front. Only La has an R_F of 0.30 and can be easily separated from the rest. With a KCl–HCl buffer (pH 1.08) La has an R_F of 0.05, Ce 0.55, whereas Nd and the others migrate with the front. The separation of Y and La is shown in Fig. 1A. To separate the heavier R.E.'s lower pH's in the stationary phase are required which is obtained by impregnation with acids. In this way Eu and Sm can be separated completely with 0.2 M $HClO_4$. The separation Y–Tb–Gd (Y front, Tb 0.57, Gd 0.16) was achieved with 0.4 M $HClO_4$ as the stationary phase and is shown in Fig. 2. All these separations, however, suffer from the fact that a residual activity of nearly 5% is irreversibly fixed on the spotting place. Since spotting of the R.E.'s was done in an aqueous solution, it was assumed that this technique disturbed the pH of the stationary phase. The R.E.'s were therefore first extracted into HDEHP and then spotted in the organic phase. This technique allowed good separations of Tb–Gd (Fig. 1B), Eu–Gd and Eu–Gd–Sm (Fig. 1C) within at most 70 min.

Although as yet only the group Tb, Gd, Eu and Sm has been examined thoroughly and at most three R.E.'s have been separated, it is reasonable to expect that this method should be valuable to separate any combination of three adjacent R.E.'s and, in favourable circumstances, even five on one plate. This can be concluded from the fact that for example Eu, Sm and Gd (Fig. 1C) have R_F's of 0.29, 0.54 and 0.73, respectively. Tb should have an R_F of approx. 1 while the R_F of Nd would be approx. 0.

Work is now in progress to examine other R.E. separations with this technique.

Institute for Analytical Chemistry, State University of Ghent (Belgium)

A. DANEELS
D. L. MASSART*
J. HOSTE

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* Research fellow of I.I.K.W. (Belgium).

Gas chromatography of inositols as their trimethylsilyl derivatives

Gas chromatography is now a standard technique in carbohydrate structure determination. Owing to their non-volatile nature, however, carbohydrates must be first converted into suitable derivatives. Methoxy and acetoxy derivatives of mono- and oligosaccharides have been analyzed successfully by gas chromatography. More recently, a trimethylsilylation technique[1] was introduced for the same purpose, and the range of applicability of gas chromatography of carbohydrates was expanded greatly. Compounds as large as a tetrasaccharide have been analyzed by gas chro

matography after trimethylsilylation, and even sugar phosphates have been chromatographed by this technique[2].

During our work on myoinositol mannosides of mycobacteria, we found that trimethylsilylation was useful not only in volatilizing the sample compounds, but also in improving separation. For example, methyl 2,3,4-tri-O-methyl-α-D-mannoside and methyl 3,4,6-tri-O-methyl-α-D-mannoside could not be separated from each other on a neopentylglycol succinate column or any other column we have tried[3]. After introduction into the mannosides of the trimethylsilyl group at the 6- and 2-positions, respectively, the compounds could be separated with ease. In a similar fashion, better separation of penta-O-methyl-*myo*-inositol isomers was achieved after trimethylsilylation[4].

In this report is described the separation of inositol isomers by gas chromatography as their trimethylsilyl derivatives. The method seems to be advantageous to that described by KRZEMINSKI AND ANGYAL[5], in which acetoxy derivatives of inositols were used.

Experimental

Gas chromatography. The apparatus used was the Aerograph Hy-Fi model A-600B, furnished with hydrogen flame detector and Brown-Honeywell Class 15 recorder. The following stationary phases were coated on Anakrom ABS (100–110 mesh, Analabs, Inc., Hamden, Conn.), in the percentage indicated: (a) QF-1, 5%; (b) SE-30, 3%; (c) Carbowax 20 M, 10%; and (d) diethyleneglycol succinate, 10%. The columns were 5 ft. × 1/8 in. (O.D.) in size. The flow rates of nitrogen and hydrogen were both 20 ml/min.

Preparation of samples. Samples were trimethylsilylated according to SWEELEY *et al.*[1]. Since inositols are not easily soluble in pyridine, the mixture was warmed to bring about complete solution. Although trimethylsilylation proceeded to some extent with the inositol in suspension, prolonged shaking (30–60 min), or warming after the addition of the reagents, was necessary for complete reaction. After the reaction, the mixture was centrifuged and portions of the supernatant were injected for analysis.

TABLE I

RETENTION TIME (MINUTES) OF TRIMETHYLSILYL DERIVATIVES OF INOSITOLS

Inositol isomer	*Column and temperature*				
	QF-1	*QF-3*	*SE-30*	*Carbowax*	*Diethylene glycol succinate*
	(150°)	(160°)	(200°)	(160°)	(146°)
allo	9.9	5.4	7.3	4.7	6.4
neo	10.4	5.9	7.5	5.0	7.7
muco	11.4	6.3	8.0	5.8	7.3
DL	13.3	7.2	9.4	7.0	9.2
scyllo	18.4	9.6	11.8	11.2	12.3
epi	20.0	11.6	11.3	10.0	13.1
myo	24.3	12.7	14.7	14.5	18.4
cis	24.5	12.7	12.8	—	—

Results and discussion

The retention times of the trimethylsilyl derivatives of inositol isomers are listed in Table I. The order of their appearance, regardless of the stationary phase used, is as follows: *allo*, (*neo–muco*), DL, (*scyllo–epi*), and (*myo–cis*). The order within the parentheses is sometimes reversed, however, depending on the stationary phase. This order is considerably different from that of the acetoxy derivatives of inositol isomers as reported by KRZEMINSKI AND ANGYAL[5], but as in that case there is no apparent correlation between retention time and configuration.

The best results were obtained with the QF-1 column. A typical separation on this column is illustrated in Fig. 1. In this system, all but the *myo–cis* pair of inositols could be separated, either partially or completely.

The present system has some advantages over the method employing acetoxy derivatives. Trimethylsilyl derivatives of inositols can be prepared quickly and the reaction mixture can be injected directly for analysis. The time needed for separation is much less than that needed for the acetoxy derivatives. Since all of the naturally occurring inositols (*neo*, DL, *scyllo*, and *myo*) can be separated completely in this system, it is suitable for their quantitative determination.

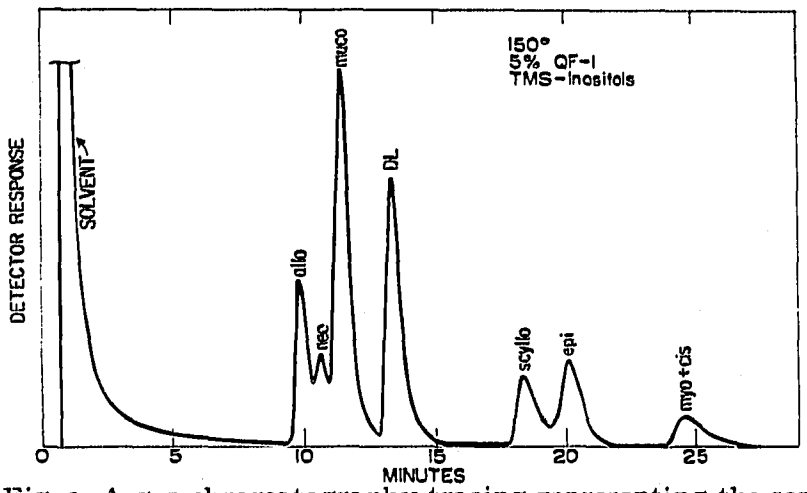

Fig. 1. A gas chromatography tracing representing the separation of trimethylsilyl derivatives of the inositols on a QF-1 column at 150°.

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Department of Biochemistry, University of California, Berkeley, Calif. (U.S.A.)

Y. C. LEE
C. E. BALLOU

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