The Solvolysis of *N*-Acetoxy-1-*N*-acetylaminonaphthalene and *N*-Acetoxy-2-*N*-acetylaminonaphthalene: Divergent Chemistry and Mutagenic Activity

Graham R. Underwood* and Catherine M. Davidson

Department of Chemistry, New York University, Washington Square, New York, New York 10003, U.S.A.

Upon solvolysis in neutral 40% aqueous acetone, *N*-acetoxy-1-*N*- and *N*-acetoxy-2-*N*-acetylaminonaphthalene exhibit completely different modes of reaction; the former reacts with nitrenium ion formation, while the latter undergoes acyl oxygen scission.

2-Naphthylamine is a potent carcinogen and mutagen; 1-naphthylamine is not.[1] Scribner *et al.*[2] have reported that the ultimate metabolites derived from these amines, namely the *N*-acetoxy-*N*-arylacetamides undergo dissociation to nitrenium ions with considerable ion pairing. Our isotopic labelling studies were in conflict with this proposal however.[3] Moreover, several workers have found acetylation, not arylamidation, of macromolecules by these agents, suggesting that alternative modes of reaction may be of importance.[4] We now report fundamental differences in the chemical behaviour of these two metabolites in aqueous solution at pH 7.

The title compounds (**1**) and (**2**) were solvolysed at 40.00 °C in acetone–water (60:40) buffered to various pH values between 5 and 10 and at an ionic strength of 0.25 M, maintained by $NaClO_4$. Kinetic data were obtained by monitoring the loss of starting material by h.p.l.c. The reactions all followed good pseudo-first-order kinetics for at least four half-lives (correlation coefficient >0.997). All pseudo-first-order rate constants could be fitted to the kinetic expression (1).

$$k_{obs.} = k_0 + k_1[OH^-] + k_2[\text{buffer}] \qquad (1)$$

These rate constants are listed in Table 1; the rates of reaction become practically pH-independent at pH $\leqslant$ 7. Because an earlier study[2] claimed the appearance of special salt effects under precisely these conditions (same substrate, solvent, temperature, and buffer, but monitoring kinetics by appearance of 'water soluble radioactivity'), we have carried out rigorous multiple linear regression analyses using the statistical method of sequential f-test[5] to demonstrate that there is only a *linear* dependence of the pseudo-first-order rate constants on the species involved. In the pH-independent region, it was possible to carry out the reactions in the absence of buffer and $NaClO_4$ and thereby demonstrate the complete lack of special and normal salt effects for the process described by k_0.

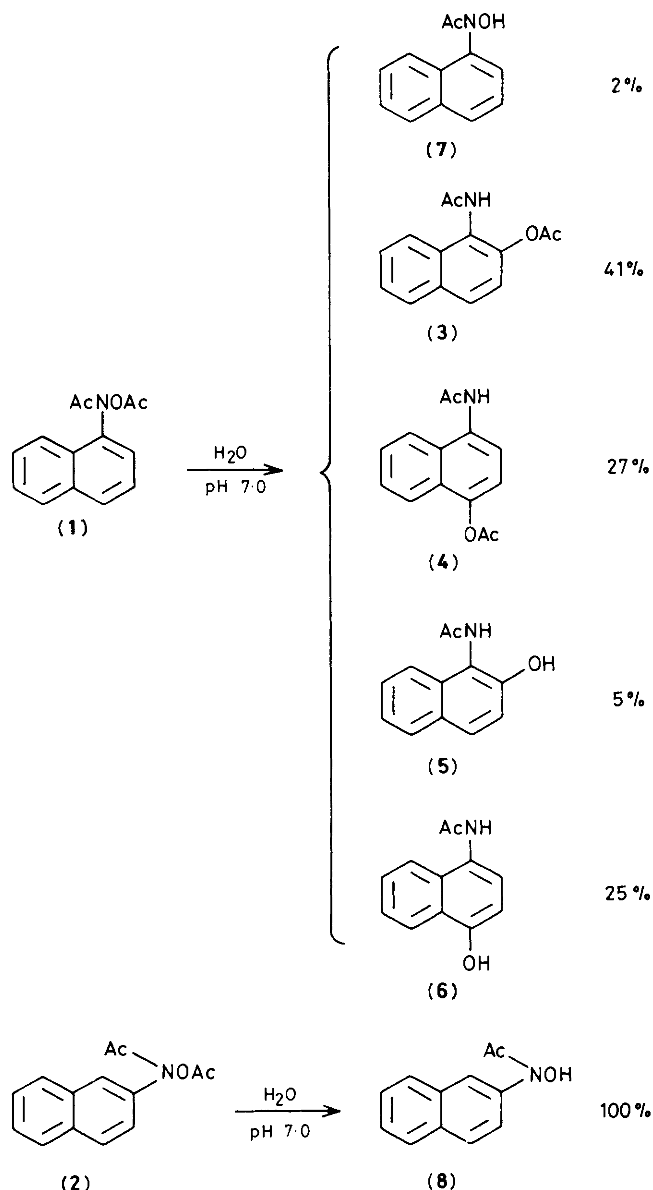
For compound (**2**), the sole products were the hydroxamic acid (**8**) and acetic acid, irrespective of the pH. When the reaction was carried out in acetone–[^{18}O]water, the isotopic label was incorporated into the acetic acid alone; none was found in the hydroxamic acid. Thus the reaction occurred with exclusive cleavage of the carbonyl–oxygen bond, evidently by a typical ester hydrolysis. Starting material recovered from this reaction after 2 half-lives showed no uptake of the label.

For (**1**), an array of products, (**3**)—(**7**), was formed. In the pH-independent region, the hydroxamic acid (**7**), although detectable, accounted for less than 2% of the products. As the

Table 1. Rate constants for the solvolysis of the *N,O*-diacetyl-*N*-naphthylhydroxylamines (**1**) and (**2**) in acetone-water (60:40) over the pH range 5—10.

Compound	$10^5 \times k_0$/s^{-1}	k_1/dm^3 mol^{-1} s^{-1}	$10^3 \times k_2$/dm^3 mol^{-1} s^{-1}
(**1**)	1.90	1.72 ± 0.09	1.75 ± 0.75[a]
(**2**)	0.15	2.16 ± 0.07	6.42 ± 0.81[a]

[a] For borate only (0.01—0.10 M). Participation by acetate, succinate, HPO_4^{2-}, and $H_2PO_4^-$ was shown to have no kinetic significance up to 0.25 M.



Scheme 1

pH of the reaction medium was increased, so did the proportion of hydroxamic acid. By comparing the product composition with the rate increase it was shown that this hydroxide-dependent process also leads exclusively to hydroxamic acid ($98 \pm 2\%$).

The products (**3**)—(**6**) are typical of those expected for a reaction taking place *via* nitrenium ion intermediates.[6] This reaction was therefore examined in somewhat more detail. When the reaction was carried out in the presence of acetate buffers (up to 0.25 M), no common-ion rate depression was observed. In the presence of [$^2H_3$]acetate buffer (0.25 M), only a small amount (<4%) of the products (**3**) and (**4**) contained [$^2H_3$]acetate, and none was found in the recovered starting material. When the starting material was labelled with oxygen-18 specifically in the acetate carbonyl, starting material recovered after two half-lives of reaction showed no scrambling of the label, but the produced (**3**) and (**4**) showed complete scrambling between the pertinent acetate oxygen atoms. Although the acetates (**3**) and (**4**) are hydrolysed slowly under the reaction conditions, the phenols (**5**) and (**6**) are the primary reaction products. This was demonstrated by extrapolation of the ratios [(**5**)]/[(**3**)] and [(**6**)]/[(**3**)] to zero reaction time.

These results clearly indicate a major difference between the reactions of (**1**) and (**2**). This is illustrated in Scheme 1. It is interesting that the derivative (**1**) of the biologically inactive 1-naphthylamine leads, at neutral pH, to nitrenium ion intermediates, while derivative (**2**) of 2-naphthylamine undergoes hydrolysis and acyl–oxygen scission. The corresponding derivative of carcinogenic 4-aminobiphenyl also shows chemical behaviour similar to that of (**2**).[7] It is perhaps relevant that evidence is now beginning to accrue[8,9] that the critical adduct between DNA and 2-aminofluorene is devoid of the *N*-acetyl group. This could formally be regarded as having resulted from acyl–oxygen scission to produce hydroxamic acid, followed by N→O acyl transfer prior to covalent binding of the *N*-acetoxy-arylamine to DNA.

We are indebted to the National Cancer Institute of the National Institutes of Health and to the Department of Energy for Grants in support of this research.

Received, 2nd July 1984; Com. 932

References

1 J. L. Radomski, *Annu. Rev. Pharmacol. Toxicol.*, 1979, **19**, 129; W. C. Hueper, F. H. Wiley, H. D. Wolfe, K. E. Ranta, M. F. Leming, and F. R. Blood, *J. Ind. Hyg. Toxicol.*, 1938, **20**, 46; J. L. Radomski, W. B. Deichman, N. H. Altman, and T. Radomski, *Cancer Res.*, 1980, **40**, 3537.
2 J. D. Scribner, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1970, **30**, 1570.
3 C. M. Scott, G. R. Underwood, and R. B. Kirsch, *Tetrahedron Lett.*, 1984, 499.
4 J. D. Scribner, N. K. Scribner, D. L. Smith, E. Jenkins, and J. A. McCloskey, *J. Org. Chem.*, 1982, **47**, 3143; E. J. Barry and H. R. Gutmann, *J. Biol. Chem.*, 1973, **248**, 2730; R. B. Kirsch, M. L. Jacobsen, R. Shapiro, and G. R. Underwood, unpublished results.
5 N. R. Draper and H. Smith, 'Applied Regression Analysis,' Wiley, New York, 1966.
6 G. Kohnstam, W. A. Petch, and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1984, 423; P. G. Gassman and J. E. Granrud, *J. Am. Chem. Soc.*, 1984, **106**, 1498.
7 G. R. Underwood and R. B. Kirsch, *Tetrahedron Lett.*, in the press.
8 C. E. Weeks, W. T. Allaben, N. M. Tresp, S. C. Louie, E. J. Lazear, and C. M. King, *Cancer Res.*, 1980, **40**, 1204.
9 B. N. Ames, E. G. Gurney, J. A. Miller, and H. Bartsch, *Proc. Natl. Acad. Sci., USA*, 1972, **69**, 3128; D. Beranek, G. White, R. Heflich, and F. A. Beland, *ibid.*, 1982, **79**, 5175; J. D. Scribner, S. R. Fisk, and K. N. Scribner, *Chem. Biol. Interactions*, 1979, **26**, 11.