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THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

PART I

TRYPANOSOMA CONGOLENSE AND *TRYPANOSOMA RHODESIENSE*

BY

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The powerful trypanocidal activity of certain phenanthridinium compounds was first demonstrated by Browning and his collaborators in 1938 (Browning, Morgan, Robb, and Walls, 1938 ; Walls, 1947a ; Browning, 1949). This activity is highly specific against African bovine trypanosomiasis caused by *T. congolense* and *T. vivax*. A single dose is usually effective and, in spite of local irritant action and the occasional late toxic effects observed in some districts, several million doses have been given. The drugs have thus gained a place in the treatment of a disease for which radical cure was previously difficult to achieve.

At the time the work described in this paper started it had been demonstrated (Walls, 1947b) that high trypanocidal activity in the phenanthridinium series is a property of quaternary salts containing a primary amino- group in the 7- and a phenyl group in the 9-position ; the activity is much enhanced by the presence of a second primary amino-group, 2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide (dimidium bromide) being particularly effective. Compounds with the amino- group in the 3-position are less active. Our work was designed to test these conclusions further, and if possible to extend them. The investigations fall into five main groups:

(*a*) To establish whether the 7-position represents the optimum location for the first primary amino- group.

(*b*) To determine the effect of variation of the 9-substituent and, in particular, whether the high activity of the 2 : 7-diamino- compounds is associated with other 9-substituents than phenyl.

(*c*) To determine the effect of modifying the primary amino- groups.

(*d*) To determine the effect of alkoxyl groups.

(*e*) To consider the relation between trypanocidal and antibacterial activity.

Materials and Methods

The methods of preparation and the chemistry of all the substances examined have been described elsewhere (see references given in the Tables).

Trypanocidal tests.—The strain of *Trypanosoma congolense* used was identical with the strain (No. III) described by Wien (1947), and killed mice in 7 to 9 days. The strain of *T. rhodesiense* used was first isolated from a human case in 1934; it killed mice in 2 to 3 days.

For the trial of a drug, groups of 5 mice (18 to 25 g.) were inoculated intraperitoneally with a suspension of infected mouse blood in glucose-saline. The inoculum of *T. congolense* contained about 1,000 trypanosomes per μl. and that of *T. rhodesiense* about 12,000 per μl. and each mouse received 0.5 ml. A single subcutaneous dose of drug, dissolved or suspended in water, was given when trypanosomes appeared in the peripheral blood of the inoculated mice; this was on the day following inoculation with *T. rhodesiense*, and after 3 to 5 days with *T. congolense*. Blood examinations were made daily. One group of mice was always kept untreated as a control, and another group was treated with dimidium bromide for comparison.

As a result of this preliminary test, drugs were classified in degrees of potency as follows:

Dose required to clear the peripheral blood of at least 80% of a group of mice	*Potency*
No activity at 50 mg./kg.	0
10 to 50 mg./kg.	1
1 to 10 mg./kg.	2
0.1 to 1.0 mg./kg.	3
0.01 to 0.1 mg./kg.	4

This rough assessment was sufficient for all those drugs with an activity against *T. congolense* of less than "3," because they could not possibly be of value in the treatment of the disease in the field. All those substances which had a higher degree of activity were assayed quantitatively against dimidium bromide (6C46) using groups of 10 mice at several dose levels. The assays were based upon the number of mice which were cleared of the infection (Hawking, 1941) and upon the survival time of the groups of treated animals (Bülbring and Burn, 1938). Both these methods are discussed by Goodwin (1944). Dimidium bromide (6C46) and phenidium chloride (129C46) have both been used in the field for the treatment of bovine trypanosomiasis and the greater efficacy of the former over the latter in the ratio of 1 to 0.33 observed by ourselves in mice is apparently found also in the field.

In vitro *antibacterial activity.*—The minimal dilutions inhibiting the growth of small inocula of a representative range of organisms were observed. Nutrient broth was used as the culture medium except for mycobacterium and corynebacterium for which a modified Long's synthetic medium and litmus milk were used. Susceptible bacteria were passaged on solid and in liquid media containing increasing concentrations of drug, in order to detect the emergence of resistant strains.

In vivo *antibacterial activity.*—The *in vivo* antibacterial activity of phenanthridinium compounds was assessed in side-by-side comparisons with sulphadiazine in groups of mice infected with *Streptococcus pyogenes* CN.10 and *Escherichia coli* CN.314. Treatment was by subcutaneous injection daily and the largest dose was that which caused no acute deaths in infected mice.

*Str. pyogenes CN.*10. Mice were infected intraperitoneally with 0.5 ml. of a suspension containing 10,000 average lethal doses. The drugs were administered immediately after infection and again six hours later on the first day; during the subsequent days injections were given at 9.30 a.m. and 5 p.m.

*E. coli CN.*314. Except that the infecting dose was one of 100 average lethal doses of organisms suspended in 5 per cent hog gastric mucin, the details of treatment and management were identical with the streptococcal-infection experiment.

Acute toxicity.—Estimates of the acute toxicity were made, usually on two groups of ten mice, and sometimes on two groups of six. Drugs were injected slowly intravenously in volumes of 0.5 ml. Since one object was to provide a guide for therapeutic experiments the mice were observed for at least 3 days. The average lethal doses (LD50) were read from a graph drawn on a log-probit scale. The results are summarized in Tables I–XI.

Results and Discussion

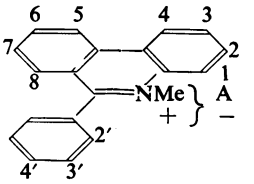
An attempt to correlate structure and antiparasite activity from the results of *in vivo* tests is largely frustrated, even within such a closely related series as that now under discussion, by the complications of absorption, distribution, metabolism, and excretion. Association of certain structural features and trypanocidal activity within this series does emerge, however, and (with the above limitation in mind) it is worth discussing the mode of action of these compounds in terms of their known chemical properties. Throughout the following discussion we have used the term "trypanocidal" to indicate that property of a drug which causes trypanosomes to disappear from infected animals when they are treated with the drug by subcutaneous injection. As we are ignorant of the exact mode of action of these compounds upon the parasites, a more accurate term might be "antitrypanosomal." This is a cumbersome word, and we have retained the older term for the purpose of this discussion with full awareness of its shortcomings.

(*a*) *The position of the amino- groups*

We have examined quaternary salts of the series with a primary amino- group in the 2-, 3-, 6-, 7-, and 8-position, and the comparative results are shown in Table I. As expected from the earlier work, the most active compounds are those with an associated 9-*p*-aminophenyl substituent, for which group the order of activity is 6-amino>2->7- $\gg$ 3-=8-, but with a 9-*p*-nitrophenyl substituent the 6-amino-compound much excels the others. It is of interest to recall that the 2-, 6-, and 8-amino- groups are in resonant positions; according to the notation of Gore and Phillips (1949), the ionic quinonoid structures are respectively of the *ortho-para*, *para-para*, and *ortho-ortho* type. From the point of view of trypanocidal activity, additional ionic resonance thus appears to be of secondary importance, for the 2- and 6-compounds do not greatly exceed the 7-compound in activity, and the 8-compound is almost inactive. The inactivity of the latter may be due to steric causes: the proximity of the 9-C atom and its substituent may prevent effective combination of an 8-amino- group with an enzyme. The original postulate that the introduction of a 7-amino- group into the 9-phenylphenanthridinium molecule confers high trypanocidal activity can thus be extended to include the 2- and 6-amino- groups.

TABLE I

EFFECT OF POSITION OF AMINO- GROUP ON TRYPANOCIDAL ACTIVITY



The figures in columns 5 and 6 represent degrees of potency (see p. 262) and the figures in parentheses the relative potencies when assayed against dimidium bromide (6C46).

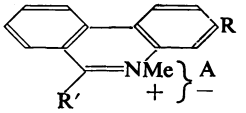
No.	Substituent	A	Ref.	*T. cong.*	*T. rhod.*	Streptococcus		i.v LD50 mg./kg.
						In vitro	*In vivo*	
522C46	2-NH_2-4′-NH_2	Br	*a*	3 (0.5)	2	+	0	9
399C48	3-NH_2-4′-NH_2	Cl	*b*	1				
563C46	6-NH_2-4′-NH_2	Cl	*a*	3 (0.7)	0	+	0	10
*129C46	7-NH_2-4′-NH_2	Cl	*b*	3 (0.3)	1	+	+	8
4C47	8-NH_2-4′-NH_2	Br	*a*	1	0	+	0	12
443C46	2-NH_2-4′-NO_2	Cl	*a*	2	2	+	0	14
63C47	6-NH_2-4′-NO_2	Cl	*a*	3 (0.3)	1	+	0	2.6
442C46	7-NH_2-4′-NO_2	Cl	*c*	1	1	+	+	4.8
65C47	8-NH_2-4′-NO_2	Cl	*a*	1	0	+	0	4.5

* Under the designations of " 897 " and " phenidium chloride " this compound has been used in the field for the treatment of bovine trypanosomiasis.

References: (*a*) Caldwell and Walls (1948); (*b*) Morgan and Walls (1938); (*c*) Walls (1947b).

TABLE II

2-AMINOPHENANTHRIDINIUM SALTS

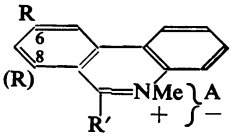


The figures in columns 5 and 6 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46). All compounds described by Caldwell and Walls (1948)

No.	R. subst.	R′	A	*T. cong.*	*T. rhod.*	Streptococcus		i.v. LD50 mg./kg.
						In vitro	*In vivo*	
523C46	NH_2	Me	Br	1	1	+	+	29
522C46	NH_2	*p*-$C_6H_4.NH_2$	Br	3 (0.5)	2	+	0	9
443C46	NH_2	*p*-$C_6H_4.NO_2$	Cl	2	2	+	0	14
3C47	NH_2	*p*-$C_6H_4.NHCO_2Et$	Br	2 (0.2)	2	+	0	7
352C47	NH_2	*p*-$CH_2.C_6H_4.NH_2$	Cl	1	0	+	±	5.6
641C46	$NH.CO_2Et$	Me	Br	0	1	+	+	37
64C47	NH.COMe	*p*-$C_6H_4.NH_2$	Cl	0	1	+	0	<5
291C46	NH.COMe	*p*-$C_6H_4.NO_2$	Cl	1	0	+	±	54
157C47	$NH.CO_2Et$	*p*-$C_6H_4.NH_2$	Cl	1	0	+	0	12
489C46	$NH.CO_2Et$	*p*-$C_6H_4.NO_2$	Cl	2	1	+	0	47
301C47	$NH.CO_2Et$	*p*-$C_6H_4.NHCO_2Et$	Cl	1	1	±	±	<5
2C47	NH.COMe	*p*-$C_6H_4.NHCO_2Et$	Cl	1	1	+	0	7

TABLE III

6- AND 8-AMINOPHENANTHRIDINIUM SALTS

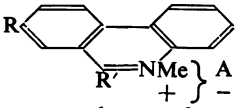


The figures in columns 5 and 6 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46). All compounds described by Caldwell and Walls (1948).

No.	R	R′	A	*T. cong.*	*T. rhod.*	Streptococcus		i.v. LD50 mg./kg.
						In vitro	*In vivo*	
31C46	6-NH_2	Me	Br	1	0	+	+	5.8
563C46	6-NH_2	*p*-$C_6H_4.NH_2$	Cl	3 (0.7)	0	+	0	9.7
63C47	6-NH_2	*p*-$C_6H_4.NO_2$	Cl	3 (0.3)	1	+	0	2.6
4C47	8-NH_2	*p*-$C_6H_4.NH_2$	Br	1	0	+	0	12.0
65C47	8-NH_2	*p*-$C_6H_4.NO_2$	Cl	1	0	+	0	4.5
9C46	6-$NH.CO_2Et$	Me	Br	1	0			

TABLE IV

7-AMINOPHENANTHRIDINIUM SALTS



The figures in columns 6 and 7 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46).

No.	R	R′	A	Ref.	*T. cong.*	*T. rhod.*	Strepto-coccus		i.v. LD50 mg./kg.
							In vitro	*In vivo*	
376C46	NH_2	Me	Br	*a*	1	0	+	±	22
359C47	NH_2	*m*-$C_6H_4.NH_2$	Cl	*b*	3 (0.3)	1	+	±	7.8
129C46	NH_2	*p*-$C_6H_4.NH_2$	Cl	*c*	3 (0.3)	1	+	+	8
442C46	NH_2	*p*-$C_6H_4.NO_2$	Cl	*a*	1	1	+	+	4.8
441C46	NH_2	*p*-$C_6H_4.NH.CO_2Et$	Cl	*a*	1	0	+	±	1.6
405C46	NH_2	*p*-$CH_2.C_6H_4.NH_2$	Cl	*d*	1	1	+	+	8.0
404C46	NH_2	*p*-$CH_2.C_6H_4.NO_2$	Cl	*d*	1	0	±	0	ca 50.0
656C46	NH_2	*p*-$CH_2.C_6H_4NHCO_2Et$	Br	*d*	1	0	±	±	5.50
167C47	NH_2	*p*-$CO.C_6H_4.NH_2$	Br	*e*	1	0	±	0	12
90C47	NH_2	*p*-$CO.C_6H_4.NO_2$	Cl	*e*	1	0	+	0	17
1C46	$NH.CO_2Et$	Me	Br	*a*	1	1			
377C46	NH.COMe	*p*-$C_6H_4.NH_2$	Cl	*a*	1	0	+	0	41
291C46	NH.COMe	*p*-$C_6H_4.NO_2$	Cl	*a*	1	0	+	+	54
292C46	NH.COMe	*p*-$C_6H_4NH.CO_2Et$	Cl	*a*	1	0	±	0	61
403C46	$NH.CO_2Et$	*p*-$CH_2.C_6H_4.NH_2$	Cl	*d*	0	0	±	0	74
166C47	$NH.CO_2Et$	*p*-$CO.C_6H_4.NH_2$	Cl	*e*	0	0	±	0	Insol.
88C47	$NH.CO_2Et$	*p*-$CO.C_6H_4NO_2$	Cl	*e*	0	0	±	0	56
555C46	$NH.CO_2Et$	*p*-$CO.C_6H_4NH.CO_2Et$	Cl	*e*	1	0	±	±	Insol.

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

(*b*) *Variations of the 9-substituent*

Results for the 2-amino- series are given in Table II, for the 6- and 8- series in Table III, and for the 7-amino- series in Table IV. It is obvious that for high trypanocidal activity *p*-aminophenyl is the most effective 9-substituent of those examined, its replacement (Table IV) by methyl (376C46), *p*-aminobenzyl (405C46), or *p*-aminobenzoyl (167C47) resulting in a considerable fall in activity. Likewise, replacement of the amino- group of the 9-substituent by other groups such as nitro in 442C46 or carbethoxyamino in 441C46 leads to loss of activity. Acylation of the 2-, 6-, or 7-amino- group usually causes a big fall in activity even if the amino- group of the 9-substituent is preserved (e.g., 64C47, 157C47, 377C46). It is noteworthy that the 2-amino- series shows a consistently higher rating than the 7-amino- series against *T. rhodesiense*, although as is characteristic of the series the activity against this species is substantially less than that against *T. congolense*, and does not always run parallel to it. The 2-amino- series is also superior against *T. congolense* although the difference is less marked, thus 523C46>376C46, 522C46>129C46, and 3C47>441C46. These relatively small differences may be associated with the possibility of additional ionic resonance in the 2-amino- series, which would stabilize the amino- group and perhaps the whole molecule. High trypanocidal activity is present, whether the amino- group is in the *meta-* (359C47) or *para-* (129C46) position of the 9-phenyl substituent; additional ionic resonance is possible for the *para-* compound but here has no obvious significance, since the *meta-* is at least equal to it in activity.

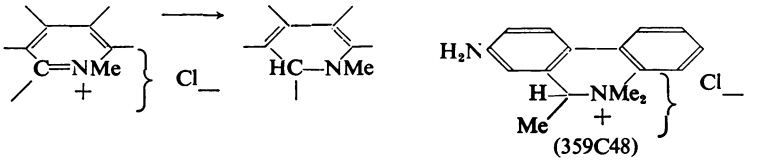
The low activity of the 9-*p*-aminobenzyl- (352C47 and 405C46) and the 9-*p*-aminobenzoyl- (167C47) compounds is striking, since they would appear to possess the features characteristic of the active types, but the following tentative explanations may be advanced.

(i) *A difference in molecular shape.*—Examination of models of the Stuart type reveals that in the 9-phenylphenanthridines the 9-phenyl group has its 1′ : 4′-axis at 60° to the 2 : 7-axis of the phenanthridine nucleus, but its rotation is obstructed by the N-methyl group so that the planes of the two ring systems are at an angle. In the 9-benzyl derivatives similar restriction of rotation of the methylene group fixes the axis of the phenyl group approximately at right-angles to the plane of the phenanthridine ring.

(ii) *A greater vulnerability to metabolic attack.*—The 9-benzyl compounds possess a chemically reactive methylene group, but since the 9-*p*-aminobenzyl compounds are among the most effective *in vivo* antistreptococcal drugs yet found in the phenanthridine series, it is difficult to associate their low trypanocidal activity with more rapid metabolism by the host than is suffered by the corresponding 9-*p*-aminophenyl compounds.

(iii) *Oxidation-reduction properties.*—A relationship between trypanocidal action and redox properties has often been sought (Bär, 1941). Many nitrophenanthridinium salts have been reduced to trypanocidal aminophenanthridinium salts by iron powder, stannous chloride, or ferrous hydroxide, the quaternary system $>C=\overset{+}{N}Me-\}\bar{Cl}$, being unaffected by the process (Walls, 1947a).

On the other hand in slightly alkaline solution under quite mild conditions this quaternary system can be reduced catalytically to the rather unstable dihydro-type (Caldwell, Copp, and Walls, 1950), a change which involves the loss of aromatic character in one of the rings.



(359C48)

This generalization does not hold for the 9-benzoyl phenanthridinium salts, which respond differently to reducing agents, a dihydrophenanthridine being formed whether iron powder or ferrous hydroxide is used ; with iron powder it is the sole product, with ferrous hydroxide the quaternary salt is the principal product. If then the benzyl compounds are converted into benzoyl compounds in the body, it is tempting to associate the relative inactivity of both series to this difference towards reducing agents. A compound (359C48) which possesses the features associated with trypanocidal activity in this series except that the quaternary group is not of the hetero-aromatic type ($>C=\overset{+}{N}Me\}Cl^-$) and which therefore cannot be reversibly reduced is devoid of trypanocidal activity.

Results that have a bearing on these questions have been obtained with the 2 : 7-diamino- compounds (Table V) among which are found the most active trypanocides of the series. The 9-methyl compound (640C46) is relatively inactive, and the known chemical reactivity of a methyl group in this situation suggests that this result may be due to its serving as a focus of metabolic attack, with consequent conversion of this compound into an inactive phenanthridone:

A somewhat similar explanation has been advanced (Walker, 1947) for the inactivity of the quinaldine analogues of the sontochin type of antimalarial.

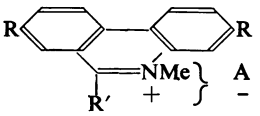
The low activity of the 9-benzoyl compound (319C49) is striking. In oxidation-reduction properties it resembles the other benzoyl compounds (90C47 and 167C47), and since this group is thus differentiated chemically from the highly active 9-phenyl compounds, it is reasonable to associate trypanocidal activity with certain redox properties. That the 9-benzyl compound (660C47) is also less active than the 9-phenyl analogue (6C46), although much more active than the 9-benzoyl compound, also supports this conclusion.

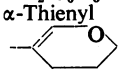
2 : 7-Diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (150C47) is the most effective compound yet found in this series for the treatment of *T. congolense* or *T. rhodesiense* infections in mice. Against the latter organism it is about as active as pentamidine isethionate, but is more toxic and does not possess the same prophylactic properties. The 9-*p*-nitrophenyl (676C46) and the 9-thienyl (621C47) compounds are slightly less effective against *T. congolense*, but the latter (621C47) has a comparatively weak action against *T. rhodesiense*.

TABLE V

2:7-DIAMINOPHENANTHRIDINIUM SALTS

The figures in columns 6 and 7 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46).



No.	R	R′	A	Ref.	*T. cong.*	*T. rhod.*	Streptococcus		i.v. LD50 mg./kg.
							In vitro	*In vivo*	
640C46	NH_2	Me	Br	*a*	1	1	+	±	15
*6C46	NH_2	C_6H_5	Br	*b*	4	3	+	0	11
150C47	NH_2	*p*-$C_6H_4.NH_2$	Cl	*c*	4 (1.5)	3	+	+	9.4
676C46	NH_2	*p*-$C_6H_4.NO_2$	Cl	*c*	4 (1.3)	3	+	+	8.4
660C47	NH_2	$CH_2.C_6H_5$	Br	*c*	3 (0.8)	1	+	+	17
319C49	NH_2	*p*-$CO.C_6H_5$		*d*	2 (0.03)	0			8.0
621C47	NH_2	α-Thienyl	Br	*c*	4 (1.3)	2	+	+	12
492C46	$NH.CO_2Et$	Me	$MeSO_4$	*a*	1	0	+	0	19
460C47	NMe_2	C_6H_5	Br	*c*	1	0	+	0	3
149C47	NH.COMe	*p*-$C_6H_4.NH_2$	Cl	*c*	1	1	±	±	8.2
25C47	NH.COMe	*p*-$C_6H_4.NO_2$	Cl	*c*	1	0	0	0	7.5
653C47	$NH.CO_2Et$	*p*-$C_6H_4.OMe$	$MeSO_4$	*c*	0	0	+	±	38
659C47	$NH.CO_2Et$	$CH_2.C_6H_5$	Br	*c*	1	0	+	±	41
620C47	$NH.CO_2Et$	α-Thienyl	Cl	*c*	1	0			
109C48	$NH.CO_2Et$		Cl	*c*	1	0			

* Under the designations of " 1553 " and " dimidium bromide " this compound has been used in the field for the treatment of bovine trypanosomiasis.

References: (*a*) Walls (1947b); (*b*) Walls (1945); (*c*) Walls and Whittaker (1950); (*d*) Caldwell Copp, and Walls.

The high activity of the triamino compound 150C47 reveals a progressive increase in trypanocidal activity as the fundamental 9-phenylphenanthridinium system is substituted with amino- groups (Table VI). 9-Phenyl-10-methylphenanthridinium chloride (44C46) is very slightly active; introduction of an amino-substituent into the phenyl group increases the activity (456C47), but if the amino- group is in the phenanthridine ring the activity is greater (Walls, 1945). With two amino- groups (e.g., dimidium bromide, 6C46) a great increase in activity results, and with three such groups the high activity of 150C47 is achieved.

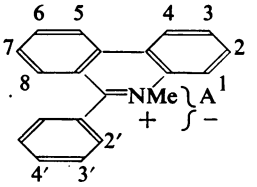
(*c*) *Modification of the primary amino- groups*

This has generally resulted in greatly diminished activity. Their replacement by amidino- (Barber *et al.*, 1947), acetamido-, ureido-, urethano- or nitro- groups (Tables II–V) greatly reduces the trypanocidal properties, but if one primary amino- group is left, particularly in the phenanthridine nucleus (3C47, Table II), considerable activity may be present. Conversion of primary into tertiary amino- groups (460C47, Table V) results in loss of activity without a corresponding loss in *in vitro* antibacterial activity; this suggests that trypanocidal and antibacterial actions may operate by a different mechanism. For trypanocidal activity some interaction

TABLE VI

EFFECT OF AMINO- GROUPS ON TRYPANOCIDAL ACTIVITY

The figures in columns 5 and 6 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46).



No.	Substituents	A	Reference	*T. cong.*	*T. rhod.*	Streptococcus		i.v. LD50 mg./kg.
						In vitro	*In vivo*	
44C46	None	Cl		0–1	0	+	0	3.5
456C47	4′-NH_2	Cl	Morgan and Walls, 1931	2	0	+	0	5.5
522C46	2-NH_2-4′-NH_2	Br	Table I	3 (0.5)	2	+	0	8.9
129C46	7-NH_2-4′-NH_2	Cl	Table II	3 (0.3)	1			8
6C46	2 : 7-diNH_2	Br	Table IV	4 (1.0)	3	+	0	11
150C47	2 : 7-diNH_2-4′-NH_2	Cl	Table IV	4 (1.5)	3			9.4

between an enzyme and a primary amino- group may be presumed (perhaps hydrogen bonding but certainly a reaction that is not possible with a tertiary amino- group). This hypothesis, which is reminiscent of that postulated by Pauling (1948) for toxin-antitoxin combination furnishes a plausible explanation of the increase in trypanocidal activity as primary amino- groups are added to the fundamental ring-system, a process, however, that does not significantly alter the *in vitro* antibacterial activity. A compound with one primary amino- group may be regarded in this respect as being univalent, a compound with two such groups as divalent, and thus anchored far more effectively to the enzyme ; a third amino- group further increases the binding-power, but not in the same proportion.

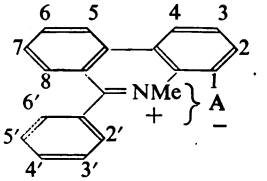
(*d*) *The effect of alkoxyl groups*

There is an outstanding exception to the generalization that modification of a primary amino-group results in profound loss of trypanocidal activity. Dr. F. C. Copp had prepared 7-methoxy-9-*p*-methoxyphenyl-10-methylphenanthridinium chloride (379C46) for another purpose, and it was unexpectedly found that this compound possessed significant activity (Table VII). Since the methoxyl group figures in many therapeutically active compounds, the synthesis of phenanthridinium salts with associated alkoxy- and amino- groups was therefore undertaken. The first of this type to be prepared (286C47), in which an "essential" amino- group had been replaced by a methoxyl group, proved to be highly active. As the size of the alkoxyl group is increased up to normal propyl, the activity increases so that 372C48 is only a little less active than dimidium bromide itself. The 7-*iso*propoxy- (117C49) and 7-butoxy- (119C49) compounds are almost inactive, and the introduction of a second amino- group (105C48) also greatly reduces the activity. The

TABLE VII

EFFECT OF ALKOXYL AND HYDROXYL GROUPS

The figures in columns 4 and 5 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46). All compounds described by Copp and Walls (1950).



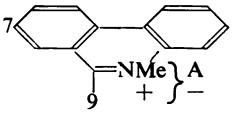
No.	Substituents	A	*T. cong.*	*T. rhod.*	Streptococcus		i.v. LD50 mg./kg.
					In vitro	*In vivo*	
214C48	2-OMe-4′-NH_2	Cl	1		+	0	2.5
322C48	2-OH-4′-NH_2	Cl	0	0			
286C47	7-OMe-4′-NH_2	Cl	3 (0.3)	0	+	±	14
671C47	7-OMe-3′-NH_2	Cl	3 (0.3)	0	+	0	16
196C48	7-OEt-4′-NH_2	Cl	3 (0.7)	0	+	0	13
372C48	7-O*n*Pr-4′-NH_2	Cl	3 (0.7)	0			18
54C49	7-O*n*Pr-3′-NH_2	Cl	3 (0.25)				6
117C49	7-O*iso*Pr-4′-NH_2	Cl	0	0			
119C49	7-O*n*Bu-4′-NH_2	Cl	0	0			
105C48	7-OMe-3′: 5′-NH_2	Cl	1	0	+	0	14
300C47	7-OH-4′-NH_2	Cl	1	0	+	0	35
725C47	7-OH-3′-NH_2	Cl	1	0	+	0	14
213C48	2-OMe-4′-NO_2	Cl	1	0	+	0	6.1
323C48	2-OH-4′-NO_2	Cl	0	0			
284C47	7-OMe-4′-NO_2	Cl	3 (0.3)	0	+	±	2.6
670C47	7-OMe-3′-NO_2	Cl	1	0	+	0	3.5
190C48	7-OEt-4′-NO_2	Cl	3 (0.3)	0	+	0	6.9
348C48	7-O*n*Pr-4′-NO_2	Cl	2	0	+	0	13.8
116C49	7-O*iso*Pr-4′-NO_2	Cl	0	0			
118C49	7-O*n*Bu-4′-NO_2	Cl	0	0			3.9
98C48	7-OMe-3′: 5′-NO_2	Cl	1	0	+	0	5.5
723C47	7-OMe	Cl	0	0	+	0	4.3
730C47	7-OH	Cl	0	0	+	+	7.8
379C46	7-OMe-4′-OMe	Cl	2	0	+	0	3.1
699C46	7-OH-4′-OH	Cl	1	0	+	+	6.4
709C47	7-OMe-4′-NH.CO_2Et	Cl	1	0	+	±	12
672C47	7-OMe-3′-NH.CO_2Et	Cl	1	0			
710C47	7-OH-4′-NH.CO_2Et	Cl	0	0	+	±	8.3
118C48	7-OH-3′-NH.CO_2Et	Cl	0	0			
93C48	7-OMe-4′-Cl	Cl	1	0	+	0	11
117C48	7-OH-4′-Cl	Cl	0	0	+	+	10

hydroxyl compounds are also inactive. A further surprising feature of the series of compounds listed in Table VII is that certain alkoxyl salts, containing a nitro- group (284C47, 190C48) in place of the primary amino- group, are hardly less active than the amino- salts. The relative order of activity of these nitro- salts differs from that of the amino- salts. The 2-alkoxyl compounds (213C48, 214C48) are comparatively inactive, thus forming a contrast to the 2-amino- compounds, which usually exceed the 7-amino- compounds in trypanocidal activity. This alkoxyl class has a more specific activity against *T. congolense* than has even the diamino- series.

TABLE VIII

OTHER ALKOXYL AND HYDROXYL COMPOUNDS

The figures in columns 4 and 5 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46). All compounds described by Copp and Walls (1950)



No.	Substituents	A	*T. cong.*	*T. rhod.*	Streptococci		i.v. LD50 mg./kg.
					In vitro	*In vivo*	
332C48	7-OMe-9-Me	Br	0	0	+	+	22
206C47	7-OH-9-Me	Cl	0	0	+	0	18
297C46	9-*p*-$CH_2C_6H_4OMe$	Cl	0	0	+	0	34
28C48	7-OMe-9-$CH_2.C_6H_4.NH_2$	Cl	0	0	+	0	7
47C48	7-OH-9-$CH_2.C_6H_4.NH_2$	Cl	0	0	+	0	14
94C48	7-OMe-9-$CH_2.C_6H_4.NH.CO_2Et$	Cl	0	0	+	0	14
149C48	7-OH-9-$CH_2.C_6H_4.NH.CO_2Et$	Cl	1	0	+	0	Insol.

These results indicate that a new factor may be operating and if these alkoxyl salts exert their action by a different mechanism from the diamino- salts it is possible that the deleterious effects that occasionally accompany the use of the diamino- salts in the field (Randall and Beveridge, 1946) might be absent for this class.

A consequence of the hypothesis that the amino- and alkoxy- groups may be concerned in " anchoring " the active molecule on to an enzyme system is that the distance between these groups is important. Compounds other than phenanthridinium salts which fulfil the structural desiderata and have the appropriate distance between groups may be found in the *iso*quinoline series. Nevertheless such compounds are practically inactive (McCoubrey and Mathieson, 1949 ; Caldwell, Walls, and Whittaker), a result which may be attributed either to the difference in weight and area of the molecules, to a difference in redox properties of the salts, or to an unfavourable electronic distribution. The area of the molecule is probably the important factor ; thus Albert (1949) regards a minimal flat area as an essential requirement, at any rate for antibacterials of the heterocyclic cationic class to which these phenanthridinium salts also belong, " in order to provide a sufficiently great area for adsorption ; it depends on the fact that increases in the number of atoms in a molecule increase its Van der Waals' attraction, whereas the kinetic energy of translation, which is the force tending to remove the molecule, is independent of molecular size."

No formal analogy can be found between the trypanocidal types and metabolites known to be essential to the growth of micro-organisms. In that they are quaternary salts, there may be said to be some analogy to aneurin, but experiments in which mice were given large daily doses of aneurin showed that this vitamin does not affect the trypanocidal action of dimidium bromide. Results in the field indicate, however, that a nutritional factor may be involved, for Stewart (1947) and Evans (1948) both

implicate grazing conditions in the delayed toxicity that is an occasional feature of its use. It is possible that the hepatotoxic properties of the drug are aggravated by the presence of plants in the grazing known to predispose animals to hepatic necrosis.

It is well known that the trypanocidal activity of organic arsenicals has been attributed to the interaction of the tervalent form with essential enzymes containing sulphydryl groups. That phenanthridinium salts might exert their action by a similar mechanism was suggested by the implication of a phenanthridinium salt, sanguinarine, as the toxic principle of argemone oil (Sarkar, 1948). This alkaloid poisons enzymes containing sulphydryl groups, an effect which may be forestalled by prior addition of BAL ; likewise the *in vivo* toxicity of sanguinarine is reduced by prior injection of BAL. Neither the trypanocidal activity nor the toxicity of phenanthridinium salts is, however, influenced by BAL.

(*e*) *Toxicity*

Most of the potent compounds examined had an intravenous toxicity to mice of 5 to 20 mg./kg. Death usually occurred, if at all, in less than an hour after injection, but there were a few exceptions. With 196C48 (Table VII), mice survived for three hours on doses which ultimately killed the whole batch. With 460C47 (Table V), 456C47 (Table VI), and 214C48 (Table VII) mice died over a period of four hours. The LD50 values given in the Tables necessarily refer to determinations made at different times with different batches of mice. For the compounds most active against *T. congolense* further LD50 (intravenous) determinations were made on the same day and with the same batch of mice (16 to 23 g.). Mortality was observed for one week ; the results are shown in Table IX. Only 196C48 showed delayed toxicity, death occurring during the first 18 hours after injection.

TABLE IX

THE TOXICITY OF THE MORE ACTIVE PHENANTHRIDINIUM DERIVATIVES
(All 9-phenyl derivatives unless otherwise indicated in column 2)

Drug	Substituents	Potency (*T. congolense*) compared with dimidium bromide (6C46)	LD50 (mg./kg.) i.v.
6C46	2:7-diNH_2	1.0	7.3
129C46	7:4′-diNH_2	0.3	8.7
676C46	2:7-diNH_2-4′-NO_2	1.3	10.1
150C47	2:7:4′-triNH_2	1.5	10.4
621C47	2:7-diNH_2-9-thienyl	1.3	13.1
660C47	2:7-diNH_2-9-benzyl	0.8	9.4
196C48	7-OEt-4′-NH_2	0.7	19.5

(*f*) *Antibacterial activity*

Phenanthridinium salts have a powerful *in vitro* antibacterial action against Gram-positive organisms and a significant but much smaller effect against Gram-negative types. Results with three representative phenanthridinium salts are shown in Table X. Unlike sulphadiazine none of the compounds is very effective against *E. coli*, but all are more active than sulphadiazine against *C. pyogenes*. After 16 passages of

TABLE X

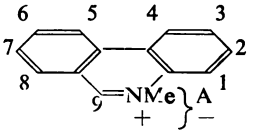
MINIMAL CONCENTRATIONS, IN MG. PER 100 ML., OF THREE PHENANTHRIDINIUM SALTS INHIBITING GROWTH OF ORGANISMS

Organism	150C47 (2 : 7 : 4′-triamino)	730C47 (7-OH-9-phenyl)	6C46 (dimidium bromide)
Str. pyogenes CN10	<0.08	0.35	0.17
,, ,, + 10% horse serum	0.08	0.7	0.17
,, ,, in whole blood ..	0.08	3.1	3.1
S. aureus, CN491	0.08	—	3.1
E. typhosa, CN512	3.1	—	50
E. coli, CN314	12.5	25	50
V. comma, CN248	1.5	—	—
S. dysenteriae, CN191	0.35	—	—
S. sonnei, CN271	3.1	—	—
S. paradysenteriae, CN225 ..	<0.04	—	6.2
Ps. aeruginosa, CN200	50	—	>200
Proteus vulgaris, CN329	3.1	—	>200
Str. agalactiae, CN1143	0.17	0.7	6.2
E. rhusiopathiae, CN353	0.17	—	1.5
M. avium, CN280	3.1	1.5	6.2
Cl. perfringens, CN1491	<0.08	—	0.17
Cl. septique, CN368	1.5	—	0.17
Cl. novyi, CN755	<0.08	—	0.08
C. pyogenes, CN1856	0.35	12.5	0.35

TABLE XI

SOME ANTIBACTERIAL COMPOUNDS

The figures in columns 5 and 6 represent degrees of potency and those in parentheses the relative potencies when assayed against dimidium bromide (6C46).



No.	Substituent	A	Reference	*T. cong.*	*T. rhod.*	Streptococci		i.v. LD50 mg./kg.
						In vitro	*In vivo*	
523C46	2-NH_2-9-Me	Br	Table II	1	1	+	+	29
442C46	7-NH_2-9-*p*-$C_6H_4.NO_2$	Cl	Table IV	1	1	+	+	4.8
405C46	7-NH_2-9-*p*-$CH_2.C_6H_4.NH_2$	Cl	Table IV	1	1	+	+	9
660C47	2 : 7-diNH_2-9-*p*-$CH_2.C_6H_5$	Br	Table V	3 (0.8)	1	+	+	17.0
621C47	2 : 7-diNH_2-9-*a*-thienyl	Br	Table V	4 (1.3)	2	+	+	12
730C47	7-OH-9-C_6H_5	Cl	Table VII	0	0	+	+	7.8
699C46	7-OH-9-*p*-C_6H_4.OH	Cl	Table VII	1	0	+	+	16
43C46	9-$CH_2.C_6H_5$	Cl	Caldwell and Walls (1948)	—	—	+	0	58
654C46	9-*p*-$CH_2.C_6H_5.NH_2$	Cl	,,	0	0	+	0	6.4
658C46	9-*p*-$CH_2.C_6H_5NH.CO_2Et$	Cl	,,	0	0	+	0	9.6

E. coli CN.314 in nutrient broth a strain emerged which grew in 100 mg. dimidium bromide per 100 ml.; originally it was inhibited at 3.1 mg. per 100 ml. Similarly after 16 passages, *Str. pyogenes* CN.10 yielded a strain inhibited at 1.25 mg. dimidium bromide per 100 ml., whereas originally the inhibiting concentration was 0.18 mg. per 100 ml.

Mechanism of antibacterial action.—The phenanthridinium salts are bactericidal to Gram-positive and Gram-negative organisms, there being a direct relation between concentration and time of exposure (Brownlee and Woodbine). Concentrations of 0.16 mg. per 100 ml. of dimidium bromide are bactericidal to *Str. pyogenes* after 30 minutes under the conditions of the test; the corresponding figures for *E. coli* are 50 mg. per 100 ml. after two hours. Of a large series of potential antagonists only the nucleic acids are effective and of these desoxyribonucleic acid is best. With *Str. pyogenes* CN.10 as the test organism and a ditch-plate technique a graded reversal began at 6.2 parts of desoxyribonucleic acid to 1 part of dimidium bromide and appeared to be complete at 50 parts to 1. The pink complex formed from phenanthridinium salts and desoxyribonucleic acid does not diffuse through a cellophane membrane although an equivalent concentration of dimidium bromide diffuses completely in two hours.

Chemotherapeutic activity in bacterial infections.—Many phenanthridinium compounds give good protection to mice infected with lethal doses of streptococci, and some protection against *E. coli*, but their high toxicity precludes their systemic use in bacterial infections. The chemotherapeutic effect may be reversed and the acute toxicity modified by the simultaneous administration of desoxyribonucleic acid (Brownlee and Woodbine). The most markedly active of the compounds *in vivo* are listed separately in Table XI. Little correlation can be found between the chemical structure of these compounds and their antibacterial properties, nor does there appear to be any obvious association of trypanocidal and *in vivo* antistreptococcal activity. The benzyl compounds (405C46 and 660C47) are the most effective antibacterials of the series, and it is interesting to note that the other member of this group 352C47 (Table II) also shows some *in vivo* activity. For comparison further representatives of this group are listed, which lack an amino- group in the phenanthridine nucleus (Table XI); they are inactive *in vivo*. Another hint of structural influence is offered by the compounds 730C47 and 699C46, and the less convincing example 710C47 (Table VII), all of which contain a phenolic group and show *in vivo* antistreptococcal activity; these compounds are almost devoid of trypanocidal activity.

Many other phenanthridine compounds, among them unquaternized phenanthridines, 9 : 10-dihydrophenanthridines and their quaternary salts (Caldwell, Copp, and Walls), and phenanthridinium salts with tertiary amino- groups, were also examined: all proved to be inactive against trypanosomes, but the quaternary salts show some *in vitro* antibacterial activity.

SUMMARY

1. A comprehensive series of phenanthridinium salts has been examined for trypanocidal activity, and it is confirmed that high activity is present in 9-phenyl-

phenanthridinium salts with two primary amino- groups, one of which must be located in the phenanthridine ring.

2. The 2-amino- surpass the 7-amino- compounds against *T. congolense*, and even more notably against *T. rhodesiense*. The 6-amino- compounds are also highly effective, but the comparative inactivity of the 8-amino- compounds is attributed to steric causes. A certain correlation may be found between the relative activity of the 2-, 3-, 6-, and 7-amino- compounds and the possibility of additional ionic resonance.

3. The 2 : 7-diamino- compounds are the most active of the series, 2 : 7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride being the most effective drug in the treatment of both *T. congolense* and *T. rhodesiense* infections in mice. Compounds of this type, but with other 9-substituents, e.g., *p*-nitrophenyl, 2-thienyl and benzyl are also highly active but the 9-benzoyl compound is much less effective.

4. Modification of the primary amino- groups, whether by acetylation or conversion to amidine or urethane groups, greatly reduces trypanocidal activity. A tertiary amino- compound corresponding to dimidium bromide is inactive.

5. 7-Alkoxy-9-*p*-aminophenylphenanthridinium salts are also highly effective against *T. congolense*. Surprisingly, the corresponding salts with a nitro- in place of the amino- group were found to be highly active.

6. An attempt has been made to determine the structural desiderata of trypanocidal activity in the phenanthridine series, and to correlate these with certain chemical factors. The inactivity of the tertiary amino- compound suggests that hydrogen-bonding between drug and an enzyme may be important. There is some evidence that trypanocidal activity is to some extent dependent upon the oxidation-reduction properties of the quaternary salts.

7. The compounds also have considerable antibacterial action, but this is not clearly related to their trypanocidal potency.

In a survey of this kind, there are a great many of our colleagues to whom we owe thanks both for practical assistance and valuable advice. We are particularly indebted to Drs. Caldwell, Copp, and Whittaker, who prepared many of the compounds; Mr. J. A. Lock, who performed the tests listed in Table IX; and to Miss S. Pluthero, Miss G. Lewis, and Messrs. P. Hankin and P. Whitfield for technical assistance.

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