

11. *Potential Trypanocides of the N-Heterocyclic Series. Part II. Analogues of Dimidium Bromide.*

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The synthesis of analogues of dimidium bromide (III; R = Ph), in which the 9-phenyl group is replaced by a *cyclohexyl*, benzyl, or heteromonocyclic nucleus, has been investigated for correlation of structure with trypanocidal activity. The phenanthridines (II) were readily obtained, but for steric reasons the *cyclohexyl* compound did not give a quaternary salt. The furyl compound (II*b*) decomposed when treated with methyl sulphate, and the pyridylphenanthridine (II*c*) yielded a pyridinium salt when treated with methyl iodide or methyl sulphate. The benzyl- and 2-thienyl-phenanthridines (II) were converted into phenanthridinium salts, hydrolysis of which gave respectively highly effective trypanocides of type (III). The dihydropranyphenanthridine (II*d*) could be quaternised, but an attempt to hydrolyse the urethane groups of the salt led to decomposition.

2:7-Diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (III; R = *p*-NH₂·C₆H₄, A = Cl) and its precursor (III; R = *p*-NO₂·C₆H₄, A = Cl) are valuable trypanocides, the former being the most active phenanthridinium compound (*T. congolense* and *T. rhodesiense*) investigated to date. The triamino-salt was also obtained by an alternative synthesis.

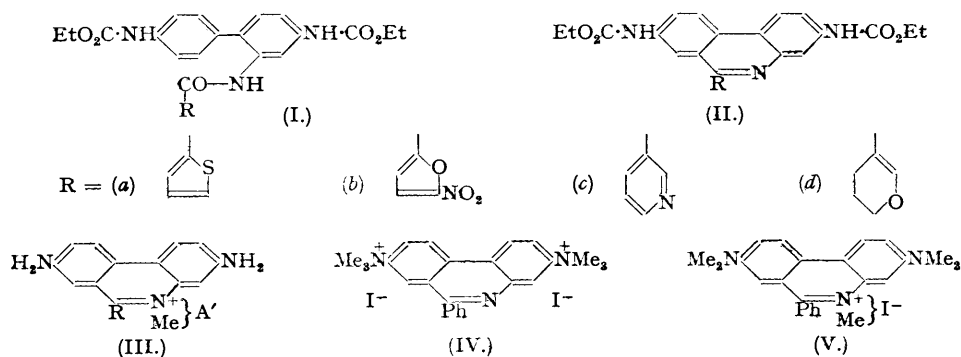
THE most active trypanocide of the phenanthridine series yet described is dimidium bromide (III; R = Ph), and it was early suggested (*J.*, 1945, 296) that its activity might be associated in part with the 2:7-arrangement of the amino-groups. The molecule contains a benzidine structure and it is well known that derivatives of benzidine are substantive towards vegetable fibres, and that a similar substantivity is frequently a property of trypanocidal drugs. It was desirable to test this hypothesis further by preparing other compounds of the series, which preserve the benzidine structure, but differ in the nature of the 9-substituent. The first compound of this type (III; R = Me) (*J.*, 1947, 68) to be tested is a much less effective trypanocide than dimidium bromide, but this may be the consequence of metabolism rather than of a reduced intrinsic trypanocidal activity. Compounds of the "Sontochin" type, which contain a 3-methylquinoline nucleus, are valuable antimalarials, but analogous compounds with a quinaldine nucleus are inactive and this striking difference is perhaps caused by the presence of the reactive α -methyl group, which may act as a focus for metabolic attack (Walker, *J.*, 1947, 1553). In the phenanthridine series the 9-methyl group is similarly highly reactive.

The 9-*cyclohexyl* compound differs little from dimidium bromide in fundamental structure and for this reason its synthesis was attempted. *cycloHexanecarboxyl chloride* and 2-amino-4'-biscarbethoxyaminodiphenyl in pyridine furnished a good yield of the *amide* (I; R = C₆H₁₁), which was cyclised by phosphoryl chloride to 2:7-*biscarbethoxyamino-9-cyclohexyl-phenanthridine* (II; R = C₆H₁₁). A quaternary salt could not be obtained from this substance: treatment with nitrobenzene-methyl sulphate at 170° and cooling gave the *sulphate* of the base, but with methyl toluene-*p*-sulphonate no reaction occurred. Even with carefully purified methyl sulphate alone, some sulphate was formed. This experience recalls the similar product obtained from 6-acetamido-2-*p*-aminostyrylquinoline by Browning, Cohen, Cooper, and Gulbransen (*Proc. Roy. Soc.*, B, 1931, 109, 53), which may very likely have been a sulphate

rather than the postulated complex with methyl sulphate. The failure of the cyclohexyl compound to yield quaternary salts is explicable by steric hindrance: models reveal that the cyclohexyl group offers much more obstruction to accommodation of a methyl group on N₍₁₀₎ than does a phenyl group.

With (III; R = CH₂Ph) an intermediate type between dimidium bromide and (III; R = Me) is under consideration; the methylene group might likewise be regarded as a point of metabolic attack but oxidation of the benzyl compound is less likely to involve the loss of the essential quaternary system. However, 7-amino-9-*p*-aminobenzyl-10-methylphenanthridinium bromide is very much less active than the corresponding *p*-aminophenyl compound (Part I). Condensation of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl and phenylacetyl chloride afforded 2-phenylacetamido-4 : 4'-biscarbethoxyaminodiphenyl (I; R = CH₂Ph) which was smoothly cyclised to 2 : 7-biscarbethoxyamino-9-benzylphenanthridine (II; R = CH₂Ph); successive quaternisation and hydrolysis converted this compound into the desired salt (III; R = CH₂Ph, A = Br), which is highly trypanocidal (*Trypanosoma congolense*) although less effective than dimidium bromide.

The effect of replacement of the 9-phenyl group of dimidium bromide by heterocyclic nuclei was also examined, readily accessible nuclei of widely different type being used, namely 2-thienyl, 5-nitro-2-furyl, 3-pyridyl, and 5 : 6-dihydro-3-pyranyl. 2-Acetylthiophen was oxidised by sodium hypochlorite to thiophen-2-carboxylic acid and the derived chloride was condensed with 2-amino-4 : 4'-biscarbethoxyaminodiphenyl to yield 2-(thiophene-2''-carboxyamido)-4 : 4'-biscarbethoxyaminodiphenyl (Ia). Cyclisation then furnished 2 : 7-biscarbethoxyamino-9-2'-thienylphenanthridine (IIa), and this was converted by the customary methods into the quaternary salt (III; R = 2-thienyl, A = Br), which is a more effective trypanocide (*T. congolense*) than dimidium bromide. The 5-nitro-2-furyl nucleus has already been associated with biologically active compounds: furacin (5-nitro-2-furaldehyde semicarbazone) is an effective antibacterial and as a trypanocide (Dodd and Stillman, *J. Pharm. Exp. Ther.*, 1944, 82, 11). 5-Nitro-2-furoic acid led, as above, to 2 : 7-biscarbethoxyamino-9-(5-nitro-2-furyl)phenanthridine (IIb), but attempts to form quaternary salts caused profound decomposition.



Petrow and Wragg (*J.*, 1947, 1410) have reported that the synthesis of 9-pyridylphenanthridines from 2-nicotinamidodiphenyls may result in low yields, even after prolonged heating with nitrobenzene-phosphoryl chloride. In the initial reaction between the amide and phosphoryl chloride, hydrogen chloride is liberated and thus a proton becomes bound to the pyridine-N. The inductive effect of this positive pole appears to decrease the electrophilic affinity of the amide-C atom and hence retards cyclisation (ring-closure being an electrophilic-substitution reaction). However, in accordance with our experience of *o*-acylamidodiphenyls with urethane substituents, cyclisation of 2-nicotinamido-4 : 4'-biscarbethoxyaminodiphenyl (Ic) proceeded under milder conditions, although the yield of the phenanthridine (IIc) was rather low (25%). Under similar conditions the methochloride of the foregoing amide was recovered unchanged, in confirmation of the retarding effect on ring-closure of a positive pole in the acyl group. With methyl sulphate in nitrobenzene at 135°, or preferably with methyl iodide in refluxing dioxan, the pyridylphenanthridine gave a gum from which, by addition of an excess of aqueous naphthalene-β-sulphonic acid, a colourless, 1'-methonaphthalene-β-sulphonate 10-naphthalene-β-sulphonate was obtained. This salt, when treated with aqueous sodium acetate, gave a yellow methonaphthalene-β-sulphonate and it is certain that this was the pyridinium salt (Petrow and Wragg, *loc. cit.*) and

not the phenanthridinium salt; since attempts at hydrolysis of the urethane groups did not lead to crystalline products, the series was not examined further. To complete the investigation with 9-heterocyclic substituents, 5:6-dihydropyran-3-carboxyl chloride (B.P. 570,974) was condensed with 2-amino-4:4'-biscarbethoxyaminodiphenyl and the resultant *amide* (Id) cyclised to 2:7-biscarbethoxyamino-9-(5:6-dihydro-3-pyranyl)phenanthridine (II*d*). Treatment of this compound with methyl sulphate, followed by metathesis with dilute hydrochloric acid, yielded a yellow *methochloride* but it was not possible to convert this into the diamino-quaternary salt since acid hydrolysis also affected the dihydropyran ring (Schniepp and Geller, *J. Amer. Chem. Soc.*, 1946, **68**, 1646).

A new approach to the production of more active trypanocides followed from the following considerations. 9-Phenyl-10-methylphenanthridinium chloride itself exhibits slight trypanocidal activity; with an amino-substituent in the diphenyl portion of the molecule this activity becomes significant, and reaches a high level with two such substituents (as in dimidium bromide), although one of the two amino-groups may then be located in the 9-phenyl group (*J.*, 1945, 297). It was of interest therefore to examine whether a third amino-group would further enhance the therapeutic effect. 2-*p*-Nitrobenzamido-4:4'-biscarbethoxyaminodiphenyl (I; R = *p*-NO₂·C₆H₄) was cyclised to 2:7-biscarbethoxyamino-9-*p*-nitrophenylphenanthridine (II; R = *p*-NO₂·C₆H₄) by phosphoryl chloride. This reacted readily in nitrobenzene with methyl sulphate to give the quaternary *methosulphate*, which afforded a good yield of 2:7-diamino-9-*p*-nitrophenyl-10-methylphenanthridinium chloride (III; R = *p*-NO₂·C₆H₄, A = Cl) when hydrolysed with sulphuric acid (*d* 1.66) at 125–130°. If the reaction temperature was increased to 150–155° some loss of the quaternary group occurred concurrently with hydrolysis of the urethane groups. Reduction of (III; R = *p*-NO₂·C₆H₄, A = Cl) with iron powder and water was unsatisfactory, and the method was not applicable to the corresponding *nitro-diacetamido*-salt. During an examination of numerous reducing agents, however, it was found that heating this salt in water with a 30% excess of ferrous hydroxide (prepared from stoichiometric quantities of ferrous sulphate and barium hydroxide) gave the *amino-diacetamido*-salt almost quantitatively, and from this 2:7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (III; R = *p*-NH₂·C₆H₄, A = Cl) was readily obtained by dilute hydrochloric acid. Similarly, by reduction with ferrous hydroxide (a method better suited to nitro-quaternary salts in general than is iron powder, a cleaner product being obtained), the salt (III; R = *p*-NO₂·C₆H₄, A = Cl) was converted directly and quantitatively into the *triamino*-salt (III; R = *p*-NH₂·C₆H₄, A = Cl). Both the nitro-diamino- and triamino-salts are highly trypanocidal, the former being at least equal to dimidium bromide in *T. congolense* infections in mice and dogs, and the latter markedly more active and somewhat less (acutely) toxic. The triamino-salt is also highly active in *T. rhodesiense* infections in mice; in this respect it much exceeds any other phenanthridinium compound yet investigated, being as active as pentamidine although more toxic. None of the compounds here described has a prophylactic action against trypanosomes comparable with that of pentamidine. In an alternative route to the triamino-salt, 4:4'-dinitro-2-*p*-nitrobenzamidodiphenyl was converted into 2:7-dinitro-9-*p*-nitrophenylphenanthridine which, unlike dinitro-compounds of the same type, did not yield quaternary salts. The corresponding *triamino*-compound, which is devoid of trypanocidal activity, was converted into the *triacetyl* derivative from which a quaternary *methosulphate* was obtained in rather poor yield. This salt was hydrolysed by dilute acid to the triamino-salt (III; R = *p*-NH₂·C₆H₄, A = Cl).

By methods similar to those already described, 4:4'-biscarbethoxyamino-2-*p*-anisoamido-diphenyl (I; R = *p*-MeO·C₆H₄) was converted into 2:7-biscarbethoxyamino-9-*p*-methoxyphenylphenanthridine (II; R = *p*-MeO·C₆H₄) and its *methosulphate*, but the product of hydrolysis of the latter, although undoubtedly containing salts of the type (III), could not be obtained crystalline owing, it is believed, to some simultaneous hydrolysis of the methoxyl group.

Modification of dimidium bromide in a different way was effected as follows. Methylation of 2:7-diamino-9-phenylphenanthridine (*J.*, 1945, 299) in aqueous methanol by methyl iodide in the presence of sodium carbonate furnished the white bisquaternary *iodide* (IV) in good yield. Pyrolysis of this salt at 180° caused loss of one mole of methyl iodide and formation of 2:7-bisdimethylamino-9-phenyl-10-methylphenanthridinium iodide (V). The corresponding *bromide* is a purple salt, bluer in tint than dimidium bromide, possessing the high antibacterial activity *in vitro* characteristic of phenanthridinium salts, but both this and (V) are practically inactive against trypanosomes. This result suggests that hydrogen-bonding, or some other reaction between drug and substrate not possible with a tertiary amine, is associated with the trypanocidal action of dimidium bromide and its analogues.

EXPERIMENTAL.

(All the products were dried to constant weight before analysis.)

2-cycloHexanecarboxyamido-4 : 4'-biscarbethoxyaminodiphenyl (I; R = C₆H₁₁).—cycloHexanecarboxyl chloride (26 g.) was added to a suspension of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl (52 g.) in dry pyridine (75 ml.), and the mixture heated on the steam-bath for 15 minutes and then poured into water (2 l.). The oil which separated soon solidified and, when crystallised from alcohol, formed colourless needles (63 g.) of the *amide*, m. p. 184—185° (Found : C, 66.1; H, 6.75; N, 9.5. C₂₅H₃₁O₅N₃ requires C, 66.2; H, 6.85; N, 9.25%).

2 : 7-Biscarbethoxyamino-9-cyclohexylphenanthridine (II; R = C₆H₁₁).—The *amide* (I; R = C₆H₁₁) (63 g.) and phosphoryl chloride (63 ml.) were heated at 130° (bath) for 45 minutes. At the outset there was vigorous evolution of hydrogen chloride, and after 15 minutes a yellow solid began to crystallise. The reaction mixture was stirred with ice and an excess of ammonia for a time, then boiled for a few minutes, and cooled, and the aqueous liquor decanted from the rock-like solid. On being heated with alcohol containing a few ml. of concentrated ammonia solution, this solid gave place to colourless prisms which, after cooling, were collected and washed with water and alcohol. The product, 2 : 7-biscarbethoxyamino-9-cyclohexylphenanthridine (55 g.), had m. p. 233—234°, unchanged on recrystallisation from alcohol-acetone (Found : C, 69.1; H, 6.65; N, 9.55. C₂₅H₂₉O₄N₃ requires C, 68.95; H, 6.65; N, 9.65%). This compound (2 g.), dissolved in nitrobenzene (15 ml.), was treated with methyl sulphate (1.2 ml.) at 170° for 10 minutes. On cooling, a yellow crystalline substance slowly separated, which was not the quaternary salt, for it was hydrolysed by 2N-sodium acetate to the starting material. It crystallised from methanol in small yellow needles, m. p. 227—228° (decomp.), and proved to be the *hydrogen sulphate* of the original base (Found : C, 55.5; H, 5.65; N, 7.8; S, 5.8. C₂₅H₂₉O₄N₃.H₂SO₄ requires C, 56.25; H, 5.8; N, 7.85; S, 6.0%). The same substance was immediately precipitated on addition of sulphuric acid to a hot nitrobenzene solution of the base.

2-Phenylacetamido-4 : 4'-biscarbethoxyaminodiphenyl (I; R = CH₂Ph).—Phenylacetyl chloride (5.5 g.) was added to a hot suspension of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl (12 g.) in chlorobenzene (130 ml.), and the mixture refluxed gently for 30 minutes. On cooling, the pure *amide* crystallised as colourless needles (15.1 g.), m. p. 204—205°, unchanged by recrystallisation from alcohol (Found : C, 68.2; H, 5.95; N, 9.15. C₂₇H₂₇O₅N₃ requires C, 67.65; H, 5.9; N, 9.1%).

2 : 7-Biscarbethoxyamino-9-benzylphenanthridine (II; R = CH₂Ph).—The *amide* (I; R = CH₂Ph) (13.2 g.) and phosphoryl chloride (40 ml.) were refluxed for 1 hour, cooled, and poured into water. After cooling, the deep-yellow solid was collected and dissolved in pyridine, and the solution diluted with water; the precipitate formed was washed thoroughly with water, followed by methanol. The *phenanthridine* (11.4 g.) had m. p. 255° (decomp.) raised to 259° (decomp.) by recrystallisation from acetone or aqueous pyridine (Found : C, 70.55; H, 5.45; N, 9.5. C₂₈H₂₅O₄N₃ requires C, 70.4; H, 5.7; N, 9.5%).

Methyl sulphate (10 ml.) was added to a solution of this base (10 g.) in nitrobenzene (80 ml.) at 170°, and the temperature maintained at 160—165° for 3 minutes. The yellow solid, which crystallised out rapidly on cooling, was collected, washed with benzene, dried, and extracted with boiling water. Addition of 2N-hydrochloric acid to the filtered aqueous solution precipitated the *methochloride* (7.2 g.), m. p. 253° (decomp.), which crystallised from methanol in small bright yellow blades, m. p. 254° (decomp.) (Found : N, 8.35; Cl, 7.15. C₂₇H₂₅O₄N₃Cl requires N, 8.5; Cl, 7.2%).

2 : 7-Diamino-9-benzyl-10-methylphenanthridinium Bromide (III; R = CH₂Ph, A = Br).—The foregoing *methochloride* (7.9 g.), concentrated sulphuric acid (28 ml.), and water (24 ml.) were heated at 150° until effervescence ceased (*ca.* 30 minutes), and then poured into water, and the red solution was carefully neutralised with aqueous ammonia. The gum which separated readily solidified; it was dissolved in water, and the filtered solution treated with potassium bromide to precipitate 2 : 7-diamino-9-benzyl-10-methylphenanthridinium bromide (5.8 g.) as a hydrate of indefinite m. p. Crystallisation from methanol yielded purple needles, m. p. 250—252° (Found : N, 10.65; Br, 20.1. C₂₁H₂₀N₃Br requires N, 10.65; Br, 20.3%).

Thiophen-2-carboxylic Acid.—2-Acetylthiophen (60 g.) (prepared according to Hartough and Kosak, *J. Amer. Chem. Soc.*, 1947, **69**, 1012) was covered with 10% sodium hypochlorite solution (500 g.) and vigorously stirred on the steam-bath. A vigorous reaction ensued and chloroform was evolved. Heating was then discontinued and, when the reaction had subsided, more hypochlorite solution was added in portions of 50 g. until chloroform was no longer evolved. After cooling of the mixture a slight excess of liquid sulphur dioxide was added, and the solution acidified with sulphuric acid and extracted with ether. The extract was shaken with sodium hydroxide solution, and the aqueous layer separated and aerated until all traces of ether were removed. Acidification with sulphuric acid precipitated thiophen-2-carboxylic acid (45—50 g.), m. p. 124—125°.

2'-(Thiophen-2-carboxyamido)-4 : 4'-biscarbethoxyaminodiphenyl (Ia).—A mixture of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl (70.5 g.), nitrobenzene (300 ml.), and thiophen-2-carboxyl chloride (30 g.) was heated in a bath at 150° for 2 hours, and the solution left overnight. The crystalline product was collected, washed with nitrobenzene, treated with hot alcohol (300 ml.), and heated for a few minutes. On cooling, the pure *amide* separated as dense colourless prisms (79 g.), m. p. 197—198°, unchanged on recrystallisation from "Cellosolve" (Found : N, 9.3; S, 7.3. C₂₃H₂₃O₄N₃S requires N, 9.25; S, 7.05%).

2 : 7-Biscarbethoxyamino-9-2'-thienylphenanthridine (IIa).—The *amide* (Ia) (79 g.) and phosphoryl chloride (80 ml.) were heated at 130—135° (bath) for 75 minutes, cooled, poured on ice, and neutralised with aqueous ammonia. The solid product was dissolved in hot pyridine, diluted with water, and cooled, and small quantities of glacial acetic acid were added with stirring until the oil solidified. A solution of this material in hot glacial acetic acid (400 ml.) was treated with concentrated hydrochloric acid (40 ml.) and, after cooling, the red needles were collected and recrystallised from glacial acetic acid. The hydrochloride thus obtained was dissolved in hot alcohol, and neutralised with the minimum quantity of concentrated aqueous ammonia (until the red colour had given place to yellow), and a seed of the product was introduced. Almost immediately the *phenanthridine* separated as pale yellow prisms (35 g.),

m. p. 227—229° (decomp.), raised to 229—230° (decomp.) on recrystallisation from alcohol-acetone (Found: N, 9.75; S 7.4. $C_{23}H_{21}O_4N_2S$ requires N 9.65; S 7.35%).

2 : 7-Biscarbethoxyamino-9-2'-thienyl-10-methylphenanthridinium Chloride.—To (IIa) (16 g.) in nitrobenzene (80 ml.) at 120°, methyl sulphate (24 ml.) was added. The mixture was heated to 135° and then cooled immediately. The orange plates (18 g.) that separated were washed with benzene and dissolved in hot water, and concentrated hydrochloric acid was added to precipitate the methochloride, which crystallised from water containing a few drops of 2N-hydrochloric acid, in fine orange needles (16 g.), m. p. 239° (decomp.) (Found: N, 8.7; S, 6.95; Cl, 7.4. $C_{24}H_{24}O_4N_2ClS$ requires N, 8.65; S 6.6; Cl, 7.3%).

2 : 7-Diamino-9-2'-thienyl-10-methylphenanthridinium bromide (III; R = 2-thienyl, A = Br) was obtained by hydrolysis of the foregoing methochloride (11.5 g.) with aqueous sulphuric acid (*d* 1.53) at 135—140°. From a solution of the crude bromide in hot alcohol a mixture of black needles and a fine crystalline precipitate separated with cooling, of which only the former were soluble in cold water. An aqueous extract was treated with potassium bromide, and the precipitated material crystallised from alcohol in deep-purple prisms and needles of 2 : 7-diamino-9-2'-thienyl-10-methylphenanthridinium bromide (5.6 g.), m. p. 256° (decomp.) (Found: N, 10.9; S, 8.3; Br, 20.5. $C_{15}H_{16}N_2SBr$ requires N, 10.85; S, 8.3; Br, 20.7%).

2-(5-Nitro-2-furoamido)-4 : 4'-biscarbethoxyaminodiphenyl (Ib).—This amide was the product of condensation of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl (75 g.) and 5-nitro-2-furoyl chloride (39 g.) in dry pyridine (150 ml.); it crystallised from glacial acetic acid in yellowish-brown prisms (95 g.), m. p. 223—225° (Found: C, 57.0; H, 4.45; N, 11.85. $C_{23}H_{20}O_8N_4$ requires C, 57.25; H, 4.55; N, 11.6%).

2 : 7-Biscarbethoxyamino-9-(5-nitro-2-furyl)phenanthridine (IIb).—The amide (Ib) (95 g.) was cyclised with phosphoryl chloride. The crude product was dissolved in hot pyridine, water added, and the precipitated black solid refluxed with glacial acetic acid (500 ml.) and cooled. The brown residue (18 g.), m. p. 275—277° (decomp.), was crystallised from pyridine, the phenanthridine separating as red prisms (15.5 g.) containing pyridine of crystallisation (11.5%); at 125° the prisms lost pyridine and became yellowish-brown, melting at 286—288° (decomp.) (Found: C, 59.6; H, 4.5; N, 12.15. $C_{23}H_{20}O_7N_4$ requires C, 59.5; H, 4.3; N, 12.05%).

2-Nicotinamido-4 : 4'-biscarbethoxyaminodiphenyl (Ic).—2-Amino-4 : 4'-biscarbethoxyaminodiphenyl (27.5 g.) was treated with nicotinic chloride hydrochloride [prepared from nicotinic acid (10 g.) and thionyl chloride] in dry pyridine (100 ml.). The reaction product gave, on being refluxed in alcohol (1000 ml.) and cooled, colourless prisms of the amide (27 g.), m. p. 228—229° (decomp.) unchanged on recrystallisation from "Cellosolve" (Found: C, 64.4; H, 5.45; N, 12.4. $C_{24}H_{24}O_5N_4$ requires C, 64.3; H, 5.35; N, 12.5%). The substance (5 g.) was heated with alcohol (25 ml.) containing methyl iodide (5 ml.) in a pressure bottle at 100° for 40 minutes, and then evaporated to dryness, and the residue extracted with hot water. From the filtered solution fine colourless needles (5.2 g.) of the methiodide, m. p. 159—161° (decomp.), of (Ic) separated on cooling; when recrystallised from alcohol the needles had m. p. 162° (decomp.) (Found: N, 9.55; I, 21.35. $C_{22}H_{21}O_5N_4I$ requires N, 9.5; I, 21.55%).

2 : 7-Biscarbethoxyamino-9-3'-pyridylphenanthridine (IIc).—(Ic) (46 g.), phosphoryl chloride (46 ml.), and nitrobenzene (46 ml.) were heated at 130° (bath) for 1 hour and then poured on ice, and the nitrobenzene distilled with steam. On neutralisation of the aqueous residue with ammonia solution and cooling, an amorphous product (41 g.) was obtained, which was digested with hot 0.5N-hydrochloric acid (650 ml.) and left overnight. Unchanged amide (10 g.) was separated by centrifugation and washed with 0.5N-hydrochloric acid, and the aqueous liquors were heated with an excess of naphthalene-β-sulphonic acid. On cooling, colourless prisms (25 g.) separated, which were washed with aqueous naphthalene-β-sulphonic acid, suspended in water, and boiled for a few minutes with a slight excess of aqueous ammonia. The precipitate was extracted with cold 0.5N-hydrochloric acid (200 ml.), and the filtered solution neutralised with aqueous ammonia; the product (9 g.) was further purified by chromatography (Al_2O_3) in acetone solution, and crystallised from alcohol. 2 : 7-Biscarbethoxyamino-9-3'-pyridylphenanthridine was obtained as almost colourless prisms and needles (7.8 g.), m. p. 196—198° (decomp.) (Found: C, 66.8; H, 5.4; N, 12.9. $C_{24}H_{22}O_4N_4$ requires C, 66.95; H, 5.1; N, 13.0%).

2 : 7-Biscarbethoxyamino-9-3'-pyridylphenanthridine 1'-Methonaphthalene-β-sulphonate.—(IIc) (10.6 g.), dioxan (50 ml.), and methyl iodide (11 ml.) were refluxed for 1 hour and then cooled, and the gum left after decantation dissolved in hot water (ca. 250 ml.) containing a few drops of glacial acetic acid. After cooling, the filtered solution was reheated, and an excess of naphthalene-β-sulphonic acid added to precipitate the 1'-methonaphthalene-β-sulphonate 10-naphthalene-β-sulphonate, which crystallised from methanol as colourless prisms (9.9 g.), m. p. 228—229° (decomp.) (Found: N, 6.45; S, 7.55. $C_{25}H_{22}O_7N_4S$ requires N, 6.5; S, 7.45%). When this substance was boiled with an excess of aqueous sodium acetate, the prisms gave place to yellow needles of the 1'-methonaphthalene-β-sulphonate which, after cooling, were collected, and recrystallised from alcohol in yellow prisms, m. p. 142° (decomp.) (Found: N, 8.7; S, 5.15. $C_{25}H_{22}O_7N_4S$ requires N, 8.6; S, 4.9%).

2-(5 : 6-Dihydropyran-3-carboxamido)-4 : 4'-biscarbethoxyaminodiphenyl (Id).—This was prepared by reaction of 2-amino-4 : 4'-biscarbethoxyaminodiphenyl (24 g.) with 5 : 6-dihydropyran-3-carboxyl chloride (10.8 g.) in dry pyridine (50 ml.). After recrystallisation from alcohol, the amide (21.5 g.) had m. p. 186—188° (Found: C, 63.55; H, 5.55; N, 9.4. $C_{24}H_{27}O_6N_3$ requires C, 63.55; H, 5.95; N, 9.25%).

2 : 7-Biscarbethoxyamino-9-(5 : 6-dihydro-3-pyranylyl)phenanthridine (IIId).—Cyclisation of (Id) with phosphoryl chloride at 130° yielded a crude mixture from which the product was isolated as a hydrobromide and then converted into the free base by aqueous ammonia. Recrystallisation from alcohol-acetone afforded a 15% yield of the phenanthridine as colourless prisms, m. p. 215—216° (Found: C, 66.2; H, 5.8; N, 10.2. $C_{24}H_{25}O_5N_3$ requires C, 66.2; H, 5.75; N, 9.65%). The substance (3.5 g.) was heated in nitrobenzene with methyl sulphate at 135° for 5 minutes, and then poured into benzene. The precipitated solid gave, by metathesis with dilute hydrochloric acid (ca. 0.1N.), the methochloride (4.1 g.) as yellow needles, m. p. 256—258° (decomp.), raised to 260° (decomp.) on recrystallisation from water (Found: N, 9.0; Cl, 7.45. $C_{24}H_{25}O_5N_3Cl$ requires N, 8.65; Cl, 7.3%).

2 : 7-Biscarbethoxyamino-9-p-nitrophenylphenanthridine (II; R = *p*-NO₂-C₆H₄).—2-Amino-4 : 4'-bis-

carbethoxyaminodiphenyl (69 g.) and *p*-nitrobenzoyl chloride (42 g.) were heated in nitrobenzene (280 ml.) at 150° for 30 minutes and then cooled, and the product that separated recrystallised from alcohol. 2-*p*-Nitrobenzamido-4 : 4'-biscarboethoxyaminodiphenyl (I; R = *p*-NO₂·C₆H₄) crystallised in yellow prisms (84 g.), m. p. 202° (Found : C, 60.85; H, 4.75; N, 11.45. C₂₅H₂₄O₄N₄ requires C, 60.95; H, 4.99; N, 11.4%). This substance (80 g.) was cyclised with phosphoryl chloride, the phenanthridine (46 g.) crystallising from pyridine in spherical aggregates of fine yellow needles, m. p. ca. 247° (decomp.) (Found : C, 63.2; H, 4.4; N, 11.75. C₂₅H₂₂O₄N₄ requires C, 63.3; H, 4.65; N, 11.8%).

2 : 7-Diamino-9-*p*-nitrophenyl-10-methylphenanthridinium Chloride (III; R = *p*-NO₂·C₆H₄, A = Cl).—(II; R = *p*-NO₂·C₆H₄) (82 g.) was treated with methyl sulphate (70 ml.) in nitrobenzene solution (500 ml.) in the same manner as the corresponding thienyl compound. The orange solid obtained was refluxed with acetone (400 ml.) for a few minutes and then cooled, and the 2 : 7-biscarboethoxyamino-9-*p*-nitrophenyl-10-methylphenanthridinium methyl sulphate (96 g.) (containing a small quantity of sulphate), m. p. ca. 238° (decomp.), collected. Recrystallisation from methanol yielded pure methosulphate as orange needles, m. p. ca. 240—241° (decomp.) (Found : C, 54.1; H, 4.45; N, 9.6; S, 5.35. C₂₇H₂₂O₁₀N₄S requires C, 54.0; H, 4.65; N, 9.35; S, 5.35%). Hydrolysis of this substance (96 g.) with aqueous sulphuric acid (*d* 1.66) at 125—130° for 30 minutes, followed by metathesis of the product with sodium chloride, gave 2 : 7-diamino-9-*p*-nitrophenyl-10-methylphenanthridinium chloride, which crystallised from water in dark purple prisms (51.5 g.), m. p. ca. 235° (decomp.) (Found : C, 63.3; H, 4.25; N, 14.45; Cl, 9.25. C₂₀H₁₇O₂N₄Cl requires C, 63.05; H, 4.45; N, 14.7; Cl, 9.35%).

2 : 7-Diacetamido-9-*p*-nitrophenyl-10-methylphenanthridinium Chloride.—Acetic anhydride (10 ml.) was added to a hot suspension of (III; R = *p*-NO₂·C₆H₄; A = Cl) (5 g.) in glacial acetic acid (50 ml.), and the whole heated for 30 minutes on the steam-bath. The cooled product was collected and recrystallised from water as fine orange needles of the nitro-diacetamido-salt (4.7 g.), m. p. > 300° (Found : N, 12.0; Cl, 7.35. C₂₃H₂₁O₄N₄Cl requires N, 12.05; Cl, 7.65%).

2 : 7-Diacetamido-9-*p*-aminophenyl-10-methylphenanthridinium Chloride.—Solutions of ferrous sulphate heptahydrate (33 g.) and barium hydroxide octahydrate (36 g.) in hot water were mixed, and the resultant sludge added to a stirred suspension of the foregoing nitro-diacetamido-salt (7.5 g.) in water (1500 ml.) at 90—95°. After being heated on the steam-bath for 30 minutes, the mixture was filtered through a preheated funnel, and the residue washed with hot water. The aqueous filtrate was again heated to 90—95° and subjected to further interaction with ferrous hydroxide, prepared from ferrous sulphate (16.5 g.) and barium hydroxide (18 g.), for 30 minutes. The hot aqueous filtrate was treated with sodium chloride to precipitate the amino-diacetamido-salt, which crystallised from water in small yellow needles (6.1 g.), m. p. ca. 280—281° (decomp.) (Found : N, 12.8; Cl, 8.2. C₂₄H₂₃O₂N₄Cl requires N, 12.9; Cl, 8.15%).

2 : 7-Diamino-9-*p*-aminophenyl-10-methylphenanthridinium Chloride (III; R = *p*-NH₂·C₆H₄, A = Cl).—(a) The amino-diacetamido-salt (6.05 g.) and 2*N*-hydrochloric acid (60 ml.) were refluxed for 1 hour, and the red solution was diluted with water (140 ml.) and neutralised carefully with aqueous ammonia; the separation of red prisms was completed by addition of sodium chloride. Recrystallisation from water (40 ml.) containing sodium chloride (0.5 g.) yielded dark red pyramids of the triamino-salt (4.7 g.), m. p. ca. 240° (decomp.) (Found : N, 16.0; Cl, 10.1. C₂₀H₁₅N₄Cl requires N, 16.0; Cl, 10.15%).

(b) Reduction of (III; R = *p*-NO₂·C₆H₄, A = Cl) (30.5 g.) with ferrous hydroxide, prepared from ferrous sulphate (224 g.) and barium hydroxide (240 g.), according to the method just described, yielded a red aqueous solution which was concentrated to ca. 500 ml. Metathesis with sodium chloride gave red prisms which, on recrystallisation as in (a), yielded dark red pyramids of the same triamino-salt (26.2 g.), m. p. ca. 240° (decomp.).

(c) Equimolecular quantities of 4 : 4'-dinitro-2-aminodiphenyl and *p*-nitrobenzoyl chloride were condensed in boiling nitrobenzene. After evolution of hydrogen chloride had ceased, the solution was cooled to allow separation of 2-*p*-nitrobenzamido-4 : 4'-dinitrodiphenyl in almost quantitative yield. Recrystallisation from aqueous pyridine furnished small yellow prisms, m. p. 234° (Found : C, 56.2; H, 2.9; N, 13.75. C₁₉H₁₂O₇N₄ requires C, 55.85; H, 2.95; N, 13.7%). When this amide was refluxed in nitrobenzene-phosphoryl chloride for several hours (B.P. 520, 273) 2 : 7-dinitro-9-*p*-nitrophenylphenanthridine crystallised almost quantitatively in cream-coloured needles; recrystallisation from nitrobenzene gave a product, m. p. 356—358° (Found : C, 58.6; H, 2.15; N, 14.35. C₁₉H₁₀O₈N₄ requires C, 58.45; H, 2.6; N, 14.35%). The foregoing powdered trinitro-compound (5 g.) was suspended in alcohol (125 ml.), concentrated hydrochloric acid (25 ml.) and crystalline stannous chloride (30 g.) were added, and the mixture was refluxed for 2 hours. After cooling, the dark red solid was collected, dissolved in hot water, separated from unchanged trinitro-compound (1.1 g.), and then stirred into excess of sodium hydroxide solution. 2 : 7-Diamino-9-*p*-aminophenylphenanthridine was thus precipitated as a yellow solid, which crystallised from alcohol in yellow plates, m. p. 246° (Found : C, 76.0; H, 5.45; N, 18.35. C₁₉H₁₅N₄ requires C, 76.0; H, 5.35; N, 18.65%). This base was extremely soluble in dilute acid, affording intense carmine solutions. It was acetylated by acetic anhydride-acetic acid; on dilution of the solution thus obtained with water an orange gel formed, which was converted into a flocculent precipitate by the addition of aqueous ammonia. Crystallisation of this product from alcohol afforded 2 : 7-diacetamido-9-*p*-acetamidophenylphenanthridine in cream-coloured wool-like needles, m. p. 312° (Found : C, 69.9; H, 5.65; N, 13.25. C₂₅H₂₃O₂N₄ requires C, 70.4; H, 5.2; N, 13.15%). A suspension of this compound (5 g.) in nitrobenzene (150 ml.) at 180° was treated with methyl sulphate (5 g.). The gum that separated was isolated by decantation and refluxed with alcohol (200 ml.). The 2 : 7 : 4'-triacetamido-9-phenyl-10-methylphenanthridinium sulphate thus left undissolved crystallised from methanol in orange plates (2 g.), m. p. 248° (decomp.) [Found : C, 63.95; H, 5.35; N, 11.05. (C₂₈H₂₅O₃N₄)₂SO₄ requires C, 63.8; H, 5.15; N, 11.45%]. Hydrolysis of this salt could be affected by refluxing it with *N*-hydrochloric acid until a clear solution was obtained (several days); more conveniently and with less serious bumping the salt (0.4 g.) was heated in suspension in 10% methanolic hydrochloric acid (15 ml.) until all had dissolved (ca. 6 hours), the solution being then evaporated to small bulk. When the residue was neutralised with aqueous ammonia the triamino-salt separated and was purified as in (a) (Found : N, 15.5; Cl, 10.1%).

2 : 7-Biscarboethoxyamino-9-*p*-methoxyphenylphenanthridine (II; R = *p*-MeO·C₆H₄).—Condensation of 2-amino-4 : 4'-biscarboethoxyaminodiphenyl and *p*-anisoyl chloride in boiling chlorobenzene furnished 2-*p*-anisamido-4 : 4'-biscarboethoxyaminodiphenyl (I; R = *p*-MeO·C₆H₄); this amide crystallised from benzene in colourless plates of indefinite m. p. 100—105° (Found : N, 8.95. C₂₆H₂₇O₆N₃ requires N, 8.9%). Cyclisation with phosphoryl chloride converted it in a high yield into the phenanthridine, which crystallised from alcohol in white microscopic needles, m. p. 190—192° (effervescence) (Found : N, 9.05. C₂₄H₂₅O₄N₃ requires N, 9.15%). Its quaternary methosulphate, m. p. ca. 230°, crystallised from water in deep-yellow needles (Found : N, 7.15; S, 5.45. C₂₈H₃₁O₈N₃S requires N, 7.2; S, 5.45%).

9-Phenylphenanthridine-2 : 7-bis(trimethylammonium) Iodide (IV).—2 : 7-Diamino-9-phenylphenanthridine (10 g.) and sodium carbonate (18 g., anhydrous) were refluxed with methanol (100 ml.), water (24 ml.), and methyl iodide (30 ml.) for 8 hours. Two layers at first appeared, but eventually mixed to a deep-purple solution from which crystals slowly separated. The crystals were collected, washed with a little methanol and then with water, and recrystallised from either of these solvents in white needles (14 g.). This salt became yellow at 160°, and with further heating blue-grey, melting at 255° (decomp.) (Found : N, 6.95; I, 40.75. C₂₅H₂₉N₃I₂ requires N, 6.7; I, 40.65%).

2 : 7-Bis(trimethylamino)-9-phenyl-10-methylphenanthridinium Iodide (V).—The foregoing bisquaternary salt (2.5 g.) was heated in a bath at 180°. Evolution of methyl iodide was observed and after 30 minutes the purple-black mass was extracted with boiling benzene. The residue crystallised from methanol in black needles (1.5 g.) (reddish-purple by transmitted light), m. p. 260—262° (decomp.) (Found : N, 8.8; I, 26.1. C₂₄H₂₆N₃I requires N, 8.7; I, 26.3%). The benzene extract contained a small amount of basic substance, which was not further investigated.

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