which separated on cooling was collected and recrystallized from water. It was obtained as flat needles; m.p. 181–182°. The yield was 7.2 g. (60%).

*Anal.* Calcd. for $C_{18}H_{18}N_{10}O_{14}$: C, 36.13; H, 3.03; N, 23.41. Found: C, 36.10; H, 3.31; N, 23.42.

The picrates obtained by procedures a, b and c showed identical absorption in the infrared.

**3-β-Ethoxyethyl-1,2,4-triazole-5-thiol.**—To a suspension of 100 g. (1.1 moles) of thiosemicarbazide in 1 l. of dry pyridine was added with stirring 136 g. (1.0 mole) of β-ethoxypropionyl chloride.[13] After standing overnight most of the pyridine was removed by heating under reduced pressure. One liter of ethanol and then 123 g. (2.2 moles) of sodium methylate were added and the solution was heated on the steam-bath overnight. The solvent was removed under reduced pressure and the residue was dissolved in 1 l. of water. After the addition of 6 *N* hydrochloric acid to *p*H 1 the solution was concentrated under reduced pressure to a volume of 500 ml. The solid which formed was collected and air-dried. The product was extracted with absolute ethanol to separate it from sodium chloride. After removal of the alcohol 52 g. (30% yield) of solid remained. A sample was recrystallized from ethyl acetate and obtained as plates; m.p. 166–167°.

*Anal.* Calcd. for $C_6H_{11}N_3OS$: C, 41.61; H, 6.40; N, 24.27. Found: C, 41.79; H, 6.22; N, 24.55.

**3-β-Ethoxyethyl-1,2,4-triazole.**—To a solution of 50 ml. of concentrated nitric acid and 100 ml. of water containing a few crystals of sodium nitrite was added with stirring 47 g. (0.27 mole) of 3-β-ethoxyethyl-1,2,4-triazole-5-thiol. The temperature was maintained near 50° during the addition. After cooling, the solution was made basic with sodium carbonate and then was concentrated to dryness by heating under reduced pressure. The residue was extracted with absolute ethanol. The alcohol was evaporated by heating on the steam-bath and the resulting oil was distilled under reduced pressure. A colorless liquid was obtained; b.p. about 130° (0.5 mm.); $n^{25}D$ 1.4785. The yield was 25 g. (66%).

*Anal.* Calcd. for $C_6H_{11}N_3O$: C, 51.04; H, 7.85; N, 29.77. Found: C, 50.65; H, 7.90; N, 29.56.

**3-β-Chloroethyl-1,2,4-triazole Hydrochloride.**—A solution of 14.1 g. (0.1 mole) of 3-β-ethoxyethyl-1,2,4-triazole and 200 ml. of 48% aqueous hydrobromic acid was heated under reflux overnight. The solvent was removed by heating under reduced pressure, and the residue was treated with 100 ml. of thionyl chloride. After heating under reflux for two hours the excess thionyl chloride was evaporated under reduced pressure. The solid residue was washed with dry benzene. It was recrystallized from ethanol–ether mixture and obtained as white plates; m.p. 120°. The yield was 7.1 g. (42%).

*Anal.* Calcd. for $C_4H_6ClN_3 \cdot HCl$: C, 28.60; H, 4.20; N, 25.01. Found: C, 28.97; H, 4.35; N, 24.86.

**3-β-Ethylaminoethyl-1,2,4-triazole Dipicrate.**—A solution of 2 g. (0.012 mole) of 3-β-chloroethyl-1,2,4-triazole hydrochloride and 5 ml. of ethylamine was allowed to stand in a stoppered bottle at room temperature for one week. The excess amine was removed by heating under reduced pressure and the residue in 10 ml. of 95% ethanol was added to 5.5 g. of picric acid dissolved in 25 ml. of ethanol. The product which formed on cooling was recrystallized from water and obtained as prismatic needles; m.p. 161°. The yield was 2.2 g. (30%).

*Anal.* Calcd. for $C_{18}H_{18}N_{10}O_{14}$: C, 36.13; H, 3.03; N, 23.41. Found: C, 36.00; H, 2.70; N, 23.68.

**3-β-Diethylaminoethyl-1,2,4-triazole Dipicrate.**—This picrate was obtained from 2 g. of 3-β-chloroethyl-1,2,4-triazole hydrochloride and 5 ml. of diethylamine in a manner similar to that described above for the preparation of 3-β-ethylaminoethyl-1,2,4-triazole dipicrate. It was recrystallized from ethanol and obtained as prisms; m.p. 160°. The yield was 2.5 g. (33%).

*Anal.* Calcd. for $C_{20}H_{22}N_{10}O_{14}$: C, 38.34; H, 3.54. Found: C, 37.93; H, 3.42.

**3-Aminoalkyl-1,2,4-triazole Salts (Table I).**—Compounds III to VII were prepared by the hydrolysis of the corresponding phthalimido compounds (Table IV) with 6 *N* hydrochloric acid.[1] The hydrochlorides of 3-β-aminopropyl-1,2,4-triazoles were hygroscopic and were converted to the sulfates in the usual manner.

Compounds VIII–XI were obtained from the corresponding picrates according to the procedure reported for the preparation of 3-β-isopropylaminoethyl-1,2,4-triazole dihydrochloride.[1]

INDIANAPOLIS, INDIANA

(13) N. A. Milas, U. S. Patent 2,369,157 [*C. A.*, **39**, 5044 (1945)].

---

[CONTRIBUTION FROM THE DEPARTMENTS OF AGRICULTURAL CHEMISTRY AND HORTICULTURE, MICHIGAN STATE COLLEGE]

# The Nature of an Oxidation Product of 3-Indoleacetic Acid[1-3]

BY WM. H. HOUFF, O. N. HINSVARK, L. E. WELLER, S. H. WITTWER AND H. M. SELL

RECEIVED OCTOBER 14, 1953

The colored product obtained by the reaction of 3-indoleacetic acid with ferric ion in an acidic solution is N-hydroxy-3-indoleacetic acid and may be formed by the hydrolysis of an intermediate resulting from the oxidation of the unsubstituted nitrogen in the indole ring.

Salkowski[4] first reported a qualitative test for 3-indoleacetic acid, in which a red color developed when ferric chloride was added to a solution of 3-indoleacetic acid in the presence of mineral acids. Later Mitchell and Brunstetter[5] placed this qualitative color reaction on a quantitative basis. Tang and Bonner[6] and Gordon and Weber[7] have modified this original quantitative procedure, the latter using ferric chloride–perchloric acid solution for color development. Further investigations in this Laboratory resulted in the isolation of a colored amorphous solid upon treatment of 3-indoleacetic acid with a solution of ferric chloride–perchloric acid.

Bonner[8] suggested that the color develops from a complex between 3-indoleacetic acid and ferric ion in acid solution. However color can be produced by a variety of oxidizing agents some of which contain no metal ions. An examination of the colored product disclosed that it was completely free of iron. These observations, along with the presence of iron II in the reaction solution, suggested that the colored species is derived from the oxidation of 3-indoleacetic acid. In addition to 3-indoleacetic acid, other indole derivatives (indole, ethyl-

(1) Journal Article No. 1561 of the Michigan Agricultural Experiment Station.

(2) This investigation was supported by the Horace H. Rackham Research Endowment.

(3) Presented before the Division of Organic Chemistry at the 124th Meeting of the American Chemical Society, Chicago, Ill., 1953.

(4) E. Salkowski, *Z. physiol. Chem.*, **9**, 23 (1885).

(5) J. W. Mitchell and B. C. Brunstetter, *Bot. Gaz.*, **100**, 802 (1939).

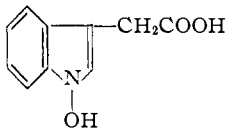
(6) Y. W. Tang and J. Bonner, *Arch. Biochem.*, **13**, 11 (1947).

(7) S. A. Gordon and R. P. Weber, *Plant Physiology*, **26**, 192 (1951).

(8) J. Bonner, "Plant Biochemistry," Academic Press, Inc., New York, N. Y., 1950, p. 442.

3-indoleacetate, 2-methyl-3-indoleacetic acid, 3-indole-carboxylic acid, 2 methylindole, 5,7-dichloro-3-indoleacetic acid, 3-indole-aldehyde and skatole) with the exception of N-substituted compounds (N-benzoyl-3-indoleacetic acid and N-carbethoxyindole) gave color reactions with oxidizing agents. These observations indicate that the colored product was formed by the oxidation of the unsubstituted nitrogen in the indole ring.

Analysis of the colored compound corresponded to the empirical formula $C_{10}H_9O_3N$. When made basic, the color of its solution changed from red to yellow. This color change was reversible upon the addition of acid, a property typical of a disubstituted hydroxylamine. A consideration of the chemical properties of the colored product, its mode of formation, and its analysis indicate the structure

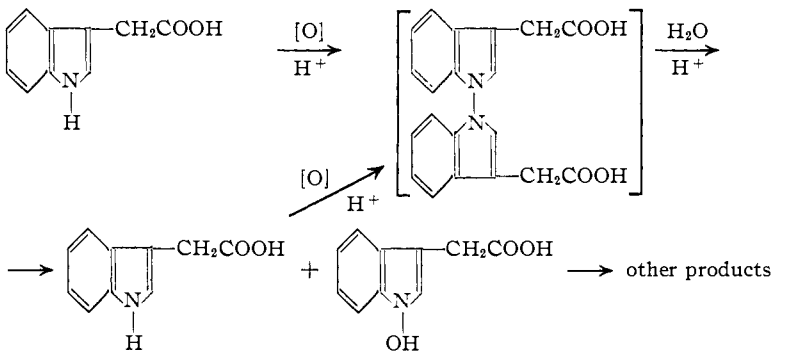



Other indole compounds having an hydroxyl group on the nitrogen atom have been reported.[9-12]

The oxidation of 3-indoleacetic acid to its N-hydroxyindole derivative involves a two-electron change. To ascertain the extent of oxidation, a standard ferric chloride solution was used to produce the colored product and the stoichiometry determined. After 45 minutes and 24 hours, respectively, one and two milliequivalents of ferric ion were consumed for each millimole of 3-indoleacetic acid. In the isolation of the colored product some 3-indoleacetic acid was found after 45 minutes which suggested the utilization of two moles of 3-indoleacetic acid for each mole of N-hydroxy-3-indoleacetic acid. This relationship explains the one-electron change. The decrease in color intensity also suggests the precipitation of N-hydroxy-3-indoleacetic acid from the reaction product. Oxidation of the regenerated 3-indoleacetic acid produced by the hydrolysis of the intermediate may explain the consumption of two milliequivalents of ferric ion for each millimole of 3-indoleacetic acid after 24 hours.

An induction period precedes the appearance of the unstable colored species in solution containing oxidizing agents. Upon standing, the color begins to disappear and deposition of a brown precipitate occurs. These observations suggest that a compound like N,N′-diindolyl-3,3′-diacetic acid may precede the formation of N-hydroxy-3-indoleacetic acid. To test this hypothesis, N,N′-diindolyl-3,3′-diacetic acid was prepared according to the Chattaway and Ingle[13] procedure. The yellow crystalline product upon treatment with acid produced immediately the characteristic red color also obtained by the oxidation of 3-indoleacetic acid. However, a much longer time was required for the color to develop by oxidizing 3-indoleacetic acid. This red color could be changed to yellow by the addition of base. This indicates that N,N′-diindolyl-3,3′-diacetic acid may be an intermediate compound which is formed during the oxidation of 3-indoleacetic acid.

The following reactions for the oxidation of 3-indoleacetic acid in acid solutions explain the observed facts




It is likely that similar reactions may take place upon the mild oxidation of other indole derivatives in acidic solution.

**Preparation of N-Hydroxy-3-indoleacetic Acid.**—3-Indoleacetic acid (2.0 g.) in 20 ml. of acetone was treated with a solution of 1.0 g. of ferric chloride in 100 ml. of 10% perchloric acid. Addition of the aqueous acid solution caused a rapid coloration and some precipitation of 3-indoleacetic acid. Sufficient acetone was added to redissolve the precipitated acid. After shaking for 1 hour, the solution was placed in the refrigerator for 24 hours. The resulting precipitate was collected and air-dried. This product was purified by dissolving in 5% sodium bicarbonate, filtering and reprecipitating by the addition of acid. This procedure yielded a red-purple non-crystalline material which could be further purified by precipitation from ethanol solution by adding water. The product had a decomposition point of 153°.

*Anal.* Calcd. for $C_{10}H_9O_3N$: C, 62.8; H, 4.71; N, 7.35; mol. wt., 191. Found: C, 62.7; H, 4.36; N, 7.58; mol. wt., 194 (neut. equiv.).

The ultraviolet absorption spectrum, measured in ethanol, had peaks at 285 mμ (log ε 3.86) and 292 mμ (log ε 3.82).

Infrared spectra of N-hydroxy-3-indoleacetic acid shows strong peaks at 3390 (NH or NOH); 3120, 3040, 2720, 2620, 2540, 2330 (carboxyl OH); 1694 (carboxyl C=O); 1620, 1555, 1517, 1490 (phenyl, conjugated C=C); 739, 750 (*ortho* disubstitution in the phenyl ring) cm.$^{-1}$. The absorption bands of N-hydroxy-3-indoleacetic acid are similar to those found for 3-indoleacetic acid.

No adduct was obtained with either picric acid or *sym*-trinitrobenzene. Ingraffia[14] has reported that N-hydroxyindole failed to combine with picric acid.

**Preparation of N,N′-Diindolyl-3,3′-diacetic Acid.**—This compound was prepared according to a procedure similar to that of Chattaway and Ingle[13] for the preparation of tetraphenylhydrazine. To 2.5 g. of 3-indoleacetic acid in 50 ml. of absolute ethanol was added a solution of 2.06 g. of sodium ethoxide in 50 ml. of the same solvent with rapid stirring. This mixture was allowed to stand for six hours and then was treated slowly with 3.63 g. of iodine. After three hours with occasional shaking, most of the ethanol was removed under vacuum and 100 ml. of ether was added along with 50 ml. of 1.0% hydrochloric acid solution (slightly less than the amount required to neutralize the sodium salt of the product). After shaking, the ethereal layer was separated and dried over anhydrous sodium sulfate. When the ether was removed, a yellow crystalline solid was ob-

(9) A. Reissert, *Ber.*, **29**, 639 (1896); **30**, 1030 (1897).

(10) F. Arndt, *Z. angew. Chem.*, **40**, 1099 (1927).

(11) A. Angeli and F. Angelico, *Atti reale accad. Lincei*, **15**, 762 (1906).

(12) G. Heller, *Ber.*, **42**, 470 (1909).

(13) B. A. Chattaway and H. J. Ingle, *J. Chem. Soc.*, **67**, 1090 (1895).

(14) F. Ingraffia, *Gas. chim. ital.*, **63**, 175 (1933).

tained which decomposed slightly at 85° and melted at 111–113.5°, and changed to a brown amorphous solid in two hours.

*Anal.* Calcd. for $C_{20}H_{16}O_4N_2$: N, 8.05. Found: N, 7.78.

**Determination of Ferric Ion Consumed.**—In each of two erlenmeyer flasks was pipetted 5.00 ml. of approximately 0.2 *N* ferric chloride in 10% perchloric acid solution. To one of these was added 0.0138 g. (0.079 millimole) of 3-indoleacetic acid. After 45 minutes, the solution was extracted with isobutyl alcohol to remove the colored product. The blank was treated in a similar manner. The blank and the reaction mixture were each titrated with standard (0.02 *N*) potassium dichromate solution. A difference of 0.083 milliequivalent of dichromate was found between the two solutions, corresponding to one milliequivalent of dichromate being required per millimole of 3-indoleacetic acid oxidized.

## Biological Properties

The biological activity of N-hydroxy-3-indoleacetic acid and the N,N′-diindolyl-3,3′-diacetic acid in stimulating growth responses in higher plants was ascertained using 3-indoleacetic acid as a control comparison.[15] In both the induction of parthenocarpy in young tomato fruits and negative curvatures of bean seedling hypocotyls, N,N′-diindolyl-3,3′-diacetic acid was equally as active as 3-indoleacetic acid. In contrast, N-hydroxy-3-indoleacetic acid showed less than 1% of the activity characteristic of 3-indoleacetic acid.

**Acknowledgment.**—The authors wish to thank Dr. M. E. Speeter of the Upjohn Company, Kalamazoo, Michigan, for the infrared and ultraviolet spectral analysis of N-hydroxy-3-indoleacetic acid.

(15) S. H. Wittwer, Univ. of Missouri Research Bull. 371 (1943).

EAST LANSING, MICHIGAN

---

[CONTRIBUTION FROM THE KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

# Pteridines from *Drosophila*. I. Isolation of a Yellow Pigment[1]

BY H. S. FORREST[2] AND H. K. MITCHELL

RECEIVED APRIL 7, 1954

A method is described for the isolation in pure crystalline form, of a yellow pigment occurring chiefly in the eyes of *Drosophila melanogaster* and in many times larger amounts in a mutant, *sepia*.

During the last 10–12 years, various attempts have been made to study the chemistry of the eye pigments of *Drosophila melanogaster*, not only because of their interest *per se* but also because of the possibility of providing further insight into the relationship between genes and biochemical processes. Provided some knowledge of the underlying chemistry is available, the group of eye color mutants in *Drosophila* would be very useful for such a study because the various genetic interrelationships are well established. This has been recognized, of course, in the pioneering work of Beadle, Tatum and Ephrussi and their co-workers[3] in which it was shown that kynurenine ($v^+$ substance) and a second compound ($cn^+$ substance) later shown by Butenandt[4] to be hydroxykynurenine are implicated in the synthesis of the so-called brown pigment of the eyes. Practically nothing is known, however, of the chemistry of the red pigments beyond an intimation of their possible pteridine nature[5] and a denial of even this allegation.[6]

Of the various groups which have made sporadic attempts to investigate these compounds[6–8] all have concentrated on the red pigments themselves, but these have been found to be extraordinarily difficult to handle from the chemical standpoint for three main reasons, firstly the difficulty of isolation in quantity, secondly that of separating efficiently several closely related compounds and thirdly that of their inherent instability. Thus the main outcome of these efforts has been the publication[9] of empirical formulas for various fractions of the red pigment complex, but no attempts have been made to relate these formulas to any chemical structure.

A new approach to this problem was made apparent by the discovery[10] of a simple chromatographic technique for the identification and approximate estimation of the fluorescent pigments in *Drosophila* and in various mutants thereof. By this method it was shown that a yellow pigment occurred in about 5 times greater amount in a mutant, *sepia*, than in *wild type* flies. This mutant does not produce red pigment and it seemed probable that the yellow substance is an intermediate or is related to an intermediate in the biosynthesis of the red pigments. It appeared that this compound would be easier to obtain in quantity, the difficulty of separating a number of very similar compounds would be eliminated and furthermore, a determination of its structure would be of considerable value in elucidating those of the red pigments if indeed it lay in the biochemical pathway toward them. Another point of interest lay in the early discovery that this compound was highly photosensitive, in contrast to the red pigments, and its possible importance in the visual processes of the flies was therefore appreciated.

Unfortunately these hoped for simplifications

(1) These investigations were supported by funds from the Rockefeller Foundation, the Williams-Waterman Fund for the Combat of Dietary Diseases and by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, contract No. N-6-onr-244, Task Order 5.

(2) U. S. Public Health Service Postdoctoral Fellow during part of the work described.

(3) *Cf.* B. Ephrussi, *Quart. Rev. Biol.*, **17**, 327 (1942).

(4) A. Butenandt, *Angew. Chem.*, **61**, 262 (1949).

(5) E. Lederer, *Biol. Rev. Cambridge. Phil. Soc.*, **15**, 273 (1940).

(6) W. K. Maas, *Genetics*, **33**, 177 (1948).

(7) G. Wald and G. Allen, *J. Gen. Physiol.*, **30**, 41 (1946).

(8) H. Heymann, F. L. Chan and C. W. Clancy, THIS JOURNAL, **72**, 1112 (1950).

(9) F. L. Chen, H. Heymann and C. W. Clancy, *ibid.*, **73**, 5448 (1951).

(10) E. Hadorn and H. K. Mitchell, *Proc. Nat. Acad. Sci.*, **37**, 650 (1951).