Metastable Ion Kinetic Energy and Collision-Induced Dissociation Study of Per-*O*-acetylated D-Trehaloses under the Conditions of Electron Impact and Chemical Ionization Mass Spectrometry

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Selected ions obtained by electron impact (at 70 and 12 eV energy) and chemical ionization (isobutane, ammonia, and methylamine) of per-*O*-acetylated D-trehaloses have been examined by metastable ion kinetic energy (MIKE) and collision-induced dissociation (CID) mass spectrometry. The predominant process of the fragmentation of molecular and quasimolecular ions is the cleavage of glycosidic linkages. The MIKE and CID spectra of the ions produced, as well as the spectra of the $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ cluster ions prove that α- and/or β-configuration of D-trehaloses does not have any influence on the fragmentation pathways.

Mass spectrometric methods have been applied successfully in the structure characterization of reducing oligosaccharides [1]. However, the nonreducing oligosaccharides have been less studied by these methods. We observed earlier that the conventional electron impact (EI) spectra reflect the deep destruction of per-*O*-methylated 1→1 linked disaccharides [2, 3]. The fragmentation routes differ substantially from those observed with the other types of interglycosidic linkages. To assist in explanation of the mass spectral behaviour of derivatives of nonreducing oligosaccharides, we decided to study the following per-*O*-acetylated D-trehaloses: α-D-glucopyranosyl-α-D-glucopyranose (*I*), α-D-glucopyranosyl-β-D-glucopyranose (*II*), and β-D-glucopyranosyl-β-D-glucopyranose (*III*).

Of the mass spectral methods the EI (at 70 and 12 eV energy) and chemical ionization (CI; isobutane, ammonia, and methylamine) modes have been chosen. The selected characteristic ions were measured by an instrument with reversed geometry using MIKE and CID methods.

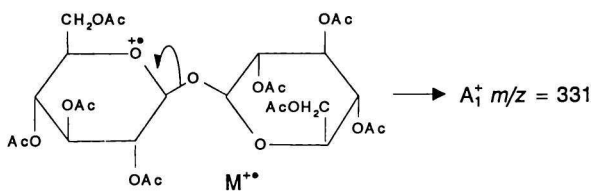
EXPERIMENTAL

The D-trehaloses *I*—*III* have been prepared according to the literature [4—6]: The α,α-D-trehalose (*I*), m.p. = 99—101 °C, $[\alpha]$(D, 20 °C, $CHCl_3$) = + 161.8°, Ref. [4] gives m.p. = 100 °C, $[\alpha]$(D, 20 °C, $CHCl_3$) = + 161.8°; α,β-D-trehalose (*II*), m.p. = 140—141 °C, $[\alpha]$(D, 20 °C, $CHCl_3$) = + 86.0°, Ref. [5] gives m.p. = 144—144.5 °C, $[\alpha]$ (D, 20 °C, $CHCl_3$) = + 83.2°; β,β-D-trehalose (*III*), m.p. = 181—182 °C, $[\alpha]$(D, 20 °C, $CHCl_3$) = − 16.6°, Ref. [6] gives m.p. = 181—182.5 °C, $[\alpha]$(D, 20 °C, $CHCl_3$) = − 16.8°.

The EI ions were produced in the ion source of a VG ZAB-2F mass spectrometer, using the direct inlet system, 70 or 12 eV energy and an ionization chamber temperature of approx. 180 °C. When measuring the CI spectra, isobutane, ammonia or methylamine were introduced into the CI ion source of a VG ZAB-2F instrument by the CI gas line, until the pressure reading at the ion gauge at the ion source housing was 10^{-4}—10^{-3} Pa. The ions under study were focussed magnetically into the second field free region (2nd FFR) of the instrument after the magnet sector. The MIKE spectra were recorded by scanning the deflection voltage of the electrostatic analyzer. The CID spectra were obtained by the same manner like MIKE, while helium was introduced as the collision reaction gas into the collision chamber of the 2nd FFR of the instrument (He collision gas pressure adjusted approx. to 50 % reduction). For better comparison the MIKE and CID spectra (Tables 1—4) have been normalized by the calculation of the percentage of the sum of intensities of the fragments.

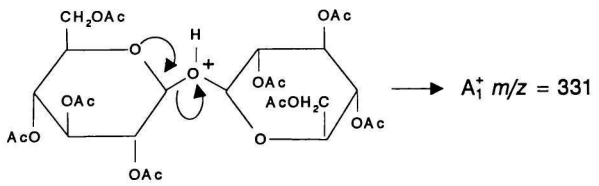
RESULTS AND DISCUSSION

The predominant process of the fragmentation of molecular and quasimolecular ions is the cleavage of glycosidic linkages giving rise to A_1 carbenium ions with m/z = 331. In the case of EI ionization the glycosidic linkage splits off homogeneously, *e.g.*



Scheme 1

The ionization of the hemiacetal oxygen atom of the "right" ring gives rise to the identical A_1 ions. The quasimolecular $[M + H]^+$ ions in the CI (isobutane) mass spectra fragment spontaneously under the production of identical A_1 ions



Scheme 2

Due to high values of proton affinities of ammonia and methylamine [7], stable $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ cluster ions are produced. The predominant way of fragmentation of cluster ions under the MIKE and CID conditions is again the production of A_1 ions

$$[M + NH_4]^+ \rightarrow A_1^+ + AcGlcp + NH_3$$
$$[M + CH_3NH_3]^+ \rightarrow A_1^+ + AcGlcp + CH_3NH_2$$

AcGlcp — 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose.

The A_1 fragments produce the secondary or deeper destruction fragments after the elimination of AcOH and CH_2CO molecules. The degree of destruction depends on the conditions of the mass spectrometric experiments. In the MIKE experiments (Table 1), the A_1 ions obtained by EI and CI (isobutane) produce the A_2 species at m/z = 271 ($A_1 - 60$), A_3 at m/z = 211 ($A_2 - 60$), and A_4 at m/z = 169 ($A_3 - 42$). The elimination of *O*-acetyl groups in the form of acetic acid or ketene molecules is less pronounced in the case of isobutane "soft" ionization, compared with the EI methods.

Table 1. MIKE Spectra of A_1 Ions (m/z = 331) of *I—III*

m/z	ΣI/% EI (70 eV)			EI (12 eV)			CI (isobutane)		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
271	60	64	60	70	59	63	91	91	93
211	10	11	9	11	9	11	2	2	2
169	30	25	31	19	32	26	7	7	5

The decomposition of A_1 ions under the CID conditions proceeds in several ways, *e.g.* A_3 ions are represented by the $[A_1 - AcOH - CH_2CO]^+$ (m/z = 229) and $[A_1 - 2\ AcOH]^+$ (m/z = 211) ion species (Table 2).

The MIKE spectra of $[M + NH_4]^+$ or $[M + CH_3NH_3]^+$ adduct ions contain the only fragment peak of the A_1 species at m/z = 331. This fact gives evidence about the very "soft" conditions during ammonia and methylamine ionizations of the molecules studied.

The CID spectra of the $[M + NH_4]^+$ ions at m/z = 696 of the per-*O*-acetylated D-trehaloses *I—III* reflect the production of A-type ions (Table 3) and the CH_3CO^+ species at m/z = 43.

Table 2. CID Spectra of A_1 Ions (m/z = 331) of *I—III*

m/z	ΣI/% EI (70 eV)			EI (12 eV)			CI (isobutane)		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
271	28	28	27	23	33	37	13	12	19
229	1	1	1	2	1	1	1	1	1
211	5	5	5	5	6	9	2	2	2
169	49	48	51	48	45	40	53	52	53
127	5	5	6	6	4	5	8	8	6
109	12	13	9	16	11	9	23	25	19

The extraordinary stability of $[M + CH_3NH_3]^+$ adducts at m/z = 710 is reflected also in the presence of $[M + CH_3NH_3 - AcOH]^+$, $[M + CH_3NH_3 - AcOH - {}^{\bullet}OAc]^+$ and $[M + CH_3NH_3 - AcOH - {}^{\bullet}CH_2OAc]^+$ species at m/z = 650, 591, and 577 (Table 4). The splitting of the glycosidic linkages gives rise to a series of A-type ions at m/z = 331 (A_1), 271 ($A_2 = A_1 - 60$), 229 ($A_3 = A_2 - 42$), 211 ($A_3' = A_2 - 60$), 169 ($A_4 = A_3 - 60$), 127 ($A_5 = A_4 - 42$), and 109 ($A_5' = A_4 - 60$). In addition, the CH_3CO^+ and $CH_3NH_3^+$ ions at m/z = 43 and 32 are well pronounced.

Table 3. CID Spectra of $[M + NH_4]^+$ Ions (m/z = 696) of *I—III*

m/z	ΣI/% *I*	*II*	*III*
331	26	30	20
271	3	3	3
229	1	1	2
211	4	3	4
169	45	43	40
109	15	13	24
43	6	7	6

Thus, the MIKE and CID spectra of the $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ cluster ions bring the valuable information about the molecular mass and type of linkages of 1→1 linked oligosaccharides, but unfortunately do not reflect different anomeric configuration of the isomers.

Table 4. CID Spectra of $[M + CH_3NH_3]^+$ Ions (m/z = 710) of *I—III*

m/z	ΣI/% *I*	*II*	*III*
650	2	3	3
591	2	3	4
577	1	2	2
331	13	10	8
271	2	2	3
229	2	1	2
211	2	1	2
169	21	20	25
127	3	2	3
109	13	9	8
43	9	12	8
32	30	35	32

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A Reinvestigation of the Ruff Degradation of a C-2 Branched Chain Saccharinic Acid

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The treatment of the calcium salt of D-isosaccharinic acid with hydrogen peroxide in the presence of ferric ions gave rise to 3-deoxy-D-*glycero*-2-pentulose, the structure of which was proved by both ^{1}H and ^{13}C NMR spectroscopy. The results are in an agreement with those obtained ninety years ago by the author of the method of shortening the carbon chain of aldoses, and offer another proof, that its intermediate is not a 2-ketoaldonic acid.

Saccharinic acids are a significant part of black liquors derived from alkaline pulping processes. From among three principal types of the saccharinic acids, α- and β-D-isosaccharinic (3-deoxy-2-*C*-hydroxymethyl-D-*erythro*- and -D-*threo*-pentonic) acids prevail in the liquors. Their formation is facilitated by the 4-*O*-substitution of the parent sugar.

Several bacterial strains [1—3] have been found to be capable of utilizing saccharinic acids including D-isosaccharinic one. Chemical ways of utilization of the acids are described in a review [4]. From among them, only the preparation of 2-deoxy-D-*erythro*-pentose from α- and β-D-glucometasaccharinic (3-deoxy-D-*ribo*- and -D-*arabino*-hexonic) acids *via* the Ruff degradation is of a practical significance [5]. The observation that also D-isosaccharinic acid undergoes the degradation was published by *Ruff* himself ninety years ago [6]. The characterization of the degradation product, however, was not sufficient; in addition to its correctly assumed structure, only the stoichiometric formula of its osazone was determined. Later, all this was like forgotten and, for a long time, one had been supposing that the decarboxylation of a 2-ketoaldonic acid was an intermediate step of the Ruff degradation [7, 8]. In 1981, *Isbell* and *Salam* [9] refuted the assumption by the preparation of D-[1-^{2}H]arabinose *via* the Ruff degradation of calcium D-[2-^{2}H]gluconate. All the data evoked our interest to reinvestigate the Ruff degradation of D-isosaccharinic acid in order to learn whether it could yield an interesting deoxyketose.

Calcium D-isosaccharinate was submitted to a modified procedure of the Ruff degradation of aldonic acids by treatment with hydrogen peroxide and ferric acetate. The modification included a change of two factors. Because of a low solubility of the starting material in water, the reaction was performed in a more diluted solution. Moreover, it was necessary to initiate the degradation by heating the reaction mixture to 50—60 °C.

The first purification of a raw product of the degradation on a cellulose column afforded a 29 % yield of a partially purified 3-deoxy-D-*glycero*-2-pentulose still having contained small admixtures of two other reducing sugars. Chromatographically pure compound was then obtained after a purification by preparative paper chromatography in an overall 8.5 % yield. We have not succeeded to isolate two accompanying saccharides as pure compounds because of about 40 % recovery of both purification steps.