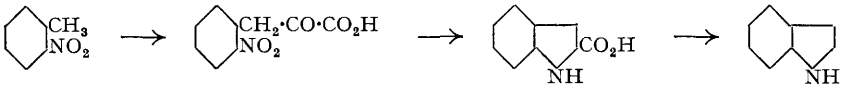
171. *Studies in the Indole Series. Part III. Improved Synthesis of Tryptophan.*

By J. Elks, D. F. Elliott, and B. A. Hems.

Indole-3-glyoxylic acid forms an anil which is smoothly decarboxylated in anisole to a product that, after hydrolysis, gives indole-3-aldehyde in high yield. This constitutes a convenient method for preparing this aldehyde from indole. By using catalytic hydrogenation in place of other methods of reduction, two of the published methods of preparing tryptophan have been improved, enabling it to be readily prepared in moderate quantity in the laboratory.

Boyd and Robson (*Biochem. J.*, 1935, **29**, 2256) prepared tryptophan by reductive hydrolysis of indolylidenehydantoin with ammonium sulphide, but we found the method very inconvenient and so decided to investigate other methods of reduction and hydrolysis. For this purpose it was necessary to prepare indole and indole-3-aldehyde in quantity.

Many methods of preparing indole are known (see summaries : Van Order and Lindwall, *Chem. Rev.*, 1942, **30**, 69; Stepanov and Polyakova, *Sintezy Dushistykh. Veshchetsv Sbornik Statei*, 1939, 127; Shorygin and Polyakova, *ibid.*, 1939, 130; *Khim. Referat. Zhur.*, 1940, No. 4, 114). A recent method (Tyson, *J. Amer. Chem. Soc.*, 1941, **63**, 2024), comprising the treatment of formo-*o*-toluidide with potassium alkoxides, has been used by us on the small scale with very satisfactory results, but for preparing larger quantities of indole the use of metallic potassium is attended by obvious disadvantages. For this reason the following series of reactions was studied :



Shorygin and Polyakova (*loc. cit.*) state that this is the best method, but we have not had access to the original paper.

Of the various methods used for condensing ethyl oxalate with *o*-nitrotoluene, that suggested by Mayer and Balle (*Annalen*, 1914, **403**, 1188) proved to be the most convenient, but their method of isolation was time-consuming and tended to destroy some of the product. A more satisfactory method of isolating the product is described in the experimental section.

The reduction and cyclisation of *o*-nitrophenylpyruvic acid to indole-2-carboxylic acid was conveniently effected by sodium hyposulphite [dithionite] (Cornforth and Robinson, J., 1942, 680) or ferrous sulphate and ammonia (Kermack, Perkin, and Robinson, J., 1921, **119**, 1625). Although, however, a number of attempts were made to reduce *o*-nitrophenylpyruvic acid catalytically, with Raney nickel as catalyst, wholly satisfactory results were not obtained by this method. The required product, indole-2-carboxylic acid, was obtained from hydrogenations carried out in both alcoholic and aqueous ammonia solutions; in the latter instance some 1-hydroxyindole-2-carboxylic acid was also obtained. Some samples of the nitro-acid tended to inactivate the catalyst, and in alcoholic solutions the temperature had to be carefully controlled, since reduction did not take place at room temperature, whereas decomposition occurred at about 80°. As the chemical methods of reduction mentioned above seemed fairly satisfactory, the catalytic method was not further studied.

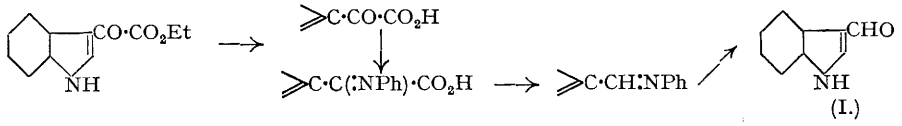
Ethyl indole-2-carboxylate was prepared in good yield on a small scale from ethyl pyruvate phenylhydrazone, prepared by reaction between ethyl methylacetoacetate and benzenediazonium chloride by treatment with a mixture of acetic and sulphuric acids, but the reaction was difficult to carry out on a larger scale.

Decarboxylation of indole-2-carboxylic acid was conveniently effected by heating, though for this purpose it was necessary that the acid should be pure and free from inorganic salts; no improvement in yield was effected by using catalysts or solvents. The above series of reactions to indole has also been studied by Cornforth and Robinson (*loc. cit.*), but they apparently used the method of Mayer and Balle without modification, and no details for the decarboxylation of indole-2-carboxylic acid are given.

Indole has been converted into its 3-aldehyde by the Reimer–Tiemann reaction (Ellinger and Flamand, *Ber.*, 1907, **40**, 3029; *Z. physiol. Chem.*, 1908, **55**, 8; Boyd and Robson, *Biochem. J.*, 1935, **29**, 555) and by reaction of indolylmagnesium iodide with ethyl formate (Majima and Kotake, *Ber.*, 1922, **55**, 3859; Potokhin, *J. Russ. Phys. Chem. Soc.*, 1927, **59**, 761; *Chem. Abs.*, 1928, **22**, 3409), but satisfactory yields were not obtained by either of these reactions. Several attempts were made to obtain the aldehyde by reaction between indole

and diphenylformamidine under various conditions, or by treating indolylmagnesium iodide with ethyl orthoformate, but these were unsuccessful.

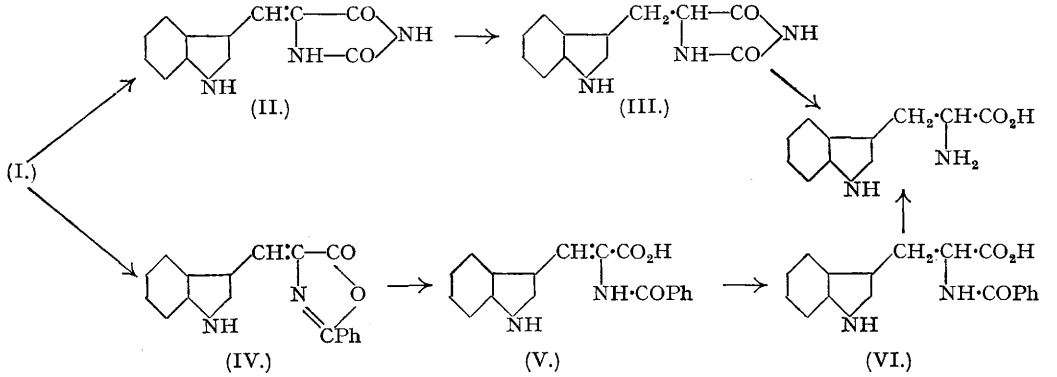
It was found that indoleglyoxylic acid readily formed an *anil* which was decarboxylated smoothly in boiling anisole to indolealdehyde anil. This was hydrolysed to indolealdehyde when the mixture was steam-distilled to remove the decarboxylation solvent. The yields at all stages were excellent, and the aldehyde so obtained was easily purified. Methyl indoleglyoxylate was first prepared from indolylmagnesium iodide and oxalic acid half ester chloride (Majima and Shigematsu, *Ber.*, 1924, **57**, 1453; Baker, J., 1940, 458), but it was later found that the ethyl ester could be readily prepared by reaction between indole and ethyl oxalate (cf. condensation of 2-methylindole with ethyl oxalate; Angeli and Marchetti, *Chem. Zentr.*, 1908, I, 739). In the presence of alcoholic sodium ethoxide, very poor yields of the glyoxylic ester were obtained; these were improved by carefully drying the reagents and by using pyridine as a catalyst in the condensation (cf. Grundmann, *Ber.*, 1937, **70**, 1148 : Blout, Fried, and Elderfield, *J. Org. Chem.*, 1943, **8**, 37). The ester after hydrolysis was converted into indole-3-aldehyde in the manner described above, the reactions being as follows :



(I.)

Condensation of the aldehyde (I) with hydantoin according to Boyd and Robson (*loc. cit.*) gave indolylidenehydantoin (II) in excellent yield, and although this substance is sparingly soluble in all solvents, a suspension in dilute sodium hydroxide solution or aqueous alcohol was readily reduced catalytically in presence of Raney nickel, to 4-3′-indolylmethylhydantoin (III), which is fairly soluble in both media; it was readily isolated in good yield by acidifying or evaporating the solution, and was obtained sufficiently pure for hydrolysis to tryptophan by baryta.

Catalytic reduction may also be applied to the preparation of tryptophan by Ellinger and Flamand's azlactone method (*loc. cit.*). The azlactone (IV), prepared according to these authors, was hydrolysed to α-benzamido-β-indole-3-acrylic acid (V), which in alkaline solution was reduced catalytically to benzoyltryptophan (VI). In this reaction Raney nickel was superior to platinum as a catalyst, since reduction with the latter was incomplete unless successive quantities of fresh catalyst were added. The hydrogenation of α-acetamidocinnamic acid with platinum oxide (Adams) catalyst, in acetic acid solution, followed by hydrolysis to phenylalanine, has been described by Herbst and Shemin (*Org. Synth.*, 1939, **19**, 67).



The dl-N-*benzoyltryptophan* so obtained was hydrolysed with concentrated baryta solution. Pure benzoyltryptophan according to Berg, Rose, and Marvel (*J. Biol. Chem.*, 1929, **85**, 207) has m. p. 104—105°, but our product, which was racemic, had m. p. 193—194°. Presumably the compound described by Berg *et al.* is not the racemic compound, since the conditions used in the benzoylation were very mild; unfortunately, the optical rotation is not recorded. The identity of the amino-acid isolated by these methods was established by colour reactions and by the preparation of the acetyl derivative, which was found to be identical in all respects with that of racemised natural tryptophan.

Experimental.

o-Nitrophenylpyruvic Acid.—The reaction conditions described by Mayer and Balle (*loc. cit*) were used, but their method of isolation was improved as follows : The dark red reaction mixture from *o*-nitrotoluene (1096 g.) was cooled to 0° and carefully acidified with a mixture of concentrated hydrochloric acid (800 c.c.) and water (500 c.c.), the temperature being kept below 10°. The alcohol was then removed in a vacuum at 30—40°, the oily residue dissolved in ether, and filtered from sodium chloride. The ethereal solution was then thoroughly shaken with two portions (8000 and 2700 c.c.) of ice-cold N-sodium hydroxide, and the combined alkaline extracts were washed with a little fresh ether and acidified below 10° with dilute hydrochloric acid (1 : 1). The acid was extracted with ether, the ether dried and evaporated, and the residue triturated with benzene. The crude product had m. p. 115° and was used in this state (yield 654 g.; 60% based on *o*-nitrotoluene consumed). The combined ethereal layers from the alkali extraction contained *o*-nitrotoluene (381 g.), which was recovered.

Ethyl Indole-2-carboxylate.—A mixture of ethyl pyruvate phenylhydrazone (20 g.) (Japp and Klingemann, *Ber.*,

1887, **20**, 2942; *Annalen*, 1888, **247**, 208) and a solution of concentrated sulphuric acid (7·5 c.c.) in glacial acetic acid (100 c.c.) was gently warmed to 45°, and the source of heat removed. An exothermic reaction took place, the internal temperature rising to 85°. The solution was allowed to cool to room temperature, and poured into ice-water (500 c.c.). The crude carbethoxyindole (yield, 10·6 g.; 58%), after being separated, washed, and crystallised from alcohol, had m. p. 122°, yielding indole-2-carboxylic acid, m. p. 202—204°, on hydrolysis with sodium hydroxide.

Alcoholic hydrogen chloride, alcoholic sulphuric acid, aqueous sulphuric acid, zinc chloride in alcohol, or hydrogen bromide in acetic acid under varied conditions were in some instances without effect on ethyl pyruvate phenylhydrazone; in others, decomposition occurred.

Indole-2-carboxylic Acid.—The method of Kermack, Perkin, and Robinson (*loc. cit.*) was used, but the following isolation procedure was more convenient for large-scale working. The reaction mixture from *o*-nitrophenylpyruvic acid (810 g.) after 12 hours' heating at 100° was cooled to room temperature and acidified, with stirring, with concentrated hydrochloric acid (10·8 l.), the temperature being allowed to rise to 50°, but not above. After standing overnight, the brown solid was filtered off, washed with water, and purified by dissolving it in an excess of hot 2N-ammonia, filtering, cooling, and acidifying the filtrate with dilute hydrochloric acid (1 : 1). The buff precipitate was filtered off, washed, and dried; a further quantity of acid was obtained from the filtrate by ether extraction. The crude product had m. p. 190—194°; yield 378 g., 60·6%.

Indole.—For successful decarboxylation it was necessary to use pure indole-2-carboxylic acid; impure acid gave very low yields. The acid described above was decolorised with charcoal and crystallised from aqueous alcohol. It had m. p. 203°. The purified acid (1000 g.) was heated at 240° in a 4-l. flask fitted with a long air-condenser until decarboxylation ceased (1¾ hours). The mass was then cooled and distilled in a vacuum. Indole distilled as a colourless liquid, b. p. 120°/2—3 mm., which rapidly solidified in the receiver. It was further purified by pouring when molten into ice-cold, well-stirred petroleum (1600 g., b. p. 80—100°). After standing for 12 hours at 0°, the indole was filtered off and dried (400 g., 55%); m. p. 51—52°.

Ethyl Indole-3-glyoxylate.—A number of modifications of the reaction procedure were investigated; the following gave the best results. To a solution of sodium (20 g.) in alcohol (250 c.c.; 0·03% H_2O) were added pyridine (dried over barium oxide; 50 c.c.), ethyl oxalate (70 g.), and indole (50 g.). The mixture was then heated on the steam-bath for 4 hours, with exclusion of moisture, cautiously at first because of frothing. The dark brown solution was then cooled to room temperature and poured, with stirring, on crushed ice (300 g.) and 2N-hydrochloric acid (800 c.c.). The yellowish-brown solid was filtered off, washed with water, dried in a vacuum over concentrated sulphuric acid, powdered, and washed thoroughly with cold ether to remove tarry impurities. The air-dried solid had m. p. 176—178° (48 g.; 50%) and was pure enough for subsequent work.

Indole-3-glyoxylic Acid Anil.—For the preparation of this compound it is preferable to use the glyoxylic acid in a moist condition. The quantity of acid to be expected from the crude ethyl indoleglyoxylate was therefore first estimated by hydrolysing a small portion by warming to 40° with five times its weight of 2N-sodium hydroxide solution. The ester rapidly dissolved, and after it had stood for ½ hour at room temperature, charcoal was added to remove a small quantity of tar, and the solution was filtered and acidified with hydrochloric acid (1 : 1). The canary-yellow precipitate was filtered off, washed, dried, and crystallised from ethyl acetate. The pure acid had m. p. 215° (decomp.), not depressed on admixture with a specimen of the acid prepared by Baker's method (*loc. cit.*).

Ethyl indole-3-glyoxylate (48 g., equivalent to 33·4 g. of acid) was hydrolysed as above, and the precipitated acid, after filtration (pump) and thorough washing with water, was added to a mixture of aniline (16·7 g.) and water (167 c.c.). The suspension was shaken and heated at 100° until a clear solution was formed. After cooling, the pink crystals were filtered off and dried in a vacuum. The *anil* had m. p. 180° (decomp.) after softening at 140° (yield 47 g., 80% calc. on crude ester) (Found: N, 10·4. $C_{16}H_{12}O_2N_2$ requires N, 10·6%).

Indole-3-aldehyde.—The anil (47 g.) was mixed with anisole (188 c.c.) and heated at about 140° (oil-bath) until evolution of carbon dioxide subsided. The mixture was then refluxed until the solid matter dissolved and gas evolution ceased (1 hour). After cooling, the brown solution was diluted with water (200 c.c.), and anisole and aniline removed by steam-distillation. The liquid then remaining was cooled, and the aldehyde (24 g.; 93%) filtered off and dried. The aldehyde, after crystallisation from aqueous alcohol, had m. p. 198°, not depressed on admixture with an authentic specimen.

4-3′-*Indolylmethylhydantoin.*—(a) Indolylidenehydantoin, prepared from indole-3-aldehyde by Boyd and Robson's method (*loc. cit.*) (5 g.), was suspended in N-sodium hydroxide (50 c.c.) and hydrogenated at room pressure and temperature in presence of Raney nickel catalyst (5 g.). The solid dissolved during the hydrogenation, which was complete in 8 hours. The catalyst was filtered off, and the filtrate acidified with dilute hydrochloric acid. The colourless, crystalline precipitate (5 g.) had m. p. 214° and was sufficiently pure for hydrolysis to tryptophan. A sample, crystallised from water, had m. p. 218—220°. Majima and Kotake (*Ber.*, 1922, **55**, 3859) prepared this substance by sodium amalgam reduction of indolylidenehydantoin and gave m. p. 220—221°.

(b) Indolylidenehydantoin (20 g.), suspended in aqueous alcohol (65% EtOH; 200 c.c.), was shaken with hydrogen at 100—110° and 50 atm., in presence of Raney nickel (20 g.) for 2 hours. The reaction mixture was allowed to cool to 80° with continued shaking, and then filtered hot and the catalyst washed with hot 65% alcohol. The filtrate was evaporated to small bulk, giving a crystalline residue (16·3 g.; 80%), m. p. 218°, of 4-3′-indolylmethylhydantoin.

dl-N-*Benzoyltryptophan.*—α-Benzamido-β-indole-3-acrylic acid (Ellinger and Flamand, *loc. cit.*) (35 g.), dissolved in N-sodium hydroxide (350 c.c.), was hydrogenated at room temperature and pressure in presence of Raney nickel (17 g.). When hydrogenation was complete (5 hours), the catalyst was filtered off, washed with water, and the filtrate cautiously acidified with dilute hydrochloric acid. The pink solid (34 g.; 96·5%) had m. p. 193—194° after crystallisation from aqueous alcohol. Difficulty was experienced in obtaining a pure sample of this *substance*, for it rapidly became pink on heating in solvents (Found: C, 69·5; H, 5·1; N, 8·7. $C_{18}H_{16}O_3N_2$ requires C, 70·1; H, 5·2; N, 9·1%).

dl-*Tryptophan.*—(a) 4-3′-Indolylmethylhydantoin (13·8 g.), barium hydroxide (cryst., 69 g.), and water (420 c.c.) were mixed and refluxed for 24 hours. The solution evolved ammonia and deposited barium carbonate. The condenser was then removed, and steam allowed to escape to expel the last traces of ammonia. After dilution of the solution to 1200 c.c., it was heated on the steam-bath, and a rapid stream of carbon dioxide passed in until the supernatant liquor was neutral to litmus. The barium carbonate, thus obtained in granular form, was filtered off and washed with boiling water (300 c.c.); the filtrate was freed from the last trace of barium ions by addition of 2N-sulphuric acid (rhodizonic acid indicator). After a further filtration the solution was evaporated to dryness in a vacuum. The residue of tryptophan (11·1 g., 90%) was washed with alcohol and dried. The m. p. was 284° (decomp.) with darkening from 260° (Found: C, 64·8; H, 5·9; N, 13·4. Calc. for $C_{11}H_{12}O_2N_2$: C, 64·7; H, 5·9; N, 13·7%). The overall yield (72%) on indolylidenehydantoin is the same as that recorded by Boyd and Robson (*loc. cit.*).

(b) *dl-N*-Benzoyltryptophan (finely powdered, 30 g.) was hydrolysed as above with barium hydroxide (165 g.) and water (900 c.c.). After removal of barium ions with 2N-sulphuric acid, the filtered solution was evaporated to dryness in a vacuum, and the residue washed with alcohol to remove benzoic acid. The pale yellow product (15 g., 75%), m. p. 274°, after crystallisation from hot water, had m. p. 282° (decomp.) with darkening from 260° (Found C, 64·7; H, 6·0;

N, 13·3%). The yield of tryptophan from α-benzamido-β-indole-3-acrylic acid is 72%, whereas Ellinger and Flamand (*loc. cit.*) recorded a 15% yield by reduction with sodium in alcohol.

The colours obtained with the Ehrlich and the Hopkins–Cole reagent from the two samples of tryptophan were identical with those obtained from natural tryptophan. The acetyl derivatives, prepared by the method of Berg, Rose, and Marvel (*loc. cit.*) from the above samples of tryptophan, had m. p. 203°, alone or on admixture with an authentic specimen of *dl*-*N*-acetyltryptophan, m. p. 203°, prepared from natural tryptophan.

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