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THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

PART II

TRYPANOSOMA CRUZI

BY

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South American trypanosomiasis (Chagas's disease), which is caused by *Trypanosoma cruzi*, has so far proved to be much less amenable to treatment with chemotherapeutic substances than African trypanosomiasis. One reason for this comparative lack of success may be that multiplication of the parasites does not take place in the peripheral blood, but as leishmania forms within the cells of the reticuloendothelial system and the muscles.

In infants, the primary infection may be acute and overwhelming, with many parasites in the peripheral blood; if the child survives, or if the infection is acquired later in life, the disease becomes chronic and circulating trypanosomes are scanty. Leishmania forms become entrenched in fibrous tissue and are difficult to kill, even with otherwise effective remedies.

Experimental infections of *T. cruzi* in mice and other laboratory animals have been used to evaluate a wide range of drugs. Certain rather toxic bismuth compounds were said to influence the course of the disease (Stein, 1933); more recently, it was found that a variety of sulphonamides, and antimony, arsenic, and bismuth compounds failed to effect a cure (McIvor, 1942; Talice and Lopez-Fernandez, 1946). Mepacrine is ineffective (Stein, 1933), but Goble (1949) has recently reported that slight activity is shown by pentaquine and other antimalarials of the 8-aminoquinoline class. Penicillin has been found by most workers to be without action (Collier and Lourie, 1943; Neghme, 1945). A fundamental advance in the chemotherapy of Chagas's disease was the introduction in 1937 of the quinoline derivative Bayer 7602 Ac (Jensch, 1937; Pratt and Archer, 1948), which has been found to be effective in clinical practice (Mazza, Basso, and Basso, 1946; Herr and Brumpt, 1939; Mazza, 1941). Its chief value is in the control of acute infections in children; it is less effective in chronic cases. A substance containing arsenic and sulphur, Bayer 9736 As, has also been used, with less favourable results. An extensive investigation of the trypanocidal activity of phenanthridine compounds (Browning, Calver, Leckie, and Walls, 1946) revealed that some of these were active against

T. cruzi infections in mice, and that, in particular, quaternary salts with urethane substituents appeared to offer considerable promise. Further synthetic work has been pursued in this direction, and the present account describes the results obtained.

METHODS

All of the drugs were tested against *Trypanosoma cruzi* infections in laboratory mice. The strain was obtained from Prof. Reichenow, of Hamburg, in 1936, and has been passaged in mice ever since. It was originally isolated in Brazil in 1926. We have used two "lines" of this strain, one of which has been kept continuously at the Wellcome Laboratories of Tropical Medicine; the other was kindly provided by Professor C. H. Browning, and was the strain used by him in his own investigations upon the action of phenanthridinium compounds. We could detect no differences in the behaviour of the two strains.

Inoculation of animals.—For the infection of a group of mice, an animal showing a fairly heavy infection in the peripheral blood was anaesthetized with ether, the thorax opened, and blood withdrawn from the heart with a sterile Pasteur pipette. The blood was diluted about five times with heparinized saline and the number of trypanosomes present estimated in a haemocytometer chamber. Quantities of 0.1 ml. of diluted blood were injected subcutaneously into the mice to be used for the test. An average inoculum for a mouse contained about 100,000 trypanosomes, and organisms appeared in the peripheral blood after 5–10 days. One group of mice was kept as a control, because although in some experiments all untreated mice died in about 20 days, it was frequently found that the infection became chronic in a proportion of the animals and they remained alive for several months, showing trypanosomes in the peripheral blood at irregular intervals. It was found that the subcutaneous injection of a heavy concentrated inoculum gave the most consistent results; the intraperitoneal and intravenous routes were less satisfactory.

Treatment with drugs.—In order to give a compound the maximum opportunity of showing any activity it possessed, we found that it was necessary to give treatment during the incubation period of the disease. All drugs were tested in this way, the first dose being given subcutaneously on the same day as inoculation with trypanosomes, and subsequent doses daily for the following four to ten days. By this time the control group were "positive" upon microscopical examination of a drop of fresh tail-blood under a coverslip, and the effect of drug treatment could be assessed by comparison with the controls. Those substances which showed some activity when given in this way were then injected into mice which had been inoculated about a week earlier and already had trypanosomes in the peripheral blood. A course of daily doses was given as before, and the effect of the treatment assessed by comparison with the controls. The dose aimed at was the highest that could be tolerated without causing serious side-reactions; if these occurred, the dose was reduced, or the treatment discontinued; thus it was sometimes necessary to suspend treatment because of necrosis at the site of injection. Drugs were dissolved or suspended in water; a volume of 0.1 ml. per 20 g. mouse was injected subcutaneously. Whenever possible, the drug was given in solution, and with some compounds of low solubility the volume of the injection was increased. When a drug was in solution, it was found to be more effective than when given in suspension in a smaller volume.

Blood examinations were made every day during treatment, and for several weeks afterwards if the drug was found to be active. Frequently it was found that treatment with a drug would tide a mouse over the acute stage of the infection, and prevent death,

but the mouse might continue to show parasites from time to time in the peripheral blood during the following weeks, the infection having passed into the chronic phase. The labour involved in daily blood examination of lightly infected mice is very great.

With such a method of assay, it is very difficult to calculate precise figures for the relative activities of drugs. We have therefore assigned arbitrary potency values, based upon the length of time after the final dose that treated mice remained free from trypanosomes in the peripheral blood. These values were estimated in terms of the following key:

T. cruzi

Treatment prevents appearance of trypanosomes in peripheral blood of all the mice in the group for	Activity
More than 2 weeks after last dose	3
1 to 2 weeks	2
Less than 1 week	1
No significant difference from controls	0

The figures for activity against *T. congolense* given in the Tables are in terms of the following key:

T. congolense

Dose required to "clear" at least 80% of a group of mice from trypanosomes in the peripheral blood. (mg./kg.)	Activity
0.01–0.1	4
0.1 –1.0	3
1.0 –10	2
10 –50	1
No activity at 50	0

All the drugs were not, of course, tested at the same time against the same batch of infected mice, and therefore the results given in Tables I, II, III and IV are not strictly comparable. However, in most of the experiments an active drug was used as a standard, and the figures are approximately correct. Some of the more active compounds were compared by using graded doses.

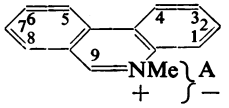
Results and Discussion

In order to determine the structural features likely to confer activity against *T. cruzi*, the large number of phenanthridine derivatives available have been tested by the above methods. The results of this preliminary survey revealed that only a few compounds other than phenanthridinium salts show activity, and that but the merest trace. All the substances mentioned in Part I have been examined in addition to those described in Table IV. The active compounds are characterized by the presence of one or other of the following structural features: (*a*) a 9-phenyl substituent, (*b*) a urethane group, and (*c*) two amino- groups. Most of the active compounds in fact combine features (*a*) and (*b*). Of over two hundred compounds that form the basis of this work, eighteen compounds showed marked suppressive action, and of these all but one (the α-thienyl compound 621C47) are 9-phenylphenanthridinium salts; eleven of them contain a urethane group, and five others two amino- groups. Phenanthridine compounds other than quaternary salts are inactive.

TABLE I

DIAMINO COMPOUNDS

Activities are expressed in terms of the key given on p. 279.



Compound number	Substituents	A	Ref.	Activity against *T. congolense*	Activity against *T. cruzi*			
					Incubation period		Established infection	
					Dose mg./kg.	Activity	Dose mg./kg.	Activity
640C46	2 : 7-diNH_2-9-Me	Br	*a*	1	8×25	0	10×25	0
660C47	2 : 7-diNH_2-9-$CH_2.C_6H_5$	Br	*b*	3	7×12.5	1	—	—
6C46*	2 : 7-diNH_2-9-C_6H_5	Br	*c*	4	8×12.5	3	14×12.5	0
676C46	2 : 7-diNH_2-9-*p*-$C_6H_4.NO_2$	Cl	*d*	4	8×25	3	10×25	0
150C47	2 : 7-diNH_2-9-*p*-$C_6H_4.NH_2$	Cl	*d*	4	9×12.5	1	10×12.5	0
621C47	2 : 7-diNH_2-9-α-thienyl	Br	*d*	4	6×12.5	2	5×12.5	0
522C46	2-NH_2-9-*p*-$C_6H_4.NH_2$	Br	*b*	3	8×3.125	2	10×3.125	0
399C48	3-NH_2-9-*p*-$C_6H_4.NH_2$	Cl	*e*	1	5×12.5	0	—	—
212C47	6-NH_2-9-*p*-$C_6H_4.NH_2$	$EtSO_3$	*b*	3	7×12.5	0	—	—
129C46*	7-NH_2-9-*p*-$C_6H_4.NH_2$	Cl	*e*	3	8×12.5	1	14×25	0
359C47	7-NH_2-9-*m*-$C_6H_4.NH_2$	Cl	*c*	3	8×12.5	1	10×12.5	0
4C47	8-NH_2-9-*p*-$C_6H_4.NH_2$	Br	*b*	1	2×12.5 6× 6.25	0	—	—
34C46	3 : 7-diNH_2-9-C_6H_5	Br	*c*	3	4×25	3	9×25	0

* These compounds are used in Africa for the treatment of bovine trypanosomiasis (*T. congolense* and *T. vivax*), 6C46 being known as "1553," or dimidium bromide, and 129C46 as "897," or phenidium chloride.

References: (*a*) Walls (1947); (*b*) Caldwell and Walls (1948); (*c*) Walls (1945); (*d*) Walls and Whittaker (1950); (*e*) Morgan and Walls (1938).

Among the diaminophenanthridinium salts are found the most effective drugs of the series for the treatment of *T. congolense* infections, but as is seen in Table I there is little relationship apparent between this property and their effect on *T. cruzi* infections. The most effective drugs of this type against the latter are the 2 : 7-diamino- salts, 6C46 and 676C46, and the 3 : 7-diamino- salt (34C46). The 9-methyl compound (640C46) is only slightly active in both *T. cruzi* and *T. congolense* infections, but a contrast is presented by the 9-benzyl compound (660C46) and the triamino- salt (150C47), both highly effective *T. congolense* drugs, which are only slightly active against *T. cruzi*. Of the other diamino- type, in which one amino-group only is located in the phenanthridine nucleus, the 2-amino- compound, 522C46, alone shows marked activity.

Since phenanthridinium salts with urethane substituents were the first of the series for which activity against *T. cruzi* was demonstrated (Browning *et al.*, 1946) a wide variety of such salts has been examined (Table II). It is confirmed that the urethane substituent usually confers a specific activity on 9-phenylphenanthridinium salts, and in this respect marked differences are again found in the relative responses of *T. cruzi* and *T. congolense* infections. Activity against *T. cruzi* in the incubation period of mouse infections can generally be demonstrated for 9-phenylphenanthridinium salts that contain both a urethano- and an amino- group or, in place of the latter, one readily convertible into it, the most consistently active type being that in which the former group is located in the *p*-position of the 9-phenyl substituent. No generalization can be made, however, for bisurethane salts: 3-carbethoxyamino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium chloride (494C46) is markedly active, but the isomeric 2-urethane compound (301C47) is only slightly so, and the bismethylurethane (77C48) corresponding to the former is inactive. Similarly the bisurethanes derived from the 2 : 7-diamino- salts of Table I are inactive, except curiously enough the 9-methyl compound (492C46), which is slightly active. Replacement of the 9-*p*-urethanophenyl group in the active compounds of Table II by benzyl, benzoyl, or alkyl leads to salts which are quite inactive even when this new 9-group carries what would appear to be a suitable substituent, such as nitro, amino, or urethane.

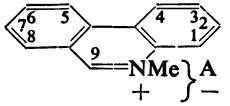
In Part I it has been recorded that powerful activity against *T. congolense* was obtained by locating a 7-alkoxyl group in the 9-*p*-aminophenylphenanthridinium molecule ; of such salts only a few show activity in *T. cruzi* infections (Table III) and that of a low order, no improvement being effected by converting the amino- into a urethano- group. Of the numerous other phenanthridine compounds tested, which included quaternary salts with substituents such as NH_2, NMe_2, OAlk, NHAc in various combinations, only a few miscellaneous 9-phenylphenanthridinium salts showed even slight activity (Table III). Of these the nitro-amino- salts form a consistent group.

So far we have discussed only the results of treatment during the incubation period of the disease. When the active compounds were examined for their effect upon established infections the results were very disappointing. In addition to the 3 : 4′-diurethano- compound 494C46 (I ; R = $NH.CO_2Et$; Browning *et al.*) only one other, the 2-amino-9-*p*-urethanophenyl compound 3C47 (II), showed much activity.

TABLE II

COMPOUNDS CONTAINING URETHANE GROUPS

Activities are expressed in terms of the key given on p. 279.



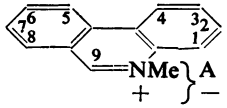
Compound number	Substituents	A	Ref.	Activity against *T. congolense*	Activity against *T. cruzi*			
					Incubation period		Established infection	
					Dose mg./kg.	Activity	Dose mg./kg.	Activity
492C46	2: 7-diNH.CO_2Et-9-Me	$MeSO_4$	*a*	1	4×50	1	8×50	2
9C46	6-NH.CO_2Et-9-Me	$MeSO_4$	*b*	1	6×25	1	14×25	1
3C47	2-NH_2-9-*p*-C_6H_4.NH.CO_2Et	Br	*b*	2	8×25	3	5×12.5	3
2C47	2-NH.COMe-9-*p*-C_6H_4.NH.CO_2Et	Cl	*b*	1	8×12.5	2	10×12.5	0
489C46	2-NH.CO_2Et-9-*p*-C_6H_4.NH_2	Cl	*b*	2	8×12.5	3	14×25	0
301C47	2-NH.CO_2Et-9-*p*-C_6H_4.NH.CO_2Et	Cl	*b*	1	7×12.5	1	—	—
494C46	3-NH.CO_2Et-9-*p*-C_6H_4.NH.CO_2Et	Cl	*c*	1	4×50	3	12×50	3
77C48	3-NH.CO_2Me-9-*p*-C_6H_4.NH.CO_2Me	Cl	*c*	1	6×12.5	0	—	—
441C46	7-NH_2-9-*p*-C_6H_4.NH.CO_2Et	Cl	*b*	1	8× 6.25	3	14× 6.25	0
292C46	7-NH.COMe-9-*p*-C_6H_4.NH.CO_2Et	Cl	*b*	1	8×25	1	14×50	0

References: (*a*) Walls (1947); (*b*) Caldwell and Walls (1948); (*c*) Walls (1946).

TABLE III

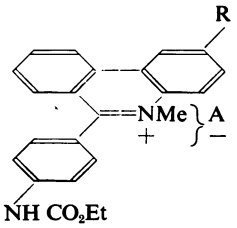
ALKOXYL COMPOUNDS AND SOME AMINO-NITRO COMPOUNDS

Activities are expressed in terms of the key given on p. 279.

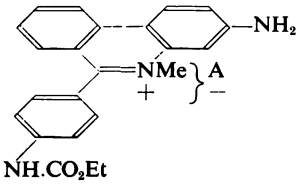


Compound number	Substituent	A	Ref.	Activity against *T. congolense*	Activity against *T. cruzi*			
					Incubation period		Established infection	
					Dose mg./kg.	Activity	Dose mg./kg.	Activity
284C47	7-OMe-9-*p*-$C_6H_4.NO_2$	Cl	*a*	3	6×12.5	1	—	—
348C48	7-OPr-9-*p*-$C_6H_4.NO_2$	Cl	*a*	2	6×12.5	1	—	—
98C48	7-OMe-9-$C_6H_3.(NO_2)_2$	Cl	*a*	1	7×12.5	1	—	—
671C47	7-OMe-9-*m*-$C_6H_4.NH_2$	Cl	*a*	3	6×12.5	1	—	—
699C46	7-OH-9-*p*-$C_6H_4.OH$	Cl	*a*	1	6×25	1	10×25	0
44C46	None	Cl		1	8× 1.5	1	10× 1.5	0
443C46	2-NH_2-9-*p*-$C_6H_4.NO_2$	Cl	*b*	2	10× 3.125	2	14× 6.25	0
63C47	6-NH_2-9-*p*-$C_6H_4.NO_2$	Cl	*b*	3	7× 6.25	1	8× 6.25	0
65C47	8-NH_2-9-*p*-$C_6H_4.NO_2$	Cl	*b*	1	7× 6.25	1	10× 6.25	0
176C48	7-NMe_2-9-*p*-$C_6H_4.NO_2$	Cl	*c*	1	6×12.5	1	—	—

References: (*a*) Copp and Walls (1950); (*b*) Caldwell and Walls (1948); (*c*) Unpublished.



(I)



(II)

It has already been mentioned that results with bisurethanes analogous to 494C46 are unpredictable, and consequently we have concentrated our efforts on 3C47, the only other compound likely to have any remedial value in Chagas's disease. Unfortunately the synthesis of 3C47 involves a large number of steps (Caldwell and Walls, 1948), and it was clearly desirable to find a more readily accessible drug. From the results of the foregoing preliminary survey it appeared that the best hope of success lay in the examination of further 9-phenylphenanthridinium salts substituted with both urethano- and amino- substituents.

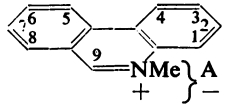
First derivatives of the 2 : 7-series were prepared in which these substituents are different (Table IV). The compound (145C48) containing both a urethano- and a primary amino- group is active in the incubation period, and it is significant that the closely related compound (177C48) in which the urethano- group is replaced by acetamido is inactive. However, the most active compound of this series (146C48) had no significant effect upon an established infection. Secondly, analogues of 3C47 were prepared in which the primary amino- group is in the 3-position, such compounds being more readily accessible than 3C47. The closest analogue to the latter, namely 74C48 (I, $R=NH_2$), proved to be as active in established infections and markedly less toxic by any route. The detailed structure of this active type was then modified by varying the alkyl group of the urethane, but replacement of ethyl by homologous groups furnished compounds which, although like 74C48 in being active in the incubation period, are much less effective in the established infection (Table IV). In the incubation period the activities are Et>*iso*Pr>Pr>Bu>Me. The compound (423C48) corresponding to 74C48, but with the urethane group in the *m*-position, is inactive even during the incubation period. More profound modification of the active type involving replacement of the urethane by other groups is still under examination, but so far has not revealed much promise. Thus the carbamido- compound (328C48) is ineffective.

It is difficult to account for the peculiar association in the phenanthridine series of the urethane group and activity against *T. cruzi*. An attempt has already been made by Walls (1946) on the lines that the urethane salts might in some way, perhaps on account of delayed metabolism compared with the diamino- salts, gain access to the infected tissues, and there be first converted into the diamino- salts which, it was considered, might be the effective trypanocides. Support for such a view might be found in the occasional correlation between *T. cruzi* and *T. congolense* activity. The much lower activity of the methyl urethanes (77C48, 329C48) might then be attributed to an expected difference in stability from other urethanes. The

TABLE IV

3-AMINO COMPOUNDS CONTAINING A URETHANE GROUP IN THE 9-PHENYL NUCLEUS

Activities are expressed in terms of the key given on p. 279. Preparation of these compounds will be described elsewhere.



Compound number	Substituent	A	Activity against *T. congolense*	Activity against *T. cruzi*			
				Incubation period		Established infection	
				Dose mg./kg.	Activity	Dose mg./kg.	Activity
146C48	2-NO_2-7-NH_2-9-C_6H_5	Cl	3	6×12.5	3	5×12.5	0
145C48	2-NH_2-7-NH.CO_2Et-9-C_6H_5	Cl	2	6×12.5	2	—	—
177C48	2-NH_2-7-NH.COMe-9-C_6H_5	$EtSO_3$	2	6×12.5	0	—	—
75C48	3-NO_2-9-*p*-C_6H_4.NH_2	Cl	2	10×12.5	1	—	—
76C48	3-NO_2-9-*p*-C_6H_4.NH.CO_2Et	$MeSO_4$	0	6×12.5	0	—	—
74C48	3-NH_2-9-*p*-C_6H_4.NH.CO_2Et	$EtSO_3$	2	6×12.5	3	5×12.5	3
329C48	3-NH_2-9-*p*-C_6H_4.NH.CO_2Me	Cl	0	6×12.5	2	6×12.5	0
357C48	3-NH_2-9-*p*-C_6H_4.NH.CO_2Pr	$EtSO_3$	3	5×20 5× 5	2 1	5×20	(Life prolonged but not cleared)
381C48	3-NH_2-9-*p*-C_6H_4.NH.CO_2*iso*Pr	$EtSO_3$	2	6×12.5	2	5×20	0
424C48	3-NH_2-9-*p*-C_6H_4.NH.CO_2Bu	$MeSO_4$	1	5×20 5×5	2 1	5×20	2
423C48	3-NH_2-9-*m*-C_6H_4.NH.CO_2Et	Cl	1	6×12.5	0	—	—
328C48	3-NH_2-9-*p*-C_6H_4.NH.$CONH_2$	Cl	1	6×12.5	1	6×12.5	0

further facts now adduced hardly support such a hypothesis, nor do the pharmacological results presented in the next paper (Part III). For instance, it is difficult to understand why 74C48 should be so much more active than 145C48, which is related to 6C46, a much more powerful trypanocide than is 3-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (399C48), the diamino- salt corresponding to 74C48. Finally, 74C48 is extremely rapidly excreted, there being no evidence that it is retained longer in the tissues than is 6C46.

SUMMARY

1. Methods of testing drugs for activity against *T. cruzi* infections in mice are discussed.

2. A large number of phenanthridinium compounds have been examined and several of them, particularly 9-phenylphenanthridinium salts with urethane substituents, proved to be active when injected during the incubation period of the disease.

3. Only a small proportion of these had any effect upon established infections, but two, namely 2-amino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium bromide (3C47) and 3-amino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium ethanesulphonate (74C48), show promise for the treatment of Chagas's disease.

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