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GAS PHASE PROTONOLYSIS REACTIONS

CHEMICAL IONIZATION MASS SPECTROMETRY OF N-NITROSAMINES

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Abstract—Gas phase protonolysis reactions of a wide variety of N-nitrosamines have been studied utilizing methane chemical ionization mass spectrometry (CIMS). N-Nitrosamines are protonated at either the N or O atoms of the N-nitroso triad (-N-N=O) leading to aminium radicals, nitrenium ions, ammonium ions, carbenium ions and various elimination reactions. A structure-CIMS reactivity relationship of N-nitrosamines is presented.

INTRODUCTION

Recently it was proposed[1] that simple electrophilic activation of N-nitrosamines via proton attack on the N-nitroso triad could be a significant pathway in the biological metabolism of these compounds. It was shown[1] that under strongly acidic conditions, alkylcarbenium ion formation was facile. Hence, the ability of N-nitrosamines to form alkylating agents could be explained using non-oxidative conditions.

The object of this investigation was to provide a better understanding of gas phase protonolysis reactions of N-nitrosamines, thus allowing comparison with solution chemistry occurring in acidic media. Chemical ionization mass spectrometry (CIMS) is ideally suited for studying protonolysis reaction in the gas phase[2a,b] and our CIMS results have established a structure-reactivity relationship regarding site of protonation on N-nitrosamines and their subsequent fragmentation.

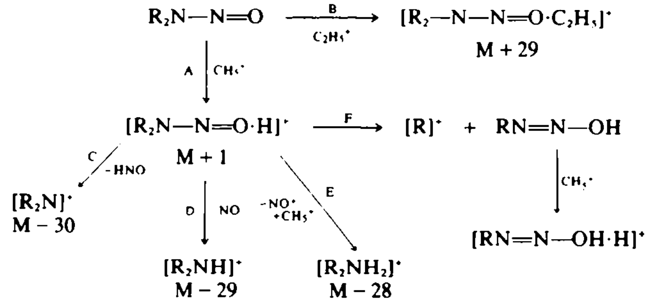
RESULTS

The gas phase protonolysis reactions of N-nitrosamines are summarized in Scheme 1, while the CIMS data for compounds **1–25** are recorded in Table 1.

The cyclic and acyclic alkyl N-nitrosamines (**1**, **2**, **4–8**) formed stable $[M+1]^+$ ions (Scheme, reaction A) utilizing methane as the reagent gas. These protonated molecular ions did not fragment further to any significant extent. Compounds **4**, **5**, **8**, **12**, **13**, **18**, **21**, **23** and **25** gave an ion at $[M+29]^+$ (Scheme 1, reaction B) resulting from electrophilic attack of the Lewis acid $[C_2H_5]^+$.

The electrophilic additions to the N-nitroso group, shown as reactions A and B, may activate these compounds for the fragmentation reactions C-F (Scheme 1). For example, an ion observed at $[M-30]^+$ (Scheme 1, reaction C) in varying amounts for **4**, **5**, **11**, **12**, **15**, **18–23** and **25** is postulated to be a nitrenium ion $[R_2N]^+$. An ion at $[M-28]^+$ observed in the CIMS of N-nitrosamines (**17** and **22**) is postulated to result from loss of NO^+ from the $[M+1]^+$ ion followed by protonation of the amine with the reagent gas to produce a substituted ammonium ion $[R_2NH_2]^+$ (Scheme 1, reaction E). Not only was heterolytic cleavage (loss of NO^+) of the protonated molecular ion observed, but several N-nitrosamines gave evidence of homolytic cleavage of the N-N bond (loss of $NO^\cdot$) (Scheme 1, reaction D). An ion at M − 29 in the CIMS of **12**, **18–23** and **25** was tentatively assigned to the aminium radical $[R_2NH]^{\ddagger}$ and was far too intense to be attributed to an isotopic fragment of the $[M-30]^+$ ion.

Another gas phase reaction of N-nitrosamines observed was that of carbenium ion formation from the protonated



Scheme 1. Protonolysis, electrophilic addition and fragmentation reactions of N-nitrosamines in the gas phase by methane CIMS.

Table 1. The methane CIMS of N-nitrosamines 1-25

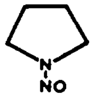
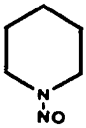
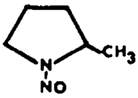
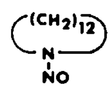
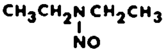
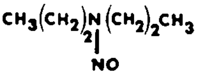
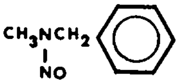
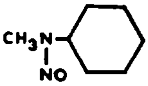
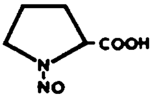
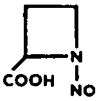
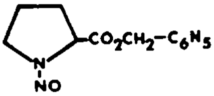
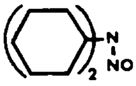
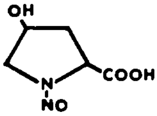
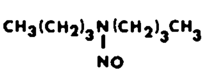
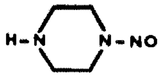
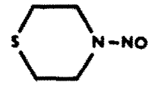
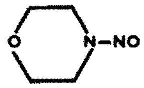
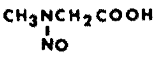
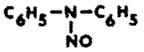
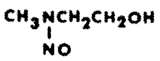
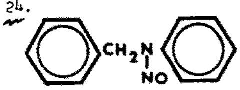
No.	Structure	Name and Parent (m/e)	m/e(relative intensity)^a	
			$[M+1]^+$	Other ions
1.		Nitrosopyrrolidine (100)	100	102(8.4), 100(6.6)
2.		Nitrosopiperidine (114)	100	116(7.8), 114(8.5)
3.		Nitroso-2-methyl-pyrrolidine (114)	100	116(9.6), 114(6.7), 70(6.0), 69(25.3)
4.		Nitrosododecamethylene-imine (212)	100	241(16.4) $[M+29]^+$, 214(13.1), 211(8.2), 182(5.0) $[M-30]^+$, 83(6.5)
5.		Nitroso-3-azabicyclo-[3.3.1]nonane (154)	100	183(12.8) $[M+29]^+$, 156(9.3), 124(6.1) $[M-30]^+$, 109(7.7)
6.	 $CH_3\underset{NO}{N}CH_3$	Dimethylnitrosamine (74)	100	74(7.8)
7.	 $CH_3CH_2\underset{NO}{N}CH_2CH_3$	Diethylnitrosamine (102)	100	104(5.3)
8.	 $CH_3(CH_2)_2\underset{NO}{N}(CH_2)_2CH_3$	Di-n-propylnitrosamine (130)	100	159(13.8) $[M+29]^+$, 130(5.1)
9.		Methylbenzylnitrosamine (150)	0	92(8.4), 91(100)
10.		Methylcyclohexyl-nitrosamine (142)	9.3	89(25.3), 83(23.7), 75(16.3), 61(100)
11.		Nitrosoproline (144)	24.7	114(14.9) $[M-30]^+$, 70(100)
12.		Nitrosoazetidine-2-carboxylic acid (130)	100	159(7.9) $[M+29]^+$, 130(5.3), 101(35.1) $[M-29]^+$, 100(85.4) $[M-30]^+$, 86(5.3), 85(5.3), 56(89.4)
13.		Nitrosoproline benzyl ester (234)	62.7	263(9.1) $[M+29]^+$, 236(8.7), 160(7.9), 119(7.9), 99(10.6), 92(8.7), 91(100), 70(53.2)
14.		Dicyclohexylnitrosamine (210)	1.0	129(15.4), 84(6.7), 83(100), 75(7.5)
15.		Nitroso-4-hydroxy-proline (160)	13.4	130(25.0)$[M-30]^+$, 112(5.4), 96 (6.2), 87(6.2), 86(100), 69(7.1), 68(61.6), 64(10.7), 61(35.7)
16.	 $CH_3(CH_2)_3\underset{NO}{N}(CH_2)_3CH_3$	Di-n-butylnitrosamine (158)	45.8	160(14.1), 116(11.9), 115(7.8), 103(17.6), 99(5.4), 86(8.7), 84(11.1), 57(100)

Table 1 (*Contd*)

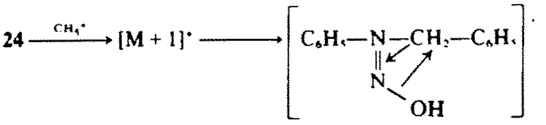
17.	 $CH_3\underset{NO}{N}CH_2CH_2Cl$	Methyl-2-chloroethyl-nitrosamine (122)	5.0	105(8.1), 100(6.4), 96(14.5), 94(46.7) [M-28]$^+$, 87(12.9) [M-Cl]$^+$, 61(27.4), 59(6.5), 58(100), 57(14.5)
18.		Nitrosopiperazine (115)	100	144(8.3) [M+29]$^+$, 117(6.1), 86(39.4) [M-29]$^{+\cdot}$, 85(41.5) [M-30]$^+$
19		Nitrosothiomorpholine (132)	56.6	105(9.4), 104(5.7), 103(100) [M-29]$^{+\cdot}$, 102(18.9) [M-30]$^+$, 88(32.1), 70(5.6), 56(9.4).
20.		Nitrosomorpholine (116)	100	118(5.1), 87(11.8) [M-29]$^{+\cdot}$, 86(7.4) [M-30]$^+$
21.	 $CH_3\underset{NO}{N}CH_2COOH$	Nitrososarcosine (118)	100	147(25.8) [M+29]$^+$, 117(8.8), 116(13.7), 89(34.6) [M-29]$^{+\cdot}$, 88(71.4) [M-30]$^+$, 73(9.9)
22.	 $C_6H_5{-}\underset{NO}{N}{-}C_6H_5$	Diphenylnitrosamine (198)	50.0	227(5.6) [M+29]$^+$, 200(11.1), 171(8.3), 170(49.9) [M-28]$^+$, 169(100) [M-29]$^{+\cdot}$, 168(30.5) [M-30]$^+$, 105(22.2), 93(19.4), 84(13.8), 77(19.4)
23.	 $CH_3\underset{NO}{N}CH_2CH_2OH$	Methyl-2-hydroxy-ethylnitrosamine (104)	100	133(5.4) [M+29]$^+$, 75(24.5) [M-29]$^{+\cdot}$, 74(5.0) [M-30]$^+$
24.		Benzylphenyl-nitrosamine (212)	0	133(6.0), 105(100), 87(5.1), 75(31.0), 74(6.0)
25.		Methylphenyl-nitrosamine (136)	100	165(5.0) [M+29]$^+$, 138(8.5), 107(7.3) [M-29]$^{+\cdot}$, 106(8.0) [M-30]$^+$

[a] Ions of relative intensity of less than 5.0 are not reported.

molecular ion (Scheme 1, reaction F). Since Olah *et al.*[1] have reported that carbenium ions formed readily in superacid from isomeric dipropyl and dibutyl nitrosamines, our results provide evidence that alkyl diazohydroxides may be the other product from this reaction. For example, N-nitrosomethylcyclohexylamine (**10**) gave the cyclohexylcarbenium ion, *m/e* 83 (24%), and an ion at *m/e* 61 (100%), which is proposed to be the protonated methylidiazonium hydroxide ion (eqn 1). Similar formation of carbenium ions and protonated alkyl diazohydroxides was observed with **14** and **16** although N-nitrosomethylbenzylamine (**9**) gave only the benzyl or tropylium ion (*m/e* 91, 100%) and no protonated methyl diazohydroxide

$$\begin{tikzcd} \mathbf{10} \arrow[r, "CH_5^+"] & \underset{m/e\ 143\ (9\%)}{[M+1]^+} \arrow[r] & \underset{m/e\ 83\ (24\%)}{[C_6H_{11}]^+} + CH_3{-}N{=}NOH \arrow[d, "CH_5^+"] \\ & & \underset{m/e\ 61\ (100\%)}{[CH_3{-}N{=}NOH\cdot H]^+} \quad (1) \end{tikzcd}$$

in its CIMS. On the other hand, N - nitroso - N - benzylaniline (**24**) did not produce the $[C_7H_7]^+$ ion as did **9** and **13**, but instead gave *m/e* 105 as the base peak and is postulated to be the phenyldiazonium ion arising from the $[M+1]^+$ ion via concerted elimination of benzyl alcohol (eqn 2).

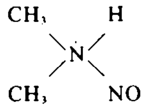


$$\longrightarrow \underset{m/e\ 105\ (100\%)}{[C_6H_5{-}N_2]^+} + C_6H_5{-}CH_2{-}OH \qquad (2)$$

The presence of an additional functional group, such as the chloro or the carboxyl group in the N-nitrosamine molecule, resulted in additional reactions due to the involvement of the adjacent group with the reagent gas ions. For example, nitrosamino acids **11**, **12** and **15** and nitrosamino ester **13**, which possess a carboxyl function on the carbon *alpha* to a nitrogen with a positive charge (nitrenium ion), eliminated carbon dioxide in the case of the acids and the benzyl carbenium ion in the case of the ester. N-Nitroso - methyl - 2 - chloroethylamine (**17**), which has a neighboring chlorine group, underwent reactions which implied that the halogen was the primary site of protonation relative to the N-nitroso triad.

DISCUSSION

Chemical studies have provided evidence for oxygen protonation of the N-nitroso triad leading to thermodynamically favored products. Alkylation of N-nitrosamines by trialkyloxonium ion or dimethylsulfate yields O-alkylated products[3,4] and X-ray structure determination of the N - nitrosodimethylamine - cupric chloride complex has shown[5] primary coordination of the copper to the oxygen of the nitroso group. As a result, stable $[M+1]^+$ ions formed from cyclic and acyclic nitrosamines, **1**, **2**, **4–8**, are probably protonated on the O atom. The formation of $[M-28]^+$, $[M-29]^{+\cdot}$ and $[M-30]^+$ ions from other N-nitrosamines may be due to oxygen ($[M-30]^+$) vs amino nitrogen ($[M-29]^{+\cdot}$) and ($[M-28]^+$) protonation of the N-nitroso triad (Scheme 1). Since calculations have shown[6,7] that the N-protonated form of dimethylinitrosamine, (**26**), has a ground state energy that is about 16 kcal higher than the O-protonated form, (**27**), the formation of the $[M-29]^{+\cdot}$ ion and the $[M-30]^+$ ion may be a consequence of this fact as well as electronic effects occurring in the gas phase.



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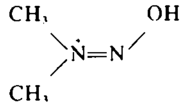

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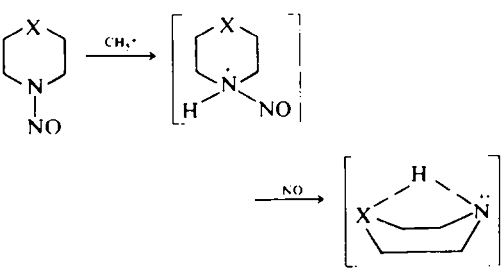
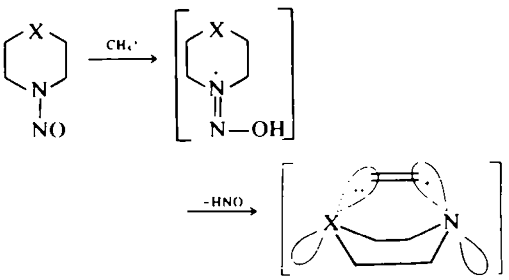
N-Nitrosamines are protonated on the amino nitrogen under photolytic conditions[7] in dilute acid to yield, after homolytic cleavage of NO$^\cdot$, the aminium radical $[R_2NH]^{+\cdot}$. Although odd electron species are infrequently observed[8,9] by CIMS, our results indicate aminium radical formation upon protonolysis of certain N-nitrosamines in the gas phase. The amount of aminium radical formed in CIMS from N-nitrosopiperidines was dependent upon the presence of a heteroatom in the 4-position of the ring. For example, photolysis of N-nitrosopiperidine (**2**) has been shown[7] to give aminium radicals in solution, while no $[M-29]^{+\cdot}$ ion was detected upon protonolysis of **2** in the gas phase. Upon replacing C-4 with a heteroatom, an increase in $[M-29]^{+\cdot}$ ion intensity was observed in the following order, S(100%) > N(39%) > O(12%) ≫ CH_2(0%) (**19**, **18**, **20** and **2** respectively). The added stabilization of 4-substituted piperidinium radicals may be due to an intramolecular H-bond between the 1,4-heteroatoms (eqn 3). A similar type of stabilization has been reported[10] in the gas phase for long chain difunctional molecules. Stabilization of radical cations may also result[11] from resonance delocalization of charge among interacting lone pair sites.



(3)

$[M-29]^{+\cdot}$

The nitrenium ions formed from N-nitrosopiperidines upon protonation of the O atom followed by loss of HNO show an increase in $[M-30]^+$ ion formation in the following order, N(42%) > S(19%) > O(7%) > CH_2(4%) (**18**, **19**, **20** and **2**, respectively). These ions may be considered as being internally solvated in the gas phase (eqn 4) by analogy to carbenium ions which possess stabilizing neighboring groups.[12] Sulfur is known to stabilize a positive charge more effectively than oxygen in the gas phase, i.e. the methylthiomethyl cation is formed in the CIMS more readily than the methoxymethyl cation.[13]



(4)

$[M-30]^+$

Summary. The CIMS results on various N-nitrosamines have shown the occurrence of several preferred fragmentations and have allowed suggestions to be made regarding the gas phase protonolysis reactions of these compounds. These reactions may be useful in providing an understanding for both the chemical and biological reactivity of N-nitrosamines.

EXPERIMENTAL

The N-nitrosamines used in this study were of analytical purity. The N-nitrosamines, **1**, **2**, **6**, **17** and **22** were obtained commercially; while **4**, **5**, **8**, **9**, **17–20**, **23–25** were gifts from Dr. L. Keefer of the National Cancer Institute, Bethesda, MD. Compounds **10**, **14**, **12** and **21** were obtained from the Eastern Regional Research Center, USDA, ARS, Philadelphia, PA and Dr. W. Lijinsky, Frederick, MD, respectively. Compounds **3**, **11**, **13** and **15** were prepared (W.G.) by the usual methods and will be reported elsewhere. CIMS were carried out on a Finnigan 1015D mass spectrometer coupled to a Systems Industries Model 150 computer system. The source was operated at a pressure of 0.9 Torr for methane. Samples were introduced via a solid probe inlet and volatilized by slowly heating the probe between 100° and 200°. N-Nitrosodimethylamine (**6**) was introduced with the use of a Granville-Phillips Co. (Boulder, Colo.) variable leak valve which was attached to the solid probe inlet of the spectrometer.

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