34. *The Adamkiewicz, Hopkins and Cole, and Rosenheim Tests for Tryptophan. An Investigation of the Configuration of the Organic Molecule responsible for the Colour Formation and its Bearing on the Constitution of Yohimbine, with a Note on the Action of Formaldehyde on Tryptophan.*

By DOUGLAS GRAHAM HARVEY, ERIC JOHN MILLER, and WILLIAM ROBSON.

If to an aqueous solution of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid,* concentrated sulphuric acid containing a trace of an oxidising agent is added so that the two liquids do not mix, the play of colours obtained at their zone of contact is similar in all respects to that obtained when tryptophan is subjected to the Adamkiewicz procedure as modified by Hopkins and Cole (*Proc. Roy. Soc.*, 1901, **68**, 21) and by Rosenheim (*Biochem. J.*, 1906, **1**, 233).

Evidence is advanced that the reaction, as described by these authors, consists of two stages : (1) the formation of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid or a derivative thereof, (2) the oxidation of the latter substance to the blue pigment which is characteristic of the reaction.

The colour reaction of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid with concentrated sulphuric acid containing an oxidising agent, and its use for detecting the presence of oxidising agents in sulphuric acids of commerce, are described.

Only those compounds possessing the structure of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid have been found to give the colour reaction mentioned when treated in the above manner.

Yohimbine behaves towards concentrated sulphuric acid containing an oxidising agent in the same way as 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid. It would thus appear that the carbomethoxy-group present in the yohimbine molecule is at C_5 † (V) and not at C_{16} as postulated by Hahn, Kappes, and Ludewig (*Ber.*, 1934, **67**, 686).

THAT a dilute aqueous solution of egg-white, when added drop by drop to concentrated sulphuric acid, produces a range of colours starting from green and passing through yellow, orange, red, and ending in violet, was first shown by Adamkiewicz (*Pfluger's Arch.*, 1874, **9**, 156). He found, moreover, that whilst the colour obtained depended upon the concentration of the protein in the final mixture, if the egg-white had been previously treated with glacial acetic acid, only a violet colour resulted. It was Adamkiewicz's view that the coloured products arose from the interaction of substances liberated from the protein by the sulphuric acid and that the acetic acid only modified the reaction.

Udransky's view (*Z. physiol. Chem.*, 1888, **12**, 395) that one of the substances involved in the colour formation was furfural was negatived by the work of Hopkins and Cole (*loc. cit.*), who showed that the reaction occurred only when the acetic acid was impure, the impurity concerned being glyoxylic acid.

That glyoxylic acid was responsible for the reaction was doubted by Rosenheim (*loc. cit.*), who emphasised that whilst a typical Adamkiewicz reaction could be obtained by the addition of " commercial " sulphuric acid to a protein or a tryptophan solution previously treated with a minimum amount of formaldehyde, no reaction could be obtained if the sulphuric acid employed was pure. Rosenheim then demonstrated that the presence of an oxidising agent, *e.g.*, ferric chloride, was a necessary adjunct for the test. These findings were confirmed in part by Dakin (*J. Biol. Chem.*, 1906, **2**, 289), who nevertheless supported the view that the impurity in the acetic acid in question was glyoxylic acid.

* Named and numbered in accordance with the suggestion of Gulland, Robinson, Scott, and Thornley (J., 1929, 1924), adopted by King and Stiller (J., 1937, 466) and by Henry (" Plant Alkaloids," 1939, p. 464).

† The numbering of the carbon atoms in the yohimbine skeleton is that proposed by Barger and Scholz (*Helv. Chim. Acta*, 1933, **16**, 1343). So far as the carboline fraction of this molecule is concerned, it differs from that suggested by Gulland *et al.* referred to above.

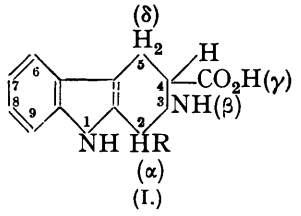
He, moreover, contended that pure glyoxylic acid reacted with tryptophan and pure sulphuric acid to give the characteristic colour.

Rosenheim's contention that the constituent of impure acetic acid which reacted with tryptophan was formaldehyde found a supporter in Voisenet (*Compt. rend.*, 1918, **166**, 789), who concluded that the pigments obtained by the Hopkins and Cole and by the Rosenheim techniques were the same substance.

In the meantime, Mottram (*Biochem. J.*, 1913, **7**, 249) had described a failure of the glyoxylic acid test as being due to the presence of an excess of oxidising agent in the sulphuric acid employed, a finding confirmed by Breidhal (*Biochem. J.*, 1915, **9**, 36), who showed that such acids could be used for the test after they had been treated with suitable amounts of reducing agents.

The realisation that the Adamkiewicz reaction might be made the basis of a method for the estimation of the tryptophan content of protein led Fearon (*Biochem. J.*, 1920, **14**, 548) to investigate the mode of formation of the blue pigment. Showing that the tests described by Hopkins and Cole and by Rosenheim depended on the use of glyoxylic acid and formaldehyde respectively, he stated that in each case two distinct coloured derivatives could be formed according to the amount of the reactant used. One product was red and the other blue, the latter arising when three molecules of formaldehyde reacted with two molecules of the amino-acid. The formula which he put forward for the latter compound was based on rather meagre evidence.

In a recent series of papers by various authors (Hahn, Barwald, Schales, and Werner, *Annalen*, 1935, **520**, 107; Hahn and Werner, *ibid.*, p. 123; Jacobs and Craig, *J. Biol. Chem.*, 1936, **113**, 759; Wadsworth and Pangborne, *ibid.*, 1936, **116**, 423; Harvey and Robson, J., 1938, 97) it has been amply demonstrated that tryptophan condenses with simple aliphatic aldehydes in aqueous solution over a wide range of p_H to yield 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acids of the general formula (I). From the extreme ease with which carboline formation proceeds—it was shown by Harvey and Robson (*loc. cit.*) that the corresponding 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid crystallises overnight from a concentrated aqueous mixture of molecular quantities of tryptophan and acetaldehyde kept at room temperature—it appears reasonable to assume that this reaction forms the first stage of the Adamkiewicz test for tryptophan. Should this hypothesis be correct, then, in the light of Rosenheim's findings, the second stage would consist in the oxidation of the carboline formed in the first stage.



(I.)

That such an interpretation of the course of the reaction is probably correct was shown as follows: When pure concentrated sulphuric acid is run into a test-tube containing a dilute acid solution of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid in such a way that the two liquids do not mix, no colour develops at their zone of contact. Should the sulphuric acid, however, contain a trace of an oxidising agent, a play of colours is obtained at the junction of the liquids similar in every respect to that seen when tryptophan is submitted to the Hopkins and Cole and the Rosenheim reactions.

Similar findings were obtained in a slightly different way. For example, when a crystal of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid was dropped into pure sulphuric acid, no colour development occurred. On the other hand, when the same compound was added to pure acid containing an oxidising agent, hereafter called the " reagent ", a blue colour commenced to develop within a few minutes, attained a maximum intensity after 15—20 minutes, and then slowly gave way to an olive-green, which in turn was replaced by a permanent yellow colour. The final solution possessed a faint green fluorescence resembling that often seen in the final stage of the Adamkiewicz reaction. During the course of this study a number of carbolines, derived not only from tryptophan but also from *r*-α-methylamino-β-3-indolylpropionic acid (Miller and Robson, J., 1938, 1910), have been tested with the " reagent " and in all cases the range of colours described above has been obtained. The reaction is exceedingly sensitive.

It will be clear from the foregoing that 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic

acid may be utilised for testing samples of concentrated sulphuric acid for the presence of oxidising agents. Tested in this way, certain samples of sulphuric acid purchased on the market and labelled " commercial " have been found to contain smaller amounts of oxidising agents than others labelled " pure ".

It became of interest, at this stage, especially in view of the bearing which the reaction was considered to have on a problem referred to below, to gain further knowledge regarding the chemical grouping in the molecule of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid which is responsible for the colour development.

Attempts were first made to isolate the pigment from the sulphuric acid mixture when the blue colour had reached its maximum intensity. When the blue solution was mixed with a large volume of water, the colour quickly disappeared and no solid could be isolated from the resulting yellow mixture. This is contrary to the experience of Fearon (*loc. cit.*), who isolated his blue pigment by such a procedure. The pigment could not be extracted from the mixture by such organic solvents as benzene, light petroleum, chloroform, and carbon disulphide, and decolorisation of the solution took place when sulphur dioxide was bubbled through it, the colour being restored by removing the sulphur dioxide with a current of nitrogen.

Such a mode of attack not offering much hope of success, an investigation was undertaken of the colour, if any, produced by the action of the " reagent " on those compounds which may be considered as forming part, or possessing a structure similar to that, of the molecule of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid.

Table of compounds tested and their colour reactions with the " reagent."

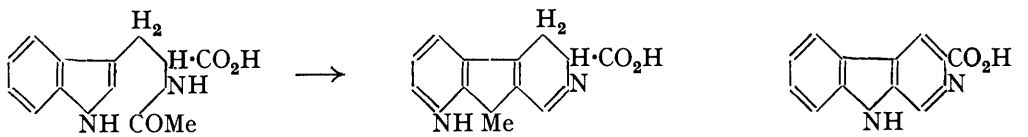
Benzene	Colourless
Proline (Dakin, *J. Biol. Chem.*, 1920, **44**, 499)	,,
Piperidine-2-carboxylic acid (Clemo and Ramage, J., 1931, 437)	,,
7-Hydroxy-1 : 2 : 3 : 4-tetrahydro*iso*quinoline-3-carboxylic acid	,,
Indole	Pale pink (permanent)
6-Methoxyindole (Harvey and Robson, J., 1938, 97)	,, ,, ,,
α-Methylindole (Fischer, *Annalen*, 1886, **236**, 126)	Colourless
β-Methylindole (*idem, ibid.*)	,,
αβ-Dimethylindole (*idem, ibid.*)	,,
Carbazole	Deep green (permanent)
l-Tryptophan (Cole, " Practical Physiological Chemistry," 9th ed., 1933)	Pale pink ⟶ weak purple ⟶ yellow with strong fluorescence
l-Tryptophan methyl ester hydrochloride (Abderhalden and Kempe (*Z. physiol. Chem.*, 1907, **52**, 207)	,, ,, ,, ,, ,,
r-α-Methylamino-β-3-indolylpropionic acid (Miller and Robson, J., 1938, 1910)	,, ,, ,, ,, ,,
Tryptamine hydrochloride (Ewins, J., 1911, **99**, 270)	,, ,, ,, ,, fluorescence less marked
2 : 3 : 4 : 5-Tetrahydro-β-carboline-4-carboxylic acid	" Carboline-blue " colour reaction
2-Methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid (Harvey and Robson, *loc. cit.*)	,, ,, ,, ,,
8-Methoxy-2-methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid (Harvey and Robson, *loc. cit.*)	,, ,, ,, ,,
2-Phenyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid	,, ,, ,, ,,
3-*Methyl*-2 : 3 : 4 : 5-*tetrahydro-β-carboline*-4-*carboxylic acid*	,, ,, ,, ,,
2 : 3-*Dimethyl*-2 : 3 : 4 : 5-*tetrahydro-β-carboline*-4-*carboxylic acid*	,, ,, ,, ,,
2-*Phenyl*-3-*methyl*-2 : 3 : 4 : 5-*tetrahydro-β-carboline*-4-*carboxylic acid*	,, ,, ,, ,,
2-*Hydroxymethyl*-2 : 3 : 4 : 5-*tetrahydro-β-carboline*-4-*carboxylic acid*	Immediate blue, quickly giving way to a deep olive-green, which in turn is gradually replaced by a greenish-yellow fluorescence
2 : 3 : 4 : 5-*Tetrahydro-β-carboline*-2 : 4-*dicarboxylic acid*	Immediate blue tinged with pink, changing to purple, then to brownish-red
Methyl 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylate hydrochloride	" Carboline-blue " colour reaction
Norharman	Yellow solution with conspicuous fluorescence
Harman (Harvey and Robson, *loc. cit.*)	,, ,, ,, ,, ,,
Harmine (Harvey and Robson, *loc. cit.*)	,, ,, ,, ,, ,,

Harmaline hydrochloride	Intense yellow-brown, changing quickly to a permanent clear amber
Tetrahydroharmine (Perkin and Robinson, J., 1919, **115**, 933)	Weak purple, changing quickly to olive-green then to yellow with a green-yellow fluorescence
N-Methyltetrahydroharmine (*idem, ibid.*)	,, ,, ,, ,, ,,
2-Methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-2-carboxylic acid (Hahn and Werner, *Annalen*, 1935, **520**, 123)	Immediate transient blue, changing to bright green, then shortly to a permanent yellow
2-*m*-Hydroxybenzyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-2-carboxylic acid (*idem, ibid.*)	,, ,, ,, ,, ,,
Norharman-2-carboxylic acid (Kermack, Perkin, and Robinson, J., 1921, 1602)	Yellow fluorescence
Norharman-4-carboxylic acid	,, ,,

Examination of the results tabulated above shows that the precursor of the blue pigment must possess a complex structure. Apart from indole and 6-methoxyindole, which yield a pink coloration, the simple molecules such as benzene, proline, piperidine-2-carboxylic acid, as might be expected, do not yield a colour with the " reagent." With carbazole a permanent deep green colour is obtained. Turning to the β-carboline bases, norharman, harman, and harmine, each gives a yellow solution characterised by a conspicuous fluorescence, and it is only when their molecules are fully hydrogenated in the pyridine ring, as in 2 : 3 : 4 : 5-tetrahydro-β-carboline, that a range of colours approximating to that described above is obtained. With the latter compound, however, the initial colour is purplish and the life period of the colour range considerably shorter than that obtained from 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid. A similar intensification of the colour obtained with the " reagent " following reduction of the compound under examination is seen on passing from norharman-2-carboxylic acid, which yields a yellow fluorescence, to 2-methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-2-carboxylic acid, which gives rise to a blue coloration, which, however, is not the same as that produced by its isomer, 2-methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid, in that it is tinged with pink and changes very rapidly to a permanent brownish-red colour.

From such findings, it would seem that, for the production of the range of colours described above, it is essential that the β-carboline nucleus be hydrogenated in positions 2, 3, 4 and 5 and carry a carboxyl group in position 4.

Whilst the exact structure of the blue pigment has not been definitely ascertained, certain indications of its probable nature exist. Norharman-4-carboxylic acid (IV) (King and Stiller, J., 1937, 466)—the second oxidation product of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid—yields with concentrated sulphuric acid a yellow fluorescence similar to that obtained when the mother substance is treated with the " reagent." The question therefore arises whether the intermediate compound, 4 : 5-dihydro-β-carboline-4-carboxylic acid (III), is the blue pigment. So far, however, all attempts to synthesise a compound (III) of such a structure from acetyltryptophan (II) by ring closure have proved unsuccessful.



(II.) (III.) (IV.)

The Action of Formaldehyde on Tryptophan.

If formaldehyde is added to (*a*) an aqueous acid solution of tryptophan, and the mixture boiled (Jacobs and Craig, *loc. cit.*), (*b*) an aqueous solution of tryptophan buffered at p_H 8·0, and the mixture kept at 38° (Wadsworth and Pangborne, *loc. cit.*), or (*c*) a solution of the sodium salt of tryptophan, and the mixture kept at 38°, the product in each case is 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid, m. p. 306°.

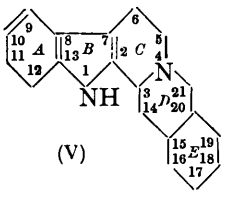
On the other hand, Homer (*Biochem. J.*, 1913, **7**, 101) has shown that, if formaldehyde is allowed to react with a neutral aqueous solution of tryptophan at 38°, a granular crystal-

line substance, m. p. 226—240°, separates after a few hours. Homer assigned to this compound the empirical formula $C_{12}H_{12}O_2N_2,2H_2O$ and showed that it can be changed very readily into a second compound, called by her the " ether oxidation product ", m. p. 324°. Ascribing to the latter compound the formula $C_{24}H_{26}O_5N_4$, she regarded it as a dimethylenetryptophan complex. In our hands the compound, m. p. 226—240°, undergoes the transformation described by Homer, but the product so obtained melts at 306°. It gives the " carboline-blue " colour reaction described above, yields norharman on oxidation with dichromate, and a mixture of it and 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid melts at 306°. The " ether oxidation product " is therefore 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid.

This finding would indicate that the compound, m. p. 226—240°, is α-hydroxymethylamino-β-3-indolylpropionic acid, *i.e.*, the simple addition product of formaldehyde and tryptophan, which substance, crystallising with one molecule of water, would have the formula $C_{12}H_{14}O_3N_2,H_2O$ and not that suggested by Homer. Baker and Happold (*Biochem. J.*, 1940, **34**, 657) state that all their preparations of this substance made according to Homer melt at 196° and that in view of the discrepancies in the analytical figures obtained for their product they doubt " whether the action of formaldehyde on *l*-tryptophan is as simple as the structures assigned by Homer suggest ". Results similar to those of Baker and Happold have been obtained in this laboratory and are due, it is believed, to the presence, in the required product, of 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid, the amount of which increases as the time of incubating the mixture of formaldehyde and tryptophan is extended.

Constitution of Yohimbine, $C_{19}H_{22}N_2(OH)(CO_2Me)$.—Apart from the light that the present work throws on the mechanism of the Adamkiewicz test, it would appear that the colour reactions given by 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid are of particular value with reference to the problem of the constitution of yohimbine.

This alkaloid, whose skeletal structure was shown by Barger and Scholz (*loc. cit.*) and by Hahn, Kappes, and Ludewig (*loc. cit.*) to be as depicted (V), reacts with the " reagent " to yield a range of colours similar in every respect to that given by the 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acids. It would appear, therefore, that the carbomethoxy-group present in the alkaloid is attached to C_5 (cf. footnote, page 153). Such a conclusion conflicts with that of Hahn *et al.* (*loc. cit.*) and of Barger (Thorpe's " Dictionary of Applied Chemistry," Suppl. Vol., 1935, 720), who are of the opinion that the carboxyl group is located at C_{16}. According to this view a suitable starting point for the synthesis of the alkaloid would be tryptophan methyl ester and not tryptophan as suggested by Hahn and Werner (*loc. cit.*). This idea is being pursued practically.



(V)

Experimental.

The " reagent " consists of pure sulphuric acid containing an oxidising agent, *e.g.*, ferric chloride, potassium persulphate, sodium nitrite, nitroprusside, or ferricyanide. Most samples of " commercial " sulphuric acid have been found suitable for the purpose described.

7-Hydroxy-1 : 2 : 3 : 4-tetrahydroisoquinoline-3-carboxylic Acid.—Strict observance of the conditions detailed by Pictet and Spengler (*Ber.*, 1911, **44**, 2030) repeatedly failed to yield this product in a pure form by the action of methylal and concentrated hydrochloric acid on tyrosine. Tests, therefore, were carried out on the amorphous compound obtained.

α-Hydroxymethylamino-β-3-indolylpropionic Acid.—*l*-Tryptophan (0·6 g.) was dissolved in water (12 ml.), formalin (2 ml.) added, and the mixture incubated at 38° for not more than 3—4 hours. The colourless, crystalline, but rather granular solid which had then separated was collected, washed with cold water, and dried in a vacuum. Yield, 60% (Found : N, 11·2; loss after drying at 130°/5 mm. for 4 hours, 13·9. Calc. for $C_{12}H_{12}O_2N_2,2H_2O$: N, 11·1; H_2O, 14·2%).

2 : 3 : 4 : 5-Tetrahydro-β-carboline-4-carboxylic Acid.—This was obtained in three ways. (1) A solution of α-hydroxymethylamino-β-3-indolylpropionic acid in dilute aqueous ammonia was boiled for 30 minutes. The solution was then clarified with charcoal, filtered, and con-

centrated to a small volume. The product separated in colourless leaflets, m. p. 306°. (2) A solution of the above amino-acid in water was boiled for 2 hours, concentrated to a small volume, and allowed to cool. The product, recrystallised from boiling water, had m. p. 306°. (3) *l*-Tryptophan (0·2 g.; 1 mol.) was dissolved in N/10-sodium hydroxide (10 ml.; 1 mol.) containing formalin (0·11 ml.; 1·1 mols.), and the mixture incubated at 38° for 15 hours. When the p_H of the cold solution was adjusted to approximately 6·5 by the addition of N/10-hydrochloric acid, 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid, m. p. 306°, was precipitated. Yield, 80%.

Norharman.—2 : 3 : 4 : 5-Tetrahydro-β-carboline-4-carboxylic acid (0·45 g.) was dissolved in boiling water (250 ml.) and treated with 10% potassium dichromate solution (30 ml.) and glacial acetic acid (6 ml.). The immediate formation of a cloudiness in the solution was followed by the evolution of carbon dioxide. Boiling was continued for about 1½ minutes, the solution then cooled, and the excess of oxidising agent removed by sulphur dioxide. The mixture was rendered alkaline with sodium carbonate solution and extracted five times with ether. The extract was evaporated to dryness, and the residue recrystallised from methyl alcohol, forming long colourless needles, m. p. 200°. Kermack, Perkin, and Robinson (*loc. cit.*) give 198·5°. The yield of the pure product was 50—60% (Found : N, 16·6. Calc. for $C_{11}H_8N_2$: N, 16·6%).

Methyl 2-Methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylate Hydrochloride.—Absolute methyl alcohol (5 ml.) in which was suspended 2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic acid (0·25 g.) was saturated with hydrogen chloride; after a few minutes the ester hydrochloride separated in long colourless needles. After some 15 minutes the product was collected, sucked dry, washed with a few drops of cold ethyl acetate, and dried over sulphuric acid (yield, quantitative). It melted at 264° (decomp.) and was extremely soluble in alcohol and water, but less so in ethyl acetate and ether (Found : N, 10·0. $C_{14}H_{16}O_2N_2$,HCl requires N, 10·0%).

2-Hydroxymethyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic Acid.—*l*-Tryptophan (0·5 g.) was dissolved in 0·1N-sulphuric acid (24·5 ml.; 1 mol.), the diethylacetal of glycollaldehyde (0·35 g.; 1·1 mols.) added, and the mixture heated in a corked vessel in a boiling water-bath for 4 hours. The solution was then clarified with charcoal, treated with aqueous ammonia to bring its p_H to about 6·0, and evaporated to a small bulk on the water-bath. The pale pink crystals which gradually separated were recrystallised from water, forming long colourless needles possessing a steel-grey sheen, m. p. 234° (Found for a specimen dried at 120°/2 mm. : C, 63·2; H, 5·8; N, 11·25. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%).

2 : 3 : 4 : 5-Tetrahydro-β-carboline-2 : 4-dicarboxylic Acid.—To *l*-tryptophan (0·5 g.) in N/10-sulphuric acid (24·5 ml.; 1 mol.) was added glyoxylic acid (25 ml. of a 1% solution; 1·4 mols.), the mixture being subjected to the treatment described in the previous preparation. When the solution, separated from a reddish tar, was concentrated to about 10 ml., pale pink crystals (0·37 g.; 50% yield) were obtained. Once recrystallised, the compound formed thin, colourless rhomboids, m. p. *ca.* 270° (decomp.) (Found for a specimen dried at 120°/2 mm. : C, 59·7; H, 4·8; N, 10·75. $C_{13}H_{12}O_4N_2$ requires C, 60·0 : H, 4·6; N, 10·76%).

3-Methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic Acid.—To a cool solution of *r*-α-methylamino-β-3-indolylpropionic acid (0·5 g.) in water (75 ml.), a slight excess (0·4 ml.; 2·3 mols.) of commercial formalin was added, and the whole incubated at 38° for 15 hours. The " solid " which separated was collected (0·11 g.), and the filtrate concentrated to a small volume on the boiling water-bath. The bundles of stout rods which settled on cooling were collected, washed with absolute alcohol, and dried in a desiccator. Concentration of the mother-liquor gave a further crop of the *carboline-carboxylic acid.* Yield, 0·40 g. (76%). On recrystallisation from water the carboline was obtained in bundles of fine rods, m. p. 208° after softening at 194° (Found for a sample dried at 130°/4 mm. : N, 11·3. $C_{13}H_{14}O_2N_2,H_2O$ requires N, 11·3%).

The " solid," recrystallised from dilute aqueous ammonia, formed sheaves of colourless needles, m. p. above 340°. With " commercial " sulphuric acid it gave a fine permanent purple colour, indicating that its structure is not the same as that of the carbolinecarboxylic acid. It is receiving further investigation.

2 : 3-Dimethyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic Acid.—A solution consisting of *r*-α-methylamino-β-3-indolylpropionic acid (0·5 g.) in N/2-hydrochloric acid (4·6 ml.; 1 mol.) and freshly distilled acetaldehyde (0·19 ml.; 1·5 mols.) was incubated overnight, neutralised with the required amount (2·3 ml.) of N-sodium hydroxide, and left to crystallise. The colourless rods were collected, washed with a little cold water, then with absolute alcohol, and dried in a desiccator. Concentration of the mother-liquor gave a further small crop. Total yield, 0·51 g. (90%). The product melted at 243—245° (Found : N, 11·4. $C_{14}H_{16}O_2N_2$ requires N, 11·5%).

2-Phenyl-3-methyl-2 : 3 : 4 : 5-tetrahydro-β-carboline-4-carboxylic Acid.—No trace of carboline could be detected on incubating for 10 days a neutral or slightly acid solution of the reactants mentioned below. When 1 mol. of acid was used, a minute yield of carboline was obtained after a similar period of time. The compound was finally prepared after the method of Jacobs and Craig (*loc. cit.*). *r*-α-Methylamino-β-3-indolylpropionic acid (0·3 g.) together with N-sulphuric acid (1·5 ml.; 1 mol.) and benzaldehyde (1·5 ml.; 10 mols.) in 75% aqueous alcohol (3 ml.) was refluxed for 20 hours on the boiling water-bath. Excess of aqueous ammonia was added to the cooled solution, and the free benzaldehyde removed with ether. When the aqueous solution was clarified with charcoal and concentrated, typical rod-like crystals of the *carboline-carboxylic acid* settled in 70% yield. After a further recrystallisation from water the pure product melted, on slow heating, at 219° (sharp) (Found: N, 8·7. $C_{19}H_{18}O_2N_2,H_2O$ requires N, 8·65%).

We desire to express our thanks to Dr. H. King for a sample of norharman-4-carboxylic acid (King and Stiller, *loc. cit.*). Part of the work herein described was done during the tenure by one of us (E. J. M.) of a Berridge Studentship awarded by the Delegacy of the University of London, King's College.

UNIVERSITY OF LONDON, KING'S COLLEGE. [*Received, November 25th*, 1940.]