XCIII.—*Indoxylic Acid*.

By ARTHUR GEORGE PERKIN.

DURING experiments on the aerial oxidation of indoxyl which had been prepared by the enzyme hydrolysis of indican (preceding paper) it appeared to be of interest to confirm the results obtained by an examination of the oxidation of synthetical indoxyl, and it seemed likely that commercial indoxylic acid would be an excellent source of this substance, which is otherwise difficult to isolate in a pure condition. Commercial indoxylic acid was for a short time placed on the market under the name of "indophor," because, owing to the readiness with which it is converted into indoxyl, and the latter by oxidation into indigotin, it was found suitable for calico-printing purposes. As the result of a search in this department of the University, two specimens bearing the correct label were discovered, but these had evidently undergone some alteration, for whereas indoxylic acid is colourless, both bottles contained a dull reddish-violet powder. Experiment showed that whereas in one of these (*A*) indoxylic acid was entirely absent, in the second (*B*) a trace still existed, but this did not yield sufficient indoxyl for a satisfactory oxidation experiment. As this decom

position of indoxylic acid appeared to be, not only remarkable, but also interesting, owing to the fact that its behaviour seemed likely to throw light on some points observed during the oxidation of indoxyl derived from indican, the substances produced were subjected to investigation.

The sample *A*, which weighed approximately 6 grams, and in which indoxylic acid was entirely absent, was the most carefully examined, and as experiment indicated the presence of a substance or substances soluble in alkali, it was in the first place completely exhausted with boiling 1 per cent. sodium hydroxide solution. The deep brown solution was acidified, the resulting brown precipitate was collected, and the filtrate (*C*) reserved for further examination. For the purpose of purification the brown precipitate was dissolved in boiling pyridine, the solution filtered from a trace of insoluble matter, and the filtrate treated at the boiling point with sufficient alcohol to precipitate an almost black substance, which was present in small quantity. This was removed by filtration, and to the clear liquid an excess of ether was added; this caused the separation of a voluminous, dull brown precipitate, which was collected and washed repeatedly with pure ether until the washings were colourless. The filtrate contained a small quantity of indirubin. The product, which resembled indigo-brown, possessed, like this substance, the property of obstinately retaining moisture, and was therefore dried at 160° :

Found, C = 67.35; H = 3.68; N = 9.94 per cent.

These analytical figures somewhat resemble those previously found for the main constituent of indigo-brown (Trans., 1907, **91**, 279), to which the provisional formula $C_{16}H_{12}O_3N_2$ (C = 68.57; H = 4.28; N = 10.00 per cent.) was assigned, and this result, together with other properties, suggest as probable that it may consist of a slightly impure form of the natural indigo-brown compound. The amount available after analysis was too small to admit of a further treatment by the fractional precipitation methods, but it was ascertained that although the main bulk of the product was homogeneous, and could only be dissolved by means of pyridine, traces of substances soluble respectively in alcohol and acetic acid were also present. It is interesting to note that crude indigo-brown also contains small quantities of more readily soluble substances, the percentage of carbon in which is somewhat lower than that of the main constituent, for which the best solvent is also pyridine. The product from indoxylic acid dissolved in alkaline solutions with a deep brown coloration, and was converted by means of nitric acid into a dull orange-yellow, amorphous product. It possessed no definite melting point, but when destructively distilled it swelled up and evolved vapour, which condensed to form a small quantity of a brown, oily matter.

That portion of the original specimen (*A*) which was insoluble in alkali was dried and repeatedly extracted with small quantities of boiling pyridine, when the main portion of the substance passed into solution, and it was observed that, whereas the first extracts possessed a deep red colour, the latter were slightly bluer, and these were accordingly kept apart. On evaporating the pyridine solutions at first obtained to a small bulk, crystals separated on cooling, and these were further purified by means of the same solvent:

Found, C = 73.19; H = 3.95; N = 10.72.

$C_{16}H_{10}O_2N_2$ requires C = 73.28; H = 3.81; N = 10.69 per cent.

This analysis, and the examination of its properties, clearly proved that this, the main constituent of the original powder (*A*), was indirubin. The small amount of substance which remained after the removal of the indirubin was crystallised from nitrobenzene, and had, as was expected, all the properties of indigotin:

Found, N = 10.75. $C_{16}H_{10}O_2N_2$ requires N = 10.69 per cent.

Attention was now directed to the aqueous filtrate (*C*) (p. 848) obtained during the isolation of the brown compound, and this was repeatedly extracted with ether and the ethereal solution evaporated. The semi-crystalline residue was digested with a small quantity of boiling benzene, the hot liquid decanted, and a brown, gelatinous product, which separated on cooling, was removed and the filtrate allowed to evaporate spontaneously. Orange-coloured needles were slowly deposited, but in too meagre an amount for efficient purification; on the other hand, as they readily gave with sulphuric acid and commercial benzene the thiophen reaction, there could be no doubt that they consisted mainly of isatin.

The residue insoluble in benzene was dissolved in a small quantity of hot alcohol, and, on cooling, crystals separated; these, after repeated purification by means of the same solvent and a final crystallisation from water, consisted of almost colourless needles, and possessed the properties of an acid. When slowly heated, this acid melted with effervescence at about 215° , but a melting point as high as 223° could be obtained by heating more rapidly. Most probably this compound is phenylglycine-*o*-carboxylic acid, and this receives support from the fact that in a patent of 1894 the Badische Anilin- & Soda-Fabrik describes the preparation of indoxyllic acid by heating the sodium salt of this substance with alkali, for it is quite likely that in such a process a trace of the original acid would escape conversion. In general physical properties it also resembled isatoic acid, $C_6H_4 \begin{smallmatrix} \text{CO-O} \\ \text{NH} \cdot \text{CO} \end{smallmatrix}$ but this, however, is said to melt at 230° .

An approximate analysis of this sample of indophor (*A*) gave the following amounts of the main constituents mentioned above.

Matter soluble in alkali	32.94 per cent.
Indirubin.....	42.37 „
Indigotin	17.30 „
Moisture	2.15 „
Ash	1.05 „

Of the matter soluble in alkali, approximately one-half (15.44 per cent.) consisted of the crude brown product above discussed, and the remainder is accounted for by the presence of other brown substances of a more soluble character, together with the phenylglycinecarboxylic acid.

The analysis of the indirubin and indigotin was carried out by a comparison of the strength and shade of the sulphonated mixture with similar solutions obtained from the chemically pure colouring matters in a Duboscq tintometer. The purity of this material was thus shown to be 89 per cent., and it was contaminated with a fibrous substance insoluble in nitrobenzene, which no doubt accounts for the deficit in the above analysis.

An examination of the sample *B*, in which a trace of indoxyl acid was found to be present, indicated that it contained the same brown substance as *A*, but to a less extent, namely, 6.41 per cent. Curiously enough the proportion of indirubin to indigotin in this specimen was found to be as 70 to 30, and this result is almost identical with that given by the investigation of the sample *A*, in which the proportion of these colouring matters was as 71 to 29. Determinations of ash and moisture gave respectively 1.38 and 4.79 per cent.

In considering the reactions which have been involved in this conversion of indoxyl acid into indigotin and indirubin, the presence of the former does not require special comment, for it is well known that indoxyl acid is readily converted into indoxyl, and this latter again into indigotin. On the other hand, with regard to the indirubin, its presence can only have originated from the simultaneous production of both indoxyl and isatin, and the question arises as to whether the isatin has been derived from the direct oxidation of indoxyl, or rather of indoxyl acid itself.

That indoxyl can be converted into isatin without an intermediate formation of indigotin has been shown by the Badische Anilin- & Soda-Fabrik (D.R.-P. 107719 of 1898) to be the case, and although air is not included among the reagents they employ, it is evident, and has been discussed in preceding communications, that the formation of indirubin during the manufacture of natural indigo can only be due to the previous production of isatin by the aerial oxidation of the indoxyl.

The quantity of indirubin, however, which can be obtained from indoxyl in this latter manner, even under the best known conditions, is but small in proportion to the indigotin which is simultaneously produced. Thus natural indigos are to be found which contain as high as from 8 to 10 per cent. of indirubin, and certain rare cases are on record in which even larger amounts have been detected, but these quantities are trifling when compared with the quantity (70 per cent.) of indirubin which was found in the colouring matter present in the indoxyllic acid product under discussion.

There is every reason to suppose that when indoxyl is oxidised by air it is preferably converted into indigotin, and it accordingly seems certain that the indoxyllic acid itself has been the direct source of the isatin necessary for the indirubin condensation which took place in the sample.*

If the constitution of ψ -indoxyllic acid is considered,

it is, of course, possible that on oxidation by air it might yield the acid,

which by elimination of carbon dioxide and a further oxidation would be converted into indigotin.

If, on the other hand, we suppose that the first product of the oxidation of the indoxyllic acid is 2-hydroxy- ψ -indoxyllic acid,

then it is easy to see that this cannot give indigotin by oxidation, but will yield isatin with elimination of carbon dioxide.

It is evident, also, that the conversion of ψ -indoxyl itself into ψ -isatin, according to the methods described by the Badische Anilin- & Soda-Fabrik (*loc. cit.*), or by the change which occurs to a small extent during the oxidation of indoxyl solutions by air, and is represented by the presence of indirubin in the indigo produced, can be similarly formulated :

From experiments on the aerial oxidation of a solution of indoxyllic acid, described in the preceding paper (p. 842), there would appear to be some evidence that indigotin is not a product of this reaction.

* It is considered that this oxidation has been due to the gradual admission of air to the samples. The investigation has yielded no evidence of the presence in the mixture of a substance known to behave as an oxidising agent, although, had this been the case, the main argument would not be affected.

Thus it was observed that when a solution containing both indoxyl and indoxylic acid was oxidised by air in the presence of a small quantity of ammonia, both compounds yielded indigotin. On the other hand, in the absence of ammonia a much smaller yield of colouring matter was produced, and there could be no doubt that this small quantity had been derived mainly, if not entirely, from the indoxyl present. When the indigo had ceased to separate, addition of ammonia and shaking with air failed to increase the amount, showing that there was no indoxylic acid present, but, instead, a deep brown solution was obtained, due to the presence of brown matter and probably also of isatin. Unfortunately, at that time the necessity for a more detailed study of the reaction did not suggest itself.

The formation of a brown substance by the oxidation of an aqueous solution of indoxylic acid is very interesting, especially when taken in conjunction with the isolation of a brown compound from the decomposition products of "indophor" (*A* and *B*) described in this paper, and which so closely resembles the main constituent of indigo-brown, for if, as is suspected, this material is identical with that present in natural indigo, the origin of its presence in the dyestuff may be easily conjectured. There is no evidence that the leaves of *indigoferae* contain a glucoside of indoxylic acid, but, on the other hand, it is quite possible that indoxylic acid might be produced from indoxyl during the fermentation of the leaf extract. During this process a very large quantity of carbon dioxide is evolved, by means of which a portion of the indoxyl may be converted into the acid, and from this by subsequent oxidation the brown compound would be produced. In connexion with this point the statement of Rawson (*Report on the cultivation and manufacture of indigo*. Mozufferpore. 1904, 86) is interesting: "the blower . . . by getting rid of carbon dioxide . . . prevents decomposition of the colouring matter into worthless brown substances, which takes place to a greater extent under other conditions."

To account for the production of brown compounds from indoxylic acid, the following reactions suggest themselves as possible, taking into account the fact that the formula $C_{16}H_{12}O_3N_2$, which has been provisionally assigned to the main constituent of indigo-brown, contains more hydrogen and oxygen than indigotin itself. Briefly, it is considered that if, as discussed above, the first product of the oxidation of indoxylic acid is 2-hydroxyindoxylic acid, two molecules of the latter may condense with elimination of water, carbon dioxide being subsequently evolved. The substance thus obtained would possess the following formula: $C_6H_4 \begin{smallmatrix} \text{C(OH)} \\ \text{NH} \end{smallmatrix} \text{---} \text{C} \cdot \text{O} \cdot \text{C} \begin{smallmatrix} \text{C(OH)} \\ \text{NH} \end{smallmatrix} \text{---} C_6H_4$.

Again, if indoxyl on oxidation is in part converted into the

compound $C_6H_4 \begin{smallmatrix} \text{C(OH)} \\ \text{NH} \end{smallmatrix} \text{---} \text{C} \cdot \text{OH}$, the same product would naturally be obtained by such a condensation, and it is suggested that the secondary reaction which was observed to take place when the indoxyl solution derived from indican (see preceding paper) was oxidised by air is due either to this or some change of a similar nature.

ADDENDUM.—Since the communication of this paper, a third sample of the “indophor” has been available for examination, and for this I am indebted to the kindness of Prof. W. Gardner, of Bradford. A qualitative investigation of this specimen which had been preserved in the museum of the Bradford Technical College since November, 1897, showed that it had undergone a similar alteration to that observed in the case of the specimens (*A* and *B*) above discussed. Thus, whereas it consisted mainly of indirubin, there was also present indigotin, the brown alkali soluble product, and a trace of indoxylie acid. There can thus be no doubt that the decomposition of indoxylie acid in these circumstances is characteristic of the substance.

CLOTHWORKERS' RESEARCH LABORATORY,
THE UNIVERSITY,
LEEDS.