119. *Synthesis of Nuclear Amidino-derivatives of 5-Aminoacridine.*

By Alan A. Goldberg and William Kelly.

(With a Bacteriological Note by Frederic Haynes.)

5-Amino-2- and -3-amidino-acridines have been synthesised for examination as bacterial inhibitors.

The high bacteriostatic activity and low tinctorial value of 5-aminoacridine hydrochloride commend its use as a general antiseptic in the place of proflavine. Since the solubility of the compound in physiological saline at ordinary temperatures is lower than is clinically desirable, it was of interest to determine whether the introduction of a nuclear amidino-substituent would improve solubility and possibly enhance antibacterial activity.

In order to obtain amidino-derivatives of 5-aminoacridine it was necessary to prepare cyanodiphenylamine-2-carboxylic acids. The condensation of *o*-chlorobenzoic acid with the three cyanoanilines did not afford the desired intermediates in satisfactory yield; this may be correlated with the observations of Tuttle (*J. Amer. Chem. Soc.*, 1923, **45**, 1916), Bogert, Hirschfelder, and Lauffer (*Coll. Trav. Chim. Czechoslov.*, 1930, **5**—**6**, 382), and Goldberg and Kelly (*J.*, 1946, 102) upon the inhibiting influence on the Ullmann reaction of negative substituents in the arylamine nucleus. 2-Chloro-5-cyanobenzoic acid and aniline, however, readily condensed to yield *4-cyanodiphenylamine-2-carboxylic acid*, which cyclised with phosphorus oxychloride to *5-chloro-3-cyanoacridine*, and this, on being heated in phenol solution

with ammonium carbonate, yielded 5-*amino*-3-*cyanoacridine*. Conversion of the aminocyanoacridine into the corresponding amino-iminoether at first presented considerable difficulty owing to the almost total insolubility of the dihydrochloride of the former in all anhydrous non-hydroxylic solvents. Success was ultimately attained by reducing the dihydrochlorides to an extremely fine state of subdivision in dioxan by continuous shaking in a closed vessel with glass beads for periods of some 15—20 days. In this manner 5-amino-3-cyanoacridine yielded the amino-iminoether, which was converted by alcoholic ammonia into 5-*amino*-3-*amidinoacridine* in excellent over-all yield. Similarly 5-cyanodiphenylamine-2-carboxylic acid yielded 5-*chloro*-2-*cyanoacridine* which by the same route was converted into 5-*amino*-2-*cyano*- and 5-*amino*-2-*amidino-acridine*. Interaction of 5-amino-2-cyanoacridine with hydroxylamine in boiling aqueous-alcoholic pyridine yielded 5-*amino*-2-*amidoximinoacridine*.

In the same manner 4- and 5-*cyano*-4′-*methoxydiphenylamine*-2-*carboxylic acids* yielded respectively 5-*chloro*-2- and -3-*cyano*-7-*methoxyacridines* which were converted by the same route into 5-*amino*-2- and -3-*amidino*-7-*methoxyacridines*.

The introduction of the nuclear amidine group into the 2- and 3-positions of 5-aminoacridine effectively increases solubility both in water and in physiological saline but inhibits the *in vitro* bacteriostatic activity against *B. proteus*, *B. pyocyaneus*, *Streptococcus pyogenes*, *Staphylococcus pyogenes aureus*, *B. coli*, *B. typhosus*, and *B. subtilis*.

Experimental.

3′-*Cyanodiphenylamine*-2-*carboxylic Acid*.—Potassium *o*-chlorobenzoate (5·0 g.), potassium carbonate (3·0 g.), *m*-cyanoaniline (3·3 g.), and copper powder (0·05 g.) were refluxed in amyl alcohol (15 c.c.) for four hours. The amyl alcohol was removed in steam, the residue filtered with carbon, and the filtrate (150 c.c.) made acid to Congo-red by addition of hydrochloric acid. The gummy precipitate was collected and crystallised twice from alcohol–benzene, 3′-*cyanodiphenylamine*-2-*carboxylic acid* being obtained in yellow prisms (1·0 g.), m. p. 204—205° [Found: N, 11·9; *M* (by titration), 234. $C_{14}H_{10}O_2N_2$ requires N, 11·8%; *M*, 238]. Prolongation of the reaction time failed to increase the yield. Closure of the acridine ring with phosphorus oxychloride gave a mixture, presumably of 5-chloro-2- and -4-cyanoacridines, and accordingly this approach was abandoned. Interaction of *o*-chlorobenzoic acid with *o*- and *p*-cyanoanilines under the same conditions did not yield the required cyanodiphenylamine-carboxylic acids in satisfactory yield.

2-*Chloro*-4-*cyanotoluene*.—This was obtained by Magidson and Travin (*Ber.*, 1936, **69**, 537) in 45% yield from 2-chloro-4-aminotoluene by a Sandmeyer reaction with cuprous cyanide; by the use of sodium nickelocyanide, however, the required product could be produced in 87% yield.

2-Chloro-4-aminotoluene (70 g.) was dissolved in water (400 c.c.) and concentrated hydrochloric acid (99 c.c.) and the sludge of hydrochloride diazotised at 0—5° with sodium nitrite (35 g. in 100 c.c. of water). The diazonium solution was added slowly to a boiling solution of sodium nickelocyanide (obtained from 125 g. of sodium cyanide and 125 g. of nickel chloride hexahydrate in 800 c.c. of water), and the mixture refluxed for a further 30 minutes after the addition had been completed. The 2-chloro-4-cyanotoluene was distilled out of the reaction mixture in a current of steam; yield, 65 g. (87%), m. p. 42—44°.

2-*Chloro*-4-*cyanobenzoic Acid*.—Oxidation of 80 g. of the foregoing cyano-compound by the method described by Magidson and Travin (*loc. cit.*) afforded 42 g. of 2-chloro-4-cyanobenzoic acid, m. p. 164—168°, which was pure enough for further use, together with 24 g. of recovered chlorocyanotoluene.

5-*Cyanodiphenylamine*-2-*carboxylic Acid*.—2-Chloro-4-cyanobenzoic acid (10 g.), potassium carbonate (10 g.), aniline (8 c.c.), amyl alcohol (40 c.c.), and copper powder (0·2 g.) were refluxed together at 150° (bath temp.) for 2½ hours, and the product isolated in the usual manner (Goldberg and Kelly, *loc. cit.*). Recrystallisation from 50% alcohol gave 5-cyanodiphenylamine-2-carboxylic acid (6·5 g.) in yellow-brown plates, m. p. 210—212°. For analysis a sample was recrystallised from aqueous alcohol and obtained in yellow rectangular plates, m. p. 214° [Found: N, 11·7; *M* (by titration), 235. Calc. for $C_{14}H_{10}O_2N_2$: N, 11·8%; *M*, 238].

5-*Chloro*-2-*cyanoacridine*.—5-Cyanodiphenylamine-2-carboxylic acid (3·7 g.) was refluxed with phosphorus oxychloride (8·5 c.c.) for 2 hours, the cooled solution quenched on an excess of powdered ice, and the stirred mixture basified with ammonia, the temperature not being allowed to exceed 2°. The precipitate was collected, washed with water, and dried in a vacuum, 5-*chloro*-2-*cyanoacridine* (3·7 g.) being obtained as a green powder, m. p. 192—194°; recrystallisation from benzene (charcoal) gave the pure compound in pale yellow needles, m. p. 197—198° (Found: N, 11·9; Cl, 14·9. $C_{14}H_7N_2Cl$ requires N, 11·7; Cl, 14·9%).

5-*Amino*-2-*cyanoacridine*.—The foregoing compound (12·1 g.) was suspended in 5% alcoholic ammonia (300 c.c.) and heated in a closed bottle at 100° for 36 hours. The precipitate was collected from the chilled mixture, and a further amount obtained by evaporation of the filtrate; the total yield was 8·0 g., m. p. 324—326°. Recrystallisation from aqueous pyridine gave 5-*amino*-2-*cyanoacridine* in orange prisms, m. p. 328—329° (Found: C, 76·7; H, 4·1; N, 19·1. $C_{14}H_9N_3$ requires C, 76·7; H, 4·1; N, 19·2%).

5-*Amino*-2-*amidinoacridine*.—Anhydrous 5-amino-2-cyanoacridine (4·5 g.) was suspended in anhydrous dioxan (80 c.c.) and anhydrous alcohol (5 c.c.), the mixture saturated with dry hydrogen chloride at 5°, and shaken with glass beads for 26 days in a sealed bottle. The solvent was pumped off at < 40°, saturated anhydrous alcoholic ammonia (130 c.c.) added to the residue, and the whole heated in a closed vessel at 50° for 72 hours. The ammonia and part of the solvent were distilled off at < 40°, the residue chilled, and the crystalline precipitate (3·9 g.) collected; this was found to be a mixture of the

mono- and the di-hydrochloride of the aminoamidinoacridine. The free base was obtained from an aqueous solution of the latter by precipitation with cold dilute sodium hydroxide; recrystallisation from aqueous methyl alcohol gave *5-amino-2-amidinoacridine* in orange-yellow needles, m. p. 305° (Found: C, 70·8; H, 5·2; N, 23·5. $C_{14}H_{12}N_4$ requires C, 71·1; H, 5·1; N, 23·7%). A 1·0% solution of the monohydrochloride in physiological saline (0·9% sodium chloride) has a pH value of 8·4 and remains clear indefinitely at 20°; it has a pale yellow colour and does not appreciably stain tissue. (A 0·1% solution of 5-aminoacridine hydrochloride in physiological saline has pH 6·8 and remains clear for 12—24 hours at 20° and then slowly precipitates long, silky, yellow needles of the aminoacridine hydrochloride which redissolve on warming. The 0·05% solution in physiological saline begins precipitating after 24—48 hours at 20° but the 0·025% solution remains clear indefinitely.)

5-Amino-2-amidoximinoacridine.—5-Amino-2-cyanoacridine (1·4 g.) was dissolved in a mixture of pyridine (15 c.c.), water (3 c.c.), and alcohol (5 c.c.), a solution of hydroxylamine (from 0·89 g. of the hydrochloride and 0·72 g. of anhydrous sodium carbonate in 3·5 c.c. of water) added, and the mixture heated on the water-bath for 1¾ hours, then cooled and diluted with water (50 c.c.); the precipitate (1·3 g.) was collected, and recrystallisation from pyridine–alcohol gave hydrated *5-amino-2-amidoximinoacridine* in felted yellow needles, m. p. 254° (decomp.). From methyl alcohol the compound separated in stout yellow prisms, m. p. 264—265° (decomp.), also containing solvent of crystallisation (Found, on material dried at 140°/2 mm.: C, 66·2; H, 4·8; N, 22·5. $C_{14}H_{12}ON_4$ requires C, 66·8; H, 4·8; N, 22·2%). L.D.$_{50}$, 50 mg./kg.; the compound has no curative influence upon mouse trypanosomiasis.

5-Cyano-4′-methoxydiphenylamine-2-carboxylic Acid (compare Magidson and Travin, *loc. cit.*).—2-Chloro-4-cyanobenzoic acid (28·8 g.), *p*-anisidine (20 g.), potassium carbonate (23 g.), copper powder (0·5 g.), and amyl alcohol (60 c.c.) were stirred at 120—125° (bath temp.) for 3½ hours and then at 130—135° for ½ hour. The crude product, isolated in the usual manner, separated as an oil which rapidly hardened; this was collected and extracted with boiling water (3 × 200 c.c.), and the insoluble material recrystallised from 50% aqueous alcohol (220 c.c.), 5-cyano-4′-methoxydiphenylamine-2-carboxylic acid (20 g.) separating in orange-brown plates, m. p. 196°.

5-Chloro-2-cyano-7-methoxyacridine.—This was prepared from 5-cyano-4′-methoxydiphenylamine-2-carboxylic acid by ring closure with phosphorus oxychloride essentially as previously reported by Magidson and Travin. The yield was practically theoretical, the crude vacuum-dried product having m. p. 225—227°.

5-Amino-2-cyano-7-methoxyacridine.—Magidson and Travin (*loc. cit.*) reported the preparation of this material by the action of absolute alcoholic ammonia on the corresponding 5-chloro-compound. Repetition of this method gave the desired *meso*-amine in only small yield, owing presumably to the low solubility of the 5-chloro-compound in the solvent employed. Reaction of the 5-chloro-compound in phenol solution with ammonium carbonate, however, gave much better results. A solution of 5-chloro-2-cyano-7-methoxyacridine (5·0 g.) in molten phenol (25 g.) was heated to 125—130°, ammonium carbonate (3·0 g.) added as fast as the effervescence allowed, and the mixture kept at this temperature for 2 hours. The mixture was cooled, acetone (100 c.c.) added, and the precipitate of hydrochloride collected, washed with acetone, suspended in water and shaken with an excess of cold 2N-sodium hydroxide and glass beads in a closed bottle for 2 hours. The yellow insoluble material (3·3 g.; m. p. 276—278°) was collected, washed, and recrystallised from pyridine–alcohol, 5-amino-2-cyano-7-methoxyacridine being obtained in orange plates, m. p. 282—284° (Found: N, 16·9. Calc. for $C_{15}H_{11}ON_3$: N, 16·9%).

2-Cyano-5-phenoxy-7-methoxyacridine.—5-Chloro-2-cyano-7-methoxyacridine (2·0 g.) was dissolved in phenol (12·0 g.) and heated at 100° for 3 hours. The melt was cooled, ether (50 c.c.) added, and the precipitate collected, suspended in water, and basified with ammonia. Collection of the precipitate and recrystallisation from alcohol gave *2-cyano-5-phenoxy-7-methoxyacridine* (2·0 g.) in fine yellow needles, m. p. 188° (Found: N, 8·8. $C_{21}H_{14}O_2N_2$ requires N, 8·6%).

2-Amidoximino-5-phenoxy-7-methoxyacridine.—The foregoing compound (5·1 g.) was dissolved in 70% alcoholic pyridine (30 c.c.), a solution of hydroxylamine (from 1·65 g. of hydrochloride and 1·35 g. of anhydrous sodium carbonate in 5 c.c. of water) added, and the mixture heated on the water-bath for 1 hour. Methyl alcohol (30 c.c.) was added to the chilled mixture, and the insoluble material collected (3·6 g.) and recrystallised from alcohol containing a little pyridine; *2-amidoximino-5-phenoxy-7-methoxyacridine* separated in felted yellow needles, m. p. 216° (decomp.) (Found: N, 11·9. $C_{21}H_{17}O_3N_3$ requires N, 11·7%).

5-Amino-2-amidino-7-methoxyacridine.—Anhydrous 5-amino-2-cyano-7-methoxyacridine (3·4 g.) was suspended in anhydrous dioxan (50 c.c.) and anhydrous alcohol (3·5 c.c.), and the mixture saturated with dry hydrogen chloride at 5° and then shaken with glass beads in a sealed bottle at room temperature for 17 days; the solvent and hydrogen chloride were pumped off at < 40°, and the residue heated in a closed vessel with anhydrous saturated alcoholic ammonia (120 c.c.) at 50—55° for 96 hours. *5-Amino-2-amidino-7-methoxyacridine monohydrochloride* (2·2 g.) separated on standing on ice in rosettes of yellow needles, m. p. 282—284° (Found: N, 17·8; Cl, 11·7. $C_{15}H_{14}ON_4$,HCl requires N, 18·4; Cl, 11·7%). The free *base*, isolated by basifying a cold aqueous solution of the hydrochloride with cold sodium hydroxide solution, was crystallised from aqueous methyl alcohol and obtained in yellow needles, m. p. 268—270° (Found: C, 67·1; H, 5·5; N, 20·5. $C_{15}H_{14}ON_4$ requires C, 67·6; H, 5·3; N, 21·0%). L.D.$_{50}$, 75 mg./kg.; the compound exerts a slight retarding influence upon the course of *Tr. equiperdum* infections in mice.

5-Amino-2-amidoximino-7-methoxyacridine.—A solution of 5-amino-2-cyano-7-methoxyacridine (3·3 g.) in pyridine (20 c.c.) and alcohol (10 c.c.) was heated on the water-bath, and a solution of hydroxylamine (from 1·85 g. of hydrochloride and 1·60 g. of anhydrous sodium carbonate in 8 c.c. of water) added. The mixture was heated at 100° for 2 hours, water (50 c.c.) added, and the precipitate collected (2·8 g.; m. p. 294°). Recrystallisation from aqueous pyridine gave *5-amino-2-amidoximino-7-methoxyacridine* in yellow plates, m. p. 296° (decomp.) (Found: C, 64·4; H, 5·1; N, 19·5. $C_{15}H_{14}O_2N_4$ requires C, 63·8; H, 5·0; N, 19·8%). L.D.$_{50}$, 50 mg./kg.; the compound has no influence upon trypanosomiasis in mice.

2-Chloro-5-cyanobenzoic Acid.—2-Chloro-5-aminobenzoic acid (60 g.; Goldberg and Kelly, *loc. cit.*), dissolved in water (185 c.c.) and hydrochloric acid (74 c.c.), was diazotised at 0—5° with a solution of sodium nitrite (25 g.) in water (75 c.c.). The diazonium solution was stirred at 0° for 20 minutes, sodium carbonate added until the reaction was no longer acid to Congo-red, and the solution added slowly to a boiling solution of sodium nickelocyanide (from 90 g. of sodium cyanide and 90 g. of nickel chloride hexahydrate in 600 c.c. of water). After the addition, the mixture was boiled for 15 minutes, hydrochloric acid added to acidity to Congo-red, the mixture heated to the boiling point for 20 minutes, cooled, and the brown sludge collected and washed with water. The residue was extracted with hot methyl alcohol (3 × 300 c.c.), the combined extracts concentrated to incipient crystallisation and cooled, and the *2-chloro-5-cyanobenzoic acid* collected (50·5 g.; m. p. 176—177°). Recrystallisation from water gave the compound in colourless silky needles containing water of crystallisation which was lost on drying at 100° to give a white powder, m. p. 178° [Found: N, 8·1; Cl, 19·3; *M* (by titration), 182. $C_8H_4O_2NCl$ requires N, 7·7; Cl, 19·5%; *M*, 181·5].

4-Cyanodiphenylamine-2-carboxylic Acid.—2-Chloro-5-cyanobenzoic acid (18·2 g.), potassium carbonate (15·2 g.), aniline (18·3 g.), amyl alcohol (40 c.c.), and copper powder (0·5 g.) were refluxed together at 150° (bath temp.) for 1½ hours. The excess of aniline and amyl alcohol was removed in steam, and the residual solution filtered (charcoal) and acidified with hydrochloric acid. The precipitate was collected, extracted several times with boiling water, and the insoluble acid crystallised from 50% ethyl alcohol (120 c.c.), *4-cyanodiphenylamine-2-carboxylic acid* being obtained in brown prisms (16·0 g.), m. p. 214—216°. Recrystallisation from aqueous alcohol gave the pure compound in brown leaves, m. p. 220—222° [Found: N, 12·1; *M* (by titration), 238. $C_{14}H_{10}O_2N_2$ requires N, 11·8%; *M*, 238].

5-Chloro-3-cyanoacridine.—4-Cyanodiphenylamine-2-carboxylic acid (15·0 g.) was gently refluxed with phosphorus oxychloride (30 c.c.) for 3 hours, the mixture quenched on an excess of powdered ice, and basified with ammonia, more ice being added in order that the temperature did not exceed 0°. The precipitate was collected, washed with water, and dried in a vacuum (15 g.; m. p. 184—186°). Crystallisation from benzene–petrol gave *5-chloro-3-cyanoacridine* in yellow-brown needles, m. p. 186° (Found: N, 11·6; Cl, 14·6. $C_{14}H_7N_2Cl$ requires N, 11·7; Cl, 14·9%).

5-Amino-3-cyanoacridine.—A solution of 5-chloro-3-cyanoacridine (8·0 g.) in phenol (80 g.) was heated at 100°, and ammonium carbonate (5·0 g.) added slowly, the mixture being heated at 100° (internal) for a further 1 hour; it was then cooled and diluted with dry ether (300 c.c.), and the precipitate collected and washed with ether. This was shaken with dilute sodium hydroxide and glass beads, the insoluble material collected (5·5 g.; m. p. 304—306°), washed with water, and crystallised from aqueous pyridine, *5-amino-3-cyanoacridine* being obtained in fine yellow needles, m. p. 313° (Found: C, 76·3; H, 3·9; N, 19·3. $C_{14}H_9N_3$ requires C, 76·7; H, 4·1; N, 19·2%).

5-Amino-3-amidinoacridine.—5-Amino-3-cyanoacridine (4·7 g.) was suspended in anhydrous dioxan (60 c.c.) and anhydrous ethyl alcohol (6·0 c.c.), the mixture saturated at 5° with dry hydrogen chloride, and the ground with glass beads in a closed bottle for 28 days at room temperature. The solvent was pumped off at 20—30°, and the residue heated at 45—50° for 72 hours in a closed vessel with anhydrous saturated alcoholic ammonia (125 c.c.). The ammonia and excess of alcohol were pumped off at < 40°, leaving a crystalline residue (4·4 g.) of the mono- and di-hydrochloride of the required amino-amidine. In order to obtain the free base, the cold aqueous solution of the foregoing mixture of hydrochlorides was shaken and very dilute sodium hydroxide added slowly with scratching; *5-amino-3-amidinoacridine* separated in yellow plates, m. p. 302—304° (decomp.) (Found: C, 70·6; H, 5·1; N, 24·0. $C_{14}H_{12}N_4$ requires C, 71·1; H, 5·1; N, 23·7%). A 1·0% solution of the monohydrochloride in physiological saline remains clear indefinitely at 20°; this solution has a pale amber colour and does not appreciably stain tissue.

4-Cyano-4′-methoxydiphenylamine-2-carboxylic Acid.—A mixture of 2-chloro-5-cyanobenzoic acid (9·4 g.), *p*-anisidine (6·6 g.), potassium carbonate (7·4 g.), copper powder (0·2 g.), and amyl alcohol (40 c.c.) was stirred at 125—130° (bath temp.) for 3 hours. The crude product was isolated in the usual manner, extracted with boiling water, and the insoluble residue recrystallised from aqueous alcohol, *4-cyano-4′-methoxydiphenylamine-2-carboxylic acid* (9·5 g.) being obtained in yellow plates, m. p. 212—214°. For analysis, a sample was recrystallised from aqueous alcohol and obtained in yellow rectangular plates, m. p. 214° [Found: N, 10·6; *M* (by titration), 265. $C_{15}H_{12}O_3N_2$ requires N, 10·4%; *M*, 268].

5-Chloro-3-cyano-7-methoxyacridine.—The foregoing acid (7·8 g.) was refluxed with phosphorus oxychloride (16 c.c.) for 3 hours, the reaction mixture quenched on ice, and the precipitate of hydrochloride collected and ground with powdered ice and dilute ammonia. The *5-chloro-3-cyano-7-methoxyacridine* (7·5 g.; m. p. 255—260°) was collected and dried in a vacuum; recrystallisation of a sample from xylene (charcoal) gave the compound in yellow plates, m. p. 270—272° (Found: N, 10·0; Cl, 13·3. $C_{15}H_9ON_2Cl$ requires N, 10·4; Cl, 13·2%).

5-Amino-3-cyano-7-methoxyacridine.—A solution of the preceding compound (6·0 g.) in phenol (40 g.) was heated with ammonium carbonate (4·5 g.) at 125—130° for 2 hours. The reaction mixture was cooled, diluted with dry ether (200 c.c.), and the precipitated hydrochloride collected. The free base (4·4 g.) was liberated by grinding with dilute sodium hydroxide and purified by dissolution in warm dilute acetic acid, filtering with carbon, and reprecipitating with dilute sodium hydroxide. Crystallisation from methyl alcohol gave *5-amino-3-cyano-7-methoxyacridine* in stout yellow prisms, which on drying at 100° lost solvent of crystallisation, giving a yellow microcrystalline powder, m. p. 302—304° (Found: C, 72·1; H, 4·6; N, 16·9. $C_{15}H_{11}ON_3$ requires C, 72·3; H, 4·4; N, 16·9%).

5-Amino-3-amidino-7-methoxyacridine.—The foregoing amine (4·4 g.) was suspended in dry dioxan (60 c.c.) and anhydrous ethyl alcohol (6·0 c.c.) and saturated with dry hydrogen chloride at 5°. The mixture was shaken at room temperature for 10 days, the solvent pumped off at 20°, and the residue heated at 40—45° with anhydrous saturated alcoholic ammonia (100 c.c.) for 96 hours. The excess of ammonia and alcohol was distilled away at 20° under reduced pressure, leaving a crystalline mixture of the hydrochlorides of the required aminoamidine and unchanged cyanoamine. The very soluble aminoamidine hydrochloride was dissolved out by lixiviation with cold water, and the filtered solution

rendered faintly alkaline to phenolphthalein with ammonia in order to precipitate the small amount of cyanoamine which it contained. Basification of the agitated filtered solution with very dilute sodium hydroxide and scratching effected precipitation of *5-amino-3-amidino-7-methoxyacridine* in microscopic yellow needles, m. p. 272—274° (Found, in material dried over phosphoric oxide at 2 mm.: C, 67·3; H, 5·4; N, 21·3. $C_{15}H_{14}ON_4$ requires C, 67·6; H, 5·3; N, 21·0%).

Bacteriological Note.—The median lethal dose ($L.D._{50}$) and maximum tolerated dose (M.T.D.) of 5-amino-2-amidinoacridine hydrochloride, 5-amino-3-amidinoacridine hydrochloride and 5-aminoacridine hydrochloride were determined for a single intraperitoneal injection into 6 weeks old white mice.

Compound.	$L.D._{50}$.	M.T.D.
5-Amino-2-amidinoacridine hydrochloride	70 mg./kg.	50 mg./kg.
5-Amino-3-amidinoacridine hydrochloride	50 ,,	40 ,,
5-Aminoacridine hydrochloride	55 ,,	45 ,,

The antibacterial activities were determined in "Lab-Lemco" broth (A) and in "Lab-Lemco" broth containing 10% normal horse serum (B). The maximum bacteriostatic dilutions of the compounds against a range of standard organisms, as indicated by total absence of observable growth after 24 hours' incubation at 37°, were found to be as follows :

	5-Amino-2-amidino-acridine.		5-Amino-3-amidino-acridine.		5-Aminoacridine.	
	A.	B.	A.	B.	A.	B.
Organism.	1 part in		1 part in		1 part in	
B. proteus	<1,000	<1000	<1,000	<1000	64,000	64,000
B. pyocyaneus	4,000	4000	4,000	1000	2,000	2,000
Streptococcus pyogenes, N.T.C. 2400	8,000	4000	8,000	4000	128,000	64,000
Staphylococcus pyogenes aureus ...	8,000	4000	8,000	4000	128,000	64,000
B. coli	16,000	8000	16,000	2000	64,000	64,000
B. typhosus	16,000	4000	16,000	4000	128,000	32,000
B. subtilis	16,000	4000	16,000	8000	128,000	64,000

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RESEARCH LABORATORIES, BRADFORD-ON-AVON, ENGLAND. [*Received, August 19th*, 1946.]