6. *Chemotherapeutic Studies in the Acridine Series. Part III. 4-Amino-, 1 : 3-, 1 : 7-, and 3 : 6-Diaminoacridines.*

By Adrien Albert and Wilfred H. Linnell.

The above acridine derivatives have been prepared in completion of an exploration of the relationship between the chemistry and the antiseptic activity of the simpler aminoacridines. 1 : 3-Diaminoacridone is easily oxidised in air, but 1 : 3-diaminoacridine is stable. The presence of 1- and 4-amino-substituents leads always to compounds which are non-fluorescent in alcoholic solution, even when the corresponding substances without these groups fluoresce actively. Furthermore the preliminary bacteriological tests indicate that all the compounds containing a 1-amino-substituent are without antiseptic effect, whereas the 4 (as well as the 5, 3, and 2)-amino-group greatly increases the antiseptic activity of the acridine molecule.

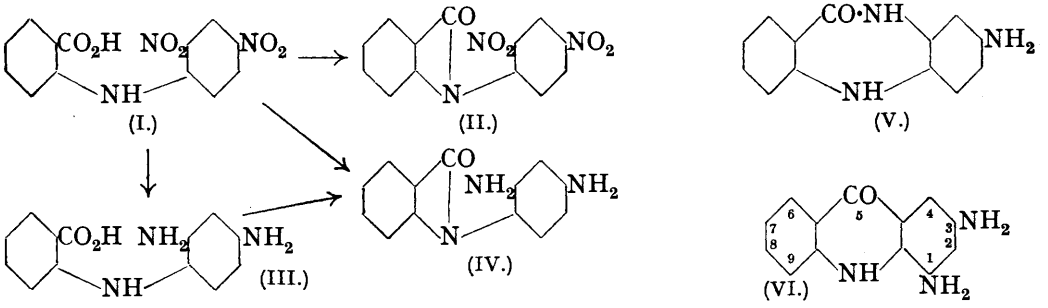
A substance described in 1885 as 1 : 3-diaminoacridone has now been found to be a diaminodiphenylaminecarboxylic acid lactam.

2-Chloro-5-nitrobenzoic acid was condensed with *m*-nitroaniline to give 4 : 3′-*dinitrodiphenylamine-2-carboxylic acid*, and 2-chlorobenzoic acid and 3 : 5-dinitroaniline similarly gave 3′ : 5′-*dinitrodiphenylamine-2-carboxylic acid*. The latter on ring closure with phosphorus oxychloride, followed by acid hydrolysis, yielded 2 : 4-*dinitroacridone*. When 4 : 3′-dinitrodiphenylamine-2-carboxylic acid was treated with phosphorus oxychloride and the usual precautions were taken to avoid hydrolysis (see Part I, J., 1936, 88), a 2% yield only of *meso*chlorodinitroacridines was obtained, from which 5-*chloro*-2 : 7-*dinitroacridine* was isolated by fractional crystallisation; the bulk of the condensation product was a mixture of 2 : 7 (*i.e.*, 3 : 8)- and (mainly) 3 : 6-dinitroacridones, since on further treatments with phosphorus oxychloride small additional amounts of 5-chloro-2 : 7-

dinitroacridine could be obtained, and complete reduction led to 3 : 6-*diaminoacridine*, a substance distinctly different from 2 : 7-diaminoacridine (Part II, J., 1936, 1614) in properties. The failure of the above dinitroacridones to yield *meso*chloro-compounds to any extent is consonant with our experience of acridones containing an odd-numbered nitro-group. 1 : 7-Dinitroacridone on acid reduction gave 1 : 7-*diaminoacridone*, but 2 : 4-dinitroacridone gave a product which was oxidised in the air so rapidly that it could not be obtained analytically pure.

1 : 7-Diaminoacridone and 4-aminoacridone (Lehmstedt, *Ber.*, 1937, **70**, 838) on reduction with sodium amalgam gave respectively 1 : 7-*diaminoacridine* and 4-*aminoacridine*. As 1 : 3-diaminoacridone (Drozdov, *J. Gen. Chem. U.S.S.R.*, 1934, **4**, 1) when similarly treated failed to give the corresponding acridine, 2-aminobenzaldehyde and 2 : 4-dinitrobromobenzene were condensed together to give 1 : 3-dinitroacridine, which on acid reduction furnished the required 1 : 3-*diaminoacridine*.

All known aminoacridones fluoresce in alcoholic solution, but the 1- and 4-amino-substituted acridines produced in this work are devoid of such fluorescence. Contrary to a published statement (Ullmann, *Ber.*, 1907, **40**, 2522) 1-aminoacridine does not fluoresce in alcoholic solution, nor do its salts fluoresce in water (Lehmstedt, private comm.).



Reduction of 2′ : 4′-*Dinitrodiphenylamine*-2-*carboxylic Acid* (I).—By vigorous reduction of this acid with tin and alcoholic hydrogen chloride, Jourdan (*Ber.*, 1885, **18**, 1444) obtained, in place of the expected diamino-acid (III), a yellow compound which he regarded as 1 : 3-diaminoacridone ("Diamidohydroacridinketon") (VI). However, when stannous chloride in cold acetic acid was used, 2′ : 4′-*diaminodiphenylamine*-2-*carboxylic acid monohydrochloride* was formed instead. When the stannichloride of this substance was heated with tin and hydrochloric acid, "Jourdan's compound" was obtained, and this was hydrolysed by methyl-alcoholic potash to the parent amino-acid (III). As 1 : 3-diaminoacridone, obtained by the reduction of 1 : 3-dinitroacridone (Drozdov, *loc. cit.*), differs in colour, solubility in alkali, melting point, and air-stability from "Jourdan's compound," and displays a fluorescence which the latter lacks, it is evident that the latter is an anhydro-compound of the type (IV) or (V). Since Drozdov (*loc. cit.*), by the action of phosphorus oxychloride on the sodium salt of (I), obtained the 1 : 2-lactam (II), it seems likely in the present case that stannous chloride functioned similarly as a condensing agent to give (IV) (cf. also Clemo, Perkin, and Robinson, J., 1924, **125**, 1779). On gentle acetylation a diacetyl compound, 2′ : 4′-*bisacetamidodiphenylamine*-2-*carboxylic acid* 1 : 2-*lactam*, was obtained, and this re-formed "Jourdan's compound" on acid hydrolysis. "7-Chloro-1 : 3-diaminoacridone" (Jourdan, *loc. cit.*) would appear likewise to be a lactam.

The pharmacological testing of these compounds was carried out by Dr. F. J. Dyer in the Pharmacological Laboratories of this College (*Quart. J. Pharm. Pharmacol.*, 1936, No. 4), and the bacteriological testing by Dr. L. P. Garrod of St. Bartholomew's Hospital (in the press). Preliminary biological tests have revealed that antiseptic activity and toxicity are not parallel in this series; for example, 2 : 7-diaminoacridine is comparable with proflavine in bacteriostatic properties but is 2·5 times less toxic, and 10 times less toxic than acriflavine; but 2-chloro-5-aminoacridine is twice as bacteriostatic as proflavine and twice as toxic. Whenever a 1-amino-group is introduced into an acridine compound, the antiseptic properties are completely destroyed.

Experimental.

Diphenylaminecarboxylic Acids.—Sodium *o*-chlorobenzoate (3 g.), 3 : 5-dinitroaniline (3 g.), precipitated copper (0·1 g.), sodium carbonate (0·45 g., ½ mol.), and 4-methyl*cyclo*hexanol (20 ml.) were refluxed for 4 hours, and the 3′ : 5′-*dinitrodiphenylamine-2-carboxylic acid* purified similarly to its isomeride (Part I). Yield, 22% of brownish-orange crystals, m. p. 263° (corr.), only slightly soluble in most solvents, soluble approx. 1 in 22 of boiling glacial acetic acid, but 90% acetic acid is more suitable for recrystallisation (Found: C, 51·85; H, 3·1; N, 13·4. $C_{13}H_9O_6N_3$ requires C, 51·5; H, 3·0; N, 13·9%). The colourless solution in sulphuric acid was turned deep orange by a trace of a nitrate. The orange-brown sodium salt was soluble in hot water and readily salted out with sodium carbonate.

Sodium 2-chloro-5-nitrobenzoate (4·5 g.), *m*-nitroaniline (4·2 g., 1½ mols.), precipitated copper (0·1 g.) and nitrobenzene (20 ml.) were refluxed for 1½ hours, and the 4 : 3′-*dinitrodiphenylamine-2-carboxylic acid* purified as above. Yield, 20% of pale brownish-yellow crystals, m. p. 229° (corr.), only slightly soluble in most solvents and best crystallised from 100 parts of boiling glacial acetic acid (Found: C, 51·7; H, 3·1; N, 13·9%). The yellow solution in sulphuric acid was unchanged by the addition of a trace of a nitrate. The bright orange sodium salt was readily salted out by sodium carbonate.

2′ : 4′-Dinitrodiphenylamine-2-carboxylic acid (2 g.) and anhydrous stannous chloride reagent (120 ml.; Part II) were left for 2 days in the cold, the lumps being broken as formed. An aqueous solution of the stannichloride was freed from tin with hydrogen sulphide and treated with sodium acetate, giving a white crystalline precipitate, soluble in sodium hydroxide solution, of 2′ : 4′-*diaminodiphenylamine-2-carboxylic acid hydrochloride* in 60% yield, m. p. 252° (corr.) (with effervescence and sublimation) (Found: C, 55·9; H, 5·2; N, 14·8; Cl, 12·4. $C_{13}H_{13}O_2N_3$,HCl requires C, 55·8; H, 5·0; N, 15·0; Cl, 12·7%). When heated for 15 hours at 140° in the air, it became in turn lilac, violet, and brown, but the m. p. remained unchanged. It was soluble in hot water, and was partly deposited on cooling, more completely on addition of sodium chloride. The free acid could not be precipitated by the use of buffers (internal salt formation?). The hydrochloride was soluble in most organic solvents without temperature gradient and was best recrystallised by solution in 85% alcohol (1—30) and addition of twice that volume of benzene. It gave a red coloration with nitrous acid, intensified by alkaline β-naphthol, although not by alkali alone. Ferric chloride gave a scarlet colour, and the phenanthraquinone test for an *o*-diamine was also given. The colourless solution in sulphuric acid was turned deep yellow by a trace of a nitrate. Instead of stannous chloride, ferrous chloride and boiling aqueous ammonia may be used as a reducing agent in the preparation of this substance.

Jourdan's Compound.—A mixture of 2′ : 4′-diaminodiphenylamine-2-carboxylic acid stannichloride (5 g.), alcohol saturated with hydrogen chloride (70 ml.), and excess of tin was refluxed for an hour and evaporated to half-volume. The product was poured into water and treated with excess of sodium hydroxide. The precipitate was taken up in hot dilute hydrochloric acid; the solution, on cooling, deposited the hydrochloride of "Jourdan's compound" in 40% yield. Ammonia liberated the free base in golden spangles having the properties described by Jourdan, as well as the following, hitherto unrecorded (Found: N, 18·4; *M*, in camphor, 244. Calc. for $C_{13}H_{11}ON_3$: N, 18·6%; *M*, 225): The substance did not fluoresce, neither did it give a precipitate in alcohol with mercuric chloride. 50% Sulphuric acid and a trace of a dichromate gave a deep brown solution, the odour of quinone becoming perceptible on warming. On diazotisation an orange solution was obtained, which became deep red on addition of alkaline β-naphthol.

The above compound (0·2 g.) and N-90% methyl-alcoholic potash (10 ml.) were refluxed for 1½ hours, and the alcohol then distilled. A filtered aqueous solution of the residue on treatment with hydrochloric acid and sodium acetate gave 2′ : 4′-diaminodiphenylamine-2-carboxylic acid hydrochloride in 50% yield, m. p. (after crystallisation from alcohol–benzene) and mixed m. p. 252°. "Jourdan's compound" (0·5 g.) and acetic anhydride (0·5 g.) were heated for 15 minutes on the water-bath, and the white precipitate of 2′ : 4′-*bisacetamidodiphenylamine-2-carboxylic acid* 1 : 2-*lactam* filtered off. Yield 92%, m. p. 307° (corr.) (Found: C, 65·8; H, 4·8; N, 13·3. $C_{15}H_{15}O_3N_3$ requires C, 66·0; H, 4·9; N, 13·6%). It was soluble in 30% alcohol (approx. 1 in 75, boiling, with 84% recovery on cooling) and in stronger alcohol without temperature gradient, almost insoluble in toluene, chloroform, and water, was not diazotisable, and gave no colour with ferric chloride. When it was warmed with sulphuric acid in alcohol, "Jourdan's compound" was re-formed.

Ring Closure and Formation of meso*Chloro-compounds.*—A mixture of 3′ : 5′-dinitrodiphenylamine-2-carboxylic acid (0·5 g.) and phosphorus oxychloride (5 ml.) was refluxed for an hour and poured into water. The precipitate was boiled with 5% hydrochloric acid for 15 minutes, dried, and dissolved in N/5-50% alcoholic potash, and the deep red solution treated with acid, giving 2 : 4-*dinitroacridone* in 94% yield. The product was dissolved in pyridine, and boiling benzene (3 vols.) gradually added; on cooling, golden-brown infusible needles were obtained, almost insoluble in acetic acid and other common solvents, but somewhat soluble in hot 20% aqueous sodium hydroxide with a dark brown colour (Found: C, 54·9; H, 2·6; N, 14·4. $C_{13}H_7O_5N_3$ requires C, 54·7; H, 2·5; N, 14·7%).

1 : 7-Dinitroacridone was produced in 61% yield (calculated on the bromonitrobenzene) by nitration of 1-nitroacridone, obtainable in excellent yield by ring closure of the corresponding nitrodiphenylamine-2-carboxylic acid (Ullmann, *Annalen*, 1907, **355**, 327).

5 : 4′-Dinitrodiphenylamine-2-carboxylic acid (2·7 g.) and phosphorus oxychloride (16 ml.) were refluxed for 8 hours, most of the solvent recovered, and the product treated with ice and ammonia. On extraction with chloroform and recrystallisation of the residue, remaining after evaporation, from benzene a 20% yield of yellow 5-*chloro*-2 : 7-*dinitroacridine* was obtained, m. p. 233° (corr.) (Found: N, 14·1. $C_{13}H_6O_4N_3Cl$ requires N, 13·85%).

Neither 2′ : 4′-dinitrodiphenylamine-2-carboxylic acid (I) nor 1 : 3-dinitroacridone furnished a *meso*chloro-compound.

2-Aminobenzaldehyde (5·2 g.), 2 : 4-dinitrobromobenzene (10·8 g.; 1 mol.), precipitated copper (0·5 g.), sodium carbonate (2·3 g.), and nitrobenzene (40 ml.) were heated in an oil-bath at 215° for 3 hours. After steam-distillation and filtration, the residue was heated with sulphuric acid (20 ml.) on the water-bath for an hour, the product poured into water (10 vols.), the solution filtered at the b. p., and aqueous ammonia added to the filtrate. The precipitated 1 : 3-dinitroacridine crystallised from pyridine in brownish-orange needles, m. p. 284° (corr.), only slightly soluble in cold dilute acids (yield, 30%).

Acid Reductions.—The reduction of 1 : 3-dinitroacridone with anhydrous stannous chloride reagent (Part II), followed by decomposition of the stannichloride with ammonia and extraction of the precipitate with alcohol, produced 1 : 3-diaminoacridone (Drozdov, *loc. cit.*), for which the following properties may now be recorded: it fluoresces bright green in alcoholic solution, is decomposed to a blue product by hydrogen sulphide, and is very readily oxidised in the air to a black insoluble product. Neither this product nor 1 : 3-dinitroacridone, when reduced with sodium amalgam by the methods described in Part II, gave any 1 : 3-diaminoacridine, which was, however, prepared in 75% yield by the action of anhydrous stannous chloride reagent on 1 : 3-dinitroacridine. The stannichloride was decomposed with excess of sodium hydroxide and the precipitate formed was extracted with alcohol. After recrystallisation from alcohol, 1 : 3-*diaminoacridine* formed air-stable, orange, feathery crystals, m. p. 225° (sealed tube, corr., decomp.), slightly soluble in boiling water and chloroform, insoluble in sodium hydroxide solution (Found: C, 74·7; H, 5·2; N, 19·5. $C_{13}H_{11}N_3$ requires C, 74·6; H, 5·3; N, 20·1%). Neither the base nor its salts fluoresce in alcohol or in water. The purple hydrochloride and the sulphate are soluble in water and the solutions are decolourised by nitrous acid, becoming orange on addition of sodium hydroxide or alkaline β-naphthol.

1 : 7-Dinitroacridone on reduction with the stannous chloride reagent gave a precipitate of the stannichloride, which was treated with ice and sodium hydroxide, more than a slight excess being avoided. The precipitate was dried at 120°, and the base extracted in dilute hydrochloric acid and reprecipitated with sodium hydroxide (slight excess), a 50% yield of 1 : 7-*diaminoacridone* being obtained; this, on recrystallisation from boiling water (1 in 2,500; 60% recovery), gave lemon-yellow needles, charring at 330°, only slightly soluble in alcohol, very soluble in alcoholic potash, and moderately in 4% aqueous sodium hydroxide (Found: C, 69·2; H, 4·7; N, 17·95. $C_{13}H_{11}ON_3$ requires C, 69·3; H, 4·9; N, 18·6%). The intense yellow-green fluorescence of an alcoholic solution was destroyed by acid; and there was no fluorescence of the base in acetic acid or of the hydrochloride in water. The diazotised solution was pale orange, becoming deep red with alkaline β-naphthol.

Reduction in the same way of the mixture of 2 : 7- and 3 : 6-dinitroacridones (see p. 22) led to a mixture of the aminoacridones, which was reduced to the corresponding diaminoacridines.

Alkaline Reductions.—4-Aminoacridone hydrochloride (0·5 g.) was reduced with sodium amalgam, and the product oxidised with ferric chloride (cf. 2 : 6-diaminoacridone; Part I). When the oxidised solution was cooled, the sodium chloride present precipitated 4-*aminoacridine* as the hydrochloride. The base was set free and crystallised from 33% alcohol, giving

a 71% yield of brownish-orange needles, m. p. 181° (corr.; sealed tube; bath at 160°). It was very soluble in alcohol and chloroform without temperature gradient, but could be recrystallised from water (Found for material dried in air at 110°: loss in a vacuum at 120°, 17·0. $C_{13}H_{10}N_2,2H_2O$ requires H_2O, 16·0%. Found for material dried in a vacuum at 120°: C, 80·3; H, 5·0. $C_{13}H_{10}N_2$ requires C, 80·4; H, 5·2). Neither the base nor its salts, which were violet, fluoresced in water or organic solvents, but a bright yellow-green fluorescence was shown in concentrated sulphuric acid.

1 : 7-Diaminoacridone, reduced as above, but not treated with acid or ferric chloride, gave a 60% yield of 1 : 7-*diaminoacridine*, which was recrystallised (1 in 2500) from boiling water, giving orange needles, m. p. 126° (corr.; sealed tube), very soluble in alcohol and pyridine, and slightly soluble in chloroform and benzene, all without temperature gradients, but insoluble in alkali. The solubility in boiling water was approx. 1 in 1,000, but more dilute solutions crystalliser better (Found: C, 74·1; H, 5·3; N, 19·7. $C_{13}H_{11}N_3$ requires C, 74·6; H, 5·3; N, 20·1%). Neither the base nor its salts gave any fluorescence in alcohol or water, but the base fluoresced yellow-green in concentrated sulphuric acid. The violet hydrochloride and sulphate were readily soluble in water and on diazotisation gave colourless solutions which became bright red with alkaline β-naphthol. In 50% sulphuric acid a trace of potassium dichromate produced a light brown coloration, whereas 1-aminoacridine gave a violet colour.

The mixture of 2 : 7 and (mainly) 3 : 6-diaminoacridones (*v.s.*) was reduced with sodium amalgam as above, and the product treated with ferric chloride. The resultant mixture was treated with sodium hydroxide, and the precipitate extracted with alcohol. On evaporation of the solvent, solution of the residue in dilute hydrochloric acid, and addition of 10% of sulphuric acid, the sulphate of 3 : 6-*diaminoacridine* was slowly deposited. The base was liberated and recrystallised from 100 parts of alcohol until the solution no longer fluoresced; yellow-brown needles, m. p. 322° (sealed tube; not sharp), were then obtained, which were slightly soluble in boiling water and benzene and very soluble in pyridine. The purple salts were very soluble in water and far less readily salted-out than those of other aminoacridines (Found: C, 74·9; H, 5·2; N, 19·6%). Neither the base nor its salts exhibited fluorescence in water or alcohol. From the mother-liquor a small quantity of 2 : 7-diaminoacridine, m. p. 355° (Found: N, 19·7), and showing a bright green fluorescence in alcohol, was obtained.

A gift of *o*-chlorobenzoic acid from Imperial Chemical Industries (British Dyestuffs Corporation, Blackley) is gratefully acknowledged.

COLLEGE OF THE PHARMACEUTICAL SOCIETY (UNIVERSITY OF LONDON),
LONDON, W.C. 1. [*Received, November 19th*, 1937.]