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GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF PERMETHYLATED ALDITOLS AND ALDONIC ACIDS

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SUMMARY

Methylation of reduced aldoses and hexuronic acids or methylation of oxidized aldoses have provided permethylated alditols and aldonates whose chromatographic mobilities on the stationary phases SE-30, QV-17, QF-1, and XE-60/EGS have been determined and the relative efficiencies of these chromatographic systems established.

INTRODUCTION

Alditols which are formed by reduction of the carbonyl group of aldoses and ketoses can be found in the lower orders of the plant kingdom such as marine algae[1], fungi[2] and lichens[3]. Identification of these acyclic polyhydric alcohols by gas–liquid chromatography (GLC) of their corresponding peracetates[4–6], pertrifluoroacetates[7] and pertrimethylsilyl ethers[8,9] has been investigated in some detail. However, the use of permethylated derivatives has been confined only to a few polyols[10].

Oxidation of the aldehydic group in aldoses or reduction of this group in uronic acids produces the corresponding aldonic acids. The common hexuronic acids, found in animal, microbial and plant polysaccharides[11], on reduction would provide the following aldonic acids:

L-Guluronic acid →D-gluconic acid
D-Mannuronic acid →D-mannonic acid
D-Glucuronic acid →L-gulonic acid
D-Galacturonic acid→L-galactonic acid

Aldonic acids, however, have the ability to exist in the cyclic 1,4- and/or the 1,5-lactone form, in which they have been identified by GLC of their corresponding pertrimethyl silyl ethers[12–14]. The trimethyl silyl esters of aldonic acids have been detected by GLC in association with the corresponding lactone forms[15]. With the formation of a single volatile derivative for each aldonic acid, hence uronic acid, the GLC examination of the reduced hydrolysate of an acidic-neutral polysaccharide would provide simultaneous identification of constituent acidic and neutral sugar residues. Also of importance is the increasing need for a convenient identification of aldonic acids in pollution and quality control when these acids are present in sulfite waste liquors[16], hydrolysates of oxidized cotton[17] and wood pulps[18].

It appeared reasonable to assume that methylation, by the Hakomori method[19], would provide for the methyl ester methyl ether derivatives of aldonic acids and the

corresponding methyl ether derivatives of alditols thereby affording volatile compounds suitable for GLC and mass spectrometric examination. Unlike the pertrimethyl silyl derivatives, these permethylated derivatives would afford ion fragments of lower mass, thus facilitating the use of low-resolution mass spectrometers[20]. To evaluate the resolution of permethylated alditols and aldonic acids by GLC four columns with liquid phases possessing diverse degrees of polarity were employed. Details of the chromatographic mobilities of these derivatives are presented in this paper.

EXPERIMENTAL

Materials

The commercial sodium salt of glucoheptonic acid and the calcium salts of glyceric, galactonic, 2-ketogluconic and 5-ketogluconic acids were used in the preparation of the corresponding permethylated derivatives. All other derivatives were formed by the oxidation or reduction of corresponding commercially available parent aldoses.

Preparation of alditols

The parent sugars (30 mg) were reduced in water (30 ml) with sodium borohydride (300 mg) for 2–4 h. After treatment with Rexyn resin 101(H^+) and concentration to dryness on a rotary evaporator, boric acid was removed by codistillation with methanol and the product thoroughly desiccated on a high-vacuum pump.

Preparation of aldonic acid salts

To the parent sugars (30 mg) in water (30 ml, 0°) containing barium carbonate (100 mg) bromine (0.1 ml) was added and the mixtures were shaken and then stored 24 h in the dark at room temperature. Excess bromine was removed by aeration and the mixtures reduced to half volume by evaporation in a round-bottom flask on a rotary evaporator. Neutral red indicator was added to the mixtures followed by the dropwise addition of aqueous barium carbonate to maintain an orange colouration (pH 8.0) for a period of 2 h. Carbon dioxide was passed into the mixtures to pH 7.0 and the barium salts dried by evaporation with the last traces of water being removed on a high-vacuum pump.

Hexuronic acids (30 mg) were reduced with sodium borohydride as in the preparation of alditols above.

Preparation of permethylated derivatives

The round-bottom flasks containing the alditols and aldonates, prepared above or as the commercial salts, were covered by rubber septa, flushed with nitrogen and dry-distilled methyl sulphoxide (30 ml) was introduced by syringe and the mixtures ultrasonicated for 1 h (ref. 21). A solution of methylsulphinyl carbanion in methyl sulphoxide (2 *M*, 20 ml) was then added to the mixtures, which were further ultrasonicated for 1 h and allowed to stand for the same period of time. The resultant mixtures were solidified by cooling and methyl iodide (10 ml) was added to complete the methylation. The reaction solutions were poured into cold dilute aqueous acetic acid to minimize possible high alkalinity and the resultant mixtures extracted with

chloroform. The combined extracts were water-washed, dried over anhydrous magnesium sulphate and evaporated to yield the permethylated derivatives.

Gas–liquid chromatography

Chromatography was carried out using a Hewlett-Packard Model 5750 gas chromatograph with hydrogen flame detectors and fitted with coiled hardened aluminum columns (180×0.45 cm) packed with (a) 10% SE-30 (w/w) on DMCS-treated Chromosorb W (60–80 mesh), (b) 1.5% XE-60/1.5% EGS (w/w) on DMCS-treated Chromosorb W (60–80 mesh), (c) 3% QF-1 (w/w) on Gas-Chrom Q, and (d) 1% OV-17 (w/w) on Gas-Chrom Q. Development was made with nitrogen at a flow-rate of 60 ml/min at the column temperature specified and retention times are quoted relative to permethylated xylitol.

RESULTS AND DISCUSSION

The alditols were obtained from the parent aldoses by reduction with sodium borohydride and the corresponding aldonic acid salts were prepared either by borohydride reduction of hexuronic acids or by buffered bromine–water oxidation of the appropriate aldoses followed by treatment with base to eliminate lactone formation. Methylation of both groups of derivatives was achieved readily by treatment with methylsulphinyl carbanion in methyl sulphoxide followed by the subsequent addition of methyl iodide[19].

The relative retention values for the permethylated derivatives of the alditols and aldonates on four stationary phases are presented in Table I. Permethylated xylitol was used as an internal standard, and its retention times on the SE-30, OV-17, QF-1 and XE-60/EGS columns, were 2.8, 4.6, 5.7 and 2.6 min respectively. Based on Rohrschneider's polarity scale[22,23] and the retention index dispersion figures for five probe solutes quoted by McReynolds[24], the liquid stationary phases used were characterized in the following order of increasing polarity: SE-30 ($\Sigma\Delta I$ 217), OV-17 ($\Sigma\Delta I$ 884), QF-1 ($\Sigma\Delta I$ 1500) and XE-60/EGS ($\Sigma\Delta I$ 2772). The chromatographic mobilities of the permethylated alditols and aldonates relative to the polarities of these liquid phases are graphically illustrated in Fig. 1. The nature of the derived products was verified by mass spectrometric analyses of the peaks from the chromatography and details of the electron impact fragmentation patterns of these derivatives will appear in a subsequent communication.

The most discrete separation of the eight permethylated alditols examined was obtained on the medium-polar liquid phase QF-1. The most poorly resolved alditols were arabinitol and xylitol derivatives on all the liquid supports tested. Permethylated glucitol and mannitol were unresolved on the non-polar phase SE-30 and on the mildly polar phase OV-17, but were moderately separated on the polar column XE-60/EGS. The four deoxyalditol derivatives examined were effectively separated on OV-17 and only on the SE-30 column were permethylated rhamnitol and fucitol not significantly differentiated.

Complete resolution of the ten aldonate derivatives was achieved by the QF-1 liquid phase. The XE-60/EGS column did not separate the arabinonate and xylonate derivatives and only moderately separated the derivatives of mannonate and gluconate. Apart from the lack of separation of permethylated arabinonate and xylonate, the

TABLE I

RELATIVE RETENTION TIMES OF PERMETHYLATED ALDITOLS AND ALDONATES ON GLC

Compound	*Liquid phase*			
	10% SE-30 (140°)	*1% OV-17 (130°)*	*3% QF-1 (110°)*	*1.5% XE-60/ 1.5% EGS (130°)*
Alditols				
Glycerol	0.11	0.07	0.08	0.11
Erythritol	0.34	0.26	0.26	0.25
Ribitol	0.83	0.70	0.60	0.65
Arabinitol	1.00	0.95	1.05	1.00
[Xylitol	1.00	1.00	1.00	1.00]
Glucitol	2.46	2.82	2.46	2.49
Mannitol	2.47	2.80	2.84	2.62
Galactitol	2.80	3.41	3.88	3.38
Deoxyalditols				
2-Deoxyribitol	0.57	0.42	0.40	0.44
Rhamnitol	1.25	1.05	1.25	1.01
Fucitol	1.29	1.21	1.47	1.19
2-Deoxyglucitol	1.50	1.54	1.39	1.28
Aldonates				
Glycerate	0.16	0.18	0.24	0.24
Erythronate	0.45	0.50	0.65	0.63
Ribonate	1.10	1.56	1.43	1.55
Arabinonate	1.41	2.16	2.95	2.63
Xylonate	1.42	2.21	2.69	2.67
Gulonate	3.33	5.49	6.32	5.94
Mannonate	3.46	5.02	7.79	6.61
Gluconate	3.51	6.75	7.08	6.72
Galactonate	4.11	8.10	11.85	9.84
Glucoheptonate	7.43	12.39	13.79	12.58
Deoxyaldonates				
2-Deoxyribonate	0.80	0.83	1.02	0.93
Rhamnonate	1.71	2.37	3.61	2.46
Fuconate	1.87	2.43	4.38	3.35
2-Deoxygluconate	2.34	2.72	3.53	3.18
Ketoaldonates				
2-Ketogluconate	2.17	3.54	3.48	4.72
5-Ketogluconate	3.18	5.28	5.38	7.68

liquid phase OV-17 afforded resolution of the other aldonates. The SE-30 column failed to achieve adequate separation of the permethylated gulonate, mannonate and gluconate, which exhibited mobilities within the range R_x 3.33–3.51, and on the same phase arabinonate and xylonate derivatives were superimposable.

The four permethylated deoxyaldonates were separated satisfactorily on the SE-30 and XE-60/EGS liquid phases. However, the rhamnonate and fuconate derivatives on OV-17 and the rhamnonate and 2-deoxygluconate derivatives on QF-1 were only moderately differentiated. All four liquid phases proved adequate for the resolution of permethylated 2- and 5-ketogluconates.

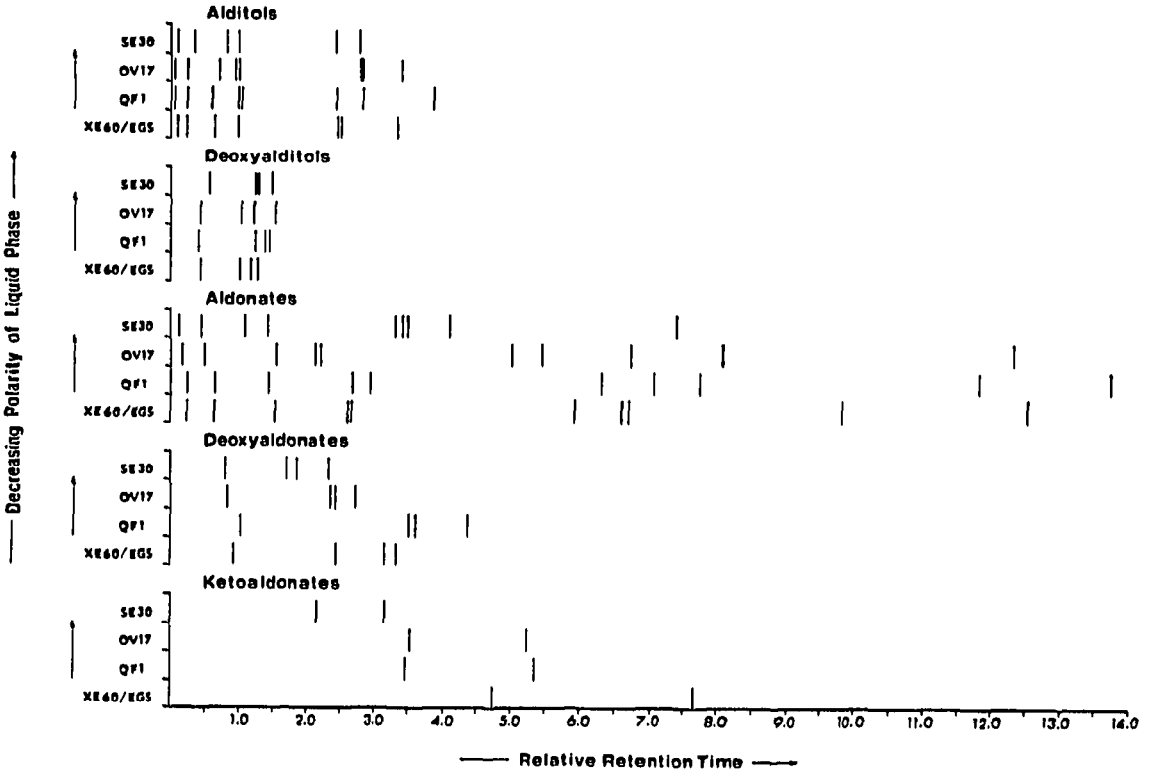

Fig. 1. Graphical representation of the chromatographic mobilities of permethylated alditols and aldonates relative to the polarity of the liquid phases.

The most effective resolution of permethylated gulonate, mannonate, gluconate and galactonate was achieved on the liquid phase QF-1, which also resolved the alditols and deoxyalditols at a faster retention time and would therefore be the obvious choice of liquid phase for analysing the products from the reduced and permethylated hydrolysate of an acidic-neutral polysaccharide. In the acidic polysaccharide alginic acid, isolated from brown seaweeds, the constituent sugar residues L-guluronic acid and D-mannuronic acid would be most effectively resolved as the corresponding gluconate and mannonate derivatives on the QF-1 column with the XE-60/EGS column offering the best alternative. In conclusion, the relative suitabilities of the stationary phases used for the separation of permethylated alditols and aldonates were ranked as follows: QF-1 > XE-60/EGS > OV-17 > SE-30, with the medium to polar phases offering the most effective resolution.

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