The Methyldiazonium Ion in Water: Competition Between Hydrolysis and Proton Exchange

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The methyldiazonium ion, generated from four different precursors, was found to undergo proton exchange with deuteriated phosphate buffer solutions.

The methyldiazonium ion is the putative ultimate carcinogen from such diverse procarcinogens as dimethylnitrosamine (DMN), various methylated hydrazines, azo- and azoxymethanes, various methyl-*N*-nitroso-*N*-acyl compounds, and various methyltriazenes.[1] It was shown a number of years ago that the intact methyl group was transferred from [$^2H_6$]-DMN to rat liver DNA and RNA, suggesting that the methyldiazonium ion, rather than diazomethane, was the methylating species.[2] We now show that the methyldiazonium ion, generated from several sources, exchanges its protons with deuteriated buffers; the presumed intermediate in the exchange is diazomethane.

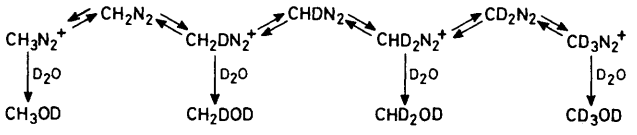
N-Acetoxymethyl-*N*-nitrosomethylamine (DMN-OAc),[3] *N*-methyl-*N*-nitrosoethyl carbamate,[4] *N*-methyl-*N*-nitrosourea,[5] and 1,3,3-trimethyltriazene[6] were used as substrates. Table 1 lists the percentages of each isotopic methanol species obtained from each of the substrates, as obtained from mass spectrometric analysis.†

† Analyses were carried out on a VG-Micromass ZAB-2F spectrometer operating in the electron impact mode at a resolution of 30,000—35,000. A peak matching unit interfaced to a VG-2035 data system was used to monitor CH_4O^+ (*m/z* 32.0262), CH_3DO^+ (33.0325), $CH_2D_2O^+$ (34.0388), CHD_3O^+ (35.0450), and CD_4O^+ (36.0513) and the reference N_2 (28.0061). Correction factors were applied for the differences in energy of the measured ions and the relative percentages of each ion were calculated based on the total amount of methanol present.

It is clear from these data that the precursor to methanol, presumably the methyldiazonium ion, suffers considerable exchange with the medium under the conditions of the experiment. Moreover, all four substrates appear to give the same intermediate, since the amount of exchange is virtually identical for all the substrates. No exchange was observed in undecomposed *N*-methyl-*N*-nitrosoethyl carbamate which was recovered from several decomposition solutions.

These data are seen to be consistent with a scheme wherein the exchange equilibrium between the diazonium ion and diazomethane is in competition with the hydrolysis of the diazonium ion to methanol (Scheme 1).

McGarrity and Smyth[8] studied the kinetics of diazomethane hydrolysis is unbuffered aqueous tetrahydrofuran. They found that the pK_a of the methyldiazonium ion in this medium was 10.0 ± 0.3 and the pseudo-first order rate constant for hydrolysis of the diazonium ion to methanol was 1.8 s^{-1}. The results suggested that the methyldiazonium ion was a sufficiently strong acid and had a sufficiently long lifetime for exchange to occur. Clearly, the specific values for the pK_a and



Scheme 1

Table 1. Distribution of deuteriated methanols from various substrates hydrolysed[a] in deuteriated phosphate buffer at pH 7.4.[b]

Substrate	CH_3OD[c]	CH_2DOD	CHD_2OD	CD_3OD
N-Methyl-*N*-nitroso-ethyl carbamate	49.5 ± 4.5	35.4 ± 3.4	11.7 ± 5.7	3.3 ± 2.2
DMN-OAc	47.4 ± 1.3	32.8 ± 0.2	14.8 ± 0.8	5.0 ± 0.2
Trimethyltriazene	47.8 ± 0.6	36.3 ± 0.6	13.2 ± <0.05	2.6 ± <0.05
N-methyl-*N*-nitrosourea	50.9 ± 0.6	34.1 ± 0.9	12.7 ± 0.4	2.3 ± 0.1

[a] Solutions of substrate (0.8 mmol) in 1 M phosphate buffer (20 ml) in D_2O, at a total ionic strength of 2.25 maintained with $NaClO_4$, were allowed to decompose for 14 h at ambient temperature (20 °C). The urea and the triazene were 100% and the other two *ca.* 75% decomposed after this period. The methanol formed was distilled out of the reaction mixture and analysed by mass spectrometry. [b] The pH meter reading was 7.0, which was corrected by 0.4 according to ref. 7. [c] Each ion was scanned at least 5 times on the mass spectrometer, and each sample run was duplicated. The errors are the standard deviation between the two runs, with each run being an average of at least 5 independent scans.

the rate constant will be different in aqueous buffers from those obtained in the mixed solvent. Interestingly, McGarrity and Smyth also reported proton exchange in the diazonium ion, although their quantitative data, at pH 6—8, which indicated a uniform distribution of $[^2H_4]$-, $[^2H_3]$-, and $[^2H_2]$-methanol, are difficult to rationalize in light of the present results.

The exchange reaction ought to be subject to a primary kinetic isotope effect. This was examined by the hydrolysis of *N*-$[^2H_3]$-methyl-*N*-nitrosoethyl carbamate in undeuteriated phosphate buffer at pH 7.4. The distribution of the deuteriated methanols was: CD_3OH 80.6 ± 1.5, CD_2HOH 13.8 ± 0.7, CDH_2OH 3.0 ± 0.3, and CH_3OH 2.7 ± 1.9%. These data clearly show that a significant isotope effect (k_H/k_D *ca.* 2.7) exists for the exchange reaction. This fact suggests that earlier data[2] on methylation of nucleic acids with $[^2H_6]$-DMN are correct since the isotope effect would have prevented significant loss of deuterons from the $[^2H_3]$-methyldiazonium ion, which is the presumed intermediate in the alkylation reaction. Thus, the isotope effect enhances the reactivity of the diazonium ion with water or with other nucleophiles at the expense of proton removal to form diazomethane. It should be pointed out that, given that the pK_a of the diazonium ion is *ca.* 10,[8] the concentration of diazomethane is very low at neutral pH.

The exchange of protons may be an excellent diagnostic tool for the intermediacy of diazonium ions, particularly in cases where it is uncertain whether the diazonium ion is a discrete intermediate on the path to the corresponding carbocation.[9]

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