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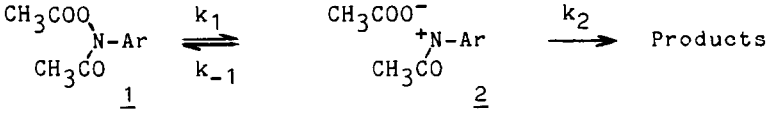
REVERSIBLY-FORMED ION PAIRS ARE NOT INVOLVED DURING THE SOLVOLYSIS OF N,O-DIACETYL-N-ARYLHYDROXYLAMINES

Catherine M. Scott, Graham R. Underwood and Robert B. Kirsch
Department of Chemistry, New York University
Washington Square,New York, New York, 10003

Abstract: Appropriate oxygen-18 labeling experiments demonstrate that N,O-diarylhydroxylamines do not undergo solvolysis *via* the reversible formation of ion pairs. This is in total conflict with the conclusions from previous indirect kinetic studies of these ultimate carcinogen models.

Aryl amines and amides constitute an extensively studied class of chemical mutagens, of which several members are carcinogenic to man[1]. Early studies involving animals showed that tumors usually developed at sites remote from the point of application, indicating that some form of metabolic activation was necessary for the induction of tumors[2]. It was reported[3] in 1960 that a major urinary excretion product of 2-acetylaminofluorene (AAF) was N-hydroxy-AAF and subsequent work[4] showed that this was a more potent carcinogen in the rat than was AAF itself. However, since N-hydroxy-AAF was not chemically reactive *in vitro* it was concluded that further activation was necessary[4]. It is now generally accepted that this subsequent activation involves O-esterification[5], followed by electrophilic attack by the "ultimate metabolite" on critical cellular macromolecules. The actual reacting form of the carcinogen has has been postulated to be a nitrenium ion[6-9], although nucleophilic attack by more stable, nonionic, species does not appear to have been ruled out totally.

Numerous esters have been implicated in this process, but we here we direct our attention to the N,O-diacetyl-N-arylhydroxylamines, which are the most widely studied models. The most direct chemical study of this class of compounds has been reported by Scribner, Miller, and Miller[7]. They monitored the rate of release of water soluble radioactivity, presumably acetate, from several N-acetoxy-(carbonyl-^{14}C)-N-arylacetamides in aqueous acetone. They found that the rate of reaction increased with nucleophile (buffer) concentration in a nonlinear manner, and levelled off at high concentrations. This was interpreted in terms of an ion-paired nitrenium ion, in equilibrium with starting material, being intercepted by the nucleophiles:



1 2

This interpretation, one among many, is predicated on the assumption that the process studied (release of acetate) parallels the rate of disappearance of starting material. However, it is known[12,13] that aryl diacylhydroxylamines rearrange to substituted phenyl acetates (ortho- and para- acetoxyarylacetmides). These compounds might well be expected to be unstable under the reaction conditions, and if they released acetate at a rate comparable with

that of decomposition of starting material, the resultant complex kinetics would render the above interpretation open to question.

The apparent facile generation of these unusual species, 2, with an electron deficient electronegative nitrogen adjacent to an electron withdrawing carbonyl, attracted our attention. Moreover the clear importance of a better understanding of the nature of the putative "ultimate carcinogen" prompted us to make a more direct study of this subject.

We have synthesized several N-acetoxy-N-arylacetamides specifically labelled with oxygen-18 in the ester carbonyl, subjected them to solvolytic conditions closely resembling those employed in the above study, and analyzed the recovered starting materials for location of the isotopic label. Goering and Levy[11] have shown that a carboxylate ion pair, sufficiently separated to be trapped by an external nucleophile, is sufficiently separated to lead to equivalence of the two carboxylate oxygens. This in turn leads to complete scrambling of the label in the starting material.

The appropriately labelled N-acetoxy-N-arylacetamides were prepared from the corresponding hydroxamic acids by treatment with O-18 labelled acetyl chloride. Mass spectral analysis is particularly informative in establishing the location of the isotopic label. The fragmentation patterns of the acetoxyarylacetamides studied are similar and provide three regions of relevance to this study. The molecular ions lose two molecules of ketene stepwise in a process represented by the shorthand:

$$M^+ \longrightarrow (M - K)^+ \longrightarrow (M - 2K)^+$$

The isotopic purity of the labelled starting materials can be obtained from the relative intensities of the molecular and (M + 2) peaks. For convenience we shall designate all fragments containing oxygen-18 with an asterisk (*), so the ratio of the intensities $^*M/(M + {}^*M)$ is a measure of the isotopic enrichment.

The fragmentation of the labelled starting materials could, *a priori*, involve initial loss of either ketene, K, or labelled ketene, $^*K$, depending on the mode of fragmentation. The relative intensities of the $^*(M - K)$ and $(M - K)$ peaks derived from those compounds in which the exact location of the label is known yields this information. Irrespective of the origin of the $(M - K)$ and $^*(M - K)$ peaks, however, the $(M - 2K)$ peaks are derived from loss of both acetyl groups such that the observed ion, $ArNHOH^+$, contains the oxygen originally bound to nitrogen in the molecular ion[14].

The compounds studied are given in table 1 together with the relevant mass spectral data. All compounds were solvolyzed in 40:60 acetone:water mixtures at 40.00° without added buffer[15], after which time the samples were cooled, and immediately chromatographed[16] and analyzed by mass spectroscopy.

From the table it can be seen that the solvolytic conditions result in a negligible decrease in the total amount of label. More important however are the data concerning the location of the label. With the exception of acetoxy acetylaminofluorene, all the labelled materials have essentially the same $^*(M - 2K)/(M - 2K)$ ratios as do the unlabelled compounds showing that no O-18 has been incorporated into the N-O oxygen. For N-acetoxy-AAF, the results are less clear-cut since the $ArNHOH^+$ peak shows the presence of *ca.* 15% of the

label, apparently due to scrambling of the label during synthesis[17]. In spite of this minor difficulty, however, it is clear that no scrambling has taken place during the reaction.

The study reported by Scribner, Miller and Miller[7] ascribed relative values to the rate constants k_{-1} and k_2 for the fluorenyl and biphenylyl systems. These ratios (k_{-1}/k_2) were 19 and 3300 respectively. Such values should lead to practically complete scrambling of the label in the acetate group.

Goering et al.[10] have pointed out that equivalence of the carboxyl oxygen atoms in the ion pair "provides a useful operational criterion for ionization because, in systems of this type, ionization that does not result in the randomization of the carboxyl oxygen atoms cannot be detected by present methods."

These results are therefore totally incompatible with reversibly-formed ion pairs being involved under these conditions, and an alternative explanation must be sought for the unusual kinetic behavior referenced above.

TABLE 1:

Apparent percentages of O-18 in relevant m.s. fragments of acetoxy aryl-acetamides. The significance of the M^+, $(M - K)^+$ and $(M - 2K)^+$ peaks is described in the text.

	PERCENTAGE OF O-18 IN:		
	$(M - 2K)^+$	$(M - K)^+$	M^+
N-Acetoxy-1-naphthylacetamide:			
Unlabelled	1.25[a]	4.0	2.0
Both carbonyls labelled	3.8	44.4	46.0[b]
Ester carbonyl labelled (3)	3.4	37.4	45.1
Recovered from solvolysis[c] of 3	3.6	37.1	42.0
N-Acetoxy-2-naphthylacetamide:			
Unlabelled	1.2	1.7	2.0
Ester carbonyl labelled (4)	0.5	39.5	49.0
Recovered from solvolysis[d] of 4	1.0	40.0	46.0
N-Acetoxy-2-fluorenylacetamide:			
Unlabelled	2.2	1.7	2.0
Ester carbonyl labelled (5)	15.0	53.0	59.5
Recovered from solvolysis[e] of 5	19.0	52.0	57.5
N-Acetoxy-4-biphenylacetamide:			
Unlabelled	3.0	1.8	2.0
Ester carbonyl labelled (6)	3.0	63.0	60.5
Recovered from solvolysis[f] of 6	3.5	61.0	62.0

a. All uncertainties are less than 1%.
b. A doubly labelled peak of 9% abundance was observed.
c. Solvolyzed for 3 half-lives, 2.5 hr.
d. Solvolyzed for 0.8 half-lives, 16 hr.
e. Solvolyzed for 2.5 half-lives, 5.0 hr.
f. Solvolyzed for 2.0 half-lives, 24 hr.

NOTES AND REFERENCES

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14. We have demonstrated that this ion does not arise from massive rearrangement, at least in the case of the 1-naphthyl derivative. N,O-diacetyl-1-naphthylhydroxylamine labelled with O-18 in both carbonyls was synthesized by treatment of the hydroxylamine with labelled acetyl chloride. The mass spectrum of this compound showed no increase in the ratio $^{*}(M - 2K)/(M - 2K)$ beyond that observed for the unlabelled compound.

15. Under these conditions, the pH, monitored throughout the reaction remained in the range 3.5 to 5.5. Parallel studies, to be reported elsewhere, indicate that the rate constants are pH-independent in this range. Solvolyses of labelled materials, conducted in the presence of buffer, gave identical results , but only the results obtained in the absence of buffer are reported here in order to remove ambiguities which may arise from ion pair interception by buffer ions.

16. Starting material was isolated by hplc over silica gel with methanol-ethyl acetate. By using unreacted starting material, in which the abundance and location of the label were known, we have shown that these conditions do not scramble or leach out the label.

17. The same $^{*}(M - 2K)/(M - 2K)$ ratios were obtained irrespective of sample purity, mass spectral conditions or extent of solvolysis.

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