543. *Experiments on the Synthesis of* Bz-*Substituted Indoles and Tryptophans. Part II.* Attempted Syntheses of* Bz-*Nitro-indoles and -tryptophans. The Synthesis of* 5-*Ethoxytryptophan and of* 4 : 5- *and* 6 : 7-*Benzotryptophan.*

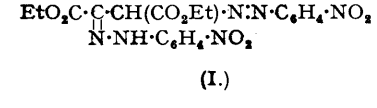
By H. N. Rydon and S. Siddappa.

Unsuccessful attempts to synthesise *Bz*-nitro-indoles and -tryptophans by a variety of methods are described. 5-Ethoxytryptophan, and 4 : 5- and 6 : 7-benzotryptophan have been synthesised, by conventional methods, for test as bacterial inhibitors.

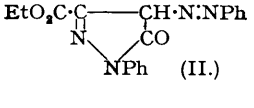
For the continuation of the study of the relation between structure and antibacterial activity of substituted indoles and tryptophans (Fildes and Rydon, *Brit. J. Exp. Path.*, 1947, **28**, 211; Rydon, *J.*, 1948, 705 *; Rydon and Long, *Nature*, 1949, **164**, 575) the nitroindoles and nitrotryptophans are desirable compounds, both for their intrinsic interest as substances containing a powerfully electron-attracting group and as intermediates for the preparation of other *Bz*-substituted indoles and tryptophans. We have, accordingly, examined several possible methods for the preparation of these substances; although we did not achieve our object it is desirable to report our findings at this stage in view of the termination of our collaboration on this subject.

Comparatively few *Bz*-nitroindoles are described in the literature. Majima and Kotake (*Ber.*, 1930, **63**, 2237) obtained 6-nitroindole by nitration of 3-carbethoxyindole, followed by hydrolysis and decarboxylation, and an unorientated nitroindole-3-aldehyde by direct nitration of the well-known indole-3-aldehyde; Bauer and Strauss (*Ber.*, 1932, **65**, 308) prepared 2 : 3-dimethyl-5-nitroindole by nitration of 2 : 3-dimethylindole, and von Walther and Clemen (*J. pr. Chem.*, 1900, **61**, 266) obtained an unorientated 2-methyldinitroindole by direct nitration of skatole. Such direct nitration methods were insufficiently flexible for our purpose, since we required all four *Bz*-nitroindoles.

The Fischer synthesis seemed more promising; various 2 : 3-disubstituted *Bz*-nitroindoles have been prepared in this way from the *o*-, *m*-, and *p*-nitrophenylhydrazones of ketones (Bauer and Strauss, *loc. cit.*; Schofield and Theobald, *J.*, 1949, 796; 1950, 1505), and Hughes, Lions, and Ritchie (*J. Proc. Roy. Soc., N.S.W.*, 1939, **72**, 309) describe the synthesis of 7-nitroindole by Fischer cyclisation of ethyl pyruvate *o*-nitrophenylhydrazone, followed by hydrolysis and decarboxylation. We have been unable to confirm the findings of the Australian workers, ethyl pyruvate *o*-nitrophenylhydrazone failing, in our hands, to cyclise under a variety of conditions; with aqueous acids the only product was pyruvic acid *o*-nitrophenylhydrazone, m. p. 222°, which appears to be the substance described by Hughes, Lions, and Ritchie (*loc. cit.*) as 7-nitroindole-2-carboxylic acid (m. p. 231°), and under non-aqueous conditions we obtained a substance isomeric with, and possibly a stereoisomeride of, the starting material. The



(I.)



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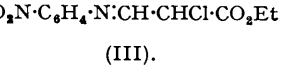


(III).

analytical figures quoted by Hughes, Lions, and Ritchie (*loc. cit.*) for 7-nitroindole-2-carboxylic acid and for 7-nitroindole are unsatisfactory and it seems doubtful whether they in fact obtained these compounds.† Similarly, attempts to cyclise the *m*- and *p*-nitrophenylhydrazones of ethyl pyruvate gave only the derivatives of pyruvic acid. Attempts to modify the structural conditions by replacing ethyl pyruvate by ethyl acetosuccinate were rendered abortive since this substance reacts with two molecules of diazotised base in the Japp–Klingemann reaction, giving (I) with diazotised *p*-nitroaniline and (II) with diazotised aniline. Similar lack of success attended attempts to prepare the *Bz*-nitrotrytophans directly by cyclisation of the nitrophenylhydrazones of the Michael adduct (XII) from acraldehyde and acetamidomalonic ester (cf. Warner and Moe, *J. Amer. Chem. Soc.*, 1948, **70**, 2763, 2765). These consistent failures to

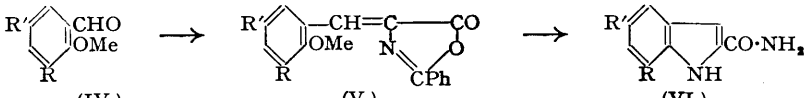
* *J.*, 1948, 705 is regarded as Part I of this series.

† In a personal communication Dr. Hughes informs us that he and his colleagues are in agreement with this conclusion.

cyclise nitrophenylhydrazones are doubtless to be ascribed to the deactivating effect of the nitro-group, although this cannot be the sole reason in view of the successful cyclisations of ketone nitrophenylhydrazones recorded by other workers (Bauer and Strauss, *loc. cit.*; Schofield and Theobald, *locc. cit.*).

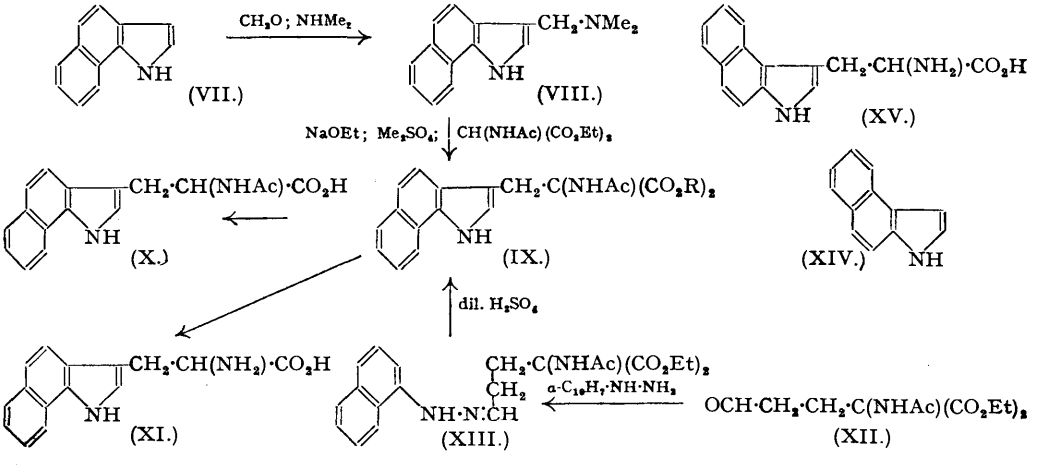
We next turned our attention to Mentzer's alleged synthesis of 2-carbethoxyindoles by the action of aromatic amines on ethyl formylchloroacetate (*Compt. rend.*, 1946, **222**, 1178). With the three nitroanilines the only products were the anils (III), and reinvestigation of two of the cases described by Mentzer, *viz.*, reaction with aniline and *p*-anisidine, showed that here, too, anils were the sole products (cf. the papers of Smith, *J.*, 1950, 1637; Robinson and Saxton, *ibid.*, p. 3136, and Taylor, *ibid.*, p. 3345, which appeared after the completion of our work).

Hill and Robinson (*J.*, 1933, 486) prepared 5 : 7-dinitroindole-2-carboxyamide (dinitrostrycholamide) (VI; R = R′ = NO_2) by the action of alcoholic ammonia on the oxazolone (V; R = R′ = NO_2) obtained from 2-methoxy-3 : 5-dinitrobenzaldehyde (IV; R = R′ = NO_2), and we attempted to prepare the 5-nitro-compound (VI; R = H, R′ = NO_2) similarly: 2-methoxy-5-nitrobenzaldehyde (IV; R = H, R′ = NO_2), prepared with some difficulty from 5-nitrosalicylaldehyde, condensed normally with hippuric acid to give the oxazolone (V; R = H, R′ = NO_2) but this did not yield any of the required indole derivative with alcoholic ammonia. In this case it appears that a single nitro-group insufficiently activates the methoxyl group.



Special interest attaches to the benzindoles and benzotryptophans as bacterial inhibitors in view of the finding (Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exp. Path.*, 1945, **26**, 160) that, in the acridine series, inhibitory activity is considerably modified by changes in the " flat " area of the inhibitor molecule. We have, accordingly, prepared the two most readily accessible benzindoles and benzotryptophans with a view to determining whether such an effect also operates in the indole–tryptophan series.

6 : 7-Benzindole (VII) was first prepared by Schlieper (*Annalen*, 1887, **239**, 229) by the Fischer cyclisation of pyruvic acid α-naphthylhydrazone with zinc chloride, followed by decarboxylation of the resulting 6 : 7-benzindole-2-carboxylic acid; the structure of the product was confirmed by two unambiguous syntheses (Pschorr and Kuhtz, *Ber.*, 1905, **38**, 217; Mayer and Oppenheimer, *ibid.*, 1918, **51**, 510, 1239). 6 : 7-Benzindole (VII), prepared in increased yield by ethanolic sulphuric acid cyclisation of ethyl pyruvate α-naphthylhydrazone, was converted into 6 : 7-benzotryptophan (XI) by the gramine method (Snyder and Smith, *J. Amer. Chem. Soc.*, 1944, **66**, 350; Albertson, Archer, and Suter, *ibid.*, p. 500; 1945, **67**, 36; cf. Rydon, *J.*, 1948, 705) thus :



6 : 7-Benzotryptophan (XI) was also synthesised by the elegant method of Warner and Moe (*J. Amer. Chem. Soc.*, 1948, **70**, 2763, 2765), cyclisation of the α-naphthylhydrazone (XIII) of

the Michael adduct (XII) from acraldehyde and acetamidomalonic ester giving an excellent yield of the intermediate (IX), identical with that obtained by the gramine method.

Precisely analogous methods were used for the synthesis of 4 : 5-benzotryptophan (XV) from 4 : 5-benzindole (XIV); the latter was first prepared by Schlieper (*Annalen*, 1886, **236**, 177) by Fischer cyclisation of ethyl pyruvate β-naphthylhydrazone, a process which we have considerably improved; its structure rests on its non-identity with 5 : 6-benzindole, the other possible product of the Fischer cyclisation, which has been prepared by another route (G.P. 516,675).

We record also (p. 2466) the synthesis of 5-ethoxyindole (cf. Hoshino and Kotake, *Annalen*, 1935, **516**, 76; Deulofeu, *Rev. brasil. Chim.*, 1938, **5**, 270) by the Fischer method from ethyl pyruvate *p*-ethoxyphenylhydrazone and its conversion into 5-ethoxytryptophan by the gramine procedure; in this case Warner and Moe's method (*loc. cit.*) was less satisfactory.

Biological tests on the indoles and tryptophans described in this paper will be reported elsewhere in due course.

Experimental.

A. *Attempted Syntheses of* Bz-*Nitrotryptophans.*—(i) *By the Fischer method.* Ethyl pyruvate *o*-nitrophenylhydrazone (10 g.; m. p. 114°; Hughes, Lions, and Ritchie, *loc. cit.*, give m. p. 106°) (Found: C, 52·65; H, 5·2; N, 16·95. Calc. for $C_{11}H_{13}O_4N_3$: C, 52·6; H, 5·2; N, 16·7%) was refluxed for 1 hour with concentrated hydrochloric acid (90 c.c.). The yellow solid (9 g., 100%; m. p. 218—220°), which crystallised on cooling, was filtered off and recrystallised from ethanol, forming yellow needles, m. p. 220°, which were clearly pyruvic acid *o*-nitrophenylhydrazone (Ciusa and Rastelli, *Gazzetta*, 1922, **52**, 121, record m. p. 211°) (Found: C, 48·7; H, 4·3; N, 18·8. Calc. for $C_9H_9O_4N_3$: C, 48·4; H, 4·0; N, 18·8%). This compound (8·5 g.) was heated under glycerol (90 c.c.) to 220° for 5 minutes; the cooled mixture was poured into water (500 c.c.), and the product extracted with ether. Distillation of the dried extract yielded only *o*-nitroaniline (3·5 g., 66%), orange rods (from light petroleum), m. p. and mixed m. p. 70°; no trace of nitroindole could be detected in the reaction product. Ethyl pyruvate *o*-nitrophenylhydrazone gave only the pyruvic acid derivative with a solution of zinc chloride in boiling concentrated hydrochloric acid, but, when it was heated (2 g.) with acetic acid (10 c.c.), containing concentrated sulphuric acid (1 c.c.) to 80° and poured into water, a yellow solid separated which crystallised from ethanol in yellow needles, m. p. 97°; this *substance* (Found: C, 52·3; H, 5·2; N, 16·9. $C_{11}H_{13}O_4N_3$ requires C, 52·6; H, 5·2; N, 16·7%) is isomeric with, and possibly a stereoisomeride of, ethyl pyruvate *o*-nitrophenylhydrazone; it was also formed by the action of cold concentrated sulphuric acid and yielded pyruvic acid *o*-nitrophenylhydrazone, m. p. and mixed m. p. 220°, on hydrolysis with concentrated hydrochloric acid.

Ice-cold 50% potassium hydroxide solution (50 c.c.) was added to a stirred solution of ethyl methylacetoacetate (20·5 g.) in ethanol (150 c.c.) at 0°. Ice-water (300 c.c.) was added, followed immediately by a diazonium solution from *m*-nitroaniline (20 g.), concentrated hydrochloric acid (60 c.c.), sodium nitrite (10·5 g.), and water (90 c.c.). The crystalline solid, which separated after a further 15 minutes' stirring, was recrystallised from hot methanol; *ethyl pyruvate* m-*nitrophenylhydrazone* (30 g., 84%) forms orange needles, m. p. 150° (Found: C, 53·3; H, 5·3; N, 16·9. $C_{11}H_{13}O_4N_3$ requires C, 52·6; H, 5·2; N, 16·7%). This substance was recovered unchanged after being heated on the steam-bath for 2 hours with acetic acid (5 vols.) and concentrated sulphuric acid (0·5 vol.); refluxing for 1 hour with concentrated hydrochloric acid (5 vols.) yielded pyruvic acid *m*-nitrophenylhydrazone, m. p. 220° (from ethanol) (Ciusa and Musajo, *Gazzetta*, 1930, **60**, 486, give m. p. 226°) (Found: N, 19·1. Calc. for $C_9H_9O_4N_3$: N, 18·8%).

Ethyl pyruvate *p*-nitrophenylhydrazone, prepared similarly in 78% yield, orange plates from ethanol, m. p. 184° (Quilico and Freri, *ibid.*, 1929, **59**, 930, record m. p. 187°) (Found: C, 52·5; H, 5·3; N, 16·7. Calc. for $C_{11}H_{13}O_4N_3$: C, 52·6; H, 5·2; N, 16·7%), was unchanged after treatment with cold concentrated sulphuric acid and after being heated with mixtures of concentrated sulphuric acid and ethanol or acetic acid; refluxing with concentrated hydrochloric acid (5 vols.), with or without the addition of zinc chloride, afforded only pyruvic acid *p*-nitrophenylhydrazone, m. p. 225° (Hyde, *Ber.*, 1899, **32**, 1815, gives m. p. 219—220°) (Found: N, 19·0. Calc. for $C_9H_9O_4N_3$: N, 18·8%).

The Japp–Klingemann reaction between diethyl acetosuccinate (30 g.) and *p*-nitroaniline (20 g.), carried out as described above, yielded 25 g. (38%) of a red solid which was very sparingly soluble in most organic solvents and separated in powdery form, m. p. 235°, from hot dioxan; this is regarded as *diethyl* p-*nitrobenzeneazo-oxaloacetate* p-*nitrophenylhydrazone* (I) (Found: N, 18·5. $C_{20}H_{20}O_8N_6$ requires N, 17·8%). A similar reaction with aniline yielded 3-carbethoxy-1-phenyl-4-phenylazopyrazol-5-one (II), which crystallised from ethanol in orange needles, m. p. 153° (Wislicenus and Scheidt, *Ber.*, 1891, **24**, 4210, give m. p. 152—154°) (Found: C, 64·25; H, 4·9; N, 16·75. Calc. for $C_{18}H_{16}O_3N_4$: C, 64·3; H, 4·7; N, 16·7%).

(ii) *By Warner and Moe's method.* Acraldehyde (3·2 g.), in magnesium-dried ethanol (5 c.c.), was added dropwise during 1 hour, with stirring, to ethyl acetamidomalonate (10·9 g.) and sodium ethoxide (from sodium, 0·05 g., and magnesium-dried ethanol, 25 c.c.), the temperature of the mixture being kept at 3—7°. After a further hour's stirring, *o*-nitrophenylhydrazine (7·5 g.) and acetic acid (1·5 c.c.) were added, and the mixture was stirred for 2 hours at 3°, then warmed to 50°, water (2 c.c.) added, and the mixture kept overnight. Dilution with water and ice-cooling precipitated a yellow oil which solidified after a few hours (12 g., 59%); crystallisation from methanol yielded *γ-acetamido-γγ-dicarbethoxy*-n-*butaldehyde* o-*nitrophenylhydrazone* as yellow needles, m. p. 40° (Found: N, 14·1. $C_{18}H_{24}O_7N_4$ requires

N, 13·7%). The corresponding m-*nitrophenylhydrazone* (63% yield), orange prisms (from aqueous acetone), m. p. 150° (Found: C, 53·4; H, 6·1; N, 13·6. $C_{18}H_{24}O_7N_4$ requires C, 52·9; H, 5·9; N, 13·7%), and p-*nitrophenylhydrazone* (73% yield), yellow plates [from benzene–light petroleum (b. p. 40—60°)], m. p. 130° (Found: C, 52·9; H, 6·0; N, 14·0%), were prepared similarly. The *p*-nitrophenylhydrazone gave only tarry material on refluxing with 5% (v/v) sulphuric acid for 4 hours, but the *m*-nitrophenylhydrazone was unaffected by this treatment. The *o*-nitrophenylhydrazone (2·0 g.) was refluxed with 5% (v/v) sulphuric acid (12 c.c.) for 3 hours; a crystalline solid (0·5 g.) separated which crystallised from aqueous ethanol in pale yellow needles, m. p. 100—101°, which proved to be the o-*nitrophenylhydrazine* salt of 1-hydroxybenzotriazole (Found: N, 29·4. $C_{12}H_{12}O_3N_6$ requires N, 29·2%).

(iii) *By Mentzer's method.* *p*-Nitroaniline (2·6 g.) and ethyl formylchloroacetate (Wislicenus, *Ber.*, 1910, **43**, 3530) (4·6 g.) were refluxed for 5 minutes; the entire mixture solidified, and digestion with ethanol gave a yellow solid (4 g., 79%); *ethyl α-chloro-β-(p-nitrophenylimino)propionate* (III) crystallised from ethanol in yellow needles, m. p. 215° (Found: C, 48·9; H, 4·2; N, 10·4. $C_{11}H_{11}O_4N_2Cl$ requires C, 48·8; H, 4·1; N, 10·35%). The same product was obtained when the heating was carried out for 90 minutes at 130° and when the reactants were heated for 4 hours in acetic acid containing a little morpholine.

The following were prepared similarly from the appropriate amines: *Ethyl α-chloro-β-o-nitrophenyliminopropionate*, yellow plates (from methanol), m. p. 101° (Found: C, 48·7; H, 4·1; N, 10·0%); *ethyl α-chloro-β-m-nitrophenyliminopropionate*, yellow plates (from methanol), m. p. 137° (Found: C, 48·75; H, 4·35; N, 10·25%); ethyl α-chloro-β-phenyliminopropionate, plates (from methanol), m. p. 82° (Smith, *loc. cit.*, gives m. p. 82°) (Found: C, 58·5; H, 5·5; N, 6·1; Cl, 15·85. Calc. for $C_{11}H_{12}O_2NCl$: C, 58·5; H, 5·3; N, 6·2; Cl, 15·7%); ethyl α-chloro-β-*p*-methoxyphenyliminopropionate, prisms (from aqueous methanol), m. p. 97° (Smith, *loc. cit.*, gives m. p. 96—96·5°) (Found: C, 56·85; H, 5·55; N, 5·6; Cl, 14·35. Calc. for $C_{12}H_{14}O_3NCl$: C, 56·4; H, 5·5; N, 5·5; Cl, 13·9%).

(iv) *By Hill and Robinson's method.* 5-Nitrosalicylaldehyde (Miller, *Ber.*, 1887, **20**, 1928) (4·2 g.) was mixed with methyl iodide (12 g.), and dry silver oxide (8·7 g.) added in small quantities under reflux; after the initial reaction had abated, the mixture was heated on the steam-bath for 1 hour, cooled, diluted with ether, filtered, and evaporated. Crystallisation from methanol gave 2-methoxy-5-nitrobenzaldehyde (IV; R = H, R′ = NO_2) as yellow needles, m. p. 80° (lit., m. p. 89°), sufficiently pure for the next stage. This aldehyde (1·8 g.), anhydrous sodium acetate (0·54 g.), hippuric acid (1·8 g.), and acetic anhydride (10 c.c.) were gently refluxed for 1 hour. The yellow solid which separated on cooling was filtered off and washed with water and ethanol (yield, 1·9 g., 59%; m. p. 225—230°); crystallisation from acetic acid gave 4-(*2-methoxy-5-nitrobenzylidene*)-*2-phenyloxazol-5-one* (V; R = H, R′ = NO_2) as pale yellow needles, m. p. 253—254° (Found: C, 62·5; H, 3·9; N, 8·9. $C_{17}H_{12}O_5N_2$ requires C, 63·0; H, 3·7; N, 8·6%).

B. *Synthesis of the Benzotryptophans.*—*6 : 7-Benzindole.* Ethyl pyruvate α-naphthylhydrazone (Hughes and Lions, *J. Proc. Roy. Soc., N.S.W.*, 1937, **71**, 475) (20 g.) was refluxed for 24 hours with anhydrous ethanol (180 c.c.) and concentrated sulphuric acid (10 c.c.). The mixture was poured on crushed ice (600 g.) and the product, which crystallised after some hours, was recrystallised from methanol. This ester (17 g., 91%; m. p. 160°; Schlieper, *Annalen*, 1887, **239**, 229, gives m. p. 170°) was refluxed for 3 hours with potassium hydroxide (20 g.) in methanol (100 c.c.); after removal of most of the methanol the residue was acidified, and the precipitated 6 : 7-benzindole-2-carboxylic acid filtered off and washed with water and a little methanol; m. p. 204° (Schlieper, *loc. cit.*, gives m. p. 204—205°); yield 14 g. (93%).

6 : 7-Benzindole-2-carboxylic acid (5 g.) was heated to 210—220° (metal-bath); decarboxylation was complete in 10 minutes and the cooled product was sublimed in a high vacuum at 130—140° (2·6 g., 66%; m. p. 179—180°). Crystallisation from aqueous methanol gave 6 : 7-benzindole (VII) in flakes, m. p. 180° (Schlieper, *loc. cit.*, gives m. p. 179—180°) (Found: C, 85·9; H, 5·4; N, 8·6. Calc. for $C_{12}H_9N$: C, 86·2; H, 5·4; N, 8·4%).

6 : 7-*Benzotryptophan.* Glacial acetic acid (15 c.c.) was added slowly to aqueous dimethylamine (33%; 15 c.c.) with cooling sufficient to keep the temperature below 5°; aqueous formaldehyde (40%; 7·5 c.c.) was added, and one-eighth of the resulting mixture added to 6 : 7-benzindole (2 g.). A clear solution was obtained after 4 hours; this was poured into 2N-sodium hydroxide (75 c.c.) and kept at 0° overnight. The resulting *3-dimethylaminomethyl-6 : 7-benzindole* (VIII) crystallised from aqueous methanol in prisms, m. p. 172° (1·7 g., 63%) (Found: C, 80·4; H, 7·2; N, 12·4. $C_{15}H_{16}N_2$ requires C, 80·4; H, 7·1; N, 12·5%). This benzogramine (1·12 g.) was added to acetamidomalonic ester (1·08 g.), dissolved in sodium ethoxide (from sodium, 0·12 g., and magnesium-dried ethanol, 15 c.c.), and treated with methyl sulphate (1·36 g.). The mixture was kept at room temperature for 24 hours and then poured into water (100 c.c.); after being kept overnight at 0°, the *ethyl α-acetamido-α-(6 : 7-benz-3-indolylmethyl)malonate* (IX; R = Et) (1·9 g., 96%; m. p. 166—167°) was filtered off; the pure compound crystallises from aqueous methanol in prisms, m. p. 171° (Found: C, 66·5; H, 6·1; N, 7·0. $C_{22}H_{24}O_5N_2$ requires C, 66·7; H, 6·0; N, 7·1%). The crude condensation product (3·5 g.) was refluxed for 4 hours with sodium hydroxide (5 g.) in water (15 c.c.) and ethanol (25 c.c.). After removal of the ethanol, the residue was dissolved in water (100 c.c.) and acidified; ether-extraction, followed by evaporation of the dried extract, yielded *α-acetamido-α-(6 : 7-benz-3-indolylmethyl)malonic acid* (IX; R = H) (1·9 g., 62%), which crystallised from ethyl acetate–petroleum (b. p. 60—80°) in prisms, m. p. 180° (Found: N, 7·9. $C_{18}H_{16}O_5N_2$ requires N, 8·2%). This acid (1·7 g.) was heated to 210° in a current of nitrogen for 30 minutes; the product, *N-acetyl-6 : 7-benzotryptophan* (X), crystallised from aqueous methanol in prisms, m. p. 235° (Found: C, 68·5; H, 5·6; N, 9·3. $C_{17}H_{16}O_3N_2$ requires C, 68·9; H, 5·4; N, 9·4%), but could not satisfactorily be hydrolysed to the parent amino-acid. Accordingly, the crude condensation product (IX; R = Et) (1 g.) was hydrolysed, as described above,

to (IX; R = H), and the crude product refluxed for 3 hours with water and concentrated hydrochloric acid (10 c.c.; 1 : 1). The mixture was cooled, filtered from a little tar, and brought to pH 5—6; the solid (0·5 g., 75%), which separated overnight, was collected by filtration and reprecipitated from dilute alkali, after use of charcoal, by adjustment of the pH to 5—6 with acetic acid. 6 : 7-*Benzotryptophan* (XI), so obtained, was a pale yellow solid, m. p. 303° (placed in bath at 280°) (Found : C, 71·0; H, 5·9; N, 10·7. $C_{15}H_{14}O_2N_2$ requires C, 70·8; H, 5·5; N, 11·0%), which was very sparingly soluble in water, giving a positive ninhydrin reaction, a green colour in the Hopkins–Cole glyoxylic reaction (Cole, " Practical Physiological Chemistry," Heffer, Cambridge, 5th edn., 1919, p. 39), and a green turning to blue in Cole's formaldehyde reaction (*op. cit.*, 9th edition, 1933, p. 80).

Alternatively, (IX; R = Et) was prepared by the following much more economical procedure : Ethyl acetamidomalonate (10·9 g.) was dissolved in sodium ethoxide (from sodium 0·05 g., and absolute ethanol, 25 c.c.), and acraldehyde (3·2 g.), in absolute ethanol (5 c.c.), was added dropwise during 1 hour, with stirring and cooling to keep the mixture at 3—7°. After a further 3 hours' stirring, acetic acid (1·5 c.c.) and α-naphthylhydrazine (7·5 g.) were added, and the mixture warmed to 50° and then kept at room temperature overnight after the addition of water (2 c.c.). Addition of more water precipitated a semi-solid mass (XIII), which was refluxed for 4 hours with 5% (v/v) sulphuric acid (105 c.c.). On cooling, a brown solid separated which, after two crystallisations from aqueous ethanol, yielded the ester (IX; R = Et) (8 g., 54%), m. p. 171°, not depressed on admixture with material prepared by the gramine method.

4 : 5-*Benzindole.* Ethyl pyruvate β-naphthylhydrazone (Hughes and Lions, *loc. cit.*) (25 g.) was refluxed for 20 hours with ethanol (150 c.c.) and concentrated sulphuric acid (15 c.c.). The mixture was poured on crushed ice (500 g.), and the crude ethyl 4 : 5-benzindole-2-carboxylate filtered off; a specimen recrystallised once from light petroleum (b. p. 60—80°) and once from aqueous methanol had m. p. 162° (Schlieper, *Annalen*, 1886, **236**, 177, gives m. p. 161°) (Found : C, 75·3; H, 5·4; N, 6·0. Calc. for $C_{15}H_{13}O_2N$: C, 75·3; H, 5·4; N, 5·9%). The bulk of the crude ester was refluxed for 3 hours with sodium hydroxide (20 g.) in methanol (150 c.c.); acidification, followed by redissolution in aqueous ammonia, decolorisation (charcoal), and reprecipitation with dilute hydrochloric acid, yielded 4 : 5-benzindole-2-carboxylic acid (15 g.; 80%), m. p. 226°, sufficiently pure for decarboxylation; the pure acid crystallised from acetic acid in flakes, m. p. 246° (Schlieper, *loc. cit.*, gives m. p. 226°) (Found : C, 73·9; H, 4·5; N, 6·5. Calc. for $C_{13}H_9O_2N$: C, 73·9; H, 4·3; N, 6·6%). The crude acid (10 g.) was suspended in dry ether (150 c.c.) and cooled in a freezing mixture while an excess of liquid ammonia was added dropwise; after being kept for 30 minutes, the ammonium salt was filtered off, dried, and heated gently over a free flame until decarboxylation was complete; distillation gave 3·9 g. (49%) of 4 : 5-benzindole (XIV), b. p. 144°/0·5 mm. The picrate, prepared in ethanol, crystallised from benzene in red needles, m. p. 202° (Found : C, 55·1; H, 3·2. Calc. for $C_{18}H_{12}O_7N_4$: C, 54·5; H, 3·0%).

4 : 5-*Benzotryptophan. Ethyl α-acetamido-α-(4 : 5-benz-3-indolylmethyl)malonate* prepared, as described above for the 6 : 7-isomer, from ethyl acetamidomalonate, acraldehyde, and β-naphthylhydrazine in 67% yield, crystallised from aqueous methanol in plates, m. p. 192° (Found : C, 66·1; H, 6·3; N, 7·4%). This ester (2 g.) was hydrolysed and decarboxylated, as described above for the 6 : 7-analogue, yielding crude 4 : 5-benzotryptophan (1 g., 78%); reprecipitation from dilute alkali by adjustment to pH 5—6 gave 4 : 5-*benzotryptophan* (XV), as a yellow solid, m. p. 275° (in bath at 260°) (Found : C, 71·2; H, 6·1; N, 10·9, 11·1%), almost insoluble even in hot water and giving an emerald-green colour in the Hopkins–Cole glyoxylic reaction and a green turning to greenish-blue in the Cole formaldehyde reaction.

In a less advantageous procedure, freshly distilled 4 : 5-benzindole (3·5 g.) was treated, as described for the 6 : 7-isomer, with dimethylamine and formaldehyde; the crude product (4·6 g., 97%) was crystallised from aqueous methanol, yielding 3-*dimethylaminomethyl*-4 : 5-*benzindole* in plates, m. p. 162° (Found : C, 80·4; H, 7·4; N, 12·6%). Condensation of this gramine (1·12 g.) with acetamidomalonic ester, as described for the 6 : 7-isomer, gave 1·7 g. (86%) of ethyl α-acetamido-α-(4 : 5-benz-3-indolylmethyl)malonate, m. p. 192° not depressed on admixture with material prepared by the alternative route.

C. *Synthesis of* 5-*Ethoxytryptophan.*—Ethyl pyruvate *p*-ethoxyphenylhydrazone (Jacobson, *Annalen*, 1922, **427**, 142) (60 g.) was refluxed for 20 hours with absolute ethanol (300 c.c.) containing concentrated sulphuric acid (5 c.c.); the dark oil, which separated on pouring on ice (800 g.), solidified when rubbed and was crystallised from methanol; it had m. p. 145—155°. This crude ethyl 5-ethoxyindole-2-carboxylate (15 g., 27%) was refluxed for 3 hours with potassium hydroxide (15 g.) in methanol (100 c.c.); removal of the methanol and acidification yielded 5-ethoxyindole-2-carboxylic acid which, crystallised from aqueous methanol, had m. p. 202—203° (Hoshino and Kotake, *Annalen*, 1935, **516**, 76, record m. p. 203—204°); the yield was 10 g. (96%). This acid (6·5 g.) was heated to 220—230° for 10 minutes; distillation of the product yielded 5-ethoxyindole (2 g., 46%), b. p. 124°/0·5 mm. This was treated as usual with dimethylamine and formaldehyde; the indole dissolved within 10 minutes, and the reaction mixture was poured into 2N-sodium hydroxide (75 c.c.) after being kept for 4 hours at room temperature. 3-*Dimethylaminomethyl*-5-*ethoxyindole* (1·6 g., 59%) was collected after being kept at 0° overnight and recrystallised from aqueous ethanol in cubes, m. p. 140° (Found : C, 71·7; H, 8·5; N, 12·6. $C_{13}H_{18}ON_2$ requires C, 71·6; H, 8·2; N, 12·8%). Condensation of this 5-ethoxygramine (2 g.) with ethyl acetamidomalonate in the usual manner afforded *ethyl α-acetamido-α-(5-ethoxy-3-indolylmethyl)malonate* (3·5 g., 98%) which crystallised from aqueous methanol in needles, m. p. 169° (Found : C, 61·6; H, 6·8; N, 7·2. $C_{20}H_{26}O_6N_2$ requires C, 61·5; H, 6·7; N, 7·2%). The crude ester (2 g.) was refluxed for 3 hours with sodium hydroxide (2 g.) in water (10 c.c.) and ethanol (12 c.c.); the alcohol was removed under reduced pressure, and the residue taken up in water, cooled to 0°, acidified, and extracted with ether. Evaporation of the dried extract and crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) yielded *α-acetamido-α-(5-ethoxy-3-indolylmethyl)malonic acid* (1·2 g., 70%) in needles, m. p.

147° (Found : N, 7·6. $C_{16}H_{18}O_6N_2$ requires N, 8·4%). The dry acid (1 g.) was heated to 165—170° for 30 minutes in a current of nitrogen; crystallisation of the residue from hot water yielded 1-*acetyl*-5-*ethoxytryptophan* (0·7 g., 80%) in needles, m. p. 195° (Found : C, 61·6; H, 6·5; N, 9·1. $C_{15}H_{18}O_4N_2$ requires C, 62·1; H, 6·2; N, 9·6%). This acetyl compound (1·3 g.) was heated at 100° for 24 hours, with exclusion of atmospheric carbon dioxide, with barium hydroxide (3·8 g.) in water (15 c.c.); boiling water (80 c.c.) was added, and barium ions removed by the addition of N-sulphuric acid. After separation of the barium sulphate, the solution was concentrated to 20 c.c. under reduced pressure; 5-*ethoxytryptophan* (0·5 g., 45%) gradually crystallised and was recrystallised from 50% aqueous ethanol, forming colourless needles, m. p. 248° (in bath at 230°) (Found : C, 62·5; H, 7·1; N, 11·2. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·4; N, 11·3%), giving a blue colour in the Hopkins–Cole glyoxylic reaction and a blue turning to purple in Cole's formaldehyde reaction.

5-Ethoxygramine (1 g.) was refluxed for 90 minutes, under dry nitrogen, with powdered sodium hydroxide (0·07 g.) and formamidomalonic ester (1·2 g.) in dry toluene (5 c.c.). Light petroleum (b. p. 40—60°) was added to the product, cooled in ice, and the solid crystallised from aqueous methanol, yielding *ethyl* α-(5-*ethoxy*-3-*indolylmethyl*)-α-*formamidomalonate* (0·5 g., 30%) as needles, m. p. 153° (Found : C, 60·8; H, 6·5; N, 7·6. $C_{19}H_{24}O_6N_2$ requires C, 60·6; H, 6·4; N, 7·45%).

Ethyl pyruvate o-*ethoxyphenylhydrazone*, yellow plates (from aqueous methanol), m. p. 87° (Found : C, 62·4; H, 7·2; N, 11·2. $C_{13}H_{18}O_3N_2$ requires C, 62·4; H, 7·2; N, 11·2%), and the p-*methoxyphenylhydrazone*, yellow rods (from aqueous methanol), m. p. 97° (Found : C, 59·4; H, 6·5; N, 11·3. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%), were prepared in 55% yield by the Japp–Klingemann method; the *p*-methoxy-compound was converted, essentially as described for the *p*-ethoxy-compound, into 5-methoxyindole, m. p. 55° (Blaikie and Perkin, *J.*, 1924, **125**, 296, record m. p. 55°), in 47% overall yield.

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