

Separation of *O*-acetyl-*O*-methyl-D-gluconitriles by gas chromatography

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Partially methylated derivatives of D-glucose have been converted to *O*-acetyl-*O*-methyl-D-gluconitriles and separated by gas chromatography. A method has been developed by means of which, using two columns of different polarity, all theoretically possible D-glucofuranose methyl ethers can be unquestionably identified.

Парциально метилированные производные D-глюкозы, были разделены газовой хроматографией после их превращения в пер-*O*-ацетил-*O*-метил-D-глюконитрилы. Был разработан метод, которым можно с применением двух колонок разной полярности точно идентифицировать все теоретически возможные частично метилированные производные D-глюкопиранозы.

Since carbohydrate *O*-methyl derivatives having the hemiacetyl hydroxy group unsubstituted are not amenable to the analysis by gas chromatography, substances of this class have been analyzed in the form of a variety of derivatives. Partially methylated derivatives of D-glucose have been separated as methyl glycosides [1], per-*O*-trimethylsilyl derivatives [2], and as the corresponding alditol per-*O*-acetates [3, 4]. The method involving the methyl glycosides of partially methylated aldoses having more than four carbon atoms in sequence is inconvenient since when both pyranose and furanose structures are possible, glycosidation of each component in the analyzed mixture may result in the formation of different proportion of up to four glycosides. This disadvantage was eliminated by *Björndal et al.* [3] who successfully separated a mixture of partially methylated derivatives of D-glucose in the form of the corresponding alditol acetates. The method has been adapted for the use in the combination with mass spectrometry [5] and has been widely used in structural investigations.

The fact that the conversion to the corresponding alditols resulted in the formation of identical products from different D-xylose methyl ethers was overcome by *Lance and Jones* [6] who prepared a series of partially methylated D-xylofuranose acetates. Aldonitriles are stable, easy-to-prepare substances and the reaction of their preparation was found to be quantitative [7—9].

Kochetkov et al. [10] described mass spectral characteristics of some partially methylated gluconitrile acetates and found the type of derivatives suitable for mass spectral identification. The gas chromatographic retention data were, however, not given in their work and, therefore, as a contribution to the methylation

analysis of substances containing D-glucose, the present work was focussed on the separation of all theoretically possible methyl ethers of D-glucopyranose by gas chromatography in the form of the corresponding D-glucononitrile acetates and the collection of their retention data.

Experimental

Gas chromatography was carried out using a Hewlett—Packard Model 5750 G gas chromatograph equipped with a flame-ionization detector. Nitrogen rid of oxygen and moisture was used as the carrier gas. The injection port was kept at 220°C and the temperature of the detector was 250°C.

The columns used (stainless steel, 304 ss, 300 cm, i.d. 2.16 mm) and the operating conditions applied were as follows: *A.* 3% QF-1 on Gas Chrom Q, 100/120 mesh (Applied Sciences Laboratories); packing density 0.54 g ml⁻¹; $p_c = 11.76798 \times 10^4$ N m⁻²; $t = 180^\circ\text{C}$; flow rate $F_c^{\text{MRC}} = 17.9$ ml min⁻¹; $n_{\text{CHUCN}} = 5100$; *B.* 3% SP-2340 on Supelcoport, 100/120 mesh (Supelco, Inc.); packing density 0.36 g ml⁻¹; $p_c = 24.51662 \times 10^4$ N m⁻²; $t = 200^\circ\text{C}$; flow rate $F_c^{\text{MRC}} = 30.0$ ml min⁻¹; $n_{\text{CHUCN}} = 7500$.

Derivatives

Methyl derivatives of D-glucose were prepared by the known procedures [11—14] and their conversion to the corresponding *O*-acetyl-*O*-methyl-D-glucononitriles was carried out in the following manner: A sample of the substance (1—5 mg) was dissolved in a solution (150 μ l) of hydroxylamine hydrochloride (100 mg ml⁻¹) in pyridine and heated at 110°C for 15 min. Acetic anhydride was added (200 μ l) and the reaction mixture was kept at 110°C for further 30 min. The mixture was cooled to room temperature and, after addition of water (1 ml), concentrated to dryness using a rotary evaporator. Chloroform (100 μ l) was added to the residue and the resulting solution was injected into the column.

Results and discussion

Applying the elaborated procedure involving the use of two columns of different polarity it is possible to identify unambiguously all theoretically possible methyl ethers of D-glucopyranose. The method may find application in the identification of the products of methylation analysis of substances containing D-glucose or, after a suitable reduction of the carboxyl group, D-glucofuranuronic acid.

O-Acetyl-*O*-methyl-D-glucononitriles are polar substances and when gas chromatographed on desactivated support materials coated with phases showing high McReynolds constants, symmetrical peaks are observed. Of the phases tested best results were obtained using methyl cyanopropyl silicone (QF-1) on which only one pair of compounds, namely 5,6-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucononitrile and 2,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-D-glucononitrile, could not be separated. The material capable of separating these two substances was found to be Supelcoport coated with SP-2340, which makes an even more polar packing and shows a sufficient thermal stability. The column, however, must be prepared very carefully since it is essential that the efficiency of $n = 7500$, required to accomplish this separation, be achieved. The number of theoretical plates was determined for

2,3,4,5,6-penta-*O*-acetyl-D-gluconitrile which appears as the last peak of the series of the substances under investigation and shows, under the given working conditions, a retention time of 62 min. On this column another pair of substances, namely 3,4,5,6-tetra-*O*-acetyl-2-*O*-methyl-D-gluconitrile and 2,5,6-*tri-O*-

Table 1
Retention times of *O*-acetyl-*O*-methyl-D-gluconitriles relative to
5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-gluconitrile (RRT)

<i>O</i> -Methyl	<i>O</i> -Acetyl	RRT	
		3% QF-1 180°C	3% SP-2340 200°C
2,3,4,6	5	1.00 (8.03 min)	1.00 (6.53 min)
2,3,4	5,6	2.23	2.27
2,3,6	4,5	2.06	2.50
2,4,6	3,5	1.44	1.40
3,4,6	2,5	2.23	2.13
2,3	4,5,6	4.04	5.11
2,4	3,5,6	3.34	3.28
2,6	3,4,5	2.67	2.77
3,4	2,5,6	5.13	5.31
3,6	2,4,5	3.56	4.07
4,6	2,3,5	2.93	2.89
2	3,4,5,6	4.79	5.26
3	2,4,5,6	7.16	8.85
4	2,3,5,6	6.70	6.81
6	2,3,4,5	4.62	4.63
	2,3,4,5,6	8.75	9.60

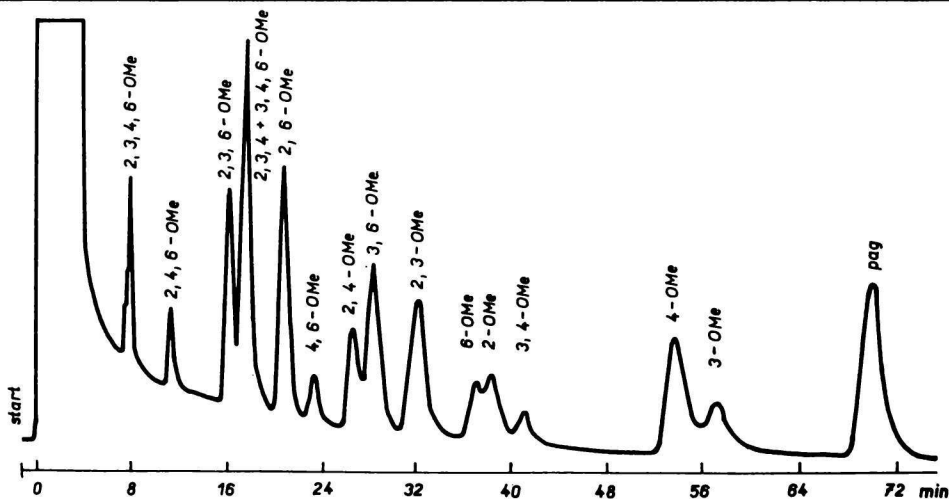


Fig. 1. Separation of *O*-acetyl-*O*-methyl-D-gluconitriles on 3% QF-1 at 180°C.
pag = 2,3,4,5,6-penta-*O*-acetyl-D-gluconitrile.

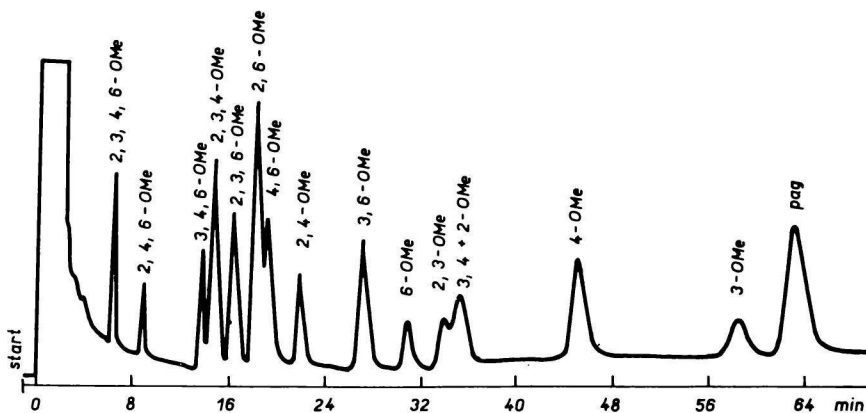


Fig. 2. Separation of *O*-acetyl-*O*-methyl-*D*-glucononitriles on 3% SP-2340 at 200°C.
pag = 2,3,4,5,6-penta-*O*-acetyl-*D*-glucononitrile.

-acetyl-3,4-di-*O*-methyl-*D*-glucononitrile, was eluted as one peak. Hence, for safe identification of all substances in the series of methyl ethers of *D*-glucopyranose both columns have to be used.

The retention times relative to 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-*D*-glucononitrile (RRT) are given in Table 1. Figs. 1 and 2 show the elution patterns observed for the mixtures of *O*-acetyl-*O*-methyl-*D*-glucononitriles.

References

1. Jones, H. G., *Methods Carbohydr. Chem.* **6**, 25 (1972).
2. Haworth, S., Roberts, J. G., and Sagar, B. F., *Carbohydr. Res.* **9**, 491 (1969).
3. Björndal, H., Lindberg, B., and Svensson, S., *Acta Chem. Scand.* **21**, 1801 (1967).
4. Jones, H. G. and Jones, J. K. N., *Can. J. Chem.* **47**, 3269 (1969).
5. Björndal, H., Hellerqvist, C. G., Lindberg, B., and Svensson, S., *Angew. Chem., Int. Ed. Engl.* **9**, 610 (1970).
6. Lance, D. G. and Jones, J. K. N., *Can. J. Chem.* **45**, 1995 (1967).
7. Deulofeu, V., *Advan. Carbohydr. Chem.* **4**, 119 (1949).
8. Easterwood, V. M. and Huff, B. J. L., *Svensk Papperstidn.* **72**, 768 (1969).
9. Varma, R., Varma, R. S., and Wardi, A. H., *J. Chromatogr.* **77**, 222 (1973).
10. Dmitriev, B. A., Backinowsky, L. V., Chizhov, O. S., Zolotarev, B. M., and Kochetkov, N. K., *Carbohydr. Res.* **19**, 432 (1971).
11. Bourne, E. J. and Peat, S., *Advan. Carbohydr. Chem.* **5**, 145 (1950).
12. Kováč, P. and Longauerová, Ž., *Chem. Zvesti* **26**, 179 (1972).
13. Kováč, P., *Chem. Zvesti* **24**, 218 (1970).
14. Kováč, P. and Longauerová, Ž., *Carbohydr. Res.* **25**, 253 (1972).

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