CLXII.—*Indican. Part I.*

By Arthur George Perkin and William Popplewell Bloxam.

According to the early researches of Chevreul (*Ann. Chim.*, 1808, **66**, 8, and 1808, **68**, 284), and of Giradin and Preisser (*Journ. Pharm.*, 1840, **26**, 344), the colouring principle of indigotin present in indigo-yielding plants was considered to consist of indigo-white,

and this theory remained uncontradicted until Schunck (*Phil. Mag.*, 1855, [iv], **10**, 74, and *ibid.*, 1858, **15**, 127) isolated from the *Isatis tinctoria* and *Polygonum tinctorium* a glucoside which he named indican. This compound, $C_{26}H_{31}O_{17}N$, on hydrolysis, gave indigotin and a sugar, indiglucin,

$$2C_{26}H_{31}O_{17}N + 4H_2O = C_{16}H_{10}O_2N_2 + 6C_6H_{10}O_6,$$

and it appeared that during the reaction the indigotin at first formed was reduced to indigo-white, and subsequently reoxidation took place. Later, however, Schunck and Römer (*Ber.*, 1879, **12**, 2311) showed that indican, when hydrolysed in the absence of air, gave a product which, when treated with oxidising agents, did not yield indigotin.

Much more recently, Marchlewski and Radcliffe (*J. Soc. Chem. Ind.*, 1898, **17**, 434) suggested that indican had possibly the formula $C_{14}H_{17}O_6N$, and that, on hydrolysis, glucose and indoxyl were formed, the latter on oxidation being naturally converted into indigotin,

$$C_{14}H_{17}O_6N + H_2O = C_8H_7ON + C_6H_{12}O_6.$$

As a result of the communication of Marchlewski and Radcliffe, Hazewinkel, the Director of the experimental station for indigo, Klaten, Java (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **2**, 512), gave an account of a research concluded in 1898, which he had hitherto considered to be to the interest of the Java planters to keep secret. In this important paper he gives proof that indican is an indoxyl glucoside, and that the sugar obtained from it is dextrose. The elaborate researches of Beyerinck, van Romburgh, and other Dutch chemists indicated that indican was far more stable than Schunck supposed, and eventually led to the isolation of this glucoside in a crystalline condition from the *Indigofera leptostachya* and *Polygonum tinctorium*, by Hoogewerff and ter Meulen (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **2**, 520). Analyses and a molecular weight determination of their substance showed that it possessed the formula $C_{14}H_{17}O_6N$, and when crystallised from water contained $3H_2O$. By passing air through a hot solution of the indican in dilute hydrochloric acid they obtained 91 per cent. of the theoretical yield of indigotin, which appeared, however, to contain some indirubin, and of the purity of the mixture they could not be certain. The identity of the sugar formed during the reaction they proposed to ascertain when they possessed sufficient glucoside for the purpose.

In a paper by Beyerinck (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **3**, 101) "On the Formation of Indigo from Woad," this chemist discusses Schunck's well-known paper on the same subject (*loc. cit.*), and points out that the indigo-yielding substance con-

tained in this plant is not, as Schunck regarded it, identical with the indican present in the *Polygonum tinctorium*. This colouring principle of woad Beyerinck names isatan, and shows that this compound, unlike indican, is decomposed in feebly alkaline solutions, whereas indican itself is stable in concentrated alkaline liquids. In the presence of acids both isatan and indican are hydrolysed, but indican with greater difficulty, a point which is specially evident when acid salts are employed. Isatase, the specific enzyme of woad, does not act on indican, and isatan, on the other hand, is unaffected both by the indigo enzyme or by common bacteria. Schunck (*Chem. News*, 1900, **82**, 176) considered that the crystalline indican of Hoogewerff and ter Meulen was not the substance obtained by him, and should not be considered as a pure variety of it, but was rather derived from it by extracting the plant with a hot solvent and the use of chemicals. He preferred to name his compound *a*-indican and theirs *b*-indican. With regard to Beyerinck's criticisms, he was at a loss to understand the views of this author.

Finally, Bergtheil (Trans., 1904, **85**, 877) did not find it possible to prepare crystalline indican in the manner described by Hoogewerff and ter Meulen.

In connexion with the study of natural indigo and its formation from the plant, which has been in progress in this laboratory for the past two years, it was necessary to obtain some quantity of pure indican for the elucidation of several points in this work. Without the possession of this substance, any exact knowledge of the efficiency of the Indian process could not be determined, for the methods of leaf analysis there employed have hitherto been based upon no standard.

The specimens of leaf investigated were collected and forwarded to us from India by Government botanists, and although it is only likely that the samples had deteriorated to some extent during the collection and drying operations, they contained considerable quantities of indican.

We are greatly indebted to Prof. Beyerinck, of Delft, and Prof. van Romburgh, of Utrecht, for specimens of crystalline indican, and we also express our thanks to Messrs. Burroughs Wellcome and Co. for very kindly undertaking the extraction of a considerable quantity of the air-dried leaves of the *Indigofera Sumatrana*, and to Dr. G. Barger for his interest in the matter.

Experimental.

For the preparation of indican, Hoogewerff and ter Meulen (*loc. cit.*) employed the *Indigofera leptostachya* and *Polygonum tinctorium*, and obtained 5 grams of the pure glucoside from 17 kilo-

grams of the plant. The essentials of their process are as follows:—The plant is extracted with hot water, certain impurities precipitated with baryta water, and the solution evaporated to dryness. A concentrated methyl-alcoholic extract of the residue is treated with ether to precipitate non-nitrogenous compounds, the purified liquid evaporated, and the product dissolved in a little water. The filtered and concentrated solution on standing deposits crystals of indican.

During a careful study of this ingenious method, both with the *Indigofera Sumatrana* and *I. arrecta*, small quantities of crystalline indican were isolated, but it was soon evident that the operations, although of a simple character, became most tedious when working with large quantities of leaf extract, and, moreover, that in our hands a considerable loss of colouring principle took place towards the end of the evaporation process. The method was accordingly abandoned, because the labour involved in preparing any large quantity of the glucoside in this manner would be very great, and because it appeared likely that by studying the solvent properties of the crystalline indican we possessed, a simpler process could be devised. Experiment indicated that acetone would be suitable for the removal of the colouring principle from the leaf, and especially so as it was observed that the viscous by-products which had been isolated from the plant in the earlier work were but slightly attacked by this solvent. As a result, this selection was justified, and the following simple method originated.

One thousand grams of the leaves and stems of the *Indigofera Sumatrana* (analysed previously and estimated to yield 3·13 per cent. of indigotin) were treated in a large bottle with 4 litres of acetone, the mixture being occasionally shaken during seven days. Up to the present, this operation has always been carried out in the cold, for although this is probably not an essential feature for success, it proceeds so satisfactorily that it has been adopted throughout.

The green-coloured acetone solution was then filtered, the residual leaf rinsed with the solvent, and the liquid evaporated in the first operations by means of a vacuum at the ordinary temperature to a volume of about 150 c.c. In subsequent experiments, however, the acetone was removed by distillation on the steam-bath in the usual manner. To this residue about ten times its volume of light petroleum was added, causing the deposition of a yellowish-brown, viscous precipitate, which was repeatedly agitated with small quantities of light petroleum until a green-coloured extract was no longer formed. The product, on treatment with water, yielded, in the earlier experiments, a pale yellow liquid containing some quantity of grey matter in suspension, which could readily be removed by filtration,

but with the more recent samples of plant the precipitate at this stage was too viscous to permit of separating the solid matter in this way. In such cases, decantation was resorted to, and the solution was clarified by agitation with ether, as but little indican is dissolved by this solvent. The clear aqueous liquid (A), after removal of dissolved ether under reduced pressure, was decanted from a small quantity of tarry deposit, treated with 10 c.c.* of $N/2$ sodium carbonate, to neutralise plant acids, and placed in a vacuum desiccator over sodium hydroxide. Within a few hours the sides of the containing vessel became coated with crystals, and in about three days a semi-solid, crystalline mass was obtained. The product was collected on a Buchner funnel and drained on porous tile; when dry, it weighed 16·40 grams. The mother liquor, again evaporated in a similar manner, gave a further 6·415 grams of the glucoside.

The residual liquid was still rich in indican, but on concentration was too viscid for filtration, and it is here that a somewhat serious loss of substance occurs. To obviate this as far as possible, the mixture, dissolved in water, was treated with finely-powdered potassium sulphate, which caused the precipitation of a brown, tarry impurity, together with some quantity of the glucoside. The solution was evaporated to dryness in a vacuum, the residue extracted with acetone, the solvent removed from the extract, and the crude indican crystallised from water. In this way, approximately 2 grams of the substance were recovered.

The residual leaf was again submitted to two extractions with acetone, by which means respectively 3·654 and 3·17 grams of indican were obtained. Accordingly, 1000 grams of leaf yielded 31·66 grams of this substance, and this quantity could have been enhanced by a further digestion of the leaf material, preferably with boiling acetone. Analysis showed, for instance, that it still contained 1·8 per cent. of glucoside, but as the object of the work was the rapid preparation of a large quantity of indican, this residue was not again extracted.

As a rule, only one digestion of the leaf with acetone was carried out, and the highest yield as yet obtained in this single operation was 32·5 grams of the substance from 1250 grams of raw material. By the adoption of a continuous method of working, a weekly return of about 30 grams of the glucoside was obtained, even during the summer, which would no doubt have been materially increased if the operations had been conducted in the winter season.

The main sources of the defective yield are, no doubt, to be ac-

* The amount of alkali necessary varies with the sample of plant employed. It is very important that sufficient be added to prevent formation of indigotin, but excess, on the other hand, causes gelatinisation.

counted for in the final mother liquors, and by the draining of the crude substance on tile, and this is impossible to avoid with so soluble a compound as indican; on the other hand, some loss undoubtedly does occur during the prolonged evaporation of the aqueous liquid in a vacuum, for this, which at first possesses a pale yellowish-brown tint, soon becomes darker, and if left for several days the discoloration rapidly increases.

The indican, when dry, consisted of an almost colourless mass of silky needles and was usually free from indigotin, but sometimes possessed a faint pink colour. For purification it was dissolved in warm water (1 gram in 4 c.c.), the solution allowed to cool in melting ice, and the crystals which separated, collected by the aid of the pump. A second crystallisation (1 gram in 5 c.c. of water) yielded, as a rule, the pure compound, which, when dry, melted at 57—58°.

The melting point given by Hoogewerff and ter Meulen for their product in this condition is 51°, and caused at first the supposition that the indoxyl glucoside present in the *I. Sumatrana* was distinct from that occurring in the plants they examined. An examination, however, of the preparations given to us by Profs. Beyerinck and van Romburgh showed that after crystallisation from water the melting point was 57—58° in each case, and it is therefore to be presumed that the figure 51° is the result of a clerical error.

Hoogewerff and ter Meulen further state that when indican, crystallised from water, is dried over sulphuric acid in a vacuum, it melts at 100—101°, and is then in the anhydrous condition. Experiment has shown, however, that although 100—101° is approximately the melting point of indican which has been dried in this manner, this is not the melting point of the anhydrous substance. For instance, when indican melting at 57—58° is heated above this point, it gradually resolidifies, and the crystalline mass thus obtained melts at 176—178°. This reaction is best observed by exposing the indican melting at 57—58° to the heat of the water-oven in a flat dish, a hard, crystalline cake being formed after some hours, and this solidification occurs much more rapidly at 110°. No decomposition, as stated by Hoogewerff and ter Meulen, beyond a slight discoloration, was observed by heating the glucoside thus treated above 100°, until near or about its melting point, but these, however, are the only details in which our work is at variance with that of these chemists.

To determine the water of crystallisation, the indican was heated for some hours in the steam-oven, then at 110°, until constant, and finally at 160°:

Found, $H_2O = 15{\cdot}25, 15{\cdot}16, 15{\cdot}07, 15{\cdot}42$.

$C_{14}H_{17}O_6N,3H_2O$ requires $H_2O = 15{\cdot}47$ per cent.

Analyses of the anhydrous indican gave:

C = 56·81; 56·72; H = 6·05; 5·94; N = 4·83.

$C_{14}H_{17}O_6N$ requires C = 56·94; H = 5·76; N = 4·75 per cent.

In an experiment during which air-dried indican previously crystallised from water was exposed in a vacuum over sulphuric acid until constant, it lost 13 per cent. in weight, corresponding to $2\frac{1}{2}H_2O$ (13·23 per cent.). On further heating to 110°, and subsequently to 160°, for a few minutes a further loss of 2·23 per cent. was experienced, corresponding approximately to $\frac{1}{2}H_2O$ (2·95 per cent.), and the total diminution in weight was thus 14·84 per cent. Indican dried in a vacuum, it was observed, had a behaviour which, as already indicated, supported the statements of Hoogewerff and ter Meulen, for it melted at about 100—101°, and remained in a fluid or semi-viscous condition after long heating to 110°. On cooling, it formed a brittle, vitreous mass containing no crystals. When subsequently heated to 160°, however, it rapidly became crystalline, although much discoloured, slight hydrolysis, no doubt, taking place at this temperature owing to the trace of water which was present. If indican dried in a vacuum is allowed to stand in moist air it approximately gains its original weight, as observed by Hoogewerff and ter Meulen, and this product, on now heating to 100°, quickly passes into the anhydrous crystalline condition.

As the purification of indican by means of water is far from economical, the following process, which succeeded better, was devised. The crude glucoside, containing water of crystallisation, was dissolved in about 3 parts of boiling absolute alcohol, the solution filtered, if necessary, by means of a vacuum, and while hot treated with boiling benzene to the point of turbidity. In case the liquid separated into two layers, a trace more alcohol was added, and the mixture allowed to cool. Small, colourless prisms soon commenced to separate, and the deposition was hastened by agitation, for otherwise the crystallisation was not complete for some hours. The product, after being collected and washed with benzene, melted sharply at 176—178°, and although possessing a faint pink colour was very nearly pure. The yield averaged 70 per cent., and a further quantity, although of inferior quality, separated from the mother liquors during distillation. A second treatment in a similar manner, employing animal charcoal, gave a colourless substance of extreme purity.

This method of purification was most useful when the glucoside was contaminated with traces of indigotin, for this, although readily removed by filtration when alcohol was used as the solvent, was not completely retained by the paper, and was a source of considerable trouble when aqueous solutions were employed.

The indican thus obtained was in the anhydrous condition, and differed considerably from the hydrated variety, being now somewhat sparingly soluble in boiling absolute alcohol and in acetone; from the former solvent it separated in colourless prisms. The production of this anhydrous form also occurs, although somewhat slowly, by digesting the hydrated glucoside with boiling absolute alcohol for a few minutes, and is deposited in this condition on cooling. Analysis of indican crystallised from benzene and alcohol gave:

C = 57·05; H = 6·16; N = 4·88.

$C_{14}H_{17}O_6N$ requires C = 56·94; H = 5·76; N = 4·75 per cent.

The general properties of the indican from the *Indigofera Sumatrana* are in agreement with those given by Hoogewerff and ter Meulen for their substance, so that the identity of the two products is evident, and no further description in this respect is necessary.

The fact that indican can be so readily isolated entirely without the aid of heat, and merely with the use of acetone, light petroleum, and ether,* is not in harmony with the contention of Schunck (*loc. cit.*), at all events in so far as the *Polygonum tinctorium* is concerned, that the crystalline glucoside is an alteration product of his amorphous indican, and consequently the terms *a*- and *b*-indican suggested by him should disappear. That Schunck obtained a concentrated form of indican from this plant cannot be doubted; moreover, the results he obtained so many years ago formed a valuable contribution to this important question.

The Analysis of Indican Solutions by Means of Isatin.—The fact that isatin condenses with indoxyl to form indirubin,

$$C_8H_7ON + C_8H_5O_2N = C_{16}H_{10}O_2N_2 + H_2O,$$

was, as is well known, discovered by Baeyer (*Ber.*, 1881, **14**, 1745), and this reaction has been employed both by Hazewinkel (*loc. cit.*) and by Beyerinck (*Proc. K. Akad. Wetensch. Amsterdam*, 1899, **2**, 120) for the characterisation of the indoxyl produced by the hydrolysis of indican. In the latter paper Beyerinck suggests that a quantitative indican determination may be based on this reaction, and as a result of this Orchardson, Wood, and Bloxam (*J. Soc. Chem. Ind.*, 1907, **26**, 4) have adopted such a method for the analysis of the indigotin-yielding content of the plant.† It was necessary therefore to determine by means of pure indican if the

* The term "chemicals" employed by Schunck (*loc. cit.*) can hardly be taken to include the use of the sodium carbonate for the neutralisation of plant acid. On the other hand, crystalline indican can be isolated without adopting this precaution, but the yield is decreased by the formation of indigotin and brown products.

† The various leaf analyses given in this paper have been carried out by this process.

reaction is quantitative, and this was carried out in the manner described by these authors. Two distinct samples of indican were employed, (*a*) purified by crystallisation from water, and rendered anhydrous by drying, and (*b*) obtained in the anhydrous condition by means of benzene and alcohol.

(*a*) 0·094	indican gave	0·083	indirubin. Theory requires	0·0835.
(*b*) 0·0992	„	0·089	„ „	0·0887.
(*b*) 0·0992	„	0·0885	„ „	0·0887.

The indirubin thus prepared was analysed.

Found C = 73·19; H = 4·02; N = 10·81.

$C_{16}H_{10}O_2N_2$ requires C = 73·28; H = 3·81; N = 10·69 per cent.

The reaction must therefore be considered as quantitative, and the suggestion of Beyerinck is thus confirmed.

Experiments on the persulphate method of analysis, originally devised by Rawson, and modified independently by Orchardson, Wood, and Bloxam (*loc. cit.*), and by Bergtheil (*J. Soc. Chem. Ind.*, 1906, **25**, 734), have more recently been carried out by Gaunt, Thomas, and Bloxam (*private communication*), employing pure indican. As a result, they were unable to obtain theoretical figures, and it accordingly appears that no method has yet been discovered by which a quantitative yield of indigotin can be obtained from this substance. Hazewinkel (*loc. cit.*) states, in regard to this point, that acid oxidising agents convert indican into indigo, and this in turn is oxidised by an excess of the reagent.

A very interesting result is obtained when nitrosodimethylaniline is employed as a source of oxygen, but the quantitative aspect of the process has not yet been determined. If indican dissolved in glacial acetic acid is treated with a small quantity of nitrosodimethylaniline no reaction appears to occur, but if a drop of hydrochloric acid be added and the mixture warmed, glistening leaflets of indigotin (Found N = 10·45) quickly separate. With the same reagents in aqueous solution, the indigotin is deposited in very minute crystals.

The Action of Acids on Indican.

It has been shown by Schunck and Römer (*loc. cit.*) that when their indican was exposed to acids in a vacuum, a compound was obtained which did not yield indigotin on oxidation, and in a previous communication (Trans., 1907, **91**, 295) such was shown to be the case when the leaf extract is boiled with acid in the absence of air. The product of the latter reaction was an amorphous brown substance, probably a mixture (found C = 66·04; H = 5·00; N = 5·67), and the clear filtrate from this on examination was shown to be free from indoxyl. These results suggested that the brown com-

pound is in part derived from indoxyl, and the point is interesting in connexion with the origin of indigo-brown.

To obtain more definite information on this subject a preliminary examination of the behaviour of indican towards boiling dilute acids has been carried out. 4·06 grams of the glucoside in 100 c.c. of water were digested at the boiling point with 3 c.c. of sulphuric acid for one and a half hours, care being taken to exclude air. The liquid, at first yellow, soon became brown, a brown, resinous product, together with a little indigotin, soon separated, and the presence of indole was observed by means of its characteristic odour. Although the reaction proceeded rapidly at first, the last traces of indoxyl disappeared somewhat slowly, and as soon as this appeared to be absent, the mixture was cooled, and the insoluble matter collected and dried.

Curiously enough, this weighed 1·815 grams, for this was almost identical with the weight of indoxyl which would be liberated by the hydrolysis of the 4·04 grams of indican. An explanation is thus given of the brown precipitate referred to above, which is obtained by the digestion of an aqueous extract of *Indigofera* leaves with acid, although, as previously discussed, the low nitrogen content of this product is due either to its contamination with a non-nitrogenous compound of the same colour, or to the condensation of the indoxyl, or products thus derived from it, with some compound of this character present in the extract.*

The crude product of the reaction was extracted with boiling alcohol, by which means a small quantity (0·08 gram) remained undissolved; this consisted chiefly of indigotin, contaminated with a trace of a brown substance soluble in pyridine. The alcoholic extract, partly evaporated, was treated with ether, causing the precipitation of a brown amorphous compound, which was collected and washed with ether, and when dry weighed 0·65 gram. By evaporating the filtrate to a small bulk, and adding much ether, a further quantity, 0·25 gram, was isolated.

Analyses of this product, dried at 160°, gave:

C = 68·10; H = 4·10; N = 9·34 per cent.

It is sparingly soluble in alcohol, and when heated it is carbonised with evolution of a small quantity of yellowish-brown vapours, which condense to form an oil of the same colour. It is readily attacked by nitric acid, forming a deep brown liquid, and this, after standing for a short time, gives, with water, an orange-yellow amorphous precipitate.

* The clear filtrate from this product contains a minute trace of an acid occurring in colourless needles, m. p. about 127—129°. This has not been yet identified and its connexion or otherwise with the indoxyl is uncertain.

It is remarkable that the percentage composition of this substance is almost identical with that which was ascribed by us to the main constituent of indigo-brown (*loc. cit.*):

C = 68·57; H = 4·28; N = 10·00 per cent.,

and that the two products are very closely allied admits of but little doubt. From the very different circumstances which surround their formation, it could hardly be anticipated that they would prove to be identical, but, on the other hand, the differences appear to be but slight. The compound in question, for which the name *indoxyl-brown* is proposed, is more soluble in alcohol than the indigo-brown substance of the above composition, and in this respect resembles the more soluble and minor constituents of this product. With boiling dilute alkalis it is but little attacked, and its hot alcoholic solution on treatment with a drop of aqueous potassium hydroxide acquires a green tint. Unfortunately, no specimens of the more soluble indigo-brown compounds were available, so that at present it is impossible to say whether they also give this coloration. The production of brown compounds by Schunck (*loc. cit.*) from his indican, and the statement of Hazewinkel (*loc. cit.*) as to the behaviour of indoxyl in this respect, have already been dealt with in a former communication, and as experiments with pure indican have thus given a similar result, it is evident that this brown formation is a characteristic reaction of indoxyl.

The suspicion already discussed, that the presence of indigo-brown in natural indigo is due to the occurrence of a secondary reaction during the process of manufacture, becomes thus considerably strengthened, and results of more recent work at present incomplete have been most suggestive in this respect.

The Sugar.—The aqueous filtrate obtained during the formation of the indoxyl-brown was neutralised with barium carbonate, filtered, the clear liquid decolorised with animal charcoal and evaporated. The pale yellow, syrupy residue gave in the usual way, with phenylhydrazine, a voluminous, yellow precipitate, which was washed with a mixture of alcohol and chloroform and finally crystallised from alcohol. This compound melted at 204—205°, and was identical with glucosazone, so that the statement of Hazewinkel (*loc. cit.*), who, however, did not employ the crystalline glucoside, that indican, when hydrolysed, yields indoxyl and dextrose, is thus confirmed.

Indigofera arrecta.

As already indicated, it was possible by the method of Hoogewerff and ter Meulen to isolate indican from the leaves of this plant, and it was evident that this substance was identical with that

present in the *I. Sumatrana* and *Polygonum tinctorium*. The *I. arrecta*, known as the Java or Natal plant, contains as a rule, in its leaf, a larger quantity of the glucoside than the ordinary Indian plant, a fact appreciated by the Dutch, and on this account, although somewhat late in the day, it is being experimented with in India for the manufacture of indigo.

Employing the acetone extraction process for the isolation of indican, satisfactory results have been obtained, but, on the other hand, the method does not proceed so well as with the *I. Sumatrana*. After evaporation of the acetone solution, treatment with light petroleum, solution in water, and removal of impurity by ether, the clear aqueous liquid containing the glucoside does not always yield crystals on evaporation in a vacuum. When this is the case, the viscous residue, dissolved in methyl alcohol, is treated with ether, to precipitate a colourless impurity; this is removed by decantation, the clear liquid evaporated, and the product dissolved in water and set aside to crystallise. Five hundred grams of the leaf (indigotin value $=0{\cdot}865$), extracted for five days in the cold with 2500 c.c. of acetone, gave 1·28 grams of the glucoside. An analysis of the substance, dried at 100°, gave:

C = 56·83; H = 5·83.

$C_{14}H_{17}O_6N$ requires C = 56·94; H = 5·76 per cent.

When crystallised from water, it melted at 57—58°, and in the anhydrous condition at 176—178°. The sugar yielded by the hydrolysis of this substance gave an osazone melting at 204—205°, which was glucosazone. The poor yield of indican obtained from this leaf, and the general difficulty of the process in regard to it, were found to be due to the presence of some quantity of a second soluble substance which hinders the crystallisation. To isolate this, the aqueous indican solution, after the ether treatment and partial evaporation, was again submitted to extraction with ether in a continuous apparatus for two days, by which means the indican was entirely removed by the ether, and could be partly recovered in the usual way. The residue obtained by the evaporation of the aqueous liquid was dissolved in methyl alcohol, and treated while hot with ether, which at first precipitated an amorphous product, but subsequently caused the deposition of crystals. These were collected and crystallised in a similar manner until colourless.

Found C = 43·56; H = 7·44.

$C_6H_{12}O_5$ requires C = 43·90; H = 7·31 per cent.

It consisted of colourless prisms readily soluble in water and alcohol, and as obtained above melted at 185—187°. This compound, which does not give an osazone, is reserved for further examination, and

we hope shortly to have accumulated sufficient material for this purpose. In a private communication, Dr. F. B. Power, of the Wellcome Research Laboratories, suggests that this is possibly a modification of quercitol, for several of these are as yet unknown.

For the isolation of indican from the leaf, experiments have been made employing cold methyl alcohol as a solvent. This removes the glucoside more readily than acetone, for a sample of the *Indigofera arrecta* (indigotin value = 1·81), after digestion with six parts of the alcohol, had then an indigotin value of but 0·27 per cent. Unfortunately, however, numerous other impurities are thus dissolved which hinder the crystallisation of the indican, so that further work in this direction was abandoned.

Summary of Results.

The results of this investigation indicate that the indoxyl glucoside contained in the leaves of the *Indigofera Sumatrana* and *I. arrecta* is in both cases identical with the indican first isolated in a crystalline condition by Hoogewerff and ter Meulen from the *Indigofera leptostachya*, and from the *Polygonum tinctorium*. By employing acetone as a solvent for the glucoside, it is easy in the case of the *I. Sumatrana* to prepare rapidly large quantities of the pure substance, in amount equal to about 3 per cent. of the air-dried leaf. With the *I. arrecta*, the process, although effective, is not so simple, and this is caused chiefly by the presence of a sugar-like compound, $C_6H_{12}O_5$, possibly a modification of quercitol, which hinders the crystallisation. As the isolation of indican by this process can be carried out entirely without the aid of heat, and no chemicals are necessary, it is evident that the contention of Schunck, in so far as his work with the *Polygonum tinctorium* is concerned, that the crystalline indican is an alteration product of his own compound, cannot be upheld. As stated by Hoogewerff and ter Meulen, indican has the formula $C_{14}H_{17}O_6N$, crystallises from water with $3H_2O$, but its melting point in this condition is 57—58°, and not 51°, as is given in their paper; this is probably a clerical error. When heated to 100°, this product gradually solidifies, with production of the anhydrous substance which melts at 176—178°. When dried in a vacuum, indican, $C_{14}H_{17}O_6N,3H_2O$, melts at about 100—101°, as shown by the above chemists, and becomes crystalline only at a much higher temperature, with slight decomposition. Anhydrous indican is readily prepared by crystallising the hydrated variety from a mixture of benzene and alcohol, and is thus obtained in colourless prisms. Solutions of indican can, by means of isatin, be quantitatively estimated as indirubin, but, on the other hand, a

theoretical yield of indigotin has not yet been produced by a combination of hydrolysis and oxidation.

On boiling indican with dilute acids, dextrose, as stated by Hazewinkel, is obtained, but the indoxyl liberated condenses with formation of brown amorphous products and with simultaneous production of a trace of indole. The main product of the reaction, and which is here termed indoxyl-brown, has a percentage composition almost identical with that of the main constituent of indigo-brown, which it very closely resembles. This point is advanced in further support of the opinion expressed in an earlier communication (*loc. cit.*), that indigo-brown is not only a derivative of indoxyl, but is also formed most probably as the result of a secondary reaction during the manufacture of indigo from the plant. A further study of this question, and other points which bear on the behaviour of indican, in circumstances allied to the actual manufacturing process on the large scale, are being investigated.

Experiments with the object of isolating the colouring principle of woad are in progress; the results at present obtained are in harmony with the statement of Beyerinck quoted in this paper.

Our thanks are due to Mr. F. Thomas for valuable help in connexion with the indirubin analyses given in this paper.

CLOTHWORKERS' RESEARCH LABORATORY,
DYEING DEPARTMENT,
THE UNIVERSITY,
LEEDS.