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SEPARATION OF ALDITOL ACETATES FROM PLASTICIZERS AND OTHER CONTAMINANTS BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Alditol acetates and contaminating plasticizers were separated on a Silar 10C glass capillary column. The retention times of a range of phthalate, adipate and sebacate esters are reported under chromatographic conditions suitable for the separation both of alditol acetates and of permethylated alditol acetates. Ways of minimizing contamination are discussed.

INTRODUCTION

Gas chromatography (GC) of alditol acetates and permethylated alditol acetates is frequently used in the analysis of polysaccharide structure. The composition of mixtures of monosaccharides in hydrolysates is determined after conversion into alditol acetates[1], and the linkages between the monosaccharide residues are determined by methylation analyses[2].

Alditol acetates and permethylated alditol acetates are best separated using capillary columns and polar phases[3]. However, many other compounds elute from polar phases with similar retention times, *e.g.*, plasticizers such as phthalate, adipate and sebacate esters, fatty acid methyl esters and chlorinated hydrocarbon pesticides. If present as contaminants in the sample, these compounds are likely to interfere with the analysis of alditol acetates and permethylated alditol acetates. Phthalate esters, the most frequently used plasticizers, are widespread pollutants of the environment and have been repoted from such diverse sources as foods[4,5], river water[6], soils[7], human tissues[8–10] and analytical-grade laboratory chemicals[11]. Phthalates have been reported to be rapidly metabolized in common laboratory animals and were thought to have low toxicity[12,13]. However, di-(2-ethylhexyl) phthalate, the most commonly used plasticizer, has recently been found to be carcinogenic in rats and mice[14].

Dudman and Whittle[15] investigated the possible interference of three common plasticizers, dibutyl phthalate, di-(2-ethylhexyl) phthalate and butylbenzyl phthalate, in the analysis of alditol acetates using columns packed with the polar polyester phases ECNSS-M and EGS/GE-XF 1150. They found that di-(2-ethylhexyl) phthalate was not

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resolved from xylitol penta-acetate and butylbenzyl phthalate was not resolved from mannitol hexa-acetate or galactitol hexa-acetate.

Recently, capillary columns coated with the polar cyanoalkyl silicone phases OV-275[16], BP75[3] and Silar 10C[17–19] have been used to separate alditol acetates and permethylated alditol acetates. We now report the retention times of a range of alditol acetates, permethylated alditol acetates, and phthalate, adipate and sebacate esters on a Silar 10C glass capillary column. These data can be used as a guide to the identity of contaminants that may be encountered in the analysis of polysaccharide structure.

MATERIALS AND METHODS

Materials

Phthalate, sebacate and adipate esters were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). *Myo*-inositol and erythritol were obtained from BDH (Poole, Great Britain). Laminaritetrose was prepared by the method of Whelan[20]. All other sugars were obtained from Sigma (St. Louis, MO, U.S.A.). Dichloromethane (extra pure, Cat. 6047) was obtained from Merck (Darmstadt, G.F.R.) and diglyme (diethyleneglycol dimethylether) (purum) from Fluka (Buchs, Switzerland). *p*-Hydroxy-benzaldehyde and vanillin were from Sigma, and syringaldehyde was from Aldrich (Gillingham, Great Britain).

Analysis of impurities in reagents

Organic solvents volatile at less than 200°C were analysed for contaminants by the direct injection of 2 μl onto a Silar 10C glass capillary column held at 180°C for 4 min then heated to 230°C at 4°C/min. Contaminants were concentrated by evaporation of the solvents[11]. Contaminants in other reagents were analysed by extraction into pure dichloromethane prior to GC.

Preparation of alditol acetates

Alditol acetates were prepared both analytically and preparatively by the method of Blakeney *et al.*[18]. Permethylated alditol acetates were prepared from cellobiose, laminaritetraose, 3-O-β-D-galactopyranosyl-D-arabinose, 1-O-methyl-α-L-arabinopyranoside, 1-O-methyl-β-D-xylopyranoside and larch arabinogalactan by a modification of the method of Jansson *et al.*[21], and the identities of the derivatives were confirmed by mass spectrometry (MS).

Gas chromatography

Alditol acetates were separated on a 28 m × 0.5 mm I.D. Silar 10C support-coated open tubular glass capillary column and on a 6 m × 0.2 mm I.D., BP-75, vitreous-silica wall-coated open tubular column (SGE-Melbourne, Australia) in a Hewlett-Packard 5710A chromatograph equipped with a flame ionization detector and a modified SGE "Unijector" capillary injection system, used in the split mode. High-purity hydrogen (less than 10 ppm oxygen) was used as the carrier gas at a flow-rate of 81 cm/sec (determined using dichloromethane). The carrier gas was further purified by passing it through two oxygen traps (Oxy-trap; Alltech Assoc., Melbourne, Australia), a drying tube filled with Linde molecular sieve 5A and a 7-μm in-line filter (Supelco, Bellafonte, PA, U.S.A.).

Two temperature programmes were used: (a) 180°C for 4 min, followed by a 4°C/min rise to 230°C, for the analysis of alditol acetates; and (b) 150°C for 4 min, followed by a 4°C/min rise to 230°C for permethylated alditol acetates. The injection port and detector were heated to 250°C and 300°C, respectively. Peak areas and retention times were recorded using a Hewlett-Packard Model 3380A reporting integrator.

RESULTS AND DISCUSSION

Gas chromatography of alditol acetates contaminated with plasticizers

The retention times of thirteen phthalate, three adipate and two sebacate esters,

TABLE I

RETENTION TIMES OF PLASTICIZERS AND ALDITOL ACETATES ON A SILAR 10C GLASS CAPILLARY COLUMN

Temperature programme: 180°C for 4 min, followed by a 4°C/min rise to 230°C.

Component	*Retention time (min)*
Diisobutyl adipate	1.8
Diisopropyl phthalate	3.4
Dimethyl phthalate	3.6
Diethyl phthalate	4.1
Diisobutyl phthalate	5.2
Di-*n*-butyl sebacate	5.7
Erythritol tetraacetate	6.0
Diallyl phthalate	6.6
Di-*n*-butyl phthalate	7.1
Di-(2-ethylhexyl) adipate	7.1
Rhamnitol pentaacetate	8.6
Fucitol penta acetate	9.0
Diisodecyl adipate*	9.9**
Ribitol pentaacetate	10.8
Arabinitol pentaacetate	11.2
Diisoheptyl phthalate	11.6***
Di-(2-ethylhexyl) phthalate	11.6
Di-(2-ethylhexyl) sebacate	11.9
Diisononyl phthalate	12.9
Xylitol pentaacetate	13.0
Deoxyglucitol pentaacetate	13.5
Di-*n*-octyl phthalate	14.2
Allitol hexaacetate	14.7
Mannitol hexaacetate	15.4
Diisodecyl phthalate*	14.9**
Galactitol hexaacetate	16.2
Dicyclohexyl phthalate	17.0
Glucitol hexaacetate	17.2
Benzylbutyl phthalate	17.5
Inositol hexaacetate	18.6
Diisotridecyl phthalate§	–

* Very broad peaks.

** Approximate midpoint of peak.

*** Small peak slightly shorter retention time but not resolved.

§ Not eluted in 25 min.

TABLE II

RETENTION TIMES OF PLASTICIZERS AND PERMETHYLATED ALDITOL ACETATES ON A SILAR 10C GLASS CAPILLARY COLUMN

Temperature programme: 150°C for 4 min followed by a 4°C/min rise to 230°C.

Component	*Retention time (min)*
Diisobutyl adipate	4.3
Diisopropyl phthalate	7.8
Dimethyl phthalate	7.8
1,4-Di-O-acetyl-2,3,5-tri-O-methylarabinitol	8.2
Diethyl phthalate	8.6
1,5-Di-O-acetyl-2,3,4-tri-O-methylarabinitol	9,5
Diisobutyl phthalate	10,5
Di-*n*-butyl sebacate	11,1
1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylglucitol	11,6
1,3,4-Tri-O-acetyl-2,5-di-O-methylarabinitol	11,9
Diallyl phthalate	12,0
1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol	12,6
Di-*n*-butyl phthalate	12,7
Di-(2-ethylhexyl)adipate	13,0
1,3,5-Tri-O-acetyl-2,4-di-O-methylarabinitol	13,4
1,3,5-Tri-O-acetyl-2,4,6-tri-O-methylglucitol	14,6
1,4,5-Tri-O-acetyl-2,3-6-tri-O-methylglucitol	16.2
1,5,6-Tri-O-acetyl-2,3,4-tri-O-methylgalactitol	17.3
Diisoheptyl phthalate	17.6
Di(2-ethylhexyl) phthalate	17.6
Di(2-ethylhexyl) sebacate	18.0
Diisononyl phthalate	19.1
1,3,5,6-Tetra-O-acetyl-2,4-di-O-methylgalacitol	19.9
Dicyclohexyl phthalate	22.8
Benzylbutyl phthalate	23.3
Inositol hexaacetate	24.9

twelve alditol acetates and ten permethylated alditol acetates chromatographed on a Silar 10C glass capillary column are shown in Tables I and II. The same elution order was also obtained on a high-polarity wall-coated open-tubular column, BP75, produced by bonding the polar phase, OV-275, on vitreous-silica. Diisoheptyl phthalate, di-(2-ethylhexyl) phthalate and di-(2-ethylhexyl) sebacate have retention times close to, but resolved from, arabinitol pentaacetate and may interfere with the detection and estimation of arabinose. Similarly diisononyl phthalate may interfere in the analysis of xylose. Dicyclohexyl phthalate and benzylbutyl phthalate have retention times close to, but resolved from, glucitol hexaacetate and may interfere with the detection and estimation of glucose.

Figs. 1 and 2 show chromatograms of plasticizers, alditol acetates and permethylated alditol acetates. The chromatograms show the ability of capillary columns to resolve a large number of plasticizers from alditol acetates. The problem of these contaminants and the need for high-resolution chromatography is increased by subjecting them to the methylation, reduction and acetylation procedures used in polysaccharide analysis. Not only do the peaks due to plasticizers remain following these reactions, but further peaks are produced, possibly owing to the formation of additional derivatives by de-esterification[9] and transesterification.

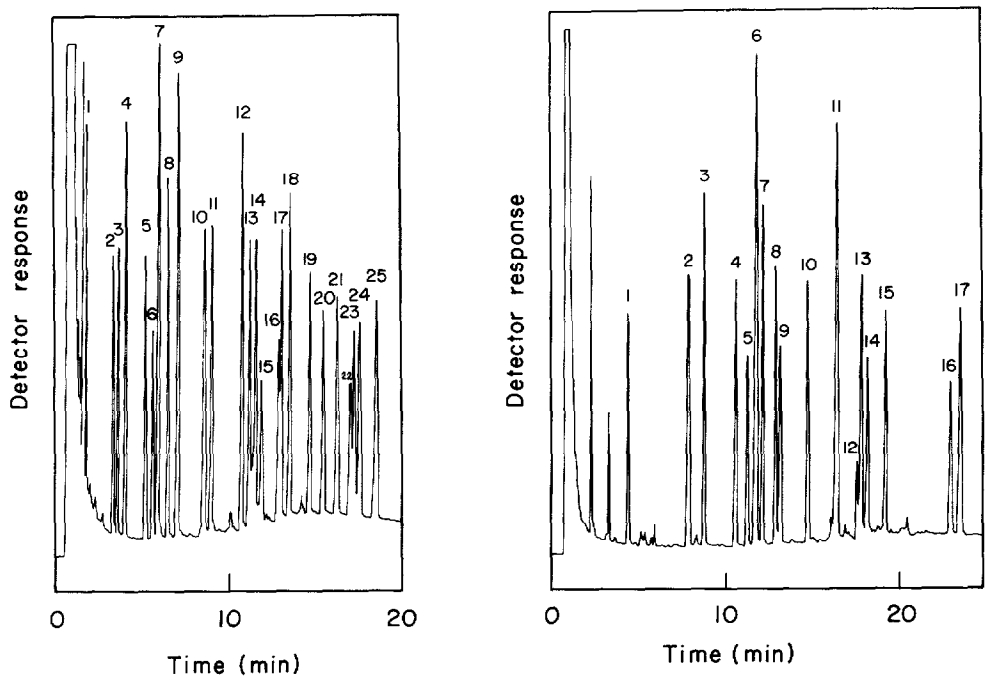

Fig. 1. Separation of alditol acetates and plasticizers on a Silar 10C glass capillary column. Temperature programme : 180°C for 4 min, followed by a 4°C/min rise to 230°C. Peaks : 1 = diisobutyl adipate ; 2 = diisopropyl phthalate ; 3 = dimethyl phthalate ; 4 = diethyl phthalate ; 5 = diisobutyl phthalate ; 6 = di-*n*-butyl sebacate ; 7 = erythritol tetraacetate ; 8 = diallyl phthalate ; 9 = di-*n*-butyl phthalate and di-(2-ethylhexyl) adipate ; 10 = rhamnitol pentaacetate ; 11 = fuctol pentaacetate ; 12 = ribitol pentaacetate ; 13 = arabinitol pentaacetate ; 14 = diisoheptyl phthalate and di-(2-ethylhexyl) phthalate ; 15 = di-(2-ethylhexyl) sebacate ; 16 = diisononyl phthalate ; 17 = xylitol pentaacetate ; 18 = 2-deoxyglucitol pentaacetate ; 19 = allitol hexaacetate ; 20 = mannitol hexaacetate ; 21 = galactitol hexaacetate ; 22 = dicyclohexyl phthalate ; 23 = glucitol hexaacetate ; 24 = benzylbutyl phthalate ; 25 = inositol hexaacetate.

Fig. 2. Separation of permethylated, peracetylated alditol acetates and plasticizers on a Silar 10C glass capillary column. Temperature programme : 150°C for 4 min, followed by a 4°C/min rise to 230°C. Peaks : 1 = diisobutyl adipate ; 2 = diisopropyl phthalate and dimethyl phthalate ; 3 = diethyl phthalate ; 4 = diisobutyl phthalate ; 5 = di-*n*-butyl sebacate ; 6 = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucose ; 7 = diallyl phthalate ; 8 = di-*n*-butyl phthalate ; 9 = di-(2-ethylhexyl) adipate ; 10 = 1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-D-glucitol ; 11 = 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-glucitol ; 12 = diisoheptyl phthalate ; 13 = diisoheptyl phthalate and di-(2-ethylhexyl) phthalate ; 14 = di-(2-ethylhexyl) sebacate ; 15 = diisononyl phthalate ; 16 = dicyclohexyl phthalate ; 17 = benzylbutyl phthalate.

The resolution of phthalate, adipate and sebacate esters on a Silar 10C glass capillary column (Figs. 1 and 2) incidentally demonstrates the suitability of high-polarity capillary columns for determining these compounds in residue analysis.

Contaminants in reagents

In addition to their possible presence in the polysaccharide samples, contaminants may also be introduced from reagents used in the analysis. Ishida *et al.*[11] reported plasticizers in a wide range of reagents, and Dudman and Whittle[15] found these contaminants in chloroform and acetic anhydride. To assess this problem reagents were

analysed directly by GC and the grades of reagents containing the lowest levels of contaminants were chosen for use in subsequent analyses. Many high-purity analytical reagents contained traces of contaminants which were extracted and thus concentrated into the sample. For example, we experienced serious contamination of alditol acetates prepared by the method of Blakeney *et al.*[18] when using diglyme[22] rather than dimethyl sulphoxide as the solvent for reduction. A control reduction and acetylation containing no carbohydrate gave many peaks. Analysis of the reagents indicated that most of the contaminants, some of which were identified as plasticizers, were present in the diglyme. These contaminants could be removed by vacuum fractional distillation of the diglyme (140°C, 360 mmHg) from sodium borohydride[23], using glassware baked at 200°C for 24 h[11].

Other possible contaminants in the sample

Biological samples may contain lipids, pesticides, phenolics and other compounds which can potentially interfere in the analysis of monosaccharides as they, or derivatives formed from them during sample derivatization, have retention times in the same range as alditol acetates and permethylated alditol acetates. The same plasticizers that interfere in the analysis of alditol acetates have been found to interfere in the analysis of fatty acids[11]. The phenolic aldehydes, *p*-hydroxybenzaldehyde, vanillin and syringaldehyde are found in plant cell walls[24] and may interfere in polysaccharide analysis since we found that when acetylated they also have retention times in the same range as alditol acetates (Fig. 3). Vanillin is close to but resolved from fucose, and syringaldehyde is poorly resolved from 2-deoxyglucose.

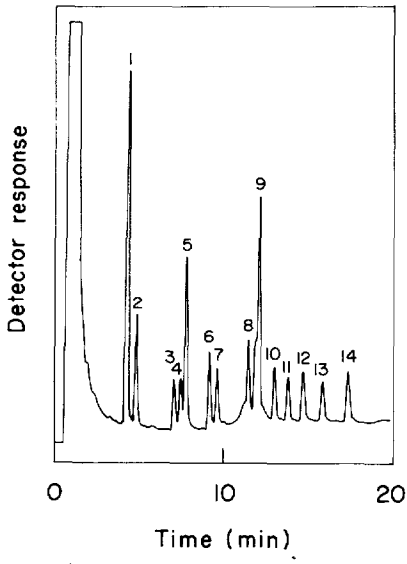

Fig. 3. Separation of the reduced and acetylated derivatives of monosaccharides and phenolic aldehydes on a Silar 10C glass capillary column. Temperature programme: 180°C for 4 min, followed by a 4°C/min rise to 230°C. Peaks : 1 = *p*-hydroxybenzaldehyde ; 2 = erythritol ; 3 = rhamnose ; 4 = fucose ; 5 = vanillin ; 6 = ribose ; 7 = arabinose ; 8 = xylose ; 9 = syringaldehyde with 2-deoxyglucose in the leading edge ; 10 = allose ; 11 = mannose ; 12 = galactose ; 13 = glucose ; 14 = inositol.

Analysis of contaminated samples

Removal of contaminating plasticizers after formation of alditol acetates is difficult because of their similar solubility and volatility. Thus, the best approach is to attempt to avoid or minimize contamination of the sample. To achieve minimum contamination it is essential to use the best possible grade of reagents and remove plasticizers from glassware by heating. Contaminants in the polysaccharide sample may be removed by a preliminary extraction with, for example, pure or purified dichloromethane or chloroform. These procedures are necessary where analysis of small samples of polysaccharides is being attempted since interference by contaminants is probably the most important factor in determining the limits of reliable detection of alditol acetates.

Some contamination of samples with plasticizers is almost inevitable because they are of such widespread occurrence. Thus reliable analysis of alditol acetates, especially at low concentrations, requires the use of a chromatographic system with the highest available resolution. Capillary chromatography on polar phases gives excellent resolution of alditol acetates from many, but possibly not all contaminants. Particular attention must be given to maintaining optimum chromatographic conditions since resolution has often been found to decline seriously with factors such as increasing column age, leading to failure to resolve some plasticizers from alditol acetates. We have found that column life is extended by using high-purity oxygen-free carrier gas, since the polar phases are oxygen sensitive[25].

It is possible to distinguish alditol acetates and permethylated alditol acetates from contaminating plasticizers by GC–MS[26] or by the use of other detection systems specific for particular compounds such as photoionization detector/flame ionization detector ratios[27,28]. However, for the routine analysis of polysaccharide structures, contamination may be minimized by using pure reagents, extracted samples and high-resolution capillary GC on polar columns.

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