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CAPILLARY GAS-CHROMATOGRAPHIC ANALYSIS OF MONOSACCHARIDES: IMPROVEMENTS AND COMPARISONS USING TRIFLUOROACETYLATION AND TRIMETHYLSILYLATION OF SUGAR *O*-BENZYL- AND *O*-METHYL-OXIMES

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ABSTRACT

Two new procedures for the gas-chromatographic analysis of monosaccharides are reported. One involves derivatization of the sugars by reaction with *O*-benzylhydroxylamine followed by trifluoroacetylation with *N*-methylbis(trifluoroacetamide) and chromatography on a DB-1701 capillary column. This technique probably provides the best resolution achieved to date of the C_3–C_6 aldoses, as well as of the corresponding alditols. Ketoses can be qualitatively analyzed by this method, but complications interfere with their quantitative analysis. The second procedure also involves initial derivatization as the *O*-benzyloxime, but is followed by trimethylsilylation with 1-trimethylsilylimidazole, and chromatography on a DB-17 column. This technique is particularly useful for C_5 sugars, C_6 ketoses, and mixtures of sugars, alditols, and/or lactones. A number of additional, critical, observations on the derivatization and capillary gas-chromatographic analysis of monosaccharides are described.

INTRODUCTION

A well established approach to the analysis of mixtures of monosaccharides involves gas–liquid chromatography (g.l.c.) of suitable derivatives, such as aldoxime trimethylsilyl ethers and alditol acetates[1]. For biological samples, proper preparation of samples is undoubtedly the primary difficulty encountered, the subsequent chromatographic analysis normally posing few problems nowadays[2]. For certain samples of chemical origin, such as those derived from formaldehyde[3,4] or from some of our new studies of transition-metal-catalyzed reactions of carbohydrates[5], the situation is reversed. These samples are generally suitable for direct derivatization, but the g.l.c. analysis can be very demanding, even with modern capillary techniques[6]. For example, it is still very difficult to resolve fully all eight of the C_6 aldoses[7]. We report here two effective, new monosaccharide g.l.c. methods based on *O*-benzyloxime trifluoroacetate and trimethylsilyl ether derivatives. In the

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course of these studies, we have also made a number of critical observations concerning the derivatization and capillary g.l.c. analysis of carbohydrates.

RESULTS AND DISCUSSION

In order to gain in-house experience with various carbohydrate g.l.c. separation methods, survey analyses of both a C_2–C_6 alditol mixture and a C_3–C_6 monosaccharide mixture were performed using several common derivatization schemes. The capillary columns (30 m × 0.25 mm i.d.) employed in these tests included most of the common stationary phases with maximum working temperatures greater than 250°: DB-5 (5% phenyl silicone, 0.25- and 1.0-μm films), DB-17 (50% phenyl silicone, 0.25-μm film), DB-210 (50% trifluoropropyl silicone, 0.25-μm film), and DB-1701 (14% cyanopropylphenyl silicone, 0.25-μm film). Temperature programs were varied to achieve optimum resolution of the test components, while limiting total analysis times to ~15–20 minutes to maximize sample throughout and minimize peak broadening. Because of our ultimate need to analyze reaction solutions containing moderate concentrations of sugars and non-volatile metal complexes, conventional split-injection techniques[6] were used throughout.

Alditol analyses. — The alditol test-mixture consisted of all of the common alditols, including galactitol, glucitol, mannitol, arabinitol, ribitol, xylitol, erythritol, and threitol, as well as ethylene glycol, propylene glycol, and furfuryl alcohol. Optimum separation of their trimethylsilyl ether (Me_3Si) derivatives was obtained using DB-5 as the stationary phase. The column with a 1.0-μm film thickness was selected for final use, because of its higher loading capacity and very slightly better resolution, although the oven temperatures needed to be ~40° higher than on the 0.25-μm column in order to obtain comparable results. Baseline resolution of all components except galactitol–glucitol is readily achieved in less than 14 minutes (*cf.* the retention times and peak widths given in Table I). The galactitol–glucitol

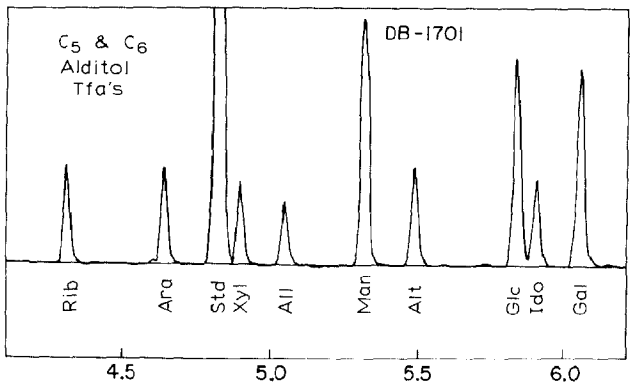

Fig. 1. G.l.c. of trifluoroacetylated alditols on DB-1701 (see Experimental section for conditions). Key: Ara = arabinitol, Rib = ribitol, Xyl = xylitol, All = allitol, Alt = altritol, Gal = galactitol, Glu = glucitol, Ido = iditol, Man = mannitol, Std = biphenyl, x-axis = time in minutes.

TABLE I

G.L.C. RETENTION TIMES FOR SELECTED COMPOUNDS AND Me_3Si AND Me_3Si *O*-METHYLOXIME MONOSACCHARIDE DERIVATIVES ON DB-5[a]

Compound[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]
$MeONH_2$	1.32	*s*-Lyxose	7.71	B-Tagatose	11.27
Me_3SiCl	1.37	*s*-Arabinose	7.74	A-Fructose	11.34
$(Me_3Si)_2O$	1.44	*a*-Arabinose	7.80	A-*xylo*-3-Hexulose	11.34
Pyridine	~1.60	*s*-Ribose	7.95	A-Sorbose	11.36
A-Glycolaldehyde	1.93	A-*erythro*-2-Pentulose	7.95	Mannono-1,5-lactone	11.38
B-Glycolaldehyde	1.96	B-*erythro*-2-Pentulose	7.97	B-Sorbose	11.46
o-Xylene	2.03	A,B-*threo*-2-Pentulose	7.98	Galacto-1,4-lactone	11.49
Ethylene glycol	2.18	*a*-Ribose	8.00	Glucono-1,5-lactone	11.50
Propylene glycol	2.24	A,B-*threo*-3-Pentulose	8.02	B-Fructose	11.53
Furfuryl alcohol	2.28	A,B-*erythro*-3-Pentulose	8.21	*s*-Allose	11.54
Imidazole	~2.45	Xylitol	8.45	*s*-Mannose	11.67
NMP	~2.55	1,6-Anhydroglucose	8.46	*s*-Glucose	11.70
Me_3SiIm	2.87	Arabinitol	8.62	*s*-Talose	11.77
Durene	3.03	*s*-6-Deoxymannose	8.65	A-*ribo*-3-Hexulose	11.77
a-Glyceraldehyde	3.26	Ribitol	8.72	α-Glucopyranose	11.78
s-Glyceraldehyde	3.33	*s*-6-Deoxyglucose	8.76	*s*-Idose	11.79
1,3-Dihydroxy-2-propanone	3.55	*s*-6-Deoxygalactose	8.84	Glucono-1,4-lactone	11.82
Glycerol	3.70	*a*-6-Deoxymannose	8.84	*s*-Galactose	11.85
1-Deoxythreitol	3.85	A-2-Deoxy-2-fluoro-glucopyranose	8.97	*s*-Altrose	11.93
1-Deoxyerythritol	3.89	*a*-6-Deoxyglucose	9.00	*s*-Glucose	11.97
Tridecane	3.99	*a*-6-Deoxygalactose	9.08	B-*xylo*-3-Hexulose	11.98
Tetradecane	4.79	*a*-Glyceraldehyde (Bn)[d]	9.22	B-*ribo*-3-Hexulose	12.04
s-5-(Hydroxymethyl)-2-furaldehyde	4.79	Octadecane	9.59	B-*arabino*-3-Hexulose	12.07
a-5-(Hydroxymethyl)-2-furaldehyde	4.83	*a,s*-2-Deoxy-*arabino*-hexulose	9.71	*o*-Terphenyl	12.10
2,5-Furandimethanol	4.88	*a,s*-2-Deoxy-*lyxo*-hexulose	9.77	*a*-Allose	12.15
Biphenyl	5.04	*a*-Glyceraldehyde (Bn)[d]	9.79	*a*-Mannose	12.15
a-Erythrose	5.10	1,3-Dihydroxy-2-propanone (Bn)[d]	9.88	*a*-Altrose	12.16
a-Threose	5.19	A-2-Deoxy-*ribo*-hexulose	9.88	*a*-Talose	12.31
s-Erythrose	5.21	B-2-Deoxy-*ribo*-hexulose	9.93	*a*-Gulose	12.33
s-Threose	5.25	*a,s*-2-Deoxy-2-fluoro-glucose	9.95	Gulono-1,4-lactone	12.39
A-*glycero*-Tetrulose	5.34	B-2-Deoxy-2-fluoro-glucopyranose	9.95	*a*-Galactose	12.41
B-*glycero*-Tetrulose	5.43	1,2-Diphenoxyethane	10.15	*a*-Idose	12.44
Threitol	5.66	A-Dendroketose	10.62	*a*-Glucose	12.47
Erythritol	5.77	B-Dendroketose	10.85	Allitol	12.80
A-Glycolaldehyde (Bn)[d]	6.11	A-Tagatose	10.89	Mannitol	12.81
B-Glycolaldehyde (Bn)[d]	6.20	A-Psicose	11.00	2,7-Anhydro-*altro*-heptulose	12.85
1-Deoxyribitol	6.24	2,7-Anhydro-*manno*-heptulose	11.04	Glucitol	13.01
5-Deoxyarabinitol	6.30	A-*arabino*-3-hexulose	11.20	Altritol	13.09
1-Deoxyarabinitol	6.32	B-Psicose	11.23	Galactitol	13.13
Bibenzyl	6.50			Iditol	13.16
s,a-2-Deoxy-*erythro*-pentose	6.35			*a*-Erythrose (Bn)[d]	13.35
Hexadecane	6.86			*A-glycero*-Tetrulose (Bn)[d]	13.43
a-Xylose	~7.6			β-Glucopyranose	13.55
a-Lyxose	7.63			*a,s*-Threose (Bn)[d]	13.60
s-Xylose	7.65			Eicosane	13.69
				Mannono-1,4-lactone	13.74
				B-*glycero*-Tetrulose (Bn)[d]	13.85

Table I (continued)

Compound[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]
s-Erythrose (Bn)[d]	14.25	*a*-*N*-Acetylmannos-amine	16.05	*a*-Glucoheptose	16.90
A-5-(Hydroxymethyl)-2-furaldehyde (Bn)[d]	15.18	*s*-*N*-Acetylgalactos-amine	16.07	*p*-Terphenyl	17.16
B-5-(Hydroxymethyl)-2-furaldehyde (Bn)[d]	15.33	*a*-*N*-Acetylgalactos-amine	16.14	A-Fructose (Bn)[d]	18.61
s-*N*-Acetylmannos-amine	15.79	A,B-*manno*-Heptulose	16.34	Tetracosane	18.65
a,*s*-*N*-Acetylglucos-amine	15.87	*s*-Glucoheptose	16.57	B-Fructose (Bn)[d]	18.74
		1,3-Diphenoxybenzene	16.61	*s*-Mannose (Bn)[d]	19.39
				s-Glucose (Bn)[d]	19.57
				a-Mannose (Bn)[d]	19.58
				a-Glucose (Bn)[d]	19.70

[a]See Experimental sections for conditions. [b]*s* = *syn*-oxime, *a* = *anti*-oxime, A,B = oxime isomers of unknown stereochemistry, NMP = *N*-methyl-2-pyrrolidinone. [c]Peak widths at baseline are: ~0.05 min at 2–3 min, 0.06–0.07 min at 5–6 min, 0.09 min at 7–9 min, 0.15 at 11–12.5 min, and 0.17 min at 13 min. [d]Me_3Si *O*-benzyloxime derivative.

pair was typically separated by better than half-height resolution. Comparable results have previously been obtained[8] on OV-101 (100% methyl silicone). Further investigation revealed, however, that the three rare hexitols could not be resolved from the three common hexitols, allitol co-eluting with mannitol, and altritol and iditol respectively coming on the leading and tailing edge of galactitol.

The six hexitols have previously been resolved as their acetates in 20–30 min analyses on[9] SE-54, Carbowax 20M, and FFAP. This has now been improved. All eleven C_4–C_6 alditols can be baseline-resolved as their trifluoroacetate (TFA) derivatives on DB-1701 *in as little as six minutes* (see Table II and Fig. 1). An equivalent separation could not be obtained on DB-210. These results show that DB-1701, like[10] OV-225, exhibits a special selectivity for structural differences of isomeric sugar trifluoroacetate derivatives. This selectivity and the 60° higher maximum working-temperature of DB-1701 (14% cyanopropylphenyl) *vs.* OV-225 (50% cyanopropylphenyl) should also make DB-1701 an ideal column for the g.l.c. analysis of oligosaccharide trifluoroacetates[11,12].

Aldose and ketose analyses. — The analysis of aldoses and ketoses is more complicated than that of alditols, because of the greater number of homologous compounds and because of the multiple structures present in solution (*e.g.*, α- and β-pyranose and α- and β-furanose). Unless there is concern with the details of these solution structures, it is common practice first to convert the sugars into open-chain carbonyl derivatives*. The traditional method of choice has been oximation[14], which yields only two peaks per sugar (the *syn* and *anti* isomers)[15]. A number of other approaches, that yield only a single peak per sugar, have been developed[1] based on conversion of monosaccharides into alditols[2f,16], aldononitriles[2e,17–19], dithioacetals[20], and aminodeoxyalditols[21]. In practice, the latter approaches are

*Detailed g.l.c. studies of aldoses in their cyclic hemiacetal form, derivatized as their trimethylsilyl ethers, are available[13].

TABLE II

G.L.C. RETENTION TIMES FOR SELECTED COMPOUNDS AND TFA AND TFA *O*-BENZYLOXIME MONOSACCHARIDE DERIVATIVES ON DB-1701[a]

Compound[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]
MBTFA	1.45	A-*glycero*-Tetrulose	8.7	A-2-Deoxy-*erythro*-pentose	12.64
Pyridine	~1.60	*s*-Erythrose	8.87	*a*-Idose	12.71
NMP	~3.00	*a*-Ribose	8.97	*s*-Xylose	12.80
Erythritol	3.33	*a*-6-Deoxymannose	8.99	A-Psicose	12.9
Threitol	3.66	*a*-Arabinose	9.24	C-*threo*-Pentulose	13.0
Ribitol	4.31	*a*-6-Deoxyglucose	9.24	Unknown	13.23
Arabinitol	4.64	B-*glycero*-Tetrulose	9.4	D-*erythro*-Pentulose	13.8
Biphenyl	4.83	C-*glycero*-Tetrulose	9.7	*s*-Allose	13.85
Xylitol	4.90	*s*-Threose	9.77	B-Psicose	13.9
Allitol	5.05	*a*-Lyxose	9.87	A-Fructose	14.4
Mannitol	5.32	*a*-Allose	10.15	D-*threo*-Pentulose	14.5
Altritol	5.49	*a*-Xylose	10.33	1,2-Diphenoxyethane	14.53
TFA–$BnONH_2$	5.5	*a*-Altrose	10.76	B-2-Deoxy-*erythro*-pentose	14.67
Glucitol	5.84	A-*threo*-Pentulose	10.8	*s*-Altrose	14.88
Iditol	5.91	*s*-6-Deoxymannose	10.95	A-Tagatose	15.0
a-Glyceraldehyde	6.05	D-*glycero*-Tetrulose	11.0	A-Sorbose	15.1
Galactitol	6.06	A-*erythro*-Pentulose	11.2	*s*-Gulose	15.27
Bibenzyl	6.44	*s*-Ribose	11.22	*s*-Mannose	15.89
A-1,3-Hydroxy-2-propanone	7.0	*a*-Gulose	11.40	*s*-Talose	15.91
s-Glyceraldehyde	7.04	*s*-6-Deoxyglucose	11.51	*o*-Terphenyl	16.21
B-1,3-Dihydroxy-2-propanone	7.3	*s*-6-Deoxygalactose	11.60	C-Psicose	16.5
C-1,3-Dihydroxy-2-propanone	7.5	*a*-Glucose	11.74	*s*-Glucose	16.50
a-Erythrose	7.63	B-*threo*-Pentulose	11.8	B-Tagatose	16.7
a-Threose	7.85	B-*erythro*-Pentulose	12.0	*s*-Idose	17.02
D-1,3-Dihydroxy-2-propanone	8.3	*a*-Mannose	12.06	*s*-Galactose	17.34
a-6-Deoxygalactose	8.67	*a*-Talose	12.23	B-Sorbose	17.8
		s-Lyxose	12.23	B-Fructose	18.0
		a-Galactose	12.35	C-Tagatose	19.1
		s-Arabinose	12.47		
		C-*erythro*-Pentulose	12.5		

[a]See Experimental section. [b]*s* = *syn*-oxime, *a* = *anti*-oxime, A, B, C, and D = oxime isomers of unknown structure, MBTFA = *N*-methylbis(trifluoroacetamide). [c]Peak widths at baseline are ~0.08 min at 4–6 min, 0.15–0.20 min at 9–13 min, and 0.20–0.25 min at 13–18 min; retention times given to only the nearest 0.1 min may not be exact with respect to nearby peaks.

usually more tedious and may also lead to loss of information (*e.g.*, in reduction to alditols) or to the formation of side-products (*e.g.*, in the preparation of aldononitriles[22]). The duplication of information provided by oxime derivatives also ensures more-reliable identification and permits quantification even if one peak is obscured.

Survey studies using a test solution containing galactose, glucose, mannose, fructose, sorbose, arabinose, xylose, glyceraldehyde, and 1,3-dihydroxy-2-propanone confirmed previous indications that sugar *O*-methyloxime trimethylsilyl ethers are best separated on methyl silicone stationary phases[3,23–25]. This approach did not, however, offer sufficient resolution (*cf.* Table I) for proper

analysis of our metal-catalyzed alditol dehydrogenation reactions[26], which sometimes lead to mixtures containing alditols, aldoses, 2- and 3-ketoses, and lactones, each in several stereochemical configurations. More effective resolution of Me_3Si *O*-methyloximes has been obtained by g.l.c.–m.s. using selective ion monitoring[23], but this technique was considered to be impractical for our routine analytical needs. An examination of *O*-trimethylsilyloxime Me_3Si derivatives[3,24] showed that they offer no apparent advantages over their *O*-methyl analogs, and suffer from the disadvantages of poorer reagent and product stability[27], greater peak overlaps, and the possibility of sugar oxidation to dicarbonyl derivatives[3].

Better resolution of monosaccharides by g.l.c. has been achieved by using oxime acetates[2a,2e,28] and trifluoroacetates[4,7,10,29]. The latter have been particularly well investigated with respect to resolution. With *O*-butylhydroxylamine as the oximating agent, Decker and Schweer[7] obtained a qualitative separation of all eight hexoses on a 50-m OV-225 column. This 38-min analysis still exhibited a number of significant, partial peak-overlaps which could again be partially circumvented by using a selective ion, chemical-ionization mass-spectrometric detector[4]. Separation of the corresponding *O*-methyloxime trifluoroacetates was reported to be less satisfactory[7], and we observed overlap of the *O*-methylaldoxime and alditol trifluoroacetates on our DB-1701 column.

It seemed likely that the resolution in this system might be improved by using *O*-benzylhydroxylamine as the oximating reagent. Previous applications of this reagent in the carbohydrate field appear to have been limited to a synthetic study[30], h.p.l.c. studies[31,32], and a few specialized g.l.c. studies[33,34] (*cf.* also the pentafluoro analog[35]). The aromatic ring of the benzyl group offered the possibility of resolving interactions with phenyl silicone stationary phases. Furthermore, the addition of seven carbon atoms to the aldose and ketose derivatives would serve to separate these sugars effectively from their corresponding non-oximatable alditols and lactones. The resulting increase in boiling point afforded by the *O*-benzyloxime derivatives is not a problem in capillary g.l.c. due to the shorter elution times *vs.* those of packed columns under otherwise comparable conditions.

Sugar Me_3Si *O*-benzyloximes were examined first. As expected, the best column for separating these non-polar aromatic derivatives was DB-17. All six C_2–C_4 aldoses and 2-ketoses are completely resolved, the six C_5 aldoses and 2-ketoses are qualitatively resolved, and at least one peak for each of the four C_6 2-ketoses is fully resolved (see Table III and Figs. 2 and 3). The separation of the eight C_6-aldoses was disappointing, however, being comparable to that of the Me_3Si *O*-methyloximes on DB-5. Nevertheless, moderately complex mixtures containing both aldoses and ketoses, together with their corresponding alditols and lactones, can be very effectively analyzed by derivatization with *O*-benzylhydroxylamine and 1-trimethylsilylimidazole followed by chromatography on both DB-5 and DB-17 (see Tables I and III).

Excellent separation of all monosaccharide aldoses was finally obtained by preparing their *O*-benzyloxime trifluoroacetate derivatives and chromatographing

TABLE III

G.L.C. RETENTION TIMES FOR SELECTED COMPOUNDS AND Me_3Si AND Me_3Si *O*-BENZYLOXIME MONOSACCHARIDE DERIVATIVES ON DB-17[a]

Compound[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]	*Compound*[b]	*Time (min)*[c]
NMP	~2.25	1,2-Diphenoxyethane	8.57	A-Fructose	13.80
Erythritol	2.42	*a*-Lyxose	9.56	A-*arabino*-3-Hexulose	13.91
C_5 Alditols	~3.23	A-*erythro*-Pentulose	9.91	A-Sorbose	14.25
Biphenyl	3.54	A-2-Deoxy-*erythro*-		A-*xylo*-3-Hexulose	14.40
a-Glycolaldehyde	3.73	pentose	10.12	B-Fructose	14.55
s-Glycolaldehyde	3.88	*a*-Ribose	10.25	B-Tagatose	14.68
Mannitol	4.42	*a*-Xylose	10.27	A-*ribo*-3-Hexulose	14.73
Bibenzyl	4.50	*a*-Arabinose	10.28	B-Psicose	14.77
Glucitol	4.56	A-*threo*-Pentulose	10.30	B-Sorbose	15.13
Galactitol	4.59	A,B-*threo*-Pentulose	10.3	B-*arabino*-3-Hexulose	15.67
α-Glucopyranose	4.87	B-*erythro*-Pentulose	10.37	B-*ribo*-3-Hexulose	15.70
Galactono-1,4-lactone	5.27	B-*threo*-Pentulose	10.55	B-*xylo*-3-Hexulose	15.83
a-Glyceraldehyde	5.27	A,B-*erythro*-Pentulose	10.60	*a*-Mannose	16.16
Mannono-1,5-lactone	5.33	*s*-Arabinose	10.73	*a*-Altrose	16.71
β-Glycopyranose	5.46	B-2-Deoxy-*erythro*-		*s*-Mannose	16.77
Glucono-1,4-lactone	5.52	pentose	10.76	*a,s*-Allose	16.82
Glucono-1,5-lactone	5.57	*s*-Lyxose	10.88	*a*-Gulose	17.15
s-Glyceraldehyde	5.71	*o*-Terphenyl	10.91	*s*-Gulose	17.39
Gulono-1,4-lactone	5.84	*s*-Xylose	11.01	*a*-Talose	17.39
1,3-Dihydroxy-2-		*s*-Ribose	11.14	*s*-Altrose	17.48
propanone	5.95	*a*-6-Deoxymannose	11.25	*s*-Galactose	17.61
2,7-Anhydro-*altro*-		*s*-6-Deoxymannose	11.91	*a*-Galactose	17.78
heptulose	6.32	*a*-6-Deoxygalactose	12.19	*a*-Glucose	17.78
Mannono-1,4-lactone	6.42	A-Dendroketose	12.32	*s*-Talose	17.79
a-Erythrose	6.95	*a,s*-6-Deoxyglucose	12.44	*s*-Glucose	18.15
A-*glycero*-Tetrulose	7.45	*s*-6-Deoxygalactose	12.46	*a,s*-Idose	18.40
s-Erythrose	7.63	A-Tagatose	12.99	A-*manno*-Heptulose	21.68
B-*glycero*-Tetrulose	7.74	B-Dendroketose	13.12	B-*manno*-Heptulose	21.89
a,s-Threose	7.88	A-Psicose	13.33	*p*-Terphenyl	22.10

[a]See Experimental section for conditions. [b]*s* = *syn*-oxime, *a* = *anti*-oxime, A, B = oxime isomers of unknown stereochemistry. [c]Peak widths at baseline are ~0.08 min at 4–6 min, 0.12 min at 7–8 min, 0.15–0.20 min at 9.5–11 min, 0.25 min at 13.5–15 min, and 0.3 min at 16.5–18 min.

them on DB-1701. *Syn* and *anti* peaks for each of the fifteen C_3–C_6 aldoses are fully resolved in less than 18 minutes, with only a few exceptions (*cf.* Table II): virtually complete overlap occurred of *syn*-mannose and *syn*-talose, *syn*-xylose and *anti*-idose, and *anti*-talose and *syn*-lyxose, and significant overlap of *syn*-lyxose and *anti*-galactose, *anti*-galactose and *syn*-arabinose, and *syn*-threose and *anti*-lyxose. Separation of the eight C_6 aldoses is shown in Fig. 4. As previously noted with the *O*-butyl oxime trifluoroacetates on[10] OV-225, there are crossovers between aldoses of different numbers of carbon atoms. It might be possible to eliminate these crossovers by using a larger perfluoroalkyl group in the esterifying agent. The ketose *O*-benzyloxime trifluoroacetates are also well resolved in general (see Table II),

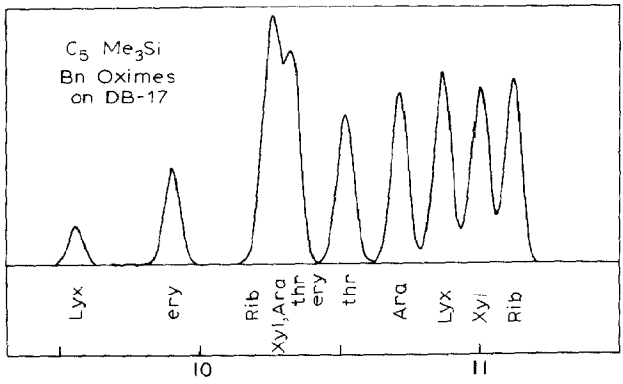

Fig. 2. G.l.c. of trimethylsilyl *O*-benzyloximes of C_5 sugars on DB-17 (see Experimental section for conditions). Key: Ara = arabinose, Lyx = lyxose, Rib = ribose, Xyl = xylose, ery = *erythro*-pentulose, thr = *threo*-pentulose, x-axis = time in minutes.

but suffer from multiple and changing peak ratios and, ultimately, decomposition *(vide infra)*. We consider that this methodology provides the best g.l.c. resolution achieved to date of the aldoses.

Derivatizations. — We have carried out a fairly detailed examination of the derivatization conditions involved in oximation, silylation, and trifluoroacetylation. This was necessitated by the new reagents employed and by the unusual nature of our sugar samples, *i.e.*, solutions in non-aqueous solvents containing transition-metal complexes. *N*-methyl-2-pyrrolidinone (NMP) has proved to be an excellent solvent for our purposes. It is unreactive, has a relatively low order of toxicity[36], dissolves most sugars to the extent of tens of $mg.mL^{-1}$, and is only a weak ligand[37] towards such catalytically active metals as Pd(II), Ru(II), and Rh(I). Sugars appear to have long-term stability in this solvent under nitrogen, and mutarotation is slow, requiring hours to days at room temperature in the absence of catalysts[38]. The boil-

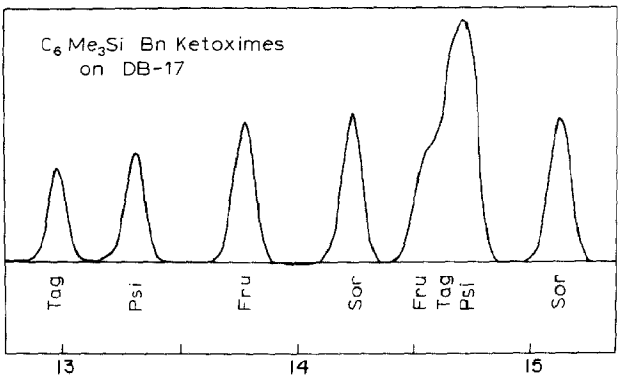

Fig. 3. G.l.c. of trimethylsilyl *O*-benzyloximes of C_6 ketoses on DB-17 (see Experimental section for conditions). Key: Fru = fructose, Psi = psicose, Sor = sorbose, Tag = tagatose, x-axis = time in minutes.

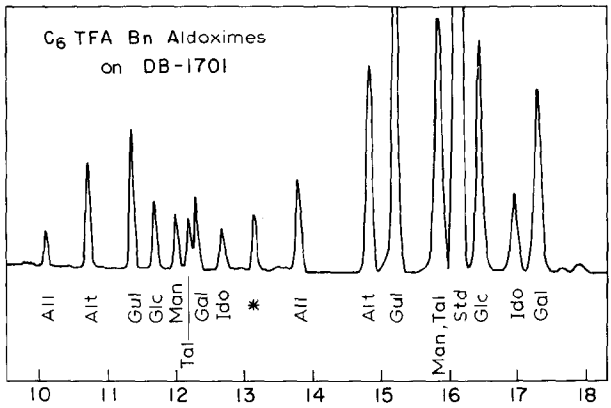

Fig. 4. G.l.c. of trifluoroacetylated C_6 *O*-benzylaldoximes on DB-1701 (see Experimental section for conditions). Key: All = allose, Alt = altrose, Gal = galactose, Glu = glucose, Gul = gulose, Ido = idose, Man = mannose, Tal = talose, Std = *o*-terphenyl, * = unknown present in all TFA *O*-benzyloxime derivatizations. x-axis = time in minutes.

ing point (202°) of NMP is a distinct advantage when using capillary g.l.c. split-injection techniques. The injector temperature needs to be kept relatively high in order to volatilize the high-molecular-weight sugar derivatives properly†. With such lower-boiling point solvents as pyridine, this can lead to significant problems in splitter linearity and precision[39]. For these reasons, NMP should serve as a good replacement for all, or most, of the pyridine normally employed in carbohydrate g.l.c. derivatization reactions, provided that proper attention is paid to the catalytic role that pyridine can have *(vide infra)*.

Oximation. — Oximation of sugars is typically carried out by treating a dried sugar sample with a solution of the appropriate hydroxylamine hydrochloride in pyridine at ~75° for at least 30 minutes[1a]. We have verified that this is the minimal derivatization time needed for the complete oximation of sugars with either *O*-methylhydroxylamine or *O*-benzylhydroxylamine under our derivatization conditions (see the Experimental section). The extent of oximation of D-glucose, a probable "worst case" example due to its very low free aldehyde content[40], was monitored *vs.* time by quenching the reaction with 1-trimethylsilylimidazole *(vide infra)* and determining the yields of the resulting Me_3Si glucose and Me_3Si glucose oxime products by g.l.c. Oximation for one hour at room temperature gave only 40% derivatization, five and ten min at 75° gave ~80 and 98% conversion respectively, and 30 min at 75° gave >99% oximation. Fructose, a representative ketose, was much more readily oximated, giving ~96% conversion after 1 h at room temp-

†In a test of detector response *vs.* injector temperature for a series of alditol Me_3Si derivatives, both absolute peak areas and relative peak areas *vs.* tetradecane increased with increasing injector temperature, asymptotically approaching a constant value when the injector temperature finally exceeded the approximate boiling point of the Me_3Si derivative (see Table IV). In this regard, it should be noted that there is a distinct advantage in having the carrier gas preheated before it enters the injector body.

TABLE IV

ESTIMATED ATMOSPHERIC PRESSURE BOILING POINTS OF CERTAIN SUGAR DERIVATIVES

Compound/Class[a]	*B.p.* (°)	*Compound/Class*[a]	*B.p.* (°)
C_2 Me_3Si, Me_3Si MeOx	150–175	C_5 Me_3Si, Me_3Si MeOx	300
C_4–C_6 TFA	<200	C_3 Me_3Si BnOx	315
C_3 Me_3Si, Me_3Si MeOx	200–225	C_6 Me_3Si, Me_3Si MeOx	325–350
C_4 Me_3Si, Me_3Si MeOx	250–275	C_4 Me_3Si BnOx	350
C_4–C_5 TFA BnOx	260–275	C_7 Me_3Si MeOx	375
C_6 TFA BnOx	265–285	C_5 Me_3Si BnOx	375
C_2 Me_3Si BnOx	275	C_6 Me_3Si BnOx	400

[a] C_n = number of carbon atoms in sugar, Me_3Si = trimethylsilylated alditols, sugars, and lactones, Me_3Si MeOx = trimethylsilyl *O*-methyloxime sugars, Me_3Si BnOx = trimethylsilyl *O*-benzyloxime sugars, TFA = alditol trifluoroacetates, TFA BnOx = *O*-benzyloxime sugar trifluoroacetates.

erature. We recommend that anyone interested in the accurate quantification of sugars *via* oximation g.l.c. procedures know where the corresponding unoximated sugar derivatives are eluted; they are generally readily detected if the oximation procedure is incomplete‡.

The *syn–anti* ratio of sugar oxime derivatives was found to differ surprisingly little from sugar to sugar, even with different carbonyl=oximating and hydroxyl=derivatizing reagents. Thus, aldoses consistently give 80–85% of the *syn* isomer with all of the derivatization schemes reported here, with the exception of glyceraldehyde (75–80% *syn*), glycolaldehyde (55–60% *syn*), and 2-deoxyaldoses. Ketoses showed more variation, giving 50–70% of the major isomer, the variation being more correlated with the sugar than with the derivatizing reagent. Consistent with this are literature reports that the *syn–anti* ratio is constant for a specific sugar derivative[10,24]. Despite this relative constancy, however, it was found that there are slight, but reproducible, variations in this ratio that depend on the temperature of the subsequent silylation reaction. For example, preparation of glucose Me_3Si *O*-benzyloxime at 75° gave a *syn–anti* ratio of 81:19, whereas preparation at room temperature gave ratios as high as 9:1. When oximation was done at 75° and the solution then cooled to room temperature before silylation, the ratio was intermediate and depended on the cooling time. Due to the almost complete lack of modern data on sugar oximes[15,22,30,41,42], it is currently impossible to evaluate the effect of such different variables as solvent, temperature, and acid–base catalysts on the kinetics and thermodynamics of oxime isomerization. Quantitative analysis

‡An extreme example of the difficulties that can be encountered is provided by 2-deoxy-2-fluoroglucose (FGlc). Complete oximation of FGlc takes over 1 h at 75° (presumably reflecting a very small percentage of aldehydo sugar present in solution due to the strong electron-withdrawing effect of the fluoro substituent). Furthermore, the *syn* and *anti* Me_3Si *O*-methyloxime isomers are co-eluted with each other and with the non-oximated Me_3Si ether of the single anomer of FGlc present in the commercial crystalline material. Hence, in the absence of anomerization, there is no obvious indication in the g.l. chromatogram that oximation is incomplete!

schemes have sometimes relied on knowledge of this *syn–anti* ratio when one of the two peaks is obscured. As shown by the foregoing data, unless proper care is taken, analysis errors could be as large as 100% if the calculated area of the large *syn* peak is based on the observed area of the small *anti* peak.

Trimethylsilylation. — A number of reagents have been used to effect trimethylsilylation[43]. The reagent of choice for carbohydrates is generally considered to be 1-trimethylsilylimidazole[44] (Me_3SiIm), although the original hexamethyldisilazane–chlorotrimethylsilane combination[14a] is still probably used more often. Me_3SiIm is reported to work better with wet sugars[45], is less corrosive, does not form precipitates during derivatization, and is readily detected by g.l.c., allowing one to ascertain that an excess of reagent is present. Another potential advantage of Me_3SiIm is that, in contrast to other silylating reagents such as *N,O*-bis-(trimethylsilyl)acetamide (BSA), Me_3SiIm does not generally silylate amines[45]. While most sugar oximes exist in open-chain forms[15,30,41], some are known to be in equilibrium with cyclic forms[22,41] containing potentially silylatable NH groups. If the sylyating reagent does not derivatize amines, the ring–open-chain oxime equilibrium should continually shift towards the open-chain form as the hydroxyl groups are silylated, preventing the formation of multiple derivatives and extra peaks during g.l.c. analysis (*cf.* the kinetic acetylation of cyclic oximes, which can interfere with the clean preparation of aldononitrile derivatives[22]). Anomerization of sugars is generally considered to be very minimal during derivatization with Me_3SiIm[45], a finding confirmed in our limited studies of this point. The Me_3SiIm silylation by-products, hexamethyldisiloxane and imidazole, are essentially inert and do not normally interfere with the g.l.c. analysis**.

While it is known that such sugars as fructose, containing tertiary hydroxyl groups, are not fully silylated by Me_3SiIm[46], it does not appear to be generally appreciated that similar complications can be encountered with sugars containing only secondary hydroxyl groups. For example, mannitol is not fully silylated by an excess of Me_3SiIm, even after 1 h at 75°. Fortunately, incomplete silylation can often be detected by the presence of extraneous peaks (that are due to partially silylated species) at retention times comparable to that of the fully silylated compound. Pyridine serves as a modest catalyst for silylation by Me_3SiIm, derivatization being somewhat more rapid in NMP–pyridine than in NMP alone. Efficient silylation of mannitol, however, requires an acidic catalyst such as HCl or chlorotrimethylsilane[43,46]. Thus, in contrast to the very slow, uncatalyzed Me_3SiIm silyla-

**The retention time of the imidazole on DB-5 was observed to increase markedly as the NMP–pyridine ratio in the injection sample was increased. Similar results were observed with solutions of other high-boiling polar compounds in high-boiling amide solvents on DB-5 columns. With 2,3-butanediol in *N,N*-dimethylformamide–dichloromethane, even peak-splitting beyond the expected *meso*-DL separation was observed. One set of peaks had a fixed retention time corresponding to the "normal" diol retention times in pure dichloromethane, while the size and retention time of the other set of peaks increased as the DMF–CH_2Cl_2 ratio was increased. This effect seems to be related to the condensation of the high-boiling amide at the head of the column, perhaps thereby temporarily generating a small section of column containing a new, low-capacity, high-polarity, liquid stationary phase.

tion of mannitol, the addition of HCl as pyridine hydrochloride to the silylation mixture leads to complete derivatization of mannitol in less than five minutes at room temperature*. As has been noted in the literature[47], when silylation follows oximation, an acid catalyst is automatically present as a carry-over from the hydroxylamine hydrochloride. Based on these findings, we recommend that the silylation of carbohydrates with Me_3SiIm always be carried out under the influence of acid catalysis unless there is a compelling reason not to do so. The Me_3Si derivatives are fairly stable, provided that an excess of the silylating agent remains present.

Trifluoroacetylation. — Although acetylation has long been a primary means of derivatizing carbohydrates for g.l.c. analysis[1], a reviw of the literature indicated that, at least for our purposes, trifluoroacetylation[1] would probably be preferable. Trifluoroacetylation has been greatly facilitated by the development of *N*-methylbis(trifluoroacetamide) (MBTFA)[48–50]. This reagent is easier to handle than trifluoroacetic anhydride, reacts more rapidly and completely, and gives rise to much more inert reaction by-products. Despite these advantages, MBTFA has only rarely been used to derivatize sugar oximes[12]. We find that MBTFA will trifluoroacetylate mannitol or glucose *O*-benzyloxime in about two to three hours at room temperature, provided that pyridine is present. In neat NMP, the reaction is very much slower. The same derivatizations require only about five minutes at 75°, but the solutions tend to turn yellow, and the peak areas decrease with further heating. Unfortunately, the completeness of derivatization is harder to verify with MBTFA than in the case of silylation, because no clear evidence for partially trifluoroacetylated derivatives could be seen by g.l.c.

Ketose oximes can also be trifluoroacetylated with MBTFA, but more than the expected two peaks per sugar are often present in the g.l.chromatogram (see Table II). Furthermore, the peak ratios exhibit a distinct time-dependence. Since MBTFA derivatizes amines faster than it does alcohols[44], it is possible that, as with some oxime acetylations[22], the MBTFA traps out cyclic forms of the oximes†. Furthermore, the TFA ketoximes decompose much more rapidly than do the TFA alditols and TFA aldoximes, as evidenced by discoloration of the derivatization solutions and disappearance of the g.l.c. peaks. Attempts to trifluoroacetylate lactones with MBTFA led to very rapid discoloration, even at room temperature, and to no discernible g.l.c. peaks at all. These decomposition reactions may be due to the presence of small proportions of trifluoroacetic acid formed by reaction with

*G.l.c. and G.l.c.–m.s. investigations show that significant proportions of chlorotrimethylsilane are present in NMP solutions of Me_3SiIm that contain a source of hydrochloric acid.

†MBTFA also derivatizes the excess of *O*-benzylhydroxylamine, giving rise to a peak in the g.l. chromatogram near that of altritol trifluoroacetate. This peak can be eliminated by converting the excess of *O*-benzylhydroxylamine into its acetone oxime before trifluoroacetylation with MBTFA.

adventitious water[‡]. While work-up of the derivatization mixtures might eliminate the decomposition problem, this defeats one of the main advantages of MBTFA, namely, the direct injection of the derivatization solutions[48]. After several hundred injections of such solutions, no deleterious effects have been noted.

Quantitative analysis. — Under optimum conditions, we have found that it is possible to determine sugars quantitatively by capillary g.l.c. with an accuracy of ~1–2%. Use of an internal standard is necessary at this level. With split-injection techniques, it also essential that the solvent be relatively high boiling and that the boiling point of the internal standard be fairly comparable to that of the unknown in order to minimize the non-linear and non-reproducible characteristics of split injections[39,51]. Estimates of the boiling points of various sugar derivatives have been made, based on standard nomograph extrapolations of known diminished-pressure boiling points[43] to atmospheric pressure and on retention times *vs.* hydrocarbons on DB-5, where separation is mainly dependent on relative vapor pressures. These are given in Table IV for assistance in choosing suitable internal standards. The values for TFA derivatives are more approximate than for the Me_3Si derivatives, as known boiling points were not available and the highly polar character of trifluoroacetates probably causes them to be eluted faster on DB-5 than would be expected based on boiling point alone. Nevertheless, the degree of increased volatility of TFA derivatives over Me_3Si derivatives is quite remarkable. There is, for example, a greater than 100° diminution in the estimated boiling point for TFA glucose *O*-benzyloxime over that of the corresponding Me_3Si *O*-benzyloxime.

While an unnatural sugar is often used as the internal standard, this option was not available to us, because any such sugar would be expected to react with the metal catalyst being used. Straight-chain alkanes can be used, but some care is required, as alkanes are not completely miscible with NMP, especially in the presence of dissolved sugars. Together with the obvious requirement that the internal standard does not overlap any other g.l.c. peaks potentially present, these constraints have proved surprisingly troublesome. A small set of acceptable, inert, aromatic hydrocarbons and ethers was finally chosen (see Table V).

A study of g.l.c. response-factors *vs.* concentrations was then made. Response factors were found more uniform if the concentration of the internal standard was fixed at a reasonably high value and the sugar concentration was varied than if the ratio of internal standard to sugar remained fixed at 1:1 and the concentrations of both were varied simultaneously. For glucose derivatized as its Me_3Si *O*-methyloxime, the response factor *vs.* 1,2-diphenoxyethane was independent of

[‡]Decomposition appeared to be more significant with aged samples of MBTFA, probably due to the presence of larger proportions of trifluoroacetic acid (evidenced by the formation of white fumes of pyridinium trifluoroacetate when aged MBTFA was added to the sugar solutions). TFA derivatives are, however, known to be relatively stable toward water itself, particularly in comparison to Me_3Si derivatives[48]. TFA aldose oximes made with fresh MBTFA are stable for at least a day at room temperature in closed vials.

TABLE V

INTERNAL STANDARDS EMPLOYED IN G.L.C.

Standard	*B.p.(°)*
o-Xylene	144
Durene	197
Biphenyl	255
Bibenzyl	284
1,2-Diphenoxyethane	~320
o-Terphenyl	337
1,3-Diphenoxybenzene	~375
p-Terphenyl	389

the glucose concentration over a range of 32–0.5 mg.mL^{-1} within the observed one sigma population error of ~1.5%. For glucose Me_3Si *O*-benzyloxime, the response factor *vs.* 1,3-diphenoxybenzene appeared to increase ~10% as the glucose concentration was decreased from 32 to 0.5 mg.mL^{-1}, but this variation is probably not statistically significant when the 3–4% random fluctuation observed at any single concentration is taken into consideration. The same glucose derivative showed a 20% variation in response factor when the (lower boiling) 1,2-diphenoxyethane was used as the internal standard, but the random errors were also higher, ~5–6% at the upper and lower concentrations. The poorer precision observed with the Me_3Si glucose *O*-benzyloxime *vs.* the corresponding *O*-methyloxime is probably due to the poorer peak shape of the former, the higher boiling point (well above the injector temperature), and, undoubtedly, an instrumental difference. It is also worth noting that (a) agreement between multiple internal standards on duplicate injections was noticeably better than between sugar derivatives and the internal standards, and that (b) the precision within a set of closely spaced injections was better than between nominally equivalent sets run on different days**. All of these observations have been confirmed in a less methodical way with a number of other alditols and sugars.

For glucose derivatized as its *O*-benzyloxime trifluoroacetate, a true, systematic variation in response factor *vs.* concentration was seen. At any given glucose concentration, the precision in the response factor *vs.* 1,2-diphenoxyethane was ~2%, but as the glucose concentration was varied from 32 to 0.5 mg.mL^{-1}, the response factor monotonically increased from 1.05 to 1.45, a 40% change! Large changes in response factors have been observed previously with sugar trifluoroacetate derivatives using an electron-capture g.l.c. detector[52]. This apparently re-

**Changes in the injector packing can have a significant effect on both the precision[51] and the relative response factors. This is an important point to keep in mind when the septum is changed (during which time, sudden carrier-gas pressure-changes can cause displacement of the injector packing) and when the injector is disassembled periodically for cleaning.

flects decomposition in the injector[7,53] and/or irreversible adsorption of the sugar derivative during chromatography, an effect that would be expected to be more pronounced at low concentrations. In the present case, the response factor was, to within a few percent, linearly related to the log of the glucose concentration over the 64-fold concentration range investigated. The rationale for this algebraic relationship is not yet clear.

Based on the factors already discussed, it seems unlikely that casual quantitative g.l.c. analysis of carbohydrates will be accurate to better than ~5–10%. If greater accuracy is desired, careful attention to many diverse details is essential. These include in-house testing of the exact derivatization scheme and instrumentation being used, proper drying of authentic sugars, and consideration of the various sources of g.l.c. errors. In the latter regard, it should be possible to improve the precision by using an autosampler and/or eliminating the use of split injections by either employing one of the alternative capillary injection techniques[6], or by using a larger-capacity, megabore (0.53 mm i.d.) column if the resultant decrease in resolution can be tolerated.

With an FI detector, the sensitivity is potentially higher for trimethylsilylation than for trifluoroacetylation, the glucose derivative peak areas being in the ratios of roughly 2.4:1.8:1 for glucose Me_3Si *O*-benzyloxime, Me_3Si *O*-methyloxime, and TFA *O*-benzyloxime, respectively. In addition, the Me_3Si *O*-methyloxime peaks on the 1.0 μm DB-5 column were much sharper than those of the Me_3Si *O*-benzyl oximes on DB-17 and of the trifluoroacetates on DB-1701. Peaks for the latter two derivatives exhibited slowly rising front-edges characteristic of column overloading, even at concentrations giving peak heights <20% of full scale at the maximum g.l.c. sensitivity setting. These considerations could obviously be altered if an electron-capture[52,53] or a nitrogen-specific[19] detector were used instead of FID.

CONCLUSIONS

The qualitative and quantitative analysis of mixtures of carbohydrates is a challenging problem, but it is made easier by the simplicity with which a variety of derivatives can be made. The ideal analytical technique and derivative will clearly vary from situation to situation. H.p.l.c. analysis can be useful, especially for oligosaccharides and certain simple mixtures of monosaccharides[1b,54]. For most analyses involving monosaccharides, however, capillary g.l.c. would seem to be the method of choice. Allowing for the fact that biological samples may involve special considerations[2], we nevertheless consider that trimethylsilylation of sugar *O*-methyloximes is still probably the most generally useful sugar g.l.c. derivatization scheme, especially when accurate quantification is desired and the mixtures are not too complex. It is rapid and is not subject to any major complicating factors. For more-complex mixtures, we consider that the new schemes reported here constitute significant improvements. For certain combinations of sugars, especially those in-

volving ketoses, C_5 sugars, and/or mixtures of aldoses, alditols, and lactones, the advantages of trimethylsilylation can be retained, but added resolution achieved, *via* the use of *O*-benzyloximes. Acetylated *O*-methyloximes[2a,2e,28], and possibly acetylated aldononitriles[2e,17–19,22], would also appear to represent good intermediate-level approaches. For the most difficult separations, however, the trifluoroacetylation of *O*-benzyloximes described here offers the best possibility for success. In addition, TFA derivatives can yield more informative mass-spectral data than do Me_3Si derivatives[55,56,*]. Quantitative analysis should also be possible using TFA *O*-benzyloximes, but accurate results will depend on the use of fresh reagents and careful multi-concentration, periodic calibrations. Should the general approaches recommended here fail, other derivatization schemes have been developed which might prove better for a particular problem[1]. Finally, the worker should not hesitate to try variations of existing schemes. For example, the trifluoroacetylation of aldonitriles does not appear to have been investigated, but it could offer high-resolution separations of aldoses as a single peak per sugar on DB-1701 or OV-225.

EXPERIMENTAL

Chemicals. — Sugars were purchased from Sigma, Pfanstiehl, or Aldrich and were dried under vacuum. Allitol and altritol were prepared by the catalytic reduction of allose and talose, respectively, using[57] hydrogen and $RuCl_2(PPh_3)_3$. NMP was Aldrich h.p.l.c. grade and was dried over 4A molecular sieves. Pyridine was reagent grade, dried over solid potassium hydroxide, or silylation grade, purchased from Pierce Chemical. Hydroxylamine and pyridine hydrochlorides were purchased from Aldrich. All other derivatizing reagents were purchased from Pierce, and stored in a refrigerator. *Caution should be used to avoid exposure of personnel to all of these derivatizing reagents.*

Stock solutions of sugars in NMP were prepared with concentrations ranging from 0.5 to 32 $mg.mL^{-1}$, typically 5–15 $mg.mL^{-1}$. G.l.c. internal-standard concentrations were normally fixed at ~10–20 $mg.mL^{-1}$, but, for test purposes, were varied from 0.5 to 32 $mg.mL^{-1}$. Solutions of derivatizing reagents were prepared in 2–4-mL Pierce Reacti-Vials® equipped with Mininert® valves to provide protection against atmospheric moisture, and stored at room temperature for up to a month. *O*-Methylhydroxylamine hydrochloride in pyridine was prepared at concentrations† of 20 $mg.mL^{-1}$†, *O*-benzylhydroxylamine at concentrations of 40 $mg.mL^{-1}$ of pyridine, pyridine hydrochloride at concentrations of 25 $mg.mL^{-1}$ of NMP, and Me_3SiIm at concentrations of 200 $\mu L.mL^{-1}$ of pyridine or, preferably, NMP.

*Preliminary, electron-ionization mass spectra of sugar *O*-benzyloxime trifluoroacetates were disappointing, however, being almost totally dominated by a single peak at mass 91, presumably the tropylium ion derived from facile cleavage of the oxime benzyl–oxygen bond. *O*-Methyloxime trifluoroacetates have apparently only been investigated mass-spectrometrically by chemical-ionization techniques[1a,4].

†Some material appears to remain undissolved, but it is probably precipitated pyridine hydrochloride since this salt is not very soluble in pyridine.

MBTFA was used neat from fresh aliquots stored for no more than a week at room temperature in small screw-capped septum vials protected with a second, larger, unpunctured, fold-over septum.

Derivatization reactions. — Derivatizations were conducted in 1.5-mL screw-cap septum vials provided with Teflon®–silicone rubber septa. In a typical derivatization, 25–50 μL of sugar solution was treated with 25–50 μL of oxime reagent, and heated in a drilled aluminum block for 30 min at ~75°, with periodic mixing. The solution was then cooled, and treated with 5–10 μL of neat MBTFA for 2–3 h at room temperature or with 50–100 μL of the Me_3SiIm reagent for 5–10 min at room temperature or 75°. For silylation without oximation, 25–50 μL of sugar solution was added to a mixture of 25 μL of pyridine hydrochloride solution and 50–100 μL of the Me_3SiIm reagent. For trifluoroacetylation without oximation, 5–10 μL of neat MBTFA was added to a mixture of 25–50 μL of sugar solution and 25 μL of pyridine.

G.l.c. analyses. — G.l.c. analyses were performed on Varian 3300 and Shimadzu GC-14A instruments. Glass inserts in the split–splitless capillary injectors were packed in the needle-penetration zone with a plug of 3% of OV-101 on Chromosorb W HP (held in place by silanized glass wool) to increase splitter reproducibility[51]. The Varian 3300 instrument provides preheated carrier gas and houses the split valve in the heated zone. The Shimadzu instrument provides no carrier gas preheating and has a room-temperature split-valve protected with a filter that was converted from molecular sieves to 35–150-mesh Columbia activated charcoal in order to lessen solvent blow-through[58]. The split and septum purge-vents were exhausted to a hood. Dry, deoxygenated helium was used as the carrier gas at linear flow-rates of ~40–35 $cm.s^{-1}$ for optimum resolution[6a] (measured at 100–150°, respectively, using butane dead-volume times). Split flows were fixed at 100 $mL.min^{-1}$ in order to ensure reproducibility of retention times and of quantification. The resulting split ratios (85–100:1) are sufficiently high to ensure good splitting characteristics[59]. Nitrogen was used as the make-up gas for the FI detectors in order to conserve helium and increase sensitivity. Injection volumes were typically 1–2 μL. Data acquisition utilized Nelson 0–1V, 20-bit A/D converters and the PE-Nelson Model 2600 IBM-PC chromatography software package. For quantitative studies, careful attention was paid to the way in which the software determined peak start and stop positions, and the way in which baselines were drawn. Retention times of closely spaced compounds were generally determined by co-injections, because retention times were found to vary slightly depending on concentrations and the presence of other components.

Capillary columns were purchased from J & W Scientific. Separations on the 1.0 μm film DB-5 column (30 m × 0.25 mm i.d.) were performed on the Varian 3300 instrument with the injector and detector temperatures set at 300° and the oven temperature programmed as follows: 140–200° at 20° min^{-1}, 200–230° at 5° min^{-1}, hold at 230° for 5 min, 230–305° at 25° min^{-1}, and hold at 305° for 4 min; total time, 21 min. Separations on the 0.25-μm film DB-1701 column (30 m × 0.25

mm i.d.) were performed on the Shimadzu GC-14A instrument with the injector and detector temperatures set at 275° and the oven temperature programmed as follows: 130–180° at 10° min^{-1}, hold at 180° for 15 min, 180–280° at 20° min^{-1}; total time, 25 min. Separations on the 0.25-μm film DB-17 column (30 m × 0.15 mm i.d.) were performed on the Shimadzu GC-14A instrument with the injector and detector temperatures set at 275° and the oven temperature programmed as follows: 150–190° at 20° min^{-1}, 190–210 at 5° min^{-1}, hold at 210° for 14 min, 210–280° at 20° min^{-1}, hold at 280° for 2 min; total time, 25.5 min. The steep temperature-ramps at the end of the runs were normally only needed if high-boiling side-components (such as triphenylphosphine from metal catalysts) were present.

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