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TRIFLUOROACETYLATION OF CARBOHYDRATES FOR G.L.C., USING *N*-METHYLBIS(TRIFLUOROACETAMIDE)

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ABSTRACT

Carbohydrates can be rapidly trifluoroacetylated in a homogeneous medium using *N*-methylbis(trifluoroacetamide). The derivatives in the reaction mixture were stable during 72 h and the mixture could be used directly for g.l.c. The use of a polar and thermally stable stationary phase (Dexsil 410) permitted analysis of mixtures of trifluoroacetylated cyclitols, hexoses, and alditols, as well as the detection of oligosaccharides up to hexasaccharides.

INTRODUCTION

Carbohydrates are amenable to g.l.c. only after conversion into suitably volatile derivatives. The requirements of quantitative yields, no isomerisation, and simple and speedy use even for small samples are now met by trimethylsilylation and acylation (acetyl, trifluoroacetyl, and heptafluorobutyryl derivatives). Methylation is used for structural investigation of polysaccharides and now is only of historical interest for g.l.c.

Alditol acetate derivatives are widely used for analysis of polysaccharide hydrolysates[1–6]. Acetylation is usually effected with acetic anhydride in the presence of such basic catalysts as pyridine[3,7,8] or sodium acetate[1,2]. Borate ions from the reduction step have to be removed, and long reaction times and vigorous heating are required. A marked increase in the reaction rate can be achieved by using 1-methylimidazole as a catalyst[9] which is not affected by borate ions. Trifluoroacetyl derivatives[10] have been used because of their higher volatility and suitability for mass spectrometry[11] due to their simple fragmentation patterns. Their preparation, using trifluoroacetic anhydride and, usually, pyridine as catalyst, requires vigorous conditions. With conversions involving acid anhydrides, excess of reagent and free acid have to be removed prior to g.l.c. in order to maintain the performance of the column[4,6,12]. This makes the derivatisation procedure unnecessarily complicated and may lead to errors in quantitative work.

Diacylamines, introduced by Donike[12] for g.l.c.[13], have the advantage that the reaction mixture may be used directly for analysis without impairing the per-

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formance of the column. Due to its relatively high volatility, the amide formed in the reaction does not interfere in the g.l.c. and is completely inert towards the acyl derivatives formed. Sullivan and Schewe[13] were the first to recommend the trifluoroacetylation of carbohydrates with *N*-methylbis(trifluoroacetamide) (MBTFA). An improved method of derivatisation is now reported and its suitability for the qualitative and quantitative analysis of complex mixtures of carbohydrates is demonstrated.

Unlike trimethylsilyl derivatives, acetyl and trifluoroacetyl derivatives can only be separated on highly polar columns. Although the volatility of trifluoroacetylated carbohydrates is significantly higher than that of the corresponding trimethylsilyl derivatives, high-temperature-resistant (up to 300°) stationary phases are required for oligosaccharide analysis. The highly polar liquid phases commonly used for alditol acetates have low thermal stabilities, for example, ECNSS-M (copolymer of ethyleneglycol succinate and cyanoethylsilicone[2]) up to 210°[2,3], OV-225 (cyanopropylphenylmethylsilicone) up to 260°[3], Silar 10C (cyanopropylsilicone) up to 250°[4,5], and SP-2330 (methylcyanopropylsilicone) up to 275°[6]. Apiezon greases have also been used[3], but their upper temperature limit (250°) is not sufficiently high. Special chiral phases used for the separation of trifluoroacetylated enantiomers[14] also have poor thermal stability. Sullivan and Schewe[13] recommended OV-17 (phenylmethylsilicone), which is stable up to 350° for trifluoroacetylated oligosaccharides. By the use of a suitable Dexsil (polycarborane siloxane) phase, it was expected that a satisfactory resolution of higher oligosaccharides and an excellent separation of hexoses, alditols, and cyclitols after trifluoroacetylation would be achieved.

RESULTS AND DISCUSSION

Of the two trifluoroacetylating reagents proposed by Donike[12], MBTFA is more volatile and reactive than bis(trifluoroacetamide) (BTFA). The poor solubility of sugars in MBTFA requires the use of a solvent[12], and pyridine was selected. The simultaneous addition of MBTFA and pyridine, as recommended by Sullivan and Schewe[13], makes the reaction speed dependent on the rate of dissolution. As carbohydrates are less soluble in an MBTFA–pyridine mixture than in pure pyridine, reaction times up to 1 h at 65° were unavoidable. More rapid reaction occurred in a homogeneous medium obtained by dissolution of the sample in a small amount of pyridine containing phenyl β-D-glucopyranoside as the internal standard and then adding MBTFA in excess. The reaction was spontaneous, but storage for 10 min at 75° ensured quantitative conversion. The resulting mixture could be used directly for g.l.c. It is important to check the quality of the MBTFA reagent before use.

Since stationary phases that are both polar and thermally stable are necessary for the g.l.c. of trifluoroacetylated carbohydrates, the choice is limited. Sullivan and Schewe[13] recommended such trifluoropropylmethylsilicones as OV-210, but

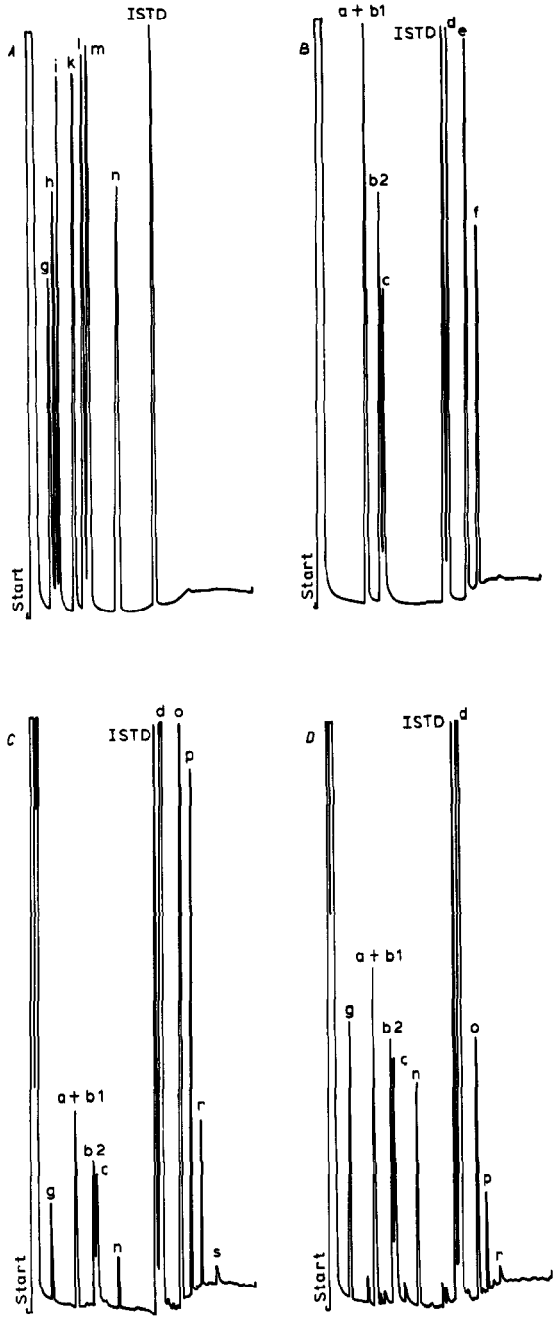

Fig. 1. G.l.c. of trifluoroacetylated carbohydrates on Dexsil 410 (see Experimental): *A*, cyclitols and alditols; *B*, mono- and oligo-saccharides; *C*, sugar content of the roots of *Cichorium intybus* L.; *D*, leaves as in *C*. Key: a, D-fructose; b, D-glucose; c, *myo*-inositol; d, sucrose; e, raffinose; f, stachyose; g, L-*chiro*-inositol; h, D-pinitol; i, L-quebrachitol; k, D-mannitol; l, D-glucitol; m, galactitol; n, *scyllo*-inositol; o, fructotriose; p, fructotetraose; r, fructopentaose; s, fructohexaose; ISTD, internal standard (phenyl β-D-glucopyranoside).

they are gradually destroyed by the reaction mixture, as indicated by column bleeding at 250°. The same was found for QF-1. Better results were obtained with Dexsil 410, a polycarborane methylcyanoethylsilicone that can be heated at least to 400° (Dexsil 300 and 400 have too low a polarity for the separation of trifluoroacetylated carbohydrates). Results using this phase are shown in Fig. 1*A,B* and illustrate the complete separation of mannitol, glucitol, and galactitol, which cannot be separated completely from each other after trimethylsilylation. The suitability of this method for plant extracts is demonstrated in Fig. 1*C,D* with the carbohydrates from the roots and leaves of *Cichorium intybus*. The conditioned column showed no bleeding, even after prolonged use, and the relative retention (Table I) remained constant for more than 500 injections. The OV-17 column[13] is less polar and of lower thermal stability than Dexsil 410.

The stability of trimethylsilylated organic compounds in g.l.c. has been studied[15]. Kim *et al.*[16] mentioned that trifluoroacetates are significantly less prone

TABLE I

MEAN RETENTION TIMES (*T*, RELATIVE TO THAT OF PHENYL β-D-GLUCOPYRANOSIDE, 14.28 MIN) AND THEIR REPRODUCIBILITY FOR TRIFLUOROACETYLATED CARBOHYDRATES ON DEXSIL 410

Compound	*n*[a]	T	*S.d.*[b] × 10^{-3}
Erythritol	64	0.134	3.6
L-*chiro*-Inositol	64	0.202	3.8
D-Pinitol	64	0.237	3.5
Ribitol	64	0.251	3.7
L-Quebrachitol	64	0 275	4.2
D-Arabinitol	64	0.285	3.1
L-Leucanthemitol	10	0.302	5.1
D-Mannitol	64	0.392	3.3
D-Glucitol	64	0.455	2.7
Galactitol	64	0.490	2 6
D-Bornesitol	10	0.518	4.0
L-Viburnitol	10	0 531	4 2
myo-Inositol	32	0 534	3 8
scyllo-Inositol	64	0.733	4.6
D-Glucose	32	0.398	3.0
D-Glucose	32	0.504	3.1
D-Fructose	32	0 400	3 1
Sucrose	64	1.017	2 1
Maltose	64	1.045	2 4
Maltose	64	1.070	2.4
Raffinose	64	1.165	8.6
Stachyose	64	1.245	11.3
Cichorium intybus			
Fructotriose	4	1.151	12.7
Fructotetraose	4	1.228	18.9
Fructopentaose	4	1.280	23.1
Fructohexaose	4	1.344	21 2

[a]Number of determinations. [b]Standard deviation.

TABLE II

STABILITY OF TRIFLUOROACETYLATED CARBOHYDRATES AND REPRODUCIBILITY OF THEIR QUANTITATIVE DETERMINATION[a]

Compound	$\bar{Q}^b$	*S.d.* $\times 10^{-2}$	*Regression Q versus t*
Erythritol	1.587	3.6	$1.590 - 1.038 \times 10^{-6}t$
L-*chiro*-Inositol	0.922	3.5	$0.921 + 0.041 \times 10^{-6}t$
D-Pinitol	1.240	1.6	$1.236 + 1.897 \times 10^{-6}t$
Ribitol	1.746	1.5	$1.748 - 1.008 \times 10^{-6}t$
L-Quebrachitol	1.270	3.1	$1.275 - 2.244 \times 10^{-6}t$
D-Arabinitol	1.260	1.8	$1.264 - 1.563 \times 10^{-6}t$
D-Mannitol	1.445	2.6	$1.446 - 0.912 \times 10^{-6}t$
D-Glucitol	1.444	2.8	$1.444 - 0.146 \times 10^{-6}t$
Galactitol	1.449	4.0	$1.449 + 0.102 \times 10^{-6}t$
scyllo-Inositol	1.157	7.1	$1.082 + 37.301 \times 10^{-6}t$
Sucrose	1.256	4.0	$1.240 + 7.233 \times 10^{-6}t$
Maltose[c]	0.953	2.2	$0.945 + 3.760 \times 10^{-6}t$
Raffinose	1.162	3.8	$1.157 + 2.031 \times 10^{-6}t$
Stachyose	1.154	3.4	$1.155 - 0.755 \times 10^{-6}t$

[a]For each substance, 64 determinations (see Experimental) were carried out within 15 min–72 h. [b]$\bar{Q}$ = mean of $area_x(t)/area_{ISTD}(t)$; the standard deviation (s.d.) and the regression line were calculated for the above period. [c]The value of Q is the sum of the Q-values of the peaks for α- and β-maltose.

to decomposition than trimethylsilyl derivatives, because they are not attacked by moisture. To prove this statement, trifluoroacetylation test mixtures were analysed after 15 min, 24, 48, and 72 h. With almost all of the carbohydrates studied, trifluoroacetylation was quantitative after 15 min (*scyllo*-inositol was an exception) and the data in Table II showed that the derivatives were completely stable within the period studied. The rate of decomposition was approx. one power of ten lower than that of trimethylsilyl derivatives[15]. Thus, trifluoroacetates are suitable for use in automatic analysers. The accuracy of the quantitative determination (±2.5%) is slightly better than that of alditol acetates[1,6].

EXPERIMENTAL

Test mixtures. — These were prepared using aqueous 40% ethanol and 2 mg/mL of (*a*) erythritol, D-pinitol, L-quebrachitol, D-mannitol, D-glucitol, galactitol, and *scyllo*-inositol; (*b*) L-*chiro*-inositol, ribitol, D-arabinitol, sucrose, maltose, raffinose, and stachyose. The other substances listed in Table I were taken from plant material. Phenyl β-D-glucopyranoside (5 mg/mL in pyridine) was used as internal standard.

Derivatisation procedure. — Test mixtures (100 μL) were added to small vials fitted into the test flasks of the automatic sampler[15], and the solvents were removed under vacuum at room temperature. Pyridine (20 μL) containing the internal standard was added to each residue, and the tube was capped with a laminated plug. The carbohydrates were dissolved by heating to 75° for 20 min. MBTFA (40

μL) was then added, each tube was sealed, heated to 75° for 10 min, and cooled, and, after 15 min, 0.8 μL was injected into a Hewlett–Packard 5835 A gas chromatograph equipped with a flame-ionisation detector, an automatic sampler 7671 A (syringe, Hamilton 701 RN), and a glass column (6 ft × 2 mm i.d.) containing 3% of Dexsil 410 (Supelco) on Chromosorb W-HP (80–100 mesh, Pierce). A single-column system with on-column injection was used with dry nitrogen as the carrier gas at 20 mL/min. Columns were conditioned at 330° for 48 h in the carrier gas stream. Temperature programme: initial temperature, 100° for 1.5 min, then 3.5°/min for 3.5 min, 6°/min for 5 min, 15°/min for 5 min, and 25°/min for 3.7 min; final temperature, 310° for 6.3 min. Injector and detector temperatures were 260° and 320°, respectively. Duration, 25 min; total analysis time (chromatography, cooling, and equilibration), 30 min. Exhaust gases from the detector were removed by application of a slight vacuum.

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