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QUALITATIVE AND QUANTITATIVE ASPECTS OF THE
OXIDATION OF 2,4-DINITROPHENYLHYDRAZINE
IN THE PRESENCE OF PYRIDINE

DISSertation
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By
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*******

The Ohio State University
1961

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PURPOSE

The purpose of this investigation was to study the interference of Cu(II) and other metal compounds in the colorimetric determination of trace amounts of aldehydes and ketones based on the formation of 2,4-dinitrophenylhydrazones. On treatment with alkali, these hydrazones yield intense wine-red solutions which are quite stable in the presence of pyridine.

Since the presence of very small amounts of copper had a marked effect, it was felt that this investigation might lead to a sensitive analytical method for copper.
INTRODUCTION

The possibility of complex formation between Cu(II) and 2,4-dinitrophenylhydrazine was investigated. The results of a spectrophotometric study by the Job method and other considerations did not support the hypothesis of interference by complex formation. However, the observed similarity in chemical behavior between 2,4-dinitrophenylhydrazine and 2,4-dinitrobenzenediazonium salts indicated the possibility of oxidation of the hydrazine by the copper. As an extension of this study, the formation of 2,4-dinitrophenylpyridinium salts from 2,4-dinitrophenylhydrazine, a suitable oxidizing agent and pyridine was investigated. Cleavage of the 2,4-dinitrophenylpyridinium ion in strong alkali results in the formation of an acid-base indicator system. The wine-red to purple color of the indicator base was found to be responsible for the interference in the colorimetric determination of carbonyls.

The great sensitivity of the reactions involved and the intense color of the indicator ion led to the study of the applicability of this sequence of reactions to qualitative and quantitative analysis.
PRELIMINARY STUDIES

A colorimetric method of determination of trace amounts of carbonyl compounds based on the formation of 2,4-dinitrophenylhydrazones and the subsequent treatment with alkali was developed by Lappin and Clark. The hydrazones are formed by addition of a solution of the carbonyl compound to an aqueous solution of 2,4-dinitrophenylhydrazine hydrochloride. Addition of alcoholic sodium or potassium hydroxide to solutions of 2,4-dinitrophenylhydrazones produces a very intense wine-red color, presumably due to resonance forms of a quinoidal ion:

The wavelength of the absorption maximum of the quinoidal ion as well as the extinction coefficient at the maximum are nearly independent of the carbonyl compound from which the hydrazone is derived. Lappin and Clark found an average extinction coefficient of $2.72 \times 10^4$ at 480 m$\mu$ for the hydrazones they studied. Carbonyl concentrations as low as $5 \times 10^{-7}$ molar can be detected by this method and an accuracy of two parts per hundred may be achieved in the range of $5 \times 10^{-6}$ to $10^{-4}$ molar carbonyl. The colors of the hydrazones are claimed to be stable over a period of days.

Boehme and Winkler were unable to apply the method described by Lappin and Clark in the determination of acetaldehyde. They
found two absorption maxima, one at 440 μm and one at 520 μm. The resulting hydrazone showed little stability, its intensity being reduced to one-third over a period of only one hour. To stabilize the color the use of pyridine was suggested as a solvent. While the use of pyridine is essential in the colorimetric determination of acetaldehyde, it is commonly used as a color-stabilizing agent in the determination of other carbonyl compounds as well.

The presence of Cu(II)-salts in the sample to be tested for carbonyl was found to interfere with the colorimetric determination of aldehydes and ketones if pyridine was used as a solvent. Addition of a solution of 2,4-dinitrophenylhydrazine hydrochloride, pyridine and sodium hydroxide in the order indicated to carbonyl-free aqueous solutions of Cu(II) produced solutions that varied in color from wine-red to purple depending on the ratio of pyridine to water in the system. The visible absorption spectra of these solutions showed two maxima, one in the range of 530 to 550 μm and one between 365 and 380 μm depending on the pyridine content. The intensely colored chromophore does not form if pyridine is eliminated from the system. It was also observed that a solution of the free base 2,4-dinitrophenylhydrazine in methanol may be used instead of the hydrochloride. Since the reaction between a carbonyl and a hydrazine function is acid-catalyzed, however, the reaction could not have produced a 2,4-dinitrophenyl-
hydrazone which might conceivably be obtained by interaction of the 2,4-dinitrophenylhydrazone and formaldehyde formed from the methyl alcohol by oxidation with cupric ion.

The first preliminary experiments suggested the possibility of complex formation between copper and 2,4-dinitrophenylhydrazone involving one of the nitrogen atoms of the hydrazine function and possibly the nitro-group in the ortho position. Pyridine was assumed to function as a ligand rather than as solvent. Even though the rather high concentration of hydroxide ion needed could not be explained assuming coordination of 2,4-dinitrophenylhydrazine and pyridine with copper, the possibility of complex formation was nevertheless investigated.

Application of Job's method of continuous variation gave inconsistent and ambiguous results mainly because the system under investigation was too complex to be treated as a two or three component system. The following purely qualitative considerations seemed to rule out complex formation: (1) The fact that other metal ions which have complexing characteristics similar to those of Cu(II) failed to produce the intensely colored material when brought to reaction under identical conditions. The ions studied were the divalent ions of zinc, cadmium, mercury(II), cobalt, nickel, palladium, iron, manganese, lead, tin and magnesium; (2) the failure of piperidine, ammonia, aliphatic amines, 2,6-dimethylpyridine and 2,4,6-trimethylpyridine to take the place of pyridine in the
reaction: (3) the observation that a mixture of alcoholic 2,4-dinitrophenylhydrazine and aqueous Cu(II) which had been allowed to stand for several hours no longer produced the purple solution upon addition of pyridine and sodium hydride.

While the experimental evidence indicated that 2,4-dinitrophenylhydrazine itself did not coordinate with copper, complex formation involving the decomposition products of the hydrazine in alkaline medium remained as a possibility. Interaction of 2,4-dinitrophenylhydrazine with sodium hydride produces mainly 6-nitro-1-hydroxy-1,2,3-benztriazole, m-dinitrobenzene and m,m'-dinitroazoxybenzene. A brown flocculent acid is also produced in increasing amounts as the pH increases, possibly owing to the action of alkali on the dinitrobenzene. Addition of some pyridine and excess sodium hydride to a solution of 2,4-dinitrophenylhydrazine in methyl alcohol produced at first a deep green color which rapidly turned dark brown and then faded within a few minutes to yield a clear amber solution. Addition of Cu(II) to the mixture of decomposition products either immediately following the addition of sodium hydride or to the clear amber solution did not result in any apparent reaction.

The results of these experiments clearly indicated no complex formation between Cu(II) and 2,4-dinitrophenylhydrazine or with any of its decomposition products in base unless an unstable intermediate had interacted with the copper to form a fairly stable
complex. The latter would have explained the fact that copper had to be present before base was added to obtain the purple color. Attempts to isolate the purple substance by rapid evaporation of the solvents, precipitation or extraction methods ended in each case with the formation of tarry materials.

The experiment which eventually led to a partial understanding of the reactions involved was the following: The 2,4-di-nitrophenylhydrazine in the reaction with copper, pyridine and base was replaced by a compound similar in structure and size. The chemical chosen was the diazonium ion of 2,4-dinitroaniline. If pyridine is added to an aqueous solution of 2,4-dinitrobenzene-diazonium salt one may envisage the formation of a so-called "Gomberg-Intermediate," \( \text{O}_2\text{N-} \begin{array}{c} \text{N=N-N=O} \end{array} \text{OH,} \) which resembles 2,4-di-nitrophenylhydrazine in the aspects important for considerations of complex formation. Indeed it was observed that the addition of Cu(II), pyridine and excess sodium hydroxide to an aqueous solution of 2,4-dinitrobenzenediazoniium sulfate produced a purple solution the absorption spectrum of which showed the two expected maxima at 530 and 370 m\( \mu \). The reaction was very vigorous and exothermic. Gas was generated during this reaction. This experiment seemed to indicate that a diazo-intermediate is involved in the reaction between 2,4-dinitrophenylhydrazine, Cu(II), pyridine and sodium hydroxide. Inasmuch as this implies oxidation of the hydrazine, however, the cupric ion is the logical choice for the
substance responsible for the formation of the diazonium intermediate.

While these additional chemical facts tended to add support to the hypothesis of complex formation, the search for the complex species came to an abrupt conclusion when it was observed that the identical purple substance could be obtained in the absence of copper simply by addition of pyridine and strong base to an aqueous solution of a 2,4-dinitrobenzenediazonium salt. The only function of the cupric ion therefore appeared to be that of an oxidizing agent. Further investigation showed this to be a correct hypothesis.
THE OXIDATION OF 2,4-DINITROPHENYLHYDRAZINE

IN THE PRESENCE OF PYRIDINE

Introduction

The preliminary studies showed that Cu(II) must have oxidized 2,4-dinitrophenylhydrazine to a diazonium compound since the reaction between Cu(II), 2,4-dinitrophenylhydrazine, pyridine and base produced results identical with those in the reaction between a 2,4-dinitrobenzenediazonium salt, pyridine and base.

The details of the interaction of 2,4-dinitrobenzenediazonium salts with pyridine will be considered in the next section of this thesis. The purpose of this part of the work is to review the research done on the oxidation of phenylhydrazines and to determine whether or not other oxidizing agents are capable of oxidizing 2,4-dinitrophenylhydrazine. The suspected diazonium intermediate is identified in all cases by the typical and highly sensitive color reaction with pyridine and alkali.

Historical

A literature survey reveals but a few rather recent references dealing specifically with the oxidation of 2,4-dinitrophenylhydrazine. The few reactions described in the literature were studied either in aqueous medium or media other than pyridine.
There are, however, a number of publications referring to the oxidation of phenylhydrazines and primary aromatic hydrazines in general.

Emil Fischer was the first to report that yellow mercuric oxide oxidizes phenylhydrazine sulfonate in aqueous acidic solution yielding small amounts of diazonium salt. He observed the evolution of nitrogen gas and was able to isolate phenol as a product. Bamberger was able to identify the diazonium intermediate formed by oxidation of phenylhydrazine with mercuric oxide by coupling the intermediate with α-naphthylamine producing an intense violet color typical of an azo-dye. If mercuric oxide is added to a solution of phenylhydrazine in ether, aniline and diphenylmercury are formed as products in addition to benzene which demonstrates the importance of the choice of solvent in these reactions.

Fischer also studied the oxidation of phenylhydrazine with Cu(II) in alkaline medium using Fehling's solution and obtained cuprous oxide, nitrogen, benzene and aniline. From the products formed he concluded that the benzene had formed from a diazonium intermediate, but that the aniline had been formed by a different mechanism. In 1885 Bayer pointed out the quantitative nature of the oxidation of phenylhydrazine with Cu(II) as oxidizing agent and suggested an azoetric method for the quantitative determination of aromatic hydrazines. He emphasized the fact that the
reaction proceeds quantitatively only in acidic medium, some aniline being formed in basic medium. According to Walter no diazonium intermediate is observed in the oxidation of phenylhydrazine by Fehling's solution, but decomposition occurs directly with evolution of nitrogen.  

Hope and Robinson oxidized hydrazino-β-gnoscopine with cupric acetate and report precipitation of cuprous oxide and evolution of nitrogen in the reaction.

Zinke used ferric chloride to oxidize β-naphthylhydrazine, obtained the corresponding hydrocarbon but is uncommitted as to the mechanism of the reaction. Walter obtained chlorobenzene in 50 percent yield instead of benzene in the oxidation of phenylhydrazine with ferric chloride in hydrochloric acid. Chlorobenzene is also formed when phenylhydrazine is oxidized by copper sulfate in the presence of concentrated hydrochloric acid.

The halogens have also been employed as oxidizing agents. The use of bromine is reported by Michaelis who isolated p-bromo-diazobenzene on treating phenylhydrazine with bromine in concentrated hydrochloric acid. The oxidation to the diazonium salt is preceded in this case by bromination of the benzene ring. Chattaway describes a method for the preparation of solid aromatic diazonium halides from primary aromatic hydrazines and the elemental halogens. He found the oxidation to the diazonium salts to be quantitative in cold glacial acetic acid. The oxidation of phenylhydrazine
with free iodine is reported by E. von Meyer,\textsuperscript{17} the use of either iodine or iodate was studied by Miller.\textsuperscript{18}

A number of metal oxides have been used to oxidize primary aromatic hydrazines. Puxeddu reports the use of lead dioxide;\textsuperscript{19} Barakat, Abdel-Wahab and El-Sadr obtained m-dinitrobenzene in 50 percent yield from 2,4-dinitrophenylhydrazine by oxidation with manganese dioxide.\textsuperscript{20} A new method of phenylation by oxidation of phenylhydrazines with metallic oxides, preferably silver oxide, in aromatic solvents was suggested by Hardie and Thomson.\textsuperscript{21} Phenylation of the aromatic solvent occurs by attack of phenyl radicals. The oxidation of phenylhydrazine in pyridine yielded a mixture of phenylpyridines. The oxidation of 2,4-dinitrophenylhydrazine was studied only in benzene and some dinitro-biphenyl was obtained.

Potassium ferricyanide oxidizes phenylhydrazine either to the corresponding hydrocarbon or to the phenyl cyanide depending on reaction conditions.\textsuperscript{22}

Bruker and Levin have recently shown that mercuric chloride oxidizes phenylhydrazine to a diazonium intermediate which reacts further to give $\textbf{\(\text{N}_2\text{Cl}\cdot\text{HgCl}_2\)}$ and finally $\textbf{\(\text{HgCl}\)}$.\textsuperscript{23}

Berka and Zyka oxidized phenylhydrazine hydrochloride and p-nitrophenylhydrazine using lead tetra-acetate as oxidant.\textsuperscript{24}

The conclusion that can be reached on the basis of the work done on the oxidation of phenylhydrazines by inorganic metal and nonmetal compounds is that a diazo-intermediate is formed which
may or may not be stable, and which can react in a variety of ways depending on the choice of solvent and reaction conditions.

**Experimental**

In the preliminary studies it was found that the compounds known to oxidize phenylhydrazine in basic solution also oxidize 2,4-dinitrophenylhydrazine in pyridine forming a diazonium intermediate which reacts with pyridine and is identified by the evolution of nitrogen and the typical, sensitive color reaction in alkali. In addition to the known oxidizing agents a number of metal and nonmetal compounds in aqueous solution and some solids were studied.

**Preparation of Reagents**

2,4-Dinitrophenylhydrazine in pyridine. A 0.01 molar solution of 2,4-dinitrophenylhydrazine in pyridine was prepared by dissolving 0.198 g. of the purified solid hydrazine in 100 ml. of anhydrous pyridine. This solution was not used more than a week after preparation because of the slow decomposition of 2,4-dinitrophenylhydrazine in pyridine.

2,4-Dinitrophenylhydrazine in methanol. A saturated solution of the purified hydrazine in carbonyl-free absolute methyl alcohol

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1For sources and purification of reagents see Appendix II.
was prepared and was not used more than a week after preparation.

**Mono-nitrophenylhydrazines in pyridine.** Solutions 0.01 molar with respect to either 2-nitrophenylhydrazine or 4-nitrophenylhydrazine were prepared by dissolving 0.153 g. of purified solid in 100 ml. of anhydrous pyridine.

**Aqueous solutions of oxidants.** The aqueous solutions of the oxidizing agents were prepared by dissolving enough of the solid material in water to prepare solutions 0.1 molar with respect to oxidant.

**Procedure**

In the study of the water-soluble substances 2 ml. of the solution of 2,4-dinitrophenylhydrazine in pyridine were added to 2 ml. of the aqueous solution of oxidant in a 10 ml. volumetric flask. In the case of the solids, a few crystals were added to 2 ml. of 2,4-dinitrophenylhydrazine in pyridine contained in a 10 ml. volumetric flask. The mixtures were shaken and heated in a water bath if necessary to bring about reaction and to expel the nitrogen formed. The mixtures were then diluted with about 2 to 3 ml. of water and 1 molar sodium hydroxide was added until the solutions turned either purple or dark brown which faded on standing to a permanent amber or yellow color. Each volumetric flask was filled to the mark with water to adjust the pyridine content to 20 percent by volume. A visible absorption spectrum was prepared with the Cary (Model 14) Recording Spectrophotometer. All
solutions had to be diluted with 20 percent (by volume) aqueous pyridine to permit measurement of the extinction for identification. In some cases it became necessary to filter the mixture first to separate any hydrous metal oxides or unreacted solid material.

The experiments were repeated with solutions of the o- and p-mononitrophenylhydrazines in pyridine using the same procedure. Oxidation of 2,4-dinitrophenylhydrazine in the absence of pyridine was investigated by mixing 2 ml. of the aqueous solution of oxidant or some solid material with 2 ml. of the saturated solution of 2,4-dinitrophenylhydrazine in absolute methanol. Two ml. of pyridine and a few drops of 1 molar sodium hydroxide solution were then added and the mixtures were diluted to 10 ml. with water. The role of the pyridine in the reaction was further studied by substituting 2,6-dimethylpyridine and piperidine for pyridine.

**Results and Discussion**

Solutions of the following compounds were found to oxidize 2,4-dinitrophenylhydrazine in pyridine at room temperature: Cu(ClO$_4$)$_2$, AgNO$_3$, AuCl$_3$, K$_2$PtCl$_6$, K$_3$Fe(CN)$_6$, CeNH$_4$(NO$_3$)$_6$, KMnO$_4$ and KIO$_4$. All reactions were rapid and accompanied by the evolution of nitrogen gas. Aqueous solutions of Hg(I) and Hg(II) failed to give a positive reaction. However, both oxidation states of mercury are capable of oxidizing 2,4-dinitrophenylhydrazine in pyri-
dine if solid HgCl₂ and yellow HgO are used. Other solids found to oxidize the hydrazine at room temperature were: Ag₂O, MnO₂, Na₃BiO₃ and I₂. Lead dioxide oxidizes 2,4-dinitrophenylhydrazine in pyridine if the mixture is heated at 100°C. on a water bath for a few minutes.

The criterion for oxidation was the formation of a deeply purple chromophore the visible absorption spectrum of which showed two maxima, one at 548 mμ and one at 370 mμ. These wavelengths refer to a solvent medium of 20 percent by volume aqueous pyridine. The spectrum agrees in detail with that of the product formed from 2,4-dinitrobenzenediazonium salts, pyridine and base.

Inasmuch as the free diazonium ion O₂N-^N₂+ is only stable in highly acidic medium, the species present at the pH of pyridine-water mixtures is believed to be the diazonium hydroxide postulated by Gomberg.

As will be explained in the subsequent section of this thesis, the purple color is due to the formation of the acid-base indicator 5-(2,4-dinitroanilino)-2,4-pentadienal from the 2,4-dinitrobenzenediazonium intermediate, pyridine and excess sodium hydroxide. This indicator system has been studied by Schwarzenbach and Sulzberger who report a color change from yellow in acid to purple in base at high pH-values and a dependence of the pH-range of the indicator on the solvent medium. In pyridine-water mixtures the indicator acid has an absorption maximum at 415 mμ (Fig. 1, C); the indicator
FIGURE 1

Visible Absorption Spectra of the Acid-Base Indicator

$5-(2,4\text{-Dinitroanilino})\text{-}2,4\text{-pentadienal}$

A : Indicator base in the absence of pyridine

B : Indicator base in 30 percent (by volume) pyridine

C : Indicator acid in aqueous pyridine
base has two maxima, one between 528 and 557 m\(\mu\), and another one between 365 and 385 m\(\mu\), the exact locations depending on the pyridine content of the system (Fig.1, A and B). In Figure 1, the absorption of the indicator base in the absence of pyridine is represented by A; the absorption in 30 percent (by volume) aqueous pyridine by B.

The pH-dependence of the indicator equilibrium was tested by addition of 1 molar hydrochloric acid to the purple solutions. The yellow indicator acid formed and the absorption maximum at 415 m\(\mu\) was confirmed. Addition of sodium hydroxide reconverted the yellow solutions to the purple color characteristic of the indicator base.

The addition of silver oxide or Cu(II)-solutions to 2,4-dinitrophenylhydrazine in methyl alcohol followed by the immediate addition of pyridine and alkali produced the same results as the addition of the same oxidizing agents to a solution of 2,4-dinitrophenylhydrazine in pyridine. After the mixtures of the oxidants and the hydrazine had been allowed to stand for a period of one hour, however, addition of pyridine and sodium hydroxide did not result in the formation of the purple color due to the slow decomposition of the diazonium intermediate.

The fact that pyridine is a reactant rather than just a solvent has been proven previously. The unique role of the pyridine as reactant is demonstrated by the failure of 2,6-dimethylpyridine or piperidine to form the highly colored indicator base when sub-
stituted for pyridine in the course of the reaction. Nevertheless pyridine affects the absorption of the indicator base of 5-(2,4-dinitroanilino)-2,4-pentadienal as a solvent, but the same effects are produced by 2,6-dimethylpyridine, piperidine, acetone, methanol and other solvents.

Addition of any substance capable of oxidizing 2,4-dinitrophenylhydrazine to solutions of the mono-nitrophenylhydrazines in pyridine resulted in the evolution of nitrogen gas, thus indicating a similar reaction. However the oxidation of these hydrazines was not followed by the typical color reaction on treatment with excess base. This strongly implies a special chemical property of the 2,4-dinitrophenyl group.

Detection of Copper(II)

The ease with which Cu(II)-solutions oxidize 2,4-dinitrophenylhydrazine in the presence of pyridine and the extreme sensitivity of the color reaction by which 5-(2,4-dinitroanilino)-2,4-pentadienal is formed from the diazonium intermediate, pyridine and alkali lend themselves to a method for detecting micro quantities of Cu(II) in the presence of a number of metals that commonly interfere with identification tests for copper.

Preparation of Aqueous Copper Perchlorate Solutions

A 0.1 molar aqueous solution of copper ion was prepared by dissolving 18.528 g. of copper perchlorate hexahydrate in enough water to make 500 ml. of solution. This solution was standardized
iodometrically at a pH of 4.28 using ammonium bifluoride as buffer and starch as indicator. The exact concentration of the solution was found to be 0.0954 moles per liter. From this standard solution others of steadily decreasing concentrations were prepared.

**Procedure**

Nine ml. of anhydrous pyridine and 1 ml. freshly prepared 0.01 molar solution of 2,4-dinitrophenylhydrazine in pyridine were added to a 50 ml. volumetric flask. One ml. aliquots of the various copper perchlorate solutions were then added and the mixtures shaken. The solutions were diluted with about 5 ml. of water to avoid precipitation of solid sodium hydroxide on addition of 1 molar base.

Two ml. of base were used for copper solutions in the range 0.1 to 0.001 molar, one ml. for copper concentrations below 0.001 molar. The quantity of base used depends somewhat on the concentration range of copper for two reasons: (1) At relatively high concentrations of copper, part of the base is used up to compensate for the hydrolysis of cupric ions; (2) at very low concentrations of copper the effect which excess base has on the colored indicator ion becomes important. The rate of fading of the purple indicator base increases with increasing hydroxide ion concentration. The precipitation of copper hydroxide at high copper and hydroxide ion concentrations may be avoided by the addition of a few drops of a concentrated tartarate solution. All solutions were then diluted to the 50 ml. mark with water. Those solutions which were too concentrated for
spectrophotometric work were further diluted with 20 percent aqueous pyridine. The visible absorption spectra of the purple solutions showed the two expected maxima for relatively high concentrations of copper, but only a single maximum at 548 m\(\mu\) for extremely low copper concentrations.

The amount of 5-(2,4-dinitroanilino)-2,4-pentadienal which is formed is proportional to the copper concentration, but the extinction coefficient of the indicator base between 528 and 557 m\(\mu\) is significantly greater than the extinction coefficient between 365 and 385 m\(\mu\). The products formed in the reaction of the excess 2,4-dinitrophenylhydrazine with alkali do not absorb appreciably between 528 and 557 m\(\mu\), but show an absorption maximum at around 380 m\(\mu\). Pyridine itself begins to absorb below 500 m\(\mu\). It is recommended that the extinction of the test solution be measured versus a reference solution identical in composition except for the copper content. This becomes very important for copper concentrations below 10\(^{-4}\) molar. The extinction was measured within five minutes after the addition of sodium hydroxide solution and again thirty minutes later. All solutions were found to fade rapidly with time, the extinction decreasing by as much as thirty percent within half an hour.

The following metals were checked for possible interference in the oxidation reaction of 2,4-dinitrophenylhydrazine with copper: Zn, Cd, Ni, Fe(III), Co, Mn, Pb(II), Sn(II) and Mg. Various combi-
nations of these and a mixture of all the metals were treated with 2,4-dinitrophenylhydrazine, pyridine and base, and the hydroxide precipitates filtered off. None of the absorption spectra of the filtrates showed a maximum between 500 and 550 μ; only the peak at 380 μ due to the decomposition products of 2,4-dinitrophenylhydrazine in alkali was observed. Addition of small amounts of copper to each combination before the addition of 2,4-dinitrophenylhydrazine, pyridine and base produced the typical purple solution. None of the common anions were found to interfere with the reaction.

A study of the sensitivity of this method for detecting copper gave the following results: When the procedure described above was followed, a 10 ml. aliquot of 2.39·10⁻⁶ molar copper perchlorate produced an extinction of 0.160 ± 0.05 with a 10 cm. cell. The same amount of copper, when present in a mixture containing 0.02 millimoles of each of the nine metals studied for possible interference, did not produce a measurable extinction. However, 2 ml. of 2.39·10⁻⁵ M copper gave an extinction of 0.200 ± 0.085 with a 10 cm. cell when added to 2 ml. of a mixture containing 0.02 millimoles of each of the other metals. The hydroxide ion concentration was doubled in the experiments involving other metals in addition to copper to assure excess base, and any hydrous metal oxides were filtered off by suction before the extinction of the solutions was measured. The
reference solution was prepared in the usual manner from the mixture of the metals but without copper.

Discussion

The sensitivity of a reaction may be described in terms of the "Limit of Identification" and the "Concentration Limit". If the symbols suggested by Feigl are used, the sensitivity of a reaction is defined by $x[S]^y$ where $S$ stands for the particular technique employed in the identification test, $y$ is the volume in ml. of test solution and $x$ is the quantity of material in gammas which is revealable by the technique $S$ when dissolved in volume $y$. The details of technique $S$ are described in the section on procedure. In the case of 10 ml. $2.39 \times 10^{-8}$ molar copper solution, $x$ equals $1.5^\gamma$ of copper ion and $y$ is equal to 10 ml. A decrease in $x$ and $y$ did not give extinctions reproducible within a detectable range in every case.

In the presence of the other nine metals, $x$ was found to be $3^\gamma$ when $y$ was taken as 4 ml. This decrease in sensitivity is presumed to be due to some adsorption of the large negatively charged indicator ion by the positively charged metal oxide precipitates.

The concentration limit is defined as the ratio of unit weight of solute in grams to volume of solvent in ml. and is given by the expression $1 : \frac{x \times 10^6}{x \times 10^6}$. If this definition is used, the concentration limits for the detection of copper by this method are $1 : 6.7 \times 10^6$ for $0.15^\gamma$ per ml. of pure copper, and $1 : 1.3 \times 10^6$ for $0.75^\gamma$ per ml.
of copper in the presence of 0.02 millimoles of each of the nine metals tested for interference.

The advantage of this method for detecting small quantities of Cu(II) is twofold: Its sensitivity is high, and Cu(II) can be detected in the presence of many metals which are found to interfere in the identification tests for copper based on precipitation reactions or complex formation. Since the principal reaction depends on the oxidizing properties of Cu(II), copper can be detected in the presence of all those metals which do not oxidize 2,4-dinitrophenylhydrazine. The divalent ions of Zn, Cd, Ni, Co, Mn, Pb, Sn and Mg do not interfere with this spectrophotometric identification of copper(II).

The reason why this method for the qualitative determination of copper does not provide a suitable basis for a quantitative method of determination is the poor reproducibility of the extinction values which appears to be due to experimental factors involved. Both the rate of mixing of the copper with the 2,4-dinitrophenylhydrazine and the times elapsing between the additions of the various reagents in the reaction sequence appear to affect the formation of the indicator ion in a quantitative manner. In addition to this, the need for a relatively high hydroxide ion concentration results in the partial precipitation of hydrous oxides with an active surface for adsorption. Inasmuch as these factors are difficult to control, this method is not suitable for a quantitative determination of Cu.
THE REACTION BETWEEN 2,4-DINITROBENZENE-DIAZONIUM COMPOUNDS AND PYRIDINE

Introduction

This portion of the investigation deals with the reaction between pyridine and the diazonium intermediate formed by oxidation of 2,4-dinitrophenylhydrazine with Cu(II) or other suitable oxidizing agents. One of the products formed in this reaction is the 2,4-dinitrophenylpyridinium ion which is easily identifiable by its characteristic cleavage reaction in strong base yielding $5-(2,4$-dinitroanilino)$-2,4$-pentadienal.

Historical

The reactions between various aryl-diazonium salts and pyridine yielding arylpyridines have been the subject of numerous investigations. Mohlau and Berger obtained a mixture of isomeric phenylpyridines by the action of a suspension of dry solid benzene-diazonium chloride in pyridine. Using the same procedure, Koenigs and Ruppelt obtained less than 1 percent of $\gamma$-4-dimethyl-amino-phenylpyridine from the dry diazonium chloride prepared from p-amino-dimethylaniline. Kuhling claimed to have obtained $\alpha$-4-nitrophenylpyridine in "good yield" by the action of dry sodium p-nitrobenzene-iso-diazotate on pyridine in glacial acetic acid, but repetition
of this work by Forsyth and Pyman showed that the product was a mixture consisting of α-, β- and γ-nitrophenylpyridines in 15, 5 and 2 percent yield respectively.32

The action of solid dry di-p-nitrobenzenediazonium sulfide on pyridine is recorded by Bamberger and Kraus who obtained a mixture of 4-nitrophenylpyridines, but no yield was reported.33

Tschitschibabin obtained a mixture of phenylpyridines in "very small" yield by the action of acetic acid on a mixture of aniline, powdered sodium nitrite and pyridine.34 Gomberg and Bachmann obtained a certain "phenylpyridine" by the addition of aqueous alkali to a mixture of aqueous benzene diazonium chloride and pyridine, but again no yield was reported.35

In a contribution by Haworth, Heilbron and Hey a new method for the preparation of arylpyridines is described.36 The method consists of the addition of an aqueous solution of a diazonium salt to an excess of pyridine. The yields vary from 20 to 80 percent depending on conditions. The work deals with the preparation of the three phenylpyridines obtained from diazotized aniline and with the nine mono-nitrophenylpyridines obtained from diazotized o-, m- and p-nitroanilines and pyridine. A free radical mechanism is suggested for the process and only C-arylation is reported in all cases. The yields reported are 75 percent for the nitrophenylpyridines obtained from p-nitroaniline and 35 percent for the nitrophenylpyridines derived from either o- or m-nitroaniline. The compound
2,4-dinitroaniline was not studied by Haworth and coworkers.

Elks and Hey obtained a mixture consisting of \( \text{\( \beta \)-naphthyl-} \) pyridines from pyridine and diazotized \( \beta \)-naphthylamine.\(^{37} \)

Phenylation of pyridine is also reported in a recent publication by Hardie and Thomson who obtained a mixture of phenylpyridines by oxidation of phenylhydrazine with silver oxide.\(^{21} \) Their work deals with the phenylation of a large number of aromatic solvents by oxidation of arylhydrazines with silver oxide in the solvent. Phenylation is believed to occur by attack of phenyl radicals.

A summary and discussion of the various reactions involving substitution on the pyridine ring may be found in a recent publication by Thomas and Jerchel.\(^{38} \)

**Discussion**

When solid dry 2,4-dinitrobenzenediazonium fluoborate is added to a large excess of anhydrous pyridine a vigorous reaction occurs whereby nitrogen is evolved and an orange solution is formed. Addition of aqueous sodium hydroxide to this solution results in the formation of 5-(2,4-dinitroanilino)-2,4-pentadienal which is known to be formed by cleavage of 2,4-dinitrophenylpyridinium compounds in strong alkali.\(^{39,40} \)

Two mechanisms seem probable for the interaction of a solid 2,4-dinitrobenzenediazonium compound with pyridine. The fact that
2,4-dinitrochlorobenzene reacts readily with pyridine to form
2,4-dinitrophenylpyridinium chloride suggests a similar mechanism
for the reaction of the solid diazonium salt with pyridine. The
mechanism for the reaction is that of an attack by the pyridine on
the positive carbon to which the chlorine is attached. Because of
stronger resonance interaction between the 2,4-dinitrophenyl group
and the pyridine than between the same group and the chlorine, the
latter is released as chloride ion. If pyridine attacks the 2,4-di-
nitrobenzenediazonium ion nitrogen is released as a result of the
more stable C-N-linkage to the pyridine ring.

The other possibility is an attack on the polarized diazonium
ion by the pyridine followed by collapse of the coupled intermediate
releasing nitrogen:

\[
\begin{align*}
O_2N-\text{C_6H_4-}-N=N=O & \quad + \quad :N \quad \rightarrow \quad \left[ \begin{array}{c}
O_2N-\text{C_6H_4-}-N=N-N \quad \text{pyridinium ion}
\end{array} \right] \quad \rightarrow \quad \left[ \begin{array}{c}
\text{N_2}
\end{array} \right] + \text{N_2}
\end{align*}
\]

Aqueous solutions of 2,4-dinitrobenzenediazonium salts also
react with pyridine to give pyridinium compounds. In this case the
intermediate formed is probably the diazohydroxide, R-N=N-OH, a
species postulated by Gomberg for his synthesis of biaryls from
diazo-compounds. The diazohydroxide is presumed to decompose ge-
genrating free aryl radicals which react with the aromatic compound
to form the biaryls. In the case of 2,4-dinitrobenzenediazonium
hydroxide in pyridine the free 2,4-dinitrophenyl radicals attack the nitrogen of the pyridine yielding 2,4-dinitrophenylpyridinium ions. However even in aqueous medium a direct interaction between the pyridine and the carbon atom in the phenyl group that bears the diazonium function cannot be ruled out.

Regardless of the mechanism involved, the 2,4-dinitrophenylpyridinium ion (I) is formed in the reaction between 2,4-dinitrobenzenediazonium salts and pyridine, and addition of alkali produces 5-(2,4-dinitroanilino)-2,4-pentadienal or its tautomeric enol form (II) which reacts with excess sodium hydroxide to yield the highly conjugated purple indicator ion (III).
The proof of the formation of the indicator anion of 5-(2,4-dinitroanilino)-2,4-pentadienal in this reaction is threefold. Firstly, the absorption spectrum showing two maxima in the visible region agreed with the spectrum of the material obtained when 2,4-dinitrophenylpyridinium chloride was treated with base. For this comparison study sodium hydroxide was added to a dilute aqueous solution of the pyridinium chloride prepared directly from 2,4-dinitrochlorobenzene and pyridine. Both of the absorption spectra agreed in turn with the spectrum of a solution of 5-(2,4-dinitroanilino)-2,4-pentadienal in sodium hydroxide. The pure solid pentadienal was directly prepared from 2,4-dinitrophenylpyridinium chloride and sodium carbonate.

Every spectrum showed a very pronounced maximum between 528 and 555 nm and one less pronounced between 365 and 385 nm, the exact wavelengths depending on the ratio of pyridine to water in the system. This solvent effect which is both bathochromic and hyperchromic in nature was found to be the same regardless of the starting material from which the purple base of 5-(2,4-dinitroanilino)-2,4-pentadienal had formed.

Secondly, addition of dilute hydrochloric acid to the purple solutions prepared either from 2,4-dinitrobenzenediazonium salts, pyridine and excess sodium hydroxide, or from a solution of 2,4-dinitrophenylpyridinium chloride and base, converted the indicator base (III) to the yellow acid form of the indi-
ctor (II) which absorbs at 415 m\(\mu\). A solution of solid 5-(2,4-dinitroanilino)-2,4-pentadienal in pyridine, acetone or other suitable solvents absorbs at the same wavelength.

Thirdly, the pentadienal was identified as glutaconialdehyde-dianil by addition of excess aniline hydrochloride to the solutions. \(^{42}\) Glutaconialdehyde-dianil has a single absorption peak at \(490\ m\mu\).

Ring cleavage of pyridinium compounds is restricted to those with strongly electron-attracting groups on the nitrogen. \(^{44}\) The presence of the strong electron acceptor \(\ce{NO2}^+\) and stabilization by resonance distribution of the anionic charge of III account for the ease with which ring opening occurs in the case of 2,4-dinitrophenylypyridinium compounds.

Mono-nitrobenzenediazonium salts do not react with pyridine and excess sodium hydroxide to yield a highly colored substance. This may be due either to the fact that direct quaternization cannot be obtained in this case or that ring cleavage is not induced by a single nitro-group on the benzene ring attached to the nitrogen of pyridine. The fact that the mononitrophenylypyridinium chlorides cannot be prepared directly from the corresponding mononitrochlorobenzenes and pyridine supports the assumption that it is impossible to obtain direct quaternization of the pyridine nitrogen. This then provides an explanation for the observation that the oxidation of the mononitrophenylhydrazines by \(\text{Cu(II)}\)
or silver oxide in the presence of pyridine did not result in the formation of 5-(2,4-dinitroanilino)-2,4-pentadienal on addition of sodium hydroxide.

Solid 2,4-dinitrobenzenediazonium salts and their aqueous solutions react with piperidine at room temperature producing an orange solution. The reaction is vigorous and accompanied by evolution of nitrogen. However, addition of sodium hydroxide does not result in an apparent reaction, the color of the solution remains unchanged. Assuming that quaternization can be obtained and that ring cleavage in strong alkali will occur, the cleavage product would still not be expected to absorb very strongly in the visible because of the lack of conjugated double bonds in the open chain.

Quaternization of the pyridine nitrogen and ring cleavage of the pyridinium ion depend not only on the nature of the group attached to the nitrogen, but also on the substituents present in the pyridine ring. Although successful with many substituted pyridines, the cleavage reaction is subject to steric hindrance in 2,6-disubstituted pyridines and to inhibition by electronegative substituents perhaps operating to prevent the quaternization step. When 2,4-dinitrobenzenediazonium salts are added to 2,6-dimethylpyridine and the mixture is heated slightly reaction occurs and nitrogen is evolved. Addition of base does not result in the formation of a highly colored material. Steric hindrance is not a factor in the ring cleavage of 3,5-disubstituted pyridines. Lukes and Jizba obtained red 5-(2,4-dinitroanilino)-
2,4-dimethyl-2,4-pentadienal from 2,4-dinitrophenyl-\(\mathbf{P}_2\mathbf{P}'\)-lutidinium chloride on treatment with sodium carbonate.

Experimental

The extinction coefficients of the colored indicator base in pure aqueous solution and in the presence of various organic solvents were obtained from pure 2,4-dinitrophenylpyridinium chloride and 1 molar sodium hydroxide. On the basis of this data the extent of nitrogen coupling in the reaction between 2,4-dinitrobenzenediazonium compounds and pyridine was calculated.

Preparation of Reagents

2,4-Dinitrophenylpyridinium chloride. This substance was prepared by the method given by Zincke.\(^{39}\) Ten parts of 2,4-dinitrochlorobenzene were dissolved in eight parts of anhydrous pyridine and the solution was heated in ordinary thin-walled test tubes at the temperature of a boiling water bath until the contents of the tubes had solidified. After the tubes had been allowed to cool, they were broken, the crude 2,4-dinitrophenylpyridinium chloride ground and the excess reagents extracted several times with anhydrous ether. Extraction was repeated until the ether layer above the 2,4-dinitrophenylpyridinium chloride was practically colorless. The product was then recrystallized twice from absolute ethanol, dried at 100° C. in a vacuum oven and stored in a desiccator over phosphorus(V)oxide. The melting points reported
in the literature for this compound fall within the range of 190 to 210° C. In the present work a melting point of 201° C. was found which agrees with the one reported by Reitzenstein.  

2,4-Dinitrobenzenediazonium fluoborate. This compound was prepared by diazotization of 2,4-dinitroaniline according to the method described by Saunders, the addition of an aqueous solution of sodium fluoborate to the cold solution of the diazonium salt, and precipitation of the water-insoluble diazonium fluoborate with anhydrous ether.

Seven grams of dry sodium nitrite were slowly added with constant stirring to 150 ml. concentrated sulfuric acid (sp.gr. 1.84), raising the temperature of the nitrosylsulfuric acid to 70° C. to ensure complete dissolution. The solution was then allowed to cool to about 35° C. While maintaining the solution at that temperature, 18 g. of 2,4-dinitroaniline were added with stirring over a period of thirty minutes. After the mixture had been allowed to react with constant stirring for two hours, it was poured on ice. The dilute aqueous solution of the diazonium salt was filtered and a solution of 15 g. sodium fluoborate in 100 ml. of water was then added to the filtrate. The 2,4-dinitrobenzenediazonium fluoborate was then precipitated with anhydrous ether, washed well with distilled water and absolute ether several times, and finally dried at room temperature in a vacuum desiccator over phosphorus(V)oxide. 2,4-Dinitrobenzenediazonium fluoborate is a pale yellow solid with no
definite melting point. It darkens and begins to decompose at temperatures above 150° C.

**p-Nitrobenzenediazonium fluoborate.** This material was prepared according to the method by Starkey. To 110 ml. of fluoboric acid in a 400 ml. beaker were added 34 g. of p-nitroaniline. The beaker was placed in ice and the solution was stirred. A cold solution of 17.0 g. of sodium nitrite in 34 ml. of water was then added drop-wise. When addition was complete, the mixture was stirred for a few minutes and then filtered by suction on a sintered glass filter. The solid p-nitrobenzenediazonium fluoborate was washed once with 25 to 30 ml. of cold fluoboric acid, twice with 95 percent ethanol and several times with ether before it was dried at room temperature over phosphorus(V)oxide.

**5-(2,4-Dinitroanilino)-2,4-pentadienal.** It was prepared by the method given by Zincke. After dissolving 10 g. of 2,4-dinitrophenyl-pyridinium chloride in 100 ml. of water, a saturated aqueous solution of sodium carbonate was slowly added until no more precipitate formed. A voluminous precipitate formed which turned from dark brown to bright red on standing. The crude 5-(2,4-dinitroanilino)-2,4-pentadienal was washed well with water, dried and recrystallized from hot acetone using some activated charcoal until the melting point of the purified material had become constant. The final product melted at 182° C. with decomposition generating some pyridine and a black tar. The C, H and N-analysis agreed well with the theoretical percentages; Calculated: C, 50.19; H, 3.45; N, 15.97; found (averages): C, 50.12; H, 3.50;
N, 15.91. An infrared absorption spectrum of 5-([2,4-dinitroanilino]-2,4-pentadienial was prepared with the Perkin-Elmer (Model 21) Recording Infrared Spectrophotometer using KBr pellets (see Appendix I).

Procedure

A 0.001 molar aqueous solution of purified 2,4-dinitrophenyl-pyridinium chloride was prepared. The amount of sodium hydroxide required for complete conversion of the pyridinium compound to the indicator base was determined by pipetting 1 ml. aliquots of the pyridinium chloride solution into different volumes of 1 molar sodium hydroxide solution contained in 50 ml. volumetric flasks. Each sample was diluted to the mark with water. In each case a timer was started as soon as the pyridinium chloride was added to the base and the solutions were shaken vigorously to assure complete mixing.

Absorption spectra over the entire visible region were obtained starting at the long wavelength limit (700 μ) 2.5 min. after zero-time of initial mixing. The spectra were prepared with the Cary (Model 11) Recording Spectrophotometer at the rate of 10 Å per second and with two matched 1 cm. cells supplied by the Beckman Company. The reference solutions were prepared by diluting identical volumes of 1 molar base as used in the sample solutions to 50 ml. with water. All spectra showed a maximum at 365 μ and another one at 528 μ. The absorption maximum at 365 μ is not useful for quantitative studies since one of the decomposition products of the indicator base in excess sodium hydroxide absorbs strongly at 360 μ. If a solution
of the indicator base in excess sodium hydroxide is allowed to stand for a long period of time until the purple color has completely disappeared and an absorption spectrum of the resulting pale yellow solution is prepared, only one maximum at 360 μm is observed.

It was found that 10 or 20 ml. of 1 molar sodium hydroxide produced identical extinctions for the pyridinium ion concentration of 2.0×10⁻⁶ at 528 μm. Thus 10 ml. of base was sufficient to completely convert the 2,4-dinitrophenylpyridinium ion to the indicator base of 5-(2,4-dinitroanilino)-2,4-pentadienal. It was noted that the solutions faded very rapidly and the decrease of extinction with time was investigated using a solution 2.0×10⁻⁵ molar with respect to pyridinium ion and 0.2 molar in hydroxide ion. Since a plot of log extinction versus time in minutes is a straight line over a period of one-and-a-half hours (Fig. 2, O%), the extinction produced by the amount of indicator ion formed at the instant of mixing may be found by extrapolating to zero time.

The solvent effects of pyridine, acetone and methanol on the absorption of the indicator base were investigated using the same procedure but adding varying amounts of these solvents. One ml. aliquots of the 0.001 molar 2,4-dinitrophenylpyridinium chloride solution were pipetted into 5, 10, 15, 20 or 25 ml. of the particular solvent which corresponds to 10, 20, 30, 40 and 50 percent by volume on the basis of 50 ml. total volume. Ten ml. of 1 molar sodium hydroxide were then added and the timer was started. All solutions were diluted to 50 ml. with water and shaken vigorously to ensure
FIGURE 2

Rate of Fading of the Indicator Ion of 5-(2,4-Dinitroanilino) -
2,4-pentadienal in Pyridine-Water Mixtures

\[ C_{I^-} = 2 \times 10^{-5} \text{ moles/liter} ; \quad C_{\text{OH}^-} = 0.2 \text{ moles/liter} \]

Percentages indicate the volume of pyridine in mixture.
complete mixing. Reference solutions were prepared so as to agree with the corresponding sample solution in pH and organic solvent content, but the 2,4-dinitropheny1pyridinium chloride was omitted.

The visible absorption spectra of the indicator base in the various solvents showed one maximum between 368 and 385 m\(\mu\) and one between 530 and 557 m\(\mu\), the latter being much stronger than the one in the lower wavelength region. All spectra were recorded at the rate of 10 \(\AA\) per second starting 2.5 min. after the addition of base at the long wavelength limit (700 m\(\mu\)) so that the extinction at the maximum between 530 and 557 m\(\mu\) was recorded approximately 5 min. after zero time of mixing. The extinctions were measured again at this wavelength 35 and 95 min. after the initial time of mixing. A plot of log extinction versus time was prepared and the extinction produced by the amount of indicator base formed at the instant of mixing was obtained by extrapolation to zero time. Figure 2 (p.40) illustrates the straight-line relationship between log \(E\) and time in min. for the solvent pyridine where the 0 % corresponds to pyridinium chloride in 0.2 molar base without pyridine present.

The data for the solvent effects of pyridine, acetone and methanol on the absorption of the indicator base are summarized in Tables 1, 2 and 3, respectively.

On addition of 0.152 g. of the stable 2,4-dinitrobenzenediazonium fluoborate to 100 ml. of pyridine in a 500 ml. volumetric flask, nitrogen was evolved vigorously and a clear red solution re-
sulted. The mixture was shaken for some time to expel the nitrogen and then diluted with pyridine to the mark. A 1 ml. aliquot of this solution was then pipetted into 50 ml. volumetric flask containing 10 ml. of 1 molar sodium hydroxide. The timer was started at the time of mixing. The solution was mixed well and diluted to the 50 ml. mark with water. An absorption spectrum was obtained in the same manner as described above. Maximum absorption for this system was recorded at 531 μ. From the time study of the decrease of extinction for this system, the extinction at the time of mixing was calculated to be 0.747.

The addition of excess sodium hydroxide to a solution of p-nitrobenzenediazonium fluoborate in pyridine produced a brown solution. This solution was diluted with water and an absorption spectrum was prepared. The only peak in the visible region was located at 410 μμ.

A 2.0·10⁻⁴ molar solution of 5-(2,4-dinitroanilino)-2,4-pentadienal in pyridine was prepared by dissolving 26.3 mg. of the purified solid in 500 ml. anhydrous pyridine. A 5 ml. aliquot of this solution was then pipetted into a mixture of 10 ml. 1 molar sodium hydroxide and 10 ml. water contained in a 50 ml. volumetric flask. The deep purple solution was then diluted to the mark with water and an absorption spectrum was prepared in the usual manner. The extinctions at 541 μμ were measured as a function of time and a plot of log extinction versus time was prepared. The points fell
<table>
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<th>Volume % Pyridine</th>
<th>5 min. ( \lambda_1 )</th>
<th>5 min. ( E_1^{95} )</th>
<th>Log ( E_1 ) + 1</th>
<th>Log ( E_1 ) + 1</th>
<th>( E_0 )</th>
<th>( \epsilon \times 10^4 )</th>
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<tr>
<td>0</td>
<td>528 m( \mu )</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

\( E_0 = E \) at zero time (extrapolated value); \( \epsilon = \) molar extinction coefficient of \( I^- \).
TABLE 2

SOLVENT EFFECTS OF ACETONE

<table>
<thead>
<tr>
<th>Volume % Acetone</th>
<th>$\lambda_{\text{I-}}$</th>
<th>$E_{\text{I-}}$ at 5 min.</th>
<th>Log $E_{\text{I-}} + 1$ at 5 min.</th>
<th>$E_0$</th>
<th>$E \times 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>528 m$\mu$</td>
<td>0.825</td>
<td>0.916</td>
<td>0.954</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.502</td>
<td>0.701</td>
<td>0.900</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.184</td>
<td>0.265</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>532 m$\mu$</td>
<td>0.884</td>
<td>0.946</td>
<td>0.975</td>
<td>4.72</td>
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<td>0.594</td>
<td>0.774</td>
<td>0.944</td>
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<td></td>
<td></td>
<td>0.270</td>
<td>0.431</td>
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<tr>
<td>20</td>
<td>536 m$\mu$</td>
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<td>0.961</td>
<td>0.995</td>
<td>4.94</td>
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<td></td>
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<td>0.582</td>
<td>0.765</td>
<td>0.989</td>
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<td>0.230</td>
<td>0.362</td>
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<td>30</td>
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<td>0.636</td>
<td>0.803</td>
<td>1.035</td>
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<tr>
<td></td>
<td></td>
<td>0.282</td>
<td>0.450</td>
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</tr>
<tr>
<td>40</td>
<td>544 m$\mu$</td>
<td>1.042</td>
<td>1.018</td>
<td>1.032</td>
<td>5.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.734</td>
<td>0.866</td>
<td>1.076</td>
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<tr>
<td></td>
<td></td>
<td>0.370</td>
<td>0.568</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>547 m$\mu$</td>
<td>1.060</td>
<td>1.025</td>
<td>1.050</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.794</td>
<td>0.900</td>
<td>1.122</td>
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<td></td>
<td>0.436</td>
<td>0.640</td>
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</tbody>
</table>

$E_0 = E$ at zero time (extrapolated value);  $E = $ molar extinction coefficient of $\text{I}^-$.  

*
### TABLE 3

#### SOLVENT EFFECTS OF METHANOL

<table>
<thead>
<tr>
<th>Volume % Methanol</th>
<th>λ&lt;sub&gt;I&lt;/sub&gt;</th>
<th>E&lt;sub&gt;I&lt;/sub&gt; 35°</th>
<th>Log E&lt;sub&gt;I&lt;/sub&gt; + 1 35°</th>
<th>Log E&lt;sub&gt;0&lt;/sub&gt; + 1</th>
<th>E&lt;sub&gt;0&lt;/sub&gt;</th>
<th>ε x 10&lt;sup&gt;4&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>528 mμ</td>
<td>0.825</td>
<td>0.916</td>
<td>0.954</td>
<td>0.900</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.502</td>
<td>0.701</td>
<td></td>
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</tr>
<tr>
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<td>0.184</td>
<td>0.265</td>
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</tr>
<tr>
<td>10</td>
<td>529 mμ</td>
<td>0.850</td>
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<td>4.54</td>
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<tr>
<td></td>
<td></td>
<td>0.282</td>
<td>0.150</td>
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</tr>
<tr>
<td>20</td>
<td>530 mμ</td>
<td>0.870</td>
<td>0.940</td>
<td>0.963</td>
<td>0.918</td>
<td>4.59</td>
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<tr>
<td></td>
<td></td>
<td>0.674</td>
<td>0.809</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.341</td>
<td>0.533</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>531 mμ</td>
<td>0.885</td>
<td>0.947</td>
<td>0.966</td>
<td>0.924</td>
<td>4.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.700</td>
<td>0.845</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.432</td>
<td>0.635</td>
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</tr>
<tr>
<td>40</td>
<td>533 mμ</td>
<td>0.916</td>
<td>0.962</td>
<td>0.970</td>
<td>0.934</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.796</td>
<td>0.901</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.620</td>
<td>0.792</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>534 mμ</td>
<td>0.922</td>
<td>0.965</td>
<td>0.974</td>
<td>0.942</td>
<td>4.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.810</td>
<td>0.908</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.650</td>
<td>0.813</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E<sub>0</sub> = E at zero time (extrapolated value) ;  \( \varepsilon \) = molar extinction coefficient of I⁻.

---

5
on the line corresponding to 10 percent (by volume) pyridine in Figure 2 (p.40), and the extrapolated extinction at the time of mixing was found to be 0.930.

Results and Discussion

From the data contained in Tables 1, 2 and 3, the molar extinction coefficients of the indicator base (I⁻) were calculated as a function of the solvent content of the systems studied. The tables contain the following information: The composition of the medium is given in volume percent of solvent in the first column on the left of each table. The wavelength of maximum absorption as a function of solvent content is found in the second column. The third column contains the actual extinctions as recorded 5, 35 and 95 minutes after the initial time of mixing; column 4 contains the corresponding logarithms which were changed to positive numbers for convenience. The fifth column contains the logarithmic values of the extinctions at zero time; the extrapolated extinction at zero time as a function of solvent content is found in the column next to the last one. From the extinctions at zero time and the concentration of the indicator base the molar extinction coefficients as a function of the solvent content were calculated with Beer's law and are tabulated in the last column.

The concentration of I⁻ is equal to the concentration of 5-(2,4-dinitroanilino)-2,4-pentadienal if one assumes complete con-
version of the acid form to the basic form of the indicator. On the basis of this assumption the extent of ring cleavage is readily obtained by comparing the extinction produced by a $2.0 \times 10^{-5}$ molar solution of the pentadienal with that produced by a solution of equally concentrated pyridinium chloride in the same solvent medium. The comparison had to be done in 10 percent pyridine inasmuch as the solid 5-(2,4-dinitroanilino)-2,4-pentadienal is practically insoluble in water. The extinctions produced by the indicator base in 10 percent pyridine and 0.2 molar base were found to be 0.930 starting with the pentadienal and 0.928 starting with the pyridinium ion. The extinctions given are those at zero time. It follows from this that the ring cleavage reaction is quantitative in excess base. This was confirmed by repeating the experiments in 30 percent pyridine and in 30 percent acetone.

Pyridine, acetone and methanol were found to affect the absorption of the indicator base I⁻ in three ways: (1) They stabilize the ion; (2) shift the wavelength of maximum absorption to higher wavelengths (bathochromic effect); and (3) increase the extinction coefficient at the wavelength of maximum absorption (hyperchromic effect). The stabilizing effect of pyridine is illustrated in Figure 2 (p.40). The change in slopes representing the rate of fading of the purple color as a function of time and solvent content indicates that the stability of I⁻ increases with increasing pyridine concentration. In the case of pyridine, 40 percent by volume is the maximum concentration in the presence of 0.2 molar sodium hydroxide.
because of the reduced solubility of the salt in pyridine-water mixtures.

The bathochromic and hyperchromic effects of pyridine, acetone and methanol on the absorption of the indicator base are graphically represented by Figures 3 and 4. The wavelength of maximum absorption varies linearly with the volume percent of solvent in the case of acetone and methanol, but not for pyridine; the extinction coefficients were found to increase linearly with increasing amounts of either of the three solvents studied. The individual points on the graphs correspond to mixtures of pyridine and methanol in the ratios indicated.

The addition of dilute hydrochloric acid to the solutions of the purple indicator base obtained either from 2,4-dinitrophenylpyridinium chloride and alkali, or from 2,4-dinitrobenzenediazonium fluoborate, pyridine and alkali, resulted in the conversion to the yellow indicator acid with the expected maximum at 415 μm. The pale yellow solutions, on shaking with aniline hydrochloride, produced the orange colored glutaconialdehyde-dianil which, when extracted with absolute ethanol, absorbed strongly at 490 μm.

The percentage yield of 2,4-dinitrophenylpyridinium ion obtained from the diazonium fluoborate and pyridine, which is a measure of the extent of coupling at the nitrogen of the pyridine ring,

---

2 A table and a figure of the data pertaining to these mixtures may be found on pages 58 and 60 respectively.
FIGURE 3

Variation in Wavelength at the Absorption Maximum with Volume Percent Organic Solvent for the Indicator Base (I⁻)
of 5-(2,4-Dinitroanilino)-2,4-pentadienal

$C_{I^-} = 2 \cdot 10^{-5}$ moles/liter; $C_{OH^-} = 0.2$ moles/liter
Wavelength (mλ)

Pyridine: Methanol
= 3:2

Pyridine: Methanol
= 4:1

Volume Percent Organic Solvent
FIGURE 4

Hyperchromic Effect of Pyridine, Acetone and Methanol on the Absorption Maximum of the Indicator Base of 5-(2,4-Dinitroanilino)-2,4-pentadienal

$C_I = 2.0 \times 10^{-6}$ moles/liter; $C_{OH} = 0.2$ moles/liter
Molar Extinction Coefficient $\times 10^{-4}$

- Acetone
- Pyridine
- Methanol

1. Pyridine: Methanol = 4:1
2. Pyridine: Methanol = 4:1
3. Pyridine: Methanol = 3:2
was calculated as follows: A 1 ml. aliquot of the solution containing 0.1524 g. 2,4-dinitrobenzenediazonium fluoborate in 500 ml. anhydrous pyridine contains \( \frac{1}{500} \times \frac{0.1524}{281.9} = 1.08 \times 10^{-6} \) moles of the diazonium ion. Addition of this aliquot to 10 ml. 1 molar sodium hydroxide and diluting to a 50 ml. volume with water produced an extinction at zero time of 0.747 in a 1 cm. cell. The pyridine content of this system corresponds to 2 percent by volume for which the molar extinction coefficient of the indicator base is \( 4.52 \times 10^4 \) (Fig. 4). It follows from a calculation with Beer's law that the concentration of indicator base produced in the reaction was equal to \( \frac{0.747}{4.52 \times 10^4} = 1.65 \times 10^{-5} \) moles per liter or \( 8.25 \times 10^{-7} \) moles in the 50 ml. volume. In consideration of the fact that the cleavage of the 2,4-dinitrophenylpyridinium ion and the removal of the proton from the indicator acid \( 5-(2,4\text{-dinitroanilino})-2,4\text{-pentadienial} \) are quantitative reactions in excess sodium hydroxide, the yield of pyridinium ion formed in the reaction between the solid 2,4-dinitrobenzenediazonium fluoborate and pyridine is \( \frac{8.25 \times 10^{-7}}{1.08 \times 10^{-6}} \times 100 \) or 76.4 percent.
SPECTROPHOTOMETRIC DETERMINATION OF
2,4-DINITROPHENYLHYDRAZINE

Historical

Various methods for detecting and determining 2,4-dinitro-
phenylhydrazine are suggested in the literature. Macbeth and Price
found that this compound is quantitatively reduced with excess
titanous chloride to 2,4-diaminophenylhydrazine, twelve equivalents
of titanous ion being consumed in the reaction. The excess of
the reducing agent is back-titrated with a standard solution of
methylen blue.

A qualitative test for aromatic nitro compounds which can
be reduced by lithium-aluminum hydride was suggested by Nelson
and Laskowski who successfully applied the method to 2,4-
dinitrophenylhydrazine. The detection depends on the color change of the
nitro compounds on reduction when they are refluxed with the hydride
in acetic anhydride.

An ultrasensitive method for the detection of 2,4-dinitro-
phenylhydrazine was developed by Kulberg and Il'mia who found a
fluorescent reaction of this compound with salicylaldehyde in 80
percent acetic acid. They report a sensitivity of 5×10⁻¹⁰ g, at
a dilution limit of 1 : 10⁶. Yamagishi and Yokoo determined 2,4-di-
nitrophenylhydrazine azometrically by oxidation with iodine in acidic medium.

Bottie and Furman determined aromatic nitro-compounds with satisfactory accuracy in amounts ranging from 0.3 to 0.2 milliequivalents by reduction with chromous chloride. An excess of standard chromous solution was added to the sample under an atmosphere of carbon dioxide and subsequently back-titrated with standard ferric-alum solution. The endpoint was detected potentiometrically.

In the indirect determination of carbonyl compounds based upon the formation of 2,4-dinitrophenylhydrazones developed by Berka and Zyka, the unreacted 2,4-dinitrophenylhydrazine is back-titrated with Chloramine-T in the presence of potassium bromide. The endpoint of the titration is detected potentiometrically.

Witry-Schwachtgen described a method in which organic compounds containing one or more nitro groups are reduced to amines by treatment with vanadium sulfate and the excess reagent is back-titrated with standard sodium dichromate.

Description of the Method

None of the known methods for quantitatively determining 2,4-dinitrophenylhydrazine are specific for this compound. All but one involve the preparation of standard solutions and titration procedures. The azometric determination suggested by Yamagishi and Yokoo depends on the measurement of very small volumes of gas
at carefully controlled conditions which renders this method tedious and liable to inaccuracies.

In the present work, a simple spectrophotometric method for the determination of 2,4-dinitrophenylhydrazine is suggested which is based on the sequence of reactions discussed in the previous sections of this thesis.

Copper perchlorate was used to oxidize the 2,4-dinitrophenylhydrazine to the diazo-intermediate. The sample solution was added to a mixture of aqueous copper perchlorate and pyridine and the indicator base of 5-(2,4-dinitroanilino)-2,4-pentadienal was obtained from the 2,4-dinitrophenylpyridinium ion by addition of excess sodium hydroxide. The extinction of the purple solution was measured at the wavelength corresponding to the solvent content of the system. There exists a direct proportionality between the extinction of the colored solution and the concentration of 2,4-dinitrophenylhydrazine in the sample solution even though not all reactions proceed quantitatively.

**Experimental**

A working curve was constructed for solutions of 2,4-dinitrophenylhydrazine in absolute methanol in the range of $8 \times 10^{-4}$ to $4 \times 10^{-6}$ molar over which Beer's law is obeyed.
Preliminary Study

Inasmuch as methanol and pyridine affect the absorption of the indicator base, a preliminary study of the bathochromic and hyperchromic shifts with mixtures of the two solvents was conducted. The extinctions produced by solutions prepared from 1 ml. 0.001 molar aqueous 2,4-dinitrophenylpyridinium chloride solution, 10 ml. of sodium hydroxide (1 molar) and the solvents methanol and pyridine in different ratios were measured. The following ratios of pyridine to methanol were investigated: 4:1 and 3:2 for 50 percent combined solvent content, and 4:1 for 25 percent total solvent. A plot of log extinction versus time was prepared and the extinctions at zero time were obtained by extrapolation (Figure 5). From the data in Table 4, the molar extinction coefficients of the indicator base in the solvent mixtures were calculated.

Preparation of Reagents

Carbonyl-free methanol. 500 ml. of C. P. methanol were refluxed with 5 g. 2,4-dinitrophenylhydrazine and a few drops of concentrated hydrochloric acid for two hours and distilled.

2,4-Dinitrophenylhydrazine in methanol. A 0.001 molar solution was prepared by dissolving 0.0991 g. of the purified 2,4-dinitrophenylhydrazine in enough carbonyl-free methanol to make 500 ml. of solution. This solution was not used more than a week. From this solution a number of others of steadily decreasing concentration were prepared by diluting aliquots of it with absolute carbonyl-free methyl alcohol.
TABLE 4

SOLVENT EFFECTS OF PYRIDINE-METHANOL MIXTURES

<table>
<thead>
<tr>
<th>Volume %</th>
<th>Ratio of Py : MeOH</th>
<th>( \lambda_1 )</th>
<th>5 min.</th>
<th>( \log E_1 + 1 )</th>
<th>5 min.</th>
<th>( \log E_0 + 1 )</th>
<th>( E_0 )</th>
<th>( \xi \times 10^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4 : 1</td>
<td>557 m(\mu)</td>
<td>1.012</td>
<td>1.005</td>
<td>1.006</td>
<td>1.014</td>
<td>5.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.986</td>
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<td>0.994</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.932</td>
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</tr>
<tr>
<td>50</td>
<td>3 : 2</td>
<td>553 m(\mu)</td>
<td>0.996</td>
<td>0.998</td>
<td>1.000</td>
<td>1.000</td>
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<td>0.908</td>
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<td>0.958</td>
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<td></td>
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</tr>
<tr>
<td>25</td>
<td>4 : 1</td>
<td>548 m(\mu)</td>
<td>0.955</td>
<td>0.980</td>
<td>0.982</td>
<td>0.960</td>
<td>4.80</td>
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<td>0.822</td>
<td></td>
<td>0.915</td>
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<td></td>
</tr>
</tbody>
</table>

Py = Pyridine; MeOH = Methanol; \( E_0 \) = E at zero time (extrapolated value)
\( \xi \) = molar extinction coefficient of 1

58
FIGURE 5

Rate of Fading of the Indicator Ion of 5-(2,4-Dinitroanilino)-2,4-pentadienal in Pyridine-Methanol-Water Mixtures

$C_{I^-} = 2.0 \times 10^{-5} \text{ moles/liter} \quad C_{OH^-} = 0.2 \text{ moles/liter}$

Percentages indicate volume of solvents in mixture.
1) Pyridine to Methanol = 4:1 (50%)
2) Pyridine to Methanol = 3:2 (50%)
3) Pyridine to Methanol = 4:1 (25%)
Aqueous copper perchlorate. A standardized 0.0954 molar solution of copper perchlorate hexahydrate in water was used.³

Aqueous sodium tartrate. A solution approximately 1 molar was prepared by dissolving 23 g. of solid sodium tartrate dihydrate in 100 ml. of water. A small amount of solid remained undissolved and the solution was filtered.

Procedure

Five ml. of 2,4-dinitrophenylhydrazine in methanol were pipetted into a mixture of 20 ml. anhydrous pyridine and 2 ml. copper perchlorate solution contained in a 50 ml. volumetric flask. The mixture was quickly shaken, diluted with approximately 10 ml. of water, and 1 ml. of the tartrate solution was added within a minute after the addition of the 2,4-dinitrophenylhydrazine to the copper-pyridine mixture. One molar sodium hydroxide was added immediately and the timer was started as soon as the first purple color appeared. Ten ml. of base were used if the concentration of 2,4-dinitrophenylhydrazine in the 5 ml. test sample was above 10⁻⁴ moles per liter, one ml. of base was added for concentrations of the test solution below 10⁻⁴ molar. All solutions were diluted to the 50 ml. mark with water following the addition of the sodium hydroxide. The extinctions were measured at 557 μ (Fig. 3, pyridine : methanol = 4 : 1 for 50 %) with the Cary (Model 14) Record-

³ For details, see p.20
ing Spectrophotometer at the rate of 10 Å per second 5, 35 and 65
minutes after the addition of base. The reference solutions were
prepared so as to agree with the test solutions in composition
except that the 2,4-dinitrophenylhydrazine was omitted. The ex-
perimental data are summarized in Table 5.

Results and Discussion

The extinctions of the amount of indicator base formed at
the instant of mixing were obtained by extrapolating the plots
of log extinction versus time in minutes to zero time (Fig. 6).
The values of the extinctions at zero time (E₀) in Table 5 are
the actual extinctions produced at the time of mixing with the
cell specified. Those extinctions based on the cell lengths of
½ or 10 cm. were changed to extinctions that would have been
produced in a 1 cm. cell. The extinctions based on an absorption
path of 1 cm. were plotted versus the molar concentrations of
2,4-dinitrophenylhydrazine in the 5 ml. test solutions (Fig. 7
p. 67). The straight-line plot indicates that Beer's law is
obeyed in the concentration range studied.

It is important that both cupric ion and pyridine be used
in excess in the reaction with the 2,4-dinitrophenylhydrazine.
The unreduced Cu(II) is complexed by the addition of tartrate to
avoid precipitation of hydrous cupric oxides upon addition of
base. The formation of such precipitates is undesirable because
<table>
<thead>
<tr>
<th>Sample</th>
<th>Millimoles 2,4-DNPH in sample</th>
<th>Vol. 1 M NaOH</th>
<th>Cell</th>
<th>$E_{I-}$</th>
<th>$\log E_{I-} + 1$</th>
<th>$\log E_0 + 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$4.0 \times 10^{-3}$</td>
<td>10 ml. ½ cm.</td>
<td>1.306</td>
<td>1.116</td>
<td>1.122</td>
<td></td>
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<tr>
<td>2</td>
<td>$3.0 \times 10^{-3}$</td>
<td>10 ml. ½ cm.</td>
<td>0.986</td>
<td>0.994</td>
<td>1.004</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$2.0 \times 10^{-3}$</td>
<td>10 ml. 1 cm.</td>
<td>1.296</td>
<td>1.113</td>
<td>1.120</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$1.4 \times 10^{-3}$</td>
<td>10 ml. 1 cm.</td>
<td>0.926</td>
<td>0.967</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$1.0 \times 10^{-3}$</td>
<td>10 ml. 1 cm.</td>
<td>0.638</td>
<td>0.805</td>
<td>0.815</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>$5.0 \times 10^{-4}$</td>
<td>10 ml. 1 cm.</td>
<td>0.328</td>
<td>0.516</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>$5.0 \times 10^{-4}$</td>
<td>1 ml. 1 cm.</td>
<td>0.332</td>
<td>0.521</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$1.0 \times 10^{-4}$</td>
<td>1 ml. 10 cm.</td>
<td>0.666</td>
<td>0.823</td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$2.0 \times 10^{-5}$</td>
<td>1 ml. 10 cm.</td>
<td>0.135</td>
<td>0.130</td>
<td>0.138</td>
<td></td>
</tr>
</tbody>
</table>

$E_0$ = $E$ at zero time (extrapolated value).

63
Absorption of the Indicator-Base as a Function of the Concentration of 2,4-Dinitrophenylhydrazine

1: \(8 \times 10^{-4}\) molar 2,4-DNPH; 10 ml. 1 molar NaOH
2: \(6 \times 10^{-4}\)
3: \(4 \times 10^{-4}\)
4: \(2.8 \times 10^{-4}\)
5: \(2 \times 10^{-4}\)
6a: \(1 \times 10^{-4}\)
6b: \(1 \times 10^{-4}\) 1 ml.
7: \(2 \times 10^{-5}\)
8: \(4 \times 10^{-6}\)
FIGURE 7

Beer's Law Plot as Working Curve in the Determination of 2,4-Dinitrophenylhydrazine
of the possibility of adsorbing some of the indicator ion on their active surfaces.

Since the rate of fading of the indicator base increases with increasing hydroxide ion concentration, a large excess of base must be avoided for very low concentrations of 2,4-dinitrophenylhydrazine. It is suggested that only 1 ml. of 1 molar sodium hydroxide be used on the basis of a total volume of 50 ml. when the test sample contains less than 0.001 millimoles of the hydrazine.

If the technique described above is used for the determination of 2,4-dinitrophenylhydrazine, the limit of identification is $\frac{1}{7}$ at a dilution limit of $10^6$.

Since the absorption of the indicator base of 5-(2,4-dinitroanilino)-2,4-pentadienal depends on the solvent content of the system, new working curves which will have different slopes must be prepared for solvent media different from the one described in the present work. The slope of the straight line in Figure 7 may be used only to calculate the concentration of 2,4-dinitrophosphylhydrazine in a medium containing 10 percent methanol and 40 percent pyridine.

In this study the extinctions were measured 5, 35 and 65 minutes after the addition of base and the extinctions at the time of mixing were obtained by extrapolation to zero time. If the extinctions at 5 min. are used instead of the extrapolated values
at zero time, the percentage error involved is not larger than 2.5 percent for any sample. It is therefore possible to determine 2,4-dinitrophenylhydrazine in the range of $8 \times 10^{-4}$ to $4 \times 10^{-6}$ molar with relatively good accuracy by measuring the extinction only once within five minutes after the addition of base to the mixture of Cu(II), pyridine and 2,4-dinitrophenylhydrazine.
CONCLUSIONS

The purpose of this investigation has been accomplished. The interference of copper(II) and other oxidizing agents in the spectrophotometric determination of carbonyl compounds has been elucidated.

A three-step sequence of reactions was established for the interaction between Cu(II), 2,4-dinitrophenylhydrazine, pyridine and alkali. No complex formation involving the copper and the 2,4-dinitrophenylhydrazine was found.

The first step of the sequence is the oxidation of the 2,4-dinitrophenylhydrazine by the copper yielding a diazonium intermediate. A number of other metals and nonmetals, both in the solid state and in aqueous solution, were investigated for their ability to oxidize 2,4-dinitrophenylhydrazine. Among those found to oxidize this hydrazine readily are silver(I), gold(III), platinum(IV), mercury(II)oxide and iodine.

The second step is the coupling of the diazonium intermediate with pyridine at the nitrogen of the pyridine ring resulting in the formation of the 2,4-dinitrophenylpyridinium ion and nitrogen. Pyridinium compounds were also obtained on addition of solid 2,4-dinitrobenzenediazonium fluoborate or aqueous solutions of 2,4-dinitrobenzenediazonium salts to excess pyridine. A mechanism for the coupling reaction between solid 2,4-dinitrobenzenediazo-
nium salts or their aqueous solutions and pyridine is suggested.

The third step of the reaction sequence consists in the cleavage of the 2,4-dinitrophenylpyridinium ion in strong alkali yielding the indicator acid 5-(2,4-dinitroanilino)-2,4-pentadienial and its conjugated highly colored indicator base in excess alkali. The formation of 5-(2,4-dinitroanilino)-2,4-pentadienial was verified in three ways: (1) The characteristic visible absorption spectra of the acid and basic forms of the indicator; (2) the pH dependence of the indicator equilibrium; (3) the interaction of the acid form with excess aniline hydrochloride to yield glutaconialdehyde-dianil.

A method for detecting copper(II) in the presence of a number of other metals commonly found to interfere in the identification tests for Cu(II) is described.

The solvent effects of pyridine, acetone and methanol on the absorption of the indicator base of 5-(2,4-dinitroanilino)-2,4-pentadienial were studied and the extinction coefficients were calculated as a function of solvent content.

A quantitative method of determination for 2,4-dinitrophenylhydrazine in the range 8·10^{-4} to 4·10^{-6} molar was developed. This method is based on the direct proportionality that exists between the concentration of 2,4-dinitrophenylhydrazine in the test solution and the extinction produced by the purple indicator ion.
APPENDIXES
Infrared Absorption Spectrum of 5-(2,4-Dinitroanilino)-2,4-pentadienal

The spectrum was obtained with the Perkin-Elmer (Model 21) Recording Spectrophotometer and KBr pellets were used.

The relative intensities of the O-H and N-H stretching absorptions at 3470 cm⁻¹ and 3310 cm⁻¹, respectively, seem to indicate that the aldo-form is predominant. The N-H stretching absorption would normally be expected at a slightly higher frequency, the shift being caused by some intramolecular hydrogen bonding to the oxygen of the neighboring NO₂-group. The sharp peak at 1670 cm⁻¹ is typical of unsaturated aldehydes which show a decrease in the carbonyl frequency located in the approximate range 1740 - 1720 cm⁻¹ for unconjugated aldehydes. The carbonyl absorption band is apparently split contributing also to the absorption at 1600 cm⁻¹ which results in an overlap with the band due to C=C bond stretching vibrations. In addition to this overlap, the unusual intensity of this band suggests conjugation of the open chain with the aryl group. The single band at 1515 cm⁻¹ and the doublet between 1310 and 1340 cm⁻¹ are due to the stretching absorptions of the C-NO₂ groups. The splitting of the peak at the lower frequency can be attributed to the intramolecular hydrogen bonding of the NO₂-group with the H on the N, thus adding further support in favor of the aldo-form.

The remaining bands are typical of the type of molecule under consideration, aliphatic and aromatic conjugated carbon-carbon double bonds; 1,2,4-substituted benzene and the skeleton of the molecule.
APPENDIX II

REAGENTS


Aniline Hydrochloride. 'Baker Analyzed' Reagent, M.P. 197-198° C.

Copper(II) perchlorate. Cu(ClO₄)₂·6 H₂O Analytical Reagent, F. Smith Chemical Co.

2,6-Dimethylpyridine. Matheson Coleman and Bell Reagent, 99 % pure.

2,4-Dinitroaniline. Matheson Coleman and Bell, M.P. 178-179° C. Purification: Recrystallization from n-butanol.

2,4-Dinitrochlorobenzene. Matheson Coleman and Bell, M.P. 50-52° C.


Ether Anhydrous. Mallinckrodt Analytical Reagent.

Methyl Alcohol. Mallinckrodt Absolute Methyl Alcohol "Analytical Reagent", B.P. 64.6° C. Purification: Preparation of Carbonyl-free Methanol by refluxing with 2,4-Dinitrophenylhydrazine hydrochloride.


2-Nitrophenylhydrazine. Prepared from 2-nitrophenylhydrazine hydrochloride by addition of excess sodium acetate to a hot aqueous solution of the hydrochloride. Purification: Recrystallization from benzene, M.P. 92° C.

2-Nitrophenylhydrazine Hydrochloride. Aldrich Chemical Co., Inc.


Piperidine. Matheson Coleman and Bell, B.P. 105-107° C.
Pyridine. 'Baker Analyzed' Reagent, redistilled and stored over potassium hydroxide pellets.

Sodium Carbonate. Mallinckrodt Analytical Reagent.

Sodium Fluoborate. Baker and Adamson Technical Grade.

Sodium Hydroxide. 'Baker Analyzed' Reagent.

Sodium Nitrite. 'Baker Analyzed' Reagent.

Sodium Tartrate Dihydrate. Matheson Coleman and Bell, Reagent.

Purification: Recrystallization with absolute ethanol from aqueous solution.
BIBLIOGRAPHY

6. E. Fischer, Ann. 190, 97 (1877).
7. E. Bamberger, Ber. 32, 1809 (1899).
9. A. Bayer, Ber. 18, 3177 (1885).
12. T. Zincke, Ber. 18, 786 (1885).
15. L. Michaelis, Ber. 26, 2190 (1893).


31. O. Kuhling, Ber. 28, 523 (1895).


33. E. Bamberger and E. Kraus, Ber. 29, 274 (1896).

34. A. E. Tschitschibabin, Ber. 37, 1373 (1904).


47. F. Reitzenstein, J. prakt. Chem. 68, 259 (1903).
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