Mass Spectrometry in Carbohydrate Chemistry. Acyclic Peracetates of Pentoses and Hexoses

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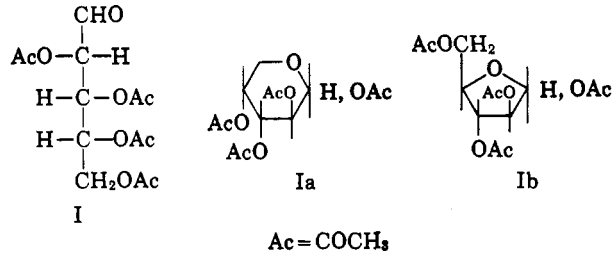
Received September 14, 1964

The mass spectra of *aldehydo*-D-arabinose tetraacetate, *aldehydo*-D-glucose pentaacetate, *aldehydo*-6-deoxy-L-galactose tetraacetate, *aldehydo*-2-deoxy-D-glucose tetraacetate, and *keto*-D-fructose pentaacetate are discussed. Interpretations of the fragmentation processes which these compounds undergo upon electron impact are presented and corroborated by the mass spectra of *aldehydo*-D-arabinose tetraacetate-d_{12} and *aldehydo*-2-deoxy-D-glucose tetraacetate-d_{12}, prepared with acetic anhydride-d_6. Some of the fragmentation patterns are in common with the mass spectra of the pyranose and furanose isomers and others are characteristic of the *aldehydo* and acyclic form of these compounds.

The mass spectra of the peracetyl derivatives of the pyranose and the furanose ring forms of monosaccharides have been systematically investigated and a detailed interpretation of their fragmentation processes presented.[1] Mass spectrometry is sensitive to structural differences such as ring size, aldoses *vs.* ketoses, and hexoses *vs.* pentoses *vs.* deoxyhexoses, whereas epimers and anomers exhibit very similar mass spectra except for minor relative-intensity differences.

Monosaccharides, especially high-carbon aldoses, can also form peracetyl derivatives in the acyclic *aldehydo* or *keto* form; for example, the structures of the three peracetyl derivatives of D-arabinose are acyclic I, pyranose Ia, and furanose Ib. The behavior upon electron impact of these acetylated sugars in the open-



chain structure has now been investigated to broaden the study of peracetyl derivatives of carbohydrates. The mass spectra of *aldehydo*-D-arabinose tetraacetate (I, Figure 1A) and its d_{12}-analog (II, Figure 1B) prepared with acetic anhydride-d_6, *aldehydo*-D-glucose pentaacetate (III, Figure 2A), *keto*-D-fructose pentaacetate (IV, Figure 2B), *aldehydo*-6-deoxy-L-galactose tetraacetate (V, Figure 3A), and *aldehydo*-2-deoxy-D-glucose tetraacetate (VI, Figure 3B) and its d_{12}-analog (VI, Ac = $COCD_3$) have been obtained and studied in detail. The interpretation of the mass spectra in Figures 1–3 are presented here; the similarities to and differences from the mass spectra[1] of the corresponding peracetylated pyranose and furanose compounds will be emphasized.

Results and Discussion

General Features of the Mass Spectra.—The mass spectra of these acyclic peracetyl derivatives do not exhibit a molecular-ion peak, nor was such a peak found in the mass spectra[1] of the cyclic peracetyl derivatives. Fragments from ion–molecule collisions are found at M + 1 (M + 2)[2] and M + 43 (M + 46). (These peaks are shown in Figure 1 only.) The M + 1 peak arises from abstraction of a hydrogen (deuterium) radical from an acetoxyl group of a neutral molecule by the molecular ion; protonated species have been found previously in the mass spectra of ethers.[3] Abstraction of an acetyl (acetyl-d_3) radical accounts for the M + 43 peak; M + 43 peaks have been reported from the mass spectra of butyl acetate and related esters[4] and from the mass spectra of peracetyl derivatives of partially methylated pentoses and hexoses.[5]

The most intense peak in these mass spectra (Figures 1–3) is the peak at m/e = 43 (46). This peak results from carbon–oxygen bond cleavage in an acetoxyl group with charge retention on carbon: CH_3CO^+ (CD_3CO^+). When charge is retained on oxygen, an M − 43 peak is formed.

Fragments from these acyclic peracetates, as from the cyclic peracetates,[1] lose acetic acid [60 (63) mass units (m.u.), CH_3COOH (CD_3COOH)] and ketene [42 (44) m.u., $CH_2{=}C{=}O$ ($CD_2{=}C{=}O$)]. One can recognize in the mass spectra, Figures 1–3, series of fragments within which the individual peaks differ by 60 and 42 m.u. Some of these series are in common with the cyclic peracetates and others are characteristic of the *aldehydo* portion of these acyclic molecules. Many of the fragmentations proposed below are corroborated by the presence of a corresponding metastable peak. (See Table I.)

Series A, B, and C.—The mass spectrum of *aldehydo*-D-arabinose tetraacetate (I), Figure 1A, exhibits the series of fragments which has been referred to as Series A.[1] The first peak of this series is found at M − CH_3CO_2, m/e = 259 (268); loss of two molecules of acetic acid, followed by the loss of ketene leads to m/e = 139 (142) and m/e = 97 (98), respectively. Series A is one possible scheme for the formation of these fragments. Peak A1 is found 72 m.u. higher (CHOAc *vs.* H) for hexoses III and IV, at m/e = 331, and 14 m.u. higher (CH_2 *vs.* H) for deoxyhexoses V and VI, at m/e = 273.

A series of peaks is found beginning with the loss of acetic acid, instead of an acetoxyl radical as in

(1) K. Biemann, D. C. DeJongh, and H. K. Schnoes, *J. Am. Chem. Soc.*, **85**, 1763 (1963).

(2) Throughout this article, the m/e assignments are followed by parentheses containing the location of the peak in the mass spectrum of the deuterated analog, when known.

(3) F. W. McLafferty, *Anal. Chem.*, **29**, 1782 (1957).

(4) J. H. Beynon, G. R. Lester, R. A. Saunders, and A. E. Williams, *Trans. Faraday Soc.*, **57**, 1259 (1961).

(5) D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **85**, 2289 (1963).

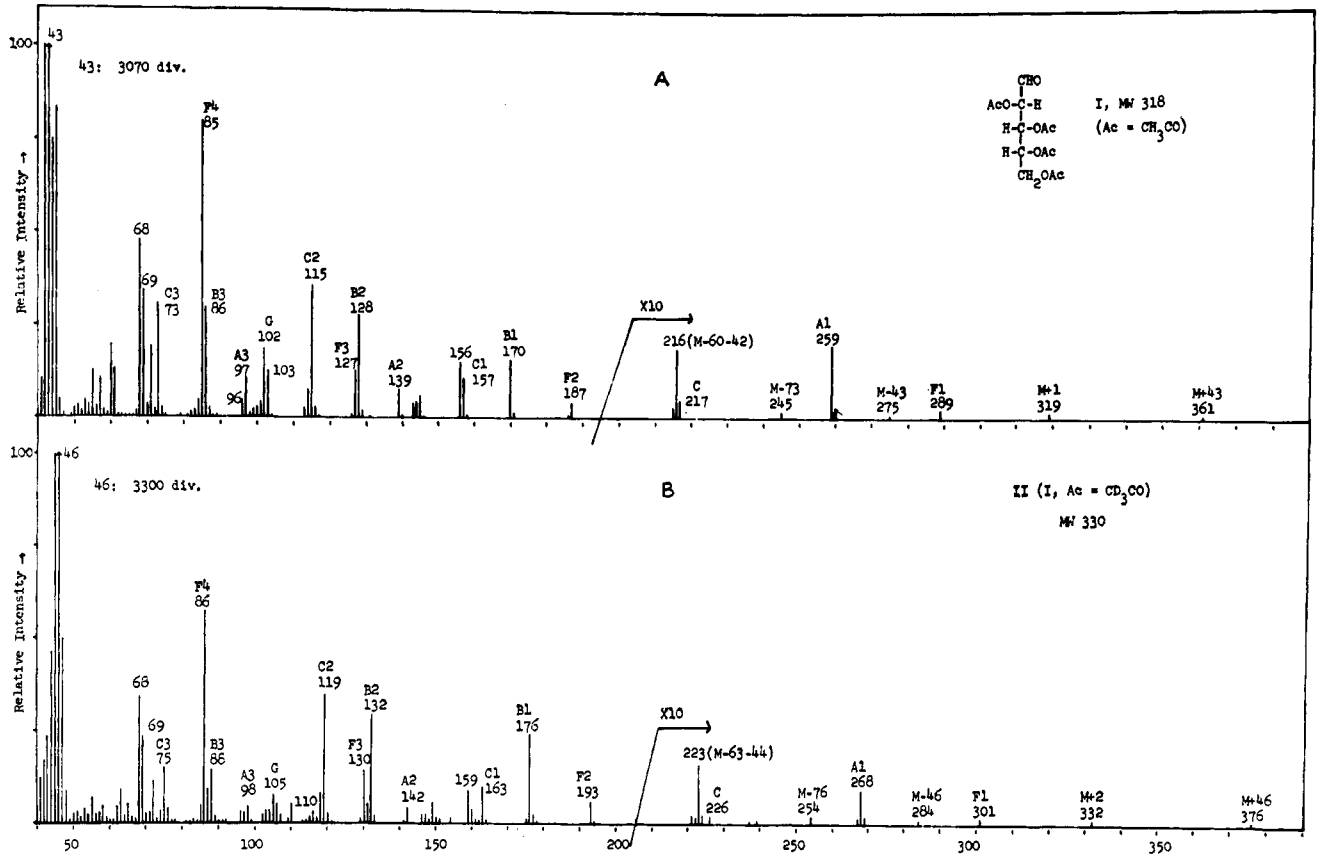


Figure 1.—(A) Mass spectrum of *aldehydo*-D-arabinose tetraacetate (I). (B) Mass spectrum of *aldehydo*-D-arabinose tetraacetate-d_{12} (II).

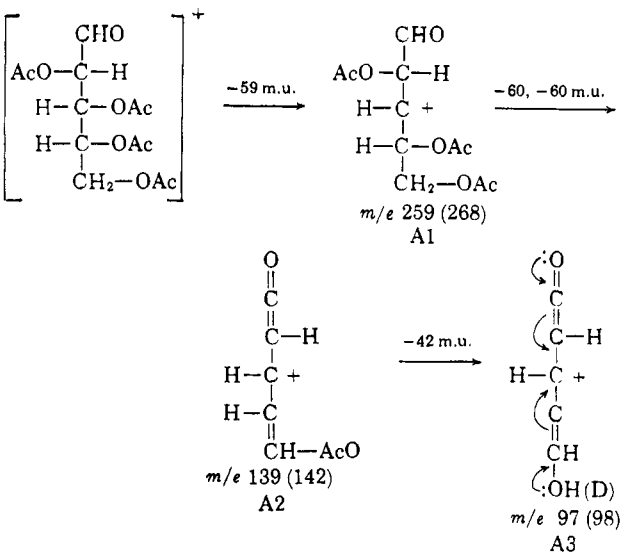
TABLE I

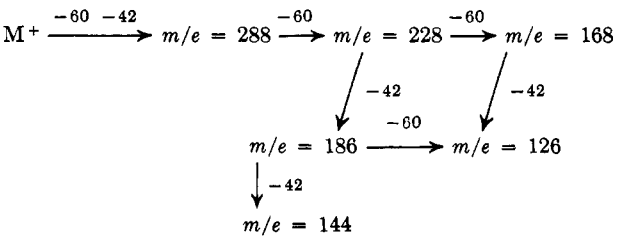
METASTABLE PEAKS IN FIGURES 1–3

	Fragmentation	Calcd.	Found
Figure 1A	115 → 73	46.3	46.4 (very weak)
	127 → 85	56.9	57.0
	145 → 85	49.8	49.9 (very weak)
	170 → 128	96.4	96.7
Figure 1B	176 → 132	99.0	99.3
	130 → 86	56.9	57.1
Figure 2A	288 → 228	180.5	180.9
	228 → 186	151.7	152.1
	228 → 168	123.8	124 (broad)
	199 → 157	123.9	
	186 → 144	111.5	111.8 (weak)
	168 → 126	94.5	94.7
Figure 2B	127 → 85	56.9	57.2
	170 → 128	96.4	96.6
	289 → 187	121.0	121.3
Figure 3A	115 → 73	46.3	46.5 (weak)
	157 → 115	84.2	84.6
	184 → 142	109.6	109.9
	303 → 201	133.3	133.8 (weak)
	332 → 273	224.5	225
Figure 3B	170 → 128	96.4	96.7
	128 → 86	57.8	57.9
Compd. VIa	176 → 132	99.0	99.2
	132 → 88	58.7	58.8

Series A, followed by losses of acetic acid and ketene. It is most prominent in the mass spectrum of *aldehydo*-D-glucose pentaacetate (III, Figure 2A). Most of these fragmentations are corroborated by metastable peaks shown in Table I. Corresponding peaks 72 m.u. lower at m/e = 216 (223), m/e = 156 (160), and m/e = 96 (97) are found in Figure 1; the facts that m/e = 156 also shifts to 159 and m/e = 96 partially stays at 96 in Figure 1B indicate that loss of $CH_3CO—O—COCH_3$, 102 (108) m.u., also occurs. Peaks at m/e = 230 and 170 in Figure 3A also are part of this series.

SERIES A





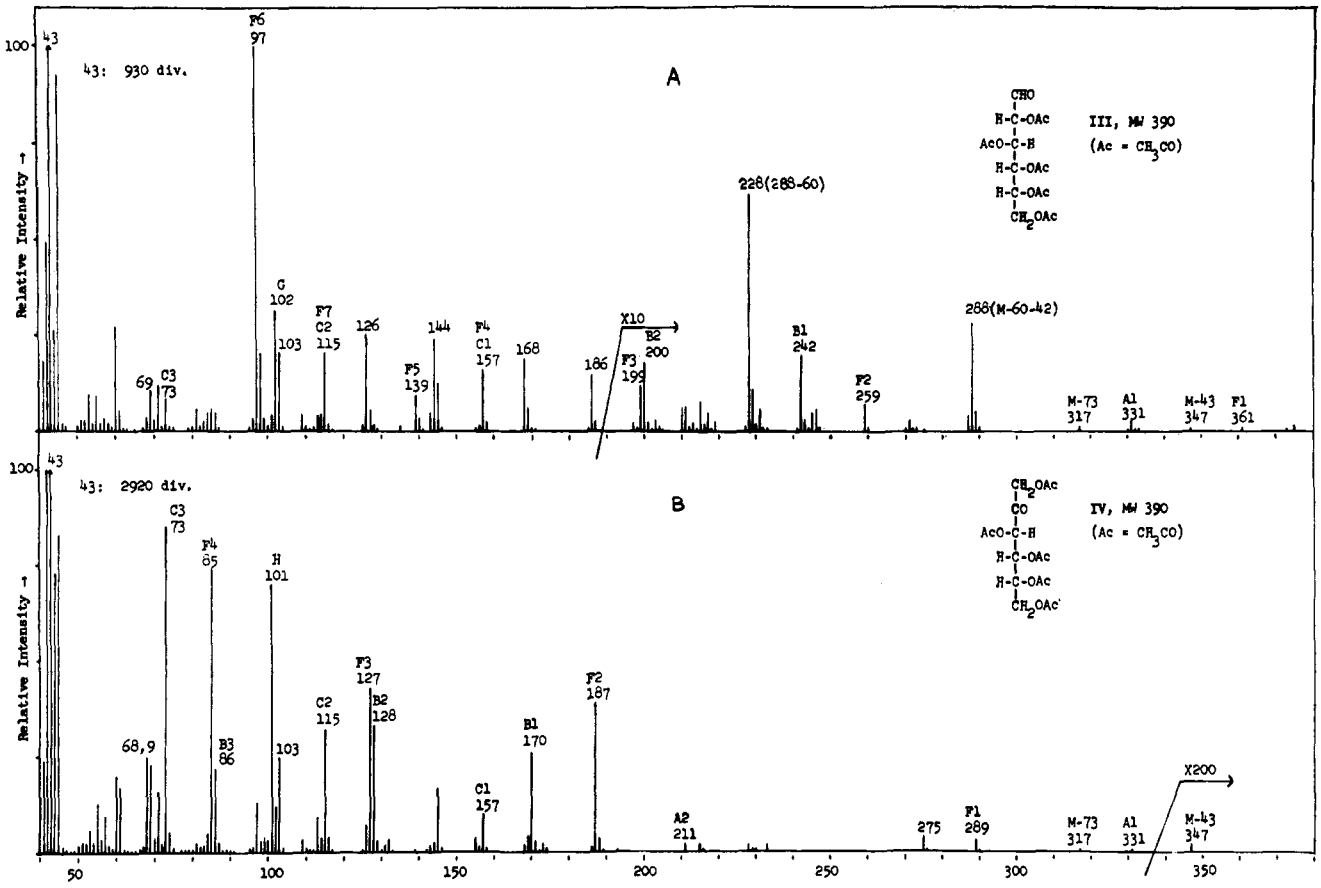
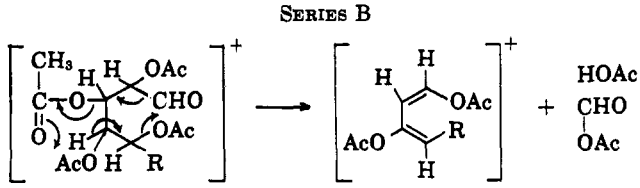
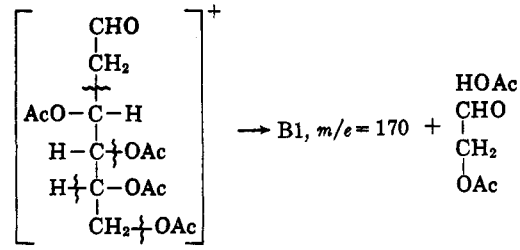


Figure 2.—(A) Mass spectrum of *aldehydo*-D-glucose pentaacetate (III). (B) Mass spectrum of *keto*-D-fructose pentaacetate (IV).

SERIES B



$m/e = 170\ (176)$
B1

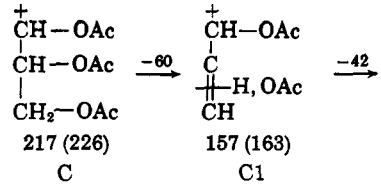


B1, $m/e = 170$ +

B1 $\xrightarrow{-42}$ $m/e = 128\ (132)$ $\xrightarrow{-42}$ $m/e = 86\ (88)$
B2 B3

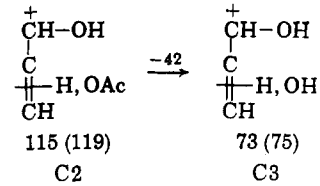
Series B is also an important fragmentation scheme of both the cyclic[1] and the acyclic peracetates of D-arabinose (R = H) with respect to mass, but not to its formation.[6] The formation of B1 is then followed by loss of two molecules of ketene, giving fragments B2 and B3. Series B is found 14 m.u. higher in Figure 3A (R = CH_3) and 72 m.u. higher in Figure 2A (R = CH_2OAc). B1 occurs at m/e = 170 in Figure 2B, the D-fructose derivative, and in Figure 3B, the 2-deoxy-D-glucose derivative. In the mass spectra of β-D-fructopyranose pentaacetate and 2-deoxy-D-glucopyranose tetraacetate, m/e = 170 is also assigned formal structure B1.[1]

The peaks of Series C in ref. 1, m/e = 157 (163), m/e = 115 (119), and m/e = 73 (75), are present in the mass spectra of the *aldehydo* derivatives also. The peak at 217 (226) in Figure 1A and B indicates that these fragments may result from the following fragmentation.

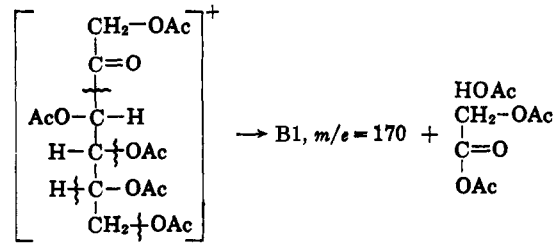


217 (226) C

157 (163) C1



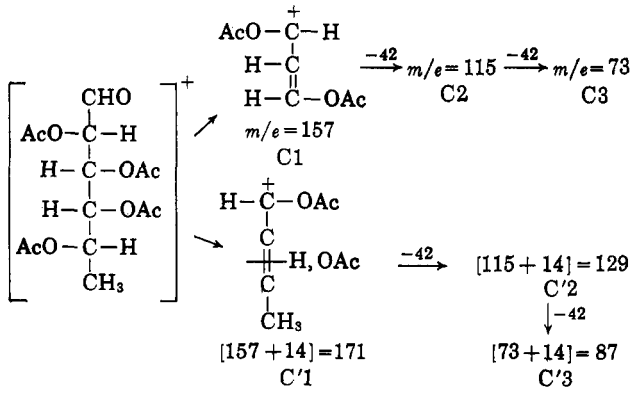
115 (119) C2 73 (75) C3



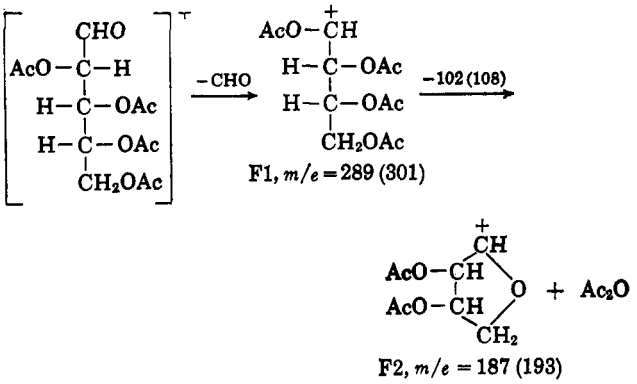
B1, $m/e = 170$ +

(6) The arrows should not necessarily imply a concerted, one-step process.

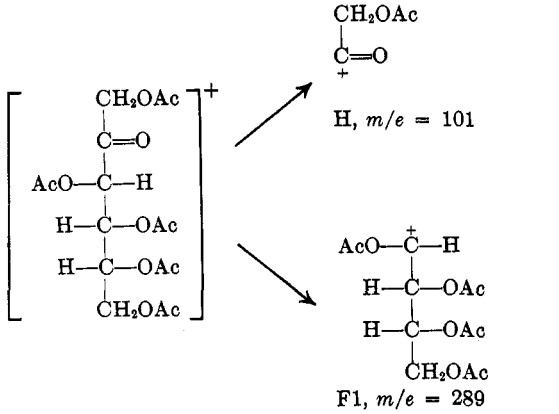
The mass spectrum of the 6-deoxyhexose, Figure 3A, indicates that Series C may be composed of three-carbon fragments both including and excluding the terminal carbon atom.



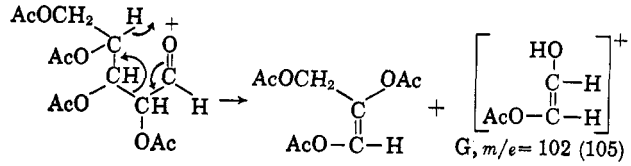
Fragmentation Involving the Carbonyl Group.—Figures 1A and B, 2A, and 3A exhibit Series F beginning at M − CHO, F1. Obviously, this series is not present in the mass spectra of the cyclic isomers[1] since the carbonyl group in the latter is in the hemiacetal form. Since F2 in Figure 1A and B is found 102 (108) m.u. below F1, acetic anhydride has been lost from F1 rather than acetic acid and ketene, 102 (107) m.u. This loss of acetic anhydride is corroborated by metastable peaks (see Table I) in the mass spectra of compounds IV and V. A mechanism for the formation of Series F from compound I follows.



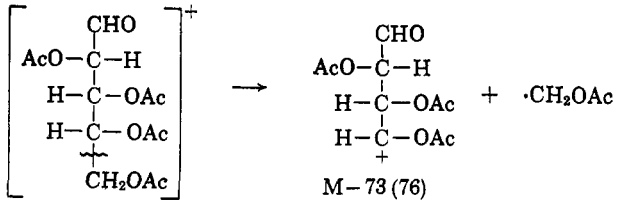
Cleavage of the C-2–C-3 bond of ketose IV, Figure 2B, yields Series F and fragment H. Fragments F2–F4 are also prominent.



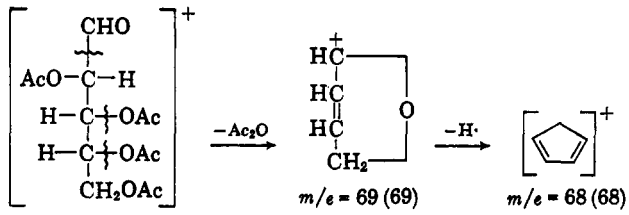
A peak at m/e = 102 (105) is prominent in Figures 1A and B, 2A, and 3A. This peak is not in the mass spectra of the cyclic isomers[1] or of 2-deoxyhexose VI and ketose IV; so it is characteristic of the *aldehydo* group with a CHOAc group at C-2. It constitutes β-cleavage with hydrogen transfer and charge retention on the enol ion.



M − 73; m/e = 68 and 69.—Expulsion of the terminal carbon atom and its substituents with charge retention on the larger fragment is a favored process for these acyclic compounds. *aldehydo*-6-Deoxy-L-galactose tetraacetate (V, Figure 3A) loses 87 m.u. because of the methyl substituent on C-5.



The peaks at m/e = 68 (68) and 69 (69) in Figure 1A and B are likely the highly stable furan and protonated furan ions. Since these peaks are shifted 14 m.u. in Figure 3A to m/e = 82 and 83, the terminal carbon atom is present. The stability of these ions could be the driving force behind their formation.[7]



General Conclusions

Now that the behavior upon electron impact of peracetyl derivatives of monosaccharides in the acyclic form as well as the pyranose and furanose ring forms has been studied, it is possible to draw general conclusions about using these mass spectra to recognize structural types. The acyclic structures can be differentiated from the cyclic structures by the presence of peaks characteristic of the carbonyl group. The mass spectra of the cyclic structures are sensitive to ring size, and furanose derivatives can be distinguished from pyranose derivatives by loss of the substituent at C-5 in the former with peaks present representing charge retention on the ring as well as on the substituent.

The mass spectra of ketoses differ from those of aldoses. In the acyclic isomers, fragmentation differences characteristic of the *aldehydo* form *vs.* the more substituted *keto* form are readily recognized. In the

(7) The zig-zag lines indicate which bonds are likely cleaved in the over-all formation of fragment 69 without implying a mechanism for the loss of the groups.

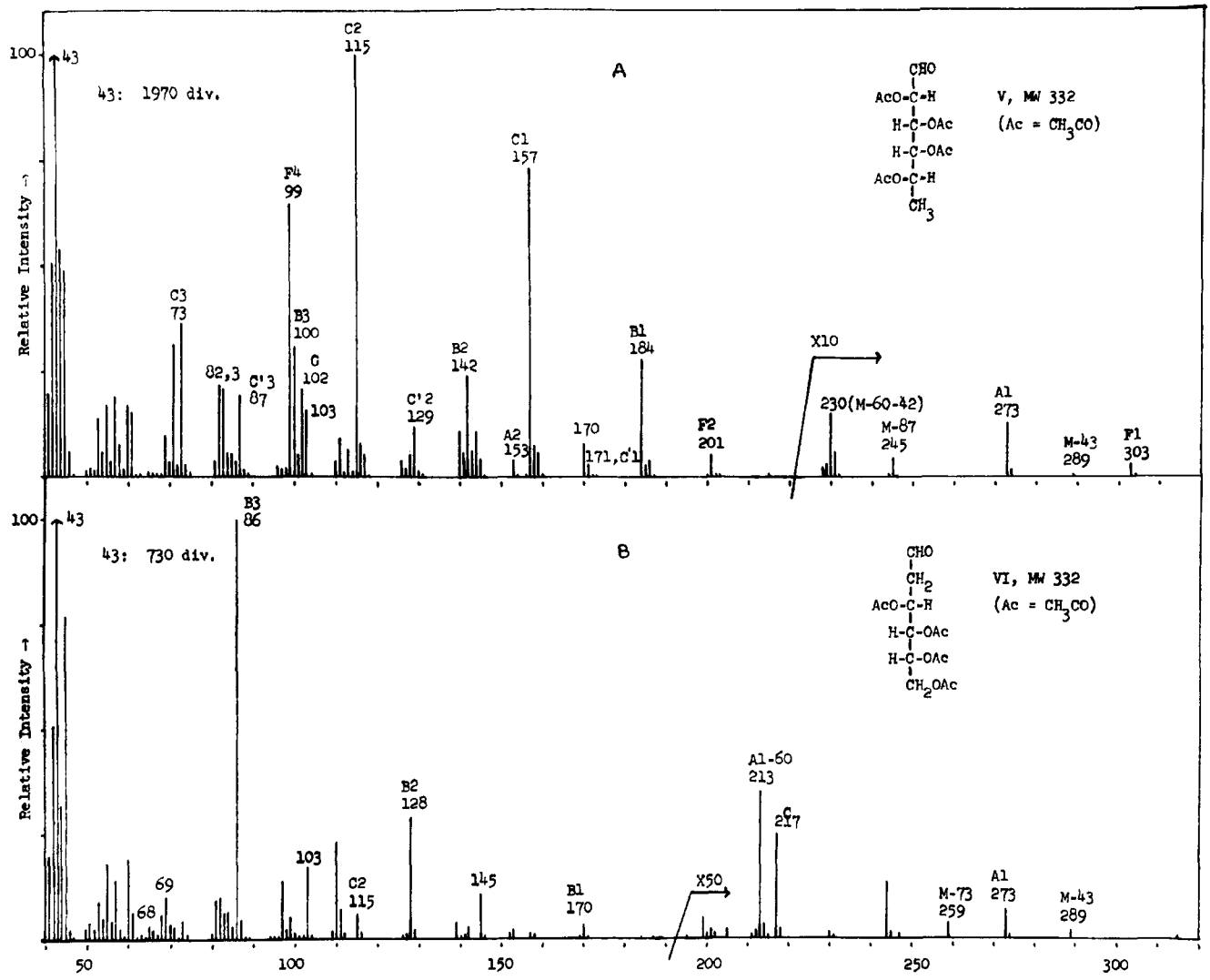


Figure 3.—(A) Mass spectrum of *aldehydo*-6-deoxy-L-galactose tetraacetate (V). (B) Mass spectrum of *aldehydo*-2-deoxy-D-glucose tetraacetate (VI).

cyclic isomers, ketoses have a ketal carbon atom more highly substituted than the acetal carbon atom of the aldoses, and this degree of substitution leads to significant variation in the corresponding mass spectra.

From the mass spectra of both the cyclic and acyclic structural types, it is possible to recognize deoxyaldoses and to distinguish, at least, between 6-deoxy and 2-deoxy isomers. The location of the deoxy-group affects various fragmentations because charge and radical nature are not so favorable on a deoxy group as on a position containing an acetoxyl group.

The mass spectra of epimeric compounds and of anomeric compounds differ in terms of intensity rather than fragmentation patterns. These intensity differences are dependent upon the conditions under which the mass spectra are determined. If mass spectra of peracetyl derivatives are to be used to recognize epimers or anomers, the spectrum of the compound in question has to be compared with the spectra of the compounds of known stereochemistry obtained under identical conditions.

Experimental

Mass Spectra.—The mass 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.; temperature of the ion source, 250°. The sample (0.5–1.0 mg.) was sublimed from a glass tube into the reservoir (3 l.).[8]

aldehydo-**Peracetates.**—The corresponding diethyl dithioacetal peracetates were demercaptalated by the procedure of M. L. Wolfrom, *et al.*[9]: *aldehydo*-D-arabinose tetraacetate (I), m.p. 111–112.5° (lit.[9] m.p. 112–114°); *aldehydo*-D-glucose pentaacetate (III), m.p. 117.5–118.5° (lit.[10] m.p. 116–118°); *aldehydo*-6-deoxy-L-galactose tetraacetate (V), m.p. 161–161.5° (lit.[11] m.p. 166–167°); and *aldehydo*-2-deoxy-D-glucose tetraacetate (VI), m.p. 100–100.5° (lit.[12] m.p. 100°).

keto-D-**Fructose Pentaacetate (IV .**—Compound IV was prepared according to the procedure of Carmer and Pacsu,[13] m.p. 69.0–69.5° (lit.[14] m.p. 69–70°).

Tetraacetates-d_{12}.—*aldehydo*-D-Arabinose tetraacetate-d_{12} (II) and *aldehydo*-2-deoxy-D-glucose tetraacetate-d_{12} were prepared by the demercaptalation[9] of D-arabinose and 2-deoxy-D-glucose diethyl dithioacetal tetraacetates-d_{12} which had been obtained by the acetylation with acetic anhydride-d_6 of the corresponding

(8) See K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 28.

(9) M. L. Wolfrom, D. I. Weisblat, W. H. Zophy, and S. W. Waisbrot, *J. Am. Chem. Soc.*, **63**, 201 (1941).

(10) M. L. Wolfrom, *ibid.*, **51**, 2188 (1929).

(11) M. L. Wolfrom and J. A. Orsino, *ibid.*, **56**, 985 (1934).

(12) J. L. Barclay, A. J. Cleaver, A. B. Foster, and W. G. Overend, *J. Chem. Soc.*, 789 (1956).

(13) F. B. Carmer and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1148 (1937).

(14) M. L. Wolfrom and A. Thompson, *ibid.*, **56**, 880 (1934).

diethyl dithioacetals: *aldehydo*-D-arabinose tetraacetate-d_{12} (II), m.p. 110.5–112°; and *aldehydo*-2-deoxy-D-glucose tetraacetate-d_{12}, m.p. 93–95.5°.

Acknowledgment.—The author would like to thank Professor K. Biemann for allowing him to use the mass spectrometer in his laboratories at the Massachusetts Institute of Technology. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

Synthesis of L-*threo*-Pentulose and 3,4,5-Tri-*O*-benzoyl-1-deoxy-L-*threo*-pentulose[1]

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Received September 24, 1964

L-*threo*-Pentulose and 3,4,5-tri-*O*-benzoyl-1-deoxy-L-*threo*-pentulose were synthesized from 3,4,5-tri-*O*-acetyl- and 3,4,5-tri-*O*-benzoyl-1-deoxy-1-diazo-L-*threo*-pentulose, respectively. The diazomethyl ketones were prepared by acetylation or benzoylation of L-threonamide, deamination to the corresponding aldonic acids, conversion to the acyl halides, and reaction with diazomethane. The acetylated diazomethyl ketone was transformed into the sirupy *keto*-acetate which on deacetylation yielded the ketopentose. The benzoylated diazomethyl ketone was reduced to the corresponding 1-deoxy derivative. Although the aldonamides of higher carbon content mutarotate in aqueous solution, the amides of the aldotetronic acids do not show this property.

In continuation of our work on the general method for the preparation of ketoses from the acetylated sugar acids with one less carbon atom,[3] we report herein the application of this method to the synthesis of L-*threo*-pentulose (VII, "L-xylulose"); we also report the synthesis of 1-deoxy-L-*threo*-pentulose as the crystalline tribenzoate (XI).

L-*threo*-Pentulose has been isolated from the urine of humans with essential pentosuria,[4–8] the inborn error of metabolism discovered by Salkowski and Jastrowitz[9] in 1892. The definitive characterization of the urinary sugar was made by Levene and LaForge[4] in 1914. The role of this ketopentose in pentose metabolism and pentosuria has recently been reviewed.[10] L-*threo*-Pentulose was first synthesized by boiling L-xylose with pyridine, removing unchanged L-xylose by crystallization, and isolating the ketose as the *p*-bromophenylhydrazone.[11]

The starting point for our synthesis was L-threono-1,4-lactone prepared by the oxidative scission of L-ascorbic acid with *p*-toluenediazonium hydrogen sulfate.[12] The lactone was converted to L-threonamide[13] (I) with liquid ammonia.[14]

In contrast to other aldonamides,[15] L-threonamide was found by us to be hydrolytically stable at room temperature. No significant change in rotation occurred during 95 hr. and the amide was recovered unchanged after this period, as shown by melting point, infrared spectrum, and X-ray powder diffraction pattern. Crum[16] observed a similar hydrolytic behavior for D-erythronamide. This lack of mutarotation for the four-carbon aldonamides may be due to the nonformation of the postulated[15] glycosylamine intermediate, which in this case would be a furanosylamine.

Acetylation of L-threonamide with acetic anhydride and zinc chloride, conditions reported to produce *O*-acetylated aldonamides,[17] gave the fully acetylated amide, *N*-acetyl-2,3,4-tri-*O*-acetyl-L-threonamide (III), rather than the *O*-acetylated product. The fully acetylated amide was also prepared by acetylation of L-threonamide with the acetic anhydride–concentrated sulfuric acid procedure used to prepare other fully acetylated aldonamides.[17] Acetylation of L-threonamide in anhydrous pyridine with acetic anhydride gave only *O*-acetylation and produced 2,3,4-tri-*O*-acetyl-L-threonamide (II) in high yield.

Benzoylation of L-threonamide with benzoyl chloride in anhydrous pyridine, as described by Lake and Glattfeld[18] for benzoylation of DL-threonamide, gave 2,3,4-tri-*O*-benzoyl-L-threonamide (VIII). In one large run under the same (presumably) conditions, the product obtained was 2,3,4-tri-*O*-benzoyl-L-threononitrile (IV), the structure of which was proved unequivocally by its conversion to and synthesis from 2,3,4-tri-*O*-benzoyl-L-threonamide. In other runs under apparently the same conditions, only the benzoylated amide was obtained. It is interesting that the infrared absorption spectrum of 2,3,4-tri-*O*-benzoyl-L-threononitrile shows no nitrile absorption at 4.5 μ, apparently because of the "quenching" effect of oxygen in the molecule.[19]

The *O*-acetylated and the benzoylated amides were converted with dinitrogen trioxide ("nitrous anhy-

(1) Paper No. 21 in the series entitled "The Action of Diazomethane upon Acyclic Sugar Derivatives"; previous communication, M. L. Wolfrom, J. C. Crum, J. B. Miller, and D. I. Weisblat, *J. Am. Chem. Soc.*, **81**, 243 (1959).

(2) Charles F. Kettering Foundation Fellow, 1959–1960, 1961; National Science Foundation Summer Fellow, 1961; Goodyear Foundation Fellow, 1963–1964; Du Pont Grant-in-Aid Recipient, 1964.

(3) M. L. Wolfrom, R. L. Brown, and E. F. Evans, *J. Am. Chem. Soc.*, **65**, 1021 (1943), and other papers of the series (see ref. 1).

(4) P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, **18**, 319 (1914).

(5) A. Hiller, *ibid.*, **30**, 129 (1917).

(6) I. Greenwald, *ibid.*, **88**, 1 (1930).

(7) P. Bálint, *Biochem. Z.*, **274**, 305 (1934).

(8) O. Touster, R. H. Mayberry, and D. B. McCormick, *Biochim. Biophys. Acta*, **25**, 196 (1957).

(9) E. Salkowski and M. Jastrowitz, *Centr. med. Wiss.*, **30**, 337 (1892.)

(10) O. Touster, *Am. J. Med.*, **26**, 724 (1959).

(11) L. von Vargha, *Ber.*, **68**, 18 (1935).

(12) R. Weidenhagen, H. Wegner, K. H. Lung, and L. Nordström, *ibid.*, **72**, 2010 (1939).

(13) K. Gätzi and T. Reichstein, *Helv. Chim. Acta*, **20**, 1298 (1937).

(14) J. W. E. Glattfeld and D. Macmillan, *J. Am. Chem. Soc.*, **56** 2481 (1934).

(15) M. L. Wolfrom, R. B. Bennett, and J. D. Crum, *ibid.*, **80**, 944 (1958).

(16) J. D. Crum, Ph.D. Thesis (The Ohio State University); University Microfilms, Ann Arbor, Mich., L. C. Card No. Mic. 58-3415; *Dissertation Abstr.*, **19**, 670 (1958).

(17) G. B. Robbins and F. W. Upson, *J. Am. Chem. Soc.*, **60**, 1788 (1938).

(18) W. W. Lake and J. W. E. Glattfeld, *ibid.*, **66**, 1091 (1944).

(19) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd. Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 265–266.