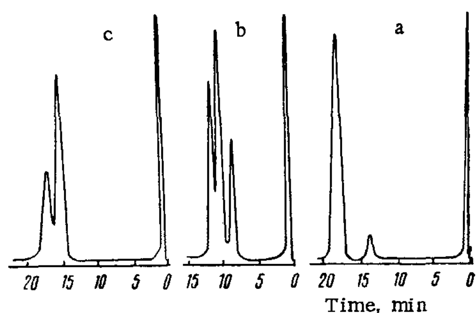

Fig. 1. Chromatograms of TMS ethers of D-digitoxose (a), D-cymarose (b), and D-boivinose (c).

TABLE 2. Relative Retention Times (R_t) of the Trimethylsilyl Ethers of the 2,6-Dideoxysugars and the Percentage Composition of the Peaks Obtained

Monosaccharide	Peaks in the order of elution					
	1		2		3	
	R_t	%	R_t	%	R_t	%
D-Digitoxose	0,71	7,5	1,00	92,5	—	—
D-Cymarose	0,46	44,2	0,58	39,3	0,63	16,5
D-Boivinose	0,81	67,8	0,90	32,2	—	—

and digitoxose, when it was treated under similar conditions, showed the same pattern. Consequently, this composition of the peaks corresponds to the equilibrium state of digitoxose under the hydrolysis conditions taken.

In view of the fact that some cardiac glycosides are sparingly soluble in water, they were dissolved in dioxane or tertiary butanol. The use of methanol and ethanol and subsequent hydrolysis in an aqueous solution of HCl led to the formation of the corresponding methyl and ethyl glycosides (about 60%) [7], leading to the appearance on the chromatogram of the additional peaks corresponding to these glycosides.

In order to determine the retention times of the TMS derivatives of the 2,6-dideoxysugars, we treated them in the same way as for hydrolysis and then silylated them and fed them into the chromatograph. The chromatograms shown in Fig. 1 were obtained for cymarose, digitoxose, and boivinose.

Table 2 gives the relative retention times and percentage equilibrium compositions of the anomers of digitoxose, cymarose, and boivinose under the hydrolysis conditions. The relative retention times are given with respect to that for the β-anomer of digitoxose (17.7 min), which was found after the chromatography of the product of the direct silylation of crystalline digitoxose, which is the β-anomeric form [8].

As can be seen from Fig. 1, under the conditions of treatment used cymarose shows three peaks, boivinose two, and digitoxose two. All these peaks are fairly well separated. A similar pattern was observed when cardenolide glycosides containing these sugars (cymarin, corchoroside, and erisimin) were treated under the conditions described.

We used the following formula [9] for the quantitative determination of the carbohydrates in the glycosides:

$$\frac{\text{Number of moles of sugar}}{\text{1 mole of standard}} = \frac{S_{sug}}{S_{st}} \cdot K, \qquad (1)$$

where S_{sug} is the area of the peak of the sugar being determined, S_{st} is the area of the peak of the standard, and K is the relevant correction factor.

In the case of glycosides containing several different sugars, one of them was taken as the standard. In the case of glycosides containing one or more identical sugars, a different sugar was taken as the standard for quantitative analysis. In this case, if the molecular weight of the genin is known, it is possible to calculate the number of sugars in the glycoside.

Using digitoxin as an example, Table 3 shows a calculation of the number of digitoxose residues in the glycoside. Knowing that the substance under investigation consisted of digitoxigenin and a sugar – digitoxose – we assumed that the number of sugar molecules in it was 1, 2, 3, and so on.

Then, from the sample weights taken, the proportion of sugar in them (in mg and in moles) was calculated and, having added the weight of the other sugar taken as standard, the number of moles of the sugar being determined per mole of the standard was calculated. By comparing the results obtained by GLC with the calculated figures, we found the number of molecules of sugar in the substance being analyzed. As the standard for the determination of digitoxose we took cymarose, and from synthetic mixtures we found the value of K for digitoxose (K = 1.45) in accordance with formula (1).

TABLE 3. Results of a Calculation of the Number of Monosaccharide Residues in a Glycoside, Using Digitoxin as Example

Substance being determined and standard	Weight of sample	Suggested number of digitoxose residues in glycoside	Amt. of sugar in the sample		No. of moles of digitoxose per mole of cymarose	
			mg	moles, × 10^{-5}	found by GLC	calcd. from the weight of the sample
Glycoside consisting of digitoxigenin and digitoxose	4,95	1	1,45	0,98	0,45	—
		2	2,31	1,56	0,72	—
		3	2,88	1,94	0,90	0,87*
		4	3,28	2,21	1,02	—
Cymarose	3,51	—	3,51	2,16	1,00	1,00

* Mean value from three different mixtures.

It can be seen from Table 3 that the molar ratio of sugars obtained agrees with that calculated for three molecules of digitoxose in digitoxin.

EXPERIMENTAL

The gas chromatography was performed on an LKhM-7A chromatograph with a flame-ionization detector. The columns used consisted of W-shaped steel tubes (internal diameter 0.4 mm, length 2 m), filled with 5% of SE-30 silicone elastomer on Diaforit (0.2-0.315 mm). The working temperature was 150°C and the carrier gas helium (60 ml/min).

Samples (10 mg each) of digitoxose, boivinose, cymarose, digitoxin, corchoroside and erisimin, and also accurately weighed mixtures of cymarose and digitoxose and of digitoxin and cymarose were dissolved in 1 ml of dioxane or tertiary butanol, 1 ml of 0.2% HCl solution was added, and the mixtures were heated in the boiling water bath for 1 h. In the hydrolysis of the glycosides, the genin was removed by extraction with chloroform. The aqueous layer was neutralized with Ag_2CO_3. The precipitate was separated off by filtration, and the resulting mixture of sugars was evaporated to dryness in vacuum.

Silylation. A dried mixture of sugars was dissolved in 0.4 ml of dry pyridine, and 0.2 ml of hexamethyldisilazane and 0.1 ml of chlorotrimethylsilane were added. After 5 min, the solvent was distilled off, and the residue was dissolved in dry benzene with filtration from the NH_4Cl. The benzene solution was evaporated to a volume of approximately 0.05 ml, and 0.3-0.4 μl was introduced into the chromatograph.

CONCLUSIONS

The possibility of the qualitative and quantitative determination of digitoxose, cymarose, and boivinose in cardiac glycosides by means of GLC has been shown.

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