## 64. *Chemotherapeutic Studies in the Acridine Series. Part IX. The Chloroaminoacridines.*

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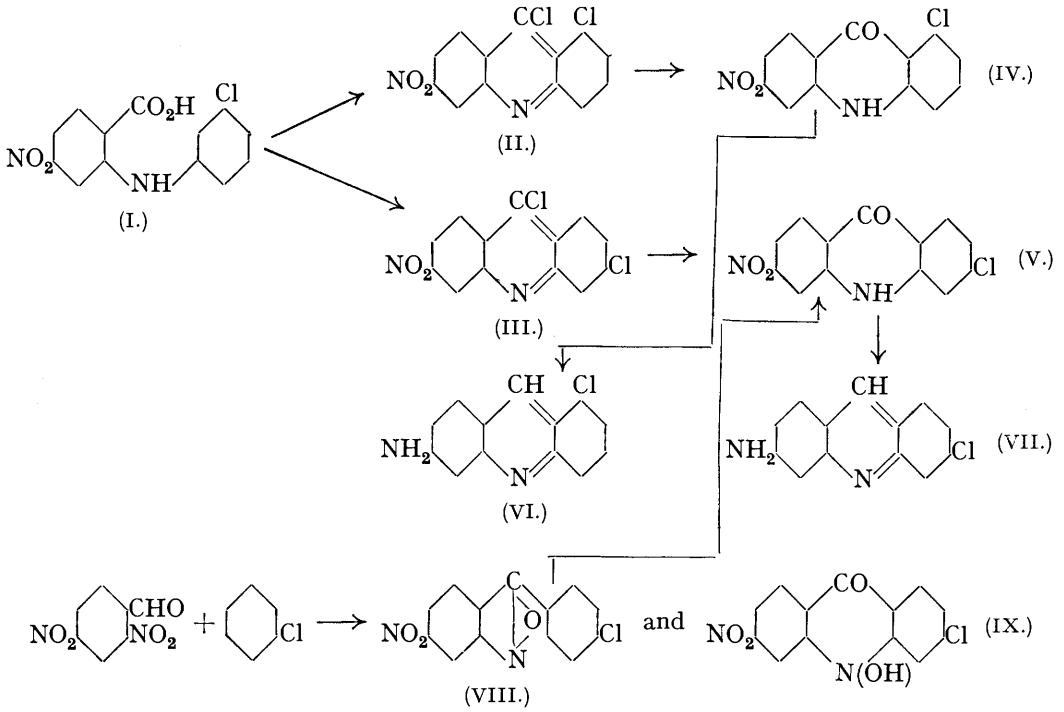
The preparation of 6- and 8-*chloro*-2-*amino*- and 6- and 8-*chloro*-3-*amino-acridine* is described. The 2- and the 3-amino-compounds were derived from the ring closure of 5- and 4-*nitro*-3′-*chlorodiphenylamine*-2-*carboxylic acid* respectively. In each case the structure of the two isomers was established by an independent synthesis of one of them, and, as in other ring closures of this type, the 6-compound had in each case a lower m. p. and was formed in greater amount than the 8-isomer. 4-*Nitro*-C-(p-*chlorophenyl*)*anthranil* has been isolated from the products of condensation of 2 : 4-dinitrobenzaldehyde and chlorobenzene, and the conversion of this anthranil into 8-chloro-2-nitroacridone by nitrous acid is in agreement with Lehmstedt's mechanism for this condensation.

Chemotherapeutic investigations of the mono- and di-amino-acridines (Albert and Linnell, J., 1936, 88, 1614; 1938, 22; Albert, Rubbo, and Goldacre, *Nature*, 1941, **147**, 332) have shown the simple monoaminoacridines, especially 2-aminoacridine, to be highly active antiseptic substances. On the other hand, many highly active antiseptics have been produced by the introduction of chlorine into the molecule of various phenols (Bechhold and Ehrlich, *Z. physiol. Chem.*, 1906, **47**, 173; Klarmann *et al.*, *J. Amer. Chem. Soc.*, 1929, **51**, 605; 1932, **54**, 3315; 1933, **55**, 2576). The activating effect of chlorine in the phenolic series has been sufficiently pronounced to warrant the study of its effect on the properties of the monoaminoacridines. The preparation of a series of chloroaminoacridines has therefore been undertaken as a further step in the development of antiseptics in the acridine series. This paper describes the preparation of 6-chloro-2-amino-, 8-chloro-2-amino-, 6-chloro-3-amino- and 8-chloro-3-amino-acridine.

*The 2-Amino-compounds.*—*3'-Chloro-5-nitrodiphenylamine-2-carboxylic acid* (I) was obtained in 38% yield, together with 18% of *p*-nitrobenzoic acid, by refluxing 2-chloro-4-nitrobenzoic acid with *m*-chloroaniline in *n*-butanol in the presence of sodium carbonate and copper during 40 hours. Poorer yields and much tar resulted when the reactants were heated for shorter periods in higher-boiling solvents (amyl alcohol, methyl*cyclo*hexanol).

Ring closure of 3'-substituted diphenylamine-2-carboxylic acids can take place in two ways, giving 6- and 8-substituted acridones. 3'-Chloro-5-nitrodiphenylamine-2-carboxylic acid was treated with excess of phosphorus oxychloride (cf. Lesnianski, *Bull. Acad. Polonaise*, 1929, 81) in the expectation of producing 5 : 6-dichloro-2-nitro- (II) and 5 : 8-dichloro-2-nitro-acridine (III), which should be separable by fractional crystallisation (cf. Albert and Linnell, *loc. cit.*; Lehmstedt, *Ber.*, 1937, **70**, 838). The *meso*-chloro-compounds, however, were largely hydrolysed to 6-chloro-2-nitroacridone (IV) and 8-chloro-2-nitro-acridone (V), compounds which, having no m. p. below 300°, did not lend themselves to separation by fractional crystallisation. The mixture was reduced by stannous chloride, yielding the chloroamino-acridones, which gave a mixture of 6-*chloro-2-aminoacridine* (VI) and 8-*chloro-2-aminoacridine* (VII) when reduced with sodium amalgam. Systematic fractional crystallisation from water and 30% alcohol separated the two products, the isomer produced in the larger proportion and having the lower m. p. being regarded as the 6-substituted compound by analogy with the findings of Albert and Linnell (*loc. cit.*) and of Lehmstedt (*loc. cit.*). This was confirmed by an alternative synthesis of 8-chloro-2-aminoacridine.




2 : 4-Dinitrobenzaldehyde was condensed with chlorobenzene in the presence of concentrated sulphuric acid, and the product isolated as described by Tanasescu (*Bull. Soc. chim.*, 1927, **41**, 528). The 8-chloro-2-nitroacridone described as the product of this condensation by Tanasescu was obtained in traces only, the main products being 4-*nitro*-C-(p-*chlorophenyl*)*anthranil* (VIII) and 8-*chloro-2-nitro*-10-*hydroxy-acridone* (IX). Lehmstedt (*Ber.*, 1932, **65**, 999) has postulated 4-nitro-*C*-(*p*-chlorophenyl)anthranil as an intermediate in the formation of 8-chloro-2-nitroacridone in the Tanasescu condensation, the conversion of anthranil into acridone taking place under the influence of nitrous acid, although he failed to isolate the phenylanthranil. We have converted 4-nitro-*C*-(*p*-chlorophenyl)anthranil into 8-chloro-2-nitro-acridone by treatment with sodium nitrite and sulphuric acid, thus confirming Lehmstedt's mechanism of the condensation. When 2 : 4-dinitrobenzaldehyde was condensed with chlorobenzene in the presence of sodium nitrite and concentrated sulphuric acid, 8-chloro-2-nitroacridone was the main product. Reduction of 8-chloro-2-nitroacridone with stannous chloride gave 8-*chloro-2-aminoacridone*, which, when reduced with sodium amalgam in the presence of alcohol and carbon dioxide (cf. Scherlin *et al.*, *J. Gen. Chem. Russia*, 1938, **8**, 880), gave 8-chloro-2-aminoacridine identical with that separated from the 6-chloro-compound by fractional crystallisation.

*The 3-Amino-compounds.*—*3'-Chloro-4-nitrodiphenylamine-2-carboxylic acid* was obtained in 23% yield when potassium 2-chloro-5-nitrobenzoate was condensed with *m*-chloroaniline, in the presence of anhydrous potassium carbonate and copper in refluxing *n*-butanol during 40 hours.

Ring closure of 3'-chloro-4-nitrodiphenylamine-2-carboxylic acid with excess of phosphorus oxychloride gave a mixture of 5 : 6-*dichloro-3-nitroacridine* and 5 : 8-*dichloro-3-nitroacridine*. Although some hydrolysis to the corresponding chloronitroacridones occurred during their isolation, it was possible to free the mixed *meso*-chloro-compounds from acridones by extraction with dry benzene, and subsequent fractional crystallisation from the same solvent separated the two entities. The compound with the lower melting point and formed in greater amount was again regarded as the 6-isomer. 5 : 6-Dichloro-3-nitroacridine was hydrolysed by boiling dilute hydrochloric acid to 6-*chloro-3-nitroacridone*, which gave 6-*chloro-3-aminoacridine* directly when reduced with sodium amalgam. Reduction of the mixed chloronitroacridones with sodium amalgam gave a mixture of 6-chloro-3-aminoacridine and 8-*chloro-3-aminoacridine*, which were separated by fractional crystallisation from dilute alcohol. The identity of the latter compound and hence that of the 6-chloro-3-aminoacridine and the *meso*-chloro-compounds was confirmed by alternative synthesis kindly carried out in the University of Sydney by Dr. Adrien Albert. 2 : 4-Dichlorobenzoic acid was condensed with *p*-phenylenediamine to give 5-chloro-4'-aminodiphenylamine-2-carboxylic acid, which underwent ring closure to give 8-chloro-3-aminoacridine when treated with concentrated sulphuric acid. The product was identical with the specimen isolated by fractional crystallisation.

The four chloroaminoacridines are being submitted to bacteriological tests, the results of which will be published elsewhere.

## Experimental.

*3'-Chloro-5-nitrodiphenylamine-2-carboxylic Acid* (I).—Sodium 2-chloro-4-nitrobenzoate (4·5 g.), anhydrous sodium carbonate (2·0 g.), *m*-chloroaniline (3·3 g.), and precipitated copper (0·1 g.) were heated in refluxing *n*-butanol (20 ml.) for 40 hours. The products of five such experiments were combined, the *n*-butanol and the excess of *m*-chloroaniline removed by steam distillation, and the aqueous residue concentrated, filtered, and cooled; the sodium salt of the product was deposited. A further quantity was obtained by acidifying the mother-liquors and extracting the precipitated acids with boiling water, to remove *p*-nitrobenzoic acid, which was deposited on cooling (3 g.; 18%). The sodium salt, decomposed with dilute hydrochloric acid, gave *3'-chloro-5-nitrodiphenylamine-2-carboxylic acid*, which crystallised from aqueous alcohol (80%) in bright orange needles, m. p. 221—222°. Yield, 38% (Found : N, 9·7; Cl, 12·0. $C_{13}H_9O_4N_2Cl$ requires N, 9·6; Cl, 12·1%).

*3'-Chloro-4-nitrodiphenylamine-2-carboxylic Acid.*—A mixture of potassium 2-chloro-5-nitrobenzoate (4·8 g.), *m*-chloroaniline (3·3 g.), anhydrous potassium carbonate (2·0 g.), and copper (0·1 g.) was treated as in the preceding preparation. After the removal of *n*-butanol and the excess of *m*-chloroaniline the hot aqueous liquid was filtered. The potassium salt of the product, remaining on the filter free from tar, was crystallised from water (1 in 200) and decomposed with dilute hydrochloric acid, giving *3'-chloro-4-nitrodiphenylamine-2-carboxylic acid*, which crystallised from dilute alcohol in pale yellow needles, m. p. 272—273° (decomp.) (Found : N, 9·8; Cl, 12·4%).

When *cyclo*hexanol replaced the *n*-butanol as solvent, much tar was formed, which, on extraction with boiling water, gave 5-nitrosalicylic acid (0·1 g.), identified by m. p. 227° and the characteristic blood-red colour with ferric chloride solution.

*Ring Closures.*—3'-Chloro-5-nitrodiphenylamine-2-carboxylic acid (5·0 g.) was refluxed with phosphorus oxychloride (50 g.) until evolution of hydrogen chloride ceased (2 hours); the excess of phosphorus oxychloride was then distilled off, and the residue rubbed with ice-water and aqueous ammonia. A chloroform extract of the lemon-yellow powder (4·8 g.) obtained deposited, on cooling, yellow needles of a mixture of dichloronitroacridines and chloronitroacridones. The whole product was therefore converted into chloronitroacridones by boiling with dilute hydrochloric acid. The mixed chloronitroacridones (2·7 g.), suspended in alcohol (125 ml.), were added gradually to boiling hydrochloric acid (25 ml.) and stannous chloride (10 g.), and the whole refluxed for 3 hours. The solution was filtered, most of the alcohol removed by distillation, and the residue cooled. The mixed tin salts deposited were decomposed with excess of 20% sodium hydroxide solution, and the resulting mixture of chloroaminoacridones washed and dried (1·9 g.). This mixture was finely powdered and suspended in N/2-sodium hydroxide (200 ml.) at 80°, and sodium amalgam (2%; 200 g.) added during 2 hours. Stirring was continued for 2 hours, and the suspension then decanted from the mercury and stirred with free access of air for 2 hours. Filtration gave 1·68 g. of mixed chloroaminoacridines. After one crystallisation from chloroform this mixture was fractionally crystallised from water, and from 30% alcohol. After some twenty crystallisations two fractions of constant m. p. were obtained, 6-*chloro-2-aminoacridine* (VI) (0·13 g.), m. p. 179—180°, and 8-*chloro-2-aminoacridine* (VII) (0·06 g.), m. p. 220—221° (mixed m. p. 212° after softening at 170°). 6-Chloro-2-aminoacridine is readily soluble in alcohol, chloroform and benzene and crystallises from hot water or dilute alcohol in yellow needles. The alcoholic solution is yellow with an intense green fluorescence (Found : N, 12·4; Cl, 15·7. $C_{13}H_9N_2Cl$ requires N, 12·3; Cl, 15·5%). The base and hydrogen chloride in dry ether gave a bright red monohydrochloride, soluble in cold water (1 in 500). 8-Chloro-2-aminoacridine is readily soluble in alcohol, chloroform and benzene, but is less soluble in water and in 30% alcohol than the 6-chloro-compound.

It crystallises in yellow needles from dilute alcohol, the alcoholic solution being yellow with an intense green fluorescence (Found : N, 12·4; Cl, 15·7%). The monohydrochloride is bright red and soluble in about 500 parts of cold water.

3′-Chloro-4-nitrodiphenylamine-2-carboxylic acid (4·0 g.) was refluxed with phosphorus oxychloride (40 g.) for 2 hours, the excess of oxychloride removed, and the residue triturated with ice-water and aqueous ammonia. The product was collected immediately, dried at 100°, and extracted with boiling dry benzene; 2·8 g. of chloronitroacridones remained, which were again treated with phosphorus oxychloride. The combined benzene extracts deposited after concentration 1·8 g. of a yellow powder, which was separated by fractional crystallisation from dry benzene into two fractions of constant m. p., 5 : 6-*dichloro*-3-*nitroacridine* (0·55 g.), m. p. 201°, and 5 : 8-*dichloro*-3-*nitroacridine* (0·15 g.) m. p. 223° (mixed m. p. 198—200°).

5 : 6-Dichloro-3-nitroacridine is soluble in benzene and chloroform, insoluble in water, and slightly soluble in alcohol. It crystallises from dry benzene in pale yellow, feathery needles, which show a strong green fluorescence in filtered ultra-violet light (Found : N, 9·85; Cl, 23·8. $C_{13}H_6O_2N_2Cl_2$ requires N, 9·6; Cl, 24·2%). 5 : 8-Dichloro-3-nitroacridine is readily soluble in chloroform and benzene and slightly soluble in alcohol. It is more readily hydrolysed than the 5 : 6-dichloro-isomer, being converted into 8-chloro-3-nitroacridone by crystallisation from undried benzene or by exposure to the atmosphere for 2 days. Crystallised from sodium-dried benzene, the compound forms feathery yellow needles which show a green fluorescence in ultra-violet light (Found : N, 9·5; Cl, 25·2%).

6-*Chloro*-3-*nitroacridone* was produced when 5 : 6-dichloro-3-nitroacridine was heated with boiling hydrochloric acid (2%; 100 ml.) for 2 hours. The insoluble product was collected, dried at 100°, and crystallised from boiling pyridine. Yield, 95%. The golden plates obtained were slightly soluble in sodium hydroxide solution and readily soluble in alcoholic potassium hydroxide to give a cherry-red solution. The compound was insoluble in aqueous acids, but slightly soluble in glacial acetic acid. It did not melt below 300° (Found : N, 10·2; Cl, 12·9. $C_{13}H_7O_3N_2Cl$ requires N, 10·2; Cl, 12·9%).

6-*Chloro*-3-*aminoacridine*.—6-Chloro-3-nitroacridone (0·5 g.), suspended in N/2-sodium hydroxide (50 ml.) at 80°, was reduced with sodium amalgam (2%; 50 g.) as described above. The product crystallised from alcohol (30%) in yellow needles, m. p. 211—212°, readily soluble in alcohol, chloroform and pyridine, slightly soluble in hot water and in ether. Yield, 66%. The alcoholic solution was yellow with an intense green fluorescence (Found : N, 11·9; Cl, 15·7. $C_{13}H_9N_2Cl$ requires N, 12·3; Cl, 15·5%). The base and excess of hydrogen chloride in ether gave a white dihydrochloride, which was converted into the violet monohydrochloride by washing with ether dried over sodium. The monohydrochloride is soluble in about 400 parts of cold water.

8-*Chloro*-3-*aminoacridine*.—The mixture of 6- and 8-chloro-3-nitroacridones obtained in the ring closure of 3′-chloro-4-nitrodiphenylamine-2-carboxylic acid was reduced with sodium amalgam and sodium hydroxide solution to a mixture of chloroaminoacridines, from which 6-chloro-3-aminoacridine and 8-*chloro*-3-*aminoacridine* were separated by fractional crystallisation from 40% alcohol. The latter is less soluble in dilute alcohol than the 6-chloro-isomer and crystallises from 40% alcohol in brown needles, m. p. 267—269°, readily soluble in alcohol and pyridine and slightly soluble in hot water and in ether; the alcoholic solution shows an intense green fluorescence (Found : N, 12·4; Cl, 15·5%). The red monohydrochloride is soluble in about 400 parts of cold water.

*Condensation of* 2 : 4-*Dinitrobenzaldehyde with Chlorobenzene*.—2 : 4-Dinitrobenzaldehyde (5 g.), chlorobenzene (20 ml.), and concentrated sulphuric acid (15 ml.) were shaken together during 36 hours. The lower layer was poured into water, and the yellow flocks produced were collected and dried. The upper layer was mixed with benzene and used to extract the dried flocks (three portions each of 300 ml. of boiling solvent). The benzene was removed under reduced pressure, and the semi-crystalline residue crystallised three times from ethyl acetate (very concentrated solutions being used) and once from absolute alcohol. The product (1 g.) was soluble in alcohol, benzene, and ethyl acetate, dissolved in alcoholic potash with a yellow colour, and crystallised in pale yellow needles, m. p. 215°. Fractional crystallisation from alcohol failed to reveal the presence of an isomer, all fractions having m. p. 215°, and the compound was identified as 4-*nitro*-C-(p-*chlorophenyl*)*anthranil* (VIII) by analysis (Found : N, 10·2; Cl, 13·2. $C_{13}H_7O_3N_2Cl$ requires N, 10·2; Cl, 12·9%) and conversion into 8-chloro-2-nitroacridone.

The benzene-insoluble portion of the product of condensation was treated with 5% sodium hydroxide solution and extracted with boiling water until the extracts were no longer red. A *trace* of insoluble material remaining crystallised from aqueous pyridine and showed the red colour with alcoholic potash characteristic of 8-chloro-2-nitroacridone. When the aqueous alkaline extracts were acidified, yellow flocks were deposited, which were further purified by dissolution in hot aqueous alkali (very dilute), filtration, and precipitation by mineral acid, this being repeated three times. Finally the product was crystallised as its sodium derivative from 5% sodium hydroxide solution. Acidification of the pure sodium derivative with dilute hydrochloric acid gave 8-*chloro*-2-*nitro*-10-*hydroxyacridone* (IX) (0·5 g.), m. p. 200°. This compound showed the properties of an *N*-hydroxyacridone (cf. Kliegl, *Ber.*, 1914, **47**, 1629; Lehmstedt, *ibid.*, 1937, **70**, 172) : it was insoluble in water and most organic solvents, slightly soluble in boiling toluene, from which it could be recrystallised, soluble in dilute aqueous alkali with the production of a red solution, but precipitable from the solution by sodium chloride or excess of alkali (Found : N, 9·8; Cl, 11·9. $C_{13}H_7O_4N_2Cl$ requires N, 9·6; Cl, 12·2%). When 0·1 g. was dissolved in N-alkali (0·5 ml.) and water (25 ml.) and shaken with methyl sulphate (0·5 g.) for 15 minutes,

yellow flocks of 8-*chloro*-2-*nitro*-10-*methoxyacridone* were formed. These, washed free from alkali, crystallised from acetone–water (4 : 1) in golden plates, decomp. 241°, soluble in acetone, alcohol and benzene, insoluble in aqueous alkali (Found : N, 9·4. $C_{14}H_9O_4N_2Cl$ requires N, 9·2%).

*Conversion of* 4-*Nitro*-C-(p-*chlorophenyl*)*anthranil into* 8-*Chloro*-2-*nitroacridone*.—The course of this reaction was followed by observing the m. p. (anthranil, 215°; acridone, above 300°) and the colour reaction with alcoholic potash (anthranil, yellow; acridone, red). Under the conditions described by Bamberger (*Ber.*, 1909, **42**, 1707) for the conversion of anthranils into acridones the reaction was incomplete. For complete conversion the anthranil (0·2 g.), concentrated sulphuric acid (4·0 ml.), and sodium nitrite (0·2 g.) were mixed without precaution against rise of temperature, which reached 40° in the initial stages. After ½ hour the mixture was poured into water, and the insoluble material collected and dried. The product, which did not melt below 300° and gave a cherry-red colour with alcoholic potash, was identified as 8-chloro-2-nitroacridone by conversion into 8-chloro-2-nitro-5-(*p*-diethylaminophenyl)acridine by heating with phosphorus oxychloride and diethylaniline as described by Lehmstedt (*loc. cit.*). This compound had the m. p. (236°) and other properties ascribed to it by Lehmstedt.

When 2 : 4-dinitrobenzaldehyde (5·0 g.), chlorobenzene (20 ml.), and sodium nitrite (0·5 g.) were treated with concentrated sulphuric acid (15 ml.), added in small quantities, the temperature being kept below 40°, and the products worked up after 24 hours as described above, 8-chloro-2-nitroacridone (1·4 g.), 4-nitro-*C*-(*p*-chlorophenyl)anthranil (0·3 g.) and 8-chloro-2-nitro-10-hydroxyacridone (0·2 g.) were obtained.

8-*Chloro*-2-*aminoacridone*.—8-Chloro-2-nitroacridone (1·0 g.), stannous chloride (10 g.), concentrated hydrochloric acid (23 ml.), and alcohol (50 ml.) were refluxed together for 1 hour. The yellow crystals of the tin salt of the base deposited on cooling were collected and triturated with excess of 20% sodium hydroxide solution. The base was washed with water and crystallised from dilute alcohol (50%), forming feathery clusters of cream plates. Yield, 70%. 8-*Chloro*-2-*aminoacridone* does not melt below 300°. It is soluble in alcohol, pyridine and glacial acetic acid and slightly soluble in hot water; the alcoholic solution shows a sky-blue fluorescence (Found : N, 11·5; Cl, 14·2. $C_{13}H_9ON_2Cl$ requires N, 11·5; Cl, 14·5%).

8-Chloro-2-aminoacridone (0·4 g.), alcohol (50 ml.), and sodium bicarbonate (10 g.) were maintained at 60—70°, and sodium amalgam (2%; 40 g.) added during 1½ hours, rapid stirring and a steady stream of carbon dioxide being maintained. The sodium bicarbonate and mercury were removed by filtration, the filter washed with alcohol, and the alcoholic solution returned to the flask, heated to 70°, and stirred vigorously for 1 hour whilst a rapid stream of air was passed through the solution. The alcohol was removed under reduced pressure, the residue extracted with boiling 2% hydrochloric acid, and the solution filtered and made alkaline with ammonia. The precipitate, dried and crystallised twice from dilute alcohol, was identical with 8-chloro-2-aminoacridine obtained from 3′-chloro-5-nitrodiphenylamine-2-carboxylic acid (m. p. and mixed m. p. 220—221°).

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