[CONTRIBUTION FROM THE INSTITUTE OF POLYTECHNICS OF OSAKA CITY UNIVERSITY]

Studies on Amino Acids. IV. The Synthesis of 3-Hydroxykynurenine

BY MUNIO KOTAKE, TAKEO SAKAN AND SIRO SENOH

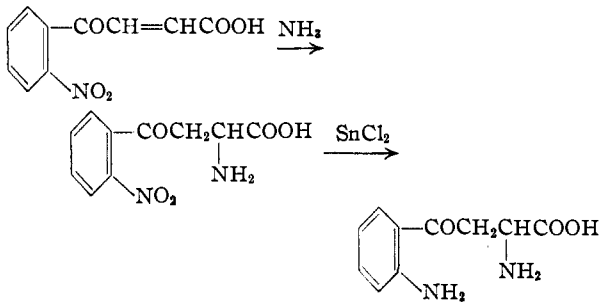
A synthesis of 3-hydroxykynurenine is described, using as starting material 2-nitro-3-methoxybenzoic acid, and involving as key step the addition of ammonia to the corresponding β-benzoylacrylic acid. The compound appears to be identical with cn+ substance and with +chromogen.

Interest in the compound, 3-hydroxykynurenine, is due to the role it plays in several important biochemical processes. Thus it has been postulated[1] and later demonstrated[2] that the substance is an intermediate in the formation of nicotinic acid from tryptophan in the metabolism of *Neurospora*. The authors[2] refer to apparently unpublished results of W. Weidel, Kaiser Wilhelm Institut für Biochemie to the effect that 3-hydroxykynurenine was isolated from larvae of *Calliphora erythrocephala* as the cn+ substance; this is a tryptophan metabolite intermediate in the formation of brown insect eye pigments, whose formation in, *e.g.*, *Drosophila melanogaster* depends upon the normal allele of the gene "cinnabar."

Recently Hirata, Nakanishi and Kikkawa[3] isolated the +chromogen from silkworm eggs; the physiological activity of this compound resembles the cn+ substance, and chemically the substance appears to be 3-hydroxykynurenine.

The literature gives no clear indication whether Mitchell and Haskins[2] obtained synthetic or natural material from Dr. Weidel; at any rate, details of a synthesis have not become available to us and thus we have applied the method which has been used by Kotake and Sakan to prepare the parent compound, kynurenine.[4]

The details of the synthesis, which proceeded with good yields, appear in the flow sheet. The acid chloride of 2-nitro-3-methoxybenzoic acid



Kynurenine

(II) was condensed with ethyl acetoacetate in the presence of sodium ethoxide to give III in almost quantitative yield as a crystalline solid. The acetyl group of III was removed with ethanolic ammonia, affording the solid benzoylacetic ester IV which was then alkylated with bromoacetate. The oily diester V on acid cleavage furnished the benzoylpropionic acid VI, whose assigned structure was confirmed by nitric acid oxidation to I. Bromination in chloroform solution with irradiation by ultraviolet light produced a bromoketonic acid (VII), convertible to the benzoylacrylic acid VIII by the action of sodium acetate. Addition of ammonia to the unsaturated linkage yielded 2-nitro-3-methoxybenzoylalanine (IX), and reduction of the nitro group followed by demethylation with hydrobromic acid led to 3-hydroxykynurenine (XI) in the form of its hydrobromide.

Free *dl*-3-hydroxykynurenine forms yellow needles that decompose at 223°; it gave positive results in each of the following tests: ninhydrin reaction, Folin–Denis test, Ehrlich diazo or ferric chloride test, Weis urochromogen reaction. The compound exhibited green fluorescence in ultraviolet light.

Biological experiments carried out by Dr. Hideo Kikkawa showed that the synthetic material acts like the cn+ substance when tested on the mutants *vermilion* and *cinnabar* of *Drosophila melanogaster*. Its physiological and chemical properties agree closely with those of +chromogen from silkworm eggs. We therefore consider both the cn+ substance and the +chromogen to be identical with 3-hydroxykynurenine.

Acknowledgment.—Our thanks are due to Dr. Hideo Kikkawa who carried out the biological tests.

Experimental

2-Nitro-3-methoxybenzoic Acid (I).—The compound was prepared by the oxidation[5] of 2-nitro-3-methoxytoluene, available from *m*-cresol.[6,7,8] A yield of 48%, based on *m*-cresol, of product decomposing at 251° was realized.

Ethyl α-(2-Nitro-3-methoxybenzoyl)-acetoacetate (II).—A mixture of 39.4 g. (0.2 mole) of I and 45.0 g. (8% excess) of phosphorus pentachloride was warmed on the steam-bath until gas evolution subsided. After vacuum distillation of phosphorus oxychloride the residue solidified and was taken up in benzene to give 120 cc. of solution. To a mixture of 80 cc. of sodium ethoxide (10.1 g. of sodium and 160 cc. of absolute ethanol) and 28.6 g. (0.22 mole) of ethyl acetoacetate was added dropwise 60 cc. of the acid chloride solution with stirring and below 5°. An additional 40 cc. of the ethoxide solution followed by 30 cc. of the acid chloride solution was added, and finally the remainder of the reagents was introduced in like fashion. After standing overnight the mixture was decomposed with ice and hydrochloric acid. Ether extraction yielded 61.4 g. of solid (almost quantitative), m.p. 62–66°, which afforded rhombic crystals upon recrystallization from alcohol. The ester gave a dark red color with ferric chloride and was soluble in bicarbonate solution.

Anal. Calcd. for $C_{14}H_{15}O_7N$: C, 54.37; H, 4.85; N, 4.53. Found: C, 54.86; H, 4.95; N, 4.75.

Ethyl 2-Nitro-3-methoxybenzoylacetate (IV).—Finely powdered ester III (30.9 g.) was gradually added to vigorously stirred 5% ethanolic ammonia, and eventually a red-

(1) H. K. Mitchell and J. F. Nye, *Proc. Natl. Acad. Sci.*, **34**, 1 (1948).

(2) F. A. Haskins and H. K. Mitchell, *ibid.*, **35**, 500 (1949).

(3) Hirata, Nakanishi and Kikkawa, *Jap. J. Genet.*, **24**, 188 (1949).

(4) M. Kotake and T. Sakan, *Proc. Imp. Acad. Tokyo*, **18**, 191 (1942); T. Sakan, *J. Chem. Soc. Japan*, **63**, 1545 (1942).

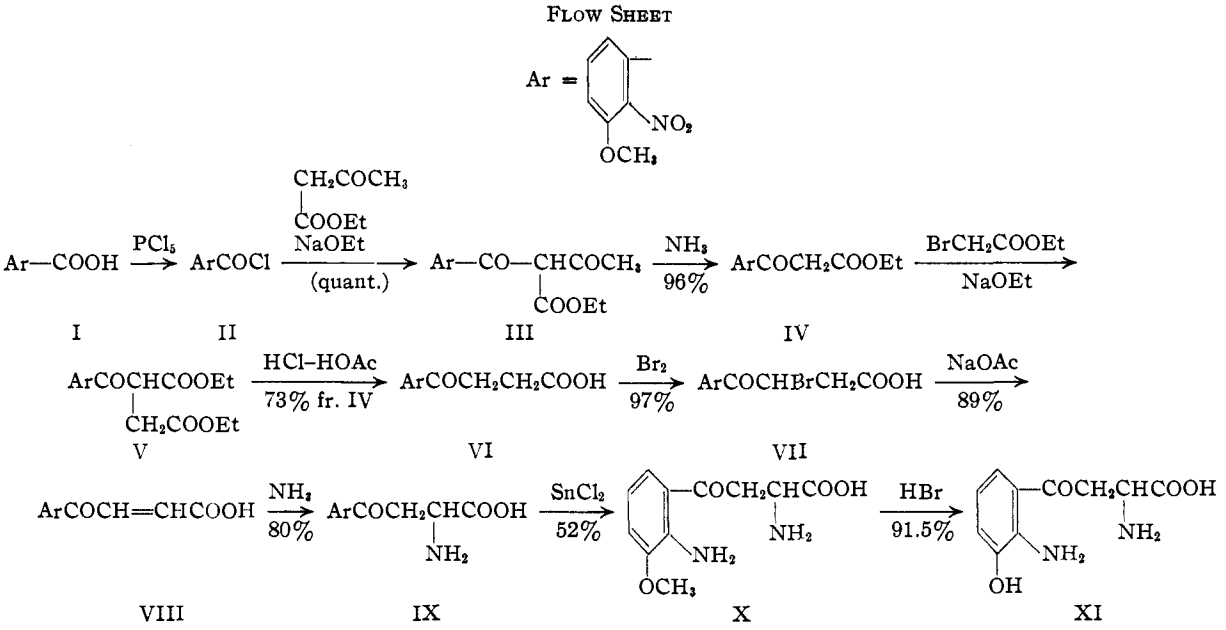
(5) Stanley, McMahon and R. Adams, THIS JOURNAL, **55**, 766 (1933).

(6) Gibson, *J. Chem. Soc.*, **123**, 1273 (1923).

(7) Verner and Jurner, *ibid.*, 2340 (1928).

(8) Hodgson and Beard, *ibid.*, **127**, 498 (1925).

FLOW SHEET



dish-brown solution resulted. Stirring was continued for 3 hours and the solution was allowed to stand overnight. The solidified mixture was acidified with dilute hydrochloric acid and extracted with ether. By bicarbonate extraction of the ethereal solution 3.0 g. of III was recovered. On evaporation of the ether 23.1 g. (96%) of crude IV was obtained, and recrystallized from ethanol; m.p. 66–66.5°. When 5.5 g. of III was treated with 20 cc. of absolute ethanol and 1 g. of concentrated sulfuric acid, 1.1 g. of IV resulted along with 2.4 g. of a solid, m.p. 125.5–126.5°, whose composition together with a positive iodoform test characterizes it as **2-nitro-3-methoxyacetophenone.**

Anal. Calcd. for $C_{12}H_{13}O_6N$: C, 53.93; H, 4.87; N, 5.24. Found: C, 54.38; H, 4.67; N, 5.65. Calcd. for $C_9H_9O_4N$: C, 55.38; H, 4.61; N, 7.18. Found: C, 55.32; H, 4.87; N, 7.47.

Diethyl α-(2-Nitro-3-methoxybenzoyl)-succinate (V).—A solution of 26.7 g. of IV in 60 cc. of dry ethanol was added to the solution resulting from 2.4 g. of sodium and 60 cc. of dry ethanol, and then 18.4 g. of ethyl bromoacetate was introduced with cooling and agitation. The mixture stood overnight, was warmed briefly on the water-bath and freed of alcohol in vacuum. The residue was taken up in water, acidified, and extracted with ether. The product appeared as a brownish-red oil that was hydrolyzed without purification.

β-(2-Nitro-3-methoxybenzoyl)-propionic Acid (VI).—The oily V described was boiled for 11 hours with a mixture of 150 cc. each of glacial acetic acid and concentrated hydrochloric acid. Carbon dioxide evolution subsided after that period of time. The solvents were removed as far as possible by vacuum distillation and the residue was extracted with a 3:1 mixture of ether and ethanol. The acidic fraction was isolated by bicarbonate extraction; the extract after acidification and standing in the cold overnight yielded crystalline VI. The product was recrystallized from boiling water (charcoal); yield 18.5 g. (73% from IV), m.p. 139–139.5°. On seeding, long standing, or long heating the substance changes to a stable, dimorphic modification, m.p. 148.5–149°. On oxidation with dilute nitric acid the compound yields 2-nitro-3-methoxybenzoic acid (I).

Anal. Calcd. for $C_{11}H_{11}O_6N$: C, 52.18; H, 4.35; N, 5.53. Found: C, 52.22; H, 4.34; N, 5.81.

β-Bromo-β-(2-nitro-3-methoxybenzoyl)-propionic Acid (VII).—A suspension of 5 g. of VI in 60 cc. of chloroform was treated with 3.2 g. of bromine by warming on the water-bath in the presence of ultraviolet light. Hydrogen bromide was evolved and after 2 hours a clear yellow solution had been formed. On further warming a bromide crystallized, and after cooling in ice this was filtered. The filtrate on concentration afforded 6.7 g. (97%) of a slightly colored product, m.p. 163–164°. Recrystallization from dilute ethanol yielded an analytical sample of VII, m.p. 163–164°.

Anal. Calcd. for $C_{11}H_{10}O_6NBr$: C, 39.76; H, 3.01; N, 4.21. Found: C, 40.08; H, 3.25; N, 4.11.

β-(2-Nitro-3-methoxybenzoyl)-acrylic Acid (VIII).—When a mixture of 5.0 g. of VII, 20 cc. of glacial acetic acid, and 5.0 g. of anhydrous sodium acetate was warmed on the water-bath for 2 hours, sodium bromide was deposited. A large quantity of water and then a little hydrochloric acid were added. After standing in the cold overnight the crystalline solid was collected and crystallized from water (charcoal). There resulted 3.2 g. (89%) of light yellow crystals, m.p. 152–153°.

Anal. Calcd. for $C_{11}H_9O_6N$: C, 52.59; H, 3.59; N, 5.57. Found: C, 52.42; H, 3.63; N, 5.69.

***dl*-β-(2-Nitro-3-methoxybenzoyl)-alanine (IX).**—When 2.5 g. of VIII was allowed to interact for 2 days with 30 cc. of 15% aqueous ammonia, 2.65 g. of fine silky needles was deposited. It was recrystallized by dissolution in a little hot water followed by dilution with ethanol. On cooling 2.15 g. (80%) of pure IX was deposited, dec. 202°. The ninhydrin reaction was yellow, changing to orange to red-dish-orange.

Anal. Calcd. for $C_{11}H_{12}O_6N_2$: C, 49.21; H, 4.47; N, 10.44. Found: C, 49.14; H, 4.56; N, 10.61.

***dl*-3-Methoxykynurenine Hydrochloride (X).**—A solution of 2.0 g. of IX in 40 cc. of glacial acetic acid was added gradually to a cold solution of 6.0 g. of stannous chloride in 30 cc. of concentrated hydrochloric acid. Following the addition the mixture was warmed for 20 minutes to 80°, when the stannic chloride double salt of X crystallized. After standing overnight the solid was collected, washed with hydrochloric acid and then with acetic acid, and dissolved in water. Tin sulfide was precipitated with hydrogen sulfide, filtered and washed repeatedly with warm water. The aqueous liquors were concentrated under reduced pressure in an atmosphere of carbon dioxide. The remaining yellow sirup was dissolved in dilute hydrochloric acid, decolorized with charcoal, and the solution was again concentrated in vacuum. On cooling the residue 1.2 g. (52%) of colorless prisms, m.p. 160°, was obtained. The ninhydrin and the diazo reaction were both positive; on boiling with alkali an odor of ammonia and a jasmine-like odor were noted; the latter appears to be due to 2-amino-3-methoxyacetophenone.

Anal. Calcd. for $C_{11}H_{14}O_4N_2 \cdot 2HCl \cdot H_2O$: C, 40.24; H, 5.48; N, 8.51. Found: C, 40.30; H, 5.38; N, 8.51.

***dl*-3-Hydroxykynurenine Hydrobromide (XI).**—A solution of 1.30 g. of X in 20 cc. of 48% hydrobromic acid was boiled gently in an atmosphere of carbon dioxide, and the escaping methyl bromide was trapped as silver bromide.

The reaction was complete after 10 hours. The mixture was diluted with water, decolorized with charcoal, and concentrated under carbon dioxide and reduced pressure to a small volume. After cooling small crystals of XI were collected on a glass filter and dried immediately over alkali in a desiccator. Dilution and reconcentration of the mother liquor yielded a second crop. The yield was 1.40 g. (91.5%), dec. 233°; the compound is readily soluble in water to give a yellow solution.

Anal. Calcd. for $C_{10}H_{12}O_4N_2 \cdot 2HBr$: C, 31.08; H, 3.62; N, 7.25. Found: C, 31.31; H, 3.50; N, 7.01.

dl-**3-Hydroxykynurenine.**—A solution of 200 mg. of XI in a little water was neutralized with dilute aqueous ammonia. Yellow needles of the free amino acid crystallized without delay. After recrystallization (charcoal) it had the decomposition point 223°; yield 101 mg. (87%). The substance is almost insoluble in alcohol, slightly soluble in cold, but readily in hot water.

Ninhydrin reaction, reddish-purple; Folin–Denis reaction, dark blue; diazo reaction, positive; ferric chloride test, blood red; Weis urochromogen reaction, positive. The compound fluoresces in ultraviolet light. When boiled with alkali it produces a jasmine-like odor. These properties agree with those shown by the +chromogen from silkworm eggs, and the behavior of the two substances on paper chromatography also showed close agreement.

Anal. Calcd. for $C_{10}H_{12}O_4N_2 \cdot H_2O$: C, 51.50; H, 5.58. Found (dried at room temperature over alkali): C, 51.46; H, 5.53. Calcd. for $C_{10}H_{12}O_4N_2$: C, 53.57; H, 5.35; N, 12.50. Found (dried for 3 hours *in vacuo* at 140°): C, 53.80; H, 5.04; N, 12.37.

OSAKA, JAPAN RECEIVED AUGUST 14, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CONNECTICUT]

The Preparation of Amino Ethers and Diamines from Chloromethyl Ether and Butadiene[1]

BY LAWRENCE H. AMUNDSEN AND WILLIAM F. BRILL

1-Chloro-5-methoxy-2-pentene and 3-chloro-5-methoxy-1-pentene, readily obtainable as the 1,4- and 1,2-addition products, respectively, of chloromethyl ether to 1,3-butadiene, react readily with ammonia and with amines to give the expected unsaturated amino ethers. With the secondary chloride, however, there is partial allylic rearrangement during amination, so that along with the expected product there is formed also a considerable quantity of the same amino ether as is obtained from the primary chloride. Catalytic hydrogenation then gives saturated amino ethers that react with hydrobromic or hydriodic acid to give salts of halogenated amines which with ammonia or amines yield diamines.

In searching for a method other than that of the von Braun synthesis[2] for preparing unsymmetrical disubstituted pentamethylenediamines, the following procedure was investigated.

$$ClCH_2OCH_3 + CH_2{=}CHCH{=}CH_2 \xrightarrow{ZnCl_2}$$

$$ClCH_2CH{=}CHCH_2CH_2OCH_3 \xrightarrow[(\text{or } NH_3)]{R_2NH} \xrightarrow{NaOH}$$

I

$$R_2NCH_2CH{=}CHCH_2CH_2OCH_3 \xrightarrow{H_2}$$

II

$$R_2N(CH_2)_5OCH_3 \xrightarrow{HX}$$

III

$$(R_2NH(CH_2)_5X)X \xrightarrow[(\text{or } R_2NH)]{NH_3} R_2N(CH_2)_5NH_2$$

IV V

It was found that while it has no definite advantages in synthesizing diamines it is clearly superior to existing methods for preparing dimethylamino[3] and diethylamino[4] ethers (III). There are no previously reported methods for making the unsaturated amino ethers (II).

The addition of chloromethyl ether to butadiene, described by Straus and Thiel[5] as a sealed tube reaction tube reaction was run in larger quantities with equally good yields by passing butadiene gas through the chloromethyl ether. Emerson, Deebel and Longley[6] used a similar procedure to add several different chloro ethers to butadiene. The amination of the 1,4-addition product (I) with ammonia, dimethylamine or diethylamine produces the expected amino ether. The 1,2-addition product, 3-chloro-5-methoxy-1-pentene may also be aminated to produce otherwise unavailable amines. However, some rearrangement to (II) occurs in the latter reaction.

The possibility of preparing unsymmetrical pentamethylenediamines from the amino ethers (II) was demonstrated by splitting N,N-diethyl-5-methoxy-1-pentanamine with hydriodic acid and aminating the resulting N,N-diethyl-5-iodo-1-pentanamine with ammonium hydroxide.

Experimental

Chloromethyl ether was prepared by the method of Marvel and Porter.[7] Amines were isolated by acidifying the reaction mixture, evaporating the solvent and freeing the amine from its salt with saturated sodium hydroxide. They were dried over sodium hydroxide pellets prior to distillation. Reductions were accomplished at 30 lb. gage pressure using a Raney nickel catalyst. Neutral equivalents were obtained by titrating the amine with 0.1 *N* hydrochloric acid using a methyl red indicator.

Preparation of the Chloromethoxypentenes.—One thousand forty-six grams (13 moles) of chloromethyl ether was placed in a 3-l. flask equipped with a reflux condenser, a thermometer extending into the liquid and a sintered glass bubbling tube. Seven grams of freshly fused zinc chloride was added. Butadiene, measured with a flow meter, was passed into the solution. By confining the flow to 0.6 mole per hour and cooling the reaction flask with running water, the temperature was kept between 30 and 40°. When 14 moles of butadiene had been passed in, the volume of the purple-brown solution became constant at twice its original volume, and the reaction was then considered complete. Unreacted chloromethyl ether was hydrolyzed by shaking the reaction mixture with water. The product was extracted with ether and washed successively with dilute sodium bisulfite solution and water. The ether was removed and the remaining light yellow liquid distilled at 10 mm. through a 4-ft. helices-packed column. At 36°, 410 ml.

(1) From the M.S. thesis of W. F. Brill, June, 1948.
(2) von Braun, *Ber.*, **43**, 2864 (1910).
(3) Clark, *J. Chem. Soc.*, **103**, 1689 (1913).
(4) Elderfield, THIS JOURNAL, **68**, 1579 (1946).
(5) Straus and Thiel, *Ann.*, **525**, 151 (1936).
(6) Emerson, Deebel and Longley, *J. Org. Chem.*, **14**, 696 (1949).
(7) Marvel and Porter, "Organic Synthesis," Coll. Vol. II, John Wiley and Sons, Inc., 1940, p. 474.