Effects of Structure on the Reactivity of α-Hydroxydialkylnitrosamines in Aqueous Solutions[1]

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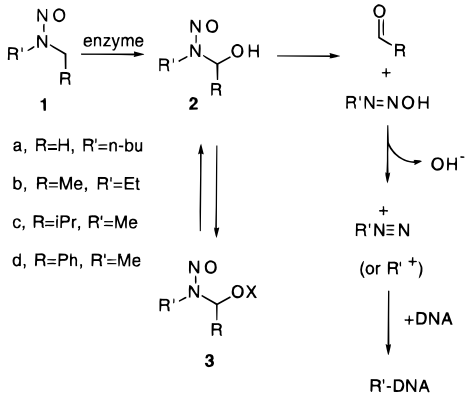
The carcinogenic activity of certain dialkylnitrosamines (**1**, Scheme 1) is thought to be a consequence of DNA alkylation by diazonium ions and carbocations that are ultimately derived from decomposition of α-hydroxydialkylnitrosamines (**2**, Scheme 1).[2,3] The α-hydroxydialkylnitrosamines are the products of enzymatic hydroxylation of dialkylnitrosamines, and it has been speculated that biological derivatives may act as transport forms (**3**, Scheme 1, X = glucuronyl, $-PO_3H^-$, or $-SO_3^-$) for the diffusion of the α-hydroxydialkylnitrosamine moiety to distant tissues where it, and the alkylating equivalents therein, might be liberated.[3] Thus, α-hydroxydialkylnitrosamines are central intermediates in the deleterious biological activities of a large number of nitrosodialkylamines. It was once believed that α-hydroxydialkylnitrosamines were too unstable to be isolated, but through the ingenious efforts of the Okada group, the syntheses and stabilities of a few such compounds were reported in the early 1980s.[4−6] Synthesis of only α-hydroxy*methyl*-alkylnitrosamines (**2**, R = H) was achieved. These compounds (**2**, R′ = Me, Et, *n*-Pr, *n*-Bu, *sec*-Bu, and *tert*-Bu) were reportedly "stable"[5] in acidic media with reactivities otherwise varying with substitution by less than a factor of 10. Since the first reports, there has been no other report of the effects of structure on the chemistry and reactivity of these important reactive intermediates in nitrosamine carcinogenesis. It was presciently suggested[7] that substitution on the hydroxymethyl group (**2**, R ≠ H) might significantly enhance the reactivity of α-hydroxydialkylnitrosamines. We report here a kinetic study of the decay of some α-hydroxydialkylnitrosamines, all derivatives of potent carcinogenic dialkylnitrosamines, that indicates that their stability is indeed dramatically affected by substitution on the hydroxymethyl group.

Synthesis of the α-hydroxydialkylnitrosamines **2a**−**d** was effected in a manner that was strategically analogous to one of the two original[4] methods, entailing triphenylphosphine reduction of the α-hydroperoxy precursors (Scheme 1, **3**, with X = OH).[8] The compound **2a** was sufficiently stable in methylene chloride to survive separation from the triphenylphosphine oxide by chromatography, as previously reported,[4−6] while **2b**−**d** were not. For kinetic studies, compounds **2b**−**d** were generated in freshly dried (from CaH) argon-purged acetonitrile by treatment, in an argon tent for **2d**, of the α-hydroperoxy precursor with 1−10 equiv of tributylphosphine. Kinetics were monitored upon mixing volumes of acetonitrile solutions, after solvent exchange[4] in the case of **2a**, with a 25-fold (or 10-fold for **2d**) excess volume of aqueous buffer using an Applied Photophysics DX17 MV stopped-flow spectrophotometer.[9]

Scheme 1



The reaction kinetics, monitored by disappearence of **2a**−**c** at 230 nm and by appearence of benzaldehyde from **2d** at 255 or 265 nm, exhibited excellent first-order behavior. Typically five determinations of k_{obsd} under a single set of conditions yielded a mean value of k_{obsd} with a standard deviation (σ_{n-1}) of ±2%. In the case of **2d**, the kinetic constants were independent of the concentrations of the reducing phosphine concentration between 1 and 10 molar equiv, and, at 10^{-3} M HCl, UV spectra of the reaction progress as a function of time indicated a clean isosbestic point at 238 nm.[10] Plots of k_{obsd} against buffer concentration containing 4−5 buffer concentrations were linear with increases of less than 15% above the value, k_0, of k_{obsd} extrapolated to the zero intercept of the buffer concentration axis. Most values of k_0 were thus obtained from such plots that generally contained two or three points.

The plots of log k_0 against pH for reactions of compounds **2a**−**d** at 25 °C, ionic strength 1 M ($NaClO_4$), 4% or 9% acetonitrile, are presented in Figure 1. Where comparable, the data for **2a** are quantitatively similar to the data previously reported for **2a** under similar reaction conditions.[4] The data in Figure 1 are consistent with rate law of eq 1, which contains three terms; a pH independent term, k_1; a term whose contribution is inversely proportional to hydrogen ion concentration, k_2; a term whose contribution is directly proportional to hydrogen ion concentration, k_3. The latter term has not been previously reported. The fit of the data to the rate law of eq 1 is indicated by the solid lines in Figure 1 for the values of the

(1) **Warning!** The α-hydroxydialkylnitrosamines and their α-hydroperoxy precursors are presumed carcinogens and must be handled accordingly.

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(8) α-Hydroperoxydialkylnitrosamines (**3**, Scheme 1, X = OH). **3b**: ^{1}H NMR ($CDCl_3$) δ 1.14, (t, *J* = 7.2 Hz, 3 H); 1.61, (d, *J* = 6.5 Hz, 3 H); 3.41−3.73, (m, 1 H); 6.33, (q, *J* = 6.4 Hz, 1 H); 8.9, (s, broad, 1 H). **3c**: ^{1}H NMR ($CDCl_3$) δ 0.87, (d, *J* = 6.8 Hz, 3 H); 1.20, (d, *J* = 6.6 Hz, 3 H); 2.00−2.19, (m, 1 H); 2.99, (s, 3 H); 5.85, (d, *J* = 9.8 Hz, 1 H); 8.72, (s, 1 H). **3d**: ^{1}H NMR ($CDCl_3$) δ 2.83, (s, 3 H); 7.26, (s, 1 H); 7.35−7.52, (m, 5 H); 10.39, (s, 1 H). α-Hydroxydialkylnitrosamines (**2**, Scheme 1). **2b**: ^{1}H NMR ($CDCl_3$) δ 1.10, (t, *J* = 7.2 Hz, 3 H); 1.64, (d, *J* = 6.4 Hz, 3 H); 3.5−3.75, (m, 2 H); 4.2 (s, broad, 1 H); 6.25, (o, *J* = 6.2 Hz, 1 H). **2c**: ^{1}H NMR ($CDCl_3$) δ 0.78, (d, *J* = 6.7 Hz, 3 H); 1.16, (d, *J* = 6.6, 3 H); 2.00−2.2, (m, 1 H); 2.97 (s, 3 H); 5.59, (q, *J* = 4.88 Hz, 1 H); 6.16, (d, *J* = 4.85 Hz, 1 H). **2d**: ^{1}H NMR ($CDCl_3$) δ 2.83, (s, 3 H); 7.23, (s, 1 H); 7.34−7.50, (m, 5 H); 11.3 (br, 1 H).

(9) In the case of **2d**, the traditional arrangement of the two-syringe mixing aparatus was replaced by introducing the substrate solution in acetonitrile, initially protected from the reaction cell by a 10−20 μL bolus of dry acetonitrile, into the reaction cell supply line by means of a T-junction and an additional filling syringe that contained the substrate solution and that could be isolated from the flow system by a third valve. The collinear ends of the T-junction were attached to the reaction cell by means of a 120 μL loop and to one of the two mixing syringes containing freshly dried (from CaH) acetonitrile.

(10) The λ_{max} (=252 nm) of the product was identical with that of benzaldehyde. HPLC analysis (C-18 column, CH_3CN/H_2O eluant) indicated a 90(±1)% yield of benzaldehyde for reactions in 10^{-3} M HCl. In the case of **2d**, a few runs with a 25-fold excess of aqueous buffer gave values of k_0 that were within 7% of those obtained in experiments in which a 10-fold excess of aqueous buffer was used.

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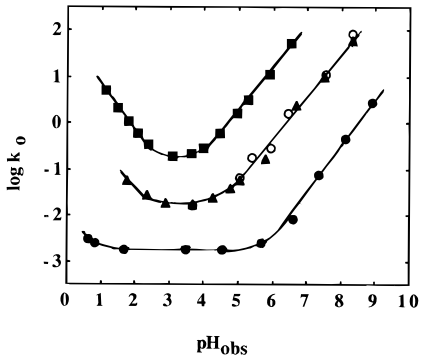


Figure 1. Plot of logarithm of k_0, the buffer independent rate constant for decomposition of α-hydroxydialkylnitrosamines, against pH at 25 °C, ionic strength 1 M ($NaClO_4$), 4% by volume acetonitrile: (●) **2a**, (▲) **2b**, (○) **2c**, and at 9% by volume acetonitrile (other conditions as stated above) (■) **2d**.

Table 1. Rate Constants for the Decomposition of α-Hydroxydialkylnitrosamines in Aqueous Solution, 25 °C, Ionic Strength 1 M ($NaClO_4$)

compd	k_1 (s^{-1})	$k_2 \times 10^9$ ($M^{-1}\,s^{-1}$)	$k_3 \times 10^3$ ($M^{-1}\,s^{-1}$)
2a[a]	0.0016	3.6	4.8
	(0.0002)	(0.3)	(1)
2b[a]	0.016	430	1900
	(0.002)	(40)	(400)
2c[a]	0.014	540	
	(0.004)	(60)	
2d[b]	0.125	20100	48000
	(0.007)	(500)	(1500)

[a] 4% acetonitrile by volume, standard errors in parentheses. [b] 9% acetonitrile by volume, standard errors in parentheses.

parameters included in Table 1.[11] In the case of **2d**, the pH independent term, k_1, contributes to the value of k_0 over a very narrow range of pH, but the nonzero value of k_1 in this case is indicated by the fact that a rate law containing only the terms k_2 and k_3 predicts minimum values of k_0 that are smaller than those observed by factors of 2 − outside the experimental uncertainty in k_0.

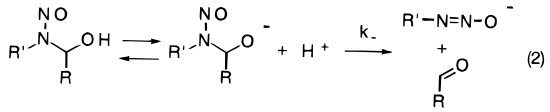
$$k_0 = k_1 + k_2/[H^+] + k_3[H^+] \qquad (1)$$

Inspection of the rate constants in Table 1 shows that the effects of structure on reactivity are appreciable. Mochizuki and co-workers earlier demonstrated[4,6] that reactivity was essentially independent of chain length for the hydroxymethyl (R = H, Scheme 1) series where R′ = Me, Et, *n*-Pr, *n*-Bu, so that the differences in reactivity in Table 1 are mainly due to the differences in R (Scheme 1). Thus the replacement of H in **2a** by CH_3 or Ph gives relative reactivities for H/CH_3/Ph of ∼1/10/80, 1/120/5600, and 1/400/10000 for k_1, k_2, and k_3, respectively.

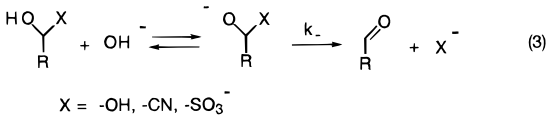
(11) The values in Table 1 were determined by a commercially available nonlinear least-squares fitting program, Enzfitter[R].

(12) A difference in pK_a of ∼0.2 units is calculated from the value for the ionization of alcohol of $\rho^* = -1.42$. Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795.

The absence of general acid/base catalysis in the region where k_2 is dominant suggests that the mechanism for k_2 involves unassisted rate-limiting decay of the conjugate base, eq 2.



Comparing the compounds with R = H and R = Ph, the latter compound is likely to be more acidic by not more[12] than 0.5 pK_a units, analogous to the difference estimated for the formaldehyde[13,14] and benzaldehyde[15] hydrates; thus, most of the 5600-fold difference in reactivity between **2a** and **2d** lies in the ≥2000-fold larger value for k_-, eq 2, for **2d**. The differences in k_- are larger by factors of ∼400, 100, and 2 than the ratios of k_- for the anionic sulfite, hydroxide, and cyanide adducts, respectively, of benzaldehyde and formaldehyde, eq 3.[16−18] However, the factor of ≥2000 is smaller than the ratios of 10^5−(5×10^6) (M^{-1} or unitless), for benzaldehyde compared to formaldehyde, of the equilibrium constants for addition of H_2O,[19,20] HCN,[18] and HSO_3^-.[15]



The similar values of k_2 for **2b** compared to **2c** indicate that greater relief of steric crowding in the k_- transition state cannot explain the differences in reactivity between R = H and Ph for the reaction in eq 2 compared to those in eq 3. The differences in the effect upon k_- of phenyl-for-hydrogen exchange between the reactions in eqs 2 and 3 are presumably due to a later transition state, with more aldehyde character, in the reaction in eq 2 compared to that in eq 3.

The data reported here indicate that there are large effects of structural changes on the lifetimes and thus the diffusibility of the alkylating equivalents in α-hydroxydialkylnitrosamines: the half-life at a physiological pH of 7.4, at which the k_2 process is dominant, is ∼9 s for **2a**, while it is ∼2 ms for **2d**. The mechanisms for the reactions k_1 and k_3 remain uncertain.

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(16) The value for k_- for the formaldehyde hydrate anion is taken from ref 13. A range for k_- from the benzaldehyde hydrate anion can be derived from $\rho = 0.5$ for the second-order rate constant for hydroxide ion catalyzed decomposition of substituted benzaldehydes, ref 14, and the pK_a of the hydrate from ref 15 and from $\rho = 0.1$ for decomposition of the substituted benzaldehyde hydrate anions in ref 14. The values for k_- from the dianionic sulfite adduct are from ref 17. Those for k_- from the anionic cyanide adduct are from ref 18 except in the case of the benzaldehyde adduct for which the conjugate acid pK_a was recalculated as in ref 15 above.

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