A PARTIAL IDENTIFICATION OF THE PRODUCTS
OF THE LIEBERMANN NITROSO REACTION

by

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Approved: Herbert D. Rhodes, Director of Thesis, May 13, 1952
To the notice of the registrar.

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Date: [Date]

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INTRODUCTION

The Liebermann Nitroso reaction was discovered in 1874 by G. Liebermann, who described as "most astonishing" the reaction he obtained when phenol, sodium nitrite, and sulfuric acid were mixed in the proper proportions. He noted the formation of a series of colors and isolated a solid dye from the reaction mixture. In furthering his investigation, Liebermann prepared a reagent consisting of a five percent solution of sodium nitrite in sulfuric acid, which he added to several different phenols to produce reactions apparently much like that with phenol itself. It has since been established that similar reactions take place with a good many phenols, and the Liebermann reaction has therefore come into rather general use as a qualitative test for phenols.

Liebermann found that when he poured the acid reaction mixture into a large amount of cold water a solid precipitate appeared, which he separated by filtration. He found this material quite stable, and noted that it was easily soluble in base to form deeply colored solutions. However, he volunteered no suggestion as to the reaction mechanism or the composition of the product.

Shortly after Liebermann's work, Baeyer and Caro, having discovered p-nitrosophenol, found that they could
produce the same colors as had Liebermann by mixing phenol with p-nitrosophenol in sulfuric acid. They felt that the product was identical with that obtained by Liebermann and suggested that the reaction proceeded by the formation of the colorless compound N-hydroxy-p,p'-dihydroxydiphenylamine, which by oxidation or further condensation gave rise to the Liebermann dye. They offered no analytical support of this proposal, however.

Liebermann was not satisfied with this explanation, and continued his investigation, although he agreed that the reaction of Baeyer and Caro was identical with his own. He stated the composition of the solid product as C_{18}H_{15}N_{3}O_{3}, although he published no analytical data, and suggested that the product of his reaction was:

\[
\text{HO-}
\text{N-}
\text{O-}
\text{O-}
\text{N-}
\text{O-}
\text{O-}
\text{O-}
\]

In 1880 A. Hirsch, studying the reactions of quinonechlorimide, reported that he had produced "exactly the same" colors as had Baeyer and Caro by treating a solution of quinonechlorimide in phenol with concentrated sulfuric acid. He also produced similar reactions with other phenols. Hirsch proposed the following reaction:

\[
\begin{align*}
\text{C}_{6}\text{H}_{4}N-\text{Cl} + \text{C}_{6}\text{H}_{5}\text{OH} & \rightarrow \text{C}_{6}\text{H}_{4}N-\text{C}_{6}\text{H}_{4}\text{OH} + \text{HCl}
\end{align*}
\]
The inference from his publications is that this product is identical with that which results from condensation of Baeyer's and Caro's \(\text{N-hydroxy-p,p}'\)-dihydroxydiphenylamine. It seems safe to assume also that Hirsch would have designated this as the product of Liebermann's reaction, as he does not question the twice-published statement that the two reactions are identical. Nevertheless, Hirsch presented no analytical evidence for the structure he proposed.

Apparently Liebermann considered his reaction product to be a single and reasonably pure compound; in 1884, however, G. Kraemer found that the solid product he obtained from the Liebermann reaction on phenol itself consisted of two fractions which he separated by virtue of their different solubility in ether\(^5\). He did not, however, offer any proposals as to the identity of the two substances.

In 1883 Richard Mühlau\(^6\) pointed out the similarity in properties between "indophenols" and Liebermann's dye, using the term "indophenols" to designate compounds of the type already discussed by Hirsch. However it was Nitzschi\(^7\) in 1889 who first stated that indophenols were formed as a product of the reaction between phenols and nitrous acid. Nitzschi sought a clarification of Weselsky's reaction between resorcinol and nitrous acid\(^8\)—a reaction which Liebermann had duplicated by the use of his nitrosyl-sulfuric acid reagent on resorcinol. As an intermediate in this
reaction Nietzski proposed an indophenol of the structure shown:

In 1902 Decher and Solonina supported Nietzski's proposals from data they obtained in research on thymol. Apparently they isolated and studied the corresponding indophenol, indophenol-N-oxide, and diphenylamine, but not under the conditions of Liebermann's reaction. Nevertheless they felt that their work left "hardly any doubt" that indophenols and their N-oxides were produced in varying amounts by Liebermann's reaction.

Until recently there have been no other investigations designed specifically to study the Liebermann reaction. It has nevertheless become a rather well-established belief among organic chemists that the reaction leads to the production of indophenols, and in the case of the reaction with phenol itself, of phenolindophenol. McElvain, Sidgwick, Vogel and others outline the reaction with phenol itself as follows:
Other authorities, however, are considerably less definite concerning the mechanism of the reaction. Whitmore's merely hints that the reaction proceeds by the oxidation of the ring hydrogen atoms of phenol, but makes no mention of the products. Karrer's discussion is even less enlightening, stating merely that the reaction is characteristic of many phenols and describing the conditions under which the reaction is observed. Certainly no sound evidence can be found in the literature to support the proposal that indophenols are formed.

In 1950, R. D. Ashmore made a study of the Liebermann reaction and obtained spectrophotometric data which he interpreted as indicative that phenolindophenol was one of several products formed in the course of the Liebermann reaction on phenol itself. His data, however, were not sufficiently conclusive as definitely to prove the presence of this compound in the mixture.

It has been the object of the present investigation, then, finally to prove or disprove the presence of phenolindophenol in the product of the Liebermann reaction with phenol, and, if possible, to identify some of the other substances formed.
In the course of his investigation, Liebermann prepared a reagent consisting of a five percent solution of sodium nitrite in concentrated sulfuric acid. He carried out his reaction by first dissolving five grams of phenol in five ml. of concentrated sulfuric acid "under good refrigeration" and slowly adding twenty grams of his reagent to the mixture under such conditions that the temperature rose to 40-50° C. and stayed in that range. He noted that the reaction mixture took on several different colors in rather rapid succession, finally becoming a beautiful royal blue. Liebermann then poured the reaction mixture into a "large volume" of cold water, whereupon the solid product appeared as a fine red-brown precipitate in the water solution.\(^1\) \(^3\)

Ashmore, in his investigation\(^15\), found that appreciable yields of the solid could be obtained only if a small amount of water were added to the phenol-sulfuric acid mixture before the Liebermann reagent was added. Subsequently a footnote in Liebermann's original publication was noted, which indicated that Liebermann himself had added a small amount of water to the phenol used in his reaction in order to prevent solidification of the phenol.

In the present investigation, early attempts to produce
Liebermann's solid product were made without the addition of water to the reaction mixture. The yields of the solid product varied from none whatever to approximately four grams, although the conditions of the reaction were essentially constant for all runs. Thus minor changes in conditions apparently alter the nature of the reaction considerably. When in later work water was added to the phenol to prevent its solidification, the reaction took a more predictable course in that the yields of the solid product were more uniform and the colors formed in the reaction mixture more reproducible. The yields of solid were no larger than in the previous runs, however.

The solid, which was a gelatinous red-brown precipitate, was filtered off in every case with a Buchner filter, dried on a porous plate and then in a desiccator over calcium chloride. In a few cases the product was dried for an hour at 110° without apparent alteration.

Ashmore was able to show chromatographically that the Liebermann solid consisted of at least three fractions; he was, however, unable to effect a satisfactory resolution of the various fractions, either by chromatography or liquid extractions of the solid product.

A part of the dried solid product was extracted with ether, and the residue extracted with acetone; by subsequent evaporation of the solvents, three solid fractions of the
product were obtained: (1) A red-brown solid, soluble in ether and alcohol and partially soluble in chloroform, soluble in concentrated sulfuric acid with a gray color and in 0.5 N NaOH with a dirty brown color; (2) A black crystalline solid, soluble in acetone and alcohol, soluble in concentrated sulfuric acid with a gray-green color and in 0.5 N NaOH with a violet color; (3) A very dark brown solid, partially soluble in alcohol, insoluble in the other common organic solvents, soluble in concentrated sulfuric acid with a blue color like that of the original reaction mixture, soluble in 0.5 N NaOH with a red-brown color. Solutions or extracts of each of these three materials were then passed through chromatographic columns as follows:

A chloroform extract of fraction (1) above was passed through a column of silica; a red band formed at the top of the column and a clear eluate was obtained. The red material remained in a compact mass upon development of the column and thus appears to be a pure compound.

Solutions of fraction (2) in acetone and in a mixture of acetone and chloroform were passed through columns of silica. Only very slight evidence of adsorption was noted.

An alcoholic extract of fraction (3) passed unchanged through a silica column.

A chloroform extract of the complete Liebermann solid was passed through a column of silica; the red material mentioned above was the only material appreciably adsorbed on
the column. Two other colored areas were also noted, however.

Further attempts to resolve the Liebermann solid chromatographically were made as follows:

Solutions of the Liebermann solid in aniline and in a mixture of aniline and chloroform were passed through columns of silica with only little adsorption and no color separation. Similar results were obtained with extracts in a mixture of absolute ethanol and chloroform. Alcoholic extracts of the solid were then passed through columns of silica mixed with Hyflo Super-Cel, and of Super-Cel alone; there was no evidence of adsorption. Another alcoholic extract was made slightly basic with aqueous sodium hydroxide, and passed through columns of silica, calcium carbonate, Hyflo Super-Cel, absorbent cotton, talc, and a mixture of talc and Hyflo Super-Cel. There was no evidence of adsorption in any of these cases.

It was decided unlikely that resolution of the Liebermann solid product could be effected by chromatographic separation on a column. One outstanding disadvantage of the method as applied to this problem is the limited solubility of the Liebermann solid in non-polar solvents, from which adsorption is most likely to take place.

Attention was next given to the filtrate remaining after separation of the Liebermann solid product from the aqueous solution. This filtrate is a clear red solution by
transmitted light, brown by reflected light—a description which applies to a good many of the solutions of Liebermann's product in water or organic solvents.

When the filtrate was treated with barium carbonate to remove sulfuric acid, the whole changed from red to blue when all the acid had been precipitated. This solution was acidified with a few drops of sulfuric acid and passed through a column of silica. There was no evidence of adsorption, and the solution was recovered unchanged from the bottom of the column.

About 800 ml. of this solution was concentrated to about half its volume by distillation at about 40° Centigrade (50-60 mm pressure); the distillate was collected and found to be pale yellow in color and to possess a distinct odor of benzoquinone. A small portion of this distillate liberated iodine from KI solution and turned red-brown on addition of aqueous NaOH solution, and therefore satisfies two qualitative tests for benzoquinone.

A positive test for ketones was obtained by adding a solution of 2,4-dinitrophenylhydrazine to a small portion of the distillate. The semicarbazone was therefore prepared directly from the water solution and compared with a semicarbazone prepared from a known aqueous solution of benzoquinone. The product obtained from benzoquinone decomposed at 241° (corrected); that obtained from the unknown decomposed at 231° (corrected). Neither product was obtained in large enough yield to permit recrystallization; nevertheless
the agreement is reasonably good, and it can be said that benzoquinone is probably one of the products of the Liebermann reaction.

The remainder of the filtrate which was concentrated by distillation as described above was allowed to stand for several weeks, after which time a rather large amount of very finely divided brown precipitate had settled out of solution. This precipitate was filtered off and the absorption spectrum of the solution determined. The curve (See Figure 1) was of no particular interest as no characteristic absorption peaks were found.
FIGURE 1

ABSORPTION SPECTRUM
OF FILTRATE FROM
LIEBERMANN REACTION

OPTICAL
DENSITY

WAVELENGTH
(MILLIMICRONS)
EXPERIMENTAL

The Liebermann Reaction:

Liebermann's reagent was prepared by adding 10 grams of sodium nitrite in small portions to 200 grams of concentrated sulfuric acid in a glass-stoppered Erlenmeyer flask; the flask was stoppered and shaken after each addition of nitrite until there was no evidence of brown fumes of NO₂. The reaction was then carried out as follows:

**Trial I:** Five grams of solid phenol was dissolved in 5 ml. of concentrated sulfuric acid which had been cooled to 0° C. Twenty grams of the Liebermann reagent was then added to this mixture with constant stirring and at such a rate that the temperature rose to 40-50° and remained in that range. A brown color was noted, which changed to green and finally to blue. After fifteen minutes the reaction mixture was poured into 800 ml. of ice-water, at which time the color changed rapidly from blue to scarlet, to red-brown, and finally to brown. A brown precipitate was evident on close examination of the mixture.

The precipitate was filtered off at once, and the filtrate allowed to stand overnight and filtered again; a total of about 0.5 g. of the dried brown precipitate was obtained.

**Trial II:** The reaction was run as above except that the quantities of reactants were doubled and the reaction
mixture poured into 400 ml. of ice-water and allowed to
stand overnight before filtering. About 3.5 g. of the dried
solid was obtained.

**Trial III:** The reaction was attempted as in Trial II
above, but no blue color developed in the reaction mixture,
and there was no yield of solid product when the mixture was
poured into water.

**Trial IV:** The reaction was run again as in Trial II,
and 1.5 g. of the solid product was obtained.

**Trial V:** The reaction was run as in Trial I, except
that the amounts of reactants used were 20 g. phenol, 20 ml.
of concentrated sulfuric acid, and 80 g. of reagent, and the
mixture was poured over 600 g. of crushed ice, allowed to
stand overnight, and filtered. The yield of dried solid
product was about 4 grams.

**Trials VI, VII, VIII:** The reaction was attempted as in
Trial V, but no blue color developed and there was no yield
of solid product when the mixture was poured into water.

**Trial IX:** At this point a footnote was found in Lieber-
mann's original paper\(^1\) in which he states that he added a
trace of water to the phenol in order to prevent its solid-
ification. Consequently, 10 g. of phenol with a trace of
water added was mixed with 10 ml. of concentrated sulfuric
acid and 40 g. of Liebermann's reagent added in small por-
tions, the conditions of temperature being those of Trial I
above. The reaction mixture took on its characteristic blue
color and a precipitate appeared at once when the mixture was poured into about a liter of ice-water. The mixture was filtered as soon as all the ice had melted, which required about two hours. The yield of dried solid was about 2 grams.

**Trial IX:** The reaction was repeated as in Trial IX, but no ice was used, although the temperature of the reactants was maintained between 40 and 50°C. The reaction proceeded normally, and about 2 g. of the dried solid was obtained.

**Liquid Extraction of Liebermann’s Solid:**

**Fraction 1:** A portion of the dried Liebermann solid was shaken with ether and allowed to stand in contact with the solvent over a period of several days, the ether being decanted frequently and replaced by fresh solvent. A clear red solution was obtained, which was shaken with BaCO₃ to remove sulfuric acid; there was little action. The solution was concentrated by distillation of the ether, and finally evaporated to dryness at room temperature. A light brown powder remained; it was found easily soluble in ether and ethanol, very slightly soluble in chloroform and benzene, insoluble in ligroin and CCl₄, soluble in concentrated sulfuric acid and in 0.5 N. NaOH.

**Fraction 2:** The residue from the ether extraction above was dissolved in alcohol; a small portion did not dissolve. The solution was shaken with BaCO₃ to remove H₂SO₄, and as before there was little action with this reagent.
The solution was filtered and the alcohol evaporated. The dark residue remaining was extracted with acetone. The acetone was evaporated from the resulting solution to yield a black, hard crystalline solid, easily soluble in alcohol, insoluble in ether, chloroform, benzene, ligroin, and CCl₄, soluble in concentrated sulfuric acid and in 0.5 N NaOH.

Fraction 2: The black residue remaining after the acetone extraction was found to be partially soluble in ethanol, insoluble in chloroform, ether, benzene, ligroin, and CCl₄, soluble in concentrated sulfuric acid and in 0.5 normal NaOH.

Chromatographic adsorption from solutions of the above fractions:

Fraction 1: A portion of the light brown solid was extracted with chloroform to produce a light orange solution. A chromatographic column was prepared by pouring finely-divided silica into a glass tube and applying suction at the bottom of the tube to produce a tightly-packed column of silica approximately 2x14 cm. in size. The silica used was Davison's Commercial Activated product, of mesh size "Thru 200." Twenty ml. of the dilute chloroform solution was introduced at the top of the column and allowed to percolate through the column at atmospheric pressure. When the solution was all on the column it was followed by 200 ml. of chloroform, and then by 10 ml. of a developing solution of 50% chloroform in ethanol. A bright red ring which had
formed at the top of the column moved rapidly about half way down the column to appear as a thin orange line. Twenty-eight ml. of a perfectly clear eluate was recovered from the column.

When the column was disintegrated it was found that "coning" had taken place—i.e., the adsorption had been most pronounced in the center of the column, where an intense red section was found, tapering outward to the edge of the column where the fine orange line was visible as mentioned above.

The adsorption was repeated as above except that 80 ml. of the chloroform solution was introduced at the top of a silica column; when this solution was all on the column, 10 ml. of a developing solution of 10% ethanol in chloroform was introduced. The bright red band moved slowly about 1.5 inches down the column. About 65 ml. of clear eluate was recovered from the column.

Fraction 2: A small portion of the black solid was dissolved in acetone, and 30 ml. of the dilute solution was passed through a column of silica as described on page 15. This solution was followed by a 10% solution of alcohol in acetone, but there was no evidence of adsorption; the solution was recovered unchanged at the bottom of the column.

A second small portion of the solid fraction 2 was dissolved in acetone, and chloroform was added until a precipitate appeared; the precipitate was then dissolved by the
addition of acetone. Twenty ml. of this solution was then passed through a column of silica. The solution was followed by ten ml. of 50% acetone in chloroform, then by 20 ml. of 25% chloroform in acetone. A pale yellow-brown diffuse zone moved down the column and washed through into the eluate. When the column was disintegrated a very small amount of adsorbed brown material was found at the top of the column.

**Fraction 3:** A dilute alcoholic extract of fraction 3 was passed through a column of silica as previously described but there was no adsorption whatever.

**Chromatographic adsorption from other solutions prepared from the Liebermann solid product:**

The Liebermann product was found to be completely soluble in aniline; a dilute aniline solution was passed through a silica column, but there was no evidence of adsorption.

Chloroform was added to another aniline solution until a precipitate appeared, which was dissolved by further addition of aniline. A portion of this solution was passed through a column of silica; there was some evidence of adsorption, but only very poor separation.

A portion of the Liebermann solid was extracted with absolute ethanol, in which nearly all the solid dissolved. The solution was diluted to twice its volume with chloroform
and passed through a column of silica. The column was developed with ten ml. of 50\% dry chloroform in absolute ethanol. The chromatogram consisted of two diffuse colored regions—a pink zone extending half way down the column, and a light brown region extending to the bottom of the column. A dark brown section was found "coned" at the bottom of the column.

A portion of the Liebermann solid was extracted overnight with dry chloroform; the solution was filtered and the filtrate passed through a column of silica. The column was subsequently developed with twenty ml. of 10\% absolute ethanol in dry chloroform (added in successive small portions), followed finally by four ml. of absolute ethanol. A diffuse light-brown region extended two inches from the top of the column, followed by a two inch zone which was light yellow-orange in color, and then by a half-inch band of a red material like those previously mentioned as having been adsorbed from chloroform solutions.

A four gram portion of the Liebermann solid was extracted with chloroform in a Soxhlet apparatus to make about 80 ml. of a solution which contained approximately half a gram of the dissolved Liebermann solid. The entire 80 ml. of solution was passed through a silica column 20x200 mm. in size; the solution was followed by 30 ml. of 10\% alcohol in chloroform, then by 20 ml. and 10 ml. portions of the same
solution. The chromatogram appeared much as described in the above paragraph except that the colors were deeper due to increased concentration. In both the above cases the red material was present in much larger amounts than the other adsorbed material, which was present only to an extent sufficient to impart color to the column.

The residue from a chloroform extraction of the Liebermann solid was extracted with absolute ethanol to form a dilute brown solution; the solution was passed through a column made up of 1:1 portions of Davison silica and Hyflo Super-Cel, a filter-aid manufactured by Johns-Manville Corp. There was little evidence of adsorption and no color separation.

Another portion of the same solution was passed through a column of Super-Cel alone which had first been heated at 130°. There was no evidence of adsorption.

A third portion of the same solution was passed through a column of calcium carbonate; the entire column took on a blue color as a result of an increase in pH of the brown solution originally introduced. There was no adsorption, however.

Some of the alcohol solution was diluted with water and made slightly basic by the addition of NaOH. The resulting solution took on a red-purple fluorescence. Portions of this basic solution were passed through columns
made up of silica, calcium carbonate, Hyflo Super-Cel, absorbent cotton, talc, and a mixture of talc and Super-Cel, but in no case was there evidence of selective adsorption.

Concentration of the filtrate from the Liebermann reaction:

The filtrate from the Liebermann reaction was concentrated by distillation at about 40° C. (50-60 mm. pressure), the total volume being reduced from about 800 ml. to half of this value. The distillate was collected and found to be pale yellow in color and to possess a definite odor of benzoquinone.

Tests for the presence of benzoquinone:

A few drops of the yellow solution above was added to a solution of KI and starch. Iodine was liberated immediately indicating the presence of quinone\(^\text{17}\).

To another portion of the yellow solution was added a few ml. of dilute aqueous NaOH; the solution immediately darkened to a red-brown color, indicating the presence of quinone\(^\text{17}\).

To a few ml. of the yellow solution was added a few drops of a solution of 2,4-dinitrophenylhydrazine; an orange precipitate of the substituted phenylhydrazone formed after a few minutes' standing, indicating the presence of a ketone.

The semicarbazone was prepared as follows:

Two grams of semicarbazide hydrochloride, 3 g. of sodium acetate, and 50 ml. of the pale yellow solution above
were placed in a test tube, shaken and cooled in ice; a precipitate formed after a short time and was filtered off and washed but not recrystallized. It melted at $231^\circ$ (corr.), with decomposition.

The above preparation of the semicarbazone was repeated with one gram of semicarbazide hydrochloride, 1.5 g. of sodium acetate, and 10 ml. of a solution of benzoquinone in water, made up to approximately the same concentration as the unknown as indicated by the depth of color. A red-brown precipitate was obtained, in appearance exactly like that obtained from the unknown, although in somewhat greater yield. It was washed but not recrystallized, and found to melt with decomposition at $241^\circ$ (corr.). The melting point is given in the literature as "about $243^\circ."

**Absorption spectrum of the filtrate:**

A portion of the filtrate was diluted and the absorption spectrum determined on the Beckmann Quartz Spectrophotometer, Model DU. The data obtained are given in Table 1 below; the corresponding absorption curve is shown in Fig. 1.

**Table 1**

<table>
<thead>
<tr>
<th>$\mu\mu$</th>
<th>O.D.</th>
<th>$\mu\mu$</th>
<th>O.D.</th>
<th>$\mu\mu$</th>
<th>O.D.</th>
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PART II -- PHENOLINDOPHENOL

DISCUSSION

In order to establish the presence of phenolindophenol in the products of the Liebermann reaction, Ashmore attempted to prepare the compound by a separate known means of preparation, intending to compare the chemical and physical properties of the indophenol with those of the Liebermann product. However, he was unable to find a completely satisfactory preparation recorded in the literature and could only assume that the method he used produced a dilute aqueous solution of phenolindophenol. To the absorption spectrum of Liebermann's product he compared the spectrum of this dilute water solution, and concluded from a similarity in the spectra that phenolindophenol was very probably produced in the course of the Liebermann reaction.

It was decided to investigate further the preparation of phenolindophenol in the hope of preparing some of the pure crystalline compound, which would form a better basis for comparison with Liebermann's product than would the solution prepared by Ashmore. A survey of the literature reveals that comparatively little work has been done on the indophenols, the most comprehensive study having been carried out by Gibba, Hall, and Clark; these men were interested in the preparation of these compounds for use as
oxidation-reduction indicators.

Two methods of preparation were investigated by these men. The first, published by Heller\textsuperscript{19}, involves the oxidation of a phenol and a p-aminophenol by hypochlorite solution at low temperature. They found this preparation unsatisfactory and suggested that hypochlorite is too powerful an oxidizing agent to yield a pure product in the above reaction.

The second method, first suggested by Hirsch\textsuperscript{20}, is carried out in two steps; a quinonechlorimide is first prepared by treating an aminophenol with hypochlorite in acid solution, and this product is then coupled with a phenol in alkaline solution to produce the desired indophenol, as follows:

\[
\text{Gibbs, Hall, and Clark found this method quite satisfactory, and describe the preparation of thirty-four indophenols.}\]

A satisfactory preparation of phenolindophenol was obtained by the above method. The immediate product of the above reaction is of course the sodium salt of the indophenol; this was decomposed with carbon dioxide to obtain the free acid. This product was recrystallized from acetone and petroleum ether (33-55°C) to form large black crystals which were washed with petroleum ether and air-dried.
A satisfactory melting point could not be obtained on this compound by the ordinary method of heating in a capillary. There appeared to be an abrupt change in the compound at approximately 150°, but at temperatures higher than 150° the product was solid. Consequently it was necessary to devise a method of heating a microscope slide so as to permit the observation of a few crystals of the material at elevated temperatures. The crystals were found to melt at 152°, the liquid immediately hardening again to a dark opaque solid.

The melting points as reported in the literature for this material are widely divergent, but several investigators have reported a melting point of 160°. Gibbs, Hall, and Clark reported an exothermic reaction at 129° in one case, and a shrinkage at 135-136° in another case; they failed to duplicate the 160° reported value.

The microscopic examination also revealed that the "black" crystals previously mentioned are actually a clear red by transmitted light, green-black by reflected light. This probably explains the statement by Gibbs that one sample of his product consisted of "two forms of minute crystals—one clear red and the other black with a greenish color by reflected light."

The absorption spectrum in the visible and near ultraviolet regions have been reported under varying conditions of pH by Schwarzenbach, Mohler, and Sorge. The absorption
spectrum of the above preparation was determined in alcoholic solution (pH approximately 8) and found to agree quite closely with one curve published by these investigators. (See Fig. 2)

As a further check on the identity of the preparation, some of the sodium salt of the indophenol was reduced with $\text{Na}_2\text{S}_2\text{O}_4$ to produce the compound 4,4'-dihydroxydiphenylamine. This product was recrystallized from a water solution of $\text{Na}_2\text{S}_2\text{O}_4$ in a nitrogen atmosphere and dried in vacuo over sulfuric acid. The melting point of this product, 174.5° (corr.), agrees well with data reported by Schneider, Heller, and Gibbs.

The chromatographic behavior of the phenolindophenol was checked by dissolving a small portion of the product in chloroform and passing the solution through a column of silica. The indophenol was adsorbed as a red band on the column, and appeared to behave identically like the chloroform-soluble fraction of the Liebermann product. Thus chromatographically the two substances appear indistinguishable, except for a rather slight qualitative color difference on the column; the phenolindophenol is a somewhat more pure red than is the Liebermann fraction.

A solution of the indophenol in chloroform and a chloroform extract of Liebermann's solid product were passed through identical silica columns and treated identically as to development and elution from the columns. The red bands
FIGURE 2

ABSORPTION SPECTRUM OF PHENOLINDOPHENOL

1 - CURRENT INVESTIGATION
2 - REFERENCE 22

WAVELENGTH (MILLIMICRONS)

OPTICAL DENSITY
FIGURE 3

1 - SPECTRUM OF PHENOLINDOPHENOL
2 - SPECTRUM OF CHLOROFORM-SOLUBLE FRAGMENT OF LIEBERMANN SOLID

WAVELENGTH (MILLIMICRONS)

OPTICAL DENSITY
were eluted with alcohol and the absorption spectra of the resulting solutions determined. (See Fig. 3) There was absolutely no agreement whatsoever between the two spectra, the chromatographic homogeneity of the two materials notwithstanding.

In a further study of chromatographic behavior, a chloroform solution of phenolindophenol was mixed with a chloroform extract of the Liebermann solid, and the resulting solution passed through a column of silica. There was no evidence of separation on development; the thin red band which formed at the top of the column remained a very concentrated section even after development had moved the red material more than twelve inches down the column.

No other red band was ever noted on any of the chromatographic columns used in the course of this investigation. Thus it appears that no phenolindophenol is present in the products of the Liebermann reaction, for this compound is quite soluble in chloroform, benzene, and other non-polar solvents in which the Liebermann solid is all but insoluble.
EXPERIMENTAL

Preparation of quinonechlorimide:

Following the directions of Gibbs, Hall, and Clark, 63 g. of NaOH was dissolved in about 200 ml. of water; the solution was cooled and poured over crushed ice. A small additional amount of ice was then added to bring the total weight of the mixture to one kg. Chlorine gas was then bubbled into the cold solution until the total weight had increased by 73 g. Twenty-seven grams of p-aminophenol was dissolved in 300 ml. of concentrated hydrochloric acid with enough water (about 400 ml.) to bring about complete solution. This aminophenol solution was poured slowly with stirring into the hypochlorite solution prepared above. A bright yellow precipitate formed immediately; it was filtered at the pump, washed with cold water, and air dried. The yield was 35 grams. This yield was somewhat greater in subsequent preparations carried out exactly as above.

Preparation of sodium indophenolate:

Trial I: Following the general procedure of Gibbs, Hall and Clark, 14.2 g. of quinonechlorimide and 9.4 g. of phenol were suspended in 150 ml. of water and the mixture cooled to 0° C. Seventy-three ml. of 3 N. NaOH solution was then added dropwise with stirring over a period of half an hour. The reaction mixture at once took on a blue color
much like that of the Liebermann mixtures. After five minutes the mixture was filtered, but only about four grams of unreacted quinonechlorimide was obtained.

One hundred grams of NaCl was then dissolved in the filtrate in an attempt to salt out the sodium salt of the phenolindophenol. After several hours standing at 0° C., the blue solution was filtered, but no precipitate was recovered. After standing overnight the solution had taken on a dirty brown color and there was still no solid in evidence.

**Trial II:** To decrease the volume of solvent present, 14.2 g. of quinonechlorimide and 9.4 g. of phenol were suspended in only 20 ml. of water containing a few small chips of ice; the solution was cooled to 0° C. Seventy-three ml. of 3 M. NaOH was then added dropwise with stirring over a period of an hour, the temperature being held at 0°. The stirring was continued for 30 minutes after all the NaOH had been added; toward the end of this 30 minute period, 15 g. of NaCl were added to salt out the product. After an hour's standing at 0° the solution was filtered and the product washed with a little ice water. The air-dried product, weighing 13 g., was a crystalline material with an iridescent green color.

**Trial III:** The preparation was repeated as above but only five ml. of water was used to suspend the reactants. No NaCl was added, and after half an hour's standing at 0°
the solution was filtered. Eleven grams of a very finely divided dark precipitate was obtained. The product was dissolved in alcohol and the solution kept at 0° for several hours. It was then filtered to yield about two grams of the solid sodium salt of phenolindophenol. To the filtrate was added 50 ml. of ether and the mixture was filtered; about four grams of the sodium salt was obtained in a very finely-divided and very voluminous form.

**Trial IV:** The reaction was repeated as in Trial III, but the 3N NaOH was saturated with NaCl before it was added to the suspended reactants. The phenol used was liquid and contained a trace of water to prevent its solidification. When the five ml. of water was added to the reactants two liquid phases appeared. As the mixture was stirred and cooled with ice to 0° C., solid phenol appeared in a very fine suspension. To this suspension was added the quinone-chlorimide and the NaOH as before. The addition of the NaOH required forty minutes and stirring was continued for forty minutes after all the base had been added. The product was filtered out at once, washed with a little saturated NaCl solution, and dried in a desiccator. The yield of the green material was 21 grams. Purification was effected by dissolving the product in 50 ml. of water and again salting out the solid sodium salt.

**Trial V:** The reaction was repeated as above except that the NaOH was added over a period of an hour, and the 14.2 g.
of quinonechlorimide was added in small portions during the first half-hour, simultaneously with the NaOH solution. Twenty-three grams of the dried impure product was obtained.

Preparation of phenolindophenol:

Trial I: Following Gibbs' directions, five grams of the purified sodium salt prepared as described previously was dissolved in 90 ml. of water. The solution was cooled to 0-5° C. and saturated with CO₂. As the gas bubbled through the blue solution the color changed to red-brown. After about ten minutes the mixture was filtered and an additional five grams of the sodium salt dissolved in the mother liquor. This solution was also saturated with CO₂ for ten minutes and filtered; the two precipitates were combined and washed with ice-water saturated with CO₂. The product appeared dark brown; the yield of the air-dried material was 8.5 grams.

This product was found to be only partially soluble in water and in benzene, and thus apparently was not homogeneous.

Trial II: Four grams of the purified sodium salt was dissolved in 80 ml. of water and the solution was cooled and saturated with CO₂. A precipitate appeared in the red-brown solution; this was filtered off and washed as before with ice water containing CO₂. About 2.2 g. of the air-dried dark-brown solid was obtained.
Trial III: One gram of the sodium salt was dissolved in 12 ml. of water, and 10 drops of glacial acetic acid was added. A precipitate formed at once, was filtered off, washed and air-dried. The yield was 0.5 grams. No melting point was noted, although the material appeared to shrink at about 135°.

Trial IV: Twenty grams of the purified sodium salt was dissolved in about 400 ml. of water, cooled to 0°, and saturated with CO₂. The gas was bubbled through the solution for about five minutes after the blue color had entirely disappeared. The precipitate was then filtered, washed repeatedly with small portions of cold water saturated with CO₂, and dried in a vacuum desiccator over CaCl₂. The product was a black finely-divided solid which appeared to be crystalline. About 12 g. of the dried solid was obtained.

About six grams of this product was dissolved in 200 ml. of acetone, and approximately a liter of petroleum ether (35-55° C.) was added to the red solution. On standing at low temperature for several hours, long black crystals formed on the sides of the beaker. These were filtered, washed with petroleum ether and air-dried.

Five grams of the crude phenolindophenol was dissolved in 1200 ml. of hot water; crystals appeared quickly as the solution cooled. The crystals were filtered off and dried in a desiccator.
Determination of the melting point of phenolindophenol:

A small portion of the product obtained above was ground to a fine powder and placed in a capillary melting-point tube. The sample was then heated in the ordinary manner in an oil bath. At approximately 150° an abrupt change was noted in the sample, which appeared to liquefy and immediately harden again. There was no further change through 200°. When the melting point tube was broken a black varnish-like coating was found on the inside of the tube. This material was soluble in NaOH solution with a blue color but not the brilliant blue of the phenolindophenol in basic solution.

Several attempts were made to determine the melting point in this manner, but it was impossible accurately to ascertain the nature of the transformation or the exact temperature at which it took place.

Five feet of 24-gauge nichrome wire was bent so as to fit between two microscope slides. The wire element was wrapped with asbestos paper in the center of which was a 3/8 inch hole, and the entire unit was placed between the two slides which were wired together. By means of a Variac transformer, 12.8 volts were placed across the nichrome element. A few crystals of the phenolindophenol were then placed on the slide adjacent to several crystals of benzilic acid, M.P. 151.5° C. The red crystals melted at a temperature only very slightly above the melting point of the benz-
ilic acid. Thus to a very close approximation the melting point of the red crystals is 152° C.

As the red crystals melted they turned dark red-brown and opaque and the liquid material solidified at once, although there was no cooling. The slide was not heated appreciably above 152° so no observation was made as to the effect of higher temperature on this red-brown solid.

Preparation of 4,4'-dihydroxydiphenylamine:

Fifteen grams of the sodium salt of phenolindophenol was dissolved in water to make a saturated solution at 30°. One hundred ml. of a 10% solution of K₂S was prepared by passing H₂S into a 10% solution of KOH for about ten minutes, until the solution had taken on a definite yellow color. Following Gibbs' directions for preparation of the diphenylamine, the K₂S solution was added dropwise with stirring to the solution of the indophenol salt. But the 100 ml. of K₂S solution was not enough to reduce all the indophenol, so a little solid Na₂S₂O₄ was added. The deep blue color disappeared and the solution took on a dirty brown color. The solution was made acid with acetic acid and cooled, whereupon brown crystals of 4,4'-dihydroxydiphenylamine separated out.

The material was recrystallized twice from water containing a little Na₂S₂O₄. The second recrystallization was carried out in an inert atmosphere of nitrogen. The crystals are white or very light tan when first formed, but
darken rapidly due to air oxidation, even when attempts are made to exclude air from the preparation.

The compound was dried in vacuo over sulfuric acid (Yield, 2.5 g.; melting point 174.5°, corr.). This melting point agrees with that reported by Schneider, Heller, and Gibbs, and serves to identify the sodium salt of the phenolindophenol from which it was prepared.

Spectrophotometric study of phenolindophenol:

A solution of purified phenolindophenol was prepared by dissolving 0.00204 g. of the compound in ethanol to make 100 ml. of a 1.02 x 10^-4 molar solution. The absorption spectrum of this solution was determined on the Beckmann Quartz Spectrophotometer, Model DU, the optical density being plotted against wavelength from 220 to 700 millimicrons. The data are given in Table 2.

Table 2

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The curve plotted from these data agrees closely with a curve obtained by Schwarzenbach, Mohler, and Sorge for phenolindophenol. (See Figure 2)
Spectrum of 4,4'-dihydroxydiphenylamine:

A 1.03 x 10^-4 molar solution of 4,4'-dihydroxydiphenylamine was prepared by dissolving 0.00207 g. of the compound in ethanol to make 100 ml. of solution. The absorption spectrum of this colorless solution was determined; the data are given in Column 1, Table 4, page 39.

After having stood overnight, the solution had developed a pale but perceptible pink color due to air oxidation of the diphenylamine. This color darkened as the solution stood for several more days. Absorption spectra were run after 24 hours, 72 hours, and six days; the data are given in Columns 2, 3, and 4, respectively, of Table 4.

Oxygen gas was then bubbled through the solution and it was allowed to stand for two more days; the absorption data are given in Column 5 of Table 4. The treatment with oxygen was repeated and the solution allowed to stand another week, and the spectrum was determined again. The data are given in Column 6 of Table 4.

All of these curves are plotted in Figure 4, together with the absorption curve for phenolindophenol, data for which are given in Column 7 of Table 4. Inspection of the curves indicates that the absorption spectrum of a solution of 4,4'-dihydroxydiphenylamine gradually approaches that of phenolindophenol due to air oxidation of the diphenylamine.
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FIGURE 4

ABSORPTION SPECTRA OF THE SYSTEM
PHENOLINDOPHENOL - 4,4'-DIDROXYOPHENYLAMINE

OPTICAL DENSITY

WAVELENGTH
(MILLIMICRONS)

700  600  500  400  300
Chromatographic behavior of phenolindophenol:

A chloroform solution of phenolindophenol and a chloroform extract of Liebermann's product were prepared. The two solutions were adjusted as to concentration so as to have approximately the same depth of color. They were then passed through silica columns (see p. 16) as nearly identical as possible.

The columns were developed with equal successive small portions of 10% ethanol in chloroform; they were allowed to stand overnight, the development proceeding by gravity.

Both columns appeared much the same after having stood overnight, a wide dark red-brown band being present about four inches down each column. Mild suction was then applied to both columns to remove residual developing reagent. Further separation then took place; the relative appearance of the columns was as shown below:

<table>
<thead>
<tr>
<th>Phenolindophenol</th>
<th>Liebermann product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very light gray</td>
<td>Pale violet</td>
</tr>
<tr>
<td>Very light orange</td>
<td></td>
</tr>
<tr>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Gray</td>
<td></td>
</tr>
<tr>
<td>Intense red</td>
<td>Intense red</td>
</tr>
</tbody>
</table>
In both cases the red material was the most concentrated of all the substances adsorbed on the column. The eluate was clear and colorless. Thus it is apparent that both the Liebermann extract and the phenolindophenol preparation consist primarily of red materials which behave similarly on adsorption on silica.

A slight difference in quality of the red colors was apparent when the two columns were compared side by side. The two red bands were eluted from the columns with ethanol. The phenolindophenol solution was red-purple, vaguely reminiscent of dilute permanganate solutions. The solution prepared from the red material from Liebermann's product was golden in color, and, when adjusted to a pH of 7.5 corresponding to that of the phenolindophenol solution, took on a pale green color.

The absorption spectra were determined for these dilute alcoholic solutions, both at pH 7.5. The data are given in Table 3. The curves, which are plotted in Fig. 3, are in no way similar.

Table 3

<table>
<thead>
<tr>
<th>μm</th>
<th>O.D. 1</th>
<th>O.D. 2</th>
<th>μm</th>
<th>O.D. 1</th>
<th>O.D. 2</th>
<th>μm</th>
<th>O.D. 1</th>
<th>O.D. 2</th>
<th>μm</th>
<th>O.D. 1</th>
<th>O.D. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>220</td>
<td>0.822</td>
<td>inf.</td>
<td>230</td>
<td>1.01</td>
<td>inf.</td>
<td>240</td>
<td>1.06</td>
<td>inf.</td>
<td>250</td>
<td>1.38</td>
<td>inf.</td>
</tr>
<tr>
<td>230</td>
<td>0.970</td>
<td>1.43</td>
<td>240</td>
<td>0.920</td>
<td>1.29</td>
<td>250</td>
<td>0.950</td>
<td>1.23</td>
<td>255</td>
<td>0.962</td>
<td>1.22</td>
</tr>
<tr>
<td>240</td>
<td>0.920</td>
<td>1.29</td>
<td>250</td>
<td>0.950</td>
<td>1.23</td>
<td>260</td>
<td>1.06</td>
<td>1.21</td>
<td>265</td>
<td>1.00</td>
<td>1.20</td>
</tr>
<tr>
<td>250</td>
<td>0.950</td>
<td>1.23</td>
<td>260</td>
<td>1.06</td>
<td>1.21</td>
<td>270</td>
<td>1.05</td>
<td>1.18</td>
<td>275</td>
<td>1.03</td>
<td>1.16</td>
</tr>
<tr>
<td>260</td>
<td>1.06</td>
<td>1.21</td>
<td>270</td>
<td>1.05</td>
<td>1.18</td>
<td>280</td>
<td>1.25</td>
<td>1.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Further investigation was made of the apparent chromatographic homogeneity exhibited by phenolindophenol and the chloroform soluble part of the Liebermann solid. A chloroform solution of phenolindophenol was added to a chloroform extract of the Liebermann solid, and the mixture was passed through a $2 \times 50$ cm. column of silica. The column was developed with successive small portions of 10% ethanol in chloroform.

The two red materials mentioned previously formed a single compact band which moved slowly down the column and was not in the least diffuse even after 36 hours, when the red ring had moved about thirty cm. down the column.
PART III -- 2,5-bis(p-hydroxyanilino)-p-benzoquinone

DISCUSSION

Whitmore describes the compound which we have designated as phenolindophenol as being a condensation product of p-aminophenol and quinone. It was thought possible then that the indophenol might be prepared directly by coupling of these two reagents. Further investigation, however, revealed that p-aminophenol and quinone react readily in alcoholic or aqueous solution to form the compound 2,5-bis-(p-hydroxyanilino)-p-benzoquinone:

\[
\text{HO} \quad \text{N} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{OH}
\]

Heller says that phenolindophenol is split by acids to form p-aminophenol and quinone which then react to form the above compound.

The presence of benzoquinone in the Liebermann product (see pp. 11, 20, 21) led to a consideration of the possibility that the substituted benzoquinone above might also be present, inasmuch as both compounds could arise from the decomposition of phenolindophenol under the acid conditions of the Liebermann reaction. Consequently, the 2,5-bis-(p-hydroxyanilino)-p-benzoquinone was prepared by the action
of p-aminophenol on a hot alcoholic solution of benzoquinone. A black crystalline material was obtained.

Comparatively little work has been done with this compound and as a consequence its melting point or derivatives were not available in the literature. It was therefore necessary to determine the percent nitrogen in the material in order to establish its identity. The nitrogen was determined by the Kjeldahl method and found to be 8.57% and 8.60% in two analyses. The theoretical value is 8.69%. This agreement coupled with the fact that the compound has reportedly been prepared by this reaction was taken as satisfactory evidence of the identity of the material.

A sharp melting point was observed at about 345°C.

This material is insoluble in all the ordinary organic solvents except ethanol in which it is very slightly soluble. It is soluble in dilute sodium hydroxide solution to give a red-brown solution, and in concentrated sulfuric acid to give a deep blue solution. In these respects and in general physical appearance the anilinoquinone resembles the fraction 3 of the Liebermann solid, described on page sixteen.

Pratt and Gibbs have published information on the absorption spectrum of 2,5-dianilinobenzoquinone. While their compound lacks the p-hydroxy groups of that currently under investigation, the basic structure is the same and a close similarity in the absorption spectra would be expected.
For comparison of the spectra, 0.00332 g. of the 2,5-bis(p-hydroxyanilino)-p-benzoquinone was dissolved in alcohol to make 100 ml. of a $1.03 \times 10^{-4}$ molar solution. The absorption spectrum was determined (See Fig. 5) and found to be quite similar to the curve published by Pratt and Gibbs (See Fig. 5A)

Fraction 3 of the Liebermann product, discussed on page sixteen, was placed in ethanol and extracted overnight; the absorption spectrum of the resulting solution was determined (See Fig. 6). The curve was found to be similar in general form and in location of absorption peaks to that obtained from the solution of 2,5-bis(p-hydroxyanilino)-p-benzoquinone. Agreement was thought to be sufficiently good to warrant further attempts to isolate the substituted quinone from the Liebermann solid. Various attempts were made to separate the material chromatographically, but a satisfactory separation was not obtained. Thus it can only be suggested that 2,5-bis(p-hydroxyanilino)-p-benzoquinone is a possible product of the Liebermann reaction; it should also be pointed out that the formation of benzoquinone and of the above-mentioned substituted quinone is consistent with the formation of phenolindophenol and the subsequent decomposition of this compound.
FIGURE 5
Reproduced from literature.

Absorption spectrum of 2,2'-diaminobenzonitrophone 4g.

Figure 5a.
FIGURE 6
ABSORPTION SPECTRUM
OF LIEBENMANN FRACTION 3

WAVELENGTH (MILLIMICRONS)

OPTICAL DENSITY

700 600 500 400 300
EXPERIMENTAL

Preparation of 2,5-bis(p-hydroxyanilino)-p-benzoquinone:

Twelve grams of quinone was dissolved in 200 ml. of hot 95% ethanol; eight g. of p-aminophenol was dissolved in 100 ml. of ethanol, and the latter solution was added to the former with stirring. The reactants are in the ratio of 3 moles of quinone to 2 moles p-aminophenol. The reaction mixture immediately turned dark brown; it was allowed to stand several days and filtered. Five grams of the dried black crystalline material was obtained. The filtrate was diluted to twice its volume with water, allowed to stand for an hour, and filtered; an additional 3.5 g. of the solid was obtained.

Determination of percent nitrogen in the above product:

One-gram samples of the black solid obtained above were digested for four hours with sulfuric acid (HgO catalyst) preparatory to a Kjeldahl nitrogen determination. The ordinary Kjeldahl procedure was followed, the ammonia being absorbed in 0.6 M boric acid.

The percent nitrogen found was 8.60% and 8.57%; the calculated value for percent nitrogen in the anilinoquinones \( \text{C}_{18} \text{H}_{14} \text{N}_2 \text{O}_4 \) is 8.69%.
Absorption spectrum of 2,5-bis(p-hydroxyanilino)-p-benzoquinone:

A 1.03 x 10^{-4} molar solution of 2,5-bis(p-hydroxyanilino)-p-benzoquinone was prepared by dissolving 0.00332 grams of the compound in ethanol to make 100 ml. of solution. The absorption spectrum was determined; the data are given in Table 5, below.

Table 5

<table>
<thead>
<tr>
<th>μm</th>
<th>O.D.</th>
<th>μm</th>
<th>O.D.</th>
<th>μm</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>220</td>
<td>2.0</td>
<td>340</td>
<td>.712</td>
<td>460</td>
<td>.795</td>
</tr>
<tr>
<td>240</td>
<td>1.90</td>
<td>360</td>
<td>.754</td>
<td>500</td>
<td>.665</td>
</tr>
<tr>
<td>250</td>
<td>1.75</td>
<td>380</td>
<td>.845</td>
<td>520</td>
<td>.597</td>
</tr>
<tr>
<td>250</td>
<td>1.68</td>
<td>370</td>
<td>.920</td>
<td>540</td>
<td>.512</td>
</tr>
<tr>
<td>260</td>
<td>1.54</td>
<td>380</td>
<td>.995</td>
<td>560</td>
<td>.418</td>
</tr>
<tr>
<td>270</td>
<td>1.36</td>
<td>390</td>
<td>1.07</td>
<td>580</td>
<td>.322</td>
</tr>
<tr>
<td>280</td>
<td>1.18</td>
<td>400</td>
<td>1.12</td>
<td>600</td>
<td>.236</td>
</tr>
<tr>
<td>290</td>
<td>1.13</td>
<td>410</td>
<td>1.13</td>
<td>625</td>
<td>.154</td>
</tr>
<tr>
<td>300</td>
<td>1.06</td>
<td>420</td>
<td>1.13</td>
<td>650</td>
<td>.104</td>
</tr>
<tr>
<td>310</td>
<td>.895</td>
<td>430</td>
<td>1.11</td>
<td>675</td>
<td>.072</td>
</tr>
<tr>
<td>320</td>
<td>.755</td>
<td>440</td>
<td>1.07</td>
<td>700</td>
<td>.055</td>
</tr>
<tr>
<td>330</td>
<td>.685</td>
<td>460</td>
<td>.925</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D.S. Pratt and H.D. Gibbs have published the absorption spectrum of 2,5-dianilinoquinone. When their data are adjusted so that a plot of wave-length against optical density is obtained (as in all absorption curves given here), then there is good agreement with the curve of the p-hydroxyanilino compound (Fig. 5). The curves can be expected to be similar but not identical, since Pratt's and Gibbs' compound does not have the p-hydroxy groups present in the compound under investigation.
Spectrum of the Liebermann Fraction 3:

A small portion of the most insoluble fraction of the Liebermann solid was shaken with 95% ethanol and allowed to remain in contact with the solvent overnight. The solution was then filtered and diluted, and the absorption spectrum determined. Data are given in Table 6. The curve plotted from these data (Fig. 6) can be seen to be somewhat similar to the curve of the p-hydroxyanilino quinone (See Fig. 5).

Table 6

<table>
<thead>
<tr>
<th>μm</th>
<th>O.D.</th>
<th>μm</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>220</td>
<td>inf.</td>
<td>250</td>
<td>270</td>
</tr>
<tr>
<td>230</td>
<td>270</td>
<td>290</td>
<td>300</td>
</tr>
<tr>
<td>250</td>
<td>370</td>
<td>280</td>
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</tr>
<tr>
<td>270</td>
<td>430</td>
<td>380</td>
<td>390</td>
</tr>
<tr>
<td>290</td>
<td>520</td>
<td>420</td>
<td>530</td>
</tr>
<tr>
<td>300</td>
<td>600</td>
<td>540</td>
<td>600</td>
</tr>
<tr>
<td>310</td>
<td>700</td>
<td>560</td>
<td>700</td>
</tr>
<tr>
<td>320</td>
<td>800</td>
<td>580</td>
<td>800</td>
</tr>
<tr>
<td>330</td>
<td>900</td>
<td>600</td>
<td>900</td>
</tr>
<tr>
<td>340</td>
<td>1000</td>
<td>620</td>
<td>1000</td>
</tr>
</tbody>
</table>

Chromatographic behavior of C_{18}H_{14}N_{2}O_{4} and Fraction 3 of the Liebermann solid:

The 2,5-substituted quinone is quite insoluble in the ordinary organic solvents; it is soluble, however, in aniline, and to some extent in acetic acid and in ethanol. Small portions of the product were dissolved in (a) aniline, (b) 50% aniline in chloroform, (c) 50% acetic acid in chloroform, and each solution was passed through a column made up of two parts by weight of silica to one part of Celite filter aid. In no case was there evidence of
adsorption.

Another small portion of the anilinoquinone was dissolved in dilute sodium hydroxide and the solution passed through a column of silica, which was found to be sufficiently acid to acidify the solution. A brown band appeared at the top of the column, probably precipitated on the column itself as a result of the change in acidity of the solution.

A small portion of the Liebermann solid was dissolved in dilute sodium hydroxide and passed through a silica column. The column was developed with water. A deep blue eluate was obtained. A brown zone remained at the top of the column; this was eluted and the absorption spectrum of the resulting solution determined; however, there was no similarity to the spectrum of the 2,5-bis(p-hydroxyanilino)-p-benzoquinone.
SUMMARY AND SUGGESTIONS FOR FURTHER INVESTIGATION

Since phenolindophenol is soluble in many common organic solvents, one of which is chloroform, it is apparent that any of the compound which was present in the Liebermann reaction product would have been extracted from the mixture by chloroform. Therefore a chloroform extract of the Liebermann solid was prepared and compared with a known chloroform solution of phenolindophenol.

Red substances which appeared to be chromatographically identical on silica columns were found to be the main constituents of both solutions. Spectrophotometric studies showed that the phenolindophenol was unchanged by adsorption on the silica column followed by elution with ethanol. However, the red material from the Liebermann reaction product, upon elution from the column and subsequent determination of the absorption spectrum of the resulting solution, led to an absorption curve which in no way resembled that of phenolindophenol. Thus it is apparent that the two red substances are not identical, and that phenolindophenol is not among the substances isolated from the product of the Liebermann Nitroso reaction.

 Phenolindophenol may be formed as an intermediate in the course of the reaction, for it has been shown that the compound decomposes into p-benzoquinone and 2,5-bis(p-hyd-
roxyanilino)-p-benzoquinone. The presence of p-benzoquinone in the filtrate of the reaction was established by the semi-carbazone derivative. The presence of 2,5-bis(p-hydroxyanilino)-p-benzoquinone was indicated by a similarity of the absorption spectrum of the pure compound to the spectrum of a part of the Liebermann product. However the pure substituted quinone was not isolated from the Liebermann solid.

Although sufficient time was not available during this investigation, the N-oxide of phenolindophenol should be investigated for similarity to the fractions of the Liebermann product. Meyer and Elbers have prepared this compound by treating phenol in acetic and sulfuric acids with pure nitric acid; the resulting compound was red-brown, soluble in alkali with a blue color and in strong mineral acids with a red-violet color. Meyer and Elbers found that the indophenol oxide is more stable to acid than is phenolindophenol. They were able to reduce the oxide to the indophenol and to the corresponding dihydroxydiphenylamine, but could not oxidize the indophenol to its oxide. It is entirely possible that the conditions of the Liebermann reaction are such that this oxide of phenolindophenol is produced.

Since quinone has been found in the Liebermann mixture, which also contains unreacted phenol, it is conceivable that the addition product of phenol and quinone, or phenoquinone, might be present among the products of the reaction. It is this material which imparts a red color to solid phenol
which has been exposed to light and air. Pratt and Gibbs
have investigated this compound and published its absorption
spectrum which might be used for reference.26

Not directly related to the original object of this in-
vestigation, but worthy of further study, is the gradual
change in the absorption spectrum of \( p,p' \)-dihydroxydiphenyl-
amine as it is oxidized to phenolindophenol (see page 40).
It will be observed that the diphenylamine has an absorption
peak at about 232 millimicrons. As the oxidation proceeds,
the optical density can be seen to decrease and the absorp-
tion peak to shift toward the shorter wavelength. In curve
6 of Figure 4, however, the optical density shows a marked
increase over that of the less-oxidized material and the peak
approaches more closely the high peak of phenolindophenol at
260 millimicrons. The reason for this shift is not apparent.

With reference to this same set of curves (Fig. 4) it
will be noted that all the curves of this redox system pass
through or near a common point at 275 millimicrons where all
optical densities are very nearly 1.28. This point might be
closely related to the "isobestic" point commonly observed
in the spectra of an acid-base indicator at varying pH.

Both phenolindophenol and the chloroform-soluble red
material from the Liebermann solid might well be investigated
as to their properties as acid-base indicators. Both com-
 pounds in dilute solution change color over a quite narrow
pH range at about pH 7.5. Phenolindophenol changes abruptly
from red to blue, and the other material from a pale yellow-brown to blue-green.
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