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THE ANTIBACTERIAL ACTIVITY OF PHENANTHRIDINE COMPOUNDS

BY

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Certain phenanthridine compounds, originally synthesized by Morgan and Walls (1938), were found by Browning, Morgan, Robb, and Walls (1938) to possess trypanocidal properties. This discovery led Walls and his colleagues (1945, 1947, 1948, 1950a, b, c, d, 1952a, b) to synthesize a large number of phenanthridine derivatives, some of which have been used with success against *T. congolense* infections of cattle. The structure-action relationships of the group have been discussed by Wien (1946), and by Brownlee, Goss, Goodwin, Woodbine, and Walls (1950), mainly from the point of view of their trypanocidal activity. At the same time the latter workers investigated the antibacterial properties of these compounds. We have collated the information they obtained on the antibacterial properties of these and related compounds, and have shown that their antibacterial properties run roughly parallel with their trypanocidal properties.

METHODS

In vitro *Activity*.—A serial dilution technique, in nutrient broth prepared from a papain digest of horse muscle, was used. *Streptococcus pyogenes* was also grown in 10% serum and in blood.

In vivo *Activity*.—Compounds were injected intravenously in volumes of 0.5 ml. into groups of 6 or 10 mice. The LD50's were read from a log-probit scale.

Sulphadiazine was used as the standard in groups of mice infected intraperitoneally with 0.5 ml. of a 1 in 50,000 suspension of an 18 hr. culture of *Streptococcus pyogenes*. Drugs were given intraperitoneally, immediately on infection, 6 hr. later, and on the morning of the following day. The doses were estimated to be below the toxic level, but occasionally they were not.

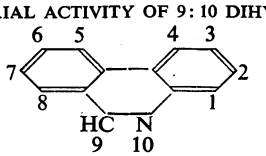
RESULTS

The therapeutic activity *in vivo* and the acute toxicity of all compounds were measured. Previous observations (Seaman, 1954) have shown that the activity against *Streptococcus pyogenes* is closely related to the general activity against many common pathogenic organisms; the results reported here are therefore confined to *Str. pyogenes*.

The compounds are grouped primarily on the basis of possession of the same major substituent on the 9 position; secondarily, on the formation

TABLE I

TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9:10 DIHYDRO PHENANTHRIDINES

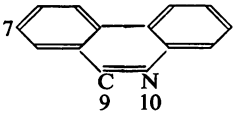


Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	7	9	10			
309C47	OH	CH_3	CH_3	25	50 (Insol)	–
368C47	OH	CH_3	CH_3CH_3I	100	5·6	–
642C46	CH_3CONH	CH_3	CH_3CH_3I	50	— (Insol)	–
491C46	C_2H_5COONH	CH_3	CH_3HCl	200	7	–
629C46	C_2H_5COONH	CH_3	CH_3CH_3I	0·4*	85	–
490C46	—	Phenyl	CH_3	3	80	–
562C46	—	Phenyl	CH_3CH_3I	1·5	21	–
610C46	CH_3O	*p*-Methoxyphenyl	CH_3	3	25 (Insol)	–
701C46	CH_3O	*p*-Methoxyphenyl	CH_3CH_3Cl	3	27·5	–
204C46	—	Benzyl	CH_3CH_3Cl	6	11·5	–
413C46	—	Benzyl	CH_3CH_3Cl	6	8·4	–

* Activity against other organisms low.

TABLE II

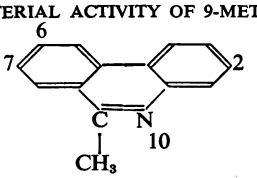
TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-ALDEHYDES AND 9-CARBOXYL PHENANTHRIDINES



Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	7	9	10			
547C47	—	CHO	—	12	— (Insol)	—
548C47	C_2H_5COONH	CHO	HCl	25	10 ,,	—
662C47	NH_2	COOK	—	200	260	—
661C47	C_2H_5COONH	COONa	—	200	34	—

TABLE III

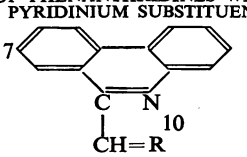
TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-METHYL PHENANTHRIDINES



Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	2	6 and 7	10			
161C47	—	7-OH	HCl	25	50 (Insol)	—
375C46	—	6-NH_2	HCl	1·5	61·5	—
630C46	C_2H_5COONH	—	HCl	200	29	—
154C46	—	7-C_2H_5COONH	HCl	200	10	—
156C46	—	7-NH_2	H_2SO_4	50	107·5	—
290C46	—	6-C_2H_5COONH	H_2SO_4	12	110·5	—
523C46	NH_2	—	CH_3Br	0·08	28·7	+
31C46	—	6-NH_2	CH_3Br	0·8	5·7	++
376C46	—	7-NH_2	CH_3Br	1·5	22·5	++
640C46	NH_2	7-NH_2	CH_3Br	0·04	14·6	+
641C46	C_2H_5COONH	—	CH_3Br	0·4	37·2	+
206C47	—	7-OH	CH_3Cl	0·08	18	—
1C46	—	7-$C_2H_5COOCONH$	CH_3SO_4	1·5	21	—
492C46	C_2H_5COONH	7-C_2H_5COONH	$CH_3CH_2SO_4$	0·4	19·2	±
9C46	—	6-C_2H_5COONH	$CH_3CH_2SO_4$	0·08	19	+

TABLE IV

TOXICITY AND ANTIBACTERIAL ACTIVITY OF PHENANTHRIDINES WITH SEMICARBAZONE, ACETOHYDRAZONE AND PYRIDINIUM SUBSTITUENTS



Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	7	=R	10			
549C47	—	$=NNHCONH_2$	HCl	50	20 (Insol)	—
550C47	C_2H_5COONH	$=NNHCONH_2$	HCl	200	— ,,	—
566C47	—	$=NNHCOCH_2N(CH_3)_3Cl$	—	0·08	11	—
575C47	C_2H_5COONH	$=NNHCOCH_2N(CH_3)_3Cl$	—	6	11·5	±
565C47	—	$=NNHCOCH_2N.Cl$	—	0·08	13	+
574C47	C_2H_5COONH	$=NNHCOCH_2N.Cl$	—	200	14	—
735C47	—	4,=CH.$C_5H_4NCH_3I$	—	0·08	13·2	+
81C48	C_2H_5COONH	4,=CH.$C_5H_4NCH_3I$	—	0·16	11·6	+
734C47	—	5,=CH.$C_5H_4NCH_3I$	—	0·08	17	+
80C48	NH_2	1,=CH.$C_5H_4NCH_3I$	—	0·16	15 (Insol)	+
82C48	NH_2	5,=CH.$C_5H_4NCH_3I$	—	0·08	17	+
79C48		5,=CH.$C_5H_4NCH_3I$	—	0·16	14	+

of series with increasingly complex substituents at positions 2 and 3, then at 6, 7, and 8 ; and, finally, on the quaternary group at 10 (see Table I).

Table I refers to compounds in which the 9:10 bond is saturated. Here the activity is generally low and the toxicity variable. The effect of a methyl group at position 10 is to decrease water solubility ; a phenyl ring at position 9 increases activity.

The compounds with an intact double bond (9–10), and a single carbon substituent (aldehyde or carboxyl) at the 9 position, possess little activity (Table II).

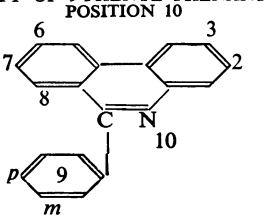
In Table III all compounds have a methyl group in position 9. They are, in general, less toxic, but when the 10 substituent is methyl there is a marked increase in activity. An interesting point is that, whereas amino groups at 2 and 7 positions confer the greatest activity (as in the trypanosome studies), those compounds with only one amino-group (2 or 7) are only as active as, and are slightly more toxic than, those without this group.

The first half of Table IV shows pairs of compounds differing only in the presence or absence of a carbethoxyamino group at position 7. This substituent lowers the activity but does not affect the toxicity, which remains at 10 to 15 mg./kg. for active phenanthridine compounds.

The compounds in Table V contain, at position 9, a phenyl group with or without substituents.

TABLE V

TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-PHENYL PHENANTHRIDINES WITH METHYL HALIDES IN POSITION 10



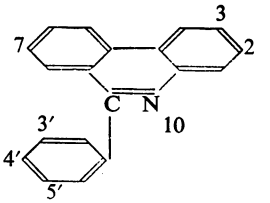
Code No.	Substituents in Positions				Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	2 and 3	6, 7, and 8	9	10			
43C46	—	—	—	CH_3Cl	0·7	3·5	−
456C47	—	—	*p*-NH_2	CH_3Cl	0·04	20	−
258C47	2-NH_2	—	*p*-NH_2	CH_3Cl	0·08	11	−
522C46	2-NH_2	—	*p*-NH_2	CH_3Br	0·16	8·7	−
443C46	2-NH_2	—	*p*-NO_2	CH_3Cl	0·08	13·8	−
75C48	3-NO_2	—	*p*-NH_2	CH_3Cl	0·35	21·5	−
64C47	2-CH_3CONH	—	*p*-NH_2	CH_3Cl	1·5	5	−
3C47	2-NH_2	—	*p*-C_2H_5COONH	CH_3Br	0·08	7	−
157C47	2-C_2H_5COONH	—	*p*-NH_2	CH_3Cl	0·17	11·7	−
489C46	2-C_2H_5COONH	—	*p*-NO_2	CH_3Cl	0·17	46	−
301C47	2-C_2H_5COONH	—	*p*-C_2H_5COONH	CH_3Cl	1·5	— (Insol)	−
494C46	3-C_2H_5COONH	—	*p*-C_2H_5COONH	CH_3Cl	0·08	32	+
730C47	—	7-OH	—	CH_3Cl	0·35	7·7	+
699C46	—	7-OH	*p*-OH	CH_3Cl	3	16·4	±
300C47	—	7-OH	*p*-NH_2	CH_3Cl	1·5	— (Insol)	−
725C47	—	7-OH	*m*-NH_2	CH_3Cl	0·35	13·7	+
710C47	—	7-OH	*p*-C_2H_5COONH	CH_3Cl	6	8·2	+
4C47	—	8-NH_2	*p*-NH_2	CH_3Br	0·7	11	−
359C47	—	7-NH_2	*m*-NH_2	CH_3Cl	0·17	7·7	+
65C47	—	8-NH_2	*p*-NO_2	CH_3Cl	1·5	4·5	−
63C47	—	6-NH_2	*p*-NO_2	CH_3Cl	0·17	2·5	−
129C46	—	7-NH_2	*p*-NH_2	CH_3Cl	0·35	6·9	+
442C46	—	7-NH_2	*p*-NO_2	CH_3Cl	0·04	4·7	+
146C48	2-NO_2	7-NH_2	—	CH_3Cl	0·08	10·7	+
6C46	2-NH_2	7-NH_2	—	CH_3Br	0·17	11	+
150C47	2-NH_2	7-NH_2	*p*-NH_2	CH_3Cl	0·08	9·4	+
676C46	2-NH_2	7-NH_2	*p*-NO_2	CH_3Cl	1·5	43·5	+
441C46	—	7-NH_2	*p*-C_2H_5COONH	CH_3Cl	0·08	1·6	+
723C47	—	7-CH_3O	—	CH_3Cl	0·08	4·3	−
284C47	—	7-CH_3O	*p*-NO_2	CH_3Cl	0·17	3·5	−
670C47	—	7-CH_3O	*m*-NO_2	CH_3Cl	0·35	2·5	−
286C47	—	7-CH_3O	*p*-NH_2	CH_3Cl	0·04	14·2	−
671C47	—	7-CH_3O	*m*-NH_2	CH_3Cl	0·08	15·5	−
379C46	—	7-CH_3O	*p*-CH_3O	CH_3Cl	1·5	3	−
709C47	—	7-CH_3O	*p*-C_2H_5COONH	CH_3Cl	0·17	12·2	−
460C47	2-CH_3CH_2N	7-CH_3CH_2N	—	CH_3Br	0·04	3·7	−
377C46	—	7-CH_3CONH	*p*-NH_2	CH_3Cl	50	41	±
292C46	—	7-CH_3CONH	*p*-C_2H_5COONH	CH_3Cl	3	61	±
25C47	2-CH_3CONH	7-CH_3CONH	*p*-NO_2	CH_3Cl	200	7·5	−
149C47	2-CH_3CONH	7-CH_3COONH	*p*-NH_2	CH_3Cl	3	8·2	−
145C48	2-NH_2	7-C_2H_5COONH	—	CH_3Cl	0·08	17	±

There is no simple relationship. The 9-phenyl structure confers high activity and the toxicities are correspondingly high, apart from such anomalies as 456C47, 494C46, and 489C46. The lowest activities are those of the 7-acetamido compounds.

The compounds in Table VI differ from those of Table V in their quaternary group. Their activity is high, possibly owing to the 9-phenyl substituent, but this activity is depressed and the toxicity greatly reduced when position 10 is

TABLE VI

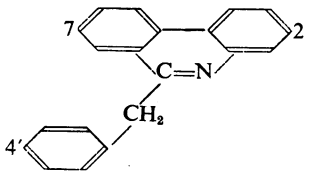
TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-PHENYL PHENANTHRIDINES



Code No.	Substituents in Positions				Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	2 and 3	7	3′, 4′, or 5′	10			
117C48	—	OH	4′-Cl	CH_3OCl	0·17	1·2	±
194C48	—	OH	4′-NO_2	CH_3OCl	0·35	5·5	—
93C48	—	CH_3O	4′-Cl	CH_3OCl	0·08	11·2	—
105C48	—	CH_3O	3′: 5′-NH_2	CH_3OCl	0·35	13·7	—
196C48	—	C_2H_5O	4′-NO_2	CH_3OCl	0·08	46·5	—
195C48	—	C_2H_5O	4′-NH_2	CH_3OCl	0·17	6·9	—
214C48	2-CH_3O	—	4′-NH_2	CH_3OCl	0·08	2·5	—
213C48	2-CH_3O	—	4′-NO_2	CH_3OCl	0·08	6·1	—
291C46	—	CH_3CONH	4′-NO_2	$CH_3CH_3SO_4$	6	50·4	+
633C46	2-C_2H_5COONH	C_2H_5COONH	4′-NO_2	$CH_3CH_3SO_4$	0·35	27·5	+
653C47	2-C_2H_5COONH	C_2H_5COONH	4′-CH_3O	$CH_3CH_3SO_4$	0·7	37·5	+
212C47	—	NH_2	4′-NH_2	$CH_3C_2H_5SO_3$	0·17	9·7	—
74C48	3-NH_2	—	4′-C_2H_5COONH	$CH_3C_2H_5SO_3$	0·08	18·5	—
177C48	2-NO_2	CH_3CONH	—	$CH_3C_2H_5SO_3$	12	25	—
188C47	2-NH_2	NH_2	—	—	50	— (Insol)	—
459C47	2-$NCH_3CH_3CH_3I$	$NCH_3CH_3CH_3I$	—	—	25	0·52	—

TABLE VII

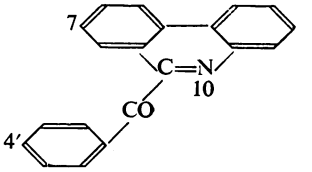
TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-BENZYL PHENANTHRIDINES



Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	2	7	4′			
44C46	—	—	—	3	51	+
654C46	—	—	NH_2	1·5	6·4	+
352C47	NH_2	—	NH_2	0·17	5·6	+
297C46	—	—	CH_3	1·5	9·6	+
658C46	—	—	C_2H_5COONH	3	34	—
47C48	—	OH	NH_2	0·17	14	+
149C48	—	OH	C_2H_5COONH	0·7	25	+
660C47	NH_2	NH_2	—	0·35	31·5	—
405C46	—	NH_2	NH_2	1·5	8	+
404C46	—	NH_2	NO_2	0·7	100 (Insol)	+
656C46	—	NH_2	C_2H_5COONH	0·35	5·5	+
28C48	—	CH_3O	NH_2	0·35	7	+
34C48	—	CH_3O	C_2H_5COONH	0·17	21	—
94C48	—	CH_3O	C_3H_7COONH	0·35	14·2	—
659C47	C_2H_5COONH	C_2H_5COONH	—	0·7	40·5	+
403C46	—	C_2H_5COONH	NH_2	6	73·5	+
555C46	—	C_2H_5COONH	C_2H_5COONH	1·5	27·5	+

TABLE VIII

TOXICITY AND ANTIBACTERIAL ACTIVITY OF 9-BENZOYL PHENANTHRIDINES



Code No.	Substituents in Positions			Inhibiting Concentration for *Str. pyogenes* mg. % (w/v)	LD50 mg./kg. (i.v.)	*In vivo* Activity
	7	4'	10			
167C47	NH_2	NH_2	CH_3Br	3	11·5	–
90C47	NH_2	NO_2	CH_3Cl	0·7	17	–
166C47	C_2H_5COONH	NH_2	CH_3Cl	6	—	–
88C47	C_2H_5COONH	NO_2	CH_3Cl	12	56	–

quaternized by dimethyl sulphate. Here also, as in Table V, the nitrophenyl compounds are slightly less active than their amino analogues.

In the benzylphenanthridines, depicted in Table VII, amino groups again increase activity and the 7-carbethoxyamino group lowers toxicity. Compounds 43C46, 297C46, and 658C46 form a series in which toxicity increases with increasing chain-length of the substituent in the benzyl group. Compounds 658C46, 149C48, 656C46, 34C48, and 555C46 form a series of *p*-carbethoxyamino-phenanthridines in which the substituent in position 7 ranges from H through hydroxyl, amino, methoxy to carbethoxyamino, with 7-methoxy having the greatest activity. The toxicity, however, is variable.

The few benzoyl phenanthridines examined (see Table VIII) exhibit only moderate activity which, with the toxicity, is increased by an amino group at position 7.

DISCUSSION

The relation between chemical constitution and biological activity in phenanthridine compounds has been discussed by Brownlee *et al.* (1950) and in the earlier work of Walls (1945, 1947). Then, however, the compounds were being assessed for their trypanocidal action and only secondarily for their antibacterial activity. The arrangement of the data then available, into series showing progressive changes in the effects on trypanosomes, led Brownlee *et al.* to consider that " little correlation can be found between the chemical structure of these compounds and their antibacterial properties." This is so much at variance with current thought that an assessment of the relevant information on the antibacterial properties of phenanthridine compounds is desirable.

Walls (1945), considering the effect of chemical structure, found that amino-groups other than those in the 9-phenyl ring, conferred anti-trypanosome activity and that this activity was reduced on acetylation. Later he found (1947) that the 9-phenyl ring was not essential. An *o*-, *m*-, or *p*-amino group in the 9-phenyl ring, however, increased the activity more than the corresponding nitro substituent. The 7-dicarbethoxyamino compounds were less active, and the 7-acetamido compounds less still, than the 7-amino compounds. The toxicity in these two classes was also lowered. The 7-carbethoxyamino-9-*p*-acetamidophenyl phenanthridines possessed very low activity.

All these points are exhibited in the antibacterial studies reported in this paper. There are certain divergencies, however, such as in Table III, where the 2:7 diamino compounds show an increase in antibacterial activity whereas the compounds with a single amino group do not; the concurrent lowering of toxicity and activity by the introduction of the 7-carbethoxyamino substituent was not always apparent: in Tables I, V, and VI there is no lowering of activity; in Tables II, III, and IV activity, but not toxicity, is lowered, and in Table VIII toxicity, but not activity, is lowered.

The effect of different quaternary groups is clearly seen in Table III, where the 10-methyl group induces the greatest activity of those tried. The recent introduction of the next higher homologue " ethidium bromide " by Watkins and Wolf (1952) confirms the activity given by a small alkyl group at the 10 position.

Brownlee *et al.* (1950) have confirmed and extended the conclusion of Walls and provided some new theoretical concepts. One of these is

that the 9-methyl compounds may be subject to metabolic attack by the host to give inactive phenanthridone products. However, the 9-methyl compounds include some which are highly active *in vivo* against *Str. pyogenes* infections in mice, and their failure in trypanosome infections is therefore unlikely to be due to their metabolic conversion into phenanthridone products. Again, the conversion of a primary amino group to a tertiary amino group leads to loss of activity *in vivo* against trypanosomes but not of *in vitro* antibacterial activity. Brownlee *et al.* (1950) suggest a difference in the antibacterial and anti-trypanosome mechanisms, but these tertiary amino compounds have also lost *in vivo* activity against *Str. pyogenes* infections. It seems more reasonable to assume that metabolic attack in the host has led to loss *in vivo* of anti-trypanosomal and of anti-streptococcal activity.

Summary

1. A series of 120 phenanthridine compounds has been examined for antibacterial activity.

2. The general conclusions on the effect of chemical constitution of phenanthridinium compounds on their activity against trypanosomes are shown to be true for antibacterial activity.

3. There is no indication that the modes of action of the phenanthridine compounds on trypanosomes and bacteria are fundamentally different.

This work has involved collaboration with many colleagues in the Wellcome Research Laboratories. We are indebted particularly to Drs. L. P. Walls, G. Brownlee, J. W. Trevan, and D. W. Adamson, and to the Wellcome Foundation for permission to publish the experimental data.

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