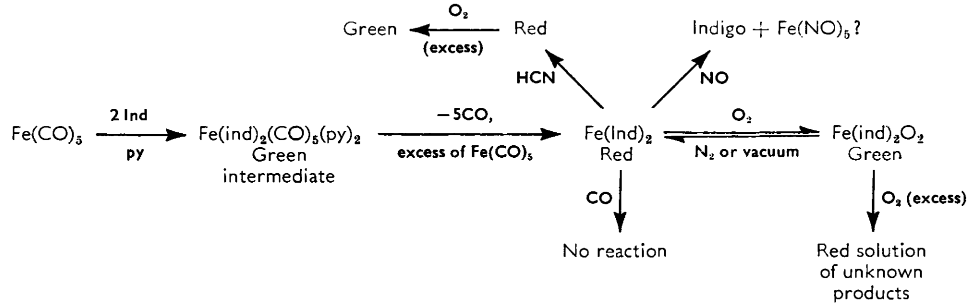
# **783.** *Iron Bisindigo, a Compound Reported to exhibit Oxygen-carrying Properties.*

By L. F. LARKWORTHY.

The iron bisindigo complex formed by reaction between iron pentacarbonyl and indigo has been found not to combine reversibly with oxygen. The apparently reversible reaction reported by earlier workers was due to an excess of iron pentacarbonyl in the reaction mixture; thus the complex is not a suitable model for hæmoglobin. Magnetic-susceptibility measurements have shown that the complex contains iron(II) in the spin-free state. This suggests a tetrahedral configuration for the apparently 4-co-ordinate metal atom, but octahedral co-ordination to neighbouring molecules is possible. On oxidation, the susceptibility decreased to a value approximately that expected for three unpaired electrons. Infrared and other studies show that reduction of one indigo molecule occurs before or during co-ordination. Apparatus for handling the complex in purified nitrogen is described.

MANY metal–indigo compounds have been prepared,[1-4] but their structures are not known. One of these, iron bisindigo, has been investigated, first, because it was reported by Kunz and Kress [2] in 1927 to combine reversibly with oxygen, and has since been widely quoted [5] as the only synthetic oxygen-carrying compound of iron. It is now known that in suitable conditions ferrous dimethylglyoxime [6] and the imidazole complexes of *meso-* and *proto-*hæm [7] also combine reversibly with oxygen.

Kunz's work may be summarised as in the annexed scheme. He prepared the iron–indigo complex by treating an excess of iron pentacarbonyl with indigo suspended in hot pyridine, and for oxygenation he used the red solution containing residual iron carbonyl obtained at the end of the preparation. It was assumed that all the indigo had been converted into the complex, and on reaction with oxygen one molecule was absorbed per iron atom present as Fe(indigo)$_2$, the red solution becoming green and much heat being evolved. By pumping out the oxygen, or by bubbling nitrogen through the solution, the original colour was restored. The reaction with oxygen could be repeated with gradual loss of efficiency. Green crystals of the formula Fe(indigo)$_2$O$_2$ were said to be isolated from the oxygenated solution.



Ind = indigo; py = pyridine; all reactions in pyridine.

Further, 5 molecules of nitric oxide were absorbed per molecule of the complex, and

---

[1] Kunz, *Ber.*, 1922, **55**, 3688; Kunz and Günther, *ibid.*, 1923, **56**, 2027; Kunz and Stühlinger, *ibid.*, 1925, **58**, 1860.

[2] Kunz and Kress, *Ber.*, 1927, **60**, 367.

[3] Kuhn and Machemer, *Ber.*, 1928, **61**, 118.

[4] Machemer, *J. prakt. Chem.*, 1930, **127**, 109.

[5] *E.g.*, Diehl, *Chem. Rev.*, 1937, **21**, 39; Martell and Calvin, "Chemistry of the Metal Chelate Compounds," Prentice Hall, New York, 1952, p. 338.

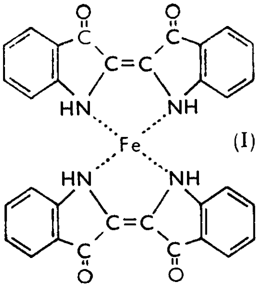
[6] Drake and Williams, *Nature*, 1958, **182**, 1084.

[7] Corwin and Bruck, *J. Amer. Chem. Soc.*, 1958, **80**, 4736.

indigo was quantitatively precipitated; and after reaction with hydrogen cyanide the compound only slowly gave a green colour with oxygen.

Kunz formulated iron bisindigo as an " addition " product (I) of iron and two molecules of *cis*-indigo; *trans*-indigo is the stable form, but *cis*-derivatives, *e.g.*, oxalylindigo, exist. The studies recorded here have been carried out to check the results of previous workers, and, when it had been found that the combination with oxygen was not reversible, to establish the structure of the complex.

*Repetition of Kunz's Work.*—The iron–indigo complex has been isolated in good yield by Kunz's method. He recorded only iron analyses for this and his other compounds, but its complete analysis agrees well with the formula Fe(indigo)$_2$. In oxygen the solid inflamed and on exposure the solution of the complex in pyridine became green almost instantaneously. Thus, the work has been done in the absence of oxygen, and apparently simple operations became necessarily elaborate: the apparatus is described in the Experimental section.


(I)

The green intermediate (see reaction scheme) has been isolated, though in poor yield. No matter at what temperature it is dried it does not reach constant weight; changes in the very complicated infrared spectrum suggest that, on drying, some decomposition occurs and pyridine may be lost, and as a result no consistent analyses can be obtained. The material is paramagnetic, with an effective magnetic moment of about 5 B.M. at room temperature. It is non-conducting in pyridine. Its highest infrared absorption in the carbonyl stretching region is at 1667 cm.$^{-1}$, though co-ordinated carbon monoxide usually absorbs at much higher frequencies, *e.g.*, at about 2000 and 1830 cm.$^{-1}$ in the terminal and bridging positions, respectively, of Fe$_2$(CO)$_9$.[8] Therefore, Kunz's conclusions concerning the nature of this intermediate do not seem valid.

Similar results were obtained when Kunz's oxygenation experiments were repeated. However, when the gas evolved under reduced pressure as the green oxygenated solution changed back to red was shaken with manganous hydroxide to estimate oxygen by Winkler's method [9] none was found. When iron bisindigo was redissolved in pyridine and treated with oxygen the red solution became green as before, but the red colour could not be restored by evacuation, and no oxygen could be detected. On addition of iron pentacarbonyl, gas was evolved and the red colour restored. Thus, the iron carbonyl was responsible for the apparently reversible reaction observed by Kunz. It had been previously reported [10] that oxygen was evolved from the oxygenated iron indigo and iron carbonyl reaction mixture: this result was in error because of manipulative difficulties and was not confirmed by the later estimations by Winkler's method.

When oxygen was passed through the reaction solution containing residual iron carbonyl, or through a solution prepared by redissolving iron bisindigo in pyridine, a microcrystalline mud was obtained instead of the beautiful green prisms reported by Kunz. On oxidation in air the solid compound became dark green and warm; the oxidation was not reversed under reduced pressure. This solid was similar to that obtained from solution, but in neither case could the materials be purified by recrystallisation; the assignment of the formula Fe(indigo)$_2$O$_2$ by Kunz seems based on insufficient evidence.

It has been confirmed that indigo is regenerated by reaction with nitric oxide, and that approximately 5 NO molecules are absorbed per iron atom, but much slow additional absorption also occurred. The reaction was similar in dimethylformamide, but only 3—4 mol. of gas were absorbed.

When an excess of dry hydrogen cyanide was distilled into a solution of iron bisindigo

---

[8] Sheline and Pitzer, *J. Amer. Chem. Soc.*, 1950, **72**, 1107.

[9] See Palmer, " Experimental Physical Chemistry," Cambridge Univ. Press, London, 1949, p. 60.

[10] Larkworthy and Nyholm, *Nature*, 1959, **183**, 1377.

in pyridine the red solution darkened and became warm, and a bright yellow solid, not reported by Kunz, soon separated. Its infrared spectrum showed the presence of CN groups and resembled that of indigo, and although it rapidly gave a pale green material in air this involved little change in the infrared spectrum. The iron content was approximately 20%. Because of its difficult preparation and ready oxidation this yellow material has not been characterised.

*Magnetic Susceptibilities.*—The oxidation state of the metal atom in an iron compound can generally be inferred from magnetic-susceptibility measurements which can be made for easily oxidisable compounds such as iron bisindigo provided sealed susceptibility tubes are used.

In many of the initial preparations of the complex some ferromagnetic impurity was present, as judged from the variation of the susceptibility with field strength. The amount of impurity was too small to affect the iron analysis, and probably arose from the decomposition of traces of iron pentacarbonyl adsorbed by the iron bisindigo during its preparation. Recrystallisation was difficult because of the poor solubility and easy oxidation of the compound, but after variation of the experimental conditions it was found that preparation according to the method in the Experimental section usually gave samples whose susceptibility was not greatly affected by the field strength; hence the concentration of impurity was extremely small, and correction for it by extrapolation of the susceptibility to infinite field was valid.[11] Two examples are given in Table 1.

TABLE 1. *Variation of gram susceptibility $\chi_g$ of iron bisindigo with reciprocal of field strength $1/H$ (H in gauss) (sample* A *at* 293° K; *sample* B *at* 265° K).

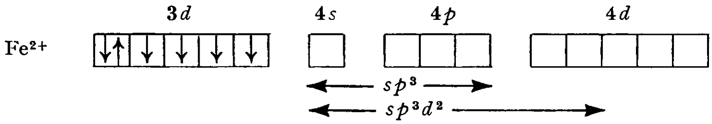
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $10^6\chi_g$ | 18·3 | 18·0 | 17·9 | 17·8 | 17·9 | 17·7 | 17·7 | 17·6 | 17·2 |
| | $10^4/H$ | 2·28 | 2·00 | 1·70 | 1·49 | 1·38 | 1·28 | 1·21 | 1·15 | 0 |
| B | $10^6\chi_g$ | 36·3 | 34·5 | 32·5 | 30·7 | 29·2 | 28·6 | | | 21·4 |
| | $10^4/H$ | 3·53 | 2·96 | 2·45 | 2·06 | 1·74 | 1·60 | | | 0 |

The effective magnetic moment was found to be 4·9 B.M. for sample A and 5·2 B.M. for sample B. In spite of almost identical iron analyses the field dependence was quite marked with sample B, whereas with A it was hardly more than is found with a normal paramagnetic compound because of non-uniform packing. However, the values for the effective moment are close enough for it to be assumed that the moment is near the spin-only value for 4 unpaired electrons, *viz.*, 4·90 B.M.

Results of temperature-susceptibility measurements for a sample with negligible field-strength variation are given in Table 2. Iron bisindigo obeys the Curie–Weiss law, with θ 25° K; thus there is little interaction between the iron atoms in the solid. From these results the effective moment at 265° K was found to be 5·1 B.M. Thus the compound contains iron(II) in the spin-free state. From the formula $Fe^{II}(indigo)_2$ the spin-free iron ion is apparently 4-co-ordinate. If so, one expects for the molecule a tetrahedral configuration because a square-planar configuration would use $3d4s4p^2$ hybridisation for bonding and give rise to a spin-only moment near 2·83 B.M. However, the magnetic

TABLE 2. *Variation of molar susceptibility of iron bisindigo with temperature.*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $1/\chi_m$ | 88·8 | 81·6 | 71·5 | 63·1 | 58·8 | 45·2 | 37·9 | 33·9 |
| Temp. (K) | 293° | 265° | 231° | 201° | 169° | 137° | 110° | 94·7° |



---

[11] Bates, "Modern Magnetism," Cambridge Univ. Press, London, 1948, p. 114.

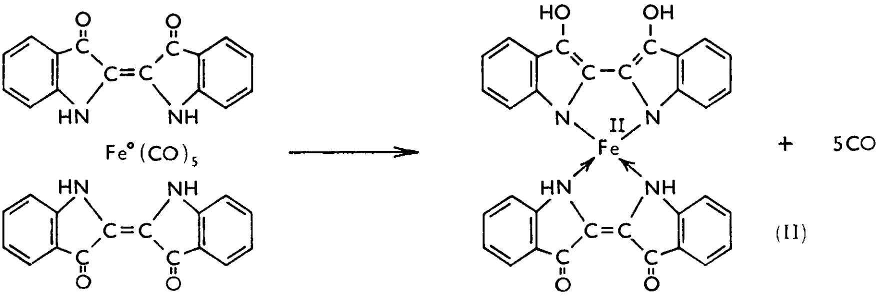
measurements do not exclude an octahedral configuration in the solid through co-ordination of the iron atom to oxygen or nitrogen atoms of adjacent chelate molecules. Only *X*-ray crystallographic determinations, for which the solid is not suitable, could distinguish between these structures.

The ferrous bisindigo was oxidised in the susceptibility tube, and the susceptibility of this product was also determined. There was slight field-dependence, as for sample A, near room temperature, and extrapolation to infinite field gave an effective magnetic moment of approximately 4 B.M. Iron(III) compounds in general have moments of 5·7—6·0 or 2·0—2·5 B.M.[12] The moment of 4 B.M. might arise because of interaction between the metal atoms in an oxygen-bridged ferric complex, or because of the use of only one 3*d*-orbital for bonding.[13] However, at low temperatures the field-dependence was too great to allow a determination of $\theta$ which might have shown whether metal–metal interaction was responsible for the reduced moment.

*Reduction of Indigo during the Formation of Iron Bisindigo.*—The magnetic measurements show that the complex contains iron(II) in the spin-free state. Gas-evolution studies have confirmed that 5 carbon monoxide molecules are evolved for every two indigo molecules present, and the infrared spectrum that it contains no co-ordinated carbon monoxide. Thus, the overall reaction may be written:

$$2\text{Indigo} + \text{Fe}^{0}(\text{CO})_5 = \text{Fe}^{\text{II}}(\text{indigo})_2 + 5\text{CO}$$

The formation of ferrous iron means that two electrons are removed from the iron atom, and co-ordination of a ferrous ion to two indigo molecules requires the displacement of two protons if a neutral molecule is to be formed. The net result could be the production of the complex with evolution of hydrogen. Kunz reported that no hydrogen was evolved, and this has been confirmed. His work was criticised by Kuhn and Machemer [3] who claimed that hydrogen ions were displaced from indigo on reaction with copper(II) salts, and suggested that the same must have occurred with iron carbonyl and indigo. Machemer [4] further claimed that the iron–indigo complex gave a green and not a red solution in pyridine. He treated ferrous acetate with indigo in pyridine and obtained a green solution, but isolated no compound. His experiment has been repeated: the pyridine solution was green and acid, and from it a yellow-green solid was isolated which was oxidised readily in air. Its structure has not been determined, but it is quite different from the complex prepared from iron carbonyl.



(II)

It is believed that in the formation of the standard complex one indigo molecule is effectively reduced by a hydrogen molecule and ferrous iron is formed. The mechanism is not known, but the complex can be imagined to be built up, as shown above, by combination of $Fe^{2+}$ and one indigo and one indigo white molecule, with the displacement of 2 protons. There is no net displacement of hydrogen from the parent indigo molecules. Formula (II) shows iron bisindigo with both indigo residues in the *cis*-configuration. Both

---

[12] Figgis and Lewis, " Modern Co-ordination Chemistry," Interscience Publ., Inc., New York, 1960, p. 406.

[13] Bayer and Hauser, *Experientia*, 1955, **XI**/**7**, 254, and other references therein.

may be *trans*, or one *cis* and one *trans*; and, according to the configuration, co-ordination of each molecule can be through two nitrogen, one oxygen and one nitrogen, or two oxygen atoms. This leads to several possible formulæ which can have different arrangements of the four hydrogen atoms. There is little evidence to show which is correct. Formula (II) has been chosen since it shows most directly the reduction of one indigo molecule and the proton redistribution. Experimental evidence for this is given below.

When strongly heated, iron bisindigo does not melt, but decomposes, and much indigo (30%) can be sublimed from it. The production of indigo in fair yield indicates that hydrogen is not lost on co-ordination, but only removed to positions on the ligand such that on decomposition indigo is re-formed.

Methylmagnesium iodide yields 4 moles of methane per mole of the complex: 2 moles of methane are obtained from 1 mole of indigo, so the complex contains 4 active hydrogen atoms in OH and/or NH groups.

Indigo is formed by reaction of the complex with nitric oxide, a reaction in which no protons are introduced.

The complex dissolves in concentrated aqueous potassium hydroxide only when heated. This, and its low molar conductance ($\sim 1\ \Omega^{-1}$ for a 0·001M-solution in pyridine, much less than expected for a uni-univalent electrolyte [14]) suggest the presence of feebly acidic hydroxyl groups.

Conductance measurements during the reaction between iron carbonyl and indigo exclude the presence in bulk of ionic species. The conductance showed little change until the green solution of the intermediate compound began to change to red. The molar conductance of the final solution, calculated by assuming that all the indigo had been converted into the complex (0·0075M), was $\sim 20\ \Omega^{-1}$. This is higher than would be expected for iron bisindigo alone and may be due to traces of ionic species, *e.g.*, carbonyl ferrates arising from reaction of the excess of carbonyl with pyridine.

The complex is readily formed in pyridine and 2,6-lutidine, less easily in dimethylformamide, and hardly at all in xylene. A basic solvent seems necessary to allow proton transfer.

The strong absorption of iron bisindigo ($\varepsilon$ 3000 at 500 m$\mu$, rising to 10,000 at 350 m$\mu$) suggests that conjugated carbonyl groups are present. Experimental difficulties have prevented more accurate measurements. On initial oxidation a broad peak developed at 720 m$\mu$ ($\varepsilon$ 13,000) which could arise from an increase in the number of carbonyl groups by oxidation of $=\overset{|}{\mathrm{C}}\mathrm{-OH}$. This agrees with the infrared results. On further oxidation the absorption at 720 m$\mu$ disappeared and a pale yellow solution remained. In an excess of oxygen these changes occurred rapidly; and much more than one oxygen molecule per iron atom was absorbed when experiments were carried out on more concentrated solutions.

Further evidence for the reduction of an indigo molecule is provided by infrared studies; some of the absorption frequencies of indigo, of indigo white, and of ferrous bisindigo and its oxidised form are given in the Experimental section. Indigo has been shown to be intermolecularly hydrogen-bonded, the NH and CO stretching absorptions appearing at 3260 and 1625 cm.$^{-1}$, respectively.[15] Indigo white shows an additional band in the hydrogen stretching region at 3450 cm.$^{-1}$, a little above the usual range (3400—3100 cm.$^{-1}$) [16] for free NH stretching vibrations in the solid state. This band also disappeared on oxidation and is therefore likely to be a hydrogen-bonded OH stretching absorption. Ferrous bisindigo shows two additional bands, at 3430 and 3360 cm.$^{-1}$, which also disappeared on oxidation, and new absorptions appeared at 1733 and 1686 cm.$^{-1}$ in the expected region for carbonyl groups in five-membered rings produced by oxidation of

---

[14] Robinson and Stokes, " Electrolyte Solutions," Butterworth's Scientific Publ., London, 1955, p. 508.

[15] Bergmann, *J. Amer. Chem. Soc.*, 1955, **77**, 1549; Weinstein and Wyman, *ibid.*, 1956, **78**, 2387; Holt and Sadler, *Proc. Roy. Soc.*, 1958, *B*, **148**, 495.

[16] Katritzky, *Quart. Rev.*, 1959, **13**, 358.

hydroxyl groups. This, and the general resemblance to the indigo white spectrum show that OH and NH groups are present, the former being obtained by reduction during the reaction with iron carbonyl.

The conductance of a 0·001M-solution of the complex in pyridine was approximately halved on reaction with oxygen. When iron carbonyl was present the same thing happened; and on evacuation the conductance increased slowly until the original colour was restored; then the conductance was somewhat higher than before reaction with oxygen. This could be repeated several times. It seems that the oxidation is analogous to the oxidation of indigo white:

$$-\mathrm{C(OH){:}\overset{|}{C}{\cdot}\overset{|}{C}{:}C(OH)}- + \mathrm{O_2} = -\mathrm{CO{\cdot}\overset{|}{C}{:}\overset{|}{C}{\cdot}CO}- + \mathrm{H_2O_2}$$

This reaction would explain the absorption of one oxygen molecule per molecule of the complex. The production of hydrogen peroxide in the autoxidation of indigo white has been demonstrated by Manchot and Herzog.[17] Kunz has reported that indigo is reduced to indigo white by iron carbonyl in wet pyridine, and in the presence of the carbonyl the hydrogen peroxide or water derived from it is used, in a similar reaction, to re-form the indigo white structure. The reducing action of iron carbonyl in rather different conditions is well known.[18] Thus, the reaction of the complex with oxygen is due to its indigo white structure and is not at all analogous to the oxygenation of hæmoglobin.

## EXPERIMENTAL

*Preparation of Iron Bisindigo.*—Resublimed indigo (3·5 g.) was weighed into vessel A (Fig. 1); pyridine (120 ml.), purified by distillation in nitrogen from barium oxide and potassium hydroxide, was added. Vessel A was attached to the main apparatus which was pumped out and filled with dried and deoxygenated nitrogen. Iron pentacarbonyl (3·5 ml.) was added at $S_1$ against a stream of nitrogen; $S_1$ was stoppered, and any air which might have entered swept out with nitrogen. The solution was stirred magnetically and heated at 90—100°. It soon began to evolve carbon monoxide, and the colour to change to green and later to red. The gas was released intermittently by opening the clip on the rubber tubing (shown broken). The reaction was usually slower than recorded by Kunz. Just before the colour change to red no solid could be seen in the solution, but very soon after this change some separated. The solution was kept at 105—110° until a fair amount of solid had separated, and was then sucked into vessel B. The solution was concentrated at 60° to about two-thirds of its volume, shielded from light, and left overnight. Vessel A was removed by cutting the rubber tubing, the part of B which was exposed to the atmosphere cleaned out, and B was then inverted. The solid was shaken down by rotating vessel B several times, and the filter (Fig. 2) was attached. Sintered-glass filters always became blocked, and it was found best to use a perforated glass disc with two pieces of strengthened filter paper (Whatman's No. 50) held in position by a Polythene ring. The crystals were filtered off by allowing the liquid to drain through under slight suction—strong suction or badly fitting paper caused great loss of the solid around the sides of the paper and could permit oxidation. The filter was fitted between vessel B and a similar flask; these could be interchanged if a considerable quantity of solid escaped.

After the filtration apparatus had been attached it was evacuated and filled with nitrogen several times. Vessel B was designed so that after inversion nitrogen could by-pass it into the filtration apparatus, and wash liquid from vessel C would flow into B to remove any residual solid as shown rather than along the by-pass. The crystals and mother liquor were run, in small quantities at a time, on to the filter. The crystals were washed with pyridine blown over from C; the solid was pumped off until it appeared dry; and the filter was filled with nitrogen and detached. The solid was pushed off the filter with the brass rod and shaken into the T-piece of the " pig " P (Fig. 2). Any lumps were crushed as much as possible (if sintered-glass filters are used small pieces of glass tend to be dislodged and contaminate the product; this was a further advantage of filter paper). The filter was re-attached and evacuated, and the solid

---

[17] Manchot and Herzog, *Annalen*, 1901, **316**, 318.

[18] Sternberg and Wender, Internat. Conf. Co-ordination Chem., *Chem. Soc. Special Publ.* No. 13, 1959, pp. 39—43.

dried by heating the T-piece in a bath at 100—110° under reduced pressure for several hours. The apparatus was again filled with nitrogen, the filter detached, and the solid shaken into the tubes of the " pig " which were then sealed under reduced pressure. The T-piece was used so that the solid could be loosely packed to a small depth and dried at one time: efficient drying in the tubes was impossible because of the depth of the solid, *e.g.*, 10 cm. for magnetic susceptibility tubes.

The design of the attachment containing the rod is shown in Fig. 2. The nozzle of a football bladder B was wired at XX to the tube from a ball-joint; a brass rod passed through the ball-joint into the bladder. There was sufficient room within the bladder for to-and-fro movement, and the ball-joint allowed some lateral movement. Small amounts of air, which had either been trapped within the bladder or had diffused into it through the rubber, contaminated the compound unless the following procedure was carried out. Before filtration the air was removed from the bladder by evacuating and refilling the apparatus several times with nitrogen; and by wiring the bladder to the rod at YY, the last time it was filled with nitrogen, evacuation of the bladder was prevented when the rest of the apparatus was pumped out. Thereby, the chance of diffusion of air into the bladder and thence to the solid was greatly decreased. When the rod was to be used the apparatus was filled with nitrogen, the wire removed, and the solid pushed into the pig. Then the bladder was rewired to the rod. The compound could be kept unchanged in the filter for several days provided it was under nitrogen at atmospheric pressure. If the filter was detached and left under reduced pressure the compound was oxidised in a few hours because of leaks where the grease in the taps had been attacked by the pyridine.

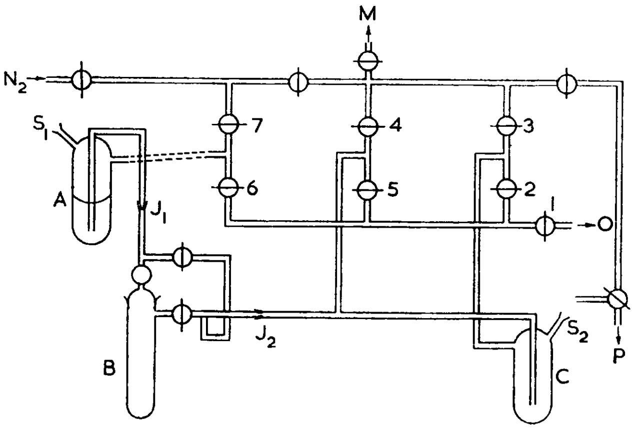


FIG. 1. *Apparatus for preparation of iron(II) bisindigo.*

M = *Manometer.* O = *Outlet through traps.* P = *Pump. A block of taps* (2—7), *allowing gas to flow in different directions, appears to have been used by Corwin and Erdman* (*J. Amer. Chem. Soc.*, 1946, **68**, 2473).

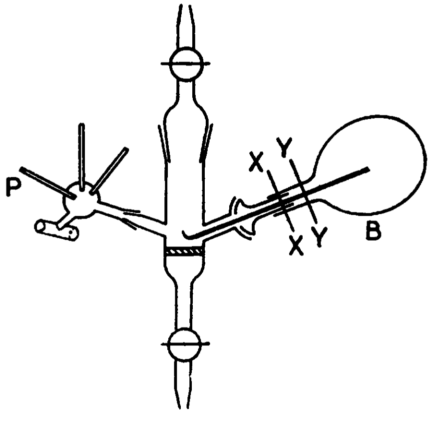


FIG. 2. *Filtration apparatus.*

B = Bladder. P = " Pig." For XX, YY see text.

In spite of the precautions the product did not have quite constant composition (Found: C, 64·9, 64·6, 62·9; H, 4·5, 4·0, 5·1; N, 8·5, 10·1, 10·1; Fe, 9·5, 9·8, 9·5. Calc. for $C_{32}H_{20}FeN_4O_4$: C, 66·2; H, 3·5; N, 9·65; Fe, 9·6%).

*Absorption Spectra.*—In the final stage of the preparation samples of the complex were sealed in Pyrex tubes of about 1 mm. wall thickness and diameter up to 1 cm. according to the quantity of solid. These tubes had to be broken open in the absence of air before almost any experiment could be carried out. This was done by notching the tube at several places and breaking it with a ball-and-socket joint incorporated in the apparatus. The apparatus used to record the absorption spectra has been described.[19] The notched tubes were weighed beforehand, and the pieces of glass collected and weighed at the end of the experiment. The tube usually broke cleanly, but there was always a possibility that small pieces of glass were lost. This was likely to be important only when weights of the order of 30 mg. were taken, *e.g.*, in the preparation of solutions for spectral measurements.

*Conductance Measurements.*—The cell had bright platinum electrodes of the dip-in type in one compartment, separated by a tap from a second compartment provided with a ball-joint for tube-breaking. The solution was tipped into the electrode compartment for resistance measurements. Rubber (pressure) tubing was needed to provide flexibility in this and other experiments: it was used as sparingly as possible and filled with freshly purified nitrogen immediately before use because of possible air-diffusion.

Some semiquantitative measurements were made during the reaction between iron carbonyl and indigo in which the temperature was kept at 90° because cooling to room temperature caused crystallisation of the green intermediate. The change in temperature increased the conductance of the pyridine, or of the final reaction solution, by a factor of less than two, which was much smaller than the conductance changes during the reaction.

*Magnetic-susceptibility Measurements.*—The solid was sealed under reduced pressure in a Pyrex susceptibility tube. It was not possible to pack the solid bit by bit in the usual way, but, as the crystals were small and the tube was evacuated, the packing seemed reasonably uniform. After the measurements on the unoxidised compound had been completed the tube was broken, the substance was oxidised in a desiccator containing oxygen, and the measurements were repeated. The glass broken from the top of the tube was too far from the centre of the magnetic field for its diamagnetism to affect the results. The temperature-susceptibility balance was of the type described by Figgis and Nyholm.[20]

*Infrared Spectra.*—These were recorded on a Grubb–Parsons double-beam grating instrument G.S. 2A with rock-salt discs. The mulling agents, Nujol and hexachlorobutadiene, were dried and were distilled under nitrogen, the former under reduced pressure. Solution studies were not possible because of the great ease of oxidation of some of the compounds and their generally poor solubility. The samples of indigo white and ferrous bisindigo were prepared (from quantities which had been stored in sealed tubes) in a dry-box through which nitrogen was passing. It was difficult to sweep the air from the ordinary type of dry-box used. The amount of oxidation was considerably reduced by working within a cardboard box inside the dry-box into which was led carbon dioxide, generated from the solid and passed through drying towers. The cardboard box was covered with a cloth, and the discs and samples were inside it. Provided no violent movements were made the carbon dioxide layer protected the compounds reasonably for several hours. Once the mull had been prepared the rate of oxidation became slow. The great change in the spectra when the samples of indigo white and ferrous bisindigo were allowed to become oxidised on the plates indicated that the compounds were little oxidised during the preparation of the samples. Some absorption frequencies of ferrous bisindigo and related compounds are given below.

| Indigo | Indigo white | Ferrous bisindigo | Oxidised Fe(indigo)$_2$ |
|---|---|---|---|
| | 3450m sharp | 3430m sharp | |
| | | 3360m sharp | |
| 3260m shoulder | 3240m very broad | 3260m | 3260w |
| 3240m | | | |
| 3030w | | 3040w | 3050w |
| | | | 1733s |
| | | | 1686m |
| 1625vs | 1628m shoulder | | |
| 1610vs | 1617m | 1613m | 1616s |
| | 1603m shoulder | 1602m | 1603s |

Some indigo was reduced under nitrogen by dehydrated sodium dithionite in a solution of sodium deuteroxide in $D_2O$. Oxygen was then bubbled through the solution to re-form the

---

[19] Davies, Hare, and Larkworthy, *Chem. and Ind.*, 1959, 1519.

[20] Figgis and Nyholm, *J.*, 1959, 331.

indigo which was purified by sublimation. The product showed a N–D stretching absorption at 2440 cm.$^{-1}$. The deuterated indigo was then used to prepare small quantities of ferrous bisindigo in which three bands appeared at 2540, 2500, and 2430 cm.$^{-1}$, corresponding to those in the hydrogen stretching region. It was hoped that deuterium analyses of the partly deuterated indigo and the ferrous bisindigo prepared from it would have confirmed that there was no net displacement of protons in the formation of the complex. Unfortunately, experimental difficulties in the handling of the complex prevented these measurements.

The indigo white was prepared by reduction of indigo with sodium dithionite in alkaline solution, followed by precipitation with dilute hydrochloric acid. All operations were carried out under nitrogen as described in the preparation of ferrous bisindigo.

*Gas-volumetric Measurements.*—The absorption of oxygen or nitric oxide by solutions of iron bisindigo was measured with a gas-burette. The nitrogen under which the solutions were prepared was removed, the gas admitted, and the total uptake measured. This was corrected for the volume of vessel and solvent.

For the reaction with methylmagnesium iodide the Grignard reagent was prepared in pyridine essentially by the method of Lehman and Basch.[21] A weighed tube of the complex was broken and allowed to fall into the Grignard solution under nitrogen, and the gas evolution measured. It would have been more satisfactory to add a solution of the complex in pyridine to the Grignard solution, but this would have rendered the apparatus much more complex.

The author thanks Professor R. S. Nyholm, F.R.S., for advice and encouragement, Dr. A. Earnshaw for carrying out the temperature-susceptibility measurements, the glass-blowing staff of the Laboratories for their help, and the Medical Research Council for a Fellowship.

SIR WILLIAM RAMSAY AND SIR RALPH FORSTER LABORATORIES,
UNIVERSITY COLLEGE, GOWER STREET, LONDON, W.C.1.
[Present address: CHEMISTRY DEPARTMENT, BATTERSEA COLLEGE OF TECHNOLOGY,
BATTERSEA PARK ROAD, LONDON, S.W.11.

[*Received, December* 12*th*, 1960.]

---

[21] Lehman and Basch, *Ind. Eng. Chem.*, 1945, **17**, 428.