IDENTIFICATION OF THE MONOSACCHARIDES OF TRITERPENE GLYCOSIDES IN THE FORM OF POLYOL TRIFLUOROACETATES

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At the present time, to determine the monomeric composition of triterpene glycosides the gas-liquid chromatography (GLC) of the silylated sugars is most frequently used [1-3]. However, under the conditions of methanolysis of saponins, each of the monosaccharides forms several isomers, which complicates their analysis. Recently, the GLC of polyol trifluoroacetates has been described in the literature [4, 5]. We have used this method to investigate the monosaccharides found in triterpene glycosides. The first stage of the study of the polyol trifluoroacetates was the determination of their relative retention times and of the calculation factors for the weight and molar methods (Table 1) [6]. As can be seen from Table 1, pentoses and hexoses have practically identical calculation factors.

TABLE 1

Trifluoroacetates of the polyols of	Relative retention times	Calculation factors for the	
		weight method	molar method
Rhamnose	0,44	1,68	1,55
Fucose	0,53	—	—
Ribose	0,74	0,90	0,91
Arabinose	0,87	1,02	1,02
Xylose	1,00*	1,00	1,00
Glucose	1,59	2,42	2,04
Galactose	1,71	2,50	2,10

*Retention time of xylose taken as the unit of calculation was 5.56 min.

Since the reduction of the monosaccharides with sodium tetrahydroborate takes place in an alkaline medium and their epimerization is therefore possible, we determined new calculation factors for the polyols (Table 2).

TABLE 2

Trifluoroacetates of the polyols of	Calculation factor for the	
	weight method	molar method
Rhamnose	0,83	0,76
Fucose	0,80	0,73
Ribose	0,93	0,93
Arabinose	0,93	0,93
Xylose	1,00	1,00
Glucose	1,65	1,38
Galactose	1,60	1,33

Table 3 gives the results of the analysis of a mixture subjected to heating under the conditions of hydrolysis and subsequent reduction. The same table gives the coefficients by which the areas of the peaks of the substances must be multiplied in order to obtain the actual weight or molar ratio of the monosaccharides.

Preliminary calculations using the best values for determining the qualitative and quantitative composition of triterpene glycosides gave satisfactory results. Detailed investigations will be published later.

EXPERIMENTAL

Chromatography was performed on a Hewlett-Packard model 5750 instrument with a flame-ionization detector. For the qualitative and quantitative determinations, a Hewlett-Packard 3370A integrator was used. The stainless-steel column, 3 m long and 3 mm in internal diameter, was filled with 1% of XE-60 on Gas Chrom Z 80/100 mesh; the carrier gas was nitrogen, 36 ml/min. The temperature of the detector was 280°C and that of the injector 240°C.

Polyol Trifluoroacetates. An excess of sodium tetrahydroborate was added to 0.1 g of a mixture of monosaccharides in water, and the reaction mixture was left overnight at room temperature. Then the reduction product was treated with Dowex-50 resin (H^+ form) which had been previously repeatedly washed free from the finest particles by decantation. The boric

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TABLE 3

Trifluoroacetates of the polyols of	Calculation factor for the weight method	Ratio of the areas of the peaks due to unit weight	Calculation factor for the molar method	Ratio of the areas of the peaks for one mole
Rhamnose	0,82	1,21	0,75	1,33
Fucose	0,91	1,10	0,83	1,21
Ribose	1,05	0,95	1,05	0,95
Arabinose	1,00	1,00	1,00	1,00
Xylose	1,00	1,00	1,00	1,00
Glucose	1,85	0,54	1,54	0,65
Galactose	1,75	0,57	1,47	0,68

acid was separated off by the evaporation of the mixture of polyols in methanol under reduced pressure. After drying over P_2O_5 overnight, the reduction product was dissolved in 0.5 ml of trifluoroacetic anhydride and 0.002 ml of absolute pyridine.

Hydrolysis of Saponins. A weighed sample of the saponins was dissolved in 2% H_2SO_4, and the solution was heated in a sealed tube at 100°C for 5 h. After cooling, the mixture was neutralized, reduced with sodium tetrahydroborate, and treated with trifluoroacetic anhydride in pyridine as described above.

SUMMARY

A method for the qualitative and quantitative characterization of the monosaccharides found in triterpene glycosides has been developed.

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