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# ISOLATION AND CHARACTERIZATION OF N-ALKYL-N-(HYDROXYMETHYL)NITROSAMINES FROM N-ALKYL-N-(HYDROPEROXYMETHYL)NITROSAMINES BY DEOXYGENATION

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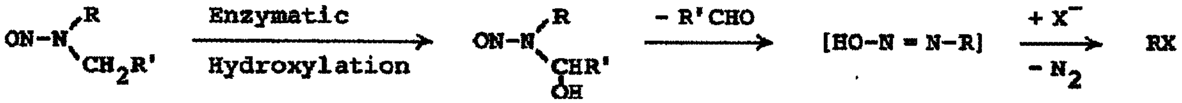
Abstract: N-Alkyl-N-(hydroxymethyl)nitrosamines, postulated intermediates in the metabolic activation of carcinogenic nitrosamines, were prepared by deoxygenation of the corresponding hydroperoxymethyl nitrosamines and characterized.

N,N-Dialkylnitrosamines (1) are carcinogens and mutagens which require metabolic activation. A postulated pathway of the activation is through α-hydroxylation.[1] The intermediate N-alkyl-N-(1-hydroxyalkyl)nitrosamines (2) decompose spontaneously by heterolysis releasing aldehydes to provide reactive electrophilic species capable of alkylating nucleophiles. They are considered to be unstable, and none of them has so far been isolated. Acylated derivatives of 2,




(1) (2)

N-alkyl-N-(1-acetoxyalkyl)nitrosamines (3), have been receiving considerable attention,[2] however, preparation of 2 from 3 was not successful.[3] On the other hand, N-alkyl-N-(1-hydroperoxyalkyl)nitrosamines (4), prepared either by nucleophilic substitution of 3 with hydrogen peroxide in acetic acid[4] or by oxygenatio of α-lithiated 1 with oxygen,[5] may serve as a potential precursor for the preparation of 2. This communication describes isolation, characterization and chemical properties of N-butyl- and N-methyl-N-(hydroxymethyl)nitrosamines, 2a and 2b, prepared from the corresponding hydroperoxymethyl nitrosamines by deoxygenation.[6]

In a typical preparative experiment, triphenyl phosphine (0.52 mmol) in $CHCl_3$ was added slowly to a $CHCl_3$ solution of 4a (0.30 mmol) while cooling with ice under a stream of nitrogen. The solution was concentrated under reduced pressure and the concentrate was chromatographed on a column of Sephadex LH-20 eluting with a mixture of $CHCl_3$ and methyl acetate (9 : 1) in an atmosphere of nitrogen. The fraction containing 2a was concentrated under reduced pressure an an appropriate aprotic solvent such as $CH_3CN$ and DMSO was added to the concentrated solution. The resulting solution was again concentrated to make a solution in the desired solvent. Similarly, a solution of 2b was prepared from 4b (0.20 mmol) by treatment with triphenyl phosphine (0.34 mmol).

2a and 2b were acetylated with acetic anhydride and pyridine at room temperature for 30 min to give 3a and 3b, respectively, whose identification was made by comparison of the NMR and IR spectra with those of the authentic samples.[7] The yields for 3a and 3b from 4a and 4b were 92% and 90%, respectively, indicating that the deoxygenation reaction proceeded almost quantitatively.

Sodium bisulfite can also be used for the deoxygenation. Aqueous bisulfite solution (1.34 M, 4.0 ml) was added to 4a (0.48 mmol), and after stirring the solution at 0°C for 1 min, the product was extracted with $CHCl_3$. It was purified by column chromatography as described above. Acetylation of the product 2a gave 3a in 81% yield calculated from 4a. In a modified manner, 2b was prepared by passing a solution of 4b (0.38 mmol) in $CHCl_3$ through a column packed with a mixture of sodium bisulfite (7 g) and celite 545 (5 g) using $CHCl_3$ saturated with water as eluting solvent. After purification and acetylation, 3b was obtained in 48% yield from 4b.

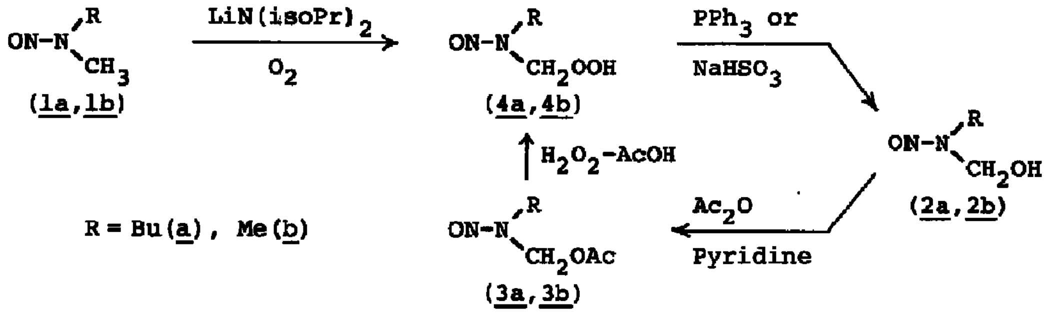


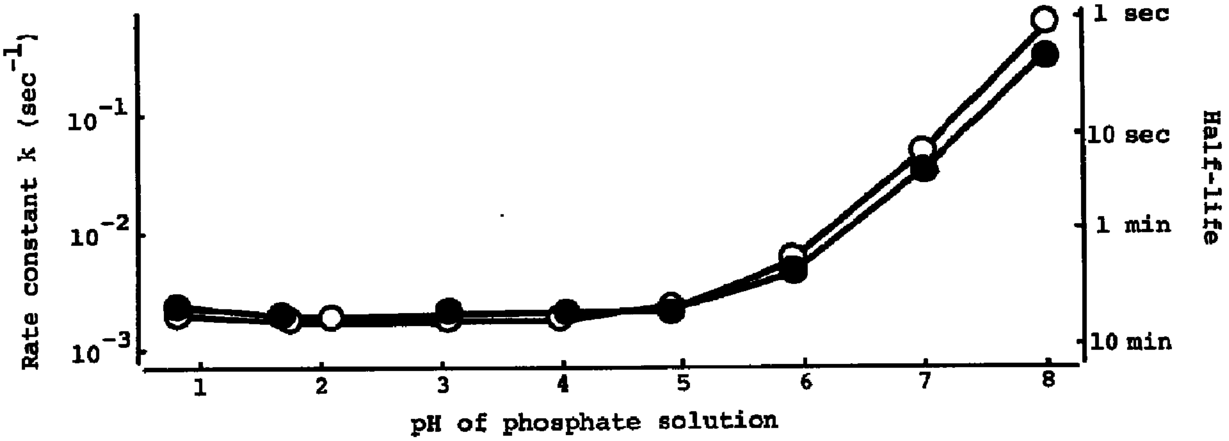

Table. NMR and UV spectral data for N-alkyl-N-(hydroxymethyl)nitrosamines

| Compound | $^1H$-NMR ($CDCl_3$) chemical shift, δ(TMS): (E) HO | (E) $CH_2$-N(N=O) | (E) -$CH_2$- | (Z) HO | (Z) $CH_2$-N(O=N) | (Z) -$CH_2$- | (E):(Z) %[a] | UV ($CH_3CN$) nm(ε)[b]: | |
|---|---|---|---|---|---|---|---|---|---|
| 2a | 4.75[c] | 5.52[d] | 3.60 | 4.13[c] | 4.83[d] | 4.16 | 81:19 | 231(6500) | 364(82) |
| 2b | 4.98[e] | 5.56[f] | 3.06 | - [g] | 4.85[f] | 3.80 | 95: 5 | 228(6000) | 359(83) |

a) (E):(Z) ratio was determined by NMR integration of peak areas. b) ε-values were approximately estimated on the basis of the concentration of acetate obtained by acetylation. c) disappeared on addition of $D_2O$. d) doublets changed to singlets by the addition of $D_2O$. e) disappeared on addition of $CD_3OD$. f) doublets changed to singlets by the addition of $CD_3OD$. g) indistinguishable under the condition examined.

The NMR and UV spectral data for 2a and 2b are shown in the table. The hydroxymethyl structure was supported by comparison of their chemical shifts observed in the NMR spectra with those of the corresponding acetoxymethyl, methoxymethyl[7] and hydroperoxymethyl compounds.[4] The structure was further confirmed on the basis of the effect of deuterium exchanges.

Figure. Rate of decomposition of N-butyl- and N-methyl-N-(hydroxymethyl)-nitrosamines in aqueous phosphate solution




The rate of decomposition in sodium phosphate solution with a constant ionic strength of 0.2 in the pH range 1 - 8 was calculated from the time dependency of the decrease of logarithmic values of the UV absorption at 228 nm due to N-NO function, using the least squares method. • for 2a, and o for 2b.

The hydroxymethyl compounds were unstable in aqueous solutions. The rate constant of the decomposition is plotted as a function of pH of the aqueous phosphate solution, as shown in the figure. No significant difference was observed between 2a and 2b. They were unstable in neutral as well as in alkaline solution, and a linear increase in the rate constant was observed at pH above 6. While, they were rather stable in acidic media, having a half-life of 5.4 min and 5.9 min for 2a and 2b, respectively, at pH 2 to 4. In ethanol, they decomposed with a similar half-life of 5.8 min and 5.7 min for 2a and 2b, respectively. The hydroxymethyl compounds were stable enough to make the isolation possible in carefully dried aprotic solvents as described above.

According to the postulated metabolic pathway, the α-hydroxy intermediate decomposes to yield an aldehyde and an alkylating species, which can be trapped by water in aqueous solution. Formaldehyde, and isomeric butanols or methanol were identified from 2a or 2b, respectively. Formaldehyde was isolated as its 2,4-dinitrophenylhydrazone and identified by comparison of the NMR and IR spectra with those of the authentic sample. The alcohols were detected by gas chromatography and identified by comparing their retention time with those of the authentic samples on two different columns.[8] Thus a solution of the hydroxymethyl compound in 0.5 ml of $CH_3CN$ or DMSO was treated with 10 ml of water for 1 h at room temperature. A portion of the solution was then treated with 2,4-dinitrophenylhydrazine reagent, and another portion was diluted with water and analyzed directly by gas chromatography. 2a (0.13 mmol) yielded formaldehyde, 1-butanol and 2-butanol in 81%, 44% and 20% yields, respectively. 2b (0.26 mmol) gave formaldehyde and methanol in 73% and 93% yields, respectively.

In the presence of thiophenol, a nucleophile other than water, its methylation or butylation was observed with 2b or 2a, respectively. 2b (0.66 mmol) was treated at room temperature with potassium thiophenolate (5 mmol) in 200 ml of sodium phosphate buffer (pH 7.4) with an ionic strength of 0.2 for 1 h. After purification of the product by column chromatography, methyl phenyl sulfide was obtained in 26% yield and identified by NMR and IR spectroscopies. Under a similar condition, 2a (0.75 mmol) gave butyl phenyl sulfide in 1.2% yield.

The result described here presents the first example of the isolation and characterization of the postulated unstable intermediate involved in the metabolic activation of carcinogenic dialkylnitrosamines. The deoxygenation reaction with primary hydroperoxy derivatives of dialkylnitrosamines proceeded almost quantitatively. The decomposition pathway of the key intermediate was also elucidated, and the alkylating activity of the intermediate was clearly demonstrated, thus substantiating the postulated metabolic activation pathway of carcinogenic dialkylnitrosamines.

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