90. *The Preparation and Therapeutic Properties of Certain Acridine Derivatives. Part IV. 5-Methylacridines, Further 5-Styrylacridines and their Quaternary Salts.*

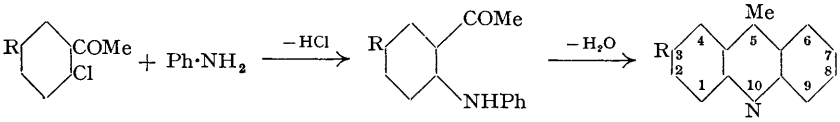
By W. Sharp, (Miss) M. M. J. Sutherland, and F. J. Wilson.

[With a Note on Antiseptic and Trypanocidal Action by C. H. Browning and (Miss) K. M. Calver.*]

This paper deals chiefly with those 5-styrylacridines containing amino-groups in both the styryl and the acridine portion of the molecule, together with certain amino-5-methylacridines. The therapeutic properties of these compounds, their quaternary salts, and the compounds described in Part III are now reported.

In Part III (this vol., p. 5) we described 5-styrylacridines and their quaternary salts containing substituents, amino- or dimethylamino-groups, in the styryl portion only. In view of the known effects of amino-groups in the acridine portion, we decided to introduce such groups into 5-methylacridine itself and into the 5-styryl derivatives, and to enhance the activity of such compounds by conversion into quaternary salts.

Jensen and Rethwisch (*J. Amer. Chem. Soc.*, 1928, **50**, 1144) prepared 4-nitro-2-acetyldiphenylamine (I, R = NO_2) from 2-chloro-5-nitroacetophenone and aniline and thence by ring closure 3-nitro-5-methylacridine (II, R = NO_2):



(I.) (II.)

This reaction, which we have improved, seemed to offer good possibilities, since substituted anilines could be used so that one ring would contain a nitro-group or, after reduction, an amino-group in position 3 and another substituent, such as an amino-group, would be present in the other ring. Albert and Linnell showed (J., 1938, 22) that an amino-group in position 3 results in a compound only slightly less active and much less toxic than one with the amino-group in position 2, which causes marked antiseptic properties.

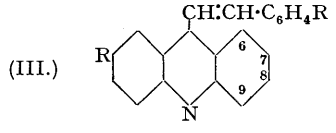
In preparing (II, R = NO_2) intermediate isolation of the diphenylamine derivative was necessary, direct condensation of the ketone with aniline to an acridine derivative proving unsuitable (compare Ullmann and Ernst, *Ber.*, 1906, **39**, 298). The intermediate diphenylamine derivatives, such as (I), do not fluoresce, but the acridine derivatives, such as (II), fluoresce in chloroform or benzene solution in ultra-violet light.

Reduction of 3-nitro-5-methylacridine with stannous chloride and hydrochloric acid gave 3-amino-5-methylacridine (II, R = NH_2), which was converted into the crystalline stable *acetyl* derivative (II, R = NHAc), and then through the *metho-p-toluenesulphonate* (II, R = NHAc, + $C_6H_4Me\cdot SO_3Me$) into *3-amino-5-methylacridine methochloride*. An ethereal solution of 3-amino-5-methylacridine showed bright green fluorescence in daylight, a phenomenon not exhibited by the corresponding nitro- or acetamido-compound, which, however, gave a bright green fluorescence in glacial acetic acid in daylight. These characteristics have been observed generally with the amino- and acetamido-compounds described in this paper.

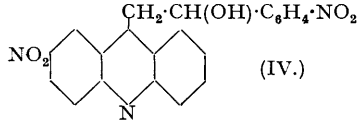
Jensen and Rethwisch state that the first stage of the reaction between 2-chloro-5-nitroacetophenone and *o*- or *p*-nitroaniline occurs, but give no details. We found, however, that, whereas the reaction with *o*-, *m*- or *p*-nitroaniline gave tarry products only, reaction ensued smoothly with *p*-aminoacetanilide, giving ultimately *3-nitro-7-amino-5-methylacridine*, the acetyl group being removed by hydrolysis during the working up. On reduction this gave 3 : 7-diamino-5-methylacridine, which was converted into the *diacetyl* derivative; the latter was converted through the metho-*p*-toluenesulphonate into 3 : 7-*diamino-5-methylacridine methochloride* (II; R = NH_2, NH_2 at 7, + MeCl). A reaction between 2-chloro-5-nitroacetophenone and *m*-aminoacetanilide was also carried out, but ambiguity as to orientation arises in this case. The reaction proceeded similarly, giving ultimately probably 3-nitro-6 (or 8)-amino-5-methylacridine (or both); this on reduction yielded 3 : 6 (or 3 : 8)-diamino-5-methylacridine (or both), which, being only moderately antiseptic, was not further investigated.

2-Chloro-5-nitroacetophenone reacted with *p*-toluidine as with aniline; the resulting *3-nitro-5 : 7-dimethylacridine* (II; R = NO_2, Me at 7) on reduction gave 3-amino-5 : 7-dimethylacridine, which was converted through the *acetyl* derivative into *3-amino-5 : 7-dimethylacridine methochloride* (II; R = NH_2, Me at 7, + MeCl).

The condensation of 3-nitro-5-methylacridine with nitrobenzaldehydes was next attempted so as to obtain 5-nitrostyryl derivatives of type (III, R = NO_2) and thence by reduction diamino-compounds. When 3-



(III.)



(IV.)

* Working with the support of the Medical Research Council at the Bacteriology and Pathology Department of the University and Western Infirmary, Glasgow.

nitro-5-methylacridine was heated with *o*- or *m*-nitrobenzaldehyde alone, there resulted α-(o-*nitrophenyl*)-β-5-(3-*nitroacridyl*)*ethanol* or α-(m-*nitrophenyl*)-β-5-(3-*nitroacridyl*)*ethanol* (IV), which compounds, in view of previous experience, were not further investigated, especially as the *p*-aminostyrylacridine, on analogy with the *p*-aminostyrylquinolines, might be expected to be of more therapeutic interest. Heated in acetic anhydride solution, 3-nitro-5-methylacridine and *p*-nitrobenzaldehyde gave 3-*nitro*-5-p-*nitrostyrylacridine* (III, R = NO_2), which was converted by reduction into 3-amino-5-*p*-aminostyrylacridine, and thence through the *diacetyl* derivative into 3-*amino*-5-p-*aminostyrylacridine methochloride*. In precisely the same way, *p*-nitrobenzaldehyde and 3-nitro-5 : 7-dimethylacridine gave 3-*nitro*-5-p-*nitrostyryl*-7-*methylacridine*, 3-amino-5-*p*-aminostyryl-7-methylacridine, the *diacetyl* derivative, and finally 3-*amino*-5-p-*aminostyryl*-7-*methylacridine methochloride* (III; R = NH_2, Me at 7, + MeCl).

The *acetyl* derivative of 3-nitro-7-amino-5-methylacridine, on heating with *p*-nitrobenzaldehyde in acetic anhydride, gave 3-*nitro*-7-*acetamido*-5-p-*nitrostyrylacridine*, which on reduction and hydrolysis gave 3 : 7-diamino-5-*p*-aminostyrylacridine. This was converted through the *triacetyl* derivative into 3 : 7-*diamino*-5-p-*aminostyrylacridine methochloride* (III; R = NH_2, NH_2 at 7, + MeCl).

Antiseptic and Trypanocidal Action. [By C. H. BROWNING and K. M. CALVER].—The results of the examination of the compounds described in Part III and in the present paper are summarised below.

Biological properties were investigated by methods previously described (*Brit. J. Exp. Path.*, 1921, **2**, 95; *Proc. Roy. Soc.*, 1929, *B*, **105**, 99). The compounds in (*A*) and (*B*) were tested as hydrochlorides; those in (*C*), being already methochlorides, were water-soluble.

(*A*) 5-*Methylacridines*. 5-Methylacridine, owing to its limited solubility, could not be examined satisfactorily for antiseptic properties; the introduction of an amino-group in position 3 yielded a substance slightly antiseptic against staphylococcus in dilute peptone water (on account of poor solubility, however, the compound was partly dispersed). 3 : 7-Diamino-5-methylacridine was more antispetic, both staphylococcus and *B. coli* being sterilised by a concentration of 1 : 10,000—1 : 20,000. The increased antiseptic properties were lost, however, when the 7-position was occupied by a methyl group instead of an amino-group. Of the amino-5-methylacridines examined, the one showing strong antiseptic properties approaching those of acriflavine and proflavine was 3 : 6 (*or* 3 : 8)-diamino-5-methylacridine; the increase in antiseptic properties indicates that it probably contains the 3 : 8-derivative (see also Albert, Francis, Garrod, and Linnell, *Brit. J. Exp. Path.*, 1938, **19**, 41). None of these substances showed trypanocidal action, and none showed marked toxicity for mice on subcutaneous injection, a dose of 5 mg. of each being tolerated by a 20-g. mouse.

(*B*) 5-*Aminostyrylacridines*. 5-*p*-Amino-, -*m*-amino-, and -*p*-dimethylamino-styrylacridines all showed poor antiseptic properties, a concentration of 1 : 10,000 having no action and the position of and substitution in the amino-group apparently having no marked effect. Trypanocidal activity was not developed in any of these compounds and they were as little toxic as those above-named.

Combinations of these two structures, namely, an amino-group in the styryl part and amino-groups in the acridine part, again showed little or no antiseptic properties. Comparison of 3 : 7-diamino-5-*p*-aminostyrylacridine with 3 : 7-diamino-5-methylacridine indicates that the *p*-aminostyryl group in position 5 depresses antiseptic action compared with the methyl group in the same position, even allowing for the relative insolubility of the former in both peptone water and serum. Also the substances were little toxic (10 mg. being tolerated by a 20-g. mouse) and showed no trypanocidal activity.

(*C*) *Influence of quaternary salt formation*. (a) *On* 5-*methylacridine derivatives*. 5-Methylacridine methochloride had considerable antiseptic action, especially for staphylococcus in peptone water (sterilisation occurring at a dilution of 1 : 40,000); although little toxic for the mouse, a dose of 5 mg. had no trypanocidal action.

3-Amino-5-methylacridine methochloride showed marked increase in antiseptic properties as compared with both 3-amino-5-methylacridine hydrochloride and 5-methylacridine methochloride, but no trypanocidal activity. 3 : 7-Diamino-5-methylacridine methochloride also showed increase in antiseptic properties (cf. 3 : 7-diamino-5-methylacridine) and trypanocidal activity appeared. Replacement of the 7-amino-group by methyl did not notably affect antiseptic properties, but the trypanocidal action disappeared, although toxicity was higher in the case of 3 : 7-diamino-5-methylacridine methochloride—maximum tolerated dose, 2 mg. for a 20-g. mouse—whereas with 3-amino-5-methylacridine methochloride and 3-amino-5 : 7-dimethylacridine methochloride 4 mg. were borne.

(b) *On* 5-*styryl compounds*. 5-*p*-Aminostyrylacridine methochloride showed increase in antiseptic properties as compared with 5-*p*-aminostyrylacridine hydrochloride, but this was very little evident in the case of the *m*-amino-derivative. There was, however, an increase, especially for staphylococcus in peptone water, in the case of 5-*p*-dimethylaminostyrylacridine methochloride. 5-*p*-Aminostyrylacridine methochloride showed slight trypanocidal activity, but 5-*m*-aminostyrylacridine methochloride showed none. 5-*p*-Dimethylaminostyrylacridine methochloride also had no trypanocidal properties, but was much more toxic—the tolerated dose for a 20-g. mouse being 0·1 mg. as compared with 2—3 mg. of the other two.

(c) *On amino*-5-*aminostyrylacridines*. In every case the quaternary compound was a much more potent antiseptic than the corresponding non-quaternary base. Also, whereas the latter lacked trypanocidal action, the methochlorides had some effect, although 3-amino-5-*p*-aminostyrylacridine methochloride and 3-amino-5-*p*-aminostyryl-7-methylacridine methochloride were somewhat more toxic. The introduction of amino-groups at position 3 or positions 3 and 7 into 5-*p*-aminostyrylacridine methochloride hydrochloride had no great effect on antiseptic or trypanocidal action or toxicity.

The work will be continued. It is proposed to attempt to prepare 2-amino- and 2 : 8-diamino-derivatives of methyl- and styryl-acridines and to investigate their therapeutic properties.

Experimental.

o-Chloroacetophenone was prepared by a modification of Thorp and Brunskill's method (*J. Amer. Chem. Soc.*, 1915, **37**, 1258). The mixture of sodium ethoxide, *o*-chlorobenzoyl chloride, and ethyl acetoacetate was kept at 0—5° for 3—4 days instead of 24 hours and the sodio-derivative of ethyl *o*-chlorobenzoylacetoacetate, thus obtained in increased yield, was heated with ammonia and ammonium chloride for 6 hours instead of 2—3 hours. Since the ester itself separated as a heavy oil, extraction with ether was omitted and the ester was washed with a little dilute sulphuric acid and used as such without drying. The overall yield of the ketone from *o*-chlorobenzoyl chloride was 54%; it boiled at 228—229°/758 mm. (lit., 227—228°/738 mm.). It was converted, as described by Thorp and Brunskill, into 2-chloro-5-nitroacetophenone, which was converted into 3-nitro-5-methylacridine by the two-stage method of Jensen and Rethwisch (*loc. cit.*) with modifications. 7 G. of 2-chloro-5-nitroacetophenone were dissolved in 7 c.c. of freshly distilled aniline in the warm, 7 g. of anhydrous potassium carbonate added, and the whole heated with stirring at 125° (± 5°) for 6 hours. While still warm, the mass was stirred with 20 c.c. of alcohol and kept overnight. The yellow solid was collected, washed with a little cold alcohol, then water, allowed to dry in air, and recrystallised from alcohol (carbon); yield, 73% of 4-nitro-2-acetyldiphenylamine, m. p. 130° (lit., 125°) (Found: C, 65·7; H, 4·7; N, 10·9. Calc.: C, 65·6; H, 4·7; N, 10·9%). Ring closure was effected with concentrated sulphuric acid (5 g. of substance, 50 c.c. of glacial acetic acid, 2·5 c.c. of sulphuric acid, 2 hours at 130°), the mixture poured into water, and the acridine precipitated with ammonia; yield 79%. The resulting 3-nitro-5-methylacridine (Found: C, 70·5; H, 4·2; N, 11·5. Calc.: C, 70·6; H, 4·2; N, 11·7%) was best recrystallised from benzene. A chloroform solution showed a brilliant yellowish-green fluorescence in ultra-violet light, but not in daylight, instantly destroyed by a drop of aniline.

3-*Amino*-5-*methylacridine*.—This was prepared as for 3-amino-5-phenylacridine (Ullmann and Ernst, *loc. cit.*), 1 g. of 3-nitro-5-methylacridine being used. The hydrochloride of the base did not, however, separate from the solution after removal of tin hydroxide. A slight excess of ammonia was therefore added, and the yellow precipitate of the free base extracted with ether. After washing with water and evaporation, the substance remained as a reddish-brown solid which could not be crystallised satisfactorily. The best procedure, which, however, did not give a pure product, was to dissolve the substance in warm alcohol, add an equal volume of warm water, boil for a few minutes (carbon), and allow the alcohol to evaporate slowly from the filtrate on the water-bath. At a certain concentration the base separated in reddish-brown crystalline tufts, which were washed with aqueous alcohol and allowed to dry; yield, 84%. It was contaminated with resinous matter and had m. p. about 200° (decomp.). The amine was soluble in the common solvents, except water, and sparingly soluble in benzene and light petroleum. The *acetyl* derivative, prepared with acetic anhydride and anhydrous sodium acetate, separated from aqueous alcohol (carbon) as a pale yellow, crystalline powder (yield, 87%), decomposing at about 260° but remaining unmelted at 360° (Found: C, 76·8; H, 5·5; N, 11·1. $C_{16}H_{14}ON_2$ requires C, 76·8; H, 5·6; N, 11·2%). It was soluble in alcohol and in glacial acetic acid, sparingly soluble or insoluble in other common solvents, and soluble in dilute hydrochloric acid.

3-*Acetamido*-5-*methylacridine metho*-p-*toluenesulphonate*, prepared from this acetyl derivative (1 g.) by heating with methyl *p*-toluenesulphonate (1 mol.) at 145° for 2 hours with occasional stirring and crystallised first from alcohol and then by precipitation of an alcoholic solution with dry ether, formed yellow-brown crystals (0·7 g.), m. p. 226° (Found: N, 6·5; S, 7·4. $C_{24}H_{24}O_4N_2S$ requires N, 6·4; S, 7·3%), easily soluble in alcohol and in water with intense greenish fluorescence. The acridinium base was precipitated as a blue-green solid on addition of ammonia to the aqueous solution. 2 G. of this salt in 3·5 c.c. of water were refluxed for 1 hour with an equal volume of concentrated hydrochloric acid, the solution evaporated to dryness on the water-bath, the residue dissolved in water, and the filtered, deep red solution exactly neutralised with dilute potassium carbonate solution. 3 G. of potassium iodide were added with shaking, a purplish-red precipitate (1 g.) of 3-amino-5-methylacridine methiodide being formed, easily soluble in alcohol, sparingly soluble in water. This precipitate was boiled with excess of freshly precipitated silver chloride in aqueous methyl alcohol for 8 hours; the deep red filtrate and washings on evaporation on the water-bath left 0·7 g. of the dark violet-red, crystalline 3-*amino*-5-*methylacridine methochloride*, which could be recrystallised from alcohol–ether (Found: N, 10·6; Cl, 13·6. $C_{15}H_{15}N_2Cl$ requires N, 10·8; Cl, 13·7%). It was slightly hygroscopic, gave a deep red solution in water, and melted above 200° (decomp.).

3-*Nitro*-7-*amino*-5-*methylacridine*.—2-Chloro-5-nitroacetophenone, *p*-aminoacetanilide, and anhydrous potassium carbonate (5 g. of each) were heated at 125° for 3 hours. The powdered brown product, after washing with water, then alcohol, gave on recrystallisation from alcohol (carbon) golden-yellow plates of 4-*nitro*-4′-*acetamido*-2-*acetyldiphenylamine*, m. p. 207° after reddening at about 120° (Found: C, 61·3; H, 4·9; N, 13·2. $C_{16}H_{15}O_4N_3$ requires C, 61·3; H, 4·8; N, 13·4%); yield, 72%. It was soluble in warm alcohol, chloroform, acetone, and glacial acetic acid, but sparingly soluble in other common solvents. To effect ring closure, 5 g. of the substance in 50 c.c. of glacial acetic acid and 2·5 c.c. of concentrated sulphuric acid were heated under reflux at 125° for 2½ hours; the mixture was poured into water containing a slight excess of ammonia, and the washed and dried dark red precipitate recrystallised twice from benzene. The resulting 3-*nitro*-7-*amino*-5-*methylacridine* was a brick-red crystalline powder, decomposing at about 270°, but still unmelted at 380° (Found: C, 66·0; H, 4·2; N, 16·4. $C_{14}H_{11}O_2N_3$ requires C, 66·4; H, 4·3; N, 16·6%); yield, 94%. It was soluble in acetone and in acids, but insoluble or sparingly soluble in other solvents. The *acetyl* derivative separated from aqueous alcohol (carbon) as a yellow crystalline powder, darkening at about 280°, but unmelted at 360° (Found: C, 65·7; H, 4·5; N, 14·2. $C_{16}H_{13}O_3N_3$ requires C, 65·1; H, 4·4; N, 14·2%); it was moderately soluble in warm alcohol and was easily deacetylated by boiling with concentrated hydrochloric acid.

3 : 7-*Diamino*-5-*methylacridine*.—The preceding nitro-amine (2 g.) was reduced in the cold by means of the anhydrous stannous chloride reagent (Albert and Linnell, J., 1936, 1614). To the deep red aqueous solution of the greenish-brown stannichloride, excess of sodium hydroxide solution was added and the brown precipitate of the diamine was collected, washed with water, and allowed to dry; yield, 1·2 g. It did not crystallise satisfactorily from solvents, and sintered at about 200° (decomp.). It was converted in good yield into the *diacetyl* derivative, a substance still unmelted at 360° and crystallising from aqueous alcohol (carbon) as a yellow-brown powder (Found: C, 71·0; H, 5·4; N, 13·7. $C_{18}H_{17}O_2N_3$ requires C, 70·4; H, 5·5; N, 13·7%). In general properties it resembled 3-acetamido-5-methylacridine. This diacetyl derivative was converted through the metho-*p*-toluenesulphonate, exactly by the same procedure as before with similar yield, into 3 : 7-*diamino*-5-*methylacridine methochloride*, which separated from alcohol–ether as a reddish-brown, crystalline, hygroscopic powder, decomp. about 200°, easily soluble in water with a deep reddish-brown colour (Found: N, 15·4; Cl, 13·1. $C_{15}H_{16}N_3Cl$ requires N, 15·3; Cl, 13·0%).

4-*Nitro*-3′-*acetamido*-2-*acetyldiphenylamine* was prepared in 62% yield in exactly the same way as the 4′-acetamido-compound described above, *m*-aminoacetanilide being used, and possessed similar properties; it formed yellow crystals, m. p. 229° (Found: C, 61·6; H, 5·0; N, 13·2. $C_{16}H_{15}O_4N_3$ requires C, 61·3; H, 4·8; N, 13·4%).

4-Nitro-2-acetyl-4'-methyldiphenylamine.—This was prepared in the same way as 4-nitro-2-acetyldiphenylamine, which it closely resembled, *p*-toluidine being used instead of aniline and heating continued only for 3 hours; yield, 77% (Found: C, 66·8; H, 5·4; N, 10·2. $C_{15}H_{14}O_3N_2$ requires C, 66·6; H, 5·2; N, 10·4%). It formed yellow crystals, m. p. 132°. From this compound, *3-nitro-5 : 7-dimethylacridine* was prepared by ring closure as before (heating at 125° for 2½ hours; crystallisation from benzene). The yellow crystals decomposed at about 235° but remained unmelted at 360°; yield, 90% (Found: C, 72·0; H, 4·9; N, 11·0. $C_{15}H_{12}O_2N_2$ requires C, 71·4; H, 4·8; N, 11·1%). The substance dissolved in dilute hydrochloric acid, was easily soluble in chloroform, in which it fluoresced bright yellow-green in ultra-violet light but only feebly in daylight, and soluble in hot alcohol, benzene, acetone, and glacial acetic acid. From it, 3-amino-5 : 7-dimethylacridine was prepared by reduction with the anhydrous stannous chloride reagent as before; the amine precipitated from the solution of the stannichloride by excess of sodium hydroxide was extracted with ether. Evaporation of the ether left a brown solid, which was purified to a certain extent by recrystallisation from aqueous alcohol (carbon) but could not be obtained pure; yield, 80%. The substance decomposed at about 170° and in general properties resembled 3-amino-5-methylacridine. Acetylation gave *3-acetamido-5 : 7-dimethylacridine* (yield, 84%), which separated from aqueous alcohol (carbon) as a pale yellow, crystalline powder, decomposing at about 250° but still unmelted at 360°; in general properties it resembled 3-acetamido-5-methylacridine (Found: C, 77·0; H, 6·3; N, 10·7. $C_{17}H_{16}ON_2$ requires C, 77·3; H, 6·1; N, 10·6%). From it was obtained, in the usual manner, *3-amino-5 : 7-dimethylacridine methochloride*, which formed, from absolute alcohol and ether, reddish-brown crystals decomposing above 200°. It was slightly hygroscopic and gave a deep red solution in water (Found: N, 10·4; Cl, 12·9. $C_{16}H_{17}N_2Cl$ requires N, 10·3; Cl, 13·0%).

α-(o-Nitrophenyl)-β-5-(3-nitroacridyl)ethanol, obtained by heating 1·19 g. of 3-nitro-5-methylacridine and 0·76 g. of *o*-nitrobenzaldehyde at 100° for 6 hours, washing the powdered product with alcohol, pyridine, then alcohol, and recrystallising it from benzene, formed small yellow crystals (1 g.), decomposing at 170° but still unmelted at 360°. It was only sparingly soluble in dilute or concentrated hydrochloric acid and the common organic solvents (Found: C, 65·0; H, 3·8; N, 10·9. $C_{21}H_{15}O_5N_3$ requires C, 64·8; H, 3·9; N, 10·8%).

α-(m-Nitrophenyl-β-5-(3-nitroacridyl)ethanol, prepared in the same way, separated from alcohol–pyridine in yellow crystals (1 g.), decomp. 175°, and closely resembled the preceding compound (Found: C, 65·0; H, 3·9; N, 10·8%).

3-Nitro-5-p-nitrostyrylacridine.—3-Nitro-5-methylacridine (2·38 g.) and *p*-nitrobenzaldehyde (1·5 g.), which, when heated alone or with zinc chloride, did not seem to react or else gave tar, were refluxed in 6 c.c. of acetic anhydride at 130° for 3 hours. The cold solid product was collected, washed with a little warm alcohol, and recrystallised from pyridine. It formed yellow crystals (1·7 g.) darkening above 250° but still unmelted at 360° and was sparingly soluble in the common solvents (Found: C, 68·2; H, 3·6; N, 11·2. $C_{21}H_{13}O_4N_3$ requires C, 67·9; H, 3·5; N, 11·3%). 3-Amino-5-*p*-aminostyrylacridine, prepared from the above compound (1·5 g.) by reduction with the anhydrous stannous chloride reagent as before, separated from the violet-red aqueous solution of the stannichloride, on addition of excess of sodium hydroxide solution, as a brown precipitate (1·2 g. after washing and drying). It could not be crystallised satisfactorily from solvents, decomposed above 200°, and in solubility resembled 3-amino-5-methylacridine. It (1 g.) was converted into the *diacetyl* derivative, which separated from aqueous alcohol (carbon) as a yellow-brown crystalline powder (1 g.), unmelted at 360° (Found: C, 76·5; H, 5·2; N, 10·5. $C_{25}H_{21}O_2N_3$ requires C, 76·0; H, 5·3; N, 10·6%). In general solubility it resembled 3-acetamido-5-methylacridine. It was converted as usual into *3-amino-5-p-aminostyrylacridine methochloride*, the intermediate metho-*p*-toluenesulphonate being obtained by using 2½ mols. of methyl *p*-toluenesulphonate. This methochloride separated from alcohol–ether as a dark purplish, crystalline powder decomposing at about 250° and easily soluble in water to a violet-red solution; yield, 0·3 g. from 1 g. of the above diacetyl derivative (Found: N, 11·4; Cl, 9·8. $C_{22}H_{20}N_3Cl$ requires N, 11·6; Cl, 9·8%).

3-Nitro-5-p-nitrostyryl-7-methylacridine was prepared as above and in similar yield, from 3-nitro-5 : 7-dimethylacridine and *p*-nitrobenzaldehyde. Although 2 mols. of *p*-nitrobenzaldehyde were used, only one reacted, evidently, as expected, with the 5-methyl group only. The substance crystallised from pyridine as a brownish-yellow powder, unmelted at 360° and sparingly soluble in the common solvents (Found: C, 68·3; H, 3·8; N, 10·9. $C_{22}H_{15}O_4N_3$ requires C, 68·6; H, 3·9; N, 10·9%). Reduction of 2 g. of this substance by the anhydrous stannous chloride reagent gave 1·5 g. of 3-amino-5-*p*-aminosytryl-7-methylacridine. Like 3-amino-5-methylacridine, which it resembled in solubility, it could not be crystallised satisfactorily, forming a yellow-brown powder, decomp. about 200°. It was converted into the *diacetyl* derivative, which separated from aqueous alcohol (carbon) as a yellow-brown powder, unmelted at 360°, resembling 3-acetamido-5-methylacridine in yield and solubility (Found: C, 76·5; H, 5·5; N, 10·2. $C_{26}H_{23}O_2N_3$ requires C, 76·2; H, 5·6; N, 10·3%). It (1 g.) was converted in the usual way into *3-amino-5-p-aminostyryl-7-methylacridine methochloride* (0·35 g.); this closely resembled the preceding methochloride in properties, decomposed above 250°, was slightly hygroscopic, and was easily soluble in water to a dark red solution (Found: N, 11·0; Cl, 9·5. $C_{23}H_{22}N_3Cl$ requires N, 11·2; Cl, 9·4%).

3-Nitro-7-acetamido-5-p-nitrostyrylacridine was prepared by heating 1·48 g. of 3-nitro-7-acetamido-5-methylacridine with 0·76 g. of *p*-nitrobenzaldehyde in 4 c.c. of acetic anhydride according to previous procedure. It separated from pyridine as a yellow-brown powder (0·95 g.), darkening above 250° but still unmelted at 360° (Found: C, 64·0; H, 3·6; N, 13·1. $C_{23}H_{16}O_5N_4$ requires C, 64·5; H, 3·7; N, 13·1%), and sparingly soluble in the common solvents. This substance (1 g.) was reduced and the base liberated, in the manner already described, was heated for 1 hour with 15% hydrochloric acid at 100° to complete deacetylation. From this solution 3 : 7-diamino-5-*p*-aminostyrylacridine was precipitated as a brown, somewhat resinous solid (0·7 g.), decomposing at about 180°, and in general solubility resembling 3-amino-5-*p*-aminostyrylacridine. It could not be crystallised satisfactorily and was (1 g.) in the usual manner converted by acetylation into 3 : 7-*bis*(*acetamido*)-5-p-*acetamidostyrylacridine* (0·9 g.), a yellow-brown crystalline powder from aqueous alcohol (carbon), unmelted at 360° and resembling 3-acetamido-5-methylacridine in general solubility (Found: C, 71·4; H, 5·4; N, 12·3. $C_{27}H_{24}O_3N_4$ requires C, 71·6; H, 5·3; N, 12·4%). It was converted into 3 : 7-*diamino-5-p-aminostyrylacridine methochloride*, the intermediate metho-*p*-toluenesulphonate being obtained by using 2½ mols. of the ester; the methiodide was a dark purplish solid slightly soluble in water, easily soluble in alcohol. The methochloride separated from alcohol–ether as a dark brown powder, decomposing at about 200° and readily soluble in water to a deep reddish-brown solution (Found: N, 15·0; Cl, 9·3. $C_{22}H_{21}N_4Cl$ requires N, 14·9; Cl, 9·4%); yield, 0·3 g. from 1 g. of the triacetyl derivative above.

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