Sequential Analysis of Oligosaccharides by Direct Chemical Ionization Mass Spectrometry

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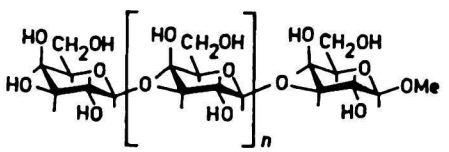
Homo- and heterooligosaccharides, composed of hexopyranose, deoxyhexopyranose, and acetamidohexose units, have been examined by direct chemical ionization (DCI) mass spectrometry. Their DCI scan, using ammonia as the reaction gas, gives rise to structurally significant ions. The information thus obtained aids significantly in the sequential analysis of the di- and trisaccharides without derivatization.

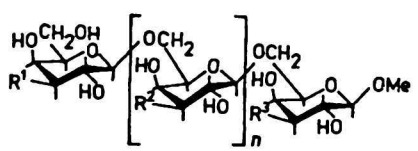
One of the major goals of mass spectrometric research in the eighties was to develop ionization methods which enable the determination of highly polar and thermally labile compounds with a minimum of decomposition. One approach has been [1, 2] to rapidly heat nonvolatile samples coated on a direct insertion probe within a chemical ionization reagent gas plasma.

The use of ammonia, possessing a relatively high value of proton affinity (863 kJ mol^{-1}) [3], serves as an excellent example of electrophilic addition resulting in the formation of intense $[M + NH_4]^+$ ions. If the molecule of the compound studied is somewhat less basic than ammonia, then a stable addition complex is formed. The presence of oxygen, or in some cases merely a centre of unsaturation, can provide electrons to form complexes with NH_4^+ ions. Since these addition complexes are formed under very mild conditions, the technique permits the observation of quasi-molecular ions from some thermally labile compounds [4]. Moreover, structurally informative fragment ions are usually present in the DCI mass spectra.

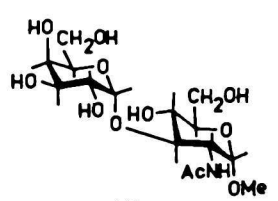
To assist in the determination of the structure of oligosaccharides without derivatization, we have measured the ammonia DCI mass spectra of a large number of oligosaccharides.



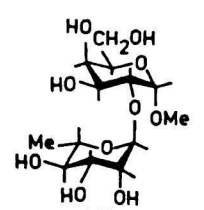
	n
I	0
II	1



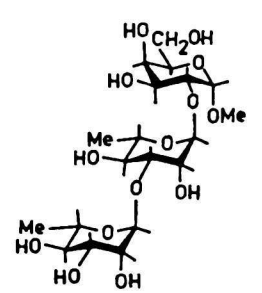
	n	R[1]	R[2]	R[3]
III	0	OH		F
IV	0	F		OH
V	1	OH	F	OH
VI	1	OH	OH	F
VII	1	F	OH	F
VIII	0	H		OH
IX	0	OH		H
X	1	OH	OH	H



XI



XII



XIII

EXPERIMENTAL

The investigated compounds *I*—*XIII* were synthesized earlier [5—8]. Methyl α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (*XIV*) obtained from Sigma Chemical Company and used as supplied. The DCI mass spectra were obtained on ELQ-400-3 mass spectrometer, using ammonia as the reaction gas. Samples were dissolved in water and introduced *via* a Vacuummetrix DCI probe.

RESULTS AND DISCUSSION

The DCI mass spectra (Table 1) of di- and trisaccharides *I*—*X*, *XII*—*XIV* exhibit intense peak of quasi-molecular $[M + NH_4]^+$ ions. Compound *XI*, bearing the acetamido group that has a higher value of proton affinity [3] exhibits, instead, an intense peak of $[M + H]^+$ ions. As an example, the DCI spectrum of methyl β-D-galactopyranosyl-(1→6)-3-deoxy-3-fluoro-β-D-galactopyranoside (*III*) is shown in Fig. 1. In addition to the quasi-molecular ions, the spectra contain a number of fragment ion peaks. The elimination of ammonia leads to the formation of $[M + H]^+$ ions. In the case of substances containing the 3-fluoro group (*III*—*VII*), the elimination of HF from cluster $[M + NH_4]^+$ ions, followed by successive elimination of methanol and ammonia takes place (Fig. 1). The elimination of methanol from $[M + NH_4]^+$ ions leads to the production of ions probably having the structure of an *N*-glycoside. We have previously studied the formation of this type of ions, using per-*O*-methylated methyl hexopyranosides as model substances [9, 10]. The fragment "sequential ions" are in the region of *m/z* = 180—242. They are produced by pathways elucidated by fast atom bombardment mass spectrometry of oligosaccharides [11]. The ions A add a molecule of ammonia, giving rise to $[A + NH_3]^+$ ion species. Pathway *B* is accompanied by a rearrangement of a hydrogen atom of one of the hydroxyl groups present in the

Table 1. Ammonia DCI Mass Spectra of Oligosaccharides *I*—*XIV*

m/z	I_r/%													
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*	*XIV*
540					69									
538			8	10										
536							2							3
521			3	1										
520					42					3				
519							1						41	
500					6									
487													15	
414														6
398										1	8			
376	100	77	100	70	11									
374						100	19							65
368										55				
359	4	13	8	7	4									
358								24	100			100	38	
357						21	6							10
356		23												
344	7	4	8	3	8									
342						11	14							
341								14	12	18		28	26	
336										26		19		
327	2	1	6											
326								3	17					
324		7			3									
310													100	
309									6	20		7		
293													61	
256					5									
242		13			19									
240	1		3	2		3		5						9
236											100			
214		100		100	100									
212	15		15	14		83	100	100				81	45	100
197		46			17	13	13							
196									21	100				
195	11	18	12			6	17	55						
180	2	30	7	11	22	14	19	13		97		8		55
179									14					
164								27	10	72	8	28	72	
162	1	12	3	3	13	3	2							
147								8		39				
145		13		3			2					8	10	10
127								8		18				

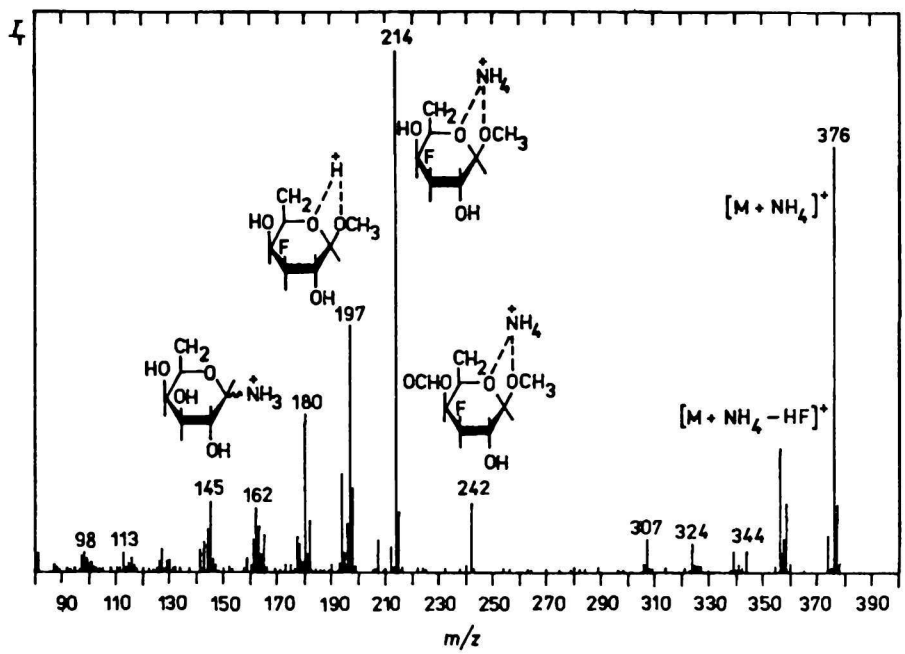
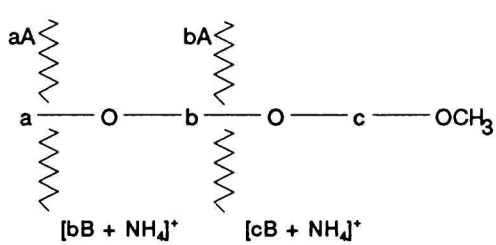


Fig. 1. DCI (ammonia) mass spectrum of methyl β-D-galactopyranosyl-(1→6)-3-deoxy-3-fluoro-β-D-galactopyranoside (*III*).

nonreducing end of the molecule [12]. It leads to the formation of ions with *m/z* 197 (protonated form) and 214 (ammonium adduct, Fig. 1). The ions with *m/z* 242 are formed *via* the *D* pathway [11], followed by addition of a molecule of ammonia.

Unfortunately, the DCI (ammonia) mass spectra of tetra- and higher oligosaccharides we have measured did not contain peaks of quasi-molecular ions. Thus, the DCI technique cannot serve as a universal method for unambiguous sequential analysis of underivatized oligosaccharides.

The DCI (ammonia) mass spectra can be used in the sequential analysis of di- and trisaccharides in the following way



$$aA = [M + NH_4]^+ - [bB + NH_4]^+ + 1 \quad (1)$$

$$bA = [M + NH_4]^+ - [cB + NH_4]^+ + 1 \quad (2)$$

The calculated mass of A ions confirms the mass of the nonreducing end of the molecule. In the case of acetamido group-containing oligosaccharides, which do not contain the cluster ions, we recommend to use for calculations the m/z values of $[M + H]^+$ and $[B + H]^+$ species, instead of *m/z* values of $[M + NH_4]^+$ and $[B + NH_4]^+$.

In conclusion, the ammonia DCI mass spectrometry is a useful tool in the structure analysis of underivatized di- and trisaccharides.

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