Carbohydrate Research, 27 (1973) 199–213

O-METHYL OXIMES OF SUGARS. ANALYSIS AS *O*-TRIMETHYLSILYL DERIVATIVES BY GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY*

ROGER A. LAINE AND CHARLES C. SWEELEY†

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823 (U. S. A.)

(Received March 23rd, 1972; accepted in revised from, September 29th, 1972)

ABSTRACT

The mass spectra of aldoses, partially methylated aldoses, deoxyaldoses, and ketoses containing 3–7 carbons, were recorded on the ethers of the trimethylsilyl *O*-methyl oxime derivatives. Each compound gave a distinctive spectrum indicating the carbon-chain length and the location of substituents. The gas–liquid chromatographic properties of most compounds in this study were also examined.

INTRODUCTION

Early analysis of sugars by mass spectrometry was carried out by DeJongh and assoc.[1–10] on acetates, isopropylidene derivatives, and dithioacetals, and by Kochetkov *et al.*[11,12] on methyl ethers. Subsequently, gas–liquid chromatography was applied to the methyl ethers[13], *O*-trimethylsilyl (TMS) derivatives[14,15], alditol acetates[16], and other volatile derivatives of carbohydrates, and the combination of g.l.c. with mass spectrometry to the TMS[17], acetates[18], methyl ethers[19], trifluoroacetates[20], *N*-butyl boronates[21], aldonitriles[22], and *O*-TMS *O*-methyl oximes (MO-TMS)[23]. A useful application of these methods was developed by Björndal *et al.*[24] for combined g.l.c. and mass spectrometry of partially methylated alditol acetates derived from permethylated oligo- or polysaccharides, a method which has broad applications in the determination of the position of linkages between sugar residues. The aldonitrile acetates[22] are also promising derivatives for this purpose because of their simplicity of preparation and asymmetry of structure. Petersson and Samuelson[25] have analyzed mixtures of partially methylated TMS methyl glycosides by mass spectrometry for determination of the linkage between sugar residues, but several g.l.c. peaks were obtained for each sugar, and ions resulting from rearrangement in the cyclic sugar derivatives made the interpretation of the results more difficult.

*This work has been supported in part by grants RR-00480 and AM-12434 from the U. S. Public Health Service, and is published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 5871.
†To whom communications should be sent.

The MO-TMS derivatives of sugars are open chain, asymmetric molecules easily prepared from the free sugars, and they exhibit distinctive g.l.c.[26] and mass spectral[23] properties. We have recorded and analyzed the mass spectra of the MO-TMS derivatives of a number of saccharides and found that this derivative is especially useful for the location of substituents on the carbon skeleton of sugars. In addition, the carbon-chain length was revealed in most cases by characteristic ion patterns. After permethylation and hydrolysis of lactose, the g.l.c.–mass spectrometric analysis of the MO-TMS derivatives of the resulting partially methylated sugars gave precise information on the location of the *O*-methyl and *O*-TMS groups. This result indicates that the use of MO-TMS derivatives may also be a rapid and useful method for the location of the linkage in disaccharides.

RESULTS AND DISCUSSION

G.l.c. of MO-TMS derivatives of various sugars. — Analysis of MO-TMS sugars by g.l.c. gave single peaks, peaks with shoulders, or a large peak followed by a small peak. Figure 1 shows the g.l.c. separation by on 3% SE-30 of the MO-TMS

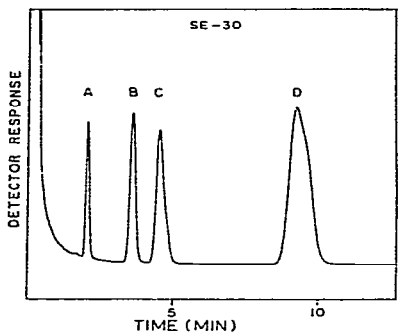


Fig. 1. Gas–liquid chromatography of MO-TMS derivatives of 2-deoxy-D-*erythro*-pentose (**10**) (A), D-ribose (**6**) (B), L-fucose (**11**) (C), and D-fructose (**13**) (D), performed on a Hewlett–Packard F & M Model 402 gas chromatograph equipped with a column (2 m × 3 mm) of 3% SE-30 on 100/120 Supelcoport. Temperatures were as follows: Flash heater, 250°; column, 170°; flame ionization detector, 250°. Carrier gas: nitrogen at a flow rate of 35 ml/min.

derivatives of 2-deoxy-D-*erythro*-pentose, D-ribose, L-fucose, and D-fructose. Similarity in the mass spectra of the two products which were obtained from some MO-TMS derivatives suggests that they represent the *syn* and *anti* forms of the *O*-methyl oxime function. In cases where only one g.l.c. peak was exhibited, it was assumed that the *syn* and *anti* forms were unresolved, but many of these could be separated by changing liquid phases. Table I lists relative retention times (R_{Rib}) for a series of MO-TMS derivatives chromatographed on the liquid phases SE-30 and OV-17. Where resolution of the *syn* and *anti* forms occurred, retention times were recorded on the early, major peak.

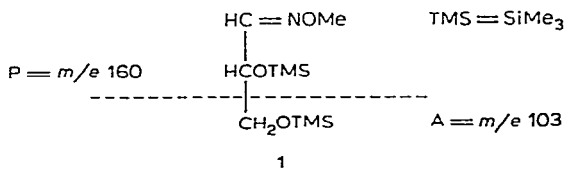
TABLE I

GAS–LIQUID CHROMATOGRAPHY OF MO-TMS DERIVATIVES AT 170°

MO-TMS derivative	*Relative retention time*	
	Liquid phase	
	3% SE-30	*3% OV-17*
D-Ribose (6)	1.000	1.000
D-Erythrose (2)	0.325[a]	0.350[a]
2,3-*O*-Methyl-D-ribose (9)	0.470[a]	0.725[a]
2-Deoxy-D-*erythro*-pentose (10)	0.6000	0.685
2-*O*-Methyl-D-ribose (7)	0.695	0.825
3-*O*-Methyl-D-ribose (8)	0.710	0.845
D-Xylose (3)	0.970	0.990[a]
L-Fucose (11)	1.360	1.220
D-Fructose (13)	2.790	2.300
D-Galactose	2.960[a]	2.460[a]
D-Glucose (4)	3.050[a]	2.600[a]
D-*glycero*-D-*gulo*-Heptose (5)	8.290[a]	5.900[a]

[a]Two peaks resolved, retention time on first, larger peak.

Mass spectrometry of aldose derivatives having carbon chains from three to seven units in length. — The mass spectra of the MO-TMS derivatives of D-glyceraldehyde (**1**), D-erythrose (**2**), D-xylose (**3**), D-glucose (**4**), and D-*glycero*-D-*gulo*-heptose (**5**) are shown in Fig. 2. Electron impact-induced cleavage between C-2 and C-3 of a given sugar derivative are labeled A and P as in the MO-TMS derivative of D-glyceraldehyde:



1

Ion P arises from homolytic cleavage with charge retention on the fragment containing C-1 and C-2 and their substituents and liberation of a free radical form of A. If the molecular ion involves ionization of the A moiety, homolytic cleavage yields ion A and the free radical form of P. Ion A in this case represents C-3 and its substituents. In sugars having a longer carbon chain, the ions resulting from cleavage between C-3 and C-4 are designated Q and B; between C-4 and C-5, R and C; between C-5 and C-6, S and D; and between 6 and 7, T and E in an analogous manner. The ions expected from this scheme of degradation for aldoses containing 3 to 7 carbons are shown in Table II. Polyhydroxy fragments containing several TMS groups tend[17] to lose trimethylsilanol ($Me_3SiOH = 90$). Fragments related by this loss are designated as primes (*e.g.*, A′, B′).

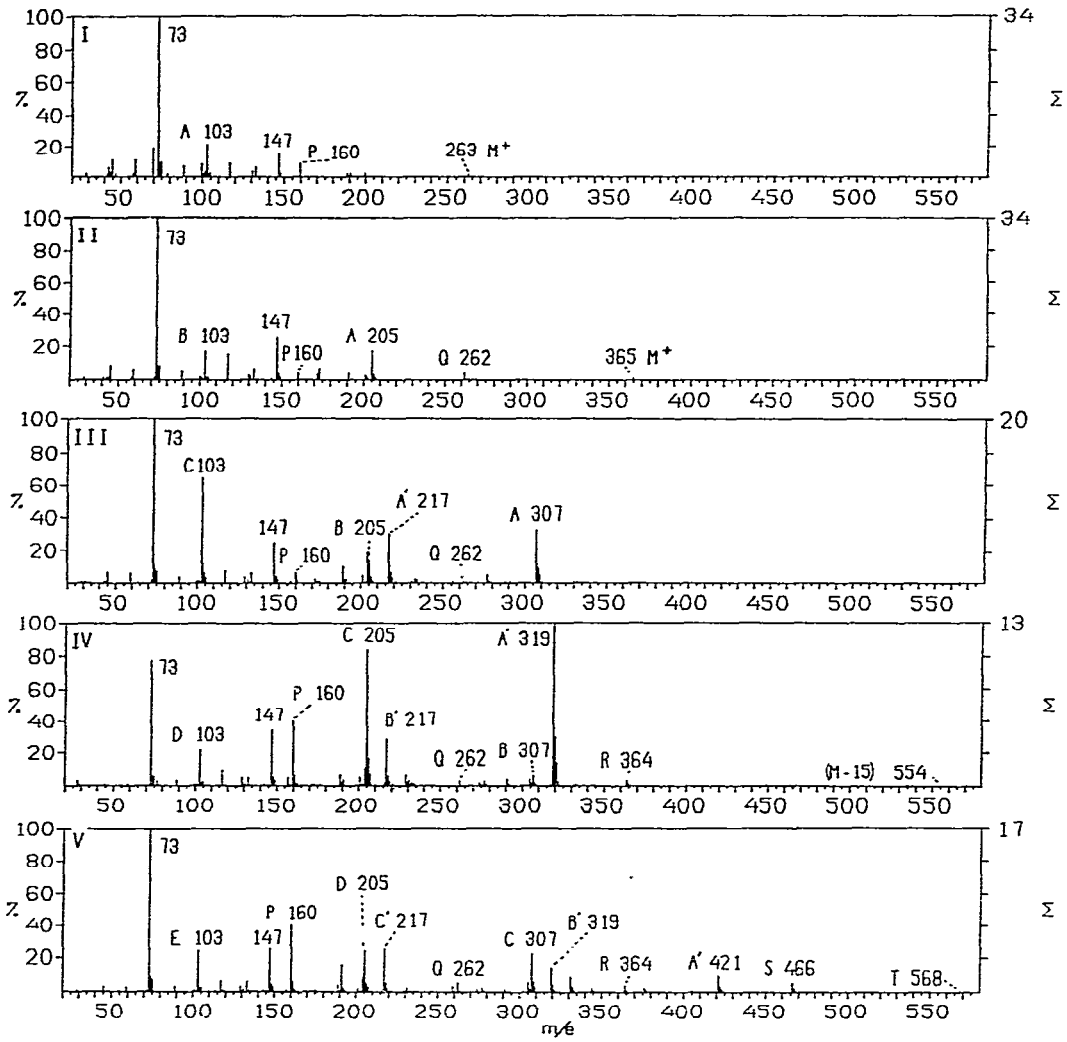
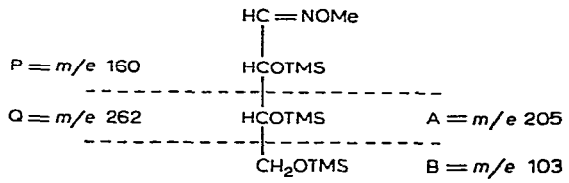


Fig. 2. Mass spectra of the MO-TMS derivatives of D-glyceraldehyde (1, mol. wt. 263, I), D-erythrose (2, mol. wt. 365, II), D-xylose (3, mol. wt. 467, III), D-glucose (4, mol. wt. 569, IV), and D-*glycero*-D-*gulo*-heptose (5, mol. wt. 671, V). Spectra were recorded on an LKB model 9000 combined g.l.c.–mass spectrometer equipped with a column (2 m × 3 mm) of 3% SE-30 on 100/120 Supelcoport. Ionizing electron energy, 70 eV. Temperature of flash heater, 250°; of column, 100–250°; of molecular separator, 250°; and ion source, 290°.

Spectrum I in Fig. 2 is that of the MO-TMS derivative of D-glyceraldehyde (1). The molecular ion is visible at *m/e* 263, and ions A at *m/e* 103 and P at *m/e* 160 represent major fragmentation pathways. Ions which appear in these spectra at *m/e* 73 and *m/e* 147 correspond to $(Me)_3Si^+$ and $(Me)_3SiO{=}Si(Me)_2$, respectively, and are characteristic of many TMS derivatives[17].

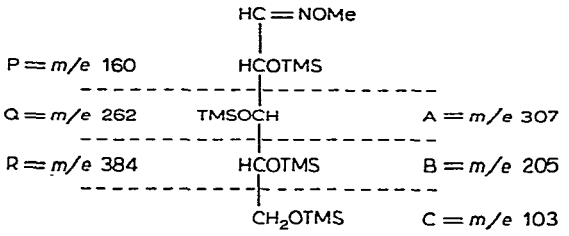
The MO-TMS derivative of D-erythrose (2) (Spectrum II) shows a molecular

ion at *m/e* 365. In comparison with the mass spectrum of D-glyceraldehyde, ion P remains at *m/e* 160, but ion A has increased to *m/e* 205, because of the additional HCOTMS group (*Δ* 102 amu). The B ion, containing C-4, is at *m/e* 103 and fragment ion Q, at *m/e* 262, is the other member of this ion pair.



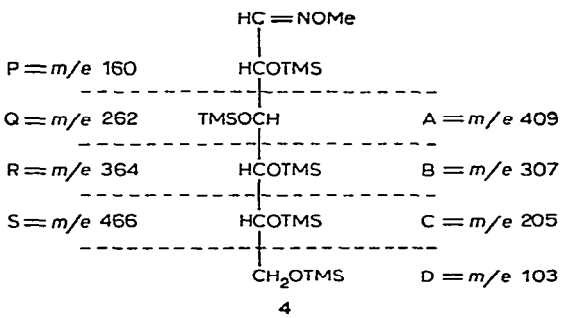
2

Pentoses, represented by the MO-TMS derivative of D-xylose (**3**) in Spectrum III (Fig. 2), exhibit an A ion at *m/e* 307, which loses trimethylsilanol to give A′ at *m/e* 217. Ions B and C are located at *m/e* 205 and 103, respectively, but the *m/e* 364 ion expected for fragmentation between C-4 and C-5 is not observed.



3

Spectrum IV (Fig. 2) represents the MO-TMS derivative of D-glucose (**4**) which has been interpreted with the use of deuterated analogs and described in detail in a previous report[23]. The ion at *m/e* 319 (A′) originates from the loss of TMSOH from ion A (*m/e* 409), and the R ion at *m/e* 364 appears in this spectrum as well as a small M-15 peak representing the loss of a neutral methyl radical from the molecular ion.



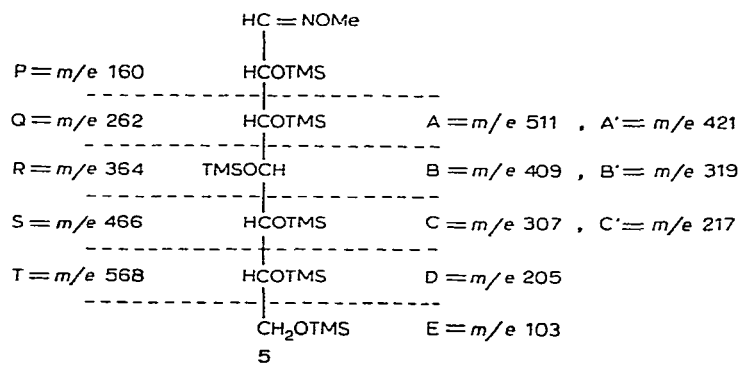
4

TABLE II

IONS (*m/e*) EXPECTED FROM ELECTRON-IMPACT-INDUCED CLEAVAGE BETWEEN CARBON ATOMS IN A SERIES OF MO-TMS DERIVATIVES OF ALDOSES FROM 3 TO 7 CARBONS IN LENGTH

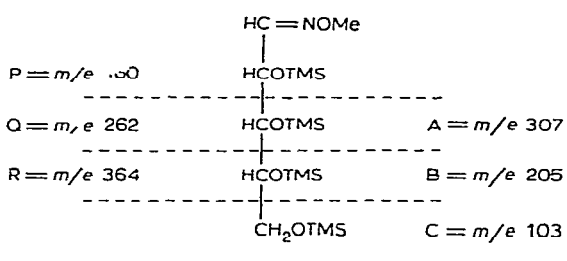
MO-TMS derivative	*Ions*	*A*	*P*	*B*	*Q*	*C*	*R*	*D*	*S*	*E*	*T*
	Fragmentation between carbons	*2,3*	*2,3*	*3,4*	*3,4*	*4,5*	*4,5*	*5,6*	*5,6*	*6,7*	*6,7*
	Carbons contained in the chain	*3+*	*1 and 2*	*4+*	*1 to 3*	*5+*	*1 to 4*	*6+*	*1 to 5*	*7*	*1 to 6*
D-Glyceraldehyde (1)		103	160								
D-Erythrose (2)		205	160	103	162						
D-Xylose (3)		307	160	205	262	103	364				
D-Glucose (4)		409	160	307	262	205	364	103	466		
D-*glycero*-D-*gulo*-Heptose (5)		511	160	409	262	307	364	105	466	103	568

The MO-TMS derivative of D-*glycero*-D-*gulo*-heptose (5) (Spectrum V, Fig. 2) is fragmented into members of all the expected series of ions (Table II). The A ion at *m/e* 511 and the B fragment at *m/e* 409 are absent, but A′ and B′ are observed at *m/e* 421 and 319, respectively.



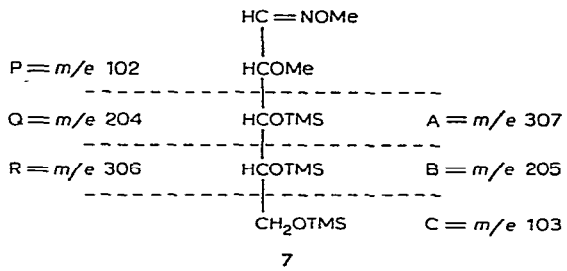
5

The distinctive patterns of ions for each of the sugars having carbon chains from 3 to 7 carbons allow identification of the length of the carbon skeleton. Sub-



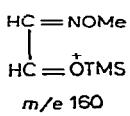
6

stituents on any of the carbons would be expected to make substantial changes in the spectra of MO-TMS saccharides. To illustrate this, spectra of several substituted and deoxy saccharides were recorded for comparison with those described above.



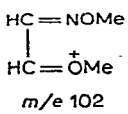
7

Mass spectra of D-*ribose and substituted analogs as MO-TMS derivatives.* — Spectra of the MO-TMS derivatives of D-ribose (**6**), 2-*O*-methyl-D-ribose (**7**), 3-*O*-methyl-D-ribose (**8**), 2,3-di-*O*-methyl-D-ribose (**9**), and 2-deoxy-D-*erythro*-pentose (**10**) were recorded. The spectrum VI (Fig. 3) of MO-TMS-D-ribose (**6**), is essentially identical to that of the derivative of D-xylose (**3**) (Spectrum III, Fig. 2), which is characteristic of aldopentoses. The *m/e* 160 ion (P) is derived from the reducing end of the sugar. When the TMS function is replaced at C-2 by Me, as in 2-*O*-methyl-D-ribose (**7**), (Spectrum VII, Fig. 3), the P ion is shifted to *m/e* 102 (*Δ* 58 amu) whereas the other member of this ion pair (A), at *m/e* 307, is unchanged.



m/e 160

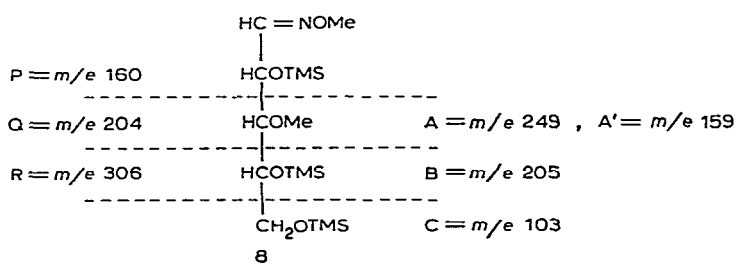
Ion P from D-ribose



m/e 102

Ion P from 2-*O*-methyl-D-ribose

This locates the *O*-methyl function at C-2, since C-1 is the reducing end of the sugar. Ion R at *m/e* 306 supports this assignment, as well as loss of *m/e* 262 (C-1 to C-3) which is shifted to *m/e* 204 (*Δ* 58 amu). The normal complement of ions B at *m/e* 205 and C at *m/e* 103 is present.



8

In 3-*O*-methyl-D-ribose (**8**) (Spectrum VIII, Fig. 3) the ion P is at *m/e* 160, as in D-ribose, but the *m/e* 307 ion is shifted to *m/e* 249 (*Δ* 58 amu). Supporting this shift is the presence of A′ at *m/e* 159. Ion B at *m/e* 205 and ion C at *m/e* 103 establish the location of the Me group at C-3, since the values of both ions would be shifted if the methyl group were at C-4 or C-5.

When C-2 and C-3 are both substituted with *O*-methyl groups, as in 2,3-di-*O*-methyl-D-ribose (**9**) (Spectrum IX, Fig. 3), ion P (*m/e* 160) shifts to *m/e* 102 (*Δ* 58 amu)

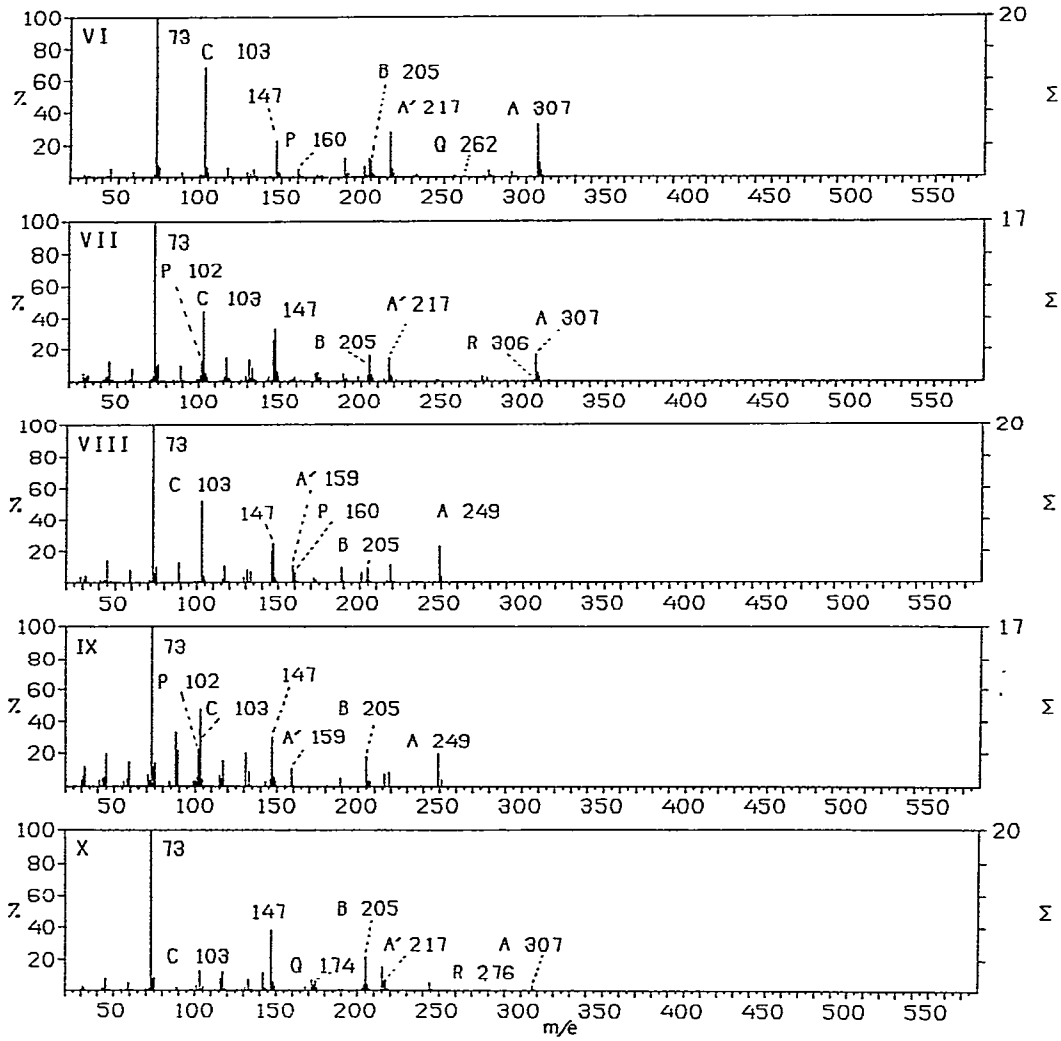
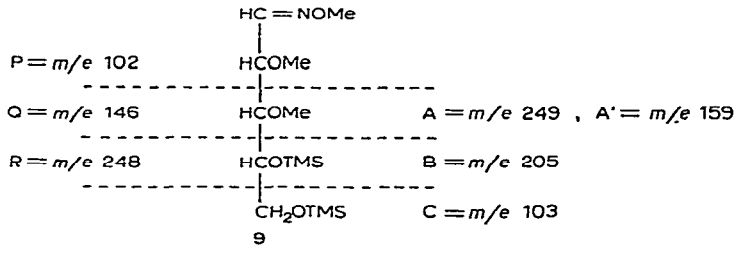


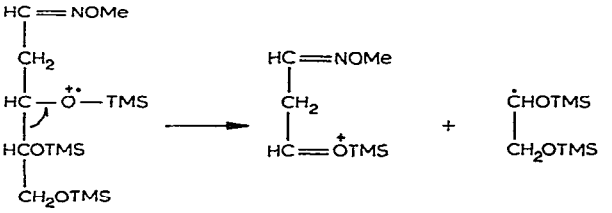
Fig. 3. Mass spectra of the MO-TMS derivatives of D-ribose (**6**, mol. wt. 467, VI), 2-*O*-methyl-D-ribose (**7**, mol. wt. 409, VII), 3-*O*-methyl-D-ribose (**8**, mol. wt. 409, VIII), 2,3-di-*O*-methyl-D-ribose (**9**, mol. wt. 351, IX), and 2-deoxy-D-*erythro*-pentose (**10**, mol. wt. 379, X). Instrument and conditions are described in the legend for Fig. 2.

and ion A (*m/e* 307) shifts to *m/e* 249 (*Δ* 58 amu). Since ion B, containing C-4 and C-5, is present at *m/e* 205 and ion C, containing C-5, is observed at *m/e* 103, the *O*-methyl groups can be definitely located at C-2 and C-3.



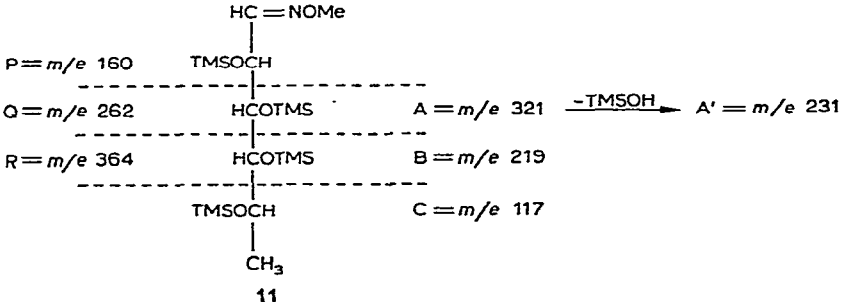
9

Spectrum X (Fig. 3) illustrates the fragmentation of 2-deoxypentoses as MO-TMS derivatives. Cleavage between C-2 and C-3 is not likely in this analog of D-ribose (**10**), because of lack of vicinal alkoxyl functions, and ion P is therefore not expected to be prominent. Ion Q is shifted, when compared with that of the spectrum of D-ribose, from *m/e* 262 to *m/e* 174 (*Δ* 88 amu) owing to replacement of OTMS by H at C-2, and ion R is shifted from *m/e* 364 to *m/e* 276 (*Δ* 88 amu) for the same reason. Ions A, B, and C occur at *m/e* 307, 205, and 103 respectively, as in D-ribose, locating the deoxy position at C-2.



10 Q = *m/e* 174

Mass spectra of MO-TMS derivatives of various saccharides. — Location of C-methyl functions in sugars can be accomplished by examining the mass spectra of the MO-TMS derivatives for *Δ* 14 amu increases in the ions. Consideration of L-fucose (**11**) (Spectrum XI, Fig. 4) as a 5-methylpentose indicates a shift of ion A



11

from *m/e* 307 to *m/e* 321 (*Δ* 14 amu), and of ions B and C to *m/e* 219 and 117, respectively. All of these changes were observed. Furthermore, since the P, Q, and R ions are present at *m/e* 160, 262, and 364, respectively, as expected for pentoses, the methyl group is located at C-5 of the sugar.

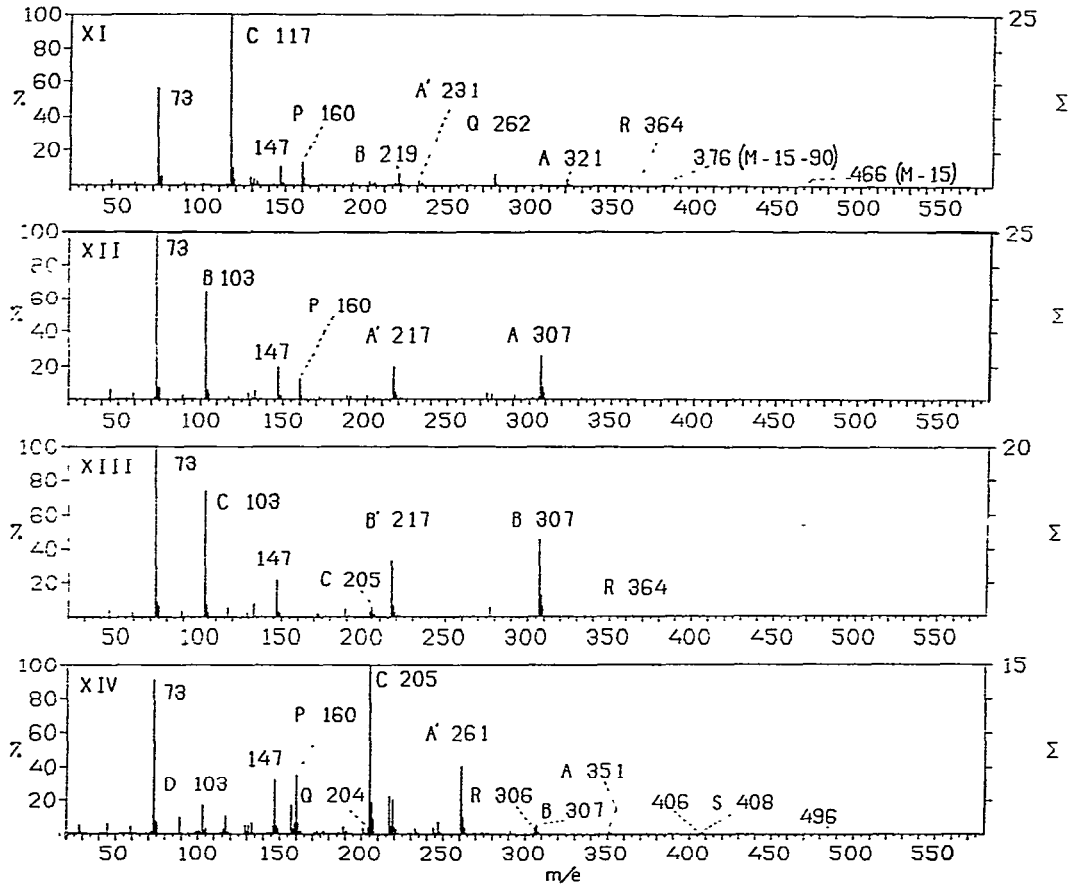
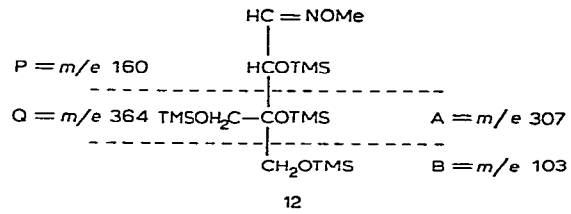


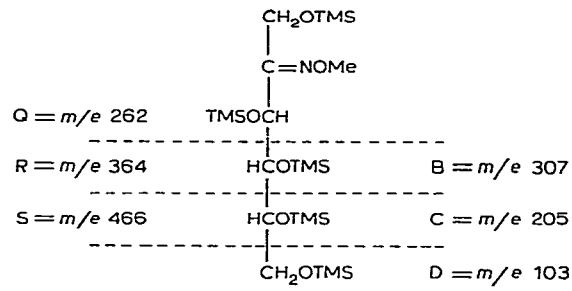
Fig. 4. Mass spectra of the MO-TMS derivatives of L-fucose (**11**, mol. wt. 481, XI), D-apiose (**12**, mol. wt. 467, XII), D-fructose (**13**, mol. wt. 569, XIII), and 3-*O*-methyl-D-glucose (**14**, mol. wt. 511, XIV). The instrument conditions are described in the legend for Fig. 2.

Indications of a branch point in such sugars as D-apiose can be seen by examining the spectra for ions missing from the normal pattern. In Spectrum XII (Fig. 4) the MO-TMS derivative (**12**) of this pentose has large *m/e* 103 and *m/e* 307 peaks, but lacks the *m/e* 205 ion, suggesting a branch of the penultimate carbon.

In spectra of 2-ketohexoses, illustrated by the MO-TMS derivative of D-fructose (**13**) (Spectrum XIII, Fig. 4), the *m/e* 160 ion (P) is absent due to the improbability of cleavage adjacent to the oxime carbon. The A ion is also missing in these compounds, which supports this contention.



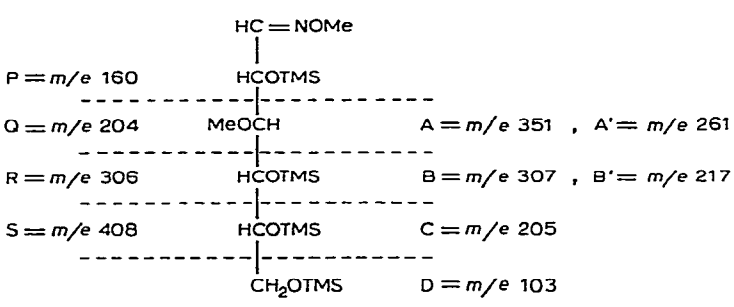
12



13

Location of methyl groups in sugars is facilitated by mass spectrometry of the MO-TMS derivatives, as previously discussed for the partially methylated derivatives of D-ribose. Locations of acyl substitution in sugars and determination of the position of linkages between sugar residues in oligo- or polysaccharides are two important applications of the methylation procedure.

In the former problem, the nonacylated hydroxyls of a sugar molecule may be blocked by formation of acetals with methyl vinyl ether[27]. Subsequent permethylation with methyl sulfinyl carbanion and methyl iodide[28] replaces the acyl with methyl groups, but leaves the base-stable acetals on the remaining hydroxyl groups, and acid-catalyzed hydrolysis removes the acetals and releases the *O*-methyl-substituted free sugars. For example, an acyl group located at O-3 of D-glucose would produce 3-*O*-methyl-D-glucose. The MO-TMS derivative (**14**) of this compound is represented by Spectrum XIV (Fig. 4). The A ion is at *m/e* 351, losing TMSOH to become A′



14

(*m/e* 261). Since ions B, C, and D occur at *m/e* 307, 205, and 103, respectively, and ion P at *m/e* 160, the methyl group is assigned to C-3 of D-glucose.

To examine the possibility that MO-TMS derivatives would be useful for the determination of the linkage in oligosaccharides, permethylation and hydrolysis of lactose was performed, and the MO-TMS derivatives of the resulting partially methylated hexoses are examined by g.l.c. (Fig. 5). The mass spectra of peaks A and B are shown in Fig. 6, Spectra XV and XVI, respectively. Definite assignments for the

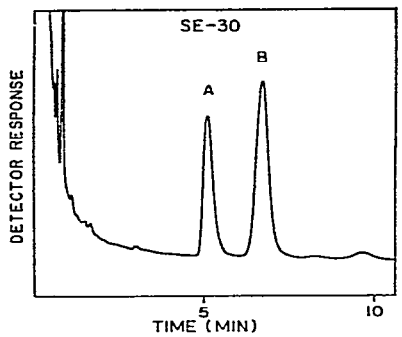


Fig. 5. Gas–liquid chromatography of the MO-TMS derivatives of partially methylated aldoses obtained by hydrolysis of permethylated lactose. Peak A corresponds to the MO-TMS derivative of 2,3,4,6-tetra-*O*-methyl-D-galactose (**15**) and peak B to that of 2,3,6-tri-*O*-methyl-D-glucose (**16**). Instrument conditions are described in the legend for Fig. 1.

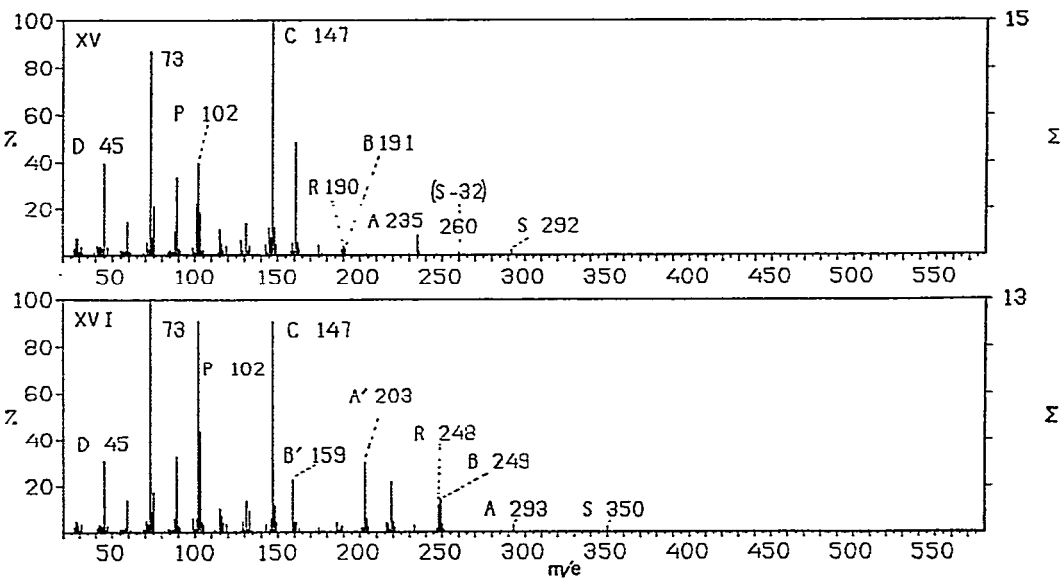
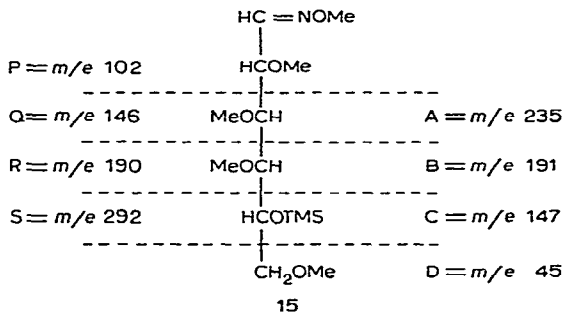


Fig. 6. Mass spectra of the MO-TMS derivatives of partially methylated aldoses derived from hydrolysis of permethylated lactose. Spectrum XV: MO-TMS derivative of 2,3,4,6-tetra-*O*-methyl-D-galactose (**15**, g.l.c. peak A, Fig. 5). Spectrum XVI: MO-TMS derivative of 2,3,6-tri-*O*-methyl-D-glucose (**16**, g.l.c. peak B, Fig. 5). Instrument conditions are described in the legend for Fig. 2.

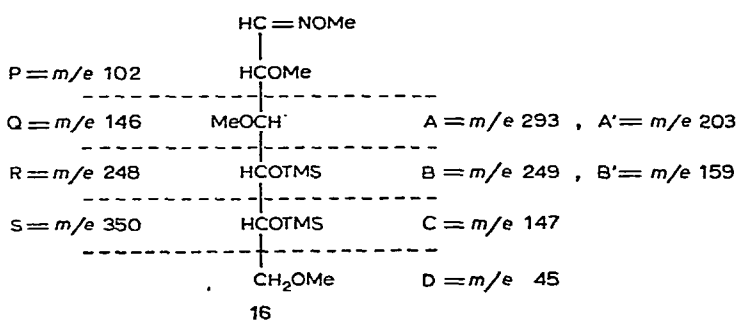
location of methyl groups can be made on the basis of the ions expected from the cleavage of the carbon chain induced by electron impact. On this basis, Spectrum XV corresponds to that of 2,3,4,6-tetra-*O*-methyl-5-*O*-trimethylsilyl-D-galactose *O*-methyl oxime (**15**). The ions observed are A at *m/e* 235 (A′ = *m/e* 145), B at *m/e* 191, C at *m/e* 147, and D at *m/e* 45.



15

Since ions P, Q, and R occur at *m/e* 102, 146, and 190, respectively, indicating methoxyl groups at C-2, C-3, and C-4, and ion S is observed at *m/e* 292 (190+102), the OTMS group is located at C-5.

Peak B of g.l.c. reported in Fig. 5 is expected to be 2,3,6-tri-*O*-methyl-4,5-di-*O*-trimethylsilyl-D-glucose *O*-methyl oxime (**16**). When compared to Spectrum XV, the mass spectrum of this compound (Spectrum XVI, Fig. 6) shows that 4 major ions shifted. These ions are as follows: A = *m/e* 293 (Δ 58 amu, confirmed by A′ at *m/e* 203), ion B at *m/e* 249 (Δ 58 amu), ion R at *m/e* 248 (Δ 58 amu), and ion S at *m/e* 350 (Δ 58 amu). These ions support the assignment of the additional OTMS group at C-4 and provide evidence for a (1→4) linkage in lactose.



16

Limited examination of liquid phases has not yet led to a useful column for the separation of closely related MO-TMS derivatives of sugars. Although the tetramethyl ethers of MO-TMS derivatives of hexoses are separated from trimethyl analogs, the various trimethyl derivatives have not been resolved sufficiently for mass spectrometric analysis of their mixture. Thus, the analysis of linkages between sugar residues in oligosaccharides by this method is, at present, limited to disaccharides.

In summary, it is clear that the MO-TMS derivatives are useful for the determination by combined g.l.c.-mass spectrometry, of the location of substituents on isolated free sugars. The derivatives can be prepared in less than 3 h after the sugars have been isolated from the saccharides, and the mass spectral interpretations are unambiguous. The major fragmentation pathways are derived from simple homolytic cleavage between carbon atoms in the sugar skeleton, allowing the ion fragments to be assigned directly to the compound being analyzed.

EXPERIMENTAL

Methods and standard compounds. — Gas-liquid chromatography was performed on a Hewlett Packard F & M Model 402 instrument equipped with columns (2 m × 2 mm) of 3% SE-30 on Supelcoport 100/120 or 3% OV-17 on Supelcoport 100/120 (Supelco, Inc., Bellefonte, Penn.) and hydrogen-flame ionization detectors, and with nitrogen as a carrier gas at 30 ml/min. The temperature was maintained isothermally at 150 or 170°.

Mass spectrometry was performed at 150° on a combined g.l.c.-mass spectrometer LKB 9000 equipped with a column (1.5 m × 3 mm) of SE-30, with helium as the carrier gas, an ionizing electron energy of 70 eV, the flash heater at 250°, the molecular separator at 250°, and the ion source at 290°. The spectra were recorded as bar graphs by means of an on-line data acquisition and processing program[29].

Standard sugars were obtained as follows: L-fucose from National Biochemicals (Chicago, Ill.); D-xylose from Nutritional Biochemicals Corp. (Cleveland, Ohio) and D-glucose as dextrose from Mallinckrodt, Inc. (St. Louis, Mo.) the partially methylated D-ribose compounds from Dr. Fritz Rottman and Mr. Lee Pike and all other sugars from Sigma, Inc (St. Louis, Mo.)

Sugar O-methyl oximes. — The sugar (1 mg) was added into a vial (Teflon-capped) with methoxylamine hydrochloride (1 mg, Supelco, Inc.) and pyridine (50 μl). This mixture was kept for 2 h at 80°. The TMS derivatives were then prepared by addition of bis-trimethylsilyltrifluoroacetamide (50 μl Regis Chemical Co., Chicago, Ill.) and heating for 15 min at 80°. Aliquots of this solution were used for injection into the g.l.c. columns.

Partially methylated aldoses from lactose. — Lactose was permethylated with methyl sulfinyl carbanion and methyl iodide[28], and hydrolyzed with 0.5M sulfuric acid for 9 h at 100°. After neutralization with barium carbonate and removal of residual ions on a small column of ion-exchange resin Dowex 50(X-8, H^+), the solution was evaporated *in vacuo* and the derivatives were prepared as just described.

ACKNOWLEDGMENTS

We thank Mr. Jack Harten for technical assistance in the operation of the mass spectrometer and Dr. Fritz Rottman ans Mr. Lee Pike for a generous gift of methylated D-ribose compounds.

REFERENCES

1 K. BIEMAN, D. C. DEJONGH, AND H. K. SCHNOES, *J. Amer. Chem. Soc.*, 85 (1963) 1763.
2 D. C. DEJONGH AND K. BIEMANN, *J. Amer. Chem. Soc.*, 85 (1963) 2289.
3 D. C. DEJONGH, *J. Amer. Chem. Soc.*, 86 (1964) 3149.
4 D. C. DEJONGH, *J. Amer. Chem. Soc.*, 86 (1964) 4027.
5 D. C. DEJONGH, *J. Org. Chem.*, 30 (1965) 453.
6 D. C. DEJONGH AND K. BIEMANN, *J. Amer. Chem. Soc.*, 86 (1964) 67.
7 D. C. DEJONGH AND S. HANESSIAN, *J. Amer. Chem. Soc.*, 87 (1965) 3744.
8 D. C. DEJONGH, *J. Org. Chem.*, 30 (1965) 1563.
9 D. C. DEJONGH AND S. HANESSIAN, *J. Amer. Chem. Soc.*, 87 (1965) 1408.
10 D. C. DEJONGH AND S. HANESSIAN, *J. Amer. Chem. Soc.*, 88 (1966) 3144.
11 N. K. KOCHETKOV, N. S. WULFSON, O. S. CHIZHOV, AND B. M. ZOLOTAREV, *Tetrahedron*, 19 (1963) 220.
12 N. K. KOCHETKOV AND O. S. CHIZHOV, *Tetrahedron*, 21 (1965) 2029.
13 A. G. MCINNES, D. H. BALL, F. P. COOPER, AND C. T. BISHOP, *J. Chromatogr.*, 1 (1958) 557.
14 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
15 C. C. SWEELEY AND B. WALKER, *Anal. Chem.*, 36 (1963) 1461.
16 J. S. SAWARDEKER, J. H. SLONEKER, AND A. JEANES, *Anal. Chem.*, 37 (1965) 1602.
17 D. C. DEJONGH, T. RADFORD, J. D. HRIBER, S. HANESSIAN, M. BIEBER, G. DAWSON, AND C. C. SWEELEY, *J. Amer. Chem. Soc.*, 91 (1969) 1728.
18 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohyd. Res.*, 5 (1967) 433.
19 J. KÄRKKÄINEN, *Carbohyd. Res.*, 14 (1970) 27.
20 O. S. CHIZHOV, B. A. DMITRIEV, B. M. ZOLOTAREV, A. YA. CHERNYAK, AND N. K. KOCHETKOV, *Org. Mass Spectrom.*, 2 (1969) 947.
21 F. EISENBERG, JR., *Carbohyd. Res.*, 19 (1971) 135.
22 B. A. DMITRIEV, L. V. BACKINOWSKY, O. S. CHIZHOV, B. M. ZOLOTAREV, AND N. K. KOCHETKOV, *Carbohyd. Res.*, 19 (1971) 432.
23 R. A. LAINE AND C. C. SWEELEY, *Anal. Biochem.*, 43 (1971) 533.
24 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610.
25 G. PETERSSON AND O. SAMUELSON, *Svensk Papperstidr.*, 71 (1968) 78.
26 E. C. HORNING AND M. G. HORNING, *Methods Med. Res.*, 12 (1970) 396.
27 A. N. DEBELDER AND B. NORRMAN, *Carbohyd. Res.*, 8 (1968) 1.
28 S.-I. HAKOMORI, *J. Biochem.* (Tokyo), 55 (1964) 205.
29 C. C. SWEELEY, B. D. RAY, W. I. WOOD, J. F. HOLLAND, AND M. KRITCHEVKSY, *Anal. Chem.*, 42 (1970) 1505.