13. *Researches in the Phenanthridine Series. Part VIII. Further Investigation of Trypanocidal Types.**

By Leslie P. Walls.

(With a Note by C. H. Browning, K. M. Calver, and M. W. Leckie.)

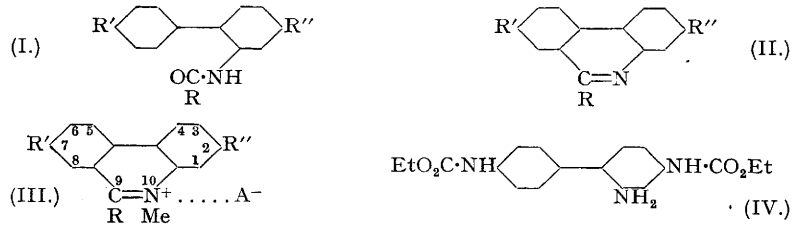
In Part VI (*J.*, 1945, 294) it has been shown that 9-phenylphenanthridinium salts with two amino-groups (cf. V; R = R′ = NH_2) exert a powerful chemotherapeutic action against *T. congolense*, whilst recently it has been further demonstrated (*Nature*, 1946, **157**, 263) that similar salts with carbethoxyamino- in place of amino-groups have some activity in *T. cruzi* infections. In this paper the effect has been studied of replacing the 9-phenyl by a 9-methyl group, and of substitution of (V) with other than amino-groups; thus in different compounds R = NH_2, NH·COMe, NH·CO_2Et, and R′ or R″ = NO_2, NH_2, NH·COMe, or NH·CO_2Et. The preparation of these types was achieved by ring-closure of the corresponding *o*-acylamido 4′-carbethoxyaminodiphenyls (I; R′ = NH·CO_2Et). This useful result is attributed to the electromeric character of the urethane group, and leads to the synthesis of (II; R = Me, R′ = NH_2, R″ = H), (II; R = Me, R′ = R″ = NH_2), and (X; R = NH_2, R′ = NO_2). The corresponding quaternary salts were readily prepared. The use of this method for the preparation of compounds with nitro- and carbethoxyamino-groups in the same molecule (cf. X; R = NH·CO_2Et, R′ = NO_2) provided a route to the quaternary salts with two different substituents referred to above. In salts with a 9-*o*-aminophenyl substituent it was not possible to convert the amino- into a carbethoxyamino-group, this failure being attributed to steric hindrance, for the 9-*p*-aminophenyl compounds readily reacted with ethyl chloroformate.

The effect of replacement of 9-phenyl by 9-methyl (cf. III; R′ = R″ = NH_2, R = Me or Ph) is to reduce the action on *T. congolense*. In the salts with mixed substituents it is found that the presence of an amino-group in the phenanthridine nucleus is essential to trypanocidal activity, which is enhanced when the 9-phenyl is substituted by *o*-, *m*-, or *p*-amino-, or by *o*- or *p*-nitro-groups.

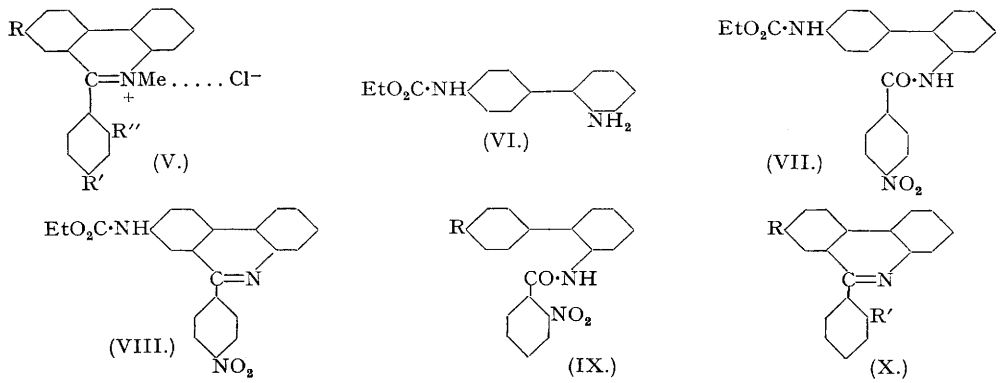
Previous work on this series has shown that in the synthesis of phenanthridines (cf. II) from acyl-*o*-xenylamines (cf. I) ring-closure is inhibited when R′ = NO_2 (Morgan and Walls, *J.*, 1932, 2225). This result was not unexpected in view of the well-known effect of a nitro-group in reducing the affinity of the positions in its ring for a katonic reagent, which the acylamido-group may be assumed to become in the presence of the normal condensing agent, phosphorus oxychloride. Indeed in (I; R = Me, R′ = NO_2, R″ = H) hydrolysis of the acetyl group proceeds much more rapidly under the reaction conditions than does ring-closure, and the yield of (II; R = Me, R′ = NO_2, R″ = H) is extremely small (*loc. cit.*). If derivatives of type (II; R = Me) are to become readily available it is clear that R′ must be a group possessing an opposite effect on substitution to the nitro-group; moreover it must be stable under the exacting reaction conditions, and in order to lead to substances likely to possess trypanocidal properties it must readily furnish an amino-group. While this work was in progress Petrow (*J.*, 1945, 19) showed how these desiderata could be satisfied by ring-closure of (I; R = Me, R′ = NH·COPh, R″ = H). The method recommended here is the location in the appropriate ring of a carbethoxyamino-group, which has a powerful electromeric effect in the opposite sense to that of the nitro-group, is very stable, and yet under appropriate conditions is readily derived from and converted into an amino-group. A further advantage with the more complex derivatives is that the melting points of carbethoxyamino-compounds are conveniently low and the solubility in organic solvents correspondingly high. That the proposed method is

* The experimental work described in this paper formed the subject of British provisional patent applications Nos. 52/44 and 53/44.

strikingly successful may be exemplified by (I; R = Ph, R′ = R″ = NH·CO_2Et) which can be converted into the corresponding phenanthridine in over 50% yield by 15 minutes' heating with phosphorus oxychloride alone; by contrast the corresponding nitro-compound (I; R = Ph, R′ = R″ = NO_2) requires over 12 hours' heating with phosphorus oxychloride–nitrobenzene at 180° for the same degree of ring-closure. As shown in Part VII, the carbethoxyaminophenanthridine compounds thus produced can be converted into quaternary salts (cf. III; R = Ph, R′ = R″ = NH·CO_2Et) which can be hydrolysed quantitatively to the important diamino-quaternary salts. Certain of the carbethoxyl derivatives are also interesting therapeutically. This gives an alternative route to the preparation of " phenanthridinium



1553 " (III; R = Ph, R′ = R″ = NH_2, A = Br; *J.*, 1945, 294) which has shown marked curative action in *T. congolense* infection of cattle in Africa (Carmichael and Bell, *Vet. Rec.*, 1944, **56**, 496; Bell, *ibid.*, 1945, **57**, 449). The intermediate required for its synthesis is (I; R = Ph, R′ = R″ = NH·CO_2Et) and is prepared as follows : 2-nitrobenzidine which is readily available is converted into 2-nitro-4 : 4′-dicarbethoxyaminodiphenyl (Lesslie and Turner, *J.*, 1933, 1588) which is reduced to the non-basic amine (IV). Benzoylation of (IV) gave the required intermediate. The amine (IV) is likewise readily acetylated and the product (I; R = Me, R′ = R″ = NH·CO_2Et) is cyclised by phosphorus oxychloride to (II; R = Me, R′ = R″ = NH·CO_2Et). Conversion of this product into a quaternary salt (III; R = Me, R′ = R″ = NH·CO_2Et, A = $MeSO_4$) and finally hydrolysis to 2 : 7-*diamino*-9 : 10-*dimethylphenanthridinium bromide* (III; R = Me, R′ = R″ = NH_2, A = Br) readily follow. This salt as expected has the deep purple colour associated also with " phenanthridinium 1553 ". The corresponding salt lacking the 2-amino-group, 7-*amino*-9 : 10-*dimethylphenanthridinium bromide* (III; R = Me, R′ = NH_2, R″ = H, A = Br) has only a light red colour. For its preparation 2-acetamido-4′-carbethoxyaminodiphenyl (I; R = Me, R′ = NH·CO_2Et, R″ = H) is condensed in high yield to 7-*carbethoxyamino*-9-*methylphenanthridine*, which is hydrolysed to the amine identical with that described by Petrow (*loc. cit.*). Successive methylation and hydrolysis give the amino-quaternary salt (III; R = Me, R′ = NH_2, R″ = H, A = Br). The facile synthesis of carbethoxyamino-compounds of this series provides an opportunity of studying the chemotherapy of phenanthridinium salts with mixed substituents. There is described here a comprehensive series of such derivatives related to " phenanthridinium 897 " (V; R = R′ = NH_2, R″ = H) a substance which possesses a powerful remedial action in *T. congolense* infections, and to a new type (1588) 7-*amino*-9-o-*aminophenyl*-10-*methylphenanthridinium chloride* (V; R = R″ = NH_2, R′ = H).



4-Nitro-2′-aminodiphenyl was prepared by an improved method which renders it readily accessible. The amino-group was smoothly carbethoxylated by the method of Lesslie and

Turner (*loc. cit.*) and then the nitro-group was reduced to give 2-*amino*-4′-*carbethoxyamino*-*diphenyl* (VI). Condensation of this amine with *p*-nitrobenzoyl chloride yielded the acyl derivative (VII) which was smoothly and rapidly converted into 7-*carbethoxyamino*-9-*p*-*nitrophenylphenanthridine* (VIII). The latter and the corresponding acetamido-compound were converted quantitatively into quaternary salts, from which was prepared a series of stable crystalline salts (V; R″ = H) in which R and R′ may be variously NH_2, NH·COMe, and $NH{\cdot}CO_2Et$.

The series related to the corresponding *o*-substituted derivatives was of particular interest, for both the corresponding *m*- and *p*- series had yielded quaternary salts with trypanocidal properties (*J.*, 1945, 294). 4-*Nitro*-2′-o-*nitrobenzamidodiphenyl* could not be converted into the phenanthridine, but condensation of 2-o-*nitrobenzamido*-4′-*carbethoxyaminodiphenyl* (IX; R = $NH{\cdot}CO_2Et$) to the corresponding phenanthridine (X; R = $NH{\cdot}CO_2Et$, R′ = NO_2) went smoothly, and conversion of the product into the quaternary salt provided a route to a similar series of salts (V; R′ = H).

In both these series the salts in which R = NH_2 were deep red, revealing the characteristic chromophoric effect of an amino-group in the 7-position.

Attempts to prepare the salts (V; R = R″ = $NH{\cdot}CO_2Et$, R′ = H) and (V; R = NH·COMe, R″ = $NH{\cdot}CO_2Et$, R′ = H) in which the carbethoxyamino-group occupies an *o*-position in the 9-phenyl ring from the corresponding salts in which R″ = NH_2 or R = R″ = NH_2 proved unsuccessful, although, as has been shown in Part VII, carbethoxylation of the amino-groups of quaternary salts of this series normally proceeds smoothly under very mild conditions. The chief contributory cause of this failure is probably steric hindrance, juxtaposition of the *o*-amino-group and the quaternary nitrogen group hindering access of the large chloroformate molecule. Acetylation of this *o*-amino-group was also inhibited; thus according to the experimental conditions 7-*amino*-9-o-*aminophenyl*-10-*methylphenanthridinium chloride* (V; R = R″ = NH_2, R′ = H) could be mono-acetylated (on the 7-amino-group) or diacetylated, a particularisation that is not afforded by any of the other diamino-quaternary salt of this series yet investigated. It may also be due to steric causes that the nitro-group in quaternary salts with a 9-*o*-nitrophenyl substituent are not smoothly reduced in aqueous solution by iron powder, a method usually most efficaceous for nitro-quaternary salts.

*Note on Chemotherapeutic Trypanocidal Action.**—The present compounds (see Table) are complementary to those previously investigated (Walls, *J.*, 1945, 294; 1946, 1031) and comparisons are made here with the action of some of the latter. The same strains of *T. congolense* and *T. brucei* were employed.

(1) *T. brucei* is less susceptible than *T. congolense* to phenanthridine compounds; and a compound exhibiting a very high curative action against infections with *T. congolense* may show little or no effect on *T. brucei* in a dose 200 times as great (No. 1588). On the other hand, slight action on *T. brucei* may be associated with relatively weak action on *T. congolense* (No. 1600).

(2) While a phenyl group in the 9-position is not essential for trypanocidal action, analogues with a methyl group in that position are less active (cf. Nos. 1596—also the corresponding bromide and iodide—and 1505; 1605—also the corresponding bromide and iodide—and 1565, *J.*, 1945, 297).

(3) When one primary amino-group is present in the 7-position and another in the 9-phenyl ring, it is practically immaterial whether the latter amino-group occupies the *o*-, *m*-, or *p*-position, so far as concerns toxicity and action on *T. congolense* (Nos. 897, 1508, 1588, *loc. cit.*).

(4) The corresponding diacetamido-derivatives (Nos. 896, 1507, and 1589) are all of low trypanocidal power.

(5) The presence of a nitro- instead of amino-group in the phenyl ring causes only slight reduction in chemotherapeutic action (cf. Nos. 1586 and 897; 1587 and 1588).

(6) Carbethoxyamino- and, still more markedly, acetamido- replacing the primary amino-group in the phenanthridine ring reduces the therapeutic action, while as a rule slightly decreasing toxicity (cf. Nos. 1600 with 1596 and the corresponding bromide and iodide; 1598 and 1581 with 1605; 1592 and 1593 with 897; 1579 and 1590 with 1588; and in previous results 1566 with 1565).

(7) When one amino-group of Nos. 897 and 1588 is replaced by carbethoxyamino- and the other by acetamido- the chemotherapeutic action is very weak (Nos. 1594, 1595, 1591).

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

Compound.	Formula (III). Substituents.	Acid radical.	Therapeutic effect in mice infected with *T. congolense* (Strain 1).* Dose in mg.‡	Result.§	*T. brucei*.† Dose in mg.‡	Result.§
1600	7-NH·CO_2Et-9-Me	Br	3·3—2	Cure	3·3	Slight
			1	Slight		
1596	7-NH_2-9-Me	Cl	1—0·5	Cure	1	0
			0·2	(Cure)		
			0·066	Slight		
1605	2 : 7-NH_2-9-Me	Cl	2—0·2	Cure	1	Slight
			0·1	(Cure)	0·2	0
			0·05	Marked—Slight		
1598	2 : 7-NH·CO_2Et-9-Me	Cl	1·66	Cure	1·66	Slight
			0·83—0·55	(Cure)		
1581	2 : 7-NH·COMe-9-Me	Cl	4	0	4	0
1593	7-NH·COMe-9-*p*-C_6H_4·NH_2	$MeSO_4$	4	Cure	4	0
			2	Slight		
1595	7-NH·COMe-9-*p*-C_6H_4·NH·CO_2Et	Cl	4	Cure	4	0
			2	Slight		
1592	7-NH·CO_2Et-9-*p*-C_6H_4·NH_2	Cl	2—0·2	(Cure)	2	0
			0·1	Slight		
1594	7-NH·CO_2Et-9-*p*-C_6H_4·NH·COMe	Cl	4	Marked	4	0
1586	7-NH_2-9-*p*-C_6H_4·NO_2	Cl	2—0·033	Cure	2	Slight
			0·017	Marked		
1590	7-NH·COMe-9-*o*-C_6H_4·NH_2	Cl	0·5	0	0·5	0
1579	7-NH·CO_2Et-9-*o*-C_6H_4·NH_2	Cl	2—0·1	Cure	2	0
			0·066	(Cure)		
			0·033	0		
1591	7-NH·CO_2Et-9-*o*-C_6H_4·NH·COMe	Cl	5	Cure	5	0
			1·66	0		
1587	7-NH_2-9-*o*-C_6H_4·NO_2	Cl	1·43—0·066	Cure	1·43	0
			0·033	Marked		
			0·017	Slight		
1588	7-NH_2-9-*o*-C_6H_4·NH_2	Cl	0·2—0·02	Cure	2	0
			0·01	(Cure)		
			0·007	Slight		
1589	7-NH·COMe-9-*o*-C_6H_4·NH·COMe	Cl	3·3	0	3·3	0

* See Browning and Calver, *J. Path. Bact.*, 1943, **55**, 393; Calver, *Trop. Dis. Bull.*, 1945, **42**, 704; treatment was given at the acme stage.

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

‡ 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.
0 = No effect.

Summary. (a) Quaternary phenanthridine compounds containing two primary amino-groups, one at least of which is situated in the phenanthridine nucleus, constitute the most powerful chemotherapeutic agents known against *T. congolense*.

(b) Reduction of trypanocidal action is produced by substitution in the amino-group or groups of the phenanthridine part of the molecule.

(c) It has been observed previously that where amino-groups are restricted to the 9-phenyl ring trypanocidal action is weak (Nos. 206, 207, III; R′ = R″ = H, R = C_6H_4·NH_2; 1052, III; R′ = R″ = H, R = $C_6H_3(NH_2)_2$, Morgan and Walls, *J.*, 1931, 2445). Accordingly it would appear that the presence of at least one amino-group in the phenanthridine part is the most important factor for trypanocidal activity: however, no compound containing such a group along with a substituted amino-group in the 9-phenyl ring has yet been examined.

Experimental.

2-Acetamido-4′-carbethoxyaminodiphenyl.—To a solution of 4-amino-2′-acetamidodiphenyl (10 g., 1 mole; Petrow, *loc. cit.*) and diethylaniline (7·1 ml., 1·01 mole) in boiling alcohol (50 ml.) ethyl chloroformate (3·5 ml., 1·01 mole) was slowly added. After the vigorous reaction had subsided the solution was refluxed for 30 minutes and then poured into 200 ml. of N-hydrochloric acid. The crystalline *product*, obtained thus in high yield, was washed with dilute hydrochloric acid and then with water. It recrystallised from spirit in white leaves, m. p. 161° (Found: N, 9·45. $C_{17}H_{18}O_3N_2$ requires N, 9·4%).

7-*Amino*-9-*methylphenanthridine*.—The foregoing compound (6·5 g.) and phosphorus oxychloride (13 ml.) were gently refluxed for 1 hour, there being vigorous evolution of hydrogen chloride during the first 15 minutes. The solution was carefully stirred into ice-water to decompose excess of phosphorus oxychloride and ammonia was added to neutrality. The solid product was purified by dissolving it in cold N-sulphuric acid, separating a small amount of insoluble amorphous matter, and adding alkali to precipitate 7-*carbethoxyamino*-9-*methylphenanthridine* as a gum which slowly set to a white solid (5·2 g.); crystallisation from spirit yielded colourless leaves, m. p. 210·5° (Found: C, 73·0; H, 5·85; N, 10·35. $C_{17}H_{16}O_2N_2$ requires C, 72·85; H, 5·7; N, 10·0%). Hydrolysis was effected by the method of Lesslie and Turner (*loc. cit.*); water (5 ml.) was added to a solution of the carbethoxyamino-compound (2 g.) in concentrated sulphuric acid (10 ml.). The temperature was raised to 150° and kept there for 15 minutes, vigorous effervescence ensuing.

After being cooled the solution was poured into water and neutralised by ammonia to liberate 7-amino-9-methylphenanthridine which crystallised from a large volume of spirit in almost white needles, m. p. 232° (Found: N, 13·25. Calc. for $C_{14}H_{12}N_2$: N, 13·45%). On being heated with acetic anhydride followed by dilution with water and neutralisation by ammonia, it was converted into the acetyl derivative, m. p. 247·5° (Found: N, 11·3. Calc. for $C_{16}H_{14}ON_2$: N, 11·2%).

7-*Carbethoxyamino*-9 : 10-*dimethylphenanthridinium Bromide* (1600).—7-Carbethoxyamino-9-methylphenanthridine (11 g.) in nitrobenzene (80 ml.) at 150° was converted by methyl sulphate (7 ml.) into the orange methosulphate. The product was characterised as the *bromide*, which crystallised from water in deep yellow plates, m. p. 230° (decomp.) (Found: N, 7·05; Br, 19·5; loss at 100°, 8·85. $C_{18}H_{19}O_2N_2Br,2H_2O$ requires N, 6·8; Br, 19·45; H_2O, 8·75%).

7-*Amino*-9 : 10-*dimethylphenanthridinium Chloride* (1596).—7-Acetamido-9-methylphenanthridine was similarly converted into the *methosulphate* which crystallised from water in clumps of yellow needles, m. p. 270—272° (decomp.) (Found: C, 57·15; H, 5·35; S, 8·7. $C_{18}H_{20}O_5N_2S$ requires C, 57·45; H, 5·3; S, 8·5%). Hydrolysis with hot 5N-hydrochloric acid followed by neutralisation and addition of potassium iodide gave brownish-red crystals of 7-*amino*-9 : 10-*dimethylphenanthridinium iodide* (1571), m. p. 261° (decomp.) (Found: N, 8·0; I, 35·85. $C_{15}H_{15}N_2I$ requires N, 8·0; I, 36·25%). The *chloride*, which is extremely soluble in water, is formed by double decomposition of the iodide with a boiling aqueous suspension of silver chloride. Evaporation of the filtrate to dryness left a gum which was converted by hot alcohol into a mass of brown prisms, m. p. 260° (decomp.) (Found: Cl, 13·3. $C_{15}H_{15}N_2Cl$ requires Cl, 13·7%). The *bromide* (1597) crystallised from water in orange-red needles, m. p. 269° (decomp.) (Found: Br, 26·35. $C_{15}H_{15}N_2Br$ requires Br, 26·4%).

2-*Amino*-4 : 4′-*dicarbethoxyaminodiphenyl* (IV).—Well-powdered 2-nitro-4 : 4′-dicarbethoxyaminodiphenyl (5·5 g.) was heated with water (20 ml.), hydrochloric acid (1 drop), and reduced iron (5·5 g.). After 1 hour the mixture was extracted with boiling spirit. The filtrate was distilled to small bulk, the non-basic *amino*-compound crystallising out in white acicular prisms, m. p. 186° (Found: C, 62·9; H, 6·15: N, 12·4. $C_{18}H_{21}O_4N_3$ requires C, 63·0; H, 6·1; N, 12·25%). When the amine was dissolved in acetic anhydride the *acetyl* compound crystallised in small white needles, m. p. 199° (Found: N, 10·95. $C_{20}H_{23}O_5N_3$ requires N, 10·9%).

2 : 7-*Dicarbethoxyamino*-9-*phenylphenanthridine*.—A solution of the foregoing amino-compound (36 g.) in nitrobenzene (100 ml.) at 150° was treated with benzoyl chloride (10·5 ml.), hydrogen chloride being evolved. After 30 minutes at this temperature, the solution was cooled and then diluted with 2 volumes of spirit. The *benzoyl* compound which separated (35 g.) recrystallised from spirit in colourless prisms, m. p. 147° (Found: C, 66·85; H, 5·6; N, 9·45. $C_{25}H_{25}O_5N_3$ requires C, 67·1; H, 5·6; N, 9·4%). This product (20 g.) was refluxed gently for 1 hour with phosphorus oxychloride (40 ml.) by which time evolution of hydrogen chloride had ceased and the solution had become yellow. On being stirred into dilute aqueous ammonia the reaction mixture was converted into a granular yellow solid, which was dissolved in 150 ml. of spirit, treated with a few drops of ammonia to discharge the orange colour, and finally with 40 ml. of water. Flocculent yellowish needles of 2 : 7-*dicarbethoxyamino*-9-*phenylphenanthridine* (13·2 g.) separated, a product surprisingly readily soluble in alcohol, but sparingly soluble in benzene. For purification advantage was taken of its slightly basic character. It was dissolved in nitrobenzene (90 ml.) and a stream of dry hydrogen chloride led into the solution, a complex with the latter being precipitated in minute yellow crystals. When a solution of this complex in alcohol was treated with dilute ammonia the base crystallised, sometimes in prisms. Recrystallisation from toluene gave prisms, and from aqueous methyl alcohol, needles, m. p. 222° (efferv.) (Found for solvent-free compound: C, 69·8; H, 5·3; N, 9·9. $C_{25}H_{23}O_4N_3$ requires C, 70·0; H, 5·25; N, 9·8%).

2 : 7-*Dicarbethoxyamino*-9-*phenyl*-10-*methylphenanthridinium methosulphate* crystallised in high yield when the foregoing substance, dissolved in nitrobenzene at 160°, was treated with a small excess of methyl sulphate, orange plates being formed, m. p. 278° (decomp.) (Found: N, 7·35; S, 5·8. $C_{27}H_{29}O_8N_3S$ requires N, 7·55; S, 5·75%). The corresponding chloride was identical with that described in the previous paper.

The methosulphate (1 g.) was dissolved in sulphuric acid (2 ml. of concentrated acid, 1 ml. of water) and heated at 135° for 20 minutes. On dilution with water and partial neutralisation a red *acid sulphate* crystallised (Found for dried salt: S, 11·85. $(C_{20}H_{18}N_3)_2SO_4,2{\cdot}5H_2SO_4$ requires S, 11·9%). It was dissolved in water, potassium bromide added, and the solution neutralised with ammonia; characteristic purple prisms of 2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide crystallised (*J.*, 1945, 294).

2 : 7-*Diamino*-9-*methylphenanthridine*.—2-Acetamido-4 : 4′-dicarbethoxyaminodiphenyl (5 g.) and phosphorus oxychloride (10 ml.) reacted vigorously with separation of a yellow crystalline precipitate. After decomposition of excess of acid chloride with water and neutralisation a pale yellow solid was obtained which was dissolved in 50 ml. of pyridine. An equal volume of hot water was added, and with cooling an almost white micro-crystalline precipitate formed (3·7 g.). Recrystallisation from nitrobenzene gave almost colourless needles of 2 : 7-*dicarbethoxyamino*-9-*methylphenanthridine*, m. p. 253° (efferv.) (Found: C, 65·7; H, 5·85; N, 11·6. $C_{20}H_{21}O_4N_3$ requires C, 65·4; H, 5·7; N, 11·45%). Hydrolysis with sulphuric acid followed by neutralisation yielded the *diamino*-compound which crystallised from

a large volume of alcohol in yellow plates, m. p. 265·5° (Found: C, 75·2; H, 6·05; N, 18·6. $C_{14}H_{13}N_3$ requires C, 75·35; H, 5·9; N, 18·85%). The *acetyl* compound formed white needles, m. p. 323—326° (decomp.) (Found: N, 13·3. $C_{16}H_{17}O_3N_3$ requires N, 13·7%).

2 : 7-*Diamino*-9 : 10-*dimethylphenanthridinium Bromide* (1580).—Reaction in nitrobenzene at 160° of 2 : 7-dicarbethoxyamino-9-methylphenanthridine with methyl sulphate gave 2 : 7-*dicarbethoxyamino*-9 : 10-*dimethylphenanthridinium methosulphate* (1582) in yellow prisms, unmolten at 320°, soluble in water but best recrystallised from alcohol (Found for dried salt: N, 8·85; S, 6·65. $C_{22}H_{27}O_8N_3S$ requires N, 8·5; S, 6·5%). The *chloride* (1598) crystallised from aqueous alcohol in microscopic yellow prisms unmolten at 320° (Found: Cl, 8·5. $C_{21}H_{24}O_4N_3Cl$ requires Cl, 8·5%). Aqueous solutions of these salts are liable to supersaturation and to setting as gels. 2 : 7-*Diacetamido*-9 : 10-*dimethylphenanthridinium chloride* (1581) was similarly prepared; orange needles, unmolten at 320° (Found: N, 12·0; Cl, 9·75. $C_{19}H_{20}O_2N_3Cl$ requires N, 11·75; Cl, 9·9%). Hydrolysis of either of these salts gave acid solutions from which on addition of potassium bromide and neutralisation 2 : 7-*diamino*-9 : 10-*dimethylphenanthridinium bromide* crystallised, m. p. 283° (Found for dried salt: N, 13·45; Br, 24·6. $C_{15}H_{16}N_3Br$ requires N, 13·2; Br, 25·15%). The *chloride* (1605) formed purple plates, m. p. 278°, extremely soluble in water (Found: Cl, 12·95. $C_{15}H_{16}N_3Cl$ requires Cl, 12·95%). The *iodide* (1606) formed sparingly soluble dark purple felted needles, m. p. 293° (Found: I, 34·95. $C_{15}H_{16}N_3I$ requires I, 34·8%).

2-*Nitro*-4′-*aminodiphenyl*.—*p*-Nitrodiphenyl (20 g.) was nitrated according to Bell and Kenyon (*J.*, 1926, 2707) and the crude mixture of dinitro-compounds (26 g.) was extracted thrice with 100 ml. of boiling spirit. The undissolved residue (12 g.) was practically pure 4 : 4′-dinitrodiphenyl, and from the extract a mixture (*ca.* 12 g.) of this substance and 2 : 4′-dinitrodiphenyl was obtained. Separation of the latter was not attempted, the mixture being employed in the next stage of partial reduction. The methods described in the literature (cf. Finzi and Bellavita, *Gazzetta*, 1934, **64**, 335) are unsuitable except for the preparation of small quantities of nitro-amine, but that used by Purdie (*J. Amer. Chem. Soc.*, 1941, **43**, 2276) for the partial reduction of 2 : 2′-dinitrodiphenyl was profitably adapted. The mixture of nitro-compounds was dissolved in boiling alcohol (180 ml.), and the solution was treated with sodium polysulphide prepared from flowers of sulphur (3·6 g.), crystalline sodium sulphide (14 g.), and water (45 ml.). After 3 hours' refluxing the solution was left overnight and then poured into water. The rather sticky red precipitate was dissolved in hot N-hydrochloric acid (180 ml., charcoal); on being cooled the solution filled with white felted needles of the hydrochloride of 2-nitro-4′-aminodiphenyl, by-products remaining in solution. The salt was washed with cold dilute hydrochloric acid, dissolved in hot water, and the solution basified to liberate the amine as a red oil which soon crystallised (7 g.). This product was pure enough for the purpose, but could be recrystallised from spirit in red prisms, m. p. 100° (Found: N, 13·25. Calc. for $C_{12}H_{10}O_2N_2$: N, 13·1%).

2-*Amino*-4′-*carbethoxyaminodiphenyl* (VI).—The foregoing nitroamine (7 g.) was converted into 2-*nitro*-4′-*carbethoxyaminodiphenyl* by the method described for the preparation of 2-acetamido-4′-carbethoxyaminodiphenyl. The product (7 g.) crystallised from spirit in transparent yellow plates, m. p. 105·5° (Found: C, 62·85; H, 4·8; N, 10·0. $C_{15}H_{14}O_4N_2$ requires C, 62·9; H, 4·9; N, 9·8%). It was reduced by an equal weight of reduced iron in acidified aqueous suspension. Extraction of the reaction mixture with hot spirit followed by removal of the solvent gave the *amine*, which was extremely soluble in alcohol, benzene, and dilute mineral acid. It recrystallised from petrol–benzene in transparent buff plates, m. p. 98° (Found: C, 70·5; H, 6·25; N, 11·05. $C_{15}H_{16}O_2N_2$ requires C, 70·3; H, 6·25; N, 10·95%).

7-*Carbethoxyamino*-9-p-*nitrophenylphenanthridine* (VIII).—2-Amino-4′-carbethoxyaminodiphenyl (27 g.) was condensed in hot pyridine solution (88 ml.) with *p*-nitrobenzoyl chloride (23 g.). The product (44·5 g.) liberated by dilution of the reaction mixture with N-sulphuric acid was extracted with dilute sodium carbonate solution, and the residue was dissolved in boiling spirit. After separation of a small amount of insoluble material and cooling, 2-p-*nitrobenzamido*-4′-*carbethoxyaminodiphenyl* (36 g.) crystallised in transparent yellow prisms, sometimes accompanied by needles, m. p. 184° (Found: C, 65·45; H, 4·75; N, 10·65. $C_{22}H_{19}O_5N_3$ requires C, 65·3; H, 4·7; N, 10·35%). The following method of ring-closure gave the best result. The acyl compound (21·5 g.), nitrobenzene (72 ml.), and phosphorus oxychloride (43 ml.) were heated in a bath at 150—160° for 1 hour and then stirred into ice-cold dilute aqueous ammonia. The solid *product* (12·8 g.) was separated from the nitrobenzene and washed successively with water, a little nitrobenzene and spirit. Crystallisation from pyridine afforded clumps of feathery yellow needles, but transparent prisms were obtained from chlorobenzene, m. p. 254° (Found: C, 68·3; H, 4·4; N, 11·0. $C_{22}H_{17}O_4N_3$ requires C, 68·25; H, 4·4; N, 10·85%). Steam-distillation of the nitrobenzene mother-liquor furnished a further quantity of the product which was best purified at the next stage. Reaction of the product in nitrobenzene solution with methyl sulphate afforded 7-*carbethoxyamino*-9-p-*nitrophenyl*-10-*methylphenanthridinium methosulphate*, which crystalllsed from water in transparent orange prisms, m. p. 209° (decomp.) (Found: N, 8·05; S, 5·75; loss at 100°, 3·25. $C_{24}H_{23}O_8N_3S,H_2O$ requires N, 7·9; S, 6·0; H_2O, 3·4%). The *chloride* was readily obtained from this salt, or by the action of ethyl chloroformate on the aqueous solution of the nitroamino-quaternary salt (see below). It crystallised from the reaction mixture in transparent yellow prisms, m. p. 243° (decomp.) (Found: N, 9·5; Cl, 8·0. $C_{23}H_{20}O_4N_3Cl$ requires N, 9·6; Cl, 8·1%).

7-*Amino*-9-p-*nitrophenylphenanthridine* was obtained by sulphuric acid hydrolysis of the urethane. It crystallised from nitrobenzene in red plates, m. p. 279° (Found: C, 72·5; H, 4·25; N, 13·5. $C_{19}H_{13}O_2N_3$ requires C, 72·4; H, 4·15; N, 13·3%). When a solution of the amine in pyridine was treated with acetic anhydride the *acetyl* compound crystallised in lemon-yellow needles; recrystallised from alcohol, m. p. 282° (Found: C, 68·4; H, 4·9; N, 10·1. $C_{21}H_{15}O_3N_3,C_2H_6O$ requires C, 68·5; H, 5·15; N, 10·4%). This compound afforded an almost quantitative yield of 7-*acetamido*-9-p-*nitrophenyl*-10-*methylphenanthridinium methosulphate*, which crystallised from water in talc-like yellow crystals, m. p. 267·5° (decomp.) (Found for dried salt: N, 8·5; S, 6·45. $C_{23}H_{21}O_7N_3S$ requires N, 8·7; S, 6·6%).

7-*Acetamido*-9-p-*aminophenyl*-10-*methylphenanthridinium Methosulphate* (1593).—A hot aqueous solution of the foregoing salt was rapidly reduced by iron powder, the filtrate depositing orange-yellow prisms of the *amino*-salt, m. p. 258° (decomp.) (Found for dried salt: N, 9·4; S, 7·3. $C_{23}H_{23}O_5N_3S$ requires N, 9·25; S, 7·05%). Acetylation with hot acetic anhydride afforded the diacetamido-salt previously described (*J.*, 1938, 395), and reaction of its aqueous solution with ethyl chloroformate converted it quantitatively into 7-*acetamido*-9-p-*carbethoxyaminophenyl*-10-*methylphenanthridinium chloride* (1595) which crystallised from water in golden-yellow plates, m. p. 200—206° (decomp.) (Found: N, 8·0; Cl, 6·75; loss at 100°, 12·15. $C_{25}H_{24}O_3N_3Cl,3·5H_2O$ requires N, 8·2; Cl, 6·9; H_2O, 12·3%).

7-*Carbethoxyamino*-9-p-*aminophenyl*-10-*methylphenanthridinium chloride* (1592) was the product of reduction of the corresponding nitro-compound in aqueous solution with iron powder, transparent orange prisms being obtained from alcohol, m. p. 297—300° (decomp.) (Found: N, 10·45; Cl, 8·45. $C_{23}H_{22}ON_3Cl$ requires N, 10·3; Cl, 8·7%). Acetylation of this salt afforded 7-*carbethoxyamino*-9-p-*acetamidophenyl*-10-*methylphenanthridinium chloride* (1594), which resembled the corresponding diacetyl and dicarbethoxyl salts in crystallising from water in two forms, *viz.*, transparent yellow prisms and microscopic needles, m. p. 213° (decomp.) (Found for dried salt: N, 9·25; Cl, 7·85. $C_{25}H_{24}O_3N_3Cl$ requires N, 9·35; Cl, 7·9%).

7-*Amino*-9-p-*nitrophenyl*-10-*methylphenanthridinium Chloride* (1586).—The corresponding acetamido-salt (2·5 g.) was hydrolysed by heating its solution in sulphuric acid (5 ml. of concentrated acid, 2·5 ml. of water) at 125° for 15 minutes. On being diluted with water the solution deposited white crystals of an acid salt, but neutralisation by ammonia and addition of sodium chloride caused dark red transparent plates of the *nitroamino*-salt to crystallise; m. p. 242° (decomp.) (Found for dried salt: N, 12·0; Cl, 9·5. $C_{20}H_{16}O_2N_3Cl$ requires N, 11·5; Cl, 9·7%).

4-*Nitro*-2′-o-*nitrobenzamidodiphenyl* crystallised from glacial acetic acid in buff plates, m. p. 230—231° (Found: N, 11·4. $C_{19}H_{11}O_4N_3$ requires N, 11·6%). Heating with phosphorus oxychloride caused profound decomposition, and from the product no crystalline substance could be isolated.

7-*Carbethoxyamino*-9-o-*nitrophenylphenanthridine*.—2-Amino-4′-carbethoxyaminodiphenyl (10 g.) in boiling chlorobenzene solution (50 ml.) condensed with *o*-nitrobenzoyl chloride (7·8 g.) to yield 2-o-*nitrobenzamido*-4′-*carbethoxyaminodiphenyl*, which crystallised (13·5 g.) from the cold reaction mixture and gave on recrystallisation from spirit almost colourless acicular prisms (10 g.), m. p. 197° (Found: C, 65·1; H, 4·6; N, 10·4. $C_{22}H_{19}O_5N_3$ requires C, 65·3; H, 4·7; N, 10·4%). Although the crude product which crystallised from the chlorobenzene reaction mixture contained a little diacyl compound it could be used without recrystallisation for the next stage. The product of reaction of the acyl compound (10 g.) and phosphorus oxychloride (20 ml.) on the steam-bath for 1 hour was liberated by cold dilute aqueous ammonia. It was purified from tarry matter by filtration of its solution in benzene (250 ml.) through a column (11 cm. × 2·5 cm. diam.) of alumina (B.D.H., for chromatographic analysis), elution being completed with a further 150 ml. of benzene. Impurities were strongly adsorbed at the top of the column, and the golden-yellow filtrate after evaporation to 100 ml. yielded bright yellow needles of 7-*carbethoxyamino*-9-o-*nitrophenylphenanthridine* (6 g.), m. p. 199° (Found: C, 68·4; H, 4·25; N, 11·3. $C_{22}H_{17}O_4N_3$ requires C, 68·25; H, 4·4; N, 10·95%); on methylation in nitrobenzene it gave 7-*carbethoxyamino*-9-o-*nitrophenyl*-10-*methylphenanthridinium methosulphate*, which crystallised from water in transparent yellow plates, m. p. 226° (decomp.) (Found: N, 7·65; S, 5·55; loss at 100°, 6·75, 6·35. $C_{24}H_{23}O_8N_3S,2H_2O$ requires N, 7·65; S, 5·8; H_2O, 6·55%).

7-*Amino*-9-o-*nitrophenylphenanthridine* was obtained by hydrolysis of the carbethoxyamino-compound with sulphuric acid. Sparingly soluble in benzene or alcohol, it crystallised from chlorobenzene in brown transparent prisms, m. p. 230° (Found: C, 71·85; H, 4·1; N, 13·2. $C_{19}H_{13}O_2N_3$ requires C, 72·3; H, 4·1; N, 13·3%). A paste of the amine (5 g.) in hot glacial acetic acid (10 ml.) was treated with acetic anhydride(4 ml.) whereupon solution rapidly took place followed by separation of the *acetyl* compound in clumps of white prisms, m. p. 287·5° (Found: C, 70·3; H, 4·3; N, 11·9. $C_{21}H_{15}O_3N_3$ requires C, 70·6; H, 4·25; N, 11·75%).

7-*Acetamido*-9-o-*nitrophenyl*-10-*methylphenanthridinium methosulphate* gave deep yellow talc-like crystals from water, m. p. 283° (decomp.) (Found: N, 8·5; S, 6·3; loss at 100°, 3·6. $C_{23}H_{21}O_7N_3S,H_2O$ requires N, 8·4; S, 6·4; H_2O, 3·6%).

7-*Acetamido*-9-o-*aminophenyl*-10-*methylphenanthridinium Chloride* (1590).—Reduction of the foregoing nitro-salt with iron powder was not satisfactory, and the following method was used. A boiling suspension of the salt (2 g.) in alcohol (40 ml.) was treated with stannous chloride (3·2 g.) and concentrated hydrochloric acid (2·8 ml.). When a clear solution had been obtained dilute ammonia was added to neutralise it partially and then alcohol was evaporated. On being cooled the solution deposited crystals of the *amino*-salt, which on recrystallisation from water furnished deep yellow matted needles (1 g.), m. p. 271·5° (decomp.) (Found: N, 9·95; Cl, 8·4; loss at 100°, 7·7. $C_{22}H_{20}ON_3Cl,2H_2O$ requires N, 10·15; Cl, 8·6; H_2O, 8·7%).

7-*Carbethoxyamino*-9-o-*aminophenyl*-10-*methylphenanthridinium Chloride* (1579).—Again, reduction of the corresponding nitro-salt with iron powder was unsatisfactory, but readily took place in alcoholic solution with stannous chloride. Addition of water to the reaction mixture caused the *product* to crystallise in an almost quantitative yield of small brown prisms which were purified by recrystallisation from water; m. p. 272° (decomp.) (Found: N, 9·9; Cl, 8·25; loss at 100°, 4·2. $C_{23}H_{22}O_2N_3Cl,H_2O$ requires N, 9·85; Cl, 8·35; H_2O, 4·25%).

From the solution of this salt in acetic anhydride golden yellow needles of 7-*carbethoxyamino*-9-o-*acetamidophenyl*-10-*methylphenanthridinium chloride* (1591) crystallised. On recrystallisation from water this salt had m. p. 203—205° (decomp.) (Found: N, 8·75, Cl, 7·2; loss at 100°, 7·65. $C_{25}H_{24}O_3N_3Cl,2H_2O$ requires N, 8·65; Cl, 7·3; H_2O, 7·4%).

7-*Amino*-9-o-*nitrophenyl*-10-*methylphenanthridinium Chloride* (1587) —7-Carbethoxyamino-9-*o*-nitrophenyl-10-methylphenanthridinium methosulphate was hydrolysed by sulphuric acid, and the reaction mixture diluted with water and neutralised by ammonia to liberate a sulphate as a red oil which slowly

crystallised. The corresponding *chloride* crystallised from water in red prisms which had the surprisingly low m. p. 86° (Found for dried salt: N, 11·65; Cl, 9·5. $C_{20}H_{16}O_2N_3Cl$ requires N, 11·5; Cl, 9·7%).

7-*Amino*-9-*o*-*aminophenyl*-10-*methylphenanthridinium Chloride* (1588).—A solution of the foregoing nitro-amino-salt in water was heated for 30 minutes with an equal weight of iron powder. The red filtrate was made just alkaline with sodium carbonate, separated from an amorphous red precipitate, neutralised with hydrochloric acid, and distilled under reduced pressure to small bulk. On being cooled the solution deposited ruby-red prisms of the *diamino*-salt, m. p. *ca.* 158° (decomp.) (Found: N, 11·4; Cl, 9·65; loss at 100°, 7·8. $C_{20}H_{18}N_3Cl$, $1{\cdot}5H_2O$ requires N, 11·6; Cl, 9·8; H_2O, 7·45%).

When an aqueous solution of this salt was stirred with ethyl chloroformate an orange precipitate formed, which on crystallisation from water gave 7-carbethoxyamino-9-*o*-aminophenyl-10-methylphenanthridinium chloride. Likewise when the salt was heated with acetic anhydride it was converted into 7-acetamido-9-*o*-aminophenyl-10-methylphenanthridinium chloride, which is almost insoluble in acetic anhydride. When either the latter compound or the diamino-salt was heated in glacial acetic acid solution with acetic anhydride no product crystallised, but, on addition of water and sodium chloride the diacetylated salt, 7-*acetamido*-9-*o*-*acetamidophenyl*-10-*methylphenanthridinium chloride* (1589) crystallised in buff plates, m. p. 240·5° (decomp.) (Found: N, 8·8; Cl, 7·2; loss at 100°, 14·7. $C_{24}H_{22}O_2N_3Cl,3H_2O$ requires N, 8·55; Cl, 7·2; H_2O, 14·6%).

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