THE CHEMISTRY OF THE CASSIA SPECIES

by

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being

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C O N T E N T S

INTRODUCTION

Research in the Organic Laboratories of the University had been actively concerned with the study of natural products from South African plants which were known to be toxic to cattle or reported as of medicinal value to man. This study had recently concentrated on the possible isolation of curare-like drugs from *Strychnos* species, and it was decided to investigate other possible sources for quaternary alkaloids.

Siddiqui and Ahmed (*Proc. Ind. Acad. Sci.*, 1935, *2*, 421) reported the isolation of the quaternary base, chacksine, $C_{12}H_{21}O_2N_3$, from the seeds of *Cassia absus*.L., and it was decided to start an investigation of other *Cassia* species.

The *Cassia* species seem to present an interesting field of study in that the native tribes use infusions of the pods as remedies for such widely different diseases as malaria, blackwater fever and dysentery. (Watt and Breyer-Brandwijk, *The Medicinal and Poisonous Plants of Southern Africa*, 1932, p. 69.)

On the other hand the *Cassias* presented a challenge in that active compounds of diverse chemical structure are reported as contained in the different

species. For example, in addition to chacksine, Wells reports a toxic alkaloid, $C_{14}H_{19}O_3N$, (Philipp.J. Sci., 1919, 14, 1.) from C. siamea.

The well known Senna pods (C. fistula) and the leaflets of C. marilandia contain sennosides A and B (cf. Stoll and Becker, Progress Chem. Natural Products, 195, 7, 248) which are glycosides of dianthraquinones, and chrysophanic acid (4:5-dihydroxy-2-methylanthraquinone) is found in C. fistula and C. bijuga.

It is of interest that the naphthaquinones have antimalarial and Vitamin-K activity, and the reported medicinal value of extracts from Cassias could well be attributed to the anthraquinone content.

It was decided to start this investigation with C. laevigatum and C. didymabotry, both of which had been introduced into South Africa from South America.

The fruits were studied initially for alkaloids and anthraquinones as glycosides. One of the first substances isolated was a colorless crystalline glycoside which promised interesting structural studies. This substance however, proved to be the rare sugar raffinose. Experiments carried out on this material before it was recognised have revealed interesting additional proof as

to its structure, several of the reported physical properties are now corrected, and these new studies permit of a more ready method of isolation than the laborious process previously employed.

Two methods of extraction were used. The crushed defatted seeds, or pods, were:-

(I) Refluxed with alcohol acidified with acetic acid.

(II) Allowed to stand, with frequent stirring, in 0.3-0.6% alcoholic hydrochloric acid.

Method I.

On removal of the alcohol under reduced pressure, and the addition of a little water and acetone, raffinose separated in the form of the crystalline penta-hydrate. The substance was obtained from both C. laevigatum and C. didymabotry.

No alkaloid or anthraquinone compounds were identified on extracting the residue with organic solvents, but on the addition of ammonium reineckate, choline reineckate was precipitated. This was identified by analysis, microscopic spot tests, the refractive indices of the crystals, and the melting point of the aurichloride.

Method II.

By this method a very small quantity of an alkaloid $C_{33}H_{47}O_{14}N_3$ was obtained from the seeds of

C. laevigatum, and the same alkaloid from the seed pods of C. didymabotry, also in very small yield. It is proposed to name the alkaloid CASSEINE.

Choline was also obtained by this method, and various substances giving characteristic color reactions with concentrated sulphuric acid were isolated from the fruits of both C. laevigatum and C. didymabotry. These were present as water soluble compounds, and were isolated either by precipitation from the alcoholic extracts of the material with ammonia, (Method I), or by hydrolysis of the residue from the alcoholic extract, with 10% hydrochloric acid, and subsequent ethereal extraction (Method II).

Method I.

The compounds which formed stable ammonium salts were precipitated on the addition of ammonia to the acid alcoholic extract of the crushed seed or pod, and subsequently hydrolysed with 10% hydrochloric acid, and isolated by ethereal extraction.

Method II.

Those which were either not acidic, or too weakly acidic to form stable ammonium salts, were obtained by hydrolysis of an aqueous solution of the syrup obtained after removal of the alcohol, and all other substances

extricable from the solution, in either an acid or alkaline condition, by either ether or chloroform. The following substances were isolated:-

FROM CASSIA DIDYMABOTRY

Origin	Method	Color with H_2SO_4	Color with Ammonia	Remarks
Pods	II	Orange-brown	Red	
Seeds	I	Red-brown	Pink	The unhydrolysed substance, in aqueous solution had a red green fluorescence. A phenolic substance was also precipitated on hydrolysis.
Seeds	II	Brilliant orange	Brick-red	An acidic substance soluble in ammonia was precipitated on hydrolysis

All these substances from C.didymabotry were very sensitive indicators, the dilute aqueous solutions turning from pink in alkaline solution, to yellow in acid solution.

FROM CASSIA LAEVIGATUM

Origin	Method	Color with H_2SO_4	Color with ammonia	Remarks
Seeds	I	Brown	Yellow	A reducing substance, probably a sugar, was formed on hydrolysis, and a phenolic substance was precipitated, which gave a deep purple solution with NaOH.
Seeds	II	Brilliant crimson turning to orange then yellow.	Insoluble	A phenolic substance was precipitated, which gave, on hydrolysis, a deep purple solution with NaOH.
This substance was an anthraquinone derivative.				

A large quantity of tarry matter was produced in all the extractions of C. laevigatum, which was not present in the extracts of C. didymabotry. When this tarry matter was steam distilled in acid solution, a substance was left which gave a brilliant yellow color with concentrated sulphuric acid, and a yellow solution

with ammonia. These colors are characteristic of the sennosides.

A detailed study was made of raffinose and the anthraquinone which was identified as 4:5-dihydroxy-7-methoxy-2-methylanthraquinone. It was present in only very small quantities and not more than 500 mg. were available for this study.

The failure to methylate the two hydroxyl groups under normal conditions was attributed to association, and the author has included in this thesis earlier studies on association promoted by hydroxyl groups. The complete exclusion of extraneous hydroxyl groups, a precaution dictated by these earlier studies, has permitted complete methylation.

PART I

R A F F I N O S E

SUMMARY

The substance obtained from C. didymabotry and C. laevigatum is proved to be raffinose, a new method for the isolation of which is described.

The stability of the pentahydrate to 85-86° has been established in contradiction to Hungerford and Nees (Ind. Eng. Chem., 1934, 26, 462) who state that it is unstable above 78°.

The structure of raffinose, as given by Hayworth, Hirst, and Ruell (J., 1923, 3125), and Charlton, Hayworth, and Hickenbottom (J., 1927, 1527), and contested by Irvine (Chem. Reviews, 1927, 4, 203), Geza and Zemplin (Ber., 1927, 60 B, 923) and Helferich and Rauch (Ber., 1926, 59 B, 2655), has been confirmed by oxidation with potassium periodate.

The stability of the 1:6-oxygen bridge under periodic acid oxidation has been established, and the stability of the sucrose oxygen bridge has confirmed the findings of Fleury and Courtois (Bull. Soc. Chem., 1945, (V), 12, 548).

A method of estimating the nature of the oxygen linkeages in polysaccharides by oxidation with periodic acid and hydrolysis with dilute acid is suggested. The 1:4-link of lactose is shown to be broken with periodic acid, but is proved stable to short time treatment with

hot 3% sulphuric acid. The 1:6-link of melibiose is proved to be stable not only to 3% hot sulphuric acid, but to two days treatment with cold 10% sulphuric acid, and to periodic acid oxidation. The sucrose link is very sensitive to dilute acid hydrolysis, but is unattacked by periodic acid.

Silver oxide is found effective in catalysing the complete benzoylation of raffinose.

A colourless crystalline substance was isolated from the seeds of both C. laevigatum, and C. didymabotry.

It contained water of crystallization which was partially lost at room temperature under reduced pressure. The melting point of the hydrate was 85°, $[\alpha]_D^{27}$ +106°.5, (water, C, 1.39) and of the anhydrous substance, 118-119°.

After hydrolysis with hot 2N sulphuric acid, the substance reduced Fehling's solution, and a preliminary examination of the hydrolysed substance by paper chromatography revealed the presence of glucose, galactose, and fructose. The presence of galactose was confirmed by the production of mucic acid on oxidation with nitric acid.

Analytical results gave the empirical formula $C_{18}H_{32}O_{16}.5\ H_2O$.

From these characteristics it was suspected that the substance was raffinose, with constants, m.p. (anhydrous) 118-119°, decomposition point of pentahydrate, 78° (Hungerford and Nees, loc. cit.) $[\alpha]_D$ +104°.5.

Raffinose was discovered by Johnston in 1843 (Chem. Soc. Mem., 1, 159) in a manna, which drops from eucalyptus mannifera, a tree which grows in Jamaica. He gave it the name melitose, the empirical formula, $C_{12}H_{22}O_{11}3H_2O$, and stated that 2 moles of water were lost at 100°, and the remainder at 130°.

The substance was further examined by Berthelot (Compt. rend., 1855, 41, 392) and by Tollens (Ber., 1885, 18, 2611), and was later found in cotton-seed. (Ritthausen, J. pr., 1884, (2), 29, 351; Bohm, J. pr., 1884, (2), 30, 37), and in beet-root molasses by Loiseux (Bull. Soc. Chim., (2) 26, 365) and by Tollens (Ber., 1885, 18, 26; Annalen, 1886, 232, 201): Littmann, (Ber., 1885, 18, 3087) and Lindet (Compt. rend., 1890, 110, 795; Bull-Soc. Chem., 1890, (3) 3, 682).

Berthelot stated that water of crystallization was lost at 108° without melting, and that the anhydrous substance was not very hygroscopic. He described a second

hydrate containing six moles of water, obtained by crystallization from dilute alcohol.
(Berthelot, Compt. rend., 1889, 109, 548;
Bull. Soc. Chim., 1889, (3) 2, 656).

The substance is now known to be a Trisaccharide, yielding galactose, glucose, fructose on complete hydrolysis, whilst the action of invertase gives melibiose and fructose and emulsin yields galactose and sucrose.

From 1894 to 1934 various methods of preparation were described, and in 1934 Hungerford and Nees (Ind. Eng. Chem., 1934, 26, 462-4) obtained raffinose on a small commercial scale from molasses by seeding heavily with the raffinose, and allowing the mixture to crystallize at 15-18° during 20 days, under slow agitation. These crystals required several recrystallizations from water before they were pure.

Raffinose was isolated from the seeds of C. didymabotry, and C. laevigatum by a new and more simplified process.

The syrup, obtained from the defatted seeds by alcoholic extraction and subsequent removal of the alcohol under reduced pressure, was diluted with an equal volume of water and two volumes of acetone were added. On

cooling overnight to 5-10° the raffinose separated out in crystalline form. On filtration, and washing with a mixture of acetone and water, a crystalline pentahydrate was obtained, containing very little ash, and needing no decolorising.

The solubility of raffinose pentahydrate has been investigated by Hungerford and Nees (loc. cit.) between 0° and 78°, and they state that above this temperature the hydrate is not stable.

The substance obtained from Cassia by acetone precipitation melted sharply at 85°.

It has not hitherto been recorded that raffinose loses two molecules of water of crystallization at room temperature, under a high vacuum forming a trihydrate. neither has the extremely hygroscopic nature of the anhydrous substance been noted, the exposed substance gaining some 10% in weight in two hours, and presenting an extremely wet appearance.

In view of this, andthe difference between the melting point of the pentahydrate from Cassia species, and that recorded for raffinose, it was not immediately apparent that the sample was in fact raffinose and a complete investigation of the substance obtained from the seeds of the Cassias was necessary.

Acetylation.

The substance acetylated extremely readily, with acetic anhydride in the presence of pyridin. The hendeca-acetyl derivative so formed had the usual indeterminate melting point of sugar derivatives, melting to a sticky blob. The apparent melting point of the substance, i.e. the production of a clear blob, was 98-99°, but on examining the substance microscopically after a slight sinter at 92°, it was found that it was already melted, but so filled with tiny bubbles as to be opaque, and apparently solid. On keeping the temperature steady at 92° for some time, the melt became clear, and this temperature must be taken as the true melting point of the acetyl derivative. The purity of the compound was confirmed by analysis. The recorded melting point for the hendeca-acetyl derivative of raffinose is 100°.

Benzoylation.

In view of the probable difficulty of completely benzoylating a sugar of this size, it was decided to use a catalyst. The substance was benzoylated in the usual manner with benzoyl chloride in sodium hydroxide solution, with the addition of a little freshly prepared silver oxide. After shaking the solution for a few minutes, the mixture became completely solid, and after leaving for one hour,

the substance was found to be fully benzoylated.

(A similar experiment carried out with glucose, without the addition of silver oxide, resulted in a mixture of partially benzoylated substances from which both the penta- and tetra-benzoyl derivatives were isolated. It was of interest that the glucose penta-benzoate, which melted at 184° when crystallized from alcohol, melted at 157° on subsequent recrystallization from acetone, and 184° on again crystallizing from alcohol. The recorded melting point of α-D-glucose penta-benzoate is 187°, and that of the β-compound 157°.

Hydrolysis

The relative stability of the oxygen bridges to attack by mineral acid, was investigated by hydrolysis, and subsequent oxidation with sodium hypoiodite. One bridge was broken giving 1.21 -CHO groups within 10 minutes heating with 3% sulphuric acid, and within 30 minutes 1.38 -CHO groups were present. Heating for one hour with 10% sulphuric acid on a boiling water-bath gave 2.12 -CHO groups.

These findings are in accordance with the results found with raffinose, which is extremely easily split into fructose and melibiose, even by citric acid (J. Chem. Soc. Japan, 1939, 60, 1127-48).

They were also confirmed by the isolation of fructosazone and melibiosazone from the raffinose after hydrolysis for 5 minutes with 3% sulphuric acid, at 90°.

The partially hydrolysed product was acetylated, and octa-acetyl melibiose was isolated from the products.

The completely hydrolysed product was benzoylated, and the mixed product resolved into its constituents by chromatography, using an alumina column. Glucose tetra- and penta-benzoate, galactose penta-benzoate, and a fructose benzoate were isolated, and identified by their melting points and mixed melting points with substances prepared from glucose, galactose, and fructose.

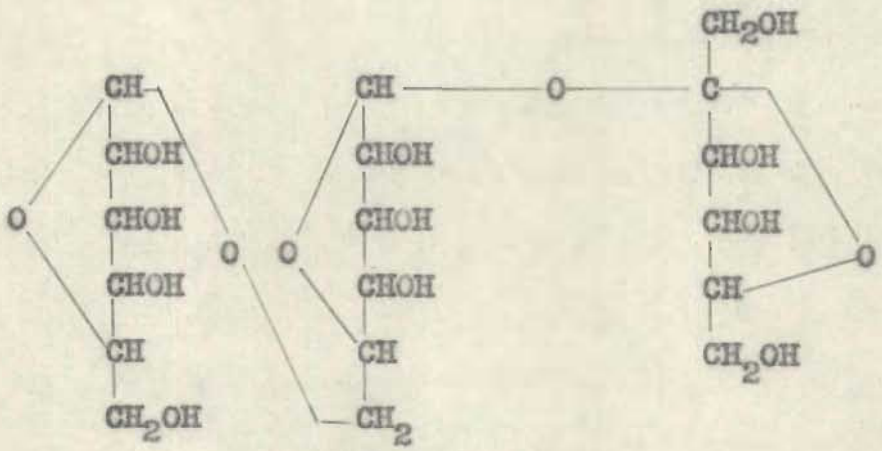
Thus the substance from Cassia has been proved to give a benzoyl derivative with the same melting point as that formed from raffinose, and to give fructose and melibiose on partial hydrolysis identified by their osazones, and glucose, galactose and fructose on complete hydrolysis, these substances being identified as their benzoyl derivatives, paper chromatography alone not being accepted as a proof of constitution.

These facts, together with the rotation and the formation of a pentahydrate, indicate that the substance is raffinose.

Further proof was afforded by an examination of the structure of the substance by periodic acid and periodate oxidation.

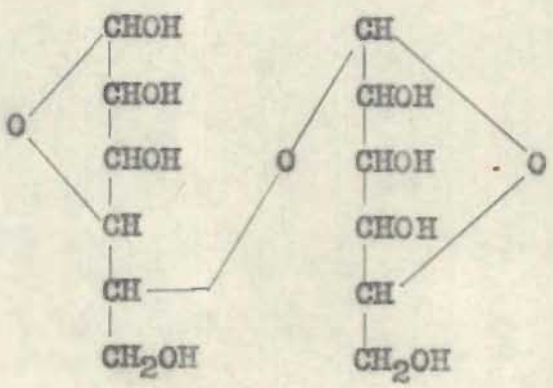
STRUCTURE OF RAFFINOSE

Haworth, Hirst and Ruell (loc. cit.) first examined the structure of raffinose by their classical method of methylation, subsequent hydrolysis, and identification of the methyl derivatives produced. They gave the sugar the structure:

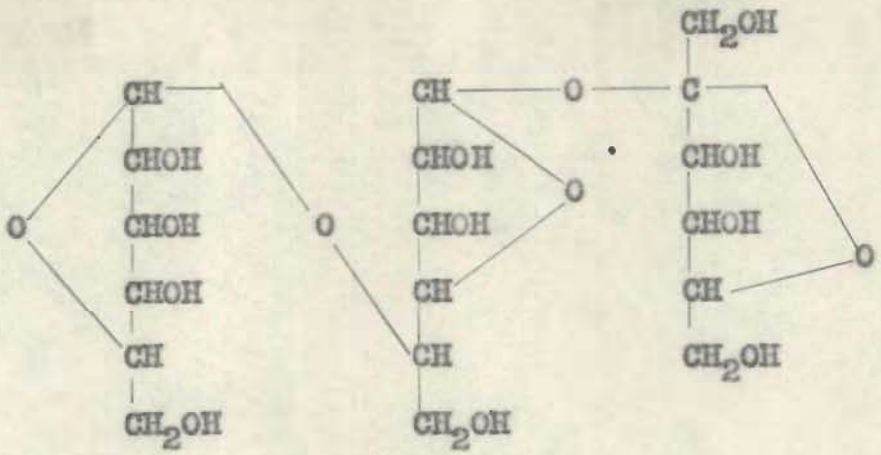


Glucose

This structure was challenged by Irvine (Chem. Reviews, 1927, 4, 203) who gave the structure for melibiose :



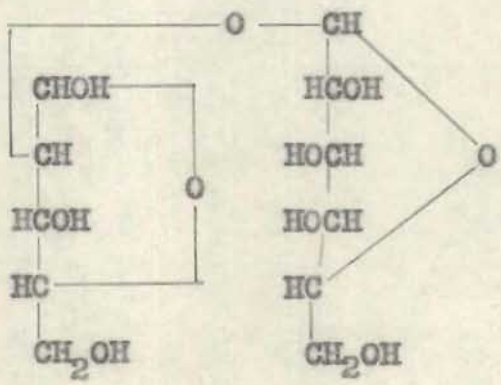
thus making the structure of raffinose :



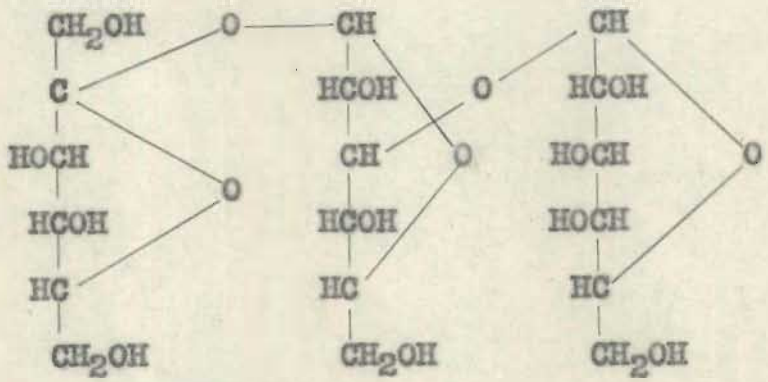
Glucose

Geza and Zemplin (Ber., 1927, 60, 923-30) said that the results of previous workers were contradictory, and approached the problem from a different angle.

They hydrolysed raffinose with top yeast, and prepared the oxime from the siropy melibiose so formed. The octaacetyl-melibo-nitrile prepared from this was a mixture, 40% of it being d-galacto-d-arabinose. This was incapable of forming an osazone, therefore they gave it the structure:



and from this they deduced that the melibiose must be 1-d-galactosido-3-d-glucose. Raffinose yields melibiose and fructose with top yeast, and galactose and cane sugar with emulsin, and has no reducing power. The structure of cane sugar having been established by Haworth, Hirst and Nicholson (J., 1927, 1513) the structure for raffinose, according to Géza and Zemplin must be :



Sucrose

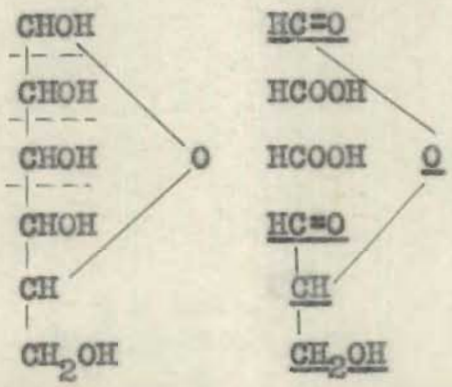
After 1927 no further work seems to have been done on the structure of this sugar.

It was decided to examine the substance obtained from the Cassia species by oxidation with periodic acid, and the periodates, a method that had not before been used. This should give very positive information as to the structure .

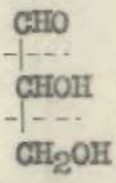
In estimating the number of oxygen atoms absorbed and the number of equivalents of acid which should be produced by one mole of any sugar, it is

necessary not only to take into account the number of glycol groupings present, but certain secondary reactions which may take place.

For instance, when glucose or galactose is oxidised, the following series of changes take place :



The underlined part being a formic ester, which will be hydrolysed giving a third acid group, and the substance



which will be further oxidised, taking two atoms of oxygen, and producing two more acidic groups, making a total of five oxygen atoms absorbed, and five acidic groups produced. These later stages may be very slow. On oxidising galactose with potassium periodate, the following results were obtained :

OXIDATION OF GALACTOSE WITH POTASSIUM PERIODATE

Time	Atoms of oxygen absorbed	Equivalents of acid produced
30 mins.	3.0	2.4
4 hrs.	3.7	3.5
11 days	4.75	4.74

The greater increase in acid produced between thirty minutes and four hours represented hydrolysis of the formic ester.

With fructose, glyoxalic acid is formed, which suffers secondary oxidation. The following results were obtained :

OXIDATION OF FRUCTOSE WITH POTASSIUM PERIODATE

Time	Atoms of oxygen absorbed	Equivalents of Acid produced
4 hrs.	3.0	2.0
1 day	3.5	2.6
3 days	3.9	2.8
5 days	3.9	2.9
21 days	3.9	2.9

Thus where a large number of glycol groups have to be oxidised, the period of oxidation may have to be extended over a number of days, and it was necessary to ensure that, under the conditions of the oxidation, the formaldehyde and formic acid produced were not decomposing.

In order to test this, glycerine was oxidised with potassium periodate over a period of 36 days. At the end of one hour 0.94 moles of formic acid had been produced.

This figure increased to 0.98 in 24 hours; 1.05 in 4 days; 1.09 in 6 days and 1.11 in 36 days. At the end of this time 2.01 atoms of oxygen had been absorbed. It is obvious that under these conditions the decomposition of the products of oxidation is negligible.

OXIDATION OF RAFFINOSE

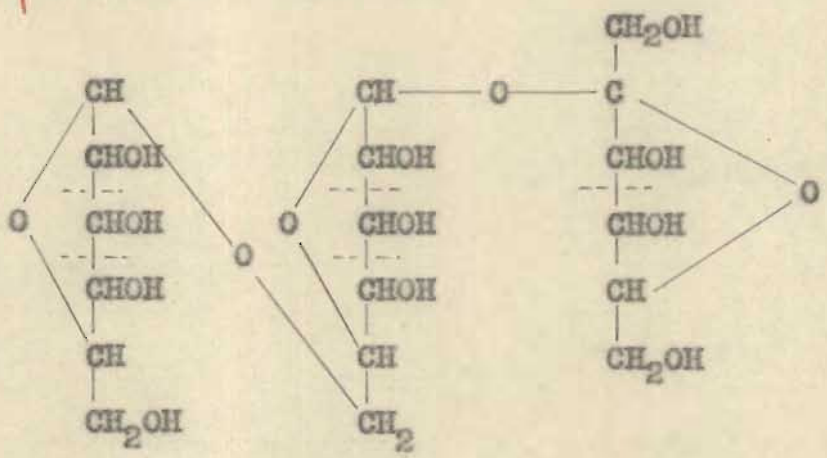
Raffinose was oxidised by both periodic acid and potassium periodate. With periodic acid 2.01 equivalents of acid were produced in 23 hours, whereas with potassium periodate the following results were obtained :

Time	Atoms of oxygen absorbed	Equivalents of Acid produced
3 hrs.	4.1	
5 hrs.		1.36
6 hrs.	4.6	
4 days	5.5	2.14
6 days		2.15
11 days	5.5	

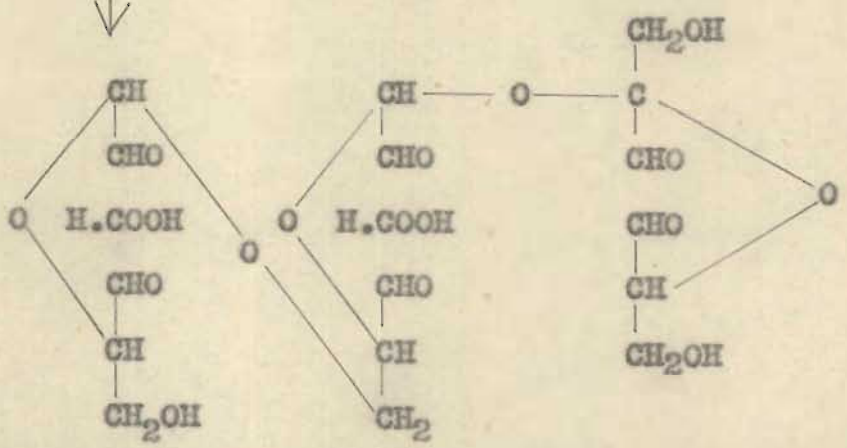
It may be assumed that the oxidation is complete in four days, with the absorption of five oxygen atoms, and the production of two molecules of formic acid. The

only structure to satisfy these conditions is that suggested by Hayworth. This is demonstrated by the following diagrams illustrating the attack by potassium periodate on the α-glycollic functions of the sugar.

HAWORTH'S STRUCTURE



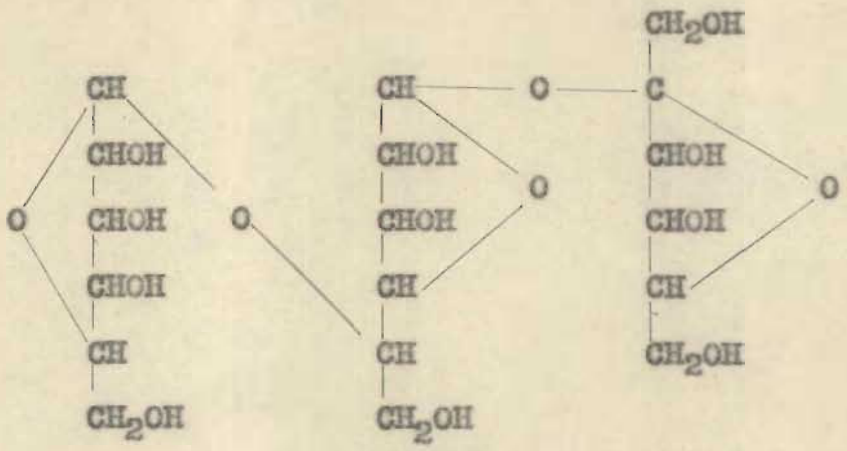
Glucose



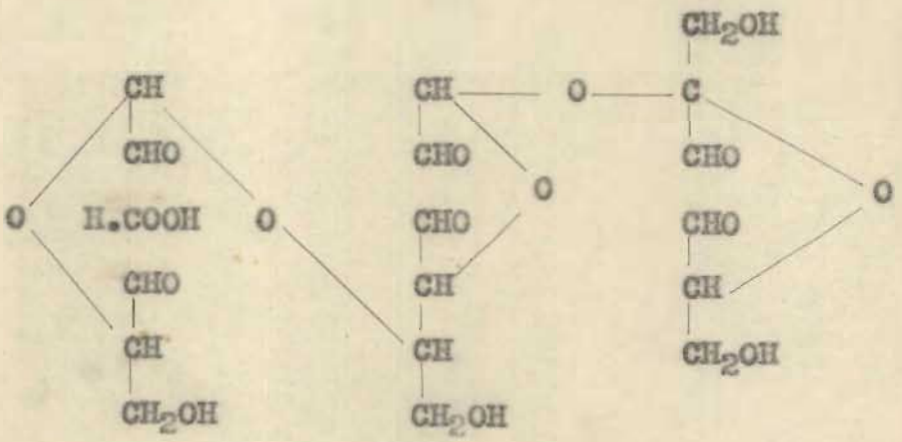
It may be seen that this structure, on oxidation with periodate, will absorb five atoms of oxygen, and give two molecules of formic acid. Moreover there is no

possibility of secondary oxidation, as there is neither formic ester, nor glyoxalic acid present.

IRVINE'S STRUCTURE:

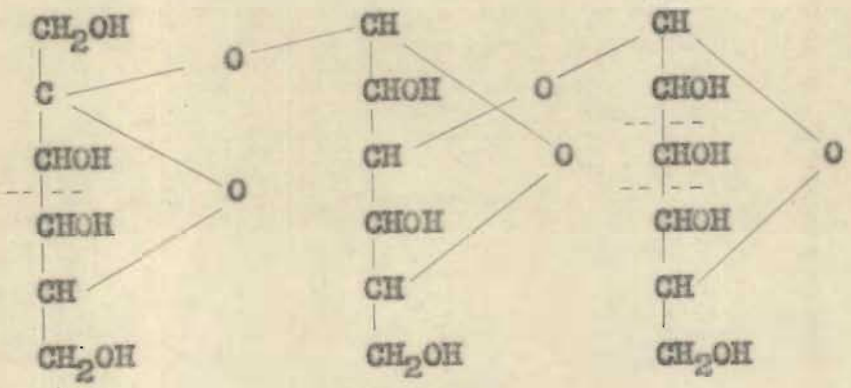


Glucose

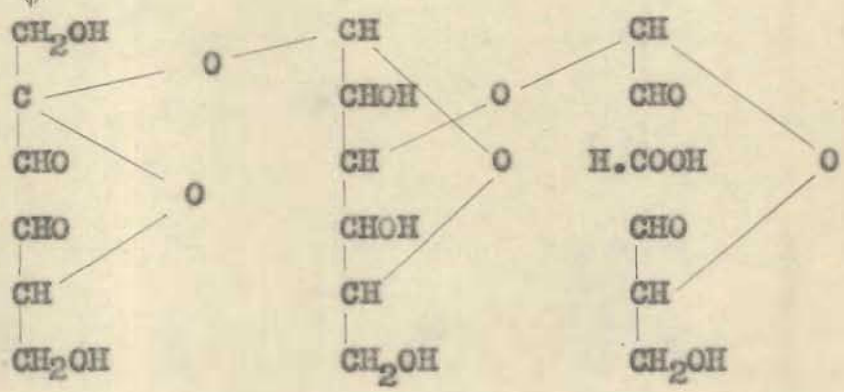


This structure will absorb four oxygen atoms, and produce one molecule of formic acid.

GEZA AND ZEMPLIN'S STRUCTURE:



Glucose



A substance with this structure will absorb three atoms of oxygen, and produce one molecule of formic acid.

STABILITY OF THE OXYGEN BRIDGES IN RAFFINOSE AND LACTOSE

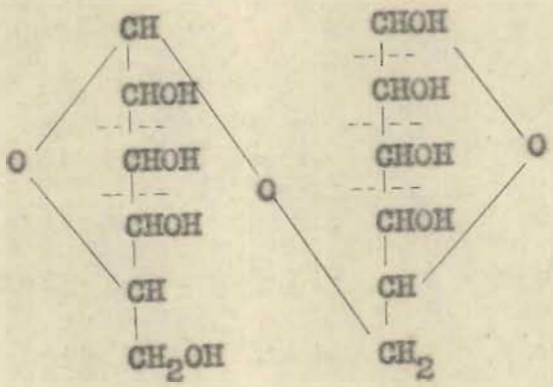
1. To Attack by Periodic Acid

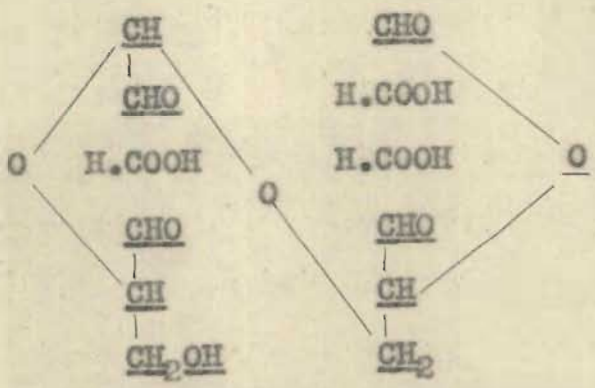
Previous to the examination of the α-glycollic functions of raffinose by periodic acid and periodate oxidation, blank determinations were carried out with lactose. A comparison of the curves on Fig. 3 (lactose) with those on Fig. 4 (raffinose) clearly demonstrates that the 1:4 oxygen bridge of lactose was rapidly broken with periodic acid, with the production of six equivalents of formic acid, whilst raffinose yielded only two equivalents of acid in 23 hours, proving that neither the sucrose nor the 1:6 oxygen bridge had been attacked.

Neither the 1:4, the 1:6, nor the sucrose bridge was attacked by periodate.

Oxidation of the mixture of melibiose and fructose produced by the partial hydrolysis of raffinose, gave a total of six equivalents of acid produced, with the absorption of eight oxygen atoms, with either potassium periodate or periodic acid. This again demonstrated the stability of the 1:6 oxygen bond with periodic acid, and confirmed the structure of melibiose. Thus, eliminating the final very slow processes of oxidation (taking more than one day), fructose gives the figures, three oxygen atoms absorbed,

and two equivalents of acid produced, (Fig. 2), leaving five oxygen atoms absorbed, and four equivalents of acid produced, by the melibiose, which is in agreement with the formula:-





The underlined part, being a formic ester, provides the fourth equivalent of formic acid.

2. To Attack by Dilute Mineral Acid:

It has already been shown that the sucrose oxygen bridge in raffinose is rapidly broken by hydrolysis with 3% sulphuric acid at 90°, whereas the 1:6 oxygen bridge of the resultant melibiose is attacked very slowly, the break within five minutes being negligible. (p. 16).

Curves "a" and "b", Fig. 5, representing the oxygen absorbed and acid produced by periodate oxidation, after hydrolysis for five minutes with hot 3% sulphuric acid, coincide with similar curves representing the results of oxidation after hydrolysis for 48 hours with cold 10% sulphuric acid, proving that the bond is also unattacked by the stronger acid.

Oxidation of lactose with periodate, both before and after treatment with hot 3% sulphuric acid, yielded only two equivalents of formic acid (Fig. 3), thus demonstrating the stability of the 1:4 oxygen bridge to the dilute mineral acid, and confirming the data found on oxidation with hypoiodite. (p.36).

These results may be summarised as follows:

Oxygen Bridge	Cold Dilute Mineral Acid	Periodic Acid
1:4 between glucose and galactose.	Unattacked	Attacked
1:6 " " "	Unattacked	Unattacked
1:2 sucrose	Attacked	Unattacked

As all these oxygen bridges are stable to periodates, consecutive oxidation with periodate, and

periodic acid, both before and after hydrolysis with cold, dilute mineral acid, should give very positive data as to the nature of the linkeages in polysaccharides.

EXPERIMENTAL

Isolation of Raffinose from Cassia Seed

The crushed, defatted seed (1000 g.) was allowed to soak in alcohol (4 l.) for ten days, when the alcoholic solution was found to contain only a very small amount of raffinose. On subsequent boiling with alcohol containing 1% acetic acid, however, the seeds yielded 0.256% of the sugar.

The alcohol was removed under reduced pressure, the temperature being kept below 40°, and the remaining brown oily residue was shaken with petroleum ether (60-80°), and left overnight. The cloudy petroleum ether was then poured off, and the residue repeatedly washed, first with petroleum ether, and then with acetone.

The remaining syrup was dissolved in a very little water, two volumes of acetone added, and the mixture kept in a refrigerator overnight, when a crop of the needle-shaped crystals of raffinose separated.

The substance was filtered off, washed with a mixture of acetone and water (2:1), and recrystallized from hot alcohol, from which it separated in the anhydrous form. The penta-hydrate (2.56 g.), was obtained by pouring off the alcohol, dissolving the anhydrous cake in a very little water, and precipitating with acetone, m.p. 85°. $[\alpha]_D^{27}$ + 106°.5 (water, C, 1.4)

Found : C, 36.3; H, 6.9%

Calc. for $C_{18}H_{42}O_{21}$: C, 36.7; H, 7.1%

The trihydrate, formed at 25° under a reduced pressure of 0.0001 mm., had m.p. 72°.

Loss in weight, 6.18%

$C_{18}H_{42}O_{21} \longrightarrow C_{18}H_{38}O_{19}$, requires loss, 6.06%

Found : C, 38.3; H, 7.0%

$C_{18}H_{38}O_{19}$ requires C, 38.7; H, 6.8%

Preparation of Mucic Acid from Raffinose

Raffinose (0.1 g.), nitric acid (3 c.c.) and water (7 c.c.) were evaporated slowly on a water-bath for half-an-hour. The syrup (2 c.c.) was cooled, and water (3 c.c.) was added. On standing, crystals of mucic acid separated, which were filtered and washed, m.p. 210° with decomposition. Mixed with mucic acid prepared similarly from lactose, m.p. 210-213° with decomposition.

Acetylation of Raffinose

Raffinose (1 g.) and pyridine (10 c.c.) previously distilled over sodium hydroxide, and acetic anhydride (8 c.c.) were mixed, and left three days at room temperature. The mixture was only very slightly discolored. A few drops of water were added to decompose the excess acetic anhydride, and after half-an-hour the mixture was poured on to ice and water, containing a little

acetic acid to dissolve the pyridine. A white precipitate separated, which was filtered and washed with water (1.32 g.)

After recrystallizing three times with petroleum ether, m.p. 92°, and from alcohol and water m. p. 91.7-92°.

Recorded m. p. 100°.

Found : C, 49.4; H, 5.6; Acetyl, 50.1%
C, 49.3; H, 5.6; Acetyl, 50.7%
Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.6; Acetyl, 48.9%

Benzoylation of Raffinose

Raffinose (0.75 g.), silver oxide (0.02 g.), benzoyl chloride (2 c.c.) and 20% sodium hydroxide (20 c.c.) were shaken for a few minutes, when the whole mass became solid. It was left for one hour, diluted with water, and extracted with ether. The ether was washed with sodium hydroxide, and then with water, dried with anhydrous magnesium sulphate, and distilled off. A white amorphous solid remained.

The substance, recrystallized from methyl alcohol, had m. p. 108-110°, recorded m. p. of hendecabenzoyl raffinose is "sintering 110-113°, an amorphous powder" (Sven Oden. Arkiv Kemi mineral. Geol. 7, No. 15, 1-22).

Hydrolysis of Raffinose with Dilute Acid

Raffinose was hydrolysed with dilute sulphuric acid, and the degree of hydrolysis was calculated from the number of aldehyde groups produced. This number was determined by oxidation with sodium hypoiodite in a solution buffered with a 2M solution of sodium hydrogen carbonate and sodium carbonate and subsequent titration with sodium thiosulphate.

Control determinations were made using glucose, and lactose.

A solution (25 c.c.) containing glucose (0.0110 g.) were added to 0.1N iodine solution (5 c.c.) in an iodine flask, the stopper moistened with potassium iodide, and the mixture left with a buffer solution (10 c.c.) in the dark for two hours. It was then diluted to 250 c.c., acidified with 2N sulphuric acid and titrated with N/100 sodium thiosulphate.

A blank determination was carried out on the iodine solution, and a control determination was made using water in place of solution.

The hydrolysed raffinose was oxidised in a similar manner.

The reducing groups were calculated from the formula:

$$-CHO \text{ groups} = \frac{N \times C \times M}{2000 \times W}$$

Where:

N = Normality of thiosulphate.

C = c.c. thiosulphate equivalent to iodine used.

M = Molecular weight of substance.

W = Weight of substance taken.

Substance	Conditions of hydrolysis	-CHO groups
Glucose		0.996
Lactose		0.993
Lactose	10 min. 3% H_2SO_4 on water-bath.	1.03
Raffinose	" " " " " "	1.21
Raffinose	30 " " " " "	1.38
Raffinose	60 min. 10% " " "	2.12

In subsequent work on the hydrolysed substance one bond was assumed to have been broken by five minutes heating with 3% sulphuric acid.

Preparation of Osazones after Hydrolysis to Melibiose and Fructose.

Raffinose (1 g.) was hydrolysed for five minutes on a boiling water-bath with 3% sulphuric acid (10 c.c.). The solution was neutralised with sodium hydroxide, and evaporated to 12 c.c. Phenylhydrazine (1.2 g.) and sodium acetate (1.8 g.) were added, and the solution heated in boiling water, when, in four minutes, fructosazone was precipitated, m. p. 205°, and identified by microscopic appearance (Recorded m. p. 205°).

Heating was continued for half-an-hour, and the liquid was allowed to cool, when an oily precipitate was formed, which, after crystallization from alcohol-water, 60:64 gave melibiosazone, m. p. 180°, seen as stars under the microscope (Recorded m. p. 178°).

Acetylation of the Partially Hydrolysed Substance. Isolation of Melibiose Octa-acetate.

Raffinose (3.75 g.) was hydrolysed by heating on a water-bath for five minutes with 3% sulphuric acid (20 c.c.), the acid removed by the addition of freshly prepared barium carbonate, and the solution evaporated to a syrup on a water-bath. This was dissolved in pyridin (40 c.c.), in which it was easily soluble, and acetic anhydride (40 c.c.) added. After three days, the solution

had become dark red, in contrast to the acetylated unhydrolysed raffinose, which remained colorless. A little water was added, and the solution left for the excess acetic anhydride to decompose. It was then poured into dilute acid and ice, and the resulting crystalline precipitate filtered off. After repeated extractions with petroleum ether, a substance was left, melting at 170-175°, and after recrystallization from ether, 177-178°. (Recorded for Melibiose octa-acetate m. p. 177°.)

Found : C, 49.9; H, 5.6; Acetyl, 51.0%
C, 50.2; H, 5.7.
Required for $C_{28}H_{38}O_{19}$: C, 49.6; H, 5.6; Acetyl, 53.7%

Benzoylation of D-Galactose.

D-galactose (1 g.), benzoyl chloride (4 c.c.), sodium hydroxide (20%; 20 c.c.) were shaken together for twenty minutes. Sodium carbonate (5 g.) was added, and the mixture shaken for a further ten minutes, after which water was added, and the mixture was extracted with ether, the ethereal extract washed with sodium hydroxide, and then with water until neutral, dried with anhydrous

magnesium sulphate, and the ether removed by distillation.

The resulting solid was washed with petroleum ether, dissolved in acetic acid and poured into water. m. p. 82°. It separated from petroleum ether as an amorphous solid, m.p. 78-82°.

Recorded for D-galactose penta-benzoate 78-82°, an amorphous solid.

Benzoylation of D-Glucose.

D-glucose (1 g.), benzoyl chloride (4 c.c.), and sodium hydroxide (20%; 20 c.c.) were shaken together for one hour. Water was added, and the mixture extracted with ether. The ethereal solution was washed first with sodium hydroxide, and then with water until neutral, dried with anhydrous magnesium sulphate, and concentrated. Crystals of the penta-benzoate, separated, m. p. 180°, after recrystallization from alcohol, m. p. 184°, after recrystallization from acetone, these crystals had m. p. 152-7°, but on again crystallizing from alcohol, had m. p. 184°.

Recorded m. p. for α-D-glucose penta-benzoate, 187°.

Recorded m. p. for β-D-glucose penta-benzoate, 157°.

D-glucose tetra-benzoate was obtained from the

ethereal mother liquor, m. p. 120°. After recrystallization from benzene/petroleum ether, m. p. 120-122°, addition compound with pyridin, m. p. 103°.
Recorded, m. p. 119-120°, for D-glucose tetra-benzoate.
Addition compound with pyridin, m. p. 103°.

Benzoylation of Fructose.

Fructose (0.5 g.), benzoyl chloride (4 c.c.), and sodium hydroxide (20%; 20 c.c.), were shaken together. When cool, benzoyl chloride (2 c.c.) and sodium hydroxide (5 c.c.) were added. The benzoylation was completed by the addition of a third volume of benzoyl chloride (0.5 c.c.) and sodium hydroxide (3 c.c.).

The mixture was diluted with water, extracted with ether, and the ethereal extract washed first with sodium hydroxide and then with water until neutral, and dried with anhydrous magnesium sulphate.

The oily residue obtained on removal of the ether was dissolved in boiling petroleum ether. On cooling an amorphous solid was deposited, m. p. 70-72°.
Recorded for fructose tribenzoate, m. p. 75°.

Benzoylation of the Completely Hydrolysed Raffinose.

Raffinose (0.94 g.) containing silver oxide (0.02 g.) was heated for four hours with 2N sulphuric acid (25 c.c.). The solution was neutralised with sodium carbonate, and made up to a 20% solution with solid sodium hydroxide. Benzoyl chloride (8 c.c.) was added. The solution became solid almost immediately, an effect not produced during a previous benzoylation of the hydrolysed product, when no silver oxide was added. In this latter case, very many benzoylation products were produced, many of which could not be identified, even after repeated chromatography through an alumina column.

To avoid the production of these partially benzoylated substances, the strength of the sodium hydroxide was made up to 20% by the addition of the solid substance, and a further quantity of benzoyl chloride (8 c.c.) was added. After a third benzoylation, the mixture was diluted with water, extracted with ether, the ether washed with water until no longer alkaline, dried with magnesium sulphate, and the ether removed by distillation.

The residue was dissolved in benzene, and poured through an alumina column (18 x 2 cm.) which had been previously wetted with the solvent. The column was

developed with petroleum ether, and eluted with benzene containing 1% alcohol.

The first fractions contained glucose penta-benzoate which, after crystallizing from ethyl alcohol, and methyl alcohol, melted at 174°, which was undepressed by the substance prepared from glucose.

The next fractions contained fructose benzoate, m. p. 72°, also undepressed by the benzoyl derivative prepared from fructose, and the last fractions contained galactose penta-benzoate m. p. 78° undepressed by the substance prepared from galactose.

From the previous incomplete benzoylation, glucose tetra-benzoate was isolated, m. p. 117-120°. Recorded for glucose tetra-benzoate, 119-120°.

Found : Benzoyl, 71.3%; Molecular weight, 568.
Calc. for $C_{34}H_{28}O_{10}$: Benzoyl, 70.5%; Molecular weight, 596.

The melting point was undepressed with the substance isolated from the benzoylation products of glucose.

Estimation of Glycol Linkages by Periodic Acid and Periodate Oxidation.

The substance to be oxidised was weighed into a volumetric flask, a small excess of oxidising reagent added, the solution made up to the standard volume, and kept in the dark. At timed intervals a measured volume was withdrawn, sulphuric acid and potassium iodide solution added, and the liberated iodine titrated with standardised sodium thiosulphate, using starch solution as an indicator.

A blank was run on the oxidising solution alone, and sufficient oxidising solution was added in the first place to ensure that the titration of the sample was always more than 80% of the blank, to make sure that enough reagent was present for complete oxidation. (If all the periodate acted, the titration would be 75% of the blank.)

Number of glycol links, or oxygen atoms absorbed = $\frac{C \times N \times M}{2000 \times W}$

Where: C = c.c. thiosulphate equivalent to iodine used.

N = Normality of thiosulphate.

M = Molecular weight of substance.

W = Weight of substance oxidised.

Where hydrolysis preceded oxidation, the solution was neutralised with sodium carbonate and washed into the

volumetric flask. The method was tested by oxidising galactose, fructose and lactose, the latter also after warming with 3% sulphuric acid, to parallel the conditions present during the oxidation of the partially hydrolysed raffinose. Lactose was not hydrolysed by the acid.

Galactose was oxidised with potassium periodate at 27° and at 22°. The effect of the small difference in temperature was found to be negligible.

The temperature range for all oxidations was 20-24°.

Estimation of Acidic Groups Produced by Periodic Acid and Periodate Oxidation.

The solution of the substance to be oxidised was made up in the same manner as for the estimation of glycol groups. Where acid groups were to be estimated during oxidation, however, any acid used for hydrolysis was previously standardised with sodium hydroxide and neutralised with this solution before oxidation.

An excess of oxidising agent was added and the solution kept in the dark. At timed intervals a measured volume was withdrawn, and sufficient ethylene

glycol (50% solution) to decompose the periodate was added. After twenty minutes the solution was titrated with standardised sodium hydroxide, using brom-thymol blue as indicator. A blank was run on the oxidising solution alone, using the same volume of ethylene glycol to destroy the periodate.

OXIDATION OF GLYCERINE

Oxidising Agent	Molality of Glycerine	Time	Oxygen Atoms Absorbed	Equivalents of Acid produced
KIO_4 0.0129N	0.00545	60 min.		0.938
		120 "		0.957
		24 hrs.		0.981
		4 days		1.052
		6 "		1.090
		36 "	2.01	1.110
HIO_4 0.1111	0.03740	60 min.		1.009
		75 "		0.984
		90 "		1.017
		105 "		1.006

OXIDATION OF GALACTOSE WITH POTASSIUM PERIODATE (0.0132N)

See Fig. 1

TEMPERATURE 22°

Molality of Galactose	Time in Minutes	Oxygen Atoms absorbed	Equivalents of acid produced
0.00236	65	3.14	
	70		2.89
	126		3.25
	180	3.59	3.38
	240	3.67	3.45
	11 days	4.75	4.74
	13 "	4.75	4.74
TEMPERATURE 27°			
0.00231	40	3.00	
	60	3.31	
	85	3.43	
	110	3.55	
	135	3.65	
	210		3.62
	240		3.67
	290	3.85	

FIG. 1.

OXIDATION OF GALACTOSE WITH KIO_4. EFFECT OF TEMPERATURE

"a" = Oxygen absorbed
"b" = Acid produced

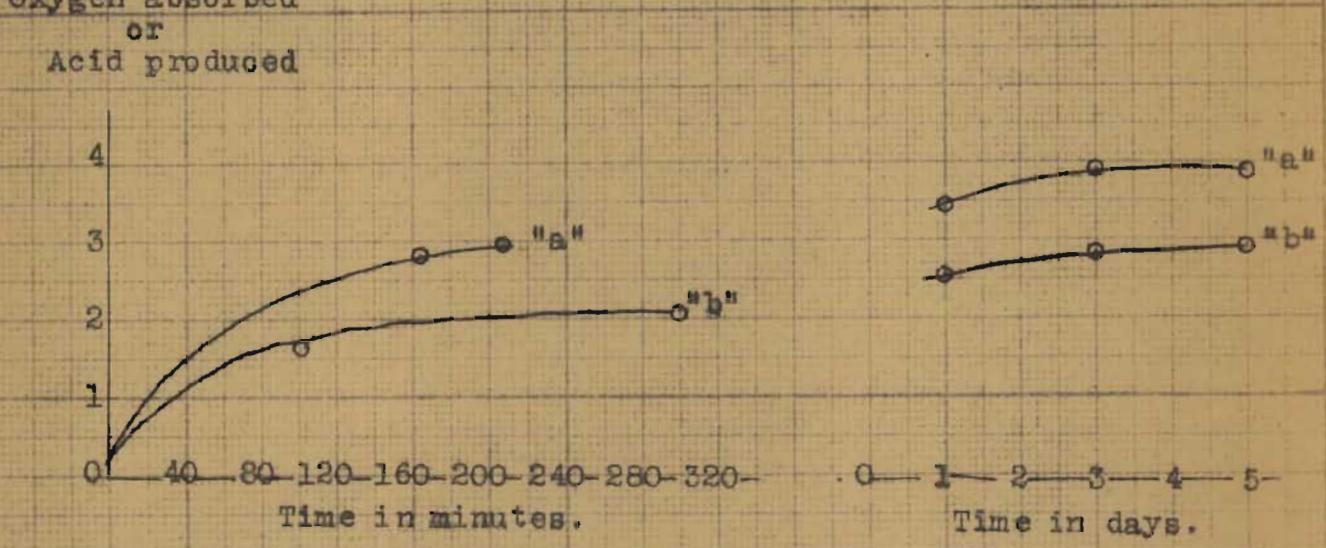
OXIDATION OF FRUCTOSE WITH POTASSIUM PERIODATE (0.0158N)

See Fig. 2

Molality of Fructose	Time in Minutes	Oxygen Atoms absorbed	Equivalents of acid produced
0.00398	105		1.67
	165	2.83	
	210	2.94	
	300		2.10
	One day	3.46	2.58
	3 days	3.90	2.85
	5 "	3.90	2.90

FIG. 2.

OXIDATION OF FRUCTOSE WITH KIO_4



"a" = Oxygen absorbed
"b" = Acid produced

OXIDATION OF LACTOSE.

See Fig. 3

Oxidising Agent	Molality of Lactose	Time in Minutes	Equivalents of Acid produced
HIO_4, 0.1082N	0.00746	50	2.15
		80	2.43
		110	2.70
HIO_4, 0.1118N	0.00761	180	3.30
HIO_4, 0.1541N	0.00660	270	4.94
		390	6.04
		23 hr.	8.46
$NaIO_4$, 0.0733N	0.00593	60	1.67
		90	1.82
		105	1.86
		128	1.96
		143	2.03
		162	2.01
		177	2.01

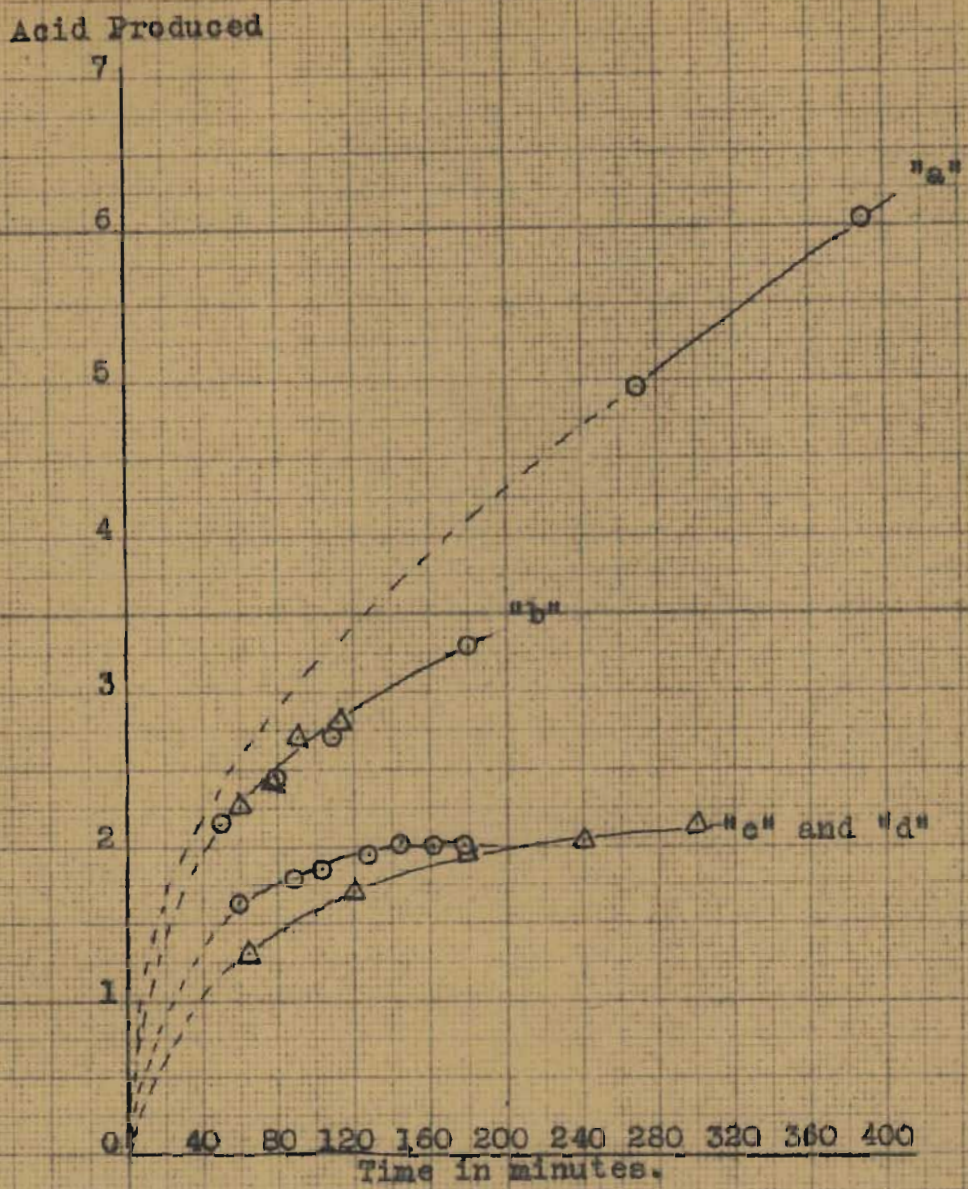
OXIDATION OF LACTOSE AFTER HEATING WITH 3% SULPHURIC ACID

See Fig. 3

Oxidising Agent	Molality of Lactose	Time in Minutes	Equivalents of Acid produced
KIO_4, 0.0158N	0.00325	65	1.31
		120	1.68
		180	1.98
		240	2.05
		300	2.17
		1500	2.28
HIO_4, 0.1120N	0.00197	60	2.25
		75	2.45
		90	2.71
		112	2.80

FIG. 3.

OXIDATION OF LACTOSE WITH KIO_4, $NaIO_4$, and HIO_4



"a" = Oxidation with HIO_4, 0.1541 N.
"b" = -o- " " " , 0.1082 N.
-△- " " " , 0.1120 N. (After heating with 3% H_2SO_4)
"c" = -o- " " $NaIO_4$, 0.0733 N.
"d" = -△- " " KIO_4, 0.0158 N.

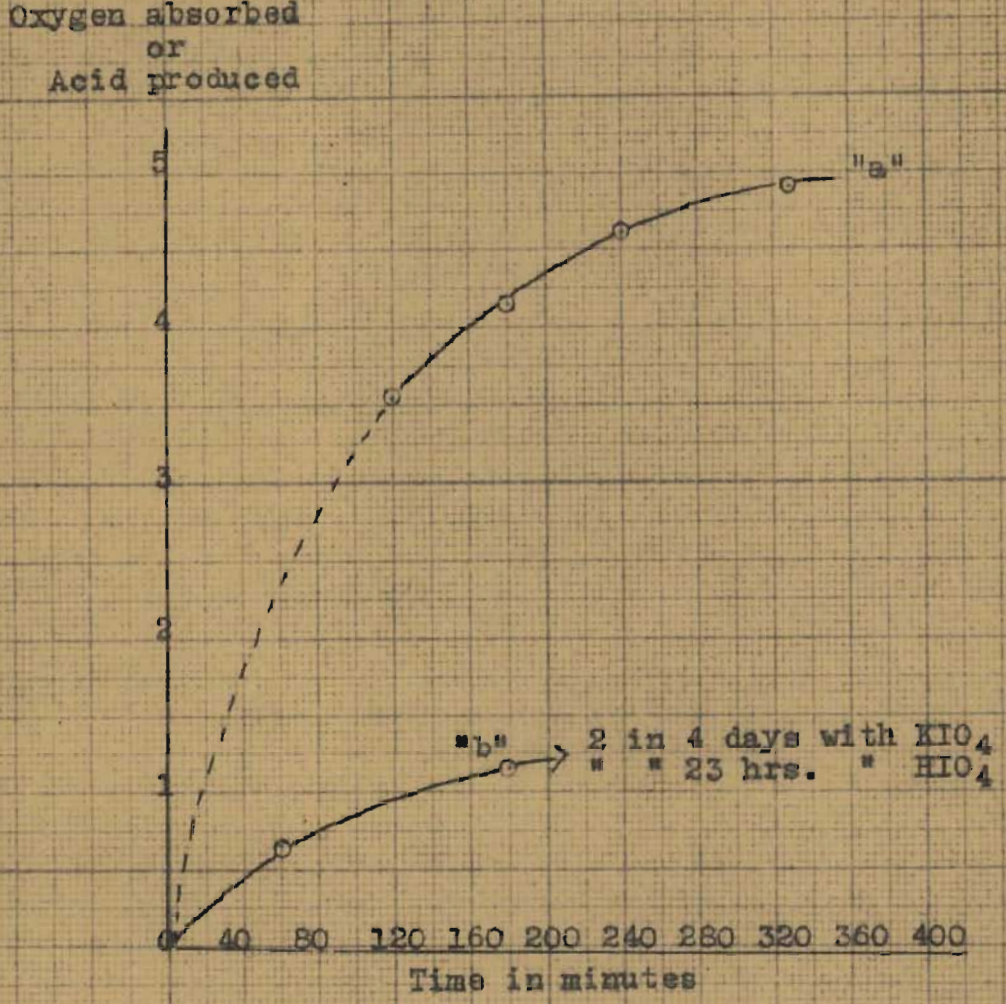
OXIDATION OF RAFFINOSE

See Fig. 4

Oxidising Agent	Molality of Raffinose	Time	Oxygen Atoms absorbed	Equivalents of Acid produced
	0.00082	180 min.	4.14	
KIO_4,		240 "	4.63	
0.0170N		300 "		1.36
		330 "	4.88	
KIO_4,	0.00058	4 days	5.50	2.14
0.0165N		6 "	5.40	2.15
$NaIO_4$,	0.00111	120 min.	3.58	
0.0212N	0.00141	120 "	3.54	
HIO_4, 0.1082N	0.00224	60 min.		0.63
HIO_4, 0.1623N	0.00258	180 min.		1.15
HIO_4, 0.1597N	0.00333	23 hr.		2.01

FIG. 4.

OXIDATION OF RAFFINOSE WITH $NaIO_4$, KIO_4 AND HIO_4



"a" = Oxygen absorbed with $NaIO_4$ and KIO_4
"b" = Acid Produced with HIO_4

OXIDATION OF RAFFINOSE AFTER HYDROLYSIS WITH 3% SULPHURIC ACID

See Fig. 5

Oxidising Agent	Molality of Raffinose	Time in Minutes	Oxygen Atoms absorbed	Equivalents of Acid produced
	0.00125	60		4.08
KIO_4,		240		5.42
0.0136N		300		5.62
		360	8.25	6.02
$NaIO_4$,				
0.0733N	0.00071	150		6.11
$NaIO_4$,				
0.0473N	0.00109	120	8.05	
HIO_4,	0.00118	75		5.19
0.1120n		90		5.38
		130		5.62
		180		6.13

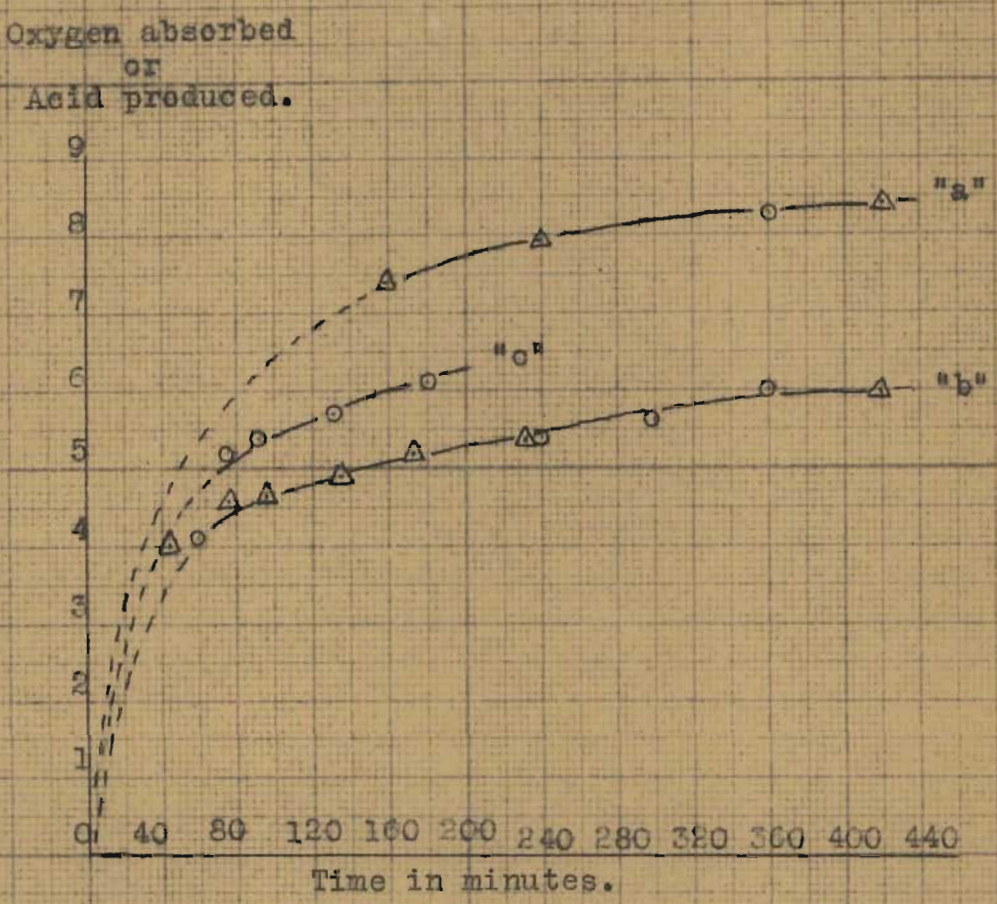
OXIDATION OF RAFFINOSE AFTER HYDROLYSIS WITH 10% SULPHURIC ACID.

See Fig. 5

Oxidising Agent	Molality of Raffinose	Time in Minutes	Oxygen Atoms absorbed	Equivalents of Acid produced
KIO_4,	0.00095	45		3.97
0.0121N		75		4.55
		95		4.62
		135		4.95
		160	7.43	
		173		5.20
		233		5.39
		240	7.93	
		420	8.40	5.95

FIG.5.

OXIDATION OF HYDROLYSED RAFFINOSE WITH KIO_4 AND HIO_4.



"a", Oxygen absorbed } with KIO_4 { --o--o after hydrolysis with 3% H_2SO_4
"b", Acid produced } with KIO_4 { --△-- " " " 10% " .

"c", Acid produced with HIO_4.

PART II

C A S S E I N E

SUMMARY

An alkaloid with the empirical formula $C_{33}H_{47}O_{14}N_3$ has been isolated from the seeds of C. laevigatum and the seed pods of C. didymabotry. It is proposed to name the alkaloid CASSEINE.

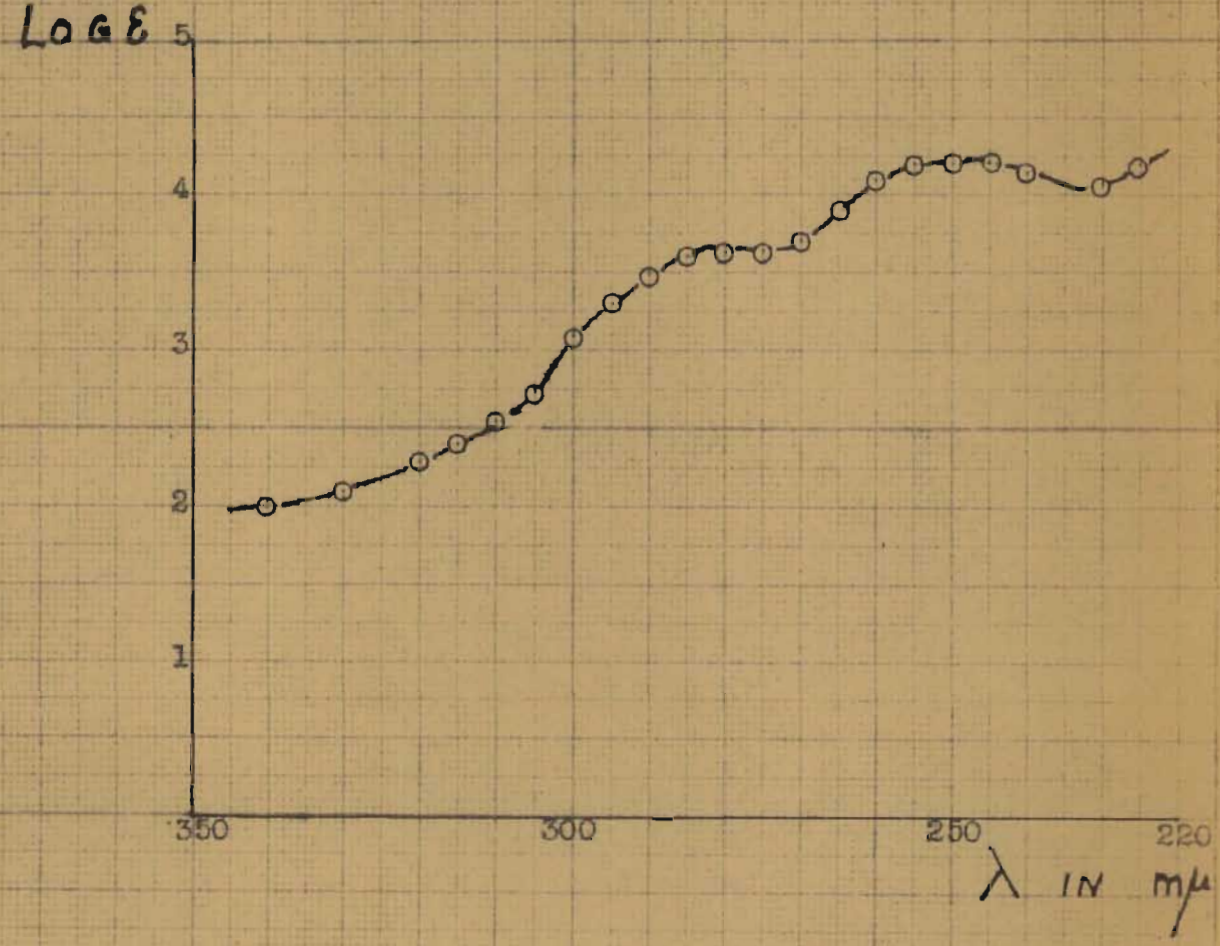
A very small quantity of an alkaloid was isolated from the seeds of C. laevigatum, and from the pods of C. didymabotry, on extraction with alcoholic hydrochloric acid, but the amount available (about 0.6 g.) was too small to allow of much structural investigation. A chromatogram over alumina indicated that only one alkaloid was obtained from C. laevigatum, and the melting point of the picrates, 149-150°, showed that the same alkaloid, hereafter referred to as casseine, was present in both species.

The U.V. extinction curve (Fig. 6), with λ_{max} 252 mμ ($\log \varepsilon_{max}$ 4.20) and λ_{max} 282 mμ ($\log \varepsilon_{max}$ 3.63), indicated that the substance was an indole derivative. A salt was formed with ammonium reineckate, casseine reineckate, which on analysis gave the empirical formula for the alkaloid of $C_{33}H_{47}O_{14}N_3$.

The addition of chlorplatinic acid gave yellow

FIG. 6.

U.V. EXTINCTION CURVE OF THE ALKALOID CASSEINE
(IN ETHYL ALCOHOL)



microcrystals of caffeine chlorplatinate, which decomposed at high temperature without melting and the analysis of this substance confirmed the empirical formula for the alkaloid. The mother liquor from the preparation of the chlorplatinate was evaporated to dryness under reduced pressure, when a purple substance was produced together with a yellowish glassy mass. The chlorplatinic acid was present in slight excess, and had become reduced to platinum, at the same time oxidising the alkaloid to the blue purple compound. Part of the mixed residue was repeatedly extracted with warm alcohol, and the colorless solution produced was filtered. On standing overnight this colorless solution deposited the purple compound.

Another part of the residue was shaken with water, and the colorless solution filtered, and left over two nights, when a purple deposit was formed, the top "skin" looking deep gold in reflected light.

The substance was sparingly soluble in hot water, making a red-purple solution. The filtered aqueous solution was decolorised by sulphur dioxide, and on evaporating the decolorised solution in air, and leaving in the air, the purple color returned. When the purple substance was heated in a melting point tube, it did not melt, but a sublimate was formed.

The substance was soluble in ammonia, and on warming the ammonia was released, and the substance was precipitated as a fine dark blue solid so that the substance is probably a week acid. It was also soluble in hydrochloric acid, and the solution gave a precipitate with Mayer's reagent.

Like indigo, the purple substance was soluble in concentrated nitric acid, giving a brilliant red solution, which became colorless on boiling, and on evaporation to dryness gave a substance soluble in water to form a soapy solution.

The purple compound probably contains a similar internal structure to indigo, as this is associated with the deep blue-purple color, turning red with nitric acid, and with the easy oxidation-reduction system.

Indigofera vetch, like Cassia, is a leguminous plant, whilst eserin, $C_{15}H_{21}O_2N_3$, also obtained from a leguminous plant, Physostigma venenosum (The Calabar bean), forms a similar dark blue compound.

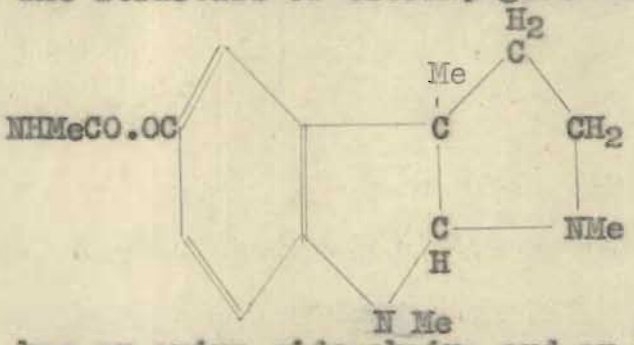
There was not sufficient of the above substance for analysis, or further investigation.

A relationship between the alkaloid and eserin is indicated by:-

(1) On removal of the elements of two sugar molecules,

suggested by the large number of oxygen atoms present, the empirical formula becomes $C_{21}H_{27}N_3O_4$ i.e. eserin + $C_6H_4(OH)_2$.

(2) The structure of eserin, given as:-



has an amino side-chain, and an indole structure.

(3) Both substances produce a dark blue substance on oxidation.

Watts (Medical and Poisonous Plants S.A.) describes a substance called Abric acid, to which he ascribes the formula $C_{21}H_{24}ON_3$ but gives no references. He states that it is obtained, together with abrine, from Abrus precatorius (the love bean). From a comparison of the empirical formulae, it is possible that the alkaloid from the Cassia plants is the tri-hydroxy derivative of abric acid, whose structure has not been elucidated. Abrine, with which it occurs, however, has been given the structure methylamino-3-indolyl-propionic acid,

and is obviously very nearly related to eserin.

Abric acid seems to be a phenyl derivative of eserin, and the alkaloid from Cassia, an hydroxy derivative of the abric acid. However, there is no evidence of the structure of abric acid, other than its occurrence with abrin, and its empirical formula.

EXPERIMENTAL

ALKALOID FROM THE PODS OF CASSIA DIDYMABOTRY

The pods, (300 g.), which had been picked in October, were crushed and extracted with 0.6% alcoholic hydrochloric acid, (2 litres), for 24 hours. The solution was made alkaline with ammonia, filtered, and the alcohol removed under reduced pressure at 40°. The syrup was poured into water and freed from the precipitated tar by filtration. The dark red filtrate gave a positive reaction with Mayer's solution, and a small precipitate with ammonium reineckate.

The solution was acidified with acetic acid, filtered, and extracted (i) with ether (ii) with chloroform, until the latter was colorless. Ammonia was then added to the aqueous solution, and it was again extracted with chloroform and the extract dried with calcium chloride. On filtration the solution became slightly milky. This was probably due to the production of the hydrochloride of the base, a phenomenon not uncommon with alkaloids in chloroform solution.

The <u>cassеine</u> (0.287 g.) was obtained in glassy flakes.

<u>Preparation of the Hydrochloride</u>.

The crude alkaloid was dissolved in a little alcohol, a drop of methyl red was added, and the solution

neutralised with N/10 hydrochloric acid. The equivalent of the crude substance was found to be 620, and this figure was probably low on account of the presence of the hydrochloride formed in the chloroform solution. On evaporation of the solution to dryness at room temperature, under reduced pressure, a glass was produced.

Preparation of the Sulphate.

The hydrochloride was dissolved in water, and silver sulphate was added drop by drop until no further precipitate was formed. A drop of the solution was tested on a watch glass with (i) silver sulphate and (ii) hydrochloric acid. Both tests were negative. The solution was filtered, and evaporated to dryness in a vacuum-desiccator. A glass was produced.

Preparation of the Chlorplatinate.

The sulphate was dissolved in water, cooled in ice, and a slight excess of a 5% solution of chlorplatinic acid was added. A copious fine yellow precipitate of casseine chlorplatinate was formed, which was filtered off and washed with ice water, in which it showed considerable

solubility. The substance was recrystallized three times from hot alcohol. It decomposed at a high temperature, without melting.

Found : C, 43.1; H, 5.3%

$2C_{33}H_{47}O_{14}N_3.H_2PtCl_6$ requires C, 43.3; H, 5.1%

The ice water, which had dissolved much of the precipitate, was evaporated to dryness in a vacuum-desiccator, and the resultant solid twice crystallized from boiling alcohol

Found : N, 4.6%

$2C_{33}H_{47}O_{14}N_3.H_2PtCl_6$, requires N, 4.6%

The analysis of the two fractions giving the same empirical formula, indicated that the original substance was homogeneous in composition.

The combined mother liquors were evaporated to dryness, and left for some weeks, when a purple substance was produced, giving a deep red solution on heating with concentrated nitric acid, and a purple solution with hot water, easily reduced with sulphur dioxide, and regaining the purple color when left in air.

Preparation of the Picrate.

The picrate was prepared by the addition of a saturated solution of picric acid to a solution of the hydrochloride in water and had m. p. 150°.

Preparation of Reineckate.

Casseine reineckate was precipitated by the addition of a solution of ammonium reineckate to a solution of the hydrochloride, and crystallized from alcohol

Found : C, 43.0; H, 5.0 %

$C_{33}H_{47}O_{14}N_3.C_4H_6N_6S_4Cr$ requires C, 43.2; H, 5.16%

ALKALOID FROM THE SEEDS OF CASSIA LAEVIGATUM

The seeds (1000 g.) were extracted with cold 0.3% alcoholic hydrochloric acid for 24 hours, the solution then made alkaline with ammonia, filtered, acidified with acetic acid, and the alcohol distilled off under reduced pressure below 40°.

The resultant syrup was dissolved in a little water, with the addition of a few drops of hydrochloric acid, and the precipitate of tarry material was filtered off.

The acid liquid was extracted (i) with ether, (ii) with chloroform, (iii) made alkaline with ammonia, and re-extracted with chloroform. From the latter solution, after drying with calcium chloride, and removing the chloroform under reduced pressure, a colorless glassy material (0.12 g.) was obtained, which gave a positive reaction with Mayer's solution, was alkaline to litmus, and soluble in dilute hydrochloric acid, the solution having an intensely bitter taste.

The substance was dissolved in chloroform, and slowly poured through previously prepared alumina (3 g.) evenly packed into a tube of 1 cm. diameter, with a plug of glass wool at the bottom, and wetted with the solvent..

The emerging liquid was examined every few drops on separate glass plates and was found to contain only traces of waxy material, negative to Mayer's reagent.

Chloroform was then poured slowly and continuously through the column, and the emerging liquid was caught in ten drop batches on separate glass plates, until they yielded no residue on evaporation of the solvent.

The solid residue found on the plates was progressive and retrogressive in quantity, showing no break, and indicating the presence of only one substance.

Preparation of the Chlorplatinate.

The material was dissolved in very dilute hydrochloric acid, cooled in ice, a solution of 5% chlorplatinic acid added, and the mixture allowed to stand in ice for one hour. The precipitated chlorplatinate was filtered off, and washed with a little ice water, in which it showed considerable solubility. Yellow micro-crystals decomposed without melting. The mother liquor deposited the same violet substance as the alkaloid chlorplatinate from the pods of C. didymabotry.

Preparation of the Picrate.

A drop of saturated solution of picric acid was added to a solution of the hydrochloride of the alkaloid. After a few minutes the picrate separated as a very fine, filter-passing precipitate, isolated by centrifuging, m. p. 149°, undepressed by the substance prepared from the alkaloid in the pods of C. didymabotry. The alkaloid was, therefore, casseine.

PART III

C H O L I N E

SUMMARY

Choline has been isolated as the reineckate, from both seeds and pods of C. laevigatum and C. didymabotry.

Choline was isolated from the seeds and pods of both C. laevigatum and C. didymabotry. It is noteworthy that, although the ripe pods contained both alkaloid and choline, the pods from the previous year, that had shed their seeds, contained choline only, the amount being almost the same as that contained in the pods of the current year.

Choline was obtained from the aqueous solution of the syrup from the alcoholic extract of the seeds or pods, after removal of all such compounds as were soluble in petroleum ether, ether or chloroform, acid, or made alkaline with ammonia.

Choline was precipitated as the reineckate, by the addition of an aqueous solution of ammonium reineckate.

The substance was identified by the aurichloride, m. p. 242-252° (recorded for choline aurichloride 243-250°), by analysis, and by the physical characteristics of the reineckate crystals.

EXPERIMENTAL

ISOLATION OF CHOLINE REINECKATE

The material was allowed to stand in 0.3% alcoholic hydrochloric acid for 24 hours, the solution decanted off, neutralised with ammonia, filtered, acidified with acetic acid, and the alcohol distilled off under reduced pressure below 40°. The resulting syrup was dissolved in water, acidified with hydrochloric acid, and repeatedly extracted with petroleum ether and then with ether, the solution made alkaline with ammonia, and re-extracted with ether. The resulting clear red aqueous solution was acidified with acetic acid, a solution of ammonium reineckate was added and the whole set aside overnight. The crystalline precipitate was dissolved in acetone, the solution filtered, and allowed to evaporate to dryness in air when very large dark red rectangular crystals of choline reineckate were obtained.

Refractive Indices

α = 1.660 ± 0.002

γ = 1.738 ± 0.002

Required by choline reineckate α = 1.658

γ = greater than, or equal to 1.734.

The reineckate was recrystallized from alcohol, with the addition of sufficient acetone to dissolve the substance. On warming gently to remove the acetone, the choline reineckate was deposited.

Found : C, 26.5; H, 4.99; N, 23.0%

Calc. for $C_{19}H_{21}ON_7S_4Cr$: C, 26.1; H, 4.96; N, 23.2%

Microscopic Examination of Crystals of Base Reineckate

From acetone on a microscope slide the base reineckate crystallized out in hexagonal plates, as well as in rosette aggregates of these plates tipped on edge, and having the appearance of needles. This is characteristic of choline reineckate.

Confirmatory Test.

The choline reineckate was heated with a mixture of acetone and water until the salt was completely hydrolysed, as indicated by the disappearance of the pink color. Two or three drops of ammonium hydroxide were added, and the preparation was heated until the ammoniacal odour disappeared. The precipitated chromium hydroxide was filtered off, and a drop of the filtrate was placed on

a microscope slide and heated to dryness. A drop of platinic chloride reagent was added, and stirred into the preparation. Into the side of this drop was drawn a very small amount of sodium iodide solution, when small black rectangular prisms were formed in the path of the sodium iodide solution. This is characteristic of choline.

References

Amelink, F., "Schema zur Mikrochemischen Identifikation von Alkaloiden."
(N.V.D.B. Centen's Uitgevers Maatschappij, Amsterdam) 1934, p.43-5.
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Preparation of the Aurichloride

The base chloride (0.037 g.) was dissolved in water, (2 c.c.) and a few drops of gold chloride (5% solution) added to the boiling solution. On refrigeration the aurichloride separated in beautiful golden plates, m. p. 242-252° with decomposition. Recorded for choline aurichloride 243-250°.

PART IV

4:5-DIHYDROXY-7-METHOXY-2-METHYLANTHRAQUINONE

SUMMARY

4:5-dihydroxy-7-methoxy-2-methylanthraquinone has been isolated from the seeds of C. laevigatum, and identified. The trimethyl ether has been prepared.

A crystalline quinonoid compound was obtained from the seeds of C. laevigatum, by extraction with alcoholic hydrochloric acid, which had the empirical formula $C_{16}H_{10}O_5$.

The substance was soluble in benzene and in ether, and sparingly soluble in acetone, from which it crystallized in yellow prisms, m. p. 205-6°. It sublimed at 245° at atmospheric pressure, forming yellow rectangular crystals, which gave a brilliant crimson color with concentrated sulphuric acid, fading rapidly to orange and then yellow.

It was insoluble in ammonia, slowly soluble in 3N sodium hydroxide, but immediately soluble on dilution, forming a bright red solution. On standing some days, this solution became slowly decolorised, and a fine red precipitate was formed. In 5N sodium hydroxide the substance was not soluble, even on long standing, but the crystals turned from gold to purple, and remained so indefinitely.

This insolubility in the stronger alkali is indicative of co-ordination of the hydroxyl groups.

The quinone was extricable from the solution in sodium hydroxide with ether, and this proved an effective method of purification, the impurities being more strongly acid.

The red deposit was also slowly soluble in ether, forming a yellow solution, and giving the original quinone on evaporation. The red substance may be a co-ordination complex.

On passing carbon dioxide through the alkaline solution, a yellow precipitate of the original substance was produced, proving the absence of any carboxylic groups.

The alkaline solution was decolorised by sodium hydrosulphite, the color returning on shaking in air.

With zinc and sodium hydroxide, the solution changed from red to yellow, and returned to red immediately in air, proving the substance to be an anthraquinone compound. The decolorising with sodium hydrosulphite is characteristic of an alpha-hydroxy-quinone.

Crimson lakes were immediately thrown out of the alkaline solution on the addition of sodium or potassium sulphate.

The quinone was not acetylated on heating at the

boiling point with acetic anhydride for one and a half hours, but a diacetyl derivative, melting at 188° was formed on heating with acetic anhydride and pyridine, indicating that alpha-hydroxyl groups only were present. This was confirmed by the observation that the substance was insoluble in cold sodium carbonate, forming a red solution on heating, and a yellow precipitate of the original quinone on cooling. Only beta-hydroxyl substances are soluble in cold sodium carbonate.

The U.V. extinction curve (Fig. 7.) showed:-

(i) A maximum extinction characteristic of the quinonoid nucleus of anthraquinone,
λ_{max} 285 mμ ($\log \varepsilon_{max}$ 4.25) (Morton and Earlam, J., 1941, 159, give
λ_{max} 276-288.5 ($\log \varepsilon_{max}$ 4.24-4.59).

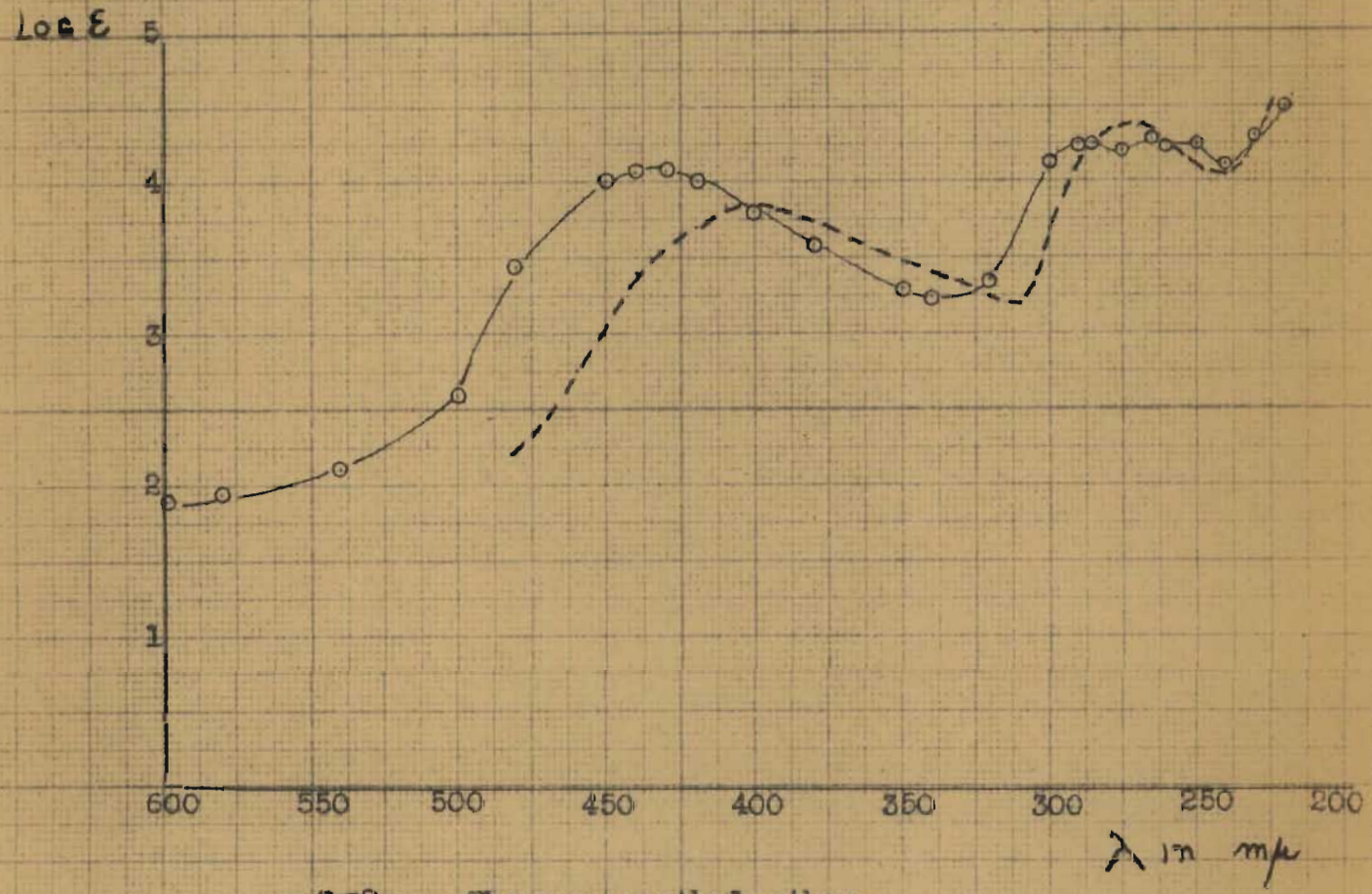
(ii) A maximum extinction characteristic of two alpha-hydroxyl groups,
λ_{max} 430 mμ ($\log \varepsilon_{max}$ 4.07) (Briggs and Paterson, J., 1952, 1718)

The infra-red absorption showed:-

(i) The absence of any frequencies characteristic of the hydroxyl group (M.St.C.Flett, J., 1948, 1441-8, has shown that no hydroxyl frequency is exhibited

FIG. 7.

U.V. EXTINCTION CURVE OF
4:5-DIHYDROXY-7-METHOXY-2-METHYLANTHRAQUINONE,
AND 4:5:7-TRIMETHOXY-2-METHYLANTHRAQUINONE,
(IN ETHYL ALCOHOL)



—o—o— The mono-methyl-ether.

- - - - - - - The tri-methyl-ether.

by 1:4-, 1:5- or 1:8-, dihydroxyanthraquinones).

(ii) The presence of frequencies due to both the unco-ordinated quinonoid group, and the co-ordinated quinonoid group of anthraquinone, i.e. 1673 cm^{-1} (25%) and 1628 cm^{-1} (85%) (M.St.C.Flett, loc. cit.)
Both hydroxyl groups are, therefore, co-ordinated with the same -C=O group.

Reduction with hydriodic acid showed the presence of one methoxyl group. The product of demethylation was passed through an alumina column. The adsorbed substance could not be removed with acetic acid, proving that a beta-hydroxyl group was now present.

Analysis showed the presence of one methyl group, but neither the parent substance, nor the acetyl derivative could be oxidised with potassium permanganate or potassium bichromate. The quinone was completely decomposed with chromic acid in acetic, or sulphuric acid solution.

The substance is thus a 1:8- or 4:5-dihydroxy-beta-methoxy-?-methylanthraquinone.

Perkin and Hummel (J., 1894, 923) isolated a substance from the root-bark of Ventilago madraspatana (a climbing plant) of empirical formula $C_{16}H_{10}O_5$, which

they characterised as a di-hydroxy-oxymethyl-methylanthraquinone. On heating to 160° with 80% sulphuric acid, they obtained "an emodin-like substance", and they concluded that the natural product was a monomethyl ether of emodin, whose structure, at this time, was unknown.

The melting point was described as being 200°, with charring, unchanged by many recrystallizations.

The color with concentrated sulphuric acid was a purple-red, the salts were purple needles, and the di-acetyl derivative melted at 185-6°.

Emodin was synthesised by Eder and Widmer (Helv.Chim.Acta. 1923, 966) and proved to be 4:5:7-trihydroxy-2-methylanthraquinone. The data recorded (loc. cit.) for the quinone from Cassia laevigatum is in agreement with the methyl ether of this substance. Moreover this quinone gave a di-acetyl derivative melting at 188°, in agreement with that obtained by Perkin by acetylating the substance from Ventilago madraspatana. The natural substances may, therefore, be assumed to be 4:5-dihydroxy-7-methoxy-2-methylanthraquinone.

The more purple color produced with sulphuric acid by Perkin's substance, and the charring on melting,

may have been due to impurities accounting for the low melting point, as, when impure, the substance from C. laevigatum gave a purple rather than a red color with the acid, and charred when melting.

In an attempt to prove that the natural substance was, in fact, an emodin methyl ether, Jowett and Potter (J., 1903, 83, 1330) methylated emodin by refluxing the substance with methyl iodide in sodium methoxide solution.

Their analyses showed the yellow crystals they obtained to be the mono-methyl ether, which they also described as melting at 200°, unchanged on further crystallization. The melting point of the di-acetyl derivative, was, however, 157°. They therefore concluded that the substance they had prepared was not identical with that obtained by Perkin and Hummel from Ventilago madraspatana. Moreover this substance was described as yielding mono- and tetra-nitro compounds, whereas, under similar conditions, the ether prepared from emodin by Jowett and Potter afforded no definite nitro-compounds.

It is notable that, although the methylation of emodin was carried out by heating in a sealed tube for 2-3 hours, or by refluxing for 24 hours, only one hydroxyl was methylated.

Later Eder and Hauser (Helv. Chim. Acta. 1925, 8, 141) methylated emodin by the action of dimethyl sulphate on the mono-potassium salt. They described the ether so obtained as melting at 203-4°, and, after sublimation, at 206-7°, unchanged on mixing with the natural emodin monomethyl ether. They do not mention the source of the natural product, it is not, therefore certain that it was the same substance as that obtained by Perkin.

Eder and Hauser also comment on the impossibility of methylating the other two hydroxyl-groups.

On treatment with 80% sulphuric acid at 160° they regenerated the emodin, thus repeating the work of Perkin.

It must therefore be assumed that the substance obtained by methylating emodin, and the product obtained by Perkin were the same.

In view of the fact that Jowett and Potter had described their acetyl derivative as melting at 157°, instead of 185-6°, as reported by Perkin, it seems a pity that Eder and Hauser did not make an acetyl derivative of their product.

We are trying, so far unsuccessfully, to obtain some emodin in order to make the mono-methyl ether, and

compare it with the product from Cassia laevigatum.

Methylation of the Quinone from Cassia laevigatum.

Anthraquinones with the hydroxyl groups in the 1-, 4-, 5-, or 8- position do not methylate readily, on account of co-ordination between the hydrogen and the quinonoid oxygen. In fact, the quinone from Cassia laevigatum showed no infra-red frequencies characteristic of an hydroxyl group.

Both Jowett and Potter, and Eder and Hauser (loc. cit.) commented on the impossibility of further methylating the mono-methyl ether of emodin, but they made their attempts in alcoholic solution.

It has been shown that the association of sulphinic and benzoic acids due to co-ordination between -OH and =O, is considerably increased by the presence of traces of water (Wright, J., 1949, 683), and it seemed likely that the presence of other -OH groups such as an alcoholic -OH might have a similar effect.

The association of benzoic acid in benzene, in the presence of various carbinols, was, therefore examined, (See Appendix).

It was found that, in every case, the presence of

small quantities of a carbinol considerably increased the association of the acid, thus, the presence of alcohol probably accounted for the failures to methylate emodin fully in alcoholic solution.

The monomethyl ether from C. laevigatum was fully methylated with methyl iodide in dry acetone solution, in the presence of calcined potassium carbonate, to form 4:5:7-trimethoxy-2-methylanthraquinone.

The U.V. extinction curve of the ether (Fig. 7) showed the maximum extinction characteristic of the quinonoid nucleus of anthraquinone, λ_{max} 275mμ (log. ε 4.35) (Morton and Earlam, loc. cit.), but the maximum extinction λ_{max} 410 mμ (log. ε 3.8), shown by the ether is not in agreement with the observations of Briggs and Paterson (loc. cit.) who give λ_{max} 356-362.5mμ (log. ε 3.36-3.88) as characteristic of anthraquinones containing no free alpha-hydroxyl groups, and λ_{max} 407.5-413.5mμ (log. ε 3.57-3.93) as characteristic of anthraquinones containing one free alpha-hydroxyl group. The insolubility of the ether, even in very dilute sodium hydroxide, and the analytical data, verify the complete methylation of the phenolic groups.

EXPERIMENTAL

Preparation of the Quinone.

The finely ground seeds of C. laevigatum were defatted with petroleum ether, and dried. The seeds (one kilo) were then put into 0.3%, or 0.6% alcoholic hydrochloric acid (4 litres). No difference in yield was observed. After 48 hours, the alcoholic solution was removed by decantation, neutralised with ammonia, and filtered from the precipitated substance. The filtrate was then acidified with acetic acid, and the alcohol removed under reduced pressure.

The resulting syrup was dissolved in water (150 c.c.) and repeatedly extracted, first with petroleum ether then with ether until the solvents were colorless.

A clear deep red solution remained, and this was boiled with hydrochloric acid (strength made up to 10%), when a dark precipitate was formed. The mixture was extracted with ether, and the ethereal solution dried with magnesium sulphate.

On evaporation of the ether, traces of a crystalline substance were produced which after recrystallization from acetone had m. p. 205°.

The seeds were re-extracted, four times, the yield increasing with each extraction. A fifth extraction gave only a few milligrams. The total time of extraction

was six weeks for a yield of 0.125 g. per kilo.

An attempt was made to hasten the release of the quinone, by moistening the seeds with water before adding the alcohol, but without success.

The substance crystallized in prisms from benzene and acetone, but was very sparingly soluble in all organic solvents. It sublimed at 135°/0.0005 mm. forming pale yellow rectangular prisms, m. p. 204°

$[\alpha]_D^{22}$ +10.7° (chloroform, C = 1)

Found : (1) C, 67.0; H, 3.9%

(2) C, 67.2; H, 4.1%

A second sample was sent for analysis. The substance was re-crystallized from a large volume of ether, in which solvent it is very sparingly soluble, and was deposited in very fine needles m. p. 205-6°.

During filtration the mixture was kept in motion, and every precaution was taken to obtain a homogeneous substance. The crystals were sent for analysis in two separate tubes, as independant substances. The results of the analyses are shown below:

Found (3) : C, 68.0; H, 4.27; OMe, 11.55%

(4) : C, 67.1; H, 4.06; OMe, 11.5%

Required by $C_{16}H_{12}O_5$: C, 67.7; H, 4.22; OMe, 10.9%

The low value for the carbon in the micro-analyses (1), (2) and (4) may have been due to the presence of the methoxy- group (See p. 81, analysis of the tri-methyl ether).

Preparation of the Acetyl Derivative.

Quinone (0.111 g.) was boiled with acetic anhydride (10 c.c.) for one and a half hours; total solution was achieved at once. The solution was refrigerated, and the resulting crystals (.0706 g.) were filtered off, washed with water, and dried. On the addition of water to the mother liquor more crystals (0.0330 g.) were slowly deposited. All were found to be unchanged quinone. (m. p. and mixed m. p. 204°).

As the total yield was 93% of the weight of quinone taken, it may be assumed that acetylation does not take place at all under these conditions.

The quinone (0.1036 g.) was heated on an oil-bath with pyridine reagent (Pyridine, freshly distilled from sodium hydroxide (30 c.c.) and acetic anhydride (10 c.c.), for half-an-hour, and then poured into ice-water. A very fine yellow precipitate (0.1069 g.) was formed, which was then separated with the aid of a centrifuge, and

washed with water.

After recrystallization from (1) alcohol and water (2) three times from alcohol, the crystals melted at 188°. The substance was soluble in benzene.

Found : C, 65.3; H, 4.3%

C, 64.9; H, 4.3%

Required for $C_6H_{10}O_5Ac_2$: C, 65.4; H, 4.3%.

Methylation of the Quinone.

Quinone (0.1062 g.), redistilled, dried acetone (15 c.c.) and methyl iodide (2 c.c.) were refluxed on a water bath with calcined potassium carbonate. The first formed deep red solution slowly became more yellow, until, at the end of an hour and a half, it was a brilliant yellow. Methyl iodide (1 c.c.) was added, and the refluxing continued. The solution gradually became paler, and after two more additions of methyl iodide (0.5 c.c.) the color remained constant. It was now a pale yellow.

The acetone and excess methyl iodide were then distilled off, water was added, and the yellow precipitate was extracted with ether, in which it was very sparingly soluble. The undissolved precipitate being suspended in ether, it was taken into solution by the addition of a

little alcohol. The yellow solid obtained on evaporation of the solvent, was crystallized from hot alcohol, and afterwards from benzene.

The resulting substance was dissolved in benzene, and passed through alumina columns until a pure chromatogram was obtained. The resultant methyl derivative crystallized from benzene in bright yellow needles, m. p. 229-230° with sublimation, the sublimate forming star like crystals.

It was insoluble in dilute sodium hydroxide, showing complete methylation, and with concentrated sulphuric acid, the color produced was an even more brilliant crimson than with the parent quinone.

Found : C, 67.5; H, 5.14; $-OCH_3$, 28.3%

$C_{18}H_{16}O_5$ requires : C, 69.2; H, 5.12; $-OCH_3$, 29.8%

The presence of three methoxy- groups may account for the low value for the carbon given by micro-analysis.

(See p. 78 where duplicate analyses of the original quinone differed by 0.9%, and p. 80 where duplicate analyses of the acetyl derivative differed by 0.4%, both these substances containing only one methoxy-group).

Attempted Oxidation of the Quinone and the Acetyl Derivative.

(1) With Cold Potassium Permanganate.

Quinone (5 mg.) was dissolved in cold 1% potassium permanganate and left four hours. It was then acidified with hydrochloric acid, extracted with ether, and purified by sublimation. The original substance was recovered having m. p., and mixed m. p. 203°.

The acetyl derivative (10 mg.) was treated as above, and after acidification, ether extraction, and recrystallization from acetone, the original substance was recovered. (m. p. and mixed m. p. 188°).

(2) With Boiling Potassium Permanganate.

Quinone (7.5 mg.) were added to a 3% solution of potassium permanganate (3 c.c.). A few crystals of potassium carbonate were added, and the solution was boiled on a water-bath for one hour. Manganese dioxide was precipitated, and the organic product was completely adsorbed on this substance. After drying, unchanged quinone was recovered from the manganese dioxide, by repeated extractions with acetone.

Oxidation of the Quinone.

(1) With Concentrated Sulphuric Acid and Potassium Bichromate.

Concentrated sulphuric acid was added to the quinone (15 mg.) and stirred mechanically in a small beaker. Finely powdered potassium bichromate was added whilst the temperature was kept about 40-50°. After a few minutes a gas was given off.

At the end of twenty minutes the mixture was poured on to ice, and extracted with ether. No insoluble substance was present, and the ether contained no substance. The solution was reduced with sulphur dioxide, and the chromium precipitated with ammonium chloride and ammonia. The solution was evaporated to dryness, and the solid extracted with acetone. Traces of a yellow oil were obtained, with a smell of vaseline, soluble in concentrated sulphuric acid to a brown solution which was not unsaturated.

(2) With Glacial Acetic Acid and Chromium Trioxide.

Quinone (60 mg.) was added to glacial acetic acid (10 c.c.) and mechanically stirred in a small beaker. Chromium trioxide (0.5 g.) were gradually added. The solution immediately darkened. A sample removed, gave a very small trace of a yellowish green crystal from an ether

extraction, after sulphuric acid acidification. The solution was stirred for one and a half hours, and left overnight, when it was diluted and extracted with ether, and dried with magnesium sulphate. On removal of the ether only a very small trace of a waxy substance was obtained.

Purification of the Quinone.

Although the quinone could be purified by sublimation in a high vacuum the process was very slow, the temperature having to be kept below 125°. The time taken to sublime 0.1 g. was 40 hours, with a loss of 0.09 g. A tarry substance was produced in the residue, formed, apparently, by the decomposition of the original material, due to the prolonged heating.

Oxidation of the impure material with potassium bichromate, proved a very effective method of purification.

The pure material was also obtained when the crude substance was dissolved in sodium hydroxide, and the alkaline solution extracted with ether.

(1) Purification by Oxidation.

Impure quinone (80 mg.), m. p. 191°, was added to water (3 c.c.) and mechanically stirred in a small beaker

with potassium bichromate (0.5 g.). Concentrated sulphuric (3 c.c.) was added drop by drop, and the mixture was stirred for half-an-hour. It was then heated on a water-bath for 20 minutes and left overnight. After dilution with water, it was extracted with ether and the ether washed with water until neutral to Congo red. After drying with magnesium sulphate, concentration of the ethereal solution gave crystals of pure quinone (45 mg.), m. p. 204°. From the mother liquor a further crop of crystals was obtained (31 mg.), m. p. 204°. The total yield of pure substance was 76.5 mg. (95.6%). It is obvious from these figures that no oxidation of the quinone has taken place.

(2) Purification by Extraction from Alkaline Solution.

Impure quinone, m. p. 176°, was dissolved in sodium hydroxide and set aside, when a red deposit was formed. The solution was heated 20 minutes in a water-bath, cooled and filtered. The filtrate was still red, i.e. the precipitation was not accelerated by heating. The red deposit gave a brilliant yellow solution with ether which, after washing with water, drying, and evaporating to dryness, gave crystals m. p. 204°. The red filtrate was reboiled, but no further precipitate was

formed. It was cooled, and extracted with ether, the ether washed with water, and dried with magnesium sulphate. On concentration crystals separated out, m. p. 204°.

APPENDIX

EFFECT OF THE PRESENCE OF AN EXTRANEOUS HYDROXYL GROUP ON THE ASSOCIATION OF BENZOIC ACID.

SUMMARY

It has been shown that the presence of very small concentrations of water in benzene considerably increases the association of benzoic acid in this solvent. (Wright. J. 1949, 683-692).

Carbinols have now been found to have a similar effect.

In order to determine the effect of hydroxy substances other than water, on the association of benzoic acid in benzene, carbinols of high molecular weight were used. By this means concentrations of hydroxyl comparable to the concentrations of water in the previous work (loc. cit.) could be accurately weighed.

The degree of association was estimated by the depression of freezing point, and the constant in the very dry solvent was confirmed by an estimation of the molecular weight of naphthalene in the presence of phosphorus pentoxide.

Benzene was dried with phosphorus pentoxide as before, and purified by fractional crystallization, until the association of benzoic acid in the solvent was unchanged by further drying. The association curve was then slightly lower than that obtained in the previous work (loc. cit.).

The association of the acid in benzene in the presence of phosphorus pentoxide was also determined, and proved to be only very slightly lower than without the presence of the drying agent. The latter could not be used in the presence of the carbinols, as it reacted with some of these substances to produce brilliantly colored compounds.

The increase in the association of the acid produced by the presence of the carbinols was comparable to that produced by the same concentration of water.

EXPERIMENTAL

	Moles per 1000 g.	ΔT	M/M_o	
	0.0202	0.063	1.64	Association of Benzoic Acid in Benzene containing 0.0158 moles Water/1000 g.
	0.0336	0.099	1.74	
	0.0581	0.160	1.86	
	0.0892	0.240	1.90	
(i)	0.0388	0.127	1.56	
	0.0719	0.224	1.65	
	0.0978	0.294	1.70	Association of Benzoic Acid in dry Benzene (See Fig. 1.)
(ii)	0.0154	0.062	1.27	
	0.0608	0.187	1.67	
	0.1060	0.317	1.71	
	0.0144	0.059	1.25	Association of Benzoic Acid in dry Benzene in the presence of P_2O_5. (See Fig. 1.)
	0.0266	0.097	1.40	
	0.0502	0.170	1.51	
	0.0169	0.057	1.52	Association of Benzoic Acid in Benzene containing 0.0155 moles p.p. ditert.benzhydrol/ 1000 g. (See Fig. 2.)
	0.1020	0.277	1.88	
	0.1298	0.356	1.87	

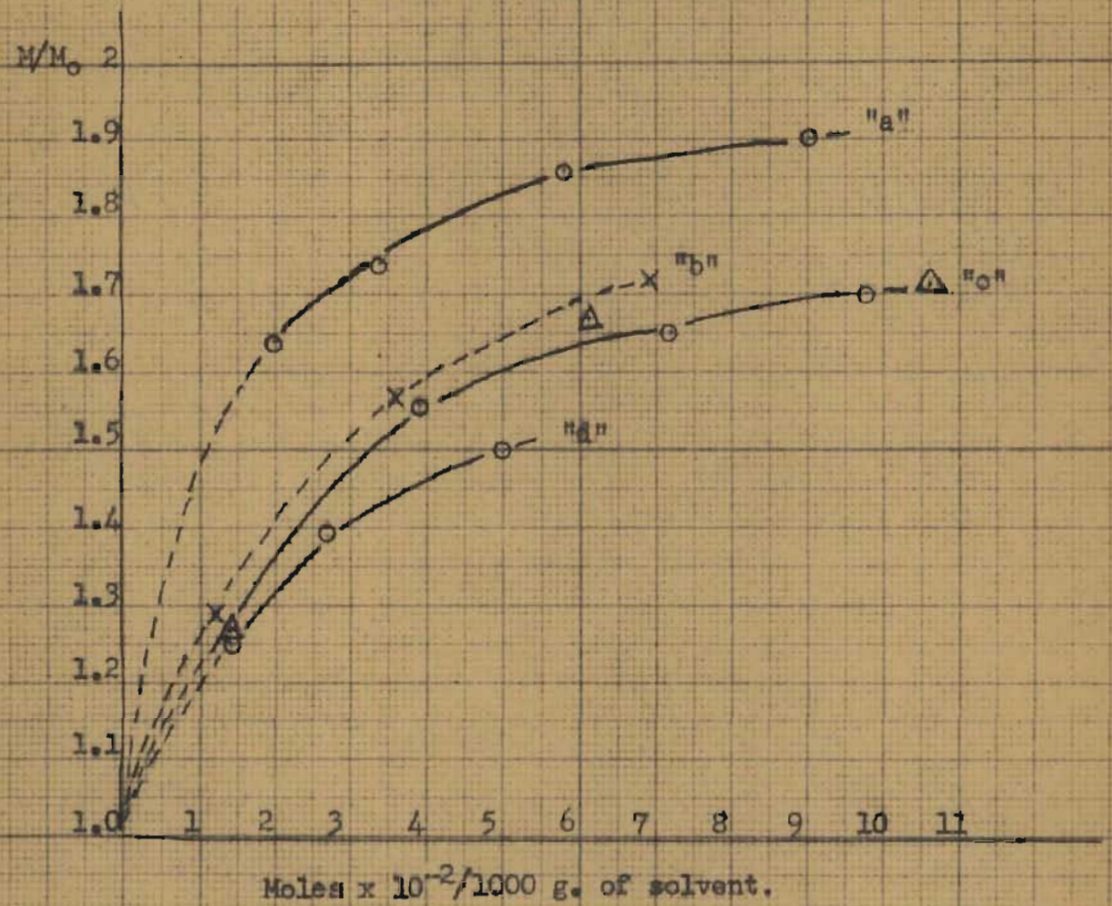
Moles per 1000 g.	ΔT	M/M_0	
0.0291	0.090	1.65	Association of Benzoic Acid
0.0452	0.129	1.79	in Benzene containing 0.0158
0.0638	0.177	1.87	moles p.p. Dianisylcarbinol/
0.0854	0.229	1.91	1000 g. (See Fig. 3.)
0.0239	0.075	1.63	Association of Benzoic Acid in
0.0483	0.141	1.75	Benzene containing 0.0139 moles
0.0770	0.218	1.81	Beta-naphthylmethylcarbinol/
			1000 g. (See Fig. 4.)
0.0316	0.091	1.78	Association of Benzoic Acid in
0.0615	0.169	1.86	Benzene containing 0.0239 moles
0.0873	0.236	1.89	Beta-naphthylmethylcarbinol/
			1000 g. (See Fig. 4.)

For purposes of calculation the freezing point lowering constant for benzene was taken as 5.12°. In order to verify the accuracy of this figure for very dry benzene the molecular weight of naphthalene was determined in this solvent.

0.0528 moles naphthalene/1000 g. benzene, in the presence of phosphorus pentoxide, gave a depression of 0.275°, giving the molecular weight of naphthalene, 126, (Calc. 128,) thus confirming the value of the constant.

FIG. 1.

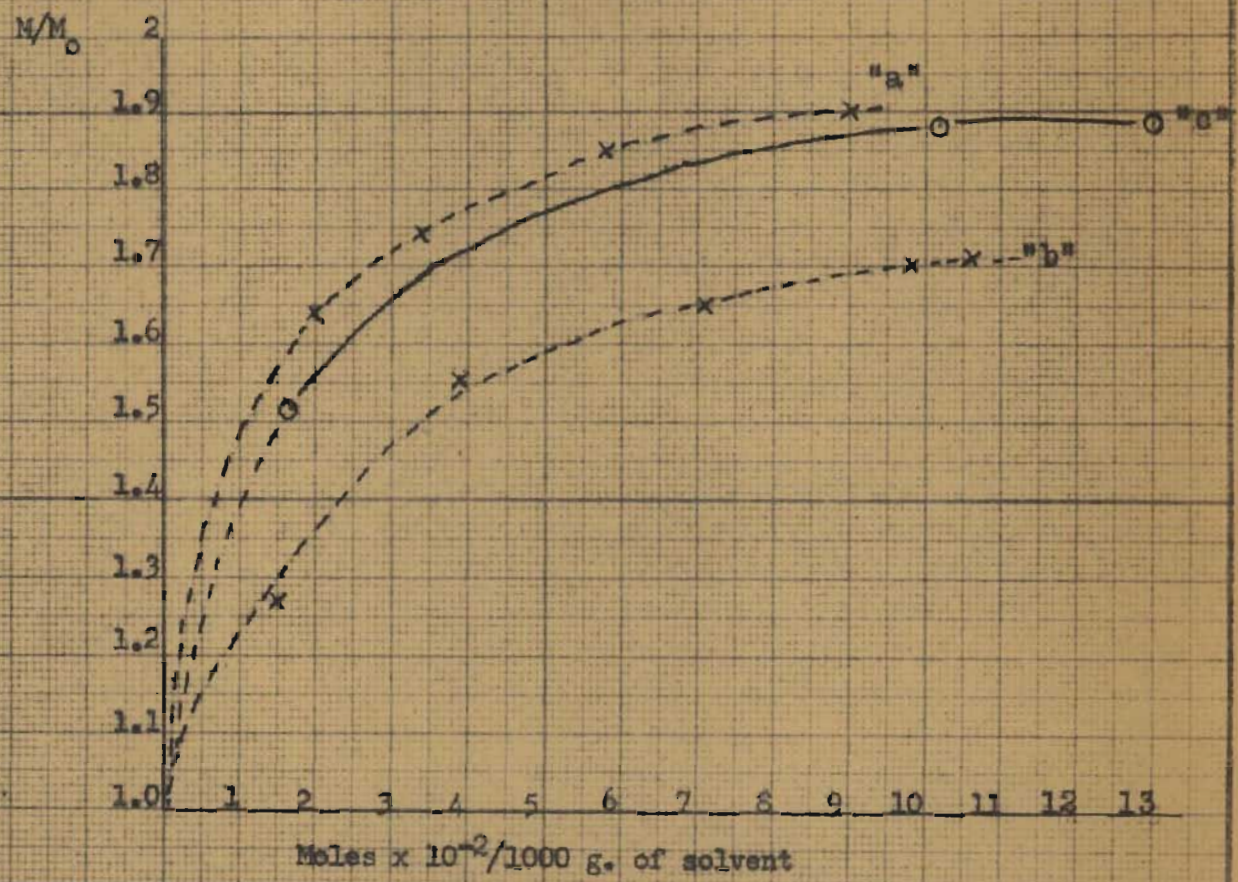
ASSOCIATION OF BENZOIC ACID IN BENZENE



(a) Benzene with 0.0158 moles of water /1000 g. of solvent
(b) -x—x-- Dry Benzene (Wright J., 1949, (146), 683)
(c) Dry Benzene (This communication) —o—o— (I) -△—△-(II)
(d) Dry Benzene containing P_2O_5

FIG.2.

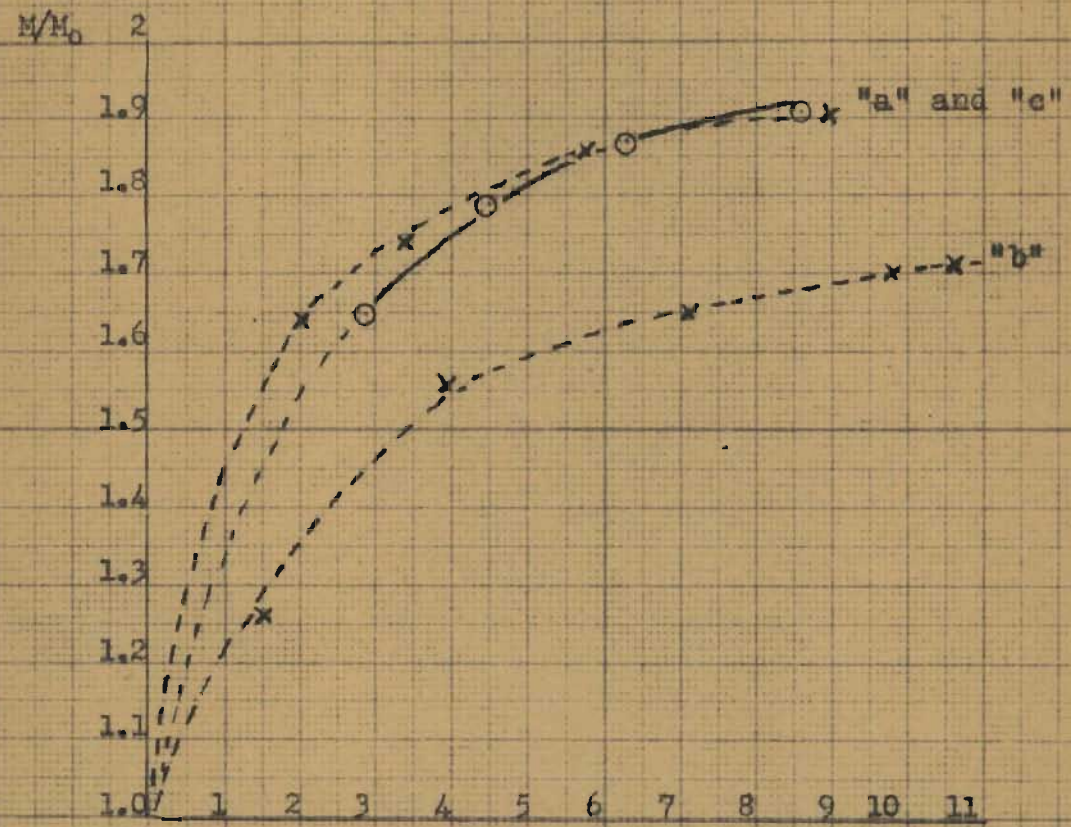
ASSOCIATION OF BENZOIC ACID IN BENZENE



(a) -x—x- Benzene with 0.0158 moles of water/1000g of solvent
(b) -x—x- Dry Benzene.
(c) -O-O- Benzene with 0.0155 moles p.p. ditertbenzhydrol/1000g. of solvent.

FIG. 3.

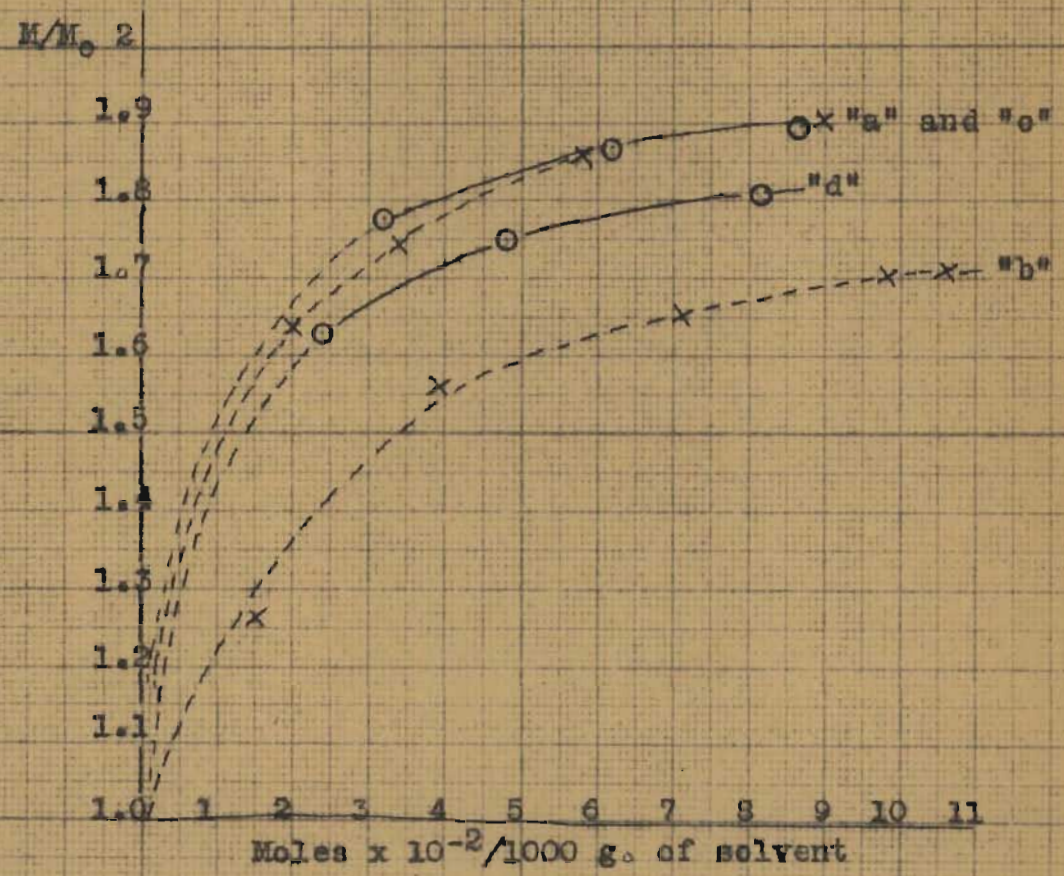
ASSOCIATION OF BENZOIC ACID IN BENZENE



(a) -x—x- Benzene with 0.0158 moles of water/1000 g. of solvent.
(b) -x—x- Dry Benzene.
(c) —⊙— Benzene with 0.0158 moles p.p. dianisylcarbinol/1000 g. of solvent.

FIG. 4.

ASSOCIATION OF BENZOIC ACID IN BENZENE



(a) -x-x- Benzene with 0.0158 moles of water/1000 g. of solvent.
(b) -x-x- Dry Benzene
(c) -o-o- Benzene with 0.0239 moles Beta-naphthyl-mecarbinol/1000 g of solvent.
(d) " " Benzene with 0.0139 " " " " " " .