141. *The Synthesis of the Nuclear-C-methylated Tryptophans. A Note on the Aldehyde Reactions for Tryptophan.*

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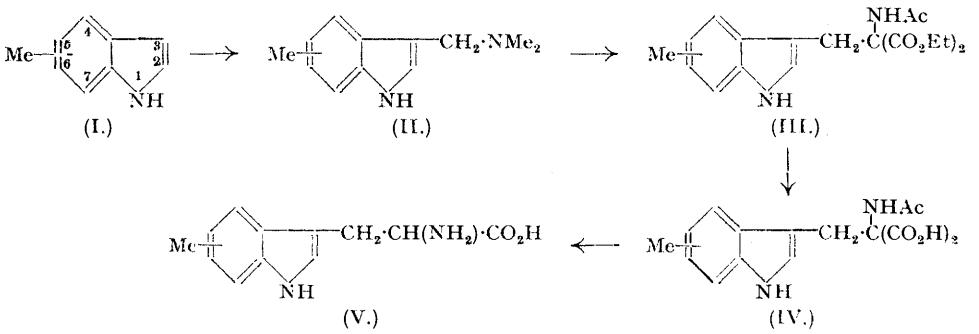
The five possible nuclear-C-methylated tryptophans have been prepared from the corresponding methylindoles by the gramine synthesis of Snyder and Smith (*J. Amer. Chem. Soc.*, 1944, **66**, 350). The colours developed by these substances in the best-known " aldehyde " reactions for tryptophan are described, and some conclusions are reached regarding the mechanism of these colour reactions.

The observation of Anderson (*Science*, 1945, **101**, 565) that 5-methyltryptophan inhibited the growth of *Bacterium coli* suggested that it would be of interest to study further the inhibitory action on bacteria of this and the other isomeric methyltryptophans, and of the related methylindoles. The preparation of these substances is recorded in the present paper; details of the biological tests, and the light the results throw on the combination of indole and tryptophan with bacterial enzymes, have been reported elsewhere (Fildes and Rydon, *Brit. J. Exp. Path.*, 1947, **28**, 211); some of these substances have proved remarkably active, *in vitro*, as inhibitors of *Salmonella typhi*.

The method used was the elegant " gramine " synthesis first worked out for tryptophan by Snyder and Smith (*loc. cit.*; cf. Albertson, Archer, and Suter, *ibid.*, p. 500) and subsequently developed by the two groups (Albertson, Archer, and Suter, *ibid.*, 1945, **67**, 36; Howe, Zambito, Snyder, and Tishler, *ibid.*, p. 38; Albertson and Tullar, *ibid.*, p. 502) :



Of the five methylindoles (I) required as starting materials only two had not previously been synthesised; these, *viz.*, 4-methylindole, which had been isolated from coal-tar by Kruber (*Ber.*, 1929, **62**, 2877), and 6-*methylindole*, were prepared by cyclisation of the corresponding formylxylidines (cf. Tyson, *J. Amer. Chem. Soc.*, 1941, **63**, 2024; *Org. Synth.*, 1943, **23**, 45). Since the present work was completed, Marion and Oldfield (*Canadian J. Res.*, 1947, *B*, **25**, 1) have described similar syntheses but report no analytical data.

All the *C*-methylindoles condensed readily with formaldehyde and dimethylamine to yield the corresponding *methyl*-3-*dimethylaminomethylindoles* (" methylgramines ") (II) and these in turn condensed with acetamidomalonic ester to give the *ethyl* 1-*acetamido*-2-(x-*methyl*-3-*indolyl*)-*ethane*-1 : 1-*dicarboxylates* (III); in the latter condensation it was found most convenient to use the modification of Albertson, Archer, and Suter (*J. Amer. Chem. Soc.*, 1945, **67**, 36), whereby the methosulphate of the gramine is made *in situ*, and essential to work in specially dried alcohol. In the one case in which it was investigated, *viz.*, the synthesis of 7-methyltryptophan, the use of acetamidocyanoacetic ester seemed to offer no advantages; Jackman and Archer (*ibid.*, 1946, **68**, 2105) have recently reported a synthesis of 5-methyltryptophan by this route. For the final stages the methods used by previous workers (direct acid hydrolysis and decarboxylation, or decarboxylation with boiling water followed by alkaline hydrolysis) proved inapplicable except in the case of the 7-methyl compound, owing to the great sensitivity of the methyl-substituted products towards acid. Success was finally achieved by hydrolysing the esters (III) to the *acids* (IV) with alkali, decarboxylating these by heating in nitrogen, and finally removing the acetyl group by alkaline hydrolysis. The overall yields (based on the methylindoles) and melting points of the methyltryptophans (V) prepared in the present work were as follows :

Substance.	Yield, %.	M. p.	
2-Methyltryptophan	46	234—235°	(Ellinger and Matsuoka, *Z. physiol. Chem.*, 1914, **91**, 45, record m. p. 234°)
4-*Methyltryptophan*	42	265—267	
5-Methyltryptophan	49	264	(Robson, *J. Biol. Chem.*, 1924, **62**, 495, records m. p. 259—263°, and Jackman and Archer, *loc. cit.*, m. p. 284—288°)
6-*Methyltryptophan*	56	258—260	
7-*Methyltryptophan*	65	296	

All these methyltryptophans, including the 2-methyl compound, gave intense colours in the formaldehyde reaction of Cole (S. W. Cole, " Practical Physiological Chemistry ", Heffer, Cambridge, 9th edition, 1933, p. 80); this was surprising, since Ellinger and Matsuoka (*loc. cit.*) state that 2-methyltryptophan gives a negative result in the glyoxylic acid test of Hopkins and Cole (*Proc. Roy. Soc.*, 1901, **68**, 21; *J. Physiol.*, 1901, **27**, 423) of which the formaldehyde reaction is supposed to be merely a modification. Accordingly, the colours given by the methyltryptophans in the best-known " aldehyde " reactions for tryptophan were investigated, with the results shown in the Table.

It is clear that Ellinger and Matsuoka's statement about the colour reactions of 2-methyltryptophan is correct; the Cole formaldehyde reaction now generally in vogue is more than a mere improved modification of the original Hopkins–Cole glyoxylic reaction, which was in general use some years ago, and confusion may arise in interpreting the earlier literature if this

	Colour and approximate position of maximum absorption in :			
	Adamkiewicz reaction.[2]	Hopkins–Cole glyoxylic reaction.[3]	Hopkins–Cole glyoxylic reaction with mercuric sulphate.[4]	Cole formaldehyde reaction.[5]
Tryptophan	Pale rose	Violet → blue. 5850 A.	Purple. 5640 A.	Violet → purple. 5680 A.
2-Methyltryptophan	Negative	Very faint blue-green on long standing.	Blue-green. General above 6500 A.	Blue-green. General above 6500 A.
4-Methyltryptophan	Pale rose	Grass-green → emerald. General below 4500 A.	Violet-blue. 5680 A.	Violet → blue. 5770 A.
5-Methyltryptophan	Rose → violet	Violet → blue. 5800 A.	Violet. 5620 A.	Blue → violet. 5650 A.
6-Methyltryptophan	Blue	Green → blue. 6000 A.	Blue. 5830 A.	Blue. 5850 A.
7-Methyltryptophan	Pale rose	Green → blue. 5850 A.	Violet-blue. 5650 A.	Blue. 5650 A.

[1] The details of the reactions are given in the experimental section.
[2] Reactions generally weak. The colours given by the 4- and the 5-methyl compound were by far the most intense.
[3] Stronger than (2) but less intense than (4) and (5). The 2-methyl compound gave only a trace of colour. The intensities of the colours given by the others was in the order 5-Me > 6-Me > 4-Me > 7-Me > tryptophan.
[4] Intense colours. Order : Tryptophan > 4- and 5-Me > 6- and 7-Me.
[5] Intense colours. 2-Me and 4-Me weakest.

is not borne in mind. It is clear from the Table that the two reactions give very similar results if both are carried out in the presence of mercuric sulphate, as always used in the Cole reaction; it seems probable that the modifying effect of mercuric sulphate is due to catalysis of further oxidation of the primary coloured products (cf. Mottram, *Biochem. J.*, 1913, **7**, 249), and it is noteworthy that copper salts have a similar action (Winkler, *Z. physiol. Chem.*, 1934, **228**, 50).

The present findings are in general agreement with the view of Fearon (*Biochem. J.*, 1920, **14**, 548) on the mechanism of these colour reactions, although the similarity of the colour reactions of 5-methyltryptophan to those of the 4-, 6- and 7-methyl compounds makes it clear that the condensation with formaldehyde to give the initial di-indolylmethane (" tryptophan red ") and bistetrahydrocarbolylmethane (" tryptophan blue ") colouring matters does not in every case involve only the C_7 positions of the indole nuclei as postulated by Fearon. The unique behaviour of 2-methyltryptophan is no doubt due to its inability to cyclise to a tetrahydrocarboline, in consequence of which it is able to give only a tryptophan red and not a tryptophan blue; it seems that the former is capable of giving rise to intense colours only in the presence of mercuric salts, as used in the Cole formaldehyde reaction but not in the Hopkins–Cole glyoxylic reaction.

Experimental.

Methylindoles (I).—2-Methylindole was prepared by the method of Fischer (" Beilstein ", 4th ed., **20**, 311) and 5-methylindole by Robson's method (*J. Biol. Chem.*, 1924, **62**, 495; cf. *Biochem. J.*, 1935, **29**, 557).

4-*Methylindole.* Crude, mainly 3-, nitro-*o*-xylene (111 g.; b. p. 95—114°/1·5 mm.) in alcohol (200 c.c.) was hydrogenated (*ca.* 5 atmos./room temp.) with 2% palladised strontium carbonate (5 g.). Distillation of the filtered product yielded 86·3 g. (91%) of mixed xylidines, b. p. 76—84°/1 mm. This mixture was heated under reflux on the water-bath for 3 hours with 90% formic acid (37 c.c.) and the product poured into water (120 c.c.); after being kept at 2° for 2 days the oily product partially crystallised and was then collected and drained on a porous tile. Crystallisation from 35% ethanol (charcoal) yielded pure *N*-formyl-2 : 3-dimethylaniline (29·6 g.; 28%), m. p. 100—102°.

This was added to warm *tert.*-butyl alcohol (200 c.c.) in which potassium (12 g.) had been dissolved, and the mixture heated in a metal-bath in a stream of dry nitrogen. *tert.*-Butyl alcohol distilled off at a bath temp. of 150—160°; decomposition of the residue set in at 340° and was completed by heating at 360° for 30 minutes; the distillate contained 6·7 g. of 2 : 3-dimethylaniline. The cooled residue in the reaction flask was treated with water and steam-distilled; the steam distillate was extracted with ether, washed successively with 2N-hydrochloric acid, water, 5% sodium carbonate, and water, dried and distilled, yielding 4-methylindole (5·7 g.; **44%**), b. p. 110°/1 mm.; the picrate crystallised from benzene in red needles with a coppery reflex, m. p. 188° (Kruber, *Ber.*, 1929, **62**, 2877, records m. p. 194—195° and Marion and Oldfield, *loc. cit.*, m. p. 194°).

6-*Methylindole.* Two 30 g. batches of formyl-*p*-xylidine were cyclised as described for the 4-isomer. Distillation of the combined products yielded 6-*methylindole* (13·1 g.; 50%) as an almost colourless oil, b. p. 111°/3 mm., which readily solidified, m. p. 28° (Found : N, 11·3. C_9H_9N requires N, 10·7%); the *picrate* crystallised from benzene in felted orange-red needles, m. p. 152° (Found : C, 50·1; H, 3·4.

$C_{15}H_{12}O_7N_4$ requires C, 50·0; H, 3·3%). Marion and Oldfield (*loc. cit.*) give m. p. 161·5° for the picrate of an unanalysed specimen of 6-methylindole, prepared similarly, which showed no tendency to crystallise.

Methylgramines (II).—These were prepared by the following general procedure. Acetic acid (15 c.c.) was added slowly to 33% (*w/v*) aqueous dimethylamine (15 c.c.), cooled in a freezing mixture, at such a rate that the temperature did not rise above 5°; 40% formaldehyde solution (7·5 c.c.) was then added, the temperature being still kept below 5°. This solution was then poured on the methylindole (13·1 g.; 0·1 mol.) and the mixture shaken. The methylindole dissolved, with evolution of heat, in the course of a minute or two and the mixture was kept at room temperature for at least 4 hours; it was then poured into 2N-sodium hydroxide, and the solid methylgramine collected after the mixture had been kept overnight in the refrigerator. The following *methyl-3-dimethylaminomethylindoles* (II) were prepared in this way :

	Yield, %.[1]	Crystalline form.	M. p.	Found, % :[4] C.	H.
2-*Methyl-*	100	Feathery clusters of needles [2]	116—117°	76·4	8·5
4-*Methyl-*	97	Leaflets [2]	128—129	76·0	8·5
5-*Methyl-*	100	Acicular prisms [3]	133	76·1	8·2
6-*Methyl-*	99	Acicular prisms [2]	117	76·0	8·7
7-*Methyl-*	100	Needles [3]	114	76·2	8·6

[1] Yield of crude material sufficiently pure for use in the next stage.
[2] From dilute ethanol. [3] From dilute methanol. [4] $C_{12}H_{16}N_2$ requires C, 76·6; H, 8·5%.

Methyltryptophans (V).—The methylgramines were condensed with acetamidomalonic ester by the following procedure. The ester (21·7 g.; 0·1 mol.) and (crude) methylgramine (II) (18·8 g.; 0·1 mol.) were dissolved in alcoholic sodium ethoxide [from sodium (2·3 g.; 0·1 g.-atom) and magnesium-dried ethanol (250 c.c.)]; methyl sulphate (25·2 g.; 0·2 mol.) was added during 5—10 minutes, and the mixture kept at room temperature for 20—24 hours and then poured into water (1000 c.c.); the condensation product was precipitated, usually as a gum which crystallised on rubbing, and was collected after being kept in the refrigerator overnight. The crude product is very nearly pure and can be used directly for the next stage. Except in the 7-methyl series the use of less rigorously dried alcohol or heating the reaction mixture reduces the yield and leads to a less pure product. The following *ethyl* 1-*acetamido*-2-(x-*methyl*-3-*indolyl*)*ethane*-1 : 1-*dicarboxylates* (III) were prepared in this way :

x.	Yield, %.[1]	Crystalline form.[2]	M. p.	Found, % :[3] C.	H.
2-	80	Minute leaflets	170—171°	63·1	6·4
4-	82	Rectangular prisms	161	63·3	6·7
5-	85	Flat prisms	136	63·5	6·7
6-	79	Rosettes of small prisms	172—173	64·2	6·9
7-	93 [4]	Rosettes of acicular prisms	127	62·7	6·5

[1] Not recrystallised. [2] From dilute ethanol. [3] $C_{19}H_{24}O_5N_2$ requires C, 63·3; H, 6·7%.
[4] A 20% excess of sodium and acetamidomalonic ester was used, and the reaction mixture was refluxed for 3 hours after having been kept at room temperature for 3 hours.

The best procedures for the subsequent stages in the synthesis of the different methyltryptophans varied, and are therefore recorded in detail.

2-*Methyltryptophan*. The crude condensation product (III) (35·9 g.) was refluxed on the water-bath for 3 hours with sodium hydroxide (25 g.) in water (120 c.c.) and ethanol (240 c.c.). Alcohol was removed under reduced pressure, and water (240 c.c.) added to the residue; after being kept in the refrigerator for some hours, the yellow precipitate was filtered off and the filtrate extracted twice with *n*-butanol–chloroform (17 : 83 *v/v*). The aqueous layer was acidified with concentrated hydrochloric acid (63 c.c.) and shaken with 17% butanol–chloroform. A copious pale orange crystalline precipitate separated (it was sometimes necessary to keep the mixture overnight in the refrigerator) and was filtered off and dried in a vacuum desiccator; 24·2 g. (80%), m. p. 142°. Crystallisation from acetone–petroleum (b. p. 60—80°) yielded 1-*acetamido*-2-(2-*methyl*-3-*indolyl*)*ethane*-1 : 1-*dicarboxylic acid* (IV) in rosettes of acicular prisms, m. p. 143° (decomp.) (Found : C, 59·2; H, 6·0. $C_{15}H_{16}O_5N_2$ requires C, 59·2; H, 5·3%).

The unrecrystallised malonic acid (2·9 g.) was heated in an oil-bath at 180° for 20 minutes in a stream of dry nitrogen; after cooling in nitrogen, the residue was treated with a hot solution of barium hydroxide (6·0 g.) in water (20 c.c.) and the mixture heated at 100° for 26 hours with exclusion of atmospheric carbon dioxide. The solution was then freed completely from barium with the required amount of 2N-sulphuric acid, and the barium sulphate filtered off and washed well with three 20 c.c. lots of hot water. The filtrate and washings were evaporated under reduced pressure to 30 c.c., and alcohol (45 c.c.) added. A first crop of 2-methyltryptophan separated after being kept for some time in the refrigerator, and two further crops were obtained by evaporation and crystallisation of the solid residues from smaller volumes of 60% alcohol; in all 1·5 g. (72%) of crude material were so obtained. Two recrystallisations from 60% alcohol gave pure 2-methyltryptophan (V) (740 mg.) in small leaflets which, dried in a vacuum desiccator over phosphoric oxide for several days at 37°, had m. p. 234—235° after previous softening (Found : C, 65·6; H, 6·4. Calc. for $C_{12}H_{14}O_2N_2$: C, 66·1; H, 6·4%).

4-*Methyltryptophan*. The crude condensation product (III) (7 g.) was refluxed on the water-bath for 4 hours with sodium hydroxide (7 g.) in water (30 c.c.) and alcohol (45 c.c.). Alcohol was removed under reduced pressure and water (100 c.c.) added to the semi-solid residue. The product was filtered to remove a little insoluble material and the filtrate acidified with concentrated hydrochloric acid

(ice-cooling); the acidified solution was thoroughly extracted with ether and the dried extract evaporated, finally in a vacuum desiccator. The glassy product (4·6 g.) was crystallised from ethyl acetate–petroleum (b. p. 60—80°), yielding 4·4 g. (74%) of the malonic acid (IV), m. p. 117—119°. A further crystallisation from acetone–petroleum (b. p. 60—80°) yielded 1-*acetamido*-2-(4-*methyl*-3-*indolyl*)*ethane*-1 : 1-*dicarboxylic acid* in small needles, m. p. 131—132° (decomp.) (Found : C, 58·9; H, 6·1%).

The once-recrystallised malonic acid (IV) (4·4 g.) was heated in an oil-bath at 160° for 30 minutes in a stream of dry nitrogen. The cooled residue was then heated at 100° for 24 hours with barium hydroxide (10 g.) in water (40 c.c.), atmospheric carbon dioxide being excluded; boiling water (200 c.c.) was added to the product and barium removed completely with the requisite amount of 2N-sulphuric acid. The barium sulphate was filtered off and washed well with hot water; evaporation of the filtrate and washings to 150 c.c. under reduced pressure gave a first crop of 4-methyltryptophan (2·12 g.), and a further 0·12 g. was obtained by concentrating the mother liquors to 30 c.c. Crystallisation of these combined products (71% yield) from hot water (taken up in 350 c.c., boiled with charcoal, filtered, and concentrated to 150 c.c.) yielded 1·80 g. of 4-*methyltryptophan* (V) in small leaflets which, dried in a vacuum desiccator over phosphoric oxide, had m. p. 265—267° after softening from 250° (placed in bath at 240°) (Found : C, 66·2; H, 6·4%).

5-*Methyltryptophan*. The recrystallised condensation product (III) (8 g.) was hydrolysed as described for the 4-methyl isomer; the glassy product was crystallised from ethyl acetate–petroleum (b. p. 60—80°), yielding the malonic acid (IV) (6·5 g.; 96%), m. p. 119—121° (decomp.). A further crystallisation from the same mixture gave 1-*acetamido*-2-(5-*methyl*-3-*indolyl*)*ethane*-1 : 1-*dicarboxylic acid* in small prismatic needles, m. p. 129° (decomp.) (Found : C, 58·6; H, 5·2%).

The once-recrystallised malonic acid (IV) (5 g.) was decarboxylated and hydrolysed as described for the 4-methyl compound; evaporation of the barium-free solution to 100 c.c. gave a first crop (1·79 g.) of 5-methyltryptophan, and a further 0·35 g. was obtained by concentrating the mother-liquors (total yield : 2·14 g.; 60%). Recrystallisation from hot water (dissolved in 200 c.c., boiled with charcoal, filtered, and concentrated to 90 c.c.) yielded 5-methyltryptophan (V) in leaflets, m. p. 264° (decomp.) after softening from 245° (Found : C, 65·7; H, 6·6%).

6-*Methyltryptophan*. The recrystallised condensation product (III) (12 g.) was hydrolysed as described for the 4-methyl compound; the glassy product (10 g.; 99%) was crystallised from ethyl acetate–petroleum (b. p. 60—80°), yielding 1-*acetamido*-2-(6-*methyl*-3-*indolyl*)*ethane*-1 : 1-*dicarboxylic acid* (IV) in rosettes of stout needles, m. p. 130—132° (Found : C, 58·8; H, 6·1%).

Hydrolysis with barium hydroxide was unsatisfactory owing to the low solubility of the barium salt of 6-methyltryptophan. Accordingly, the crude malonic acid (IV) (2·5 g.) was decarboxylated as usual and then heated at 100° for 23 hours with a solution of potassium hydroxide (2·0 g.) in water (25 c.c.). Water (10 c.c.) and acetic acid (2·05 c.c.) were added to the hot solution which was then left to crystallise in the refrigerator overnight. The crude 6-methyltryptophan (1·30 g.; 73%) was filtered off, washed, and dried in a vacuum desiccator. Recrystallisation from hot water (charcoal) yielded 6-*methyltryptophan* (V) (0·75 g.) in small shining leaflets, m. p. 258—260° after softening from 245° (Found : C, 66·1; H, 6·2%).

7-*Methyltryptophan*. The crude condensation product (III) (10 g.) was refluxed for 3 hours with sodium hydroxide (8 g.) in water (50 c.c.) and alcohol (100 c.c.). Alcohol was removed under reduced pressure, and the residue treated with water (50 c.c.) and concentrated hydrochloric acid (50 c.c.) and refluxed for 3 hours. After filtration from a little tar, the solution was brought to pH 5—6 with 2N-sodium hydroxide and rapidly filtered from an immediate dirty precipitate. The white solid deposited after the solution had been seeded and kept overnight was filtered off and dried in a vacuum desiccator (6·0 g.; 99%). Recrystallisation from water (dissolved in 1000 c.c., boiled with charcoal, filtered, and concentrated to 300 c.c.) yielded 3·5 g. of fairly pure 7-methyltryptophan, m. p. 283—285° (decomp.), a further 0·75 g. being obtained by concentrating the mother liquors to 100 c.c. For final purification, 6·0 g. of such material was dissolved in N-hydrochloric acid (120 c.c.) and extracted 5 times with *n*-butanol–chloroform (17 : 83 *v/v*). The residual aqueous solution was filtered through a wet paper, and 2N-sodium hydroxide (60 c.c.) added; the methyltryptophan (3·0 g.) crystallised in rosettes of pointed needles. Recrystallisation from water yielded 2·35 g. of pure 7-*methyltryptophan* (V) in small leaflets, decomp. 296° after softening from 264° (Found : C, 66·3; H, 6·6%). 7-Methyltryptophan (200 mg.) was dissolved in 2N-sodium hydroxide (2·0 c.c.) and treated with six 0·1 c.c. lots of acetic anhydride, the mixture being shaken well after each addition; the product was kept at 37° for 2 hours and then at 0° overnight. The solid (200 mg.; 83%) was filtered off, washed with water, and crystallised from water; N-*acetyl*-7-*methyltryptophan* formed feathery aggregates of needles, m. p. 217—218° (Found : C, 64·1; H, 6·4. $C_{14}H_{16}O_3N_2$ requires C, 64·6; H, 6·1%).

In another series of experiments, acetamidocyanoacetic ester (2·9 g.) and 7-methylgramine (II) (3·2 g.) were added to alcoholic sodium ethoxide [from sodium (0·40 g.) and absolute alcohol (25 c.c.)]. The mixture was refluxed on the water-bath while a mixture of ethyl iodide (2·66 g.) and absolute alcohol (10 c.c.) was added during 15 minutes. The mixture was refluxed for 22 hours and the alcohol removed under reduced pressure. The residue was treated with water and extracted with chloroform; evaporation of the dried chloroform extract, washed with 2N-hydrochloric acid, 10% sodium hydrogen carbonate, and water, yielded 3·55 g. (67%) of a glass; crystallisation from dilute ethanol gave *ethyl* 1-*acetamido*-1-*cyano*-2-(7-*methyl*-3-*indolyl*)*ethane*-1-*carboxylate* in small prisms, m. p. 183° (Found : C, 65·6; H, 6·5. $C_{12}H_{19}O_3N_3$ requires C, 65·2; H, 6·1%). This crude condensation product (3·3 g.) was refluxed for 19 hours with potassium hydroxide (3·1 g.) in water (10 c.c.) and alcohol (10 c.c.). After distillation of the alcohol, water was added to bring the volume to 50 c.c., and a precipitate removed. The filtrate was treated with 2N-hydrochloric acid to maximum precipitation and then kept in the refrigerator overnight. The solid (1·54 g.) was filtered off and refluxed for 3 hours with water (15 c.c.), some tar and 7-methylindole being formed; sodium hydroxide (1·2 g.) was added and refluxing continued for 19 hours. Water (10 c.c.) was added, and the solution filtered; addition of acetic acid (1·72 c.c.) precipitated crude 7-methyltryptophan (1·2 g.; 52%) which was filtered off, dissolved in water (20 c.c.) containing

sodium hydroxide (0·75 g.), boiled with charcoal, filtered, and reprecipitated by adding alcohol (10 c.c.) and acetic acid (1·12 c.c.). After the mixture had been kept in the refrigerator overnight the precipitate (780 mg.) was filtered off and recrystallised from boiling water, yielding pure 7-methyltryptophan in small shining leaflets, decomp. 296° after softening from 264°.

Colour Reactions of the Methyltryptophans.—The reactions were carried out as follows, the absorption bands being observed with a Hartridge reversion spectroscope after dilution, if necessary, with 50% *v/v* sulphuric acid.

(A) *Adamkiewicz reaction* (*Pfluger's Arch.*, 1874, **9**, 156). 1 C.c. of a solution of the methyltryptophan (1 mg./c.c.) was mixed with 1 c.c. of glacial acetic acid, underlayered with 2 c.c. of concentrated sulphuric acid, and finally mixed.

(B) *Hopkins–Cole glyoxylic reaction* (S. W. Cole, *op. cit.*, p. 39). 1 C.c. of a solution of the methyltryptophan (1 mg./c.c.) was mixed with 1 c.c. of the " glyoxylic reagent ", underlayered with 2 c.c. of " AnalaR " sulphuric acid, and finally mixed.

(C) *Hopkins–Cole glyoxylic reaction with mercuric sulphate.* As (B) but with addition of 2 drops of the mercuric sulphate used in (D).

(D) *Cole formaldehyde reaction* (S. W. Cole, *op. cit.*, p. 80). 1 C.c. of a solution of the methyltryptophan (1 mg./c.c.) was mixed with 1 c.c. of water, 2 drops of 1/500 formalin, and 2 drops of 10% mercuric sulphate in 10% sulphuric acid, underlayered with 2 c.c. of " AnalaR " sulphuric acid, and finally mixed.

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