# Chemical Ionization Mass Spectrometry of Doxylamine and Related Compounds

**Walter A. Korfmacher†, Claude L. Holder, James P. Freeman and Ronald K. Mitchum‡**
Department of Health and Human Services, Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079, USA

**Aubrey B. Gosnell**
Henderson State University, Arkadelphia, Arkansas 71923, USA

**The chemical ionization mass spectrometric (CIMS) analysis of doxylamine, *N,N*-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine, and related compounds, using both ammonia and methane as reagent gases, is discussed. The two reagent gases did not produce the same major fragment ion for doxylamine. Mechanisms for the fragmentation of doxylamine under either ammonia or methane CIMS conditions are proposed. The mechanisms explain the observation of an *m/z* 182 fragment ion for doxylamine analyzed under methane CIMS conditions and an *m/z* 184 product ion detected under ammonia CIMS conditions.**

## INTRODUCTION

Doxylamine succinate, *N,N*-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine succinate, a component of the drug Bendectin, has been subjected to several studies regarding its safety and metabolic products.[1–6] In the course of a recent metabolic study of doxylamine by Slikker *et al.*,[2] chemical ionization mass spectrometric (CIMS) techniques were used to examine the metabolites.

Ammonia CIMS has recently been found to be advantageous for the analyses of biologically derived molecules.[6–8] Gielsdorf and Schubert[6] have used both methane and ammonia CIMS methodology for the analysis of doxylamine and several of its metabolic products. Therefore, ammonia CIMS was investigated as a method for the analysis of the doxylamine metabolites found in this study.[2,3] During this work, it was noted that doxylamine behaved quite differently under ammonia CIMS conditions than under methane CIMS conditions.[2,3] This report describes a study of the mass spectrometry of doxylamine and related compounds under CIMS conditions with an emphasis on ammonia as the reagent gas.

## EXPERIMENTAL

All CIMS experiments were performed with a Finnigan-MAT 4023 mass spectrometer system incorporating the standard PPINICI (Pulsed Positive Ion Negative Ion Chemical Ionization) electronics and an electron impact/chemical ionization (EI/CI) source. Except where noted, all samples were analyzed via a Vacumetrics DCI probe incorporating a platinum filament and a heating ramp of 60 s. The remaining samples were analyzed by the packed column gas chromatographic/mass spectrometric (GC/MS) technique with the CI gas as the make-up gas. The GC carrier gas was helium set at a flow rate of 20 $cm^3$/min. The GC column was a 6 ft, 2 mm i.d. glass column packed with 1.5% OV17 + 1.95% OV210 on 100/120 mesh Chromosorb WHP. Typically, 1–2 $\mu$l of sample were injected and the oven temperature was held at 60 °C for 2 min and then programmed to 240 °C at 15 °C/min. All analyses were in the positive ion mode. The reagent gas (methane or ammonia) was set to an uncorrected nominal pressure of 0.25 Torr. The source temperature was 270 °C for methane CIMS experiments and 200 °C for ammonia CIMS experiments.

### Gas phase studies

Two sets of gas phase gas reaction experiments were performed. In the first gas phase experiment, a gas chromatograph/flame ionization detector (GC/FID) was modified by inserting a 0.1 m, $\frac{1}{4}$ in (6 mm) o.d., 4 mm i.d. empty glass GC column into the oven and placing a 85 mm by 3 mm i.d. Teflon tube into the FID chamber. The glass column acted as a reaction chamber and the Teflon tube was used to collect the products as they passed through the FID detector chamber (the FID was not activated). The gas flow for each experiment was 10 $cm^3$/min. The injector, column oven and detector temperatures were set at the same temperature for a given experiment. Three temperatures were selected: 200 °C, 250 °C and 300 °C. The other variable was the reactant gas; three gases were selected: ammonia, helium and P-5 (5% $CH_4$ in argon). For each experiment, 50 $\mu$l of a benzene solution containing 1 mg/$cm^3$ of doxylamine (free base) was injected into the GC via the injector. The products were collected for 30 s after the injection. The products were analyzed using a separate conventional GC/FID system. Where noted in the text, the products were confirmed by GC/MS analysis using the

† Author to whom correspondence should be addressed.
‡ Current address: Quality Assurance Division, US Environmental Protection Agency, PO Box 15027, Las Vegas, Nevada 89114, USA.

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system described above in the methane CIMS mode. Under the described GC/MS conditions, base-line separation of the products was obtained.

In the second gas phase experiment, 500 $\mu$g of doxylamine (free base) was put into the loading cap of a reaction apparatus. The reaction apparatus consisted of a stainless steel isolation valve connected to a 0.5 in (13 mm) o.d. × 20 cm stainless steel tube connected to a 0.25 in (6 mm) o.d. × 5 cm stainless steel tube. The 0.25 in o.d. tube was used as the 'loading cap' and the product collection point. The overall volume of the assembly was 50 $cm^3$. After loading the doxylamine into the loading cap and removing the solvent by a stream of nitrogen gas, the loading cap was attached to the assembly. The lower part of the assembly including the loading cap was inserted into a dry ice/acetone bath. Then the assembly was evacuated ($10^{-3}$ Torr) for 30 min. Next the assembly was shut off from the vacuum line and brought to room temperature. Then, the assembly was briefly pumped down to $10^{-3}$ Torr again and then 10 Torr of ammonia was added to the reaction apparatus. The reaction apparatus was removed from the vacuum line and heated to 250 °C for 30 min in a GC oven. While still hot, the apparatus was removed from the GC oven and the loading cap of the assembly was placed into a dry ice/acetone bath for 20–30 min. The assembly was then taken out of the dry ice/acetone bath and allowed to return to room temperature. At this point, the loading cap was removed from the apparatus and the reaction products were obtained by adding 250 µl of benzene to the loading cap and then removing the benzene solution. The benzene solution was analyzed by GC/MS, using methane as the reagent gas.

As a comparison experiment the above experiment was repeated, but 10 Torr of helium (instead of ammonia) was added to the assembly before heating it.

### Reagents

The deuterium-labelled compounds were received from J. Althaus (NCTR). Doxylamine succinate was purchased from J. T. Baker Chemical Company. The other reference compounds used in this study were synthesized by this laboratory as described elsewhere.[3]

## RESULTS AND DISCUSSION

Figure 1 compares the ammonia CIMS results (a) with the methane CIMS results (b) for doxylamine. In both cases, a strong $[M+1]^+$ ion ($m/z$ 271) was observed. However, for methane CIMS the major fragment ion was $m/z$ 182 while it was $m/z$ 184 for ammonia CIMS. The $m/z$ 182 ion found in the methane CI analysis was assumed to be the $[C_{13}H_{12}N]^+$ fragment resulting from cleavage at the ether linkage in doxylamine. The identity of the $m/z$ 184 ammonia CIMS fragment was not immediately clear.

A series of related compounds were analyzed by the ammonia CIMS and methane CIMS methods in an effort to determine the structure of the $m/z$ 184 ion and the behavior of related compounds. Table 1 summarizes the data from these analyses. Holder *et al.*[3] have discussed the results of additional doxylamine-related compounds which also formed the $m/z$ 184 ion under ammonia CIMS conditions.

As shown in Table 1, not only doxylamine, but also the *N*-oxide of doxylamine and the monodesmethyldoxylamine displayed the same major fragment ion, $m/z$ 182 and $m/z$ 184 under methane and ammonia CIMS conditions, respectively. Therefore, 1-phenyl-1-(2-pyridinyl)ethanol was analyzed by these methods to

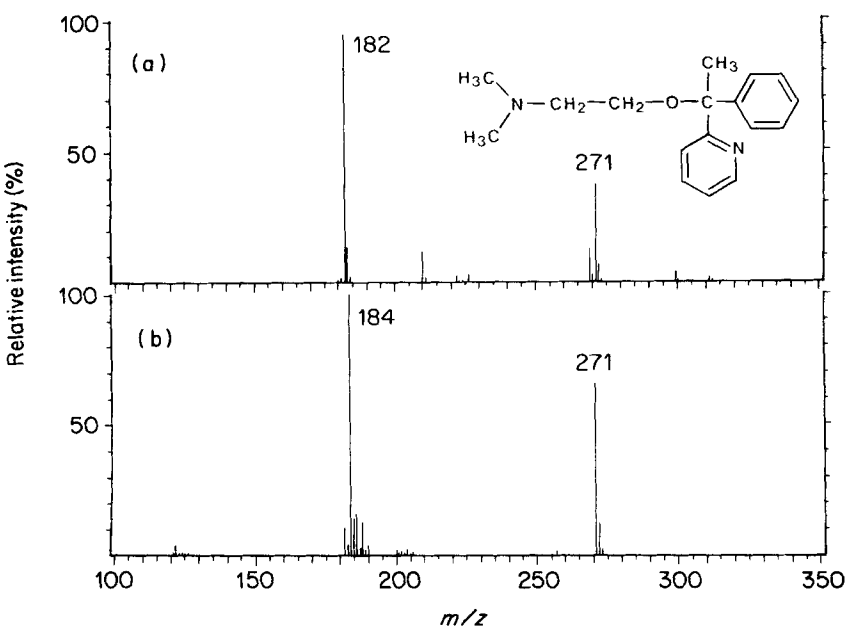



**Figure 1.** (a) Methane and (b) ammonia CI mass spectrum of doxylamine.

**Table 1. Relative ion intensities (%) of doxylamine and related compounds by positive ion methane and ammonia CIMS**

| Compound | Formula | Mol. wt[a] | Relative intensities (%) of major ions[b] Methane CIMS | Ammonia CIMS |
|---|---|---|---|---|
| Doxylamine | $C_{17}H_{22}N_2O$ | 270 | 271(31), 269(13) 210(12), 182(100) | 271(66), 184(100) 182(12) |
| Doxylamine-*N*-oxide | $C_{17}H_{22}N_2O_2$ | 286 | 287(13), 271(19) 269(10), 210(12) 182(100) | 287(29), 271(70) 184(100), 182(12) |
| Monodesmethyl-doxylamine | $C_{16}H_{20}N_2O$ | 256 | 257(20), 210(10) 182(100) | 257(100), 184(20) |
| 1-Phenyl-1-(2-pyridinyl)-ethanol[c] | $C_{13}H_{13}NO$ | 199 | 200(20), 182(100) 122(33) | 200(100), 184(31) 122(62) |
| 1-Phenyl-1-(2-pyridinyl)-ethanol-$d_3$ ($CD_3$)[c] | $C_{13}H_{10}NOD_3$ | 202 | 203(24), 185(100) 125(10) | 203(100), 187(40) 125(23) |
| 1-Phenyl-1-(2-pyridinyl)-ethanol-$d_5$ ($C_6D_5$)[c] | $C_{13}H_8NOD_5$ | 204 | 205(31), 187(100) 122(20) | 205(30), 189(100) 122(10) |
| 1-Phenyl-1-(2-pyridinyl)-ethylene[c] | $C_{13}H_{11}N$ | 181 | 210(23), 182(100) | 184(74), 182(100) |
| 1-Phenyl-1-(2-pyridinyl)-ethane[c] | $C_{13}H_{13}N$ | 183 | 212(18), 184(100) | 184(100) |

[a] Molecular weight.
[b] Only ions with relative intensities of at least 10% are listed in the table. The relative intensity of the ion is listed in parentheses after the observed ion.
[c] Analyzed by packed column GC/CIMS.

determine if it also would fragment in the same manner. As shown in Table 1, 1-phenyl-1-(2-pyridinyl)ethanol also produced *m/z* 182 and *m/z* 184 as major fragment ions under methane and ammonia CIMS conditions, respectively.

In order to demonstrate the identity of these fragment ions, 1-phenyl-1-(2-pyridinyl)ethanol-$d_3$ with the deuterium atoms in the methyl group was analyzed by these methods. The results, listed in Table 1, show that 1-phenyl-1-(2-pyridinyl)ethanol-$d_3$ produced fragment ions at *m/z* 185 and *m/z* 187 when analyzed by the methane and ammonia CIMS methods, respectively. These analyses clealy demonstrated that the fragment ions of interest still contained the methyl group.

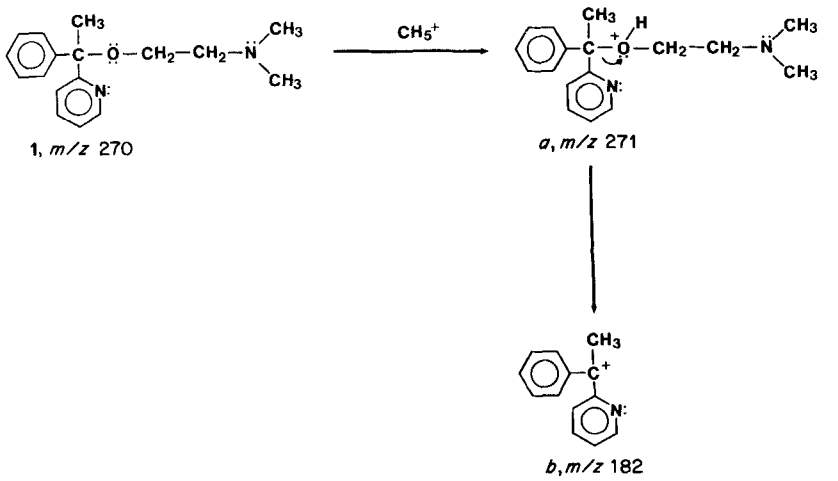
Additional evidence for the assignment of the fragment of interest was obtained by the methane and ammonia CIMS analysis of 1-phenyl-1-(2-pyridinyl)ethanol-$d_5$, where the phenyl ring was deuterated. As shown in Table 1, the methane and ammonia CIMS analysis of 1-phenyl-1-(2-pyridinyl)ethanol-$d_5$ produced a fragment ion at *m/z* 187 and *m/z* 189, respectively, showing that the phenyl ring is also part of this fragment ion.

In addition, 1-phenyl-1-(2-pyridinyl)ethylene was analyzed under CIMS conditions. Under methane CIMS conditions, the expected $[M+1]^+$ ion, *m/z* 182, was observed for this compound. Under ammonia CIMS conditions, this compound still displayed $[M+1]^+$ ions at *m/z* 182, but ions at *m/z* 184 were also observed, presumably due to the same ion as found for the other larger doxylamine-related molecules when analyzed under ammonia CIMS conditions. The final compound in Table 1, 1-phenyl-1-(2-pyridinyl)ethane, produced the expected $[M+1]^+$ ion at *m/z* 184 under both ammonia and methane CIMS conditions.

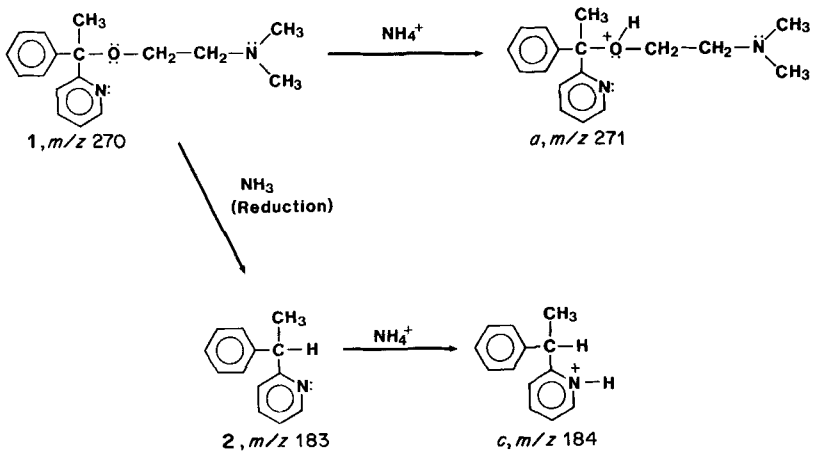
Although the formation of the *m/z* 184 fragment ion for doxylamine and several related compounds under ammonia CIMS conditions was reported previously by Gielsdorf and Schubert,[6] they did not indicate a structure for this fragment ion and they did not propose a mechanism for its formation. They did, however, state that one of their observed doxylamine degradation products was 1-phenyl-1-(2-pyridinyl)ethane. As evidence, they showed that the compound had an empirical formula of $C_{13}H_{13}N$ by high-resolution mass spectrometry and they listed EI and both ammonia and methane CIMS data for this compound. Under both ammonia and methane CIMS conditions, this compound gave an $[M+1]^+$ ion at *m/z* 184. These results agree well with those listed in Table 1 for 1-phenyl-1-(2-pyridinyl)ethane.

The proposed mechanism for the production of the two major product ions for doxylamine under methane CIMS conditions is shown in Scheme 1. Protonation of doxylamine (**1**) by $CH_5^+$ gives the $[M+1]^+$ product (*a*) which fragments producing the *m/z* 182 ion (*b*).

Under ammonia CIMS conditions, a different mechanism is proposed for doxylamine (Scheme 2). As shown in Scheme 2, doxylamine could follow two




**Scheme 1**




**Scheme 2**

pathways. In one pathway, it would be protonated by the $NH_4^+$ to give the $[M+1]^+$ ion (*a*). In the second pathway, doxylamine is reduced by ammonia to 1-phenyl-1-(2-pyridinyl)ethane (**2**) which is then protonated by $NH_4^+$ to give the *m/z* 184 product ion (*c*). This latter pathway provides a two-step route for doxylamine (**1**) to be converted to the *m/z* 184 product ion (*c*).

Formation of the 1-phenyl-1-(2-pyridinyl)ethyl cation, *m/z* 182 (*b* in Scheme 1) was not observed under ammonia CIMS conditions. Because of the small difference in the proton affinities of ammonia and doxylamine, formation of the *m/z* 182 ion by fragmentation of the protonated molecular ion (*m/z* 271) would not be expected.

The proposed methane CIMS mechanism is straightforward and needs no discussion. The ammonia CIMS mechanism hinges on the reduction step (**1** to **2** in Scheme 2). Although reproduction of the mass spectrometric conditions outside of the mass spectrometer were impossible in this laboratory, two different experiments were designed to approximate certain aspects of the mass spectral conditions. In one experiment, a flowing reaction chamber was created by the use of a modified GC/FID system. Doxylamine was injected using three temperatures and three reaction gases. The products from this system were analyzed by a conventional GC/FID system and confirmed by GC/MS analysis. The results obtained in this experiment are shown in Table 2. This data set shows

**Table 2. GC column gas phase reaction of doxylamine[a]**

| Reaction gas (carrier gas) | Oven temp. (°C) | % of total product[b] A[c] | B[d] | C[e] | D[f] |
|---|---|---|---|---|---|
| Helium | 200 | 100.0 | — | — | — |
| | 250 | 99.5 | — | — | 0.5 |
| | 300 | 94.9 | 0.7 | — | 4.5 |
| P-5 | 200 | 99.5 | — | — | 0.5 |
| (5% $CH_4$ in argon) | 250 | 99.6 | — | — | 0.4 |
| | 300 | 98.8 | 0.2 | — | 1.0 |
| Ammonia | 200 | 100.0 | — | — | — |
| | 250 | 99.2 | 0.1 | — | 0.7 |
| | 300 | 93.4[g] | 0.8[g] | 1.0[g] | 4.8[g] |

[a] See Experimental section for details.
[b] '—' means none detected, analysis by GC/FID.
[c] Unchanged doxylamine.
[d] 1-Phenyl-1-(2-pyridinyl)ethanol.
[e] 1-Phenyl-1-(2-pyridinyl)ethane.
[f] 1-Phenyl-1-(2-pyridinyl)ethylene.
[g] Identity and relative concentrations confirmed by GC/MS.

that some of the doxylamine was reduced to 1-phenyl-1-(2-pyridinyl)ethane (**2** in Scheme 2) in a gas phase reaction with ammonia at 300 °C. On the other hand, none of this product was observed with helium or P-5 gas (5% $CH_4$ in argon) at 300 °C or with ammonia at the lower temperatures. In the second experiment, doxylamine was placed in a stainless steel reaction chamber and 10 Torr of ammonia gas was added to the chamber. This assembly was heated to 250 °C for 30 min in a GC oven. The products were analyzed by GC/MS. In this experiment, it was found that 9.2% of the doxylamine was reduced to 1-phenyl-1-(2-pyridinyl)ethane. When the same experiment was repeated with 10 Torr helium added to the reaction chamber, only 2.5% of the doxylamine was found to be converted to 1-phenyl-1-(2-pyridinyl)ethane.

Although not reproducing the conditions of the mass spectrometer, these two experiments demonstrated that doxylamine can be reduced by ammonia to produce 1-phenyl-1-(2-pyridinyl)ethane via gas phase reaction at elevated temperatures. In the source of a mass spectrometer operating under CI conditions, the concentration of neutral reagent gas is in great excess to the reagent ion concentration, thus affording the possibility for this reduction reaction to occur. Thus, the results of these two experiments give support for the ammonia reduction step proposed in Scheme 2.

From the data in Table 1, it can be deduced that the *m/z* 182 and *m/z* 184 ions are *b* (Scheme 1) and *c* (Scheme 2), respectively. Table 1 also shows that when **2** (Scheme 2) was synthesized, it was able to be protonated to give *c* under either ammonia or methane CIMS conditions. Thus, the data listed in Table 1 agree well with the proposed mechanisms.

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