Spectrophotometric Determination of Acid Hydrazides *via* Nickel (II)-Catalyzed Hydroxamic Acid Formation

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Abstract □ The reaction of phenyl acetate with hydroxylamine to form acetohydroxamic acid is catalyzed by nickel (II). Kinetic studies revealed that of the two steps in this process (the first being formation of *O*-acetylhydroxylamine and the second its conversion to acetohydroxamic acid), it is the second step that is catalyzed by nickel (II). Formation of a five-membered chelate ring between nickel (II) and *O*-acetylhydroxylamine was postulated to account for the catalysis. This hypothesis suggested that acid hydrazides, which are structurally similar to *O*-acylhydroxylamines, should also undergo hydroxylaminolysis catalyzed by nickel (II). This predicted catalysis was observed with four acid hydrazides. A spectrophotometric method for acid hydrazides was developed based upon their nickel (II)-catalyzed conversion to hydroxamic acids, followed by treatment with ferric ion to give the colored ferric hydroxamate complex. Isonicotinic acid hydrazide can be determined in the 10^{-4}–10^{-2} *M* concentration range in aqueous solutions without interference from isonicotinic acid.

Keyphrases □ Acid hydrazides—nickel (II)-catalyzed hydroxamic acid formation, ferric hydroxamate colorimetric analysis □ Nickel (II)-catalyzed hydroxylaminolysis—in spectrophotometric analysis of acid hydrazides □ Ferric hydroxamate complex formation—in spectrophotometric analysis of acid hydrazides □ Colorimetry—analysis, acid hydrazides

The "ferric hydroxamate" method for the determination of carboxylic acid derivatives is based on Eq. 1:

$$RCOX + NH_2OH \rightleftarrows RCONHOH + HX \qquad \text{(Eq. 1)}$$

The carboxylic acid derivative RCOX (X is OR, NHR, SR, Cl, or OCOR) is converted to the corresponding hydroxamic acid, which is then complexed with ferric ion, yielding a color suitable for spectrophotometric measurement. This approach has been widely applied to esters, amides, anhydrides, *etc.* Under the usual alkaline reaction conditions, carboxylic acids themselves do not react.

A recent detailed study (1) of the reported acid (2) and nickel (II)-catalyzed (3) reactions of carboxylic acids to form hydroxamic acids resulted in the development of analytical methods for carboxylic acids based upon their direct nickel (II)-catalyzed conversion to hydroxamic acids in aqueous solution (4). During this study the question arose whether nickel (II) might be capable of catalyzing the reaction of an ester with hydroxylamine. The answer to this question led to the analytical method for acid hydrazides discussed in this paper. To clarify the chemistry of the hydrazide method, a brief summary of the ester study will first be presented. Because acid hydrazides are relatively resistant to base-catalyzed hydroxamic acid formation, the conventional procedure is inapplicable to their determination.

EXPERIMENTAL

Materials—All chemicals were, unless otherwise stated, analytical reagent grade and were used directly. Ferric perchlorate hexahydrate[1], $Fe(ClO_4)_3 \cdot 6H_2O$, was used directly. Phenyl acetate[2] was distilled at atmospheric pressure, b.p. 193–194° [lit. (5) b.p. 193–195°]. Benzhydrazide was synthesized by the method of Gatterman and Wieland (6). The product was recrystallized from water, m.p. 111–112° [lit. (7) m.p. 112°]. Acethydrazide was prepared by the method of Curtius and Hofman (8) and recrystallized from ether, m.p. 65° [lit. (8) m.p. 67°]. Isonicotinic acid hydrazide[3] was recrystallized from methanol, m.p. 169–170° [lit. (9) m.p. 171°]. *p*-Nitrobenzhydrazide[4] was recrystallized from methanol, m.p. 209–210° [lit. (10) m.p. 210°]. Semicarbazide hydrochloride[4] was recrystallized from aqueous methanol, m.p. 175–180° dec. [lit. (11) m.p. 173° dec.]. Acetophenylhydrazide[4] was recrystallized from aqueous methanol, m.p. 127–128° [lit. (12) m.p. 128°].

All water was redistilled from alkaline permanganate. Standard pH buffer solutions were prepared according to Bates (13). Ferric perchlorate stock solution (1.5 *M*) was prepared by dissolving 693.45 g. of ferric perchlorate hexahydrate and 217 ml. of 60% perchloric acid in enough water to make 1 l. This solution was stored in the dark. Ferric perchlorate reagent solution (0.15 *M*) was prepared by diluting 100.0 ml of ferric perchlorate stock solution to 1 l. with methanol. Phenyl acetate stock solutions in methanol were freshly prepared before use.

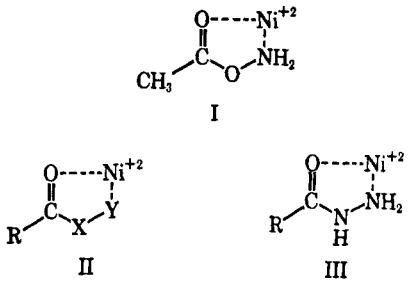
Apparatus—Spectral measurements were made with a spectrophotometer[5] fitted with thermostatted cell compartments set at 25°. The pH measurements were made with either a pH meter[6] with scale expander and equipped with a combination electrode[7] or a pH meter[8] with a high-temperature combination electrode[9].

Procedures—*Phenyl Acetate Kinetics*—Rates of phenol release were measured by direct monitoring of the reaction solution in the spectrophotometer, the absorbance at 275 nm. being recorded as a function of time. The initial concentration of ester was about 6×10^{-4} *M*. Apparent first-order rate constants were obtained from plots of $\log (A_\infty - A_t)$ against time, where A_t is the absorbance at time t, and A_∞ is the absorbance when the reaction is essentially complete.

Rates of hydroxamic acid production were measured in solutions initially about 6.5×10^{-3} *M* in phenyl acetate. The reaction mixture was shaken well and poured into a 25-ml. glass-jacketed buret, through the jacket of which 25.0° water was circulated. The 1.0-ml. samples were each run into 20 ml. of ferric perchlorate reagent solution at recorded times. After standing, protected from light, for 1 hr., the solution absorbances were measured at 530 nm. (14). Apparent first-order plots were made as already described. The jacketed-buret technique for withdrawing samples allows kinetic study of reactions with half-lives as short as 30 sec., which would not be possible with sampling by pipet.

Acid Hydrazide Rate Behavior—The dependence of hydroxamic acid production on time was determined as follows. A solution was prepared by mixing 20.0 ml. of 4.0 *M* hydroxylamine hydrochloride, 10.0 ml. of 0.4 *M* nickel chloride, and 50 ml. of water. The pH was adjusted to 6.5 with saturated sodium hydroxide solution. Then 1.0 ml. of a 0.5 *M* aqueous solution of the acid hydrazide was added, the volume was brought to 100 ml. with water, and the pH of the final solution was adjusted to 6.50. Three-milliliter portions of this solution were sealed in 5-ml. glass ampuls. The ampuls were placed in a 90.5° water bath (or a boiling water bath for routine analytical work). Ampuls were removed at 5-min. intervals, im-

[1] G. F. Smith Co.
[2] Aldrich Chemical Co.
[3] Pfizer Chemical Co.
[4] Eastman Organic Chemicals.
[5] Cary model 14 or 16.
[6] Radiometer, model 25.
[7] Sargent S 30072-15.
[8] Orion, model 801.
[9] Fisher 13-639-90.



I

II

III

mediately immersed in an ice water bath to quench the reaction, and stored in a refrigerator freezer until all samples were withdrawn. The ampuls were brought to 25°, and 1.0 ml. of the contents of each ampul was transferred to separate foil-wrapped flasks containing 20.0 ml. of ferric perchlorate reagent solution. After 1 hr., the absorbance at 530 nm. was measured. From a plot of A_{530} against reaction time, the optimum reaction time (corresponding to time of maximum absorbance) was selected for the acid hydrazide.

Analytical Procedure—To 5.0 ml. of sample solution containing 10^{-4}–10^{-2} *M* acid hydrazide was added 2.0 ml. of 4.0 *M* hydroxylamine hydrochloride and 1.0 ml. of 0.4 *M* nickel chloride. The pH was adjusted to 6.50 with saturated sodium hydroxide and the volume was brought to 10.0 ml. with water. Five milliliters of this solution was sealed in a 10-ml. glass ampul. This procedure was repeated with a standard solution of an authentic sample of the acid hydrazide having approximately the same concentration as the unknown solution. Both ampuls were placed in a boiling water bath. At the optimum reaction time (as determined in the preceding paragraph), both ampuls were removed and immersed in an ice water bath. They were brought to 25°, and 1.0 ml. of the contents of each ampul was pipeted into 20.0 ml. of ferric perchlorate reagent solution contained in a foil-wrapped flask. After 1 hr., the absorbance of each solution was measured at 530 nm. in a 2-cm. cell. The concentration of acid hydrazide in the unknown solution, C_u, was calculated with Eq. 2:

$$C_u = C_s\left(\frac{A_u}{A_s}\right) \qquad \text{(Eq. 2)}$$

where C_s is the concentration of the standard solution and A_u and A_s are the absorbances of the unknown and standard solutions, respectively.

Alternatively, a Beer's law working curve may be prepared by subjecting graded concentrations of standard solutions to this procedure, concomitantly treating the sample solution in the same way, and then reading the sample concentration from the plot of absorbance *versus* standard solution concentration.

RESULTS AND DISCUSSION

Nickel-Catalyzed Hydroxamic Acid Formation from Phenyl Acetate—Activated acyl compounds were shown by Jencks (15, 16) to react with hydroxylamine in a two-step process, the first reaction giving an *O*-acylhydroxylamine (Eq. 3) which then reacts with hydroxylamine to yield the hydroxamic acid (Eq. 4)[10]:

$$RCOOR' + NH_2OH \rightarrow RCOONH_2 + R'OH \qquad \text{(Eq. 3)}$$

$$RCOONH_2 \xrightarrow{NH_2OH} RCONHOH \qquad \text{(Eq. 4)}$$

Phenyl acetate, the substrate in the present study, is sufficiently labile to permit kinetic studies at 25°. The progress of the first step of the reaction was followed by monitoring the absorbance change at 275 nm., which is caused by the production of phenol. The second step was followed by measuring the ferric hydroxamate color; *O*-acetylhydroxylamine does not give a color with ferric ion. Good first-order behavior was observed for both the phenol release and hydroxamic acid production. Plots of apparent first-order rate constants against total hydroxylamine concentration, at constant pH, showed positive curvature, suggesting a rate term higher than the first order in hydroxylamine. The simplest assumption is that the rate equation contains terms first order and second order in hydroxylamine, as in Eq. 5:

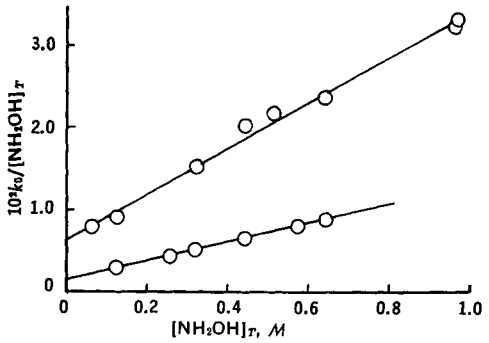


Figure 1—*Apparent second-order rate constants for phenol production (top line) and hydroxamic acid formation (bottom line) from phenyl acetate in the absence of nickel (II), plotted as a function of total hydroxylamine concentration (25°, pH 6.05, ionic strength 1.0* M).

$$v = k'[CH_3COOPh][NH_2OH] + k''[CH_3COOPh][NH_2OH]^2 \qquad \text{(Eq. 5)}$$

Equation 5 can be transformed into Eq. 6, where k_2 and k_3 are functions of pH and the dissociation constant of hydroxylamine, and where $[NH_2OH]_T = [NH_2OH] + [NH_3OH^+]$:

$$\frac{v}{[CH_3COOPh]} = k_{obs.} = k_2[NH_2OH]_T + k_3[NH_2OH]_T^2 \qquad \text{(Eq. 6)}$$

Plots of $k_{obs.}/[NH_2OH]_T$ (an apparent second-order rate constant) against $[NH_2OH]_T$ are linear, showing that Eq. 5 is the rate equation for this system. Figure 1 shows these plots for phenol release and hydroxamic acid production in the absence of nickel (II). Both the second-order constant k_2 (intercept) and the third-order constant k_3 (slope) are larger for phenol release than for hydroxamic acid production. The second step of the process, formation of acetohydroxamic acid from *O*-acetylhydroxylamine, is the rate-determining step under these conditions.

In the presence of nickel chloride, the rate of acetohydroxamic acid formation is increased. This same graphical treatment gave Fig. 2, whose notable feature is the superposition of the lines for phenol release and hydroxamic acid formation. Analysis of this plot shows that the phenol release rate is slightly decreased by nickel (II) [probably because Ni(II) ties up the nucleophile NH_2OH in nickel–hydroxylamine complexes], but that Ni(II) increases the rate of hydroxamic acid formation. It appears, in fact, that Ni (II) catalyzes the second step sufficiently so that the first step becomes rate limiting, hence the identical rate behavior seen in Fig. 2 for the two steps[11].

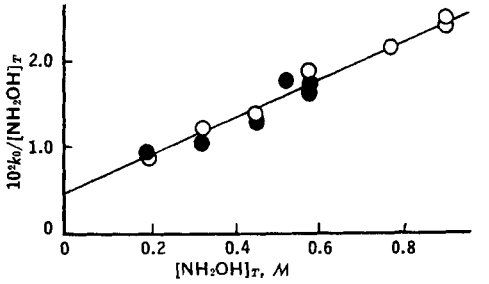


Figure 2—*Apparent second-order rate constants for phenol production* (○) *and hydroxamic acid formation* (●) *from phenyl acetate in the presence of 0.04* M *nickel chloride, plotted as a function of total hydroxylamine concentration (25°, pH 6.05, ionic strength 1.0* M).

[10] Notari (17) presented evidence for *O*-acetylhydroxylamine formation in the reaction of ethyl acetate with hydroxylamine.

[11] A more detailed kinetic analysis was made (18) but is not necessary for the present purpose.

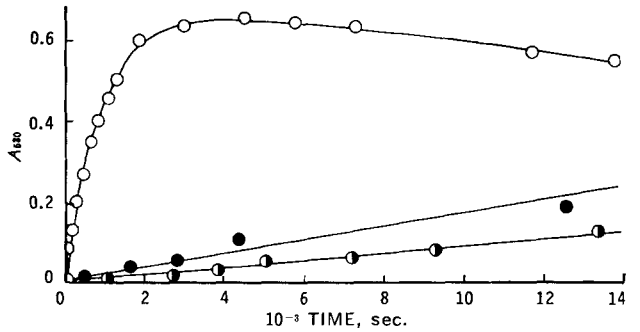


Figure 3—*Absorbance of the ferric benzohydroxamate complex as a function of time for the reaction of 0.005* M *benzhydrazide with 0.8* M *hydroxylamine [90.5°, ionic strength 1.0* M, *1% (v/v) methanol]. Key:* ○, *pH 6.75, 0.04* M *nickel chloride;* ●, *pH 11.52, no nickel chloride;* and ◑, *pH 6.75, no nickel chloride.*

It is proposed that the nickel (II) catalysis of the conversion of *O*-acetylhydroxylamine to acetohydroxamic acid occurs through a five-membered chelate ring as in I. This chelation will polarize the carboxyl function, rendering attack by hydroxylamine easier at the acyl carbon.

Nickel (II) Catalysis in Analysis of Hydrazides—The hypothesis that chelation by nickel (II) as in Structure I is responsible for catalysis of hydroxamic acid formation from phenyl acetate suggests that other carboxylic acid derivatives of suitable structure, such as II where Y is a group capable of coordination to a metal ion, might similarly be made labile to nucleophilic attack upon chelation with nickel (II). Acid hydrazides have such a structure, and the presumed chelate of nickel (II) with an acid hydrazide is shown in Structure III.

Figure 3 is a plot of absorbance due to the ferric hydroxamate complex (that is, a measure of hydroxamic acid production) against time for benzhydrazide in the presence and absence of nickel (II). The predicted catalysis obviously occurs[12]. (Figure 3 also shows data under alkaline conditions, revealing why the conventional ferric hydroxamate method is not useful for hydrazides.) The slow decrease in absorbance after initial catalysis may be the result of a nickel (II)-catalyzed hydrolysis of benzohydroxamic acid. The rate of hydroxamic acid formation catalyzed by nickel (II) increases with pH, but precipitation of nickel hydroxide occurs above pH 7.

A colorimetric determination of an acid hydrazide, based upon this nickel (II)-catalyzed conversion to the hydroxamic acid, uses these three steps: (*a*) determination of the optimum reaction time, corresponding to the time to reach maximum hydroxamic acid yield, as in Fig. 3; (*b*) treatment of the sample solution and a standard solution of the same hydrazide with a nickel–hydroxylamine reagent for the predetermined reaction time; and (*c*) development of the ferric hydroxamate color. The following optimum reaction times were found at 90.5° with 0.8 *M* hydroxylamine, 0.04 *M* nickel chloride, and pH 6.5: benzhydrazide, 5000 sec.; isonicotinic acid hydrazide, 1500 sec.; acethydrazide, 2500 sec.; and *p*-nitrobenzhydrazide, 2700 sec. Table I shows absorption data for standard solutions of isonicotinic acid hydrazide; over this concentration range the calibration curve is linear, the least-squares line for all of the data being $A_{530} = 143.86C + 0.0025$, with the standard deviation from the line being 0.01.

Isonicotinic acid (5.3×10^{-3} *M*) itself gave no measurable color when subjected to the same reaction conditions as its hydrazide. Semicarbazide and acetophenylhydrazide also did not react. Because of its different basis from many current methods for isonicotinic acid hydrazide (many of these are based on oxidation–reduction reactions), the nickel (II)-catalyzed hydroxamic acid method described here may find use in systems where redox reactions are inapplicable. As a general method for the determination of acid hydrazides, this method represents an extension of the ferric hydroxamate method to another functional grouping by taking advantage of a mechanistic study to suggest a new analytical reaction.

[12] This, however, does not mean that Structure III must be correct. Several structures have been proposed for metal–hydrazide complexes (19, 20).

Table I—Calibration Data for Nickel (II)-Catalyzed Hydroxamic Acid Formation from Isonicotinic Acid Hydrazide[a]

10^3c, *M*	A_{530}[b]	$\bar{A}_{530}$
0.564	0.076, 0.073, 0.078, 0.076, 0.093, 0.074	0.078
1.69	0.238, 0.238, 0.240, 0.244 — —	0.240
2.82	0.403, 0.414, 0.404, 0.408, 0.428, 0.403	0.410
3.95	0.578, 0.578, 0.587, 0.572, 0.585, 0.604	0.584
5.64	0.830, 0.802, 0.799, 0.803, 0.800, 0.798	0.805

[a] 90.5°, pH 6.5, 0.8 *M* hydroxylamine, 0.04 *M* nickel chloride, reaction time 1500 sec. [b] Measured in 2-cm. cells; 1 ml. of reaction mixture added to 20 ml. of 0.15 *M* $Fe(ClO_4)_3$.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 13, 1971, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication October 18, 1971.

Supported by the National Institute of Dental Research Training Program (DE-171) and National Institutes of Health General Research Support Grant RR 05456.

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