123. *The Nature of the Amino-group in Aminoacridines. Part II. Evidence from Chemical Reactions.*

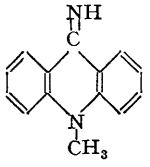
By Adrien Albert and Bruce Ritchie.

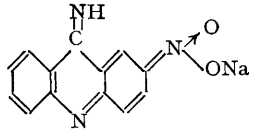
Examination of the chemical reactions of the five monoaminoacridines revealed no correlation as striking as that between ionisation and antisepsis (Part I). Nevertheless, the biologically outstanding isomerides (5-, 2-, and 1-) showed the greatest chemical individuality, particularly the first, which behaved distinctively on diazotisation, alkaline hydrolysis, hydrogenation, reaction with aldehydes and with methyl iodide.

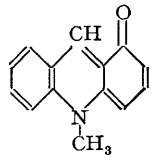
Because of the highly electrophilic nature of the acridine nucleus, the amino-group was readily detached from the salts of all the isomerides by aniline and by acid at 160°.

Reactions with Aldehydes.—When refluxed in alcohol with benzaldehyde, 3- and 4-aminoacridines gave almost quantitative yields of the anils, but the 1- and 2-isomerides reacted only to a small extent, and the 5-isomeride not at all. Salicylaldehyde, which reacts more vigorously with amines, gave a quantitative yield with the 2-isomeride, but the 5- still failed to react. The abnormally low reactivity of 2- and 5-aminoacridines suggests that in alcohol they exist in equilibrium with non-reacting imino-forms (VI*c* and V*c*, Part I). Since 4-amino-2-phenylquinoline reacts with salicylaldehyde (Feist and Kulinski, *Arch. Pharm.*, 1936, **274**, 310), the total lack of activity of 5-aminoacridine is attributed to a summation of this tautomeric effect and a steric hindrance akin to cases noted by Jensen and Rethwisch (*J. Amer. Chem. Soc.*, 1928, **50**, 1144), *viz.*, acridine-5- and 3-methylquinoline-4-carboxylic acids, which, unlike quinoline-4-carboxylic acid, cannot be esterified with alcohol and acid. In its slow reactivity with benzaldehyde, 1-aminoacridine gives further evidence of the " ortho-effect " (Part I).

Action of Methyl Iodide.—When 1-, 2-, 3-, and 4-aminoacridines were refluxed with methyl iodide in methanol (acridine does not methylate under these conditions), only the primary amino-group became methylated. On the other hand, 5-aminoacridine gave an almost quantitative yield of *5-amino-10-methylacridinium iodide*, which may also be regarded as the hydriodide of *5-imino-10-methylacridane* (XI), into which it is reversibly converted on basification. 4-Aminoquinoline and 4-aminopyridine also methylate on the ring nitrogen under these conditions (Tschitschibabin, *Ber.*, 1921, **54**, 822; 1925, **58**, 1708) and it is likely that in all these cases the usually unreactive tertiary ring nitrogen is reacting as a secondary amine, because of tautomerism (V*c*, Part I). The above acridane compound is incapable of tautomerism and sufficiently resembles " 5-amino-acridine " in colour and fluorescences as to suggest that the latter is really 5-iminoacridane (V*c*, Part I). (*5-Dimethylaminoacridine* differs markedly from these substances, but *5-methylaminoacridine* is similar.) Another case of prototropy involving an imino-group in the 5-position is provided by *3-nitro-5-aminoacridine* (red), which gives the purple nitronate (XII) with alcoholic alkali, whereas 2-nitro-5-aminoacridine, in which rearrangement of bonds is impossible, gives no colour change.

(XI.) 

(XII.) 

 (XIII.)

Methylation on the ring nitrogen takes place at 180° with 2-, 3-, 4-, and 5-acetamidoacridines, but the 1-isomeride does not react because of the " ortho-effect."

Diazotisation.—Although 2-, 3-, and 4-aminoacridines diazotised and coupled normally, as did their 10-methobromides, yet 1-aminoacridine formed a precipitate with sodium nitrite and hydrochloric (or acetic) acid and did not couple with β-naphthol. The analogously constituted 8-aminoquinoline, however, diazotised and coupled normally. 5-Aminoacridine and its methobromide did not diazotise by the more usual methods, but highly acid conditions (cf. 4-aminopyridine; Königs, *Ber.*, 1924, **57**, 1175) were effective and an intense red colour was obtained with alkaline β-naphthol. This resistance to diazotisation apparently arises from the high ionic resonance of the monoacid salts of 5-aminoacridine which do not allow of true $-\overset{\oplus}{N}H_3$ groups arising in any quantity.

Hydrogenation.—On hydrogenation (Raney nickel), the aminoacridines all added two atoms of hydrogen but at different rates and always more slowly than acridine itself. As will be seen from the table below, the rate of reduction places the isomerides in the same order as their basicity, and 2 : 5-diaminoacridine, presumably because of its higher basicity ($pK = 10{\cdot}5$), shows marked resistance to hydrogenation. Dr. B. Breyer of this University informs us that aminoacridines require highly negative potentials for polarographic reduction and the reaction is thermodynamically irreversible. 3-Aminoacridane has now been obtained analytically pure and has characteristics different from those assigned to it in the literature.

Total absorption of hydrogen (ml.) at 760 mm. and 20° of 1 g. of acridine compound and 1 g. of Raney nickel catalyst (itself responsible for 10 ml.) in 98% alcohol. (The monoaminoacridines require 120 ml. for 2H; acridine requires 130 ml.)

Substance. Time (mins.).	2.	5.	10.	20.	40.	60.	80.	120.	180.
Acridine	65	125	137	140	140				
1-Aminoacridine	—	65	90	120	132	135	135		
3-Aminoacridine	—	—	55	85	120	127	135	135	
4-Aminoacridine	—	—	55	85	120	127	135	135	
2-Aminoacridine	—	—	—	40	65	85	95	120	135
5-Aminoacridine	—	—	—	—	50	65	75	95	125
2 : 5-Diaminoacridine	—	—	—	—	—	—	5	7	10

Action of Alkali.—When the aminoacridines were boiled with 5N-potassium hydroxide, 5-aminoacridine alone was hydrolysed, evolving ammonia and giving 11% of 5-hydroxy-acridine in 2 hours. Similarly, 5-methylamino- and 5-dimethylamino-acridines gave 24 and 68% yields, respectively. On the other hand, 2-aminoacridine could be fused with moist sodium hydroxide for 2 hours at 200° without decomposition.

Action of Aniline and of Acid.—When the hydrochlorides of the five aminoacridines were refluxed with aniline, or heated with concentrated hydrochloric acid (2 hours at 160—200°), ammonia was split out and the corresponding phenylaminoacridines or hydroxyacridines, respectively, formed. No special significance attaches to the differences in yields obtained, as it was not possible to devise conditions where homogeneity was identical. However, all ten reactions went with a readiness that reveals an attraction exerted by the highly conjugated acridine nucleus on the electrons forming the $\overset{\oplus}{C}$–NH_3 bond in the salts. This electrophilic effect is small in the benzene ring but larger in the naphthalene and the quinoline series (cf. Franzen and Kempf, *Ber.*, 1917, **50**, 101; Claus and Howitz, *J. pr. Chem.*, 1893, **47**, 432; **48**, 177). 2-*Phenylaminoacridine* was thus prepared by a method excluding contamination with the 4-isomeride, and melts 55° higher than Besthorn and Curtman's specimen (*Ber.*, 1891, **24**, 2042).

Experimental.

Reactions with Aldehydes.—The aminoacridine (1 g.) and benzaldehyde (0·6 g. = 1·1 mol., freed from acid and redistilled) or salicylaldehyde (0·7 g., similarly purified) were refluxed in alcohol (15 ml.) for 1½ hours. Only in the case of the 1-isomeride did a deposit form during the reaction. The mixture was made alkaline with sodium carbonate solution, steam-distilled until no more aldehyde passed over, and cooled. The solid was filtered off, dried in a vacuum, and analysed by steam-distilling a portion (1 g.) in the presence of excess dilute sulphuric acid into a known amount of N/2-hydroxylamine hydrochloride, previously made neutral to methyl-orange. The hydrochloric acid liberated by the aldehyde was back-titrated with N/2-sodium hydroxide, with constant shaking. The yield of anil was calculated from these analyses, whereas pure specimens were obtained in duplicate experiments. 1-*Benzylideneaminoacridine* (28% yield), yellow crystals from benzene on addition of light petroleum, m. p. 149° (151° corr.); the yield is greatly increased if one drop of piperidine is used as a catalyst (cf. Glen, Sutherland, and Wilson, J., 1936, 1484) (Found: N, 9·9. $C_{20}H_{14}N_2$ requires N, 9·9%). 2-Benzylideneaminoacridine (20% yield) resisted purification. 2-*Salicylideneaminoacridine* (95% yield), orange crystals from alcohol, m. p. 229° (236° corr.) (Found: N, 9·2. $C_{20}H_{14}ON_2$ requires N, 9·4%). 3-*Benzylideneaminoacridine* (86% yield), yellow crystals from *cyclo*hexane or methanol, m. p. 148° (Found: N, 10·0%). 4-*Benzylideneaminoacridine* (92% yield), orange crystals from 50 parts of alcohol, m. p. 179° (182° corr.) (Found: N, 10·1%).

1-Nitroacridine was prepared (Jensen, *J. Amer. Chem. Soc.*, 1927, **49**, 1049) by treating the black condensate from 2-aminobenzaldehyde and *o*-bromonitrobenzene with sulphuric acid. It was later found that the black condensate could be recrystallised from ether (60% yield) in long, orange needles, m. p. 120°, of 2-*nitrodiphenylamine-2′-aldehyde* (Found: C, 64·6; H, 4·0. $C_{13}H_{10}O_3N_2$ requires C, 64·5; H, 4·2%).

Action of Methyl Iodide.—Acridine (0·9 g.), 1-nitroacridine (1·3 g.), and the five aminoacridines (0·97 g.) were severally refluxed with methyl iodide (2·9 g., 4 equivs.) and methanol (2·9 g.) for 4 hours. The first two were practically unchanged, but 1-, 2-, 3-, and 4-aminoacridines gave uncrystallisable mixtures of partly methylated products that were completely precipitated by ice-cold N/20-sodium carbonate and were hence free from quaternary compounds. On the other hand, 5-aminoacridine deposited 5-amino-10-methylacridinium iodide in 85% yield on cooling, and this, after being washed free from a little 5-dimethylaminoacridine with acetone, was recrystallised from 80 parts of water, giving bright yellow crystals, m. p. *ca.* 305° (decomp.). These were treated with cold, dilute sodium hydroxide, and the precipitate, dried at 130°, was identified as 5-imino-10-methylacridane (see p. 461) of which the m. p. and mixed m. p. were identical with those of a specimen prepared from the methylation of 5-acetamidoacridine (see below). The methiodide or the imine, boiled for an hour with N/20-sodium carbonate, was quantitatively decomposed into ammonia and 10-methylacridone (m. p. and mixed m. p. with authentic specimen, 202°).

Methylation of Acetamidoacridines.—Nitrobenzene (100 ml.) was heated to 180°, and the appropriate acetamidoacridine (10 g.) and methyl *p*-toluenesulphonate (10 g.; 1·25 mols.) added. The mixture was stirred at 180° for ½ hour, cooled, treated with benzene (250 ml.), and filtered after a day. The precipitate, which in no case contained a diazotisable amine, was washed with benzene and heated with hydrobromic acid (60 ml. of 25% HBr) on the water-bath for 2 hours. The hydrolysate was neutralised with sodium hydroxide, chilled well, and the crude amino-10-methylacridinium bromide filtered off, dissolved in water (500 ml.) at 50°, treated with sodium bicarbonate (2 g.), filtered at 50° from tar and unmethylated product, and finally mixed with hydrobromic acid (2·7 ml. of 48% HBr) and well chilled. After separation of the crystals, a second crop was obtained by concentration. 1-Acetamidoacridine and 1-nitroacridine were not methylated by this method, the latter being partly oxidised to 1-nitroacridone. Acridine was methylated quantitatively.

2-*Amino*-10-*methylacridinium bromide* was further purified by dissolution in water (200 ml.) and treatment at room temperature with sodium carbonate (2·5 g.), a tarry impurity being coagulated on charcoal before filtering. The filtrate was acidified with hydrobromic acid, chilled, and the bright scarlet crystals (75% yield) recrystallised from 1·5 parts of water (charcoal) (90% recovery); m. p. 236° (243° corr.; sealed tube). They were soluble in water, giving an orange solution having an intense green fluorescence when dilute (Found: C, 55·3; H, 4·9; N, 9·0; Br, 26·0. $C_{14}H_{13}N_2Br,H_2O$ requires C, 54·7; H, 4·9; N, 9·1; Br, 26·05%). Sodium hydroxide at high temperatures, but not at 0°, gives an immediate precipitate of the carbinol base. The bromide diazotises and couples with β-naphthol to give an intense blue precipitate, soluble in alcohol to a port-wine coloured solution.

3-*Amino*-10-*methylacridinium bromide* was finally recrystallised from water (10 parts) or alcohol (60 parts); yield, 70% of red crystals giving a red, non-fluorescent solution in water (Found: C, 58·3; H, 4·5; N, 9·8. $C_{14}H_{13}N_2Br$ requires C, 58·1; H, 4·5; N, 9·7%). Diazotisation and coupling with β-naphthol gives a port-wine colour. Sodium hydroxide instantly gives a yellow precipitate of the carbinol base which dissolves in ether and regenerates the original substance on acidification. 4-*Amino*-10-*methylacridinium bromide* was finally recrystallised from water (30 parts); yield, 80% of violet crystals, m. p. 267° (sealed tube). It gives a purple, non-fluorescent solution in water, but is only sparingly soluble in alcohol (blue solution) (Found: C, 54·8; H, 4·8; N, 9·3%). Diazotisation and coupling with β-naphthol gave a brownish-red colour. Sodium hydroxide acts as with the 3-isomerides. Both the 3- and the 4-isomeride are dibasic, giving yellow bromide-hydrobromides with excess of hydrobromic acid. The 2- and the 5-isomeride are only monobasic. 5-*Amino*-10-*methylacridinium bromide* was finally recrystallised from boiling water (8 parts); yield, 75% of yellow crystals, m. p. approx. 305° (decomp.). It dissolves in 50 parts of cold water to a yellow solution with a greenish-blue fluorescence that becomes pure blue on dilution (Found: C, 58·2; H, 4·4; N, 9·85%). Sodium hydroxide (but not ice-cold sodium carbonate) instantly liberates the yellow carbinol base, 5-*amino*-5-*hydroxy*-10-*methylacridane*, which is very soluble in cold alcohol with a green fluorescence, becoming blue on dilution (Found, for material dried in a vacuum at 20°: C, 74·6; H, 6·1. $C_{14}H_{14}ON_2$ requires C, 74·3; H, 6·2%). The bright yellow aqueous solution is only faintly alkaline. When boiled with dilute sodium carbonate it evolves ammonia and is entirely

converted into 10-methylacridone in an hour. When dried at 130° for 30 mins., it gives 5-*imino*-10-*methylacridane* (XI), an orange substance, m. p. 134—136° (sealed tube), very soluble in benzene (Found : C, 80·5; H, 5·7. $C_{14}H_{12}N_2$ requires C, 80·8; H, 5·8%). Both the anhydro- and the carbinol-base regenerate the original salt with acid.

5-Chloro-3-nitroacridine (B.P. 441,007) was treated with ammonium carbonate in phenol as described for 5-chloro-2-nitroacridine (Albert and Gledhill, *J. Soc. Chem. Ind.*, 1942, **61**, 159); yield, 93% of 3-*nitro*-5-*aminoacridine hydrochloride*, which was recrystallised from 55 parts of water, forming bright yellow crystals, soluble in 300 parts of cold water without fluorescence (Found : C, 56·8; H, 3·8; N, 15·3. $C_{13}H_9O_2N_3$,HCl requires C, 56·6; H, 3·7; N, 15·2%). The base is bright red and melts at *ca.* 300° (decomp.). It is sparingly soluble in benzene with a green fluorescence that is abolished by a drop of alcohol. The temperature gradients in nitrobenzene and 60% aqueous pyridine are good. Although insoluble in aqueous sodium hydroxide, it gives a purple colour (XII) and green fluorescence in N-alcoholic sodium hydroxide.

When this was reduced with nascent ferrous carbonate (cf. Albert and Gledhill, *loc. cit.*), 3 : 5-*diaminoacridine* was obtained (75% yield) as orange crystals from dilute alcohol, m. p. 229—230° (sealed tube), moderately soluble in boiling water and very soluble in alcohol to a yellow solution with a green fluorescence (Found : C, 74·2; H, 5·4; N, 20·0. $C_{13}H_{11}N_3$ requires C, 74·6; H, 5·3; N, 20·1%). The hydrochloride is very soluble in water to an orange, non-fluorescent solution and is precipitated by alcohol. Excess of concentrated hydrochloric acid produces a colourless solution with a violet fluorescence, as happens with the 2 : 5-isomeride (dihydrochloride formation). The sulphate is sparingly soluble. The salts diazotise and couple with β-naphthol (red).

5-Methylaminoacridine.—5-Phenoxyacridine (6·8 g.; Drozdov, *J. Gen. Chem. U.S.S.R.*, 1935, **5**, 1576), methylamine hydrochloride (2·5 g.), and phenol (30 g.) were heated for ½ hour at 120°, cooled, and treated with ether (50 ml.). The precipitate was dissolved in water (75 ml.) and treated with hydrochloric acid (5 ml.). The hydrochloride that separated on standing (85% yield) was collected and basified, giving 5-*methylaminoacridine*, which was recrystallised quickly (to prevent hydrolysis) from 8 parts of 50% alcohol; canary-yellow crystals, m. p. 173—174° (sealed tube), somewhat soluble in water, soluble in 3·5 parts of alcohol without temperature gradient, and only moderately soluble in benzene or acetone (Found : C, 81·0; H, 6·0. $C_{14}H_{12}N_2$ requires C, 80·8; H, 5·8%). The alcoholic solution has a greenish-blue fluorescence. The hydrochloride is the same shade of yellow as the base and is soluble in 2 parts of boiling and 10 parts of cold water. It shows no fluorescence by daylight, but ultra-violet light shows a green fluorescence that becomes bright violet on dilution. It is not affected by boiling with N-hydrochloric acid for an hour. *5-Dimethylaminoacridine hydrochloride*, similarly prepared from dimethylamine hydrochloride, was purified by dissolving in water (5 ml.) and adding acetone (100 ml.). After standing over-night, the hydrochloride was obtained in 75% yield as orange crystals, m. p. *ca.* 275° (decomp.). It is soluble in one part of water; the solution has no fluorescence by daylight and a blue-green one by ultra-violet light, but only when dilute (Found : N, 11·0. $C_{15}H_{14}N_2$,HCl requires N, 10·8%). When boiled with N/1-hydrochloric acid, it hydrolyses to acridone to the extent of 25% in an hour (5-aminoacridine is perfectly stable under these conditions), and sodium hydroxide acts similarly even in the cold. The base was therefore liberated from the salt with dimethylamine. It was yellow, and the alcoholic solutions did not fluoresce. Its general instability prevented its further examination.

Alkaline Hydrolyses.—The above two amines and 5-aminoacridine (0·1 g.) were separately boiled with potassium hydroxide (10 ml. of 5N, made up in 80% alcohol) and the evolved alkaline vapours were led into hydrochloric acid (25 ml. of N/50). The volume of boiling liquor was kept constant with alcohol, and after 2 hours the acid was back-titrated with N/50-alkali. The contents of the flask were then examined for 5-hydroxyacridine (acridone) which was found in quantities that tallied with the amounts of volatile bases titrated (see p. 460). When similarly treated, 1-, 2-, 3-, and 4-aminoacridines did not hydrolyse. When 5-aminoacridine was treated as above, but with omission of the potassium hydroxide, it still hydrolysed (1·25% per hour, observed over 8 hours). Catalysis is due here to hydroxyl ions derived from the base itself.

Acid Hydrolyses.—The amine (0·5 g.) was heated with concentrated hydrochloric acid (10 ml.) for 2 hours at 160—200° in a sealed tube (several such tubes of 1-aminoacridine exploded). Except in the case of the 5-isomeride, on cooling, the contents were distilled with sodium hydroxide, and the ammonia evolved was determined by titration. The flask residue was then filtered, the filtrate made faintly acid with acetic acid, and the precipitated hydroxyacridines dried and weighed, good correspondence with the ammonia values showing that diacridylamines were not formed; yields, 42, 70, 95, 98, and 10% of 1-, 2-, 3-, 4-, and 5-hydroxyacridines, respectively. 5-Hydroxyacridine was separated from unchanged 5-aminoacridine by extraction with dilute acetic acid in which the former is insoluble.

4-*Hydroxyacridine* was obtained in yellow needles from its blue solution in dilute alcohol, m. p. about 250° (decomp.) (Found : C, 79·5; H, 4·7; N, 7·35. $C_{13}H_9ON$ requires C, 80·0; H, 4·7; N, 7·2%). It gives a green solution in absolute alcohol and a yellow solution in benzene. The blue form is probably similar in constitution to 10-methylacrid-4-one (XIII) which is also blue (Nitzsche, *Angew. Chem.*, 1939, **52**, 517). The sodium salt gives a deep red, the hydrochloride a deep brown, aqueous solution without fluorescence. The m. p. of 2-hydroxyacridine, not previously recorded, was 285° (sealed, uncorr.). The other hydroxyacridines agreed with the properties given in the literature.

Anilinolyses.—The amine hydrochloride (1 g.) and aniline (10 ml.) were refluxed for 2 hours, sodium hydroxide (0·2 g.) added, the liquid steam-distilled, and the distillate extracted with ether. The phenylaminoacridines show little fluorescence either as bases or as salts. 1-*Phenylaminoacridine*, 75% yield, scarlet crystals from alcohol (orange solution), m. p. 187·5° (191° corr.), very soluble in benzene (Found : N, 10·4. $C_{19}H_{14}N_2$ requires N, 10·4%). Being a very weak base it is insoluble in dilute acetic acid, but gives a red solution in dilute hydrochloric acid. 2-*Phenylaminoacridine*, 45% yield, orange-yellow crystals from toluene, m. p. 231° (238° corr.) (Found : C, 84·4; H, 4·9; N, 10·4. $C_{19}H_{14}N_2$ requires C, 84·4; H, 5·2; N, 10·4%). The scarlet hydrochloride was sparingly soluble in cold, more soluble in boiling, water. The acetate was soluble in cold water. 3-*Phenylaminoacridine*, 95% yield, yellow crystals from toluene, m. p. 229° (236° corr.) (Found : N, 10·5%). It gives a purple solution in dilute hydrochloric or acetic acids. 4-*Phenylaminoacridine*, 55% yield, red crystals with a blue glance, m. p. 220° (decomp.) (Found : N, 10·2%). It gives a pure blue solution in dilute acids and forms a blue sodium salt in alcoholic sodium hydroxide. 5-*Phenylaminoacridine*, 95% yield, orange-yellow crystals from alcohol, m. p. 224° (230·5° corr.) (Found : 84·1; H, 5·2; N, 10·5%). The hydrochloride is orange and sparingly soluble in water, the acetate is more soluble. 5-Aminoacridine *base* treated with aniline as shown gave only a 15% yield.

Hydrogenations.—These were carried out in the apparatus described by the authors (*Proc. Roy. Soc. N.S.W.*, 1941, **74**, 373). 1- and 2-Aminoacridanes agreed with the properties assigned to them by Clemo, Perkin, and Robinson (J., 1924, **125**, 1784) and Scherlin, Bras, Jakubowitsch, Worobjowa, and Ssergejew (*Annalen*, 1935, **516**, 218), respectively. The latter authors prepared 3-aminoacridane (" 2-aminodihydroacridine " in their numbering) by the action of sodium on 3-nitroacridone. Their product is described as bright yellow, m. p. 191—192° (introduced at 190°), and soluble with a dark red colour in dilute hydrochloric acid; they analysed it only for nitrogen (14·34%). Our product consists of pale yellow plates from alcohol, m. p. 184—185° (sealed tube; 187—188° corr.), and soluble without colour in acids. The m. p. is independent of the temperature at which the specimen is introduced (Found : C, 79·4; H, 6·1; N, 14·1. 3-Aminoacridane, $C_{13}H_{12}N_2$, requires C, 79·5; H, 6·2; N, 14·28%. 3-Aminoacridine, $C_{13}H_{10}N_2$, requires C, 80·4; H,

5·2; N, 14·43%). When exposed to the air for a month, the surfaces of our crystals gave a red colour with hydrochloric acid and the m. p. of the sample had fallen to 180°. It would appear that the Russian authors' specimen was mainly 3-aminoacridine.

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