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IMPROVED GAS CHROMATOGRAPHIC SEPARATION OF ENANTIOMERIC CARBOHYDRATE DERIVATIVES USING A NEW CHIRAL STATIONARY PHASE

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ABSTRACT

Pentylated cyclomaltohexaose (α-cyclodextrin) can be used as a chiral stationary phase for capillary g.l.c. in the range 40° to >200°. Strong enantioselective molecular interactions are observed towards trifluoroacetylated carbohydrates, methyl glycosides, 1,4- and 1,5-anhydroalditols, polyols, and polyhydroxy acid methyl esters. Base-line resolution of enantiomers is achieved after only a few minutes.

INTRODUCTION

The absolute stereochemistry of carbohydrates from natural sources is usually determined by measuring optical rotation. Often the "natural configuration" of the constituents of polysaccharides is assumed, although carbohydrates with unusual configurations have been found, particularly in bacterial polysaccharides[1].

G.l.c. of volatile enantiomeric carbohydrate derivatives has been carried out on chiral polysiloxane stationary phases. Thus, the chiral phase XE-60-L-valine (*S*)-α-phenylethylamide[2] is suitable for trifluoroacetylated methyl aldohexosides, and other chiral polysiloxanes have been suggested for derivatives of aldopentoses[3] and polyols[4]. Using these methods, the ratio of D- and L-galactose in snail galactans was determined[5]. Chirasil-val has also been used as a stationary phase[6,7] for the g.l.c. of carbohydrate enantiomers, and g.l.c. has been applied to diastereomeric derivatives of carbohydrates[8–13]. These latter methods suffer from low volatility of the derivatives and low separation factors due to the high column temperatures necessary for elution of the derivatives.

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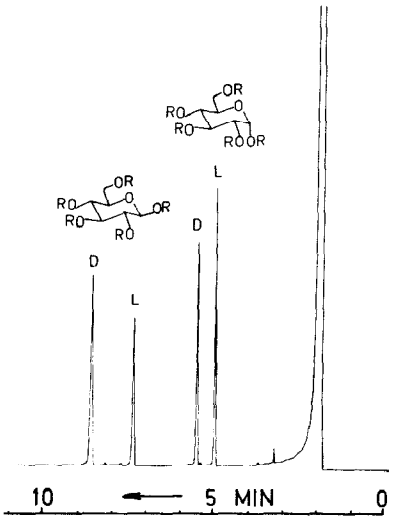
Although numerous applications of cyclomalto-oligosaccharides (cyclodextrins) in liquid chromatography of enantiomers are known[14], there are few reports of the use of cyclomaltoheptaose as a chiral phase in gas–solid chromatography[15] and of methylated cyclomaltoheptaose as a stationary phase in capillary g.l.c.[16,17].

RESULTS AND DISCUSSION

We now report the use of fully pentylated cyclomaltohexaose (α-cyclodextrin) as a stationary phase for the g.l.c. of carbohydrate enantiomers. As shown in Fig. 1, the D and L forms of trifluoroacetylated α- and β-glucopyranose have large separation factors and similar results are obtained for most of the other hexoses and pentoses (Table I). In Fig. 2, the behaviour of the trifluoroacetylated methyl α- and β-galactopyranosides is shown and complete separation was achieved in only a few minutes. The separation factors of methyl glycosides are listed in Table II and for some polyhydroxy compounds in Table III (see also Figs. 3 and 4). Trifluoroacetylated D- and L-arabinitol have been also separated on columns with XE-60-L-valine-(*R*)-α-phenylethylamide[4].

As an alternative to acid hydrolysis in methylation analysis, reductive depolymerisation was introduced by Gray *et al.*[18,19]. The reaction of fully substituted carbohydrates with triethylsilane in the presence of a Lewis acid converts pyranosides into 1,5-anhydroalditols and furanosides into 1,4-anhydroalditols. After trifluoroacetylation, the enantiomers could be separated (Table III and Fig.



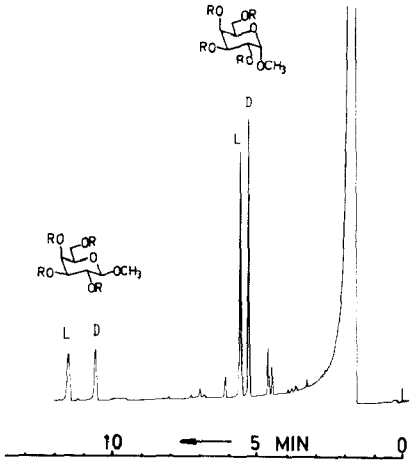


Fig. 1. Separation of enantiomeric α- and β-glucopyranose (R = CF_3CO) on pentylated cyclomaltohexaose at 115° (carrier gas, H_2 at 1 bar; 40-m capillary column).

Fig. 2. Separation of enantiomeric methyl α- and β-galactopyranosides (R = CF_3CO) at 120° (other details as in Fig. 1).

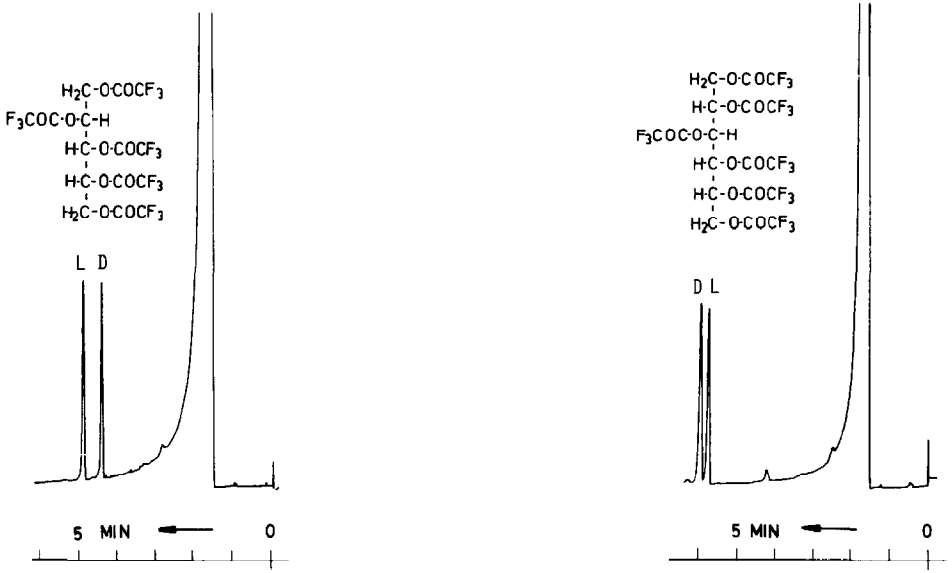


Fig. 3. Separation of enantiomers of arabinitol at 110° (other details as in Fig. 1).

Fig. 4. Separation of enantiomers of glucitol at 110° (other details as in Fig. 1).

TABLE I

SEPARATION FACTORS (α), ORDER OF ELUTION, AND COLUMN TEMPERATURE FOR TRIFLUOROACETYLATED ENANTIOMERIC CARBOHYDRATE DERIVATIVES ON PENTYLATED CYCLOMALTOHEXAOSE

Compound[a]	*α-Value*	*Configuration of the component in the first peak*	*Column*	
			Temp (°)	*Length (m)*
Glyceraldehyde	1.021	D	72	40
Erythrose	1.033	D	80	40
α-Ribose (*p*)	1.064	D	115	40
β-Ribose (*p*)	1.150	L	115	40
β-Ribose (*f*)	1.020	D	115	40
α-Galactose (*p*)	1.035	D	120	40
β-Galactose (*p*)	1.059	D	120	40
β-Galactose (*f*)	1.070	L	120	40
α-Glucose (*p*)	1.117	L	115	40
β-Glucose (*p*)	1.166	L	115	40
α-Allose (*p*)	1.171	D	120	20
β-Allose (*p*)	1.064	D	120	20
α-Talose (*p*)	1.099	D	110	20
α-Mannose (*p*)	1.000		100	20
β-Mannose (*p*)	1.110	L	100	20
α-Gulose (*p*)	1.000		120	20
β-Gulose (*p*)	1.043	D	120	20

[a] *p*, Pyranose; *f*, furanose.

TABLE II

SEPARATION FACTORS (α), ORDER OF ELUTION, AND COLUMN TEMPERATURE FOR TRIFLUOROACETYLATED ENANTIOMERIC METHYL GLYCOSIDES ON PENTYLATED CYCLOMALTOHEXAOSE (40-m COLUMN)

Compound[a]	*α-Value*	*Configuration of the component in the first peak*	*Temp. (°)*
Xylose (α-*p*)	1.020	D	90
Arabinose (α-*p*)	1.099	D	90
Arabinose (β-*p*)	1.093	D	90
Lyxose (α-*p*)	1.019	L	90
Lyxose (β-*p*)	1.030	D	90
Ribose (α-*p*)	1.071	D	100
Ribose (β-*p*)	1.043	D	100
Fucose (α-*f*)	1.035	D	90
Fucose (α-*p*)	1.057	L	90
Fucose (β-*f*)	1.026	D	90
Fucose (β-*p*)	1.033	D	90
Mannose (α-*p*)	1.036	D	100
Idose (α-*p*)	1.032	D	100
Idose (β-*p*)	1.023	L	100
Galactose (α-*p*)	1.070	D	110
Galactose (β-*p*)	1.106	D	110
Glucose (α-*p*)	1.035	L	110

[a] *p*, Pyranose; *f*, furanose.

TABLE III

SEPARATION FACTORS (α), ORDER OF ELUTION, AND COLUMN TEMPERATURE FOR TRIFLUOROACETYLATED ENANTIOMERIC 1,5-ANHYDROALDITOLS, 1,4-ANHYDROALDITOLS, POLYOLS, AND METHYL ALDONATES ON PENTYLATED CYCLOMALTOHEXAOSE (40-m COLUMN)

Compound[a]	*α-Value*	*Configuration of the component in the first peak*	*Temp. (°)*
1,5-Anhydrofucitol	1.050	D	90
1,4-Anhydroribitol	1.064	L	90
1,5-Anhydroarabinitol	1.045	D	90
1,4-Anhydroxylitol	1.030	D	90
1,5-Anhydrolyxitol	1.030	D	100
1,5-Anhydroglucitol	1.037	L	120
1,5-Anhydrogalactitol	1.080	D	120
1,5-Anhydromannitol	1.019	D	120
Glucitol	1.033	L	110
Arabinitol	1.111	D	110
Mannitol	1.013	D	100
Methyl mannonate	1.038	D	100
Methyl galactonate	1.087	D	100
Methyl glycerate	1.043	D	90
Dimethyl tartrate	1.071	L	90

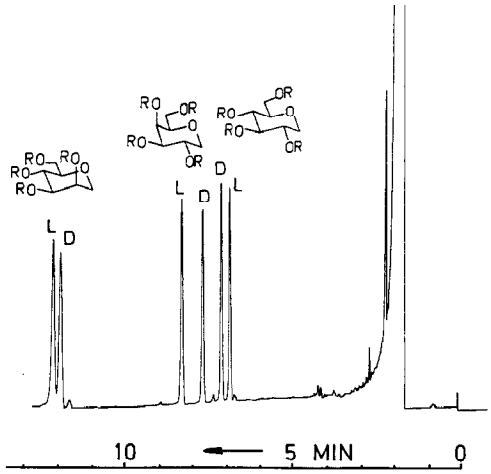


Fig. 5. Separation of enantiomeric 1,5-anhydroglucitol, 1,5-anhydrogalactitol, and 1,5-anhydromannitol (R = CF_3CO) at 120° (other details as in Fig. 1).

5). Although these results are preliminary, they indicate that it should be possible to identify simultaneously the structure and absolute configuration of the constituents of a polysaccharide. Moreover, in reductive depolymerisation, the cyclic structure of the sugars is retained and each constituent of a polysaccharide is represented by only one peak in the gas chromatogram.

Reduction of ribose and xylose with triethylsilane produces non-chiral 1,5-anhydroalditols, but it is possible to isomerize these sugars to chiral 1,4-anhydroalditols by using moist trimethylsilyl trifluoromethanesulfonate as a Lewis acid.

The enantiomers of trifluoroacetylated glyceraldehyde, erythrose, methyl mannonate, methyl galactonate, methyl glycerate, and dimethyl tartrate can also be separated on perpentylated cyclomaltohexaose (Table III).

Hydrogen bonding between the pentylated cyclomaltohexaose and trifluoroacetylated carbohydrates is not possible and it is assumed that the large number of asymmetric centres induces enantioselectivity. It seems unlikely that inclusion phenomena, as discussed in liquid chromatography for chiral recognition of enantiomeric drugs[14], play an important role in the gas chromatographic separation. It is more probable that dipole–dipole interactions are responsible for separation of trifluoroacetylated carbohydrate enantiomers since neither methylated nor trimethylsilylated carbohydrate derivatives can be separated.

EXPERIMENTAL

Preparation of derivatives. — Methyl glycosides were prepared by heating samples (~1 mg) of carbohydrates for 60 min at 100° in methanolic 1.5M hydrogen chloride (0.5 mL) in a ReactiVial (Ventron). Trifluoroacetylation was performed with trifluoroacetic anhydride (50 μL) in dichloromethane (200 μL) for 60 min at

100°. After removing the excess of reagents, the samples were dissolved in dichloromethane and used for gas chromatography. 1,5-Anhydroalditols were prepared as described by Gray *et al.*[18,19].

Pentylated cyclomaltohexaose. — A solution of cyclomaltohexaose in dimethyl sulfoxide was treated[20] with 1-bromopentane in the presence of 3 equiv. of NaOH. The resulting hexakis(2,6-di-*O*-pentyl)cyclomaltohexaose was further boiled under reflux with 1-bromopentane in the presence of NaH in tetrahydrofuran solution for 5 days. The resulting product was purified by silica gel chromatography and investigated by g.l.c. and mass spectrometry after reductive depolymerization[21].

Preparation of glass capillary columns. — Pyrex glass capillaries were coated according to the static procedure[22], using a 0.2% solution of pentylated cyclomaltohexaose in CH_2Cl_2 after treatment[23] of the inner glass surface with Silanox (Cabot Corp.).

Gas chromatography. — A Carlo Erba Model 2101 gas chromatograph with split injection and a flame-ionization detector was used.

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