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TRYPANOCIDAL ACTION OF PHENANTHRIDINE COMPOUNDS: EFFECT OF CHANGING THE QUATERNARY GROUPS OF KNOWN TRYPANOCIDES

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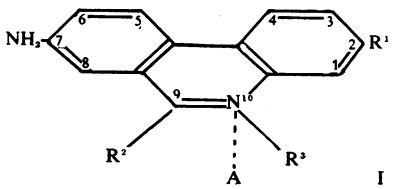
Woolfe (1952) showed that the trypanocidal action of dimidium bromide (2:7-diamino-10-methyl-9-phenylphenanthridinium bromide; I, R^1=—NH_2, R^2=phenyl, R^3=—CH_3, A=—Br) is profoundly affected by changing the quaternary group. The corresponding ethyl quaternated compound (I, R^1=—NH_2, R^2=phenyl, R^3=—C_2H_5, A=—Br), to which the name ethidium bromide has been given, was far more effective as a trypanocide



I

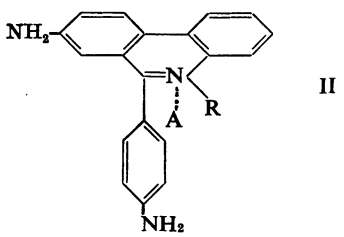
in mice against all the African species of trypanosomes used. Field trials in Africa have shown that ethidium is more active than dimidium against *Trypanosoma congolense* infections in cattle (Wilde and Robson, 1953; Wilson and Fairclough, 1953; Ford *et al.*, 1953a; Karib *et al.*, 1954; Milne and Robson, 1955) and against *T. vivax* infections in cattle (Ford *et al.*, 1953b; Unsworth, 1954a, b). In numerous experiments ethidium has also been shown to be less toxic than dimidium to cattle (Burdin and Plowright, 1952; Burdin, 1953; Ford *et al.*, 1954; Wilson, 1954). It was considered important, therefore, to try the effect of changing the quaternating group of other phenanthridinium compounds which, in the methyl-quaternated form, had been found to possess trypanocidal activity.

METHODS

The techniques used were the same as those described previously (Woolfe, 1952). Toxicities were determined by subcutaneous injection in mice. Trypanocidal

TABLE I

THE TRYPANOCIDAL ACTIVITY OF PHENIDIUM DERIVATIVES



II

Compound	Structure		LD50 mg./kg. (s.c.)	Trypanocidal Activity (Dimidium Bromide=1)			
	R	A		*congolense*	*brucei*	*rhodesiense*	*gambiense*
Phenidium chloride	methyl	Cl	60	0·3	Delayed death at 40 mg./kg.	Delayed death at 32 mg./kg.	Delayed death at 40 mg./kg.
RD2057	ethyl	Cl	100	0·5	,, ,,	0·1	,, ,,
RD2081	*n*-propyl	Cl	100	0·25	,, ,,	0·2	,, ,,
RD2075	allyl	Cl	70	0·6	,, ,,	0·15	,, ,,

activities recorded were determined by giving a single subcutaneous dose to mice which, after inoculation with diluted blood of infected mice, showed a moderate degree of parasitaemia. The Median Curative Dose (CD50) was estimated as the dose necessary to remove parasites from the peripheral blood of 50% of mice for a period of not less than one month.

RESULTS

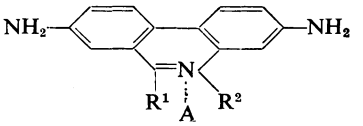
In the Tables which follow, the activity of each new compound (where activity was found) has been compared on the basis of the CD50 with that of dimidium bromide, the activity of which was taken as unity. For instance, if in one test the CD50 of dimidium bromide was 0.7 mg./kg., and that of the new compound 0.35 mg./kg., the activity would be given as 2.0. Figures in the Tables represent the means of all the tests carried out, although when a particular compound showed complete inactivity against any trypanosome species the test was not repeated.

Derivatives of Phenidium

Phenidium chloride (7-amino-9-*p*-amino phenyl-10-methylphenanthridium chloride; I, R^1=—H, R^2=*p*-aminophenyl, R^3=—CH_3, A=—Cl) was used against *T. congolense* in Africa until it was replaced by the more active compound, dimidium. However, phenidium is less toxic than dimidium, and the view has been expressed that phenidium

TABLE II

THE TRYPANOCIDAL ACTIVITY OF 2:7-DIAMINO PHENANTHRIDINIUM COMPOUNDS



Group	Compound	Structure			LD50 mg./kg. (S.C.)	Trypanocidal Activity (Dimidium Bromide = 1)			
		R^1	R^2	A		*congolense*	*brucei*	*rhodesiense*	*gambiense*
a	Dimidium bromide	phenyl	methyl	Br	85	1	1	1	1
	Ethidium bromide	,,	ethyl	Br	(110)	(10)	(20)	(11)	(50)
	RD1427	,,	*n*-propyl	Br	(100)	(6)	(35)	(12)	(40)
	RD1446	,,	allyl	Br	(115)	(6)	(20)	(18)	(30)
b	150C47	*p*-amino phenyl	methyl	Cl	>200	0·4	<0·1	<0·1	<0·1
	RD1698	,,	ethyl	Cl	140	1·5	5	2·5	3
	RD1629	,,	*n*-propyl	Cl	140	2·2	9	6	2·5
	RD1595	,,	allyl	Cl	120	3·2	2	6	3
c	676C46	*p*-nitro phenyl	methyl	Cl	120	0·5	0·4	0·6	0·4
	RD1662	,,	ethyl	Cl	140	0·6	5	3	3
	RD1601	,,	*n*-propyl	Cl	280	0·6	3	6	4
	RD1542	,,	allyl	Cl	*ca.* 400	2·5	6	8	8
d	660C47	benzyl	methyl	Br	80	0·85	0·12	0·3	0·2
	RD2607	,,	ethyl	Br	90	3·5	1·2	4	8
e	621C47	2-thienyl	methyl	Br	[12 i.v.]	[1·3]		[<1]	
	RD2482	,,	ethyl	Br	100	14	5	12	7
f	492C46	methyl	methyl	SO_4CH_3	70	0·1	Inactive at 32 mg./kg.	Delayed death at 32 mg./kg.	Delayed death at 32 mg. kg.
	RD2234	,,	ethyl	Br	125	0·125	Delayed death at 32 mg. kg.	0·03	,,
	RD2319	,,	allyl	I		0·03	Inactive at 32 mg. kg.	Delayed death at 32 mg./kg.	,,

Figures in parentheses (x) are taken from Woolfe (1952). Figures in brackets [x] are taken from Brownlee *et al.* (1950).

may have been discarded prematurely (Burdin, Plowright, and Purchase, 1952). As I found -ethyl, -propyl, and -allyl to be the most useful replacement quaternating groups in dimidium, with activity reduced by longer chains, only these groups were tried for phenidium.

The results obtained are shown in Table I. It will be seen that phenidium itself was less than one-third as active as dimidium against *T. congolense*, and almost inactive against *T. brucei*, *T. rhodesiense* and *T. gambiense*. Changing the quaternating group to -ethyl (RD 2057) or -allyl (RD 2075) increased activity against *T. congolense* about twofold, but the propyl derivative (RD 2081) had substantially the same activity as had phenidium. All three derivatives had increased activity against *T. rhodesiense*, though they were still inferior to dimidium. Activity against *T. brucei* and *T. gambiense* was little changed.

Derivatives of Known 2:7-*Diaminophenanthridinium Compounds*

Brownlee *et al.* (1950) published results of tests on a number of phenanthridinium compounds, some of which they found to be more active than dimidium. These latter compounds were recently tried in the field in Africa (Goodwin and Chandler, 1952; Goodwin and Unsworth, 1952; Neal and Karib, 1954). I had, unwittingly, duplicated some of the original work, and had agreed, in the main, with the published results, though not finding 150C47 and 676C46 (see Table II) to be more active than dimidium. However, I thought that it would be useful to try the effect of changing the quaternary group on some of these compounds, and several derivatives were made. I tried some of the less active compounds as well as those said to be more active than dimidium; Table II summarizes the results obtained. For comparison, the dimidium derivatives are included in the same Table.

Group (a), 9-Phenyl Derivatives.—As was shown previously, the trypanocidal activity of the parent compound, dimidium bromide, is increased manyfold in the ethyl, propyl and allyl quaternated compounds.

Group (b), 9-p-Aminophenyl Derivatives.—Brownlee *et al.* (1950) found the methyl quaternated compound to possess trypanocidal activity greater than that of dimidium; we found it less active than dimidium. However, the ethyl, propyl, and allyl quaternated compounds were several times more active than dimidium against *T. congolense*, *T. brucei*, *T. rhodesiense*, and *T. gambiense*.

Group (c), 9-p-Nitrophenyl Derivatives.—Again Brownlee *et al.* stated the methyl quaternated compound to be more potent than dimidium, and again we differed. Against *T. congolense* the ethyl and propyl compounds were less active than dimidium, but the allyl derivative was more active. Against the other trypanosome species all three new compounds were more active than dimidium or the parent methyl compound.

Group (d), 9-Benzyl Derivatives.—Only the ethyl derivative was prepared. This appeared to be considerably more active than the methyl derivative.

Group (e), 9-Thienyl Derivatives.—The ethyl quaternated compound had activity of a very high order against *T. congolense*, and it was more active than dimidium against all the species of trypanosomes used.

Group (f), 9-Methyl Derivatives.—The parent methyl compound had negligible activity. Slight but definite activity against *T. congolense* and *T. rhodesiense* resulted after ethyl quaternation; allyl quaternation was less effective.

It is obvious from the results given, that the effect of the quaternary group on the trypanocidal activity of phenanthridium compounds is of major importance. The enhancement of activity produced by replacing a methyl group by, say, ethyl is greatest when the original compound itself has high activity; thus in the 9-phenyl series ethidium is very much more active than dimidium, but in the 9-methyl series the ethyl compound has only slight activity. We are still investigating the reason for this increase in activity.

Summary

1. The effect on trypanocidal activity of replacing the methyl quaternating group of known phenanthridinium compounds by ethyl, propyl, or allyl has been tried.

2. Trypanocidal activity was always enhanced by such changes, and the increase in activity was greatest with compounds originally of high activity.

I am grateful to Dr. T. I. Watkins, who supplied all the compounds here reported; the chemistry of this work will be published elsewhere. My thanks are due, too, to Dr. M. R. Gurd, who supplied the toxicity figures for the various compounds.

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