395. *The Synthesis of 5- and 7-Methoxytryptophan, and of Some Derivatives.*

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5- and 7-Methoxytryptophan have been synthesised from the corresponding indolylmethylenehydantoins by the method described by Elks, Elliot, and Hems (*J.*, 1944, 629). These amino-acids condense with acetaldehyde to give carboline-acids, which are readily oxidised to two harmine analogues, *viz.*, 7-methoxy-2-methyl-β-carboline * and 9-methoxy-2-methyl-β-carboline, previously synthesised by Späth and Lederer by a different route (*Ber.*, 1930, **63**, 2102).

DURING some metabolic studies on tryptophan it was necessary to synthesise 5- and 7-methoxytryptophan. The only known analogue appears to be 6-methoxytryptophan, synthesised by Harvey and Robson (*J.*, 1938, 97) in their studies on the possible biological precursors of harmine; the success of the methods then employed has led to the adoption of similar lines of attack in the present study.

5- and 7-Methoxyindoles and their respective 3-aldehydes were synthesised by Blaikie and Perkin (*J.*, 1924, **125**, 296), and the same general methods have now been followed, although improved yields of 5-methoxyindole and of both the aldehydes have been achieved by decarboxylation of the ammonium 5-methoxyindolecarboxylate in glycerol, and by application of the Boyd and Robson (*Biochem. J.*, 1935, **29**, 555) modifications to the Ellinger–Reimer–Tiemann reaction. No such improvement could be obtained in the preparation of 7-methoxyindole; even after prolonged decarboxylation of the ammonium salt, significant, recoverable quantities of 7-methoxyindole-2-carboxylic acid remained in the melt.

The aldehydes were condensed with hydantoin in boiling piperidine (cf. Boyd and Robson, *Biochem. J.*, 1935, **29**, 2257), and both 5- and 7-methoxyindolylmethylenehydantoin were obtained in good yield and purity.

It was hoped to carry out reduction and hydrolysis of the indolylmethylenehydantoin in a single step, as described by Boyd and Robson (*Biochem. J.*, 1935, **29**, 2257), by using ammonium sulphide solution and ammonia solution at 100°, but although a product was obtained from 5-5′-methoxyindolylmethylenehydantoin which had many properties of a tryptophan, it proved very difficult to free it from organic and inorganic impurities. That it contained at least 60% of 5-methoxytryptophan was proved by the isolation of 2 : 3 : 4 : 5-tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic acid,* when it was treated with acetaldehyde in aqueous solution.

In view of the difficulties in purification and the attendant loss at each crystallisation, the above method was abandoned in favour of that of Elks *et al.* (*loc. cit.*), whereby the reduced hydantoins were readily formed by catalytic hydrogenation, and were then hydrolysed by hot aqueous baryta to the methoxytryptophans in good yields and purity.

* The numbering of β-carboline in this paper is that used by Cook *et al.*, *J.*, 1951, 1203.

The two amino-acids reacted with aqueous acetaldehyde giving 2 : 3 : 4 : 5-tetrahydro-7-methoxy- and 2 : 3 : 4 : 5-tetrahydro-9-methoxy-2-methyl-β-carboline-4-carboxylic acids, although the 7-methoxytryptophan condensed with acetaldehyde far less readily than did the 5-methoxy-isomer. Both these carboline acids were oxidised by acidic dichromate solution to the harmine analogues, *viz.*, 7- and 9-methoxy-2-methyl-β-carboline, which had been synthesised by Späth and Lederer (*loc. cit.*) from the acetylmethoxytryptamines.

The amino-acids and the derived carboline acids give some characteristic colour reactions, although 2 : 3 : 4 : 5-tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic acid failed to give a blue colour with " impure " sulphuric acid (cf. Harvey, Miller, and Robson, *J.*, 1940, 153), or " pure " sulphuric acid with added ferric chloride; it thus differed from the 8-methoxy- and 9-methoxy-analogues, both of which gave similar blue colours. On application of this test to the tryptophans (including 6-methoxytryptophan) green, yellow, or green-yellow colours were obtained, with the exception of 7-methoxytryptophan which yielded, somewhat suprisingly, a deep blue colour.

The apparently anomalous behaviour of 2 : 3 : 4 : 5-tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic acid towards sulphuric acid–ferric chloride suggests that the C_7 position of the carboline nucleus may require to be unsubstituted for the formation of the characteristic blue colour hitherto described (cf. Rydon, *J.*, 1948, 705). However, the corresponding 5-methoxytryptophan gives a fairly typical glyoxylic acid reaction, thereby suggesting that the tryptophan colour production may not involve the intermediate formation of the corresponding carboline.

The transient nature of the carboline blue colour—which in most cases often lasts only minutes even under carefully controlled conditions—has rendered the chemical isolation of the responsible complex difficult.

Experimental.

M. p.s are uncorrected. All micro-analyses by Drs. Weiler and Strauss.

5-Methoxyindole.—5-Methoxyindole-2-carboxylic acid (Blaikie and Perkin, *loc. cit.*) was converted into the ammonium salt, and this (33 g.) was decarboxylated by heating it in glycerol (10 ml.) at 200—210° for 1 hour. The hot melt was poured into aqueous sodium hydrogen carbonate (10 g. in 250 ml. of water), the mixture cooled, and the aqueous layer decanted off. The semi-crystalline indole was boiled with water (100 ml.), again cooled, and the solid extracted with successive quantities of hot ether, hot benzene, and hot ethanol. Concentration of the extracts yielded 5-methoxyindole as a yellow-brown oil, which rapidly solidified (20—21 g.; 85—90%), and was then pure enough for the next step. Recrystallisation from light petroleum (b. p. 60—80°) gave leaflets, m. p. 55°. The indole formed a picrate, red needles, m. p. 144° (Blaikie and Perkin, *loc. cit.*, gave 145°) (Found : N, 15·0. Calc. for $C_9H_9ON,C_6H_3O_7N_3$: N, 14·9%).

7-Methoxyindole.—7-Methoxyindole-2-carboxylic acid (10 g.) (Blaikie and Perkin, *loc. cit.*) was converted into the ammonium salt which was decarboxylated at 230—240°. 7-Methoxyindole was obtained as a pale yellow oil (4·5 g.) after distillation *in vacuo*. The picrate formed bright red needles, m. p. 150—151° (decomp.) (Found : N, 14·5%) (Blaikie and Perkin record m. p. 155°).

5-Methoxyindole-3-aldehyde.—5-Methoxyindole (20 g.), ethanol (96%; 400 ml.), and chloroform (250 g.) were stirred and heated under reflux, and a solution of potassium hydroxide (350 g.) in water (400 ml.) added at such a rate that gentle refluxing was maintained (4—6 hours). The mixture was stirred for a further 2 hours and then set aside overnight. Potassium chloride was removed by filtration and the solution steam-distilled. The distillate yielded unchanged 5-methoxyindole (2·5 g.) and 3-chloro-6-methoxyquinoline (5·5 g.), m. p. 73° (Found : N, 7·2. Calc. for $C_{10}H_8ONCl$: N, 7·24%). When cooled and concentrated, the mother liquor and aqueous ethanolic tar extracts yielded yellow needles of 5-methoxyindole-3-aldehyde (7·0 g.) which had m. p. 178° after recrystallising from dilute ethanol (Found : N, 7·9. Calc. for $C_{10}H_9O_2N$: N, 8·0%).

7-Methoxyindole-3-aldehyde.—7-Methoxyindole (12 g.) treated as described above for 5-methoxyindole yielded, after recrystallisation from aqueous ethanol, colourless needles of the aldehyde, m. p. 159—161° (2·6 g.) (Found : N, 8·1%). The distillate yielded 7-methoxyindole (3·0—3·5 g.) and needles of 3-chloro-8-methoxyquinoline (0·8 g.), m. p. 83—84° (Found : N, 7·28. Calc. for $C_{10}H_8ONCl$: N, 7·24%).

5-5′-Methoxyindolylmethylenehydantoin.—5-Methoxyindole-3-aldehyde (3·8 g.), hydantoin (2·3 g.), and piperidine (redistilled; 10 ml.) were boiled under reflux for 40 minutes. Dilution with water and acidification (Congo-red) yielded stout yellow needles of *5-5′-methoxyindolylmethylenehydantoin* (4·4 g., 95%). After recrystallisation from acetic acid or pyridine the product had m. p. 302° (Found : N, 15·9. $C_{13}H_{11}O_3N_3$ requires N, 16·3%).

5-7′-Methoxyindolylmethylenehydantoin.—Similarly 7-methoxyindole-3-aldehyde (2·15 g.) yielded yellow needles of *7-7′-methoxyindolylmethylenehydantoin* (2·25 g., 73·1%) which after recrystallisation from acetic acid or pyridine had m. p. 295—300° (Found : N, 16·8%).

5-5′-Methoxyindolylmethylhydantoin.—5-5′-Methoxyindolylmethylenehydantoin (1·0 g.), sodium hydroxide solution (N.; 10 ml.), and Raney nickel (*ca.* 10 g.) were shaken with hydrogen at normal

temperature and pressure for a total of 20·5 hours, and the solution was filtered and carefully acidified with dilute acid. Pale pink or colourless crystals of *5-5′-methoxyindolylmethylhydantoin* (0·79 g., 77%), m. p. 192—202°, were formed when the vessel was scratched. Recrystallisation from dilute ethanol yielded colourless prisms, m. p. 206—208° (Found: N, 16·3. $C_{13}H_{13}O_3N_3$ requires N, 16·2%).

5-7′-Methoxyindolylmethylhydantoin.—Similarly 5-7′-methoxyindolylmethylenehydantoin (2·0 g.) yielded colourless leaflets of *5-7′-methoxyindolylmethylhydantoin* (1·62 g., 80%) which after recrystallisation had m. p. 247—249° (Found: N, 16·2%).

5-Methoxytryptophan.—Method 1. 5-5′-Methoxyindolylmethylenehydantoin (4·9 g.), ammonium sulphide solution (16% A.R.; 100 ml.), and ammonia solution (3%; 40 ml.) were heated at 100—105° for 10 days in a closed bottle. The brown, clear liquid was filtered from a little undissolved material, evaporated to dryness *in vacuo*, and extracted with carbon disulphide and absolute ethanol, and then with hot water (300 ml.) containing a little ammonia solution. The aqueous extract was clarified (charcoal) and concentrated to a small bulk (5—10 ml.) whence addition of excess of ethanol and long storage at 0° caused the formation of a pale brown precipitate (1·25 g., 26%). Repeated attempts to crystallise the product from pyridine and ethanol removed most of the colour but did not result in crystallisation; the product had m. p. 249° (Found: C, 55·8; H, 6·0; N, 10·0. $C_{12}H_{14}O_3N_2$ requires C, 61·5; H, 6·0; N, 12·0%).

Some of the crude amino-acid (0·100 g.) was dissolved in water, clarified with charcoal, and treated with acetaldehyde (0·1 ml.). A colourless crystalline product was produced (61%); on recrystallisation this gave long thin needles (m. p. 230—232°) which appeared to be identical with the 2 : 3 : 4 : 5-tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic acid prepared from 5-methoxytryptophan by an alternative route described below (Found: C, 60·6; H, 6·1; N, 10·2. Calc. for $C_{14}H_{16}O_3N_2$: C, 64·6; H, 6·15; N, 10·8. Calc. for $C_{14}H_{16}O_3N_2,H_2O$: C, 60·4; H, 6·5; N, 10·18%).

Method 2. 5-5′-Methoxyindolylmethylhydantoin (1·0 g.), barium hydroxide (4·4 g.), and water (27 ml.) were heated under reflux for 24 hours. Carbon dioxide was passed into the boiling solution, which was then filtered and the remaining barium ion removed with 2N-sulphuric acid. The final filtrate and washings were combined and concentrated to a small bulk, treated with excess of ethanol and pyridine, and cooled to 0°. After long storage *5-methoxytryptophan* was obtained as practically colourless crystals (0·55 g., 61%), which after recrystallisation formed needles, m. p. 238—240° (decomp.) (Found: C, 60·1; H, 5·8; N, 11·8%).

7-Methoxytryptophan.—From 5-7′-methoxyindolylmethylhydantoin (3 g.), by a procedure similar to Method 2 (above) for 5-methoxytryptophan, very pale yellow needles of *7-methoxytryptophan* (2·3 g., 85%) were obtained. On recrystallisation these formed needles, m. p. 255—264° (Found: C, 60·1; H, 5·8; N, 11·8%).

2 : 3 : 4 : 5-Tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic Acid.—5-Methoxytryptophan (0·2 g.), dissolved in water (50 ml.), was treated with acetaldehyde solution (50%; 0·8 ml.) and then set aside overnight at room temperature. The *carboline-carboxylic acid* (0·176 g.) which separated as white needles had m. p. 231—233° (decomp.) (mixed m. p., with the product from crude tryptophan prepared by method 1, was 234—236°) (Found: C, 56·8; H, 6·8; N, 9·75. $C_{14}H_{16}O_3N_2,2H_2O$ requires C, 56·75; H, 6·7; N, 9·5%).

2 : 3 : 4 : 5-Tetrahydro-9-methoxy-2-methyl-β-carboline-4-carboxylic Acid.—The reaction between 7-methoxytryptophan and acetaldehyde appeared to proceed far less readily than in the case of 5-methoxytryptophan.

By method 1, a product was obtained only on considerable concentration of the reaction mixture in one experiment, or after storage for a week or so in another. The second crop from the first preparation was used for the oxidation (below), but the second preparation is recorded here.

Method 1. 7-Methoxytryptophan (0·2 g.), dissolved in water (50 ml.), was treated with acetaldehyde solution (50%; 0·8 ml.), and set aside at room temperature. No product was obtained until after a week or so, when white nodules (84 mg.), m. p. 249—258°, appeared. Analysis and m. p. indicated that this was probably a mixture, although some *carboline acid* was undoubtedly present.

A second crop (micro-plates) (40 mg.; m. p. 256—258°) was obtained on concentration (Found: C, 61·0; H, 6·7; N, 10·25. $C_{14}H_{16}O_3N_2,H_2O$ requires C, 60·5; H, 6·5; N, 10·1%).

Method 2. 7-Methoxytryptophan (0·1 g.), dissolved in water (60 ml.) and hydrochloric acid (4·3 ml.; 1 mole), was treated with acetaldehyde solution (50%; 0·5 ml.) and set aside for several days, then heated on the water-bath and concentrated to a very small bulk, and ethanol and pyridine added. The product (57 mg.) had m. p. 240—246° (Found: C, 64·1; H, 6·0; N, 10·2. $C_{14}H_{16}O_3N_2$ requires C, 64·6; H, 6·15; N, 10·8%).

7-Methoxy-2-methyl-β-carboline.—The corresponding tetrahydro-carbolinecarboxylic acid (0·2 g.), dissolved in boiling water (60 ml.), was treated with aqueous potassium dichromate (10% w/v; 10 ml.) and glacial acetic acid (2 ml.). The mixture was boiled for 1 minute and cooled, and sodium sulphite solution (10%; 13 ml.) added. The solution was made alkaline with sodium carbonate and extracted ten times with ether. Evaporation to dryness yielded a yellow crystalline compound (65 mg., 39%), which was recrystallised from methanol, forming prisms, m. p. 267—269° of the carboline (Found: C, 73·2; H, 5·85; N, 13·1. Calc. for $C_{13}H_{12}ON_2$: C, 73·6; H, 5·7; N, 13·2%). (Späth and Lederer, *loc. cit.*, reported m. p. 273—274°.)

9-Methoxy-2-methyl-β-carboline.—The corresponding tetrahydro-carbolinecarboxylic acid (0·1 g.) from the second crop of the first preparation, dissolved in boiling water (60 ml.), was treated with aqueous potassium dichromate (10%; 5 ml.) and glacial acetic acid (1 ml.). The mixture was boiled for 1 minute and cooled, and sodium sulphite solution added (10%; 7·5 ml.). The product (41 mg.; 49%) was

isolated exactly as with the 7-methoxy-compound and was recrystallised from methanol, forming colourless plates, m. p. 238—239° (Spāth and Lederer, *loc. cit.*, reported 239—240°) (Found : C, 73·0; H, 5·9; N, 13·2%).

Colour Reactions.—Details of the two reactions are given in the table.

Compound.	Glyoxylic acid reaction.*	"Carboline blue" test.†
5-Methoxytryptophan	Violet ring	Yellow
2 : 3 : 4 : 5-Tetrahydro-7-methoxy-2-methyl-β-carboline-4-carboxylic acid	Pale green ring	Bright yellow —> brown
6-Methoxytryptophan	Blue ring —> purple	Yellow —> green; blue on long keeping with excess of $FeCl_3$ ‡
2 : 3 : 4 : 5-Tetrahydro-8-methoxy-2-methyl-β-carboline-4-carboxylic acid	Blue ring surmounted by purple	Yellow; purple —> green with excess of $FeCl_3$ ‡
7-Methoxytryptophan	Blue ring surmounted by purple	Blue
2 : 3 : 4 : 5-Tetrahydro-9-methoxy-2-methyl-β-carboline-4-carboxylic acid	Blue ring surmounted by purple	Bluish-green

* The amino-acid or carboline (0·5—1·0 mg.) was dissolved in water (2 ml.), glyoxylic acid solution (4 drops) was added, and the solution underlayered with concentrated sulphuric acid (2—3 ml.; AnalaR).

† A crystal (0·1 mg.) of the amino-acid or carboline acid was added to concentrated sulphuric acid (3 ml.; AnalaR), containing a trace of ferric chloride. If no colour appeared aqueous ferric chloride (0·01 ml.; 10%) was added and the solution shaken. Ferric chloride was used as the oxidant throughout Mercuric sulphate, normally used in the Hopkins–Cole test (*Proc. Roy. Soc.*, 1901, **68**, 21; *J. Physiol.*, 1901, **27**, 423), did not cause the formation of a blue colour when it was added to a sulphuric acid solution of a carboline-carboxylic acid.

‡ The trace of ferric chloride originally added was insufficient and more was therefore added.

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