The change in state between E and E* in the native enzyme may be described as a change in hydration or, more probably, a conformational change of the native enzyme. Although the binding and proton absorption data require that E be the major form when protonated and E* be the major form when unprotonated, the introduction of inhibitor (or substrate) into the system transforms all of the enzyme to EHI^+ (or EHS^+), which explains the pH-independent catalytic steps (k_2 and k_3) in the high pH region. The conformation change thus is related to the binding of small molecules at the enzyme active site but not to the catalytic process *per se*. A pH-dependent intramolecular competitive inhibition of the active site will explain the data on the transformation given both here and elsewhere.[4] Although this (conformational) change drastically affects the activity of chymotrypsin through an inhibition of binding, it does so only in a negative way.

(14) National Institutes of Health Predoctoral Fellow.
(15) The authors thank Professor F. J. Kézdy for assistance with the mathematical formulation.

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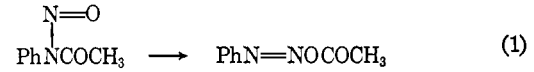
On the Mechanism of Aromatic Arylation with Nitrosoacetanilide

Sir:

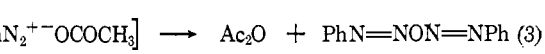
Nitrosoacetanilide (**1**) has long been known as a source of phenyl radicals[1] and has found particular application in studies of homolytic phenylation of aromatic compounds.[2,3] It has been established that the rate-determining step in these reactions is the first-order isomerization (eq 1) leading to benzenediazo acetate.[4] However, the detail of subsequent processes has been less clear. Two major difficulties challenged early mechanistic ideas. First, early schemes show the high yields of acetic acid arising by way of the acetyloxy radical, in spite of the known instability of this intermediate.[5] Second, the initial adduct of phenyl radicals to benzene (*i.e.*, the phenylcyclohexadienyl radical, **2**) is cleanly oxidized to biphenyl, while in other systems disproportionation and dimerization lead, in addition, to hydroaromatic products.[6]

The difficulties referred to have led to suggestions of concerted[4,7] or cage[8] processes. However, a more satisfactory mechanistic interpretation, which has received general acclaim,[9] was advanced recently by Rüchardt and his collaborators[10] and was successfully extended to the Gomberg reaction.[11] In this new scheme, outlined below for the phenylation of benzene, the acetic acid is formed in a nonradical process involving the ion-pair form[12] of the diazoacetate. Furthermore, rapid oxidation of radical **2** by a high stationary-state concentration of the phenyldiazotate radical

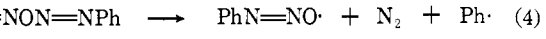

(1)

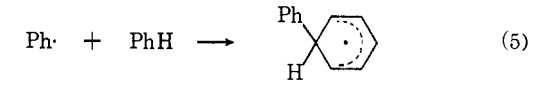

(2)

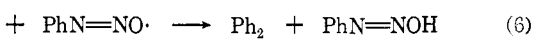
Initiation

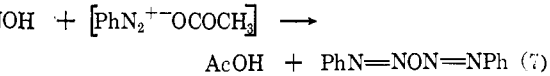

(3)

Major product-forming sequence


(4)

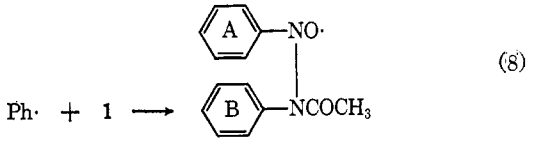

(5)


(6)


(7)

(PhN=NO·) accounts for the failure to observe disproportionation or dimerization products from **2**. This mechanism received added support when a stable radical was detected in the reaction by electron spin resonance (esr),[13] and its spectrum was interpreted in terms of the phenyldiazotate structure (a_N = 1.67, 11.61; a_H = *o*-, −2.60; *m*-, 0.89; *p*-, −2.73). The larger nitrogen splitting has subsequently been assigned to the nitrogen atom bonded to oxygen, by means of ^{15}N labeling experiments.[14]

Our interest[15] in the scavenging of phenyl radicals by C-nitroso compounds (to give nitroxide radicals) led us to propose that the radical detected by Rüchardt and Binsch[13] might, in fact, have structure **3**. Any hyperfine splitting by the protons in ring B may have been too small to have been resolved.


(8)

We have redetermined the esr spectrum of the stable radical from the decomposition of nitrosoacetanilide in benzene (Figure 1) *and have now been able to obtain*

(1) W. S. M. Grieve and D. H. Hey, *J. Chem. Soc.*, 1797 (1934).
(2) See, for example, G. H. Williams "Homolytic Aromatic Substitutions," Pergamon Press, Oxford, 1960, Chapter 4.
(3) R. Ito, T. Migita, N. Morikawa, and O. Simamura, *Tetrahedron*, **21**, 955 (1965).
(4) R. Huisgen and G. Horeld, *Ann. Chem.*, **562**, 137 (1949).
(5) See, for example, C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957, p 493.
(6) D. F. DeTar and R. A. J. Long, *J. Am. Chem. Soc.*, **80**, 4742 (1958); E. L. Eliel, S. Meyerson Z. Welvart, and S. H. Wilen, *ibid.*, **82**, 2936 (1960); D. H. Hey, M. J. Perkins, and G. H. Williams, *J. Chem. Soc.*, 5604 (1963); 3412 (1964); D. I. Davies, D. H. Hey, and M. Tiecco, *ibid.*, 7062 (1966).
(7) R. Huisgen and G. Sorge, *Ann. Chem.*, **566**, 162 (1950).
(8) E. L. Eliel, M. Eberhardt, O. Simamura, and S. Meyerson, *Tetrahedron Letters*, 749 (1962).
(9) E. L. Eliel, J. G. Saha, and S. Meyerson, *J. Org. Chem.*, **30**, 2451 (1965); B. Capon, M. J. Perkins, and C. W. Rees, "Organic Reaction Mechanisms 1965," John Wiley and Sons, Inc., New York, N. Y., 1966, p 154. A related mechanism has been advanced, with little supporting evidence, for homolytic phenylation by N-phenyl-N′-tosyloxydiimide N-oxide: E. A. Dorko and T. E. Stevens, *Chem. Commun.*, 871 (1966).
(10) C. Rüchardt and B. Freudenberg, *Tetrahedron Letters*, 3623 (1964).
(11) C. Rüchardt and E. Merz, *ibid.*, 2431 (1964).
(12) P. Miles and H. Suchitzky, *Tetrahedron*, **18**, 1369 (1962).
(13) G. Binsch and C. Rüchardt, *J. Am. Chem. Soc.*, **88**, 173 (1966).
(14) G. Binsch, E. Merz, and C. Rüchardt, *Chem. Ber.*, **100**, 247 (1967).
(15) G. R. Chalfont, D. H. Hey, K. S. Y. Liang, and M. J. Perkins, *Chem. Commun.*, 367 (1967).

essentially the same spectrum by mixing benzene solutions of nitrosobenzene and N-bromoacetanilide (Figure 2). We consider that the most probable structure for the radical responsible for this spectrum is therefore **3**; in the second reaction this is considered to arise by addition of the nitrogen-centered radical **4** to nitrosobenzene (eq 9).[16] The ^{15}N experiments already cited[14] once more allow assignment of the larger nitrogen coupling in radical **3** to the nitrogen atom bonded to oxygen. The value of 11.7 gauss seems very reasonable for nitrogen in this environment, by analogy with other data on nitroxide radicals.[17]

$$\underset{\mathbf{4}}{\dot{\mathrm{N}}\mathrm{COCH_3}} + \mathrm{PhN{=}O} \longrightarrow \mathbf{3} \qquad (9)$$

Reaction of N-bromo-*p*-chloroacetanilide with nitrosobenzene gives a radical whose esr spectrum is indistinguishable from that of **3**, consistent with the hypothesis of negligible splitting by the ring B protons. Reactions of N-bromoacetanilide with *p*-chloronitrosobenzene, *p*-nitrosotoluene, and 2-methyl-2-nitrosopropane also give stable radical species, the esr spectra of which have been resolved into 50, 42, and 9 lines, respectively.[18] These reactions appear to constitute the first examples of addition of organic nitrogen-centered radicals to the nitroso group, though there are now numerous reports of additions by carbon-[19] and oxygen-centered[20] radicals.

Assuming that structure **3** correctly represents the structure of the stable radical in the nitrosoacetanilide reaction, this species could then adopt the role of oxidant in a modified mechanism such as that outlined below. This finds close analogy with the mechanism proposed for the decomposition of benzoyl peroxide in the presence of diphenyl nitroxide.[15]

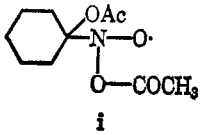
Initiation

$$\mathrm{PhN{=}NOCOCH_3} \longrightarrow \mathrm{Ph\cdot} + \mathrm{N_2} + \mathrm{\cdot OCOCH_3} \qquad (10)$$

$$\mathrm{Ph\cdot} + \mathbf{1} \longrightarrow \mathbf{3} \qquad (8)$$

(16) The esr spectrum developed on mixing the reagents, though photolysis of the bromo amide by stray laboratory light may have been instrumental in radical production. At room temperature, the presence of silver powder did not increase the radical concentration. In a control experiment, no radical spectrum was formed from nitrosobenzene and molecular bromine. Spectra were recorded on a modified Varian V4502 spectrometer generously placed at our disposal by Dr. G. R. Wilkinson of the Physics Department of this college. We are also indebted to Dr. Yvonne Rees for a helpful appraisal of various sources of phenylacetamido radicals.

(17) For example, compound i has a_N = 16.2 gauss in methylene chloride: J. W. Lown, *J. Chem. Soc., Sect. B*, 441 (1966). This value might be expected to be reduced slightly in **3** by delocalization of the unpaired electron over the aromatic ring (*cf.* ref 18).



i

(18) The last of these (nine lines) appeared as a triplet of triplets (a_N = *ca.* 16 and 1.5 gauss) and is ascribed to *t*-BuN(O·)N(Ph)$COCH_3$. This radical has not yet been obtained free from di-*t*-butyl nitroxide, despite attempts to avoid direct photolysis of the nitrosobutane (see ref 19). The spectrum in Figure 1 has been analyzed in detail[13,14] and involves considerable coincidence of lines. There must also be coincidences in the spectra of the chloro- and methyl-substituted radicals and analysis of the spectra of these and related nitroxides will be given in the full paper.

(19) A. Mackor, Th. A. J. W. Wajer, Th. J. de Boer, and J. D. W. van Voorst, *Tetrahedron Letters*, 2115 (1966), and references therein.

(20) A. Mackor, Th. A. J. W. Wajer, Th. J. de Boer, and J. D. W. van Voorst, *ibid.*, 385 (1967).

FIG. 1

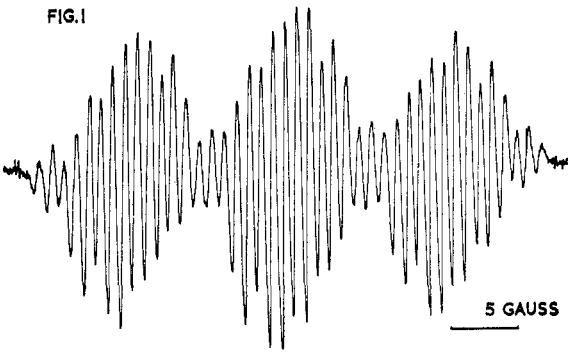


FIG. 2

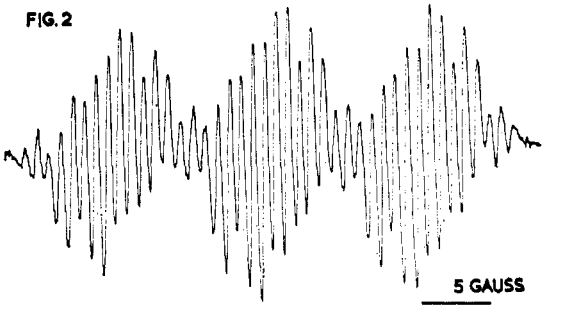


Figure 1. Esr spectrum of the radical formed during the decomposition of N-nitrosoacetanilide in benzene.

Figure 2. Esr spectrum of the radical formed on mixing benzene solutions of N-bromoacetanilide and nitrosobenzene.

Main product-forming sequence

$$\mathrm{Ph\cdot} + \mathrm{PhH} \longrightarrow \mathbf{2} \qquad (5)$$

$$\mathbf{2} + \mathbf{3} \longrightarrow \mathrm{Ph_2} + \mathrm{PhNOH}\ (\mathrm{PhNCOCH_3}) \qquad (11)$$

$$\mathrm{PhN{=}NOCOCH_3} + \mathrm{PhNOH}\ (\mathrm{PhNCOCH_3}) \longrightarrow \mathbf{3} + \mathrm{Ph\cdot} + \mathrm{N_2} + \mathrm{AcOH} \qquad (12)$$

Reaction 12 may represent more than one discrete step. The reduced yield of biphenyl with high initial concentrations of nitrosoamide might be explained in terms of increased removal of phenyl radicals in reaction 8, leading to unidentified by-products.

Other examples of phenylation reactions in which product formation is dominated by the presence of relatively stable radical intermediates have also been documented.[21] In the light of the present work, the identity of any such species in the Gomberg reaction[11] merits reinvestigation.

Acknowledgment. We thank the Science Research Council for financial support (to G. R. C.), and Professor D. H. Hey for valuable discussions.

(21) D. H. Hey, M. J. Perkins, and G. H. Williams, *J. Chem. Soc.*, 110 (1965); M. J. Perkins, *ibid.*, 5932 (1964).

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A New Versatile Ketone Spin Label

Sir:

The study of molecular systems by esr spectroscopy is contingent on the presence within the system of un-