X. THE CONDENSATION OF TRYPTOPHANE AND OTHER INDOLE DERIVATIVES WITH CERTAIN ALDEHYDES.

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The oxidation of tryptophane by methods other than that adopted by Hopkins and Cole [1902] gives rise to coloured amorphous compounds. These observers, employing ferric chloride as the oxidising agent, isolated two substances, the one having the formula C_9H_7ON and afterwards identified by Ellinger [1906] as β -indolealdehyde; the other an interesting crystalline base, the analysis of which gave results in accordance with the formula $C_{12}H_{10}N_2$. This method of oxidation was used in the course of the present investigation with a view to the isolation and identification of the base $C_{12}H_{10}N_2$. Unfortunately, however, even though the conditions were varied as much as possible, none of this substance could be obtained. Ellinger, when preparing β -indolealdehyde by this method, had also failed to obtain Hopkins' base. In trying to ascertain the extent to which the ether used for extraction of indolealdehyde had been directly or indirectly responsible for the production of the base, Hopkins noticed that a crystalline derivative was formed when an aqueous solution of tryptophane was in contact with ether which had been heated locally with a glass rod. This compound however did not prove to be the much sought after base $C_{12}H_{10}N_2$, but a new substance of an acidic nature. At his suggestion the properties of the new substance, which may be conveniently spoken of as the "ether oxidation substance," were examined. This work led to an investigation of the direct action of formaldehyde, glyoxylic acid and glyoxal on tryptophane, and the action of trioxymethylene, in the presence of a condensing agent, on indole, skatole, indolepropionic acid, indoleacetic acid and tryptophane. It was found that the direct action of formaldehyde and glyoxylic acid on tryptophane led to the formation of *colourless* crystalline compounds; in these cases the *amino*-group of tryptophane was the reactive group (Section A). Glyoxal reacted directly with

tryptophane in the presence of an oxidising agent to form a coloured derivative (Section B). For interaction with the *imino*-group of indole derivatives, the presence of a condensing agent is necessary, and the resulting products are all deeply *coloured* substances (Section C).

SECTION A.

I. *Preparation of the ether oxidation substance.*

(a) An aqueous solution of tryptophane was poured into a large wide-mouthed bottle fitted with a bung, and sufficient ether was added to form a layer a few mm. deep. In preliminary experiments the ether was heated locally by plunging a red-hot glass rod into the bottle and keeping it there a few minutes. Very pungent fumes were evolved, accompanied by a considerable rise in temperature. The process was repeated several times, and the bottle was well shaken and allowed to stand for a short time, when a crystalline deposit slowly formed. This somewhat tedious process was replaced by the following simple arrangement.

(b) The bung was pierced with four holes through which were passed a straight wide tube open at both ends, two electrodes, and an exit tube of narrow bore. The electrodes were connected inside the bottle by means of a platinum spiral placed just above the surface of the ether. The wide tube was arranged so as to have one end close to the platinum spiral. One end of the narrow tube was flush with the bung inside the bottle, the other end was connected with the vacuum pump and a slow current of air was drawn through the apparatus entering by the wide tube.

Outside the bottle the electrodes were connected with a battery sufficiently powerful to make the platinum spiral red-hot. The electric current was then switched off, and the current of air was regulated so that the heat developed during the incomplete combustion of the ether was sufficient to keep the platinum wire glowing. As soon as the layer of ether had disappeared, a further supply of ether was added, and the process repeated. The bottle was then disconnected, corked and left to stand at a temperature of 38° for several hours, during which time a crystalline deposit slowly formed. This substance proved to be the same as that prepared in (a).

(c) In another experiment a mixture of ether vapour and steam was passed over red-hot glass, and the ensuing vapours were passed through a solution of tryptophane; under these conditions no ether oxidation substance

was formed. Thus it is evident that, to bring about the production of this substance, it is necessary for the tryptophane to be in contact with the reactive principle at the moment of its formation. The pungent fumes evolved during the slow incomplete combustion of ether were passed through a wash bottle containing water, and on examination were found to contain both hydrogen peroxide and formaldehyde.

(d) An attempt was made to prepare the substance by the action of formaldehyde and hydrogen peroxide on tryptophane. A crystalline derivative was obtained, but it did not prove to be the ether oxidation substance (*post*, p. 107).

In (a) and (b) the yield of crystalline product was 65 per cent. of the original tryptophane. The substance melts and decomposes at 324° , is colourless, crystalline, slightly soluble in water, alcohol and phenol, insoluble in cold and hot benzene, xylene, toluene, ethyl acetate, aniline, nitrobenzene, pyridine, amyl alcohol and ethylene dibromide. It is soluble in boiling glacial acetic acid: with concentrated hydrochloric acid it darkens considerably, and on evaporation *in vacuo* crystals separate out, which can be recrystallised from ether and alcohol and melt at 285° . Several analyses of this product were made, but failed to give concordant results. The substance can be boiled with dilute hydrochloric and sulphuric acids (5%) without any decomposition taking place. It is precipitated by the mercury sulphate reagent, gives the glyoxylic but not the bromine tests, and does not darken with concentrated sulphuric acid. It is soluble in alkalis, and can be precipitated by means of acetic acid. The use of caustic alkalis is to be avoided, owing to the pigmentation which ensues. Its sodium salt cannot be recrystallised from water owing to hydrolysis.

The substance does not benzoylate easily, and does not form a naphthalene sulphochloride derivative. On reduction with phosphorus and hydriodic acid in a sealed tube at 280° it did not give products suitable for analysis. It is unattacked by alkaline permanganate. Fuming nitric acid reacts with the substance to yield a bright yellow nitro-compound which is soluble in water, giving a yellow solution capable of dyeing silk. On the addition of alkali to the yellow solution, the colour changes to deep red. Oxidation of the substance with chromium trioxide and glacial acetic acid, and with potassium bichromate, gave rise to brown amorphous substances. With concentrated sulphuric acid no colour reaction is produced unless a trace of formaldehyde be added. The purple colour produced under these conditions is identical with that produced by the addition of a trace of glyoxylic acid to the suspension of the substance in water.

A. The original crystalline compound prepared from solutions of recrystallised tryptophane by using methods (a) and (b) was filtered from the mother liquor, washed well with water, alcohol and ether, dried and analysed.

(1) Dried at the ordinary temperature *in vacuo* :

0.1600 g.; 0.3575 g. CO₂, 0.0880 g. H₂O. C=60.94, H=6.11 %.
 0.1230 g.; 12 c.c. moist nitrogen at 15° C. and 768 mm. N=11.42 %.
 C₂₄H₂₆O₅N₄·H₂O requires C=61.49, H=6.04, N=11.96 %.

(2) Heated to a temperature of 155° :

0.1533 g.; 0.3730 g. CO₂, 0.0870 g. H₂O. C=66.35, H=6.36 %.
 0.1710 g.; 0.4180 g. CO₂, 0.0860 g. H₂O. C=66.66, H=5.63 %.
 0.1050 g.; 11.6 c.c. moist nitrogen at 15° C. and 768 mm. N=13.00 %.
 0.2080 g.; 21.2 c.c. moist nitrogen at 7° C. and 776 mm. N=12.90 %.
 C₂₄H₂₄O₄N₄ requires C=66.66, H=5.61, N=12.96 %.

B. The compound was twice dissolved in ten per cent. sodium carbonate solution and precipitated with dilute acetic acid. The constant melting point 324° was obtained. The crystalline precipitate was washed well with water, alcohol and ether, dried at various temperatures and analysed.

(1) Dried *in vacuo* at the ordinary temperature :

0.1200 g.; 0.2683 g. CO₂; 0.0645 g. H₂O. C=61.10, H=6.02 %.
 0.1080 g.; 11.1 c.c. moist nitrogen at 22° C. and 767 mm. N=11.95 %.
 C₂₄H₂₆O₅N₄·H₂O requires C=61.49, H=6.04, N=11.96 %.

(2) Dried at temperatures between 110° and 150° :

0.1187 g.; 0.2804 g. CO₂; 0.0645 g. H₂O. C=64.42, H=6.04 %.
 0.1014 g.; 0.2395 g. CO₂; 0.0549 g. H₂O. C=64.41, H=6.05 %.
 0.1252 g.; 0.2950 g. CO₂; 0.0672 g. H₂O. C=64.3, H=6.01 %.
 0.1914 g.; 20.9 c.c. moist nitrogen at 18° C. and 748 mm. N=12.51 %.
 0.1912 g.; 20 c.c. moist nitrogen at 11.5° C. and 757.2 mm. N=12.43 %.
 C₂₄H₂₆O₅N₄ requires C=64.00, H=5.84, N=12.45 %.

(3) Heated to temperatures between 150° and 200° :

0.1037 g.; 0.2513 g. CO₂; 0.0545 g. H₂O. C=66.13, H=5.89 %.
 C₂₄H₂₄O₄N₄ requires C=66.66, H=5.61, N=12.96 %.

It is thus evident that after solution of the ether oxidation substance in alkali and subsequent precipitation by acidification, the product precipitated has the same composition as when originally formed from the mother liquor.

It is of importance to note that the change in composition from C₂₄H₂₆O₅N₄·H₂O (B (1)) to C₂₄H₂₆O₅N₄ (B (2)) is of a reversible nature. That is to say, the ether oxidation substance previously heated to 140° will readily take up water again to give the monohydrate. But having effected the change

from $C_{24}H_{28}O_5N_4$ (B (2)) to $C_{24}H_{24}O_4N_4$ (B (3)), it is not possible to get the reverse reaction to take place. In other words, at temperatures above 150° the ether oxidation substance loses the elements of water as the result of some internal molecular rearrangement. The compound $C_{24}H_{24}O_4N_4$ does not undergo further change at temperatures from 200 – 220° , but it is decomposed at 225° , and carbon dioxide, ammonia and skatole are evolved.

II. *The condensation of formaldehyde and tryptophane.*

If a few cubic centimetres of formalin be added to a concentrated solution of pure tryptophane, and the reaction mixture be kept at a temperature of 38° for several hours, a crystalline derivative is deposited from the solution.

The compound melts and decomposes at 225 – 240° ; it is slightly soluble in water and alcohol, insoluble in benzene and its homologues, amyl alcohol, chloroform, ethyl acetate and ethylene dibromide; it is of a slightly yellow colour which becomes intensified if the substance be kept at a temperature of 150° for a short time, or if it be left exposed to the air at ordinary room temperature for some days. On being boiled with water, dilute acids and weak alkalis, it is readily hydrolysed, formaldehyde is liberated and the ether oxidation product is formed. With concentrated sulphuric acid the substance gives a purple colour, which is intensified if the suspension of the substance in water be heated and cooled before adding the concentrated acid.

The condensation product was formed as follows:

(a) By the interaction of tryptophane and samples of formaldehyde obtained (1) from Merck's, (2) from Kahlbaum's, and (3) from Schering's formalin, (4) by distilling Merck's trioxymethylene in a current of hydrogen or nitrogen and collecting the issuing gases in air-free water.

(b) The formaldehyde vapours evolved in (4) were passed into a solution of tryptophane from which all air had been dispelled by a current of hydrogen.

In each experiment a crystalline derivative melting at 235 – 240° was formed.

On account of the hydrolysis which takes place with water, acids and alkalis, the substance could not be purified by being dissolved in alkalis and re-precipitated by acid. For purposes of analysis it was filtered from the mother liquor, washed well with water, alcohol and ether, and dried at different temperatures. Analyses of samples prepared at different times and dried at the same temperature indicated constancy of composition.

(1) Of the substance dried *in vacuo* :

0.1208 g.; 0.2537 g. CO₂; 0.0700 g. H₂O. C=57.30, H=6.49 %.
 0.1135 g.; 0.2377 g. CO₂; 0.0658 g. H₂O. C=57.10, H=6.56 %.
 0.1340 g.; 0.2820 g. CO₂; 0.0763 g. H₂O. C=57.33, H=6.38 %.
 0.1175 g.; 10.7 c.c. moist nitrogen at 16° C. and 760 mm. N=10.81 %.
 0.1512 g.; 14.4 c.c. moist nitrogen at 23° C. and 763.4 mm. N=10.95 %.
 C₁₂H₁₂O₂N₂ · 2H₂O requires C=57.14, H=6.35, N=11.11 %.

(2) Of the substance dried at temperatures between 110° and 130° :

0.1045 g.; 0.2355 g. CO₂; 0.0600 g. H₂O. C=61.45, H=6.43 %.
 0.1135 g.; 0.2540 g. CO₂; 0.0635 g. H₂O. C=61.04, H=6.26 %.
 0.1265 g.; 12.7 c.c. moist nitrogen at 25° C. and 765 mm. N=11.63 %.
 C₁₂H₁₂O₂N₂ · H₂O requires C=61.49, H=6.04, N=11.96 %.

(3) The substance was heated to 160° for ten minutes, but no analyses could be made owing to decomposition which began to take place; there was considerable carbonisation together with the elimination of skatole.

Analyses were made of the crystalline derivative formed by the hydrolysis of the formaldehyde product :

(1) *By water*. The derivative melted at 324°, and when mixed with some of the ether oxidation substance the melting point remained unchanged. The substance was dried at 110°.

0.1127 g.; 0.2678 g. CO₂; 0.0575 g. H₂O. C=64.80, H=5.72 %.
 0.1055 g.; 0.2475 g. CO₂; 0.0518 g. H₂O. C=63.99, H=5.50 %.
 0.1064 g.; 0.2520 g. CO₂; 0.0572 g. H₂O. C=64.50, H=5.98 %.
 0.1340 g.; 14.8 c.c. moist nitrogen at 19° C. and 768.4 mm. N=12.94 %.

(2) *By Na₂CO₃ solution*. The derivative melted at 324°.(a) Of the substance dried *in vacuo* :

0.1028 g.; 0.2290 g. CO₂; 0.0565 g. H₂O. C=60.80, H=6.16 %.

(b) Of the substance dried at 110° :

0.1000 g.; 0.2347 g. CO₂; 0.0525 g. H₂O. C=64.09, H=5.83 %.
 0.1135 g.; 12.3 c.c. moist nitrogen at 21° C. and 768 mm. N=12.74 %.

These results are in accordance with the assumption from the melting point that the product of hydrolysis is the ether oxidation substance (C₂₄H₂₆O₅N₄ · H₂O and C₂₄H₂₆O₅N₄).

It is thus evident that, although the product formed by the condensation of formaldehyde with tryptophane is not the same as the ether oxidation substance, yet there is a close connexion between these two which can be demonstrated by the fact that on hydrolysis the former is readily converted into the latter substance.

III. *The action of formaldehyde and hydrogen peroxide on tryptophane* gave rise to a crystalline compound which proved to be the formaldehyde product II, and not the ether oxidation substance.

IV. *The condensation of glyoxylic acid with tryptophane.*

To an aqueous solution of tryptophane was added glyoxylic acid in the proportion of one molecule of tryptophane to one molecule of glyoxylic acid. A crystalline substance was deposited from the solution.

The substance was slightly soluble in water and alcohol, insoluble in ether, petroleum ether, benzene and its homologues, amyl alcohol, ethyl acetate and ethylene dibromide. It is soluble in alkalis, moderately soluble in dilute acids, readily in concentrated acids. It is precipitated by the mercury sulphate reagent. It gives the glyoxylic reaction and also the bromine colour tests characteristic of tryptophane. It does not give any colour reaction with concentrated sulphuric acid; on the addition of a trace of formaldehyde or of glyoxylic acid solution, the glyoxylic colour is formed; while on the addition of a trace of hydrogen peroxide a claret colour is obtained.

The substance, whether separated from the mother liquors and repeatedly washed with water, alcohol and ether, or recrystallised from hot water and from alcohol, melted at 324° , and when mixed with some of the ether oxidation substance the melting point was unaffected. That the derivative under discussion was not the ether oxidation substance was shown by the fact that when dissolved in sodium carbonate solution and the solution acidified with acetic acid, the *acid sodium salt* was precipitated and not the original condensation product. Analyses of the original acid (*A*), and of the acid sodium salt (*B*) were made and the results obtained were in accordance with the formulae $C_{13}H_{12}O_4N_2$ for the acid and $C_{13}H_{11}O_4N_2Na \cdot 4H_2O$ for the sodium salt.

A. Analyses of the original product (m.p. 324°).

(1) Of the substance dried in vacuum desiccator:

0.1238 g.; 0.2693 g. CO_2 ; 0.0542 g. H_2O . C=59.34, H=4.89 %.
0.1858 g.; 18 c.c. moist nitrogen at $21^{\circ}C$. and 777.3 mm. N=11.21 %.

(2) Dried at 150° :

0.1008 g.; 0.2193 g. CO_2 ; 0.0433 g. H_2O . C=59.35, H=4.82 %.
0.2820 g.; 26.2 c.c. moist nitrogen at $20^{\circ}C$. and 757.2 mm. N=10.70 %.

(3) Heated to 200° :

0.1055 g.; 0.2287 g. CO_2 ; 0.0465 g. H_2O . C=59.12, H=4.99.
(1), (2) and (3). $C_{13}H_{12}O_4N_2$ requires C=59.97, H=4.66, N=10.77 %.

(4) Heated to temperatures between 205° and 220° for ten minutes, carbon dioxide being evolved.

0.1184 g.; 0.2875 g. CO_2 ; 0.0575 g. H_2O . $\text{C}=66.23$, $\text{H}=5.44\%$.

0.1525 g.; 16.8 c.c. moist nitrogen at 14.5°C . and 755.3 mm. $\text{N}=12.94\%$.

$\text{C}_{24}\text{H}_{24}\text{O}_4\text{N}_4$ (ether oxidation substance) requires $\text{C}=66.66$, $\text{H}=5.61$, $\text{N}=12.96\%$.

It is evident from the above analyses that at temperatures between 15° and 200° the glyoxylic condensation product has a constant composition; at 220° it is converted, with loss of carbon dioxide, into the ether oxidation substance.

(5) If the glyoxylic compound be heated for a short time at a temperature of 225 – 230° it decomposes, yielding carbon dioxide, ammonia and skatole (cf. I, p. 105).

B. The acid sodium salt (see above) was twice recrystallised from water and analysed.

0.3735 g.; 26.1 c.c. moist nitrogen at 20.8°C . and 757.3 mm. $\text{N}=8.08\%$.

0.1289 g.; 9 c.c. moist nitrogen at 21.5°C . and 761.9 mm. $\text{N}=8.02\%$.

0.2048 g.; 0.406 g. Na_2SO_4 . $\text{Na}=6.42\%$.

0.3700 g.; 0.0562 g. Na_2CO_3 and 0.0762 g. Na_2SO_4 . $\text{Na}=6.59$ and 6.67% .

0.8122 g.; 0.1542 g. Na_2SO_4 . $\text{Na}=6.15\%$.

From these determinations the molecular weight of the substance = 355.

0.6074 g.; 0.4890 g. anhydrous residue,

$\therefore$ in the acid salt there are 3.84 mols. water.

0.1033 g.; 0.0528 g. H_2O ; 0.1612 g. CO_2 .

Putting in the correction for the Na_2CO_3 remaining } $\text{C}=44.20$, $\text{H}=5.72\%$.
in the boat (0.0150 g.)

$\text{C}_{13}\text{H}_{11}\text{O}_4\text{N}_2\text{Na} \cdot 4\text{H}_2\text{O}$ requires $\text{N}=7.91$, $\text{C}=44.07\%$.

$\text{H}=5.37$, $\text{Na}=6.50\%$.

M.W. 354.3.

From these results it is obvious that the glyoxylic condensation product and the ether oxidation substance are two distinct compounds, in spite of the fact that the compounds when taken separately and also when mixed together have the same melting point, viz. 324° . It is obvious that the glyoxylic compound readily changes to the ether oxidation substance on being heated to a temperature of 205° . (The ether oxidation substance is not affected under the same conditions.) At 225° both are decomposed into carbon dioxide, ammonia, skatole, etc.

There is thus an interesting connexion between the formaldehyde compound, the glyoxylic condensation product, and the ether oxidation substance. The last-named substance can be produced from the formaldehyde product by hydrolysis, and from the glyoxylic condensation product by the application of heat.

It is probable from the following reasons that these compounds described under I, II and IV are formed by the simple process of condensation between the $-\text{NH}_2$ group of tryptophane and the $-\text{CHO}$ group of the reacting aldehydes:

1. The ease with which the reactions take place (the presence of a condensing agent is unessential).

2. The failure to produce naphthalene sulphochloride derivatives points to the substitution of the hydrogen of the amino-groups.

3. The fact that the compounds so obtained are colourless points to the condensation having taken place as a result of the activity of the amino- and not the imino-group (*post*, p. 112).

4. Corresponding compounds could not be obtained with indole, skatole, indoleacetic and indolepropionic acids, i.e. with indole derivatives in which there is no *amino*-group.

For reaction to take place with these compounds, the presence of a condensing agent is necessary, and the resulting products are intensely coloured (*post*, p. 112).

Analyses of the three compounds I, II, IV are in accordance with the following formulae:

(a) The formaldehyde product	...	...	$\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2 \cdot 2\text{H}_2\text{O}$.
(b) The glyoxylic product...	...	...	$\text{C}_{13}\text{H}_{12}\text{O}_4\text{N}_2$.
" " " " above 205°	...	...	$\text{C}_{24}\text{H}_{24}\text{O}_4\text{N}_4$.
(c) The ether oxidation substance	...	...	$\text{C}_{24}\text{H}_{26}\text{O}_5\text{N}_4$.
" " " " above 150°	...	...	$\text{C}_{24}\text{H}_{24}\text{O}_4\text{N}_4$.

The mechanism involved in the production of these three substances may be briefly described as follows:

I. Since the *ether oxidation product* is in no way an oxidation product of tryptophane, but is closely connected with the formaldehyde condensation product, it is suggested that in the formation of the ether oxidation substance nascent formaldehyde is the active principle (p. 102, I c):

On heating this substance to a temperature of $150\text{--}200^\circ$, molecular water and not water of crystallisation is lost. After such a process the composition of the substance corresponds to the formula $\text{C}_{24}\text{H}_{24}\text{O}_4\text{N}_4$, and can be represented graphically as

II. *The formaldehyde condensation product* is formed as follows :

III. *The glyoxylic condensation product* is produced in a similar manner :

It has been shown that the ether oxidation substance is readily formed (a) from the formaldehyde condensation product by hydrolysis, (b) from the glyoxylic condensation product by the application of heat. For the former process (a) there are two possibilities to be considered, viz. :

(1) That during hydrolysis the molecules are linked together by means of the addition of the elements of water, thus :

(2) That during the hydrolysis tryptophane and nascent formaldehyde are re-formed, and that these react as in the ether oxidation process to form the substance $C_{24}H_{28}O_5N_4$.

(b) In the case of the production of the substance $C_{24}H_{24}O_4N_2$ from the glyoxylic product by the application of heat, it is evident that two molecules of the latter each lose a molecule of carbon dioxide, and at the temperature at which the reaction takes place condensation of these two molecules occurs, accompanied by a rearrangement of the bonds into the more stable configuration of the ether oxidation substance.

A study of the properties of these three compounds throws some light on the mechanism of the Adamkiewicz reaction: a discussion of this point will be dealt with in the following paper [Homer, 1913, p. 117, Table I].

SECTION B.

The condensation of glyoxal with tryptophane.

If an aqueous solution of tryptophane heated to a temperature of 38° be treated with the calculated quantity of glyoxal in the presence of hydrogen peroxide, a crystalline chocolate-coloured deposit slowly forms.

This coloured substance is of an acidic nature and is slightly soluble in water and in alcohol. It dissolves in alkalis, and is moderately soluble in

dilute acids, and with the exception of acetic acid readily soluble in concentrated acids. It is precipitated by the mercury sulphate reagent. With concentrated sulphuric acid it gives a brown colour which in the presence of hydrogen peroxide changes to claret. In the presence of a trace of formaldehyde, a brown colour is produced which is changed to the colour of bromine on the addition of an oxidising agent.

Analyses of the substance gave the following results :

(1) Of the substance recrystallised from water and

(a) Dried at 110° :

0.1030 g.; 0.2325 g. CO₂; 0.0463 g. H₂O. C=61.60, H=5.04 %.

0.1223 g.; 0.2780 g. CO₂; 0.0560 g. H₂O. C=61.99, H=5.13 %.

0.1025 g.; 0.2346 g. CO₂; 0.0485 g. H₂O. C=62.40, H=5.25 %.

0.2268 g.; 21.6 c.c. moist nitrogen at 24° C. and 767.5 mm. N=10.9 %.

C₂₆H₂₂O₆N₄ · H₂O requires C=61.90, H=4.81, N=11.1 %.

(b) Heated to 130° :

0.1006 g.; 0.2360 g. CO₂; 0.0412 g. H₂O. C=64.00, H=4.62 %.

0.1080 g.; 10.7 c.c. moist nitrogen at 21° C. and 768 mm. N=11.51 %.

(2) After solution in Na₂CO₃ and precipitation by acetic acid :

0.1430 g.; 0.0565 g. H₂O; 0.3403 g. CO₂. C=64.90, H=4.43 %.

0.1060 g.; 10.2 c.c. moist nitrogen at 20° C. and 769 mm. N=11.24 %.

(3) Crystallised from alcohol and heated to 110° :

0.1017 g.; 0.0441 g. H₂O; 0.2390 g. CO₂. C=64.15, H=4.86 %.

0.1290 g.; 12.5 c.c. moist nitrogen at 19° C. and 750.5 mm. N=11.1 %.

(4) Crystallised from pyridine :

0.1060 g.; 0.0441 g. H₂O; 0.2505 g. CO₂. C=64.45, H=4.63 %.

Ib. 2, 3, 4 :—C₂₆H₂₂O₆N₄ requires C=64.2, H=4.53, N=11.5 %.

(5) Heated to 160° :

0.1006 g.; 0.0410 g. H₂O; 0.2459 g. CO₂. C=66.66, H=4.53 %.

0.1150 g.; 0.0460 g. H₂O; 0.2830 g. CO₂. C=67.07, H=4.46 %.

0.1890 g.; 19.6 c.c. moist nitrogen at 16° C. and 749.6 mm. N=12.01 %.

C₂₆H₂₀O₆N₄ requires C=66.66, H=4.32, N=11.97 %.

Taking into consideration the necessity for the presence of an oxidising agent, and also the fact that the substance produced is intensely coloured, it is highly probable that in this reaction, besides the simple aldehyde condensation occurring in the reactions described in Section A, there has also been elimination of hydrogen accompanied by complex ring formation.

Possible equation :

Any attempt to ascertain the constitution of this substance has been postponed for the present.

SECTION C.

THE PRODUCTS FORMED BY THE CONDENSATION OF ALDEHYDES
WITH THE IMINO-GROUP OF INDOLE DERIVATIVES.I. *The action of formaldehyde on indole derivatives in presence of a condensing agent.*

In connexion with the work on the condensation of formaldehyde with tryptophane, an attempt was made to prepare corresponding derivatives with indole, skatole, indolepropionic and indoleacetic acids, but without success. However, it is well known that under suitable conditions, in the presence of concentrated sulphuric acid, a solution of formaldehyde reacts with the above-mentioned compounds to yield coloured substances. Concentrated sulphuric acid being known to be both an oxidising and a condensing agent, the question arose as to whether the colour effect was due to a process of simple condensation between formaldehyde and the above-mentioned substances, which have an imino-group in common, or was in any way dependent upon the oxidising power of the acid.

In order to test this suggestion, it was thought advisable to avoid the use of sulphuric acid and attempts were made to form condensation products of indole derivatives with trioxymethylene, using zinc chloride and alcohol saturated with hydrochloric acid gas as the condensing agents (with these condensing agents an aqueous solution of formaldehyde could not be used).

II. *Derivatives formed by the interaction of trioxymethylene and indole derivatives, using zinc chloride as the condensing agent.*

Indole, and indoleacetic acid reacted readily at temperatures below 100° to produce purple-coloured products. Skatole and indolepropionic acid under the same conditions gave brown products. Owing to scarcity of material, the products from indoleacetic and indolepropionic acids could not be analysed. The indole and skatole derivatives were investigated.

Tryptophane treated in the same way did not react until heated to a much higher temperature (160°); the resulting product was of a *deep brown* colour. The reaction was accompanied by a certain amount of decomposition of tryptophane. As it was impossible to free the product of the reaction from the results of carbonisation, no further investigation of this substance has been made.

Preparation of the trioxymethylene derivatives of skatole and indole. One part by weight of indole or skatole was mixed with the same weight of trioxymethylene and ground together with one part by weight of freshly powdered and freshly ignited zinc chloride. The process of grinding the reaction mixture gave rise in the former case to the formation of a purple colour. The reaction mixture was heated on a water-bath to a temperature of 80° for half an hour, and then transferred to an oil bath and the temperature raised to 150° for a few minutes. On cooling, the coloured mixture was treated with hydrochloric acid solution (one part concentrated acid to one part water) and allowed to stand for some hours. The residue was in each case repeatedly boiled with dilute hydrochloric acid, and then with water until free from formaldehyde and hydrochloric acid. The coloured substance was finally washed with water, alcohol and ether, dried and analysed.

It was impossible to purify the products further on account of their insolubility in all liquids other than concentrated sulphuric acid. Constancy of composition was denoted by analyses of samples prepared at different times and under slightly different conditions as regards the proportion of zinc chloride used. It is interesting to note that the indole compound is of a purplish colour, the skatole compound is brown. They are both insoluble in ordinary solvents, in alkalis and in acids with the exception of concentrated sulphuric acid, in which they are appreciably soluble, and concentrated hydrochloric acid in which they are slightly soluble. In concentrated sulphuric acid the indole derivative gives a reddish violet colour which on dilution becomes bluish and finally disappears owing to the decreasing solubility of the substance as the acid becomes more dilute. The skatole compound under the same conditions gives rise to a brown colour.

(1) Analyses of the indole derivative prepared on different occasions were made¹.

- A. 0.1026 g.; 0.0475 g. H_2O ; 0.2702 g. CO_2 . C=71.85, H=5.20 %.
- B. 0.1057 g.; 0.0518 g. H_2O ; 0.2783 g. CO_2 . C=71.80, H=5.49 %.
- A. 0.1325 g.; 9.4 c.c. moist nitrogen at 14°C . and 753.5 mm. N=8.32 %.
- 0.1500 g.; 11.0 c.c. moist nitrogen at 15.5°C . and 747.3 mm. N=8.34 %.
- B. 0.2074 g.; 14.2 c.c. moist nitrogen at 13°C . and 755.3 mm. N=8.07 %.

Analysis of the indole derivative after being heated to 205° for fifteen minutes gave the following result:

0.1045 g.; 0.0502 g. H_2O ; 0.2750 g. CO_2 . C=71.78, H=5.38 %.

Hence the compound is stable to heat at temperatures not exceeding 205° .

$\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2$ requires C=72.41, H=5.74, N=8.04 %.

¹ These compounds are extremely difficult to burn and this probably accounts for the slightly low values obtained.

(2) Analyses of the skatole derivative gave the following results¹:

0.1161 g.; 0.0658 g. H₂O; 0.3120 g. CO₂. C=73.30, H=6.35 %.
 0.1083 g.; 0.0575 g. H₂O; 0.2910 g. CO₂. C=73.30, H=5.94 %.
 0.1685 g.; 9.8 c.c. moist nitrogen at 13.5° C. and 758.5 mm. N=7.13 %.
 0.1820 g.; 10.8 c.c. moist nitrogen at 11.5° C. and 765 mm. N=7.15 %.
 C₂₃H₂₄O₃N₂ requires C=73.40, H=6.38, N=7.42 %.

The condensations may therefore be represented by the equations:

It is thus obvious that in the presence of a condensing agent trioxymethylene reacts with the imino-group of the indole nucleus to form coloured bodies.

III. *Derivatives formed by the interaction of trioxymethylene and indole derivatives, using a solution of hydrochloric acid gas in alcohol as the condensing agent.*

A description of the condensation products with indole, skatole and tryptophane formed under these conditions will form the subject-matter of a future paper. It may be stated here that they are intensely coloured substances, and that the indole derivative is the only one of the three which will give a purple colour with concentrated sulphuric acid.

The derivatives formed by the action of trioxymethylene on indole derivatives in the presence of a condensing agent, whether it be concentrated sulphuric acid, or zinc chloride, or an alcoholic solution of hydrochloric acid gas, are all characterised by their insolubility in solvents other than concentrated mineral acids.

A discussion as to the significance of activity of the *imino*-group of indole derivatives in the production of the specific colour reaction known as the Adamkiewicz reaction, will be found in the paper following this [Homer, 1913, p. 116].

During the course of the work under consideration it has been noticed that tryptophane itself under certain conditions of oxidation gives rise in the process of time to the formation of pigments having definite absorption bands. The properties both chemical and physical of these pigments, and of those formed from normal breakdown products of tryptophane, are being investigated, and will form the subject of a future paper. At this

¹ These compounds are extremely difficult to burn and this probably accounts for the slightly low values obtained.

point it may be observed that in nearly every case these substances are themselves brown in colour, and their solutions in organic solvents are also brown, but the presence of acid changes the colour from brown to purple.

SUMMARY.

1. Tryptophane by virtue of its $-NH_2$ group reacts directly with nascent formaldehyde, formaldehyde and glyoxylic acid to form colourless crystalline compounds.

2. Glyoxal will react with tryptophane in the presence of an oxidising agent to form an intensely coloured compound.

3. Indole derivatives, by virtue of the $-NH$ group in the nucleus, will react with formaldehyde and trioxymethylene *in the presence of a condensing agent* to form substances of intense colour and marked insolubility in ordinary solvents other than concentrated mineral acids.

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