The Synthesis of 3-Hydroxykynurenine (+Chromogen)*

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+Chromogen (W-substance) (1) of *Bmbyx mori*, corresponding to the cn$^+$-substance of *Drosophila* and A-substance of *Ephestia*, is widely distributed among insects and animals belonging to *Arthropods*. It is considered to be an important intermediate in the conversion of tryptophan to various pigments[1] and nicotinic acid,[2] and prior to its isolation in a pure crystalline state, a structure corresponding to 3-hydroxy-kynurenine (2-amino-3-hydroxybenzoylalanine) (VIII) was postulated[3] in 1948. H. Kikkawa[4] and we have recently

*) These results were presented before the Third Annual Meeting of the Chemical Society of Japan at Kyoto, April, 1950. At the same Meeting, S. Seno and T. Sakan (Osaka University) also reported the synthesis of +chromogen by applying the Kotake-Sakan method for the synthesis of kynurenine.

(1) H. Kikkawa, *Sanshi-shikenjo Hokoku* (*Reports of Sericultural Exp. Stat.*), **11**, 311 (1943). *Kagaku no Ryoiki*, **3**, 2 (1949).

(2) F. A. Haskins and H. K. Mitchell, *Proc. Nat. Acad. Sci.*, **35**, 500 (1949).

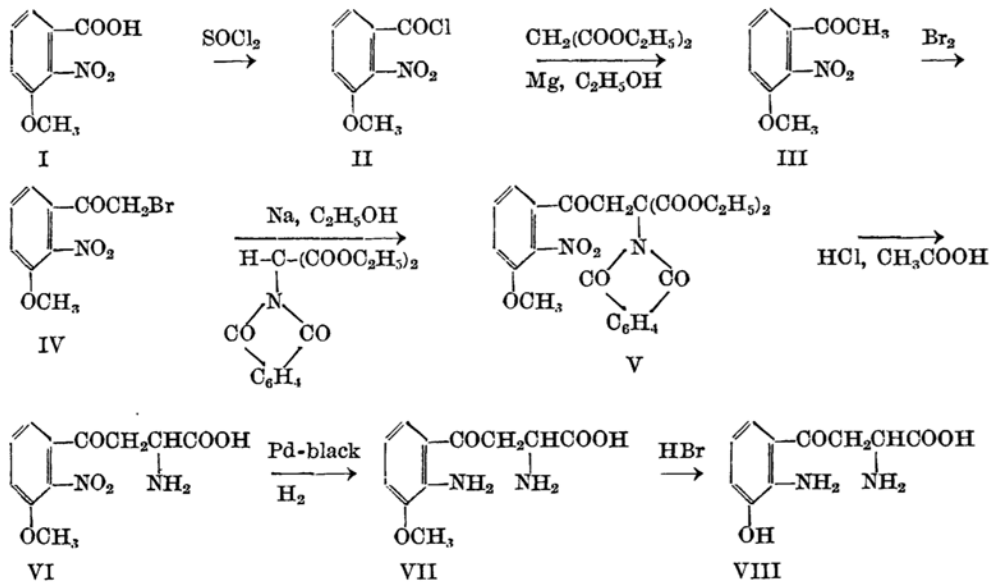
(3) H. K. Mitchell and J. F. Nyc, *ibid.*, **34**, 1 (1948).

(4) Y. Hirata, K. Nakanishi and H. Kikkawa, *J. of Genetics* (Japan) (in press). *Science* (U. S. A.) (in press).

succeeded, for the first time, in isolating 50 to 60 mg. of light-yellow crystals from 400 g. of new-laid eggs of *Bombyx mori*,[5] positive to the qualitative and biological tests of +chromogen. In the following paper the synthesis of 3-hydroxykynurenine is reported. The synthesized sample was identical with the natural specimen (excepting optical behavior[6]), thus establishing the structure of +chromogen to be 3-hydroxykynurenine.

modified procedure of Butenandt *et al.*[9] for the synthesis of kynurenine (2-amino-benzoylalanine) was applied, resulting in 2-amino-3-methoxybenzoylalanine (VII). This was finally converted into 2-amino-3-hydroxy-benzoylalanine (VIII) hydrobromide by hydrolysing with hydrobromic acid. The treatment of the hydrobromide with sulfuric acid and then with barium hydroxide gave free DL-2-amino-3-hydroxy-benzoylalanine. Excepting the conversion of IV to V, in which several by-products were obtained, every step in this synthesis proceeded quite smoothly with a high yield.

Fig. 1



The starting substance for the synthesis of 2-amino-3-hydroxy-benzoylalanine was 2-nitro-3-methoxybenzoic acid (I), which was prepared by the method of Nyc and Mitchell[7] from 3-methoxy-benzoic acid. 2-Nitro-3-methoxy-benzoic acid was converted into its chloride (II) and then to 2-nitro-3-methoxy-acetophenone (III). The latter step was carried out by submitting the chloride to the method employed in the synthesis of p-nitroacetophenone,[8] an intermediate in the synthesis of chloromycetin. After bromination of 2-nitro-3-methoxyacetophenone, which yielded 2-nitro-3-methoxyphenacyl bromide (IV), the suitably

The synthetic sample possessed the following natures, thus proving the identity with the natural substance, +chromogen. The ninhydrin, xanthoprotein and diazo (Ehrlich's) reactions were positive. The alkaline solution, with a deep yellow color, gave a jasmine-like odor when heated, and a dilute solution of potassium permanganate also changed its color into deep yellow (urochromogen test of Weiss). Furthermore, employing the ninhydrin or Ehrlich's reagents or the light blue fluorescence as means for location, paper chromatography with various solvents could be used for the identification of 3-hydroxykynurenine. The solvents used were butanol, lutidine, phenol and the butanol-acetic acid mixture, each giving satisfactory results.

(5) It is reported in the forementioned reference (2) that W. Weidel has isolated the substance from *Calliphora erythrocephala*, but the details seem to be yet unreported.

(6) Natural specimen: $[\alpha]_{4561}^{17} = -30°$ (approximately).

(7) J. F. Nyc and H. K. Michell, *J. Am. Chem. Soc.* **70**, 1847 (1948).

(8) Walter and Hauser. *ibid.*, **68**. 1386 (1946); L. M. Long and H. O. Troutman, *ibid.*, **71**, 2474 (1949).

(9) A. Butenandt, W. Weidel, R. Weichert and W. von Derjugen, *Z. physiol. Chem.*, **279**, 27 (1943).

Experimental

2-Nitro-3-Methoxy-benzoyl-chloride (II).—2-Nitro-3-methoxy-benzoic acid (I) (m.p. 255°) was prepared by the method of Nyc and Mitchell[7] from 3-methoxy-benzoic acid. After converting 2-nitro-3-methoxy-benzoic acid into its chloride with thionyl chloride, the excess reagent was completely removed by evaporation *in vacuo*. The chloride could be recrystallized from carbon tetrachloride (m.p. 76°), but the crude sample was directly submitted to the following procedure.

2-Nitro-3-methoxy-acetophenone (III).—This compound was prepared by a slight modification of the method of Long and Troutman[5] for the synthesis of *p*-nitro-acetophenone. 3.15 g. (0.13 mole) of freshly ground magnesium turnings and a solution of 0.5 cc. of dry carbon tetrachloride in 3cc. of absolute ethanol were placed in a 500cc. flask and as the reaction began to occur, 35cc. of dry chlorobenene was added. A solution of 21g. of diethyl malonate (0.13 mole), 20cc. of dry chlorobenzene and 15cc. of absolute ethanol was added while shaking and cooling at such a rate as to keep the temperature at about 65°. When the removal of the cooling bath did not result in a rise in temperature, the mixture was heated to 85° on a water-bath until the reaction ended (two hours). The solution was cooled and a solution of 24g. (0.11 mole) of II in 35cc. of dry chlorobenzene was added by stirring and cooling so as to keep the temperature below 35° (usually, cooling was unnecessary). The mixture gradually changed into a brown gelatinous mass and the stirring was continued for 30 minutes. The flask was cooled in an ice-bath and a solution of 10cc. of concentrated sulfuric acid in 70cc. of water was added. The lower layer, saturated with sodium sulfate, was discarded and the chlorobenzene layer was slightly warmed, whereby precipitates of magnesium sulfate appeared. This was filtered and the chlorobenzene layer of the filtrate was concentrated *in vacuo* until the solvent was almost completely removed. The residue was hydrolysed with a mixture of 40cc. of acetic acid, 5cc. of hydrochloric acid and 30cc. of water for 5 hours and then ice-cooled. A crystalline mass was obtained, which was filtered and boiled with a solution of 15g. of sodium bicarbonate in 150cc. of water to remove the acidic by-products. The residue was crystallized from acetic acid. Yield, 20.5g. (93%), m.p. 129°.

2-Nitro-3-methoxy-phenacyl Bromide (IV).—To a solution of 20.5g. (0.15 mole) of III in 150cc. of acetic acid, some aluminium chloride and equimolecular bromine (1.7g.) dissolved in 50cc. of acetic acid were added. By slightly warming the mixture for 30 minutes the reaction ended. A crude crystalline mass was obtained by pouring the mixture into cold water. This was filtered and water was gradually added to its acetic acid solution. 22.5g. (78%) of IV was obtained as white crystals by adding water until a brown syrupy mass gradually began to precipitate. m.p. 84–86°.

2-Nitro-3-methoxyphenacyl-phthalimidomalonic Ester (V).—The sodium salt of phthalimidomalonic ester was prepared by the method of Sörensen[10]. 2.1g. (6.9 millimoles) of phthalimidomalonic ester was added to a solution of 167mg. (7.3 millimoles) of sodium in 10cc. of absolute ethanol and dissolved by slight warming. The ethanol was completely evaporated *in vacuo* and 10cc. of dry acetone was added twice, each time being removed *in vacuo*. The residue was completely dried *in vacuo* at 145° for one hour and a half and finally dissolved in 140cc. of dry acetone, giving a yellow-orange solution. 2.0g. (7.3 millimoles) of IV in 10cc. of dry acetone was added and after refluxing the mixture for 15 hours, it was ice-cooled for 5 hours to complete the precipitation of sodium bromide. This was discarded and the acetone evaporated *in vacuo*. The residual brown syrup containing V was directly submitted to the following procedure.

2-Nitro-3-methoxy-benzoylalanine (VI).—The syrup was refluxed on the water-bath with a mixture of 15cc. of hydrochloric acid and 15cc. of acetic acid for two hours and after further addition of 10cc. of hydrochloric acid, refluxed for two additional hours, by which the evolution of carbon dioxide ceased. The cooled mixture was filtered and the filtrate evaporated to dryness *in vacuo*. Dry acetone was added, whereby a considerable amount of crystalline glycine hydrochloride (identified by means of paper chromatography and analysis of nitrogen) remained undissolved. The acetone solution was concentrated and 15cc. of water was added. Acetone was further removed from the mixture, and the residue, a turbid aqueous solution, submitted to centrifugal separation. The clear solution was concentrated somewhat *in vacuo*, whereby white crystals (m.p. 197°) appeared. They were discarded and the residue was evaporated and dried *in vacuo*, resulting in 45 mg. of 2-nitro-3-methoxy-benzoylalanine hydrochloride (2.2%, calculated on the basis of IV).

2-Amino-3-methoxy-benzoylalanine hydrochloride (VII).—The aqueous solution of 45 mg. of VI-hydrochloride was reduced, using palladium black as catalyst, and the solution, possessing a blue-green fluorescence, was evaporated *in vacuo* after the addition of a few drops of concentrated hydrochloric acid. 37mg. (80%). m.p. (decomp.) 158°.

3-Hydroxykynurenine (+chromogen) (VIII).—A solution of 37mg. of VII-hydrochloride in 20cc. hydrobromic acid and 10cc. acetic acid was refluxed for three hours. The removal of the solvent *in vacuo* yielded white needles, m.p. (decomp.) 230°, with a positive diazo reaction (Ehrlich's). The hydrobromide was dissolved in dilute sulfuric acid and somewhat concentrated under carbon dioxide. The solution was neutralized with barium hydroxide and the barium sulfate discarded. By concentrating the filtrate

(10) S. P. L. Sörensen, *Z. physiol. Chem.*, **44**, 454 (1905).

in vacuo and cooling, 22mg. (77%) of+chromogen was obtained as yellow needles. m.p. (decomp.) 217°. *Anal.* Calc. for $C_{10}H_{12}O_4N_2$: C, 53.57; H, 5.36. Found (dried for 3 hours at 140°): C, 53.16; H, 5.84. The sample thus obtained gave all the qualitative natures expected for+chromogen and was positive to biological tests using the Malpighian tubes of *Drosophila*. Details regarding biological tests are to be published elsewhere.

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