

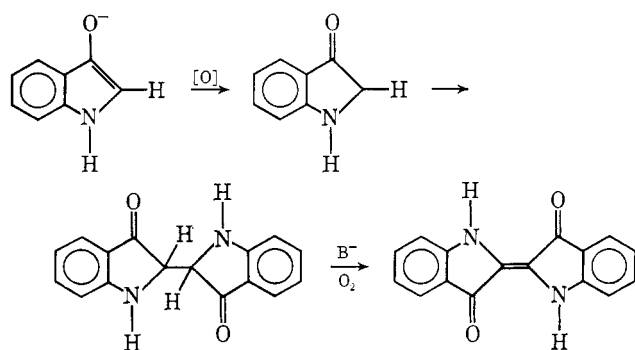
Oxidation of Carbanions. IV. Oxidation of Indoxyl to Indigo in Basic Solution¹

Glen A. Russell and Gerd Kaupp

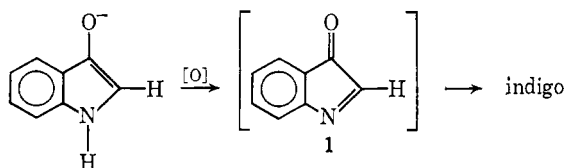
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Abstract: By a combination of kinetic, stoichiometric, and spectrometric measurements it has been demonstrated that the reaction of indoxyl with oxygen in basic solution involves leucoindigo as an intermediate. A precursor to leucoindigo that is diverted to nonindigo products by either excess oxygen or base is involved. This precursor is most likely the indoxyl radical. Leucoindigo is most likely formed by the coupling of indoxyl radicals. However, a coupling reaction between indoxyl radicals and the indoxyl anion has not been excluded. Organic one-electron acceptors, such as nitrobenzene, duroquinone, or 2-chloro-2-nitropropane, can be used to bring about the oxidative dimerization of indoxyls that are resistant to oxygen (*e.g.*, thioindoxyl). The use of these reagents is particularly beneficial in instances where the indigo itself is readily oxidized by air or peroxide, *e.g.*, N,N'-dimethyl-indigo. The reduction of indigo to indigo radical anion and thus to leucoindigo can be readily demonstrated by esr spectroscopy. Similar reductions of indirubin and thioindigo are readily observed. The reductions of thioindigo or indigo by hydrazobenzene in methanolic base are particularly noteworthy.

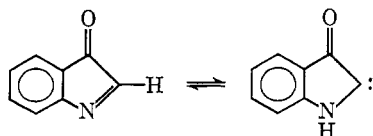
The conversion of indoxyl to indigo by the action of oxygen in basic solution is an interesting problem for mechanistic speculation. Conversion of indoxyl anion to the indoxyl radical followed by coupling to leucoindigo is an eminently reasonable mechanism.



The literature contains the speculation that indigo can be formed by the coupling of indolone (1).^{2,3}



However, the decarboxylation of 2-carboxyindolone yields not indigo but instead indoxyl and dehydroindigo.⁴ Nevertheless, it is difficult to dismiss the indolone intermediate completely since a number of indigo-forming reactions can be reasonably formulated as proceeding *via* the indolone or its tautomeric carbene.



(1) Reactions of Resonance Stabilized Carbanions. XXXI. This work was supported by a grant from the National Science Foundation.

(2) W. Madelung and P. Siegert, *Chem. Ber.*, **57**, 228 (1924).

(3) O. Neunhoeffer, "Handbuch der Katalyse VII," Springer-Verlag, Vienna, 1943, p 795.

(4) O. Neunhoeffer and G. Lehmann, *Chem. Ber.*, **94**, 2965 (1961).

Among these reactions are the formation of indigo by the reaction of *o*-nitrobenzaldehyde with acetone in basic solution,⁵ by the reduction of *o*-nitrobenzoyl-acetic acid by base and glucose,⁶ by the basic dithionite reduction of 1-*o*-nitrophenyl-2-nitroethanol,⁷ by the acidic hydrolysis of the dimethyl acetal of *o*-amino-phenylglyoxal,⁸ by the treatment of 2-anilinoindoxyl with base or acid,⁹ by the treatment of 2-oximinindoxyl with sulfuric acid,¹⁰ or by the action of various reducing agents on 2-chloroindolone.¹¹ Since in many cases the yield of indigo is improved if indoxyl is also present (for example, in the reduction of 2-chloroindolone¹²) it may be that indoxyl is itself an intermediate in some of these processes. Furthermore, it should be recognized that the intermediate(s) in the indigo synthesis in acidic solution may not necessarily be the same as in basic medium.

A free radical oxidation sequence has been proposed in which a peroxy radical is the precursor to leucoindigo (Scheme I).¹³

Other schemes can be imagined. In view of other results from this laboratory¹⁴ the interaction of the indoxyl radical with the indoxyl anion deserves consideration. Other coupling reactions can be written in which the indolone reacts with the anion or the radical of indoxyl.

Formation of indigo by a base-catalyzed condensation between isatin (an indoxyl oxidation product) and indoxyl can be eliminated completely from consideration. This reaction leads not to indigo but to the isomeric indirubin systems.¹²

In the present work we have tried to devise experimental tests to prove or disprove some of the possible mechanisms.

(5) A. von Baeyer and V. Drewsen, *ibid.*, **15**, 2856 (1882); I. Tanasescu and A. Georgescu, *Bull. Soc. Chim. Fr.*, [4], **51**, 234 (1932).

(6) A. von Bayer, *Chem. Ber.*, **13**, 2254 (1880); **14**, 1741 (1881).

(7) J. Harley-Mason, *J. Chem. Soc.*, 2907 (1950).

(8) E. Ziegler and T. Kappe, *Angew. Chem.*, **76**, 921 (1964).

(9) R. Pummerer and M. Göttinger, *Chem. Ber.*, **43**, 1376 (1910).

(10) H. Wieland and E. Gmelin, *ibid.*, **41**, 3512 (1908).

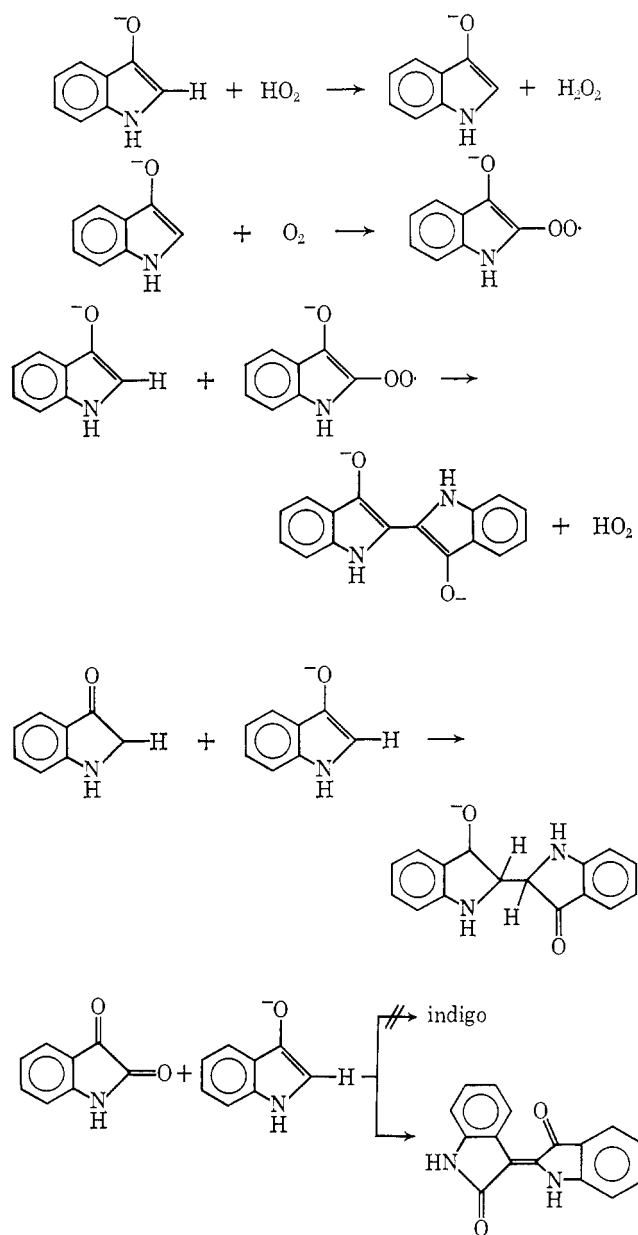
(11) A. von Bayer, *ibid.*, **11**, 1296 (1878); **12**, 456 (1879).

(12) A. Wahl and P. Bagard, *Bull. Soc. Chim. Fr.*, [4], **7**, 1090 (1910).

(13) S. Cotson and S. J. Holt, *Proc. Roy. Soc., Ser. B*, **148**, 506 (1958).

(14) G. A. Russell and W. C. Danen, *J. Amer. Chem. Soc.*, **90**, 347 (1968).

Scheme I



Results

Stoichiometry. The oxidation of indoxyl presents certain experimental complications. Indoxyl and leucoindigo are readily oxidized, even in the absence of base or solvent. To circumvent this problem we have generated indoxyl and leucoindigo by the *in situ* saponification of the O-acetates in basic solution. In solution indigo is readily oxidized by base and oxygen or by alkali metal peroxides. This is a particularly bothersome problem in the synthesis of easily soluble indigos, such as N,N-dimethylindigo. Indigo itself has a low solubility in methanol and as a crystalline precipitate is quite stable to oxygen and base in this medium. In aqueous solution indigo readily forms a colloidal suspension which makes isolation a problem and increases the ease of further oxidation. In the presence of strong bases, such as potassium *t*-butoxide in *t*-butyl alcohol, the oxidation of indoxyl does not even yield indigo, although crystalline indigo is stable under the reaction conditions.

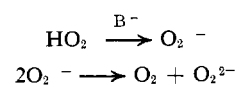
We have employed as standard oxidation conditions the reaction of the O-acetates of indoxyl or leucoindigo with sodium methoxide in methanol. The crystalline indigo formed is readily isolated and is inert to further oxidation. Indoxyl itself is not destroyed by the reaction medium in the absence of oxygen. The medium itself is not oxidized appreciably under the reaction conditions. Solutions of leucoindigo were formed by the presaponification of O,O'-diacetyl-leucoindigo in a nitrogen atmosphere (O,O'-diacetyl-leucoindigo dissolves slowly in methanol). O-Acetyl-indoxyl dissolves rapidly in methanol and it was found that the same yield of indigo is found if saponification is conducted in the presence of oxygen or if saponification is performed under a nitrogen atmosphere prior to the admission of oxygen. Table I demonstrates

Table I. Oxidation of Indoxyl or Leucoindigo in Basic Methanol Solution^a

Indoxyl, mmol	Sodium methoxide, mmol	Reaction volume	Reaction time, min	Oxygen absorbed, mmol, 15 min	Yield of indigo, %
2.04	8.35	15	15	1.7 ^b	81
2.45	4.95	7.5	30	1.7 ^b	85
2.53	5.10	8.7	1500	2.3 ^b	85
1.29	2.58	17.7	30	1.1	87
1.30	11.7	17.7	30	1.2	76
1.28 ^c	2.58	17.7	30	1.2	89
2.48	4.95	8.7	1500	<i>d</i>	83
1.15 ^e	23.4	35.5	15		98
1.16 ^e	23.4	35.5	15	<i>f</i>	100
1.17 ^e	23.4	35.5	15	<i>f,g</i>	100
0.46 ^e	4.17	5	1500	0.6 ^{b,d}	95
0.45 ^e	4.75	5	1500	0.5 ^{b,d}	98

^a At ca. 25°. An oxygen saturation pressure of 745 mm was employed in a rapidly agitated reaction flask. ^b Not corrected for volume changes due to partial pressure of solvent vapors. ^c In presence of 9.7 mmol of nitrobenzene. ^d Oxygen allowed to slowly diffuse into a reaction flask filled with nitrogen. During most of the oxidation the oxygen pressure approached zero. Oxygen uptake after 1500 min. ^e Leucoindigo. ^f Oxygen pressure 3300 mm. The saponified solutions of leucoindigo were sealed in thin-walled bulbs and broken in a Parr "hydrogenation" flask containing oxygen and solvent. ^g In the presence of 2.36 mmol of sodium peroxide generated by the oxidation of hydrazobenzene.

that the conversion of indoxyl to indigo requires 2 mol of oxygen/mol of indigo formed. The conversion of leucoindigo to indigo itself requires 1 mol of oxygen. Thus, if leucoindigo is an intermediate in the conversion of indoxyl to indigo, the conversion of indoxyl to leucoindigo requires 0.5 mol of oxygen/mol of indoxyl. However, the results do not require that the peroxide anion (O₂²⁻) or hydrogen peroxide are the initial products. The superoxide ion (O₂⁻) or hydroperoxy radical (HOO·) may be first formed. In methanol solution they would give rise to the peroxide anion. The indicated stoichiometry is in agreement



with that measured previously by Cotson and Holt.¹³ These workers were able to isolate 1.75 mol of hydrogen peroxide/mol of indigo formed.

The stability of indoxyl, leucoindigo, and indigo to base under the reaction conditions is indicated by the

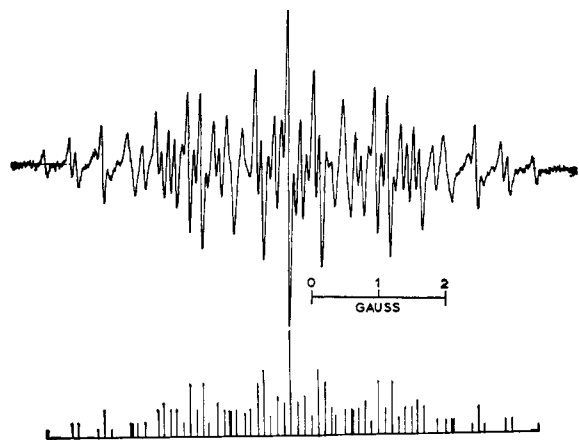
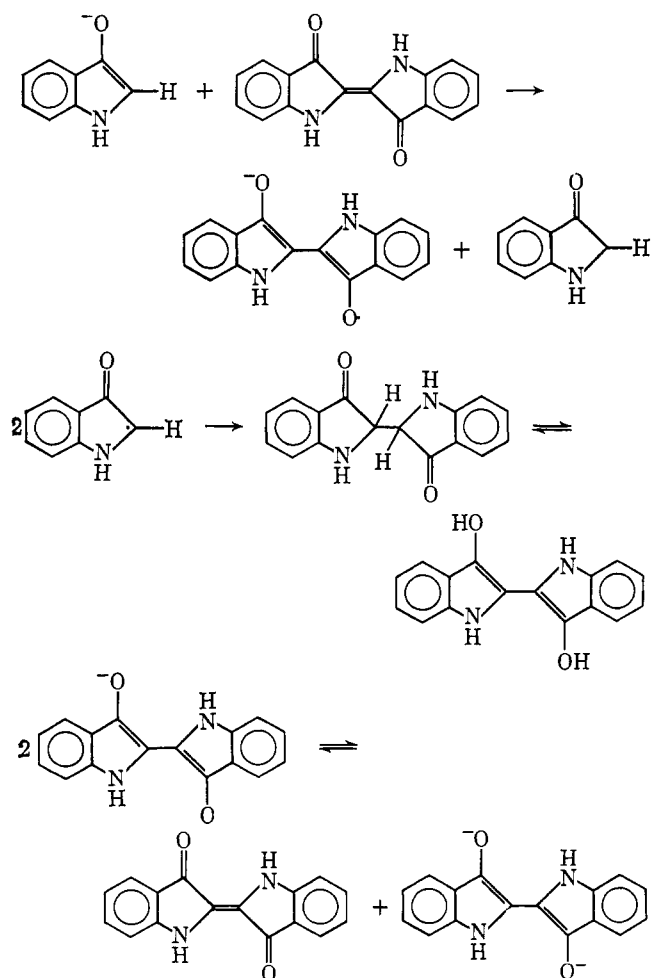


Figure 1. Thioindigo radical anion formed from the reaction of thioindigo with thioindoxyl acetate in methanol containing 1.4 *M* sodium methoxide.

experiments summarized in Table I in which oxygen was allowed to slowly diffuse into the reaction vessel. The yield of indigo was not decreased even though the indoxyl or leucoindigo was exposed to the basic

Scheme II



solution for a number of hours. Qualitatively, the absorption of oxygen was extremely fast (a few seconds) for the leucoindigo oxidations and was possibly limited by the rate of oxygen diffusion into the solution. Since the rate of oxidation of indoxyl appeared to proceed at a more moderate rate, it is obvious that the detection

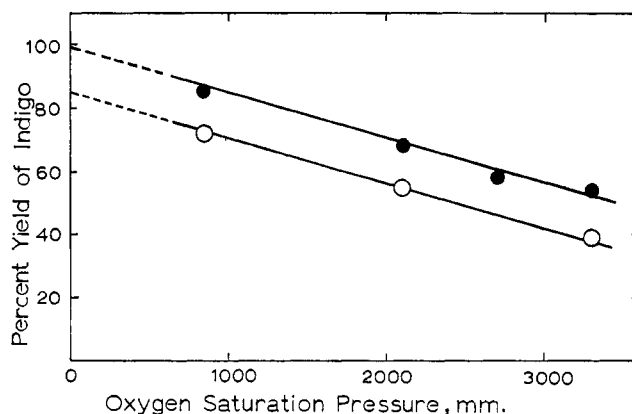


Figure 2. Final yield of indigo in the autoxidation of indoxyl (0.066 *M*) in a methanol solution at 25–26°: ●, 0.066 *M* sodium methoxide; ○, 0.132 *M* sodium methoxide.

of leucoindigo as an intermediate in the conversion of indoxyl to indigo is a difficult task.

Demonstration of the Intermediacy of Leucoindigo.

It has been claimed that incomplete oxidation of indoxyl yields leucoindigo.¹⁵ However, this does not constitute proof that the leuco compound is an intermediate since in basic solution indoxyl will reduce indigo readily.¹⁵ By esr spectroscopy the indigo radical anion is readily detected and upon work-up the leucoindigo will result (Scheme II). The same oxidation–reduction sequence is observed for thioindoxyl and thioindigo and in Figure 1 is given the esr spectrum of thioindigo radical anion observed from this combination in basic methanol solution (the esr of indigo radical anion has been previously published¹⁶).

The oxidation–reduction reactions of Scheme II hold a key for demonstrating the intermediacy of leucoindigo in the oxidation. Experimentally, treatment of indoxyl in basic methanol with a trace of oxygen did not yield significant amounts of the indigo radical anion as detected by esr spectroscopy. Blank experiments demonstrated that the addition of small amounts of indigo (approximately equivalent to the oxygen absorbed) to a solution of indoxyl in basic methanol produced a strong esr signal for the indigo radical anion. Similar results were observed for the oxidation of thioindoxyl.

These observations exclude the formation of indigo by the coupling of indolone since this should lead rapidly to indigo radical anion. The results also exclude the formation of indigo *via* the coupling of indolone and indoxyl radical since this mechanism would also lead directly to the indigo radical anion.

A reaction leading to leucoindigo is strongly supported. Additional oxygen does lead to the indigo or thioindigo radical anions detectable by esr spectroscopy, presumably as a result of oxidation of the first formed leucoindigo.

Evidence for a Precursor to Leucoindigo. The yield of indigo was studied as a function of oxygen pressure and concentration of base and the concentration of

(15) Badische Anilin- und Soda-Fabrik, German Patent 164,509 (1905); *Chem. Zentr.*, 76, 1753 (1905). In the abstract of this patent it is quite correctly stated that indigo is capable of serving as the oxidizing agent for the conversion of unreacted indoxyl to leucoindigo.

(16) G. A. Russell, R. Konaka, E. T. Strom, W. C. Danen, G. Kaupp, and K.-Y. Chang, *J. Amer. Chem. Soc.*, 90, 4646 (1968); see also F. W. Heineken, M. Bruin, and F. Bruin, *J. Chem. Phys.*, 37, 452 (1962).

Table II. Reaction of Indoxyl Anions with One-Electron Acceptors in Methanol Solution, 25–27°

Substrate, mmol	Acceptor, mmol	Sodium methoxide, mmol	Volume, ml	Reaction time	Yield of indigo, %
Indoxyl (2.28)	Nitrobenzene (9.7)	2.28	12	12 hr	89
Indoxyl (1.4)	Nitrobenzene (9.7)	11.7	10	7 hr	85
Indoxyl (1.4)	Azobenzene (5.4)	11.7	10	43 hr	95
Indoxyl (1.4)	Duroquinone (2.8)	11.7	10	23 hr	92
Indoxyl (2.0)	2-Chloro-2-nitropropane (4.0)	2.0	21	1 hr	88
Indoxyl (1.5)	2-Chloro-2-nitropropane (1 ml)	7	15	5 min	100
N-Methylindoxyl (2.1)	Nitrobenzene (9.7)	2.1	10	200 hr	<i>a,b</i>
N-Methylindoxyl (1.05)	Duroquinone (2.62)	8.8	9	15 min	84 ^b
N-Methylindoxyl (2.0), indoxyl (2.1)	Nitrobenzene (9.7)	4.1	12	24 hr	55 ^c
Thioindoxyl (5.7)	Potassium ferricyanide (24)	25 (NaOH)	3 (CH ₃ OH)- 30 (H ₂ O)	1 hr	77 ^d
Thioindoxyl (1.5)	Nitrobenzene (16)	3.2 (KOC(CH ₃) ₃)	10 (<i>t</i> -BuOH)	2 hr	<i>d</i>

^a Only after exposure to oxygen. ^b N,N-Dimethylindigo. ^c No N,N-dimethylindigo detected; monomethylindigo was detected. ^d Thioindigo.

indoxyl. The yield decreased with an increase in the oxygen pressure and in the concentration of base. On the other hand the yield of indigo increased as the concentration of indoxyl was increased. The simplest interpretation of these results is that the anion of

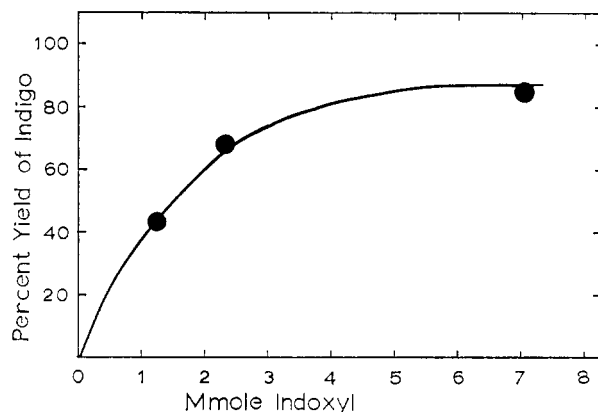
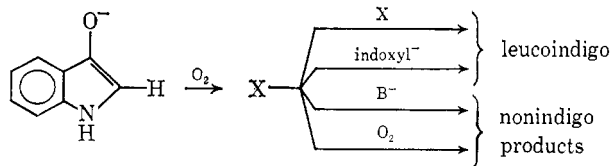


Figure 3. Final yield of indigo in the autoxidation of indoxyl in methanol solution (35.5 ml) at 25–26°, oxygen saturation pressure 2100 min. The concentration of sodium methoxide employed was twice that of indoxyl acetate.

indoxyl is converted to an intermediate X that either dimerizes, or reacts with indoxyl, to yield leucoindigo. The reactions leading to leucoindigo compete with the destruction of X by oxygen or by excess base. In



t-butyl alcohol containing potassium *t*-butoxide indoxyl is rapidly oxidized but indigo is not formed. Here, the high basicity of the *t*-butoxide ion apparently destroys X quantitatively under conditions where preformed indigo is stable.

Figure 2 demonstrates the effect of the oxygen saturation pressure and methoxide ion concentration

on the oxidation in methanol solution. Figure 3 demonstrates the effect of indoxyl anion concentration at a standard oxygen pressure. The major product of the oxygen trapping reaction was apparently isatin or anthranilic acid derived from the isatin. Indirubin formation was not a competing process under the standard conditions employed in this work.

We have previously mentioned that the indoxyl anion is not destroyed by the action of methoxide ion in the absence of air. It is also possible to demonstrate that unhydrolyzed indoxyl acetate is not a reactant under the reaction conditions. When indoxyl acetate was saponified with 0.5 equiv of base, a rapid absorption of 0.68 equiv of oxygen occurred in 15 min. At this time the addition of another 0.5 equiv of base resulted in the absorption of 0.35 equiv of oxygen with a total yield of 60% of indigo.

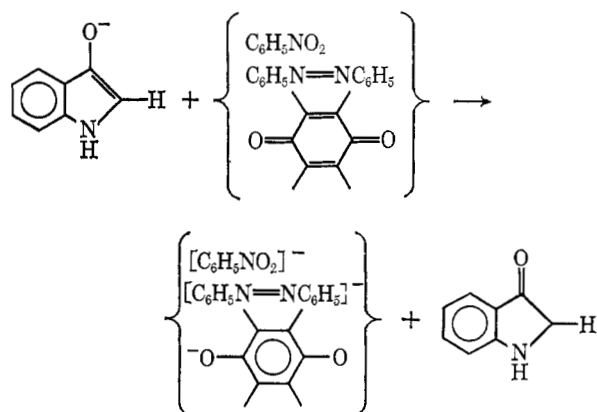
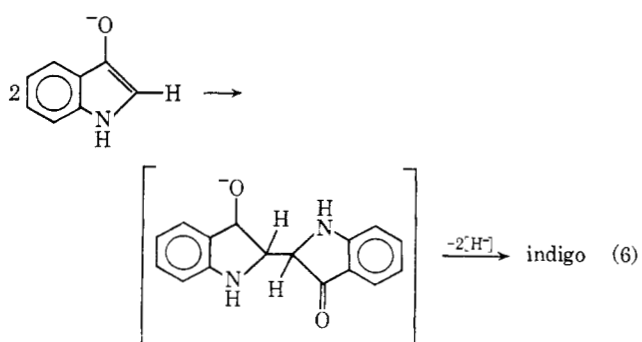
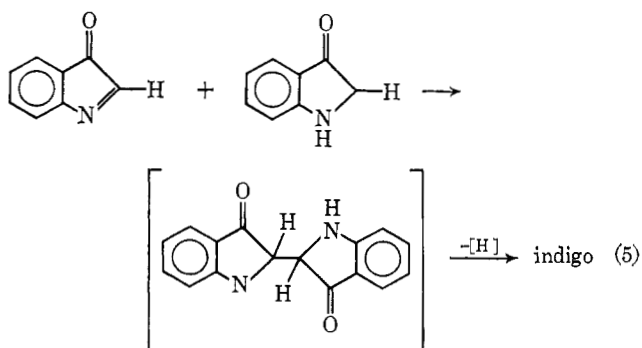
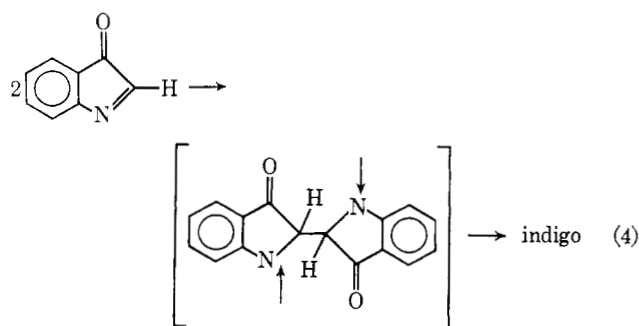
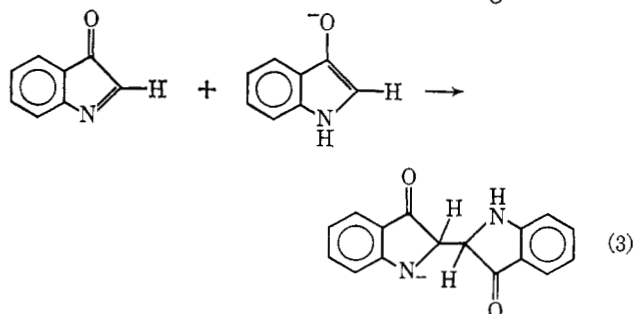
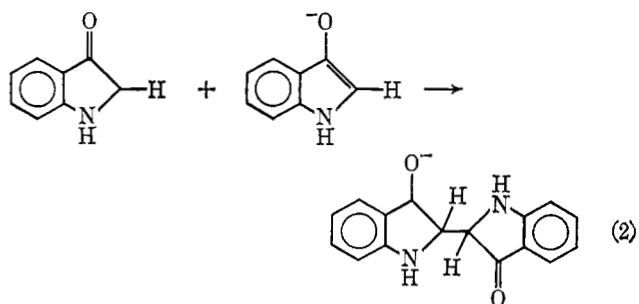
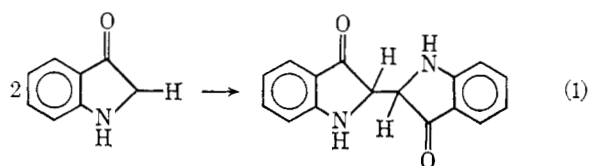
Among the likely candidates for intermediate X are the indoxyl radical or the indolone. Reactions 1–3 could be involved in the formation of leucoindigo. Reactions 4 and 5 would not lead to leucoindigo as an intermediate and are dismissed from further consideration as is reaction, 6 which does not involve a precursor to leucoindigo consistent with the experimental observation.

Oxidation of Indoxyl by One-Electron Transfer Reagents. If the oxidation of indoxyl in basic solution involves the indoxyl radical, the indigo synthesis should be operable with various organic one-electron acceptor molecules¹⁷ as well as with the commercial inorganic oxidants such as potassium ferricyanide. Table II lists some results obtained by use of the electron acceptors nitrobenzene, azobenzene, and duroquinone. In each case the radical anion of the acceptor molecule could be detected under the reaction conditions except for the azobenzene radical ion which readily undergoes disproportionation in methanol solution.¹⁶

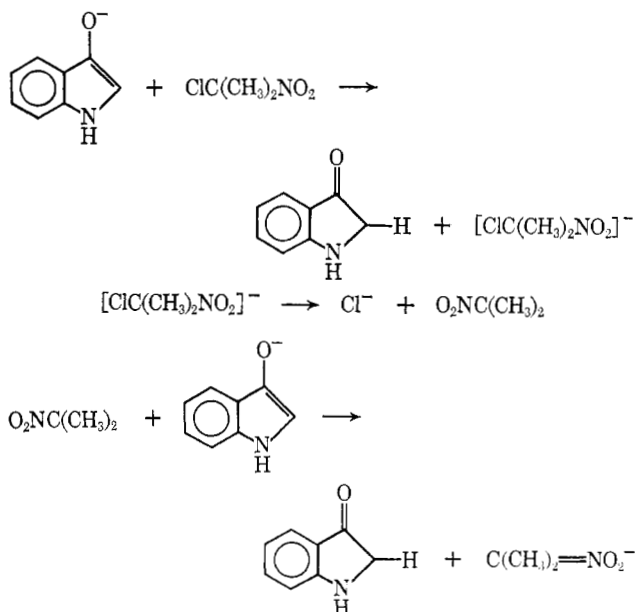
2-Chloro-2-nitropropane proved to be an excellent oxidation agent for indoxyl. The reaction may well involve the processes of Scheme III plus similar reactions involving leucoindigo.

Evidence for 2-chloro-2-nitropropane functioning as an electron acceptor and for the instability of the 2-

(17) G. A. Russell, E. G. Janzen, and E. T. Strom, *J. Amer. Chem. Soc.*, **86**, 1807 (1964).



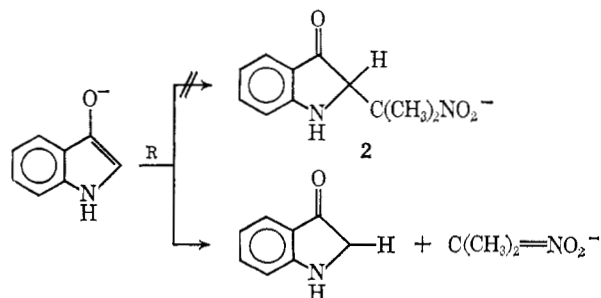
Scheme III



chloro-2-nitropropane radical anion has been presented previously.¹⁴ The reaction discussed herein is similar to that observed between 1,1,1-trinitroethane and the anion of 2-nitropropane or *n*-butyl mercaptan.^{14,18}



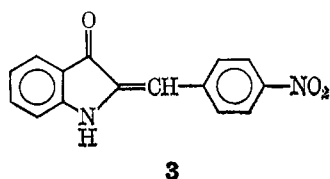
Our results suggest that the 2-nitro-2-propyl radical ($\text{R}\cdot$) reacts with the indoxyl anion by electron transfer and not by addition. Alternately 2 may be formed readily but reversibly.



Since the *p*-nitrobenzyl radical readily adds to carbanions, such as the anion of 2-nitropropane,¹⁴ we have examined the reactions of the indoxyl anion with

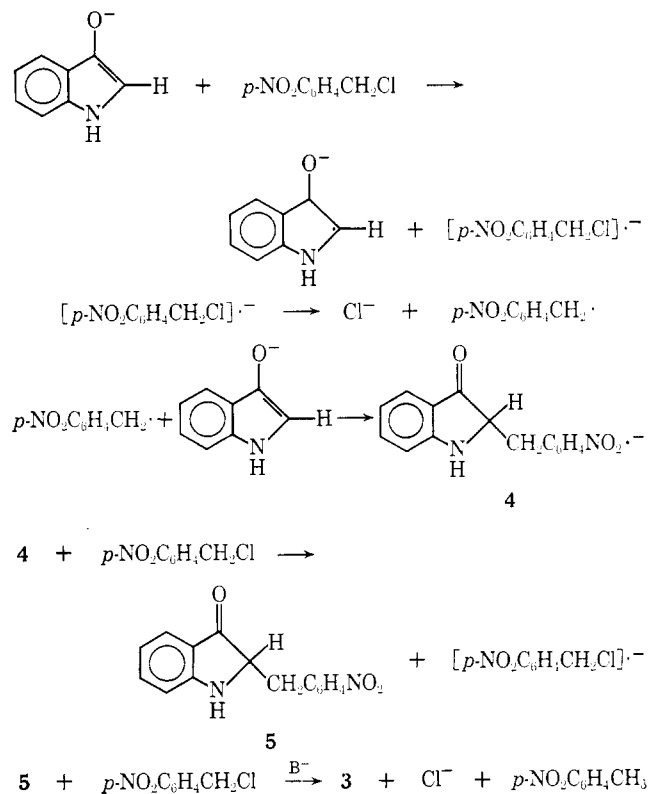
(18) L. Zeldin and H. Shechter, *J. Amer. Chem. Soc.*, **79**, 4708 (1957).

100% excess of *p*-nitrobenzyl chloride. This reaction gave no indigo but instead compound **3** was isolated. This product is consistent with reaction Scheme IV. However, other schemes can be imagined, for example,

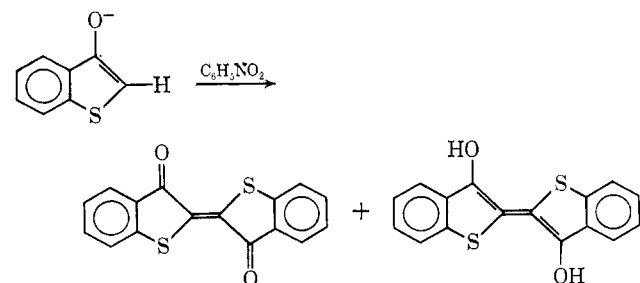


the reaction of indoxyl anion with *p*-nitrobenzyl chloride by an S_N2 displacement followed by oxidation-reduction reactions.

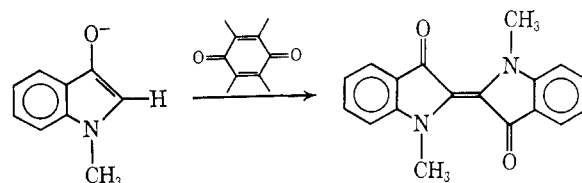
Scheme IV



We have been able to apply the use of one-electron acceptors to the synthetic formation of indigos. Thus, thioindoxyl (which is autoxidized very slowly in basic solution) is readily converted by nitrobenzene into a mixture of thioindigo and leucothioindigo. The leucothioindigo is easily oxidized by air and base to thioindigo.

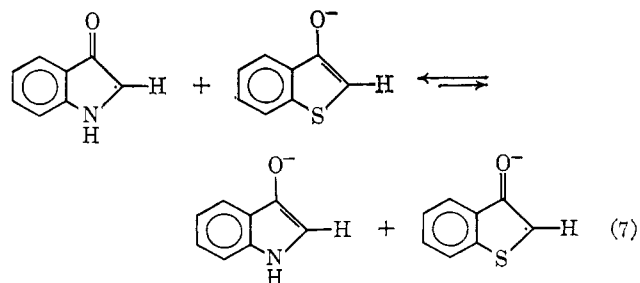


N,N-Dimethylindigo is readily formed in 84% yield by treatment of *N*-methylindoxyl with duroquinone in basic solution. The durosemiquinone anion can be detected by esr spectroscopy as a reaction product.



With nitrobenzene at 25° the reaction stops at the leuco stage from which a good yield of the indigo can be obtained by autoxidation of the basic solution (the rate of oxidation of the indigo is appreciable but the leucoindigo oxidizes much more rapidly). The autoxidation of *N*-methylindoxyl in basic methanol yields only a mixture of *N*-methylisatin and *N*-methylanthranilic acid. We believe that this reflects an increased efficiency in oxygen trapping of the *N*-methylindoxyl radical due to a decrease in the rate of the dimerization process.

Attempts to Trap Indoxyl Radicals by Anions. We believe that all of our results implicate the indoxyl radical as one of the precursors of indigo in the autoxidation process. Two possible routes to leucoindigo exist, reactions 1 and 2. In an attempt to differentiate these processes, we have attempted to trap the indoxyl radical by various anions. It has been shown that unsaturated anions can be excellent traps for radicals provided that the resulting radical anion is quite stable. The anion of 2-nitropropane has been shown to be an excellent trap for several radicals.¹⁴ However, the presence of the lithium salt of 2-nitropropane had but a slight effect on the indigo yield from indoxyl. Thus, the yield of indigo from the experiment summarized in entry 1 of Table I decreased from 81 ± 2% to 64% when the oxidation was conducted in the presence of 11.9 mmol of the lithium salt of 2-nitropropane. A possible conclusion is that the indoxyl anion is a much better trap for the indoxyl radical than is the anion of 2-nitropropane. The oxidation of indoxyl in the presence of an excess of the anion of thioindoxyl was an obvious experiment. Since the anion of thioindoxyl is quite stable to molecular oxygen, it appears that the equilibrium of reaction 7 will lie far to the left. The reaction should thus involve as monomeric species the indoxyl anion, the indoxyl radical, and the thioindoxyl anion. Reaction of 1.4 mmol of indoxyl with oxygen in methanol containing 6.9 mmol of free thioindoxyl and



10.7 mmol of sodium methoxide yielded (15 min) 0.6 mmol of dyestuffs (calculated for mol wt of indigo) with the absorption of 2.25 mmol of oxygen. Thioindigo could be detected as well as the mixed indigo in the reaction product (mass spectrometry). However, the major product was indigo. If the indoxyl radical had been trapped exclusively by the anion of thioindigo a total oxygen uptake of 2.8 mmol and a dyestuff yield

of ~ 1.4 mmol would have been expected. The thioindoxyl anion is thus not an efficient trap for indoxyl radical, at least when competing with the indoxyl anion at a five- to tenfold lower concentration.

Autoxidation of indoxyl (1.1 mmol) with N-methylindoxyl (5.6 mmol) in methanol containing sodium methoxide (13.6 mmol) failed to produce any of the indigos. N-Methylisatin was an important oxidation product (3.4 mmol). It appears that the major oxidation products were monomethylindigo and dimethylindigo and that under the reaction conditions these soluble indigos were oxidized further. The results can possibly be explained by the concurrent formation of both the indoxyl and N-methylindoxyl radicals. On the other hand, the complete absence of indigo seems more reasonably explained as arising from a more effective trapping of indoxyl radicals by N-methylindoxyl anion than by the indoxyl anion.

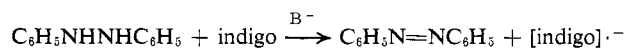
Electron Spin Resonance Experiments. Indigo, thioindigo, N,N'-dimethylindigo, or indirubin in pure methanol containing sodium methoxide does not give an esr signal except after very long periods of time. Use of solvent that has been exposed to oxygen for some time leads to formation of the radical anions. A variety of reducing agents will form the radical anions. The strongest signals were observed *via* use of hydrazobenzene. Table III lists some other experiments in

Table III. Electron Spin Resonance Examination of Various Reaction Mixtures in Methanol Containing 1.4 M Sodium Methoxide at 25–27°

Reducing agent ^a	Oxidizing agent	Signal obsd, comments
Indoxyl	Indigo	Indigo \cdot^- , immediate, strong
Thioindoxyl	Indigo	Indigo \cdot^- , immediate, strong
Thioindoxyl	Thioindigo	Thioindigo \cdot^- , immediate, strong
Indoxyl	Thioindigo	Indigo \cdot^- , immediate, strong
Indoxyl	Indirubin	Mixture of radical anions, indigo \cdot^- plus indirubin \cdot^-
Thioindoxyl	Indirubin	Mixture of radical anions, thioindigo \cdot^- plus indirubin \cdot^-
Leucoindigo	Trace of O ₂	Indigo \cdot^- , immediate, strong
N-Methylindoxyl	Nitrobenzene	Nitrobenzene \cdot^-
N-Methylindoxyl	Duroquinone	Duroquinone \cdot^-
Hydrazobenzene	Indigo	Indigo \cdot^- , very strong
Hydrazobenzene	Thioindigo	Thioindigo \cdot^- , very strong
Hydrazobenzene	Indirubin	Indirubin \cdot^- , very strong
Hydrazobenzene	Dehydroindigo	Indigo \cdot^-

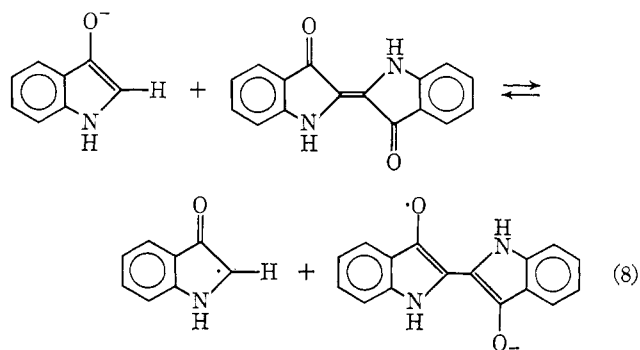
^a Indoxyl or leucoindigo generated by saponification of O-acetates in esr cell.

which indigo was reduced to the radical anion, particularly by the indoxyl anion.



The results of Table III must be considered in regard to the over-all description of indigo synthesis. Since the indigoid dyes are readily reduced by the indoxyls, it is necessary to consider reaction such as (8) after oxidation has produced significant amounts of the dyestuff. Since leucoindigo is very rapidly oxidized to indigo, reaction 8 will be important whenever the amount of oxidation has been significant.

We have performed many experiments aimed at direct experimental observation of the indoxyl radical. Unfortunately the best techniques for the generation of α -keto radicals¹⁹ (ceric ion oxidation, acidified aqueous

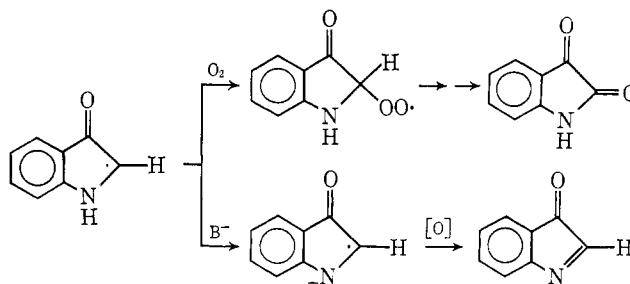


solution) could not be applied because of solubility problems and the basicity of indoxyl.

Flow experiments were performed wherein indoxyl, N-methylindoxyl, or thioindoxyl in basic methanol solution were mixed with oxidizing agents in methanol. Using a trace of oxygen, iodine, or potassium ferricyanide, it was observed that no esr signal was seen at very fast flows. Stopped flow led to the production of the indigo radical anion (indigo plus one electron) after a few minutes for indoxyl or N-methylindoxyl or after a few hours from thioindoxyl. We thus conclude that if the indigo synthesis involves the indoxyl radical that the radical is scavenged readily and never achieves steady-state concentrations in the range of 10^{-6} M, a concentration readily detected by esr spectroscopy.

Discussion

The formation of indigo by the oxidation of indoxyl in basic solution certainly appears to involve the indoxyl radical as an intermediate. The effects of oxygen pressure, concentration of base, and concentration of indoxyl anion on the yield of indigo are quite consistent with the intermediacy of the radical. The effect of excess base in decreasing the yield of indigo can perhaps be rationalized by the formation of indolone which in the presence of base and oxygen may be converted to nonindigo products. The oxidation proceeds in the



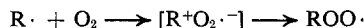
absence of excess base and monomeric dianion or radical anion intermediates can thus be eliminated.

A definite experimental distinction between the coupling of two indoxyl radicals (reaction 1) and the coupling of the indoxyl radical and indoxyl anion (reaction 2) has not been achieved. Conclusive evidence for reaction 2 might have been obtained if the radical could have been trapped by the 2-nitro-2-propyl anion or the thioindoxyl anion. However, such trapping occurred inefficiently, if at all. Perhaps the indoxyl anion is itself a superior trap for the indoxyl radical. Reaction 3 has not been rigorously excluded. However, the effect of oxygen concentration seems more

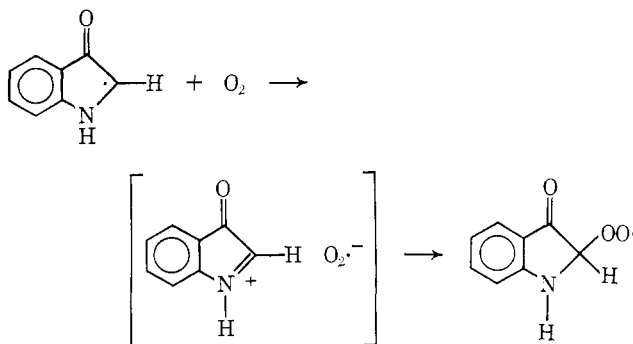
(19) G. A. Russell and J. P. Lokensgard, *J. Amer. Chem. Soc.*, **89**, 5059 (1967).

consistent with reactions 1 or 2 rather than 3. Reaction 3 is not possible in the oxidations of thioindoxyl or N-methylindoxyl, reactions that appear to be similar to the oxidation of indoxyl itself.

Reaction 1 appears to be the most reasonable explanation of the mechanism of indoxyl oxidation in basic solution. The coupling of radicals in the presence of oxygen is not a common process. In the present case, it may reflect a low reactivity of the indoxyl radical toward molecular oxygen. Polar effects can be very important in the reaction of radicals. We have previously suggested that alkyl and phenyl radicals differ greatly in their reactivity toward molecular oxygen.²⁰



Here, charge transfer in the transition state is much more favorable for R = alkyl than for R = C₆H₅. In a similar fashion it might be expected that the indoxyl radical would be less reactive than an ordinary alkyl radical toward molecular oxygen. Charge transfer in the transition state would yield an indolone-type structure that might be expected to possess the antiaromaticity of a cyclopentadienone.²¹



Experimental Section

General Oxidative Procedure. Commercial O-acetylindoxyl (Eastman Kodak Co.) was used without purification. The acetate was placed in the side arm of a creased flask attached to a wrist action shaker and to an oxygen buret.²² Solutions of base and solvent in the creased flask were saturated with oxygen at the desired pressure, the indoxyl acetate was added from the side arm, and the wrist action shaking started. In a modification of this procedure, samples of indoxyl acetate in the desired solvent were sealed under vacuum in a thin-wall bulb with a 8-mm rod as a handle. The rod was inserted through the stopper of a Parr hydrogenation flask filled with base and solvent. The solvent was saturated with oxygen and the bulb broken underneath the surface of the vigorously shaken solution. Indigo formed in the oxidation was isolated by filtration after neutralization. The purity of the indigo was checked by tlc. Analytical tlc was performed on methylene chloride solutions using silica coated plates. Using a mixture of benzene (2 parts by volume)-acetonitrile (1 part by volume) gave a round spot for indigo and provided good separation from indirubin. Extraction of indigo with hot pyridine gave a violet solution if the material contained >0.001% indirubin. However, tlc of the methanolic mother liquors from the oxidation proved to be an even more sensitive test for indirubin.

The oxidation of N-methylindoxyl, thioindoxyl, and leucoindigo were conducted in a similar fashion as were tests for the stability of indigo. Peroxide ion (O₂²⁻) was generated *in situ* by the oxidation of hydrazobenzene in methanol solution.²³ ESR spectra were

recorded in quartz flat cells using an E-3 Varian Associates spectrometer as described previously.^{16,19}

N-Methylindoxyl acetate was prepared by the reaction of N-methylanthranilic acid and chloroacetic acid.²⁴ A yield of 33% of colorless crystals, mp 57° (lit.²⁴ mp 57°), was obtained.

Thioindoxyl acetate was prepared from *o*-methylmercaptobenzoic acid and chloroacetic acid.²⁵ The material was distilled before use at 165° (18 mm) since the material slowly turned red on storage.

Thioindoxyl was prepared from 246 mg (1.4 mmol) of thioindoxyl acetate in 20 ml of 2 *N* sodium hydroxide under a nitrogen atmosphere.²⁵ The solution was refluxed 10 min and then neutralized with concentrated hydrochloric acid. Thioindoxyl crystallized as colorless needles, mp 60°. The pmr spectrum in carbon tetrachloride showed no impurities and no enol content. Thioindoxyl could be weighed in air without colorization. However, upon storage for several weeks under nitrogen, a violet color developed.

O,O'-Diacetylleucoindigo. Indigo was reduced with sodium dithionite and acetylated with acetic anhydride according to the procedure of Vorlaender and Drescher²⁶ to yield 39% of colorless crystals (from acetonitrile) which develop a violet color at 205°, mp 214–216° dec; ir (KBr) 3401 (NH), 1740 cm⁻¹ (C=O); mass spectrum (70 eV) showed a parent peak at *m/e* 348. Other abundant peaks over *m/e* 200 occurred at 307, 291, 264, 247, 235, 218, and 205. Metastable peaks were observed at *m/e* 270 (348 → 307), 228 (307 → 264), 210 (291 → 247 or 264 → 235), 193 (247 → 218), and 181 (235 → 205).

Thioindigo was prepared according to the procedure of Friedlaender by the ferricyanide oxidation of thioindoxyl in 79% yield, mp >300°.²⁵ No impurities were detected by tlc or mass spectrometry. The parent peak, *m/e* 296, was observed.

N,N'-Dimethylindigo was prepared according to the procedure of Ettinger and Friedlaender²⁴ by oxidation of N-methylindoxyl in aqueous ammonia solutions. Recrystallization from heptane under nitrogen yielded 28% of needles, mp ≈ 180°. No impurities were found by tlc or mass spectrum (parent peak *m/e* 290). Alternately N,N'-dimethylindigo was prepared by reaction of 1.05 mmol of N-methylindoxyl acetate in 9 ml of methanol containing 8.8 mmol of sodium methoxide with 2.2 mmol of duroquinone under nitrogen. After 15 min the mixture was acidified with hydrochloric acid and chromatographed on silica gel with chloroform to yield 0.44 mmol (84%) of pure N,N'-dimethylindigo.

Indirubin was prepared by a process similar to that employed by von Bayer by the condensation of indoxyl and isatin.⁶ A solution of 2.2 g of indoxyl acetate in 45 ml of methanol, 1.9 g of isatin, and 2.9 g of sodium carbonate was stirred under nitrogen for 30 min. The mixture was allowed to stand for 24 hr and then filtered and the crystalline product washed with methanol. Soxhlet extraction by ethanol was very inefficient but yielded brown needles, mp >300°, free of indigo by tlc (methylene chloride on silica gel; ir (KBr) 3344 and 3165 (NH) and 1664 cm⁻¹ (C=O); mass spectrum (70 eV), parent peak at *m/e* 262.

Dehydroindigo was prepared by the lead dioxide oxidation of indigo according to the procedure of Kalb.²⁷ Brown crystals with mp 216° were obtained. Upon cooling the melt, crystals, mp 235–240°, are formed. Kalb reported that indigo sublimed at 195° upon heating dehydroindigo. We did not observe the formation of indigo. No gas was evolved upon melting. The mass spectrum of dehydroindigo gave major peaks at *m/e* 260 (parent), 232, 204, 202, 200, 199, 149, 130, 104, 102, 100, and 97. Metastable peaks at 207 (260 → 232) and 179 (232 → 204) were observed.

Reaction of Indoxyl with 2-Chloro-2-nitropropane. To a mixture of 263 mg (1.5 mmol) of indoxyl acetate and 7 mmol of sodium methoxide in 15 ml of methanol (nitrogen atmosphere) there was added 1 ml of 2-chloro-2-nitropropane *via* a hypodermic syringe. A blue precipitate formed immediately. After shaking for 5 min the mixture was acidified with concentrated hydrochloric acid and filtered to yield 196 mg (100%) of indigo whose purity was demonstrated by tlc and ir.

Reaction of Indoxyl with *p*-Nitrobenzyl Chloride. To 350 mg (2.0 mmol) of indoxyl acetate and 2.0 mmol of sodium methoxide in 100 ml of methanol (nitrogen atmosphere) there was added 686 mg (4.0 mmol) of *p*-nitrobenzyl chloride in 10 ml of deoxygenated methanol *via* a hypodermic syringe. After 1 hr, the mixture was exposed

(20) G. A. Russell and R. F. Bridger, *J. Amer. Chem. Soc.*, **85**, 3765 (1963).

(21) C. H. DePuy and C. E. Lyons, *ibid.*, **82**, 631 (1960).

(22) G. A. Russell, E. G. Janzen, A. G. Bemis, E. J. Geels, A. J. Moye, S. Mak, and E. T. Strom, *Advances in Chemistry Series*, No. 51, American Chemical Society, Washington, D. C., 1965, p 112.

(23) G. Kaupp and G. A. Russell, *Chem. Ber.*, **101**, 1729 (1968).

(24) L. Ettinger and P. Friedlaender, *ibid.*, **45**, 2074 (1912).

(25) P. Friedlaender, *Justus Liebigs Ann. Chem.*, **351**, 390 (1907); C. J. Holt and N. P. Sadler, *Proc. Roy. Soc., Ser. B.*, **148**, 481 (1958).

(26) D. Vorlaender and B. Drescher, *Chem. Ber.*, **34**, 1854 (1901).

(27) L. Kalb, *ibid.*, **42**, 3642 (1909).

to oxygen. However, oxygen was not absorbed and indigo was not formed. Acidification with aqueous hydrochloric acid produced 2-(*p*-nitrobenzal)indolinone, mp 273°. Recrystallization from acetonitrile gave 190 mg (35%), mp 285–286° (lit.²⁸ mp 273–274°);

ir (KBr) 3340 (NH), 1680 (C=O), and 1510 and 1335 cm⁻¹ (NO₂); mass spectrum (70 eV), parent peak at *m/e* 266.

Anal. Calcd for C₁₃H₁₀N₂O₃: C, 67.66; H, 3.79; N, 10.52. Found: C, 67.70; H, 3.84; N, 10.54.

Reaction of Tetramethylhydrazine with Iodine

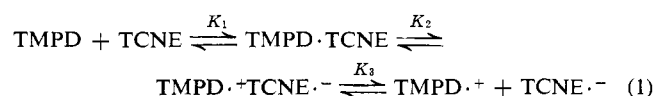
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Contribution from the Department of Chemistry, The University of Nebraska, Lincoln, Nebraska 68508. Received January 9, 1969

Abstract: In nonpolar solvents tetramethylhydrazine (TMH) and iodine form a charge-transfer complex. At low TMH to iodine ratios the complex probably has 1:1 stoichiometry but at higher ratios a 2:1 TMH to iodine complex may be formed. In middle polarity solvents solutions of TMH and iodine yield esr spectra which are broadened by electron exchange due to the reaction of TMH·⁺I₂⁻ ion pairs with uncomplexed TMH. The rate constant for electron exchange is estimated to be 9 × 10⁹ l./mol sec. The change in *g* factor as a result of ion pairing is noted. In polar solvents the solutions give highly resolved esr spectra characteristic of TMH·⁺. It is postulated such spectra are due to the free, solvated TMH·⁺ ion. The mechanism of the one-electron oxidation of TMH by iodine appears to be as shown in eq 7.

The esr spectrum of the tetramethylhydrazine radical cation (TMH·⁺) has been published.^{2,3} In our earlier communication³ we showed that TMH·⁺ could be generated by the reaction of tetramethylhydrazine (TMH) with iodine. It is the intent of this paper to discuss this reaction in greater detail.

The interaction of N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) with tetracyanoethylene (TCNE) was studied by Liptay, Briegleb, and Schindler.⁴ They found that in nonpolar solvents the two reactants formed a charge-transfer complex. In polar solvents the uv visible spectra showed that only the radical cation and radical anion of TMPD and TCNE, respectively, were formed. In solvents of intermediate polarity, however, the charge-transfer complex, radical-ion pairs, and free, solvated radical ions were in simultaneous equilibrium (eq 1). The con-



clusions of Briegleb and his coworkers were based on an involved analysis of electronic spectra. They were also able to estimate *K*₁, *K*₂, and *K*₃ as a function of the solvents used.

There is considerable current interest in one-electron oxidations because of their importance in biological processes. Several other groups⁵ presented electronic absorption and esr data consistent with the type of reaction shown in eq 1 but none of these studies showed all the steps of the equilibrium. Recently the oxidation of magnesium octaethylporphin with iodine and several other one-electron oxidizing agents was reported.⁶ A

qualitative dependence of the oxidation on solvent was noted.

The present study describes the system of TMH and iodine. Our data indicate an equilibrium similar to the type observed by Briegleb, *et al.*,⁴ but there are significant differences in techniques and conclusions.

Experimental Section

Materials. Tetramethylhydrazine was prepared by the method of Beltrami and Bissell.⁷ The compound was purified by distillation (bp 68–69° (730 mm)) and before each run by glpc (5 ft × 0.25 in. Apiezon L column, operated at 62°, 50 cc/min He flow rate). The purity was checked by nmr (singlet at 2.22 ppm in CCl₄, internal TMS).

Iodine was sublimed and was stored over phosphorus pentoxide.

Solvents. Chloroform (reagent grade) was passed through a column of aluminum oxide to remove ethanol. Dichloromethane and acetonitrile were heated at reflux over phosphorus pentoxide, then distilled. Dimethoxyethane was heated at reflux with sodium and was then distilled. Dioxane was purified according to method a given by Fieser.⁸ Carbon tetrachloride, 2-propanol, *t*-butyl alcohol, acetone, methanol, and *n*-hexane were either reagent grade or spectroscopic quality and were used without further purification.

Diphenylpicrylhydrazyl (DPPH) (Aldrich Chemical Co.) was recrystallized from benzene. The first crystals formed were discarded and the remaining crystals were dried *in vacuo* at 80° for at least 16 hr, mp 127–128°.

Anal. Calcd for C₁₃H₁₂N₂O₆: C, 54.85; H, 3.05; N, 17.70. Found: C, 55.4; H, 3.1; N, 17.1.

Tetracyanoethylene (TCNE) (Eastman White Label) was recrystallized from methylene chloride and then sublimed *in vacuo*, mp 194–200° in a sealed tube.

Measurements. Ultraviolet spectra were obtained on a Cary Model 14 spectrophotometer. The sample compartment was thermostated by circulation of water from a constant temperature bath. Both 1- and 10-cm matched fused silica cells were used in the measurements.

Electron spin resonance spectra were obtained with a JEOLCO 3BSX spectrometer employing 100-KHz field modulation. The modulation amplitudes were small with respect to line widths unless otherwise noted.

(6) J.-H. Fuhrhop and D. Mauzerall, *ibid.*, **90**, 3875 (1968).

(7) R. T. Beltrami and E. R. Bissell, *ibid.*, **78**, 2467 (1956).

(8) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed, D. C. Heath and Co., Boston, Mass., 1957, p 285.

(1) To whom requests for reprints should be addressed.

(2) S. F. Nelsen, *J. Am. Chem. Soc.*, **88**, 5666 (1966).

(3) W. H. Bruning, C. J. Michejda, and D. Romans, *Chem. Commun.*, **11** (1967).

(4) W. Liptay, G. Briegleb, and K. Schindler, *Z. Elektrochem.*, **66**, 331 (1962).

(5) H. Kainer and A. Überle, *Chem. Ber.*, **88**, 1147 (1955); J. W. Eastman, G. Engelsma, and M. Calvin, *J. Am. Chem. Soc.*, **84**, 1339 (1962); J. Isenberg and S. L. Baird, *ibid.*, **84**, 3803 (1962).