Communications to the Editor

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SOLVOLYSIS OF N-NITROSO-N-(1-ACETOXYALKYL)ALKYLAMINES IN PHOSPHATE BUFFER: CHARACTERIZATION AND MUTAGENICITY OF N-NITROSO-N-(1-PHOSPHONOOXYALKYL)ALKYLAMINES

Masataka Mochizuki,*[a,b] Takako Anjo,[a] Naoko Sekiguchi,[a,b] Atsuko Ikarashi,[b] Atsuko Suzuki,[b] Yuko Wakabayashi,[a] and Masashi Okada[a]

Tokyo Biochemical Research Institute,[a] Takada 3-41-8, Toshima-ku, Tokyo 171, Japan and Kyoritsu College of Pharmacy,[b] Shibakoen 1-5-30, Minato-ku, Tokyo 105, Japan

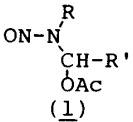
The kinetics of solvolysis of some α-acetoxy nitrosamines in phosphate buffer solution was investigated and their mutagenic products were identified. N-Nitroso-N-(1-acetoxyalkyl)alkylamines were decomposed in two ways in aqueous phosphate buffer solution: O-acyl fission yielded α-hydroxy nitrosamines which were decomposed into aldehydes and alcohols, while O-alkyl fission gave a resonance hybrid of α-N-nitroso carbonium and iminium ions which, when trapped with phosphate, afforded N-nitroso-N-(1-phosphonooxyalkyl)alkylamines. They were stable in neutral and alkaline aqueous solutions, and were mutagenic in Salmonella typhimurium TA1535 and Escherichia coli WP2 and WP2 hcr⁻.

KEYWORDS —— α-acetoxy nitrosamine; α-phosphonooxy nitrosamine; N-nitroso iminium ion; mutagenicity; phosphate buffer solvolysis

α-Acetoxy nitrosamines (1) are masked compounds of α-hydroxy nitrosamines (2), active intermediates in the metabolic activation of carcinogenic N-nitrosodialkylamines.[1] An anomalous behavior was observed in their hydrolysis: in phosphate buffer solution, some of them changed to new compounds which were mutagenic and retained UV absorption due to the N-NO group. The present paper describes the kinetics of the solvolysis and characterizes the products.

The rate of decomposition of N-nitroso-N-(1-acetoxyalkyl)alkylamines in phosphate buffer solutions at different pHs was determined as a pseudo-first order reaction from the decrease in their UV absorption maxima at 228-235 nm. Both the pH of the buffer solution and the kind of alkyl group affected the rate. Generally, compounds with a primary acetoxy group (acetoxymethyl) were more stable than those with a secondary one, and acetoxymethyl nitrosamines with a normal alkyl chain were more stable than those with a branched α-carbon. When α-acetoxy nitrosamine decomposes through α-hydroxy nitrosamine, one expects the formation of alcohol and aldehyde and no strong UV absorption.[2] However, in phosphate buffer solutions some α-acetoxy compounds gave products with UV absorption at a maximum similar to

Chart 1. Typical α-Acetoxy Nitrosamines Which Gave α-Phosphonooxy Nitrosamines in Reaction with Phosphate



(1)

	R	R'
a	Me	Pr
b	Et	Me
c	Pr	Et
d	Bu	Pr

	R	R'
e	tert-Bu	H
f	sec-Bu	H
g	isoPr	H

	RR'
h	$-(CH_2)_3-$
i	$-(CH_2)_4-$

that of α-acetoxy nitrosamines. The production of the new compounds in phosphate buffer solutions occurred in secondary α-acetoxy nitrosamines (1a-1d), cyclic α-acetoxy nitrosamines (1h, 1i), and primary acetoxymethyl nitrosamines with a branched alkyl group as tert-butyl (1e), sec-butyl (1f), and isopropyl (1g) (Chart 1), but seldom occurred in acetoxymethyl compounds with a normal alkyl chain. The formation of the product was dependent on the pH of the phosphate buffer solution, and was maximal in the pH range of 7-9.

The rate of hydrolysis was also determined in aqueous solutions other than phosphate buffer solutions. Table I shows that after the reaction there was no strong UV absorption in borate or Tris buffer solutions, or in water, although the half-life of hydrolysis was similar. This indicates that the product was derived from the reaction of an intermediate with phosphate, and that the rate was independent of the solutes used. The formation of the product did not affect the rate of solvolysis, indicating that the rate determining step was the decomposition

Table I. Effect of Solutes on Solvolysis of α-Acetoxy Nitrosamines at pH 7

Concentration (M):
2.75×10^{-4} (1b)
2.76×10^{-4} (1e)

Buffer solutions	Final absorption 1b	Final absorption 1e	Half-life (min) 1b	Half-life (min) 1e
0.2 M phosphate	0.939	0.993	8.7	321
0.2 M borate	0.099	0.017	8.3	304
0.2 M Tris	0.097	0.021	8.2	293
water	0.100	0.011	8.2	307

of the α-acetoxy nitrosamines. The rate constant was independent of the concentration of phosphate, which again indicates that the rate determining step is present prior to the reaction with phosphate. The final absorption was dependent on the concentration of phosphate: its increase resulted in an increase in the yields of new products, and a plot of reciprocal values of the final absorption versus reciprocal values of the concentration of phosphate was linear as in Fig. 1. The linearity may be explained by the mechanism in Chart 2. For example, the calculated value[3] indicates that 65% of 1b changed to the N-nitroso iminium intermediate, and 92% of the intermediate was trapped with phosphate. The yield expected from the calculation, 56%, was the same as the actual yield of isolation, 56% as described below. The reaction procedure was determined by both UV spectra and HPLC (LiChrosorb RP-18, CH_3CN-phosphate buffer solution). As the peak heights of α-acetoxy nitros-

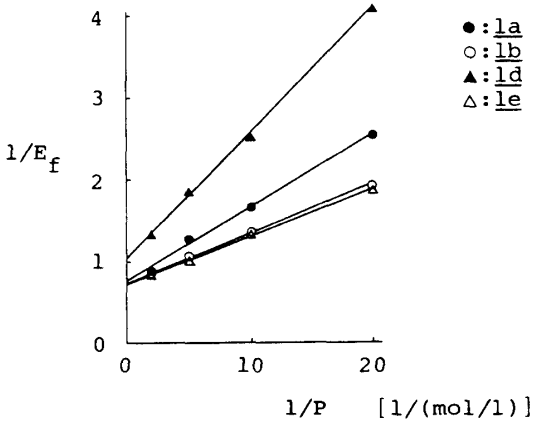


Fig. 1. Double Reciprocal Plot of the Concentration of Phosphate (P) at pH 7 and the Final Absorption (E_f)

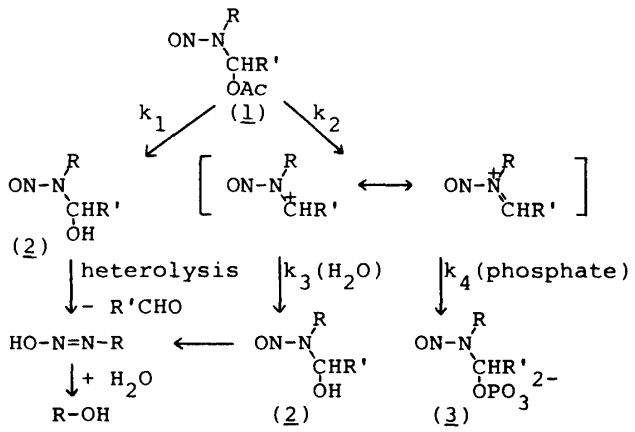
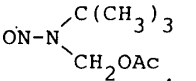


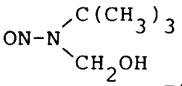
Chart 2. Possible Mechanism of the Decomposition of α-Acetoxy Nitrosamines in Phosphate Solution

amine in HPLC decreased, new peaks increased. The rate constants measured from the changes in UV spectra were identical with those obtained from HPLC, and the rate of formation of the product was also identical with the rate of decomposition of the α-acetoxy nitrosamines. Since the rate of formation of the products was not related to the concentration of phosphate, but to the rate of decomposition of α-acetoxy nitrosamines, the reaction proceeds by an S_N1 mechanism with elimination of the α-acetoxy group as the rate determining step.

The decomposition of an α-acetoxy nitrosamine (1e) and the corresponding α-hydroxy nitrosamine (2e) was compared (Table II). In 1e, the final UV absorption increased with the increase of phosphate concentration, whereas in 2e, the product with the chromophore was not formed at any concentration of phosphate. Thus, in the case of α-hydroxy nitrosamine the decomposition through iminum and carbonium ions is not a major pathway. The major pathway is through heterolysis by releasing aldehydes to alkyldiazohydroxide.

Table II. Final UV Absorption after the Decomposition of α-Acetoxy and α-Hydroxy Nitrosamines in Aqueous Buffers at pH 7

1e  2.75×10^{-4}M

2e  3.5×10^{-4}M

Buffers	1e	2e
0.5 M phosphate	1.207	0.085
0.2 M phosphate	0.993	0.103
0.1 M phosphate	0.765	0.092
0.05 M phosphate	0.548	0.089
0.2 M Tris	0.021	0.084
0.2 M borate	0.017	0.091

The product was purified by crystallization as sodium salt or as cyclohexylammonium salt, and was characterized as α-phosphonooxy nitrosamine (3). As an example, a product from the solvolysis of 1b in phosphate buffer solution is N-nitroso-N-(1-phosphonooxyethyl)ethylamine (3b) which is isolated as cyclohexylammonium salt in 56% yield.[4] 3b was stable in acetonitrile, ethanol and basic aqueous solution, but in acidic solution, it decomposed by acid catalysis. 3b was also a good substrate for an alkaline phosphatase. The salt of 3b was converted by acid catalysis to an α-methoxy nitrosamine by treating it with methanol. This indicates the involvement of N-nitroso iminium ions derived from the phosphonooxy compound.[5]

The mutagenicity of the products was assayed in Salmonella typhimurium TA1535 and Escherichia coli WP2 and WP2 hcr$^-$. Fig. 2 shows two comparisons of the activities of the corresponding α-phosphonooxy and α-acetoxy nitrosamines. The

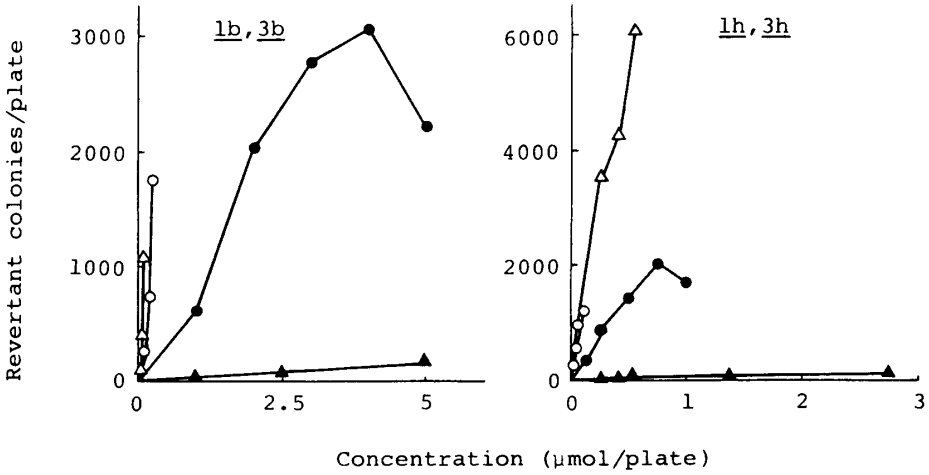


Fig. 2. Mutagenicity of α-Acetoxy and α-Phosphonooxy Nitrosamines in S. typhimurium TA1535 (○:1, △:3) and E. coli WP2 hcr$^-$ (●:1, ▲:3)

activity of the isolated α-phosphonooxy nitrosamine accounts for the total mutagenic activity of the reaction mixture. The α-phosphonooxy nitrosamines were directly mutagenic in all strains tested, and the strength was greater in S. typhimurium and less in E. coli compared with the activity of the corresponding α-acetoxy nitrosamines.[6] α-Acetoxy N-nitrosamines are more strongly mutagenic in the salmonella strain than in the E. coli strains,[6] whereas the mutagenicities of α-hydroxy, α-hydroperoxy and α-oxo nitrosamines are stronger in the E. coli strains than in the salmonella strain.[7,8] These differences in mutagenicity can be partly explained by the intermediate formation of a product such as a phosphate ester, as shown in the present paper. In phosphonooxymethyl nitrosamines, a compound with tert-butyl is not mutagenic, and one with sec-butyl or isopropyl is only weakly mutagenic with a pattern similar to a secondary phosphonooxy compound: stronger in S. typhimurium and weaker in E. coli than the corresponding acetoxymethyl compounds. A possible involvement of the carbonium and iminium ions in the metabolic activation of carcinogenic N-nitrosodialkylamines is a subject of our research in progress.

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2) M. Mochizuki, T. Anjo, and M. Okada, Tetrahedron Lett., 21, 3693 (1980).
3) Using C_0 as the initial concentration of α-acetoxy nitrosamine, P as the concentration of phosphate, and as the molar absorption coefficient of the product, the yield Y is $Y = [k_2/(k_1+k_2)][k_4P/(k_3+k_4P)]$ and the final absorption E_f is $E_f = \varepsilon C_0 Y$, which is $1/Y = \varepsilon C_0/E_f = (1+k_1/k_2)(1+k_3/k_4/P)$ and $1/E_f = C_1/P+C_2$ where $C_1 = (1/\varepsilon/C_0)(1+k_1/k_2)k_3/k_4$ and $C_2 = (1/\varepsilon/C_0)(1+k_1/k_2)$. Since C_1 and C_2 are constants, the double reciprocal plot of the final absorption and the concentration of phosphate gives a linear line as in Fig. 1. The constant C_2 is related to the initial rate determining competition of O-acyl (k_1) and O-alkyl (k_2) fissions, and C_1 is related to the competing reaction of N-nitroso iminium and carbonium ions with water (k_3) and with phosphate (k_4). In 2a, C_1 was 0.061 and C_2 was 0.75, and when $C_0 = 2.7 \times 10^{-4}$ M and $\varepsilon = 7600$, $k_1/k_2 = 0.54$ and $k_3/k_4 = 0.081$. The calculated yield Y was 56% when P was 0.5 M.
4) mp 141-145 °C (from AcOEt as fine needles). ^{1}H-NMR (in CD_3OD) δ from TMS: 1.12 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.70 (3H, d, J=7Hz, $CHC\underline{H}_3$), 3.62 (2H, q, J=7Hz, $C\underline{H}_2$),6.44 (1H, d, q, J=8Hz, 7Hz, NC$\underline{H}$OP). IR (KBr): 1460 (N-NO), 1163, 1090 cm^{-1}. UV λmax(in ethanol) nm (ε): 229 (7600), 356 (85). Anal. Calcd for $C_4H_9N_2O_5P$-$(C_6H_{11}NH_3)_2$-H_2O:C,46.37;H,9.48;N,13.52. Found:C,46.08;H,9.40;N,13.29. The formation of this compound was reported quite recently in N. Frank, and M. Wiessler, Carcinogenesis, 7, 365 (1986).
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