Ozonolysis of Original (+)-Camphene-10-C^{14} and Formaldehyde Isolation.—This procedure was that of Roberts and Yancey.[4] The crystalline formaldehyde methone was collected and recrystallized twice from methanol–water; m.p. 192–193° This material was "counted" at zero disintegrations/min.[20]

Partial Racemization of (+)-Camphene-10-C^{14}.—The procedure used was that of Roberts and Yancey.[4] A mixture of 7.3 g. of (+)-camphene-10-C^{14}, $[\alpha]_D$ +42.3° (benzene), 1.5 ml. of redistilled (*in vacuo*) pyruvic acid and 30 ml. of acetonitrile[31] was heated in a sealed tube immersed in refluxing bromobenzene vapor, b.p. 156°. The time of heating varied from 1–3 hr. The mixture was cooled, added to 200 ml. of water, neutralized with 20 ml. of 10% aqueous sodium carbonate and extracted with ether (3 × 100 ml.). The ether extracts were combined, dried over magnesium sulfate and the ether distilled. The residue was sublimed at 10 mm. affording 4.1 g. (56%) of camphene.

Ozonolysis of Camphene.—This procedure was the same as that of Harries.[32] The crystals of dimethylnorcampholide were dissolved in ether and allowed to crystallize, giving snow-white crystals, m.p. 95–96°; reported 96.0–96.5°,[32] 95–96° [33]

Acknowledgment.—The senior author is indebted to the Michigan Memorial Phoenix Project for a grant which enabled Dr. Goodrow to undertake the initial extensive research on this problem in 1957.

(31) Eastman Organic Chemicals, yellow label, No. P-488, purified by refluxing with phosphorus pentoxide and distilling.

(32) C. Harries and B. J. Palmen, *Ber.*, **43**, 1432 (1910); *cf.* ref. 5.

(33) F. W. Semmler, *ibid.*, **42**, 246 (1909).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

Application of Mass Spectrometry to Structure Problems. XIV.[1] Acetates of Partially Methylated Pentoses and Hexoses

BY DON C. DE JONGH AND K. BIEMANN

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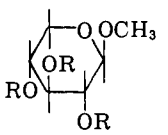
The mass spectra of the acetates of various mono-, di-, tri- and tetra-O-methyl derivatives of xylose, glucose and mannose are presented and interpreted. It is possible to relate the characteristics of the spectra to the number and position of the methoxyl groups in these molecules and to distinguish between pyranoses and furanoses. Some of the perdeuterioacetates have been prepared to corroborate the assignments; a convenient procedure for the preparation of sugar acetates on a 1 mg. scale is described.

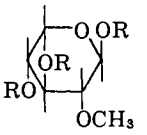
Recently[1] we have shown that the mass spectra of the polyacetates of hexoses and pentoses can be interpreted in terms of the structural characteristics of these compounds—such as molecular size, ring size and variation in the degree of the substitution—but not so much in terms of the stereochemistry. Because of the role which partially methylated sugars play in the determination of the structure of polysaccharides and derivatives of monosaccharides,[2] in addition to naturally occurring methyl sugars, it was of interest to explore the possibility of locating mass spectrometrically a methoxyl group within a sugar molecule.

Fully acetylated derivatives (*e.g.*, of monomethylxyloses, monomethylglucoses and monomethylmannoses) were chosen because of their volatility, thermal stability and ease of preparation on a micro scale. The separability of these derivatives from each other and the acetylating reagents by gas chromatography makes it possible to prepare a sample for mass spectrometry using as little as 1 mg. of the O-methyl sugar, a technique which might also prove useful in working up a mixture of acetylated O-methyl sugars without prior separation into the individual components.

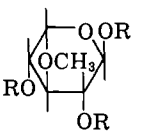
The availability of four of the five possible monomethylxyloses, namely, Ia, IIa, IIIa and IVa, provided a rather complete series of compounds differing in the position of the methoxyl group and in one case (IVa) also differing in the ring size. Of these, the 1-O-methyl derivative was expected to behave on electron impact quite differently from the others as the methoxyl group is a glycosidic one rather than an ordinary methyl ether, as in the 2-O-methyl and 3-O-methyl isomers. Furthermore, 5-O-methylxylose as a furanose differs in ring size and should also give rise to a very different spectrum in analogy to the previously reported acetates of pyranoses and furanoses.[1] However, even the most closely related compounds of this series, the 2-O-methyl and 3-O-methyl derivatives, also exhibit very different mass spectra, indicating that the position of the substituent at the ring can be established mass spectrometrically.



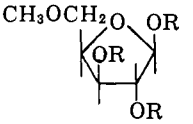
Ia, R = H
Ib, R = CH_3CO



IIa, R = H
b, R = CH_3CO



IIIa, R = H
b, R = CH_3CO



IVa, R = H
b, R = CH_3CO
c, R = CD_3CO

The presence of a peak at *m/e* 259 (M-31) in the spectrum (Fig. 1) of methyl β-D-xylopyranoside triacetate (Ib), a peak absent in the spectra of the other isomers (Fig. 2 through 4), is due to the loss of the glycosidic methoxyl group from C-1, a process leading to a secondary carbonium ion next to an ether oxygen which provides considerable stabilization. Loss of the methoxyl in the isomers IIb, IIIb, IVb would lead instead to a simple secondary or primary carbonium ion lacking any additional stabilization, and is thus not able to compete with the loss of the acetoxyl group from C-1; the latter process gives rise to the M-59 peak in those isomers, which is in turn absent in the spectrum of Ib. This observation is in agreement with the earlier one, namely, that the 1-acetoxyl group is exclusively lost from polyacetates of pentoses or hexoses on electron impact.[1]

The remaining part of the spectrum of Ib, with the exception of the peaks at mass 171, 230, 291 and 333, is very similar to the spectrum of α-D-xylopyranose tetraacetate (the similar spectrum of the epimeric ribose derivative has been published previously[1]). In most of the significant fragments of sugar acetates, the substituent at C-1, or that entire carbon atom, is

(1) Part XIII: K. Biemann, D. C. De Jongh and H. K. Schnoes, *J. Am. Chem. Soc.*, **85**, 1763 (1963).

(2) (a) H. O. Bouveng and B.Lindberg, *Advan. Carbohydrate Chem.*, **15**, 53 (1960); (b) W. Pigman, "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1960.

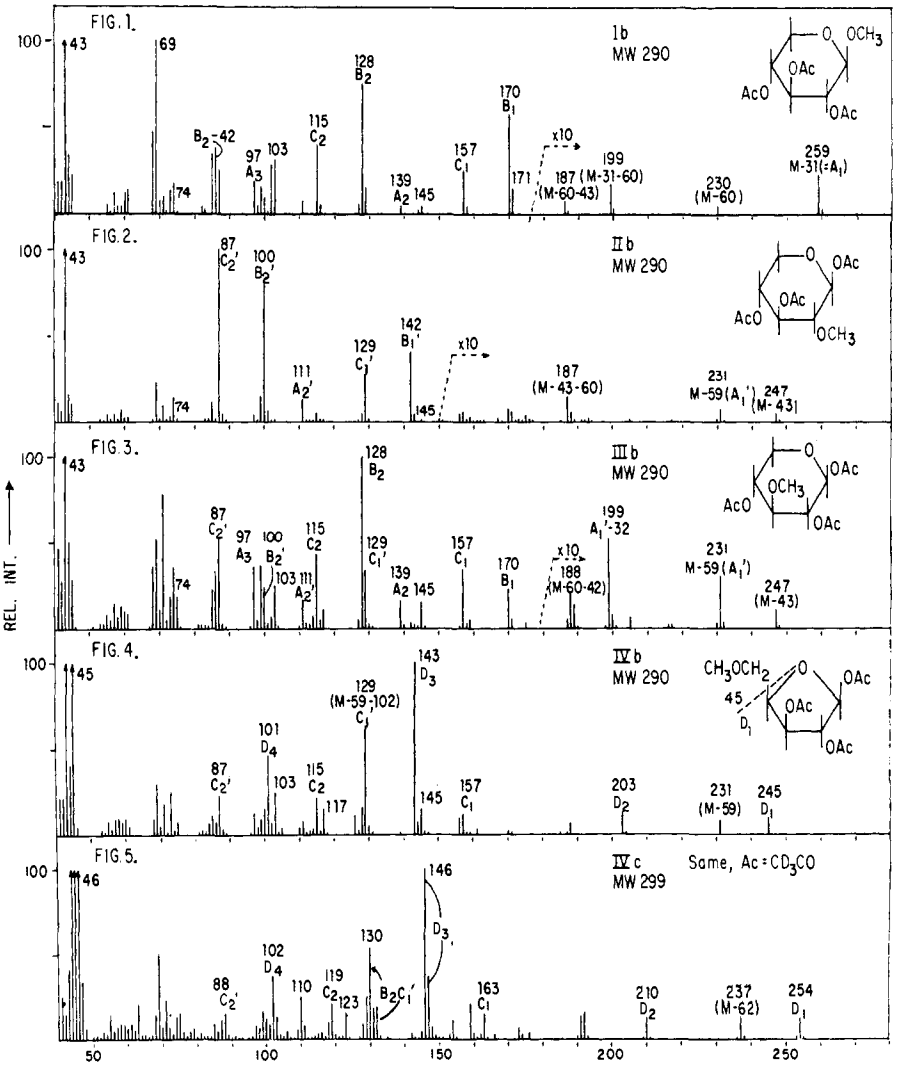
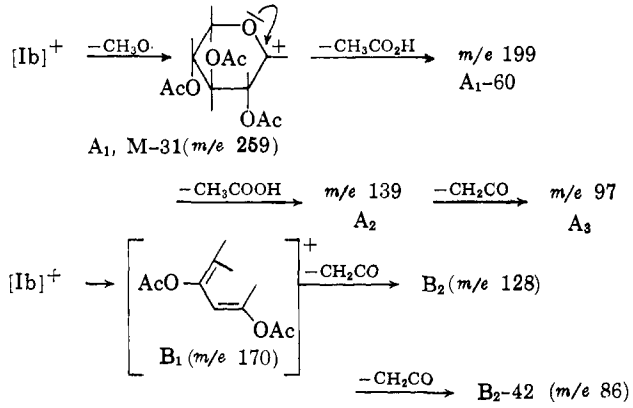


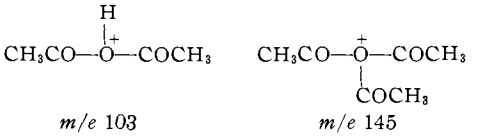
Fig. 1.—Mass spectrum of methyl β-D-xylopyranoside triacetate (Ib).
Fig. 2.—Mass spectrum of 2-O-methyl-β-D-xylopyranose triacetate (IIb).
Fig. 3.—Mass spectrum of 3-O-methyl-β-D-xylopyranose triacetate (IIIb).
Fig. 4.—Mass spectrum of 5-O-methyl-β-D-xylofuranose triacetate (IVb).
Fig. 5.—Mass spectrum of 5-O-methyl-β-D-xylofuranose triacetate-d_9 (IVc).

lost, and the mass of these fragments is therefore the same whether derived from a 1-acetoxyl or from a 1-methoxyl derivative. Thus, the peaks of group A are found at mass 259, 199, 139 and 97, corresponding to the loss of one and two molecules of acetic acid (60



m.u.) and one molecule of ketene (42 m.u.) from the ion formed upon loss of the 1-methoxyl group.[3] The intense peaks at mass 170 and mass 128 are analogous[3] to the fragments of group B in the spectra of the polyacetates discussed previously[1] (details of the first step are discussed later in connection with Vb).

The species of mass 157 and mass 115 are an indication that the three-carbon fragments, C_1 and C_2, bearing two acetoxyl groups are present here also (their nature is discussed later in connection with the more highly methylated compounds IX, X and XI) and the peaks at mass 103 and mass 145 represent di- and triacetyloxonium ions.[1]



The fragments of mass 171 and 230 (Fig. 1) correspond to those of mass 243 and 302 in the spectrum of methyl α-D-mannopyranoside tetraacetate (V, Fig. 6). The origin of these fragments will be discussed below along with the spectrum of V because of the availability of the isotopically labeled analog Va (Fig. 7).

A further characteristic of the mass spectra of acetylated methyl glycosides are peaks at M + 1 and M +

(3) The details of these fragmentations have been suggested in the earlier paper (ref. 1).

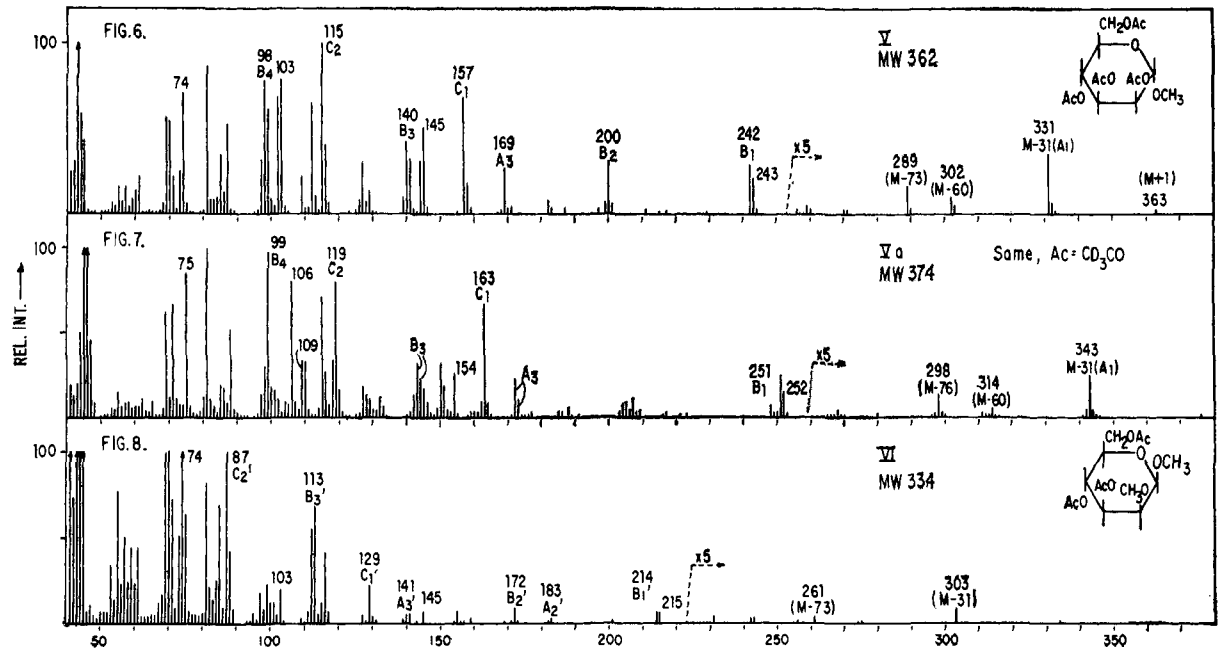
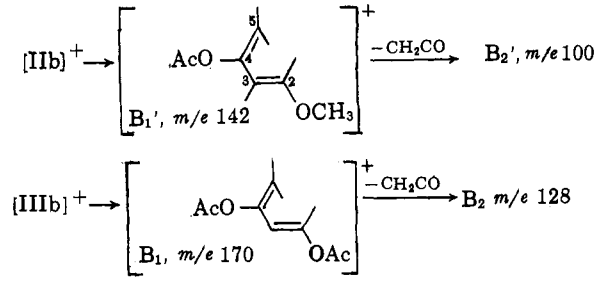


Fig. 6.—Mass spectrum of methyl α-D-mannopyranoside tetraacetate (V).
Fig. 7.—Mass spectrum of methyl α-D-mannopyranoside tetraacetate-d_{12} (Va).
Fig. 8.—Mass spectrum of methyl 2-O-methyl-β-D-mannopyranoside triacetate (VI).

43, *e.g.*, at *m/e* 291 and 333 in the spectrum of Ib and 363 and 405 in the spectrum of V. While the formation of a protonated species of ethers and esters ("M + 1" peak) is well known,[4] and even M + 43 peaks have been observed in the mass spectra of butyl acetate and related esters,[5] it is somewhat surprising to find these species also in the spectra of these large molecules determined at relatively low sample pressure. The absence of these peaks in the spectra of sugar acetates, other than those derived from methyl glycosides, indicates a particular tendency of the latter to form complex oxonium ions. The correctness of this assignment is borne out by the spectrum of Va in which this peak shifts to M + 46 (CD_3CO). These peaks, which are of the order of magnitude of the "M + 1" peak at *m/e* 363 in Fig. 6 and the "M + 2" peak (uptake of D, implying that the abstraction of deuterium from the acetyl groups is favored over the abstraction of hydrogen from the ring or the methoxyl group) at *m/e* 376 in Fig. 7, are not shown in these figures to conserve space; the relative magnitude of these peaks varies with sample size and may remain undetected if the spectrum was determined with a very small sample (which was the case with VI). It is worth pointing out that the spectra of acetylated methyl sugars, in analogy to the previously discussed polyacetates,[1] fail to exhibit a peak for the molecular ion because of its great tendency for fragmentation.

A comparison of the mass spectra of the triacetates of the methyl ethers IIa, IIIa and IVa with the spectra[1] of pentopyranose tetraacetates reveals that the replacement of an acetoxyl group by a methoxyl group does not appreciably change the fragmentation of the molecule, except that a process analogous to the elimination of ketene from an acetoxyl group is not possible in the case of a methoxyl group. Thus Fig. 2 is quite similar to the spectrum of a pentopyranose tetraacetate—such as the previously reported one of ribopyranose tetraacetate (Fig. 8 in ref. 1)—with the exception that many of the characteristic peaks are found 28 mass units lower (the mass difference between CH_3COO and CH_3O).[6] This finding corroborates the fragmentation mechanisms advanced previously,[1] according to which most fragments retain C-2 and, in most cases, also the substituent attached to it.

The most interesting aspect of a comparison of the mass spectrum of the 2-O-methyl derivative IIb with the 3-O-methyl isomer IIIb is the clear indication that in the more involved fragmentation processes leading to the fragments of group A and B it is the substituent at C-3 which is preferentially eliminated. In the region below mass 180, the spectrum (Fig. 3) of IIIb is, therefore, quite similar to the spectrum of a pentopyranose tetraacetate or, for that matter, of the methyl glycoside Ib. The elimination of the substituent at C-3 is most specific in the fragments of series B, as evidenced by the intense peaks at *m/e* 142 and 100 in Fig. 2 and at 170 and 128 in Fig. 3, respectively.

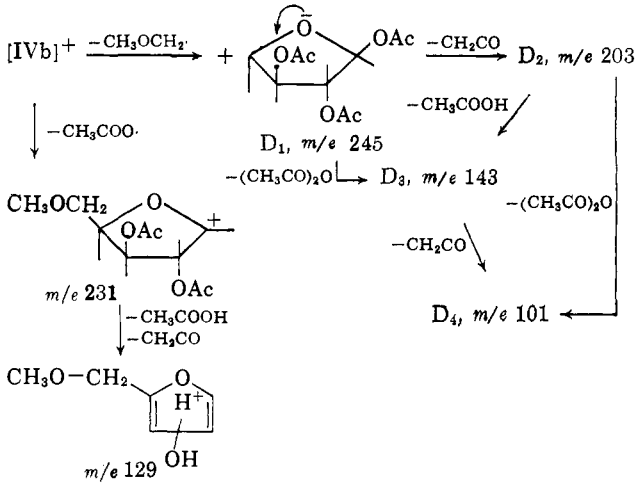


The elimination of the substituent at C-3 is less specific in the fragmentation of series A (*e.g.*, Fig. 3 shows a fragment A_2' at *m/e* 111 (139–28) in addition to A_2 at *m/e* 139) and least in the fragments of series C (their nature is shown below), as indicated by the occurrence of reasonably intense peaks at mass 129 (C_1', 157–28) and 87 (C_2', 115–28) as well as at *m/e* 157 and 115. The greater specificity of the elimination of the C-3 substituent in IIb compared with IIIb seems to indicate a greater ease for the loss of a molecule of

(4) F. W. McLafferty, *Anal. Chem.*, **29**, 1782 (1957).
(5) J. H. Beynon, G. R. Lester, R. A. Saunders and A. E. Williams, *Trans. Faraday Soc.*, **57**, 1259 (1961).
(6) In an effort to facilitate comparison of the spectra with those of the polyacetates (ref. 1) the fragments retaining the methoxyl group, and thus appearing 28 mass units below the corresponding ones of the polyacetates or of those methyl derivatives from which the methoxyl group is eliminated, are indicated with a prime. Double prime refers to fragments containing two methoxyl groups in place of two acetoxyl groups in the corresponding fragment derived from polyacetates.[1]

acetic acid than of methanol. Thus when the 3-position is occupied by methoxyl, to a certain extent acetic acid may be lost from another position in the course of a variant of the normal fragmentation process.

Turning now to the spectrum (Fig. 4) of the furanose derivative IVb, we find, in analogy to the earlier reported case of β-D-galactofuranose pentaacetate,[1] that the mass spectrum is quite different from the pyranoid isomers. Loss of the substituent from either of the two carbon atoms adjacent to the ether oxygen is particularly favored and gives rise to the majority of the fragments formed from such a compound. Thus loss of C-5 in IVb gives rise to a series of peaks (D) beginning with the initially formed ion of mass 245, which may lose all three acetoxyl groups to form ions of mass 203, 143 and 101. From the labeled derivative IVc (Fig. 5) it might be inferred that these ions arise by more than one pathway, involving either loss of ketene and acetic acid, or acetic anhydride, as the major component of D_3 is found in Fig. 5 at *m/e* 146 rather than at *m/e* 147 and of D_4 at 102 rather than 103.

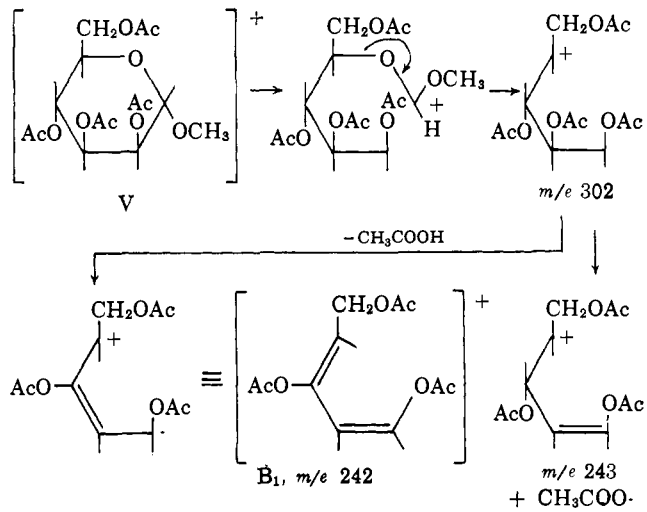


Loss of the substituent at C-1 gives rise to the peak at mass 231 and elimination of the two remaining acetoxyl groups as acetic acid and ketene forms the ion of mass 129, which might be viewed as a protonated furan derivative. The proposed fragmentation scheme is corroborated by the spectrum (Fig. 5) of the trideuterioacetate IVb. The spectrum also shows that part of the species at mass 129 is due to a fragment of type C_1' as it shifts to mass 132 and must thus contain one trideuterioacetyl group.

The mass spectra of the acetyl derivatives of methylated hexoses further corroborate the interpretation presented above for the spectra of the various monomethylxylose triacetates. The spectrum of methyl α-D-mannopyranoside tetraacetate (V) (Fig. 6) exhibits peaks at the same mass as shown in the spectrum of α-D-mannopyranose pentaacetate (Fig. 6 in ref. 1) because of the preferential loss of the substituent at C-1, eliminating the only difference between the two compounds. As the annotation of these peaks is the same as that used in the earlier paper[1] (and also above for the related pentopyranoside Ib), the nature of the fragments need not be repeated here. Only those species not exhibited by the spectrum of the pentaacetate shall be discussed, namely, the peaks at mass 243, 302, 363 and 405. The last two represent "M + 1" and "M + 43" peaks (the latter are not shown for reasons stated earlier), the origin of which was mentioned in connection with the spectrum of Ib and is supported by the presence of these peaks at 366 ("M + 2") and 420 (M + 46) in the spectrum of the trideuterioacetyl derivative Va.

The assignment of the nature of peaks 302 and 243, which are nominally represented as M-60 and M-60-59 and could be interpreted simply as loss of acetic acid followed by the loss of an acetoxyl group, becomes more clear on inspection of the spectrum of the trideuterioacetate Va (Fig. 7). Here the peaks shift to mass 314 and 252, corresponding to M-60 and M-60-62. Thus the loss of 60 mass units in the first step is not due to the loss of one molecule of acetic acid involving an acetoxyl group, while the second step is, in fact, loss of CH_3COO and CD_3COO, respectively. This process is obviously associated with the presence of a 1-methoxyl group as it is also found in the spectrum of Ib (at *m/e* 171) and VI (at *m/e* 215) and in the spectrum (not shown) of methyl β-D-glucopyranoside tetraacetate, which is quite similar to the epimer V and also exhibits a peak at mass 243. It will be noted that analogous peaks are absent in the spectra of the methyl ethers (Fig. 2–5 and 9–13).

The acetal grouping must, therefore, be primarily responsible for this difference, and fragmentation of the C-1, C-2 bond with retention of the positive charge at C-1 would seem to be a reasonable first step. Such a cleavage is a very favored process as the resulting carbonium ion is particularly well stabilized by the two neighboring ether oxygens. Cleavage of the C-5–O bond in the manner shown below results in the elimination of a neutral molecule, methyl formate (mass 60), and a new radical-ion of mass 302 still containing all four acetoxyl groups, in accordance with its shift to mass 314 in the deuterated compound (this ion may gain further stabilization by cyclization to a substituted tetrahydrofuran ion-radical, involving the oxygen atom attached to C-2). Further elimination of an acetoxyl radical gives rise to an unsaturated carbonium ion of mass 243 without increasing the over-all number of radicals and ions, a prerequisite for an energetically favorable fragmentation process. Alternatively, a molecule of acetic acid can be eliminated instead of the acetoxyl radical leading to the fragment (B_1) of mass 242.



The mass spectrum of methyl 2-O-methyl-β-D-mannopyranoside triacetate (VI, Fig. 8) is analogous to the spectrum of V with the exception that practically all peaks are found 28 mass units lower with a few exceptions, such as the peaks at 103 and 145. These are in turn of lower intensity, presumably because of the decreased number of acetyl groups available in this molecule.

The spectra of 2-O-methyl- and 3-O-methylglucopyranose tetraacetates (VII and VIII, Fig. 9 and 10) indicate again the preferential elimination of the substituent at C-3 inasmuch as the spectrum (Fig. 9) of

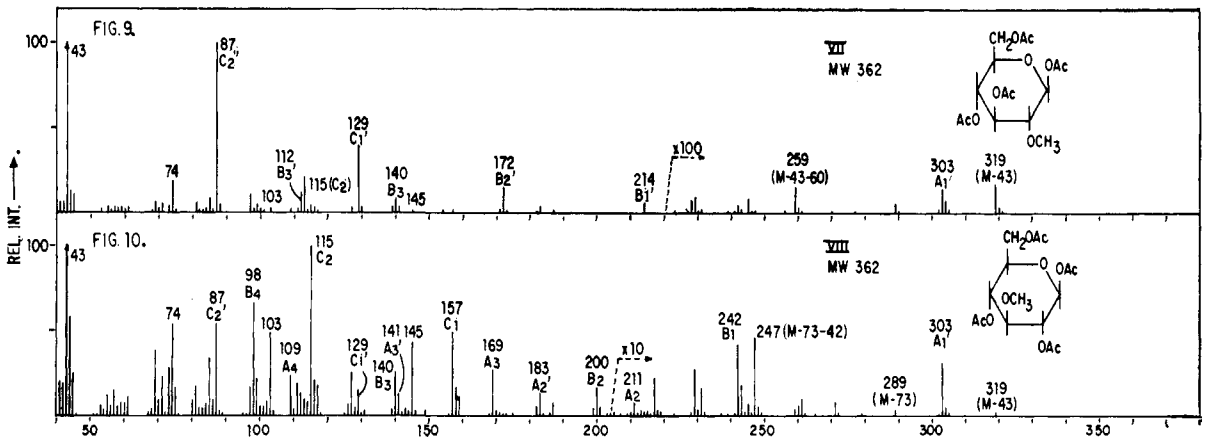


Fig. 9.—Mass spectrum of 2-O-methyl-β-D-glucopyranose tetraacetate (VII).
Fig. 10.—Mass spectrum of 3-O-methyl-β-D-glucopyranose tetraacetate (VIII).

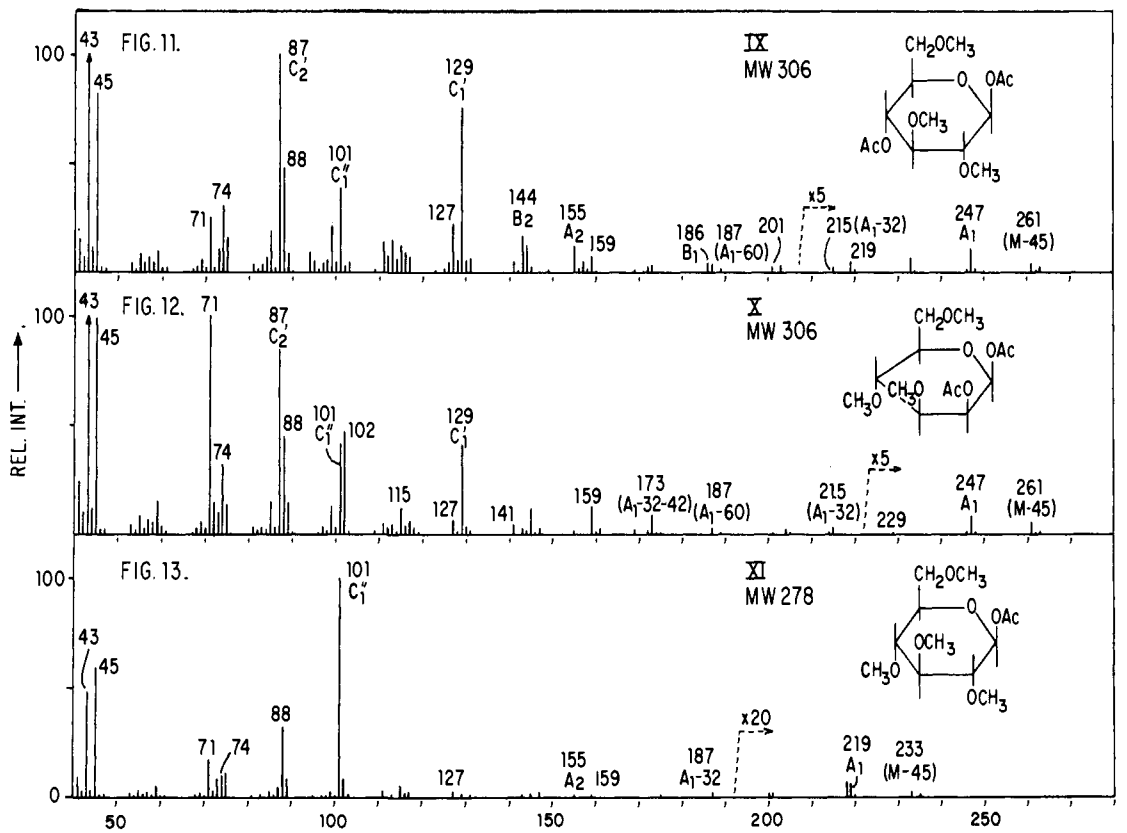


Fig. 11.—Mass spectrum of 2,3,6-tri-O-methyl-β-D-glucopyranose diacetate (IX).
Fig. 12.—Mass spectrum of 3,4,6-tri-O-methyl-β-D-mannopyranose diacetate (X).
Fig. 13.—Mass spectrum of 2,3,4,6-tetra-O-methyl-β-D-glucopyranose acetate (XI).

VII exhibits peaks, most of which have retained the methoxyl group and are thus found 28 mass units lower than those of hexopyranose pentaacetates, or the 1-O-methyl or 3-O-methyl derivatives. The high intensity of the three-carbon fragments (C_1' and C_2') points to an increased tendency of fragmentation of the C-1, C-2 bond with retention of the positive charge at C-2 in the case of the 2-methoxyl derivatives (note also the rather high abundance of these fragments in Fig. 2). The similarity of the mass spectrum (Fig. 10) of VIII with the spectrum of β-D-glucopyranose pentaacetate (Fig. 2 in ref. 1), at least with respect to the mass of most of the fragments, is again a striking one; but as in the case of the corresponding xylose derivative IIIb, a series of peaks, differing by 28 mass units from the major fragments, indicates partial retention of the methoxyl group in favor of loss of an acetoxyl group. The mass spectrum (not shown) of the trideuterioacetyl analog VIIIa supports the assignments made for the spectrum of VIII.

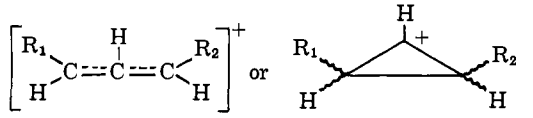
The acetates of mono- and di-O-methylated carbohydrates discussed above represent a group intermediate between the polyacetates of unmethylated sugars and the mono- and diacetates of more highly methylated derivatives, frequently encountered in the determination of the structure of oligosaccharides *via* permethylation and hydrolysis.[2] The mass spectra of some derivatives of this type, namely, 2,3,6-tri-O-methyl-β-D-glucopyranose diacetate (IX, Fig. 11), 3,4,6-tri-O-methyl-β-D-mannopyranose diacetate (X, Fig. 12) and 2,3,4,6-tetra-O-methyl-α-D-glucopyranose acetate (XI, Fig. 13) were determined.

These spectra show, first of all, that isomers such as IX and X, differing only in the relative position of the methoxyl groups at C-2 and C-4 (from many of the previous examples[1] it can be safely assumed that epi-

merization at C-2 alone would not appreciably alter the spectrum), can easily be distinguished on the basis of their mass spectra. In addition, one observes, on comparison with the spectra discussed above and in the previous paper,[1] a gradual decline in abundance of the ragments of series A and B. This is not unexpected as they are due to species deriving their stability from unsaturation originating from elimination of one or more molecules of acetic acid on electron impact. As the elimination of methanol from methyl ethers is much less favorable, the corresponding peaks in Fig. 11–13 are of much lower intensity. With increasing number of acetoxyl groups, the intensity of the CH_3CO^+–ion at *m/e* 43 decreases also and the di- and triacetyloxonium ions of *m/e* 103 and 145 disappear.

A new group of peaks of low intensity is due to loss of the substituent at C-5 (CH_2OCH_3) to give *m/e* 261 (for IX and X) followed by the elimination of ketene and/or acetic acid and methanol to form fragments of mass 261, 219, 201, 159 and 127. The corresponding peaks hardly show in the spectrum (Fig. 13) of XI, with exception of the peak at *m/e* 233 (M-45), because of the high abundance of the species of mass 101.

On the other hand, the three-carbon fragments (series C)[1,6] become even more significant as the elimination of



Fragment

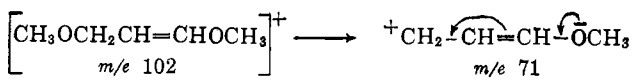
C_1: R_1, R_2 = CH_3CO_2 (*m/e* 157)
C_2: $R_1 + R_2 = CH_3CO_2 + OH$ (*m/e* 115)
C_1': $R_1 + R_2 = CH_3CO_2 + CH_3O$ (*m/e* 129)
C_1'': R_1, R_2 = CH_3O (*m/e* 101)
C_2': $R_1 + R_2 = HO + CH_3O$ (*m/e* 87)
C_3: R_1, R_2 = HO (*m/e* 73)

CH_3COOH is not involved in their formation. In addition, both the stabilizing effect of the electron-donating methoxyl groups and the decreasing opportunity for further fragmentation (such as elimination of ketene from acetoxyl groups) add to the predominance of these peaks in the spectra of the polymethyl derivatives, reaching an extreme in the case of XI (see *m/e* 101 in Fig. 13), a compound in which all three carbon atoms (C-2, C-3 and C-4) believed to be present in that fragment are substituted by methoxyl. In IX and X both methoxyl and acetoxyl groups are attached to these carbon atoms, and their spectra (Fig. 11 and 12) exhibit intense peaks at *m/e* 129, 101 and 87 due to the ions indicated above.

Aside from fragments of mass 45 and M-45 due to the CH_3OCH_2– moiety attached to C-5, the most significant fragmentation processes not operative in the polyacetates of unmethylated hexoses and pentoses lead to ions having retained only two carbon atoms of the ring

$$[CH_3OCH{=}CHOCOCH_3]^+ \longrightarrow [CH_3OCH{=}CHOH]^+$$
m/e 116 *m/e* 74

$$[CH_3OCH{=}CHOCH_3]^+$$
m/e 88



These fragments seem to become more important with increasing methoxyl and decreasing acetoxyl content in both the entire molecule and the fragment itself; the corresponding fully acetylated (or hydroxylated) species derived from polyacetates such as

$$[CH_3COOCH{=}CHOCOCH_3]^+ \longrightarrow$$
m/e 144

$$[CH_3COOCH{=}CHOH]^+ \longrightarrow [HOCH{=}CHOH]^+$$
m/e 102 *m/e*60

are thus absent from the spectra of polyacetates.[1] The higher abundance of these two-carbon fragments probably is caused by the tendency of carbon–carbon bonds next to ether oxygen to cleave on electron impact and by the greater electron-donating power of a methoxyl group if compared with an acetoxyl. In addition, the inability of these derivatives to undergo some of the fragmentations characteristic of the polyacetates (see above) increases the relative abundance of the two-carbon fragments.

Thus the trimethyl diacetate X, containing one 2-acetoxy-3-methoxy grouping, one 3,4- and one 4,6-dimethoxy grouping, exhibits (Fig. 12) peaks at *m/e* 88, 74, 102 and 71, the last one being the most intense. A metastable peak at 49.6 (calcd. 49.4) confirms the formation of the fragment of mass 71 by loss of methoxyl from mass 102. The same peaks are found in the spectrum (Fig. 13) of XI, but the peak at *m/e* 71 is now lower than the one at *m/e* 88, probably because there are now two possibilities for the formation of the latter peak, the 2,3- and the 3,4-dimethoxy groupings. Their over-all intensity appears lower because of the dominating peak at *m/e* 101.

Similarly, the absence in Fig. 11 of a considerable peak at *m/e* 102 and the low intensity of *m/e* 71 (which is here probably due to an ion such as $^+CH{=}CHCH_2OCH_3$) is in agreement with the absence of a 4,6-dimethoxy grouping in IX while the peak at *m/e* 88 is due to two methoxyls on adjacent carbon atoms.

Such considerations involving the two-carbon and three-carbon fragments, combined with the information regarding the substituents at C-1 and C-6 derivable from the presence or absence of peaks at M-31, M-45, M-59 and 45 make it possible to place the methoxyl and acetoxyl groups in such molecules.

Since sugars obtained on hydrolysis of a methylated polysaccharide are best acetylated before being separated by the application of chromatographic techniques[2a] and are often obtained in minute quantities, the mass spectra of these acetates should be useful in that phase of polysaccharide chemistry as well as in the determination of the structure of newly discovered, naturally occurring O-methyl sugars.

Experimental

Mass Spectra.—The spectra were determined with a CEC 21–103C mass spectrometer, equipped with a heated stainless steel inlet system operated at 170°, ionizing potential 70 e.v., ionizing current 50 μamp., temperture of the ion source 250°. The sample (~0.5–1.0 mg.) was sublimed from a glass tube into the reservoir (3 l.).[7]

Acetylation Procedure.—Compounds Ib, IIb, IIIb, IVb, IVc, V, Va, VII, VIII, VIIIa, IX, X and XI, were prepared from the corresponding mono-, tri- and tetramethyl monosaccharides. One milligram of the sugar was sealed in an ampoule with 30 μl. of pyridine and 15 μl. of acetic anhydride (acetic anhydride-d_6 for the preparation of compounds IVc, Va and VIIIa). The reaction mixture was heated for 1 hr. on a steam bath.[8] The ampoule was opened and the reaction mixture was injected onto a gas chromatography column (3% SE-30 on silanized Gaschrom-P using temperatures of 150–200°, or 8% Apiezon L on silanized Gaschrom-P, at 230°) from which the acetylated compound was collected.

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(7) For a more detailed description see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 28.

(8) In the preparation of compounds VII, VIII and VIIIa, the reaction mixture was left at room temperature for 20 hr.