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Note

Separation of neutral and amino sugars by capillary gas chromatography

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Analysis of neutral sugars of the glycoproteins by gas chromatography (GC) and of the amino sugars from the same sample by ion-exchange chromatography was the most widely accepted procedure[1] until Niedermeir[2] first separated the hexosamines in glycoproteins by GC of the alditol acetate derivatives. Although glucosamine was separated from galactosamine by this method with good resolution, mannosamine was not separated from galactosamine. Also, rhamnose was not resolved from fucose and ribose was not resolved from arabinose. These workers used a $6 \times \frac{1}{4}$ in. glass column packed with 1% ECNSS-M on Gas-Chrom A. Griggs *et al.*[3] reported identical results using Gas-Chrom P (100–200 mesh) precoated with a mixture of 0.2% ethylene glycolsuccinate, 0.2% stabilized ethylene glycol adipate and 1.4% silicone XE-60 with temperature programming, beginning at 150°C with a program rate of 1°C/min to a final temperature of 205°C. A separation of 6 neutral and 2 amino sugars was reported by Metz *et al.*[4] who used OV-225 and determined the optimum hydrolysis conditions for their recovery from glycoprotein samples. Niedermeir and Tomana[5] in a subsequent study reported an effective separation of the alditol acetates of the three hexosamines using a polyamide (Poly A 103) liquid phase, but galactose was not separated from glucose and rhamnose and ribose were not included in the known mixture. A common constituent of acidic polysaccharides of plant gums and hemicelluloses has been identified as 4-O-methyl-D-glucuronic acid. One of the procedures[6] for its determination involved reduction of the polysaccharide before hydrolysis and the 4-O-methyl glucose thus formed was subsequently identified as its alditol acetate derivative. Holzer *et al.*[7], using a 20 m × 0.3 mm glass capillary column coated with a 9:1 mixture of N-propionyl-L-valine-*tert.*-butylamide polysiloxane and Witoconol LA23, with temperature programming from 90–200°C at 6°C/min, reported a separation of alditol acetates of the common neutral sugars. However, when this procedure was applied to the analysis of plant gums and hemicelluloses which contain 4-O-methyl-D-glucuronic acid, the alditol acetates of mannose and 4-O-methyl-D-glucose were not fully separated[7].

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In the present report a chiral stationary phase was used for the first time during gas–liquid chromatographic (GLC) analysis of a mixture of neutral sugars and the three hexosamines. By using a longer glass capillary column of 35 m × 0.3 mm coated with the same mixture, a complete separation of the alditol acetates of the common neutral sugars including 3-O-methyl- and 4-O-methyl-D-glucitols was accomplished. An application of this method for the separation of the alditol acetates from the hydrolysate of reduced polysaccharide from *Daemonorops* species is described.

MATERIALS AND METHODS

GC was carried out on a Varian Aerograph 2000, adapted for glass capillary work. In addition, the alditol acetates were analyzed by GLC–mass spectrometry (MS) using an LKB instrument. Identification was done by a comparison of retention time data and mass spectral fragmentation patterns with those of known standard. The glass capillaries were drawn from Pyrex glass, having a I.D. of 0.3 mm. For the analysis of the alditol acetates, columns of 20–35 m were etched with 5% KHF_3 solution[8] and deactivated using the Carbowax 20M method[9]. The column was then coated with a 0.2% stationary phase, consisting of 90% N-propionyl-L-valine-*tert.*-butylamide polysiloxane and 10% Witconal LA 23 as surfactant using the static method. The column was conditioned at 230°C with a low helium carrier gas flow-rate. For the analysis of the alditol acetates the column was operated at helium flow-rates between 4 and 6 ml/min and temperatures up to 200°C.

Solutions (0.05 *M*) of alditol acetates of neutral sugars were purchased from Regis (Morton Grove, IL, U.S.A.) while the remaining monosaccharides were derivatized by the procedure outlined in the *Operation Manual* supplied by the above company. Polysaccharide material (B-fraction) was isolated from benzene extracted hemicellulose of *Daemonorops* species after releasing it during the delignification by the method of Whistler *et al.*[10]. The purified polysaccharides were reduced by reaction of the propionated methyl ester with lithium borohydride in tetrahydrofuran, hydrolyzed with sulfuric acid, reduced with sodium borohydride and acetylated by the procedure of Dutton and Kabir[6].

RESULTS AND DISCUSSION

Chiral polysiloxane phases were introduced by Frank *et al.*[11,12] for the separation of enantiomeric amino acids. The polarity of the phase and its thermal stability make it useful for the analysis of a variety of compounds[13].

Gas chromatograms of the mixture of alditol acetates of the 13 common sugars and the three hexosamines is shown in Fig. 1. The peaks were identified by co-chromatography and GLC–MS. The results showed that except for the acetates of D-mannitol and 4-O-methyl-D-glucitol, all of the other alditol acetates were well separated by this method. The procedure can be applied for the identification of sugars in the glycoproteins which contain one or more neutral and amino sugars.

In order to obtain a complete separation of D-mannitol and 4-O-methyl-D-glucitol, the sample mixture of 13 alditol acetates was injected in a 35 m × 0.3 mm glass capillary column. The results presented in Fig. 2 showed that all of the components were fully separated. The longer column used in this study prolonged the

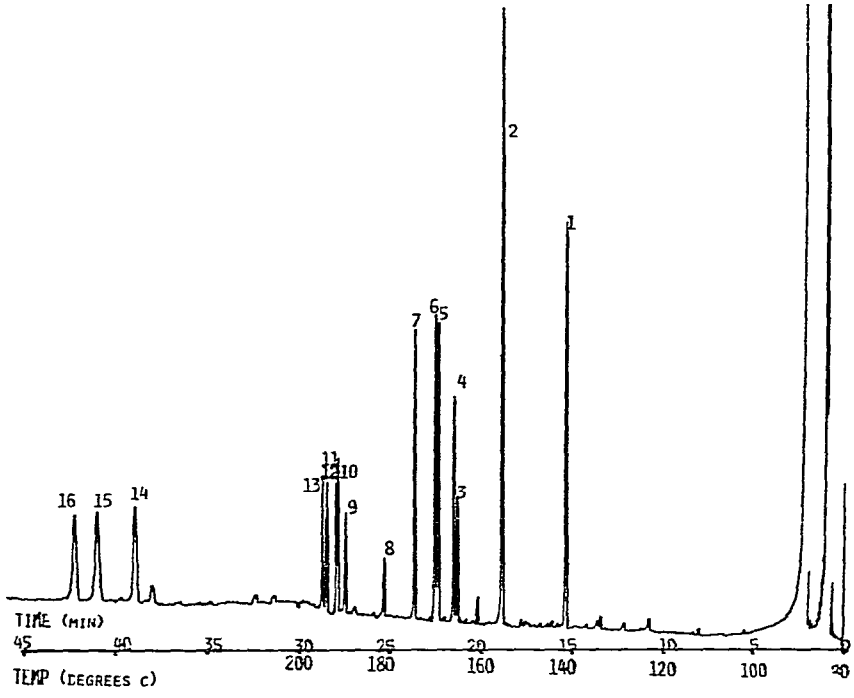

Fig. 1. Gas chromatograph of alditol acetates of the neutral sugars and the three hexosamines. Column: 20 m × 0.3 mm glass capillary column coated with 9:1 mixture of N-propionyl-L-valine-*tert*-butylamide polysiloxane and Witconal LA 23. Temperature: 80–200°C at 4°C/min for 30 min and isothermal at 200°C. Helium pressure: 18 p.s.i., flame-ionization detector. Peaks: 1 = erythritol; 2 = D-2-deoxyribitol; 3 = L-rhamnitol; 4 = L-fucitol; 5 = ribitol; 6 = arabitol; 7 = xylitol; 8 = D-2-deoxyglucitol; 9 = 3-O-methyl-D-glucitol; 10 = 4-O-methyl-D-glucitol; 11 = D-mannitol; 12 = D-galactitol; 13 = D-glucitol; 14 = N-acetyl glucitol; 15 = N-acetyl-galactitol; 16 = acetyl mannitol.

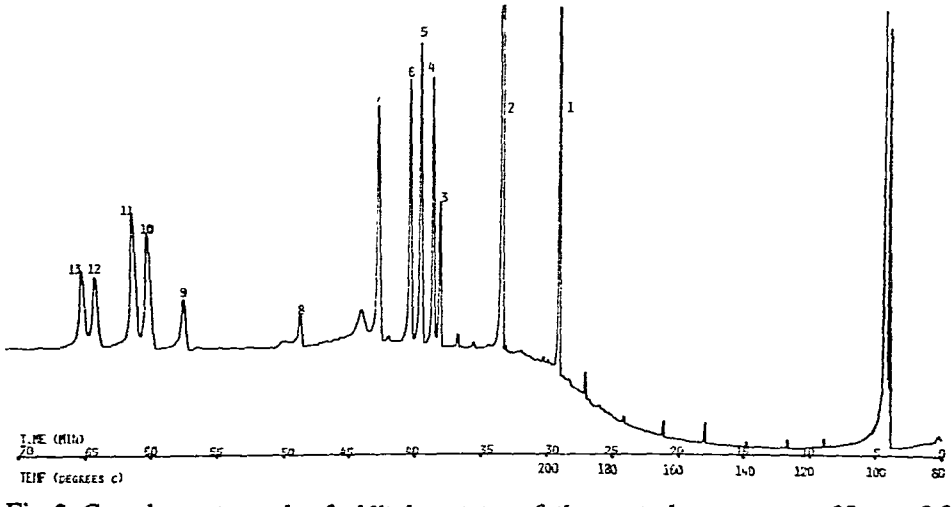

Fig. 2. Gas chromatograph of alditol acetates of the neutral sugars on a 35 m × 0.3 mm glass capillary coated with the same mixture as in Fig. 1. Temperature program: 80–200°C at 4°C/min and isothermal at 200°C; Peaks 1–13 same as in Fig. 1.

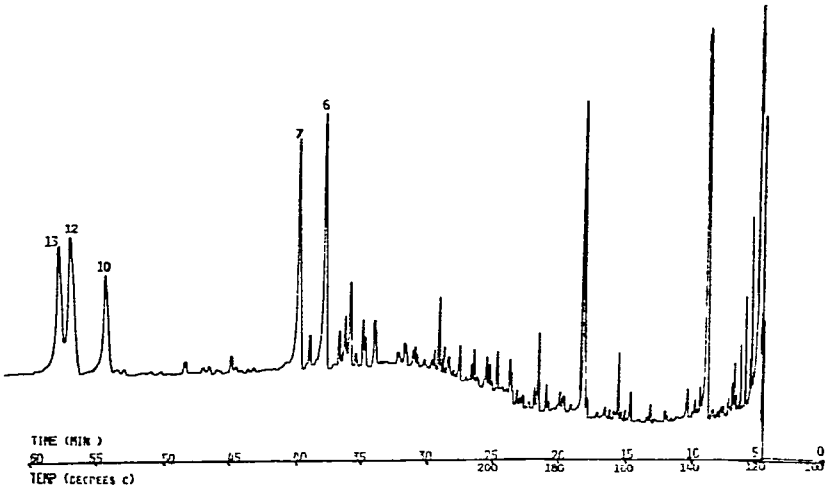

Fig. 3. Gas chromatograph of alditol acetates from the hydrolysate of reduced polysaccharide from *Daemnorops* species. Column, helium pressure and peaks were the same as in Fig. 2. Temperature program: 100–200°C at 4°C/min and isothermal at 200°C.

analysis time in comparison with Fig. 1 but a complete separation of all sugars was obtained. This procedure would be recommended for the analysis of sugars in plant gums or hemicelluloses which may contain 4-O-methyl-D-glucuronic acid and one or more neutral sugars.

The GLC profile of the alditol acetates of the sugars from the hemicellulose of *Daemonorops* species reported in Fig. 3 showed the presence of arabinose, xylose, 4-O-methyl-D-glucuronic acid, galactose and glucose. The results reported[7] earlier with the 20-m capillary column showed that D-mannitol and 4-O-methyl-D-glucitol were so close to each other that without mass spectral fragmentation pattern, the two could not be identified. In this study, however, by GLC alone or by co-chromatography, the two components could be easily identified. The components which emerged between 120 and 180°C before the alditol acetates may be the result of impurities in the polysaccharide or from the reagents used in the isolation of the sugars. The advantage of the chiral phase in the analysis of alditol acetates is its potential thermal stability as well as good resolving power.

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