Journal of Chromatography, 205 (1981) 460–463
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,405

Note

Gas chromatography of inositols as their hexakis-O-acetyl derivatives

GEORGE CLIFFORD JOHN IRVING
Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T., 2601 (Australia)
(Received October 7th, 1980)

The initial separation of isomeric inositols by gas–liquid chromatography (GLC) was as their hexakis-O-acetyl derivatives[1]. This method was quickly superseded by trimethylsilylation[2] and still later by trifluoroacetylation[3]. The latter two methods were obviously preferred because of their ease of reaction, the relatively high volatility of the derivatives and the superior resolution of the isomers. These methods, however, are not without drawbacks when applied to the impure inositol extracts obtained from such natural sources as soils.

Oades[4] was critical of trimethylsilylation on the grounds that it is not quantitative and that in contrast to acetyl derivatives many TMS derivatives of carbohydrates cannot be crystallized. Therefore he preferred the use of acetylation in a GLC study of alditols prepared from hydrolysates of soil carbohydrates.

In a paper published in 1949 Bourne *et al.*[5] showed that trifluorocetic anhydride acts as a promoter of ester formation between hydroxy compounds and carboxylic acids, and they used it to prepare carboxylic esters of a number of carbohydrates and phenols. The reaction requires only the anhydride, the hydroxy compound and the carboxylic acid and proceeds rapidly to completion at room-temperature. The conclusion to be drawn from the observations of Bourne *et al.* is that the use of trifluoroacetic anhydride should be restricted to pure hydroxy compounds since the presence of any impurities containing carboxylic acid groups would result in the synthesis of the wrong ester.

Attempts, in this laboratory, to prepare hexakis-O-trifluoroacetyl derivatives of inositols which had been isolated from soil hydrolysates yielded inconsistent results, and in some instances peaks which could be attributed to hexakis-O-trifluoroacetyl inositols were observed to disappear from the sample after periods as short as 1 h. These difficulties, considered together with the above facts suggested that a re-evaluation of the use of hexakis-O-acetyl inositols for GLC might be appropriate.

EXPERIMENTAL

Materials

The trifluoroacetic anhydride (laboratory reagent grade) and the acetic acid (analaR grade) were obtained from BDH (Poole, Great Britain). The hexitols were commercial products obtained from various suppliers and the inositols, with the exception of the *myo*-inositol (E. Merck Darmstadt, G.F.R.), were prepared and purified, in this laboratory and elsewhere, using recognised methods.

0021-9673/81/0000–0000/$02.50

Derivatization of inositols and hexitols

Standard solutions of the hexakis-O-acetyl inositols and hexakis-O-acetyl hexitols were prepared from the free polyol using the method of Bourne *et al*[5]. 5.0 mg of polyol was suspended in 0.04 ml glacial acetic acid and 0.1 ml trifluoroacetic anhydride added. The reaction was gently warmed to initiate reaction which proceeded vigorously to completion. After 10 min at room-temperature the reaction mixture was evaporated to dryness using rotary-film evaporation. For injection into the chromatograph the residue was dissolved in 0.5 ml methylene chloride and an aliquot withdrawn. Hot glacial acetic acid was used as a solvent for hexakis-O-acetyl *scyllo*-inositol[1]. The individual inositols and hexitols gave single peaks by GLC; complete acetylation was therefore inferred. The peak for *myo*-inositol corresponded exactly with that for an authentic sample of hexakis-O-acetyl *myo*-inositol.

Chromatography

A Pye GCD gas–liquid chromatograph equipped with FID and fitted with coiled stainless-steel columns (2.5 m × 4.0 mm I.D.) packed with 0.5% QF-1 plus 0.5% LAC-2R-446 was used. The column temperature was maintained at 195°C and the injection port and detector at 325°C and 270°C respectively. The carrier gas flow-rate was 40 ml nitrogen/min, the sample volume 0.25 μl and the attenuation $32 \cdot 10^{-10}$ A/V.

RESULTS AND DISCUSSION

Krzeminski and Angyal[1] separated the eight inositol isomers into six peaks using LAC-1R-296 on a 1.15-m column. They were unable to resolve *muco*- from *myo*-inositol and only partially resolved the *neo*- from the *chiro*-isomer. The use of a 3.5-m column just resolved *neo*- from *chiro*- but still did not resolve *muco*- from *myo*-. Steward[6] using ECNSS-M was unable to resolve *neo*- from *chiro*- and unfortunately omitted *muco*- from his standard mixtures.

QF-1 had been used as a stationary liquid in GLC studies of hexakis-O-acetyl alditols[7,8] and Lee and Ballou[c] recommended its use for trimethylsilyl derivatives of inositols. A trial with 1.0% QF-1 on Chromosorb W HP (80–100 mesh) resolved hexakis-O-acetyl derivatives of *neo*- and *chiro*- but the *neo*-peak overlapped the *myo*-peak. Since LAC-1R-296 was known to resolve *neo*- from *myo*[1], a mixed stationary liquid consisting of LAC-2R-446 and QF-1 was investigated. LAC-2R-446 has similar polarity to LAC-1R-296[10].

Fig. 1 illustrates the results obtained with a synthetic mixture of the five naturally occurring inositol isomers. Resolution, with the exception of the slight overlap of *neo*- and *chiro*-, is complete. Further studies showed that 0.7% QF-1 plus 0.3% LAC-2R-466 completely resolved the five isomers, but the column efficiency was reduced from 1256 to 1098 theoretical plates/m. 0.5% QF-1 plus 0.5% LAC-2R-466 was therefore the preferred stationary liquid.

Table I lists the relative retention times of the inositols as well as those of the hexakis-O-acetyl derivatives of mannitol, galactitol and glucitol. The peaks for the three hexakis-O-acetyl alditols overlap the *chiro*- to *myo*- region and therefore should be absent from any inositol mixture assayed.

The use of the method of Bourne *et al*.[5] to acetylate pure samples of inositols

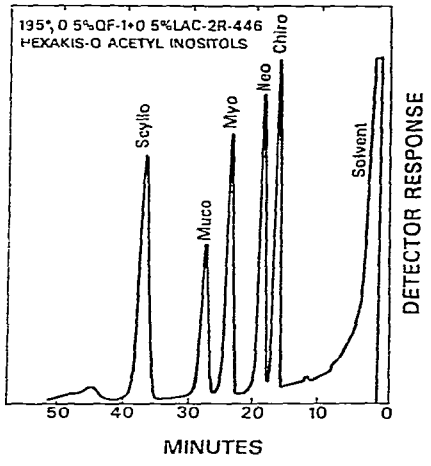

Fig. 1. Gas-liquid chromatograph of a mixture of the five naturally occurring inositols. Stationary phase was 0.5% QF-1 plus 0.5% LAC-2R-466 on Chromosorb W HP (80–100 mesh). Column temperature was 195°C.

TABLE I

RELATIVE RETENTION TIMES OF HEXAKIS-O-ACETYL DERIVATIVES

Isomer	*Relative retention**
Inositol isomer	
chiro-	0.72
neo-	0.81
myo-	1.00
muco-	1.17
scyllo-	1.53
Hexitol isomer	
Mannitol	0.69
Galactitol	0.79
Glucitol	0.87

* The times are calculated relative to that of hexakis-O-acetyl *myo*-inositol which had an observed retention time of 25.0 min on the 0.5% QF-1 plus 0.5% LAC-2R-446 column (PYE model GCD, 2.5 m × 4.0 mm I.D.) at 195°C.

and hexitols illustrates its potential as a procedure for derivatizing polyhydroxy compounds of assured purity and therefore it could find wider use in GLC studies of carbohydrates. Acetylation by other means is clearly required for less pure samples and one of the most promising would be that using 1-methylimidazole as catalyst[11]. Acetylation of sucrose is complete within 10 min at room temperature[11]. If the inositols exhibit the same high reaction rate then the method will offer an advantage over trimethylsilylation since the preparation of hexakis-O-trimethylsilyl *neo*-inositol requires 24–48 h[12].

The resolution of the GLC method described in this note appears, on the basis of relative retention times, to equal the best achieved by using TMS derivatives[12] and therefore provides a useful alternative to the TMS procedure. Since the order of

elution of the hexakis-O-acetyl inositols differs from that of the corresponding TMS derivatives it also provides a means of confirming peak assignment. In contrast to earlier methods using hexakis-O-acetyl inositols[1,6] it provides a means of completely resolving the five naturally occurring inositols.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. D. J. Cosgrove for samples of the inositols and the hexitols, Dr. E. A. Schwinghamer for a sample of mannitol and Dr. M. L. Tonnet for the LAC-2R-446.

REFERENCES

1 Z. S. Krzeminski and S. J. Angyal, *J. Chem. Soc.*, (1962) 3251.
2 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
3 T. Ueno, N. Kurihara and M. Nakajima, *Agr. Biol. Chem. (Tokyo)*, 31 (1967) 1189.
4 J. M. Oades, *J. Chromatogr.*, 28 (1967) 246.
5 E. J. Bourne, M. Stacey, J. C. Tatlow and J. M. Tedder, *J. Chem. Soc.*, (1949) 2976.
6 J. H. Steward, *Ph. D. Thesis*, University of Adelaide, Adelaide, 1970.
7 W. J. A. VandenHeuvel and E. C. Horning, *Biochem. Biophys. Res. Communs.*, 4 (1961) 399.
8 J. A. Hause, J. A. Hubicki and G. G. Hazen, *Anal. Chem.*, 34 (1962) 1567.
9 Y. C. Lee and C. E. Ballou, *J. Chromatogr.*, 18 (1965) 147.
10 W. O. McReynolds, *J. Chromatogr. Sci.*, 8 (1970) 685.
11 R. Wachowiak and K. A. Connors, *Anal. Chem.*, 51 (1979) 27.
12 W. W. Wells, T. A. Pittman and H. J. Wells, *Anal. Biochem.*, 10 (1965) 450.