20. *Chemotherapeutic Studies in the Acridine Series. Part I. 2 : 6- and 2 : 8-Diaminoacridines.*

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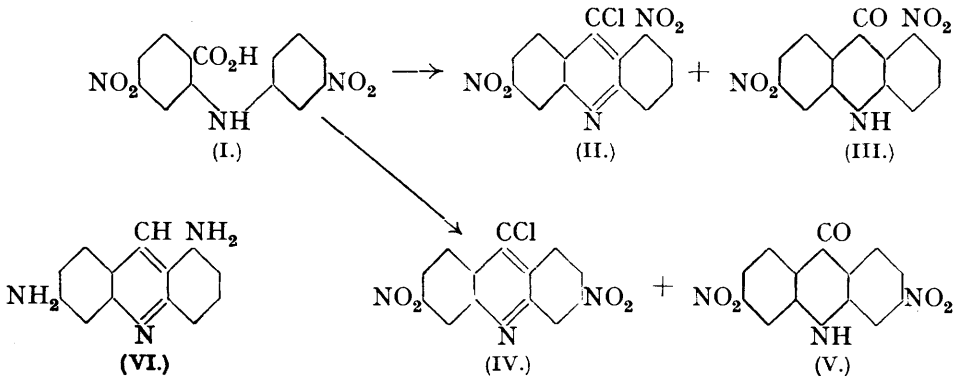
THE use of acridine derivatives as bactericides was first suggested by Browning and co-workers (*Brit. Med. J.*, 1917, 73), who introduced proflavine (2 : 8-diaminoacridine sulphate) and the corresponding methochloride (acriflavine). In 1922, Browning, Cohen, Gaunt, and Gulbransen (*Proc. Roy. Soc.*, 1922, *B*, **93**, 329) showed by synthesis that the bactericidal

properties of proflavine were due to the acridine molecule as a whole, and were not shown by corresponding derivatives of pyridine, quinoline, hydroquinoline or naphthaquinoline. In addition, they tried the effects of various substituents in the molecule of 2 : 8-diaminoacridine, but the products rarely equalled and in no case excelled the parent compound in activity. In 1930, Bogert and co-workers (*Coll. Czech. Chem. Comm.*, 1930, **2**, 383) made and tested 3 : 7-diaminoacridine. Beyond this, no attempt has been made to investigate the chemotherapeutic effects of placing the amino-groups elsewhere in the ring.

It was therefore decided to attempt the preparation and to investigate the bactericidal properties of the twenty possible diaminoacridines, of which seventeen are unknown. This work will provide an opportunity for studying ring closure in the series.

The 2 : 6-*Diaminoacridine Series.*—5 : 5′-*Dinitrodiphenylamine-2-carboxylic acid* (I) was obtained in 28% yield, together with 30% of *p*-nitrobenzoic acid, by condensing sodium 2-chloro-4-nitrobenzoate with *m*-nitroaniline in presence of copper. When *cyclo*hexanol replaced the 4-methyl*cyclo*hexanol used as solvent, another by-product, 5 : 5′-dinitrodiphenic acid (m. p. 289°; anhydride, m. p. 266°), was obtained in quantities up to 18%. Tuttle (*J. Amer. Chem. Soc.*, 1923, **45**, 1906) obtained 4 : 4′-dinitrodiphenic acid from the condensation of 2-chloro-5-nitrobenzoic acid with diethyl-*m*-phenylenediamine. The constitution of the 5 : 5′-dinitrodiphenylamine-2-carboxylic acid was confirmed by an alternative synthesis from *m*-bromonitrobenzene and 4-nitroanthranilic acid.

Although several nitrodiphenylamine-2-carboxylic acids have been reduced to the corresponding amino-acids (Graebe, *Annalen*, 1893, **276**, 41; Ullmann, *ibid.*, 1907, **355**, 351), reduction of the above dinitrodiphenylaminecarboxylic acid gave 3 : 3′-*diaminodiphenylamine*. The loss of carbon dioxide in this reaction is not an isolated instance (cf. Seidel *et al.*, *Monatsh.*, 1902, **23**, 434) : Blanksma (*Rec. trav. chim.*, 1905, **24**, 320) suggested, for simple benzoic acids, that the deciding factor is a nitro-group meta to the amino-group and para to the carboxyl. The above example, and others shortly to be published, suggest that this contention applies also to the *N*-alkylanthranilic acids. 3 : 3′-Diaminodiphenylamine was characterised by its *diacetyl* derivative and by its identity with the product of reduction of 3 : 3′-*dinitrodiphenylamine* obtained by the condensation of *m*-nitroaniline with *m*-bromonitrobenzene. This dinitrodiphenylamine was obtained in better yield by a method similar to that used for analogous substances by Goldberg (*Ber.*, 1907, **40**, 4541), notwithstanding that Ryan and Glover (*Proc. Roy. Irish Acad.*, 1918, **34**, *B*, 97) failed to make it by the Goldberg reaction.



Ring closure in *m*-substituted *N*-arylanthranilic acids may follow alternative routes, and 5 : 5′-dinitrodiphenylamine-2-carboxylic acid may yield both 2 : 6- and 2 : 8-acridine derivatives. Closure by means of excess of phosphorus oxychloride (cf. Lesnianski, *Bull. Acad. Polonaise*, 1929, 81) favours the formation of the 2 : 6-compound, for a quantitative yield of a mixture of acridine compounds was obtained, of which the principal member was the required 5-*chloro*-2 : 6-*dinitroacridine* (II), accompanied by 2 : 6-*dinitroacridone* (III) and smaller amounts of the corresponding 2 : 8-compounds (IV and V), which formed approximately 10% of the products. When less phosphorus oxychloride was used and

the product was treated with warm water (cf. Magidson, *Ber.*, 1933, **66**, 866), a quantitative yield was obtained of 2 : 6- and 2 : 8-dinitroacridones free from chlorinated products, but no advantage was to be gained by following this route owing to the difficulty of separating these isomerides. The chlorodinitroacridines were separated by fractional crystallisation. 5-Chloro-2 : 6-dinitroacridine, unlike the 5-chloroacridine of Graebe (*Annalen*, 1893, **276**, 48), does not evolve a vapour attacking the mucous membranes, but in the solid condition it causes considerable irritation when allowed to remain in contact with the skin. Its constitution is confirmed by the series of compounds formed in the subsequent reactions, a series closely mirrored by those formed by similar transformation of the isomeric 2 : 8-compound, which led to well-known end-products.

On hydrolysis of (II) with dilute acid, 2 : 6-dinitroacridone was quantitatively formed. Reduction of this with stannous chloride gave a good yield of 2 : 6-*diaminoacridone* as its stannichloride. The base and the 2 : 8-isomeride (*v.i.*) differ markedly in physical properties but are very similar in chemical properties.

On reduction with sodium amalgam, 2 : 6-diaminoacridone gave 2 : 6-*diaminoacridine* (VI). This differed from its 2 : 8-isomeride by not exhibiting fluorescence in any solvent, but closely resembled it in chemical properties. Its bactericidal properties are under investigation.

The 2 : 8-*Diaminoacridine Series.*—5-Chloro-2 : 8-dinitroacridine (IV) resembled the 2 : 6-isomeride in physical and chemical properties very closely, but differed in not exhibiting fluorescence in ultra-violet light. Hydrolysis with dilute acids gave 2 : 8-dinitroacridone (V). The constitutions of these two substances follow from analogy with the corresponding 2 : 6-isomerides, and from the reduction of 2 : 8-dinitroacridone with stannous chloride to 2 : 8-diaminoacridone (Schöpff, *Ber.*, 1894, **27**, 2316). Further reduction with sodium amalgam and aqueous sodium hydroxide gave 2 : 8-diaminoacridine in 90% yield.

Experimental.

5 : 5′-*Dinitrodiphenylamine-2-carboxylic Acid* (I).—Condensation of 2-chloro-4-nitrobenzoic acid with *m*-nitroaniline by the usual methods (Jourdan, *Ber.*, 1885, **18**, 1448; Cohn, *Monatsh.*, 1901, **22**, 385; Ullmann, *Annalen*, 1907, **355**, 330; Perkin *et al.*, J., 1924, **125**, 1784) either failed or gave insignificant yields. Sodium 2-chloro-4-nitrobenzoate (4·5 g.), *m*-nitroaniline (5·6 g.; 2 mols.), freshly precipitated copper (0·1 g.), and 4-methyl*cyclo*hexanol (20 ml.) were refluxed together for 1 hour; sodium carbonate (2 g.) was added and after steam-distillation the liquid was filtered. The sodium salt that separated from the filtrate yielded on acidification 5 : 5′-*dinitrodiphenylamine-2-carboxylic acid* (I), which crystallised from alcohol in brownish-orange crystals, m. p. 263° (corr.), almost insoluble in petroleum spirit, dilute mineral acids, and hot and cold water, sparingly soluble in benzene, toluene, and chloroform, and soluble in boiling glacial acetic acid (approx. 1 in 20) and 97% alcohol (approx. 1 in 29, boiling; 1 in 120, cold) (Found: C, 51·7; H, 3·0; N, 14·2. $C_{13}H_9O_6N_3$ requires C, 51·5; H, 3·0; N, 13·9%). Increasing the quantities of material treated at a time decreased the yield considerably, as did also the use of other varieties of solvent or copper. The almost colourless solution of the acid in sulphuric acid becomes yellow on addition of a trace of a nitrate. The alkali salts are freely soluble in hot, and sparingly in cold, water, and are readily salted out. The sodium salt consists of reddish-orange longitudinal plates. The *silver* salt is bright yellow, soluble in aqueous ammonia, and insoluble in boiling water (Found: Ag, 26·0. $C_{13}H_8O_6N_3Ag$ requires Ag, 26·3%). The calcium salt forms orange-brown crystals, soluble in hot, insoluble in cold water. The above acid, m. p. and mixed m. p. 263°, was similarly obtained in 4% yield by refluxing *m*-bromonitrobenzene, 4-nitro-2-aminobenzoic acid, sodium carbonate, and precipitated copper in 4-methyl*cyclo*hexanol for 16 hours and fractionally crystallising the calcium salts of the acids remaining after steam-distillation.

Reduction of the above acid with sodium sulphide, yellow ammonium sulphide, sodium hyposulphite, or stannous chloride gave no trace of amino-acid.

3 : 3′-*Diaminodiphenylamine.*—5 : 5′-Dinitrodiphenylamine-2-carboxylic acid (6 g.) was added to a hot solution of stannous chloride (35 g.) in 10% hydrochloric acid (100 ml.). The solution was concentrated and cooled and the stannichloride was collected, washed with ether, and dissolved in hot water. After removal of tin with hydrogen sulphide the filtrate was saturated with sodium acetate and the precipitated *amine* (yield, 68%) was repeatedly crystallised

from boiling water (1 in 105), forming white needles, m. p. 94·5—95°, soluble in cold alcohol, hot benzene, and toluene, but precipitated from the latter with petroleum spirit (Found : C, 71·9; H, 6·7; N, 21·2. $C_{12}H_{12}N_2$ requires C, 72·4; H, 6·6; N, 21·1%). The base decomposes on even gentle warming, becoming pink on exposure to the air; it is diazotisable and then couples with β-naphthol (scarlet). The salts with mineral acids are freely soluble. The solution in concentrated sulphuric acid becomes intense brown with a trace of a nitrate, and bright pink with a trace of ferrous sulphate and a drop of hydrogen peroxide solution. The aniline sulphate–dichromate test gives a brown precipitate, an ethereal extract of which is purple and becomes green when well acidified. The 3 : 3′-*bisacetyl* derivative, formed by the action of acetic anhydride in ether (yield, 95%), formed minute white crystals, m. p. 211° (corr.) (from 35% alcohol), insoluble in hot water, soluble in boiling alcohol (approx. 1 in 15 of 97%) and boiling glacial acetic acid, but the recovery on cooling from both of these was poor (Found : N, 14·4. $C_{16}H_{17}O_2N_3$ requires N, 14·9%). The amine is readily regenerated by boiling hydrochloric acid; both substances fluoresce brightly in ultra-violet light, the amine violet and the bisacetyl derivative bluish-white.

3 : 3′-*Dinitrodiphenylamine.*—A mixture of *m*-nitroacetanilide (5·4 g.), *m*-bromonitrobenzene (6·1 g.; 1 mol.), precipitated copper (0·1 g.), potassium iodide (0·1 g.), potassium carbonate (2·1 g.), and nitrobenzene (20 ml.) was maintained at 140° for 10 hours, then refluxed and saponified (cf. Goldberg, *loc. cit.*). Yield, 50%. Crystallisation of the product from nitrobenzene, and glacial acetic acid (1 in 8), gave orange crystals, m. p. 186·5° (corr.), fairly soluble in ether and boiling alcohol (Found : C, 55·5; H, 3·65; N, 16·5. $C_{12}H_9O_4N_3$ requires C, 55·6; H, 3·5; N, 16·2%). Unlike certain isomerides, it gave no colour in sulphuric acid with sodium nitrite, but the light yellow solution in alcohol became orange on addition of alcoholic potash. The dinitro-compound (0·2 g.) was dissolved in glacial acetic acid (5 ml.), quickly cooled, and treated with A.R. zinc needles (1·0 g.) and concentrated hydrochloric acid, drop by drop, until the solution was decolorised. The resultant solution was treated with sodium hydroxide, shaken out with ether, and the 3 : 3′-diaminodiphenylamine recrystallised; it did not depress the m. p. of a specimen made from 5 : 5′-dinitrodiphenylamine-2-carboxylic acid.

5-*Chloro*-2 : 6-*dinitroacridine* (II).—A mixture of 5 : 5′-dinitrodiphenylamine-2-carboxylic acid (7·6 g.) and phosphorus oxychloride (60 g.) was refluxed for 4 hours and distilled to dryness at 160°. The residue was cooled in a desiccator, powdered, and added to a well-stirred mixture of ice and water, kept faintly alkaline with ammonia. After filtration, the solid was dried and extracted with chloroform. The insoluble portion consisted of a mixture of 2 : 6- and 2 : 8-dinitroacridones (III and V) (9% yield) and was used to supplement the raw material in subsequent condensations. The chloroform solution gave 6·7 g. (88%) of a mixture of 5-*chloro*-2 : 6-*dinitroacridine* (principally) and the corresponding 2 : 8-dinitro-compound (IV). These were separated by repeated fractional crystallisation from benzene (1 in 40). The pure substance (II) was finally obtained in lemon-yellow needles, m. p. 200—203° (corr.), and fluoresced brightly in ultra-violet light (Found : C, 51·8; H, 2·1; N, 13·7. $C_{13}H_6O_4N_3Cl$ requires C, 51·4; H, 2·0; N, 13·8%). It is practically insoluble in water, mineral acids, or light petroleum, but dissolves in chloroform, toluene and benzene (approx. 1 in 10), and is less soluble in ether and alcohol; none of the solutions fluoresce. It is soluble in cold *N*/5-alcoholic potash to a brown solution, from which water precipitates it, and insoluble in cold 5*N*-aqueous sodium hydroxide. Both these reactions distinguish it from the corresponding acridone. In moist air, it is readily hydrolysed, and both alkaline solutions and undried organic solvents have the same action, which is most readily brought about by boiling dilute mineral acids. When it was treated for ½ hour with excess of 10% hydrochloric acid, 2 : 6-*dinitroacridone* (III) was quantitatively formed; this was washed with boiling 2% sodium carbonate solution, extracted with boiling water, dried, and dissolved (1 in 30) in 1% alcoholic potash, and the filtered solution poured into boiling *N*-hydrochloric acid. The bright yellow powder obtained (Found : C, 54·3; H, 2·6; N, 14·5. $C_{13}H_7O_5N_3$ requires C, 54·7; H, 2·5; N, 14·7%) is capable of sublimation, and is insoluble in mineral acids and all common solvents, but slightly soluble in pyridine, aniline, and molten phenol : none of its solutions show fluorescence. Aqueous alkalis dissolve only minute amounts with the production of a red-brown colour, and from these solutions mineral acids, or ammonium chloride, but not sodium chloride, reprecipitate it. The solution in alcoholic potash is wine-coloured, and excess of the solvent causes the potassium compound to be partly salted out as blood-red crystals; a dilute solution is not immediately precipitated on addition to water (distinction from the chloro-compound). 2 : 6-Dinitroacridone is not formed by the action of hot sulphuric acid on 5 : 5′-dinitrophenylamine-2-carboxylic acid, but may be obtained direct from the latter by Ullmann's acid chloride method (Ullmann, *loc. cit.*).

2 : 6-*Diaminoacridone*.—2 : 6-Dinitroacridone (5 g.) was slowly added to a boiling solution of stannous chloride (48 g.) in the same weight of fuming hydrochloric acid, and the mixture heated for ½ hour; on cooling, 8·4 g. of the ether-insoluble stannichloride were deposited. These crystals, together with a second crop, were dissolved in water and poured into sodium hydroxide solution at 0°. The precipitated amine was extracted in boiling 5% sulphuric acid, which, on filtration and cooling, deposited the insoluble sulphate. (This method of freeing an ether-insoluble amine from its stannichloride depends, first, on the low temperature of the alkali preventing the formation of metastannic acid and, secondly, on the ready hydrolysis of tin sulphate in boiling solution to an insoluble substance.) The *base*, liberated by alkali, was dissolved in alcohol, filtered, and the alcohol recovered. Yield, 75% of a bright yellow solid, which on twice crystallising from boiling water (approx. 1 in 4,000) gave greenish-yellow needles, m. p. 306° (bath at 300°), soluble in pyridine, aniline, phenol, glacial acetic acid, and alcohol (approx. 1 in 150, equally hot or cold), and sparingly soluble in benzene, nitrobenzene, toluene, and chloroform (Found for base dried at 80° : C, 66·5; H, 4·8; N, 17·6; loss at 120°, 3·5. $C_{13}H_{11}ON_3,\frac{1}{2}H_2O$ requires C, 66·7; H, 5·2; N, 17·95; H_2O, 3·8%). Unlike the 3 : 7-isomeride (cf. Bogert, *loc. cit.*), but like the white 2 : 8-isomeride, it is insoluble in 20% sodium hydroxide solution. It is diazotisable (light red solution) and then couples with β-naphthol (bright red). Aqueous and alcoholic solutions of the base fluoresce yellow-green. The fluorescence of an alcoholic solution disappears on boiling and reappears on cooling; the addition of one drop of hydrochloric acid intensifies it, but ammonia has no effect. Glacial acetic acid solutions are devoid of fluorescence. The hydrochloride is freely soluble.

2 : 6-*Diaminoacridine* (VI).—2 : 6-Diaminoacridone (0·5 g.) and *N*-hydrochloric acid (5·0 ml.) were stirred slowly on a water-bath at 80° and *N*/2-sodium hydroxide (50 ml.) was added at once, followed by 2% sodium amalgam (50 g.) during 2 hours. The temperature still being maintained, the rate of stirring was increased so as to beat air into the mixture, which after 2 hours was acidified with sulphuric acid and treated with ferric chloride (about 0·2 g.) until a blue spot test with potassium ferrocyanide was given. After a further ½ hour's heating, the mixture was brought to the boiling point, filtered, and concentrated to 40 ml., air being excluded. Concentrated sulphuric acid (4 ml.) was added and the sparingly soluble, bright red sulphate obtained was recrystallised three times from boiling water in the presence of decolorising carbon and sulphuric acid. The base, liberated in 50% yield with sodium hydroxide, repeatedly crystallised from boiling water (approx. 1 in 100), and dried in a vacuum at 100°, formed brownish-orange felted needles (yield, 23%), m. p. 213—216° (decomp.) (bath at 200°), soluble in pyridine, slightly soluble in cold water, and very soluble in alcohol, in which the 2 : 8-isomeride is far less soluble; in all other common solvents 2 : 6-*diaminoacridine* was practically insoluble (Found : N, 20·0. $C_{13}H_7N_3$ requires N, 20·1%). None of its solutions exhibit fluorescence. It has a bitter taste, stains the skin bright yellow, is slightly hygroscopic, and tends to oxidise, especially in warm air, becoming deeper in colour. The hydrochloride is readily soluble, forms a yellow precipitate with formaldehyde, and gives a violet diazo-solution which couples with β-naphthol (wine-red).

5-*Chloro*-2 : 8-*dinitroacridine* (IV).—This was isolated from the first mother-liquors of the 2 : 6-dinitro-isomeride (*v.s.*) and, purified by repeated fractional crystallisation from benzene (1 in 50), formed brownish-yellow rosettes of needles, m. p. 247—248·5° (corr.), which profoundly depressed the m. p. of the 2 : 6-dinitro-isomeride. It is soluble in the same solvents as the latter, gives the same distinguishing reactions with alkalis, and is hydrolysed by the same reagents to lemon-yellow 2 : 8-dinitroacridone (V). The latter substance exhibits physical and chemical properties qualitatively identical with those already described for its 2 : 6-isomeride. The solubility in 1% alcoholic potash is twice as great as that of the 2 : 6-isomeride, but the colours of the solutions are very similar, whereas Ullmann (*loc. cit.*) found a considerable difference in the colours of alcoholic potash solutions of the 6- and 8-nitroacridones (1- and 3- in his nomenclature). Reduction of 2 : 8-dinitroacridone by the method described for the 2 : 6-isomeride gave 2 : 8-diaminoacridone, which displayed all the properties ascribed to it in the literature, as well as the following, hitherto unrecorded : the solubility in boiling 97% alcohol is approx. 1 in 320, in boiling water, approx. 1 in 4000, the latter being suitable for recrystallisation. The aqueous solution shows a violet fluorescence whether hot or cold, but the violet fluorescence displayed in alcoholic solutions between the concentrations of 1 in 3000 and 1 in 10^7, and reaching a maximum at approx. 1 in 500,000, disappears temporarily on heating. This fluorescence is changed to intense greenish-blue by a trace of hydrochloric acid, but ammonia is without effect. In glacial acetic acid solution, the fluorescence is intense blue-green and disappears on standing. In acid solution 2 : 8-diaminoacridone gives an orange-

yellow precipitate with formaldehyde. It is diazotisable (light red solution) and then couples with β-naphthol (bright red). The sulphate consists of bright orange-yellow crystals soluble approx. 1 in 7000 in cold water and readily hydrolysed, but appreciably soluble in boiling water in the presence of a little excess acid. The 2 : 8-*dibenzylidene* derivative formed golden satiny crystals from nitrobenzene, m. p. approx. 370° (decomp.), readily hydrolysed by cold dilute acids (Found : N, 10·4. $C_{27}H_{19}ON_3$ requires N, 10·5%). The 2 : 8-*bisacetyl* derivative formed an ivory-white powder, slightly soluble in hot 60% acetic acid and insoluble in all other solvents; it does not melt below 350° (Found by the method of Phillips, *Ind. Eng. Chem. Anal. Ed.*, 1934, 321 : CH_3·CO, 27·25. $C_{17}H_{15}O_3N_3$ requires CH_3·CO, 27·8%). The 2 : 8-*bisbenzoyl* derivative, obtained by the action of benzoyl chloride on the amine in pyridine, formed a light, buff-coloured, micro-crystalline powder, insoluble in all solvents and not melting below 350° (Found : N, 9·3. $C_{27}H_{19}O_3N_3$ requires N, 9·7%). When 2 : 8-diaminoacridone (4·1 g.), suspended in aqueous sodium hydroxide (1500 ml. of 2%), was treated with sodium amalgam (400 g. of 2%) under the conditions described for the 2 : 6-isomeride, and air was beaten into the mixture for 6 hours longer, 2 : 8-diaminoacridine (proflavine base) was deposited without the necessity for the use of ferric chloride. After cooling, it was filtered off as orange-yellow needles, m. p. 277—280° (3·33 g.; 90% yield). On recrystallisation from boiling water, the m. p. rose to 281° and was not lowered by an authentic specimen of m. p. 281—282°.

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