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Short communication

Separation of acyclic derivatives of sugar enantiomers using gas chromatography on chiral stationary phases of permethylated α-, β- and γ-cyclodextrin

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Abstract

Gas–liquid chromatography on monosaccharide single peak derivatives have been run in order to improve methods for the determination of absolute configuration. Eight out of eleven investigated enantiomeric pairs of trifluoroacetylated alditols or aldose diethyl dithioacetals were separated using capillary columns containing chiral stationary phases of permethylated α-cyclodextrin (α-Dex 120), β-cyclodextrin (β-Dex 120) and γ-cyclodextrin (γ-Dex 120).

Keywords: Enantiomer separation; Monosaccharides; Saccharides

1. Introduction

For determination of the absolute configurations of monosaccharides gas–liquid chromatography has been commonly used in the last two decades for separation of enantiomers. The developed methods are based on (1) separation of achiral enantiomer derivatives using a chiral stationary phase [1–6], or (2) separation of the diastereomeric compounds obtained by treatment of the sugars with a chiral reagent, usually an optically active alcohol, using non-chiral GLC columns [7–11]. However, separation of enantiomeric sugars as their diastereomeric glycosides gives rise to multiple-peak chromatograms and also in the approach with direct resolution on a chiral stationary phase, each enantiomer of a monosaccharide or monosaccharide glycoside, usually trifluoroacetylated, produces a chromatogram in which up to four forms may be present. One way to circumvent the multiplicity is to reduce the sugars to alditols but, when converted to an acyclic alditol some sugars lose their chirality, since a symmetrical polyol (a *meso* compound) is formed. Some sugars also give the same alditol as, e.g., D-arabinose and D-lyxose. For preservation of the chirality the aldoses can be converted to, for example, aldononitriles or aldose diethyl dithioacetals.

For the approach, one sugar, one derivative, we have evaluated some commercially available columns carrying chiral stationary phases for separation of acyclic sugar derivatives. Pentoses, 2-deoxypentose, hexoses, 6-deoxyhexoses and 3,6-dideoxyhexoses have been included. The latter are common

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constituents of bacterial cell-wall lipopolysaccharides.

2. Experimental

2.1. General

PTFE-lined screw-capped glass tubes (100×13 mm) were used for sample preparation, and for GLC analysis 1 μl of sample solution was injected, throughout. An HP 5890 gas chromatograph equipped with an autoinjector and a flame ionization detector was used. The chiral GLC columns examined were fused-silica columns (30 m×0.25 mm): α-Dex 120, β-Dex 120 and γ-Dex 120, coated with 0.25 μm 20% permethylated α-, β- and γ-cyclodextrin, respectively, in 80% poly-(35% diphenyl–65% dimethyl)-siloxane (Supelco, Bellefonte, PA, USA). Helium carrier-gas velocity was maintained at 30 cm/s with a split ratio of about 20:1. The chiral columns were run at 100°C for trifluoroacetylated alditols, at 120°C for trifluoroacetylated aldose diethyl dithioacetals (unless otherwise stated) and using a temperature program (160°C for 1 min, 2.5°/min to 200°C and hold for 30 min) for trimethylsilylated aldose diethyl dithioacetals. The injector and detector interface temperatures were 250°C. The different sugar derivatives were run separately as well as enantiomeric pairs in a D/L-ratio of 2:1 together with the internal standard (D-galactose). A Nermag R 10-10 instrument was used for mass spectrometry.

2.2. Sample solutions

Stock solutions of each enantiomeric monosaccharide; arabinose, fucose, galactose, glucose, lyxose, mannose, rhamnose, ribose, 2-deoxyribose and xylose (2.0 mg/ml) in methanol–water (1:4), were kept at 5°C. Prior to analysis, samples were quenched twice with methanol (0.5 ml) and evaporated to dryness.

Samples of 3,6-dideoxyhexoses were prepared by hydrolysis of lipopolysaccharides (10 mg/ml) with aqueous trifluoroacetic acid (50 m*M*) for 1 h at 100°C. After cooling the hydrolysed sample was quenched with methanol and centrifuged (20 000 *g* at 4°C for 4 min) to remove the precipitated polysaccharide. The supernatant was evaporated to dryness before being reconstituted to the original volume in methanol–water (1:4). That procedure yielded almost pure 3,6-dideoxyhexoses, which occurred terminally in the lipopolysaccharides. The lipopolysaccharides used were prepared from *Salmonella enterica* serovar *typhimurium* (strain SH4809), *S. enterica* serovar *enteritidis* (strain SH1262), and from *Escherichia coli* O111, and contained as terminal sugar: abequose (3,6-dideoxy-D-galactose), tyvelose (3,6-dideoxy-D-mannose), and colitose (3,6-dideoxy-L-galactose), respectively.

2.3. Preparation of alditol trifluoroacetates

A sample (0.3 ml; in total 0.1–0.6 mg of monosaccharide) in 1 *M* NH_4OH containing sodium borohydride (1 mg/ml) was kept for 30 min at room temperature (≈22°C). The sample was then repeatedly quenched with methanol containing 10% acetic acid and with methanol. After each addition the sample was evaporated to dryness with a stream of dry air.

The dry sample was dissolved in acetonitrile (0.10 ml), containing 10% trifluoroacetic acid, and trifluoroacetic anhydride (0.10 ml) was then added. Following mixing by vortexing for 15 s the solution was heated at 60°C for 15 min and after cooling taken to dryness with a weak stream of dry N_2. Dichloromethane (0.3 ml) was added and following vortexing for 15 s the clear solution was transferred to a small PTFE-lined screw-cap tube and kept at 5°C or analysed directly.

2.4. Preparation of trifluoroacetylated aldose diethyl dithioacetals

Trifluoroacetic acid (12 μl) and ethanethiol (50 μl) were added to a sample (0.1–0.6 mg sugar) and following mixing by vortexing for 15 s the sample was incubated at room temperature (≈22°C) for 30 min. The sample was then quenched five times with methanol (0.5 ml) and evaporated to dryness.

Trifluoroacetylation of aldose diethyl dithioacetals was carried out as described above. Trimethylsilylated derivatives were prepared by the addition of 0.45 ml silylating reagent (trimethylchlorosilane–

hexamethyldisilazane–pyridine, 1:2:3) to the dry dithioacetal, and then heated for 20 min at 50°C. After centrifugation (1500 *g* for 3 min), the clear solution was collected and used for GLC analysis.

2.5. Preparation of peracetylated aldononitriles

Aldononitrile acetate derivatives were prepared essentially as described [12]. To a sample of sugar (0.4–2.0 mg) was added 0.3 ml of a pyridine–methanol (4:1) solution containing hydroxylamine–HCl (32 mg/ml) and 4-(dimethylamino)-pyridine (DMAP, 40 mg/ml). Following mixing by sonication for 1 min the sample was heated at 75°C for 25 min and, after being cooled to room temperature, acetic anhydride (1.0 ml) was added, sonicated for 1 min, and heated for 15 min at 75°C. After cooling 1,2-dichloroethane (2.0 ml) was added. Two extractions with 1 *M* HCl (1.0 ml) and three with water (1.0 ml) removed excess reagents. After the last extraction, the tube was centrifuged at 1500 *g* for 3 min and remaining water was removed. The dichloroethane phase was transferred to a dry tube and evaporated to dryness. The sample was reconstituted in dichloromethane (400 μl).

Attempts to prepare trifluoroacetylated aldononitriles by a similar procedure were unsuccessful, most probably because of the highly exothermic reaction of trifluoroacetic anhydride in the presence of hydroxylamine–HCl and DMAP. Also, attempts to prepare a derivative of trifluoroacetylated aldononitrile by deacetylation of peracetylated aldononitrile derivatives, subsequently trifluoroacetylated, were unsuccessful.

3. Results and discussion

3.1. Gas–liquid chromatography of trifluoroacetylated alditols

Trifluoroacetylated alditols were analyzed on chiral capillary columns of fused-silica coated with derivatised α-cyclodextrin (α-Dex 120), β-cyclodextrin (β-Dex 120) and γ-cyclodextrin (γ-Dex 120). Galactose, ribose and xylose pairs could not be separated as they are meso forms. The chiral alditols behaved somewhat differently on the different Dex 120 columns. Those derived from 2-deoxyribose were separated only with the α-Dex 120 column, whereas those from rhamnose and mannose were resolved only on the β-Dex 120 and γ-Dex 120 columns, respectively. For glucose the D-form eluted before the L-form on the α-Dex 120 column, but in the reverse order on the β-Dex 120 and γ-Dex 120 columns. The alditols of D- and L-lyxose appeared in the chromatograms on the same positions as the corresponding D- and L-enantiomers of arabinose, alditols which are the tautomeric forms of D- and L-lyxitol. The D-form of arabinose (and lyxose) as well as the D-form of 3,6-dideoxy-galactose was eluted before the L-form on all three columns. In contrast, the fucitol enantiomers were unresolved on all three columns. A summary of the retention data is given in Table 1. Those that did not separate are included for comparison of retention times.

Identifications of the GLC peaks were made using

Table 1
GLC data for trifluoroacetylated alditols of sugar enantiomers on three chiral columns: α-Dex 120, β-Dex 120 and γ-Dex 120

Parent sugar	α-Dex 120		β-Dex 120		γ-Dex 120	
	t_R^a	α^b	t_R	α	t_R	α
Ara	0.71	1.08	0.73	1.09	0.67	1.05
Fuc	0.51	1.00	0.47	1.00	0.42	1.00
Gal	1.00	–[c]	1.00	–	1.00	–
Glc	0.86	1.01	0.86	1.05*[d]	0.81	1.06*
Lyx	0.71	1.08	0.73	1.09	0.67	1.05
Man	0.65	1.00	0.63	1.00	0.60	1.04*
Rha	0.45	1.00	0.43	1.04	0.40	1.00
Rib	0.57	–	0.47	–	0.50	–
2-Deoxy-Rib	1.12	1.02	1.24	1.00	1.08	1.00
Xyl	0.84	–	0.78	–	0.85	–
Abe/Col	1.22	1.04	1.35	1.05	1.08	1.07
Tyv[e]	1.06	–	1.14	–	0.94	–

[a] t_R, Retention time for the D-form derivative relative to that of trifluoroacetylated galactitol, which eluted at about 10 min (α-Dex), 12 min (β-Dex), and 14 min (γ-Dex).

[b] α, Separation factor, t'_{R2}/t'_{R1}, where $t'_{R2} > t'_{R1}$ and are the corrected retention time for the slower and the faster component (D- or L-derivative, see below), respectively. Corrected retention time (t'_R) is the retention time for a component subtracted from the retention time for solvent. If $\alpha < 1.005$ it is given as 1.00.

[c] –, Not applicable, the relative retention time given for comparison.

[d] *, The D-form eluted before L-form for all derivatives except for those which are marked with an * or had α-value 1.00.

[e] The L-form (3,6-dideoxy-L-mannose, ascarylose) was not available.

GLC–MS. The mass spectra of the alditol trifluoroacetates have the advantage of being easy to interpret and have been described previously [13].

3.2. Gas–liquid chromatography of trifluoroacetylated aldose diethyl dithioacetals

No pair of trifluoroacetylated aldose diethyl dithioacetals was resolved on α-Dex 120 and only the lyxose enantiomers were separated on β-Dex 120 (α 1.010). On γ-Dex 120 the only pairs of sugar enantiomers which were resolved were those from lyxose, xylose and 3,6-dideoxygalactose (Table 2). The arabinose derivatives were not resolved but were well separated from those of lyxose. Consequently, GLC on the γ-Dex 120 column of trifluoroacetylated aldose diethyl dithioacetals makes it possible to distinguish between lyxose and arabinose in addition to the identification of D- and L-forms of lyxose and xylose. However, this kind of derivative does not resolve the enantiomers of galactose, ribose and fucose, which were also unresolved as alditols. The electron impact mass fragmentation of the trifluoroacetylated aldose diethyl dithioacetals was found essentially corresponding to the mass fragmentation of acetylated aldose diethyl dithioacetals [14] with a significant molecular ion. For a pentose, as an example, the molecular ion (m/z=640) loses a CH_3CH_2S radical (m/z=61) and/or one, two or three CF_3CO_2H groups (m/z=114) which gives significant ions at m/z 579, 526, 465, 412 and 237. The corresponding fragments were found also for hexoses, deoxyhexoses and deoxypentoses. The $(CH_3CH_2S)_2CH$ ion (m/z=135) obtained on cleavage between aldose C-1 and C-2 is present in all spectra.

The different trimethylsilylated aldose diethyl dithioacetals were eluted at 20–40 min on the α-Dex 120, β-Dex 120 or γ-Dex 120 columns but none of eleven enantiomeric pairs (see Table 1) was resolved.

Table 2
GLC data for enantiomers of trifluoroacetylated aldose diethyl dithioacetals on γ-Dex 120

Parent sugar	120°C		130°C	
	t_R^a	α^b	t_R	α
Gal	1.00	1.00	1.00	1.00
Ara	0.77	1.00	0.82	1.00
Lyx	0.90	1.02	0.95	1.02
Xyl	0.79	1.03	0.84	1.02
Abe/Col	n.d.[c]	n.d.	2.93	1.01

[a] t_R, Retention time for the D-form derivative relative to that of trifluoroacetylated D-galactose diethyl dithioacetal which eluted at about 27 min (120°C) and 15 min (130°C). D-Lyxose, D-xylose and abequose were all eluted before the corresponding L-form. Galactose and arabinose are included for comparison of retention times.

[b] α, Separation factor, $t'_{R2}t'_{R1}$, where $t'_{R2}>t'_{R1}$ and are the corrected retention time for the slower and the faster component (D- or L-derivative, see below), respectively. Corrected retention time (t'_R) is the retention time for a component subtracted from the retention time for the solvent. If $\alpha<1.005$ it is given as 1.00.

[c] n.d, Not determined.

3.3. Gas–liquid chromatography of peracetylated aldononitriles

By the method used for preparation of peracetylated aldononitriles [12], a single significant derivative was obtained for each monosaccharide. However, no enantiomeric pair was resolved on the chiral columns; α-Dex 120, β-Dex 120 or γ-Dex 120. These results are in agreement with earlier findings that, so far reported, only trifluoroacetylated sugar enantiomers are useful for GLC on chiral columns [1,3].

4. Conclusion

For the present approach of one sugar, one derivative, the chiral columns tested cannot serve as a general replacement of existing methods in the determination of the absolute configurations of sugars. There are two reasons for this, the separation factors are in several cases low and only some pairs of sugars are resolved on each column. However, for at least eight different sugars these derivatives and columns can be useful and can be complementary to other methods.

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