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# SYNTHESIS AND SOLVOLYSIS OF METHYL(ACETOXYMETHYL)NITROSAMINE. SOLUTION CHEMISTRY OF THE PRESUMED CARCINOGENIC METABOLITE OF DIMETHYLNITROSAMINE

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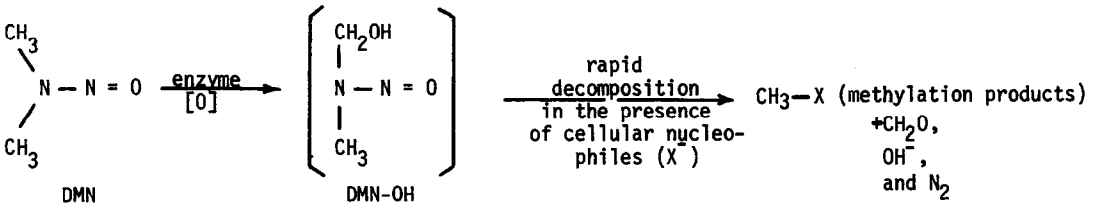
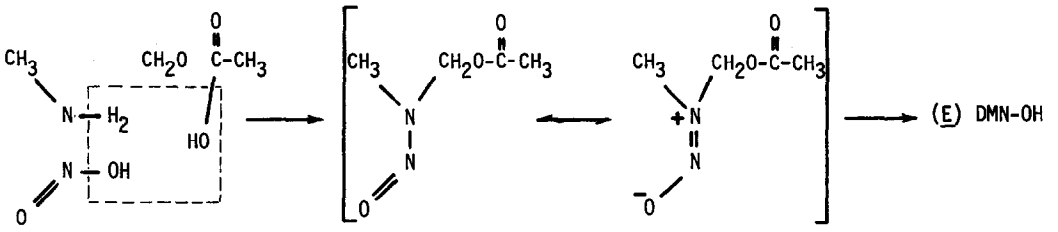
The chemistry of the α-nitrosamino carbinols has been the subject of considerable speculation, largely because of the suggestion that they might serve as critical metabolic intermediates in dialkylnitrosamine carcinogenesis.[1] According to the generally accepted[2] nitrosamine activation pathway, which is illustrated in Scheme I for dimethylnitrosamine (DMN), such compounds are predicted to be unstable at intermediate (physiological) pH's, suffering rapid rearrangement to relatively energetic alkylating agents[1,2]. Unfortunately, these predictions have been impossible to verify because direct experimental data concerning this class of compounds appear to be totally absent from the literature.




Scheme I. Accepted mechanism of DMN metabolism[2]

We report here the highlights of our continuing laboratory survey of compounds having this oxidation state. In particular, we describe a convenient preparation of methyl(acetoxymethyl)nitrosamine (DMN-OAc), a new compound which should play an important role in a variety of independent tests of the postulated biological activation pathway shown in Scheme I. In addition, we present data on the solvolysis of DMN-OAc which we believe provide the first experimental insights into the solution chemistry of an α-nitrosamino carbinol.




(E) DMN-OAc

*Starting material* - The synthesis of DMN-OAc was accomplished in one step by modification of the versatile route to this oxidation state developed by Eiter, *et al.*[3] Simply by omitting the methanol from these authors' preparation of methyl(methoxymethyl)nitrosamine, the acetate was isolated in low but satisfactory yield. In a typical synthetic run, an aqueous solution of sodium nitrite (1.2 moles in 200 ml) was added dropwise to a solution of paraformaldehyde and methylamine hydrochloride in glacial acetic acid (0.66 moles each in 1 liter) at room temperature over a period of 20 hrs. Neutralization with saturated $Na_2CO_3$, extraction with $H_2CCl_2$, and distillation($bp_{32}$ 113°) gave the yellow product in 17% yield.[4] The structural proof rests on the following observations: the uv spectrum exhibited a typical nitrosamine absorption [$\lambda_{max}^{H_2O}$352nm(ε79), 227(ε7120); $\lambda_{max}^{CH_2Cl_2}$361nm]; the ir spectrum (film) indicated the ester carbonyl (1750 $cm^{-1}$); three singlets in the nmr spectrum ($CDCl_3$, TMS) at δ2.12 (3H), 3.08 (3H), and 6.15 (2H) ppm were assigned to the acetoxy protons, the N-methyl group, and the methylene protons of the E conformer,[5] respectively. [In addition, three singlets presumably due to the corresponding protons of the Z conformer,[5] which collectively accounted for 5% of the total integrated nmr spectrum, were found at δ2.04 (3H), 3.88 (3H), and 5.32 (2H), respectively]. The ms data were also consistent with the assigned structure: m/e (70 ev) 132 (5%, $M^+$), 102 (3%, $M^+$-NO), 73 (37%), 60 (4%), 43 (100%), 42 (46%), 41 (6%), and 30 (8%).

*Stability of DMN-OH* - The metabolic activation product of DMN is usually assumed to decompose quite rapidly[1,2] after being generated in the liver. When we hydrolyzed 0.28 mmole DMN-OAc at 21° with 600 μg hog liver esterase (Sigma Chemical Co.) in 25 ml of 67 mM phosphate (pH 7.0), the uniform consumption rate of the starting material was found by gc[4b] to be $3.6_5(\pm 0.2_3^6)$ μmole/min (halflife 40.4 min), whereas the uniform disappearance rate of the 345-355 nm uv peak (presumably a measure of [DMN-OAc] + [DMN-OH]) was 3.36 ($\pm$0.06[6]) μmole/min

(halflife 42.4 min). While it is not as yet clear whether the apparent differences between these rates are significant, the data strongly suggest that DMN-OH is too short-lived to be isolated[7] under these conditions. Furthermore, when the pH *vs* stability profile of DMN-OAc was measured by uv at 21° in various phosphate buffers, a roughly Gaussian curve with a maximum at pH 5.1 (halflife 20 days) was found; the similarity of these results to those for ethyl acetate[9] (maximum stability at pH 5.6) suggests that hydrolysis of the ester group is essentially rate-limiting under non-enzymatic conditions as well. Finally, our conclusion that DMN-OH is very unstable in aqueous solutions is further supported by our failure to observe nmr signals attributable to it in any of the experiments described below.

*Solvolysis products* - According to Scheme I, the products of DMN-OH decomposition should mirror DMN's observed metabolite distribution. Thus, rearrangement of the hydroxy compound should lead with loss of $N_2$ and $OH^-$ to both formaldehyde and a methylating agent, with the latter species being relatively indiscriminate[10] in its attack on cellular nucleophiles (as evidenced by the fact that DMN methylates the guanine residues of liver nucleic acids *in vivo* at both the O-6 and N-7 positions, while methyl methanesulfonate is specific for N-7 under these conditions[11]). Hydrolysis of DMN-OAc in 0.1 N $D_2SO_4$ proceeded with gentle effervescence to an equimolar (by nmr[8]) mixture of acetic acid, formaldehyde, and methanol (identified by nmr, gc, ms, and/or dimedone test), as predicted. In basic solution (1M 4-methylmorpholine in $D_2O$, pH 9.4), hydrolysis of 0.9M DMN-OAc gave the same three products, but the yield of methanol was substantially smaller than that of the $D_2SO_4$ reaction, and both 4,4-dimethylmorpholinium ion and a trace of methyl acetate[12] were identified in the reaction mixture (by nmr and gc, respectively, as well). Thus, consistent with the biochemical results, the methylating intermediate in this reaction showed significantly greater preference for amine and acetate than for water, but it was considerably less selective than dimethyl sulfate, which attacked only the amine under these conditions.

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WARNING: UTMOST CAUTION MUST BE USED IN HANDLING N-NITROSO COMPOUNDS: THE HAZARD IS INSIDIOUS, IN THAT ADVERSE EFFECTS OF EXPOSURE MAY NOT BECOME APPARENT FOR MANY YEARS.[1,2]

## REFERENCES AND FOOTNOTES

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[4] The product was homogeneous by gc: (a) 10% SP-1000 on 80/100 Chromosorb WAW, 10' by 1/8", S.S., oven temp 70° for 4 min, then 8° $min^{-1}$ to 170°, He flow 30 ml/min, retention time 18.8 min [double distillation was necessary to eliminate traces of methyl (methoxymethyl)-nitrosamine, retention time 12.7 min, presumably produced after deaminative conversion of some methylamine to methanol]; (b) a column capable of accepting aqueous solutions of DMN-OAc directly was 15% DEGS on 80/100 Chromosorb WAW-DMCS, 6' by 1/8" O.D., S.S., oven temp 160°, He flow 30 ml/min, retention time 4.5 min.

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[6] These values are the 95% confidence limits of the slopes.

[7] The effect of esterase on DMN-OH is of course not known, but its isomer, methylazoxymethanol, could be isolated under similar conditions of enzymatic acetate hydrolysis[8].

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[12] This product was apparently formed by direct methylation of acetate, since no evidence for ester formation could be found in methanol-acetate solutions at pH 9.4.