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ON THE SOLVOLYSIS OF N-ACETOXY-N-ACETYLAMINOBIPHENYL: THE COMPLETE LACK OF NITRENIUM ION INVOLVEMENT.

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Abstract: The solvolysis of N-acetoxy-4-acetylaminobiphenyl in 40% buffered aqueous acetone has been studied. Two processes have been identified, both leading exclusively to the formation of hydroxamic acid. By the use of O-18 labeling, both have been shown not to involve the intermediacy of nitrenium ions.

The ultimate carcinogen derived from aryl amines and amides is generally regarded as being a nitrenium ion(1). Support for the involvement of such a species derives partly from the nature of the products formed in vivo and in vitro(2), and partly from kinetic studies(3,4). Product studies clearly demonstrate that reaction with DNA takes place at nucleophilic sites, but give absolutely no information regarding the nature of the reactive species. Mechanistic studies have been limited to two kinetic analyses. In a recent study(4), the methanesulfonate esters of several simple N-hydroxyacetanilides were rearranged in chloroform-d_1. The products, and a Hammett linear free energy relation (rho = -9.24), were clearly indicative of nitrenium ion involvement

However the most frequently employed models for the ultimate carcinogens are the N-acetoxy-N-arylacetamides. The acetate is a far poorer leaving group than is the methanesulfonate and the mode of reaction of these derivatives is far from clear. Novak and Brodeur(5) used spin trapping techniques to provide convincing evidence for the involvement of radicals during the reaction of substituted N-pivaloyloxyacetanilides in benzene.

Scribner et al(3) reported that, in aqueous solution, several carcinogenic N-acetoxy-N-arylacetamides underwent solvolysis with reversible formation of nitrenium ion ion pairs. This conclusion was reached on the basis of a non-linear response of the observed rate constant to added buffer salts. Such behavior was interpreted as a "special salt effect", indicative of the partitioning of an intermediate ion pair between product formation and return to starting material.

On the other hand, we found evidence(6) which was in conflict with the involvement of ion pairs under conditions identical with those employed in in the latter study(3). In particular, we argued that if these model compounds were, in fact, dissociating to ion pairs, then the two oxygen atoms of the acetate ion should become equivalent, and recombination to form the starting material, an absolute requirement of the observation of a special salt effect, should take place equally at either oxygen atom(7,8). By labeling the starting material specifically at one of these oxygens, recovering starting material from the reaction mixture, and analyzing by mass spectrometry, we were able to show that no such return from ion pairs was involved(6).

We now report that the solvolysis of the carcinogen N-acetoxy-4-acetylaminobiphenyl (AAABP) takes place exclusively by yet another pathway, namely by heterolytic cleavage of the acyl-oxygen bond. All reactions were carried out in 40% buffered aqueous acetone at 40°, conditions identical with those employed previously(3,6). The medium was buffered to different pHs between 5.0 and 10.0 using acetate, phosphate and borate buffers. Unless otherwise indicated, the ionic strength was maintained constant at a value of 0.25M with $LiClO_4$ added as necessary. The reaction was monitored by sampling at appropriate intervals and analyzing by high performance liquid chromatography (hplc). Reactions were monitored for at least three half-lives, and were first order with respect to AAABP. Serial dilution of the buffers at several pHs (5.50, 7.00, 7.92, 9.57 and 9.82) revealed only minor participation by buffer ions. A rigorous analysis of the data, however, indicated that there was no statistically-significant curvature of these plots with respect to any of the participants. The apparent nature of the participation by borate ions was complex, but this presumably is due to the polymerization of the buffer under these conditions(9). The rate data for the solvolyses taking place in the presence of acetate and phosphate buffers can be fitted to the equation(10):

$$k_{obs} = k_{H_2O} + k_{OH}[OH] + k_{PO4}[HPO_4^{=}] \quad \text{.....eq.1}$$

The rate constants thus obtained are:

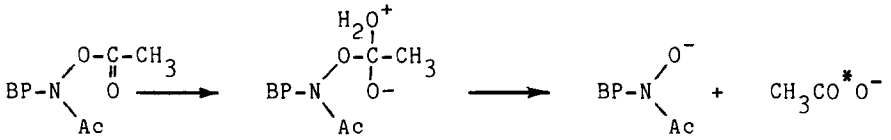
$$k_{H_2O} = (1.49 \pm 0.39) \times 10^{-6} \text{ sec}^{-1}$$
$$k_{OH} = 2.02 \pm 0.05 \text{ M}^{-1}\text{sec}^{-1}$$
$$k_{PO4} = (0.78 \pm 0.15) \times 10^{-5} \text{ M}^{-1}\text{sec}^{-1}$$

The linear dependence of the rate constants on $[HPO_4^{=}]$ suggests that there is no "special salt effect" under these conditions but it must be borne in mind that these reactions were carried out under constant-ionic-strength conditions. It is more difficult to rule out a special salt effect unequivocally because, since the reaction is pH-dependent, it is desirable to

buffer the medium. However, when reactions were carried out in the absence of buffer, the pH, monitored throughout the reaction, remained within the range 5.5 to 6.5. In this range the reaction is essentially pH independent. The addition of lithium perchlorate to these reactions resulted only in a minor normal salt effect ($b = 4 \times 10^{-7}\ M^{-1}sec^{-1}$). Thus, at least for the pH-independent reaction, there is no special salt effect.

The sole products formed at all pHs were acetic acid and the biphenyl acetohydroxamic acid: no phenols, nitrosobiphenyl(11), biphenyl acetamide(12) or rearranged starting material(4) could be detected. The reaction was also carried out in out in H_2O^{18} at pH 7.0, and the products and starting material were collected after one half-life of reaction, and were analyzed by mass spectrometry. No O-18 appeared in the hydroxamic acid, but the produced acetic acid showed essentially complete incorporation of the label. Thus the reaction occurs with exclusive acyl-oxygen cleavage and there is no evidence whatsoever to suggest the involvement of either heterolytic or homolytic cleavage of the N-O bond under these conditions.

The starting material recovered from this reaction was also shown to contain no label, suggesting that the tetrahedral intermediate, presumably involved in this ester hydrolysis, breaks down to products more rapidly than it can undergo symmetrization(13) and return to starting material.



These reactions were carried out under conditions identical with those previously employed(3) from which it was deduced indirectly that nitrenium ions were involved. The fact that nitrenium ions are not involved at all with this compound under these conditions necessitates further investigation of the chemistry of this important class of model carcinogens. In particular any attempts to prepare carcinogen - DNA adducts which necessitate the scission of the nitrogen - oxygen bond must take into account this alterative, dominant and undesirable mode of reaction.

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