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THE ANTIVIRAL ACTION OF PHENANTHRIDINIUM COMPOUNDS

BY

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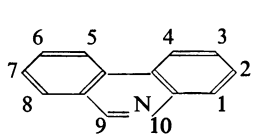
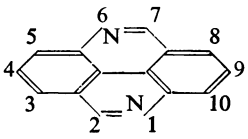
Dickinson and Codd (1952) reported that a phenanthridinium compound, 2:7-bis-(2-dihydroglyoxalinyl)-9-phenylphenanthridine hydrochloride dihydrate, inhibited the development of a bacteriophage of *Pseudomonas pyocyanea* at a much lower concentration than that having any apparent effect on the growth curve of the host. The activity was not due to the direct inactivation of free phage, and the phenomenon seemed of sufficient interest to warrant further investigations of the phenanthridines. About a hundred phenanthridines and related compounds had been prepared by our colleagues in the chemistry division of the Research Department, and these compounds were investigated for their activity against (1) Pb bacteriophage of *Ps. pyocyanea*; (2) influenza A virus in eggs (two compounds in mice); (3) Gram-positive and Gram-negative bacteria; (4) Rous sarcoma virus I in chicks (selected compounds only). The compounds were classified into eight groups, and results, summarizing those for all the tests, are given for the following 23 compounds:



Groups 1–7



Group 8

Group 1

1095.—9-Anilinophenanthridine.

1279. — 9-Anilino-10-methylphenanthridinium chloride hydrochloride.

1280. — 9-(N-Carbethoxyanilino)-10-methylphenanthridium methanesulphonate.

Group 2

Phenidium.—7 - Amino - 9 - *p* - aminophenyl-10-methylphenanthridinium chloride (Morgan and Walls, 1938).

Group 3

481. — 2:7-Diamino-10-methyl-9-phenylphenanthridinium bromide (Walls, 1945).

1602. — 2:7-Diamino-10-*n*-amyl-9-phenylphenanthridinium chloride (Watkins, 1952).

1470. — 2:7-Diamino-10-*n*-hexyl-9-phenylphenanthridinium chloride (Watkins, 1952).

1446. — 10-allyl-2:7-diamino-9-phenylphenanthridinium bromide (Watkins, 1952).

Group 4

1390.—2:7 - Diamino - 9 - *p* - aminophenyl - 10-methylphenanthridinium bromide (Walls and Whittaker, 1950).

1343. — 2:7-Bisglucosylamino-9-*p*-aminophenylphenanthridine (Walls and Whittaker, 1950).

1662. — 2:7-Diamino-10-ethyl-9-*p*-nitrophenylphenanthridinium chloride (B. Application No. 5926/52).

1601. — 2:7 - Diamino - 9 - *p* - nitrophenyl - 10-propylphenanthridinium chloride (B. Application No. 5926/52).

Group 5

2202. — 2:7-Diamino-9:10-dimethylphenanthridinium bromide (Walls, 1947).

1098.—2:7-Diamino-9-butylphenanthridine.

1120. — 2:7-Diamino-9-butyl-10-methylphenanthridine methobromide.

Group 6

1099. — 2:7-Dicarbethoxyamino-9:10-dimethylphenanthridinium methosulphate (Walls, 1947).

1097. — 9-Butyl-2:7-biscarbethoxyamino-10-methylphenanthridinium methosulphate.

Group 7

1367. — 2:7 - Bis - (2 - dihydroglyoxalinyl) - 9 - phenylphenanthridine trihydrochloride dihydrate.

1393. — 2:7 - Bis - (2 - dihydroglyoxalinyl) - 10-methyl-9-phenylphenanthridinium chloride dihydrochloride tetrahydrate.

742. — 2:7-Bis-(N-phenylamidino)-9-phenylphenanthridine monohydrate.

Group 8—Diazapyrenes (Fairfull, Peak, Short, and Watkins, 1952).

1015. — 2:7-Diphenyl-1:6-diazapyrene-1:6-bismethiodide dihydrate.

1428. — 2:7-Di-(4-aminophenyl)-1:6-diazapyrene-1:6-bis-methomethane-sulphonate.

1408. — 4:9-Diamino-2:7-diphenyl-1:6-diazapyrene-1:6-bis-methochloride.

The compounds or their salts were soluble at 1% (w/v) in water. An exception, No. 1343, was supplied as the glucoside, which was soluble.

Apart from the question of any correlation between structure and activity, the series offered an opportunity for the further assessment of the value of bacteriophage as a test virus for screening possible antivirus agents. A bacteriophage test is very convenient if one considers the phage as one particular test virus and if one is aware of the limitations of such tests.

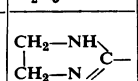
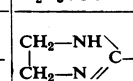
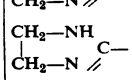
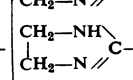
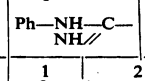
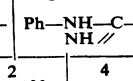
METHODS

Ps. Pyocyanea Phage.—The method used was described by Dickinson (1948) and modified by Chantrill *et al.* (1952). Tenfold dilutions of the compound under test were made in a synthetic medium and inoculated with host bacterium *Ps. pyocyanea* and phage Pb. After incubation for 24 hours the cultures were spotted on an indicator plate, previously sown with the sensitive host culture; this plate was then incubated for 24 hours. Provided that the host had grown in the dilution tube, an area of "no lysis" denoted that the phage had not grown in that tube. Results were expressed as:

$$\frac{\text{Log. titre phage inhibition}}{\text{Log. titre host inhibition}}$$

TABLE I

ANTIVIRUS ACTIVITY OF THE PHENANTHRIDINES

No.	Structure of Compound 2	7	9	10	*Ps. pyocyanea* Phage: Log. Phage Titre/ Log. Host Titre	Antibacterial Action	Influenza A: Toxic Dose (micromol./egg)	Influenza A: Dilution of Toxic Dose Active (Reciprocal)	Rous Sarcoma (Chick Test)	Acute Toxicity LD50 in Mice (Subcutaneous mg./g.)
Group 1:										
1095			PhNH		3/3	0	>10	—	—	>0·5*
1279			PhNH	CH_3	4/4	+	—	—	—	>0·5
1280			$PhNCO_2C_2H_5$	CH_3	6/6	+	>20	0	—	>0·5
Group 2:										
Phenidium		NH_2	*p*-$NH_2C_6H_4$	CH_3	5/3	+++	10	0	0T	0·09
Group 3:										
481	NH_2	NH_2	Ph	CH_3	5/4	+++	5–10	?2	PT	0·07
1602	NH_2	NH_2	Ph	C_5H_{11}	5/4	+++	1·2	4	+T	0·1
1470	NH_2	NH_2	Ph	C_6H_{13}	6/4	+++	0·62	4	+T	0·07
1446	NH_2	NH_2	Ph	$CH_2CH{=}CH_2$	5/4	+++	2·5	2	—	0·14
Group 4:										
1390	NH_2	NH_2	*p*-$NH_2C_6H_4$	CH_3	5/3	++	10	?2	—	>0·2
1343	NH_2	NH_2	*p*-$NH_2C_6H_4$	(as glucoside)	4/3	++	>20	0	—	1·5
1662	NH_2	NH_2	*p*-$NO_2C_6H_4$	C_2H_5	6/3	+++	5–10	4	—	0·14
1601	NH_2	NH_2	*p*-$NO_2C_6H_4$	C_3H_7	4/<3	+++	5	2	—	0·28
Group 5:										
2202	NH_2	NH_2	CH_3	CH_3	5/3	+++	10	0	—	0·07
1098	NH_2	NH_2	C_4H_9		3/3	+	20	0	—	0·08
1120	NH_2	NH_2	C_4H_9	CH_3	5/3	+++	5	0	P	0·07
Group 6:										
1099	C_2H_5COONH	C_2H_5COONH	CH_3	CH_3	4/3	++	1·2	0	—	0·15
1097	C_2H_5COONH	C_2H_5COONH	C_4H_9	CH_3	5/3	+++	0·3	?2	0T	0·05
Group 7:										
1367			Ph		5/<3	+	2·5	4	PT	0·2
1393			Ph	CH_3	<3/<3	—	>2·5	0	—	0·04
742			Ph		4/4	0	5	0	—	>0·9
	1 2	4	6	7 9						
Group 8	see formula page 139									
1015	CH_3 Ph		CH_3	Ph	3/<3	+	0·62	0	PT	0·0025
1428	CH_3 *p*-$NH_2C_6H_4$		CH_3	*p*-$NH_2C_6H_4$	5/3	+	5	2–4	PT	0·04
1408	CH_3 Ph	NH_2	CH_3	Ph NH_2	5/<3	+++	5	4	—	0·06

0=Inactive. P=Partial activity. T=Toxic. —=Not tested. ?=Activity not demonstrated in every test. *=I.P. figure, S.C. not available. +, ++, +++=Degree of activity (see Methods).

Influenza A Virus.—The methods described by Chantrill *et al.* (1952) were used. For *in ovo* tests the compound was introduced into the allantoic sac of 10-day-old fertile eggs; one hour later about 100 egg-infective doses of influenza A virus (PR8 strain) were injected by the same route. After 48 hours' incubation the allantoic fluid was tested for its ability to agglutinate fowl cells. Control tests for false haemagglutination and haemagglutination inhibition were always made. Fourfold dilutions of the compounds were tested, starting at M/20 and including a dose which was not toxic to the egg. Three eggs per dilution were usually tested, but any equivocal results were confirmed by further tests using twofold dilutions of the compounds. Results, expressed as a factor, referred to the dose in micromols. which was toxic to the chick embryo; thus a factor of 4 denoted that the compound was active at one-quarter the toxic dose.

Nos. 481 and 1367 were tested against mouse-adapted influenza A virus *in vivo*. Mice were infected intranasally with 100 infective doses of virus, the drugs being given twice daily subcutaneously, starting the day before infection. Survivors were killed seven days after infection and the lungs examined.

General Antibacterial Action.—The simple ditch-plate technique was considered sufficiently accurate for this series of compounds. A ditch containing 1/1,000 of the compound was made in a 5% blood-agar plate and the following organisms streaked across it: (1) *Streptococcus haemolyticus* (Richards); (2) *Staphylococcus aureus,* 663; (3) *Proteus vulgaris,* N.C.T.C. 5887; (4) *Pseudomonas pyocyanea.* 10; (5) *Salmonella aertryke*; (6) *Shigella shigae*; (7) *Shigella sonnei*; (8) *Corynebacterium pyogenes*; (9) *Streptococcus agalactiae,* N.C.T.C. 6175; (10) *Haemophilus pertussis*; (11) *Brucella abortus,* N.C.T.C. 6059. After 24 hours' incubation at 37° C. the width of the zone of inhibition was measured in mm.

Zone diameters were summarized for publication as: 0 (no inhibition against any organisms), + (slight inhibition, usually on the ditch only, against Gram-positive organisms and Nos. 8–11), ++ (inhibition of up to 10 mm. against Gram-positive organisms and Nos. 8–11), +++ (inhibition of 10–20 mm. against Gram-positive organisms and Nos. 8–11, with a slight inhibition against the Gram-negative organisms Nos. 3–7).

Rous Sarcoma Virus.—The method using young chicks was described by Dickinson and Thompson (1952). Both virus and drug solutions were inoculated subcutaneously on to the parietal peritoneal membrane, the first drug dose being given 24 hours after the virus. After eight daily doses of drug, the chicks were killed and the membranes examined visually for the presence of lesions produced by the virus. Assessments of 0–3 were given and compared with untreated controls; results were only considered significant if "p" <0.05. Chicks were weighed daily to see whether the drugs were toxic; sometimes toxic symptoms occurred—e.g., haemorrhagic membranes.

Results

Results for all tests appear in Table I; the subcutaneous LD50 for mice is included, by courtesy of Dr. M. R. Gurd, as a measure of the acute toxicity of the compounds. No compound was active against influenza A virus at less than a quarter of the dose toxic to the chick embryo. One of the most promising, 1367, was inactive in mice at the maximum tolerated dose (Table II). Furthermore, several of the active compounds, 1367, 1408, 1470, 1602, were inactive against larger inocula (10,000 E.I.D.) of influenza virus. The results of the Rous sarcoma virus tests (Table III) also indi-

TABLE II

ACTION OF 481 AND 1367 AGAINST INFLUENZA A VIRUS IN MICE

Dosage mg./g./Day	Deaths Due to Toxicity (Within 2 Days)	Deaths Due to Virus	Infected Lungs of Survivors
481:			
0·02 ..	0/27	13/27	11/14
0·05 ..	2/12	Test discontinued*	—
0·1 ..	7/12	,, ,,	—
1367:			
0·02 ..	0/12	5/12	7/7
0·05 ..	1/9	6/9	1/2
0·1 ..	1st dose toxic	Test discontinued*	—
Controls ..	0/12	7/12	5/5

* This dose was toxic, many mice dying within two days and others obviously suffering from toxic effects.

TABLE III

ACTION OF PHENANTHRIDINES AGAINST ROUS SARCOMA VIRUS IN CHICKS (SUBCUTANEOUS ROUTE OF INFECTION)

Treatment commenced 24 hr. after infection

Compound	Daily Dose by Subcutaneous Route for 8 Days	No. of Chicks	Average Gain in Weight: Treated/Control*	Non-specific Deaths or Toxic Deaths	Parietal Peritoneum Examined 9–11 Days After Infection	
					Individual Assessments (Maximum 3)	Average Assessment
1st test:						
1602 ..	0·3 μg./g.	5	$0{\cdot}59_{11}$ T	0	0, 0·5, 0, 1, 1	0·5†
1470 ..	0·3 μg./g.	5	$0{\cdot}68_{11}$ T	0	0·5, 0, 0, 0, 0	0·1†
1097 ..	0·3 μg./g.	5	$0{\cdot}55_{11}$ T	0	1, 3, 3, 3, 3	2·6
Controls	—	5	—	1 N S	3, 3, 3, 3	3·0
2nd test	(dose 0·002 micromol./g. body wt.)					
Phenidium	0·6 μg./g.	6	$0{\cdot}60_{10}$ T	1 T	2·5, 3, 1·5, 1·5, 3	2·3
481 ..	0·6 μg./g.	6	$0{\cdot}65_{10}$ T	1 T	1, 1, 2·5, 1, 1·5	1·4†
1470 ..	0·6 μg./g.	6	$0{\cdot}79_{10}$ T	0	0, 0, 0, 0, 0, 0	0†
1120 ..	0·5 μg./g.	6	$0{\cdot}89_{10}$	0	1·5, 1·5, 1, 1·5, 1, 1·5	1·3†
1367 ..	0·8 μg./g.	6	0·72 T	1 N.S.	1, 2, 2·5, 1, 2	1·7‡
1015 ..	1·0 μg./g.	6	0·79 T	0	1·5, 2, 2, 1·5, 1·5, 0·5	1·5†
1428 ..	0·9 μg./g.	6	0·73 T	0	0·5, 1·5, 2, 1, 1·5, 1	1·25†
Controls	—	12	—	0	3, 2, 2, 3, 2, 1·5, 2, 2·5, 2, 3, 1, 2	2·2

* Suffix to ratio refers to the period of days over which the average weight gain was calculated. † "p" = <0·001. ‡ "p" = <0·05. T = Toxic.

cate that the compounds are active only at a dose toxic to the host. No. 1367 had previously been reported as inactive at the maximum dose which was not toxic to the chick (Dickinson and Thompson, 1952).

The high ratios in the anti-phage tests were probably due to the relative resistance of the bacterial host, *Ps. pyocyanea*. This organism, like the other Gram-negative bacilli, was much less susceptible than the Gram-positive organisms. Three compounds, 1095, 1393, and 1098, were inactive against the host at the lowest dilution (1/1,000) tested and also inactive against phage. Three other compounds, 1279, 1280, and 742, although showing activity against the host, were inactive against phage at the highest drug concentration which permitted host growth.

Discussion

Little can be deduced regarding the correlation of structure and activity. Brownlee *et al.* (1950) found that the phenanthridines possess marked antibacterial activity, but that their high toxicity precludes their use in bacterial infections. Toxicity to the host probably accounts for the inactivity of 1367 in mice; even in eggs the therapeutic ratio is only 4. Egg toxicity figures vary, apparently quite independently of other activities, from 0.3 to >20 micromol./egg. The compounds which are not toxic to eggs are inactive against influenza, but compounds markedly toxic to eggs are not necessarily active.

No compound found active against influenza is inactive against phage, but some compounds of equal activity against phage are inactive against influenza. There is no quantitative relationship, but the bacterial host is unlikely to respond similarly to the fertile egg. One can only say that the antiviral activity of this class of compounds was detected in the first instance by the phage screening test. It would have been "missed" by the usual egg tests, where it is not usual to test such narrowly spaced dilutions as are necessary to detect the anti-influenza action of the phenanthridines.

Although of no obvious chemotherapeutic interest the phenanthridines provide a useful tool for investigations into bacteriophage production. No. 1367 is being used for such work; it behaves anomalously in that it would be expected to be less active than 1393, rather than more, because the ring nitrogen is not quaternized.

Summary

1. Twenty-three phenanthridines and related compounds were tested against influenza A virus in eggs, against Pb phage of *Ps. pyocyanea*, and against a range of bacteria.

2. Many compounds possessed marked anti-phage action, up to 1,000 times that on the bacterial host. The host, *Ps. pyocyanea*, and other Gram-negative bacilli were much less susceptible than the Gram-positive bacteria.

3. Several of the compounds active against phage possessed slight anti-influenza activity in eggs; the most promising of these compounds was, however, inactive in mice.

4. Seven of nine compounds had a suppressive action against Rous sarcoma virus in chicks, but only at a toxic dose.

The authors wish to acknowledge the interest of Mr. C. E. Coulthard and the late Sir Jack Drummond. They thank Drs. W. F. Short, T. I. Watkins, and A. E. S. Fairfull of the Chemistry Division for the compounds, and Dr. M. R. Gurd of the Pharmacology Division for the mouse toxicity figures.

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—— and Whittaker, N. (1950). Ibid., 41.

Watkins, T. I. (1952). Ibid., 3059.