558. *Potential Antituberculosis Agents of the Indole Series.*

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A series of indole derivatives has been prepared and tested for antibacterial activity. Antituberculous activity *in vivo* was found to be limited to 3-formylindole thiosemicarbazone.

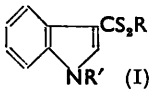
3-Formylindole thiosemicarbazone has recently been reported to have antituberculous activity in mice.[1] This observation, made independently in these laboratories some years ago, stimulated us to prepare indole analogues of several known antituberculosis agents.

The thiosemicarbazones of 2- and 3-formylindole and of 3-indolylglyoxylic acid [2] were prepared by the usual method which, however, failed with ethyl 3-indolylglyoxylate.

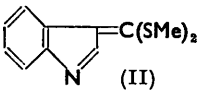
Indole-2-carboxyhydrazide was readily prepared [3] by the action of an excess of hydrazine hydrate on the ester; attempts to prepare the 3-carboxyhydrazide and the 2- and 3-carboxyamide similarly from the esters were unsuccessful, so these derivatives were obtained through the acid chlorides. Ethyl 3-indolylglyoxylate on the other hand readily gave the hydrazide and the known amide.[4]

Indole-2- and -3-thiocarboxyamide were prepared from the corresponding nitriles, themselves obtained in good yield by the dehydration of the amides with phosphorus oxychloride. Dehydration of 3-formylindole oxime [5] with thionyl chloride also gave a high yield of the 3-nitrile. Majima *et al.*[6] prepared this nitrile by the reaction of cyanogen chloride with 3-indolylmagnesium iodide, but use of the more reactive cyanogen bromide in our hands gave only an intractable gum.

Indole-2-thiocarboxyamide was readily prepared from the corresponding nitrile by the pyridine–hydrogen sulphide method of Fairfull, Lowe, and Peak,[7] but the 3-thioamide was obtained only by carrying out the reaction under pressure. An attempt to prepare the 3-thioamide from the amide and phosphorus pentasulphide in pyridine merely caused dehydration and gave the corresponding nitrile in excellent yield.



(I)



(II)

R·C(OEt):NH₂Cl (III)

R·C(NH₂):N·NHPh (IV)

Indole-3-thiocarboxyamide was also obtained by the action of cold methanolic ammonia on methyl indole-3-dithiocarboxylate (I; R = Me, R′ = H). This ester was obtained from indole-3-thiocarboxymorpholide (itself prepared from 3-formylindole, morpholine, and sulphur by the Willgerodt–Kindler reaction) by treatment with methyl iodide followed

[1] Weller, Sell, and Gottshall, *J. Amer. Chem. Soc.*, 1954, **76**, 1959.
[2] Elks, Elliott, and Hems, *J.*, 1944, 629.
[3] Piccinini and Salmoni, *Gazzetta*, 1902, **32**, I, 252.
[4] Baker, *J.*, 1940, 458; Oddo and Albanese, *Gazzetta*, 1927, **57**, 827.
[5] Pschorr and Hoppe, *Ber.*, 1910, **43**, 2549.
[6] Majima, Shigematsu, and Rokkatu, *Ber.*, 1924, **57**, 1455.
[7] Fairfull, Lowe, and Peak, *J.*, 1952, 742.
[8] Peak and Stansfield, *J.*, 1952, 4067.

by hydrogen sulphide in pyridine.[8] Treatment of the crude dithio-acid (I; R = R′ = H)[9] with methyl sulphate and alkali gave, not the expected ester (I; R = Me, R′ = H), but a dimethylated product for which the indolenine structure (II) is considered more probable than the 1-methyl alternative (I; R = R′ = Me).

2-Cyanoindole was readily converted into the imidic ester hydrochloride (III; R = 2-indolyl) by the Pinner reaction in ether solution, but the 3-indolyl isomer was formed only slowly at elevated temperatures in the presence of an excess of hydrogen chloride.

The hydrochloride of *N*-indole-2-carboxyimidoyl-*N*′-phenylhydrazine (IV; R = 2-indolyl) was obtained from the imidic ester (III; R = 2-indolyl) by reaction with phenylhydrazine in ethanol at room temperature. The use of boiling chloroform as a solvent led to contamination of the product with ammonium chloride. Treatment of the 2-thioamide with phenylhydrazine in pyridine containing mercuric chloride gave the same product.[11]

Indole deriv.	Activity *in vitro* against M.tb.H37 Rv. (Maximum effective dilution, w/v) Dubos medium	Proskauer & Beck medium
2-$CS{\cdot}NH_2$	1 : 20—40,000	1 : 1,280,000
2-$CO{\cdot}NH{\cdot}NH_2$	<1 : 20,000	1 : 40—80,000
2-$CH{:}N{\cdot}NH{\cdot}CS{\cdot}NH_2$	1 : 320,000	1 : 1,280,000
2-$C(NH_2){:}N{\cdot}NHPh$	<1 : 10—20,000	1 : 40—80,000
3-$CH{:}N{\cdot}NH{\cdot}CS{\cdot}NH_2$	1 : 320,000	1 : 320,000
3-$C(NH_2){:}N{\cdot}NHPh$	1 : 20,000	1 : 80,000

The hydrazine (IV; R = 3-indolyl) and the corresponding hydrochloride were similarly prepared from the imidic ester (III; R = 3-indolyl).

Although several of the compounds described were found to have considerable antituberculous activity *in vitro*, only 3-formylindole thiosemicarbazone was significantly active *in vivo* when tested in mice. *In vitro* results are listed in the Table (compounds not mentioned were not active at 1 : 20,000). None of the compounds showed marked activity against a small range of Gram-positive and Gram-negative bacteria.

Experimental

2- *and* 3-*Formylindole Thiosemicarbazone*.—The crude aldehyde[12] was crystallised from aqueous methanol to give 2-*formylindole* as buff-coloured plates, m. p. 141—142° (Found : C, 73·8; H, 5·1; N, 10·2. C_9H_7ON requires C, 74·5; H, 4·8; N, 9·7%). Its *thiosemicarbazone*, dark buff needles (from 50% aqueous ethanol) had m. p. 229° (decomp.) (Found : C, 55·7; H, 4·7; N, 25·0; S, 14·6. $C_{10}H_{10}N_4S$ requires C, 55·0; H, 4·6; N, 25·7; S, 14·7%). Similarly, the 3-aldehyde[13] gave the thiosemicarbazone[1] as colourless needles, m. p. 226—227° (decomp.) (Found : C, 55·4; H, 4·6; N, 25·4; S, 14·7%).

3-*Indolylglyoxylic Acid Thiosemicarbazone*.—Ethyl 3-indolylglyoxylate (5 g.) was hydrolysed as described by Elks *et al.*,[2] and the resulting moist acid in ethanol (40 ml.) gave the *thiosemicarbazone* (3·3 g., 82·5%) as yellow needles (from 50% aqueous ethanol), m. p. 203—204° (decomp.) (Found : C, 47·2; H, 4·6; S, 11·1. $C_{11}H_{10}O_2N_4S,H_2O$ requires C, 47·1; H, 4·3; S, 11·4%).

3-*Indolylglyoxylhydrazide*.—Hydrazine hydrate (2·5 g.) and ethyl 3-indolylglyoxylate[2] (5 g.) in boiling methanol (100 ml.) gave the *hydrazide* (4·65 g., 99%), yellow needles (from ethanol), m. p. 221—222° (decomp.) (Found : C, 59·1; H, 4·5; N, 20·7. $C_{10}H_9O_2N_3$ requires C, 59·1; H, 4·5; N, 20·6%).

Indole-2-carboxyamide.—Indole-2-carbonyl chloride[14] (derived from the acid, 29 g.) in dry ether (500 ml.) was added to a stirred solution of liquid ammonia (200 ml.) in dry ether (1 l.). After 24 hr. the white solid was collected and washed with water. The residue (15 g., 52%), crystallised from 50% aqueous ethanol, gave the *amide* as colourless needles, m. p. 235° (Found : C, 67·9; H, 5·4; N, 16·8. $C_9H_8ON_2$ requires C, 67·5; H, 5·0; N, 17·5%).

2-*Cyanoindole*.—Indole-2-carboxyamide (5 g.) and phosphorus oxychloride (25 ml.) were

[9] Oddo and Mingoia, *Gazzetta*, 1926, **56**, 782.
[10] Cf. Jerschel and Fischer, *Annalen*, 1951, **514**, 85.
[11] Cf. van der Burg, *Rec. Trav. chim.*, 1955, **74**, 257.
[12] Taylor, *Helv. Chim. Acta*, 1950, **33**, 164.
[13] Tyson and Shaw, *J. Amer. Chem. Soc.*, 1952, **74**, 2273.
[14] Johnson, *ibid.*, 1945, **67**, 427.

heated under reflux for 5 min., hydrogen chloride being copiously evolved. The cooled solution was poured on crushed ice (200 g.) and ammonia (50 ml.), further ammonia being added to maintain alkalinity. The brown solid (3·8 g., 86%) was collected and crystallised from 33% aqueous ethanol, to give the *nitrile* as fawn crystals, m. p. 101° (Found: C, 76·5; H, 4·0; N, 19·4. $C_9H_6N_2$ requires C, 76·1; H, 4·2; N, 19·7%).

Indole-2-thiocarboxyamide.—Dry hydrogen sulphide was bubbled through a solution of 2-cyanoindole (7·6 g.) in pyridine (25 ml.) and triethylamine (7·5 ml.) for 3 hr. Dilution with water gave a yellow solid (9·2 g., 98%), which crystallised from 70% aqueous ethanol to give the *thiocarboxyamide* as fawn needles, m. p. 209° (Found: C, 61·4; H, 4·9; N, 15·9. $C_9H_8N_2S$ requires C, 61·4; H, 4·5; N, 15·9%).

Ethyl Indole-2-carboxyimidate Hydrochloride.—Dry hydrogen chloride (5·5 g.) was absorbed into a solution of 2-cyanoindole (17·8 g.) in dry ether (75 ml.) and dry ethanol (8 ml.) at 0°. After 3 days at 0° the crystals were collected and washed with dry ether, to give crude *ethyl indole-2-carboxyimidate hydrochloride* (19 g., 67%) which crystallised from chloroform (450 ml.) as pale buff needles, m. p. 151·5° (Found: N, 13·1; Cl, 15·6. $C_{11}H_{13}ON_2Cl$ requires N, 12·5; Cl, 15·8%).

N-*Indole-2-carboxyimidoyl*-N′-*phenylhydrazine.*—(*a*) Phenylhydrazine (1 ml.) was added to a suspension of ethyl indole-2-carboxyimidate hydrochloride (2·2 g.) in dry ethanol (20 ml.). The mixture was warmed slightly to dissolve the reactants and then kept for 3 days at room temperature. The solid (0·9 g., 28%) that had separated was filtered off, and recrystallised from ethanol, to give the *phenylhydrazine hydrochloride* as colourless needles, m. p. 129° (Found: C, 61·7; H, 6·8; N, 17·2; Cl, 10·9. $C_{15}H_{15}N_4Cl,C_2H_6O$ requires C, 61·4; H, 6·3; N, 16·9; Cl, 10·7%). Removal of the ethanol of crystallisation was not effected by further drying and attempted crystallisation from glacial acetic acid gave products of varying and higher m. p.s with unsatisfactory analyses.

(*b*) Ethyl indole-2-carboxyimidate hydrochloride (2·2 g.) was added to a solution of phenylhydrazine (1 ml.) and triethylamine (1·4 ml.) in dry ethanol (15 ml.). After 48 hr. at room temperature, the solution was diluted with water. The precipitated *phenylhydrazine*, that rapidly solidified (2·15 g., 87%), formed from benzene fawn crystals, m. p. 187° (Found: C, 72·3; H, 5·8; N, 22·5. $C_{15}H_{14}N_4$ requires C, 72·0; H, 5·6; N, 22·4%). Ethanolic hydrogen chloride gave the hydrochloride, m. p. and mixed m. p. 129°.

(*c*) Indole-2-thiocarboxyamide (1 g.) was added to a solution of mercuric chloride (1·54 g.) and phenylhydrazine hydrochloride (0·82 g.) in dry pyridine (30 ml.). After 10 minutes' boiling under reflux mercuric sulphide was removed and the filtrate cooled and diluted with water. The precipitated phenylhydrazine (1·2 g., 85%) was collected and recrystallised from benzene, m. p. and mixed m. p. 187° (Found: C, 72·5; H, 5·1; N, 22·4%).

Indole-3-carboxylic Acid.—The following modification of Majima's method [15] gave reproducible results. Indole (11·8 g.) in anhydrous ether (20 ml.) was added slowly at 0° to the Grignard reagent from magnesium turnings (4·8 g.) and ethyl iodide (32 g.) in anhydrous ether (100 ml.). Reaction was completed by 30 minutes' stirring at room temperature and then 1·5 hours' boiling under reflux. The resulting two-layer mixture was cooled to 0° and then added slowly, with stirring, to a large excess of powdered solid carbon dioxide. After evaporation of excess of carbon dioxide, water (100 ml.) was added cautiously with cooling, and the resulting mixture was acidified with glacial acetic acid. The ether layer was separated after the removal of a little magnesium powder, and the aqueous layer was extracted with ether. The combined extracts were washed with dilute sodium carbonate solution, dried ($MgSO_4$), and evaporated *in vacuo*, to give the crude acid (12 g., 75%). Trituration with ether and light petroleum, followed by recrystallisation from 40% aqueous ethanol, gave colourless needles of the pure acid (4·6 g., 29%), m. p. 220—224° (decomp.), almost insoluble in ether. [Majima [15] gives m. p. 220° (decomp.).]

Indole-3-carboxyamide.—Crude indole-3-carbonyl chloride was prepared by stirring indole-3-carboxylic acid (13 g.) with thionyl chloride (130 ml.) at room temperature for 18 hr. The excess of thionyl chloride was evaporated *in vacuo* and the solid residue repeatedly evaporated with ether (50 ml. portions). The residue in anhydrous ether (150 ml.) was filtered and added to a cooled solution of liquid ammonia (100 ml.) in anhydrous ether (500 ml.). The resulting solution was left for 18 hr. The solid residue was collected, washed with water, and crystallised from water, to give the cream-coloured *amide* (6·8 g., 52%), m. p. 196—197° (Found: C, 66·9; H, 4·7; N, 17·3. $C_9H_8ON_2$ requires C, 67·5; H, 5·0; N, 17·5%).

[15] Majima, *Ber.*, 1922, **55**, 3861; 1930, **63**, 2237.

Indole-3-carboxyhydrazide.—Indole-3-carbonyl chloride, prepared from the acid (2 g.) as above, in anhydrous ether (20 ml.) was added to a stirred solution of hydrazine hydrate (2·4 g.) in dioxan (20 ml.) at 0°. After 30 min., the precipitate was collected and washed with dilute sodium hydroxide solution and water. The crude *hydrazide* (1·15 g., 53%) crystallised from ethanol as plates, m. p. 224—226° (decomp.) (Found: C, 62·0; H, 5·4; N, 24·2. $C_9H_9ON_3$ requires C, 61·7; H, 5·2; N, 24·0%).

3-*Cyanoindole.*—(*a*) Indole-3-carboxyamide was dehydrated with phosphorus oxychloride (43 ml.) as for the 2-isomer, to give the nitrile (7·3 g., 95%), fawn needles (from 33% ethanol), m. p. 177—178° (Majima [6] gives m. p. 178°) (Found: C, 75·7; H, 4·5; N, 19·8. Calc. for $C_9H_6N_2$: C, 76·1; H, 4·2; N, 19·7%).

(*b*) 3-Formylindole oxime (34 g.) was suspended in anhydrous ether (200 ml.), and thionyl chloride (110 ml.) added at such a rate that refluxing was maintained. When the reaction had subsided the ether and excess of thionyl chloride were removed *in vacuo*, leaving the crude nitrile (28·5 g., 94%) which crystallised from 33% aqueous ethanol as fawn needles, m. p. and mixed m. p. 177—178°.

Indole-3-thiocarboxyamide.—(*a*) Triethylamine (5 ml.) was added to the 3-nitrile (10·9 g.) in dry pyridine (30 ml.), and the whole saturated with dry hydrogen sulphide at room temperature, and then heated at 160° for 16 hr. (sealed tube). Dilution with water gave a dark oil which was extracted with ether. The extracts were dried (Na_2SO_4) and evaporated to an oil that crystallised (8·9 g., 66%). Recrystallisation from water and then toluene gave the *thiocarboxyamide* as pale yellow plates, m. p. 148—149° (decomp.) (Found: C, 61·3; H, 4·7; N, 15·6; S, 17·6. $C_9H_8N_2S$ requires C, 61·4; H, 4·5; N, 15·9; S, 18·2%).

(*b*) Methyl indole-3-dithiocarboxylate (0·5 g.) in methanol (20 ml.) was saturated with ammonia at room temperature and set aside in a stoppered vessel for 24 hr. Evaporation of the methanol *in vacuo* and trituration of the residual oil with toluene–light petroleum gave the crude thioamide (0·26 g.), m. p. and mixed m. p. 148—149° (decomp.) after crystallisation from toluene.

Indole-3-thiocarboxymorpholide.—3-Formylindole (17·5 g.), sulphur (5·8 g.), and morpholine (19·5 g.) in ethanol (45 ml.) were refluxed for 2 hr. The resulting solution was cooled to 0°; after the separation of a little amorphous material the product crystallised (21·4 g., 72%). Recrystallisation from ethanol gave the *morpholide* as yellow prisms, m. p. 171—172° (Found: C, 63·0; H, 5·3; N, 11·2. $C_{13}H_{14}ON_2S$ requires C, 63·4; H, 5·7; N, 11·4%).

Methyl Indole-3-dithiocarboxylate.—Methyl iodide (3 ml.) and the morpholide (10 g.) in dry acetone (50 ml.) were refluxed for 20 min., during which an oil separated, then cooled, and treated with dry pyridine (10 ml.). The mixture was then saturated with dry hydrogen sulphide and kept for 18 hr., the acetone removed *in vacuo*, and the residue poured on crushed ice containing dilute hydrochloric acid. The resulting brown oil was extracted with ether and, after being dried (Na_2SO_4), the ether was evaporated, to leave an oil which crystallised on trituration with ethanol (4·9 g., 58%). Recrystallisation from benzene gave the *ester* as yellow needles, m. p. 108—109° (Found: C, 57·9; H, 4·4; N, 6·8; S, 30·7. $C_{10}H_9NS_2$ requires C, 57·9; H, 4·4; N, 6·8; S, 30·9%).

3-*Dimethylthiomethyleneindolenine.*—The crude oily dithio-acid obtained by the action of carbon disulphide (30 g.) on 3-indolylmagnesium iodide (from indole, 23·6 g.) by the method of Oddo and Mingoia [9] was shaken with 12% w/v aqueous potassium hydroxide (100 ml.), and the resulting red solution filtered. Treatment of this solution with dimethyl sulphate (20 ml.) gave an insoluble black gum (1·5 g.), which crystallised under ether. Recrystallisation from light petroleum gave yellow needles, m. p. 107—109° (mixed m. p. with methyl indole-3-dithiocarboxylate 63—65°), which were probably 3-*dimethylthiomethyleneindolenine* (Found: C, 59·7; H, 4·9; N, 6·4; S, 29·4. $C_{11}H_{11}NS_2$ requires C, 59·7; H, 5·0; N, 6·3; S, 29·0%).

Ethyl Indole-3-carboxyimidate Hydrochloride.—Dry hydrogen chloride was passed into a solution of 3-cyanoindole (10 g.) in tetrahydrofuran (100 ml.) and dry ethanol (4·2 ml.) for 2·5 hr. at 45—50°. After 14 days at 30—35°, the product (15·1 g., 96%) was collected and recrystallised from glacial acetic acid to give the *ester hydrochloride*, m. p. 173° (decomp.) (Found: C, 58·7; H, 6·3; N, 12·0; Cl, 16·1. $C_{11}H_{13}ON_2Cl$ requires C, 58·8; H, 5·8; N, 12·5; Cl, 15·8%).

N-*Indole-3-carboxyimidoyl*-N′-*phenylhydrazine.*—(*a*) Ethyl indole-3-carboxyimidate hydrochloride (2 g.) was warmed with phenylhydrazine (1·9 ml.) in dry ethanol (40 ml.) to obtain complete dissolution. Ammonium chloride was removed after 16 hr. and the filtrate evaporated to a sticky residue which solidified under chloroform (yield, 0·7 g., 27%). It was collected, washed with a little water, and crystallised from glacial acetic acid, to give the solvated *phenylhydrazine hydrochloride* as colourless rhombs, m. p. 251° (decomp.) (Found: C, 59·2; H, 5·5;

N, 15·9; Cl, 10·5. $C_{15}H_{15}N_4Cl,C_2H_4O_2$ requires C, 58·9; H, 5·5; N, 16·2; Cl, 10·3%). (*b*) The imidate hydrochloride (6·4 g.), phenylhydrazine (2·85 ml.), and triethylamine (4·0 ml.) in dry ethanol (40 ml.) were kept at room temperature for 16 hr. Dilution with water (150 ml.) gave an oil which solidified at 0°. The product (3·7 g., 66%) crystallised from ethyl acetate–ether, to give the *phenylhydrazine* as brown rhombs, m. p. 168—170° (decomp.) (Found : C, 72·4; H, 5·8; N, 22·9. $C_{15}H_{14}N_4$ requires C, 72·0; H, 5·6; N, 22·4%). Recrystallisation of the crude product from benzene gave a metastable *form* of the phenylhydrazine as brown plates, m. p. 154° (decomp.) (Found : C, 72·2; H, 6·2; N, 22·4%), which reverted to the stable form, m. p. and mixed m. p. 168—170°, after a few weeks at room temperature.

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