OPTIMIZATION OF THE HYDROLYSIS PROCEDURE IN THE GLC ANALYSIS OF THE QUANTITATIVE RATIOS OF MONOSACCHARIDES IN STEROID GLYCOSIDES

L. I. Strigina

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To determine the quantitative ratios of monosaccharide residues in triterpene and steroid glycosides, wide use is made of GLC [1-3]. A necessary condition for successful GLC analysis is the complete cleavage of all the glycosidic bonds in the course of the preceding acid hydrolysis. The glycosides are therefore subjected to prolonged (5-20 h) heating with 2-5% H_2SO_4, HCl, or Kiliani's mixture [1, 4]. On comparing the results of the GLC analysis of the monosaccharide compositions of a number of pennogenin glycosides with those of ^{13}C NMR spectroscopy, we found that the poor solubility of the initial glycosides and of the progenins formed in aqueous solutions of acids may lead to a considerable lowering of the result for the monosaccharide residue attached to the C-3 of the aglycone. With the aid of TLC we have shown that when these glycosides are heated with 3 N H_2SO_4 for 5 h at 100°C in an ampul, partial precipitation and resinification of pennogenin β-D-glucopyranoside takes place. Consequently for the hydrolysis of glycosides sparingly soluble in water — polygonatosides C_1 and C_2 and progenins II and III — we have used an aqueous ethanolic solution of H_2SO_4 [5, 6]. Here we describe details of the hydrolysis procedure and give correction factors to allow for the degree of degradation of the individual monosaccharides under the conditions of hydrolysis.

Samples (10 mg each) of polygonatosides C_1 and C_2 and progenins II and III were each dissolved in 3 ml of redistilled MeOH, 3 ml of 6 N H_2SO_4 was added, and the mixture was heated in a sealed ampul in the boiling water bath for 3 h. After cooling, each solution was transferred to a conical flask, 3 ml of H_2O was added, and it was evaporated in vacuum to its original volume, this operation being repeated three times. The resulting solution was boiled under reflux for 45 min and was then neutralized with Dowex 1 × 4 (HCO_3^-), and the monosaccharides were eluted with MeOH (0.25 liter). The aqueous and methanolic solutions were combined and evaporated to dryness, the residue was dissolved in 10 ml of H_2O, the solution was extracted with $CHCl_3$ (6 × 5 ml) to eliminate the aglycone and was then evaporated and the residue was dried in the vacuum pistol over $CHCl_3$ for 6 h; the monosaccharides were converted into the corresponding aldononitrile peracetates [7]. GLC analysis was performed on a Tsvet-5 instrument (FID) fitted with glass columns (91 × 0.5 cm) filled with 5% NPGS on Chromosorb W/45-60 mesh (t° 150→225°C, 4°C/min) at a rate of flow of carrier gas of 33 ml/min. To introduce a correction for degradation, a standard mixture of monosaccharides containing 10^{-4} mole each of glucose and arabinose and 2×10^{-4} moles of rhamnose was kept under the hydrolysis conditions and converted into the aldononitrile peracetates (CF Ara 0.98; CF Rha 0.91; CF Glc 1.00).

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