Tetrahedron Letters No. 49, pp 4269 - 4272, 1977. Pergamon Press. Printed in Great Britain.

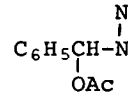
ALKYLATION BY α-ACETOXY-N-NITROSAMINES: MODELS FOR N-NITROSAMINE METABOLITES

Paul L. Skipper,* Steven R. Tannenbaum,*
Jack E. Baldwin,** and Allen Scott**

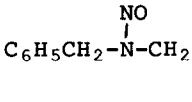
*Department of Nutrition and Food Science and
**Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139 U.S.A.

(Received in USA 26 July 1977; received in UK for publication 13 October 1977)

We recently reported[1] mutagenicity studies of two α-oxidized nitrosamines, N-methyl-N-(α-acetoxybenzyl)-nitrosamine (I) and its isomer N-acetoxymethyl-N-benzyl-nitrosamine (II), the corresponding alcohols of these two acetates being presumed metabolites of methylbenzylnitrosamine, a potent esophageal carcinogen.[2]



(I)



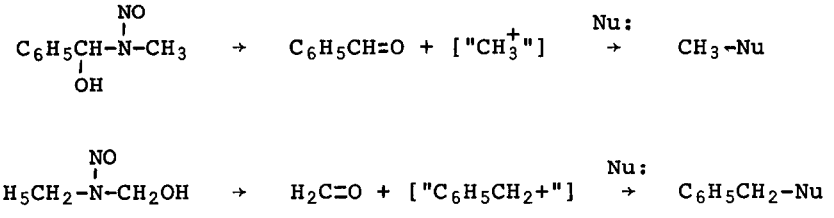
(II)

We observed that I was strongly mutagenic in the Ames assay (*Salmonella typhimurium* TA1535), whereas II was inactive. In addition, I was three orders of magnitude more toxic than II. Since I is expected to be a methylating agent and II a benzylating agent, variable interaction of these two nitrosamines with critical cellular targets is an obvious possible explanation for their different biological actions. It has been demonstrated that N-methyl-N-acetoxymethyl-nitrosamine, an identically functionalized nitrosamine, could be hydrolyzed in aqueous media with concomitant methylation of nucleophiles other than water.[3] We were thus encouraged to pursue this explanation using chemical systems designed to simulate a cellular environment and to highlight differences in reactivity between I and II, especially selectivity toward nucleophiles with particular emphasis on the nucleic acids. We report here our preliminary observations.

Nitrosamines I and II[4] were hydrolyzed (hog liver esterase E.C. No. 3.1.1.1, Sigma) in an aqueous medium at ambient temperature in the presence of either

α-aminopyridine (α-AP) or 2,4-dinitrophenol (2,4-DNP), which served to trap the electrophilic intermediates arising from fragmentation (Scheme I) of the short-lived α-hydroxynitrosamines.[3]

Scheme I

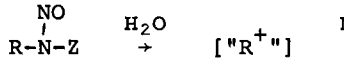


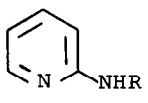
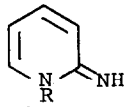
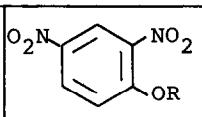
The two nucleophiles were chosen on the basis of structural features analogous to those of nucleic acids. They were used in a concentration of 0.1 M in solutions buffered to pH 8 (α-AP) or pH 9.5 (2,4-DNP).[5] Reaction products were analyzed for yield and isomer distribution directly by high-pressure liquid chromatrography using ion-exchange or reverse-phase modes. Authentic materials were synthesized according to published procedures for use as standards. A typical experiment involved mixing 10-20 μmol of nitrosamine in 2 ml of nucleophile solution with 10-20 units of enzyme preparation and allowing the mixture to stand at room temperature. Since the nitroso compounds were only very slightly soluble, the end of reaction was conveniently determined by observing their disappearance as a separate phase. The validity of this approach was verified in two experiments by measuring the yield of aldehyde (quantitatively, as predicted by Scheme I) and in a third by measuring the yield of alcohol (resulting from reaction with water and equaling the difference between 100% and the sum of other product yields). Results of these experiments are presented in the following table, as well as some data similarly obtained for nitrosomethylurea (NMU) and nitrosobenzylurea (NBU).

Considering first the experiments involving α-aminopyridine, it is clear that benzylation by II is a much higher yield reaction than methylation by I. This is not surprising, considering the relative reactivities of the two electrophilic intermediates; the yield of products is a direct function of the ability of the electrophile to select (in preference to water) for the more nucleophilic, but much less available, amine. The same trend is exhibited by the nitrosoureas, suggesting that very similar intermediates are involved. A high degree of selectivity is also exhibited by II and by nitrosobenzylurea, vis-a-vis the two nucleophilic sites of α-AP, whereas I and NMU are quite indiscriminate. This again may be ascribed to the relative reactivities of the intermediates, although preference for the amino group is somewhat surprising, as this site is the less basic of the two[6] and thus expected to be the less nucleophilic. We feel that hydrogen bonding plays a critical role.

Benzyl chloride and α-AP react in 50% aqueous ethanol to yield preferentially α-benzylaminopyridine (2.8:1). However, in the aprotic solvent dimethyl formamide, the reverse is observed--α-benzylaminopyridine is *dis*favored by at least 50:1.

Yield (%) of Products from the Reaction:



Nitrosocompound				
I: R = CH_3 Z = -CHOAc C_6H_5	1.2	1.5	2.7	0.7
II: R = $C_6H_5CH_2$ Z = $-CH_2OAc$	26	0.9	27	0.7
NMU: R = CH_3 Z = $-CONH_2$	0.8	0.6	1.4	--
NBU: R = $C_6H_5CH_2$ Z = $-CONH_2$	3.3	0.8	4.1	--

The alkylations of 2,4-DNP are not directly comparable with those of α-AP because of the different pH values, but they do serve to demonstrate that oxygen nucleophiles can successfully compete with water for the electrophiles derived from both I and II. This point is vital because much evidence now correlates alkylation of nucleic acids on oxygen (*e.g.*, O^6 of guanine) with tumor induction.[7] We intend to examine this in more detail as we turn our attention to the interactions of I and II with polynucleotides.

The feasibility of employing α-acetoxynitrosamines as models for the metabolites of N-nitrosamines should be clear from our experiments. We expect they will prove to be especially useful if the nitrosamine is unsymmetrical, and the pattern of enzymatic oxidation is thus impossible to control.

References

1. S. R. Tannenbaum, P. Kraft, J. E. Baldwin, and S. Branz, Cancer Letters, 2, 305 (1977).
2. H. Druckrey, R. Preussmann, S. Ivankovic, and D. Schmal, Z. Krebsforsch., 69, 103 (1967).

3. P. P. Roller, D. R. Shimp, and L. K. Keefer, Tetrahedron Letters, 25, 2065 (1975).
4. For details of the synthesis of these two compounds, see reference 1.
5. The higher pH in the latter was necessary to obtain a 0.1 M concentration.
6. H. C. Longuet-Higgins, J. Chem. Phys., 18(3), 275 (1950).
7. B. Singer, Nature, 264, 335 (1976).