Pergamon

Tetrahedron, Vol. 53, No. 31, pp. 10671-10676, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00687-X

Secondary α-Deuterium Kinetic Isotope Effects in the Decomposition of Simple α-Acetoxydialkylnitrosamines: Nitrosiminium Ion Intermediates

Hongliang Cai and James C. Fishbein*

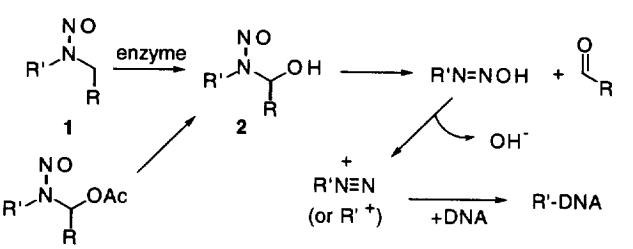
Dept. of Chemistry, Wake Forest University,

Winston-Salem, North Carolina, USA, 27109

Abstract: Secondary α-deuterium kinetic isotope effects for the pH independent decay of two (N-nitrosoalkylamino)methylacetates (**4**) and two (N-nitrosoalkylamino)propyl acetates (**5**) have been determined at 52 °C in aqueous solution, ionic strength 1 M. In all cases the isotope effects are $k_{obsd}^{H}/k_{obsd}^{D}$ ~ 1.1 - 1.2 (per hydrogen). It is concluded that the direction and magnitude of the isotope effects are inconsistent with mechanisms involving rate limiting reaction at the carbonyl group; and, are consistent with the formation of N-nitrosiminium ions in, or prior to, the rate limiting step.

Introduction

Dialkylnitrosamines (**1**, Scheme 1) are mutagenic and/or carcinogenic because they are believed to undergo metabolic activation to form relatively unstable α-hydroxydialkylnitrosamines (**2**). These decompose to ultimately yield diazonium ions that directly or ultimately alkylate DNA (Scheme 1).[1] α-Acetoxydialkylnitros-

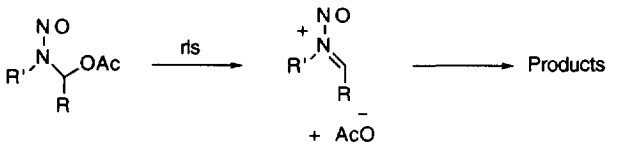


1 **2** **3**

Scheme 1

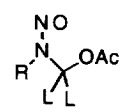
amines (**3**, Scheme 1) are widely used in the study of nitrosamine carcinogenesis as agents that likely generate α-hydroxynitrosamines in vitro and in vivo (Scheme 1).[1] The chemistry of α-acetoxydialkylnitrosamines is thus of significant practical interest.

A recent publication on the mechanism of decomposition of α-acetoxydialkynitrosamines summarized kinetic, structure-reactivity and product studies that ruled out a number of mechanisms and concluded that the mechanism for the pH-independent solvolysis involves formation of a N-nitrosiminium cation as in eq. 1(rls = rate limiting step).[2] Secondary α-deuterium kinetic isotope effects are sensitive probes of mechanism



(1)

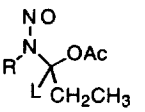
and transition state structure that are potentially capable of differentiating between the nitrosiminium ion mechanism of eq 1 and certain alternatives.[3] Thus, an analysis of secondary α-deuterium kinetic isotope effects in the present reaction that might complement the previous analysis appeared warranted. The kinetic effects of isotopic substitution have been determined for the four compounds, below (L = H, D) In summary, the



4

a: R = i-Pr

b: R = n-Bu



5

a: R = iPr

b: R = n-Bu

direction and magnitude of these isotope effects are consistent with mechanisms involving formation of N-nitrosiminium ions in, or prior to, the rate limiting step and are inconsistent with some other alternatives that were rejected in the previous study. These appear to be the first α-deuterium kinetic isotope effects reported involving formation of iminium ions in, or prior to, the rate-limiting step.

Experimental

Chemicals were standard ACS, reagent grade or better. In the cases of acetic acid and triethylamine, further purification by distillation was carried out. The solvent CH_2Cl_2 was dried by distillation from CaH_2. Water, for analytical procedures and kinetics, was distilled in glass.

Synthesis. The protiated and deuteriated methyl acetates, 4-H_2 and 4-D_2, respectively, were synthesized from the imines (cyclic trimer forms) as described previously.[2] The deuteriated cyclic trimer form of the imine required for 4a-D_2 and 4b-D_2 was synthesized using an aqueous CD_2O solution purchased (99% isotopic purity) from Cambridge Isotope Labs (Cambridge, Mass.). The ^{1}H-NMR of the product 4a-D_2, 4b-D_2 and 5a-D indicated no signal for protium at the region of chemical shift where the α-methyl protons appear so that the product contained less than 3% protium in the α position.

(N-nitroso-butylamino) methyl acetate (4a-H). Synthesis and analytical data were published previously.[2]

(N-nitroso-isopropylamino) methyl acetate (4b-H). Synthesis and analytical data were published previously.[2]

(N-nitroso-isopropylamino) methyl acetate (4a-D_2). This material was made following the procedures previously published.[2] ^{1}H-NMR ($CDCl_3$): δ E isomer (68%) 1.15, (d, 6H); 2.10, (s, 3H); 4.85, (m, 1H); Z isomer (32%) 1.45, (d, 6H); 2.05, (s, 3H); 4.85, (m, 1H). ^{13}C-NMR ($CDCl_3$): δ E isomer (68%) 169.8; 72.7; 44.4; 20.9; 19.1; Z isomer (32%) 170.0; 61.1; 54.7; 20.6; 21.9.

(N-nitroso-butylamino) methyl acetate (4b-D_2). ^{1}H-NMR ($CDCl_3$): δ E isomer (86%) 0.90, (t, 3H); 1.28, (m, 2H); 1.45, (m, 2H); 2.13, (s, 3H); 3.55, (t, 2H); Z isomer (14%) 1.00, (t, 3H); 1.45, (m, 2H); 1.78, (m, 2H); 2.05, (s, 3H); 4.25, (t, 2H). ^{13}C-NMR ($CDCl_3$): δ E isomer (86%) 170.4; 43.3; 28.6; 21.0; 20.4; 13.7; Z isomer (14%) 166.4; 52.0; 30.9; 20.7; 19.8.

(N-nitroso-isopropylamino) propyl acetate (5a-H). ^{1}H-NMR ($CDCl_3$): δ E isomer (77%) 1.00, (t, 3H); 1.14, (d, 3H); 1.21, (d, 3H); 2.12, (m, 2H); 2.30, (s, 3H); 4.81, (m, 1H); 6.63, (t, 1H); Z isomer (23%) 0.88, (t, 3H); 1.55, (d, 3H); 1.59, (d, 3H); 1.75, (m, 2H); 2.09, (s, 3H); 4.28, (m, 1H); 7.05, (t, 1H). ^{13}C-NMR ($CDCl_3$): δ E isomer (77%) 169.6; 83.4; 45.3; 26.2; 21.0; 18.90; 18.87; 9.4; Z isomer (23%) 168.7; 74.9; 49.9; 24.6; 23.84; 23.73; 20.6; 8.7. Anal. calcd: C, 51.05; H, 8.57; N, 14.88. Found: C, 51.18; H, 8.47; N. 14.75.

(N-nitroso-butylamino)propyl acetate (5b-H). ^{1}H-NMR ($CDCl_3$): δ E isomer (92%) 0.92, (t, 3H); 0.98, (t, 3H); 1.30, (m, 4H); 2.20, (s, 3H); 2.21, (m, 2H); 3.27, (m, 1H); 3.70, (m, 1H); 6.90, (t, 1H); Z isomer (8%) 1.81, (m, 4H); 4.05, (m, 2H); 6.98, (t, 1H); other peaks are either overlapped with proton peaks of the E isomer or undetectable. ^{13}C-NMR ($CDCl_3$): δ E isomer 169.4; 86.6; 42.1; 29.0; 25.6; 20.9; 20.5; 13.6; 9.2. Anal. Calcd: C, 53.45; H, 8.97; N, 13.85. Found: C, 53.53; H, 8.91; N, 13.81.

(N-nitroso-isopropylamino) propyl acetate (5a-D). ^{1}H-NMR ($CDCl_3$): δ E isomer (77%) 1.00, (t, 3H); 1.14, (d, 3H); 1.21, (d, 3H); 2.12, (m, 2H); 2.30, (s, 3H); 4.81, (m, 1H); Z isomer (23%) 0.88, (t, 3H); 1.55, (d, 3H); 1.59, (d, 3H); 1.75, (m, 2H); 2.09, (s, 3H); 4.28, (m, 1H). ^{13}C-NMR ($CDCl_3$): δ E isomer (77%) 169.6; 45.3; 26.2; 21.0; 18.90; 18.87; 9.4; Z isomer (23%) 168.7; 24.6; 20.6; 8.7.

(N-nitroso-butylamino) propyl acetate (5b-D) (92% deuteriated). ^{1}H-NMR ($CDCl_3$): δ E isomer (90%) 0.92, (t, 3H); 0.98, (t, 3H); 1.30, (m, 4H); 2.20, (s, 3H); 2.21, (m, 2H); 3.27, (m, 1H); 3.70, (m, 1H); 6.90, (m, 1H); Z isomer (10%) 1.81, (m, 4H); 4.05, (m, 2H); other peaks are either overlapped with proton peaks of the E isomer or undetectable. ^{13}C-NMR ($CDCl_3$): δ E isomer 169.4; 86.6; 42.1; 29.0; 25.6; 20.9; 20.5; 13.6; 9.2.

The deuteriated imine required for 5-D was synthesized as described earlier[4] using deuteriated aldehyde that was synthesized by minor modification of the Nef reaction[5] of nitropropane.

Nitropropane-1,1-D_2. A mixture of nitropropane (50 ml.) and deuterium oxide (50 ml.) containing 3 drops of NaOD (40 wt. % solution in D_2O) was refluxed for two days. Then the layer of deuteriated nitropropane was separated and distilled off. The yield of colorless liquid was 26 ml. The product was 80% deuterated by ^{1}H-NMR analysis. ^{1}H-NMR ($CDCl_3$): δ 1.02, (t, 3H); 2.03, (q, 2H); 4.35, (m, 2H).

Propanal-1-D. Nitropropane-1,1-D_2 (26 ml.) was dissolved in 290 ml. of ice-cold 15% sodium hydroxide in a seperatory funnel. The solution was added slowly dropwise to a beaker containing 53.2 ml. sulfuric acid dissolved in 355 ml. water. The sulfuric acid solution was cooled in an ice bath and stirred continuously while the solution of nitropropane-1,1-D_2 was added. After the addition was completed, the reaction was stirred an additional 20 min. The product, propanal-1-d (92% D) was purified by distillation. ^{1}H-NMR ($CDCl_3$): δ 1.11, (t, 3H); 2.47, (q, 2H); 7.29, (t, 1H).

Kinetics. Kinetic runs were carried out in 3 ml of aqueous solution in a quartz cuvette stoppered with a rubber septum. The kinetics of decay were monitored at 240 nm Hewlett Packard 8452A, Milton Roy 3000, or Milton Roy 1001$^+$ spectrophotometers all of which were thermostated by circulating water baths. For compounds 4a-H_2, 4b-H_2, 4a-D_2, 4b-D_2 , 5a-H, 5b-H and 5a-D the rate constants were determined by best fit

of the absorbance decay curve to a single exponential decay expression. In the the case of the propylacetate (5b-D), the compound was contaminated with 8% protium as indicated by ^{1}H-NMR. The rate constant for decay of the deuteriated material was determined by best fit of the absorbance decay curve to the expression in eq 2 in which f_1 and f_2 were the fractions of protiated and deuteriated material, respectively, as indicated by ^{1}H-NMR

$$A = f_1DA(e^{-k_Ht}) + f_2DA(e^{-k_Dt}) + C \quad (2)$$

and k_H was the value of the rate constant for the pure protiated compound.

Results and Discussion

Rate constants for decay of the α-acetoxydialkylnitrosamines **4** and **5** were measured in 0.05 M cacodylic acid buffer, pH = 6.31. The mean value for each compound is reported in Table 1, in which k_{obsd}^H and k_{obsd}^D are for the protiated and deuteriated compounds, respectively. Control experiments indicated that, as reported previously[2], the rate constants k_{obsd}^H were essentially independent of the cacodylic acid buffer concentration. The value of k_{obsd}^H increased less than 3% above the rate constant extrapolated to zero buffer concentration as buffer concentration was varied from 0.050 to 0.20 M. Further, the rate constants k_{obsd}^H are independent of pH at this pH and temperature. This is indicated by the fact that the buffer independent rate constants[2] determined at 52 °C from experiments at pH = 5.7 and 7.5 were within ±5% of the value determined at pH = 6.31.

Table 1. Secondary α-Deuterium Kinetic Isotope Effects on the Solvoloysis of α-Acetoxydialkylnitrosamines in Aqueous Solutions, 52 °C, Ionic Strength 1M ($NaClO_4$), 0.05 M Cacodylic Acid Buffer (50% anion).

Coumpound	$10^4 \times k_{obsd}^H$ sec^{-1} (σ_{n-1})	$10^4 \times k_{obsd}^D$ sec^{-1} (σ_{n-1})	k_{obsd}^H/k_{obsd}^D per hydrogen (σ_{n-1}')[a]
4a	1.65[b]	1.17[b]	1.19
	(0.020)	(0.015)	(0.021)
4b	0.524[b]	0.388[b]	1.16
	(0.0035)	(0.0030)	(0.012)
5a	88.1[b]	75.8[b]	1.16
	(1.12)	(0.94)	(0.021)
5b	71.9[c]	63.2[c]	1.14
	(0.32)	(0.46)	(0.0097)

a. σ_{n-1}' is calculated as $\sigma_{n-1}' = [\sqrt{[((\sigma_{n-1})^H/k_{obsd}^H)^2 + ((\sigma_{n-1})^D/k_{obsd}^D)^2]}]*k_{obsd}^H/k_{obsd}^D$.

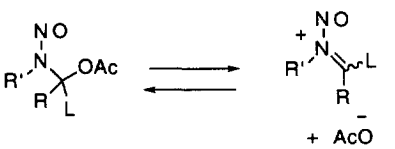
b. Based on three experiments.

c. Based on five experiments.

Inspection of the rate constants for the protiated compounds, k_{obsd}^{H}, in Table 1 indicates that the rate constants for the compounds **4** are smaller than those for the compounds **5** by factors of between 20 and 150. The large increase in reactivity upon replacement of a hydrogen by an alkyl group at the central carbon has been noted previously.[2]

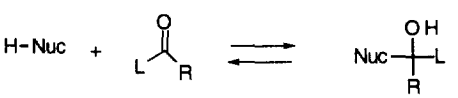
The secondary α-deuterium kinetic isotope effects measured for the decay of the four α-acetoxy-nitrosodialkylamines are summarized in Table 1. Each value is based on three or five rate constant determinations from runs of cuvettes containing alternately protiated and deuteriated compounds that were monitored sequentially in cycles. The secondary α-deuterium isotope effects, $k_{obsd}^{H}/k_{obsd}^{D}$ (Table 1), are in all cases greater than 1.0, outside experimental error.

The direction and magnitude of the α-secondary deuterium isotope effects observed in this study are consistent with the rate limiting formation of N-nitrosiminium cations. It is expected that the change in zero-point energy difference between protium and deuterium in going from the sp^3-hybridized starting material to the sp^2-hybridized iminium ion should manifest a "normal" ($k_H/k_D > 1$) effect.[6] In the case of reactions involving iminium ions specifically, there is little experimental evidence concerning this expectation. It is, however, confirmed by the inverse α-deuterium secondary isotope effects of $k_H/k_D \sim 0.83$ on the rate constants for hydroxide ion and water attack of benzylidene iminium ions that entails the microscopic reverse, sp^2 to sp^3, of the transformation in the present study.[7] The maximum effect expected, that for the equilibrium in eq 3, is unknown and neither theoretical nor experimental values for the equilibrium formation of other iminium ions



(3)

appear to have been determined. A reasonable model, about which there has been considerable investigation, is the isotope effect on the equilibrium formation of tetrahedral adducts from aldehydes, eq. 4 (H-Nuc = protonated nucleophile), which ranges between $K_H/K_D = 1.25 - 1.4$.[8] A similar range, with perhaps a slightly higher



(4)

upper bound,[9] may be expected to include the value for the effect on the equilibrium in eq 3, so that the values in Table 1 of $k_H/k_D > 1.1$ are consistent with significant rehybridization at the carbon atom of the nascent iminium ion in the mechanism of eq 1.

The magnitudes of the isotope effects in Table 1 are inconsistent with a rate limiting reaction at the carbonyl group of the ester for which a small or negligible isotope effect is expected. The isotopic substitution is in a remote, γ, position relative to the site of reaction in such a mechanism and the position is isolated by saturated atoms. Thus the effect of isotopic substitution upon the rate of such a reaction is expected to be negligible.[9] β-Deuterium isotope effects on ionization equilibria, manifesting the effectively electropositive

character of deuterium relative to protium are small, typically $K_H/K_D \leq 1.05/D$, by comparison to the effects summarized in Table 1.[9,10] γ-Deuterium isotope effects are expected to be still smaller.

This conclusion, based on the secondary α-deuterium kinetic isotope effects, is consistent with the previous rejection of a "carbonyl attack" mechanism that was deduced from structure-reactivity effects, activation parameters and the trapping of a putative N-nitrosiminium ion intermediate by azide ion.[2]

There may be a difference in the isotope effects for the reactions of the methyl acetates compared to the propyl acetates - the isotope effect for **4a** is larger than that for **5a** and that for **4b** is likewise larger than that for **5b**. However, the similarity in magnitude of the isotope effect for **4b** and **5a** suggests the data may be of too limited accuracy to be certain. This possible difference is not inconsistent with the possibilities that there are differences either in the transition states or the rate-limiting step for methyl acetates compared to the propyl acetates, as was suggested previously for the methyl and ethyl acetates on the basis of differences in structure reactivity correlations.[2]

This work was supported by grants R01 CA52881 and K04 CA62124 from the National Cancer Institute of the National Institutes of Health.

References

1. Lawley, P.D, in *Chemical Carcinogens*; Searle, C.D., ed.; ACS Monograph 182, American Chemical Society; Washington, D.C, **1984**. Lijinsky W., Chemistry and Biology of N-nitroso Compounds, Cambridge University Press, Cambridge, **1992**.

2. Rajamaki, M.; Vigroux, A.; Chahoua, L.; Fishbein, J.C.*J. Org. Chem.* **1995** *60*, 2324. Revis, C.; Rajamaki, M.; Fishbein, J.C. *J. Org. Chem.* **1995**, *60* 7733.

3. *Isotopes in Organic Chemistry*, vol. 7, Secondary and Solvent Isotope Effects, E. Buncel, C.C. Lee, eds. **1987**, Elsevier, New York

4. Rajamaki, ref. 2.

5. Noland, W.E. *Chem. Revs.* **1955** *55*, 137. Leitch, L.C. *Can. J. Chem.* **1955** *33*, 400.

6. Westaway, K.C. in ref. 3.

7. Archila, J.; Bull, H.; Lagenaur, C.; Cordes, E.H. *J. Org. Chem.* **1971** *36*, 1345.

8. do Amaral, L.; Bull, H.G.; Cordes, E.H. *J. Am. Chem. Soc.* **1972** *94*, 7579. do Amaral, L.; Bastos, M.P.; Bull, H.G.; Cordes, E.H. *J. Am. Chem. Soc.* **1973** *95*,7369. Hogg, J.L.; Rodgers, J., Kovach, I.; Schowen, R.L. *J. Am. Chem. Soc.* **1980** *102*, 79. Young, P.R.; McMahon, P.E. *J. Am. Chem. Soc.* **1979** *101*,4679.

9. Melander, L.; Saunders, W.H. *Reaction Rates of Isotopic Molecules*, **1987**, p. 197. R.E. Krieger, Florida

10. Northcott, D.; Robertson, R.E. *J. Phys. Chem.* **1969** *73*, 1559. Van der Linde, W.; Robertson, R.E. *J. Am. Chem. Soc.* **1964** *86*, 4505. Bary, Y.; Gilboa, H.; Halevi, E.A. *J. Chem. Soc. Perkin Trans. 2* **1979**, 938. Paobo, M.; Bates, R.G.; Robinson, R.A. *J. Phys. Chem.* **1966** *70*, 540.

(*Received in USA* 15 *April* 1997; *revised* 2 *June* 1997; *accepted* 6 *June* 1997)