225. *Researches in the Phenanthridine Series. Part VII. Quaternary Salts with Urethane and Urea Substituents.**

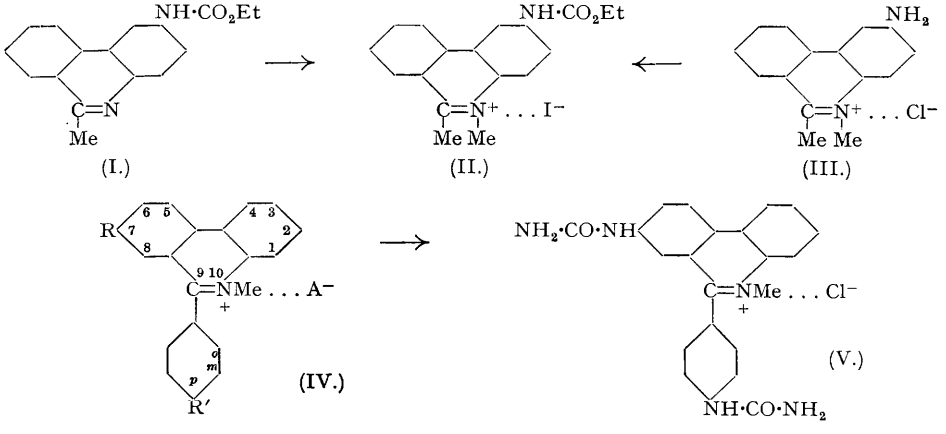
By Leslie P. Walls.

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

Previous work has shown that 9-phenylphenanthridinium salts with two amino-groups are powerful trypanocides. Methods have been devised whereby these compounds can be converted into urethanes and ureas. The products are stable crystalline salts, which do not possess much trypanocidal activity in *T. congolense* infections. In some of them, however, a new feature appears : an alleviation of the symptoms in mice due to the important South American parasite, *T. cruzi*, which is resistant to the common trypanocides.

A comprehensive study of the phenanthridine series has revealed so far (*J.*, 1945, 294) that quaternary salts of 9-phenylphenanthridine with two amino-groups possess marked curative action in *T. congolense* infections. This trypanosome is resistant to arsenicals and other well-known trypanocides, and the results obtained seemed sufficiently significant to justify an examination of quaternary salts in which the substituent amino-groups are modified. In general, acetylation of these groups has a striking dystherapeutic effect. However, it was decided to examine the corresponding carbalkoxyamino- and carbamido-derivatives because these groups differ in many respects from acylamido-groups. When present in a benzene ring they direct substituents to the *ortho* and *para* positions even in the presence of concentrated sulphuric acid (compare Dikshoorn, *Rec. Trav. chim.*, 1929, **48**, 527); carbalkoxyamino- are far more resistant to hydrolysis than are acylamido-groups. The protection thus afforded the susceptible amino-groups might be reflected in a greater persistence of such compounds in the body of the host, the active diamino-salts being slowly liberated. It is appreciated that so little is known about the mode of action of trypanocides that arguments of this kind have doubtful validity. A further valuable property of these compounds as drugs would be the marked reduction in colour compared with the diamino-salts.

The preparation of the carbalkoxyamino-derivatives may be by two routes. Thus 3-amino-9-methylphenanthridine (Morgan and Walls, *J.*, 1932, 2228) may be converted into the carbethoxyamino-compound (I), and the product methylated by known methods to yield 3-*carbethoxyamino*-9 : 10-*dimethylphenanthridinium iodide* (II), or the amino-quaternary salt (III) in aqueous soluton may be stirred with ethyl chloroformate for



a few minutes and then treated with potassium iodide. By the latter method the diamino-quaternary salts (compare IV; R = R′ = NH_2, A = Cl) referred to in previous papers of this series have been converted into the corresponding dicarbethoxyamino-salts. These yellow salts are stable, and crystallise well from water, although several of them are markedly thixotropic.

In aqueous solution reaction between the diamino-quaternary salts (compare IV) and cyanic acid is slow, and owing to simultaneous decomposition of the acid yields of the dicarbamido-compounds (compare V) are

* The experimental work described in this paper formed the subject of British provisional patent applications Nos. 14478/43 and 17811/44.

small. The low reactivity of the amino-groups in these salts is shown also by their inertness to nitrourea (Davis and Blanchard, *J. Amer. Chem. Soc.*, 1929, **51**, 1794). Cyanic acid is more stable in 50% acetic acid than in aqueous solution, for reaction went to completion when a solution of (IV; R = R′ = NH_2) in this medium was treated with potassium cyanate. The reverse method, formation of the carbamido-compounds followed by methylation, is unsatisfactory owing to the extremely sparing solubility of the carbamides. This substitution of the amino-groups by carbamido-groups likewise leads to stable crystalline salts, of diminished colour, which have not the bitter taste of the diamino-salts.

*Note on Chemotherapeutic Trypanocidal Action.**—The biological properties of the compounds referred to here were investigated on the same lines as in a previous report (Walls, *J.*, 1945, 294). Nos. 1543, 1544, 1569, 1575, 1566, and 1567 are the carbethoxyamino-compounds corresponding to the acetyl derivatives of the same bases (Nos. 896, 893, 1507, 1574, 1554, and 1162) already described. There is generally no striking difference in the toxicity of corresponding compounds in the two series. Both usually have a much lower chemotherapeutic action on *T. congolense* and *T. brucei* than the unsubstituted amino-compounds. The difference is strikingly shown with nos. 1543 and 897, 1566 and 1565, 1569 and 1508, 1575 and 1573; in each pair less than one-hundredth of the maximum tolerated dose of the unsubstituted amino-compound cures infections with *T. congolense* in a proportion of animals, whereas the corresponding carbethoxyamino-derivatives have at most a slight therapeutic effect in a dose which is one-sixth of the maximum tolerated. The carbomethoxyamino- and also the carbamido-derivatives do not differ greatly from the corresponding carbethoxyamino-analogues (cf. 1544 with 1585 and 1584; 1543 with 1583). It is noteworthy, however, that the carbethoxyamino-compound No. 1544 and the corresponding methanesulphonate, in a dose of 3·0—1·2 mg., has a definite therapeutic action on *T. cruzi* in mice (Browning, Calver, Leckie, and Walls, *Nature*, 1946, **157**, 263). Infections with this trypanosome are refractory to other classes of trypanocidal drugs and apart from certain other phenanthridine compounds respond only to Bayer 7602 Ac.

	Formula (IV).		Therapeutic effect in mice infected with *T. congolense* (Strain 1).[1]		*T. brucei*.[2]	
Compound.	Substituents.	Acid radical.	Dose, mg.[(a)]	Result.	Dose, mg.[(a)]	Result.
1543	7-NH·CO_2Et-9-*p*-C_6H_4·NH·CO_2Et	Cl	1·43	Cure	1·43	O
			0·22—0·066	Slight—O		
1544	3-NH·CO_2Et-9-*p*-C_6H_4·NH·CO_2Et	Cl	2·85*—1·43	Cure	1·85 *	Cure
			0·5	Slight	1·25	Cure—Slight
					0·5	O
1569	7-NH·CO_2Et-9-*m*-C_6H_4·NH·CO_2Et	Cl	2·5	Cure	2·5	Slight
			1	Marked—Slight		
1575	3-NH·CO_2Et-9-*m*-C_6H_4·NH·CO_2Et	Cl	1	Slight	1	O
1566	2 : 7-NH·CO_2Et-9-Ph	Cl	1	Cure	0·5	O
			0·2	Slight		
1567	3 : 7-NH·CO_2Et-9-Ph	Cl	10 *	Cure	10 *	Slight
			2 *	Slight		
1585	3-NH·CO_2Me-9-*p*-C_6H_4·NH·CO_2Me	Cl	5—1	Cure	5	Cure
			0·33	Slight	1·66	Slight
1599	3-NH·CO_2Et-9-Me	I	2	(Cure)	2	O
			1	Marked—Slight		
1583	7-NH·CO·NH_2-9-*p*-C_6H_4·NH·CO·NH_2	Cl	2	Slight	2	O
1584	3-NH·CO·NH_2-9-*p*-C_6H_4·NH·CO·NH_2	Cl	3·3*—2·5	Cure	1·66	Slight
			1·66	Slight	1	O

* 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; Calver, *Trop. Dis. Bull.*, 1945, **42**, 704; treatment was given at the acme stage.

[2] 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.

Experimental.

7-Carbethoxyamino-9-p-carbethoxyaminophenyl-10-methylphenanthridinium Chloride (1543).—The diamino-quaternary salts are insoluble in ethyl chloroformate and reaction does not occur in absence of solvent. A solution of 7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (2 g.; *J.*, 1938, 396) in hot water (40 ml.) was cooled rapidly, and before crystallisation occurred a small excess of ethyl chloroformate (1·4 ml.) was stirred in vigorously. The colour of the solution rapidly faded from deep red to yellow and in a few minutes yellow crystals of the *product* separated (2·2 g.). This salt was rather sparingly soluble in water from which it crystallised in transparent deep yellow prisms, m. p. 242° (decomp.) (Found for dried salt: N, 9·0; Cl, 7·35. $C_{26}H_{26}O_4N_3Cl$ requires N, 8·75; Cl, 7·4%).

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

The following compounds were similarly prepared from the corresponding diamino-quaternary salts. The m. ps. are dependent on the rate of heating, being with decomposition and effervescence. Most of the salts contain solvent of crystallisation, but analyses refer to dried salts. 3-*Carbethoxyamino*-9-p-*carbethoxyaminophenyl*-10-*methylphenanthridinium chloride* (1544) crystallised from water in sparingly soluble buff-coloured needles, m. p. 261° (Found : N, 8·85; Cl, 7·25%). Double decomposition with silver methanesulphonate afforded the corresponding ***methanesulphonate*** in pale yellow prisms readily soluble in water (Found : N, 7·5. $C_{27}H_{29}O_7N_3S$ requires N, 7·8%). 7-*Carbethoxyamino*-9-m-*carbethoxyaminophenyl*-10-*methylphenanthridinium chloride* (1569) crystallised from alcohol in yellow needles, m. p. 219° (Found : N, 8·85; Cl, 7·2%). 3-*Carbethoxyamino*-9-m-*carbethoxyaminophenyl*-10-*methylphenanthridinium chloride* (1575) crystallised from water in pale yellow needles, m. p. 245° (Found : N, 9·15; Cl, 7·55%). 2 : 7-*Dicarbethoxyamino*-9-*phenyl*-10-*methylphenanthridinium chloride* (1566) crystallised from alcohol in orange-yellow needles, markedly thixotropic, m. p. 242°. The solution showed brilliant green fluorescence (Found : N, 8·75; Cl, 7·45%).

3 : 7-*Dicarbethoxyamino*-9-*phenyl*-10-*methylphenanthridinium Chloride* (1567).—This *salt* was purified by dissolving it in hot glacial acetic acid and then diluting with several volumes of hot water. On being cooled the solution deposited minute yellow needles, m. p. 267° (Found : N, 8·85; Cl, 7·45%). 3-*Carbomethoxyamino*-9-p-*carbomethoxyaminophenyl*-10-*methylphenanthridinium chloride* (1585) was prepared similarly with methyl chloroformate. Yellow needles, m. p. 318—323° (decomp.), from water (Found : Cl, 7·9. $C_{24}H_{22}O_4N_3Cl$ requires Cl, 7·85%).

3-*Carbethoxyamino*-9-*methylphenanthridine* (I).—3-Amino-9-methylphenanthridine was readily carbethoxylated by the method of Lesslie and Turner (*J.*, 1933, 1588). The amine (3 g.) and diethylaniline (2·2 g.) were dissolved in absolute alcohol (20 ml.) and treated with ethyl chloroformate (1·9 g.) whereupon there was an immediate precipitation of minute yellow needles. After being refluxed for 20 minutes the solution was poured into water, neutralised by ammonia, and steam-distilled to remove diethylaniline. The product remained in the still as a white crystalline powder (3·1 g.) which was recrystallised from methyl alcohol, forming slightly discoloured prisms, m. p. 179—180° (Found : C, 73·15; H, 5·65; N, 9·75. $C_{17}H_{16}O_2N_2$ requires C, 72·9; H, 5·7; N, 10·0%).

3-*Carbethoxyamino*-9 : 10-*dimethylphenanthridinium Iodide* (II, 1599).—When the foregoing compound (2 g.) was dissolved in nitrobenzene and treated at 150° with methyl sulphate (1 ml.), the quaternary methosulphate crystallised in high yield. Since this salt and the corresponding chloride are extremely soluble in water, the product was characterised as the *iodide*, which crystallised from that solvent in yellow plates, m. p. 209° (decomp.) (Found : N, 6·35; I, 28·15; loss at 100°, 6·0. $C_{18}H_{19}O_2N_2I$, 1·5 H_2O requires N, 6·25; I, 28·25; H_2O, 6·0%).

7-*Carbamido*-9-p-*carbamidophenyl*-10-*methylphenanthridinium Chloride* (V, 1593).—The corresponding diamino-quaternary salt was dissolved in dilute hydrochloric acid, but with either 2 or 4 equivalents of acid at 0·2N or N the yield of reaction with potassium cyanate was very small, decomposition of cyanic acid being the predominant reaction. The following method proved successful : the diamino-salt (2 g.) was dissolved in acetic acid (10 ml. each of glacial acid and water), cooled in ice-water, and treated with potassium cyanate (0·5 g.) dissolved in a small quantity of water. The quality of the cyanate was previously proved by its almost quantitative conversion of aniline into phenylurea. Small crystals began to separate after a few minutes, and after 12 hours the *product* (1·4 g.) was collected, a further yield (0·6 g.) of less pure substance being obtained by dilution of the mother liquor with dilute hydrochloric acid. The salt crystallised from water in deep yellow thin plates, m. p. 265° (decomp.) (Found for dried salt : N, 16·65; Cl, 8·55. $C_{23}H_{20}O_2N_5Cl$ requires N, 16·6; Cl, 8·4%). 3-*Carbamido*-9-p-*carbamidophenyl*-10-*methylphenanthridinium chloride* (1584) was similarly prepared in high yield; it crystallised from water in matted yellow needles, m. p. 276° (decomp.) (Found for dried salt : N, 16·3; Cl, 8·55%).

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CHEMICAL RESEARCH LABORATORY, DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH,
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