22. *The Synthesis of* r-6-*Methoxytryptophan and of Harmine, with a Note on the Action of Acetaldehyde on Tryptophan.*

By Douglas Graham Harvey and William Robson.

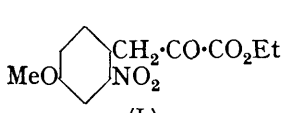
The synthesis of r-6-*methoxytryptophan*, which may be regarded as the biological precursor of the *Harmala* alkaloids, has been effected. Attempts to demethylate the new amino-acid have been unsuccessful and it would appear that before the hydroxy-derivative can be obtained a new method of synthesising tryptophan will be necessary. It is shown that acetaldehyde will condense with tryptophan in aqueous solution at the ordinary temperature to form 3-methyl-3 : 4 : 5 : 6-tetrahydro-4-carboline-5-carboxylic acid, this substance being identified by its oxidation to harman. The corresponding 6-methoxy-derivatives have also been studied.

The researches of Kermack, Perkin, and Robinson (J., 1921, **119**, 1617), who showed that harman is produced by the oxidation of the reaction product of *l*-tryptophan and acetaldehyde, of Spath and Lederer (*Ber.*, 1930, **63**, 112), who transformed acetyl-6-methoxytryptamine into harmaline, and of Akabori and Saito (*ibid.*, p. 2245), who condensed tryptamine with acetaldehyde to obtain tetrahydroharman, would almost certainly indicate that the amino-acids 6-hydroxytryptophan and its corresponding methoxy-derivative are the biological precursors of the *Harmala* alkaloids.

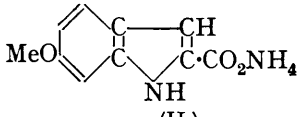
The possible occurrence of 6-hydroxytryptophan in the plant has been mooted by Barger (*Ann. Reports*, 1919, 157); that it may have even wider distribution in nature is suggested by the communication of Abderhalden and Baumann (*Z. physiol. Chem.*, 1908, **55**, 412) on the presence of an unidentified hydroxytryptophan in a tryptic digest of casein. Nothing, however, is known of these amino-acids and since their isolation and

the study of their properties would help to clarify our knowledge regarding the mode of formation of the alkaloids *in vivo*, and moreover, yield data facilitating a search for the amino-acids themselves in plant and animal products, it was decided to attempt their synthesis. The present communication deals with the synthesis of 6-*methoxytryptophan* and its transformation into harmine. At one time it was hoped that demethylation of the former compound would yield the required hydroxy-amino-acid. Up to the present, however, efforts in this direction have proved unavailing and a new line of attack would appear essential.

6-Methoxyindole, which was described by Kermack, Perkin, and Robinson (*loc. cit.*) in their attempt to obtain 6-methoxytryptophan, has now been prepared as follows: *o*-Nitro-*p*-toluidine was converted through *o*-nitro-*p*-cresol (Manske, "Organic Syntheses," 396) into *o*-nitro-*p*-tolyl methyl ether (cf. Kermack, Perkin, and Robinson, *loc. cit.*), which was condensed with ethyl oxalate in dry ether in the presence of potassium ethoxide (Wislicenus and Thoma, *Annalen*, 1924, **436**, 24; Blaikie and Perkin, J., 1924, **125**, 296). The resulting unstable potassio-derivative of ethyl *o*-nitro-*p*-methoxyphenylpyruvate (I)



(I.)



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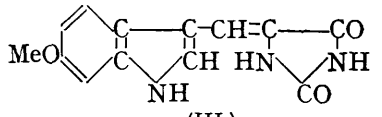
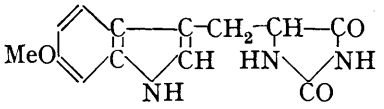
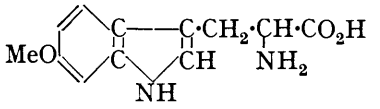


(III.)

was converted into ammonium 6-methoxyindole-2-carboxylate (II) in excellent yield by dissolving it, immediately it was isolated, in cold water and after 15 minutes treating the filtered solution with ferrous sulphate and ammonia. Decarboxylation of the product proceeded most satisfactorily in hot glycerol, and 6-methoxyindole-3-aldehyde was then obtained by Boyd and Robson's modification (*Biochem. J.*, 1935, **29**, 555) of the Ellinger–Reimer–Tiemann method. The remaining stages closely followed the hydantoin method of synthesising amino-acids as modified by Boyd and Robson (*Biochem. J.*, 1935,

(IV.)

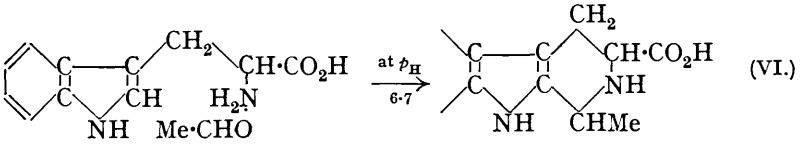




(V.)

29, 542, 546). Condensation of the aldehyde with hydantoin was effected in boiling piperidine, and the reduction of the resulting 5-(6′-*methoxyindolal*)*hydantoin* (III) accomplished by treating a solution of it in pyridine with hydrogen sulphide at 100° for 70 hours. Cleavage of the saturated *hydantoin* (IV) by heating it in an ammoniacal solution at 100° gave the required 6-methoxytryptophan (V) in good yield and purity.

Before the conversion of the amino-acid to the "alkaloidal" stage was attempted, an investigation of the best conditions for the condensation of acetaldehyde and tryptophan was carried out. Kermack, Perkin, and Robinson (*loc. cit.*) in their synthesis of harman mixed these substances in dilute acid solution and obtained their product by immediately oxidising the mixture. Akabori and Saito (*loc. cit.*) and Hahn and his co-workers (*Ber.*, 1934, **67**, 2031; *Annalen*, 1935, **520**, 107) studied the effect of buffered solutions on the "biological" condensation of tryptamine hydrochloride with various aldehydic and ketonic compounds, and Jacobs and Craig (*J. Biol. Chem.*, 1936, **11**, 759) in their investigation into the constitution of lysergic acid condensed *l*-tryptophan with various aldehydes, again in acid solution. It is now found that, if a molar quantity of freshly distilled acetaldehyde is added to a cold saturated aqueous solution of *l*-tryptophan

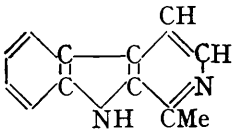


(VI.)

(p_H 6·7) and the mixture is kept overnight at room temperature, 3-methyl-3 : 4 : 5 : 6-tetrahydro-4-carboline-5-carboxylic acid (VI) separates. Evaporation of the mother-liquor

and addition of alcohol to the concentrate gives a further crop of the carboline, the yield becoming quantitative.

From the ease with which this reaction takes place it would appear that we were approaching the " biological " conditions under which the formation of the alkaloids takes place *in vivo*. At the moment, however, we do not attach much importance to the reaction, for the following reasons. Treatment of the carboline derived from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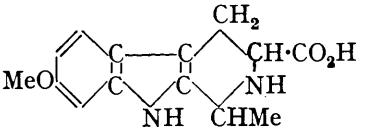
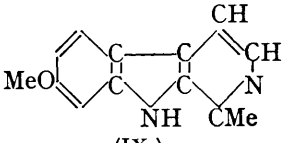
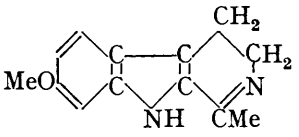
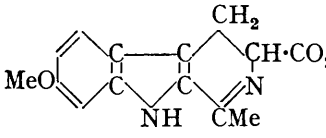

(VIII.)


(IX.)

tryptophan with the most gentle oxidising agents yielded harman (VII) in 70% yield, whereas the corresponding 11-*methoxy-carboline* (VIII) yielded harmine (IX) in only a 40% yield. It would appear, then, that the presence of the methoxy-group effects some difference in the behaviour of the carbolines towards oxidising agents, although no evidence has been obtained of a compound resembling harmaline (X) or 11-methoxy-3-methyl-5 : 6-dihydro-4-carboline-5-carboxylic acid (XI) in the reaction mixture. Moreover,

(X.)




(XI.)

attempts to decarboxylate 3-methyl-3 : 4 : 5 : 6-tetrahydro-4-carboline-5-carboxylic acid by the methods available for the degradation of amino-acids to amines have been unsuccessful and this would seem to indicate that the tetrahydropyridine ring is formed after, rather than before, decarboxylation has occurred; in other words, the amine and not the amino-acid may be the precursor of these alkaloids in the plant.

Experimental.

o-*Nitro*-p-*cresol*.—The suspension obtained by cooling a solution of *o*-nitro-*p*-toluidine (100 g.) in warm 10% sulphuric acid (1500 ml.) to 0° was diazotised with sodium nitrite (50 g. in water, 130 ml.) (cf. Manske, *loc. cit.*), added during 20 minutes, and the red mixture cautiously added to boiling dilute sulphuric acid (1000 ml. of conc. acid in water, 2000 ml.). When the tar had coagulated, the yellow solution was rapidly cooled. The cresol, collected next day, crystallised from water in large yellow needles, m. p. 77—78° (yield, 55—60%).

o-*Nitro*-p-*tolyl Methyl Ether*.—To a well-cooled solution of *o*-nitro-*p*-cresol (75 g.) in aqueous caustic soda (30 g. in 220 ml. of water), methyl sulphate (100 ml.) was added in 10 ml. portions, the temperature being kept below 50°. The solution was then heated on the water-bath until the red colour had practically disappeared (15 minutes), excess of caustic soda added (25 g. in 100 ml. of water), and the whole heated on the water-bath for 5 minutes. A washed and dried ethereal extract of the product gave on distillation a fraction, b. p. 150°/20 mm., as a pale yellow, viscous liquid consisting of practically pure *o*-nitro-*p*-tolyl methyl ether (yield, 90—95%); this rapidly solidified in needles (m. p. 15·5°) when cooled in ice.

Potassium Derivative of Ethyl o-*Nitro*-p-*methoxyphenylpyruvate* (I).—A suspension of potassium ethoxide prepared from potassium (7·8 g.) and dry alcohol (15 ml.) in dry ether was well cooled, and ethyl oxalate (29·2 g.) slowly added with vigorous shaking, followed, after cooling in ice–salt for 20 minutes, by an ethereal solution of *o*-nitro-*p*-tolyl methyl ether (33·4 g.), added in 10 ml. portions without rise in temperature. The dark red solution was kept at room temperature for 48 hours, and the crystalline potassium derivative then collected, washed with ether, and immediately treated as described in the next section (yield, 70—80%).

Ammonium 6-*Methoxyindole*-2-*carboxylate* (II).—A solution of the potassium derivative (100 g.) in water (1000 ml.) was after 15 minutes extracted with ether, filtered, and diluted to 1500 ml. with water, and 80 ml. of aqueous ammonia (*d* 0·880) added, followed by a hot

solution of ferrous sulphate (900 g. in 1300 ml. of water). The whole was warmed for ½ hour, boiled for ½ hour, and filtered hot, the ferric oxide sludge washed with hot water, and the combined filtrate and washings concentrated; ammonium 6-methoxyindole-2-carboxylate (pale pink plates) was obtained in 50—60% yield.

6-Methoxyindole.—The preceding salt (7·5 g.) was carefully heated in glycerol for 2—3 hours at 200—210°, the mass poured into water and cooled at 0° for 6 hours, and the indole recrystallised from hot ligroin, forming plates (3·9 g.), m. p. 92°.

6-Methoxyindole-3-aldehyde.—A boiling solution of 6-methoxyindole (15 g.) in 96% alcohol (300 ml.) and chloroform (115 ml.) was treated with a solution of potassium hydroxide (150 g. in 180 ml. of water) at such a rate that a steady but not too vigorous reaction was maintained. The solution was boiled for a further 4 hours and kept at room temperature overnight. The potassium chloride was then removed and washed with 96% alcohol, and the washings and filtrate steam-distilled. After the alcohol and chloroform had passed over, the receiver was changed and the distillation continued for a further 20 hours, by which time all the unchanged 6-methoxyindole and 3-chloro-7-methoxyquinoline had distilled. The hot liquor in the flask was filtered from the coagulated tar and set aside to cool. The tar was dissolved in alcohol, the solution poured into water, the alcohol removed by steam-distillation, and the resulting mixture filtered. The filtrate deposited a further crop of aldehyde. The remaining tar was submitted to the same process a second time. The yellow needles of 6-methoxyindole-3-aldehyde which separated from all the solutions were combined and recrystallised from hot water; they were then obtained practically colourless. Yield, 4·5 g. or 25% of the theoretical. The aldehyde (Found: N, 8·2. Calc.: N, 8·0%) had m. p. 186° (Kermack, Perkin, and Robinson give 186°) and gave a slight colour with Ehrlich's reagent and a deep pink colour with warm 50% sulphuric acid.

3-Chloro-7-methoxyquinoline.—The steam-distillate from the above preparation was shaken six times with ether, and the extracts concentrated and shaken several times with small quantities of dilute acid. The acid washings were made alkaline with 10% caustic soda solution, and the 3-chloro-7-methoxyquinoline collected and recrystallised from 50% alcohol. It had a pleasant smell characteristic of the substituted 3-chloroquinolines and melted at 93—94°. Yield, 2 g. from 15 g. of 6-methoxyindole.

5-(6′-Methoxyindolal)hydantoin (III).—6-Methoxyindole-3-aldehyde (3·5 g.), hydantoin (2·3 g.; 1·2 mols.), and freshly distilled piperidine (10 ml.) were heated under reflux for 35 minutes, a bright yellow, crystalline solid beginning to separate after 15 minutes. The resulting semi-solid mass was poured into water and made slightly acid with acetic acid, the liquid filtered, and the solid collected, washed with methyl alcohol and with hot water, and recrystallised from pyridine–water and also from glacial acetic acid, forming stout yellow prisms or blunt needles (3·7 g.) of *5-(6′-methoxyindolal)hydantoin*, m. p. 311—315° (Found: N, 16·5. $C_{13}H_{11}O_3N_3$ requires N, 16·3%). It was practically insoluble in absolute ethyl and methyl alcohols, ether, benzene, xylene and ligroin and gave, when heated with Ehrlich's reagent, a very pale pink colour which faded rapidly on cooling.

5-(6′-Methoxyindolylmethyl)hydantoin (IV).—A solution of 6′-methoxyindolalhydantoin (1·0 g.) in pyridine (20 ml.) was saturated with hydrogen sulphide at 0° and heated in a closed bottle at 100° for 21 hours; fresh pyridine was then added (20 ml.), and the process of saturation with hydrogen sulphide and heating repeated. The pyridine was finally removed in a vacuum, and the residue treated with 100 ml. of absolute alcohol. The alcoholic solution was filtered (the unchanged 6′-methoxyindolalhydantoin only amounted to 0·1—0·2 g.), diluted to 200 ml. with water, boiled with a little animal charcoal, and again filtered. The filtrate on standing deposited 0·45 g. of practically colourless *5-(6′-methoxyindolylmethyl)-hydantoin*. This, on recrystallisation from 50% alcohol, formed large plates, m. p. 220° (Found: N, 16·1. $C_{13}H_{13}O_3N_3$ requires N, 16·2%). Yield (based on recovered unreduced hydantoin), 50%.

6-Methoxytryptophan (V).—A mixture of 6′-methoxyindolylmethylhydantoin (3·2 g.), aqueous ammonia (6 ml., *d* 0·880), and water (150 ml.) (after treatment with hydrogen sulphide for 5 minutes to prevent oxidation) was heated in a closed bottle for 72 hours at 100—110°. The solution was evaporated to dryness in a vacuum and the residue was extracted, first with alcohol to remove sulphur and unchanged hydantoin, and then with hot water to remove the amino-acid. The aqueous extract was concentrated to 20 ml. and treated with 60 ml. of absolute alcohol. The thick crust of *6-methoxytryptophan* which formed on the surface was removed, and the filtrate concentrated to 10 ml. and treated with a further 60 ml. of absolute alcohol; a second crop of less pure amino-acid was then

precipitated, the total yield being 1·9 g. (60%). Recrystallisation from water gave well-defined hexagonal plates. Like *r*-tryptophan, the 6-methoxy-derivative was intensely sweet to the taste, and insoluble in alcohol, ether, and benzene. It darkened and shrank at 246—250°, melted at 263—268°, and frothed with charring at 274°. It gave with the Hopkins–Cole reagent a greenish-blue colour, changed by a few drops of ferric chloride solution to purplish-red; dilution with water produced a vivid green-blue solution. Bromine water gave a characteristic rose-pink colour, readily removed by amyl and butyl alcohols (Found for material dried at 110°/15 mm.: C, 61·9; H, 6·1; N, 11·9. $C_{12}H_{14}O_3N_2$ requires C, 61·5; H, 5·9; N, 12·0%).

Attempted Demethylation of r-6-*Methoxytryptophan.*—The tryptophan was (1) heated for 3·5 hours with 35% hydrochloric acid at 150°, (2) dissolved in dilute sulphuric acid (5 g. of acid : 5 ml. of water) and boiled under reflux for 2 hours in an atmosphere of nitrogen, (3) heated with N/10-sulphuric acid (1 mol.) for 7 hours at 160°. A dark brown, amorphous product was isolated in the first case and greenish amorphous masses in the other two.

3-*Methyl*-3 : 4 : 5 : 6-*tetrahydro*-4-*carboline*-5-*carboxylic Acid* (VI).—A solution of *l*-tryptophan (0·3 g.) in water (10 ml.) was cooled, treated with freshly distilled acetaldehyde (1 ml.), and kept in a corked bottle at room temperature overnight. Colourless opaque needles (0·2 g., m. p. 290° after preliminary darkening at 280°) had then formed, and the mother-liquor gave, on concentration and addition of a large excess of alcohol, a further crop of the carboline (yield, practically theoretical). Recrystallised from a small volume of water by the addition of alcohol, it formed practically colourless needles, m. p. 295—299° (Found for material dried at 110°/15 mm.: C, 68·6; H, 6·1; N, 12·0. Calc. for $C_{13}H_{14}O_2N_2$: C, 67·8; H, 6·1; N, 12·2%).

3-*Methyl*-4-*carboline* (*Harman*) (VII).—To a boiling solution of the preceding acid (0·25 g.) in water (65 ml.), 10% potassium dichromate solution (12·5 ml.) was added, and then glacial acetic acid (2·5 ml.). The solution was boiled for 1 minute, cooled, treated with dilute sodium sulphite solution to remove the excess of oxidising agent, made strongly alkaline with sodium carbonate, and shaken with ether. The extracts were evaporated to dryness, leaving a mass of pale yellow needles (0·152 g.), m. p. 238° after recrystallisation from alcohol (Jacobs and Craig give m. p. 238°). The base gave salts which fluoresced bright blue in aqueous alcoholic solution.

11-*Methoxy*-3-*methyl*-3 : 4 : 5 : 6-*tetrahydro*-4-*carboline*-5-*carboxylic Acid* (VIII).—*r*-6-Methoxytryptophan (0·5 g.) was dissolved in water (50 ml.), treated with acetaldehyde (1 ml.), and warmed gently for 10 minutes. The colourless hexagonal rods formed (0·42 g.) were removed, the filtrate concentrated to 1 ml., and a further crop of the *carboline* precipitated by the addition of a large excess of alcohol (yield, approximately theoretical). The carboline resembled the demethoxycarboline (m. p. 295—299°) in properties, but melted at 244—246° (Found for material crystallised from water and dried at room temperature: loss at 120°/3 mm. after 4 hours, 6·1. $C_{14}H_{16}O_3N_2,H_2O$ requires H_2O, 6·5%. Found for material crystallised from water and air-dried: C, 62·5; H, 6·5. $C_{14}H_{16}O_3N_2$ requires C, 64·6; H, 6·2%. Found for material crystallised, in opaque yellow needles, from water by the addition of a large volume of alcohol and dried at 110°: N, 10·75. $C_{14}H_{16}O_3N_2$ requires N, 10·8%).

11-*Methoxy*-3-*methyl*-4-*carboline* (*Harmine*) (IX).—The above carbolinecarboxylic acid (0·1 g.) was oxidised in the same way as the acid (VI) (water, 30 ml.; 10% potassium dichromate solution, 5·0 ml.). The residue obtained from the dried ethereal extracts was recrystallised from methyl alcohol and water, giving colourless needles of harmine, m. p. 260—261° (Found: N, 13·1. Calc. for $C_{13}H_{12}ON_2$: N, 13·2%). Yield, 40%. The base formed characteristic salts with dilute mineral acids (*e.g.*, the hydrochloride) which gave a blue fluorescence in aqueous-alcoholic solution.

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