carbon moiety in the transition state resembles the free carbanion. Because the carbanion was also used as the model of the transition state in the HMO treatment of our exchange reaction, we expect that the value of α should also be reflected in the effective value of β for the HMO correlation in the same way that this effective value of β reflects the carbonium ion character of the transition state in HMO correlations of solvolysis reactions.[6,17] The correlation of Fig. 1 corresponds to an effective value of β for our exchange reaction of -49 kcal.[18]

Several decades ago, McEwen[19] presented a list of pK's of some hydrocarbons based on his experiments and those of Conant and Wheland.[20] These pK's have been found[21] to provide a satisfactory correlation with the HMO method and the same theoretical model used in this paper; however, the effective value of β for that correlation is only -22 kcal. At face value, these results imply that α for the exchange rates has the impossible value of 2.2! Analysis suggests strongly that the trouble lies in McEwen's pK assignments. These values were assigned on the basis of exchange reactions of hydrocarbons with alkali metal salts of hydrocarbons. With the less acidic hydrocarbons, no measurement of equilibrium constant was possible, the direction of the equilibrium (by color or by carbonation) being used to assign arbitrarily a ΔpK of 2 units between adjacent hydrocarbons in a series. It seems probable that McEwen's scale is seriously compressed for pK's of hydrocarbons above the early twenties. In the intervening years since McEwen's paper, his pK's have been widely quoted and used, and few serious attempts have been reported to obtain more quantitatively reliable equilibrium constants for hydrocarbons.[22] Such experiments are now in progress in our laboratory, but a report of results at this time would be premature. Meanwhile, however, a revised scale of acidities can be established *tentatively* on the basis of the MO correlations, the more soundly established pK's of comparatively acidic hydrocarbons and the assumption that the Brönsted α equals unity for our exchange reaction. This treatment leads to eq. 9, from which the pK's in Table II are derived. Note that the use of a smaller value for the Brönsted α would result in further spreading of the acidity scale.

$$pK = 84 - 35\Delta M_i \qquad (9)$$

TABLE II

REVISED ACIDITIES OF HYDROCARBONS

Hydrocarbon	pK, eq. 9	pK, exptl.
Fluoradene	10	11–12[a]
Cyclopentadiene	14	14–15[b]
Indene	23	~21[c]
Fluorene	31	
4,5-Methylenephenanthrene	31	
Cycloheptatriene	45	
2-Methylanthracene	57	
Toluene	59	
Methane	84	

[a] In water; H. Rapoport and G. Smolinsky, *J. Am. Chem. Soc.*, **82**, 934 (1960). [b] R. E. Dessy, Y. Okuzumi and A. Chen, *ibid.*, **84**, 2899 (1962). [c] Ref. 19.

(17) M. J. S. Dewar and R. J. Sampson, *J. Chem. Soc.*, 2946, 2952 (1957); ref. 5, sec. 12.3.

(18) Effective values of β of this magnitude are given by several types of HMO correlations. Pertinent to the present discussion is the value -55 kcal., given by correlations of polarographic reduction potentials of hydrocarbons in which carbanions are also involved.[5]

(19) W. K. McEwen, *J. Am. Chem. Soc.*, **58**, 1124 (1936).

(20) J. B. Conant and G. W. Wheland, *ibid.*, **54**, 1212 (1932).

(21) A. Streitwieser, Jr., *Tetrahedron Letters*, **No. 6**, 23 (1960).

(22) As one noteworthy exception, the equilibrium acidity of 9-phenylfluorene is apparently well established (pK = 18.49) in aqueous sulfolane: C. H. Langford and R. L. Burwell, Jr., *J. Am. Chem. Soc.*, **82**, 1503 (1960).

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

Application of Mass Spectrometry to Structure Problems. XIII.[1] Acetates of Pentoses and Hexoses[2]

BY K. BIEMANN, D. C. DEJONGH AND H. K. SCHNOES

RECEIVED DECEMBER 31, 1962

The mass spectra of a number of monosaccharide polyacetates (aldohexoses, ketohexoses, deoxyhexoses, pentoses, pyranoses and furanoses) are discussed and a detailed interpretation of the fragmentation processes which these molecules undergo is presented. The various types mentioned above can be differentiated on the basis of their characteristic fragmentation, but it is more difficult to distinguish epimers. The proposed fragmentation processes are supported by the spectra of isotopically labeled compounds, mainly trideuterioacetates.

During the last few years the usefulness of mass spectrometry as a technique for the determination of the structure of complex organic molecules has been demonstrated, and the method has been applied successfully to a wide variety of compounds.[3] Areas having received more detailed attention encompass lipids,[4a] steroids,[4a,b,c] amino acids,[4d,e] peptides[4d,e] and alkaloids[4f]; the number of compounds whose structure was elucidated, in part or entirely, by mass spectrometry is already considerable.[3]

Very little has been done in the area of carbohydrate chemistry, and only a few sporadic notes have been published by Reed and collaborators. Their first note[5] reports the results of appearance-potential measurements on the $C_6H_{11}O_5^+$ ion derived from α- and β-methylglucopyranosides and four disaccharides. The result, namely, a slightly lower appearance potential for the ion derived from α-glycosides, is interpreted as being due to a weakening of the bond as compared with β-glycosides. In view of the small differences reported and the difficulties inherent in the determination of accurate appearance potentials with conventional instruments, the technique seems to be of little practical

(1) Part XII: K. Biemann, H. K. Schnoes and J. A. McCloskey, *Chem. Ind.* (London), 448 (1963).

(2) Presented in part at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962. For a preliminary communication see ref. 1.

(3) For a summary see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

(4) For recent reviews see (a) R. Ryhage and E. Stenhagen, *J. Lipid Research*, **1**, 361 (1960); (b) S. Bergström, R. Ryhage and E. Stenhagen, *Svensk Kem. Tidskr.*, **73**, 11 (1961); (c) C. Djerassi, H. Budzikiewicz and J. M. Wilson, "Proc. Intern. Congress Hormonol Steroids, Milano, May, 1962, Academic Press, Inc., New York, N. Y., in press; (d) K. Biemann, *Chimia*, **14**, 393 (1960); (e) E. Stenhagen, *Z. anal. Chem.*, **181**, 462 (1961); (f) ref. 3, Chapter 8.

(5) P. A. Finan, R. I. Reed and W. Snedden, *Chem. Ind.* (London), 1172 (1958).

applicability. In an attempt to explore the potential usefulness of mass spectrometry as a tool for the elucidation of the structure of carbohydrates, these authors obtained the mass spectrum of permethylated laminarin (an oligosaccharide) and concluded that the appearance of groups of peaks spaced an average of 204 mass units apart (200–208) is due to the expulsion of 2,4,6-tri-O-methylglucose residues.[6] More recently, the mass spectrum of methyl β-D-glucopyranoside has been reported along with a brief interpretation.[7]

It was, therefore, of interest to undertake a more systematic investigation of the mass spectra of carbohydrates and their derivatives, as it was expected they might yield structural information otherwise more difficult to obtain, particularly in view of the extreme sensitivity of the mass spectrometer which permits the use of samples of a fraction of a milligram or much less. The major technical problem presented by carbohydrates is their very low vapor pressure and appreciable heat sensitivity. Although it has been shown that spectra of rather non-volatile compounds may be obtained[6,8] if they are introduced directly into the ion source of the spectrometer, we have investigated not only free pentoses and hexoses but also some of the derivatives in which the polar hydroxyl groups are esterified or etherified.[2] Such derivatives are sufficiently volatile to be introduced into a conventional inlet system of the mass spectrometer. The present paper describes the results obtained with acetates of pentoses and hexoses, but methyl ethers and isopropylidene derivatives were also found to be very suitable and will be discussed in later papers. All these derivatives have different characteristics with respect to their mass spectra and the choice between these may depend on the kind of information required; most can be learned from a consideration of the spectra of more than one derivative.

The fact that peracetates are easily prepared on a very small scale and that they can be separated and purified by gas chromatography[9] makes them attractive as derivatives suitable for mass spectrometry. It was for these reasons that we have focused our attention first on this group.[10]

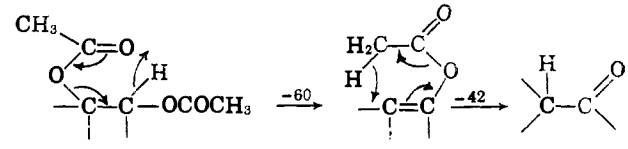
The spectra of a considerable number of hexopyranose pentaacetates were first determined to obtain some information on the general aspects of the fragmentation of these compounds. As was to be expected, epimers exhibit relatively similar spectra because in these monocyclic compounds the stereochemical differences are of little influence on the fragmentation except in those cases where the interactions of large substituents occupying an unfavorable conformation make themselves felt in appreciable intensity differences (these will be discussed later in this paper). Structural differences that express themselves in the size of substituents or of the entire molecule naturally lead to significant variations in the mass spectra, as borne out by comparison of the corresponding derivatives of hexoses, deoxyhexoses and pentoses, or of aldoses *vs.* ketoses. Probably the most valuable aspect of the spectra to be discussed is the sensitivity of the method to ring size, which makes it possible to distinguish furanose derivatives from pyranose derivatives (Fig. 13 and 14).

The spectra of typical representatives of all these types have been determined and interpreted, and the conclusions drawn were substantiated by the mass spectra of deuterated analogs, most of which were obtained by acetylation of the corresponding sugars with acetic anhydride-d_6. It is worthwhile to draw attention to the fact that the shifts of the peaks produced in the spectra of the deuterated compounds (see Fig. 3, 4 and 9) are very clean and complete in spite of the sometimes very high D/H ratio. This is in general not necessarily so, but in the present cases the method of synthesis precludes scrambling of deuterium in the product, and the location of the isotope (in isolated methyl groups) prevents non-specific migration on electron impact.

Because of the predominant occurrence of hexopyranoses, the spectra of their pentaacetates shall be outlined first, followed by a discussion of the influence on the spectra of a change in degree or type of substitution of the pyran ring and finally of a change in ring size.

Hexopyranoses, Deoxyhexopyranoses and Pentopyranoses.—The mass spectrum (Fig. 2) of β-D-glucopyranose pentaacetate (I) shall be considered in detail as a typical example of a hexopyranose pentaacetate. As expected of such a highly substituted molecule, the molecular ion (mass 390) is of very low intensity and can hardly be detected under normal operating conditions. The only observable peaks in the higher mass range are due to loss of the substituents and fall, therefore, at *m/e* 347 ($M-CH_3CO$), *m/e* 331 ($M-CH_3CO_2$) and *m/e* 317 ($M-CH_3CO_2CH_2$). By far the most intense peak is found at *m/e* 43 (CH_3CO^+), except in the trideuterioacetates where it is displaced to *m/e* 46. This peak is about 10–12 times as intense as the second most intense peak which is the reference peak (= 100 arbitrary divisions) in Fig. 1–14. The relative intensities of these peaks are indicated as an example in Fig. 1–4.

A very important mode of fragmentation of these polyacetates (corroborated in many instances by the occurrence of a corresponding metastable peak) is the loss of acetic acid (60 mass units), a process well known for most esters of acetic acid, and the loss of ketene (42 mass units). This latter process seems to be greatly facilitated if preceded by loss of acetic acid; the resulting double bond seems to play a significant role in the elimination of ketene, for which we would suggest the mechanism



rather than a simple 1,2-elimination, producing a hydroxyl group. The arguments in favor of the conclusion are primarily based on deuteration experiments discussed below. Furthermore, we have observed that in the mass spectra of various aliphatic polyacetates, the elimination of 102 mass units in a given step is much more pronounced in compounds containing two acetoxy groups in a 1,2- or possibly a 1,3-relationship, but not if the acetoxy groups are farther apart.

In addition to these small peaks at the high mass end of the spectrum shown in Fig. 2 (note that the peaks above mass 255 are shown ten times their actual size) there can be recognized mainly four series of fragments within which the individual peaks differ by 60 and 42 mass units.

(6) P. A. Finan and R. I. Reed, *Nature*, **184**, 1866 (1959).

(7) R. I. Reed, W. K. Reid and J. M. Wilson, "Symposium on Mass Spectrometry," Oxford, September, 1961.

(8) K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 2005 (1962).

(9) J. A. VandenHeuvel and E. C. Horning, *Biochem. Biophys. Res. Comm.*, **4**, 399 (1961).

(10) In addition to the spectra shown in Fig. 1–14, those of β-D-altropyranose pentaacetate; the tetraacetates of α-L-sorbopyranose, β-D-glucopyranose, methyl and phenyl β-D-glucopyranoside, α-L-arabinopyranose, α-D-xylopyranose; the perdeuteriopentaacetates of β-D-galactopyranose, β-D-galactofuranose, β-D-fructopyranose; methyl 2-O-methyl-β-D-mannopyranoside triacetate and cladinose diacetate were determined. These spectra, even if not specifically mentioned, are in agreement with and support the conclusions drawn in this discussion.

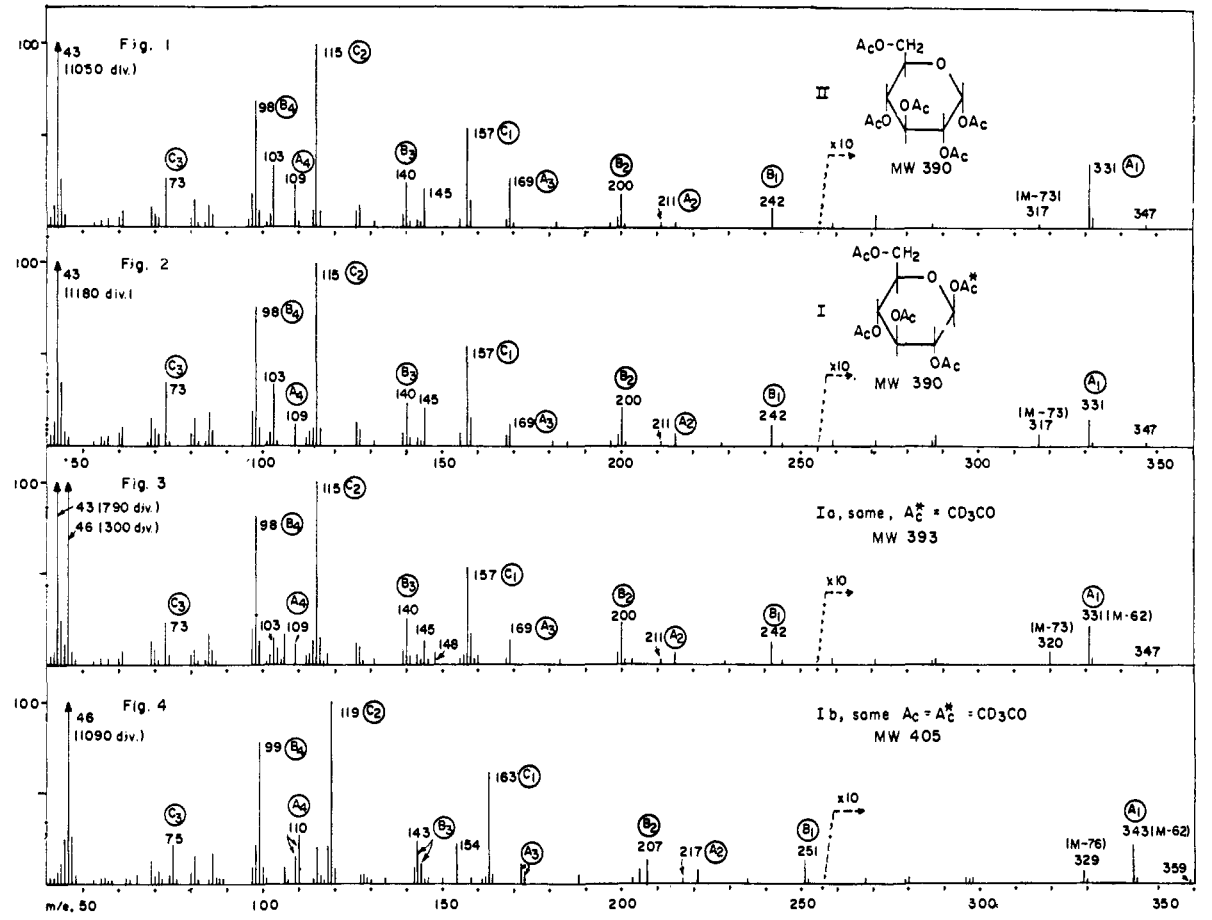


Fig. 1.—Mass spectrum of α-D-glucopyranose pentaacetate (II).
Fig. 2.—Mass spectrum of β-D-glucopyranose pentaacetate (I).
Fig. 3.—Mass spectrum of 1-trideuterioacetyl-β-D-glucopyranose tetraacetate (Ia).
Fig. 4.—Mass spectrum of β-D-glucopyranose pentaacetate-d_{15} (Ib).

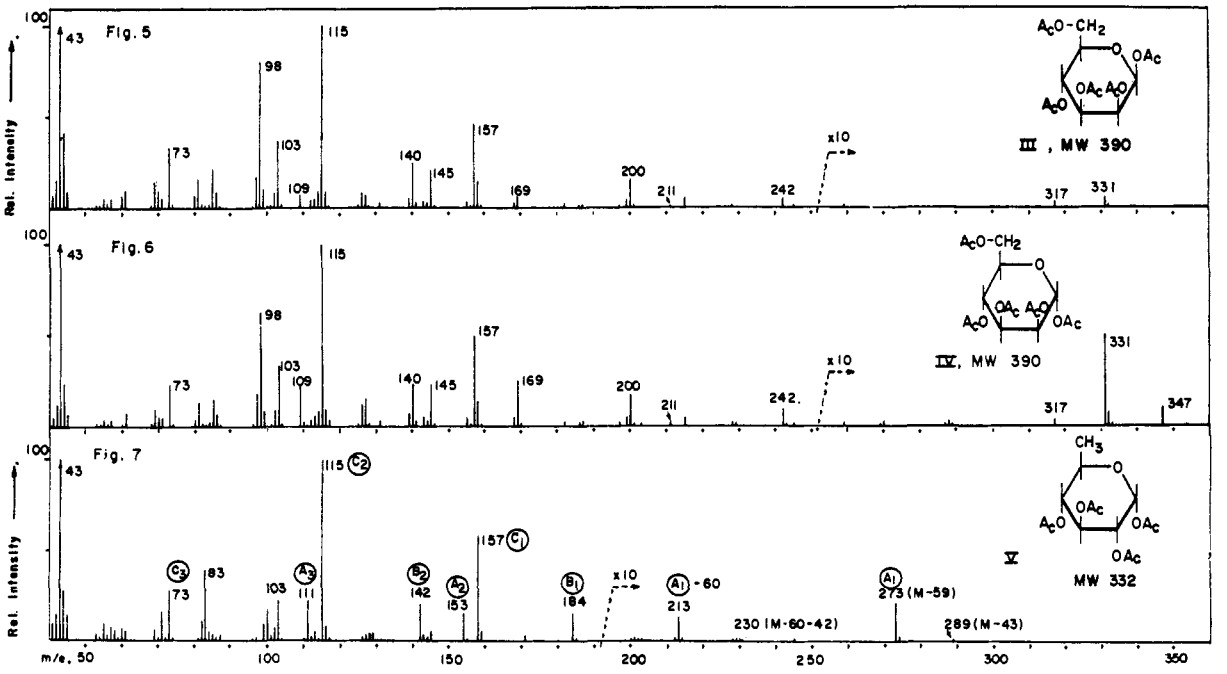


Fig. 5.—Mass spectrum of β-D-mannopyranose pentaacetate (III).
Fig. 6.—Mass spectrum of α-D-mannopyranose pentaacetate (IV).
Fig. 7.—Mass spectrum of α-D-6-deoxyglucopyranose tetraacetate (V).

The first series (A) begins with the peak A_1 at mass 331, which was formed from the molecular ion by loss of an acetoxy group. Specific loss of the 1-acetoxy group, rather than random loss of any one of the five acetoxy groups present, was anticipated because the resulting carbonium ion would be resonance-stabilized

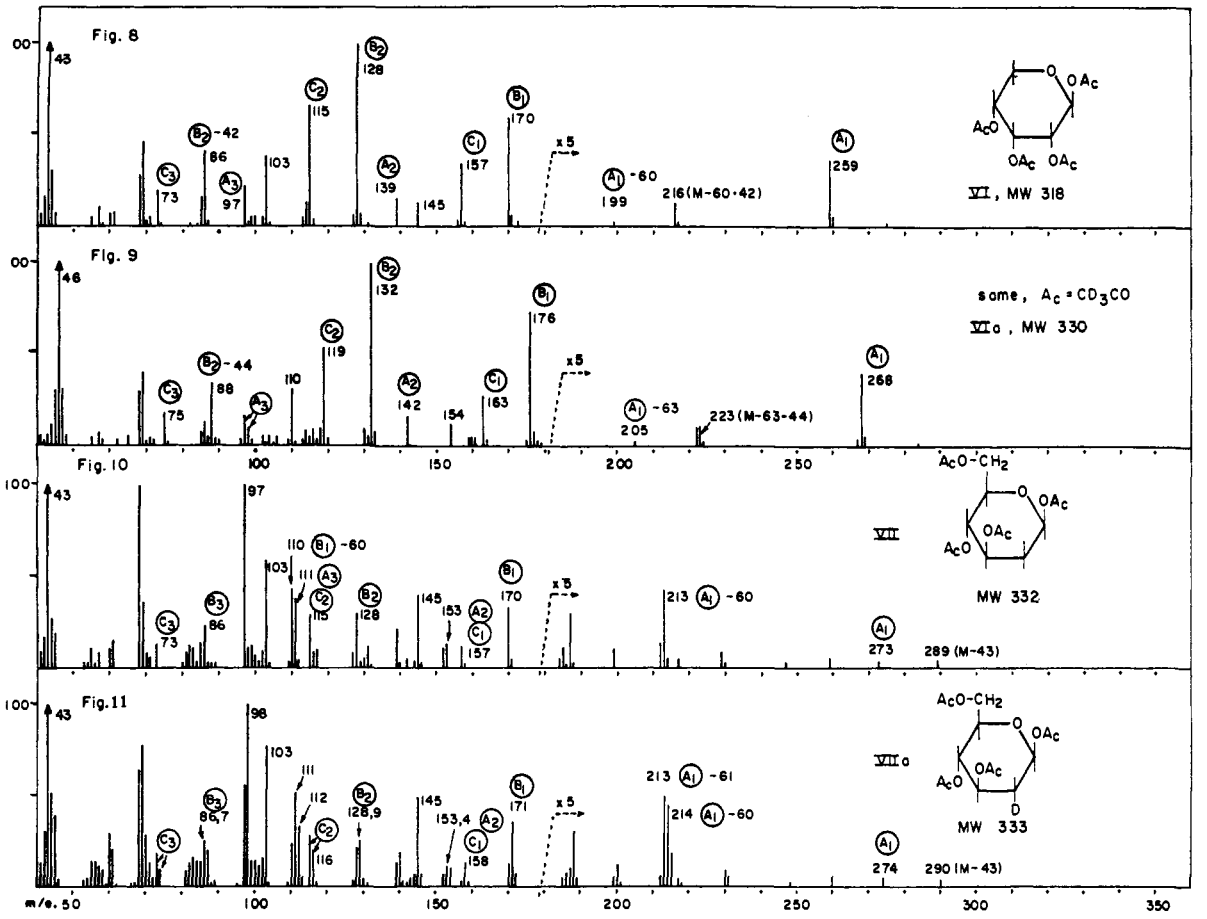


Fig. 8.—Mass spectrum of β-D-ribopyranose tetraacetate (VI).
Fig. 9.—Mass spectrum of β-D-ribopyranose tetraacetate-d_{12} (VIa).
Fig. 10.—Mass spectrum of β-D-2-deoxyglucopyranose tetraacetate (VII).
Fig. 11.—Mass spectrum of β-D-2-deuterio-2-deoxyglucopyranose tetraacetate (VIIa).

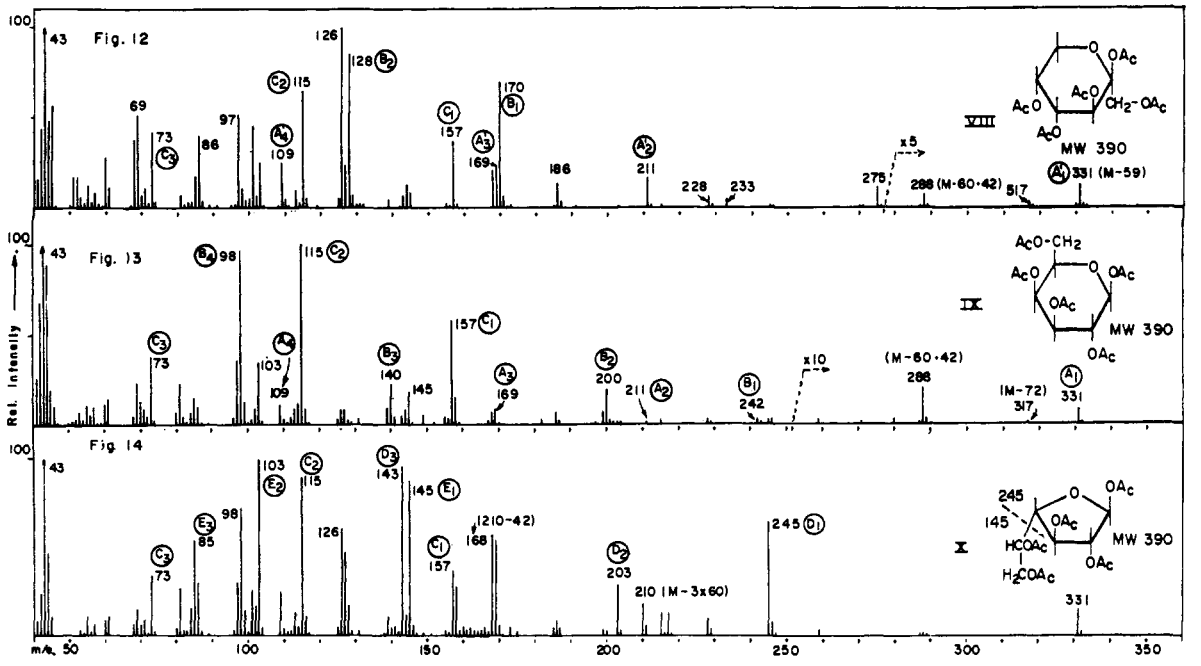


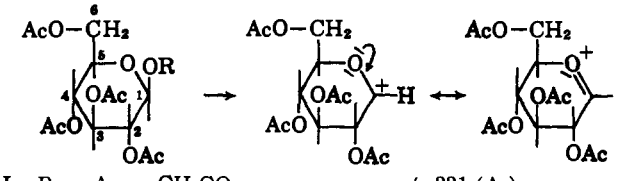
Fig. 12.—Mass spectrum of β-D-fructopyranose pentaacetate (VIII).
Fig. 13.—Mass spectrum of β-D-galactopyranose pentaacetate (IX).
Fig. 14.—Mass spectrum of β-D-galactofuranose pentaacetate (X).

by the free electron pairs of the ether oxygen. To support this assumption, 1-trideuterioacetyl-β-D-glucopyranose tetraacetate (Ia) was prepared. Its spectrum (Fig. 3) clearly indicates that it is almost exclusively (>97%) the CD_3COO group (*i.e.*, the acetoxyl at C-1) that is lost in the formation of fragment A_1

which appears at *m/e* 331 as in the spectrum (Fig. 2) of the unlabeled pentaacetate I. In addition, all the peaks of a series A are found at the same *m/e* in Fig. 2 and 3.

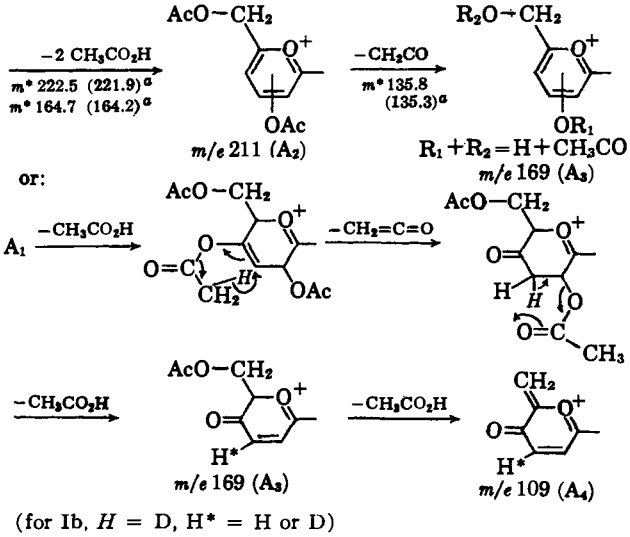
Further loss of two molecules of acetic acid gives rise to the admittedly very small peak at mass 211 (A_2), a "pyronium" ion, and the next step (elimination of ketene) leads to fragment A_3 of mass 169. The process leading to this ion must be somewhat more complex, or even more than one, as judged from the spectrum (Fig. 4) of the perdeuterioacetyl derivative Ib. While all the peaks discussed up to now appear in the spectrum of Ib completely displaced for three mass units per acetyl group (with the exception of the peak of mass 288, which shifts to 298, as anticipated for the loss of $CD_3COOH + CD_2{=}C{=}O$), the fragment of mass 169 appears distributed over mass 172 and 173, instead of being found exclusively at *m/e* 173 as would be expected upon loss of one acetoxy group, two molecules of acetic acid and one molecule of ketene. The only process by which one additional deuterium atom can be lost in such a sequence is outlined in Scheme A, namely, that the elimination of ketene involves the transfer of a deuterium atom (the italicized H) to carbon (*e.g.*, C-3) rather than oxygen, and the removal of that deuterium atom in the next elimination of acetic acid, in this case, CD_3COOD. (The analogous process involving the abstraction of the corresponding hydrogen atom cannot be distinguished from the 1,2-elimination of ketene.) Further elimination of a molecule of acetic acid from C-6 leads to the species A_4 of mass 109 (109 and 110 in the deuterated compound).

SCHEME A



m/e 331 (A_1)

I, R = Ac = CH_3CO
Ia, R = CD_3CO, Ac = CH_3CO
Ib, R = Ac = CD_3CO
Ic, R = CH_3, Ac = CH_3CO
Id, R = C_6H_5, Ac = CH_3CO
Ie, R = H, Ac = CH_3CO



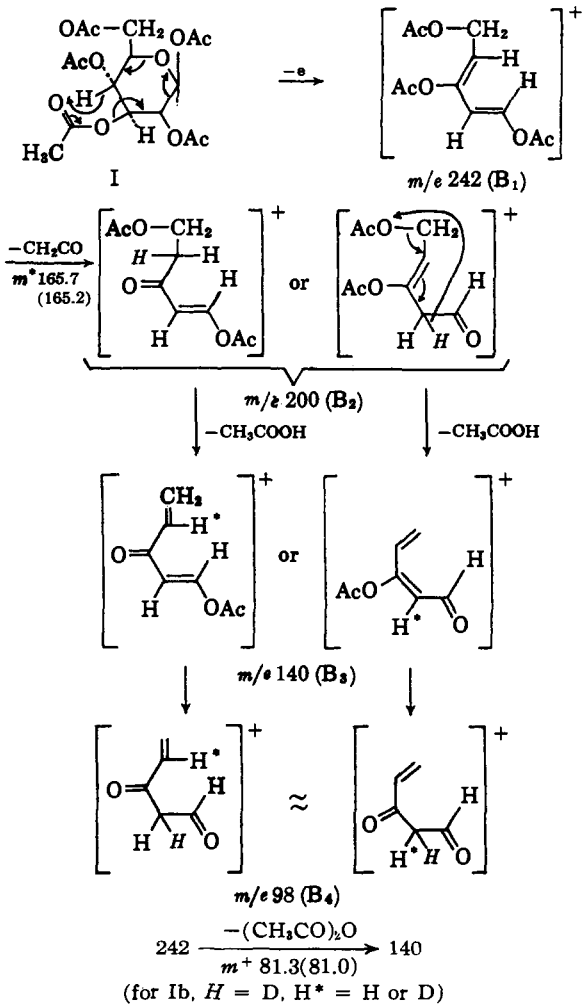
(for Ib, *H* = D, H* = H or D)

Corresponding metastable peaks in the spectrum of I (calculated value in parentheses).

It is worth noting that this fragmentation series A, which begins with mass 331, is also observed, even more pronounced, in the spectra of the methyl and phenyl glucoside tetraacetates Ic and Id, thus indicating that the loss of the substituent at C-1 increases in the series: acetoxy, methoxy, phenoxy (the intensity of *m/e* 331 is 1.5%, 3% and 30% of the intensity at *m/e* 115 in the spectra of I, Ic, and Id, respectively). On the other hand, the peaks of series A are of lower intensity in β-D-glucopyranose tetraacetate (Ie). These fragments can also be recognized in related pyranoses, such as Fig. 7, 8 and 10, where they appear at correspondingly lower mass due to the variation in the size of the C-5 or C-2 substituent.

In most of these spectra (Fig. 1–3, 5, 6 and 13) there is a second group of peaks (B) that begins at mass 242 (Fig. 2) and is followed by peaks at *m/e* 200, 140, and 98. This fragment must contain C-5 including its substituents because the peaks of group B are found 58 mass units ($CH_3CO_2CH_2$ *vs.* CH_3) lower in the spectrum of 6-deoxyglucopyranose tetraacetate (V, Fig. 7) and 72 mass units lower ($CH_3CO_2CH_2$ *vs.* H) in the spectrum of pentose tetraacetates (*e.g.*, Fig. 8). It seems to be formed by elimination of C-1 plus the ether oxygen in simultaneous elimination of one molecule of acetic acid,[11] most probably by the path outlined in Scheme B.

SCHEME B

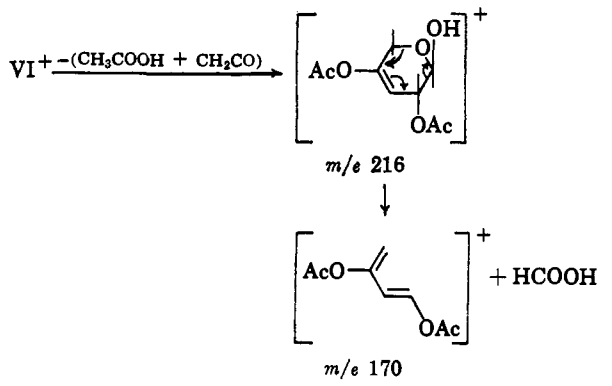


(for Ib, *H* = D, H* = H or D)

The arrows shown in the formation of fragment B_1 in the above scheme should not necessarily imply a concerted one-step process. This fragmentation may just as well be a stepwise one instead, particularly

(11) In some of the epimers of glucose, this process must involve a *trans*-elimination of acetic acid, which might at a first glance seem to be unfavorable. One has to keep in mind, however, that the double bond formed in the resulting ion has a one-electron π-bond, thus leading to a "flexible" double bond.

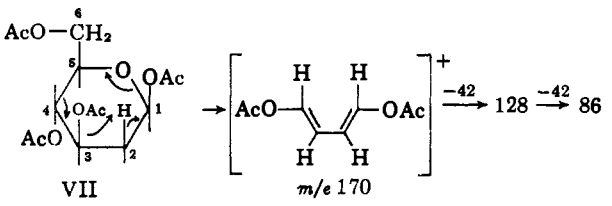
as in the spectrum (Fig. 8) of β-D-ribopyranose tetraacetate (VI) there is a metastable peak with a maximum at *m/e* 134.5 (*ca.* 134) corresponding to the loss of 46 mass units from *m/e* 216 to form a fragment (B_1) of mass 170, suggesting the sequence



The detailed mode of further elimination of acetic acid and ketene from B_1 is depicted in accordance with the retention of deuterium in the case of the perdeuterio derivative Ib. Here again after the third step, the peaks are doublets differing by one mass unit (*m/e* 143 and 144 in Fig. 4), which can best be explained by the removal of a deuterium atom during the elimination of acetic acid by the process discussed above. If this deuterium atom is at a carbon atom also bearing hydrogen, splitting of those peaks must occur. It might be pointed out that this fact could also be explained by invoking a one-step elimination of acetic anhydride (resulting in the loss of six deuterium atoms) in the fragmentation parallel to the two-step process, namely, elimination of acetic acid and ketene (1,2) (which involves only the loss of five deuterium atoms).

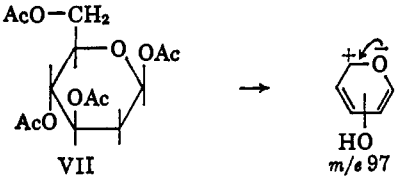
From the spectra which we have determined, one can deduce that this fragmentation process (B) seems to be most significant if C-5 (with respect to ring atoms) is unsubstituted as is the case in the pentopyranoses or in fructopyranose. In these compounds, the peak corresponding to mass 242 is found at mass 170, and those resulting from further fragmentation are similarly displaced.

The fragmentation sequence depicted in Scheme B seems to be sensitive to substituents at C-2. The peaks of this series (B) are practically absent in the spectrum (Fig. 10) of 2-deoxyglucopyranose tetraacetate (VII). In that molecule a variation of this fragmentation results in a fragment of mass 170, encompassing C-1 through C-4 after elimination of the ether oxygen and C-5 and C-6. The reason for this variation might be that the first step in the frag-



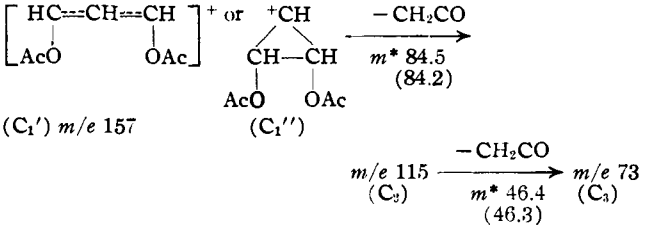
mentation leading to the species of mass 242 in the glucose derivative is the cleavage of the C-1, C-2 bond with retention of the positive charge at C-2 where it is initially stabilized by the acetoxy group. As this group is absent in the 2-deoxy derivative, cleavage of the analogous (next to the ether oxygen) bond, namely, C-4, C-5, is very much preferred and initiates the fragmentation leading to the fragment of mass 170 depicted above.

The most intense peak in the spectrum (Fig. 10) of VII is found at *m/e* 97, corresponding to a fragment formed by loss of C-6, two molecules of acetic acid and one molecule of ketene. Such a process could yield a highly stabilized pyronium ion



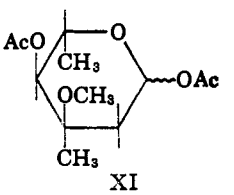
In agreement with this assignment is the partial displacement of this peak to *m/e* 98 in the 2-deuterio derivative VIIa as the elimination of acetic acid may there involve either H or D of C-2.

In many of these spectra two very intense peaks are found at mass 157 and mass 115, accompanied by a less intense one at mass 73. From the spectra of the fully deuterioacetylated compounds (Fig. 4 and 9), it can be deduced that the fragment of mass 157 contains two acetoxy groups as it shifts to mass 163 in the perdeuterio analogs. It must therefore contain three carbon atoms in addition to the two acetoxy groups and should thus have one of the two structures



The allyl ion C_1' might be favored over the cyclopropyl ion C_1'' because in addition to better stabilization it contains a double bond which would facilitate the elimination of ketene (see above) to lead to the abundant fragment C_2 of mass 115 (119 in the deuterio derivative). Further elimination of ketene leads to the peak C_3 at *m/e* 73 (75 upon deuteration); the lower intensity of this fragment is in line with the hypothesis that 1,2-elimination is less likely than 1,4-elimination involving a double bond.

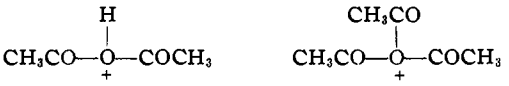
There is thus not much doubt about the composition of the fragments of series C, and it is now of interest to recognize the part of the molecule from which they are derived. As there is a peak at *m/e* 157 in the spectrum (Fig. 12) or fructopyranose pentaacetate, C-1 (with respect to ring atoms) cannot be present any longer because in that case the mass of this fragment would be 58 or 72 mass units higher depending on which one of the substituents ($OCOCH_3$ or CH_2O-$COCH_3$) is retained. The presence of a peak at *m/e* 157 in the spectra of pentose tetraacetates (*e.g.*, VI, Fig. 8) excludes C-6 of aldohexoses (*e.g.*, I). The analogous fragment at *m/e* 143 in the spectrum of cladinose diacetate (XI) indicates that C-3 is included in this type of fragment.



Finally, fragment C must include C-2 because it shifts in part from *m/e* 157 in VII (Fig. 10) to 158 in VIIa (Fig. 11), which is deuterated at C-2. This conclusion is supported by the spectrum of methyl 2-O-methyl-

mannopyranoside triacetate (IIIa)[12] in which a peak is found at mass 129, thus requiring the presence of one acetoxy and one methoxy group in fragments of series C. It seems, therefore, one can draw the conclusion that there must be present on three consecutive carbon atoms, namely, C-2, C-3, C-4, those functional groups required by the mass of this fragment.

Finally there are in all these polyacetates two peaks of considerable intensity at mass 103 and 145. The latter was first thought to represent two carbon atoms with two acetoxy groups which upon elimination of ketene could give mass 103, but the spectrum of the deuterated species clearly shows that this is not the case as they shift to mass 154 and mass 110, respectively, indicating the presence of three and two acetyl groups, respectively. These two fragments must, therefore, represent di- and triacetyloxonium ions

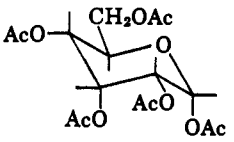


m/e 103 *m/e* 145

While these species seem to be rather unusual at a first glance, their formation is not so surprising if one keeps in mind that molecules such as H_2O, NH_3, CH_3OH, H_2S, CH_3COOH, etc., if eliminated, never carry the positive charge except when split off in the protonated form (by simultaneous abstraction of a proton from the molecule) as H_3O^+, NH_4^+, $CH_3OH_2^+$, H_3S^+, $CH_3COOH_2^+$, etc.[13] Thus, the fragment of mass 103 involves an analogous process in which protonated acetic anhydride is eliminated (deuterated acetic anhydride-d_6 in the trideuterioacetates). The species of mass 145 goes one step further, namely, abstracting an acetonium ion rather than a proton. The excellent stabilization of the positive charge and the high concentration of acetoxy groups within these molecules are certainly the main reasons for the appearance of these peaks. The 1-acetoxy group seems to participate preferentially in the formation of the fragments of mass 103 and 145 as the peaks at *m/e* 106 and 148 in the 1-trideuterioacetate Ia (Fig. 3) are more intense than expected on the basis of statistical participation. The formation of the species of mass 145 does not even seem to contain exactly the same two acetoxy groups present in the fragment of mass 103 because the 103:106 and the 145:148 ratios in Fig. 3 would have to be more nearly the same if the latter arises from the former by addition of CH_3CO^+ instead of H^+.

From the above discussion it becomes clear that one can recognize from the mass spectrum the presence of a hexopyranose pentaacetate and that it is possible to distinguish them from deoxyhexopyranose tetraacetates. In the latter case, one can also deduce the "deoxy" position, at least differentiate between 6-deoxy and other deoxyhexoses. It is similarly possible to recognize pentose derivatives and to distinguish them from deoxypentoses.

As was pointed out at the outset of this discussion and can be noticed by comparison of Fig. 1, 2, 5, 6, and 13, it is, however, more difficult to get some insight into the stereochemistry of such molecules, an important aspect of carbohydrate chemistry. Only if the steric arrangement is such that the molecule is excessively crowded, does one observe appreciable changes in the intensity of certain peaks. The increased intensity of the M-59 peak (at *m/e* 331) in the mass spectrum of the pentaacetates of α-D-glucopyranose (Fig. 1) and of α-D-mannopyranose (Fig. 6), as contrasted to the spectrum of the corresponding β-isomer (Fig. 2 and 5), is best interpreted by the more hindered position of the acetoxy group in the α-anomer. Similarly, the same peak is quite intense in the spectrum of α-D-altropyranose pentaacetate (XII), which has two large axial substituents in either one of the



XII

two possible conformations. Another example is the fragment of mass 288 in the spectrum (Fig. 13) of the β-D-galactopyranose pentaacetate, which might be due to the closeness of the 4- and 6-acetoxy groups which are *cis*. Thus it might be possible to deduce the stereochemistry at C-1 in a pair of anomeric acetates by comparison of the intensity of the M-59 peaks.[14] Obviously this will be done only in very special cases, for example, if the material available is insufficient for other approaches such as optical rotation and n.m.r. spectroscopy.

Ketopyranoses *vs.* Aldopyranoses.—In contrast to the small changes in the mass spectrum upon epimerization of one or more centers, the spectra of derivatives of ketoses differ appreciably from those of the aldose derivatives discussed above.

Pentaacetates of 2-ketohexopyranose differ from the corresponding aldose derivatives in principle only by an interchange of substituents at C-1 and C-5 of the tetrahydropyran ring; the mass spectra (*e.g.*, Fig. 2 and 12) of such pairs are quite different, mainly because the ketose derivative, *e.g.*, β-D-fructopyranose pentaacetate (VIII), has a more highly substituted ketal carbon atom and mass spectrometrically resembles more to a certain extent a pentose rather than a hexose. (The latter fact is borne out by the peak at mass 170 which is fragment B_1 and thus of the same mass as, for example, in arabinose tetraacetate. Elimination of two moles of ketene leads to the fragments of mass 128 and 86.) Most other peaks are similar to the ones found in the spectra discussed previously, *e.g.*, A_1'–A_4', which correspond to A_1–A_4 of the aldose analogs except that the hydrogen at C-1 and the CH_2-OAc group at C-5 are interchanged. The most intense peak, however, is found at mass 126. An attractive mechanism involves the elimination of acetic anhydride between C-1 and C-4, reminiscent of the formation of anhydro derivatives of hexoses. The resulting bicyclic ion, upon elimination of all the remaining acetyl groups either as ketene or as acetic acid, followed by cleavage of the C-3, C-4 bond, gives a highly conjugated oxonium ion-radical of mass 126.

It is worth noting that a peak at mass 126 does occur in most of the hexose spectra although it is there of very low intensity.

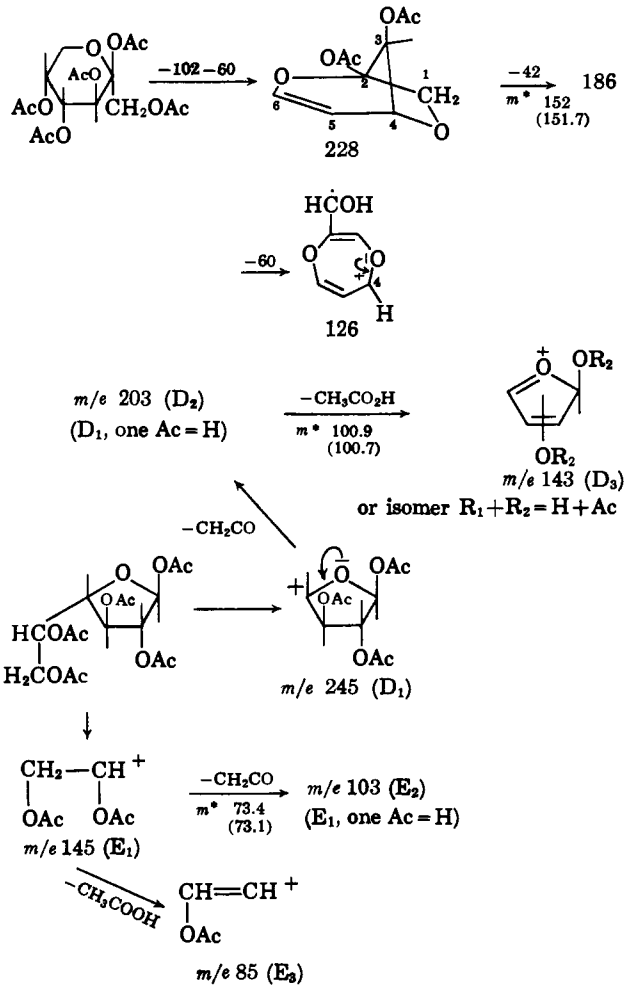
Pyranoses *vs.* Furanoses.—One of the questions frequently arising in carbohydrate chemistry, namely, whether a certain derivative is a pyranose or furanose, was expected to be most easily solved by mass spectrometry. This is in fact the case as shown by comparison of β-D-galactopyranose pentaacetate (IX, Fig. 13)

(12) D. C. DeJongh and K. Biemann, to be published.

(13) For a discussion of these processes see F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959), or ref. 3, Chapter 3.

(14) The relative intensities of some of the peaks seem to be somewhat less reproducible than is normally the case with mass spectra. It is thus necessary to take care to reproduce the experimental conditions (*e.g.*, temperature of inlet system and ion source of the spectrometer) if spectra for close comparison, as mentioned above, are to be obtained. These fluctuations are, however, not of a magnitude to interfere with qualitative interpretations not concerning subtle stereochemical questions.

and its furanoid isomer (X, Fig. 14). These two compounds, while isomeric, differ both in the size of the ring and the "side chain." In the furanose, a two-carbon chain is attached to the tetrahydrofuran ring next to the ether oxygen, which greatly facilitates cleavage of the C-4, C-5 bond; retention of the positive charge at C-4 leads, therefore, to the intense fragment at mass 245. Charge retention at C-5 results in a fragment of mass 145 and both these species decompose further by consecutive loss of 60 and 42 mass units. Thus the peaks at *m/e* 245, 203, 143, 145, 103 and 85 are accounted for.



These assignments are corroborated by the mass spectrum of the corresponding perdeuterioacetate of X in which the fragment of mass 245 is displaced to 254 (three deuterioacetyl groups) while the one at mass 145 is now found at mass 151 (two deuterioacetyl groups). Part of the species of mass 145 and 103 shifts, however, to mass 154 and 110, respectively, thus indicating that also here, as in all other polyacetates, di- and triacetyl oxonium ions are formed. The other peaks mentioned above shift accordingly and also in this case are observed doublets differing, in one mass unit, for those fragments that have experienced loss of acetic acid and ketene in consecutive steps (*e.g.*, the fragment of mass 143 shifts thus to 146 and 147). Finally, the relatively high intensity of the M-59 peak found at *m/e* 331 should be pointed out. It seems to indicate that loss of a substituent from a carbon atom bearing an ether oxygen is more favored in five-membered rings (tetrahydrofurans) than in the six-membered homologs.

Other furanoses exhibit analogous spectral characteristics, and additional pairs (pyranose *vs.* furanose) of methyl and isopropylidene derivatives will be discussed in later papers.[12,15]

From the mass spectra discussed in this paper, it seems to be clear that such data may be useful not only for the characterization of a sugar, a problem that seems to be solved at present mainly by paper chromatographic techniques if it has to be done on a very small scale, but also in the determination of the structure of a newly discovered sugar. Since those are now most frequently isolated as metabolites of microörganisms, they often contain additional carbon atoms, either extending the chain or as methyl groups. It is particularly these characteristics which are recognized more easily from the mass spectrum and may aid in the determination of the gross structure, the knowledge of which is a prerequisite for deducing the stereochemical details by other methods. While trideuterioacetates have been used in this work mainly for the purpose of supporting proposed fragmentation processes, it is obvious that such derivatives would be very useful also in the determination of the structure of the parent sugar.

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 μa., temperature of the ion source 250°. The sample (~0.5–1.0 mg.) was sublimed from a glass tube into the reservoir (3 l.).[16]

1-Trideuterioacetyl-β-D-glucopyranose Tetraacetate (Ia).—A solution of 50 mg. of β-D-glucopyranose tetraacetate in 0.2 ml. of pyridine and 0.3 ml. of acetic anhydride-d_6 (Merck and Co., Ltd.) was stirred for 6 hr. at room temperature. After addition of an equal volume of water the aqueous solution was neutralized with sodium bicarbonate and extracted with chloroform. After removal of the chloroform, the crystalline residue was recrystallized from ethanol; m.p. 131.0–131.5° (β-D-glucopyranose pentaacetate melts at 132°).

β-D-Glucopyranose Pentaacetate-d_{15} (Ib).—When free β-D-glucose was treated with acetic anhydride-d_6 as described above, the penta-(trideuterio)-acetate Ib was obtained, m.p. 133–134° and 132–133° when mixed with authentic non-deuterated material (I).

β-D-2-Deuterio-2-deoxyglucopyranose Tetraacetate (VIIa).—A small sample (<50 mg.) of methyl 2-deuterio-2-deoxyglucopyranoside[17] was hydrolyzed by heating to 100° with 1 ml. of 0.01 *N* hydrochloric acid.[18] The residue obtained after neutralization with sodium bicarbonate and evaporation was heated for 30 min. on a steam-bath with 0.2 ml. of acetic anhydride and 50 mg. of anhydrous sodium acetate. Water was added and after neutralization of the solution it was extracted with chloroform. The residue obtained after removal of the chloroform was purified by gas chromatography (at 165°; 3% SE-30 on silanized Gaschrom-P). The non-deuterated analog VII was prepared by acetylating β-D-2-deoxyglucose (Calbiochem) in the same manner.[19]

β-D-Ribopyranose Tetraacetate-d_{12} (VIa).—D-Ribose was deuterioacetylated as described above for glucose, except that the reaction mixture was kept at 0° overnight. The product was recrystallized from petroleum ether (90–100°) and melted at 109–110° (reported[20] 110° for the ordinary acetate).

β-D-Galactofuranose Pentaacetate-d_{15} (Deuterio-X).[21]—To 5.0 ml. of boiling pyridine and 1.5 ml. of acetic anhydride-d_6, 0.3 g. of D-galactose was added and the mixture refluxed until homogeneous. The solution was then cooled, an equal volume of water added and repeatedly extracted with chloroform. The washed and dried chloroform phase was evaporated and the furanose (deuterio-X) was isolated by gas chromatography (at 180°; 3% SE-30 on silanized Gaschrom-P), selecting the fraction whose retention time matched authentic X.

Acknowledgment.—We are indebted to Prof. R. U. Lemieux for many of the compounds whose spectra are discussed in this paper, to Dr. A. S. Perlin for a

(15) D. C. DeJongh and K. Biemann, to be published.
(16) For a more detailed description see ref. 3, p. 28.
(17) Kindly provided by Prof. R. U. Lemieux.
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sample of IIIa and to Dr. R. B. Morin for cladinose. This investigation was supported by a research grant (RG-5474) of the National Institutes of Health, Public Health Service.

[CONTRIBUTION FROM THE JAMES BRYANT CONANT LABORATORY OF HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

On the Mechanism of the Oxidative Cleavage of Phenyl-*t*-butylcarbinol with Chromic Acid[1]

BY JOHN J. CAWLEY AND F. H. WESTHEIMER

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Chromic acid partially oxidizes phenyl-*t*-butylcarbinol to pivalophenone and partially cleaves it to benzaldehyde and *t*-butyl alcohol. The present work was designed to decide whether the oxidative cleavage occurs directly (to produce a *t*-butyl cation) or indirectly, by way of a rearrangement to form the oxonium ion A, $C_6H_5-CH=\overset{+}{O}-C(CH_3)_3$; subsequent hydrolysis of A would yield benzaldehyde and *t*-butyl alcohol. The latter pathway has now been ruled out by experiments in which the alcohol has been labeled with ^{18}O. The oxidative cleavage of labeled carbinol in ordinary water leads exclusively to the production of unlabeled *t*-butyl alcohol. Prior work had shown that the hydrolysis of the *t*-butyl oxoniun salt of benzaldehyde, A, proceeds without rupture of the alkyl–oxygen bond. Therefore, if the cation A were an intermediate in the oxidation–reduction process, the label would have been found in the *t*-butyl alcohol. Confirmatory experiments were conducted with excess ^{18}O in the solvent rather than in the carbinol, and with anisyl-*t*-butylcarbinol rather than phenyl-*t*-butylcarbinol.

The chromic acid oxidation of alcohols has now been shown to proceed by way of a chromic acid ester of the alcohol as intermediate.[2-4] The ester undergoes internal oxidation–reduction to produce ketone and a chromium compound of valence IV.[5] A possible pathway for the subsequent reduction of the derivative of tetravalent chromium, applied to a particular example, is shown in eq. 1–3 below.

In 1948, Mosher and Whitmore[6] discovered that chromic acid oxidizes methyl-*t*-amylcarbinol in part with cleavage to acetaldehyde and (presumably) *t*-amyl alcohol. Mosher has further postulated that, in this and similar reactions,[7] the alcohol is oxidized by chromic acid to an intermediate with a positively charged electron-deficient oxygen atom; the intermediate is assumed to cleave spontaneously to form a tertiary carbonium ion.

In a further investigation of the problem, it was discovered that the cleavage of phenyl-*t*-butylcarbinol is caused by a derivative of tetravalent or pentavalent chromium[8]; that fraction of the oxidation which is caused by hexavalent chromium yields pivalophenone.

$$C_6H_5-CHOH-R + Cr^{VI} \longrightarrow C_6H_5CO-R + Cr^{IV} \quad (1)$$

$$Cr^{VI} + Cr^{IV} \longrightarrow 2Cr^{V} \quad (2)$$

$$C_6H_5-CHOH-R + Cr^{V} \longrightarrow C_6H_5CHO + ROH + Cr^{III} \quad (R = t\text{-butyl}) \quad (3)$$

The evidence for this pathway rests both on the primary deuterium isotope effects observed in the oxidation,[8] and on the effect of manganous ion on the reaction. The chromic acid oxidation of alcohols "induces" the oxidation of manganous ion[5] to manganese dioxide; kinetic analysis shows that the active oxidant for Mn^{++} is Cr^{IV}, which is thereby swept from solution by the process

$$Mn^{++} + Cr^{IV} \longrightarrow Mn^{III} + Cr^{III} \quad (4)$$

Cr^{V} arises from Cr^{IV}, so that reaction 4 eliminates both of the intermediate valence states of chromium from solution. Since manganous ion diminishes or suppresses[8] the cleavage of phenyl-*t*-butylcarbinol, the cleavage must be caused by the oxidation of the alcohol by a compound of pentavalent or possibly tetravalent chromium.

The present paper shows that the cleavage does not occur by way of a rearrangement of phenyl-*t*-butylcarbinol to the cation A. The hypothetical reactions may be formulated as occurring by way of an ester of an acid of pentavalent chromium, as

$$C_6H_5-\underset{\underset{OH}{|}}{CH}-C(CH_3)_3 + Cr^{V} \rightleftarrows \quad (5)$$

$$C_6H_5-\underset{\underset{\underset{CrO_2H_2}{|}}{O}}{\overset{\overset{H}{|}}{CH}}-C(CH_3)_3 \dashrightarrow C_6H_5-\overset{\overset{H}{|}}{C}=\overset{+}{O}-C(CH_3)_3 + Cr^{III}$$

A

This scheme was initially not without merit, since Winstein[9] has discovered that when migration of a group, R, to oxygen takes place during the acetolysis of compounds of the type $(CH_3)_2\underset{\underset{R}{|}}{C}-O-O-COC_6H_4NO_2$, *t*-butyl migrates more rapidly than does phenyl.

The proof that the mechanism outlined in eq. 5 does not apply to the oxidative cleavage depends on labeling the oxygen atom of phenyl-*t*-butylcarbinol with ^{18}O. Proper control experiments show that if the cation A were involved, the reaction sequence would transfer the oxygen atom to the *t*-butyl alcohol; the label is not in fact so recovered.

The conclusions drawn from these experiments are in accord with those of Lansbury, *et al.*,[10] who inferred that the oxidative cleavage must occur by the direct production of a carbonium ion. They observed that phenyl apocamphylcarbinol is oxidized without accompanying cleavage and correlated this finding with the difficulty of establishing a positive charge at a bridgehead.[11] The rearrangement is also ruled out by their experiments, since the apocamphyl group rearranges, in the Baeyer–Villiger reaction, in preference to phenyl.[12]

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