# Part I) The oxidation of typical carbohydrates
# Part II) Glucosides related to indigo

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A THESIS IN TWO PARTS

I.

# THE MECHANISM OF THE OXIDATION OF TYPICAL CARBOHYDRATES.

II.

# GLUCOSIDES RELATED TO INDIGO.

## PART I

## THE OXIDATION OF TYPICAL CARBOHYDRATES.

## PART II

## GLUCOSIDES RELATED TO INDIGO.

being a Thesis presented by

JAMES CRAIK, M.A.,B.Sc.,

to the University of St. Andrews
in application for the degree of Ph.D.

ST. ANDREWS. APRIL 1924.

# DECLARATION.

I hereby declare that the following Thesis is based on the results of experiments carried out by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Chemical Research Laboratory of the University of St. Andrews , under the direction of Principal Irvine, C.B.E., LL.D., F.R.S., and Dr. A.K.Macbeth.

[redacted]

## CERTIFICATE.

I certify that Mr. James Craik spent four Terms (1921-22) as a Research Student under my direction, and taking into account the subsequent six terms certified by Dr. Macbeth he is entitled to submit a Thesis for the degree of Ph.D.

[redacted]

Principal.

I certify that Mr. James Craik spent six Terms (1922-24) as a Research Student under my direction.

[redacted]

Supervisor.

ST. ANDREWS.

# CAREER.

I matriculated as an Arts and Science Student in the University of St. Andrews in October 1916. In December 1917 I undertook military service and served as a combatant officer until November 1919. At that date I returned to the University and resumed my studies.

In July 1920 I graduated Master of Arts (Mathematics and Chemistry) and in June of the following year I completed the final B.Sc. examination with Special Distinction in Chemistry.

Subsequent to graduation I was elected by the Council of the Society of Chemical Industry to the Latham Research Fellowship and in this capacity spent a year (1921-22) in the Chemical Research Laboratory of St. Andrews where I carried out a research on the oxidation of carbohydrates. The subject was selected for me by Mr C. F. Cross, F.R.S., and the work was directed by Principal Irvine.

In October 1922 I was awarded a grant by the Department of Scientific and Industrial Research and commenced a research on Glucosides related to Indigo. This part of the work along with the previous research undertaken as Latham Fellow is now submitted as a Ph.D. Thesis.

My admission as a Research Student in the University dates from October 1921.

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## PART I.

## PART II.

# PART I.

## THE MECHANISM OF THE OXIDATION OF TYPICAL CARBOHYDRATES WITH HYDROGEN PEROXIDE AND HYPOCHLOROUS ACID.

# INTRODUCTION.

# INTRODUCTION.

The standard literature of the sugars contains numerous references to the oxidation of carbohydrates with acid and alkaline media .In particular exhaustive studies have been made of the products formed on subjecting carbohydrates to the action of nitric acid , potassium permanganate , dichromate , silver oxide and other common oxidisers .

It may be recalled that J.U.Nef and his collaborators (Annalen, 1907, 357, 214 ; 1910, 376, 1 ; 1914, 403, 204 .) spent nine years investigating the oxidation of the simple sugars in the presence of alkali and made a detailed study of the behaviour of carbohydrates dissolved in aqueous alkaline hydroxides towards oxidisigg agents such as air, hydrogen peroxide and the oxides of mercury, silver, and copper . It was ascertained (Annalen , 1914, 403, 204 ) that the oxidation of 47 sugars resulted in the formation of carbon dioxide , formic, glycollic, oxalic and d,l-glyceric acids, four trihydroxy-butyric acids, eight tetrahydroxy-valeric acids and eight pentahydroxy-hexoic acids . These results speak sufficiently for the complexity of the oxidation changes involved .

At an earlier date Cross, Bevan and Smith had pointed out ( *T.*, 1898, *73*, 463 ) that only one reference ( C.Wurster, *Ber.*, 1887, *20*, 2613 ) could be found in the literature where the effect of hydrogen peroxide on the sugars had been tested and the author dismissed the topic with the remark that glucose and cane sugar were remarkably stable towards this reagent. On the other hand, Fenton ( *T.*, 1894, *65*, 899; 1895, *67*, 48; 1896, *69*, 546 ) oxidised tartaric acid by means of hydrogen peroxide in the presence of ferrous salts with surprising results, and later applied the same reaction to the oxidation of polyhydric alcohols (*T.*, 1899, *75*, 1 ). As Cross, Bevan and Smith stated in the paper already mentioned, these results disclose several points of interest with regard to the mode of action of hydrogen peroxide more particularly the oxidation of the residue $-\mathrm{CH(OH)}-\mathrm{CH(OH)}-$ to $-\mathrm{C(OH)}{:}\mathrm{C(OH)}-$ . They therefore undertook the oxidation of a few typical carbohydrates by hydrogen peroxide in the presence of iron salts but, apart from this development , there has been practically no further attempt to apply neutral oxidising agents to the carbohydrates.

The purpose of the present work was to study in detail the mechanism of the oxidising action of hydrogen peroxide and hypochlorous acid on non-reducing sugars with the ultimate intention of applying the results to starch and cellulose. These two reagents were selected as they are strong oxidisers and, as one of them is neutral and the other a weak acid, they are unlikely to cause profound molecular alteration in the sugars employed. In addition, both reagents are applied technically to cellulose.

In the present research the oxidation reactions were studied under conditions in which the ratio of the available oxygen atoms to each $C_{12}$ unit in the carbohydrate varied between $\frac{1}{2}$, 1, 2 and 5. In addition to non-reducing sugars it was found necessary to use controls in which glucose and fructose were used. The progress of the reactions was traced:-

1. polarimetrically,
2. by determining the rate of disappearance of the available oxygen,
3. by determining any change in acidity,
4. by estimation of variations in the reducing power on Fehling's solution,

5. by the action of phenylhydrazine acetate at 20°, 60° and 100° on the products of the oxidation,
6. by isolation and identification of the individual products.

Considering the ultimate object in view the oxidations were allowed to proceed at ordinary temperature in all cases.

It was thought advisable to start with saccharides of simple and known constitution and for this reason sucrose was selected as a test substance in view of its non-reducing properties . In addition the possibility was open that this compound containing as it does one $\gamma$-sugar residue in combination would possess the property of combining directly with one atom of oxygen without molecular rupture ( Irvine, Fyfe and Hogg , T., 1915, 107, 524 ) In addition, lactose and maltose were selected for experiment as representative of reducing disaccharides differing in essential structure from sucrose .

A survey of the results taken in conjunction with recent developments in the chemistry of starch and cellulose quickly showed that they had no direct bearing on the oxidation of cellulose and it was deemed advisable to introduce another series of test substances. The oxidation of $\beta$ -glucosan was accordingly undertaken as, although this component is not present in cellulose , it bears, as an anhydride possessing the formula $C_6H_{10}O_5$ , a closer resemblance to the group of polysaccharides than do any of the sugars proper. In addition the compound is now the most accessible of the anhydro-sugars and may be employed in large-scale experiments . The principal results obtained in this section of the work are now described.

## THE ACTION OF HYDROGEN PEROXIDE ON DISACCHARIDES .

### SUCROSE , MALTOSE , AND LACTOSE .

In the absence of a catalyst the above disaccharides were stable at ordinary temperatures towards hydrogen peroxide but in the presence of ferrous sulphate oxidation proceeded rapidly even at romm temperature. In the case of sucrose where the ratio of available oxygen was over ½ atom per $C_{12}$ unit it was ascertained that the oxidising agent attacked one of the primary alcoholic groups and converted it into the carboxyl group. The acid thus formed effected inversion of the remaining sucrose and the scission products then underwent independent oxidation , to give a complex series of acids. This development of free organic acid increased rapidly as the amount of active oxygen was increased. Formic and acetic acids were then produced in quantity, but there was no evidence of the presence of gluconic acid, the molecular rupture giving essentially acids with two and three carbon atoms in the chain. Judging from the action of phenyl hydrazine on the products of the change laevulinic acid was present, a surprising result considering the conditions .

The precipitates yielded by phenyl hydrazine were somewhat indefinite and consisted mainly of phenylglucosazone together with mixed hydrazides of organic acids, the separation of which was difficult. The combined results of the experiments proved:-

1. If direct addition of oxygen to the sucrose molecule takes place the product is not neutral but acidic.
2. The fructose residue in sucrose although of the $\gamma$ -type does not undergo oxidation in the same manner as $\gamma$ -methylglucoside.
3. The formation of free acid precedes hydrolysis.
4. Once hydrolysis commences the oxidation becomes rapid and is ultimate .

No part of the evidence obtained seems to have any bearing on cellulose problems beyond the fact that any reaction which liberates a reducing sugar in the presence of hydrogen peroxide leads to rapid destruction of the sugar molecule .

In pursuing a parallel series of experiments on maltose and lactose it was apparent that the reagent attacked the reducing group of the sugar with the formation of maltobionic acid and lactobionic acid respectively . As in the case of sucrose these acid products effected the hydrolysis of the parent disaccharides , and oxidation of the fragments ensued. Such changes are notoriously complex and hydrogen peroxide was abandoned in favour of hypochlorous acid as the possibility was not excluded that this reagent might act in an entirely different manner .

## THE ACTION OF HYPOCHLOROUS ACID ON DISACCHARIDES.

### SUCROSE , MALTOSE AND LACTOSE .

In the principal experiments the amount of hypochlorous acid was adjusted so that one atomic proportion of oxygen was available for each $C_{12}$ unit of the carbohydrates As before the disaccharides employed were sucrose, maltose and lactose . With these bioses no organic acids were formed as products of oxidation, the result offering a sharp contrast to that obtained with hydrogen peroxide. Sucrose was remarkably stable towards hypochlorous acid; in fact , when the oxidation was carried out in the presence of a base to neutralise the hydrochloric acid formed by the decomposition of the oxidising acid , 97.6 per cent of the original weight of sucrose persisted after complete utilisation of the hypochlorous acid . Even in the absence of a base reaction was comparatively slow and no acidity was developed . The final value of the specific rotation of the oxidised solutions did not coincide with that calculated from the composition of the residual sugar as determined from the copper-reducing power. Similar results were obtained with maltose and lactose, the same optical lag being recorded in all the experiments. In the case of lactose the specific rotation actually diminishes in place of increasing.

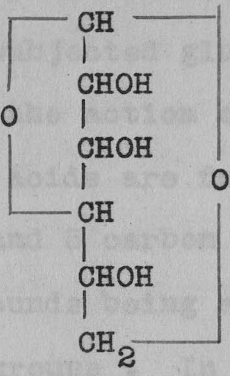
In view of these results the general conclusion may be drawn that hypochlorous acid acts on the disaccharides through the preliminary formation of an addition compound. Attempts to isolate such a compound were naturally unsuccessful but the combined physical evidence is strongly in favour of its existence .

## THE ACTION OF HYDROGEN PEROXIDE AND HYPOCHLOROUS ACID ON $\beta$ -GLUCOSAN.

In the absence of a catalyst , $\beta$ -glucosan proved to be stable towards hydrogen peroxide , practically no change being detected even after the lapse of several days but in the presence of ferrous salts reaction was rapid especially in the earlier stages . Both volatile and non-volatile acids were formed and simultaneously the glucosan was partially converted into glucose .

Whem hypochlorous acid was used the reaction was profoundly modified as the atomic ratio of oxygen increased . Thus , with one atom of oxygen per $C_6$ unit, very little change was detected . The specific rotation at the beginning and at the end of the reaction was the same, but at an intermediate stage ( about two hours after the start ) the value of $[\alpha]_D$ showed a diminution from $-68.6^\circ$ to $-55.2^\circ$ . In all probability this fall in the optical activity followed by a return to the original value is due to the temporary addition either of oxygen or of hypochlorous acid to the anhydro-sugar . By increasing the ratio of oxygen to two atoms per $C_6$ unit a remarkable result was obtained as the glucosan was partially converted to glucose although no organic acids were formed . Finally with five atoms of available oxygen per $C_6$ unit acidity was developed equivalent to 1/20 normal acid and the conversion into glucose was accelerated .

These results have an important bearing on the oxidation of cellulose . $\beta$ -Glucosan has been shown to possess the structure

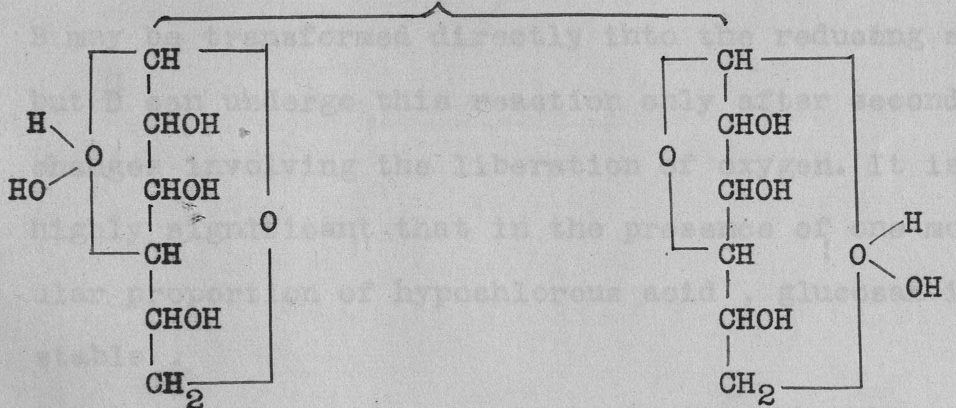


Even if Pictet's views as to the relationship of glucosan to cellulose are not accepted the former compound must be regarded as being closely related to the unit of the polysaccharides. Now glucosan is not readily hydrolysed even on heating with sulphuric acid to regenerate glucose, yet the results of the present research have shown that in the absence of stable acids the anhydro-ring can be opened out at ordinary temperatures and that glucose is then formed. This points clearly to the idea of a preliminary addition of hypochlorous acid to one of the ring-forming oxygen atoms (probably that connecting the terminal carbons ) . If this view be correct , a necessary consequence is that the oxidation of β -glucosan should follow an entirely different course when occasioned by oxidising agents which do not admit of molecular addition. The point has been established in the course of a parallel research, carried out by Irvine and Oldham , who subjected glucosan dissolved in methyl alcohol to the action of silver oxide. Essentially three acids are formed containing respectively 3 , 4 and 5 carbon atoms in the chain, one of these compounds being a ketonic acid containing two hydroxyl groups . In this type of oxidation no glucose is liberated and the molecule suffers progressive disruption.

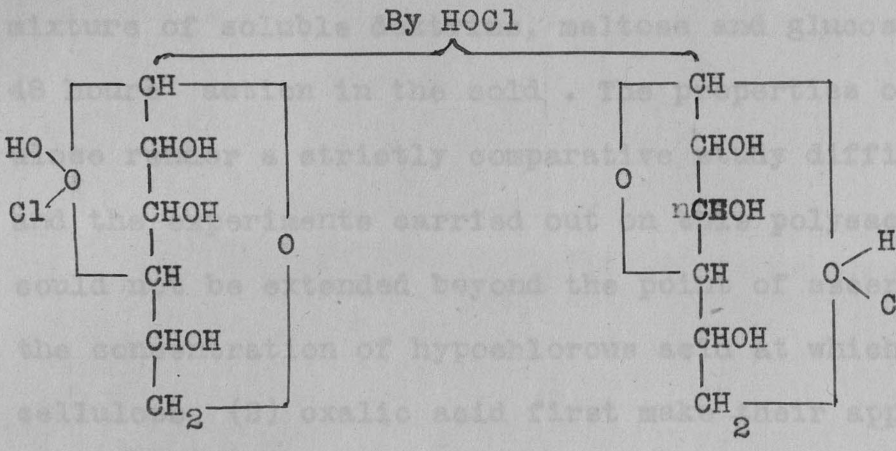
## Action of Hypochlorous Acid on Starch and Cellulose.

The most important result disclosed in the experiments just described is that dilute hypochlorous acid is capable of effecting at ordinary temperatures the hydrolysis of n-saccharides or anhydro-sugars . Emphasis must be laid on the example of β -glucosan which requires prolonged boiling with with 4-5 per cent. sulphuric acid in order to open the anhydro-ring yet undergoes the same change at 16° in the presence of 1.8 per cent. hypochlorous acid. Both reagents effect hydrolysis yet the mechanism of the change cannot be identical in both cases . The opening of a ring compound is generally explained by preliminary addition and applying this principle to the hydrolysis of glucosan we have the alternatives:-

By $H_2SO_4$



**A.** **B.**



C. D.

Types A and C may reasonably be excluded as the product is the butylene-oxide form of glucose; the compound B may be transformed directly into the reducing sugar but D can undergo this reaction only after secondary changes involving the liberation of oxygen. It is highly significant that in the presence of one molecular proportion of hypochlorous acid , glucosan is stable .

Evidently support would be lent to the above views if it could be shown that polysaccharides behaved in the same manner as glucosan and underwent hydrolysis with low concentrations of hypochlorous acid. In the a case of starch this has been confirmed and as much as 80-90 per cent. of the compound is converted into a mixture of soluble dextrins, maltose and glucose after 48 hours' action in the cold . The properties of cellulose render a strictly comparative study difficult and the experiments carried out on this polysaccharide could not be extended beyond the point of ascertaining the concentration of hypochlorous acid at which (1) oxycellulose, (2) oxalic acid first make their appearance. The results which are given in the experimental part are capable of considerable theoretical discussion but this would be premature in the case of a preliminary research of this nature.

# EXPERIMENTAL PART.

## EXPERIMENTAL .

### The Action of Hydrogen Peroxide on Sucrose:

### (a) Without a catalyst:-

Typical experiments only are quoted. The sucrose solution used in these experiments was 1/6 molar ( 5.7 per cent. ). Three experiments were carried out with the ratio of available oxygen contributed by the hydrogen peroxide in the proportion of 1, 2 and 5 atoms per $C_{12}$ unit. There was no rise in temperature on mixing; the optical activity remained constant; no action on Fehling's solution was developed and no organic acids were formed. It was therefore evident that sucrose was quite stable to hydrogen peroxide in the absence of a catalyst.

### (b) With a catalyst:-

Fenton and Jackson ( <u>J.C.S.</u>,<u>T</u>., 1899, <u>75</u>, 1 ) oxidised polyhydric alcohols with hydrogen peroxide in presence of ferrous sulphate . They used the catalyst in the proportion of 1/10 atom $Fe^{\cdot\cdot}$ in crystalline ferrous sulphate per molecule of the alcohol. Similar ratios were used in the present work , the concentration of the sucrose solution being 1/6 molar . The oxidations were studied under conditions in which the oxygen ratio per $C_{12}$ unit varied between 1/4 , 1/2 , 1 and 2 . Unfortunately, owing to the turbidity and brown colour of the solution , caused by the oxidation of the ferrous salt , it was impossible to study the progress of the reaction polarimetrically . The disappearance of active oxygen and the development of acidity were determined by direct titration . When the decomposition of the hydrogen peroxide was complete a portion of the liquid was evaporated to dryness under dimished pressure at a bath temperature of about 35° . On extracting the residue with 80 per cent. alcohol at the boiling point and distilling the solvent in a high vacuum a syrup was obtained. The copper-reducing power of this syrup was determined before and after hydrolysis .

As these experiments were carried out on exactly similar lines it is most convenient to display the results in tabulated form.

| Ratio of oxygen in $H_2O_2$ per $C_{12}$ unit. | 1/4 | 1/2 | 1 | 2 |
|---|---|---|---|---|
| Rise in Temperature (on mixing) | | | | |
| Initial | 18° | 19° | 15° | 17° |
| Maximum | 21° | 22° | 21° | 20° |
| Acidity developed N/1 Acid . | N/22.3 | N/20 | N/20 | N/20 |
| Time to completion. | 25 mins . | 30 mins. | 30 mins. | 45 mins. |
| Ratio of Copper values before and after hydrolysis. | - | 68.1% | 68.1% | 68.1% |

## The Action of Hydrogen Peroxide on Lactose in presence of Ferrous Sulphate.

(a) 0.463 Gram of crystalline ferrous sulphate was dissolved in 5c.c. of water and added to 100 c.c. of a sixth molar solution of lactose ; this was well mixed with a solution of hydrogen peroxide (previously titrated ) containing 0.266 gram of available oxygen. The temperature rose on mixing from 15.5° to 19° . The acidity developed and the rate of disappearance of active oxygen were determined at regular intervals.

| Time in minutes. | Acidity | Disappearance of oxygen. |
|---|---|---|
| 10 | N/17 | 43.8% of total. |
| 90 | N/14.9 | 97.0% of total. |
| 120 | N/14 | 99.5% of total. |
| 150 | N/13.7 | 100 % of total. |

(b) With two atoms of oxygen the rise in temperature on mixing was from 19° to 21.5° and at the end of two hours a sudden rise to 30° took place.

| Time in minutes. | Acidity | Disappearance of oxygen. |
|---|---|---|
| 10 | N/18.4 | 16.1% of total |
| 30 | N/17.8 | 24.3% |
| 90 | N/9.5 | 92.2% |
| 120 | N/7.7 | 99.3% |
| 150 | N/7.3 | 100% |

(c) By using a smaller proportion of the oxidiser, viz:- 1/2 atom oxygen per molecule of lactose, the reaction followed the same course as above but the oxidation was not so complete.

| Time in minutes. | Acidity | Disappearance of oxygen. |
|---|---|---|
| 10 | N/19.6 | 72.6% of total. |
| 30 | N/17.6 | 87.5% |
| 90 | N/17.1 | 92.2% |
| 120 | N/15.4 | 96.8% |
| 150 | N/15.3 | 100 % |

In each case the residual sugar was isolated as already described for sucrose . Analysis of these products showed that there was an increase in molecular rupture during oxidation, as the amount of oxygen was raised from 1/2 atom to 2 atoms , thus:-

| Proportion of Oxygen per $C_{12}$ unit. | Reducing Power before Hydrolysis . |
|---|---|
| 1/2 | 80 % of that of glucose. |
| 1 | 80.3 % " |
| 2 | 95.72 % " |

The oxidation of Maltose with Hydrogen Peroxide in presence of Ferrous Sulphate .

(a) One atom available oxygen to one molecule Maltose:- 100 C.c. maltose solution containing 5.7 grams of pure maltose were mixed with 5 c.c. of ferrous sulphate solution containing 0.463 grams crystalline ferrous sulphate . 25 C.c. of hydrogen peroxide solution ( 0.266 grams available oxygen ) were added and the mixture well stirred with a thermometer . The temperature rose from 16° to 19°. An examination at intermediate stages showed:-

| Time in minutes. | Acidity . | Disappearance of Available Oxygen. |
|---|---|---|
| 10 | N/13.7 | 40 % of total. |
| 90 | N/10.4 | 96.3% " |
| 120 | N/10.3 | 99.1 % " |
| 150 | N/10.3 | 100 % " |

(b) Two atoms available oxygen:-

On mixing the solutions of maltose and hydrogen peroxide the temperature showed an increase of 3°, and after 90 minutes from the start there was another rise from 14.5°( original temperature ) to 30°, The figures shown below were recorded:-

| Time in minutes. | Acidity. | Disappearance of oxygen . |
|---|---|---|
| 10 | N/18.5 | 18.1 % of total. |
| 30 | N/12.3 | 42.3 % " |
| 90 | N/8 | 98.1 % " |
| 120 | N/7.9 | 99.3 % " |
| 150 | N/7.85 | 100 % " |

(c) By decreasing the amount of available oxygen to 1/2 atom the rate of the reaction was:-

| Time in minutes. | Acidity. | Disappearance of oxygen . |
|---|---|---|
| 10 | N/23 | 82.8 % of total. |
| 30 | N/21.27 | 92.18% " |
| 90 | N/21.4 | 93.75% " |
| 120 | N/19.7 | 94.53% " |
| 150 | N/19.6 | 100 % " |

In all cases the residual products were isolated after removal of volatile acids and inorganic salts .The syrup obtained was tested with Fehling's solution before and after hydrolysis . A comparison of the two copper values showed:-

| Atomic Proportion of oxygen. | Reducing Power before Hydrolysis. |
|---|---|
| 1/2 | 69.6% of that of glucose. |
| 1 | 79.93% " |
| 2 | 84.0 % " |

Oxidation of Disaccharides with Hypochlorous Acid:

The hypochlorous acid was prepared by a private method devised by Principal Irvine which yielded a solution free from chlorine and more highly concentrated than that obtained by ordinary processes .

The progress of the oxidation was now traced polarimetrically in addition to the controls employed with hydrogen peroxide .

(a) The Action of Hypochlorous Acid on Sucrose:-

In this series of experiments 1 atom of available oxygen was used per $C_{12}$ unit . 50 C.c. of sucrose solution ( 5.7 per cent. ) were mixed at room temperature, with 50 c.c. of hypochlorous acid solution ( 0.87per cent. ) The reaction was slow , 1 atomic ratio of oxygen requiring almost 24 hours for complete utilisation . No organic acids were formed , the acidity developed being exactly equivalent to the hydrochloric acid from the decomposition of the hypochlorous acid used . There was a steady decrease in the value of the specific rotation as shown below:-

| Time in hours from start. | $[\alpha]_D$ | Disappearance of active oxygen. | |
|---|---|---|---|
| 0 | + 66.6 ° | 3.44% | of total. |
| 2 | + 61.7 ° | - | |
| 4 | + 55.7 ° | 68.7 % | " |
| 7.5 | + 54.7 ° | 88.0 % | " |
| 22 | + 54.0 ° | 100 % | " |

On isolating the residual sugar after all oxidation had ceased, the copper value was 23.3 per cent. of that obtained on complete hydrolysis . The specific rotation calculated on this hydrolysis should have been $[\alpha]$ + 46.4° . The actual drop in optical activity was $[\alpha]_D + 66.6^\circ \longrightarrow 54^\circ$ .

(b) <u>The Action of Hypochlorous Acid on Lactose.</u>

In the following experiments 1 atom of available oxygen was allowed per $C_{12}$ unit.

50 C.c. of lactose solution ( 1/6 molar ) were mixed with 50 c.c. of hypochlorous acid ( containing 0.87 per cent. HOCl ). The reaction proceeded very slowly extending over a period of 48 hours. During this time no organic acids were formed and the specific rotation changed from $[\alpha]_D$ + 52.5° to + 47.6° .

| Time in hours from start. | $[\alpha]_D$ |
|---|---|
| 0 | + 52.5° |
| 2 | + 51.5° |
| 6 | + 51.3° |
| 20 | + 51.3° |
| 44 | + 49.6° |
| 48 | + 47.6° |

On isolation of the residual sugar, analysis gave a copper-reducing value of 81.9 per cent. of that determined after hydrolysis. This partial hydrolysis of lactose should give the optical change $[\alpha]_D + 52.5° \rightarrow +66.6°$ in place of + 47.6°.

(c) <u>Action of Hypochlorous Acid on Maltose.</u>

For each $C_{12}$ unit, 1 atom of available oxygen from the hypochlorous acid was used.

The solutions were mixed as before in the above proportions . The reaction was extremely slow , the maltose reacting completely with the hypochlorous acid only in 80 hours . No organic acids were found as products of the oxidation . Polarimetric readings showed a gradual decrease from $[\alpha]_D$ $+134° \rightarrow +123.5°$

| Time in hours. | $[\alpha]_D$ |
|---|---|
| 0 | + 134° |
| 24 | + 125.9° |
| 48 | + 119.6° |
| 80 | + 123.5° |

The total product was isolated and the reducing power determined before and after hydrolysis, the ratio being 69.0 per cent. Here again the analytical results are not consistent with the optical activity and the general conclusion may be drawn that the oxidising agent acts on the sugar through the preliminary formation of an addition compound .

## OXIDATION OF $\beta$ -GLUCOSANS

### (a) The Action of Hydrogen Peroxide on $\beta$ -Glucosan.

(1 Atom available oxygen in hydrogen peroxide per $C_6$ unit. )

In the absence of a catalyst hydrogen peroxide gave negative results . After standing for seven or eight days there were only slight traces of acidity developed and over 50 per cent. of the hydrogen peroxide still remained . In presence of ferrous sulphate the reaction proceeded fairly rapidly .

60 C.c. of a 2.7 per cent. solution of glucosan containing ferrous sulphate were mixed with 60 c.c. of hydrogen peroxide solution ( 0.266 per cent . available oxygen ). The reaction extended over a period of 24 hours . The acidity developed was equivalent to 1/23 normal acid , almost half of which was due to volatile acids. The progress of the reaction can be followed from the table below;-

| Time | Acidity(N/1 acid) | Disappearance of oxygen. |
|---|---|---|
| 10 minutes | N/40 | 45.9 per cent. |
| 40 " | N/38.4 | 51.9 " |
| 60 " | N/37 | 57.8 " |
| 16 hours | N/27.4 | 94.3 " |
| 20 " | N/25.9 | 95.1 " |
| 21 " | N/25.3 | 96.2 " |
| 24 " | N/23.2 | 100.0 " |

A portion of the liquid was evaporated to dryness under diminished pressure . The residue was boiled under reflux condenser with 80 per cent. ethyl alcohol, the solution filtered and evaporated to a syrup in a vacuum. This residual product reduced Fehling's solution and the copper values determined before and after treatment with dilute acid showed that the oxidation had yielded

1. Glucose ..............................10.7 parts
2. Unchanged glucosan ....................48.3 parts
3. Non-volatile acids ....................41.0 parts.

(b) The Action of Hypochlorous Acid on β -Glucosan.

Owing to the prolonged time necessary for the preparation of pure hypochlorous acid it was decided to utilise bleaching powder as the source of the oxidising agent . A good specimen of bleaching powder was shaken for a considerable time with distilled water . After standing for a few hours the insoluble calcium salts were filtered off and the calcium hypochlorite present in the filtrate determined by analysis . The solutions of the carbohydrate and calcium hypochlorite were mixed in the desired proportions and the hypochlorite was then decomposed by blowing carbon dioxide through the solution from time to time until the oxidation was complete .

(1) 1 Atom available oxygen in hypochlorite per $C_6$ unit.

100 C.c. of a 2.7 per cent. solution of $\beta$ -glucosan were mixed with 100 c.c. of hypochlorite solution containing available oxygen in the above proportions. No variation in temperature took place on mixing or during the reaction . The total disappearance of oxygen extended over a period of 20 hours and during that time no acidity was developed . The optical activity underwent the following change:-

| Time. | $[\alpha]_D$ |
|---|---|
| 0 hours | -68.6° Start. |
| 2 " | -55.2° Minimum. |
| 24 " | -68.6° Final value. |

On isolating the residual products, a syrup was obtained which did not reduce Fehling's solution . This was to be expected since the final value of $[\alpha]_D$ was -68.6°.

(2) 2 Atoms available oxygen per $C_6$ unit.

The solutions were well mixed and carbon dioxide blown through the solution from time to time . The polarimetric readings indicated a fall in the specific rotation from $[\alpha]_D$ -68.6° to -43.7° after 27 hours .

| Time. | $[\alpha]_D$ |
|---|---|
| 0 hours | -68.6° |
| 6 " | -51.1° |
| 24 " | -42.9° |
| 27 " | -43.7° |

The residual products when isolated showed a reducing action on Fehling's solution . On the basis of titrations before and after hydrolysis the composition of the mixture was

| | |
|---|---|
| Glucose .................... | 1part |
| Unchanged Glucosan ......... | 3.44 parts . |

(3) 5Atoms available oxygen provided for each $C_6$ unit.

In these proportions the reaction proceeded more vigorously . Within [illegible] 10 minutes of mixing the temperature rose from 16° to 39°. Acidity was developed to the extent of 1/20 normal acid . Polarimetric readings were impossible during the intermediate stages owing to the turbidity of the solution and evolution of gas . At the end of 50 hours when the oxidation ceased the specific rotation had changed from $[\alpha]_D$ -68.6° to -27°.

100 C.c. of the solution were neutralised and evaporated to dryness . The residue was boiled under reflux condenser with alcohol , filtered and evaporated under diminished pressure to a syrup which reduced Fehling's solution . The copper reducing value found after boiling with dilute acid showed that a 30.2 per cent. conversion of glucosan to glucose had taken place during the oxidation .

Glucose ...................... 1 part
Unchanged glucosan .......... 3.3 parts .

The Action of Hypochlorous Acid on Starch .

(a) 0.87 grams hypochlorous acid ( from calcium hypochlorite ) per 2.7 grams of starch:

A solution of 5.4 grams of Japanese potato starch in 100 c.c. of water was mixed with 100 c.c. of hypochlorite solution , the concentration of which was adjusted to give the above proportion of available oxygen . The reaction was very slow , the hypochlorous acid being utilised only after the lapse of several days . On examining the solution polarimetrically it was found to be dextro-rotatory , the value of $\alpha$ increasing as the available oxygen was used . Only a very slight acidity was observed at the end of the reaction.

When all oxidation had ceased , 100 c.c. of the solution were evaporated to dryness under diminished pressure . The residue was boileed under reflux condenser with alcohol , filtered and the alcoholic solution evaporated to a syrup under reduced pressure . From 2.7 grams of starch the syrup obtained in this way weighed 1.9581 grams , showing that about 75 per cent. of the starch had been transformed into soluble products . The syrup reduced Fehling's solution and the copper reducing power was 33.7 per cent. of that obtained after boiling with dilute acid .

Precipitates produced by the action of phenyl hydrazine , as was the case in the earlier stages of this work , were somewhat inconclusive , but indicated that maltose together with its oxidation products was present .

(b) 1.74 Grams of hypochlorous acid ( from hypochlorite solution ) per 2.7 grams starch .

In these proportions over 90 per cent. of the starch reacted . The results were very similar to those quoted above and examination of the residual group gave a copper reducing power equal to 90 per cent. of that obtained on complete hydrolysis .

## The Action of Hypochlorous Acid on Cotton Cellulose.

(1) 0.87 Gram hypochlorous acid ( from calcium hypochlorite solution ) per 2.7 grams of cellulose.

Cotton cellulose was found to be remarkably stable to hypochlorous acid when ~~the~~ present in the above proportions and when the concentration was 0.87 grams of hypochlorous acid per 100 c.c.. After 48 hours the hypochlorous acid was exhausted but an examination of the oxidation products showed that 86 per cent. of the cellulose still remained intact . Only traces of oxycellulose and oxalic acid were found .

(2) 1.74 Gram hypochlorous acid per 2.7 grams of cellulose.( Concentration of acid = 1.74 grams per 100 c.c.. )

Even in these proportions the change was not extensive although the formation of oxycellulose and oxalic acid was increased . The weight of the residue from 2.7 grams of cellulose was 2.0711 grams showing a loss in weight of 23.2 per cent.

(3) 4.35 Grams of hypochlorous acid ( from hypochlorite solution ) per 2.7 grams of cellulose .

2.7 Grams of cellulose were mixed with 100 c.c. of acid of 4.35 per cent. concentration . At the end of the reaction which extended over almost 48 hours , the residue and filtrate were examined. 20 C.c. of the filtrate gave on evaporating to dryness 1.9120 grams of residue . Calcium and chlorine were estimated in another 20 c.c. and the results found were:

Calcium: = 0.69 ; Chlorine: = 0.80 gram . Difference 0.41 Gram. No volatile acids were found as products of the oxidation .

From 2.7 grams of cellulose the residue weighed 1.9532 grams , showing a loss in weight equivalent to 27.66 per cent. The residue in this case was not fibrous but amorphous .. It was partially soluble in alkaline solution to the extent

| | |
|---|---|
| soluble .............. | 8.86 parts |
| insoluble .............. | 1.14 parts. |

From this alkaline solution the oxycellulose could be reprecipitated by means of acids , salts or alcohol . It gave no blue colouration with iodine in potassium iodide . Concentrated sulphuric acid attacked and dissolved it giving a dark brown solution . It reduced Fehling's solution on boiling .

# PART II.

## GLUCOSIDES RELATED TO INDIGO.

### (a) SYNTHESIS OF THIOINDICAN.

### (b) CONDENSATION REACTIONS OF INDOXYL AND 3-OXY(1)THIONAPHTHEN.

# INTRODUCTION.

# INTRODUCTION.

## THE CONSTITUTIONAL STUDY OF INDICAN AND THIOINDICAN.

It may be recalled that Irvine and Rose (T., 1906, 89, 814 ) pointed out that the complete constitutional study of a glucoside involved the consideratinn of three factors:-

(1) The identification of the constituent sugar and of the molecule or molecules with which it is combined.

(2) The determination of the configuration of the glucoside. This is necessary as derivatives of sugars the formation of which involves condensation of the reducing group may exist in $\alpha$- or $\beta$- stereoisomeric forms.

(3) A study of the oxygen-linkage present in the sugar residue. (See Irvine. T.,1923, 123, 898).

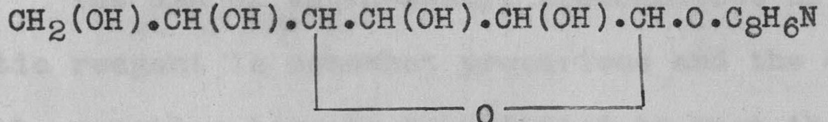
These factors which were developed by Irvine and his pupils in their studies of artificial glucosides were first applied to natural compounds by Irvine and Rose (loc. cit.). Recently in the course of parallel work Macbeth and Pryde (T., 1922, 121, 1660 ) have shown that the important glucoside "Indican" is constituted on the same structural model as salicin and $\alpha$ methylglucoside as it may be converted into the same butylene-oxide form of tetramethyl glucose.

The present research was originally designed to verify this structure for indican synthetically and to supplement the above investigation, the results of which were incomplete owing to experimental difficulties.

Indican was first isolated from Isatis tinctoria, Polygonum tinctorium and other plants by Schunck (Phil. Mag., 1855, iv, 10, 74; 1858, iv, 15, 127), but its correct formula was not established until many years later (Marchlewski and Radcliffe, J.Soc. Chem. Ind., 1898, 17, 434). It was shown by these authors that on hydrolysis indican yielded glucose and indoxyl, the latter undergoing oxidation into indigotin. This was confirmed by other workers including Ter Meulen (Rec. trav. chim., 1905, 24, 444 ) who by means of enzyme hydrolysis established the nature of the component sugar as d-glucose as did A.G.Perkin (T., 1905, 91, 1715 ). Although substantial proof was available that indican is an indoxyl glucoside and that the component sugar is d-glucose there was until recently no direct evidence as to the internal oxygen-linking of the sugar . The work described by Macbeth and Pryde (loc. cit. ), to which reference has already been made, involved the conversion of indican into a tetramethyl derivative which behaved as a normal methylated glucoside. The compound was subjected to simultaneous hydrolysis and condensation by treating with methyl alcohol containing 1 per cent. of hydrogen chloride, and this resulted in the formation of the corresponding methyl glucosides according to the scheme:

$$\begin{tikzcd}
& \text{Tetramethyl indican} \arrow[dl] \arrow[dr] & \\
\text{Indoxyl derivatives} & & \text{Tetramethyl glucose} \arrow[d] \\
& & \alpha\text{- and }\beta\text{-tetramethyl methylglucosides.}
\end{tikzcd}$$

The alkylated glucoside actually obtained was a mixture of the $\alpha$- and $\beta$- forms of tetramethyl methylglucoside and subsequent hydrolysis with aqueous acid thereafter yielded a crystalline tetramethyl glucose which was readily identified as the 2:3:5:6- or butylene-oxide compound. From these results it would appear that indican is derived from a molecule of d-glucose condensed with indoxyl, the internal linking of the sugar being of the butylene-oxide type. The following structure has therefore been assigned to the glucoside:



$$CH_2(OH).CH(OH).\underbrace{CH.CH(OH).CH(OH).CH}_{O}.O.C_8H_6N$$

and in view of the optical properties of the glucoside and its behaviour towards enzymes it is evident that ~~a~~ the compound like most natural glucosides is derived from $\beta$-glucose.

It will be granted that, wherever possible, constitutional formulae should be verified by synthesis and in the cases of Salicin, Helicin, Populin (Irvine and pupils ) and Arbutin (Macbeth and Mackay, T., 1923, **123**, 717 ) this confirmation has been obtained. The present work was therefore undertaken with the object of synthesising indican, bht considering the impossibility of identifying tetramethyl indican ,

( which is a glass ) it was necessary to proceed by the interaction of tetra-acetyl bromoglucose and derivatives of indoxyl. A condensation of this nature secures the necessary conditions of introducing the correct type of glucose in the correct stereoisomeric form.

## Practical Methods of Applying Tetra-acetyl bromoglucose.

The use of tetra-acetyl bromoglucose as a synthetic reagent is somewhat precarious and the conditions of the reaction have to be modified to meet the exigencies of each individual case. This is in part revealed by a study of the standard literature which contains numerous references to the synthesis of glucosides. Thus, Mauthner (J. pr. Chem., 1910, ii, 82, 271-274 ) carried out a successful condensation of methyl syringate dissolved in aqueous sodium hydroxide by shaking for 24 hours with an ethereal solution of tetra-acetyl bromoglucose . In this way he obtained methyl tetra-acetyl glucosyringate, $C_{24}H_{30}O_{14}$ , which was hydrolysed by 6 per cent. barium hydroxide solution giving a glucosyringic acid identical with that produced by the oxidation of syringin. In the following year the same author

( J. pr. Chem., 1911, ii, 83, 556-560 ) published an account of the synthesis of glucovanilic acid and of gluco-p-hydroxybenzoic acid both of which were obtained by condensing the corresponding methyl ester( dissolved in aqueous sodium hydroxide ) with tetra-acetyl bromoglucose (dissolved in ether ). The synthesis of picein also described by Mauthner ( J. pr. Chem.,1913, ii, 88, 764-770 ) involves another variation as it was accomplished by the gradual addition of aqueous sodium hydroxide to a solution of p-hydroxy acetophenone and tetra-acetyl bromoglucose dissolved in acetone, the temperature being restricted to 16°.

It may also be recalled that Fischer and Strausz (Ber., 1912, 45, 2468 ) effected the condensation of tetra-acetyl bromoglucose and resorcinol by shaking an alkaline solution of the latter with an ethereal solution of the glucose derivative. In this way they obtained the crystalline tetra-acetyl ~~bromoglucose~~ derivative of resorcinol-d-glucoside but only to the extent of approximately 3.5 - 7.5 per cent. of the theoretical amount . After hydrolysis of the acetyl groups by means of barium hydroxide the crystalline unsubstituted glucoside was obtained.

A few years later Fischer and Bergmann (Ber., 1917, 50, 711 ) introduced an entirely novel procedure by condensing l-menthol and tetra-acetyl bromoglucose in the presence of quinoline at a temperature of about 100°. From 50 grams of tetra-acetyl bromoglucose they obtained 15.7 grams of the pure tetra-acetyl glucoside which yielded approximately 10 grams of the crystalline unsubstituted glucoside on hydrolysis with dilute barium hydroxide solution.

For the conversion of sitosterol into its glucoside, Salway (T.,1913, 103, 1022 ) agitated dry ethereal solutions of sitosterol and tetra-acetyl bromoglucose with freshly precipitated silver oxide. A similar method is described by Fischer (Ber., 1917, 50, 1055, see also Ber., 1919, 52, 197 ) who was successful in synthesising mandelonitrile glucoside but it may be remarked that Fischer does not appear to have explored this reaction fully . The above typical examples have been quoted to indicate the customary use of tetra-acetyl bromoglucose and emphasise that the selection of a working method depends largely on the properties of the compound with which the reagent is to interact . In the particular synthesis under discussion this is indoxyl which is somewhat inaccessible and possesses highly inconvenient properties.

## Preparation of Indoxyl.

Indoxyl, which was discovered by Baumann and Tiemann in 1879 and synthesised by Baeyer in 1881, was first described as an oil soluble in water which could not be obtained in a sufficiently pure state to give satisfactory analytical figures. In 1902 however Vorländer and Drescher (Ber., 1902, 35, 1701 ) isolated the compound in yellow crystals by decomposing indoxylic acid with warm water in an atmosphere of coal gas. Subsequently Friedländer and Risse (Ber., 1914, 47, 1919 ) found a more convenient method for its extraction from the indoxyl melt. They added the roughly powdered solid to 25 per cent. acetic acid, filtered the warm fluorescent solution and kept it in an ice-bath for several hours. The indoxyl then separated in long yellow crystals .

The literature contains no experimental details for the preparation of the indoxyl melt as the processes are secret. It is clear however that phenyl glycine-o-carboxylic acid, when fused with certain proportions of sodium or potassium hydroxide or probably sodamide, gives a melt rich in indoxyl. The initial stages of the present research thus involved the preparation of phenylglycine-o-carboxylic acid which was easily formed by condensing anthranilic acid with monochloracetic acid in the presence of aqueous potassium hydroxide. In this way a considerable quantity of the starting material was prepared. Fusions were attempted under varying conditions (1) in an open crucible, (2) in an atmosphere of coal gas and (3) under diminished pressure. It was found that a melt rich in indoxyl was best prepared by carryingout the fusion in a high vacuum at a temperature of 230-250°. A green fluorescent solution resulted when the powdered material was added to a 25 per cent. solution of acetic acid but the yield of crystalline indoxyl was exceedingly poor as the solution contained only 1 gram of indoxyl per 100 c.c. The methods for estimating the indoxyl content are discussed in the experimental part of this thesis .

Several attempts were made to effect the condensation of indoxyl with tetra-acetyl bromoglucose both reagents being employed in solution as recommended by Fischer and Strausz (loc. cit.). These were uniformly unsuccessful but showed that only crystalline indoxyl could be employed and, further, that large quantities of material would be necessary. Owing to the difficulty in obtaining sufficient quantities of crystalline indoxyl and in view of the ease with which oxidation to indigo took place it was decided that the method of synthesising indican should be standardised in advance by performing a parallel condensation with tetra-acetyl bromoglucose and a non-sugar residue unlikely to cause similar difficulties. Attention was therefore directed to "thioindoxyl" or 3-oxy(1)thionaphthen. This substance, although not so reactive as indoxyl is similar in many respects; it oxidises with comparative ease to the red dyestuff "thioindigo" but is much more stable and more easily prepared than indoxyl.

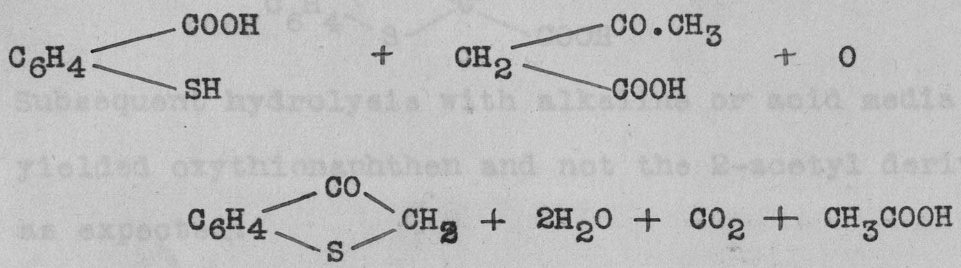
### Preparation of 3-Oxy(1)Thionaphthen.

The preparation of 3-oxy(1)thionaphthen involves several features of interest. Investigations carried out by Smiles and Ghosh (T., 1915, 107, 1377 ) led to the assumption that concentrated sulphuric acid solutions of certain aromatic mercaptans or disulphides contained the corresponding sulphenic acids, these substances being formed either by the oxidation of the mercaptan or by hydrolysis of the disulphide . These reactions appeared to be reversible and may be represented as follows:

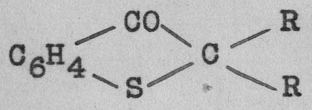
$$\mathrm{Ar.SH} + \mathrm{O} \rightleftarrows \mathrm{Ar.SOH}$$

$$\mathrm{Ar.SH} + \mathrm{Ar.SOH} \rightleftarrows \mathrm{Ar.S.S.Ar} + H_2O$$

The application of this hypothesis led to the synthesis of thioxanthones from o-thiolbenzoic acid and derivatives of benzene. Smiles and Ghosh (loc. cit.) then ascertained whether this type of condensation could be effected with substances containing the keto methylene group. The interaction of o-thiolbenzoic acid and malonic or acetoacetic acid in concentrated sulphuric acid yielded 3-oxy(1)thionaphthen when equimolecular proportions were used. The 3-oxy(1)thionaphthen was converted to "thioindigo" if the temperature rose above 70°.

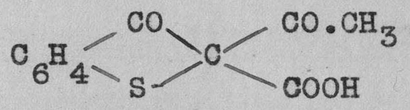


When the ester was used in place of the corresponding acid the acetyl derivatives of oxythionaphthen were formed in the preliminary stages of the reaction, these substituent groups being ultimately removed by the hydrolytic action of the hot sulphuric acid



R = $.CO_2Et$ or $.CO.CH_3$

Further light was thrown on these reactions by Smiles and McClelland (T., 1921, 119, 1810 ). In the case of ethyl acetoacetate it was shown (T., 1912, 101, 572; 1915, 107, 1379 ) that 2-thiolbenzoic acid and sulphuric acid at 90° gave "thioindigo", small quantities of oxythionaphthen and methylcoumarin. Experiments carried out at lower temperatures yielded none of these compounds but gave a product which, from its properties, appeared to be the acid:



Subsequent hydrolysis with alkaline or acid media yielded oxythionaphthen and not the 2-acetyl derivative as expected.

The experimental details given by Smiles and McClelland (<u>loc.cit.</u>) for the preparation of the 3-oxy(1)thionaphthen are discussed in another part of this work, but it may be stated that it was necessary to make further alteration on the method before obtaining the yield mentioned by these authors.

# CONDENSATION OF 3-OXY(1)THIONAPHTHEN WITH TETRA-ACETYL BROMOGLUCOSE AND ISOLATION OF TETRA-ACETYL THIOINDICAN.

The work now described may be synopsised in the scheme:

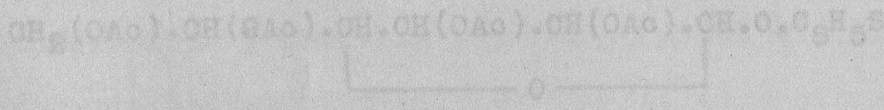
3-oxy(1)thionaphthen and tetra-acetyl bromoglucose

Condensation in
quinoline at
105°

**SYNTHESIS OF THIOINDICAN.**

Reacetylation

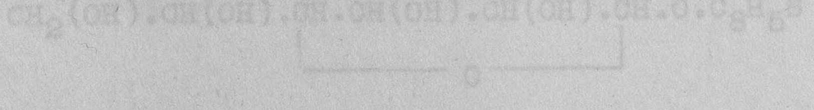
Tetra-acetyl, 3-oxy(1)thionaphthen glucoside



$CH_2(OAc).CH(OAc).CH.CH(OAc).CH(OAc).CH.O.C_8H_5S$

Hydrolysis by means of barium hydroxide or ammonia

THIOINDICAN



$CH_2(OH).CH(OH).CH.CH(OH).CH(OH).CH.O.C_8H_5S$

## CONDENSATION OF 3-OXY(1)THIONAPHTHEN WITH TETRA-ACETYL BROMOGLUCOSE AND ISOLATION OF TETRA-ACETYL THIOINDICAN .

The work now described may be synopsised in the scheme:

3-oxy(1)thionaphthen and tetra-acetyl bromoglucose

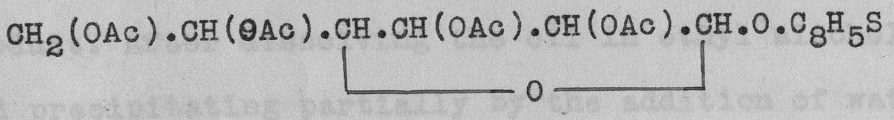
Condensation in quinoline at 105°

$\downarrow$

Reacetylation

$\downarrow$

Tetra-acetyl, 3-oxy(1)thionaphthen glucoside



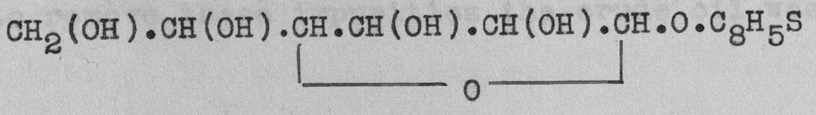
$$\mathrm{CH_2(OAc).CH(OAc).\underbrace{CH.CH(OAc).CH(OAc).CH}_{O}.O.C_8H_5S}$$

$\downarrow$

Hydrolysis by means of barium hydroxide or ammonia

$\downarrow$

THIOINDICAN



$$\mathrm{CH_2(OH).CH(OH).\underbrace{CH.CH(OH).CH(OH).CH}_{O}.O.C_8H_5S}$$

Preliminary experiments showed that the most favourable method of effecting the initial condensation was the process described by Fischer and Strausz. When a mixture of tetra-acetyl bromoglucose (1 part) and 3-oxy-(1)thionaphthen, ($1\frac{1}{2}$ parts) were heated in the presence of quinoline and the resulting product reacetylated a very dark red brown oil was obtained. On repeated precipitation from 60-70 per cent. ethyl alcohol this oil finally yielded delicate white needles consisting of the tetra-acetyl derivative of the required glucoside. The compound was soluble in organic solvents generally, behaved as a normal acetylated glucoside and showed $[\alpha]_D^{18^\circ} = +7.4^\circ$ in acetone. The yield of pure acetylated glucoside was however small and difficulty was experienced in obtaining crystalline crops from the crude product. After dissolving the oil in ethyl alcohol and precipitating partially by the addition of water, very impure crystalline material was isolated in small amounts. The red colour of thr oil constituting the main product was probably due to oxidation of the thioindoxyl during the condensation and in an endeavour to remove these impurities the crude oil was subjected to steam distillation and thereafter reacetylated. Although after this treatment nucleation with the crystalline glucoside did not promote solidification complete analysis showed that the resulting syrup was essentially tetra-acetyl thioindican. It was free from bromine, Fehling's solution was reduced only after treatment with hydrochloric acid and thioindoxyl and glucose were identified as the products of hydrolysis. It was therefore justifiable to conclude that the syrup consisted of the tetra-acetyl glucoside which was thus obtained in two forms (a) crystalline (b) non-crystalline .

## Alkaline Hydrolysis of the crystalline Tetra-acetyl Thioindican by means of Barium Hydroxide.

In the course of preliminary experiments the conditions were established whereby the acetyl groups could be removed quantitatively by the action of N/5 barium hydroxide at 25°. The reaction was however slow and in subsequent deacetylations the temperature was maintained at 60°. In this way, the parent glucoside was isolated in the crystalline state (m.p. 73.5°). The compound was soluble in alcohol and acetone but insoluble in petroleum ether, and was readily hydrolysed being thereby converted into glucose and thioindoxyl . The method just described was accompanied by much experimental loss and only small quantities of product were obtained .

### Alkaline Hydrolysis of the Non-crystalline Tetra-acetyl Thioindican by means of ~~means~~ Ammonia.

The syrup was dissolved in dry ethyl alcohol and the solution saturated with dry ammonia in the cold (Fischer., Ber.,1919, 52, 197 ). After standing for about ten hours the excess of ammonia was removed and the alcohol distilled under diminished pressure. The acetamide and other acetylated by-products were removed by extracting with ether, the glucoside remaining undissolved. On repeated precipitation from absolute ethyl alcohol the crude product finally yielded delicate white needles consisting of the parent glucoside and possessing the same properties as those isolated from the alkaline hydrolysis of the tetra-acetyl derivative by means of barium hydroxide.

# CONDENSATION REACTIONS OF INDOXYL AND 3-OXY(1)THIONAPHTHEN.

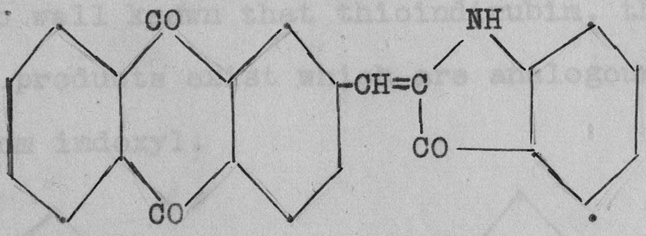
## CONDENSATION REACTIONS OF INDOXYL AND THIOINDOXYL.

As an extension of the present research it was decided to study in some detail other condensation reactions of indoxyl and thioindoxyl. It was shown by Baeyer (Ber., 1881, 14,1745 ) that indoxyl condenses with isatin to form indirubin

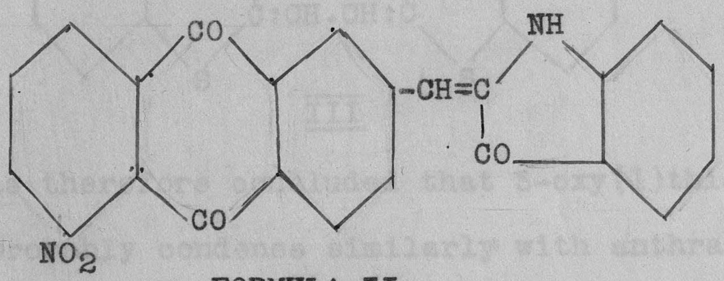
$$C_8H_7ON + C_8H_5O_2N = C_{16}H_{10}O_2N_2 + H_2O$$

and this reaction has been employed for the characterisation of the indoxyl produced by the hydrolysis of indican (Beyerinck, Proc. K. Akad.Wetensch., Amsterdam, 1899, 2, 120; Hazewinkel, ibid., 1900,2, 512). Orchardson, Wood and Bloxam (J.Soc.Chem.Ind., 1907, 26, 4 ) confirmed the suggestion of Beyerinck that this reaction is quantitative by applying it to estimate the purity of an authentic crystalline specimen of indican.

It has now been ascertained that indoxyl reacts quantitatively in a parallel manner with derivatives of anthraquinone to give a series of condensation compounds characterised by their intense colours, high melting point and sparing solubility. The indoxyl was provided from the acetic acid solution obtained in the preparation of indoxyl already mentioned and the indoxyl present was estimated by one of the following methods: (1) by oxidation in alkaline solution to indigo, (2) by condensation with isatin, (3) by condensation with piperonal. Typical condensation products were formed by the interaction of indoxyl with (a) 2,aldehyde anthraquinone (I) and (b) 5,nitro 2,aldehyde anthraquinone (II). These were easily condensed by boiling under a reflux condenser in the presence of concentrated hydrochloric acid, the linkage taking place between the aldehyde group of the anthraquinone derivative and the 2 position of the indoxyl ring .



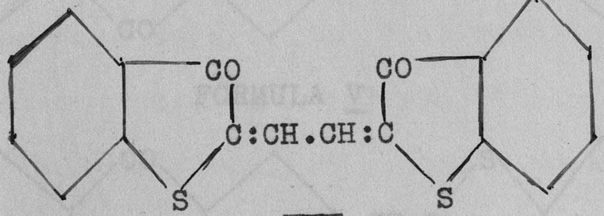
FORMULA I.



FORMULA II.

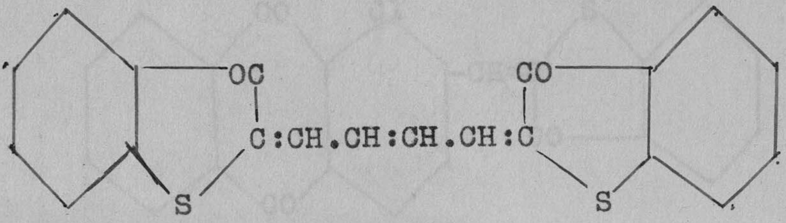
Both products were very stable, sparingly soluble and had extremely high melting points. Their formation can therefore be used to estimate indoxyl either in solution or as one of the hydrolytic products obtained from a glucoside of the indican type.

Again, 3-oxy(1)thionaphthen like indoxyl is known to condense quantitatively with various compounds containing an aldehyde group. For example Friedländer and Risse (Ber., 1914, 47, 1919 ) showed that in presence of acetic or hydrochloric acid very stable, insoluble compounds were formed by the combination of thioindoxyl with such substances as Glyoxal (III) or Maleindialdehyde (IV). It is also well known that thioindirubin, thioindigo and other products exist which are analogous to those formed from indoxyl.



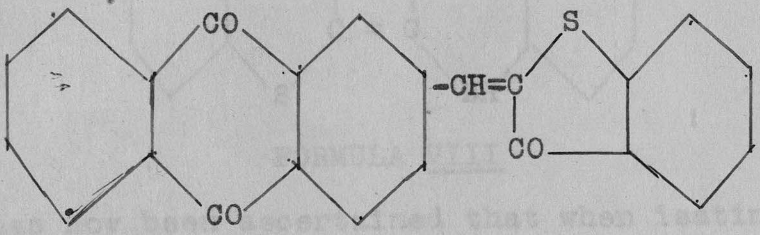
III

It was therefore concluded that 3-oxy(1)thionaphthen would probably condense similarly with anthraquinone derivatives. Starting from 2,methyl anthraquinone the

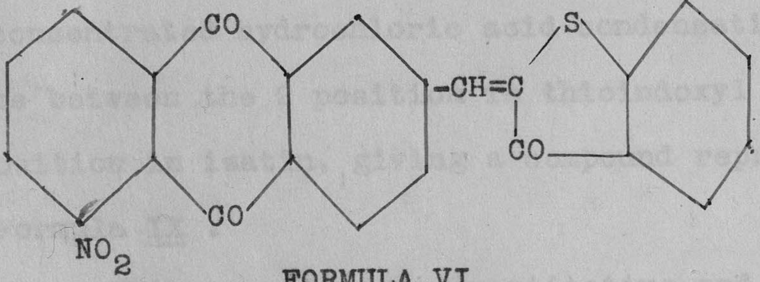


FORMULA IV

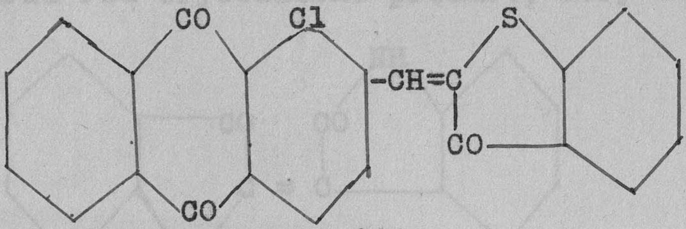
2,aldehyde and 5,nitro 2,aldehyde compounds were prepared; and the 1,chloro 2,aldehyde was prepared from the corresponding 2,methyl compound, The aldehyde and its nitro- and chloro- derivatives were all successfully condensed with 3-oxy(1)thionaphthen to give stable insoluble substances formulated respectively by V , VI and VII below.



FORMULA V

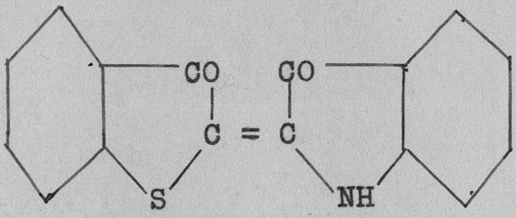


FORMULA VI



FORMULA VII

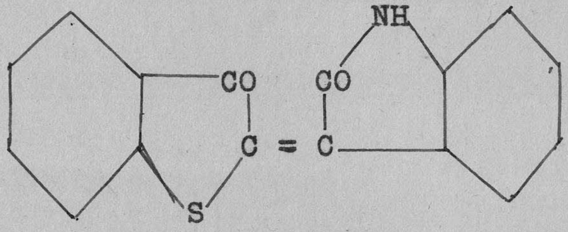
In addition a direct condensation was carried out with 3-oxy(1)thionaphthen and isatin. It may be recalled that the anilide of isatin gives a condensation product in which the linkage couples the 2 positions as shown in Formula VIII .



FORMULA VIII

It has now been ascertained that when isatin and thioindoxyl dissolved in alcohol are boiled in the presence of concentrated hydrochloric acid condensation takes place between the 2 position in thioindoxyl and the 3 position in isatin, giving a compound represented by Formula IX .

This reaction was quantitative and yielded a beautiful red crystalline product, very stable, and



FORMULA IX

insoluble in most organic reagents. All the reactions now described form the basis of excellent methods for the estimation of thioindoxyl .

## EXPERIMENTAL PART .

# <u>EXPERIMENTAL PART .</u>

## EXPERIMENTAL.

### Preparation of Pure Tetra-acetyl Bromoglucose.

There are certain standard methods for the preparation of tetra-acetyl bromoglucose but as considerable quantities were required for the present research, it was advisable to adopt the quickest and most practical one. J.K.Dale (J.Amer. Chem.Soc., 1916, 38, 2187 ) treated glucose with acetic anhydride saturated with hydrobromic acid gas and on dissolving the resulting syrup in chloroform, washing the solution and evaporating to a thick syrup, obtained the crystalline product on the addition of excess of petroleum ether. Fischer (Ber., 1916, 49, 584 ) prepared glucose pentacetate in a separate operation and treated it with the commercial solution of hydrobromic acid in glacial acetic acid. The results from this preparation were very poor, but more success was attained when glacial acetic acid saturated at 0°C. with pure dry hydrogen bromide was used in place of the commercial solution. The quickest and most reliable method of preparation however, is that dependent on the use of acetyl bromide. 40 Grams of acetyl bromide were added to 10 grams of powdered glucose and after preserving for about 10 minutes in the cold, a vigorous reaction ensued most of the glucose having dissolved at the end of 20 minutes. The resulting solution when poured into 300 c.c. of water gave a precipitate which soon hardened. This was washed twice with sodium bicarbonate solution, then with water until the washings were free from acid. The white solid mass was dissolved in ether, the solution dried for 10 minutes over sodium sulphate, when on removing most of the solvent, the addition of petroleum ether caused the separation of an oil which crystallised spontaneously within twelve hours.

Rapid recrystallisation from a little amyl alcohol gave a pure crystalline specimen of tetra-acetyl bromoglucose. Prepared in this way, and if kept in a vacuum desiccator over calcium chloride or soda lime the compound can be stored for several months, but otherwise it decomposes abruptly.

The yields and purity of the final product showed certain variations in different preparations, and as a rule 10 grams of glucose gave from 8 to 12 grams of the acetylated bromo-compound .

## PREPARATION OF INDOXYL.

As has already been mentioned various attempts were made to prepare crystalline indoxyl before even modified success was achieved. When 10 grams of the potassium salt of phenylglycine were fused in an open crucible with 20 grams of sodium or potassium hydroxide, until the temperature rose slowly to 250°,an orange-coloured melt was obtained. All attempts to extract crystalline indoxyl from this melt were however unsuccessful and it was decided that the salt of phenylglycine was not the best starting material.

Further attempts were made commencing from phenylglycine-o-carboxylic acid. This compound was readily prepared in large quantities from o-aminobenzoic acid and chloracetic acid. 55 Grams of anthranilic acid and 38 grams of chloracetic acid were dissolved in 400 c.c. of water containing 45 grams of potassium hydroxide, and the solution warmed under a reflux condenser for two hours at a tempreature of 60° to 80°. Partial separation of the phenylglycine-o-carboxylic acid took place during this time and on acidifying slightly with hydrochloric acid the remainder of the product was precipitated. This was filtered, dried and used for the next stage without further purification. A number of fusions was carried out varying the proportions of alkali from 1 - 3 parts to 1 part of the sodium or potassium salt of the carboxylic acid. In different experiments the fusions were conducted in an open crucible, in an atmosphere of coal gas and in a high vacuum. The best melt was obtained by fusing 50 grams of dry phenylglycine-o-carboxylic acid with 125 grams of finely-powdered potassium hydroxide (as dry as possible ) in the presence of a little sodamide. The operation was carried out under diminished pressure in a glass flask which was heated in an air bath, at first slowly and thereafter at 230-250° for ten minutes .During the reaction the colour changed to bright orange and finally to lemon yellow throughout. The presence of a white core in the centre of the melt showed that the fusion was not complete.

The melt prepared in this way was roughly powdered and added to 900 c.c. of a 25 per cent. solution of acetic acid kept at a temperature of 60-70°. The beautiful green fluorescent liquid was filtered while still warm to remove slight traces of indigo which had been formed. Crystalline indoxyl separated in small amount after standing in ice overnight and this method requires further modification in order to give good results. 100 C.c. of the acetic acid solution were treated so as to estimate the indoxyl content (1) by oxidation in alkaline solution to indigo, (2) by the formation of indirubin with isatin, and (3) by means of other condensation reactions .

## Estimation of Indoxyl in the Acetic Acid Solution.

The experimental data now quoted are those given by estimations conducted on different mother liquors. The majority of these solutions were found to contain approximately 1 gram of indoxyl per 100 c.c., but, on some occasions, only half of this amount was present.

### (1) By oxidation to indigo.

50 C.c. of the mother liquor were rendered alkaline with excess of sodium hydroxide and on aspirating air through this solution for 30 minutes the indoxyl was completely oxidised to indigo . The indigo was collected in a Gooch crucible, washed, dried and weighed . 50 C.c. of the mother liquor yielded 0.4825 gram of indigo, showing that 100 c.c. of the liquid con-

tained 0.9800 gram of indoxyl in solution .

(2) By condensation with isatin to form indirubin.

0.25 Gram of isatin, 300 c.c. of water and 50 c.c. of indoxyl solution were ~~boiled~~ heated under a reflux condenser, the flask being immersed in boiling water. An atmosphere of carbon dioxide was maintained in order to prevent any oxidation to indigo . 15 C.c. of concentrated hydrochloric acid were added and the heating continued for one hour . Separation of indirubin soon commenced and at the end of the reaction the liquid was cooled and filtered . The product was washed with a little 1 per cent. solution of warm sodium hydroxide and a few drops of 4 per cent. solution of acetic acid, dried and weighed. 50 C.c. of the mother liquor gave 0.4220 gram of indirubin. 100 C.c. of the original solution contained 0.4286 gram of indoxyl .

(3) By condensation with piperonal.

2 Grams of piperonal, 300 c.c. of water and 100 c.c. of the indoxyl solution were heated under a reflux condenser in presence of 15 c.c. of concentrated hydrochloric acid in a manner similar to the above. A

dirty orange coloured precipitate soon formed and the heating was continued for two hours. At the end of this time the liquid was cooled, and the precipitate collected, washed and dried. The yield of the condensation product was 2.12 grams showing that 100 c.c. of the original solution contained 0.9964 gram of indoxyl .

## Attempt to condense Indoxyl in solution with Tetra-acetyl Bromoglucose.

Only one attempt was made to condense the indoxyl present in the acetic acid solution with tetra-acetyl bromoglucose as it was considered unlikely that the reaction would be successful unless pure crystalline indoxyl were employed.

800 C.c. of the acetic acid solution containing in all about 8 grams of indoxyl were neutralised with sodium hydroxide. To this were added 50 c.c. normal sodium hydroxide and 20 grams of tetra-acetyl bromoglucose in 600 c.c. of ether. After shaking on a mechanical shaker for 24 hours at room temperature another 20 c.c. of normal sodium hydroxide were added and the shaking continued for an additional period of 24 hours. At the end of this time the ethereal solution was separated and evaporated to a syrup. This was heated on the water bath with about 200 c.c. warm water when most of the material went into solution. The solution was very dark, almost black, and an attempt to isolate the desired product

gave only negative results.

The 1 per cent. solution of indoxyl in acetic acid was however utilised, as subsequently described, for the preparation of a new series of condensation products (cf. pages 80-84).

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## PREPARATION OF 3-OXY(1)THIONAPHTHEN.&

3-oxy(1)thionaphthen (thioindoxyl) was prepared by Smiles and McClelland (T., 1921, 119, 1813 ) on adding 12 grams of ethyl acetoacetate very slowly to a mixture of 10 grams of 2,thiolbenzoic acid and 120 grams of concentrated sulphuric acid. The temperature was kept at 28-30° and the authors claimed that the reaction was over after 35 minutes, after which time the solution was poured on to crushed ice, filtered and the precipitate washed. When treated with a current of steam in presence of a little dilute sulphuric acid the substance yielded 3-oxy(1)thionaphthen. From 10 grams of thiolbenzoic acid they isolated 5 grams of oxythionaphthen.

In the course of the work now described the above preparation was carried out repeatedly, but in no case were more than 2.5 grams isolated. If the temperature were raised to 40-45° however, and the reaction allowed to continue for two hours the yield of oxythionaphthen was increased to 5 grams. It was not advisable to prepare more than this quantity in one experiment but if kept in an atmosphere of carbon dioxide the

the product could be preserved for a few days.

## CONDENSATION OF 3-OXY(1)THIONAPHTHEN WITH TETRA-ACETYL BROMOGLUCOSE.

### (1) Attempted condensation in ether with silver oxide.

14 Grams of tetra-acetyl bromoglucose (2 mol.) and 5 grams of 3-oxy(1)thionaphthen (1 mol.) were dissolved in dry ether. 5Grams of freshly precipitated silver oxide (1¼mol.) were added and the mixture was agitated on a mechanical shaker for 24 hours. After adding a drop of nitric acid to coagulate the suspension of silver compounds the ethereal solution was filtered, washed and evaporated. The residue was very red in colour and consisted chiefly of thioindigo. No trace of the tetra-acetyl glucoside could be detected. The yield of sitosterol-d-glucoside obtained by Salway by this method was also low and the experiment may have been on too small a scale to give .a positive result. It was also apparent that a considerable quantity of the thioindoxyl underwent oxidation to thioindigo during the experiment.

## A Synthesis of Tetra-acetyl, 3-oxy(1)thionaphthen Glucoside ( "Thioindican" ) .

(2) Condensation in presence of quinoline:

25 Grams of pure tetra-acetyl bromoglucose and 13 grams of 3-oxy(1)thionaphthen along with 10 grams of quinoline were heated on an oil bath to a temperature of 105-110°. The air in the flask was displaced by carbon dioxide to minimise oxidation to thioindigo. During the reaction the mixture turned red-brown in colour and finally became dark brown. After about two hours the warm viscous mass was poured into 300 c.c.(approximately) ice water and brought into solution in ether. The ethereal layer was washed with a litre of normal sulphuric acid to remove quinoline, then with sodium bicarbonate solution and finally with water until free from acid. After evaporation of the solution the excess of thioindoxyl was removed as quickly as possible by a rapid steam distillation, and when the water had been removed under greatly reduced pressure, the residue consisted mainly of acetylated and partially acetylated derivatives of the required glucoside. In order to acetylate the latter fully, the residue was dissolved in

60 c.c. of pyridine and 60 c.c. of acetic anhydride were added, the solution being allowed to stand in the cold for 24 hours. It was then poured into ice water and the oil which separated was extracted with ether. As before the ethereal solution was washed with sulphuric acid, sodium bicarbonate and water, and on distilling away the ether a red-brown mass remained. This was dissolved in about 150 c.c. of warm alcohol and, after cooling, treated with water until permanent turbidity was just commencing. In the course of a few hours a brown solid separated which on repeated crystallisation from 60-70 per cent. alcohol yielded beautiful white needles of the tetra-acetyl glucoside. The yield of the pure product was invariably very poor only about 2.5 grams having been isolated from the best experiment.

Subsequent experiments yielded a very dark syrup which could not be crystallised. After various unsuccessful attempts to obtain crystals by precipitation with water and petroleum ether from the alcoholic solution it was decided to subject the whole product to another steam distillation in the hope that by-products which might hinder crystallisation would be removed. This was carried out and after removal of the water the syrup was reacetylated as before, as the steam distillation causes partial deacetylation. Although a few crystals were isolated from this reacetylated syrup the yield was practically negligible.

Tetra-acetyl thioindican melts sharply at 106°. The compound which is soluble in alcohol, ether, acetone, pyridine and benzene but insoluble in water, shows only a slight dextro-rotation in acetone $[\alpha]_D^{18^\circ} = 7.36^\circ$.

Analysis of the substance gave:

$C = 54.7$; $H = 4.81$; $S = 6.68$.

$C_{22}H_{24}O_{10}S$ requires: $C = 55.0$; $H = 5.00$; $S = 6.66$ per cent.

Quantitative Estimation of Acetyl groups with standard Barium Hydroxide.

0.2640 Gram of substance was dissolved in 15 c.c. warm alcohol (absolute), 30 c.c. of barium hydroxide solution (0.174 N) were added and the mixture kept at 25° for 6 hours. The excess of barium hydroxide was titrated with hydrochloric acid (0.114 N) using phenolphthalein as an indicator.

25.2 C.c. 0.114 N hydrochloric acid were required to neutralise the excess of barium hydroxide. Hence 13.49 c.c. barium hydroxide solution were required to hydrolyse the acetyl groups in 0.2640 gram of substance, the theoretical amount required for the tetra-acetyl derivative being 12.64 c.c.

## Hydrolysis of the Tetra-acetyl Derivative to give the free Glucoside.

0.8268 Gram of the crystalline tetra-acetyl product was dissolved in 40 c.c. of absolute alcohol, warmed to 60°. 45 C.c. (0.174 N) barium hydroxide solution at 60° were added to the above. By this means the hydrolysis proceeded rapidly but was accompanied by slight colouration. Carbon dioxide was bubbled into the warm solution, the barium carbonate being filtered and washed with alcohol. The united filtrates on evaporation under diminished pressure gave a syrup mixed with white crystals. To isolate the glucoside this was extracted with absolute ethyl alcohol and a little water added to the warm solution. On cooling, crystals were deposited which were coloured slightly red. Recrystallisation from ethyl alcohol did not remove this red colour but on boiling for a few minutes with animal charcoal beautiful long white needles were obtained. These melted sharply at 73.5°. The yield was very poor, only 0.13 gram being isolated. They were soluble in alcohol and acetone but insoluble in petroleum ether and water. The rotation value, although showing very slight dextro properties, was too small at the low concentration available to give a reliable reading. The substance possessed the property of a glucoside as it did not reduce Fehling's solution until after hydrolysis with acid. It was also proved that it contained the thioindoxyl residue, which was easily identified after the hydrolysis by its characteristic properties.

As so little of the product was available it was decided to attempt an estimation of the thioindoxyl content by simultaneous hydrolysis and condensation with isatin (cf. page 58 of this thesis) according to the method outlined for estimating indican in plant extracts (Orchardson, Wood and Bloxam, loc.cit.). The acid concentration was increased and the time of refluxing much extended but the results obtained with the glucoside were much too low, owing to the low melting point of the substance and its insolubility in water, both of which factors make hydrolysis extremely difficult. The characteristic red condensation product was obtained but the yield was only 47.2 per cent. of the theoretical amount. 150 C.c.of water, 0.1 gram of isatin and 0.09 gram of the glucoside were boiled under a reflux condenser with 60 c.c. of hydrochloric acid for seven hours. After cooling,the red product was collected in a Gooch crucible, washed with water, dried and weighed. 0.09 Gram of the glucoside gave 0.038 gram of the condensation product. The theoretical amount required was 0.0845 gram.

Treatment of reacetylated syrup.

When all attempts to crystallise the reacetylated syrup failed it was examined to ascertain whether it was essentially glucosidic. It did not reduce Fehling's solution until boiled with hydrochloric acid and thioindoxyl was identified as one of the products of hydrolysis. The syrup was entirely free from bromine. An acetyl estimation was carried out with barium hydroxide solution. 0.5039 gram of the syrup was dissolved in 25 c.c. absolute alcohol at 60°, and 20 c.c. of warm barium hydroxide solution (0.34 N) were added. When the hydrolysis was complete the excess of barium hydroxide was titrated with hydrochloric acid (0.117 N).0.5039 gram of the syrup required 11.23 c.c. of 0.34 N. barium hydroxide, the theoretical volume being 10.5c.c.

## Action of Ammonia on the Reacetylated Syrup.

Since the syrup proved to be essentially glucosidic and gave satisfactory figures for the acetyl content it was justifiable to subject it to treatment with ammonia.

2 Grams of the syrup were dissolved in absolute alcohol and the solution was saturated in the cold with thoroughly dried ammonia gas, special precautions being taken to exclude atmospheric moisture. The solution was allowed to stand for ten hours, when the excess of ammonia was removed and the alcohol distilled under greatly reduced pressure at a bath temperature of 20°. Acetamide and other acetylated bodies were removed by extracting at the boiling point with ether. A very dark syrup remained which was dissolved in a mixture

of petroleum ether and ethyl alcohol at the boiling point. As ~~at~~ on cooling nothing separated most of the solvent was therefore removed and petroleum ether was added slowly at first and then in excess. A red solid was precipitated which on repeated crystallisation from absolute ethyl alcohol yielded fine white needles of the parent glucoside. These melted sharply at 73.5°. They were soluble in alcohol and acetone but insoluble in water and petroleum ether. The new glucoside possessed feebly dextro-rotatory properties but the specific rotation was too small for a definite value to be ascribed.

Analysis of the pure product gave:

$C = 53.61$; $H = 5.24$

$C_{14}H_{16}O_6S$ requires: $C = 53.84$; $H = 5.13$ per cent.

## CONDENSATION REACTIONS OF INDOXYL.

Reference has been made to the preparation of acetic acid solutions containing approximately 1 gram of indoxyl per 100 c.c. and it was decided to utilise this material for condensation with anthraquinone,2, aldehyde and its derivatives. Owing to the ease with which the formation of indirubin takes place with isatin it was reasonable to expect that analogous condensations would proceed smoothly.

### (1) Condensation of 2,aldehyde anthraquinone with Indoxyl.

### (a) Preparation of 2,aldehyde anthraquinone from 2,methyl anthraquinone (Ullman and Klingenberg, Ber., 1913, 46, 712 ).

17.75 Grams of 2,methyl anthraquinone were dissolved in 17.75 grams of nitrobenzene at 145°. To this solution 38.4 grams (12.4 c.c.) of bromine were gradually added from a dropping funnel. The temperature of the oil bath was kept at 145-150° until the end of the reaction when it was raised to 160°. Bromination extended over a period of seven hours and after about three quarters of the reagent had been added crystals of the dibromide began to settle at the foot of the flask, which finally became filled with a mass of crystals. It is essential that the addition of the bromine be carried out very slowly otherwise the tribromo-compound is formed.

After cooling, the mass was treated with alcohol, filtered and again boiled with alcohol. By this method 27.77 grams (91.8 per cent. of the theoretical amount ) of the dibromo-methyl anthraquinone were obtained, sufficiently pure for conversion to the aldehyde.

27.77 Grams of the crude dibromide were warmed at 125-130° with 278 grams of concentrated sulphuric acid in a large flask. Hydrogen bromide was evolved from the orange yellow solution, and was removed by a current of air. After three hours the reaction was complete. Ice was added to the cooled solution, the aldehyde filtered and washed with water (hot) . The residue, still moist, was boiled with water and a little ammonium hydroxide to remove the carboxylic acid, and the aldehyde, free from acid, was thereafter extracted 6-8 times with dilute sodium bisulphite solution at the boiling point. On addition of concentrated hydrochloric acid to this solution the aldehyde separated in needles. From 17.7 grams of 2, methyl anthraquinone 13.2 grams of product (m.p. 185-186°) were obtained, this being 70 per cent. of the theoretical amount.

(b) <u>Condensation with Indoxyl.</u> (Fofmula <u>I</u>) .

100 C.c. of the indoxyl acetic acid solution (containing 0.98 gram of indoxyl) and 1.8 grams of 2, aldehyde anthraquinone were heated under a reflux condenser for two hours in the presence of 15 c.c.. of concentrated hydrochloric acid, a stream of carbon dioxide being passed through the solution to minimise oxidation to indigo. A large crop of red crystals was formed and, after cooling, these were filtered, washed with water and dried in a steam oven. The product was insoluble in most organic reagents with the exception of glacial acetic acid in which it was only sparingly soluble at the boiling point. Recrystallisation from this solvent yielded red needles which melted at 327°.In boiling alcohol the product gave a very faint characteristic purple tint. The condensation was quantitative, 2.58 grams of the compound being isolated.

An analysis of the recrystallised substance gave:

C = 78.50; H = 3.67; N = 4.10

$C_{22}H_{13}NO_3$ requires: C = 78.60; H = 3.7 ; N = 3.99 per cent.

### (2) Condensation of 5,nitro 2,aldehyde anthraquinone with Indoxyl.

(a) Preparation of 5,nitro 2,aldehyde anthraquinone from 2,aldehyde anthraquinone. (Eckert, Monatsh. 1914, 35, 289).

5 Grams of the aldehyde derivative were dissolved in 50 grams of concentrated sulphuric acid and after cooling the solution was treated with 3 c.c. of nitrating acid (50% sulphuric acid and 50% nitric acid). After standing for three hours the mixture was poured on ice. The nitro-aldehyde was obtained pure after one recrystallisation from glacial acetic acid. It crystallised in pale yellow needles which melted at 268°.

(b) Condensation with Indoxyl. (Formula II)

100 C.c. of the indoxyl solution, containing 0.80 gram of indoxyl, were heated under a reflux condenser with 2 grams of the nitro-aldehyde of anthraquinone, together with 15c.c. of concentrared hydrochloric acid.

As before, the experiment was carried out in an atmosphere of carbon dioxide. A dark brown precipitate soon separated and the heating was continued for one hour. After cooling the product was filtered, washed with alcohol and water, and finally dried at 100°. The compound separated in dark brown needle shaped crystals from a very large excess of boiling glacial acetic acid , but was insoluble in all other organic solvents. The recrystallised product decomposed without melting at a temperature of about 315°. Yield:- Quantitative. Analysis of the recrystallised product gave:

C = 69.55; H = 3.15; N = 7.12

$C_{23}H_{12}N_2O_5$ requires: C = 69.69; H = 3.03; N = 7.07 per cent.

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## CONDENSATION REACTIONS OF 3-OXY(1)THIONAPHTHEN.

### (1) 3-oxy(1)thiomaphthen with 2,aldehyde anthraquinone.

(Formula V)

Equivalent weights of 3-oxy(1)thionaphthen and anthraquinone aldehyde, dissolved in acetic acid, reacted quantitatively in the presence of a few drops of concentrated hydrochloric acid. The product was recrystallised from excess of boiling glacial acetic acid forming bright yellow needles melting at 320°.

Analysis gave:-

$$C = 74.81;\ H = 3.15;\ S = 8.77$$

$C_{23}H_{12}SO_3$ requires: $C = 75.0$ ; $H = 3.26$; $S = 8.70$ per cent.

With the exception of glacial acetic acid, the substance was insoluble in organic reagents.

### (2) 3-oxy(1)thionaphthen with 5,nitro 2,aldehyde anthraquinone. (Formula VI).

Nitroaldehyde anthraquinone and thioindoxyl in acetic acid solution, condensed easily in the presence of concentrared hydrochloric acid when heated under a reflux condenser for one hour. The condensation product was deposited in yellow needles, and the yield was quantitative. When recrystallised from glacial acetic acid the product melted at 275° . Like the other condensation products obtained from indoxyl and thioindoxyl it was extremely insoluble in organic reagents.

A sulphur estimation showed:-

$$S = 7.70$$

$C_{23}H_{11}NSO_5$ requires: S = 7.74 per cent.

(3) Thioindoxyl with 1,chloro 2,aldehyde anthraquinone.

(a) Preparation of 1,chloro 2,aldehyde anthraquinone from 1,chloro 2,~~aldehyde~~ methyl anthraquinone.

Starting from the 1,chloro 2,methyl compound, the aldehyde derivative was prepared in two stages: (1) the formation of the dibromomethyl anthraquinone and (2) the conversion of the dibromide into the aldehyde. The experimental details were similar to those already given for the preparation of the 2,aldehyde anthraquinone from the methyl compound.

(b) Condensation .

The chloro-compound condensed readily with thioindoxyl when the acetic acid solutions were heated in the presence of concentrated hydrochloric acid and a quantitative yield of small yellow needles was obtained. The product was recrystallised from glacial acetic acid .

Analysis gave:

$$Cl = 8.79;\ S = 7.9$$

$C_{23}H_{11}O_3SCl$ requires: $Cl = 8.82;\ S = 7.95$ per cent.

The substance decomposed without melting at 264°.

(4) Condensation of 3-oxy(1)thionaphthen with Isatin. (Formula IX) .

Isatin and thioindoxyl underwent quantitative condensation to give a characteristic bright red crystalline compound. 0.8 Gram of isatin and 0.8 gram of thioindoxyl were dissolved in alcohol and boiled under a reflux condenser for one hour in the presence of concentrated hydrochloric acid. The product which was purified by recrystallisation from a large excess of boiling glacial acetic acid was insoluble in most organic solvents but was found to be sparingly soluble in ethyl alcohol. It did not melt below 330° .The yield was 1.4 grams.

A sulphur estimation gave:-

S = 11.49

$C_{16}H_9SNO_2$ requires: S = 11.47 per cent.

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In conclusion, the author wishes to express his indebtedness to Principal Irvine, F.R.S., and Dr. A. K.Macbeth for the interest they have taken in the work throughout its course; and to Mr. C.F.Cross, F.R.S., who selected the topic for the first part of this research.

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PH. D. THESIS — JAMES CRAIK