made basic with concentrated ammonium hydroxide and extracted with chloroform. The chloroform extracts were evaporated and the ergotamine obtained was submitted to mass spectrometry for isotopic analysis.

Acknowledgment. We are indebted to Professor K. L. Rinehart and Dr. C. E. Snipes, Roger Adams Laboratory, University of Illinois, Urbana, for the mass spectral analyses and to Professor J. C. Vederas, Department of Chemistry, University of Alberta, Edmonton, for advice on the ^{18}O feeding technique. Support of this work by the U.S. Public Health Service through NIH Research Grant CA 17482 is gratefully acknowledged.

Registry No. Ergotamine, 113-15-5.

Reductive Formylation of *N,N*-Dimethyl-*p*-nitrosoaniline by Glyoxylic Acid. Evidence for a Hydroxamic Acid Intermediate[1]

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Received August 28, 1980

We recently reported that nitroso aromatics react rapidly with glyoxylic acid in aqueous solution to produce *N*-hydroxyformanilides.[2] During an attempt to apply this reaction for the synthesis of a previously unknown and highly reactive hydroxamic acid **2**, we found that the final product was actually the corresponding formanilide **3**. The direct condensation of glyoxylic acid and *N,N*-dimethyl-*p*-nitrosoaniline (**1**) to give **3** is not possible, so it was of interest to determine how **3** is produced. We now provide evidence which indicates that production of the formanilide **3** follows the initial production of the hydroxamic acid **2** (Scheme I). Since a similar reductive reaction was not observed for the *N*-hydroxyformanilides previously reported,[2] the *p*-dimethylamino substituent in **1** represents a special case of the nitroso–glyoxylate reaction.

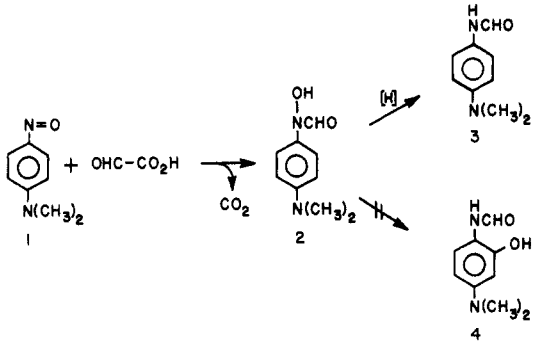
After discovering that the product isolated from the reaction of **1** with glyoxylic acid in aqueous solution was not the hydroxamic acid **2**, we suspected that it might be the isomeric rearrangement product **4**. However, all spectral data indicated that the product was identical with an authentic sample of the formanilide **3**. Typical yields of the formanilide **3** from synthetic-scale reactions were in the 50–60% range after column chromatography and recrystallization. Reactions carried out in more dilute solutions under suitable conditions were found to give **3** in quantitative yields as shown by high-pressure LC analysis. We do not have any evidence for the production of **4** as a secondary reaction product.

The use of TLC and a special high-pressure LC analytical technique[3] enabled us to detect an intermediate in the reaction of the nitroso compound **1** with glyoxylic acid. That this intermediate is the hydroxamic acid **2** is supported by all available evidence and is consistent with our previous report.[2]

The effect of pH upon the rate of formation of this hydroxamic acid intermediate (probable structure **2**) and its subsequent conversion to the final product **3** were studied by high-pressure LC. It was observed that at pH 2.0 the conversion of the intermediate **2** to **3** was quite slow and that a high concentration of the intermediate **2** was produced. Analysis by TLC of similar reactions conducted at pH 2.0, but at higher substrate concentrations, indicated the presence of a compound with a slightly lower R_f than was observed for **3**. Spraying of the TLC plate with 1% $FeCl_3$ resulted in an immediate violet color reaction for this compound. The formation of violet-colored complexes with Fe^{3+} is highly characteristic of hydroxamic acids.[4] Attempts to isolate this hydroxamic acid **2** from pH 2.0 reactions were unsuccessful due to its highly unstable nature.

At pH 3.0 the intermediate hydroxamic acid **2** was still detectable by TLC analysis of reactions. As the pH was increased further, the intermediacy of **2** could be detected only by high-pressure LC analysis since the rate of conversion of **2** to **3** increases greatly with increasing pH. The

Scheme I. Pathway for Reductive Formylation of *N,N*-Dimethyl-*p*-nitrosoaniline (1) by Glyoxylic Acid



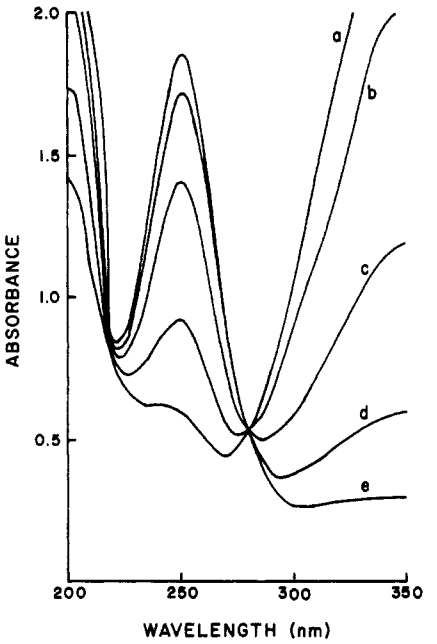


Figure 1. Change in UV spectrum during the reaction of *N,N*-dimethyl-*p*-nitrosoaniline (**1**) with glyoxylic acid at pH 2.0. An initial reaction mixture containing 0.125 mM **1** and 5.0 mM glyoxylic acid in 2.0 mL of 0.05 M, pH 2.0, KH_2PO_4 buffer was placed in a 1.0-cm path length quartz cell and scanned at the indicated times: a, 1.0 min; b, 5.0 min; c, 15 min; d, 30 min; e, 60 min.

(1) This investigation was supported by Grants CA-21992 and CA-23492 from the National Cancer Institute and by Research Career Development Award ES-00038 to M.D.C. from the National Institute of Environmental Health Sciences, DHEW.

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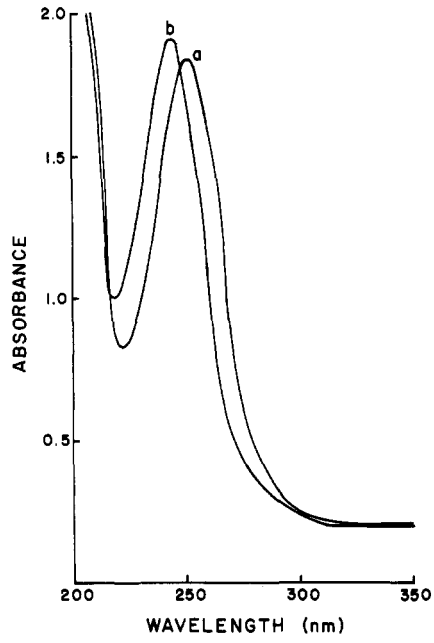


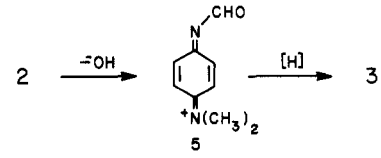
Figure 2. Change in UV spectrum resulting from the conversion of hydroxamic acid 2 to the final product 3. Contents of the UV cell described in Figure 1 was treated with 67 μL of 1 N NaOH. Curve a was obtained prior to the addition of NaOH and curve b was obtained 4 h after the addition of NaOH which caused a pH increase to 3.0.

optimal pH for the overall rate of conversion of 1 to 3 was 4.0. At pH 7.0, the conversion of 1 to 3 was quite slow, and above pH 7.0 other unknown reactions compete with the title reaction.

UV analyses were made on the reaction of 1 with excess glyoxylic acid at pH 2.0, along with simultaneous high-pressure LC analyses. Figure 1 illustrates the effect of the conversion of 1 to 2 upon the UV spectra. After 1 h the UV scan (curve e in Figure 1) is reasoned to be that of the proposed intermediate 2. High-pressure LC analysis at this same time supported this proposal in that the intensity of the peak thought to be due to 2 suggested that it was nearly pure. Thus, the UV spectrum after 1 h (Figure 1) should be very close to that of pure 2, which is not available for comparison. Attempts to synthesize 2 have failed; and it is noteworthy that 2, related hydroxamic acids, and the parent arylhydroxylamine have never been previously reported, although the parent arylhydroxylamine has been reported as a transient species in the enzymatic reduction of 1.[5] Even though we are confident that we succeeded in producing nearly pure 2 in situ at pH 2.0, this contention cannot yet be proven due to the instability of 2 toward normal isolation techniques.

A further study was conducted on 2 produced at pH 2.0 in the presence of excess glyoxylate. After addition of sufficient NaOH to raise the pH to 3.0, the reaction was followed by high-pressure LC and UV methods. Immediately after this pH jump, high-pressure LC analyses demonstrated the conversion of 2 to 3 with time. After 4 h at pH 3.0, the high-pressure LC peak due to 2 had disappeared, with a corresponding appearance of a single strong peak known to be due to 3. The percent yield of 3 in this experiment was 96% based upon the original nitroso substrate 1 employed in this experiment. The simultaneous change in the UV spectrum during this time consisted of a shift in the λ_{max} from 250 to 245 nm (Figure 2). Such a slight wavelength shift is what we expected

Scheme II. Explanation for Reactivity of Hydroxamic Acid 2 Produced by Condensation of *N,N*-Dimethyl-*p*-nitrosoaniline (1) with Glyoxylic Acid



since other formanilide/*N*-hydroxyformanilide pairs available to us display similar relative differences in their UV spectra in aqueous solution.

The conversion of 2 to 3 is a reductive process. Since the overall conversion of 1 to 3 is generally quantitative, the reducing agent must be glyoxylic acid. This is supported by our observation that glyoxylate could not be quantitatively converted to 3 alone. We also found that the conversion of 2 to 3 could be brought about by formaldehyde, which implies that the aldehyde group is a suitable reducing agent for 2. On the other hand, formaldehyde would not react with the nitroso substrate 1 to give 2, 3, or any other product detectable by high-pressure LC. The uniqueness of glyoxylic acid in its reaction with nitroso aromatics was previously discussed.[2]

Since it is most probable that the title reaction proceeds via the reduction of the intermediate hydroxamic acid 2, we investigated the possibility that other *N*-hydroxyformanilides might be reduced by glyoxylic acid, although at slower rates. Prolonged incubation (4 h) of *N*-hydroxyformanilide and *N*-hydroxy-*N*-(*p*-methoxyphenyl)formamide with a 20-fold excess of glyoxylic acid at 50 °C both at pH 3 and pH 6 failed to give any detectable reduction of these substrates to their corresponding amides. In view of this finding we conclude that the reaction of *N,N*-dimethyl-*p*-nitrosoaniline (1) with glyoxylic acid is a special case of the more generalized reaction between nitroso aromatics and glyoxylate. The high reactivity of the hydroxamic acid 2 is explained on the basis that loss of $^{-}$OH is facilitated by resonance interaction from the dimethylamino substituent to give a quinonimine reactive intermediate 5 (Scheme II). The relative stability of 2 at pH 2 is readily explained on the basis of protonation of the dimethylamino group, which retards the formation of 5. Evidently a highly polarized quinoid system such as 5 is subject to rapid reduction. Our expectation that $^{-}$OH might attack the quinoid ring of 5 to produce the simple rearrangement product 4 was not observed.

One other research group has reported on the reaction of 1 with α-oxo acids that leads to anilides. They employed α-keto acids such as the nitrophenylpyruvates and obtained nitrophenylacetanilides.[6] Surprisingly, pyruvic acid would not produce the acetanilide expected on the basis of their work. These Roumanian workers proposed a mechanism that depended on some very speculative redox interactions to yield *p*-(dimethylamino)phenylhydroxylamine and nitrophenylacetaldehydes, which would then condense to give the reported products. They provided no evidence for the production of such intermediates. It is possible that the mechanism we have described for the glyoxylic acid reaction with 1 is also the mechanism by which the phenylpyruvates form anilides with 1.

Experimental Section

N,N-Dimethyl-*p*-nitrosoaniline, *N,N*-dimethyl-*p*-phenylenediamine, and dicyclohexylcarbodiimide were obtained from Aldrich Chemical Co. The first was purified by chromatography

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on silica gel (E. Merck) with $CHCl_3$. Glyoxylic acid monohydrate was obtained from Sigma Chemical Co. and desferal mesylate was a gift from CIBA Pharmaceutical Co. High-pressure LC analyses were achieved by use of Waters Associates Model 6000A solvent delivery system, Model 440 absorbance detector (254 nm), and Model U6K septumless injector. Melting points were taken on a calibrated Thomas-Hoover Unimelt. UV spectra were obtained on a Beckman Model 35 spectrophotometer, IR spectra were obtained on a Perkin-Elmer Model 180 spectrophotometer, 60-MHz NMR spectra were obtained on a Varian EM 360 A spectrometer, and mass spectra were obtained on a Hewlett-Packard 5980A mass spectrometer. Elemental analyses were performed by Galbraith Laboratories.

Synthesis of *p*-(Dimethylamino)formanilide (3). To a solution of 2.7 g (0.02 mol) of *N,N*-dimethyl-*p*-phenylenediamine in 50 mL of anhydrous Et_2O, which was stirred with a magnetic stirring bar, was added in a single portion 6.2 g (0.02 mol) of dicyclohexylcarbodiimide in 30 mL of anhydrous Et_2O. A solution of 1.5 g (0.03 mol) of 90% formic acid in 10 mL of Et_2O was then added to the reaction over the course of 2 min. Further stirring was continued for 20 min and then the reaction mixture was filtered. The filtrate was dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by chromatography and recrystallized as described below to give 1.5 g (46%) of **3** as white plates, mp 106.5–107.5 °C (lit.[7] mp 104.5–105.5 °C).

Product from Reaction of *N,N*-Dimethyl-*p*-nitrosoaniline (1) with Glyoxylic Acid. To a solution of 18.4 g (0.20 mol) of glyoxylic acid monohydrate in 1.0 L of water, adjusted to pH 6.0 with 10% NaOH, was added 15.0 g (0.10 mol) of **1** dissolved in 100 mL of 95% ethanol. The reaction mixture was stirred at ambient temperature for 4 h and then extracted twice with 500 mL of Et_2O. The combined Et_2O extract was dried (Na_2SO_4) and evaporated in vacuo. The residue was dissolved in 100 mL of $CHCl_3$ and chromatographed on silica gel (60 × 2.6 cm bed size) with 500 mL of $CHCl_3$, 500 mL of 1% MeOH/$CHCl_3$, and finally 1.0 L of 2% MeOH/$CHCl_3$. Those fractions containing the single reaction product were combined and evaporated to give a yellow solid. Repetition of this chromatographic procedure gave 12 g of a pale yellow solid that showed a single spot on TLC (5% MeOH/$CHCl_3$ with silica gel plates). Recrystallization twice from benzene/hexane (5:1) gave 9.6 g (58% based on mol wt 164) of fine white plates, mp 106.5–107.5 °C. The UV, IR, NMR, and mass spectral data were identical with those found for authentic **3**, which was prepared as described above. The C, H, N analysis was consistent with $C_9H_{12}N_2O$.

General Procedure and Analytical Methods for Reaction of 1 with Glyoxylic Acid. To 40 mL of 0.05 M KH_2PO_4 buffer of the desired pH was added 23 mg (0.25 mmol) of glyoxylic acid monohydrate. The pH of the resulting solution was readjusted to the desired pH with 0.05 M K_2HPO_4 and the solution then brought to 50.0 mL final volume with the desired buffer to yield a 5.0 mM solution of glyoxylic acid. Aliquots of 5.0 mL were placed in test tubes and equilibrated to the desired reaction temperature. The title reaction was initiated by the addition of 50 μL of a solution of either 1.9 mg/mL or 3.8 mg/mL of **1** in 95% ethanol to give a reaction concentration of 0.125 or 0.25 mM, respectively. At the desired time after the start of the reaction, a 10-μL aliquot was injected directly onto a high-pressure LC system consisting of a Waters μBondapak C_{18} column (30 × 3.9 mm i.d.) with 30% MeOH buffered to pH 3.5 with 0.01 M KH_2PO_4 and containing 0.01% desferal mesylate as the elution solvent at a flow rate of 1.5 mL/min. For quantitative determination of **3**, the peak heights at 254 nm were measured and compared to those of pure standard. The retention times in this high-pressure LC system were as follows: **1**, 5.0 min; **2**, 4.57 min; **3**, 2.73 min. The investigation of the reaction by UV spectrophotometry was conducted with **1** at 0.125 mM initial concentration. Simultaneous high-pressure LC analyses were made to correlate high-pressure LC peaks to **1**, **2**, or **3**. Analyses of this reaction by TLC were conducted in a similar manner, except that the concentration of **1** was generally 5 mM with either 5 or 10 mM glyoxylic acid. R_f values on silica gel plates with 5% MeOH/$CHCl_3$ were as follows: **1**, 0.70; **2**, 0.24; **3**, 0.37. The zones were visualized by ultraviolet quenching; in addition the zone corresponding to **2** gave an immediate violet color with 1% $FeCl_3$ that faded to a yellow spot within several minutes.

Registry No. **1**, 138-89-6; **2**, 75767-78-1; **3**, 18606-63-8; *N,N*-dimethyl-*p*-phenylenediamine, 99-98-9; dicyclohexylcarbodiimide, 538-75-0; glyoxylic acid, 298-12-4.

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Specific Deuteration of δ-Aminolevulinic Acid by Pyridoxal-Catalyzed Exchange and Analysis of Products Using an Aqueous Lanthanide Nuclear Magnetic Resonance Shift Reagent

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Received September 4, 1980

Samples of δ-aminolevulinic acid (ALA, $H_2NCH_2COCH_2CH_2CO_2H$) with specific isotopic labels have long been of use in studies of heme biosynthesis.[1] While a number of methods are known for specific incorporation of ^{14}C or ^{15}N into ALA,[1,2] none of the published chemical syntheses[1-3] lends itself to substitution of hydrogen isotopes specifically at only one position. This is especially true because most syntheses terminate with an acid-catalyzed hydrolytic removal of an acyl protecting group from the amino nitrogen. This procedure exchanges the protons at both C-3 and C-5 with the aqueous medium,[4] thus altering any deuterium or tritium labeling pattern that might have been introduced at an earlier step. Simple base-catalyzed exchange of ALA with water also fails because the protons at C-3 and C-5 exchange at almost equal rates with a variety of catalysts.[4,5]

We have investigated methods for catalyzing ALA–water hydrogen exchange which lead to regiospecific incorporation of isotope. The use of a water-soluble aldehyde to form a transient Schiff base at the amino group of ALA in situ was anticipated to be effective in accelerating exchange at C-5. Specifically, we turned to pyridoxal, whose biochemical functions include acidifying the proton on the α carbon of α-amino acids.[6]

Results and Discussion

Incubation of ALA with approximately 0.1 molar equiv of pyridoxal in a pyridine buffer at pH 4.4 was found to catalyze the exchange of the hydrogens of the C-5 methylene with those of the aqueous medium much faster than the corresponding exchange at the C-3 methylene. This discrimination results from the transient formation of an

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