before it is mixed with the substrate results in complete inactivation of the enzyme. Similar results are obtained if citric acid-phosphate buffer is used at pH 4.6; if this pH is employed in the viscosimetric assay of streptococcal hyaluronidase, abnormal curves are always obtained and no quantitative assays can be made. It appears that hyaluronidase from either group A or group C streptococci differs from the enzymes obtained from testis, *Cl. welchii* and staphylococci in that it is rapidly destroyed at pH 4.6, but is relatively stable at pH 7.0.

SUMMARY

1. Hyaluronidase from *Cl. welchii* is not readily inactivated by molecular oxygen, does not require

oxygen for its activity and does not produce conditions which will either oxidize reduced rosinduline or reduce indophenol during its activity on the hyaluronate at pH 7.0.

2. The presence or absence of Cu has no influence on the activity of hyaluronidase from *Cl. welchii*.

3. Hyaluronidase derived either from *Cl. welchii* or testis is partially inactivated by KMnO_4 and by iodine; this inactivation is not reversed by $\text{Na}_2\text{S}_2\text{O}_4$.

4. Hyaluronidase derived either from group A or group C streptococci is rapidly inactivated at pH 4.6, but is relatively stable at pH 7.0. This inactivation explains previous reports of the anomalous viscosimetric behaviour of streptococcal hyaluronidase.

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The Antibacterial Action of some Diphenyl Derivatives

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The observations recorded in this paper are a continuation of those previously reported; similar methods have been used (Faulkner, 1943, 1944).

EXPERIMENTAL

The anti-bacterial test. In the standard experiment organisms are incubated at 37° in broth containing the substance being investigated, and subcultured on to solid media after 24 and 48 hr. to test survival. To test bacteriostatic action the organisms are sown on to agar-medium in which the bactericide is incorporated in a concentration 1:2500.

Organisms used. All the organisms used were freshly isolated from material submitted for bacteriological examination, and were as follows: *Staph. aureus*, three strains; haemolytic *Streptococcus* Group A, three strains; *C. diphtheriae*, four strains all virulent to guinea-pigs; *Diplococcus pneumoniae*, two strains; *Streptococcus faecalis*, three strains; Gram-negative diplococci, two strains; *Klebsiella pneumoniae* (Friedlander's bacillus); and seven strains of Gram-negative intestinal bacilli: *Esch. coli*, *Shigella paradysenteriae*, *Shigella sonnei* (*B. dysenteriae* Sonne), *Salmonella typhimurium*

(*B. aertrycke*), *Salmonella* sp. *oranienburg* type, *Salmonella schottmuelleri* (*Bact. paratyphosum* B) and *Eberthella typhosa*.

Compounds used. The starting-point of this series of experiments was diethylstilboestrol, i.e. 4-OH.C₆H₄CEt:CEt.C₆H₄.OH-4'. Most of the substances tested contain two benzene rings; a few compounds were included containing three benzene rings, or only one, but none of these showed any antibacterial activity. Many of the compounds are not soluble in the concentrations used. In these cases they were used as a fine suspension obtained by adding an ethanol solution to broth.

RESULTS

Table 1 summarizes the results of experiments with the most active compounds. Such a compilation does not give a complete picture, as it does not take into account the number of different organisms against which the substance is active, or the variations in the relative susceptibilities of the organisms to the compounds. In general the order of decreasing susceptibility is: *C. diphtheriae*; haemolytic *Streptococcus* and *Diplococcus pneumoniae* approxi-

Table 1. *List of most active compounds related to diphenyl arranged approximately in order of diminishing bactericidal activity*

Compound	Groups separating the rings	Groups attached to the rings	Greatest dilution at which bactericidal
1. 4-Hydroxy- α : β -diethylstilbene	—C ₂ H ₅ :C ₂ H ₅ —	OH	1: 600,000
2. 4:4'-Dihydroxy- α : β -diethylstilbene (diethylstilboestrol)	—C ₂ H ₅ :C ₂ H ₅ —	OH OH	1: 500,000
3. 4:4'-Dihydroxy- γ : δ -diphenylhexane (hexoestrol)	—CH ₂ Et:CH ₂ Et—	OH OH	1: 500,000
4. 4:4'-Dihydroxy- α -isopropylstilbene	—CC ₃ H ₇ :CH—	OH OH	1: 100,000
5. 4:4'-Dihydroxy- α -n-propylstilbene	—CC ₃ H ₇ :CH—	OH OH	1: 100,000
6. 3:3'-Dihydroxydiphenylhexadiene	—CC ₂ H ₄ :CC ₂ H ₄ —	OH OH	1: 50,000
7. Diazoaminobenzene	—N ₂ :NH—	—	1: 50,000
8. 4:4'-Dihydroxydiphenylhexadiene (dienoestrol)	—CC ₂ H ₄ :CC ₂ H ₄ —	OH OH	1: 50,000
9. 4-Hydroxystilbene	—CH:CH—	OH	1: 50,000
10. o-Benzylphenol	—CH ₂ —	OH	1: 10,000
11. p-Benzylphenol	—CH ₂ —	OH	1: 10,000
12. o-Hydroxydiphenyl	—	OH	1: 10,000
13. p-Hydroxydiphenyl	—	OH	1: 10,000
14. Diphenylamine	—NH—	—	1: 10,000
15. Diphenylmethane	—CH ₂ —	—	1: 10,000
16. Diphenylether	—O—	—	1: 10,000
17. 4:4'-Dihydroxydiphenyl	—	OH OH	1: 10,000
18. 3:3'-4:4'-Tetrahydroxy- γ : δ -diphenyl-n-hexane	—CH ₂ Et:CH ₂ Et—	OH OH OH OH	1: 10,000
19. Dibenzylether	—CH ₂ :O:CH ₂ —	—	1: 10,000
20. 2:4-Diaminoazobenzene (chrysoidine)	—N:N—	NH ₂ NH ₂	1: 10,000
21. Diphenyl	—	—	1: 10,000
22. 4:4'-Dihydroxy- α -ethylstilbene	—C ₂ H ₅ :CH—	OH OH	1: 10,000
23. Dimethoxy- α : β -diethylstilbene	—C ₂ H ₅ :C ₂ H ₅ —	CH ₃ O CH ₃ O	1: 10,000
24. 4:4'-Dihydroxy- β : γ -diphenylbutane	—CHCH ₃ :CHCH ₃ —	OH OH	1: 10,000

Table 2. *Comparative activities of the five most active diphenyl compounds for various susceptible organisms*

Organism	Compound				
	4-Hydroxy- α : β -diethylstilbene	Diethylstilboestrol	Hexoestrol	4:4'-Dihydroxy- α -isopropylstilbene	4:4'-Dihydroxy- α -n-propylstilbene
	Greatest dilution at which bactericidal				
<i>C. diphtheriae</i>	1: 600,000	1: 200,000	1: 500,000	1: 100,000	1: 100,000
<i>Streptococcus pyogenes</i>	1: 400,000	1: 300,000	1: 100,000	—	—
<i>Diplococcus pneumoniae</i>	1: 100,000	1: 50,000	1: 100,000	—	—
<i>Staphylococcus aureus</i>	1: 250,000	1: 50,000	1: 50,000	1: 10,000	1: 10,000
<i>Streptococcus faecalis</i>	—	1: 50,000	1: 50,000	1: 10,000	1: 10,000
<i>Neisseria catarrhalis</i>	—	1: 500,000	—	—	—
<i>Neisseria pharyngis</i>	1: 5,000	1: 5,000	—	—	—

mately similar; *Staph. aureus*; *Streptococcus faecalis*. Many of the substances were bacteriostatic to organisms which they did not kill. The effect on Gram-negative organisms is discussed later.

Of all the substances tested the only one superior to diethylstilboestrol and hexoestrol is 4-hydroxy- α : β -diethylstilbene. These three compounds have a marked superiority over all others tested and are bactericidal to at least one type of organism in a dilution of 1: 500,000 (see Table 2).

In addition to the compounds listed in Table 1 the following were bactericidal in concentrations between 1: 10,000 and 1: 2500:

(25) stilboestrol dipropionate, (26) dibenzylamine, (27) 4:4'-dihydroxy-stilbene, (28) hexoestrol dimethyl ether, (29) benzidine, (30) hydrazobenzene, (31) azobenzene, (32) di-*p*-tolylamine, (33) dibenzyl, (34) dibenzyl ketone.

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The following compounds showed neither bactericidal nor bacteriostatic action in a concentration of 1: 2500:

m-diphenylbenzene; *p*-diphenylbenzene; phenylmethyl ether; phenylethyl ether; benzylmethyl ether; benzylethyl ether; diphenylamine sulphonic acid; barium diphenylamine sulphate; (sym) diphenyl urea; triphenylamine; triphenylmethane; phenyl-ethylene; triphenyl-ethylene; triphenyl-chloro-ethylene; diphenyl-ethylene (stilbene); stilbene dibromide; 2:2'-dihydroxy- α : β -diethylstilbene; 4:4'-dimethoxy- α : β -dichloro-stilbene; and stilboestrol dibutyrate.

DISCUSSION

The one feature common to all the active compounds tested is the presence of two benzene rings. These rings may be separated by one or more radicals and may have one or more radicals attached to

themselves. But diphenyl itself possesses weak bactericidal properties, being lethal to pneumococci in a dilution of 1:10,000 and to three strains of *C. diphtheriae* in a dilution of 1:2500.

The most active compounds contain one or more hydroxyl groups (i.e. the phenolic structure) but this is not essential. One OH group is better than two OH groups in the following pairs of compounds: nos. 1 and 2; 12 or 13 and 17; also 9 and 27. This is not evident from the data given in Table 1 but is apparent when detailed results are compared: for instance *p*-hydroxy-diphenyl is lethal to *Staph. aureus* in a concentration of 1:10,000, but dihydroxy-diphenyl is only bacteriostatic to this organism in a concentration of 1:2500. Comparison of nos. 18 and 3 shows that increasing the number of hydroxyls from two to four decreases the activity.

activity, but there seems to be no correlation between the degree of bactericidal activity and their oestrogenic properties as recorded by Emmens (1941, 1942). The present observations confirm those of Brownlee, Copp, Duffin & Tonkin (1943), who used some 30 stilbene derivatives, four of which were tested in the present experiments. The best compound found by these authors is the same as that recorded above, namely, 4-hydroxydiethylstilbene.

SUMMARY

1. Bactericidal activity is recorded in 34 compounds of the diphenyl or stilbene type.

2. Five compounds are active in a concentration of 1:100,000 or less; the most efficient is 4-hydroxy- α : β -diethylstilbene.

Table 3. *Comparative activities of the five compounds active against the intestinal Gram-negative bacilli*

(The compounds were dissolved or suspended in nutrient broth. Bacteriostatic action was tested in a dilution of 1:2,500.)

Organism	Compound				
	<i>o</i> -Benzyl-phenol	<i>p</i> -Benzyl-phenol	<i>o</i> -Hydroxy-diphenyl	<i>p</i> -Hydroxy-diphenyl	Diphenyl-amine
	Greatest dilution at which bactericidal				
<i>Esch. coli</i>	1:10,000	1:5,000	1:5,000	1:2,500	Bacteriostatic
<i>Shigella paradyseenteriae</i>	1:5,000	1:5,000	1:5,000	1:2,500	1:5,000
<i>Shigella sonnei</i>	1:5,000	1:5,000	1:5,000	1:2,500	1:2,500
<i>Salmonella typhimurium</i>	1:5,000	1:5,000	1:5,000	Bacteriostatic	Bacteriostatic
<i>Salmonella sp. oranienburg</i>	1:2,500	1:2,500	1:5,000	None found	None found
<i>Eberthella typhosa</i>	1:5,000	1:5,000	1:5,000	Not tested	1:2,500
<i>Salmonella schottmuelleri</i>	1:5,000	1:2,500	1:5,000	None found	Bacteriostatic
<i>Klebsiella pneumoniae</i>	1:5,000	1:2,500	1:5,000	1:2,500	None found

The position of the hydroxyl groups makes some difference: if the hydroxyls of diethylstilboestrol are transferred to position 2 the resulting compound is quite inactive. Two compounds containing a propyl group (4 and 5) are more effective than the corresponding compound containing an ethyl group (22). Also two ethyl groups (3) are better than two methyl groups (24).

Of all the compounds tested only five exert any inhibition on the growth of the Gram-negative intestinal bacilli (Table 3). It is noteworthy that *p*-hydroxy-diphenyl appears to differentiate between the *Salmonellae* and the other Gram-negative bacilli.

The bactericidal action of diethylstilboestrol and of 4-hydroxydiethylstilbene on tubercle bacilli is the subject of a separate communication (Faulkner, 1944).

Some of the compounds used possess oestrogenic

3. These substances are most active against Gram-positive organisms; the susceptibility decreases in the order: *C. diphtheriae*, *Diplococcus pneumoniae*, haemolytic streptococci, *Staphylococcus aureus*, *Streptococcus faecalis*.

4. Only five substances were found to exert any inhibitory action on the Gram-negative intestinal bacilli tested.

5. The most efficient bactericides contain one or more hydroxyl groups in the molecule, but this configuration is not essential.

I am indebted to Prof. E. C. Dodds for compounds nos. 1, 6, 8, 9, 18, 22, 27, and 2:2'-dihydroxy- α : β -diethylstilbene; to Sir Robert Robinson for nos. 4, 5, 24, also 4:4'-dimethoxy- α : β -dichlorostilbene; and to Boots Pure Drug Co. Ltd. for eight compounds. I express my thanks to Dr A. I. Messer for continued help during this investigation, and to Prof. J. H. Gaddum for much advice both in planning and in interpreting the experiments.

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