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Note

Identification of acetates of monosaccharide hydrazones by mass spectrometry*

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Hydrazones are useful derivatives for the identification and isolation of reducing carbohydrates, mainly those crystallizing only with difficulty or that have not been crystallized (such as the tetroses and the 2- and 3-pentuloses). Hydrazones and their acetates are of special importance because of their potential antitumor activity[1–3].

Attempts to use mass spectrometry for the identification of the condensation products of saccharides with substituted hydrazones without derivatization were unsuccessful. Although some short-chain molecules gave reproducible spectra, hydrazones of pentoses and hexoses were thermally destroyed under the conditions of the measurements. A solution to this problem was found in derivatization of the compounds, and we now describe the mass-spectrometric fragmentation of peracetates of substituted hydrazones of some typical monosaccharides, including 2- and 3-ketoses and glycolaldehyde. The following compounds were studied: glycolaldehyde (2,4-dinitrophenyl)hydrazone acetate (**1**), 2,3-di-*O*-acetyl-DL-glyceraldehyde (2,4-dinitrophenyl)hydrazone (**2**), 2,3,4-tri-*O*-acetyl-*aldehydo*-D-erythrose (4-nitrophenyl)-hydrazone (**3**), 2,3,4,5-tetra-*O*-acetyl-*aldehydo*-D-arabinose (2,4-dinitrophenyl)hydrazone (**4**), 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-galactose (4-nitrophenyl)hydrazone (**5**), 1,3-diacetoxy-2-propanone (2,4-dinitrophenyl)hydrazone (**6**), 1,3,4-tri-*O*-acetyl-*keto*-L-*glycero*-tetrulose (2-nitrophenyl)hydrazone (**7**), 1,3,4,5-tetra-*O*-acetyl-*keto*-D-*erythro*-pentulose (2-nitrophenyl)hydrazone (**8**), 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose (2,4-dinitrophenyl)hydrazone (**9**), and 1,2,4,5-tetra-*O*-acetyl-*keto*-*erythro*-3-pentulose (2,5-dichlorophenyl)hydrazone (**10**).

The stability of the molecular ions of ketoses is greater than that of aldoses, and decreases with increase in the number of carbon atoms in the sugar chain. This is clearly demonstrated in Table I, which shows, for example, the presence of an intense peak of the molecular ion in the spectra of hydrazones **8** and **10** derived from a 2- and a 3-pentulose, whereas the M^+ peak could not be found in the spectrum of the aldose derivative **4**.

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*Hydrazones and their derivatives. Part I.

TABLE I

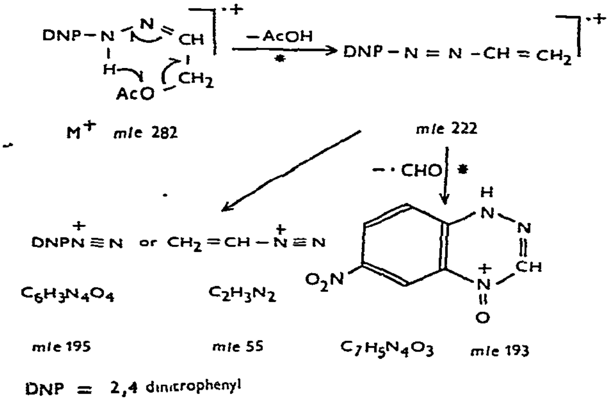
IONS CHARACTERISTIC OF THE FRAGMENTATION OF THE CARBOHYDRATE PART OF A MOLECULE OF A HYDRAZONE ACETATE

Ions	*Aldoses*					*2-Ketoses*				*3-Ketose*
	1	2	3	4	5	6	7	8	9	10
	2[a]	*3*	*4*	*5*	*6*	*3*	*4*	*5*	*6*	*5*
M^+	+	+	+			+	+	+		+
$[M-60]^+$	+	+	+	+	+	+	+	+	+	+
$[M-60-42]^+$		+	+	+	+	+	+	+	+	+
$[M-2\times60]^+$		+	+	+	+	+	+	+	+	+
$[M-2\times60-42]^+$			+	+	+		+	+	+	+
$[M-3\times60]^+$				+	+		+	+	+	+
$[M-2\times60-2\times42]^+$				+	+			+	+	+
$[M-3\times60-42]^+$				+	+			+	+	+
$[M-4\times60]^+$					+				+	
$[M-3\times60-2\times42]^+$					+				+	
$[M-4\times60-42]^+$					+				+	
$[M-60-73]^+$			+					+		
$[M-60-73-42]^+$			+					+		
$[M-2\times60-73]^+$								+		
$[M-2\times60-73-42]^+$								+		
$[M-60-145]^+$				+					+	
$[M-60-145-42]^+$				+					+	
$[M-2\times60-145]^+$									+	
$[M-2\times60-145-42]^+$									+	
$[M-60-217]^+$					+					
$[M-60-217-42]^+$					+					

[a] Number of carbon atoms in the chain.

The disintegration of the molecular ions occurs preferentially in the carbohydrate part; it commences with splitting off of the *O*-acetyl groups, either in the form of an ·OAc radical or in that of a molecule of acetic acid, giving rise to a pair of peaks of the ions $[M-59]^+$ and $[M-60]^+$. Thus, the molecular weight of compounds of this class, the spectra of which do not contain the peak of the molecular ions, can be calculated indirectly by adding the values 59 and 60 to those of $[M-59]^+$ and $[M-60]^+$, respectively. The splitting off of the *O*-acetyl group results in a series of consecutive eliminations of the remaining *O*-acetyl groups. In Table I, the fragments thus formed are noted. Their formation is characterized by 1,2-elimination of acetic acid, and of ketene from the *O*-acetyl group attached to the double-bonded carbon atom[4,5]. The ions $[M-59]^+$ disintegrate in an analogous way, giving peaks having an *m/e* value one unit higher. The number of eliminations can be clearly seen in the spectra, as most of them are accompanied by metastable transitions. In this way, the number of *O*-acetyl groups present in the molecule can conveniently be determined. For instance, the presence of the peak for the ions $[M-(3\times60)-(2\times42)]^+$ confirms the presence of five *O*-acetyl groups in a molecule of an acetylated hydrazone. These eliminations follow the same course, regardless of whether the monosaccharide is an aldose or a ketose.

Interpretation of the spectra of *N*-deuterio analogs of **1** and **6** showed that, in the first phase of the framentation, a 1,4-elimination of acetic acid with the participation of the amine hydrogen atom takes place. Scheme 1 shows this elimination occurring in the molecule of glycolaldehyde (2,4-dinitrophenyl)hydrazone acetate (**1**). The disintegration that takes place subsequently is atypical, owing to the lack of any other possibilities. The peak at *m/e* 55 is the secondmost intense peak (after the one at *m/e* 43 of the ions $CH_3{-}C{\equiv}O^+$). Among the less intense peaks in the spectrum of **1** are those of the ions $[M-59]^+$, $[C_8H_7N_3O_2]^+$, and $[C_5H_3NO]^+$, at *m/e* 223, 177, and 93, respectively.

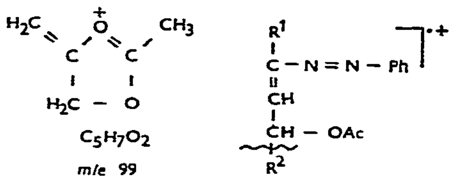


Scheme 1.

In addition to the elimination of a molecule of acetic acid and one of ketene, a pathway characteristic of the disintegration of 1,3-diacetoxy-2-propanone (2,4-dinitrophenyl)hydrazone (**6**) is the rupture of $[M-60]^+$ ions to give peaks at *m/e* 99 ($[C_5H_7O_2]^+$) (see Scheme 2). The stability of these ions, the secondmost intense ions in the spectrum of this compound, may lie in the possibility of cyclization; ketene is not split off from these ions.

Ions of the same type are not formed in the fragmentation of 2,3-di-*O*-acetyl-DL-glyceraldehyde (2,4-dinitrophenyl)hydrazone (**2**). The excess of energy of the ions $[M-60]^+$ is released, after elimination of a molecule of acetic acid, by loss of a molecule of NO, to give rise to the ions $[C_9H_6N_3O_3]^+$ at *m/e* 204 ($m^* = 177.8$ for $234 \rightarrow 204$), followed by cleavage of an $\cdot NO_2$ radical; in this way, the ions $[C_9H_6N_2O]^+$ at *m/e* 158 ($m^* = 122.4$ for $204 \rightarrow 158$) are formed. Among the less intense peaks are those of the ions $[C_7H_5N_2O_3]^+$, $[C_7H_7N_2O_4]^+$, and $[C_9H_5N_4O_3]^+$ at *m/e* 165, 183, and 217, respectively.

The ions $[M-60]^+$ formed by 1,4-elimination from peracetates of substituted hydrazone peracetates of aldotetroses, aldopentoses, aldohexoses, 2-pentuloses, and



Compound	R^1	R^2
3	H	CH_2OAc
4	H	$CHOAcCH_2OAc$
5	H	$(CHOAc)_2CH_2OAc$
8	CH_2OAc	CH_2OAc
9	CH_2OAc	$CHOAcCH_2OAc$

Scheme 2.

2-hexuloses are disintegrated through a series of new, diagnostically important pathways. In the case of aldoses, the cleavage occurs at the C-3–C-4 linkage, and, for 2-pentuloses and 2-hexuloses, at the C-4–C-5 linkage (see Scheme 2), to give the ions listed in Table I. The ions $[M-60]^+$ formed from the aldotetrose hydrazone acetates lose a radical of mass 73, but this cleavage does not occur with 2-tetruloses. For aldopentoses, 2-pentuloses, aldohexoses, and 2-hexuloses, the leaving radical has a mass of 145, 73, 217, and 145, from which it follows that, for 3-hexuloses, it should have a mass of 73. Thus, the location of the hydrazone residue, and, consequently, that of the carbonyl group in any monosaccharide studied as a hydrazone, can be determined. The ions $[M-60-\cdot R^2]^+$ subsequently eliminate a molecule of ketene or an $\cdot NO_2$ radical from the nitrophenyl group. For ketoses, an additional elimination of the acetyl group from O-1 takes place. All pathways discussed are well pronounced, and are accompanied by metastable transitions.

The mode of fragmentation described for compounds **3**, **4**, **5**, **8**, and **9** unambiguously confirms that the sugar residue of these compounds exists in the acyclic form. Another proof of their open-chain structure lies in the fact that the ions $[M-42]^+$, which are preferentially formed in the fragmentation of *N*-arylamide acetates[6], were not found in the spectra of these compounds. For **4** and **5**, the facts described are in agreement with chemical proofs[7,8] of the acyclic nature of the sugar moiety of these compounds.

Interpretation of the spectrum of 2,3,4-tri-*O*-acetyl-*aldehydo*-D-threose (2,5-dichlorophenyl)hydrazone, and comparison of its fragmentation with that of **3**, revealed that substitution on the benzene ring does not alter the mode of fragmentation of this class of compounds, for which the criteria given in Table I are valid.

EXPERIMENTAL

The mass spectra were obtained at 70 eV with an MCh 1306 spectrometer. Depending upon the volatility of the compounds, the temperature at the site of evaporation was 40–70°, and that in the ionizing chamber was 120–130°. Exact mass measurements were made by using an MS 902-S instrument working with a resolution power of 20,000.

Acetylated hydrazones were prepared as described in the literature, and showed the following physical constants: **1**, m.p. 152–153° (lit.[9] m.p. 152°); **2**, m.p. 134–136° (lit.[10] m.p. 125°); **3**, m.p. 140–142° (ref. 2); **4**, m.p. 156–157° (lit.[7] 153–154°); **5**, m.p. 193–194° (lit.[8] m.p. 194–195°); **7**, syrup, $[\alpha]_D^{20}$ $-104°$ (c 0.5, ethyl acetate; ref. 3); **8**, m.p. 67–69° (ref. 3); **9**, m.p. 110–111° (ref. 2); and **10**, m.p. 97–99° (ref. 3).

Compound **6**, known only as a syrup[10], was obtained crystalline in the following way. To a mixture of acetic anhydride (3.8 ml) and dry pyridine (7.6 ml) was added 1,3-dihydroxy-2-propanone (2,4-dinitrophenyl)hydrazone (3 g) at 0°, and the mixture was kept for 2 h at 0° with occasional shaking. After an additional 16 h at 3°, the solution was evaporated under diminished pressure at 30°, and ethanol (70 ml) was added to the syrupy residue. The product **6** (3.6 g, 91.5%) crystallized slowly on scratching with a glass rod; after two recrystallizations from the same solvent, it melted at 86–88°.

Anal. Calc. for $C_{13}H_{14}N_4O_8$: C, 44.07; H, 3.98; N, 15.82. Found: C, 44.08; H, 4.15; N, 16.03.

N-Deuteration of compounds **1** and **6** was accomplished by three evaporations of a suspension of the compound in CH_3OD (99.9% D). For each, the degree of deuteration was 55%.

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