riboflavin from the green fermentation product. The latter compound was then easily isolated by filtration and was washed with methanol and acetone. The compound (600 mg.) thus obtained remained green in the dry state, but upon exposure to air in the presence of water it was readily oxidized to an orange substance which from chromatographic analysis appears to be identical with 6,7-dimethyl-9-(2′-hydroxyethyl) isoalloxazine.

Kuhn and Ströbele[6] reported that one mole of half-reduced flavin could form quinhydrone-like complexes with one mole of flavin (chloroflavin, light green) with one mole of half-reduced flavin (verdoflavin, dark green) or with one mole of dihydroflavin (rhodoflavin, red).

In view of the ready air oxidation to yellow flavin of the green and red substances observed during the fermentation, it appeared possible that these substances are molecular complexes of the kind observed by Kuhn and Ströbele.

Evidence in support of this hypothesis was obtained by showing that when 100 mg. of the green compound (equivalent to 175 μM of presumed complex) was suspended in 2.0 ml. of water and shaken in air for 4 hours in a Warburg respirometer at 26°, 40 μM of oxygen was consumed. No further oxygen uptake occurred even after shaking overnight. The observed oxygen uptake corresponds to 91% of the theoretical uptake expected from a molecular complex composed of one mole of half-reduced and one mole of oxidized flavin. Further evidence in support of the above hypothesis was obtained by showing that when an alkaline solution of the purified bacterial flavin is treated under anaerobic conditions with a solution of sodium hydrosulfite, a dark green precipitate is formed, corresponding to the verdoflavin reported by Kuhn. Addition of concentrated acid caused formation of a bright red solution (rhodoflavin), which, on dilution with water and gradual admission of air became light green (chloroflavin).

It appears very probable, therefore, that the red and green precipitates observed early in the fermentations are in fact quinhydrone-like complexes of partially reduced 6,7-dimethyl-9-(2′-hydroxyethyl)-isoalloxazine.

(6) R. Kuhn and R. Ströbele, *Ber.*, **70**, 753 (1937).

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The Iodination of Tyrosine and its Derivatives

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Early attempts to iodinate tyrosine in various alkaline media were generally unsatisfactory. In recent years, however, tyrosine has been converted into 3,5-diiodotyrosine in good yields by reaction with iodine monochloride[2] and with iodine and ethylamine.[3] Bauer and Strauss[4] reported that iodine did not react with 3-nitrotyrosine in alkaline solutions or in the presence of mercuric oxide. With iodine monochloride, they obtained 3-iodo-5-nitrotyrosine in small yield.

A new method was developed recently for the iodination of phenols and aromatic ethers.[5] This method involved the reaction of the aromatic compound with iodine and hydrogen peroxide in the presence of a strong mineral acid. As part of a research program which has had to be abandoned, this method was used very satisfactorily in the iodination of tyrosine derivatives. Good yields of monoiodo derivatives were obtained from 3-nitrotyrosine and *o*-methyltyrosine and of 3,5-diiodotyrosine from tyrosine.

(1) U. S. Department of Agriculture, Pasadena, California.

(2) (a) P. Block, Jr., and G. Powell, THIS JOURNAL, **65**, 1430 (1943); (b) E. T. Borrows, J. C. Clayton and B. A. Hems, *J. Chem. Soc.*, 5185 (1949).

(3) J. H. Barnes, E. T. Borrows, J. Folks, B. A. Hems and A. G. Long, *ibid.*, 2824 (1950).

(4) (a) H. Bauer and E. Strauss, *Ber.*, **68B**, 1108 (1935); (b) **69**, 245 (1936).

(5) (a) L. Jurd, *Australian J. Sci. Research*, **2A**, 595 (1949); (b) **3A**, 587 (1950).

Experimental[6]

3,5-Diiodotyrosine.—Powdered iodine (2.8 g.) was suspended in a solution of tyrosine (2.0 g.) in glacial acetic acid (14.0 cc.) and 36 *N* hydrochloric acid (8.0 cc.). Thirty per cent. hydrogen peroxide solution was then added in small portions with shaking during five minutes until the iodine color had disappeared, the temperature of the reaction being maintained at 60–65°. Approximately 1.3 cc. of the hydrogen peroxide solution was required. The yellow solution was cooled and diluted successively with water (10 cc.), 0.880 *M* ammonia solution (9.0 cc.), and 10% sodium hydrogen sulfite solution (5 cc.). 0.880 *M* ammonia solution was then added dropwise until crystallization began. 3,5-Diiodotyrosine separated in flat, almost colorless needles. It was collected, washed with water and alcohol and air-dried, m.p. 198° (lit. 201° (cor.)[2a]) (3.4 g., 71%).

3-Iodo-5-nitrotyrosine.—Concentrated nitric acid (3.0 cc.) was added to a suspension of 3-nitrotyrosine (6.0 g.) and powdered iodine (3.4 g.) in 95% alcohol (45 cc.). Thirty per cent. hydrogen peroxide solution was then added in 0.5-cc. portions until the color of iodine had disappeared. 4.0 cc. of hydrogen peroxide solution was required and the heat of the reaction maintained the temperature at 45–50° throughout the addition. The reaction mixture was heated to 70° for five minutes, diluted with water (30 cc.) and treated with concentrated ammonia, added dropwise, until a heavy yellow solid separated. After standing at 0° for two hours, the solid was collected, washed with a small quantity of water and alcohol and heated under reflux with alcohol (30 cc.) for ten minutes. On cooling, the crystalline solid was collected. It was dissolved in hot dilute hydrochloric acid and precipitated with ammonia (5.6 g., 60%, m.p. 220°). For analysis the 3-iodo-5-nitrotyrosine was recrystallized from water and separated in golden-yellow needles, m.p. 224–226° dec. (lit. 225–226°).

Monoiodo-*o*-methyltyrosine.—Concentrated sulfuric acid (1.0 cc.) and finely powdered iodine (1.48 g.) were added to a suspension of *o*-methyltyrosine hydrogen sulfate[7] (3.40 g.) in alcohol (15 cc.). The reaction mixture was maintained at 50° and treated slowly with 30% hydrogen peroxide solution (1.4 cc.) during ten minutes. The temperature was raised to 65° for five minutes, the solution then was diluted with water (10 cc.) and adjusted to *p*H 7.5 with concentrated ammonia when crystallization of the product began. Ten per cent. aqueous sodium hydrogen sulfite (3.0 cc.) was added and the mixture was cooled. The white crystalline mass was collected, washed with alcohol, and air-dried (3.12 g., 84%, m.p. 220–221°). Recrystallized from water, the monoiodo-*o*-methyltyrosine separated in colorless needles, m.p. 222°.

Anal. Calcd. for $C_{10}H_{12}INO_3 \cdot {}^1/_2H_2O$: C, 36.3; H, 4.0; I, 38.5; N, 4.2. Found: C, 36.3; H, 4.0; I, 39.2; N, 4.4.

(6) All melting points are uncorrected.

(7) L. D. Behr and H. T. Clarke, THIS JOURNAL, **54**, 1630 (1932).

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The Chemistry of Nitroacetic Acid and its Esters. III. The Synthesis of Tryptamine from Ethyl-α-nitro-β-(3-indole)-propionate[1]

BY DOUGLAS A. LYTTLE AND DAVID I. WEISBLAT

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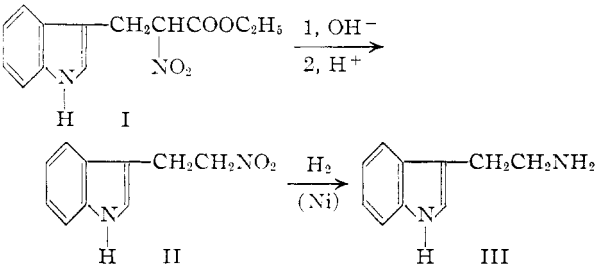
Ethyl α-nitro-β-(3-indole)-propionate (I) is a key intermediate in our synthesis of *dl*-tryptophan from ethyl nitroacetate[2a] or ethyl nitromalonate[2b] and

(1) D. I. Weisblat and D. A. Lyttle, U. S. Patent 2,616,896.

(2) (a) D. A. Lyttle and D. I. Weisblat, THIS JOURNAL, **69**, 2118 (1947); (b) **71**, 3079 (1949).

gramine. The scheme below represents a convenient method for the preparation of tryptamine from this compound (I)



Hydrolysis of I is carried out at room temperature using alcoholic sodium hydroxide. A solid sodium salt is collected, dissolved in water and treated with an excess of acid to give 3-(2-nitroethyl)-indole[1,3] (II) in 83% yield. The melting points we found for two dimorphic forms of this compound (55.5–56.1° and 68.3–69.2°) agree with those reported by Noland and Hartman[3] (56.5–57° and 68–68.5°). Catalytic reduction of II (Raney Ni) gave tryptamine, isolated as the hydrochloride, in 81.6% yield.

Experimental

3-(2-Nitroethyl)-indole (II).—A solution of sodium hydroxide (32.0 g., 0.8 mole) in 64 ml. of water was added to 78.6 g. (0.30 mole) of ethyl α-nitro-β-(3-indole)-propionate (I) in 200 ml. of ethanol. The resulting solution was allowed to stand at room temperature for 44 hours. Solvent was removed under reduced pressure until the flask contained a mass of solid, semi-crystalline material. Ethanol (400 ml.) was added, the mixture was slurried well and the solid was collected on a filter. It was washed with ethanol, then with ether and dried on the filter. The solid was dissolved in 600 ml. of water, the solution was cooled in ice and acidified by slowly adding 20% hydrochloric acid until the *p*H was between 4 and 5. Crystalline material began to separate and the flask was cooled at 4° overnight. The light pink crystals were collected, washed with water and dried under vacuum. The yield of 3-(2-nitroethyl)-indole was 47.4 g. (83.2%). Material of analytical purity was obtained from the "practical grade" product described above by dissolving it in 200 ml. of ethanol, treating the solution with charcoal, filtering and adding 95 ml. of warm water to the warm (60°) filtrate. The flask was allowed to cool slowly to room temperature and was then placed in the refrigerator for 2 days. The large, sparkling plates were collected on a filter and washed with two 40-ml. portions of 50% ethanol. The product, dried under vacuum, weighed 39.14 g. (68.5%) and melted at 55.5–56.1°. Two months later the melting point of the same material was 68.3–69.2°. It was shown by analysis and infrared spectra that these are dimorphic forms.

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.14; H, 5.30; N, 14.73. Found: C, 62.80; H, 5.20; N, 14.71.

Tryptamine (3-(2-Aminoethyl)-indole) (III).—In a Parr rocking autoclave of 250-ml. void volume was placed 25.8 g. of 3-(2-nitroethyl)-indole, 102 ml. of ethanol, approximately 3.8 g. of Raney nickel catalyst and hydrogen under a pressure of 1520 p.s.i. at 23°. The autoclave was heated as rapidly as possible. Very little reduction occurred until the temperature reached 85°, when it was complete in about 3 minutes. Heating was discontinued, the temperature continued to rise to 100°, and the autoclave was allowed to cool slowly to room temperature. Catalyst was removed by filtration and the filtrate was concentrated under vacuum to 75 ml. An excess of 10% alcoholic hydrogen chloride was added, causing crystalline material to precipitate. The mixture was chilled at 4° overnight, the crystals were collected on a filter and washed with a little cold ethanol, then ether. The light brown, crystalline material weighed 21.77 g. (81.6%), m.p. 240° dec. An analytical sample was prepared by recrystallizing the above material twice from ethanol; m.p. 242–248°.

Anal. Calcd. for $C_{10}H_{13}ClN_2$: C, 61.06; H, 6.66; N, 14.25. Found: C, 61.33; H, 6.41; N, 14.23.

The reduction was also carried out under the same conditions in the presence of a slight excess of acetic acid. The solvent was removed under reduced pressure and the residual solid was recrystallized from ethanol to give 60% of pure tryptamine acetate, m.p. 134–135°. Free base prepared from the acetate melted at 114–115° (uncor.).

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(3) W. E. Noland and P. J. Hartman, THIS JOURNAL, **76**, 3227 (1954).

Hydroxylation of Benzene in Aqueous Solution in the Presence of Hydroxylamine Hydrochloride

BY JAN O. KONECNY

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The formation of *o*-nitrosophenol at room temperature by the action of aqueous hydrogen peroxide, hydroxylamine hydrochloride and copper sulfate on benzene or phenol has been described in the literature.[1] Other *o*-nitrosophenols have been prepared[2] in the same manner from numerous aromatic hydrocarbons, phenols and their derivatives. *o*-Nitrosophenols, formed in small quantities, are identified readily owing to their formation of characteristic and intensely colored complexes[2] with the ions of transitional metals. The reaction has been attributed to the formation of –NOH radicals and their attack on the organic molecule. In order to explain the seemingly specific catalytic action of copper ions, it has been postulated[3] that they react with and stabilize the free radicals.

The present investigation revealed that ferrous sulfate, cuprous chloride and metallic copper also catalyzed the reaction. Traces of *o*-nitrosophenol were found to be formed from benzene even in the absence of a catalyst, and under such conditions the reaction rate was increased by exposing the solution to X-rays. Phenol, however, reacted readily with the solution of hydroxylamine hydrochloride and hydrogen peroxide. The formation of –NOH radicals in the system has so far remained conjectural. However, ample evidence is available concerning the formation of hydroxyl radicals from hydrogen peroxide by the action of short wave radiation,[4] of copper[5] and iron salts[6] and metallic silver and mercury.[7] It has been shown[4,6,8] that such systems oxidize benzene to phenol. It also has been shown[9] that hydroxylamine hydrochloride is oxidized to nitric acid by hydrogen peroxide, and it is very probable that nitrous acid is formed in the reaction as an intermediate. *p*-Nitrosophenol[9] has been prepared by the action of these reagents on phenol, and the formation of *o*-nitrosophenol from phenol and nitrous acid is described in this work.

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(7) J. Weiss, *Trans. Faraday Soc.*, **31**, 1547 (1935).
(8) J. O. Konecny, THIS JOURNAL, **76**, 4993 (1954).
(9) C. Wurster, *Ber.*, **20**, 2631 (1887).