Superoxide-Promoted Oxidation Reactions of Aniline and N-Methylaniline in Dimethyl Sulfoxide

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The chemistry of superoxide was investigated in reference to its reactions with primary and secondary aromatic amines. Two aromatic amines (aniline and *N*-methylaniline) reacted extensively in aprotic solutions containing potassium superoxide. In the case of aniline, *trans*-azobenzene and 4-nitrodiphenylamine were the major products, with smaller amounts of 4-aminodiphenylamine, 4-nitrosodiphenylamine, and *p*-(phenylazo)diphenylamine also being produced. With *N*-methylaniline, both oxidation and demethylation occurred, leading to the isolation of *N*-phenylformamide, aniline, and smaller amounts of azobenzene and 4-nitrodiphenylamine. Both superoxide and hydrogen peroxide alone were unable to convert either 4-aminodiphenylamine to its nitro and nitroso derivatives or *N*-phenylformamide to aniline. Solutions containing potassium *tert*-butoxide in place of superoxide produced the same products and oxygen was required for the reaction. Taken together, these results indicated that primary and secondary reducing aromatic amines are readily ionized by superoxide in aprotic solutions and then oxidized in a process involving molecular oxygen, leading to products whose structures suggest that processes such as radical recombination, N-oxidation, and N-demethylation have taken place.

Superoxide, one-electron reduced molecular oxygen, is known to be produced in aerobic organisms (for review see ref 1 and 2). Numerous studies have implicated superoxide generation with lipid peroxidation,[3,4] cytotoxicity,[5-9] certain drug toxicities,[10,13] and tissue inflammation.[14,15] While the debate regarding the importance of superoxide in vivo continues, the chemistry of the radical remains to be completely characterized. A recent review by Sawyer and Valentine describes the types of reactions that superoxide is likely to carry out.[16] In protic solutions, certainly a major reaction is the dismutation to hydrogen peroxide and oxygen; however, in both aprotic and protic solvents, other reactions are known to occur. Superoxide can act as a base and this property is responsible for initiating the complex reactions with ascorbic acid and α-tocopherol.[17,18] It can also act as one-electron reductant with certain quinones and metals.[19-24]

Superoxide has also been shown to act as an oxidant. In particular, oxidation of iron complexes has been studied.[24,25] Sawyer and colleagues have studied the reactions of superoxide in aprotic conditions with several classes of organic compounds, including some of biological interest. In addition to the examples mentioned above, they have found that superoxide is an effective initiator of oxidation of basic reducing compounds with readily transferable hydrogen atoms such as dihydrophenazines, reduced flavins, hydrazines, and hydroxylamine.[26] This paper further explores the reactions of superoxide with basic reducing molecules. During the course of this work Frimer and co-workers[27] reported the characterization of the products resulting from the reaction of superoxide with aniline in benzene. Our work is in agreement with theirs with regard to the major products of the aniline reaction and mechanistic conclusions. We also report two minor products of the reaction and have examined the reactivity of *N*-alkylarylamines using *N*-methylaniline as a model.

Results and Discussion

In agreement with Frimer and colleagues we found the major products of the aniline reaction with superoxide to be I (63 μmol) and III (19.7 μmol). The mass fragmentation pattern is that of the trans isomer of I.[28,29] We

Table I. Physical Characteristics of Reactants and Products

compd	R_f	t_R OV-17 (min)[c]	λ_{max} (nm)	M^+, base peak
I	0.91[a]	15.0	228, 320, 347 (EtOH)[b]	182, 77
II	0.61[a]	26.5	400, 275 (CH_3CN)	273, 168
III	0.33[a]	21.7	392, 258 (EtOH)	214, 214
IV	0.60	20.2	417, 260 (CH_3CN)	198, 167
V	0.39	13.7	242 (CH_3CN)	121, 121
VI	0.42	19.6	285 (95% EtOH)	
aniline	0.51	6.2		
N-methylaniline	0.70	7.2		

[a] The developing solvent was CCl_4/CH_3CN, 10:1. In all other cases, $CHCl_3/CH_3CN$/benzene, 10:1:1, was used. [b] These absorbance maxima are characteristic of *trans*-azobenzene.[29] [c] Conditions were as follows: injection port 250 °C; oven 60 °C for 3 min then 60-300 °C at 12°/min; 300 °C for 8 min; manifold 285 °C. He flow approximately 20 mL/min.

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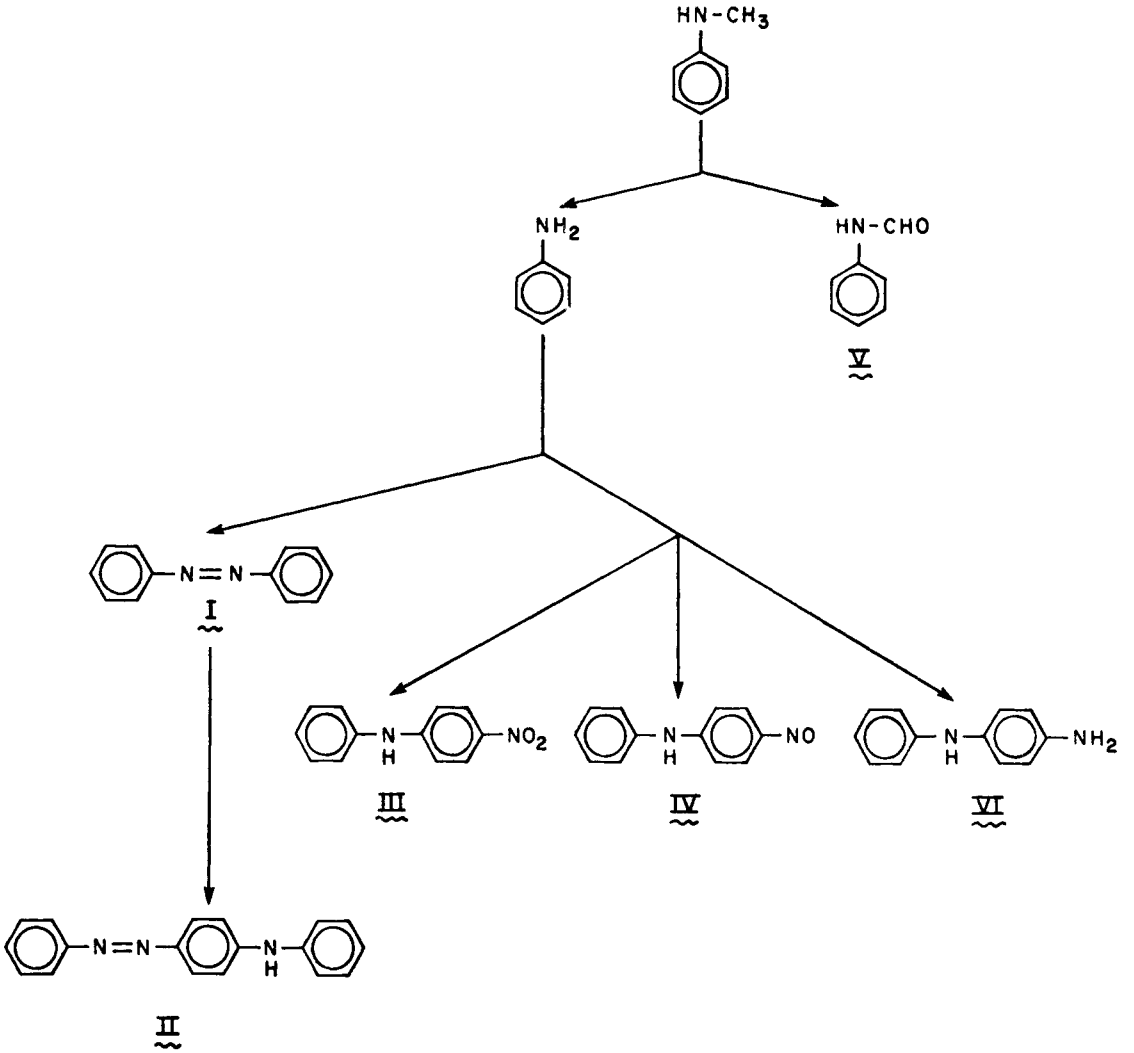
Scheme I. Products Isolated from the Aniline and *N*-Methylaniline Reaction with Superoxide



characterized the reaction further identifying the trimeric product II (1.1 μmol) and IV (3.1 μmol) and VI (3.3 μmol). The conversion of IV to III with superoxide took place very slowly, and so while it is reasonable to assume that some of III resulted from the oxidation of IV, it is also likely that IV results from the reaction of nitrosobenzene and aniline. We did not find any nitrobenzene as Frimer reports; however, they used an excess of superoxide and in our case the amine was in excess. III is indicative of the presence of nitrobenzene and we found it as a major product. Reaction of VI with H_2O_2 in Me_2SO produced a small amount of IV exclusively. The five products identified in this reaction accounted for 90% of the total. The role of oxygen in these reactions was seen in our reaction under argon. We continuously purged the flask and observed a much diminished yield with superoxide. Evidently the purging was not efficient enough to remove all the O_2 generated from the dismutation of the superoxide conjugate acid. Again, consistent with Frimer no reaction was observed with *tert*-butoxide under argon where O_2 is not generated during the course of the reaction.

Our finding that *N*-methylaniline undergoes oxidation and demethylation is interesting in that oxidation of this relatively inactivated position has not been previously reported. Among the identified products of the *N*-methylaniline reaction, the two products present in greatest yield were aniline (18.4 μmol) and phenylformamide (V) (9.4 μmol). In addition to these, much smaller amounts of dimeric aniline-based products were formed, namely, azobenzene (I) (0.79 μmol), 4-nitrodiphenylamine (III) (1.6 μmol), and 4-nitrosodiphenylamine (IV) (0.3 μmol). The relative quantities of these dimeric aniline-based products present in the *N*-methylaniline reaction mixture paralleled those seen in the aniline reaction, in that I and III were of the highest yield. However, in contrast to the aniline reaction, the identified products of the *N*-methylaniline reaction accounted for only 40% of the total products seen in the GC.

Experimental Section

Gas chromatography was carried out on a Perkin-Elmer 900 spectrometer using OV-17 (3%) on Supelcoport (80/100 mesh). Two instruments were used for GC/MS. A Finnigan MAT 212 double-focusing instrument with a fused silica capillary column (SE-52) and a Hewlitt-Packard 5992A quadripole instrument with a fused silica capillary column (Carbowax 20M) were used. Silica

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GF plates (1000 and 250 μm) were used for TLC with $CHCl_3$/CH_3CN/benzene (10:1:1) (I) or CCl_4/$CHCl_3$ (10:1) (II) as the developing solvent.

Aniline and *N*-methylaniline were vacuum distilled before use and stored under N_2. Phenylformamide was recrystallized twice in petroleum ether (bp 35–60 °C) and dried in vacuo. 4-Aminodiphenylamine was recrystallized once in CCl_4 and then again in petroleum ether (bp 35–60 °C). The crystals were dried in vacuo and stored in a desiccator, protected from light.

4-Nitrosodiphenylamine was purified by column chromatography on silica gel (70–230 mesh) using CH_2Cl_2/CH_3CN (10:1) as the eluting solvent. The crystals that formed upon evaporation were dried in vacuo and stored desiccated. All other compounds were used without further purification.

General Reaction Conditions. Solutions of 18-crown-6-ether (0.15 M) in Me_2SO were made and stored over 4-Å molecular sieves before use. Freshly crushed potassium superoxide was added to an aliquot of the Me_2SO solution, the mixture was shaken for 15 min, and the superoxide concentration was determined as described.[30] The superoxide solutions used in these experiments ranged between 0.08 and 0.12 M superoxide by assay. A typical experiment had 50 μL of amine (0.54 mmol of aniline, 0.47 mmol of *N*-methylaniline) added to 1 mL of the superoxide solution, which contained from 0.08 to 0.12 mmol of superoxide. The reaction was allowed to proceed for 24 h and then was quenched by the addition of the 0.2 mL of H_2O. For preparative work, the reaction was scaled up so that 0.5 mL of amine (5.4 mmol of aniline, 4.7 mmol of *N*-methylaniline) was added to 5 mL of superoxide solution. Reactions of amines with HOOH in Me_2SO were carried out by adding the amine and 0.5 mL of 30% HOOH to 0.5 mL of Me_2SO. This resulted in amine and HOOH concentrations of 0.18 and 4.4 M, respectively. Reaction times were 24 h.

Anaerobic reactions were carried out in sealed 100-mL flasks. Argon was dried and rendered oxygen-free by passage through a gas purifier (Matheson No. 6406). Reaction solutions were purged by bubbling with argon both prior to addition of aniline and during the reaction. All anaerobic and corresponding control reactions were quenched after 24 h by the addition of 0.5 mL of 0.2 N HCl.

Column chromatography was carried out using glass columns (30 × 1.9 cm). When silica was used, the elution solvent was CH_2Cl_2/CH_3CN (10:1). Depending on the starting material various color-containing fractions were isolated and then subjected to TLC. Solvent system I was used except in the aniline case where solvent system II was used on the first color-containing fraction eluted from the column. Standard graphs of peak area vs. weight were constructed for each compound via GC analysis. The product recoveries were calculated and were used in determining product yields.

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Registry No. I, 17082-12-1; II, 101-75-7; III, 836-30-6; IV, 156-10-5; V, 103-70-8; VI, 101-54-2; $PhNH_2$, 62-53-3; PhNHMe, 100-61-8; KO_2, 12030-88-5; HOOH, 7722-84-1.

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Notes

Crystal Structure of 3-(N^{α}-Tritylmethionyl)benzotriazole 1-Oxide, a Synthon in Peptide Synthesis

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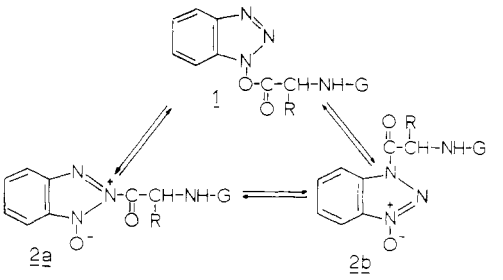
1-Hydroxybenzotriazole (HOBt) is widely used in peptide synthesis as an additive to DCC, because it is an effective racemization suppressant,[1] and sometimes as a catalyst.[2] The recognized intermediate implicated is the benzotriazolyl ester 1 with an IR carbonyl absorption band at around 1820 cm^{-1}. In solution ester 1 exists in equilibrium with an amide form which exhibits an IR carbonyl absorption at around 1740 cm^{-1}. Isolated crystalline compounds are in either one form or the other.[1,3a] Two amide forms **2a** and **2b** (Scheme I) have been postulated[1] with

Scheme I



2a preferred and associated with the 1740-cm^{-1} absorbance.[3] Horiki,[4] based on kinetic investigations, concluded in favor of **2b** whereas Davies et al.[5] used **2a** to accommodate seemingly anomalous results with *N*-methylamino acids.

Nevertheless it was not unambiguously known whether the amide with the carbonyl absorption band at 1740 cm^{-1} had structure **2a** or **2b**. Clarification of the issue would allow a better understanding of the mechanism of the racemization-suppressing and, sometimes, -promoting effect of HOBt. We thus decided to undertake an X-ray

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