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RAPID GAS CHROMATOGRAPHIC ANALYSIS OF ALDOSES AS THEIR DIETHYL DITHIOACETAL TRIMETHYLSILYLATES

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SUMMARY

A simple gas chromatographic method has been developed for the rapid determination of aldoses, which gives single peaks for the aldoses. A sample of an aldose or a mixture of aldoses is first mercaptalated with a mixture of ethanethiol and trifluoroacetic acid (2:1) and then trimethylsilylated. Gas chromatographic analysis of the derivatized products on a SCOT SF-96 column permits the analysis of aldoses at the 10^{-6} mole level with a coefficient of variation of less than 2%. The total analysis time, including derivatization, is within 2 h. Some applications of this method are presented.

INTRODUCTION

The large number of peaks obtained on gas chromatography (GC) of aldose derivatives, due to anomeric and ring structural isomerization, has stimulated the study of derivatization methods which give single peaks for aldoses. Among several methods hitherto developed[1–4], the alditol acetate method[1] has been the most widely used, but is laborious, consisting of several processes, including treatment with sodium borohydride, desalting, evaporation and acetylation.

In a previous paper[5] we reported the use of the dithioacetal method for simultaneous determination of conjugated aldehydes in the products of periodate oxidation of pyranose sugars. As a continuation of this work, we have extended this derivatization to the analysis of aldoses. This paper described a simple and rapid GC method for the analysis of aldoses as their diethyl dithioacetal trimethylsilylates.

EXPERIMENTAL

Materials

All the aldose samples were from commercial sources. The samples of oligo- and polysaccharides, trifluoroacetic acid, hexamethyldisilazane and *myo*-inositol were obtained from Wako (Osaka, Japan). Ethanethiol and trimethylchlorosilane were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All samples and reagents were

of the highest grade available. Pyridine was dehydrated by heating it under reflux with barium oxide, and distilled before use.

Apparatus

Isothermal gas chromatography was performed on a Shimadzu 4BMPF instrument equipped with a hydrogen flame ionization detector. A sodium chloride-treated glass capillary column (a SCOT column, 50 m × 0.28 mm I.D.) coated with SF-96 was used at 225° throughout the work, and was supplied by Gasukuro Kogyo (Tokyo, Japan). The flow-rate of the carrier gas (nitrogen) was regulated at 1.5–2.0 ml/min by use of a 100:1 splitter. The eluate was continuously mixed with the scavenger gas (nitrogen), flow-rate 50 ml/min, and the mixture was introduced to the detector. Peaks were integrated by a Shimadzu E1A integrator.

Analysis of aldoses

An aqueous solution (100 μl) of 0.01 *M* *myo*-inositol (internal standard) was added to a sample of an aldose (10^{-8}–10^{-5} mole) contained in a small glass reaction tube (5 × 0.5 cm I.D.), and the mixture was evaporated to dryness under reduced pressure in a desiccator containing sodium hydroxide. A mixture of ethanethiol and trifluoroacetic acid (2:1, 20 μl) was added, the reaction vessel closed tightly with a polyethylene stopper, the residue in the tube dissolved by gentle swirling and the resulting solution kept for 10 min at 25°. Pyridine (50 μl) was added, followed by hexamethyldisilazane (100 μl) and trimethylchlorosilane (50 μl), and the mixture was incubated for 30 min at 50° with occasional shaking. The mixture was centrifuged, and the aldose was analyzed by injecting a 1–10 μl sample of the supernatant into the GC column. In parallel with this determination, a mixture of a known amount of an authentic specimen of the aldose and of the same amount of *myo*-inositol as used in the above determination was prepared and was analyzed by the same procedure. The amount of the aldose in the sample was calculated as the ratio of the aldose to *myo*-inositol relative peak area for the sample to that for the authentic specimen, multiplied by the amount of aldose in the latter. Simultaneous determination of a number of aldoses was possible by reference to a mixture of authentic aldoses.

Hydrolysis of carbohydrate materials

A sample of a carbohydrate material (*ca.* 10^{-6} mole) was dissolved in 2 *M* trifluoroacetic acid (100 μl) contained in a small glass tube. The tube was then flushed with nitrogen for a few minutes, sealed and heated for 5 h on a boiling water-bath. The tube was opened, and the hydrolysate was evaporated to dryness under reduced pressure in a desiccator containing sodium hydroxide. The residue was subjected to GC analysis as described above.

RESULTS AND DISCUSSION

Aldoses lose their asymmetric centre at C-1 on derivatization to dithioacetals, so that the resulting dithioacetals have no steric isomers based on the C-1 configuration. At the same time, differences in structure among the products are reduced due to the absence of rings, making difficult the resolution of the GC peaks of isomeric aldose derivatives. Thus, pentoses and hexoses were well separated in groups,

but isomeric aldoses were only poorly resolved on ordinary packed columns[6]. In order to overcome this problem, capillary columns coated with various liquid phases were tried, and eventually a reasonable separation was obtained when a SCOT SF-96 column was used. Fig. 1 shows a chromatogram for a mixture of 14 diethyl dithioacetal trimethylsilylates of common aldoses. Of the derivatives of the three pentoses

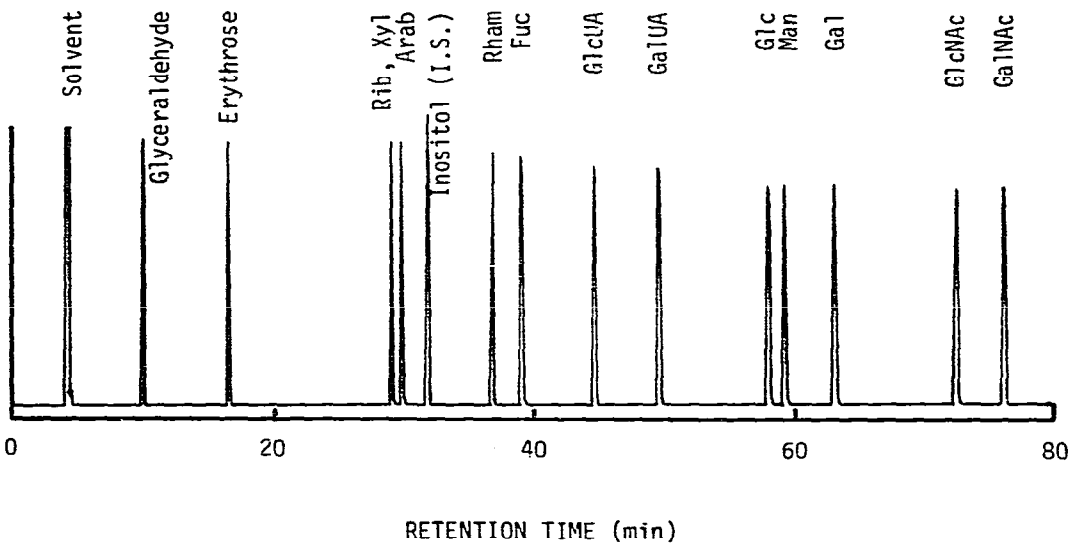

Fig. 1. Gas chromatographic separation of diethyl dithioacetals of various aldoses on SCOT SF-96 column. Arab: L-arabinose, Rib: D-ribose, Xyl: D-xylose, Gal: D-galactose, Glc: D-glucose, Man: D-mannose, Fuc: L-fucose, Rham: L-rhamnose, GalUA: D-galacturonic acid, GlcNAc: D-glucuronic acid, GalNAc: N-acetyl-D-galactosamine, GlcNAc: N-acetyl-D-gulcosamine.

examined (L-arabinose, D-ribose and D-xylose), those of L-arabinose and D-ribose were separated from each other, and those of L-arabinose and D-xylose were also mutually separable, but those of D-ribose and D-xylose were almost indistinguishable on this column. However, the latter pair could be resolved by using a SCOT Ucon oil HB 2000 column (40 m, obtainable from the same source), as shown in Fig. 2, although their retention times were almost doubled.

Although the main product of mercaptalation of an aldose is the dithioacetal, isomeric monothioacetals and thioglycosides may be also formed as by-products, especially on prolonged reaction or at high acid concentration, as pointed out by Williams and Jones[6]. For the optimization of the conditions for dithioacetal formation, the nature of the acid catalyst, its concentration and the reaction time were investigated using L-arabinose and ethanethiol. Bulkier mercaptans could be used, but their dithioacetal derivatives had larger retention times and the analysis became tedious.

Among the various acids examined, trifluoroacetic acid was the most suitable. Stronger acids such as hydrochloric acid, trifluoromethanesulphonic acid and boron trifluoride yielded larger amounts of by-products, and a weaker acid such as acetic acid did not effect dithioacetal formation. Sulphuric and fluorosulphonic acids were immiscible with ethanethiol, and formed two-layer mixtures. These results were the same as those observed for the analysis of the products of periodate oxidation[5].

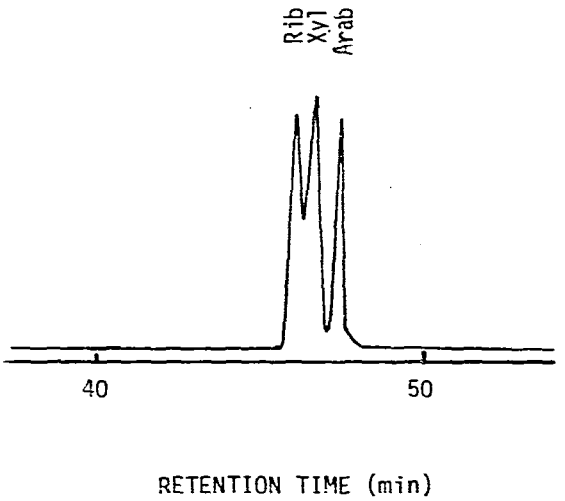

Fig. 2. Separation of the pentose derivatives on a SCOT Ucon oil HB 2000 column. Abbreviations as in Fig. 1.

Fig. 3 shows the effect of the concentration of trifluoroacetic acid on the yield of L-arabinose diethyl dithioacetal. The dithioacetal was obtained in almost quantitative yield at ethanethiol:trifluoroacetic acid volume ratios between 2 and 5. At lower concentrations of the acid, dithioacetal formation was incomplete, whereas at higher

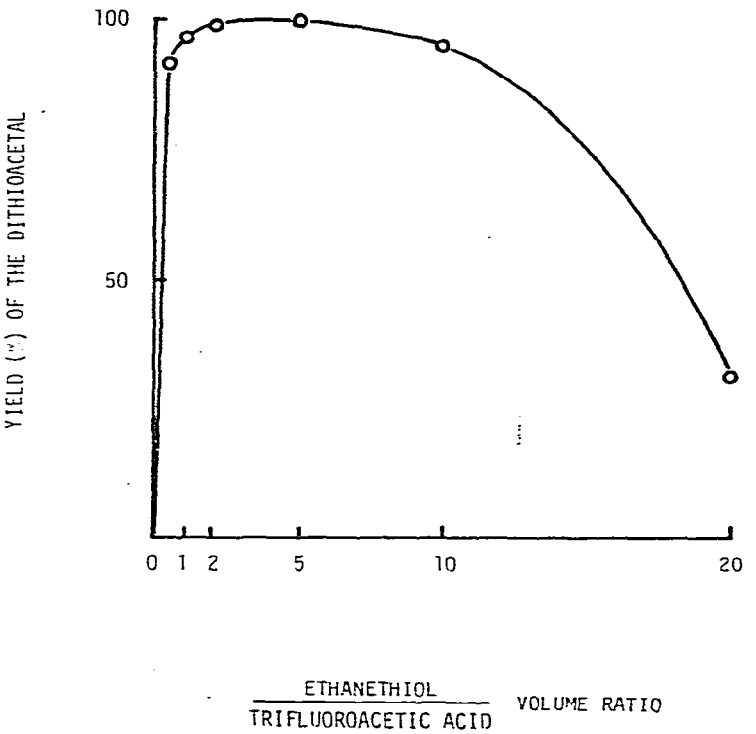

Fig. 3. Effect of the concentration of trifluoroacetic acid on mercaptalation of L-arabinose.

concentrations the proportions of by-products increased. Fig. 4 indicates the course of dithioacetal formation from L-arabinose using a 2:1 mixture of ethanethiol and trifluoroacetic acid at 25°. The reaction was very rapid, being complete in 5 min, but the dithioacetal formed gradually decomposed after 10 min.

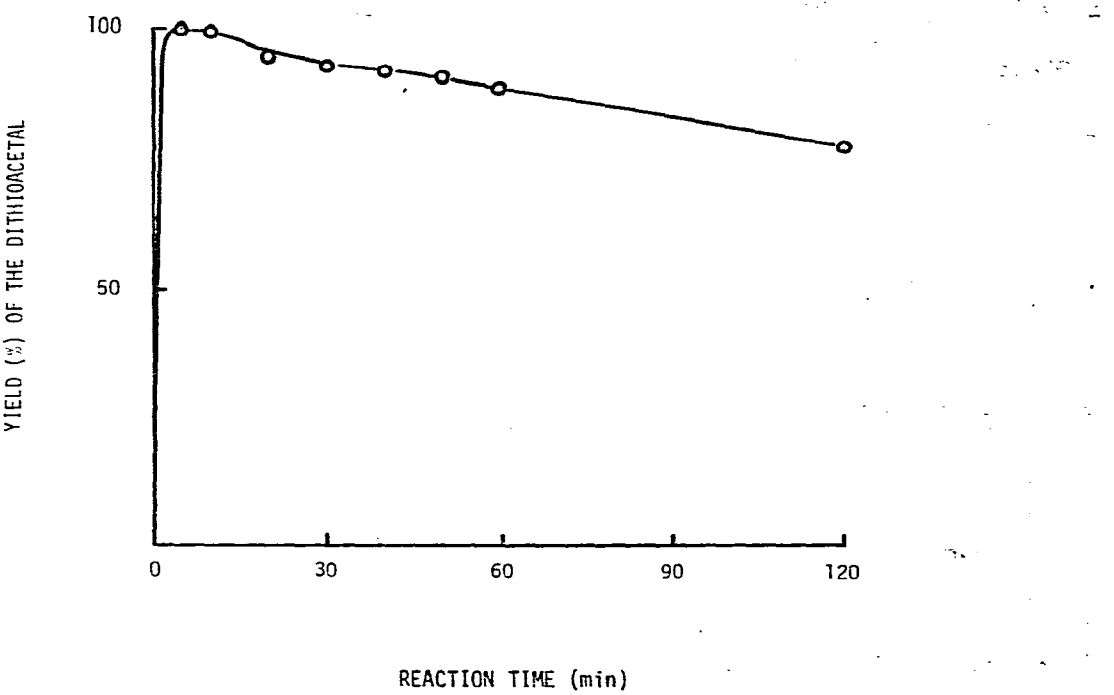

Fig. 4. Reaction course of mercaptalation of L-arabinose with a mixture of ethanethiol and trifluoroacetic acid (2:1) at 25°.

On the basis of these results, a procedure for rapid analysis of aldoses was devised, as described in the Experimental section. The conditions for trimethylsilylation was based on the results reported previously[5]. *myo*-Inositol used for the internal standard had an appropriate retention time for the analysis of aldose mixtures. Because of its poor solubility in pyridine, it was added at the sample preparation stage, but not after mercaptalation. It was sufficiently stable under ordinary conditions for hydrolysis of carbohydrate materials.

The calibration curves for pentoses, hexoses and methylpentoses showed excellent linearity between 10^{-8} and 10^{-5} mole when detected by a flame ionization detector. The limit of detection could be lowered to the 10^{-10} mole level by use of a flame photometric detector, at 394 nm, although linearity was not observed. For aldose mixtures the total molar amount should not exceed 10^{-5} mole, otherwise the amounts of aldoses may be underestimated, because of a decrease in the relative molar ratio of ethanethiol to total aldoses. Table I indicates that these aldoses were determined accurately and with high reproducibility.

Fig. 5 shows some applications of the dithioacetal method to the analysis of constituent monosaccharides in oligo- and poly-saccharides. The molar ratios of

TABLE I

ACCURACY AND PRECISION OF THE DETERMINATION OF TYPICAL ALDOSES

Aldose	*Amount of sample added (mole)*	*No. of determinations*	*Average value found (mole)*	*Coefficient of variation*
L-Arabinose	$1.00 \cdot 10^{-7}$	7	$1.00 \cdot 10^{-7}$	3.9
	$1.00 \cdot 10^{-6}$	5	$1.00 \cdot 10^{-6}$	1.7
D-Glucose	$1.01 \cdot 10^{-7}$	7	$1.04 \cdot 10^{-7}$	3.9
	$1.01 \cdot 10^{-6}$	5	$1.00 \cdot 10^{-6}$	1.6
L-Rhamnose	$1.03 \cdot 10^{-7}$	7	$1.00 \cdot 10^{-7}$	5.7
	$1.03 \cdot 10^{-6}$	5	$1.01 \cdot 10^{-6}$	1.9

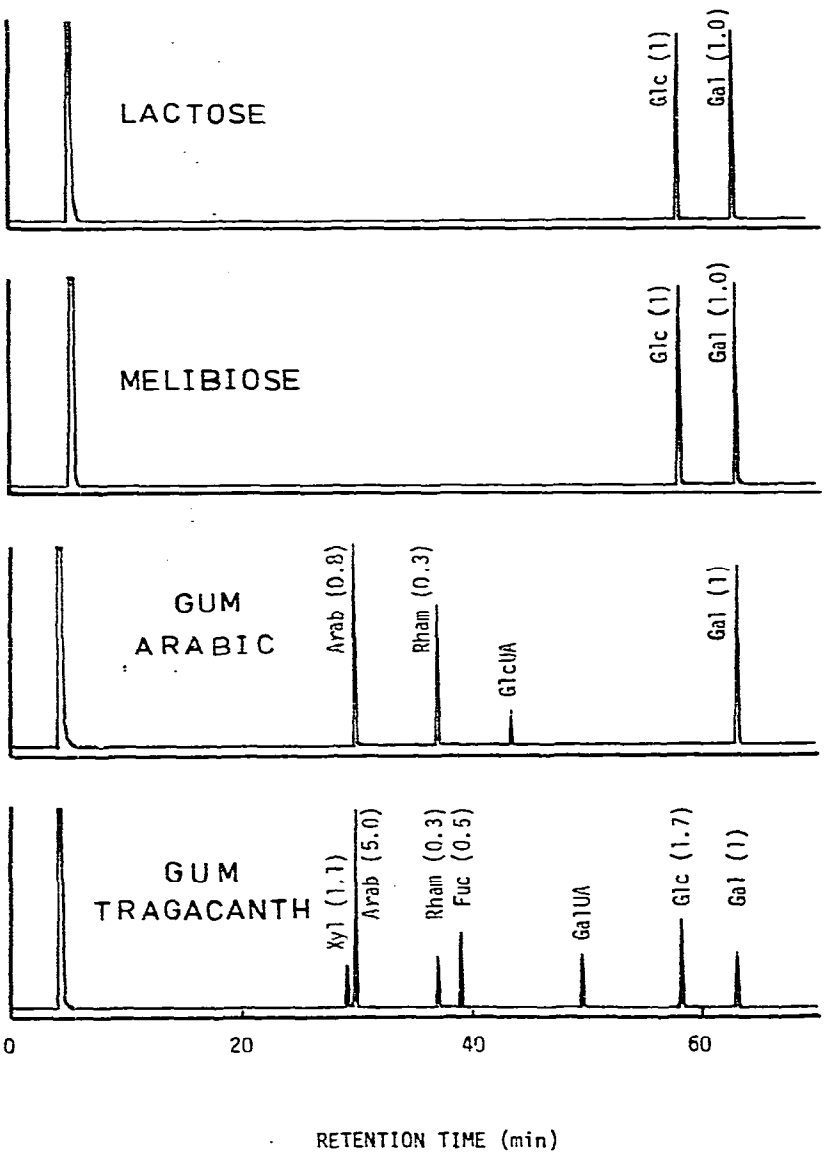

Fig. 5. Analysis of the constituent monosaccharides of selected oligo- and poly-saccharides. Abbreviations as in Fig. 1.

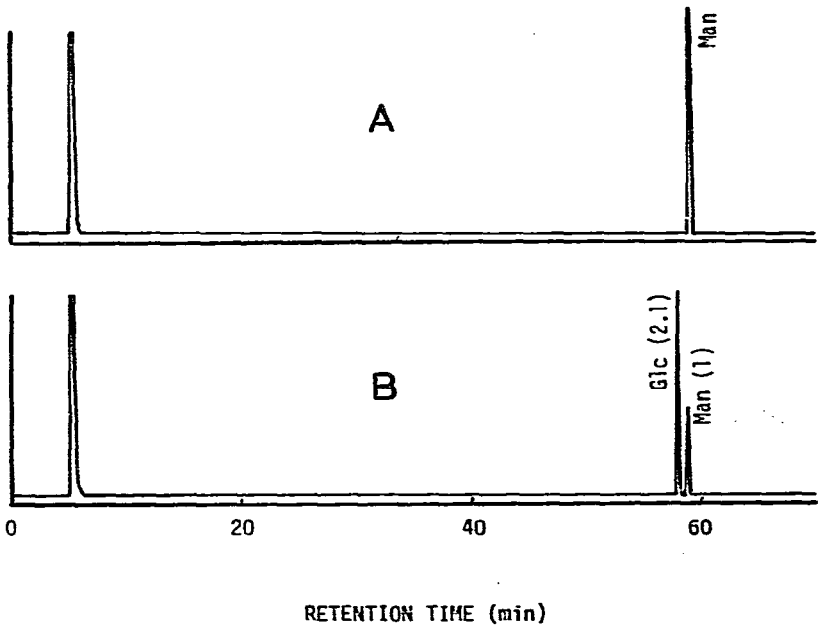

Fig. 6. Molybdic acid-catalyzed epimerization of D-mannose. A, In the absence of molybdic acid (blank), 100°, 5 h; B, with 1% molybdic acid, 100°, 5 h.

D-galactose relative to D-glucose were as calculated for both oligosaccharides (lactose and melibiose). The relative molar proportions of constituent monosaccharides in gum arabic were in approximate agreement with those reported[7], but the monosaccharide composition of gum tragacanth was rather different from that previously described[8]. A large amount of D-glucose which was not reported in the literature was detected in this preparation, possibly due to contamination with polyglucoses such as cellulose and starch.

It is known that molybdic acid catalyzes the epimerization of aldoses. Examination by the dithioacetal method indicated that approximately two thirds of D-mannose was converted into D-glucose by heating its aqueous solution containing a trace of molybdic acid for 5 h at 100°, whereas no appreciable change was observed in the absence of molybdic acid under the same conditions (Fig. 6). This result is consistent with that obtained by high-performance liquid chromatography[9].

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