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Reduction of azides under conditions of desorption–chemical ionization or fast-atom-bombardment mass-spectrometry

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ABSTRACT

Under conditions of desorption–chemical ionization or fast-atom-bombardment mass-spectrometry, the azido groups in some carbohydrate derivatives and other substances undergo apparent reduction to amino groups. Experimental evidence is provided to corroborate the reduction, and possible explanations are proposed for the phenomenon.

INTRODUCTION

Organic azides have been widely employed as synthetic starting materials[1]. The azido group can be introduced readily into aliphatic, aromatic, and heterocyclic systems, and can be transformed into other functional groups and utilized for the synthesis of heterocyclic compounds. The azido group has achieved great importance also in carbohydrate and nucleoside chemistry. For example, displacement of sulfonyloxy groups by azide is one of the most widely used synthetic routes to amino sugars[2]. Also, photolysis of primary azides in sugar systems, followed by mild hydrolysis of the resultant imino derivatives, is an excellent method for the preparation of aldehydo sugars[3]. Some glycosyl azides afford in good yield, on irradiation with u.v. light, the corresponding next-lower aldoses[4]. In the area of chemical synthesis of complex oligosaccharides, an excellent method for the synthesis of α-glycosides of 2-amino sugars is the azide method[5], in which 2-azido sugars are employed; for example, the method has been applied to the synthesis of the oligosaccharide determinants of blood-group substances[6]. By far, the best known example of an azido derivative in the nucleoside field is 3′-azido-3′-deoxythymidine (AZT), which has been shown to be an effective inhibitor of human immunodeficiency virus type-1 (HIV-1), the causative agent of AIDS[7]. In this article, we report on an observation made during the course of mass-spectrometric analysis of some carbohydrate derivatives and other substances containing azido

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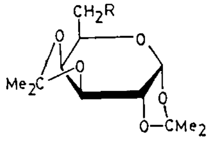
groups. It was found that, under conditions of desorption–chemical ionization (d.c.i.) or fast-atom-bombardment (f.a.b.) mass-spectrometry, the azido groups undergo apparent reduction to amino groups. Similar behavior under electron-impact (e.i.) mass-spectrometry has been reported by Wentrup[8] for a number of heterocyclic azides. This worker attributed the formation of the reduction products to "thermal fragmentation to nitrenes, which by H-capture, gave amines". To our knowledge, this phenomenon has not been discussed, except in a review by Miller and Jones[9], or investigated further. The results of experiments to corroborate the reduction are presented. The observation that some compounds undergo chemical reduction during chemical-ionization mass-spectrometry (c.i.–m.s.) has been reported by several groups[10–12]. The reduction of double bonds under c.i. conditions has been extensively studied; for example, Das *et al.*[13] proposed that a reduction occurred in a bimolecular reaction between cyclic adducts ($[M + N_2H_7]^+$) of diethyl fumarate or diethyl mesaconate and ammonia. Experiments with other conjugated carbonyl compounds indicated that in these cases linear adducts might be involved in the reduction of the double bonds in question[14]. McEwen and Rudat[15] have shown that there is a large population of radicals in the plasma in c.i. experiments. It has been suggested that hydrogen radicals are responsible for the reduction of carbon–carbon double bonds[16]. Madhusudanan *et al.*[16] found that source conditions which favored the production of radicals resulted in an increase in the intensity of ions ($[M + 2H + NH_4]^+$), resulting from reduction of compounds containing double bonds, relative to the corresponding nonreduced pseudomolecular ions ($[M + NH_4]^+$). Furthermore, when tetracyanoquinonedimethane, a powerful radical scavenger, was introduced into the source in c.i. experiments using ammonia as reagent gas, the intensity of the $[M + 2H + NH_4]^+$ ion decreased relative to that of the $[M + NH_4]^+$ ion. These results show a direct relationship between the concentration of radicals in the c.i. source and the observed abundance of ions attributed to the reduction of double bonds, and imply the involvement of radicals in the reduction process.

Reduction of analytes has been reported to occur also in f.a.b. mass spectrometry[17,18]. Radicals have been implicated also in the reduction process under f.a.b. conditions[19,20]. Schröder *et al.*[21] proposed that ionization under f.a.b. conditions occurs in a manner similar to ionization under c.i. conditions. Therefore, it is reasonable to expect the reduction of a particular analyte to occur in either ionization mode.

RESULTS AND DISCUSSION

The ammonia d.c.i. spectral data of compounds **1**, **3–6**, **8**, and **10** are presented in Table I and representative spectra are shown in Figs. 1A and 2A. All of the spectra showed $[M + H]^+$ ions and most of the spectra have $[M + NH_4]^+$ ions. The presence of either or both of these ions is expected under c.i. conditions. However, of special note, are ions that occur at an m/z value corresponding to $[M + H - 26]^+$. If all the azido groups of compounds **1**, **3–6**, **8**, and **10** were reduced to primary amino groups, they would be expected to give $[M + H]^+$ ions at m/z values corresponding to those of the observed $[M + H - 26]^+$ ions (see Table I). Most of the spectra listed in Table I have



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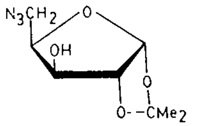

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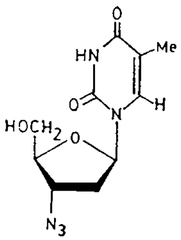

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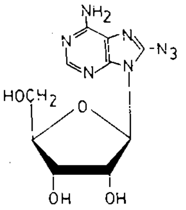

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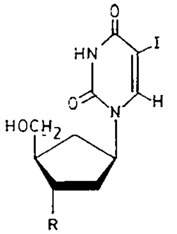

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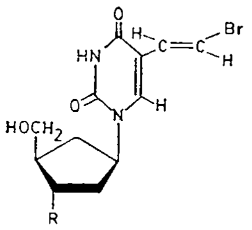

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9 R = NH_2

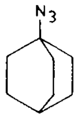

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TABLE I

Relative intensities of selected ions in ammonia d.c.i. mass spectra of compounds **1**, **3**–**6**, **8**, and **10**[a]

Ion	*Compound*[b]						
	1 (*285*)	**3** (*215*)	**4**[c] (*267*)	**5**[d] (*308*)	**6**[e] (*377*)	**8**[e] (*355*)	**10** (*177*)
$[M + NH_4]^+$	22	52	1		3	1	
$[M + H]^+$	3	8	15	28	6	15	2
$[M + H - 26]^+$	100	100	3	62	2	3	100
$[M + H - 28]^+$	61	66		5			67

[a] The relative intensities of the ions in the d.c.i. spectra of the compounds in Tables I, II, and IV are strongly dependent on source temperature and sample concentration in the source. [b] Mol. wt. in parentheses. [c] Base peak m/z 127. [d] Base peak m/z 151. [e] Base peak m/z 112.

also an ion at an m/z value corresponding to $[M + H - 28]^+$. This ions corresponds to the loss of molecular nitrogen from the $[M + H]^+$ ion. The loss of molecular nitrogen from an azido group has been observed previously under e.i. mass spectrometry conditions[9].

The methane d.c.i. mass spectra of compounds **1**, **3**–**6**, **8**, and **10** were recorded also and representative spectra are shown in Figs. 1B and 2B. Not all of the compounds

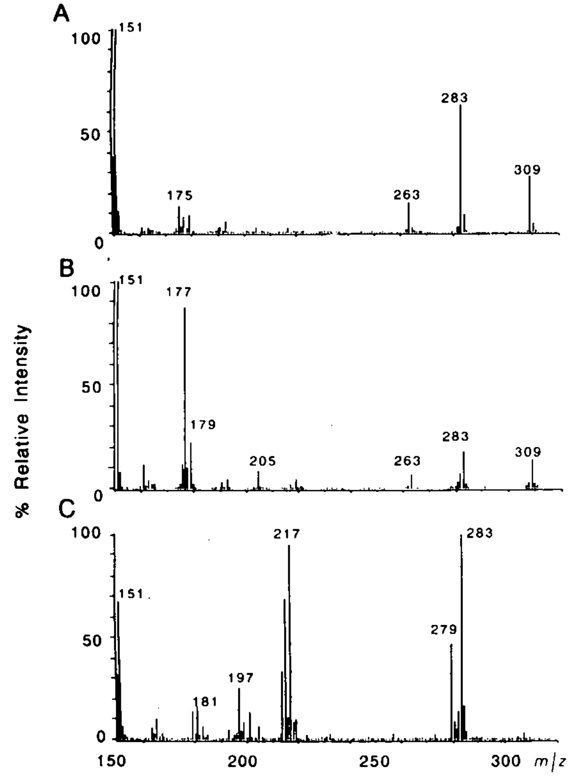


Fig. 1. (A) Ammonia d.c.i. mass spectrum of compound **5**; (B) methane d.c.i. mass spectrum of compound **5**; (C) f.a.b. mass spectrum of compound **5**.

studied exhibited ions in the molecular-ion region, presumably because extensive fragmentation occurred under methane d.c.i. conditions. The compounds that gave useful spectra under methane d.c.i. conditions are presented in Table II. All of the spectra show an $[M + H]^+$ ion as well as an ion at an m/z value corresponding to $[M + H - 26]^+$. Once again there appeared to be a reduction of the azido groups to primary amino groups. Attempts to obtain f.a.b. spectra of compounds **1**, **3–6**, **8**, and **10** were only partially successful. None of the compounds exhibited ions at m/z values corresponding to $[M + H]^+$. However, $[M + H - 26]^+$ ions appeared in the spectra of **1**, **3**, and **5** (see Table III, and Figs. 1C and 2C).

Two proposals to account for the observation of the $[M + H - 26]^+$ ion in the various spectra of organic azides are shown in Scheme 1. In route (a), it is proposed that the protonated azide (the $[M + H]^+$ ion) loses nitrogen to afford the ion at $[M + H - 28]^+$, which is reduced by hydrogen radicals in the source to yield the ion at $[M + H - 26]^+$. This proposal implies that reduction is exceedingly fast. In route (b), the thermal decomposition of the azide to a nitrene is postulated to occur. The nitrene could undergo protonation to the $[M + H - 28]^+$ ion of route (a) or the nitrene could undergo

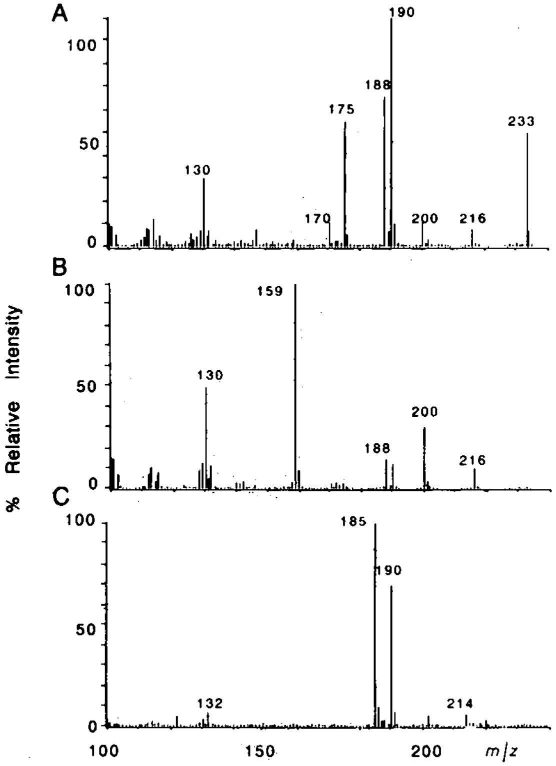


Fig. 2. (A) Ammonia d.c.i. mass spectrum of compound **3**; (B) methane d.c.i. mass spectrum of compound **3**; (C) f.a.b. mass spectrum of compound **3**.

TABLE II

Relative intensities of selected ions in methane d.c.i. mass spectra of compounds **3**–**6** and **8**[a]

Ion	*Compound*[b]				
	3[c] (*215*)	**4**[d] (*267*)	**5**[e] (*308*)	**6**[f] (*377*)	**8**[f] (*355*)
$[M + H]^+$	10	19	14	10	35
$[M + H - 26]^+$	12	2	17	11	6
$[M + H - 28]^+$	14		7		

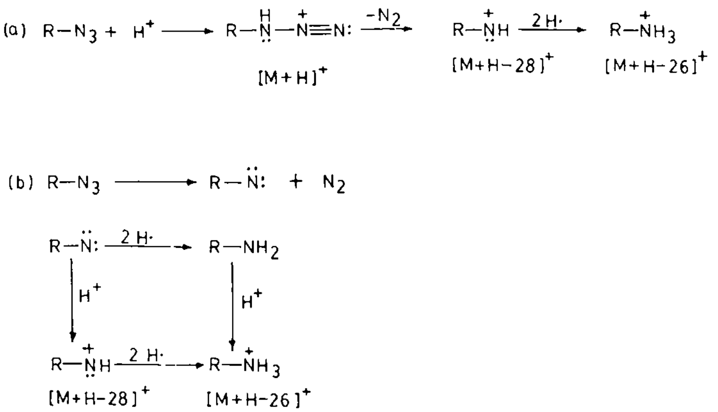
[a] See footnote to Table I. [b] Mol. wt. in parentheses. [c] Base peak *m/z* 159. [d] Base peak *m/z* 127. [e] Base peak *m/z* 151. [f] Base peak *m/z* 112.

TABLE III

Relative intensities of a selected ion in f.a.b. spectra of compounds **1**, **3**, and **5**

Ion	*Compound*[a]		
	1[b] *(285)*	**3**[b] *(215)*	**5** *(308)*
$[M + H - 26]^+$	11	69	100

[a] Mol. wt. in parentheses. [b] Base peak *m/z* 185.



Scheme 1. Two possible routes for the formation of the $[M + H - 26]^+$ ion in the mass spectra of organic azides.

TABLE IV

Relative intensities of selected ions in ($^2H_3$)ammonia d.c.i. mass spectra of compounds **1**, **3**–**6**, **8**, and **10**[a]

Ion[b]	*Compound*[c]						
	1 *(285)*	**3** *(215)*	**4**[d] *(267)*	**5**[e] *(308)*	**6**[f] *(377)*	**8**[f] *(355)*	**10** *(177)*
$[M + X + ND_4]^+$	80	43			6	2	
$[M + X + D]^+$	3	4	3	36	10	16	1
$[M + X + D - 24]^+$	100	100	3	55	4	3	61
$[M + X + D - 28]^+$	75	12		2	1		100

[a] See footnote to Table I. [b] The values of X are 0,1,2,5,2,2, and 0 for compounds **1**, **3**–**6**, **8**, and **10**, respectively. [c] Mol. wt. in parentheses. [d] Base peak *m/z* 130. [e] Base peak *m/z* 157. [f] Base peak *m/z* 115.

reduction by hydrogen radicals to the primary amine. On protonation, the latter would then afford the $[M + H - 26]^+$ ion. In the case of the ammonia d.c.i. spectra, two other explanations might be contemplated, namely, substitution of the azido group by ammonia, and the loss of hydrazoic acid from the $[M + NH_4]^+$ ion. Substitution by ammonia of other groups has been reported[22–24]. However, since ions having an *m/z* value corresponding to $[M + H - 26]^+$ were observed also in the methane d.c.i. spectra and the f.a.b. spectra of the azides, neither of which provide a source of ammonia, the substitution of the azido group by ammonia was not considered a possible route to the $[M + H - 26]^+$ ion. The observation of $[M + H - 26]^+$ ions in the methane d.c.i. or the f.a.b. mode of ionization also obviates the explanation in these cases based on the loss of hydrazoic acid from $[M + NH_4]^+$.

Further evidence in support of the proposals outlined in Scheme 1 was provided by an examination of the d.c.i. spectra of some of the azides using ($^2H_3$)ammonia as the reagent gas (see Table IV). If reduction of the azido groups to primary amino groups indeed occurs, then the ion resulting from the reduction of the azido group would appear at an *m/z* value corresponding to $[M + X + D - 24]^+$, where X equals the number of exchangeable hydrogen atoms in the parent molecule. The pseudomolecular ions corresponding to $[M + H]^+$ and $[M + NH_4]^+$, observed in the ammonia d.c.i. spectra, will now appear at $[M + X + D]^+$ and $[M + X + ND_4]^+$, respectively, in the spectra obtained with ($^2H_3$)ammonia as the reagent gas. The loss of nitrogen from the pseudomolecular ion should be reflected by the $[M + X + D - 28]^+$ ion, and indeed it is present in the spectra of those compounds, namely, **1**, **3**, and **10**, that showed intense $[M + H - 28]^+$ ions in the ammonia d.c.i. spectra. However, in those compounds, namely, **4**–**6** and **8**, that showed low-intensity ions in the ammonia d.c.i. spectra, the $[M + X + D - 28]^+$ ion was barely above background or could not be differentiated from ions arising from incomplete exchange of hydrogen for deuterium atoms. Compounds **1** and **3** were anomalous in that they showed $[M + X + D - 27]^+$ ions in their spectra of relative intensity 41 and 51%, respectively. The presence of these ions is attributed to rearrangement of the nitrene intermediate to an imine, which has an exchangeable hydrogen atom that is not present in the nitrene intermediate itself (see *Eq. 1*).

$$R\text{–}CH_2\text{–}\ddot{N}: \rightarrow R\text{–}CH{=}NH \qquad (1)$$

This ion was not observed in the spectrum of compound **10** because rearrangement of a hydrogen atom in the manner shown in *Eq. 1* is not possible. The results recorded in Table IV are consistent with the reduction of the azido to an amino group.

Exchange was observed also under f.a.b. conditions as was evidenced by the shift of the $[M + H - 26]^+$ ion present in the f.a.b. spectrum of **3** when D_2O was added to the matrix. The mass shift is analogous to that observed in the ($^2H_3$)ammonia d.c.i. spectrum of **3**, except that exchange was less complete in the f.a.b. experiment, with ions also present at *m/z* 192 and 193, as well as at *m/z* 194.

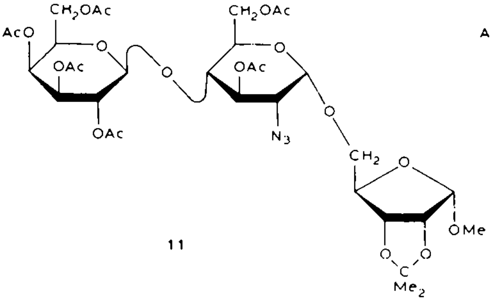
The ammonia d.c.i. spectra of amines **2**, **7**, and **9**, the reduction products of azides, **1**, **6**, and **8**, were examined also. The pseudomolecular ion $[M + H]^+$ of these amines was expected to be identical in structure with the $[M + H - 26]^+$ ion of the corresponding azides, provided that the azido group had been converted into an amino group without rearrangement of the carbon skeleton. The mass-analyzed ion kinetic energy (MIKE) spectra[25] of the $[M + H]^+$ ions of the three amines proved to be virtually identical with the MIKE spectra of the $[M + H - 26]^+$ ions of the three corresponding azides. These experiments provided further evidence that the azido groups are converted into amino groups under d.c.i. conditions using ammonia as the reagent gas and that the reactions occurred without rearrangement of the carbon skeleton.

The mass of the $[M + H - 26]^+$ ion, formed in the methane d.c.i. experiments with **4**, **5**, and **8** was determined by peak-matching experiments using ions of perfluorokerosene as mass markers. The experimentally determined masses agreed with those calculated within 1.4 millimass units. The mass of the $[M + H - 26]^+$ ion of **3**, formed in a f.a.b. experiment using 1:5 (v/v) glycerol–thioglycerol as matrix, was determined also. The matrix ions, $[2\text{ glycerol} + H]^+$ at m/z 185 and $[\text{glycerol} + \text{thioglycerol} + H]^+$ at m/z 201, were used as reference ions. The calculated and experimentally observed masses were in agreement. These results provided further evidence that the azido groups are converted into amino groups under d.c.i. and f.a.b. conditions.

The trisaccharide **11** was examined also by ammonia d.c.i. and f.a.b. mass spectrometry. Under the former conditions, the pseudomolecular ions $[M + NH_4]^+$ and $[M + H]^+$, and the fragment ions $[M + H - 26]^+$ and $[M + H - 28]^+$, were observed. However, in the f.a.b. experiment using glycerol as matrix, a pseudomolecular ion was not present, but the fragment ion $[M + H - 26]^+$ was observed. The tetrasaccharide **12** under ammonia d.c.i. conditions showed only the pseudomolecular ion $[M + NH_4]^+$, but the fragment ions observed in the spectrum of **11** were absent. Under f.a.b. conditions using thioglycerol as matrix, an $[M + H]^+$ ion and the fragment ion $[M + H - 26]^+$ were present in the spectrum. Thus, in any mass spectral examination of a complex oligosaccharide containing an azido group, it is prudent to perform the experiments using both f.a.b. and ammonia d.c.i. conditions.

EXPERIMENTAL

All mass spectra were obtained on a VG Analytical ZAB-E mass spectrometer equipped with a VG-11-250J data system. The f.a.b. spectra were acquired by use of an 8-KeV Xe atom beam. The sample ions were accelerated at 7 kV from an ambient temperature source. An exponential down-scan of the magnet was used, and scan speeds varied from 2.0 to 4.0 s per decade, depending on the mass range over which spectra were acquired. The d.c.i. spectra were acquired by use of a 7-kV accelerating voltage. The electron-beam energy was ~70 eV, and the source temperature was maintained at 200°. The ammonia reagent gas was used at a pressure that resulted in a 20:1 ratio of $[NH_4]^+$ to $[NH_4\text{–}NH_3]^+$. The methane reagent gas was used at a pressure that resulted in a 1:1 ratio of $[CH_5]^+$ to $[C_2H_5]^+$. The scanning mode and speed were the same in d.c.i. as in f.a.b. High-resolution experiments were performed at a resolution of ~4500. Hydrogen–deuterium exchange experiments in f.a.b. were done by adding 2 μL of D_2O to the probe tip after the sample and matrix had been applied.



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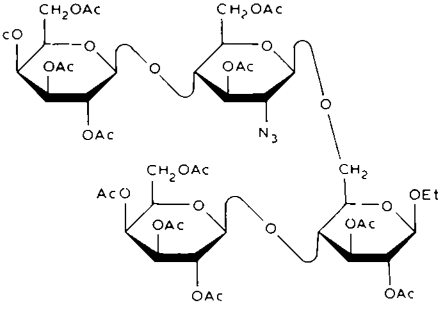

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Compounds **4**, **5**, and **10** were obtained from the Aldrich Chemical Co. The synthesis of the 3′-azido compounds, **6** and **8**, and their conversion into the corresponding amines, **7** and **9**, respectively, have been described in a recent publication[26]. The preparation of samples of the azides **1** and **3**, and the reduction of **1** to the amine **2**, were performed as described previously[27]. Trisaccharide **11** and tetrasaccharide **12** were samples available[28].

ACKNOWLEDGMENTS

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