*Journal of Chromatography*, 194 (1980) 410–415
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,780

## Note

# Separation of monosaccharides as their alditol acetates by capillary column gas–liquid chromatography

G. HOLZER* and J. ORÓ
*Departments of Biophysical Sciences and Chemistry, University of Houston, Houston, TX 77004 (U.S.A.)*
and
S. J. SMITH and V. M. DOCTOR
*Department of Chemistry, Prairie View A & M University, Prairie View, TX 77445 (U.S.A.)*
(Received February 26th, 1980)

Rapid separation and quantitative estimations obtained by gas–liquid chromatography (GLC) make it ideally suited for a number of constituents in biological materials.

Separation of a wide range of carbohydrates from mono- to tetrasaccharides as their trimethylsilyl derivatives was first reported by Sweeley *et al.*[1] and applied for quantitative estimation by Sawardeker and Slonekar[2] who used a column coated with Carbowax 20M, and accomplished a good separation of all the anomers of D-glucose from D-galactose and D-mannose, but pentoses and deoxyhexoses were incompletely resolved. The fact that each monosaccharide gave more than one peak due to the formation of anomeric derivatives led to a search for a means of eliminating this complication. The simplest procedure reported to date is the removal of the anomeric center by reduction to the alditols which are then readily acetylated and separated by GLC[3,4]. These methods, however, did not resolve D-glucitol and galactitol. Little progress was made for 4 years subsequent to these studies until ECNSS-M, a copolymer of ethylene glycol succinate and cyanoethyl silicone was introduced as the liquid phase by Sawardeker *et al.*[5]. This procedure was capable of resolving all the common alditols through the hexitols in 70 min, when programmed from 155 to 190°C. Since that time the method has been used for the determination of neutral aldoses in wood hemicellulose[6], plantcell-wall polysaccharides[7] and glycoproteins[8–10]. The liquid phase, however, has a low maximum operating temperature and often columns packed with this material have a short lifetime.

A common constituent of the acidic polysaccharides of plant gums[11–15], hemicellulose constituent of grasses[16] and wood pulps[17] has been identified as 4-O-methyl-D-glucuronic acid. There are no satisfactory methods available for its determination because the linkage between uronic acid and the aldose sugars in the polysaccharide are more resistant toward acid hydrolysis than the linkages between neutral sugars units[18] and if conditions resulting in a complete cleavage are used, a considerable decomposition of the uronic acid cannot be avoided. One of the alternative routes involves reduction of the uronic acid function in the polysaccharide before hydrolysis. The presence of 4-O-methyl-D-glucose was then identified in the

0021-9673/80/0000–0000/$02.25 

acid hydrolysate by converting it to the alditol acetate derivative and comparing its retention time with the peak of the reference sample using GLC[16,17,19]. These procedures however, did not separate galactitol hexaacetate from 4-O-methyl-glucitol pentaacetate[16], or galactitol hexaacetate from glucitol hexaacetate[19] or 4-O-methyl-D-glucitol pentaacetate from D-mannitol hexaacetate[16,17,19] and, in general, were slow because the column packings were unstable at higher temperatures.

In the present report a chiral stationary phase was used for the first time during the GLC analysis of alditol acetates. Chiral polysiloxane phases were introduced by Frank *et al.*[20–21] 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[22]. For the separation of alditol acetates, glass capillaries were coated with N-propionyl-L-valine–*tert.*-butylamide polysiloxane. The alditol acetates of the common neutral sugars and the three common hexaacetates of the aldehexoses from the pentaacetates of 3-O-methyl- and 4-O-methyl-D-glucitols can be resolved within 25 min on this column. The application of this procedure to plant gums and hemicellulose 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 a LKB 9000 instrument. Identification was done by a comparison of retention time data and mass spectral fragmentation patterns with those of known standards. The glass capillaries were drawn from Pyrex glass, having an I.D. of 0.30 mm. For the analysis of the alditol acetates, columns of 10–30 m were etched with 5% $KHF_2$ solution[23] and deactivated using the Carbowax 20M method[24]. The column was then coated with a 0.2% stationary phase, consisting of 90% N-propionyl-L-valine–*tert.*-butylamide polysiloxane and 10% Witconal LA23 as surfactant using the static method. The column was conditioned at 230° 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 220°C.

Polysaccharide from frankincense was prepared by the method of Jones and Nunn[14] while the procedure of Jones and Thomas[15] was employed for the isolation of asafoetida polysaccharide. Specific rotations of both the polysaccharides were within 5% of the reported values[14,15]. 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.*[25]. The purified polysaccharides were reduced by reaction of the propionated methyl ester with lithium borohydride in tetrahydrofuran, hydrolyzed with $H_2SO_4$, reduced with sodium borohydride and acetylated by the procedure of Dutton and Kabir[19].

## RESULTS AND DISCUSSION

The gas chromatogram of 13 alditol acetates is shown in Fig. 1. The peaks were identified by co-chromatography and by GLC–MS. Under the operating conditions, the acetates of D-mannitol and 4-O-methyl-D-glucitol are not fully separated, however for all the other alditol acetates a complete separation was obtained. This

is apparently the first report where a complete separation of acetates of L-rhamnitol from L-fucitol and ribitol from L-arabinitol was obtained by GLC in less than 25 min. Earlier procedures using 3% ECNSS-M[25] or a mixture of 0.2% ethylene glycol adipate and 1.4% silicone XE-60[27] with temperature programmed from 155°C at 1°C/min could not separate these compounds. The analysis time can be further shortened without significant loss of resolution by operating the column at a higher starting temperature or an increased resolution may be obtained by using longer capillaries but with prolonged analysis time.

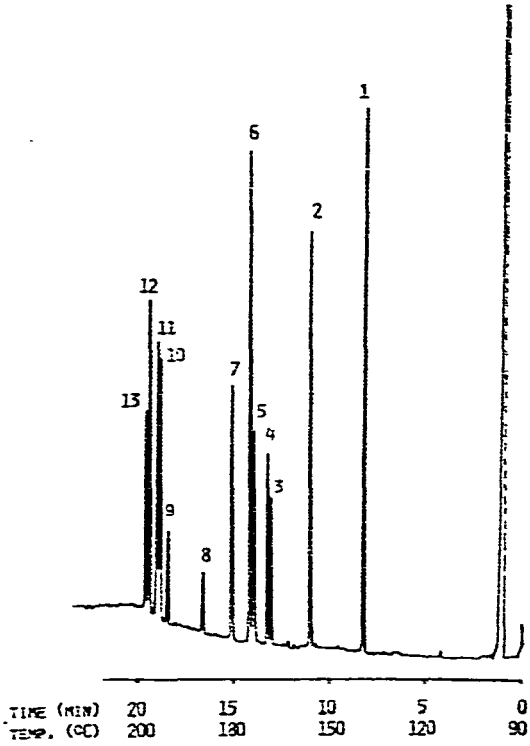


Fig. 1. Gas chromatogram of alditol acetates on a 20 m × 0.3 mm glas capillary column coated with a 9:1 mixture of N-propionyl-L-valine-*tert.*-butylamide polysiloxane and Witconol LA23. Temperature program: 90–200°C at 6°/min; helium pressure; 18 p.s.i., flame-ionization detector. Peaks: 1 = erythritol, 2 = D-2-deoxyribitol, 3 = L-rhaminitol, 4 = L-fucitol, 5 = ribitol, 6 = L-arabinitol, 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.

The gas chromatograms of the alditol acetates from the hydrolysate of reduced asafoetida and frankincense polysaccharides are shown in Figs. 2 and 3. The GLC profiles of the two samples were very similar in sugar composition with 4-O-D-methyl glucuronic acid as a major constituent of both the samples. The asafoetida polysaccharide contained about 2% rhamnose and the principal sugar was arabinose[28] while the frankincense polysaccharide showed only trace amounts of rhamnose and fucose with galactose as the principal sugar[14]. These results are in agreement with earlier reports where the separations of the sugars were performed by column chro-

matography[14,28]. The GLC profile of the alditol acetates of the sugars from the hemicellulose of *Daemonorops* species is reported in Fig. 4. The results showed the presence of rhamnose, arabinose, xylose, 4-O-methyl-D-glucuronic acid, galactsoe and glucose. The report by Blake and Richards[16] on the uronic acid of the hemicelluloses showed 4-O-methyl-D-glucuronic acid as the common constituent of plants and trees and was isolated from the acidic component in the acid hydrolysate by ion-exchange separation. This procedure[16] required a large starting sample and the isolated material was identified by paper chromatography. The results presented in Figs. 2 and 4 showed one or more unidentified components which emerged between 100 and 140°C. These may have resulted from impurities present in the polysaccharide or in the reagents used for its isolation. No attempt was made during the present study to identify them.

The advantage of the chiral phase in the analysis of alditol acetates is its potential thermal stability, as well as good resolving power for the acetates. The GLC determination of the aldohexoses and 4-O-methyl-D-glucuronic acid can be

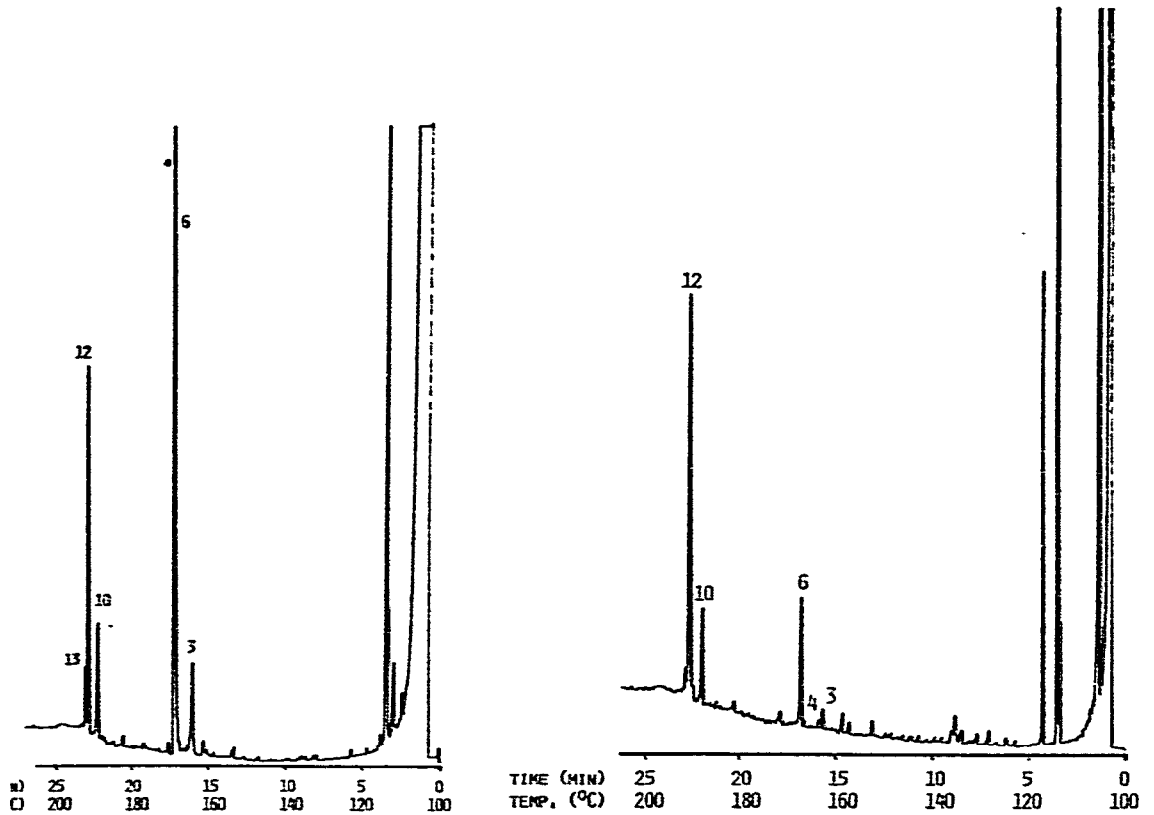


Fig. 2. Gas chromatogram of alditol acetates from the hydrolysate of reduced asafoetida polysaccharide. Column and peaks as in Fig. 1. Temperature program: 100–200°C at 4°/min; helium pressure: 18 p.s.i.

Fig. 3. Gas chromatogram of alditol acetates from the hydrolysate of reduced polysaccharide of frankincense. Column and peaks as in Fig. 1. Temperature program: 100–200°C at 4°/min; helium pressure: 18 p.s.i.

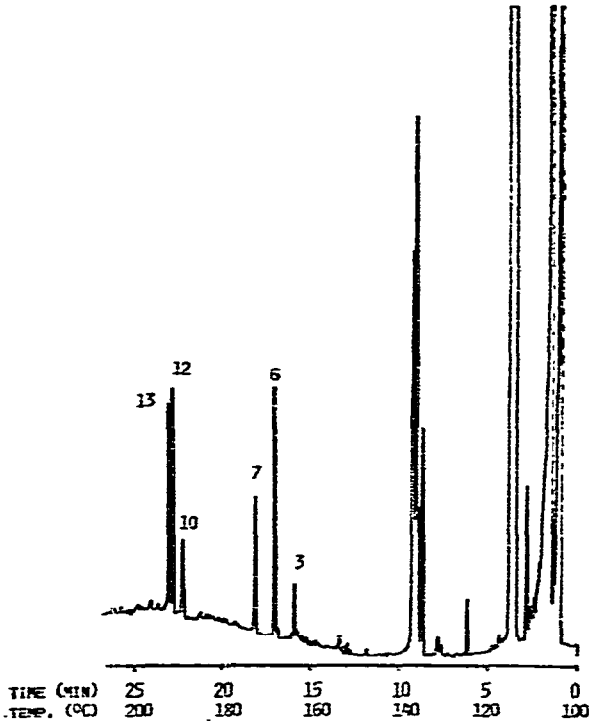


Fig. 4. Gas chromatogram of alditol acetates from the hydrolysate of reduced polysaccharide from *Daemonorops* species. Column and peaks as in Fig. 1. Tempeature program: 100–200°C at 4°/min; helium pressure: 18 p.s.i.

carried out in less than 25 min with a satisfactory separation for routine quantitative analysis.

## ACKNOWLEDGEMENTS

We would like to acknowledge the generous gift of N-propionyl-L-valine-*test.*-butylamide polysiloxane by Dr. H. Frank.

This work was supported by the Robert A. Welch foundation, Houston, TX, Grant No. RR0894-01A1 from the National Institutes of Health, and Grant NGR-44-005-002 from the National Aeronautics and Space Administration, Washington, DC.

## REFERENCES

1 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
2 J. S. Sawardeker and J. H. Sloneker, *Anal. Chem.*, 37 (1965) 1602.
3 S. W. Gunner, J. K. N. Jones and M. B. Perry, *Chem. Ind. (London)*, (1961) 255.
4 S. W. Gunner, J. K. N. Jones and M. B. Perry, *Can. J. Chem.*, (1961) 1892.
5 J. S. Sawardeker, J. H. Sloneker and A. Jeanes *Anal. Chem.*, 37, (1965) 1602.
6 E. P. Crowell and B. B. Burnett, *Anal. Chem.*, 39 (1967) 121.
7 P. Albersheim, D. J. Nevins, P. D. English and A. Karr, *Carbohyd. Res.*, 5 (1967) 340.

8 J. H. Kim, B. Shome, T. Liao and H. G. Pierce, *Anal. Biochem.*, 20 (1967) 258.
9 T. Takahashi, P. Ramchadramurthy and I. E. Liener, *Biochim. Biophys. Acta*, 133 (1967) 123.
10 W. F. Lemhardt and R. J. Winzler, *J. Chromatogr.*, 34 (1968) 471.
11 E. V. White, *J. Amer. Chem. Soc.*, 70 (1948) 367.
12 F. Smith, *J. Chem. Soc.*, (1951) 2646.
13 L. Hough, J. K. N. Jones and W. H. Wasman, *J. Chem. Soc.*, (1952) 796.
14 J. K. N. Jones and J. R. Nunn, *J. Amer. Chem. Soc.*, 77 (1955) 5745.
15 J. K. N. Jones and G. H. S. Thomas, *Can. J. Chem.*, 39 (1961) 192.
16 J. D. Blake and G. N. Richards, *Carbohyd. Res.*, 14 (1970) 375.
17 E. Sjostrom, S. Juslin and E. Seppala, *Acta Chem. Scand.*, 23 (1969) 3610.
18 T. E. Timell, *Advan. Carbohyd. Chem.*, 19 (1964) 271.
19 G. G. S. Dutton and S. Kabir, *Anal. Lett.*, 4 (1971) 95.
20 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
21 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 167 (1978) 187.
22 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
23 R. A. Heckmann, C. R. Green and F. W. Best, *Anal. Chem.*, 50 (1978) 2157.
24 L. Blomberg, *J. Chromatogr.*, 115 (1975) 365.
25 R. L. Whistler, J. Bachrach and D. R. Bowman, *Arch. Biochem.*, 19 (1948) 25.
26 J. H. Sloneker, *Meth. Carbohydr. Chem.*, 6 (1972) 20.
27 L. J. Griggs, A. Post, E. R. White, J. A. Finkelstein, W. E. Moeckel, K. G. Holden, J. E. Zarembo and J. A. Weisbach, *Anal. Biochem.*, 43 (1971) 369.
28 L. Be'zanger-Beauguesne and J. Chosson, *Ann. Pharm. Fr.*, 16 (1958) 665.