THE HUMIN FORMED BY THE ACID HYDROLYSIS OF PROTEINS VIII. THE CONDENSATION OF INDOLE DERIVATIVES WITH ALDEHYDES[1]

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Introduction

Osborne[2] in 1910 called attention to the black humin or melanin which is formed upon the acid hydrolysis of proteins as a source of error in the quantitative analysis of protein material, inasmuch as the amino acids causing humin formation were unknown. Since that time Gortner and his students[3] have demonstrated that the black humin formation of protein hydrolysis is due to the presence of tryptophan in the protein molecule and that the reaction probably involves the condensation of the tryptophan with an aldehyde, inasmuch as products similar in physical and chemical properties can be prepared by heating tryptophan with aldehydes in the presence of 20% hydrochloric acid, and also inasmuch as the addition of aldehydes to proteins undergoing acid hydrolysis usually increases the amount of acid-insoluble humin which is formed. Apparently most proteins contain a small amount of an unknown aldehydic constituent which reacts with the tryptophan to produce the black humin.

Gortner and Holm[3c] also showed that this reaction between tryptophan and aldehydes was in reality a reaction with the indole nucleus and that, by properly controlling the amount of aldehyde present in the reaction, it might be utilized as a means of quantitatively determining the amount of tryptophan in a protein.

That this is actually the case has been recently demonstrated by Holm and Greenbank.[4]

It thus appears to be definitely proved that of all the known hydrolytic products of proteins, tryptophan alone is involved in the formation of the black humin, and that humin formation requires a second component which in all probability is an aldehyde. However, the nature of the chemical reaction which is involved in humin formation and the structural

[2] Osborne, *Am. J. Physiol.*, **26**, 305 (1910).

[3] (a) Gortner and Blish, THIS JOURNAL, **37**, 1630 (1915). (b) Gortner, *J. Biol. Chem.*, **26**, 177 (1916). (c) Gortner and Holm, THIS JOURNAL, **39**, 2477 (1917); (d) **42**, 821 (1920). (e) Holm and Gortner, *ibid.*, **42**, 632 (1920); (f) **42**, 2378 (1920). (g) Gortner and Norris, *ibid.*, **45**, 550 (1923).

[4] Holm and Greenbank, *ibid.*, **45**, 1788 (1923).

configuration of the humin molecule still require elucidation. The present investigation has, therefore, been directed along these lines.

Inasmuch as it has already been shown that humin formation is a reaction of the indole nucleus with an aldehyde we have in most instances used substituted indoles in place of the extremely expensive tryptophan.

The condensation of aldehydes and ketones with indoles has been previously studied by a number of investigators.[5] Examination of these papers brings out certain important facts.

1. There are at least two types of compounds formed having very different properties. The formation of one or the other of these depends on the strength of the condensing agent. The relation of these compounds is best shown by the equilibrium equation

I.   Rosindole type.   II.   Indolidene-methane type.

Compounds of Type I are non-basic, colorless, stable, while compounds of Type II are distinctly basic, highly colored and more or less unstable. Both are readily oxidized.

2. The hydrogen atoms in the indole ring have the following order of decreasing activity: β-, α-, N-. If the β hydrogen is present, condensation will take place on the β carbon. If the β hydrogen is substituted there may rarely be condensation of the α carbon. Very rarely has condensation on the N atom been reported.

3. The methyl group in α-methylindole probably does not take part in ordinary condensations.

The work that has been reported on the formation of humin by various workers[3,6] gives two general views of the nature of humin formation. First it has been shown that humin-like substances may be made by oxidation of pure amino acids and other pure compounds with hydrogen peroxide. On the other hand, some have evidence that condensation is the only reaction involved in humin formation. Between these extremes

[5] (a) Fischer, Ber., 19, 2988 (1886); (b) Ann., 236, 116 (1886). (c) Degen, Ann., 236, 151 (1886). (d) Roder, Ann., 236, 164 (1886). (e) Schlieper, Ann., 236, 174 (1886). (f) Fischer and Wagner, Ber., 20, 815 (1887). (g) Fischer, Ann., 242, 372 (1887). (h) Renz and Loew, Ber., 36, 4326 (1903). (i) Freund and Lebach, Ber., 36, 308 (1903); (j) 38, 2640 (1905). (k) Freund, Ber., 37, 322 (1904). (l) Scholtz, Ber., 46, 1082 (1913); (m) 46, 2138 (1913). (n) Plancher, Giorn. chim. ind. applicata, 2, 459 (1920). (o) Homer, Biochem. J., 7, 101 (1913). (p) Oddo, Gazz. chim. ital., 41, I, 255 (1911). (q) Fürth and Lieben, Biochem. Z., 109, 124 (1920).

[6] Ref. 5 q. F. Samuely, Beitr. chem. physiol. path., 2, 355 (1902). Adler and Wiechowski, Ber., 55B, 3030 (1922). Maillard, Ann. chim., [9] 5, 258 (1916). Roxas, J. Biol. Chem., 27, 71 (1916).

is the view that the humin of protein hydrolysis is the product of condensation between certain amino acids and aldehydes, with or without oxidation.

Experimental Part

The Materials.—We have used in the present work the following indole derivatives: *l*-tryptophan (β-indole-α-aminopropionic acid); α-methylindole; α-phenylindole; α,β-diphenylindole; and α-methyl-β-ethyl-indole. The tryptophan was prepared by the tryptic digestion of casein; the other indoles were synthesized in this Laboratory by the conventional methods. The aldehydes used were benzaldehyde, *p*-hydroxybenzaldehyde, *o*-hydroxybenzaldehyde, *o*-chlorobenzaldehyde, cinnamaldehyde, 2,4,6-trinitrobenzaldehyde, *o*-tolylaldehyde, anisaldehyde, *m*-nitrobenzaldehyde, and *p*-dimethylaminobenzaldehyde. With the exception of formaldehyde, aliphatic aldehydes were not used because of their tendency to polymerize or condense in strong acid solution and form materials difficult to purify.[7]

Condensations with Alpha-Methylindole

Phenyl-α-methylindolidene-methane Acid Sulfate.—Freund and Lebach[5i] were unable to isolate this product by their method of passing dry hydrogen chloride into absolute alcohol solution.

To 0.5 g. of α-methylindole dissolved in 2 or 3 cc. of benzaldehyde, concd. sulfuric acid was added on a stirring rod a little at a time until the entire mass was almost solid. The reaction product was washed thoroughly with benzene and ether, recrystallized from warm glacial acetic acid and washed in ether. It forms clusters of long, five-edged, orange-colored needles, which slowly darken when heated above 100° and melt at 172–173° (corr.) to a dark melt which changes form and swells to a solid at 175°.

Analysis. (Subs. dried for a short time at 100° and then in a vacuum.) Calc. for $C_{16}H_{15}O_4NS$: C, 60.57; H, 4.73; O, 20.18; N, 4.41; S, 10.09. Found: C, 60.73; H, 3.79; O, 21.08; N, 4.25; S, 10.03.

Mol. wt. (in glacial acetic acid). Calc.: 317. Found: 301, 288.

This is, therefore, the acid sulfate and has the formula
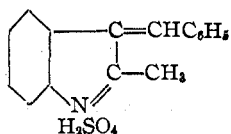
It is entirely stable in the air at room temperature. It is readily soluble in alcohol, acetone or ethyl acetate giving a pale yellow solution, slightly soluble in cold acetic acid, readily soluble in hot acetic acid giving an orange-colored solution, insoluble in ether, benzene or chloroform, and very slightly soluble in water.

When dissolved in a neutral solvent such as alcohol it soon turns red. This is due to the fact that it dissociates and forms the benzylidene-dimethyl-ketole which oxidizes to rosindole. When it is precipitated from alcohol by the addition of water, colorless crystals are formed.

FREE BASE.—It was not possible to obtain the free phenyl-α-methylindolidene-methane in crystalline form. On trituration with ammonium hydroxide and extraction with ether, a pale yellow solution and a colorless residue are obtained. On evaporation this solution gave an amorphous yellow powder which readily dissolved in concd. sul-

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[7] Compare Ref. 3 e, pp. 637–638.

furic acid and soon formed the characteristic acid sulfate crystals which melted at 172–173° (corr.). Because of the fact that it was impossible to recrystallize the free base an analysis was not attempted.

Hydrolysis to Benzylidene-di-α-methylindole.—An aqueous suspension of the acid sulfate mentioned above was boiled with water for a few minutes. Benzaldehyde was given off and white crystals were deposited. These were washed with alcohol and recrystallized from benzene as cubes that darkened at 230° and melted sharply at 243° (corr.) (Fischer reports 243°).

Analysis. Calc. for $C_{25}H_{22}N_2$: N, 8.00. Found: 7.90.

Therefore this is Fischer's benzylidene-dimethylketole. When these crystals were dissolved in benzaldehyde and a little sulfuric acid was added while the mixture was stirred, the orange-colored crystals of phenyl-α-methylindolidene-methane acid sulfate separated at once.

These changes are represented by the equilibrium
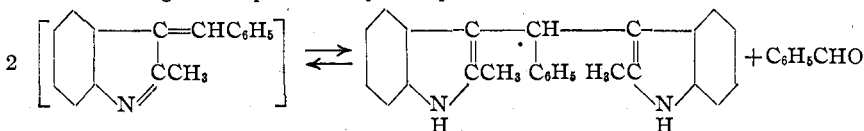
Phenyl-α-methylindolidene-methane.   Benzylidene-di-α-methylindole.

p-Dimethyl-aminophenyl-α-methylindolidene-methane.—This was prepared according to the method of Freund and Lebach. Into a solution of 1.5 g. of p-dimethyl-aminobenzaldehyde and 1.3 g. of α-methylindole in absolute alcohol, dry hydrogen chloride was passed to saturation. A red, sticky mass was formed which was amorphous and whose base did not show a melting point.

The hydrochloride was dissolved in water and the free base precipitated with sodium hydroxide. It was then washed in the centrifuge free from chlorides, filtered and dried.

Analyses. Calc. for $C_{18}H_{18}N_2$: N, 10.68. Found: 10.22; 10.55.

The base is entirely insoluble in ether and since it showed no further change when hydrogen chloride was passed into it for 12 hours, it seemed probable that this was a rearrangement product and not the simple condensation product. No further condensation was obtainable with benzaldehyde.

Advantage was then taken of the fact that α-methylindole and p-dimethyl-amino-benzaldehyde are mutually soluble. Dry hydrogen chloride was passed into a mixture of 0.65 g. of α-methylindole and 0.75 g. of p-dimethyl-aminobenzaldehyde while the liquid was constantly stirred. It soon changed to a hard mass. This material is a brilliant gold by reflected light and purple-red in solution. It is slightly soluble in water, readily soluble in alcohol, acetic acid and acetone, insoluble in benzene, ether and ligroin, and gives amorphous salts with picric acid, mercuric chloride and platinic chloride. The hydrochloride gradually melts between 150° and 160°.

The base was prepared by adding dil. sodium hydroxide solution to a solution of the salt and extracting with ether. The ethereal solution when concentrated was deep red, but yellow when dilute. The melting point of the free base depends upon the rate of heating, but is about 100°.

The alcoholic solution is a sensitive indicator, colorless in alkali, and red-purple in acid; it is red at P_H 6.48 and colorless at P_H 7.58. Inasmuch as its color changes are the reverse of those of phenolphthalein and extremely brilliant, and also since the neutral point is apparently very near P_H 7.0, it should be a valuable indicator for acidimetric titrations, especially for titrating an alkaline solution.

This material is changed to an infusible product by dissolving it in alcohol and passing in dry hydrogen chloride for a few hours. The base is no longer soluble in ether.

Undoubtedly the compound as prepared by passing dry hydrogen chloride into alcohol is a humin-like material and not the simple condensation product.

Ferrocyanide salts were made of both the infusible product and the hydrochloride of melting point 150–160°. These salts both form infusible, dark red, amorphous powders, showing by analysis the composition, $(Base)_4.H_4Fe(CN)_6.2H_2O$.

o-Chlorophenyl-α-methylindolidene-methane Hydrochloride.—A solution of 0.33 g. of α-methylindole in absolute alcohol saturated with hydrogen chloride, and 0.36 g. of o-chlorobenzaldehyde was added directly while the mixture was stirred. Golden-yellow, rhombic needles quickly separated. They were filtered off, washed with absolute alcohol saturated with hydrogen chloride and with absolute ether, and dried at 100°; m. p. 192° (corr.). (Freund and Lebach reported 194°.) The yield was almost quantitative.

o-Hydroxyphenol-α-methylindolidene-methane.—A solution of 1.0 g. of α-methylindole in the minimum amount of absolute alcohol was saturated with •hydrogen chloride and 0.92 g. of salicylaldehyde was added slowly while the liquid was stirred and cooled. The hydrochloride was isolated exactly as in the previous experiment except that the washing with alcohol was omitted. Golden, diamond-shaped wedges were obtained which melted at 202° (corr.). (Freund and Lebach reported 202°.) The yield was 1.71 g., or 90%. When these crystals were triturated with ammonium hydroxide, filtered off and washed, the free base was obtained as a light yellow powder which melted sharply at 186° (corr.). (Freund and Lebach reported 185°.)

The acid sulfate was obtained exactly as with the benzaldehyde product, and the free base from this substance melted at 185° (corr.). When dry hydrogen chloride was passed through an alcohol solution for an hour a violet-colored material was obtained which gives a base melting at about 305°. This is the humin.

Anisyl-α-methylindolidene-methane Hydrochloride.—To a solution of 0.33 g. of α-methylindole in absolute alcohol saturated with hydrogen chloride 0.34 g. of anisaldehyde was added directly. The crystals separated quickly, were filtered off, washed and dried. Almost a quantitative yield was obtained of beautiful, golden, irregular plates which darkened at 155° and melted sharply at 181° (corr.).

Analysis. Calc. for $C_{17}H_{16}ON.HCl$: N, 4.95. Found: 5.0.

Cinnamyl-α-methylindolidene-methane Hydrochloride.—To a solution of 0.33 g. of α-methylindole in absolute alcohol saturated with hydrogen chloride 0.33 g. of cinnamaldehyde was added directly. Very fine needles suddenly precipitated. When these were recrystallized from absolute alcohol saturated with hydrogen chloride, flowers of golden needles were formed which blackened at 160°, contracted at 180°, and melted gradually to a black mass at 195–205° (corr.).

Analysis. Calc. for $C_{18}H_{16}ON.HCl$: N, 5.00. Found: 5.1.

o-Tolyl-α-methylindolidene-methane Hydrochloride.—To a solution of 0.33 g. of α-methylindole in absolute alcohol saturated with hydrogen chloride 0.30 g. of o-tolylaldehyde was added. After the mixture was cooled for a few minutes an almost quantitative yield of yellow, prismatic needles was precipitated. These were washed with cold, absolute alcohol saturated with hydrogen chloride, and dried at 100°. They darkened at 165° and melted sharply at 178° (corr.).

Analysis. Calc. for $C_{17}H_{16}N.HCl$: N, 5.2. Found: 5.3.

p-Hydroxyphenyl-α-methylindolidene-methane.—One-half g. of α-methylindole and 0.46 g. of p-hydroxybenzaldehyde were separately dissolved in absolute alcohol saturated with hydrogen chloride and the solutions mixed. The mixture required considerable cooling and stirring before crystallization began. The orange-red plates and wedges formed were filtered off, washed with ether and dried at 100° and in a vac-

uum; m. p., 189° (corr.); yield, 0.82 g., or 84%. The crystals are slightly soluble in water.

Analysis. Calc. for $C_{16}H_{13}ON.HCl$: N, 4.2. Found: 4.3.

These crystals were triturated with ammonium hydroxide. At first an orange-yellow crystalline powder remained but when this was dried it changed to a bright red powder that again became yellow at 185° and melted to a red liquid at 208° (corr.).

m-Nitrophenyl-α-methylindolidene-methane.—One-third g. of α-methylindole and 0.38 g. of *m*-nitrobenzaldehyde were separately dissolved in absolute alcohol saturated with hydrogen chloride and the solutions mixed. After a few minutes a small amount of brown powder of no apparent crystalline form was precipitated. Ether seemed to decompose the precipitate, and it was washed only with absolute alcohol saturated with hydrogen chloride. It showed no melting point but decomposed explosively at a high temperature.

Analysis. Calc. for $C_{16}H_{12}O_2N_2$: N, 10.6. Found: 10.1.

This is apparently the free base and not the hydrochloride.

2,4,6-Trinitrophenyl-α-methylindolidene-methane.—In attempting to prepare this product by the usual procedure using absolute alcohol saturated with hydrogen chloride no condensation product was obtainable, but the 2,4,6-trinitro-benzaldehyde was recovered almost quantitatively as colorless rectangular and diamond-shaped prisms which are probably an isomeric form melting at 90° (corr.).[8]

The nitro groups so interfered with the condensation[9] that it was necessary to use concd. sulfuric acid as a condensing agent.

One-third g. of α-methylindole and 0.60 g. of 2,4,6-trinitro-benzaldehyde were separately dissolved in the minimum quantity of concd. sulfuric acid. The solutions were mixed and the orange-colored condensation product was precipitated with dil. sulfuric acid. The product is somewhat soluble in hot water and was hydrolyzed by the water to the free aldehyde and indole. The orange-colored powder was dried at 100°. It was then recrystallized from glacial acetic acid as clusters of minute, orange-colored needles which melted at 152° (corr.). They are unstable at 100° on long drying.

Analysis. Calc. for $C_{16}H_{10}O_6N_4$: N, 15.8. Found: 15.5.

It was not found possible to isolate the hydrochloride or hydrosulfate.

Condensations with Alpha-Phenylindole

Phenyl-α-phenylindolidene-methane.—1. In Benzaldehyde. To a solution of 0.5 g. of α-phenylindole in 2 cc. of benzaldehyde, concd. sulfuric acid was added on a stirring rod while the liquid was constantly stirred. A deep orange color developed as with α-methylindole but there was no crystallization. When the reaction mixture was poured into ether or benzene to remove the excess of benzaldehyde no solid was formed but when poured into concd. sulfuric acid a small solid deposit was obtained. The main portion was dissolved in alcohol, the solution filtered, and the filtrate was diluted with water. A white crystalline precipitate was obtained which was filtered, washed, and recrystallized from acetone; m. p., 262–263° (corr.). This is Fischer's "benzylidene-Pr₂-phenylindol."

2. In Concd. Sulfuric Acid. One g. of α-phenylindole and 0.6 cc. of benzaldehyde were dissolved in 6 cc. of concd. sulfuric acid. The solution immediately became bright orange in color. It was poured into 27.5 cc. of cold 40% sulfuric acid, the mixture was cooled and diluted further with 30% sulfuric acid while it was kept cool. It was then further diluted with ice water until there was complete precipitation of the orange-

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[8] Compare Forster and Coulson, *J. Chem. Soc.*, **121**, 1988 (1922).

[9] Scott and Cohen, *ibid.*, **121**, 2035 (1922).

colored product. This was filtered, washed free from sulfate ion and dried at 100°. No crystals could be obtained. The powder was washed with a little alcohol saturated with hydrogen chloride. It then melted to a black mass at 182–184°. Probably it was not perfectly pure; yield 1.3 g. or 93%.

PROPERTIES.—It is very unstable in solvents. In 95% or absolute alcohol it dissolves readily but soon precipitates the colorless benzylidene-di-α-phenylindole. In ethyl acetate it dissolves to form a colorless solution but when 5 drops of concd. hydrochloric acid are added the solution becomes orange in color, and on long standing a white powder is precipitated, the liquid becoming green. The colorless solution in benzene gradually becomes green. It forms a colorless solution in anhydrous ether. It is slightly soluble in ligroin but a white residue is soon formed. It is entirely insoluble in water, but when a suspension is boiled a white product is precipitated, and benzaldehyde is given off. The white product is benzylidene-di-α-phenylindole. The same results are obtained with alkali. It is sparingly soluble in 1:1 sulfuric acid to a perfectly stable orange-colored solution. It is stable in concd. sulfuric acid and sparingly soluble in concd. hydrochloric acid forming a stable solution. It dissolves rather readily in hot glacial acetic acid, but on standing the solution turns green and a white precipitate separates. Benzylidene-di-α-phenylindole dissolves in concd. sulfuric acid giving an orange-colored solution but does not crystallize and when dissolved in ethyl acetate and two drops of concd. hydrochloric acid are added the change to orange takes place.

Analysis. Calc. for $C_{21}H_{15}N$: N, 5.0. Found: 4.78.

An analysis for sulfur showed that only a trace was present. This is probably due to occluded sulfuric acid rather than to the presence of a true salt.

Therefore, this substance shows the same equilibrium as does phenyl-α-methylindolidene-methane, but it is much more unstable and cannot form salts.

For preparing the α-phenylindole products with substituted benzaldehydes the same procedure was used as with α-methylindole. In most cases, however, crystallization did not take place readily or even at all, and the materials required precipitation with some other reagent.

Recrystallization was not successful because of the instability in solvents and the instability of the salts.

p-Hydroxyphenyl-α-phenylindolidene-methane.—Separate solutions of 0.32 g. of α-phenylindole and 0.20 g. of *p*-hydroxy-benzaldehyde in the minimum quantity of absolute alcohol saturated with hydrogen chloride were mixed and the mixture was stirred and cooled but no crystallization took place. However, a deep orange-color was developed, indicating the presence of a condensation product in the solution. Three drops of water were added. Crystallization began slowly and after an hour the clusters of long, red prisms were filtered off, washed with 90% alcohol saturated with hydrogen chloride and dried at 100°. These contracted to a black substance at about 170° and then showed no true melting point.

Analysis. Calc. for $C_{21}H_{15}ON.HCl$: N, 4.2. Found: 4.3.

The filtrate and washings were treated with water until complete precipitation had taken place. The precipitate was a red powder which contracted at 170° and melted to a black mass at 196–198° (corr.).

Cinnamyl-α-phenylindolidene-methane.—To a solution of 0.32 g. of α-phenylindole in absolute alcohol saturated with hydrogen chloride was added directly 0.22 g. of cinnamic aldehyde. After the mixture had been stirred and cooled for a few minutes it became a solid mass of microscopic red crystals. These were filtered off and washed with 90% alcohol saturated with hydrogen chloride. Within a few minutes they be-

came very dark brown. When dried at 100° they soon changed to a brown powder, and the hydrochloric acid was lost. This powder melted at 146–148° (corr.).

Analysis. Calc. for $C_{23}H_{17}N$: N, 4.6. Found: 4.4.

o-Hydroxyphenyl-*α*-phenylindolidene-methane.—Since this could not be obtained crystalline from absolute alcohol, other solvents were tried. Dry hydrogen chloride was passed into absolute ether and benzene solutions of the reaction materials, but red polymerization products were obtained which were probably similar to those isolated by Keller[10] and by Oddo.[11] Butyl alcohol gives no better results than does ethyl alcohol.

The following method was, therefore, adopted. To 0.32 g. of *α*-phenylindole dissolved in the minimum quantity of warm absolute alcohol saturated with hydrogen chloride was added 0.20 g. of salicylaldehyde and the mixture was stirred. No precipitate formed, even after long standing. A few drops of concd. hydrochloric acid were added until precipitation began. After an hour the solution had solidified with the separation of minute needles. These were filtered off and washed with concd. hydrochloric acid. They were then dissolved in absolute alcohol saturated with hydrogen chloride and reprecipitated. They were dried on a suction filter and then further dried at 100–105° for a short time. They contracted at 165° and then gradually changed to a black, hard material without any melting point.

Analysis. Calc. for $C_{21}H_{15}ON.HCl$: N, 4.2. Found: 4.18.

These crystals are the true hydrochloride but they are unstable, and on long heating at 100° they disintegrate to a powder.

o-Chlorophenyl-*α*-phenylindolidene-methane.—To 0.32 g. of *α*-phenylindole dissolved in absolute alcohol saturated with hydrogen chloride was added 0.23 g. of *o*-chlorobenzaldehyde while the mixture was cooled and stirred. The deep orange color was produced but crystallization could not be started. An orange-colored powder was precipitated by the addition of a little water, washed with 66% alcohol saturated with hydrogen chloride and dried. It could not be recrystallized from alcohol saturated with hydrogen chloride or from glacial acetic acid. Since, like all the other condensation products of *α*-phenylindole, it readily decomposes in ordinary solvents, crystallization was not possible. The substance melts at 212–214° (corr.).

Analysis. Calc. for $C_{21}H_{14}NCl$: N, 4.48. Found: 4.6.

Anisyl-*α*-phenylindolidene-methane.—To 0.32 g. of *α*-phenylindole dissolved in absolute alcohol saturated with hydrogen chloride was added 0.23 g. of anisaldehyde while the mixture was cooled and stirred. No crystals could be obtained from the orange-colored solution. Water was added until precipitation began; the red powder thus formed was washed with 50% alcohol saturated with hydrogen chloride, and dried at 100°. No definite melting point was obtained.

Analysis. Calc. for $C_{22}H_{17}ON$: N, 4.50. Found: 4.9.

p-Dimethyl-aminophenyl-*α*-phenylindolidene-methane.—A brilliant purple solution of 0.32 g. of *α*-phenylindole and 0.25 g. of *p*-dimethyl-aminobenzaldehyde in the minimum quantity of concd. sulfuric acid was precipitated with water very slowly, care being taken to avoid an undue rise in temperature. A deep purple precipitate was formed which did not dissolve in an excess of water. This was filtered off and dried, triturated with ammonium hydroxide and washed in the centrifuge until free from sulfates. The purple powder, dried at 100°, melted at 155° (corr.).

Analysis. Calc. for $C_{23}H_{20}N_2$: N, 8.64. Found: 8.90.

o-Tolyl-*α*-phenylindolidene-methane.—To 0.32 g. of *α*-phenylindole dissolved in

[10] Keller, *Ber.*, **46**, 726 (1913).

[11] Oddo, *Gazz. chim. ital.*, **43**, I, 385 (1913).

warm absolute alcohol saturated with hydrogen chloride was added 0.20 g. of o-tolyl-aldehyde. It was impossible to obtain crystals under any conditions used. The brown-yellow powder was precipitated with a little water, but it was not obtained entirely pure. The analysis was made after a second fractional precipitation.

Analysis. Calc. for $C_{22}H_{17}N$: N, 4.75. Found: 5.10.

m-Nitrophenyl-α-phenylindolidene-methane.—Separate solutions of 0.32 g. of α-phenylindole and 0.25 g. of *m*-nitrobenzaldehyde were mixed. No crystals could be obtained, so the yellow powder was precipitated with a few drops of water. It seemed very unstable and had to be dried at a low temperature. No good melting point could be obtained as the material was probably not perfectly purified. The analysis indicated a lack of purity.

Analysis. Calc. for $C_{21}H_{14}O_2N_2$: N, 8.58. Found: 8.1.

2,4,6-Trinitrophenyl-α-phenylindolidene-methane.—Separate solutions of 0.32 g. of α-phenylindole and 0.40 g. of 2,4,6-trinitro-benzaldehyde in absolute alcohol saturated with hydrogen chloride were mixed and the mixture was quickly precipitated with a little water to prevent the aldehyde crystallizing as an isomeric form. An orange-colored powder was obtained which was unstable on standing and rapidly decomposed at 100°. It was impossible to obtain the material dry in a good condition. The analysis indicates a loss of water, possibly with a corresponding increase in nitrogen.

Analysis. Calc. for $C_{21}H_{12}O_6N_4$: N, 13.45. Found: 14.9.

Condensations

With Alpha-Beta-Diphenylindole

In Absolute Alcohol.—α,β-Diphenylindole was dissolved in the minimum quantity of absolute alcohol saturated with hydrogen chloride and one molecular equivalent of benzaldehyde was added. There was no change in color or any other indication of re-action. But on long standing the solution became slightly brown by transmitted light and brilliant green by reflected light. Two drops of water were added and a heavy white precipitate immediately formed. This was filtered off, washed with cold, absolute alcohol saturated with hydrogen chloride and with ligroin, and dried. The white powder showed no true melting point but softened at 170°. When this was redissolved in ben-zene and precipitated with ligroin a gray powder was obtained which melted at 123° (corr.). The fact that the substance is colorless and is strongly fluorescent in solution shows that it is not one of the usual condensation products, but is of an entirely differ-ent nature. It was not further investigated.

In Concentrated Sulfuric Acid.—The same green solution was obtained but no product was isolated.

In Concentrated Hydrochloric Acid.—When each reagent was dissolved in concd. hydrochloric acid and the solutions were mixed together neither color nor precipitate was obtained. This indicates the lack of any condensation reaction analogous to those given by mono substituted indoles.

With α-Phenyl-β-Methylindole

In Absolute Alcohol.—α-Phenyl-β-methylindole was dissolved in absolute alcohol saturated with hydrogen chloride, and one molecular equivalent of benzaldehyde was added. A red color was immediately developed, indicating a condensation of the or-dinary type. Cooling and stirring did not cause precipitation, but a solid brown mass was obtained by the addition of one drop of water. After being filtered, washed and dried, the brown powder melted at 190° (corr.). It was readily soluble in ether and al-cohol giving a brown solution which showed a strong green fluorescence. When the

substance was dissolved in benzene and the solution poured into ligroin a gray precipitate was formed which melted at 221–223° (corr.), was difficultly soluble in alcohol, and showed the usual green fluorescence in benzene. The solution was not affected by the addition of acid or alkali.

In **Concentrated Hydrochloric Acid.**—No condensation took place in concd. hydrochloric acid. Both attempts at condensation indicated that an ordinary 1:1 condensation product cannot be formed. The compounds which were formed were not analyzed because they could not be obtained in crystalline form, and no criterion of purity could be established.

With Tryptophan

In **Absolute Alcohol.**—To 0.60 g. of tryptophan dissolved in absolute alcohol saturated with hydrogen chloride was added 0.36 g. of salicylaldehyde. Stirring and cooling did not cause precipitation. The solution was evaporated on the steam-bath and the brown, amorphous powder was soluble in dil. hydrochloric acid and alcohol, giving a red solution. It was purified by solution in alcohol and precipitation with several volumes of ether. The gray powder was filtered off, washed with ether and dried at 100°. The yield was 0.80 g. or 80% of that calculated for a hydrochloride of the 1:1 condensation product. The powder was not soluble in ether or alcohol after trituration with ammonium hydroxide.

Nitrous acid splits off the amino nitrogen in acetic acid solution and gives an orange-colored precipitate.

The ether-purified product was changed to an insoluble material without melting.

Analysis. Found: C, 61.19; H, 5.60; O, 18.3; N (total), 6.9; N (amino), 3.17; Cl, 8.03.

This corresponds to the formula $C_{12}H_{24}O_5N_2Cl$. The product has no acid properties and it is probably esterified. It gives the Voisenet[12] reaction for tryptophan. It probably is not the simplest condensation product but is changed by some secondary reaction, as tryptophan is very sensitive to reagents. The constitution was not further studied.

Three-tenths g. of tryptophan was condensed with an excess of salicylaldehyde in absolute alcohol as described above. The resulting product is almost insoluble in alcohol, acetic acid, hydrochloric acid, and dil. sodium hydroxide solution. It has the appearance of a typical humin and is not readily purified.

When condensed in concd. sulfuric acid the same kind of orange-colored product was obtained; but this was not purified.

Humins

Preparation.—The preparation of the indole humins was carried out either directly with the aldehyde and the indole or, with the same results, by boiling the intermediate 1:1 condensation product with an excess of the aldehyde in 20% hydrochloric acid. The intermediate product always precipitated from the hydrochloric acid before the humin formation began. Then, apparently, the intermediate dissolved in the excess of aldehyde and as the temperature rose the solution became black and the humin separated. There seemed to be no change after the first few hours of boiling, but the time of boiling was extended to allow for complete change.

[12] Voisenet, *Bull. soc. chim.*, **33**, 1198 (1905).

The following general procedure was used in all the preparations. The indole derivative was dissolved in 25 to 40 parts of concd. hydrochloric acid by warming and 2.5 molecular equivalents of the aldehyde were added directly if a liquid, or first dissolved in 15 to 25 parts of concd. hydrochloric acid if a solid. The solution was then diluted to 20% concentration of hydrochloric acid when the orange-colored intermediate always precipitated. After the mixture had boiled for 18 hours it was filtered hot, the humin was crushed and washed with hot 20% hydrochloric acid, then with water, dried and pulverized.

Table I lists the humins made in this series together with certain of their physical properties. They will hereafter be referred to by number.

In practically every case the yield was almost quantitative for one molecule of the indole and two molecules of the aldehyde. The filtrate was always red and as the solution cooled a small amount of red amorphous material was precipitated.

The lowest yields were obtained from 2,4,6-trinitro-benzaldehyde and a large amount of red, amorphous material was left in the mother liquor.

The humins made from p-dimethyl-aminobenzaldehyde were especially different in their solubilities. No. 18, from α-methylindole, was readily soluble in dil. acid, while No. 17, from α-phenylindole was not soluble in dil. acid to an appreciable extent.

No. 21 was a gray powder which was readily soluble in all organic solvents and showed none of the properties of the other humins. However, No. 22 shows properties much like the rest of the humins.

No humin could be prepared from α,β-diphenylindole.

Purification.—From Table I five humins were selected on the basis of their solubilities for purification. These were Nos. 2, 12, 14, 18 and 20. Each has such solubility that a different method is necessary for the purification.

No. 2. α-Methylindole and p-Hydroxybenzaldehyde.—The humin was dissolved in 0.5% sodium hydroxide solution, forming a deep red solution which left no appreciable residue when filtered. It was then precipitated with a considerable excess of hydrochloric acid. The precipitate was too flocculent to be centrifuged but it filtered clear on a hardened filter paper. It was not peptized by washing with water and was washed free from chloride ion. This treatment was repeated. The amorphous powder, dried at 100°, is slightly soluble in water yielding a pink solution.

No. 12. α-Methylindole and o-Tolylaldehyde.—The crude humin was triturated with ammonium hydroxide and dissolved in ether. The ether solution was thoroughly washed with water, dried and evaporated. The amorphous, yellow powder was redissolved in ether, and the solution was poured into ligroin. The amorphous, gray-yellow powder was washed with ligroin and dried at 100°. It was just as satisfactory to pour the ether solution into methyl alcohol, in which the humin is almost insoluble. The powder began to contract at 185° and continued slowly until it completely melted without decomposition at 255° (corr.).

No. 14. α-Methylindole and Anisaldehyde.—Two g. of the humin was dissolved

TABLE I

SOLUBILITIES OF HUMINS

No.	Source of humin	Solubility of the hydrochloride									Solubility of the base in ether	Fusibility
		Acid	Alkali	Alcohol	Ether	Acetone	Benzene	Phenol	Aniline	Chloro-acetic		
1	α-Phenylindole and p-hydroxybenzaldehyde....	i	s	s	i	s.s	i	v.s	s	s	i	High temp.
2	α-Methylindole and p-hydroxybenzaldehyde...	i	s	s	i	s.s	i	v.s	s	s	i	+
3	α-Phenylindole and salicylaldehyde...........	i	s	s	i	s.s	i	s	s	s	i	High temp.
4	α-Methylindole and salicylaldehyde...........	i	s	s	i	s.s	i	s	s	s	part sol.	+
5	α-Phenylindole and o-chlorobenzaldehyde......	i	i	i	i	i	i	s	s.s	s.s	i	+
6	α-Methylindole and o-chlorobenzaldehyde.....	i	i	i	i	i	i	s	s	s	s	+
7	α-Phenylindole and cinnamaldehyde...........	i	i	i	i	s.s	i	s	s	s	i	+
8	α-Methylindole and cinnamaldehyde..........	i	i	i	i	s.s	i	v.s	s	s	part sol.	+
9	α-Phenylindole and 2,4,6-trinitro-benzaldehyde	i	s.s	i	i	s	i	s	s	s	i	Fusible decomp.
10	α-Methylindole and 2,4,6-trinitro-benzaldehyde	i	sol. in s.s NH₃	i	i	s	i	s	s	s	i	Fusible decomp.
11	α-Phenylindole and o-tolylaldehyde...........	i	i	i	s.s	i	s.s	s.s	s.s	s.s	part sol.	+
12	α-Methylindole and o-tolylaldehyde...........	s.s in acetic	i	i	s.s	s	s.s	v.s	s	v.s	v.s	+
13	α-Phenylindole and anisaldehyde.............	i	i	s.s	i	s.s	i	v.s	v.s	v.s	i	+
14	α-Methylindole and anisaldehyde.............	i	i	s.s	i	s.s	i	v.s	v.s	v.s	part sol.	+
15	α-Phenylindole and m-nitrobenzaldehyde......	i	i	i	i	s.s	i	s	s	s	i	+
16	α-Methylindole and m-nitrobenzaldehyde......	i	i	i	i	s.s	i	s	s	s	s.s	decomp.
17	α-Phenylindole and p-dimethyl-aminobenzaldehyde.........	s.s	i	i	i	s.s	i	s	v.s	s	i	infus.
18	α-Methylindole and p-dimethyl-aminobenzaldehyde.........	v.s	i	i	i	s.s	i	s	v.s	s	i	infus.
19	α-Phenylindole and benzaldehyde.............	i	i	i	i	i	i	s	s	s	i	infus.
20	α-Methylindole and benzaldehyde.............	i	i	i	i	i	i	s	s	s	i	infus.
21	α-Phenyl-β-methylindole and benzaldehyde....	s.s	i	s.s	s	·s	s	v.s	v.s	v.s	s	+
22	α-Ethyl-β-methylindole and benzaldehyde.....	i	i	s.s	i	s	s.s	s	s	s	s	+
23	α-Ethyl-β-methylindole and formaldehyde.....	i	i	i	i	i	i	i	i	i	i	infus.

The solubility symbols have the following meanings: i = insoluble; s = soluble; s.s = slightly soluble; v.s = very soluble. "+" means readily fusible. "Fusible" means that the product melts to a liquid but none of the samples melt below 200° and some of them melt at a much higher temperature. These are not sharp melting points.

in 60 cc. of 95% alcohol and the solution was filtered. There was considerable residue. The filtrate was poured into 2 volumes of water but no precipitate formed. A small amount of sodium chloride was added and precipitation began slowly. After 24 hours the precipitate was filtered, dried, ground to a powder and extracted with water until free from chloride ion.

No. 18. α-Methylindole and p-Dimethyl-aminobenzaldehyde.—The powder, which had already been precipitated from acid solution by sodium hydroxide, was re-dissolved in very dilute hydrochloric acid, the solution filtered and precipitated with a slight excess of sodium hydroxide. The precipitate was washed nearly free from chloride ion in the centrifuge. The last traces of chloride ion could not be removed by centrifug-ing because the precipitate began to peptize. The precipitate was then dried, ground to a powder and extracted with water for 24 hours. It then gave no test for chloride ion.

No. 20. α-Methylindole and Benzaldehyde.—Two g. of the humin which had already been extracted with alcohol and ether was dissolved in a small amount of phenol and the solution poured into several volumes of glacial acetic acid. No precipitate formed. The solution was diluted with five volumes of water without precipitation. On the addition of 5 cc. of concd. hydrochloric acid the entire solute was immediately precipitated in large flocks. This was filtered on hardened filter paper, thoroughly washed, dried, ground to a powder and extracted with alcohol until no coloring material was dissolved by the alcohol. This residue was then dried at 100°.

The humins numbered from 24 to 40 inclusive were obtained from various sources in this Laboratory. Each was prepared and purified in somewhat different fashion from the others. Although it would require a description of these processes for the work to be repeated exactly, space will not per-mit these details and it must be stated that generally they were purified by extraction with several organic solvents or were dissolved and reprecip-itated when possible.

TABLE II

SOLUBILITIES IN TWO GROUPS OF SOLVENTS

No.	Source of Humin	Solubility in pyridine, aniline, phenol	Solubility in chloro-acetic acid
24	Phenyl-α-phenylindolidene-methane and benzaldehyde	s	s
25	α-Phenylindole and excess of benzaldehyde	s	s
26	α-Phenylindole and 2 equiv. of formaldehyde	s	s
27	α-Phenylindole and excess of formaldehyde	s	s
28	α-Phenylindole and large excess of formaldehyde	s	s
35	Fibrin (no added aldehyde)	i	s
36	Fibrin (no added aldehyde)	i	s
29	Tryptophan and 2 equiv. of formaldehyde	i	i
30	Tryptophan and excess of benzaldehyde	i	i
31	α-Methylindole and 2 equiv. of formaldehyde	i	i
32	α-Methylindole and large excess of formaldehyde	i	i
33	Tyrosine and excess of benzaldehyde	i	i
34	Fibrin and formaldehyde	i	i

Table II gives an arrangement of these varied products into three classes according to their solubility in two groups of solvents. Phenol, pyridine

and aniline constitute one group of solvents, since solubility in one means solubility in the others.

When dissolved in chloro-acetic acid, all of the solutes are reprecipitated on dilution with water except the fibrin humins.   These require the addition of a little hydrochloric acid to cause coagulation.

Analyses.—In order to determine whether any constancy existed in the analyses of these humins a series of determinations was made as shown in Table III.   The table is divided into four groups of humins, according to source.

TABLE III

CONSTANCY OF ANALYSES

No.	Source of humin	C %	H %	N %
40	α-Methylindole and 1 equiv. of formaldehyde	75.88	5.52	8.09
31	α-Methylindole and 2 equiv. of formaldehyde	73.58	5.81	7.62
32	α-Methylindole and large excess of formaldehyde	71.78	5.46	5.33
26	α-Phenylindole and 2 equiv. of formaldehyde	86.08	5.52	6.08
27	α-Phenylindole and excess of formaldehyde	85.76	5.58	4.65
28	α-Phenylindole and large excess of formaldehyde	85.54	5.87	5.68
25	α-Phenylindole and excess of benzaldehyde	89.42	5.04	3.81
29	Tryptophan and formaldehyde	63.89	4.68	11.31
30	Tryptophan and excess of benzaldehyde	71.55	7.03	6.92
				2.21 (amino N)

Nitrogen analyses were made on the protein (fibrin) humins to determine whether any constancy in analysis could be obtained.   The acid-insoluble humin from No. 36 contained 9.84, 9.78% of nitrogen; that from No. 38 contained 7.30%; the acid-soluble humin from No. 37 contained 8.0%, and that from No. 39, 10.0%.

It is evident that humins of a constant analysis can be obtained from a given hydrolysis, but different treatments, as in the case of the fibrin humins, give very different results, both in the soluble and the insoluble humins.

From Table III it is evident that α-phenylindole and benzaldehyde have given the only oxygen-free humin (No. 25), the percentages calculated for $C_{28}H_{19}N$ being C, 91.06; H, 5.15; N, 3.79; as compared with 89.42%, 5.04% and 3.81%, respectively, found.

However, the α-phenylindole-formaldehyde humins contain only 2.5 to 3.0% of oxygen, which is only one oxygen atom for every two nitrogen atoms.

From an examination of Table I it will be observed that the base of Humin 12 (α-methylindole and o-tolylaldehyde) shows the best solubility in ether and this makes purification of this humin most possible.   After purification by precipitation in ligroin a complete analysis was made in duplicate with the following average results.

Calc. for $C_{25}H_{21}N$: C, 89.55; H, 6.27; N, 4.18.   Found: C, 88.90; H, 6.32; N, 3.90.

Since this proved to be the true theoretical value for 1 indole + 2 aldehyde − 2H$_2$O, only the nitrogen was determined for the other four well-purified humins.

Humin No.	2	14	18	20
Formula............	C$_{23}$H$_{17}$O$_2$N	C$_{25}$H$_{21}$O$_2$N	C$_{27}$H$_{27}$N$_3$	C$_{23}$H$_{17}$N
N, % calc............	4.20	3.81	10.70	4.56
Found........	4.40	3.75	10.00	4.60

Each analysis conforms closely to the theory that two molecules of any of the aromatic aldehydes used condense with one molecule of α-methylindole to form a molecule containing no oxygen.

Molecular Weights.—Advantage was taken of the fact that the protein humins were soluble in monochloro-acetic acid and the molecular weights were determined in this solvent. Mameli[12a] determined the cryoscopic constant for this acid and his figures show it to be a rather satisfactory solvent for molecular weight work. The following constants are recorded in the literature: m. p., 61.2°; K, 5210; heat of fusion, 42.55 cal.

Preparation of the Solvent.—Two hundred and fifty cc. of Baker's c. p. monochloro-acetic acid was distilled. The first 30 cc. was discarded when the thermometer had become constant at 187.0°. The next 200 cc. distilled within 0.5° range of temperature. All moisture was excluded. The freezing point was 61.9°. Table IV gives a record of the results with fibrin humins.

TABLE IV

MOLECULAR WEIGHTS OF FIBRIN HUMINS

Humin	Sample G.	Solvent G.	F. p. of solvent °C.	F. p. of solution °C.	Δ °C.	Mol. wt.
36	0.1053	14.7	5.727	5.642	0.085	439
	.2144	..	...	5.554	.173	439.5
38	.2103	14.0	5.530	5.430	.100	780
	.3362	..	...	5.361	.169	740

The two samples used contained a very small amount of ash and the values found probably represent very closely the true value in this solvent. The molecular weight of No. 38 is almost double that of No. 36.

Molecular weights of the indole humins were then determined. Since these substances are soluble in pyridine, aniline and phenol, any of these solvents might be used. It was impossible to obtain pyridine of sufficiently constant boiling point, probably due to the presence of methyl pyridine, so its use was abandoned. Aniline was reported by Ampola and Rimatori[13] as a satisfactory solvent for cryoscopic work. However, the values of K, as reported, are very different at different concentrations of the solute; m. p., −5.96°; K, 5870.

[12a] E. Mameli, *Gazz. chim. ital.*, [2] **39**, 579 (1909).

[13] Ampola and Rimatori, *Gazz. chim. ital.*, [1] **27**, 35 (1897).

Preparation of the Solvent.—Two hundred and fifty cc. of Merck's Blue Label aniline was fractionated. The first 30 cc. was discarded when the thermometer had become constant at 180.75° and 732 mm.; 200 cc. was then distilled with a maximum fluctuation of 0.1°; freezing point, —6.0°. A smaller fraction was then distilled which showed a boiling-point fluctuation of ±0.01°.

Ebullioscopic Method.—The apparatus of McCoy[14] was tried but the readings were rather variable and gave molecular weights varying between 25 and 40. These were obviously abnormal, so the method was abandoned.

Cryoscopic Method.—Table V gives the results as obtained from two different humins.

<div align="center">

TABLE V

MOLECULAR WEIGHTS OF TWO HUMINS

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Humin	Sample G.	Solvent G.	F. p. of solvent °C.	F. p. of solution °C.	Δ °C.	Mol. wt.
24	0.0495	15.0	1.550	0.597	0.953	20
24	.0500	20.0	1.530	.505	1.025	14
26	.0714	15.0	3.324	2.792	0.532	40

These results must be entirely erroneous, since the minimum theoretical weight must be about 300. Fig. 1 was constructed from the values given
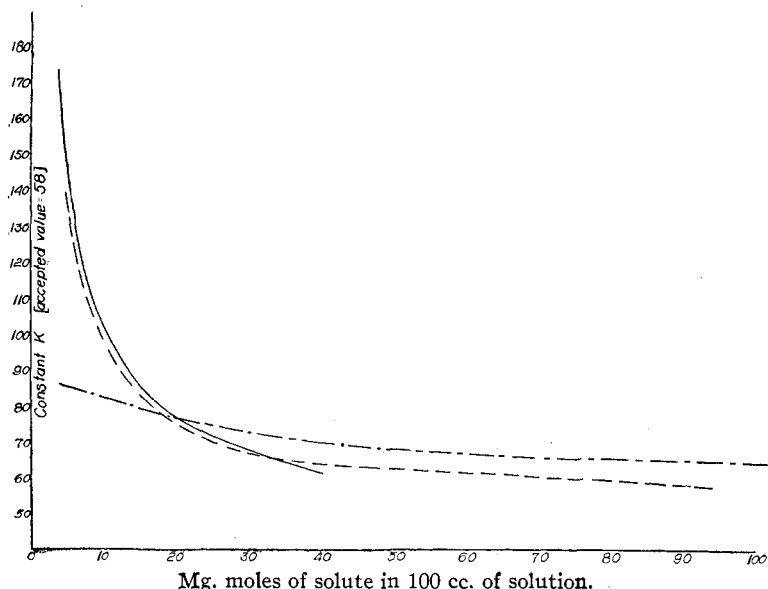

Mg. moles of solute in 100 cc. of solution.
Fig. 1.—The variation of K for aniline with varying concentration of solute.
Caprylic alcohol, ———. Dimethylaniline, — — —. Quinoline, — - —.

by Ampola and Rimatori. It is at once evident why such low values were obtained with these humins. When the concentration of the solute is less than 5 mg.-moles per 100 cc. of the solvent, the constant K rises very rapidly for some substances. In this work only 1 to 2 mg.-moles of the

[14] McCoy, Am. Chem. J., 23, 353 (1900).

humin was used in 100 cc. of the solvent, assuming the minimum molecular weight of 369. In fact, later determinations show that the molecular weight is much higher than 369. Consequently, aniline cannot be satisfactorily used as a solvent in this work and it was abandoned.

Eykmann[15] used phenol as a solvent for molecular-weight determinations and obtained very satisfactory results. Indeed, he recommended it as one of the best solvents for cryoscopic work. The following constants are reported in the literature: m. p., 42.5–43.0°; K, 7200; sp. ht., 0.561; heat of fusion, 24.93 cal.

Preparation of the Solvent.—Five hundred cc. of crystalline u. s. p. phenol was distilled. The first 100 cc. was discarded and the next 250 cc. distilled within a range of ±0.025°; m. p., 42.6°. Table VI gives the results of a series of determinations.

TABLE VI

MOLECULAR WEIGHTS IN PHENOL

Humin	Sample G.	Solvent G.	F. p. of solvent °C.	F. p. of solution °C.	Δ °C.	Mol. wt.
24	0.1023	20.0	5.328	5.283	0.045	818
26	.0850	20.0	5.341	5.324	.017	1880
	.1760	..	...	5.300	.041	1545
	.1810	20.1	5.447	5.408	.039	1629
14	.1650	20.0	5.441	5.346	.095	625
18	.2393	20.1	5.452	5.349	.103	833
	.4162	..	...	5.252	.200	796
12	.1044	10.05	4.778	4.688	.090	840
	.2118	..	...	4.596	.182	838

Because of the high molecular weight and the rather low solubility it is not advisable to try to obtain greater depressions. Since under these conditions a variation of 0.001° makes a difference of 30 in the molecular weight found, these figures represent the molecular weight with an error of less than 10%. It is evident that the humin molecule is not the simplest that can be formed from the condensation of two molecules of the aldehyde with one molecule of the indole but it must be considered as a product of polymerization or further condensation of two or more of these simplest molecules. If the molecular weight is calculated on the basis of the condensation of two molecules of the indole with four molecules of the aldehyde and the elimination of four molecules of water, the following comparison of figures can be made.

Humin No.	24	14	18	12
Calc.	734	734	786	670
Found	818	625	814	839

Nos. 14 and 18 show no sign of association in phenol, while Nos. 24 and 12 appear to be considerably associated. No. 26, with an average molec-

[15] Eykmann, Z. physik. Chem., 4, 496 (1889).

ular weight of 1600, is evidently a different type of compound, due to the fact that formaldehyde was used instead of an aromatic aldehyde. The formaldehyde humins contain oxygen, showing that they are actually different chemically.

It was decided to take the molecular weight of No. 12 in benzene as a check on the value found in phenol. The following constants for benzene are reported in the literature: K,[16] 5000; f. p., 5.3°; sp. ht., 0.423; heat of fusion, 30.6 cal.

Preparation of the Solvent.—Merck's highest purity, crystallizable benzene "for molecular-weight determinations" was used without further purification. Humin No. 12 and 10 g. of solvent, f. p. 6.043°, were used.

Sample G.	F. p. of solution °C.	Δ °C.	Mol. wt.
0.1109	5.993	0.050	1132
.2136	5.953	.090	1200

Therefore, No. 12 has a tendency to associate further and, in benzene, shows a molecular weight nearly four times the minimum calculated value.

The Constitution of the Humins.—The changes of α-methylindole and some of the condensation products when subjected to various treatments were studied.

α-Methylindole in 20% Hydrochloric Acid.—A solution of 1 g. of α-methylindole in 35 cc. of 20% hydrochloric acid was refluxed in an atmosphere of carbon dioxide for five hours. The solution was made slightly alkaline with sodium hydroxide, and steam-distilled; 0.3 g. of pure α-methylindole was recovered in 1 liter of distillate. Only a trace of brown, amorphous residue was formed and no other products were detected. It is probable that no changes take place in the indole nucleus during humin formation in 20% hydrochloric acid.

Changes in Phenyl-α-methylindolidene-methane Acid Sulfate.—When this was heated to 140–150° for a few hours a loss of one molecular equivalent of water took place and it is evident that the new compound is sulfonated. It forms an ammonium salt readily.

When phenyl α-methylindolidene-methane acid sulfate is boiled with 30% sulfuric acid or with 20% hydrochloric acid for 24 to 48 hours, or when exposed to the light of a tungsten lamp for 24 hours in an alcohol or acetic acid solution, fundamental changes take place and amorphous, red-to-violet products are obtained. Solubilities, melting points and other properties are greatly altered while the analysis is about constant. It is significant that some of these products condense with benzaldehyde to form humins more readily than does the phenyl-α-methylindolidene-methane.

It is of further significance that the phenyl-α-methylindolidene-methane

[16] Raoult, *Ann. chim. phys.*, [6] **2**, 66 (1884).

remains unchanged for days in cold concd. sulfuric acid or in benzaldehyde with concd. sulfuric acid.

These, together with other observations are interpreted as indicating that humins are formed from these intermediates only after a rearrangement has taken place.

The Chemical Reactions of the Humins

Resistance of the Humins to Oxidation, Reduction, Alkali Fusion and Hydrolysis.—Three different humins (Nos. 20, 10 and 12) were thoroughly studied. They are entirely stable toward alkaline permanganate and are completely broken down by refluxing with chromic oxide in glacial acetic acid, the only product isolated being benzoic acid. They are entirely stable to fusion with potassium hydroxide at 240–250° and are unchanged by heating with water at 225° for four hours. However, with dil. nitric acid an oxidized product was obtained.

One g. of crude humin (No. 12) was partially dissolved in 35 cc. of concd. nitric acid and the mixture was diluted with 53 cc. of water. After three days of refluxing, there was considerable deposit of red material and the acid was red with dissolved material. The solution was diluted and set aside for a few hours. On filtration, a red residue and a pale yellow filtrate were obtained. The residue was washed free from acid, dried, suspended in water and treated with a slight excess of sodium hydroxide. It was completely and immediately dissolved to a red solution which was filtered and the acid material was reprecipitated with a slight excess of nitric acid. After thorough washing it was dried at a low temperature; yield, 0.5 g. It appears to be rather unstable in alkaline solution when warmed. A second preparation, refluxed for two days, yielded exactly 0.5 g.

This material is soluble in alkali, ammonium hydroxide and acetone, sparingly soluble in glacial acetic acid, alcohol and ether, and insoluble in benzene.

The filtrate was concentrated in a vacuum to dryness and the residue was extracted with ether. A considerable yield of yellow oil was obtained which became very viscous when cold, and when scraped with a rod it liberated nitrogen oxides. It could not be made to solidify, even at low temperatures and on long standing.

The alkali-soluble material is reduced to a colorless material with zinc dust and ammonium hydroxide on the water-bath. It is not precipitated from alkaline solution by carbon dioxide, yet it dissolves very slowly in sodium bicarbonate. It does not give Liebermann's nitroso reaction or the test for a nitro group (this test applies also to nitroso, azo and azoxy groups). It does give a slight test for an oxime with Tollen's reagent. There was no reaction with phenylhydrazine, and no color was produced with ferric chloride.

The silver and barium salts are non-crystalline. The following analyses were made.

Found: Ag, 23.7; Ba, 11.2.

The result with the silver salt requires a monobasic acid with a molecular weight of 367, while that with the barium salt requires a dibasic acid of molecular weight 1265 or nearly four times that of the acid required by silver instead of two times, as would be expected. Both of these salts decompose violently when ignited.

The material does not titrate well as an acid. The purified acid material contained by analyses C, 57.2%; H, 4.2%; O, 32.3%; N, 6.3%. This requires an empirical formula of $C_{21}H_{19}N_2O_9$, as contrasted with $C_{25}H_{21}N$, for the original humin. Obviously, a simple oxidation of the methyl group does not take place.

Bromination.—A chloroform solution of the pure base of Humin 12 was treated with a slight excess of bromine in chloroform. The color of the solution changed immediately to black but no precipitate formed. The solution was evaporated, the residue dried, powdered, washed with acetic acid, and dried at 100° for several hours; it was a black, amorphous material.

   Analysis. Calc. for $C_{25}H_{21}N.Br_2$: Br, 33.7. Found: 33.4.

Therefore there is one active double bond for each indole nucleus in the humin, and this is probably the bond between the nitrogen and the α carbon. This compound, treated with sodium in alcohol, caused the bromine to be split off as sodium bromide and regenerated a bromine-free compound which was indistinguishable from the free base.

Treatment with Nitrous Acid.—In view of the work of Plancher and Bettinelli[17] and of Scholl[18] it seemed possible that nitrous acid might act on the α-methyl group if still free in the humin molecule. Several procedures were tried without success. This behavior is quite different from that of the intermediate, o-tolyl-α-methylindolidene-methane, which readily forms an alkali, soluble material which is reprecipitated from alkaline solution by carbon dioxide.

Basicity of Humins.—In the presence of acids, No. 12 (as well as all humins) is insoluble in ether and benzene but on trituration with ammonium hydroxide solution it becomes very soluble in both solvents. This shows definitely a salt formation with strong acids, and the freedom from a basic nitrogen group.

An attempt was made to form the picrates in ether and benzene solutions. In each case the solution became dark but no definite salt was isolated. When, however, an ether solution is treated with a little dry hydrogen chloride in ether an immediate precipitate, undoubtedly an unstable salt, is formed.

Summary and Conclusions

Five new condensation products have been prepared from α-methyl-indole and aromatic aldehydes. These are of the indolidene-methane

[17] Plancher and Bettinelli, *Gazz. chim. ital.,* [1] **29,** 106 (1899).

[18] Scholl, *Ber.,* **23,** 3490 (1890).

type.   An entirely new series of compounds of the indolidene-methane type has been prepared from α-phenylindole.   Only one condensation product of α-phenylindole had been previously described and that was of the rosindole type.   All of these have been used as intermediates in the preparation of humins.

It has been shown that some indole derivatives and aldehydes condense in acid media to form humin materials which are very similar in properties to the humins obtained from the acid hydrolysis of proteins.   Those indole derivatives which most readily form such humins are α-methylindole, α-phenylindole, α-ethyl-β-methylindole and tryptophan, while α-phenyl-β-methylindole and α,β-diphenylindole do not form humins with aromatic aldehydes.   Substitution in the aromatic aldehydes causes small differences in the solubility, fusibility and yield of the humins, but the general nature of the humin is the same throughout.   Each humin from α-methylindole and an aromatic aldehyde is always more soluble than the corresponding humin from α-phenylindole.   Further differences in the solubility of the humins is summarized in Table II.   It is of interest that α-phenylindole is the only material tried which gives a soluble humin with formaldehyde.   Tryptophan does not give a soluble humin with either formaldehyde or benzaldehyde while the humins from protein hydrolysis are soluble in monochloro-acetic acid and dilute alkali (peptization?).

The humins from indoles plus aromatic aldehydes are in general decidedly basic, although they do not form picrates or platinum salts.   Boiling in 20% hydrochloric acid as a method of preparation probably excludes the possibility of condensation on the indole nitrogen and the ring nitrogen is probably in the imidene form.

Humins do not react with nitrous acid while phenyl-α-methylindolidene-methane does react giving what is probably a formoxime on the α carbon atom; so probably the methyl group is no longer free in the α-methylindole humins.

Forty humins have been prepared and described.   Analyses of a part of these bring out the following conclusions.

1.   The humins from protein hydrolysis cannot be prepared so that they are constant in composition.   This substantitates the conclusions of previous workers.

2.   All formaldehyde humins have some oxygen in the molecule, while the humins from α-methylindole and α-phenylindole with aromatic aldehydes contain no oxygen and the analyses correspond to the calculated value for one molecular equivalent of indole plus two of aldehyde minus two of water.

3.   Tryptophan forms, with both formaldehyde and benzaldehyde, humins which contain oxygen aside from that in the carboxyl group, and

the humin with benzaldehyde gives an analysis corresponding to one tryptophan group plus two of benzaldehyde without the splitting out of any water. This same humin contains by analysis 2.21% of amino nitrogen.

Molecular-weight determinations show that the humins from protein hydrolysis have molecular weights of the same order as the humins pre-
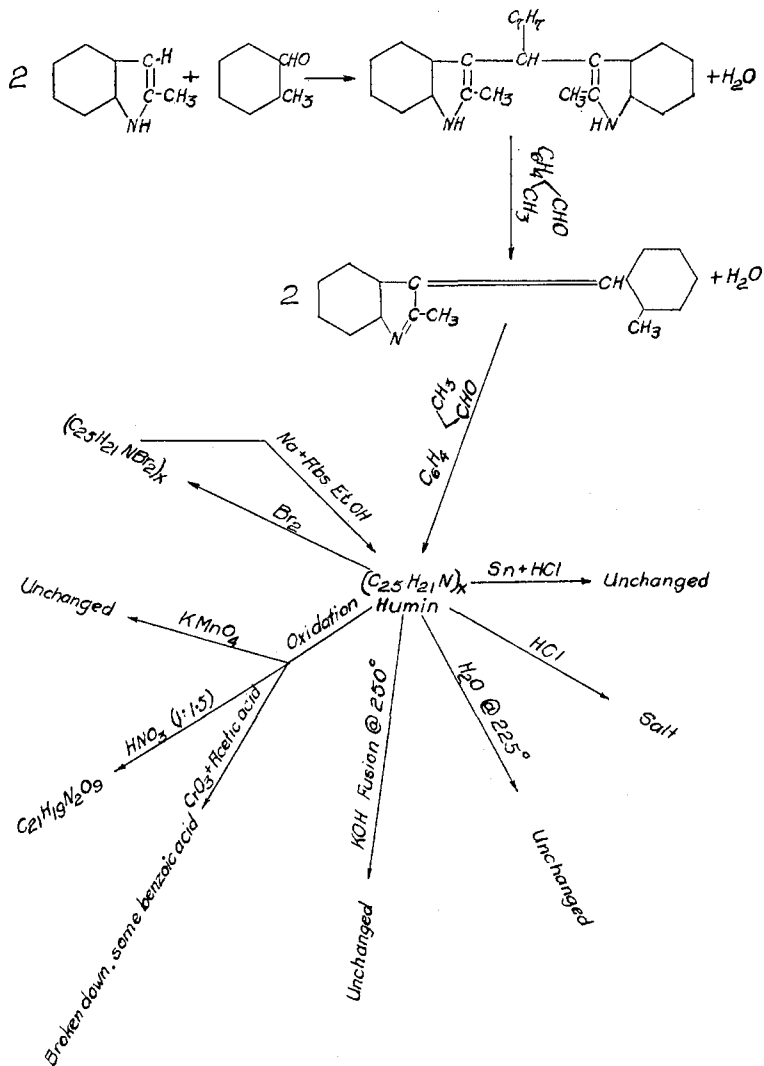

Fig. 2.

pared from α-methylindole and aromatic aldehydes, the molecular weights ranging between 500 and 1000. The molecular weight of the humins from α-phenylindole and formaldehyde is much higher, being about 1600.

The humin molecule is thus shown to be not the simplest compound that can be formed from one molecular equivalent of indole plus two of aldehyde, but a complex of two to four times that size. It is very possible that this is simply an association in the solvents phenol and benzene, since it is a common occurrence for the more basic indolidene derivatives to polymerize the solvents. On the other hand, the inactivity of the humins and their behavior toward solvents are evidence of high molecular weight. For the present the humin from α-methylindole and o-tolylaldehyde can be represented by the formula $(C_{25}H_{21}N)_X$, where X is not greater than 4.

The synthesis and chemical properties of the humin from α-methylindole and o-tolylaldehyde can be represented by Fig. 2.

In short, compounds of similar inactivity and complexity to the humin resulting from the acid hydrolysis of proteins can be prepared from substituted indoles and aromatic or aliphatic aldehydes. All of the accumulated data point to the conclusion that such artificial humins are not simple molecular condensations but that it is possible to isolate at least two intermediate products. These intermediate products have been purified and analyzed, and their structures have been ascertained. These products belong to the rosindole and indolidene methane groups. The final humin results by the condensation of one molecule of aldehyde with the indolidene methane derivative, but during this condensation some rearrangement apparently takes place, for the resulting humin is extremely resistant to drastic chemical treatment such as alkaline fusion and permanganate oxidation. The data so far available do not permit us to decide as to the structural formulas.

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THE DECOMPOSITION OF DIHYDROXYMALEIC ACID[1]

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Among the various theories which have been advanced to explain the photosynthesis of optically active sugars in nature, the one involving the condensation of glycolaldehyde deserves more careful scrutiny.[2] A prerequisite for such a study is more definite knowledge of the condensation of glycolaldehyde to tetroses and hexoses.[3] A symmetric orientation

[1] This paper embodies a portion of the work carried out at the suggestion of Dr. H. A. Spoehr while the author was a fellow of the Carnegie Institution of Washington at the Coastal Laboratory, Carmel-by-the-Sea, California.

[2] Reinke, *Ber.*, **14**, 2148 (1881). (b) Lippmann, *Ber.*, **24**, 3306 (1891). (c) Fincke, *Biochem. Z.*, **61**, 157 (1914). (d) Nef, *Ann.*, **376**, 7, 115 (1910).

[3] Fenton, (a) *J. Chem. Soc.*, **65**, 899 (1894); (b) **67**, 48, 774 (1895); (c) **69**, 546 (1896); (d) **71**, 375 (1897); (e) **73**, 71 (1898); (f) **81**, 426 (1902); (g) **87**, 817 (1905). (h) Fischer and Landsteiner, *Ber.*, **25**, 2549 (1892).