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GAS AND LIQUID CHROMATOGRAPHIC BEHAVIOUR OF SOME ACETYLATED GLUCOSIDES*

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SUMMARY

The gas chromatographic (GC) separation of a series of anomeric peracetylated alkyl- and arylglucosides on Apiezon L (2% and 3%) supported on Chromosorb W AW DMCS was studied. The 3% phase loading showed satisfactory separations of anomers of most of the glycosides. High-performance liquid chromatography, particularly in the reversed-phase mode with Radial-Pak C_{18}, proved better for the separation not only of those compounds which could be separated by GC but also of those, such as *p*-biphenylyl- and cetyltetra-O-acetyl-D-glucosides, which presented difficulties in GC on Apiezon L.

INTRODUCTION

Gas chromatographic (GC) separations of sugars and related compounds have been achieved using methyl ethers[1,2], trimethylsilyl (TMS) ethers[3–5], acetates[5–7], trifluoroacetic acid (TFA) derivatives[5,8], etc. The relationship between mobility on the GC column and structural features of some carbohydrate derivatives, *e.g.*, TMS and acetyl derivatives of pentopyranoses and pentopyranosides[3,6] or TMS, TFA and acetyl derivatives of glycosides in pyranose and furanose configurations[5], have also been reported.

Recently, high-performance liquid chromatography (HPLC) has also been used for the separation of carbohydrates. HPLC separations of derivatized carbohydrates have been reported by McGinnis and Fang[9,10], Yoshida *et al.*[11] and many others. Wells and Lester[12] and Valent *et al.*[13] studied the separations of peracetylated oligosaccharides and partially methylated/ethylated oligosaccharides, respectively, using reversed-phase liquid chromatography.

The glycosidic linkage in glycosides and di- and oligosaccharides seems to be very important as the formation of many biologically interesting compounds depends on the spatial arrangement of the functional group at C-1. Consequently, the separation, identification and retention behaviour of glycosidic anomers on GC and HPLC columns would provide useful information for studies of carbohydrates and

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also facilitate the evaluation of different chromatographic systems as a means of monitoring glycosidation reactions. As studies of peracetylated glycosides have received little attention with respect to chromatographic behaviour, it was considered useful to study this aspect of the behaviour of some alkyl- and arylglycosides and a few dissaccharides by GC and HPLC.

This paper reports the separations of anomers of a series of peracetylated alkyl- and arylglycosides by GC using Apiezon L on Chromosorb W AW DMCS support, and by HPLC using normal- and reversed-phase modes, on silica and Radial-Pak C_{18} bonded phase, respectively. Some preliminary findings with Carbowax 20M on Chromosorb W AW are also reported.

EXPERIMENTAL

Materials

The peracetylated alkyl- and arylglycosides used were prepared in this laboratory[14].

Gas chromatography

Hewlett-Packard gas chromatographs, Model 700 equipped with a flame ionization detector and with temperature programming, and Model 5840A with a flame ionization detector and an HP 5840A data terminal, were used. The columns were packed with 2% and 3% Apiezon L on Chromosorb W AW DMCS (60–80 mesh) (1.8 m × 4.0 mm I.D.) and 5% Carbowax 20M on Chromosorb W AW (1.22 m × 4.0 mm I.D.). Nitrogen was used as the carrier gas at flow-rates between 30 and 40 ml/min.

High-performance liquid chromatography

A Waters Assoc. Model ALC/GPC 202/R401 liquid chromatograph, equipped with a Model 440 UV detector and a Model R401 refractive index detector, was used with a U6K injector and a Model 6000A pump. The following columns as supplied by Waters Assoc. were employed: (a) μPorasil (10 μm), 30 cm × 3.9 mm I.D., stainless steel, and (b) Radial-Pak C_{18} (10 μm), 10 cm × 8 mm I.D., HDPE, fitted in an RCM-100 radial compression module.

All solvents were purified to HPLC requirements and filtered through a 0.5 μm Millipore fluorocarbon membrane filter before use in the HPLC system.

All separations were carried out isocratically at room temperature.

RESULTS AND DISCUSSION

Separation of anomeric pairs by GC

Relative retention times are presented in Tables I and II for a series of alkyl- and aryl-D-glycosides. 1,2,3,4,6-Penta-O-acetyl-D-glucopyranose was used as a standard.

Alkylglucosides. It is clear from Table I that the anomeric methyl- and ethylglucosides could not be separated on the 3% Apiezon L column at 230 or 200°C. However, anomers of methylglucoside could be separated at 170°C (10.88 and 12.19 min for the α- and β-anomers, respectively) and those of ethylglucoside at 180°C

TABLE I

RELATIVE RETENTION TIMES OF α- AND β-ANOMERS OF ALKYLGLUCOSIDES ON APIEZON L COLUMNS

Retention times relative to glucose pentaacetate. The values in parentheses are the actual retention times in min.

Compound	*3% Apiezon L*				*2% Apiezon L, 220°C*	
	200°C		*230°C*			
	α-	β-	α-	β-	α-	β-
2,3,4,6-Tetra-O-acetyl-D-glucopyranoside:						
Methyl-	0.83	0.83	0.71	0.71	0.92	0.92
Ethyl-	0.82	0.90	0.76	0.76	0.96	0.96
Propyl-	1.10	1.20	0.95	1.02	1.39	1.39
Butyl-	1.50	1.77	1.23	1.37	1.82	1.82
tert.-Butyl-	1.10	1.21	0.95	1.02	1.30	1.30
Hexyl-	2.74	4.71	2.13	3.32	3.15	4.13
Cetyl-	1.19	No pk	1.19	No pk	No pk	No pk
1,2,3,4,6-Penta-O-acetyl-D-glucopyranose	1.00 (3.53)	1.00 (3.53)	1.00 (1.32)	1.00 (1.32)	1.00 (1.30)	1.00 (1.30)

TABLE II

RELATIVE RETENTION TIMES OF α- AND β-ANOMERS OF ARYLGLUCOSIDES ON APIEZON L COLUMNS

Compound	*3% Apiezon L*				*2% Apiezon L, 220°C*	
	220°C		*230°C*			
	α-	β-	α-	β-	α-	β-
2,3,4,6-Tetra-O-acetyl-D-glucopyranoside:						
Phenyl-	3.17	3.75	4.01	4.72	1.54	1.78
Benzyl-	3.43	4.53	4.87	5.58	2.69	3.08
2-Phenylethyl-	5.25	5.87	–	–	3.17	4.18
p-Chlorophenyl-	6.80	7.41	5.68	6.44	3.75	4.42
Phenyl-1-thio-	6.42	7.83	5.44	6.59	3.65	3.65
3-Phenylpropyl-	9.58	12.25	7.25	9.35	4.52	5.68
p-Biphenylyl-	No pk	No pk	No pk	No pk	No pk	No pk
2,3,4,6-Tetra-O-acetyl-D-galactopyranoside:						
Phenyl-	5.47	7.79	6.44	8.31	1.92	2.46
2-Phenylethyl-	6.58	8.75	8.55	11.46	2.98	3.94
1,2,3,4,6-Penta-O-acetyl-D-glucopyranose	1.00 (1.50)	1.00 (1.50)	1.00 (1.32)	1.00 (1.32)	1.00 (1.30)	1.00 (1.30)

(6.97 and 7.96 min for the α- and β-anomers, respectively) on the 3% column. The anomers of cetylglucoside were retained on the column, probably because of the nature of the stationary phase. All other alkylglucosides showed increasing retention times with increased chain length of the aglycone moiety at C_1, and the anomers were separated satisfactorily on the 3% Apiezon L column.

A 2% loading of Apiezon L gave virtually no separation of the anomers of any of the alkylglucosides, with the exception of hexylglucosides (retention times 4.10 and 5.37 min for the α- and β-anomers, respectively).

Arylglucosides. Although almost all of the arylglucosides showed separation of their anomeric pairs on the 2% Apiezon L column (Table II), the α- and β-anomers of phenyl-1-thioglucoside could not be resolved. Also, the anomers of *p*-biphenylyl-glucoside were retained on both the 2% and 3% columns. Thus, with the exceptions given above, the 3% Apiezon L column could separate anomeric pairs of all other compounds at both temperatures, *i.e.*, 220 and 230°C, and with sufficiently large differences in the retention times (Table II).

Relationship between retention times and structure. Ferrier[3,6] studied the relationship between the retention times and the structures of acetyl and TMS derivatives of pentopyranoses and pentopyranosides. Subsequently, Yoshida *et al.*[5] also reported on this relationship for glycoside derivatives on six stationary phases. In this study, a series of peracetylated, anomeric glycosides were chromatographed on Apiezon L.

It is clear from Tables I and II that the α-anomer in all the pairs of anomers studied eluted before the corresponding β-anomer. This observation is in agreement with that reported by Yoshida *et al.*[5]. The bulky groups at C_1 do affect the mobility, *e.g.*, the behaviour of anomers of *p*-biphenylylglucosides on the Apiezon L column. The mobility decreased in the order phenyl-, benzyl-, 2-phenylethyl-, phenyl-1-thio-, *p*-chlorophenyl- and 3-phenylpropylglucosides, although phenyl-1-thio- and *p*-chlorophenylglucosides do not show any regularity. Yoshida *et al.*[5] observed for the conformation–retention time relationship that the anomers of methyl-D-galactopyranosides eluted before those of the methyl-D-glucopyranosides. However, in our study, a comparison of the retention times of phenyl- and 2-phenylethyl-D-gluco- and galactopyranosides under the conditions employed showed that the retention times of galactopyranosides are longer than those of the corresponding glucopyranosides. This observation is in accordance with that of Ferrier[6] for the mobilities of tetra-O-acetylpentopyranoses on 20% Apiezon L on firebrick.

TABLE III

RETENTION TIMES OF PHENYL- AND *tert.*-BUTYL-2,3,4,6-TETRA-O-ACETYL-D-GLUCOPYRANOSIDES

Column: 5% Carbowax 20M at 190°C. Nitrogen carrier gas flow-rate: 20 ml/min.

Compound (aglycone)	*Retention time (min)*	
	α-Anomer	*β-Anomer*
Phenyl-	4.45	6.00
tert.-Butyl-	11.32	14.33

TABLE IV

RETENTION TIMES OF PERACETYLATED GLUCOSIDES ON μPORASIL COLUMN

Mobile phase: $CHCl_3$–CCl_4 (60:40). Flow-rate: 1.2 ml/min. Detection: UV (280 nm).

Compound	*Retention time (min)*	
	α-Anomer	*β-Anomer*
2,3,4,6-Tetra-O-acetyl-D-glucopyranoside:		
Phenyl-	3.33	3.87
Benzyl-	8.93	9.37
p-Chlorophenyl-	3.40	3.73
Phenyl-1-thio-	3.55	4.12
p-Biphenylyl-	3.00	3.27
2,3,4,6-Tetra-O-acetyl-D-galactopyranoside:		
Phenyl-	5.73	7.40
2-Phenylethyl-	9.07	10.13

Carbowax 20M. A few trials with 5% Carbowax 20M on a Chromosorb W AW column (4 ft × $^1/_4$ in. I.D.) at 190°C showed excellent separations of the anomers of phenyl- and *tert.*-butylglucosides (Table III). The sequence of elution of the α- and β-anomers is the same as that observed on the Apiezon L column.

High-performance liquid chromatography

Separations on a μPorasil column. The results are presented in Tables IV and V. Anomers of arylglucosides could be separated satisfactorily on μPorasil with chloroform–carbon tetrachloride (60:40) as the eluent at a flow-rate of 1.2 ml/min. However, the anomers of glucose pentaacetate could not be separated under these conditions.

Similarly, the separation of anomers of alkylglucosides (Table V) on μPorasil,

TABLE V

RETENTION TIMES OF PERACETYLATED GLUCOSIDES ON μPORASIL COLUMN

Mobile phase: light petroleum (b.p. 60–80°C)–ethyl acetate (1:1). Flow-rate: 0.3 ml/min. Detection: refractive index.

Compound	*Retention time (min)*	
	α-Anomer	*β-Anomer*
2,3,4,6-Tetra-O-acetyl-D-glucopyranoside:		
Methyl-	23.13	24.86
Ethyl-	20.40	22.40
Propyl-	19.20	20.80
Butyl-	18.07	19.50
tert.-Butyl-	19.05	20.85
Hexyl-	16.87	18.22
Cetyl-	16.13	18.93

TABLE VI

RETENTION TIMES OF PERACETYLATED DISACCHARIDES ON (A) μPORASIL AND (B) RADIAL-PAK C_{18} COLUMNS

μPorasil column: mobile phase, light petroleum–ethyl acetate (1:1); flow-rate, 0.3 ml/min. Radial-Pak C_{18} column: mobile phase, methanol–water (65:35); flow-rate, 1.5 ml/min. Detection refractive index (× 16).

Compound	*Retention time (min)*	
	A	*B*
Sucrose octaacetate	39.30	5.25
β-Maltose octaacetate	31.35	5.75
α-Lactose octaacetate	39.15	3.50
α-Cellobiose octaacetate	38.92	2.00*

* Solubility in methanol–water not satisfactory.

using light petroleum–ethyl acetate (1:1) as the eluent at a flow-rate of 0.3 ml/min was satisfactory, although glucose pentaacetate anomers could not be separated.

With both the alkyl- and arylglucosides, the α-anomer eluted first, but the retention times decreased with increase in the chain length at C_1 in alkylglucosides.

Separation of disaccharides. A mixture of some disaccharide peracetates was chromatographed on a μPorasil column with light petroleum–ethyl acetate as the eluent at a flow-rate of 0.3 ml/min (Table VI). Although maltose octaacetate could be separated from the peracetates of sucrose, α-lactose and α-cellobiose, the separation of these three compounds was not satisfactory.

TABLE VII

RETENTION TIMES OF PERACETYLATED ALKYL- AND ARYLGLUCOSIDES ON RADIAL-PAK C_{18} COLUMN

Mobile phase: methanol–water (65:35). Flow-rate: 1.5 ml/min. Detection: UV (254 nm), refractive index (× 16).

Compound	*Retention time (min)*	
	α-Anomer	*β-Anomer*
2,3,4,6-Tetra-O-acetyl-D-glucopyranoside:		
Phenyl-	10.50	8.25
Benzyl-	6.88	5.75
p-Chlorophenyl-	11.00	8.50
Phenyl-1-thio-	14.50	9.50
p-Biphenylyl-	25.62	18.75
Methyl-	3.50	3.10
Ethyl-	4.25	3.50
Propyl-	6.50	5.50
Butyl-	9.50	7.25
tert.-Butyl-	8.12	5.50
2,3,4,6-Tetra-O-acetyl-D-galactopyranoside:		
Phenyl-	7.12	4.75
2-Phenylethyl-	8.50	6.63

TABLE VIII

CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF TETRA-O-ACETYLATED ARYL- AND ALKYLGLUCOSIDES

*Aglycone**	*μPorasil column*			*Radial-Pak C_{18} column*		
	k'		α	k'		α
	α-	β-		α-	β-	
Phenyl-	0.28	0.49	1.75	4.25	3.13	1.36
Benzyl-	2.43	2.74	1.13	2.44	1.88	1.30
p-Chlorophenyl-	0.31	0.43	1.39	4.50	3.25	1.32
Phenyl-1-thio-	0.37	0.58	1.57	6.25	3.75	1.67
p-Biphenylyl-	0.54	0.77	1.43	11.81	8.38	1.41
Methyl-	0.71	0.84	1.18	0.75	0.55	1.36
Ethyl-	0.54	0.69	1.28	1.13	0.75	1.51
Propyl-	0.41	0.53	1.29	2.25	1.75	1.29
Butyl-	0.35	0.46	1.39	3.75	2.63	1.43
tert.-Butyl-	0.46	0.60	1.30	3.06	1.75	1.75
Hexyl-	0.29	0.40	1.37	–	–	–
Cetyl-	0.20	0.41	2.05	–	–	–
Phenyl-	1.20	1.85	1.54	2.56	1.38	1.86
2-Phenylethyl-	2.49	2.90	1.16	2.25	2.32	1.40

* The first twelve are glucopyranosides and the last two are galactopyranosides.

Separations on Radial-Pak C_{18} column. Reversed-phase HPLC proved superior to the normal-phase technique and gave excellent separations of anomeric pairs of alkyl- and arylglucosides on a Radial-Pak C_{18} column with methanol–water (65:35) as the eluent at a flow-rate of 1.5 ml/min (Table VII). The *tert.*-butylglucosides eluted faster than the *n*-butylglucosides. The elution sequence of the anomers of glycosides on the μPorasil column was reversed on the RP C_{18} column, as expected. The glucose pentaacetate anomers were not separated even on this column.

The three peracetylated disaccharides, that were not separated on μPorasil could be readily separated on the Radial-Pak C_{18} column (Table VI).

Peracetylated phenylmaltoside. The separation of the α- and β-anomers of phenyl hepta-O-acetyl-D-maltoside was tried on both columns. The retention times were found to be α- = 24.45 min and β- = 23.32 min on μPorasil [with light petroleum–ethyl acetate (1:1) as the eluents, flow-rate 0.3 ml/min] and α- = 2.5 min and β- = 3.5 min on RP C_{18} [methanol–water (65:35) as the eluent, flow-rate 1.5 ml/min]. Obviously the elution sequence of the anomers is reversed in this instance compared with that for all other glycosides.

Table VIII gives the capacity factors, k', and separation factors, α, for various glycosides. A comparison of the separation factors indicates a generally better performance of the C_{18} column for the separation of anomeric pairs of acetylated glycosides.

CONCLUSION

The GC and HPLC behaviours of peracetylated alkyl- and arylglucosides show certain regularities in relation to their structural features. Although GC can be used for the separation of less volatile esters (acetates) of glycosides, HPLC, particularly in the reversed-mode, seems to be a more efficient technique for such separations.

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