Table IV. Values of 10^3k (in M^{-2} s^{-1}) for Iodination of Butyric and Caprylic Acids

acid	time, min 0	1	2	3	4	5
$CH_3CH_2CH_2CO_2H$	2.96	2.80	2.50	2.34	2.20	2.09
$CH_3(CH_2)_6CO_2H$	2.92	2.39	2.04	1.79	1.61	1.50

ond-order rate constants (k) were calculated by eq 8 according to the stoichiometric eq 1-5.

$$\ln\left(\frac{a-x}{b-(x/2)}\right) = \frac{k}{2}(a-2b)t - \ln\left(\frac{b}{a}\right) \quad (8)$$

Here, a and b are the initial concentrations of the aliphatic acid and iodine, respectively, and $x/2$ is the molar concentrations of consumed iodine at time t.

As typical examples, the $k \times 10^3$ M^{-2} s^{-1} values for the iodination of butyric and caprylic acids are shown in Table IV, where the k values at time 0 are obtained by extrapolation and used in Table III.

Products and Conversion Curves of Products. A 1,2-dichloroethane solution (50 mL) containing a mixture of aliphatic acid (85 mmol) and iodine (6.5 mmol) was thermostated at 80 °C, and a thermostated dichloroethane solution of $ClSO_3H$ (32 mmol) was introduced to start the reaction. Aliquots (2 mL), after treatment with aqueous $Na_2S_2O_3$, were esterified by an ethereal solution of diazomethane. The yields were calculated by GLC as described below. The residual mixture in the flask was esterified by refluxing with methanol (30 mL) for 8 h. The ester solution obtained was successively washed with water and aqueous $Na_2S_2O_3$, dried (Na_2SO_4), and distilled in vacuo after removal of methanol and 1,2-dichloroethane. The resulting methyl esters of α-iodo acids were identified and their amounts estimated by means of GLC with a Yanagimoto GCG-550 gas chromatograph equipped with a hydrogen-flame ion detector and a copper column packed with PEG 20M (10%) on Chromosorb WAW (60-80 mesh), with methyl caprate as an internal standard. The isolated methyl esters of α-iodo acids were identified by GLC and NMR (a 60-MHz Hitachi, R-24B NMR spectrometer) in comparison with the authentic samples. IR spectra were measured with a Perkin-Elmer Model 337 spectrophotometer (Table II). For long-chain aliphatic acids, a solution of 1,2-dichloroethane (50 mL) containing aliphatic acid (50 mmol), iodine (12.5 mmol), and $ClSO_3H$ (50 mmol) was reacted analogously at 80 °C for 2 h. α-Iodo acid produced was esterified as described above and analyzed by means of GLC using PEG 20M and Silicon OV 17 (5%) on Shimalite W 201D (80-100 mesh) and employing methyl caprate and dodecane as internal standards.

The yields estimated by the actual isolation of methyl esters of α-iodo acids were ca. 30-40% lower than those by GLC because of a loss in the esterification with methanol and in the distillation. For example, the yield of methyl α-iodobutyrate [85-86 °C (31 mm)] was ca. 59% (93.6% by GLC), and the yield of methyl α-iodocaprylate [81-83 °C (0.2 mm)] was ca. 62% (100% by GLC). The direct vacuum distillation of prepared α-iodo acids results in a considerable decomposition and gave darkly colored product, the yield being 30% lower, e.g., 63% for α-iodobutyric acid [110-114 °C (3.2 mm)].

Registry No. Acetic acid, 64-19-7; propionic acid, 79-09-4; butyric acid, 107-92-6; isobutyric acid, 79-31-2; isovaleric acid, 503-74-2; diethylacetic acid, 88-09-5; α-methylvaleric acid, 97-61-0; caprylic acid, 124-07-2; capric acid, 334-48-5; lauric acid, 143-07-7; myristic acid, 544-63-8; palmitic acid, 57-10-3; stearic acid, 57-11-4; iodine, 7553-56-2; chlorosulfonic acid, 7790-94-5; methyl α-iodoacetate, 5199-50-8; methyl α-iodopropionate, 56905-18-1; methyl α-iodobutyrate, 73651-35-1; methyl α-iodoisobutyrate, 67194-53-0; methyl α-iodoisovalerate, 73635-60-6; methyl α-iodocaprylate, 73635-61-7; methyl α-iodocaprate, 73635-62-8; methyl α-iodolaurate, 73635-63-9; methyl α-iodomyristate, 73635-64-0; methyl α-iodopalmitate, 73635-65-1; methyl α-iodostearate, 67810-48-4; α-iodobutyric acid, 7435-10-1.

Reaction of Nitroso Aromatics with Glyoxylic Acid. A New Path to Hydroxamic Acids[1]

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In aqueous solution substituted aromatic nitroso compounds react rapidly with glyoxylic acid to produce *N*-hydroxyformanilides and CO_2. The reaction is nearly quantitative for all nitroso aromatics investigated and serves as a convenient synthetic route to *N*-hydroxyformanilides. This reaction follows second-order reaction kinetics overall and is unimolecular in each of the two reactants. The reaction is strongly inhibited by organic cosolvents but is not affected by hydroquinone, H_2O_2, catalase, superoxide dismutase, or O_2. The rate of reaction was found to increase with increasing electron donation by ring substituents. Possible ionic reaction mechanisms are presented in which the nitroso group behaves as a nucleophile.

Hydroxamic acids have received considerable attention in recent years as the result of the discovery of their role in the biochemical toxicology of many drugs and other chemicals. Their production by the microsomal oxidation of amide-containing chemicals explains in large part the mechanism by which the latter exert toxic effects on living systems.[2] Our interest in this structural group arose from earlier studies on a unique family of natural products that contain the hydroxamic acid functionality.[3] More recently we have elucidated a new biochemical pathway for the production of aromatic hydroxamic acids through the interaction of thiamine-dependent enzymes with aromatic nitroso compounds.[4,5] During the course of these investigations we discovered a purely chemical reaction that was totally unexpected. We now describe this novel reaction between nitroso aromatics 1 and glyoxylic acid (2) that

(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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Table I. Structure Assignments and Physical Data for Hydroxamic Acids 5

compd	R	mp, °C	λ_{max}[a] (ϵ)	t_R,[b] min
5a	4-H	69.5-70	250 (10 700)	3.3
5b	4-Br	122-122.5	260 (16 200)	6.1
5c	4-Cl	122.5-124 dec	258 (14 100)	5.5
5d	3-Cl	129.5-130.5	255 (12 200)	5.6
5e	4-CH_3	105.5-106.5	253 (11 600)	4.2
5f	2-CH_3	72-73	238 (6000)	3.6
5g	4-F	111.5-112.5	249 (9700)	3.4
5h	4-OCH_3	75-77	254 (10 800)	3.4
5i	4-CO_2Et	122.5-123	281 (17 800)	6.1

[a] In EtOH; value of λ_{max} in nanometers. [b] High-pressure LC retention time.

leads to the producton of *N*-hydroxyformanilides **5**.

Results

Our initial observation of the production of the *N*-hydroxyformanilide **5c** resulted during a study of the action of yeast pyruvate decarboxylase on 4-chloronitrosobenzene (**1c**) in the presence of glyoxylic acid. We suspected that such a product would result in the presence of this enzyme but were very surprised to discover this product in control incubations that lacked pyruvate decarboxylase. Further investigations also established that yeast pyruvate decarboxylase did not catalyze the conversion of 4-chloronitrosobenzene (**1c**) to the *N*-hydroxyformanilide **5c** and that the cofactors thiamine pyrophosphate and Mg^{2+} had no significant effect on this reaction.

Detection of the hydroxamic acid product was first achieved through the use of a high-pressure LC method specially developed in this laboratory for hydroxamic acid analyses.[6] This technique was employed throughout this study to determine the kinetic parameters of the generalized reaction between nitroso aromatics and glyoxylic acid. The chromatographic retention times of the hydroxamic acid products are recorded in Table I.

Assignment of the *N*-hydroxyformanilide structure **5** (Table I) to the products resulting from the reaction of glyoxylic acid and the corresponding nitroso aromatic was made by spectral methods on the products isolated in pure form from large-scale reactions. Most notable was the $(M - 16)^{+\cdot}$ ion in the mass spectrum of each product, which is characteristic of hydroxamic acids.[7] The NMR spectrum of each product was consistent with the hydroxamic acid structure, as was the IR spectrum, which characteristically displayed a strong C=O stretching band at about 1680 cm^{-1} for the hydroxamate carbonyl group.[8] As expected for hydroxamic acids, each product gave an intense violet complex upon treatment with methanolic $FeCl_3$ both in solution and on silica TLC plates. Final confirmation of the structure of hydroxamic acid products **5a** and **5c** was made by comparison to synthetic products prepared by more conventional methods.[3,9,10] This was achieved by the reaction of the corresponding arylhydroxylamine with formic acid and dicyclohexylcarbodiimide (DCC). Although the DCC condensation method[10] and the use of acetic formic anhydride[11] would be considered more usual methods for the preparation of formanilides and thus *N*-hydroxyformanilides, we found that the present reaction of a nitroso aromatic with glyoxylic acid in an aqueous solvent gave much more readily purified *N*-hydroxyformanilide products. For that reason all remaining *N*-hydroxyformanilides were prepared by the glyoxylate method.

Table II. Effect of Solvent on the Rate of Reaction between 4-Chloronitrosobenzene and Sodium Glyoxylate

solvent system	rel rate[a]	solvent system	rel rate[a]
pH 6.0, 0.05 M KH_2PO_4	1.00	50% MeOH	0.06
		MeOH	0.00
pH 6.0, 0.20 M KH_2PO_4	0.96	95% EtOH	0.00
		Me_2SO	0.10
pH 6.0, 0.05 M sodium citrate	0.97		
distilled water	0.86		

[a] Relative rate of *N*-hydroxyformanilide production.

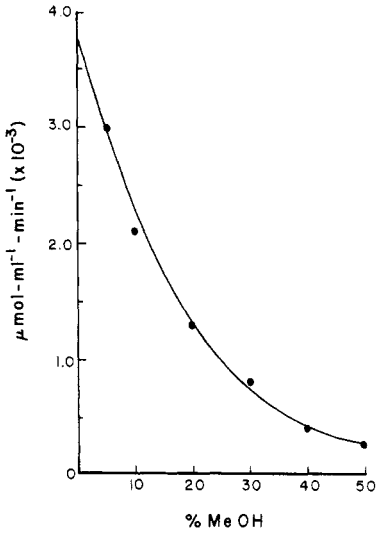

Figure 1. Effect of methanol as a cosolvent on the rate of reaction of nitrosobenzene with sodium glyoxylate. Rates are expressed as initial rates for the reaction with 0.5 mM nitrosobenzene and 2.0 mM sodium glyoxylate at 30 °C and pH 6.0 (0.05 M KH_2PO_4 buffer prepared in aqueous methanol solutions of the indicated composition).

A study of the effect of pH upon the title reaction indicated that the reaction occurred over a wide pH range of 3.0–9.0 with a broad maximum centered at pH 6.0. Buffer strength did not significantly affect the pH dependency, nor did the nature of the buffer, since citrate buffer resulted in nearly the same reaction rate as phosphate buffer when investigated at identical pH (Table II).

The reaction between nitroso aromatics and glyoxylic acid to give *N*-hydroxyformanilides was found to be highly dependent on the nature of the solvent, as shown in Table II. Most significant was the observation that this reaction is almost totally restricted to aqueous solvents. The addition of slight amounts of organic cosolvents was found to have a strong inhibitory effect on the rate of the reaction. This effect is best illustrated in Figure 1 in which a reciprocal relationship between the rate of hydroxamic acid production and the methanol content of the solvent is displayed. All studies indicated to have been conducted in aqueous systems were actually conducted in 1% ethanol solutions, since dissolution of the nitroso aromatic substrates in an organic solvent was necessary prior to their addition to the aqueous system.

In previous studies we had never observed a reaction between a nitroso aromatic and pyruvic acid to give a hydroxamic acid without the intervention of pyruvic acid decarboxylase.[12] Thus, the direct chemical reaction be-

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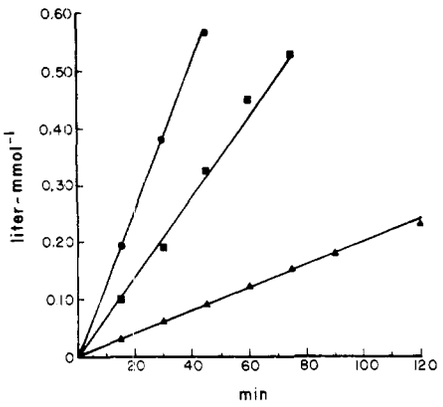

Figure 2. Plots of the second-order rate equation vs. time for the reactions of three different nitroso aromatics with sodium glyoxylate. The equation $1/(a-b)\ln[b(a-x)/a(b-x)] = k_2t$ was plotted as a function of time, where a is the initial concentration of sodium glyoxylate, which was 5.0 mM, b is the initial concentration of the nitroso aromatic, which was 0.10 mM, and x is the amount of product at time t. All reactions were carried out as described in the Experimental Section for this study: ●, 4-methylnitrosobenzene; ■, nitrosobenzene; ▲, 4-chloronitrosobenzene.

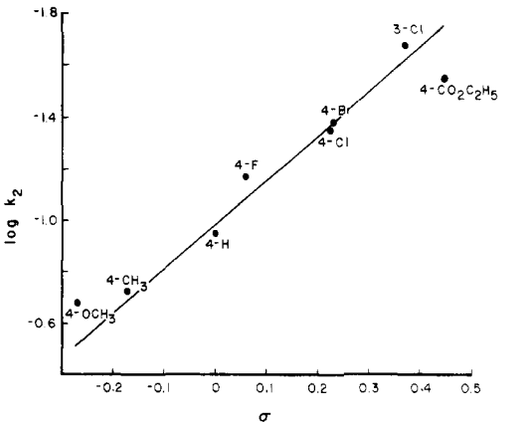

Figure 3. Plot of the logarithms of the second-order rate constants for the title reaction vs. Hammett σ constants.

Table III. Release of $^{14}CO_2$ from the Reaction of 4-Methylnitrosobenzene with Sodium [1-^{14}C]Glyoxylate

[4-MeC_6H_4NO], mM	dpm of protosol	molar ratio of $^{14}CO_2$ to hydroxamic acid
0	50	
0.10	18 800	0.92
0.25	41 050	0.81
0.50	97 900	0.97

tween a nitroso aromatic and an α-oxo carboxylic acid is unique to glyoxylic acid, at least under the mild conditions employed in this study.

In an attempt to discern a possible mechanism by which *N*-hydroxyformanilides are produced from the reactions of nitroso aromatics with glyoxylic acid, we conducted numerous additional studies. Nitrosobenzene (**1a**) would not react with formaldehyde, acetaldehyde, glyoxal, formic acid, or oxalic acid to give any detectable hydroxamic acid product under conditions analogous to those employed with glyoxylic acid as the substrate. In addition, phenylhydroxylamine did not yield any hydroxamic acid upon reaction with glyoxylic acid, formaldehyde, formic acid, or oxalic acid. This evidence, coupled with the failure to detect any intermediate arylhydroxylamine during the reaction of the nitroso aromatics with glyoxylic acid, eliminated any mechanism involving an initial redox reaction to produce the arylhydroxylamine as a necessary intermediate. We also interpreted these studies to disprove the possibility that the title reaction proceeds through the presence of decomposition products formed in glyoxylic acid or formed during the incubation with the nitroso aromatic.

The incubation of nitrosobenzene with glyoxylic acid in the presence of superoxide dismutase or catalase had no effect upon the reaction; likewise, incubations conducted under an inert (N_2) atmosphere or in the dark proceeded at the usual rate. Furthermore, the addition of H_2O_2 to the incubations did not increase the reaction rate. These studies eliminated the possibility that an active oxygen species participated in the reaction. The presence of hydroquinone in the reaction mixtures did not affect the rate of the reaction, which suggests that the reaction is not of the free-radical type.[13]

The reaction between glyoxylic acid and nitroso aromatics followed second-order reaction kinetics, with the reaction kinetics being first order with respect to each of the reactants. The range of concentrations investigated was from 0.25 to 10.0 mM for glyoxylate and from 0.10 to 1.0 mM for the nitroso aromatic reactant. That the title reaction follows the simple second-order rate equation was established for three different nitroso aromatic substrates by plotting the rate equation vs. time (Figure 2). The computed second-order rate constants at 30 °C and pH 6.0 were found to be 0.034 ± 0.0012 L/(mol s) for 4-chloronitrosobenzene, 0.119 ± 0.010 L/(mol s) for nitrosobenzene, and 0.214 ± 0.003 L/(mol s) for 4-methylnitrosobenzene. We did find it necessary to conduct these time-course studies under experimental conditions that prevented the possibility of loss of the nitroso aromatic substrate by volatilization.

A plot of the logarithm of the second-order rate constants for the formation of the hydroxamic acids vs. Hammett σ values[14] suggests a fairly good correlation between the rate of reaction of the various nitroso aromatic substrates and the electron-donating properties of the ring substituents (Figure 3). Most obvious is the fact that the rate of the title reaction increases with electron donation by ring substituents. This electronic effect is not particularly large, since the relative rate of reaction increased only tenfold between the slowest and fastest reacting substrates that were investigated.

An investigation of the reaction of sodium [1-^{14}C]glyoxylate with 4-methylnitrosobenzene (**1e**) proved that the carbonyl carbon of the *N*-hydroxyformanilide **5e** came from the aldehyde (C-2) carbon of glyoxylate, that CO_2 came from the C-1 carbon, and that CO_2 was released in a 1:1 stoichiometric ratio to hydroxamic acid production (Table III). The hydroxamic acid product **5e** resulting from the radiotracer experiments was partially purified and found not to contain any significant amount of the ^{14}C label.

Discussion

In this study we found that nitroso aromatics will react with glyoxylic acid in aqueous solutions to produce the corresponding *N*-hydroxyformanilides **5** and an equimolar

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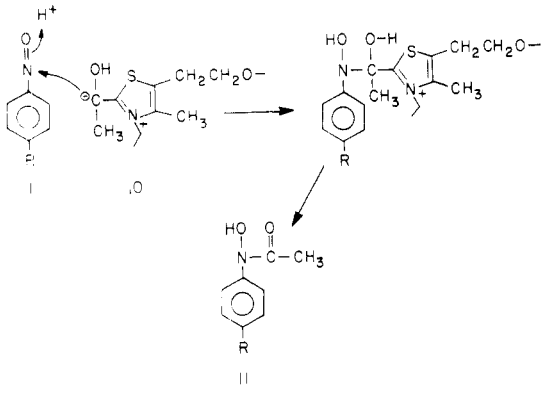
Scheme I. Proposed Mechanism for the Pyruvate Decarboxylase Catalyzed Conversion of Nitroso Aromatics to *N*-Acetyl-Type Hydroxamic Acids[12] [a]



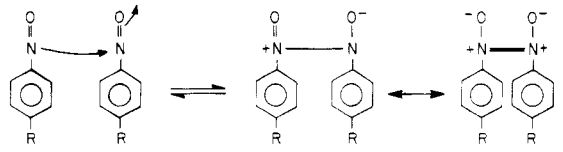
[a] Structure 10 is a partial representation of the enzyme-bound intermediate considered to be the thiamine-stabilized acyl carbanion (also known as active acetaldehyde).[15]

amount of CO_2. The conversion of nitroso aromatics to the hydroxamic acids appears to be quantitative based on those substrates investigated to date. Radiotracer studies proved that CO_2 arose from the C-1 portion of glyoxylate and the hydroxamate carbonyl from the C-2 of glyoxylate. Under the conditions employed, the reaction was highly specific for glyoxylic acid alone, since no analogous reaction was observed with pyruvic acid and other aldehydes and carboxylic acids. The possibility that this reaction might be mediated by an initial reduction of the nitroso aromatic to the hydroxylamine oxidation state was eliminated; furthermore, no intermediates of this bimolecular reaction could be detected by high-pressure LC analysis. All data generated in this investigation suggest that the reaction proceeds by an ionic rather than a radical mechanism.

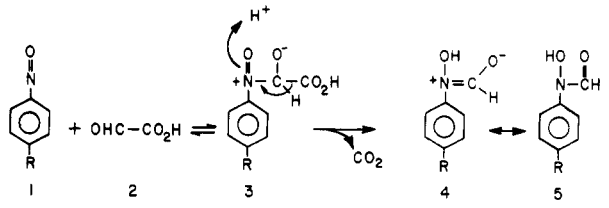
We previously discovered that nitroso aromatics are converted to *N*-acetyl-derived hydroxamic acids 11 through a condensation with pyruvic acid that is catalyzed by pyruvic acid decarboxylase.[12] From a mechanistic viewpoint, this biochemical reaction is unrelated to the chemical reaction between nitroso aromatics and glyoxylic acid. On the basis of Breslow's mechanism[15] for thiamine-catalyzed reactions, a reasonable mechanism for the pyruvate decarboxylase mediated production of hydroxamic acids is nucleophilic attack of the stabilized acyl carbanion 10 on the nitroso group (Scheme I). In the absence of enzyme, no such stable acyl carbanion can be produced from pyruvic acid, and thus no reaction can occur. It is equally unreasonable to expect glyoxylic acid to be converted to an acyl carbanion, especially under the conditions employed in the title reaction. Subsequently, we suspect that the mechanism for the chemical reaction between nitroso aromatics and glyoxylic acid is one in which the nitroso nitrogen serves as a nucleophile. This proposal is supported by our observation that electron donation by ring substituents increased the reaction rate.

The best known chemical reactions of the nitroso functional group are those in which it serves as an electrophile in condensations with nucleophiles such as active methylene compounds,[16] enamines,[17] amines,[18] and hydroxylamines.[19] For such reactions, available evidence indicates that the rate of nucleophilic attack upon substituted nitrosobenzenes decreases with increasing electron donation by substituents,[18] which is opposite from the substituent effects on the reaction of nitroso aromatics with glyoxylate. However, one well-known property of nitroso compounds that clearly illustrates the potential nucleophilic character of this group is their strong tendency to form dimers.[20] This dimerization process results from the interaction of the nonbonding electron pair on the nitroso N with another nitroso group (Scheme II).

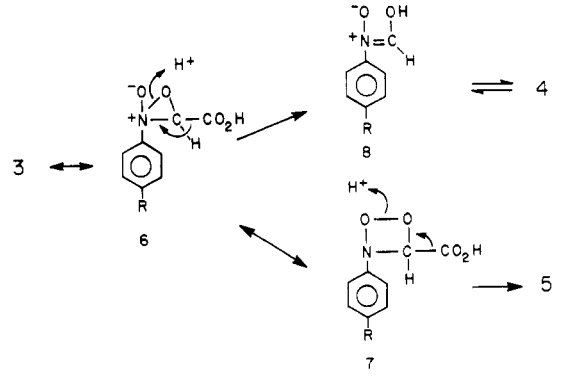
Scheme II. Dimerization Reaction of Nitroso Aromatics



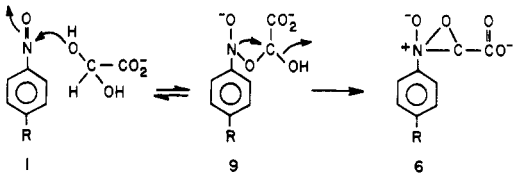
Scheme III. Proposed Nucleophilic Mechanism for the Reaction of Nitroso Aromatics with Glyoxylic Acid



Scheme IV. Possible Intermediates in Proposed Mechanism



Scheme V. Alternate Mechanism for the Production of an Oxaziridine *N*-Oxide



The initial step for the title reaction could be a nucleophilic attack of the nitroso group on the aldehyde carbon of glyoxylic acid to give the "mixed dimer" 3 (Scheme III). Irreversible decarboxylation of 3 would give 4, which is a resonance structure of the product hydroxamic acid 5. The oxaziridine *N*-oxide 6 and the dioxaz-

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etidine **7** are possible resonance structures for the "mixed dimer" **3**, and either could give rise to the hydroxamic acid **5** by undergoing decarboxylation (Scheme IV). Aue and Thomas proposed structures similar to **6** and **7** to explain the peracid oxidation of 3-alkoxyoxaziridines, which produced esters and aliphatic nitroso compounds.[21] That reported reaction is similar to the reverse of the reaction of nitroso aromatics with glyoxylate. Neither the oxaziridine *N*-oxide nor the dioxazetidine ring system is known to be a stable structure. The possibility that the title reaction might occur as the result of an initial 2 + 2 cycloaddition to give the dioxazetidine **7** cannot be ruled out at present.

The oxaziridine *N*-oxide **6** could also arise by an alternate mechanism (Scheme V).[22] Nucleophilic attack by a hydroxyl oxygen of hydrated glyoxylic acid on the nitroso group could give intermediate **9**. Displacement of ^-OH by the nitroso nitrogen would result in the oxaziridine *N*-oxide **6**. This mechanism could explain the affect of ring substituents upon the rate of the title reaction if the rate-determining step was the conversion of **9** to **6**.

The very facile condensation of nitroso aromatics with glyoxylate appears to be specific only for this aldehyde. The failure of pyruvic acid to undergo an analogous reaction is most readily explained on the basis of the lower reactivity of a ketone carbonyl toward nucleophiles in comparison to the aldehyde carbonyl group. Although we could not detect any reaction between nitrosobenzene and formaldehyde under the mild reaction conditions employed, Bamberger did report a trace of **5a** upon reaction of these two substrates in highly concentrated alcoholic solutions.[23] This great difference in reactivity between glyoxylate and other aldehydes must be the result of irreversible decarboxylation forcing the reaction to completion in the case of glyoxylate.

We have found the reaction of nitroso aromatics and glyoxylic acid to be a very convenient method for the synthesis of *N*-hydroxyformanilides. In comparison to other methods, the title reaction is much simpler to carry out, can be optimized to produce the product in nearly quantitative yields, and, as a result, gives a product that is very readily purified by recrystallization alone.

This investigation developed from a program dealing with the chemical and biochemical mechanisms by which nitroso aromatics produce a highly toxic action on living systems, including their carcinogenic properties. In view of the fact that glyoxylic acid is known to be present in rather high concentrations in certain tissues,[24] it will be necessary to determine if this reaction between nitroso aromatics and glyoxylic acid is a significant reaction in biochemical systems.

Experimental Section

All nitroso aromatic and arylhydroxylamine compounds were prepared from the corresponding commercially available nitro aromatics by the general methods of Lutz and Lytton.[25] Glyoxylic acid monohydrate and sodium glyoxylate were obtained from Sigma Chemical Co., as were all enzymes employed in this study. Desferal mesylate was a gift from CIBA Pharmaceutical Co. High-pressure LC analyses were achieved by use of a Waters Associates Model 6000A solvent delivery system, Model 440 absorbance detector, and Model U6K septumless injector and a μ-Bondapak C_{18} column. Melting points were taken on a calibrated Thomas-Hoover Unimelt. Ultraviolet spectra were obtained on a Beckman Model 25 spectrophotometer, infrared spectra were obtained on a Perkin-Elmer Infracord, 60-MHz NMR spectra were obtained on a Varian EM 360A spectrometer, and mass spectra were obtained on a Du Pont 21-492 instrument. Elemental analyses were performed by Galbraith Laboratories. Liquid scintillation counting was conducted with a Beckman LS 100C.

Synthesis of *N*-Hydroxyformanilides 5a–i. Compounds **5a** and **5c** were synthesized by both methods A and B, while compounds **5b** and **5d–i** were prepared by Method B.

Method A.[10] To a solution of 0.02 mol of the arylhydroxylamine in 50 mL of anhydrous Et_2O, which was stirred with a magnetic stirring bar and cooled in an ice bath to 0 °C, was added in a single portion 6.2 g (0.03 mol) of dicyclohexylcarbodiimide in 20 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 5 min. Further stirring and cooling was continued for 15 min, and then the mixture was filtered, the filter cake was washed with 20 mL of Et_2O, and the combined Et_2O portion was extracted with a solution of 1.2 g (0.03 mol) of NaOH in 30 mL of ice-cold H_2O. The Et_2O layer was washed with 10 mL of H_2O, which was combined with the base extract and then extracted with 20 mL of Et_2O. The pH of the aqueous portion was adjusted to 5 with 5% HCl and then extracted twice with 40 mL of Et_2O. The combined Et_2O extract was dried (Na_2SO_4) and evaporated in vacuo to give a brown residue which, following decolorization with 0.5 g of Norit (neutral) in 10 mL of acetone, was recrystallized twice from acetone/Et_2O/hexane (1:2:3) to give white crystals. The yields for **5a** and **5c** were 1.3 (47%) and 1.4 g (41%), respectively. Melting points and λ_{max} values are recorded in Table I (for **5a**, lit.[26] mp 68–69 °C). The NMR, IR, and mass spectral data were consistent with the assigned structures, and satisfactory elemental analyses (C, H, N) were obtained for **5a** ($C_7H_7NO_2$) and **5c** ($C_7H_6ClNO_2$).

Method B. A solution of 9.2 g (0.10 mol) of glyoxylic acid monohydrate in 500 mL of H_2O was adjusted to pH 6 with 10 M NaOH and heated to 40 °C in a water bath. To this solution was added 5 mmol of the nitroso aromatic as a solution in a minimal amount of EtOH, and the reaction was stirred for 2 h and then treated with 50 g of NaCl. The aqueous reaction was extracted twice with 300 mL of Et_2O, and the combined Et_2O extract was dried (Na_2SO_4) and evaporated to give a white to pale yellow solid. Recrystallization once from acetone/Et_2O/hexane gave the desired products in high purity in the following amounts and yields: **5a** ($C_7H_7NO_2$), 462 mg (68%); **5b** ($C_7H_6BrNO_2$), 800 mg (74%); **5c** ($C_7H_6ClNO_2$), 600 mg (70%); **5d** ($C_7H_6ClNO_2$), 557 mg (65%); **5e** ($C_8H_9NO_2$), 612 mg (81%); **5f** ($C_8H_9NO_2$), 390 mg (52%); **5g** ($C_7H_6FNO_2$), 513 mg (66%); **5h** ($C_8H_9NO_3$), 392 mg (47%); **5i** ($C_{10}H_{11}NO_4$), 786 mg (75%). Compounds **5a** and **5c** were identical with those prepared by method A. All compounds gave satisfactory elemental analyses for C, H, and N, and their NMR, IR, and mass spectral analyses were consistent with the assigned structures. Melting points and λ_{max} values are recorded in Table I.

General Reaction and Analytical Methods. Sodium glyoxylate was dissolved in 0.05 M, pH 6.0, KH_2PO_4 buffer, the pH of the resulting solution was adjusted to 6.0 with 0.05 M KH_2PO_4, and the solution was then diluted to the desired concentration of glyoxylate with 0.05 M, pH 6.0, KH_2PO_4 buffer. Aliquots of generally 10.0 mL were placed in 10 cm × 22 mm (i.d.) test tubes which were stoppered with silicone stoppers, placed in a water bath, and equilibrated to the desired reaction temperature. The reaction was initiated by the addition of the nitroso aromatic as a solution in 95% ethanol. The concentration of the nitroso aromatic in the ethanolic solution was such that the volume of addition of the solution was 0.01 that of the buffered glyoxylate solution, generally 100 μL. Following the substrate addition, the mixture was rapidly shaken and then allowed to react at the desired temperature for a carefully timed period of either 10 or 15 min. Thirty seconds prior to the desired total incubation period, a 10 μL aliquot was taken with a syringe and loaded into

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the injector of the high-pressure LC system. The injector lever was turned exactly at the lapse of the desired time, which caused the aliquot to rapidly mix with the methanolic elution solvent and be transported to the high-pressure LC column.

The high-pressure LC system consisted of a Waters μ-Bondapak C_{18} column (30 cm × 3.9 mm i.d.) with 50% 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.[6] Detection was made at 254 nm, and the signal peak height of the *N*-hydroxyformanilide product converted to micromoles by comparison with the peak height of a known amount of the standard *N*-hydroxyformanilide. Standard calibration curves for each product were prepared immediately prior to the analysis and were always linear in the range in which the studies were conducted.

Special Reaction Method for Time-Course Studies. For those studies that required sequential analyses at given times exceeding 15 min, the above general methodology was modified in the following manner. A 25-mL, round-bottomed flask (Kontes K-294000) fitted with a syringe-needle adapter (Kontes K-275750) was filled with 30.0 mL of 0.05 M, pH 6.0, KH_2PO_4 buffer containing 5.0 mM sodium glyoxylate. After equilibration to 30 °C in a heated water bath, the flask was placed in a New Brunswick Model G-24 shaker and further allowed to equilibrate to 30 °C for about 15 min. The reaction was initiated by the addition of 3 μmol of the nitroso aromatic in 300 μL of ethanol. Rapid mixing was achieved within 15 s by agitation at 400 rpm, after which the agitation rate was lowered to 200 rpm. Aliquots for analysis were taken by temporarily stopping the agitation and then inserting a high-pressure LC syringe needle through the septum.

Radiotracter Experiments with Sodium [1-^{14}C]Glyoxylate. Approximately 50 μCi of sodium [1-^{14}C]glyoxylate (specific activity 8.33 mCi/mmol; Amersham Corp.) was diluted with 499 mg of sodium glyoxylate to give sodium [1-^{14}C]glyoxylate with a specific activity determined to be 9.18 μCi/mmol. The diluted sodium [1-^{14}C]glyoxylate was dissolved in 5.0 mL of H_2O and kept frozen until needed. Incubations were conducted under N_2 in Thunberg tubes by placing 10.0 mL of pH 5.0, 0.05 M, KH_2PO_4 buffer containing 50 μL (0.48 μCi total radioactivity, 52 μmol) of the diluted sodium [1-^{14}C]glyoxylate solution in the reaction tube. The top reservoir was charged with 200 μL of Protosol (New England Nuclear), and the reaction was initiated by the addition of sufficient 4-methylnitrosobenzene as a 100-mM solution in EtOH to achieve the desired reaction concentration. The tubes were incubated in a water bath at 25 °C for 4 h, and then 100 μL of 10% H_2SO_4 was carefully added through the side arm of the tube. After further incubation for 4 h, the Protosol was quantitatively transferred into 12 mL of Aquasol (New England Nuclear) in a glass LSC vial and counted after chemiluminescence effects had disappeared (16 h). Counting efficiency was determined by recounting each vial after the addition of 45 000 dpm of [^{14}C]toluene standard. High-pressure LC analysis of the reaction product was used to quantitatively determine the amount of *N*-hydroxy-*N*-(4-methylphenyl)formamide (**5e**) produced during the incubation. The isolation of **5e** from a large-scale radiotracer experiment (60 mL with 4-methylnitrosobenzene at 0.50 mM) conducted in an identical manner was achieved by extraction twice with 60 mL of Et_2O after the 4-h incubation period. The combined Et_2O extract was washed with 50 mL of saturated NaCl solution, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel (11 cm × 9 mm bed size) with 10 mL of $CHCl_3$ and then 50 mL of 2% MeOH/$CHCl_3$. Fractions containing **5e** were identified by the use of high-pressure LC analysis and were combined and evaporated. The residue was dissolved in 2.0 mL of EtOH, of which 0.5 mL was analyzed by LSC in Aquasol. High-pressure LC analysis indicated that the yield of **5e** was 3.3 mg (73% of theory).

Registry No. **1a**, 586-96-9; **1b**, 3623-23-2; **1c**, 932-98-9; **1d**, 932-78-5; **1e**, 623-11-0; **1f**, 611-23-4; **1g**, 352-15-8; **1h**, 1516-21-8; **1i**, 7476-79-1; **2**, 298-12-4; **5a**, 31335-69-0; **5b**, 1836-27-7; **5c**, 1613-88-3; **5d**, 4070-53-5; **5e**, 73747-07-6; **5f**, 73747-08-7; **5g**, 456-07-5; **5h**, 73747-09-8; **5i**, 73747-10-1; sodium glyoxylate, 2706-75-4.

Nitrous Acid Deamination of Axial and Equatorial *trans*-2-Decalylamines in Mixed Solvents[1,2]

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The nitrous acid deamination of the axial *trans,trans*-2-decalylamine and the equatorial *trans,cis*-2-decalylamine has been performed in water, dioxane, and sulfolane, each containing increasing concentrations of acetic acid. The nitrosation conditions were found to destroy substantial proportions of the elimination and alcohol products, and discrepancies in earlier work may be due to this phenomenon; the alcohols, but not the alkenes, can be nearly completely regenerated by ebullition of nitrogen through the solution, a procedure which is strongly recommended for future work. It is shown that the stereochemical results as well as the acetate/alcohol distribution are essentially identical for the two amines in solutions in nonhydroxylic solvents containing low concentrations of acetic acid; the retained substitution product (60–65% of the total and consisting of about one-half alcohol and one-half acetate) is believed to be formed by collapse of an ion pair containing the carbocation, an acetate ion, and a water molecule produced in the diazotization, whereas the inverted product, which is very largely acetate, is believed to arise by collapse of an inverted ion pair. Very little of the small yield of inverted alcohol is formed by solvent attack on the backside of some intermediate since greatly increasing the concentration of water in the solvent has a negligible influence on the proportion of inverted alcohol produced. The stereochemical results for the two amines diverge as the solutions in nonhydroxylic solvents become richer in acetic acid and the divergence becomes more pronounced with increasing water content of the acetic acid; the more hydroxylic the solvent, the greater the equatorial/axial ratio of substitution products. These results are attributed to unpairing of ion pairs as the hydroxylic nature of the solvent increases; the resulting, symmetrically solvated cations form mainly equatorial products. In the mixtures of acetic acid with nonhydroxylic solvents, only those ion pairs (or their precursors) in which the cation has become stereochemically inverted appear to undergo unpairing.

Ever since the principles of conformational analysis were developed and applied to six-member alicyclic systems,[5] nucleophilic substitution reactions in cyclohexane derivatives have intrigued organic chemists. It is now known

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