Carbohydrate Research, 90 (1981) 127–130
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

The use of 1-methylimidazole as a solvent and catalyst for the preparation of aldononitrile acetates of aldoses

CHYI C. CHEN AND GARY D. MCGINNIS
Forest Products Utilization Laboratory, Mississippi State University, Mississippi State, Mississippi 39762 (U.S.A.)

(Received July 28th, 1980; accepted for publication, September 29th, 1980)

Carbohydrates cannot be analyzed directly by gas–liquid chromatography (g.l.c.) because of their low volatility and their thermal instability. Consequently, carbohydrates are converted into more volatile derivatives, such as the trimethylsilyl ethers[1,2], alditol acetates[3], and aldononitrile acetates[4–6]. For g.l.c., the major advantage of the trimethylsilyl ethers is their ease of formation; however, they give multiple peaks for individual reducing-sugars. In contrast, both the alditol acetate and aldononitrile acetate derivatives give single peaks for individual reducing-sugars, but, generally, 40–100 min is needed for preparation of the derivatives. The aldononitrile acetates are excellent derivatives for carbohydrates, not only because of their chromatographic properties, but also because they provide excellent electron-impact and chemical-ionization mass spectra that are particularly suitable for structural identification.

The object of this study was to determine whether 1-methylimidazole could be used as a solvent and catalyst for the preparation of aldononitrile acetates of sugars.

EXPERIMENTAL

Preparation of aldononitrile acetates. — A stock solution was prepared by dissolving hydroxylamine hydrochloride (5 g; the weight used in the derivatization was approximately the same as that of the sugars) and, as the internal standard, D-glucitol (1 g) in 1-methylimidazole (100 mL). The stock solution (0.5 mL) was added to a 6.3-mL vial (closable by a vinyl-lined screw-cap) that contained the sugars (25 mg; 5 mg of each) in 1-methylimidazole (0.5 mL). The vial was kept for 5 min in a heating block at 50°, and then removed; acetic anhydride (0.3 mL) was added, and, after 5 min, chloroform (1 mL) was added, and the solution was washed with water (3 × 2 mL), and dried (anhydrous sodium sulfate); the sample was then ready for g.l.c. analysis.

Separation and identification. — The sample was analyzed by g.l.c. in a stainless-steel column (1.83 m × 3.17 mm) packed with 3% of OV-225 plus 2.5% of high-efficiency 9BP on Supelcoport (80–100 mesh) (Supelco, Inc., Bellefonte, PA). Aldononitrile acetates were eluted isothermally at 210°, with helium as the carrier gas at 25 mL/min. For comparison, aldononitrile acetates were also prepared by the method of Vercellotti *et al.*[4]. Electron-impact mass spectra of the aldononitrile acetates were recorded with a DuPont model 21-490B gas–liquid chromatography–mass spectrometry instrument at an ionization potential of 70 eV. The instrument was fitted with a stainless-steel column (1.83 m × 3.17 mm) packed with 5% of OV-17 on Wbmcs (60–80 mesh). The sugar derivatives were eluted isothermally at 230°.

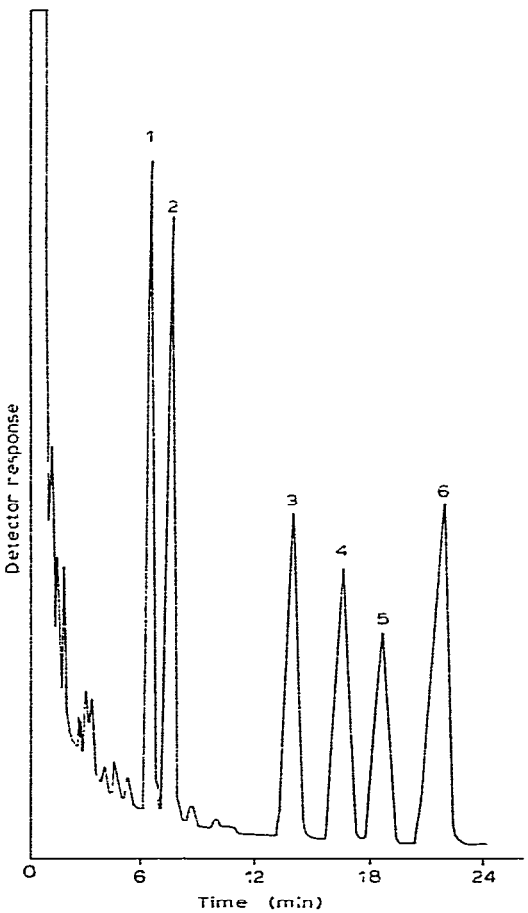

Fig. 1. Gas–liquid chromatogram of aldononitrile acetates: 1, L-arabinose; 2, D-xylose; 3, D-mannose; 4, D-glucose; 5, D-galactose; and 6, D-glucitol. [Stainless-steel column (1.83 m × 3.17 mm) with 3% of OV-225 plus 2.5% of high-efficiency 9BP on Supelcoport (80–100 mesh) at 210°.]

RESULTS AND DISCUSSION

The use of 1-methylimidazole as an acetylating catalyst was reported by Connors and co-workers[7,8]. They used it as the catalyst, base, and solvent for acetylation, and found that it is much more effective than pyridine for acetylating alcohols, glycols, phenols, and sugars.

Usually, the formation of aldononitrile acetates requires 40–100 min. In the present study, it was found that the formation of aldononitrile acetates of aldoses was complete in less than 10 min when 1-methylimidazole was used instead of pyridine.

A typical gas–liquid chromatogram of the derivatives of some aldoses (L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose) is shown in Fig. 1. The retention times of the derivatives were identical with those of aldononitrile acetates prepared by another method[4]. These derivatives were further confirmed as being aldononitrile acetates by g.l.c.–m.s. The molecular ions were not observed; however, all mass spectra showed the characteristic M — 59 (-OAc) and M — 73 ($-CH_2OAc$) ions, and matched well with available reference spectra[9]. To test the reproducibility of the method, five preparations were made from each sugar, 5 mg of each sugar being used for derivatization. One of the preparations was arbitrarily chosen for the calculation of the conversion factors for each sugar, using the peak area (height × width at half-height), and D-glucitol as the internal standard. The amount of sugar recovered in the other four preparations was calculated, based on the conversion factors; the results are shown in Table I.

After being washed with water, the chloroform fraction is colorless; however, it becomes colored should an excess of acetic anhydride be used. Although coloration does not seem to affect derivatization, extra peaks may appear near the peaks of the pentoses. If this happens, the interfering peaks may be obviated by lessening the proportion of acetic anhydride.

The method appears to provide a rapid and convenient way in which to quantify neutral sugars.

TABLE I

REPRODUCIBILITY OF THE METHOD

Compound	*Retention time (min)*[a]	*Amount*[b] *added (mg)*	*Amount recovered (mg) Experiment number*			
			1	*2*	*3*	*4*
L-Arabinose	6.4	5.0	4.8	5.1	5.2	5.3
D-Xylose	7.5	5.0	4.7	4.8	5.0	4.7
D-Mannose	14.0	5.0	4.8	4.6	4.6	5.1
D-Glucose	16.7	5.0	5.2	4.8	4.9	5.1
D-Galactose	18.7	5.0	4.9	4.6	4.8	5.0
D-Glucitol	21.8	5.0	5.0	5.0	5.0	5.0

[a]Same as original[4]. [b]D-Glucitol was used as the internal standard.

REFERENCES

1 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
2 A. E. PIERCE, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, IL 61105.
3 J. S. SAWARDEKER, J. H. SLONEKER, AND A. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
4 B. W. LI, T. W. COCHRAN, AND J. R. VERCELLOTTI, *Carbohydr. Res.*, 59 (1977) 567–570.
5 T. P. MAWHINNEY, M. S. FEATHER, G. J. BARBERO, AND J. R. MARTINEZ, *Anal. Chem.*, 101 (1980) 112–117.
6 F. R. SEYMOUR, E. C. M. CHEN, AND S. H. BISHOP, *Carbohydr. Res.*, 73 (1979) 19–45.
7 R. WACHOWIAH AND K. A. CONNORS, *Anal. Chem.*, 51 (1979) 27–30.
8 K. A. CONNORS AND N. K. PANDIT, *Anal. Chem.*, 50 (1978) 1542–1545.
9 J. SZAFRANEK, C. D. PFAFFENBERGER, AND E. C. HORNING, *Carbohydr. Res.*, 38 (1974) 97–105.