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REPORT
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COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Shinjiro Sato final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

May 31 1917

R. W. Hatcher
Chairman

William S. Cooper

J. H. Lucey

REPORT
of
Committee on Thesis

The undersigned, acting as a Committee of
the Graduate School, have read the accompanying
thesis submitted by Shinjiro Sato
for the degree of Master of Science
They approve it as a thesis meeting the require-
ments of the Graduate School of the University of
Minnesota, and recommend that it be accepted in
partial fulfillment of the requirements for the
degree of Master of Science.

R. W. Hatcher
Chairman

William S. Cooper
J. H. H. H.

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THE RECOVERY OF INDIGO FROM POLYGONUM TINCTORIA AND
METHODS OF PURIFICATION OF CRUDE INDIGO.

A thesis submitted to the Faculty of the Graduate
School of the University of Minnesota.

by

Shinjiro Sato

in partial fulfillment of the requirements for
the degree of Master of Science

June, 1917.

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INTRODUCTION.

For the past thirty years the production of natural indigo has been diminishing because of the competition with the synthetic indigo which is replacing it in a surprising rate of increase in the world market. It is immensely interesting to trace back the development of synthetic indigo, showing the possibilities of scientific researches in their application to the practical industry. Before synthetic indigo was placed on the market some of the German dye manufacturers had spent an enormous sum of money, and long years of research; they won the prize for which they were struggling, and they now seem to monopolize the supplies of indigo for the world. What has been the matter with the manufacture of the natural indigo? What caused the industry to diminish while the demand for the dye has been steadily increasing. The explanation is, that those people who are associated with the indigo manufacture had unanimously neglected to see the value of research. No person has ever carried out a systematic investigation on the subject as patiently and conscientiously as some of the German chemists, like Baeyer who spent fifteen years continuously for the establishment of constitution, indigotin and of its formula, and two or three chemists who devoted their lives to the development of the synthetic indigo. Unfortunately, in the natural indigo there has been no progress; the old medieval process is still in existence today without much modification, and it seems that this industry which has existed for three hundred years will be completely driven out of the market before very long if the present method of manufacture is kept in use.

The present work was begun with the purpose of contributing to the slightest degree, to the manufacture of the natural indigo. The writer has firm faith in the possibilities of this industry which has the vital economical as well as social bearings upon the lives of those who have been and are still depending more or less on the welfare of the industry. For instance, in the Province of Tokushima, Japan, to say nothing about those millions of people in India and other parts of the world, the people depend chiefly on the indigo plantation for their annual household expenses. The necessary cultivation of the plant in Tokushima is between the two seasons, namely, between the wheat and rice crops: there is no other crop which can be substituted for the indigo plant if the industry is destroyed by the competition with the synthetic indigo. What would be the fate of their lives if such is the case?

Taking all these facts into consideration, this work was undertaken, and the results obtained are not merely laboratory procedures or academic interests. It is hoped that they may be of practical application for the indigo industry. Of course, this work is far from a complete and exhaustive research, and needs further investigations. There are a few items that have been impossible to take up in the present work, though they are vitally connected with the indigo industry, such as a relationship between the yield of the plant and the character of soil and fertilizers, investigating the efficiency of the indigo as a dye, and possibilities of making many substituted dyes derived from the indigo. These subjects may be taken up in the future investigations.

Part I.

HISTORY OF INDIGO.

Indigo was probably one of the earliest known coloring matters. It was used by ancient Egyptians, being found in the mummy cloths prepared at least five thousand years ago. It was well known to the Greeks and Romans who imported it from India. A method of indigo production as we know it now was described by Marcopalo in the thirteenth century. It was, however, the Dutch traders who introduced it from the East Indies into Europe, and began its use on a large scale in the beginning of the sixteenth century. The increasing manufacture of the dye met a serious blow by law prohibiting the manufacture of the "fugitive dye". The cause of this law was purely commercial jealousy on the part of the wood cultivators who were the instigators of the law. In 1737, France was the first nation in Europe to make it lawful to use the dye, and from this time its valuable properties became known gradually throughout Europe.

The introduction of indigo into Japan was probably in the latter part of the seventeenth century, it having been imported through China, and from that time to this the plant has been cultivated, especially in the Province of Tokushima where this industry is exclusively carried on ever since its importation. It became one of the most profitable industries of the province and its role has been the chief factor in controlling the financial affairs of the province. At one time it turned over from four to five million dollars to the provincial treasury.

However, its industry began to decline, as in India with the introduction of the synthetic indigo to the market.

THE INDIGO PLANT.

The variety of the indigo plant, which the writer employed for the production of indigo, is known as *Polygonum tinctoria*. The seed was secured from Japan and was planted in flats in the greenhouse. The young plants, when about six inches high, were transplanted to an open field on the University Farm. It required almost four months to reach the full growth on the soil of University Farm, Minn. The height of the plant was about eighteen to twenty inches. This was not a normal growth when compared with the plant grown in the province of Tokushima where it grows about thirty-six inches.

The leaves of the plant only are concerned in the manufacture of indigo, for the coloring matters are contained exclusively in them. From the standpoint of the indigo industry there are three vital points that we must take into consideration with the plant.

(1) Weight of plant yielded per acre.

(2) The percentage of leaf given by the plant.

(3) The indigatin producing value of the leaf.

According to Rawson (1) good plants yield 40% of leaf. Leake (2) has found 62%, whereas the plant cultivated by the writer gave 68.50%. There is also a great variation in the amount of the plant yielded by an acre of land according to the character of the soil, difference in environment influences, etc. A fair average crop in India may be taken at 50 to 60 cwts. per acre, whereas the writer obtained approximately 8.10 tons of the

(1) The Cultivation and Manufacture of Indigo in Bengal.

(2) J. Soc. Chem. Ind. 1907, 26, 1174.

green matter per acre.

The writer conducted experiments to ascertain the relative growth of the plant by using different fertilizers when the cost of the fertilizers should be the same in each case. Three kinds of fertilizers were employed, namely sodium nitrate, dried blood and acid phosphate, each fertilizer being applied to the plants in five rows. It was found at the time of maturity that the growth of the plant was just about the same in all cases. From this experiment the writer does not wish to make a definite conclusion, for it was the first time that the plant has ever grown in this part of the country, and consequently it was not adapted to the soil and to the climate. It is, however, his earnest desire to find out a relationship between the growth of the plant and the indigo content on one hand, and the character of fertilizers on different soils on the other.

The content of indican in the leaf varies at different periods of the year. Rawson⁽³⁾ found that in one instance May 28th the indigo content was .20%; on Aug. 25th this had risen to .76%. As the plant grows, the young leaves contain more coloring matter than the old ones on the same plant. Finally there is a gradual increase in coloring matter in the leaves from the bottom of the plant upward.

The composition of leaf and stem composing the indigo plant varies greatly. According to Rawson⁽⁴⁾ the mean result of twenty determinations gave in round numbers 40% of leaf and 60% of stem. On this basis the composition of the whole green plant will be as follows:

(3) (L. C.)

(4) (L. C.)

Water-----	75.00%
Nitrogenous matters-----	4.27"
Woody fibre-----	.60"
Carbohydrates and other organic matter-	8.87"
Mineral matter (Ash)-----	2.12"
	<u>100.00%</u>

THE CHEMISTRY OF NATURAL INDIGO MANUFACTURE.

I. Review of Previous Investigation.

In the beginning of the Nineteenth Century the coloring principle of indigotin present in the indigo plant was supposed to be indigo-white, and this theory remained uncontradicted until 1855 when the celebrated plant chemist Schunck⁽¹⁾ proved that the coloring principle was a glucoside which he named indican. He isolated indican from the *Isatis tinctoria* (wood), *Polygonum tinctoria*, and *Indigofera tinctoria*.

In order to explain a little further the method by which indigotin is prepared from the glucoside, it is necessary to discuss something about the constitution of indican, and the chemical reactions by which indigotin is formed.

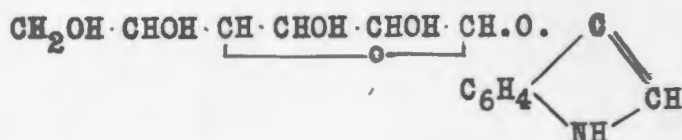
Generally speaking the glucosides are condensation products of at least one molecule of a hexose, pentose, or other carbohydrate with a molecule of another substance of acid or basic character. Among the latter may be found phenolic substances, amino compounds, and some other carbohydrates. The generally adopted formula for glucosides is as follows:

$\text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CH} \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{CH} \cdot \text{O} \cdot \text{R}$, in which R represents a phenolic or basic body combined with the glucoside residue. According to Marchlewski and Radcliffe⁽²⁾ the indican cannot possibly contain the whole molecule of indigotin in any form whatever. They believe that the formation of indigotin is caused by a condensation process simultaneously with the hydrolysis of the indican,

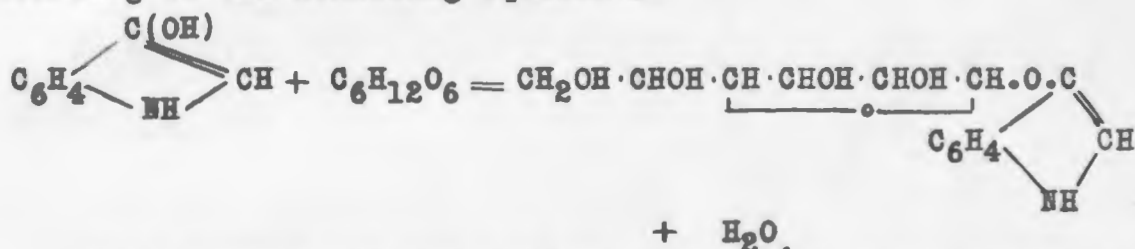
(1) Phil. Mag. 1855 (IV) 17, 74, and 1858, 15, 127.

(2) J. Soc. Chem. Ind. 1898, 17, 431.

and they went further to say that the original substance of indigotin is indoxyl, and that the constitution of indigotin is expressed by the formula:-



in which a molecule of indoxyl appears to be condensed with one molecule of glucose with the elimination of one molecule of water, the latter being formed from a hydrogen atom from the glucose and the hydroxyl group of the indoxyl or vice versa according to the following equation:-



This formula for the indican explains the properties of indican so far as the formation of indigotin and glucose under the influence of hydrolysis and subsequent oxidation is concerned. As a result of the communication of Marchlewski and Radcliffe, Hazewinkel, the director of the experimental station for indigo, Klaton, Java⁽³⁾ gave an account of his research in which he arrives at the same conclusion with Marchlewski and Radcliffe that indican is an indoxyl glucoside, and that the sugar obtained from it is dextrose.

The assumption that indican is the glucoside of indoxyl is also very probable from physiological consideration. According to the two above mentioned chemists⁽⁴⁾ the formula proposed is analogous to that of indoxyl sulphuric acid, a sulphuric acid

(3) Proc. K. Akad. Wetensch. Amsterdam, 1900, 2, 512.

(4) (L. C.)

ether of indoxyl which appears among the secretions of the animal organism, probably a product of the decomposition of albuminoid substances. The authors assume that certain species of plants have a similar physiological function in that they decompose their albumen in a similar manner with the result that the degradation product of albumen appears in the form of indoxyl which is subsequently neutralized by condensing with glucose. This explanation seems very probable because plant and animal organisms very often do work up their albumen on analogous lines.

Hoogewerff and ter Meulen⁽⁵⁾ isolated indican in a crystalline condition from the *Indigofera leptostachya* and *Polygonum tinctoria* as follows: the leaves are digested in boiling water; the extract is evaporated in vacuo; the residue is taken up by methyl alcohol; precipitated with ether and after removing the precipitate, the clear liquid evaporated, and the residue is taken up with hot water. The filtered and concentrated solution on cooling deposits well defined crystals. They obtained 5 grams of pure indican from 17 kilos of the leaves of *Polygonum tinctoria*.

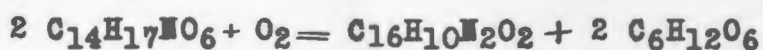
Perkin and Bloxam⁽⁶⁾ prepared pure indican from *I. sumatrana* and *I. Arrecta* by treating the leaves with cold acetone and treating the extract with light petroleum. They obtained 31.66 grams of indican out of 1000 grams of leaves.

When thus obtained indican $C_{14}H_{17}O_6N$ crystallizes from water in spear-shaped crystals which contain three molecules of

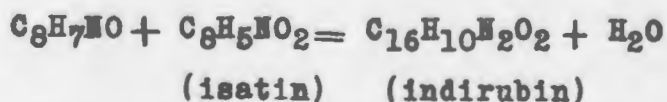
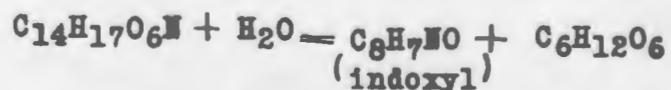
(5) Proc. K. Akad. Wetensch. Amsterdam, 1900, 2, 520.

(6) Chem. Soc. Trans. 1907, 91, 1715.

water of crystallization. It melts at 57-58°C. When heated in a test tube, or on platinum foil, purple fumes are given off, but in an atmosphere of carbon dioxide this does not take place. By passing a current of air through a hot solution of indican in dilute hydrochloric acid containing a little ferric chloride, 91% of the indican can be converted into indigotin according to the following equation:-



It has been shown by Baeyer⁽⁷⁾ that indoxyl readily condenses with aldehydes and ketones, forming the indogenides, and Hazewinkel⁽⁸⁾ furthermore demonstrated for the first time that indican is an indoxyl glucoside and partly identified this substance by means of its condensation products with isatin, benzaldehyde, and pyruvic acid and determined the quantitative reactions for the indirubin thus formed. Perkin and Bloxam⁽⁹⁾ and also Gaunt, Thomas, and Bloxam⁽¹⁰⁾ found independently that when indican dissolved in water is added to a boiling solution of isatin, acidified with a little hydrochloric acid, and the operation is carried in an atmosphere of hydrogen or carbon dioxide, the yield of indirubin is quantitative according to the following equation:-



This method offers a ready means, not only for the analysis of the crystalline glucoside or indican, but also for the estimation of the amount which is present in aqueous infusions of the

leaf. More recently Perkin and Thomas⁽¹¹⁾ obtained quantitative amount of P-nitrobenzaldehyde-indogenide by the condensation of indoxyl with P-nitrobenzaldehyde, and, that this reaction could also be employed for the analysis, both of crystalline indican and that present in the leaf extract.

When indican is hydrolysed with acid in the presence of an oxidising agent, it does not appear to give a quantitative yield of indigotin. According to Hazewinkel⁽¹²⁾ that acid oxidising agents convert indican into indigo, and this in turn is oxidised by an excess of the reagent. Bloxam and Leake⁽¹³⁾ studied the oxidising effect of ammonium persulphate and found that the process was far from quantitative since with pure indican, it gave only 82% of the theoretical. Perkin and Thomas⁽¹⁴⁾ studied the effect of the hydrolysis of solutions of indican with acid during the aspiration of air through the liquid, under varying conditions of temperature and concentration. The most satisfactory yield of the coloring matter (93.5%) was produced when air was passed during 8 hours through a solution of 5. grams of the indican in 850 c.c. of water acidified with 15 c.c. of 33% HCl, and the temperature maintained at 60 . It seems probable that since the pure indican did not give quantitative yield of indigotin, according to their results some secondary reaction occurs although the nature of chemical change is not known. The function of indigo-brown may be the explanation.

According to the results which will be shown later, the

- (7) Ber. 1881, 14, 1745.
- (8) Proc. K. Akad. Wetensch Amsterdam, 1900, 2, 520.
- (9) Chem. Soc. Trans. 1907, 91, 1715.
- (10) J. Soc. Chem. Ind. 1907, 26, 1174.
- (11) Chem. Soc. Trans. 1909, 95, 795.
- (12) Proc. K. Akad. Wetensch. Amsterdam, 1900, 2, 520.
- (13)

acid extraction in the presence of oxidising agent such as ammonium persulphate gave the most favorable yield of indigo from the green leaves and the writer questions very much whether the works mentioned above was accurate. The problem remains as a further interesting study.

II. The Indigo Enzyme.

The fact that the hydrolysis of glucosides is caused by their specific enzymes is also applied to the hydrolysis of indican by the indigo enzyme called "indimulsin" the name of which was first suggested by Hazewinckel. He investigated the properties of the indimulsin and proved for the first time that indican is a glucoside of indoxyl. It was, however, Schunck who first discovered the presence of this enzyme in the green leaf, and it was later elaborately investigated by the Dutch chemists, especially by Beylrinck, who isolated the enzyme as a snow-white highly active powder. Ammonia destroys the enzyme. Emulsin slowly hydrolyses indican, but the intensity of its action is only one twentieth of that of the "indimulsin".

To isolate the indimulsin from the fresh leaf is rather difficult, probably because the presence of tannin in the leaf. The writer tried to isolate the enzyme by pounding the leaves with hide powder which fixes the tannin, and then extracted with alcohol. The product thus obtained was a green amorphous substance which did not show any indication of enzyme action when

(13) Dalsingh, Serai Report, 1904.

(14) Chem. Soc. Trans. 1909, 95, 795.

applied it for the fermentation of an extract of the leaves.

A study of the hydrolysis of pure indican by means of the enzyme and subsequent oxidation of the indoxyl solution with air under varied conditions has been made by Thomas, Perkin, and Bloxam⁽¹⁵⁾. The results of these investigations show that the hydrolytic action of the enzyme proceeds somewhat rapidly, and that by using 2 grams of the enzyme and 1 gram of indican under a dilute solution, the reaction was complete after 2 hours' digestion at 50 C.

Much attention should be paid to the degree of the acidity of the indican solutions which are the progress of fermentation, A trace of sulphuric acid present during the fermentation inhibits the decay of the indoxyl, and also prevents theocclusion of the indoxyl by the enzyme powder as frequently occurs.

III. Bacterial Fermentation.

The hydrolysis of the indican by the action of its specific enzyme, indimulsin, is the main factor in the manufacture of indigo. It is well known fact that bacteria which are present exert a similar action although in minor degree.

Aluars in 1887, was the first man to isolate from an extract of indigo plant an organism *Bacillus indigogenus* which was capable of producing the fermentation. Bergtheil⁽¹⁶⁾ considers that at least one organism capable of producing indigo fermentation is invariably found in large quantities in an infusion of the plant. The manufacture of indigo in Japan is

(15) Chem. Soc. Trans. 1909, 95, 829.

(16) " " " 1904, 85, 877.

carried out chiefly by the application of the bacterial action which causes the fermentation of the leaves.

Part II.

LABORATORY METHODS OF PRODUCING INDIGO FROM THE PLANT.

QUESTIONS INVOLVED.

The problems to be included in the proposed investigation are conveniently divided into three parts:-

- I. To investigate a method which yields the maximum amount of indigo from the green leaf.
- II. To investigate a method which yields the maximum amount of indigo from the air dried leaf.
- III. To find a method of purification on crude indigo manufactured in the Province of Tokushima.

PRODUCTION OF INDIGO FROM THE GREEN LEAF.

The purpose of this investigation is to study the various environment influences upon the yield of indigo. It involves the following considerations:-

1. Degree of cell division of the plant tissues.
2. Choice of the duration of steeping period.
3. Choice of an optimum temperature.
4. Choice of a proper medium for the extraction.

General Methods of Procedure.

(a) The production of indigo from the indigo plant is a simple matter and consists mainly of two process:

- (1) A steeping of the plant with water (fermentation).
- (2) Followed by the oxidation of the water extract with air in a separate vessel.

The leaves are cut into small chips, and placed in a large glass cylinder having six to seven liter capacity. For

every hundred grams of the leaves, add two liters of water which is strongly acidified with hydrochloric acid. Let the content of the cylinder stand 6 hours at least for the hydrolysis of indican in the leaves. The extract is completely removed from the leaves, and transferred into another cylinder of the same size, and is now ready to oxidise. 50 c.c. of 5% ammonium persulphate solution are added to the extract and the oxidation is completed by the continuous aspiration of air through it. The indigo gradually settles down to the bottom, and the oxidation is complete in about three hours. While the oxidation is in progress, several tests should be made at different intervals to see whether the oxidation is complete or not. To apply the test, a few c.c. of the extract are poured into a test tube and a few drops of the ammonium persulphate solution added. If the color of the extract becomes blue or bluish green, the oxidation is still incomplete, but if no change of color is seen, the extract is straw yellow color, the oxidation is complete. Heat the extract to boiling, and let it stand until flakey indigo settles down to the bottom. There is a three-fold object in boiling the extract:

- (1) It prevents putrefaction which rapidly takes place in warm season, and would result in loss of the dye.
- (2) It dissolves some of the brown matters which are mixed with the coloring matter and thus produces a finer quality.
- (3) It causes indigo particles to settle more rapidly.

Decant the clear liquid off, filtered through a hardened

filter. A hardened filter paper is absolutely necessary in this operation because the indigo sticks so hard that it is very difficult to remove if an ordinary filter paper is employed. The precipitate is thoroughly washed with hot water until free from acid, then with alcohol until no more reddish colored washing comes through the filter. Now remove the filter paper, and dry the precipitate in a drying-oven at 70 - 75 C. The indigo thus prepared is a crude compound, and must be purified from the subsequent analysis.

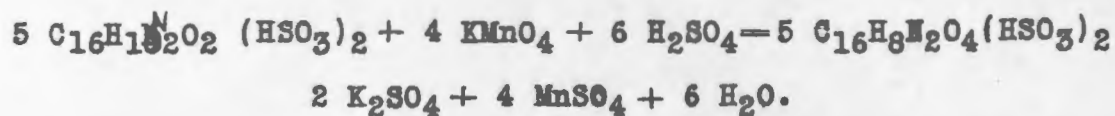
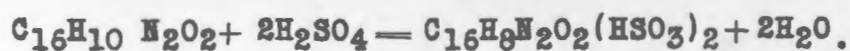
(b) Purification of the crude indigo.

The details of this process will be explained later in connection with the method used in purifying a crude indigo prepared in the Province of Tokushima.

(c) Method of analysis.

There has been much dispute over the different methods of analysis proposed by many chemists, and there still seems to be some question as to the best and most practical method to be adopted. The matter is of considerable importance, since a correct estimate of the efficiency of the process of indigo manufacture depends upon the reliability of the method of determining the indigotin obtainable from the plant.

What seems to the writer to be the most accurate and simplest method of indigo analysis is based upon the titration of a solution of the sulphonated indigo with potassium permanganate by which the sulphonated indigo is oxidised according to the following equations:-



This method of estimating indigotin has been previously very carefully studied by the writer⁽¹⁾, and was used in all the work reported in this thesis.

(1) A thesis presented for the degree of B.S., The State College of Washington. pp. 38-47.

Degree of the Division of Plant Cells upon the Yield of Indigo.

Experiment No. I.

Most of indigo manufacturers so far as the writer knows have been over-looking the fact that the fine division of the plant tissue is one of the important factors in determining the yield of indigo from the green leaves. The finer the tissues are cut, the better the yield of the coloring matter, because the cells in the leaves have a better chance to come in closer contact to each other, whereby the activity of the enzyme becomes greater during the fermentation period. The result is, as shown in the following table, a better yield of indigo.

<u>Sample No.</u>	<u>Condition of leaf.</u>	<u>% of indigotin found.</u>
1	whole leaf	.067%
2	cut leaf about 1/2 in.	.13 %
3	ground leaf	.14 %

In this experiment the leaves were fermented at a room temperature for 48 hours. The percentage yield of indigotin was based on the weight of the green matter used, and the same method of calculation is used throughout the subsequent experiments.

Determination of Duration of Steeping Period.

Experiment No. II.

Crookes⁽²⁾ states that a steeping period of nine to ten hours, at ordinary temperatures, is sufficient, whereas Rawson⁽³⁾ claims that ten to eleven hours is sufficient for the average external temperature, and Bergtheil⁽⁴⁾ thinks ten hours¹

(2) Handbook of dyeing and Calico Printing.

(3) Report on Cultivation and Manufacture of Indigo.

(4) Indigo Research Station Sirsiah, 1906.

steeping is enough. The writer believes that many matters must be taken into consideration in this connection, such as the species of plant used, the manner in which the leaves are subjected to the steeping process, etc. as well as the prevailing external temperatures. Consequently, there is no way of fixing a standard period of steeping for all cases. As a rule the steeping hours will be comparatively briefer in the country like India where warmer temperature prevails than in Minnesota. With the *I. Arrecta* the steeping should vary from thirteen hours to fifteen hours at 32.2 C, whereas *I sumatrana* gives the best result in twelve hours' steeping. The leaves of *Polygonum tinctoria* are not easily wet by water, and for an hour or two at ordinary room temperature, no action takes place. When examined closely, they are covered by an immense number of small hairs which are the chief cause of water repelling. In order to facilitate the action of steeping in the following experiment the leaves were cut into small size and then subjected to treatment with water at a temperature of 50°C. and in acid medium.

<u>Sample No.</u>	<u>Duration of steeping</u>	<u>% of indigotin obtained</u>
1	1	.160%
2	3	.166%
3	6	.160% (?)
4	16	.183%
5	33	.220%
6	38	.175%

The figures show that the fermentation during the first six hours gives identical yields, and does not show any appreci-

able increase of yield until eighteen hours' steeping. The yield becomes maximum at thirty-three hours' steeping. After that the yield decidedly decreases.

Determination of the Optimum Temperature.

Experiment No. III.

The extraction of indigo plant with hot water has been used for many years in some parts of India. Perkin⁽⁵⁾ claims that the hot water extract gives the best result and that the indigo produced is of a superior quality. Nagai⁽⁶⁾ also proposes hot water extraction method. He uses cold water first and gradually raises the temperature to boiling while the fermentation process is in progress. However, the writer cannot accept their methods for a simple reason that the activity of the indimulsin stops instantly as soon as the temperature is raised up to the water boiling point. It must be borne in mind that the indimulsin is destroyed at a temperature between 60° and 74°C. The writer attempted to extract indigo with hot water according to their respective methods partly from curiosity and partly as a mere trial, but no trace of indigo was obtained. The color of the extract was straw yellow and it, of course contained no coloring principle at all. Experiments were then conducted by digesting the cut leaves in water at varying temperatures, for 35 hours. The following table shows a relative yield of indigo at different temperatures:-

<u>Sample No.</u>	<u>Temperature</u>	<u>% of indigotin obtained.</u>
1	30°	.176%
2	40°	.190%
3	50°	.178%

The extraction was carried out in an acid medium, and ammonium persulphate was employed as an oxidising agent. The experiment has been carried out just like the previous experiments throughout the operation. From the above table it is evident that the optimum temperature is 40° C. Bergtheil⁽⁷⁾, however, claims an optimum temperature being 49° - 51° C.

Comparison of the quantity of indigo obtained from an extract of the plant by the action of an acid and an oxidising agent with that obtained by an alkaline extract and oxidising agent.

Experiment No. IV.

The question of the total amount of indigotin obtainable from an extract of the plant by an acid medium or by an alkaline medium has been very important one and has not as yet, been settled. It has long been known that the oxidation of indoxyl solutions, in so far as the crude fermented factory liquid is concerned, is facilitated by the presence of ammonia or lime water, and this view has been confirmed by Rawson and Bergerinck. According to Thomas, Perkin, and Bloxam, the use of a small amount of either of these reagents during the oxidation of the indoxyl was beneficial, and gives an increase of about 5% in the yield of indigo. Both ammonia and lime water when used in suitable amount inhibits some secondary change of indoxyl in the course of the oxidation, although the chemical nature of this change is, as yet, not definitely known. But in using either of the reagents, an appreciable amount of indirubin is produced, and

(6) Lectures on Awa Indigo, pp. 54 - 55. (Published in Japanese)

(5) Chem. Soc. Trans. 1907, 91, 435.

(7) J. Chem. Soc. Trans. 1904, 85, 886.

for the formation of the indirubin, isatin is absolutely indispensable, and it is very likely that, in the presence of the above reagent, an excessive production of indirubin is formed, and the result is that the yield of indigotin is decreased under these circumstances. For the facts thus presented, and from a series of experiments which will be described presently, the writer cannot agree with these chemists at all.

The following series of the experiments were conducted at a temperature of 30° C., steeping for 24 hours, and using 50 C.C. of 5% solution of ammonium persulphate for the oxidation. Hydrochloric acid was used for the acid medium, and ammonia for the alkaline medium.

<u>Sample No.</u>	<u>% of indigotin obtained.</u>
1. { (a) acid medium	.34%
{ (b) alkaline medium	.12%
2. { (a) acid medium	.38%
{ (b) alkaline medium	.13%
3. { (a) acid medium	.23%
{ (b) alkaline medium	.10%

From the above results it is evident that extraction in an acid medium gives much better yield of indigotin than in the alkaline medium. Moreover, the quality of the dye prepared in the acid medium is superior to that of the alkaline medium. It is curious enough that indigo does not separate out at all in the latter case even after the oxidation is complete, as it does in the case of the acid medium. However, when the extract is

acidified, then the separation of indigo takes place slowly.

Perkin and Thomas⁽⁸⁾ observed another interesting phenomenon, namely that when hydrochloric acid is replaced by an equivalent amount of sulphuric acid, a much lower result yield of indigo is obtained under similar conditions. The decreased yield may be due to the fact that the enzyme action is more favored by the hydrochloric acid, or else some portion of indoxyl is converted into substances other than indigo in the course of the fermentation. Another difference was observed by the same chemists that whereas in the case of hydrochloric acid the filtrate (after indigo being separated) has a pale yellow color, that containing sulphuric acid has a browner and darker tint.

(8) Chem. Soc. Trans., 1909, 95, 795.

ESTIMATION OF INDIGOTIN FROM AIR DRIED LEAVES.

I. Discussion as to the Existence of Indigotin in the Dry Leaf.

Perkin⁽¹⁾, in his discussion of a product obtained in the manufacture of natural indigo, shows that the air dried leaf contains little or no indican, since it gives no corresponding reaction for the indican. Perkin believes that indican does not exist in the dried leaf. On the other hand, Orchardson, Wood, and Bloxam⁽²⁾ proved the presence of indican, and obtained indigo by extracting the dried leaf with hot water and oxidizing the extract with ammonium persulphate in an acid medium. Accordingly they held a view that the indican in the dry leaf exists in the same manner as in the green leaf.

After a series of experiments on the dry leaf had been carried out, the writer became convinced that neither of these chemists were right because:

(a) The indican was not destroyed or lost, as Perkin supposed, but it has been transformed into indoxyl, by the enzyme action, and then finally into indigo during the process of the sun drying. It is a common occurrence during the season of indigo growth that the young leaves from the plant when exposed to the sun turn to deep blue color in a few minutes. If they are placed in a hot drying oven, no change of color takes place because the enzyme is destroyed by a hot temperature. This phenomenon of the color change leads the writer to believe that the production of indigotin is the final step in the leaves, and that what Perkin failed to observe: and

(1) Chem. Soc. Trans. Mar. 1916, 213.

(2) J. Soc. Chem. Ind. Jan. 15, 1907, 7 - 8.

(b) It naturally follows that, since indigotin is already formed in the dry leaves; it cannot be extracted by hot water for indigotin is insoluble in it. Therefore, the hot water extraction method by the joint chemists is entirely inpracticable.

II. Method of Obtaining Indigo from the Dry Leaves.

Since the insolubility of the coloring matter in the dry leaf has been established, the first step is to convert it into a soluble form, whereby the amount of indigo may be determined. The only way to make it soluble is to convert it into what is known as indigo white which forms a brownish yellow solution. For this purpose the writer tried many different commonly known reducing agents, such as sulphurous acid, stannous chloride, zinc with hydrochloric acid, etc. All of these acid reducing agents had no effect at all on the dry leaf. But a totally different effect was produced by action of alkaline reducing agents such as zinc and caustic alkalies, glucose and alkalies and some others. After a series of careful observations, the writer found a method for the manufacture of indigo from dry leaves, and it is his earnest hope that his method may be of some service to the industry of indigo. Many chemists had previously failed to obtain a satisfactory result, and the problem of indigo manufacture from the dry leaves has been unsettled even at the present time at least so far as Japan is concerned.

The following procedure has been found to give the best results: Grind the leaves to a very fine powder, and place from 20 - 25 grams of sample with a sufficient amount of zinc powder

in a 1 1/2 liter beaker containing 750-800 c.c. of a 5% solution of caustic soda. Warm the content of the beaker on a steam bath for an hour, and then heat to boiling by free flame and let it boil for 15 minutes. While boiling, prepare a coarse cloth filter, and filter immediately through the cloth. The residue is again boiled with the caustic soda solution in order to insure all the indigo is completely dissolved out, filtering through the same filter. Wash the residue with the hot caustic soda solution for several times receiving the filtrate in the same beaker. Let the filtrate cool. It will be a brownish yellow solution, covered with a thin blue sheet on the surface, which gradually turns to deep blue liquid when the air is passed through it. Now acidify the solution with dilute sulphuric acid. A deep blue heavy flocculent precipitate is formed. Let it stand over night. Decant off the clear liquid which has a golden yellow tint, and filter through a hardened paper. Wash the precipitate with water until free from acid reaction, and continue the washing with alcohol until no more reddish tint comes through the filter. The precipitate is dried in a drying oven at 70°C. The dry substance thus produced is a crude indigo which is an amorphous black mass. It may then be submitted to purification, and analysed for the estimation of the indigotin content in the sample. The methods of purification and of analysis are precisely the same as in the case of the crude indigo from the green leaves, as previously described.

To determine the strength of caustic soda which effects the maximum yield of indigotin.

Experiment No. V.

<u>Sample No.</u>	<u>Strength of NaOH</u>	<u>% of indigotin obtained</u>
1	1%	.54%
2	2%	.83%
3	3%	.84%
4	4%	.90%
5	5%	.91%
6	6%	.90%
7	7%	_____
8	8%	.72%
9	9%	.54%

From these results, it is evident that the maximum amount of indigo is obtained when the strength of 5% caustic soda solution is used. The variation from 4% to 6% solutions in the indigo yield is very slight indeed, but beyond 6% solution it decidedly decreases the yield.

Other Methods of Extracting Indigo from Dry Leaves.

Acetic-sulphuric acid was also used as a solvent, and indigotin obtained by this solvent is compared with that obtained by the alkaline reducing method as shown in the following table. The difficulty with the acetic sulphuric acid was that it dissolved many impurities with indigo, and then in turn combined with the latter, and the compound thus formed was very poor indigo. Acetic sulphuric extraction was also carried out in a soxhlet extraction but no indigo was obtained at all.

A third and by far the best method which as the writer has found was that the leaves are first treated with alcohol which dissolves out pigments other than indigo, and the non-pigment leaves were submitted to the reducing method mentioned above. The result, as indicated below, gave not only the best yield, but also a superior quality of indigo.

<u>Method.</u>	<u>% of indigotin obtained.</u>
Reduction by 5% NaOH and zinc.-----	.91%
Acetic sulphuric acid extraction method.-----	.25%
Alcohol extraction followed by the alkaline reduction method.-----	1.08%

PURIFICATION OF THE CRUDE INDIGO MANUFACTURED IN THE
PROVINCE OF TOKUSHIMA, JAPAN.

I. Character of Indigo.

The manufacture of indigo in the province of Tokushima is carried out by a fermentation method by which the plant tissues are submitted to decay rather than extracting the coloring principle from it. The indigotin content prepared in this method runs from 2% to 7%. The impurities, which run very high are composed chiefly of plant tissues and mineral matters. On account of such high content of the impurities and also of low grade quality, it is most important to find out a suitable and economical method for its purification. Two or three methods have been suggested, which are described below, were tried out by the writer on a sample of crude indigo obtained from his father's farm in Tokushima, Japan.

II. Methods and Processes of Purification.

1. Reduction methods.

(a) Reducing the crude indigo with caustic alkalies and zinc. The process is just precisely the same as in the treatment of the dry leaves which are previously mentioned.

(b) Hydrosulphite method.

For one part indigo use eight parts of caustic soda solution (19%), twenty parts strong hydrosulphite solution⁽¹⁾, and ten parts of water.

(1) For the preparation of hydrosulphite solution, see Indigo M L B, by H. A. Metz and Co. 72 pp.

The mixture is heated to boiling and the boiling continued for 25 minutes. The solution is rapidly filtered through a hardened paper, and the later steps of operation are carried out practically in the same manner as in the case of the alkaline reducing method.

2. Dissolving indigo by solvents.

Indigo is separated by dissolving in solvents, such as acetic sulphuric acid, aniline, nitro benzene, and so forth. None but acetic sulphuric acid was used in this investigation because the other solvents are too expensive for this purpose, and whenever a method is impractical for the commercial preparation of the dye, the writer did not take it into any consideration at all.

The method by acetic sulphuric acid solvent as follows:- About 5 grams of the fine powdered indigo were weighed into a beaker containing 100 c.c. of acetic sulphuric acid (100 parts of glacial acetic acid and 4 parts of concentrated sulphuric acid)⁽²⁾ and the mixture heated on a steam bath for an hour. The solution was then filtered through glass wool to a beaker containing 1000 c.c. of water. The indigotin gradually settles down to the bottom of the beaker. After it stands for some time, decant off the clear liquid, and the indigotin is filtered through a hardened paper. Wash the indigotin with hot water until free from acid, and followed the washing by alcohol until

(2) Zeitsch. farb. text. chem., 1903, 10, 189.

no more reddish solution passes through the filter. Dry the indigotin in a drying oven at 105°C. It may be necessary to repeat the same operation for twice or three times to obtain chemically pure indigotin, but for ordinary determination of its purity one operation is sufficient.

The following figures show a relative yield of indigotin obtained by the different methods of purification:-

<u>Sample No.</u>	<u>% of indigotin obtained.</u>
1. Reduction by caustic soda and zinc.-----	2.58%
2. Reduction by hydrosulphite.-----	2.11%
3. Acetic sulphuric acid method.-----	3.71%

Obviously the acetic sulphuric acid show the best result, and this method of purification is therefore, recommended by the writer.

NOTES ON SOME CONSTITUENTS OF NATURAL INDIGO.

Indigo, as it is commercially called, is a mixture of various preparations of several substances most of which has no tinctorial value. In addition to the indigotin natural indigo contains varying proportions of indirubin, or indigo red, indigo brown, indigo gluten, and the remainder is organic and mineral matter of no practical value. The amount of the indigotin present in crude product varies from 2% to 80%.

(a) Indirubin or indigo red.

It is now confirmed that the indirubin originates from the indican existing in the leaves of the various species of Indigofera, and is due to no second constituent of the plant, its production being due to the condensation of isatin with indoxyl. At one time indirubin was considered to be a valuable constituent of natural indigo, but it is now known that such is not the case. It contains from 2% to 9% in crude indigo.

(b) Indigo brown.

After careful studies by Chevreul, Perkin, and Bloxam and Rawson, it was proved that the constituents of indigo brown are derived from indoxyl during the manufacture of indigo from the plant. The fact that indican itself, when boiled with dilute acid, produces the very similar indoxyl brown, and the isolation of brown substances from indigo prepared by the hydrolysis of pure indican in the laboratory, agrees with this suggestion. When indigo brown is sulphated, a dilute solution shows a dark brown color, and is attacked by permanganate. This sulphated indigo brown is responsible for the dirty end point after ob-

served when titrating a crude indigo sulphonic acid without purification. It was found impossible to obtain a sharp end point when this impurity is contained in indigo. Therefore, it is absolutely necessary to remove this impurity in order to obtain accurate results. Pyridine is the best solvent for indigo brown. It contains from 5% to 10% in all indigos.

(c) Indigo gluten.

In crude indigo, indigo gluten exists in combination with mineral matter, possibly as calcium compound. It is removed by means of dilute mineral acid, During the sulphonation of indigo it goes into solution, and it is impossible to tell by color observation whether it is carried away with the other impurities or remains associated with the indigotin. It does not in any way obscure the end point of the titration with the permanganate. This point escapes very easily the notice of workers who may think the clearness of the end point is necessarily an indication of the product oxidised by the reagent. The indigo gluten is more easily oxidised than indigotin sulphonic acid, and its presence, therefore, increases the consumption of the reagent, and so it gives rise to error in the estimation of indigotin.

SUMMARY.

1. Indigo exists only in the green leaf of *Polygonum tinctoria*. Hence the leaves are only concerned in the manufacture of indigo.
2. The coloring principle in the green leaf is known as indican which is a glucoside of indoxyl. The hydrolysis of the indican produces the indoxyl and glucose, and when the indoxyl is oxidised, indigotin is produced.
3. The best method of obtaining indigo from the green leaves must be such that the plant tissues are in the finest state of division, and the extraction with water is carried out in an acid medium, for from 30 to 33 hours with the optimum temperature at 40°C.
4. In the air dried leaves the indican has been converted into indigotin by the drying process. The indigotin is separated out by converting it into indigo white by means of alkaline reducing agents only. The indigo white is converted into indigotin by oxidation followed by the precipitation with an acid. The maximum yield of indigotin is obtained when a 5% solution of caustic soda and a sufficient quantity of zinc powder are used.
5. In the purification of the crude indigo manufactured in the province of Tokushima a mixture of acetic and sulphuric acids as a solvent gives the best result.

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