Carbohydrate Research, 119 (1983) 241–247
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

High-pressure liquid chromatography of dimethylphenylsilyl derivatives of some monosaccharides

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(Received May 18th, 1982; accepted for publication, February 24th, 1983)

G.l.c. has long been used[1,2] to separate the components of mixtures of volatile derivatives of monosaccharides and to identify anomeric forms. Ion-exchange[3] or gel-filtration[4] techniques have been applied to monosaccharides and oligosaccharides, but normally are time-consuming methods. For maximal sensitivity, such methods use destructive methods of detection, which is a major disadvantage when only small amounts of precious material are available.

High-pressure liquid chromatography (h.p.l.c.) offers an alternative approach, since it is more rapid and normally uses non-destructive detectors. Refractive-index detection has been used for monosaccharides[5,6] and oligosaccharides[7], but the method is only sensitive to ~20 μg. The mass detector (a destructive system of detection) can give a ten-fold increase in sensitivity[8], combined with improved stability and amenability to gradient elution. Non-specific u.v. detection at or below 200 nm has also been used[9], but requires solvents of high purity (and hence high cost).

The use of carbohydrate derivatives containing a suitable chromophore allows the use of u.v. detectors operating at higher wavelength, and also cheaper sol-

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0008-6215/83/$ 03.00

vents and detectors (fixed wavelength). Such u.v.-absorbing derivatives should (*a*) be easy to prepare in high yield, (*b*) absorb strongly at a convenient wavelength, (*c*) be compatible with preferred solvent systems, (*d*) allow ready regeneration of the carbohydrate precursor, and (*e*) be suitable for n.m.r. spectroscopy and/or mass spectrometry. Several derivatives have been proposed, including acetates[10], benzoates[11], 4-nitrobenzoates[12], and benzoylated benzyloximes[13], but a drawback to their use is the time-consuming preparation. We now report on dimethylphenylsilyl derivatives of some simple carbohydrates which can be prepared easily and are amenable to h.p.l.c. using a simple isocratic system and inexpensive solvents.

The use of silyl derivatives in the analysis of carbohydrates[2] is well established, but the commonly used trimethylsilyl derivatives are not suitable for h.p.l.c. because of their high volatility, susceptibility to hydrolysis, and poor u.v. absorbance. Such monoalkyl dimethylsilyl derivatives as (1,1-dimethylethyl)dimethylsilyl derivatives[14] are more stable towards hydrolysis but are not u.v.-absorbing, whereas an aromatic group confers intermediate stability and useful u.v. absorbance. Therefore, dimethylphenylsilyl derivatives were selected for study.

The initial method of dimethylphenylsilylation (100-mg scale, see Experimental) gave fully derivatised material contaminated with (*a*) substantial proportions of partially derivatised material (slower-running bands in t.l.c.) and (*b*) a fast-running product, the proportion of which was dependent on the amount of water present in the *N*,*N*-dimethylformamide used as reaction solvent. When sufficient

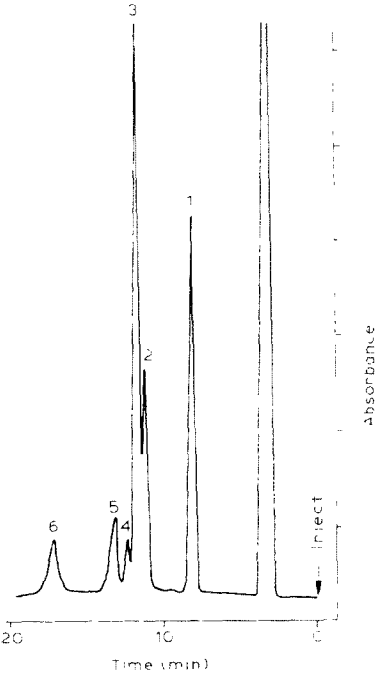

Fig. 1. H.p.l.c. of dimethylphenylsilylated pentoses (ethyl acetate–hexane, 1:99; at 1.5 mL/min). 1, D-Xyl; 2, D-Rib; 3, L-Ara, D-Rib, D-Lyx, D-Xyl; 4, L-Ara; 5, D-Lyx, and 6, L-Ara.

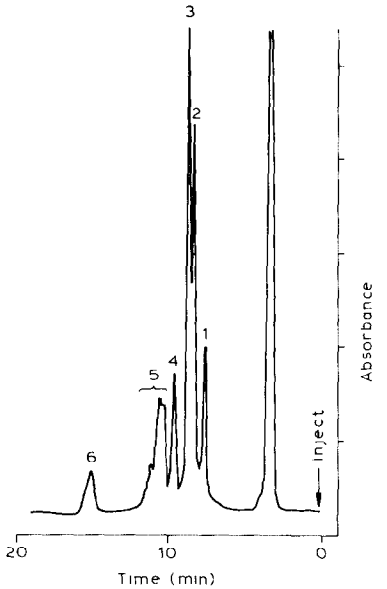

Fig. 2. H.p.l.c of dimethylphenylsilylated hexoses (conditions as in Fig. 1): 1, D-Glc; 2, D-Alt, D-Glc; 3, D-Gal, D-Man; 4, D-Gal; 5, D-Alt, D-Gal, D-Man; 6, D-Alt.

water was present, the only product was the fast-running compound, which is probably tetramethyldiphenyldisiloxane. By using dry solvent, the amount of this impurity can be kept to 10%, whilst the addition of a controlled amount of water after silylation reduced the quantity of partially derivatised material. For routine analysis on the 1–10 mg scale, a modified method was used (see Experimental).

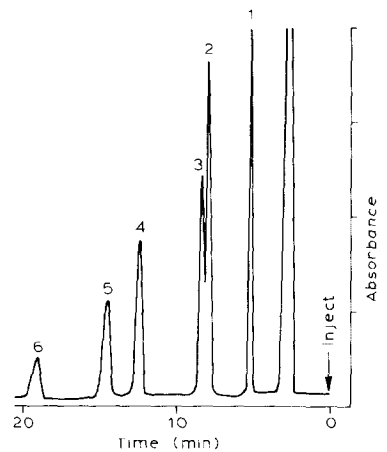

Fig. 3. H p.l.c. of dimethylphenylsilylated methyl glycosides (ethyl acetate–hexane, 1:49; at 1.5 mL/min): 1, Me-β-D-Glc*p* (*k'* 2.3); 2, Me-β-D-Xyl*p* (*k'* 3.6); 3, Me-β-D-Gal*p* (*k'* 3.8); 4, Me-α-D-Glc*p* (*k'* 5.6); 5, Me-α-D-Xyl*p* (*k'* 6.4); and 6, Me-α-D-Gal*p* (*k'* 8.4)

T.l.c. indicated that an ethyl acetate–hexane system was suitable for column use. For monosaccharide derivatives, a 1:99 mixture gave the best separation of the various anomeric forms and ring types, and maximum separation from the impurity (Table I). Alditol derivatives required a less-polar system (1:199 mixture), and disaccharides and methyl glycosides more-polar systems (3:197 and 1:49 mixtures, respectively), to allow quantitative analysis. Maximisation of resolution of the components of a mixture may require fine adjustment of the composition of ethyl acetate–hexane mixtures. Quantification can be achieved even though all of the peaks were not fully resolved (Figs. 1 and 2). The fact that more than one peak per carbohydrate is obtained can be useful for identifying carbohydrates of biological origin, and methods for analysing and quantifying overlapping peaks have been described[15].

Good separation of anomeric pairs of methyl glycosides was obtained (Fig. 3), and the sensitivity of the system was such that the equivalent of 440 ng of methyl β-D-glucopyranoside could be detected at 254 nm, or 250 ng at 260 nm, using maximum electronic amplification. A further increase in sensitivity can be obtained by concentration of the hexane solution or by detection at 210 nm, but this requires the use of purer solvents to ensure low levels of baseline noise.

EXPERIMENTAL

Materials. — *N,N*-Dimethylformamide (AnalaR) was purified[16], and stored over molecular sieves. Ethyl acetate (spectroscopy grade, BDH Chemicals Ltd.), chlorodimethylethylphenylsilane (Cambrian Chemicals Ltd.) and hexane (h.p.l.c. grade, Rathburn Chemicals) were used directly.

Dimethylphenylsilylation (cf. *ref. 14*). — (*a*) *Large scale.* To a solution of monosaccharide (100 mg) in dry *N,N*-dimethylformamide (2.5 mL) was added imidazole (2.5 equiv. per OH group), and the mixture was heated at 100° for 1 h, cooled to 0–4°, and treated with chlorodimethylphenylsilane (1.2 equiv. per OH group) for 6 h at ambient temperature. The product was extracted with hexane (2 × 3 mL), and the combined extracts were washed with water (3 × 3 mL), dried (Na_2SO_4), and concentrated using a stream of nitrogen. The resulting oil was stored at −20°. T.l.c. (Kieselgel 60 F_{254}; ethyl acetate–hexane, 1:9) of the product thus obtained, followed by fluorescence-quenching densitometry (Perkin–Elmer MPF4 fluorimeter with scanning attachment), revealed, mainly, fully dimethylphenylsilylated products, together with significant proportions of impurity (probably tetramethyldiphenyldisiloxane) and incompletely dimethylphenylsilylated products, as exemplified by the following data.

Products from (*a*) D-glucose: R_t 0.52 (11.1%, impurity), 0.46 and 0.42 (80.9%), 0.11 (8%, partially derivatised material); n.m.r. data (100 MHz, $CDCl_3$, external Me_4Si): δ 4.63 (d, $J_{1,2}$ 4 Hz, pyranose H-1α), 4.38 (d, $J_{1,2}$ 7 Hz, pyranose H-1β): αβ-ratio ~1:1. (*b*) D-Galactose: R_t 0.55 (10.3%), 0.45 and 0.39 (82.9%), 0.09 (6.8%); n.m.r. data: δ 4.35 (d, $J_{1,2}$ 7 Hz, pyranose H-1β), 4.92 (d, $J_{1,2}$ 4 Hz, pyranose H-1α), 5.08 (d, $J_{1,2}$ 4 Hz, furanose H-1); ratios 4:2:1. (*c*) Methyl α-D-

glucopyranoside: R_F 0.54 (11.8%), 0.34 (72.1%), 0.09 and 0.04 (16.1%); n.m.r. data: δ 4.18 (d, $J_{1,2}$ 4 Hz, H-1). (*d*) Methyl β-D-glucopyranoside: R_F 0.53 (8.0%), 0.44 (80.1%), 0.19 and 0.10 (11.9%); n.m.r. data: δ 3.90 (d, $J_{1,2}$ 7 Hz, H-1).

The n.m.r. data are for products purified by a modification of a flash chromatographic method[17], using silica gel 60 and ethyl acetate–hexane (1:9).

(*b*) *Routine application.* To a solution of carbohydrate (1–10 mg) in *N*,*N*-dimethylformamide (150 μL) contained in a screw-cap septum vial was added imidazole solution (0.33 g/mL, *N*,*N*-dimethylformamide, 200 μL). After heating at 100° for 1 h and subsequent cooling in ice, chlorodimethylphenylsilane (70 μL) was added. After 6–18 h at ambient temperature (or 1 h at 100°), the product was extracted with hexane (2 × 200 μL), and used directly (or after concentration) for analysis or stored at −20°.

T.l.c. as in (*a*), with detection by u.v. light or the 1-naphthol reagent[18], revealed that the products contained fully derivatised carbohydrates, impurity (R_F ~0.58), and only traces of slow-moving products. The following carbohydrates were derivatised [R_F values of the main product(s) are given in brackets]: D-glucose (0.52), D-galactose (0.50), D-mannose (0.49), D-altrose (0.50), D-tagatose (0.51), D-fructose (0.51), D-ribose (0.47), L-arabinose (0.45 and 0.49), D-xylose (0.45 and 0.49), D-lyxose (0.46), L-rhamnose (0.48), L-fucose (0.49), methyl α-D-glucopyranoside (0.38), methyl β-D-glucopyranoside (0.47), methyl α-D-galactopyranoside (0.31), methyl β-D-galactopyranoside (0.39), methyl α-D-

TABLE I

H P L C DATA[a] FOR DIMETHYLPHENYLSILYLATED MONOSACCHARIDES

Carbohydrate	*Isomers*							
	1		*2*		*3*		*4*	
	k′	(%)	k′	(%)	k′	(%)	k′	(%)
L-Arabinose	4.1	(31)	4.6	(34)	5.9	(35)		
D-Ribose	3.6	(34)	4.0	(66)				
D-Lyxose	4 1	(80)	4.9	(20)				
D-Xylose	2.9	(62)	4.1	(38)				
D-Altrose	2.6	(34)	3.7	(39)	5.5	(27)		
D-Galactose	3.0	(62)	3.3	(25)	3.8	(13)		
D-Glucose	2.4	(38)	2.8	(62)				
D-Mannose	3.0	(88)	3.5	(12)				
D-Fructose	2.3	(11)	3.1	(78)	3.2	(10)	3.9	(1)
D-Tagatose	1.9	(2)	2.3	(42)	2.8	(51)	3.2	(5)
L-Fucose	2.4	(2)	2.6	(44)	3.3	(44)	3.7	(10)
L-Rhamnose	2.8	(4)	3.2	(9)	3.7	(87)		
Methyl α-D-glucopyranoside	~10							
Methyl β-D-glucopyranoside	3.4							
Methyl α-D-galactopyranoside	~20							
Methyl β-D-galactopyranoside	10							

[a]Eluant, ethyl acetate–hexane, 1:199.

TABLE II

H.P.L.C. DATA[a] FOR DIMETHYLPHENYLSILYLATED DISACCHARIDES

Disaccharide	*Isomers*			
	1		*2*	
	k′	(%)	k′	(%)
Lactose	2.8	(27)	4.4	(73)
Maltose	2.5	(62)	3.7	(38)
Sucrose	2.7	(100)		

[a]Eluant, ethyl acetate–hexane, 3:197.

TABLE III

H.P.L.C. DATA[a] FOR DIMETHYLPHENYLSILYLATED ALDITOLS

Carbohydrate	k′	*Carbohydrate*	k′
Erythritol	1.7	D-Glucitol	1.5
Ribitol	1.5	Galactitol	1.5
Xylitol	1.7	D-Mannitol	2.3
D-Arabinitol	2.1		

[a]Eluant, ethyl acetate–hexane, 1:99.

xylopyranoside (0.33), methyl β-D-xylopyranoside (0.39), maltose (0.41 and 0.44), lactose (0.41), sucrose (0.44), erythritol (0.50), ribitol (0.51), xylitol (0.49), D-arabinitol (0.50), D-glucitol (0.50), galactitol (0.50), and D-mannitol (0.50).

U.v. spectra. — These were recorded on a Hilger and Watts Ultrascan instrument equipped with 10-mm pathlength cells, using hexane solutions of the purified derivatives: λ_{max} 254, 260, 265, and 270 nm; the middle two peaks had ε values ~50% greater than those of the other two peaks.

H.p.l.c. — The system used comprised an Altex 110 A pump, Rheodyne 7125 sample-injection valve fitted with 20-μL sample loop, Cecil CE 272 spectrophotometer fitted with a 20-μL flow cell, and a Watanabe Servocorder. A Partisil 5 column (25 cm × 4.6 mm i.d., Whatman) was eluted with ethyl acetate–hexane (1:49–1:199, depending on the nature of the carbohydrates derivatised). Injections were typically of 3 μL if the hexane extract was not concentrated. The retention times (k') of the derivatives (relative to that of an unretained compound) and the isomeric compositions (based on the combined areas of peaks) are recorded in Tables I–III. Typical analyses of mixtures are shown in Figs. 1–3.

The same column has been used over several months, giving excellent reproducibility in terms of k' and efficiency with various samples.

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