

78. *Researches in the Phenanthridine Series. Part VI. The Relationship between Structure and Trypanocidal Properties.**

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(with a Note by C. H. BROWNING, K. M. CALVER, and M. W. LECKIE).

Certain amino-substituted quaternary salts of this series possess trypanocidal properties, and the effect of variation in number and position of the substituents has been investigated. An improvement has been effected

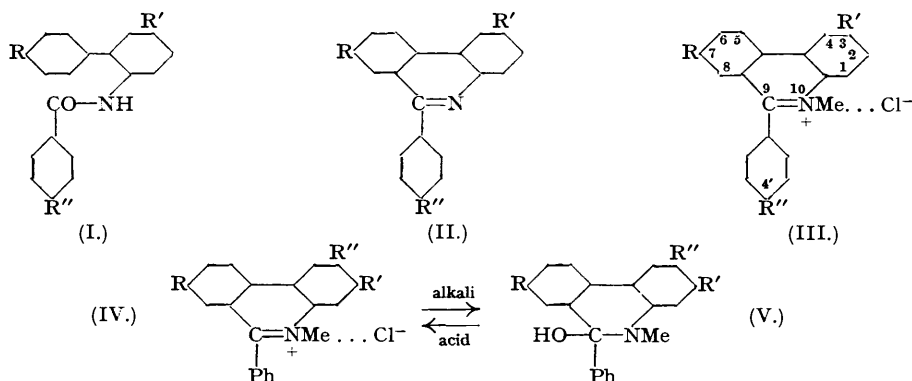
* Much of the work described in this paper forms the subject of B.P.P. 511,353 and 520,273 and recent provisional specifications.

in the method of ring closure of acyl-*o*-xenylamines (I→II), and it has been found that the facile preparation of nitro-quaternary salts such as 2 : 7-dinitro-9-phenyl-10-methylphenanthridinium chloride (IV; R = R' = NO₂, R'' = H), followed by reduction, offers an alternative and generally preferable route to the trypanocidal types. In order to obtain a similar bromo-substituted compound it has been shown that bromination of 4'-nitro-2-acetamidodiphenyl occurs as expected in the 5-position.

Activity against *T. congolense* is well maintained in all the diamino-quaternary salts except when both amino-substituents are in the 9-phenyl group. Pronounced action against *T. brucei* is only found in (IV; R = R' = NH₂, R'' = H), where the amino-groups are in the "benzidine" position. Acetylation of the amino-groups results always in diminution in activity.

THE discovery that 7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (III; R = R' = NH₂, R' = H), referred to as No. 897, exerts a curative action in *T. congolense* infections in mice and cattle (Browning, Morgan, Robb, and Walls, *J. Path. Bact.*, 1938, **46**, 203; Browning, Browning, and Robb, *ibid.*, 1940, **50**, 371; Hornby, Evans, and Wilde, *J. Comp. Path. Ther.*, 1943, **53**, 269; Carmichael and Bell, *ibid.*, 1944, **54**, 49) has led to the investigation of analogous compounds. A valuable improvement in method has resulted from the observation that in the conversion of acyl-*o*-xenylamines, *e.g.*, (I), into the corresponding phenanthridines, *e.g.*, (II), by phosphorus oxychloride, both the rate of condensation and the yield of product are increased by the addition to the reagents of an inert, high-boiling liquid miscible with them. Nitrobenzene answers the purpose well, and its function is simply thermal, in that it permits reaction to be performed at 180–200°. In several of the examples given below the yield of phenanthridine derivative has been increased by this means from a small amount to over 50%.

An alternative route to the desired amino-quaternary salts, *e.g.*, (IV; R = R' = NH₂, R' = H), has been found in the facile reduction of the nitro-quaternary salts (compare IV; R = R' = NO₂, R' = H), which themselves are readily prepared directly from the nitrophenanthridines. For instance, 3- and 7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride, (III; R' = R'' = NH₂, R' = H) and (III; R = R' = NH₂, R' = H), described by Morgan and Walls (*J.*, 1938, 389), are both more conveniently prepared by this method, which is shorter than the alternative by two stages, protection of amino-groups by acetylation and subsequent deacetylation. Moreover, a further disadvantage of the older method is evaded: with several of the diamino-phenylphenanthridines, notably 2 : 7-diamino-9-phenylphenanthridine (see below), the two amino-groups are not severally and quantitatively monoacetylated, and the isolation of the desired compound in which both amino-groups are protected is not readily accomplished. By the addition of alkali to aqueous solutions of the nitro-quaternary salts, crystalline pseudo-bases are precipitated, *e.g.* (V; R = R' = NO₂, R' = H), which are reconverted by dissolution in dilute acids into the original salts. These salts are almost colourless, highly crystalline substances of indefinite melting-decomposition point which dissolve in dilute acid, but are subject to partial hydrolysis to pseudo-bases by water alone. Reduction to the corresponding amino-quaternary salts proceeds very smoothly with iron and water, preferably in the absence of acid, but catalytic hydrogenation under pressure generally involves nuclear reduction, a phenomenon which is probably associated with high reactivity of the $>C^+NMe$ grouping.



Modification of the structure of the trypanocidal type (III; R = R' = NH₂, R' = H) has been effected in the following ways:

(i) *Elimination of one amino-group.* The compound (III; R'' = NH₂, R = R' = H) has already been described (Morgan and Walls, *J.*, 1931, 2447) and is devoid of trypanocidal properties. Its isomeride (III; R = NH₂, R' = R'' = H) has now been prepared by the improved methods.

(ii) *Substitution of the 9-phenyl group with m-amino- in place of p-amino-.* In view of the promising trypanocidal properties of the compounds (III; R = R' = NH₂, R' = H) and (III; R' = R'' = NHAc, R = H) it was desirable to determine whether a favourable therapeutic effect would follow when the substituent R'' = NH₂ or NHAc was in the *m*-position. The synthesis of these types was readily effected by the new methods.

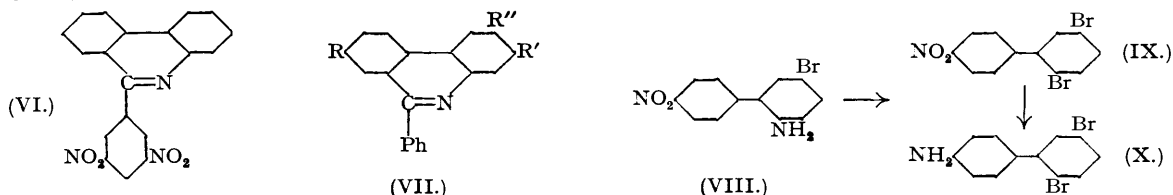
(iii) *Placing of both amino-groups in the 9-phenyl substituent.* 3'' : 5''-Dinitrobenzamidodiphenyl was condensed by phosphorus oxychloride to 9-3' : 5'-dinitrophenylphenanthridine (VI). Methylation of this dinitro-

compound readily afforded the *methochloride*, which was reduced by stannous chloride to 9-3' : 5'-*diamino-phenyl-10-methylphenanthridinium chloride*. An attempt to prepare this salt by the alternative route involving reduction of (VI) to the diamino-compound and protection of the amino-groups by acetylation before methylation failed owing to lack of reactivity of the diacetamido-compound with the usual methylating agents.

(iv) *Placing of both amino-groups in the phenanthridine part of the molecule.* Isolation of a small yield of 3 : 7-dinitro-9-phenylphenanthridine (VII; R = R'' = NO₂, R' = H) from the ring-closure of 5 : 4'-dinitro-2-benzamidodiphenyl has already been reported (J., 1938, 389). The yield has been increased to over 50% by the use of a phosphorus oxychloride-nitrobenzene mixture. Conversion of the product into the desired quaternary salt (IV; R = R'' = NH₂, R' = H) was effected by the alternative routes: methylation, followed by reduction with iron powder and water; or reduction to 3 : 7-diamino-9-phenylphenanthridine (VII; R = R'' = NH₂, R' = H), protection of the amino-groups by acetylation, and then methylation, followed by hydrolysis to 3 : 7-diamino-9-phenyl-10-methylphenanthridinium chloride (IV; R = R'' = NH₂, R' = H).

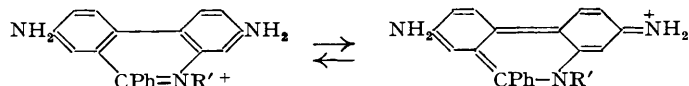
The isomeric type (IV; R = R' = NH₂, R'' = H) was prepared by a similar series of reactions from 4 : 4'-dinitro-2-aminodiphenyl (Finzi and Bellavita, *Gazzetta*, 1938, 68, 77). The final product, 2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide, is of particular interest because of its amino-groups being in the "benzidine" position. It was expected to possess enhanced substantive characteristics, which should be reflected in its chemotherapeutic properties. The salt crystallises in almost black prisms, purple by transmitted light. The crystals and their aqueous solution closely resemble permanganate in appearance.

(v) *Introduction of a halogen atom into the active molecule.* The toxicity of several acridine antimalarials and antiseptics is known to be diminished by the introduction of a halogen atom. A representative of this type was obtained as follows. Bromination of 4'-nitro-2-acetamidodiphenyl afforded a monobromo-derivative. Hydrolysis of this compound yielded an amine (VIII), which was converted by Hodgson and Walker's method



(J., 1933, 1620) into a dibromonitro-compound (IX), and the latter by successive reduction and oxidation into respectively the dibromo-amino-compound (X) and 2 : 5-dibromobenzoic acid. Hence, bromination had occurred in the 5-position.* The amine (VIII) was *p*-nitrobenzoylated, and the product (I; R = R'' = NO₂, R' = Br) cyclised to 3-bromo-7-nitro-9-*p*-nitrophenylphenanthridine (II; R = R'' = NO₂, R' = Br). Conversion into the desired quaternary salt (III; R = R'' = NH₂, R' = Br) was effected through the diacetamido-derivative (II; R = R'' = NHAc, R' = Br); the alternative route was not satisfactory.

The colour relationships of the salts described in this paper conform generally with those described in Part V (J., 1938, 389); thus salts of amines derived from (VI) are not markedly coloured: those from (VII; R = R'' = NH₂, R' = H) are orange, and those from (II; R = R'' = NH₂, R' = Br) deep red like the corresponding bromine-free compounds. That the diamino-salts referred to in sections (i) and (ii), in which the *p*-amino-group is either missing or is replaced by a *m*-amino-group, are deeply coloured shows that an amino-group in the 3- or the 7-position, particularly the latter, of the phenanthridine molecule has a marked chromophoric effect, a fact difficult to explain on a benzenoid-quinonoid resonance theory. Reference has already been made to the intense colour of salts derived from the "benzidine" type (IV; R = R' = NH₂, R'' = H). Here benzenoid-quinonoid resonance may be postulated (compare J., 1931, 2447) :



According to Kumler and Daniels (*J. Amer. Chem. Soc.*, 1943, 65, 2190), bacteriostatic properties in the sulphonamide and acridine series are associated with this type of resonance, but so far in this series no clear conclusion can be drawn that this type of resonance specifically favours either bacteriostatic or trypanocidal action.

Note on Chemotherapeutic Trypanocidal Action.†

The results of the investigation of these amino-quaternary compounds for trypanocidal action in experimentally infected mice are shown in the table, the methods employed being those to which references are given. Two species of trypanosomes have been used, *viz.*, *T. brucei* and *T. congolense*, one strain of each. The former is allied to the causal agents of African trypanosomiasis of man and behaves similarly towards drugs. The latter affects cattle and other domestic animals in Africa, but not man; its behaviour towards

* Since going to press (VIII) and its acetyl derivative have been described by Case (*J. Amer. Chem. Soc.*, 1945, 67, 118), and their constitution proved by substantially the same method.

† Work done with the support of the Medical Research Council in the Department of Bacteriology and Pathology, The University and Western Infirmary, Glasgow.

drugs differs strikingly from that of *T. brucei*, since most classes of compounds which are highly active against *T. brucei* have little or no effect on *T. congolense*. It must be noted that in assessing the chemotherapeutic activity of a substance, toxicity for the mammalian host is the limiting factor; hence the largest doses used approach the maximum which the animal will tolerate without serious toxic effects. The most striking features of the present series are as follows. For comparison results obtained under similar conditions with compounds 893, 894, and 897 (Nos. 19, 20, and 23 in Morgan and Walls, *loc. cit.*) are given.

(1) All the amino-compounds show distinct trypanocidal action except that in which the amino-substituents are restricted to the 9-phenyl group (1052).

(2) *T. congolense* is more strongly influenced as a rule than *T. brucei*, each infection being treated at the most susceptible stage—*e.g.*, Nos. 1508, 1568, which are the analogues of No. 897 with the amino-group of the 9-phenyl radical in the 3'- instead of the 4'-position; also, 1505 and 1506, 1565 and 1553, and 1573. This is in agreement with our previous findings in the phenanthridine series.

(3) Comparison of the structure of the more effective compounds (894; 1505, 1506; 1508, 1568; 1573; 1565, 1553) indicates that action is obtained with various positions of the amino-groups. The 2:7-diamino-compounds (1565, 1553), which possess a "benzidine" type of structure, are the most active against both species of trypanosome.

(4) Acetylation of the two amino-groups in the active substances greatly reduces the therapeutic efficiency even where the solubility is not markedly diminished, cf. Nos. 894, 893; 1508, 1507; 1573, 1574. In the table the orientations of the compounds are denoted as in (III), the Cl- being replaced by the anion A-

Compound.	Formula.	Therapeutic effect in mice infected with			
		<i>T. congolense</i> (Strain I). ¹		<i>T. brucei</i>	
		Dose, in mg. ^(a)	Result.	Dose, in mg. ^(a)	Result.
897	NH ₂ = 7 : 4', A = Cl	0.033	Cure	1	(Cure)
		0.01	(Cure)		
896	NHAc = 7 : 4', A = Cl	0.5	O	1	O
894	NH ₂ = 3 : 4', A = Cl	0.2—0.02	Cure	0.2	Slight
		0.01	Slight—O		
893	NHAc = 3 : 4', A = Cl	2.5	(Cure)	2.5	Cure
				1.25	Marked
1505	NH ₂ = 7, A = Cl	0.5—0.1	Cure	0.5	Cure
		0.016	Marked	0.33	Slight
1506	NH ₂ = 7, A = I	0.33—0.05	Cure	0.33	Marked
		0.016—0.01	Slight		
1504	NHAc = 7, A = Cl	10 †	Cure	10	O
		2.5	Marked		
1508	NH ₂ = 7 : 3', A = Cl	1.6—0.022	Cure	1.6	Cure
		0.01—0.005	(Cure)	0.33	Marked
		0.0033	Slight		
1568	NH ₂ = 7 : 3', A = I	1.3—0.02	Cure	0.66	(Cure)
		0.01	(Cure)		
1507	NHAc = 7 : 3', A = Cl	5	(Cure)	3.3	O
		2.5	Slight		
1573	NH ₂ = 3 : 3', A = Cl	0.33	Cure	0.5	O
		0.033—0.01	(Cure)		
1574	NHAc = 3 : 3', A = Cl	2.5	Slight	2.5	O
1061	NO ₂ = 3' : 5', A = Cl	0.5	(Cure)	2	O
		0.25	Slight		
1052	NH ₂ = 3' : 5', A = Cl	0.5	Slight	2	O
1053	NHAc = 3' : 5', A = Cl	0.5	Slight	1	O
1542	NH ₂ = 3 : 7, A = Cl	0.66	Cure	0.33	Cure
		0.01	(Cure)	0.05	Marked
1162	NHAc = 3:7, A = Cl	1	O*	2	Slight—O
1565	NH ₂ = 2 : 7, A = Cl	0.33—0.005	Cure	0.33	Cure
		0.0033	(Cure)	0.16—0.05	(Cure)
1553	NH ₂ = 2 : 7, A = Br	0.33—0.005	Cure	0.33	Cure
		0.0025	Marked	0.1	(Cure)
1554	NHAc = 2 : 7, A = Cl	0.5	Slight	0.5	O
1141	NH ₂ = 7 : 4', Br = 3, A = Cl	0.25—0.05	Cure	1	Slight
		0.02	Slight		
1142	NHAc = 7 : 4', Br = 3, A = Cl	0.5	O	2*	O

* Maximum solubility.

† Partly undissolved.

(a) Dosage is reckoned per 20 g. of body weight, 1 c.c. of solution being injected subcutaneously: the highest dose shown is not less than half the average maximum tolerated.

The terms used to designate degrees of trypanocidal action are as follows:

Cure = Complete sterilisation of infection.

(Cure) = Cure effected only in a proportion of the animals treated.

Marked = Absence of parasites from blood for 10 days or longer.

Slight = Disappearance of parasites from blood for several days.

O = No effect.

¹ See Browning and Calver, *J. Path. Bact.*, 1943, **55**, 393; treatment was given at the acme stage.

² See Browning *et al.*, *ibid.*, 1934, **39**, 75; 1938, **46**, 203; the strain of trypanosome used was "Paris III" and treatment was given 24 hours after inoculation.

EXPERIMENTAL.

3-Nitro-9-*p*-nitrophenyl-10-methylphenanthridinium Methosulphate.—A solution of 3-nitro-9-*p*-nitrophenylphenanthridine (3 g.; J., 1938, 839) in nitrobenzene (30 ml.) was boiled to expel moisture, cooled to 180°, and methyl sulphate (3 g.) added. When the reaction had subsided the solution was boiled for 30 mins. and then distilled in steam. The residual liquor was filtered from a small quantity of unchanged material, and on being cooled deposited the *methosulphate* in high yield in buff-coloured plates of indefinite melting-decomposition point (Found: N, 9.15. $C_{21}H_{17}O_5N_3S$ requires N, 8.9%).

The 7-nitro-methosulphate was similarly prepared and converted into the *methochloride* by heating its aqueous solution with hydrochloric acid; the salt crystallised from water in pale yellow prisms (Found: N, 10.85; Cl, 9.15. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 9.0%).

Reduction in aqueous solution by iron filings of either of the foregoing salts was rapid. The red filtrate was made just alkaline with ammonia, filtered from a small amount of amorphous material, made just acid with hydrochloric acid, and evaporated to small bulk. On neutralisation, the corresponding diamino-quaternary salt crystallised in high yield.

7-Nitro-9-phenylphenanthridine.—A solution of 4'-nitro-2-aminodiphenyl (20 g.) in hot nitrobenzene (40 ml.) was treated with benzoyl chloride (9 ml.), and boiled gently until evolution of hydrogen chloride ceased. On being cooled, 4'-nitro-2-benzamidodiphenyl was deposited in high yield; it crystallised from benzene in colourless acicular prisms, m. p. 165.5° (Found: N, 8.85. $C_{19}H_{14}O_3N_2$ requires N, 8.8%). This benzoyl derivative (15 g.), phosphorus oxychloride (30 g.), and nitrobenzene (45 g.) were heated together under reflux at 170–180° for 12 hours. The product was carefully stirred into water, and a complex of 7-nitro-9-phenylphenanthridine and acid separated from the nitrobenzene. When the complex was heated with aqueous alkali the desired product (14 g.) was liberated; it crystallised from pyridine in fine yellow, silky needles, m. p. 237° (Found: N, 9.4. $C_{19}H_{12}O_2N_2$ requires N, 9.35%). When nitrobenzene was omitted from the reaction mixture, only about a quarter of this yield resulted, most of the diphenyl derivative being recovered unchanged.

This base was converted by the above method into the methosulphate, and addition of hydrochloric acid to its solution precipitated the *methochloride*, which crystallised from boiling water in stout, buff-coloured prisms (Found: N, 8.25; Cl, 9.8. $C_{20}H_{15}O_2N_2Cl$ requires N, 8.0; Cl, 10.1%).

7-Amino-9-phenyl-10-methylphenanthridinium chloride (1505) was obtained by reduction of the foregoing salt with iron powder and water. As before, addition of ammonia to the aqueous solution of the filtrate precipitated a brown amorphous impurity. Evaporation of the slightly acid solution to small bulk afforded the quaternary salt in large brown transparent rhombs, m. p. 235.5° (decomp.) (Found: N, 8.65; Cl, 10.95. $C_{20}H_{17}N_3Cl$ requires N, 8.7; Cl, 11.05%). The *iodide* (1506) crystallised from water in transparent red prisms, m. p. 188–190° (decomp.) (Found: C, 57.9; H, 4.1; N, 7.15; I, 31.0. $C_{20}H_{17}N_3I$ requires C, 58.2; H, 4.1; N, 6.9; I, 30.85%).

On being warmed with acetic anhydride, the chloride was converted into the *acetyl* derivative (1504); it crystallised from a large volume of water in yellow plates, m. p. 275° (decomp.) (Found: C, 73.0; H, 5.4; N, 7.75; Cl, 9.9. $C_{22}H_{19}ON_2Cl$ requires C, 72.85; H, 5.25; N, 7.7; Cl, 9.8%).

7-Nitro-9-*m*-nitrophenylphenanthridine.—4'-Nitro-2-*m*-nitrobenzamidodiphenyl, prepared from 4'-nitro-2-aminodiphenyl and *m*-nitrobenzoyl chloride in nitrobenzene solution, crystallised from glacial acetic acid in small white needles, m. p. 187° (Found: N, 11.5. $C_{19}H_{13}O_5N_3$ requires N, 11.55%). This compound (25 g.), phosphorus oxychloride (50 g.), and nitrobenzene (75 g.) were heated under reflux from a bath at 180° for 12 hours. On being stirred into water, the desired product (19.5 g.) separated directly from the nitrobenzene layer. After filtration it was washed successively with nitrobenzene, water, and hot methylated spirit, and finally crystallised from nitrobenzene or a large volume of pyridine in matted, cream-coloured needles, m. p. 269° (Found: N, 12.45. $C_{19}H_{11}O_4N_3$ requires N, 12.2%). Its *methochloride*, prepared as for similar salts, crystallised from water in transparent, buff-coloured prisms which disintegrated on exposure to air owing to loss of water of crystallisation (Found, for dehydrated salt: N, 10.65; Cl, 8.95. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 9.0%).

7-Amino-9-*m*-aminophenyl-10-methylphenanthridinium chloride (1508), obtained from the foregoing salt by reduction with iron and water, crystallised from water in red prisms, m. p. 147–149° (decomp.) (Found: C, 71.2; H, 5.5; N, 12.65; Cl, 10.6. $C_{20}H_{18}N_3Cl$ requires C, 71.5; H, 5.35; N, 12.5; Cl, 10.6%). The *iodide* (1568) crystallised from water in red prisms, m. p. 240° (decomp.) (Found: I, 29.55. $C_{20}H_{18}N_3I$ requires I, 29.75%). The *diacetyl* derivative (1507) crystallised from water in minute yellow prisms, m. p. 237.5° (decomp.) (Found: C, 68.75; H, 5.2; N, 10.25; Cl, 8.55. $C_{24}H_{22}O_2N_3Cl$ requires C, 68.65; H, 5.25; N, 10.0; Cl, 8.45%).

Except where otherwise stated, the following compounds were prepared by similar methods, all in excellent yields.

3-Nitro-9-*m*-nitrophenylphenanthridine.—5-Nitro-2-*m*-nitrobenzamidodiphenyl formed almost colourless needles from glacial acetic acid, m. p. 190.5° (Found: N, 11.6. $C_{19}H_{13}O_5N_3$ requires N, 11.55%), and the *phenanthridine* almost white, matted needles from nitrobenzene, m. p. 269° (Found: N, 12.5. $C_{19}H_{11}O_4N_3$ requires N, 12.2%). When the latter compound (5 g.) in nitrobenzene solution (30 ml.) was heated with methyl sulphate (3 ml.), the very sparingly soluble methosulphate crystallised almost quantitatively, so steam-distillation was unnecessary. The salt was separated, washed with nitrobenzene and several times with hot benzene. It was then heated in suspension in aqueous ammonia, and the yellow pseudo-base thus liberated was dissolved in *n*-hydrochloric acid. On being cooled, the solution deposited the rather sparingly soluble *methochloride* in cream-coloured, matted needles, the yield being almost theoretical (Found: N, 10.55; Cl, 8.6. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 9.0%).

3-Amino-9-*m*-aminophenyl-10-methylphenanthridinium Chloride (1573).—This *chloride* crystallised from water in golden-yellow needles, m. p. 199–202° (decomp.) (Found: N, 12.45; Cl, 10.4. $C_{20}H_{18}N_3Cl$ requires N, 12.5; Cl, 10.6%). Its *diacetyl* derivative (1574) separated from water as rather ill-defined, pale yellow prisms, m. p. 215–217° (decomp.) (Found: N, 10.2; Cl, 8.2. $C_{24}H_{22}O_2N_3Cl$ requires N, 10.0; Cl, 8.45%).

9-3' : 5'-Dinitrophenylphenanthridine (VI).—2-3' : 5''-Dinitrobenzamidodiphenyl, obtained by condensation of excess of *o*-xenylylamine with 3 : 5-dinitrobenzoyl chloride in hot pyridine, crystallised from benzene in yellow plates, m. p. 185° (Found: N, 11.7. $C_{19}H_{13}O_5N_3$ requires N, 11.55%), and the *compound* (VI) crystallised from pyridine or nitrobenzene in small, buff prisms, m. p. 294° (Found: N, 12.0. $C_{19}H_{11}O_4N_3$ requires N, 12.2%). Its *methochloride* (1061) crystallised from water in buff, feathery needles (Found: N, 10.8; Cl, 8.65. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 9.0%).

9-3' : 5'-Diaminophenyl-10-methylphenanthridinium Chloride (1052).—The foregoing salt (4 g.) was well powdered and suspended in alcohol (100 ml.) and concentrated hydrochloric acid (20 ml.). Rapid reduction occurred on addition of stannous chloride (16 g.), dissolution of the nitro-compound being followed by crystallisation of a yellow stannichloride. When the aqueous solution of this salt was partly neutralised stannic hydroxide was precipitated; complete neutralisation caused the *diamino-methochloride* to crystallise in brownish plates (2.5 g.), m. p. 241° (decomp.) (Found: C, 72.2; H, 5.45; N, 12.95; Cl, 10.2. $C_{20}H_{18}N_3Cl$ requires C, 71.55; H, 5.35; N, 12.5; Cl, 10.6%). Its *diacetyl* derivative (1053) crystallised from water as pale yellow prismatic needles, m. p. 227° (decomp.) (Found: C, 68.65; H, 5.1; N, 10.1; Cl, 8.05. $C_{24}H_{22}O_2N_3Cl$ requires C, 68.65; H, 5.25; N, 10.0; Cl, 8.45%).

3 : 7-Dinitro-9-phenylphenanthridine.—5 : 4'-Dinitro-2-benzamidodiphenyl (23 g.), nitrobenzene (69 ml.), and phosphorus oxychloride (46 g.) were heated in a bath at 180° for 18 hours. The phenanthridine (12.3 g.) separated from the nitrobenzene layer when the product was stirred into water. On steam-distillation of the nitrobenzene mother-liquor a crudely crystalline residue was left which consisted largely of unchanged diphenyl derivative, and from which a further yield of the phenanthridine could be obtained by retreatment with nitrobenzene-phosphorus oxychloride mixture.

3 : 7-Diamino-9-phenylphenanthridine.—A suspension of the foregoing dinitro-compound in alcoholic hydrochloric acid was reduced by stannous chloride. When the red solution obtained was poured into aqueous sodium hydroxide solution it furnished the diamino-compound as a pale yellow crystalline precipitate, very soluble in alcohol; it was best crystallised from benzene, forming clusters of discoloured prisms, m. p. 194° (Found : C, 80.0; H, 5.3; N, 14.6. $C_{19}H_{15}N_3$ requires C, 80.0; H, 5.25; N, 14.7%). Its diacetyl derivative crystallised from alcohol in almost colourless prisms, m. p. 266° (Found : C, 74.5; H, 5.25; N, 11.4. $C_{23}H_{19}O_2N_3$ requires C, 74.8; H, 5.15; N, 11.4%).

When this acetyl compound (1 g.) in hot nitrobenzene (25 ml.) was treated with methyl *p*-toluenesulphonate, the solvent removed by steam-distillation, and the residual aqueous liquor treated with sodium chloride, 3 : 7-diacetyl-amido-9-phenyl-10-methylphenanthridinium chloride (1162) crystallised in yellow plates, m. p. 285° (decomp.) (Found : C, 68.8; H, 5.4; N, 10.2; Cl, 8.4. $C_{24}H_{22}O_2N_3Cl$ requires C, 68.65; H, 5.25; N, 10.0; Cl, 8.45%).

3 : 7-Diamino-9-phenyl-10-methylphenanthridinium chloride (1542) was obtained from the diacetyl salt by hydrolysis with concentrated hydrochloric acid. Neutralisation with ammonia liberated the diamino-chloride as a brown gum, which slowly crystallised. Recrystallisation from water furnished brown prisms, m. p. 268° (decomp.) (Found : C, 71.8; H, 5.15; N, 12.7; Cl, 10.45. $C_{20}H_{18}N_3Cl$ requires C, 71.55; H, 5.35; N, 12.5; Cl, 10.6%).

3 : 7-Dinitro-9-phenyl-10-methylphenanthridinium chloride crystallised from dilute hydrochloric acid in buff prisms (Found : N, 10.75; Cl, 9.45. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 9.0%). When its aqueous solution was treated with ammonia, the pseudo-base 3 : 7-dinitro-9-phenyl-10-hydroxy-10-methyl-9 : 10-dihydrophenanthridine was precipitated. It was sparingly soluble in alcohol or benzene, but crystallised in golden-yellow needles, m. p. 221—223° (decomp.), on addition of hot alcohol to its pyridine solution (Found : N, 11.05. $C_{20}H_{18}O_5N_3$ requires N, 11.15%). The dinitro-quaternary salt was readily reduced to the corresponding diamino-salt by iron filings and water.

2 : 7-Dinitro-9-phenylphenanthridine.—4 : 4'-Dinitro-2-benzamidodiphenyl, prepared from 2-amino-4 : 4'-dinitrodiphenyl and benzoyl chloride in hot chlorobenzene, separated from glacial acetic acid solution in almost colourless prisms, m. p. 234° (Found : N, 11.8. $C_{19}H_{13}O_6N_3$ requires N, 11.55%). By the standard procedure it afforded the phenanthridine in about 50% yield, unchanged diphenyl compound being recovered; pale yellow needles were obtained by crystallisation from nitrobenzene (Found : N, 12.2. $C_{19}H_{11}O_4N_3$ requires N, 12.2%). Reduction of this compound in alcoholic suspension with stannous chloride-hydrochloric acid, or with hydrogen under pressure using a platinum-black catalyst, furnished the diamine, which crystallised from alcohol in golden-yellow prisms, m. p. 198° (Found : C, 79.8; H, 5.45; N, 14.7. $C_{19}H_{15}N_3$ requires C, 80.0; H, 5.25; N, 14.75%).

2 : 7-Dinitro-9-phenyl-10-methylphenanthridinium chloride crystallised from water in buff-coloured prisms (Found : N, 10.7; Cl, 8.65. $C_{20}H_{14}O_4N_3Cl$ requires N, 10.6; Cl, 8.95%). The pseudo-base crystallised from acetone in small red prisms, m. p. 186—188° (decomp.) (Found : N, 10.9. $C_{20}H_{15}O_5N_3$ requires, N, 11.15%).

2 : 7-Diamino-9-phenyl-10-methylphenanthridinium Bromide (1553).—The permanganate-like solution from the reduction of the foregoing dinitro-quaternary salt (20 g.) with iron filings and water was diluted to ca. 600 ml., treated with potassium bromide (6 g.), and made just alkaline with ammonia. It was then purified chromatographically from a purple-black by-product by filtration through a column (8 cm. long by 4 cm. diam.) of talc. The filtrate was neutralised, and concentrated under reduced pressure to 200 ml. On being cooled, the bromide crystallised in purple-black, elongated plates (first crop 15 g.), m. p. 241—243° (decomp.) (Found : C, 62.8; H, 4.95; N, 11.35. $C_{20}H_{18}N_3Br$ requires C, 63.15; H, 4.75; N, 11.05%). When the solution was chromatographed without addition of potassium bromide, and the filtrate evaporated to dryness under reduced pressure a purple-black gum was obtained, and on being boiled with alcohol this rapidly afforded purple-black plates of the chloride (1565), decomp. ca. 253° (Found : Cl, 10.45. $C_{20}H_{18}N_3Cl$ requires Cl, 10.6%). This salt was extremely soluble in water, and boiling acetic anhydride rapidly converted it into the diacetamido-chloride (1554); aqueous solutions of this compound were liable to set to a jelly, but from aqueous alcohol (1 : 1) minute yellow needles separated, m. p. 260° (decomp.) (Found : N, 9.9; Cl, 8.5. $C_{21}H_{22}O_2N_3Cl$ requires N, 10.0; Cl, 8.45%).

5-Bromo-4'-nitro-2-acetamidodiphenyl.—A solution of 4'-nitro-2-acetamidodiphenyl (21 g.) in glacial acetic acid (210 ml.) was added with stirring to a solution of bromine (14.7 g.) in the same solvent (147 ml.). After 24 hours the solid product was collected; it recrystallised from glacial acetic acid in small yellow prisms (23.5 g.), m. p. 214° (Found : N, 8.55; Br, 23.5. $C_{14}H_{11}O_3N_2Br$ requires N, 8.4; Br, 23.9%). This acetyl compound was refluxed for 6 hours with alcoholic hydrochloric acid (1 : 1), the hydrochloride separated, and decomposed with alkali, yielding the amine (VIII), which crystallised from alcohol in orange-red, acicular prisms, m. p. 152° (Found : N, 9.45; Br, 27.0. $C_{12}H_9O_2N_2Br$ requires N, 9.55; Br, 27.3%). Hodgson and Walker's modification of the Sandmeyer reaction converted this amine into 2 : 5-dibromo-4'-nitrodiphenyl (IX), which crystallised from alcohol in almost colourless prisms, m. p. 98° (Found : N, 4.25; Br, 44.2. $C_{12}H_9O_2NBr_2$ requires N, 3.9; Br, 44.8%).

2 : 5-Dibromo-4'-aminodiphenyl (X), obtained quantitatively from the nitro-compound by reduction with iron and acidified water, crystallised from alcohol in light brown prisms, m. p. 95° (Found : N, 4.5. $C_{12}H_9NBr_2$ requires N, 4.3%). It was oxidised slowly by alkaline permanganate, and the only product isolated was 2 : 5-dibromobenzoic acid, m. p. 156°, alone or in admixture with an authentic specimen (cf. Claus and Weil, *Annalen*, 1892, 269, 223).

5-Bromo-4'-nitro-2-*p*-nitrobenzamidodiphenyl crystallised from nitrobenzene in pale yellow prisms, m. p. 245° (Found : N, 9.6; Br, 18.0. $C_{18}H_{12}O_5N_3Br$ requires N, 9.5; Br, 18.1%).

3-Bromo-7-nitro-9-*p*-nitrophenylphenanthridine was obtained in high yield by the nitrobenzene-phosphorus oxychloride method; pale yellow needles from nitrobenzene, m. p. 348° (Found : N, 10.15; Br, 18.5. $C_{19}H_{16}O_4N_3Br$ requires N, 9.9; Br, 18.85%). Catalytic reduction of this compound with hydrogen caused removal of the bromine atom, so the following method was used : the well-powdered compound (5 g.) was suspended in 40 ml. of a glacial acetic acid solution of stannous chloride saturated with hydrogen chloride (compare Thiele and Dimroth, *Annalen*, 1899, 305, 114). After 24 hours the red salt that had separated was removed and converted into the 7-amino-compound, which crystallised from pyridine-benzene in yellow prisms, m. p. 265° (Found : C, 62.9; H, 3.95; N, 12.1; Br, 22.0. $C_{19}H_{11}N_3Br$ requires, C, 62.65; H, 3.85; N, 11.55; Br, 22.05%). The diacetyl compound crystallised from alcohol in hard, colourless, solvated cubes, or from nitrobenzene in clusters of white microscopic needles, m. p. 301° (Found : C, 62.0; H, 4.0; N, 9.45; Br, 17.6. $C_{23}H_{18}O_2N_3Br$ requires C, 61.6; H, 4.0; N, 9.35; Br, 17.85%).

3-Bromo-7-amino-9-*p*-aminophenyl-10-methylphenanthridinium Chloride (1141).—Although methyl sulphate caused profound decomposition of 3-bromo-7-acetamido-9-*p*-acetamidodiphenylphenanthridine in hot nitrobenzene, yet with methyl *p*-toluenesulphonate smooth condensation took place. After steam-distillation of the solvent, the crude residue was hydrolysed with hydrochloric acid, and on neutralisation the diamino-quaternary chloride crystallised in transparent red prisms, m. p. 265° (decomp.), which were purified by recrystallisation from water (Found : C, 57.75; H, 3.75; N, 10.4; Cl, 8.35. $C_{20}H_{17}N_3ClBr$ requires C, 57.9; H, 4.1; N, 10.15; Cl 8.55%). The diacetyl compound (1142

crystallised from a large volume of alcohol in solvated, pale yellow, matted needles, m. p. 213° (decomp.). After removal of solvent the salt rapidly absorbed 4 mols. of water from the atmosphere (Found, for hydrated salt: C, 50.7; H, 5.15; Cl, 5.9; Br, 13.3; loss on heating, 12.35. $C_{24}H_{21}O_2N_3ClBr_4H_2O$ requires C, 50.5; H, 5.1; Cl, 6.2; Br, 14.0; H_2O , 12.6%).

Most of the quaternary salts described above are hydrated, the degree of hydration often depending on the humidity of the atmosphere. Consequently, analyses refer to the dried salts unless otherwise stated.

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