The Antibacterial Action of some Stilbene Derivatives

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Early types of antibacterial substances were not specifically antibacterial, but were usually toxic to all living cells, and were of value only in so far as they could be employed without serious damage to the host. The search for more suitable antibacterial agents resulted in the preparation and testing of many substances of widely different constitution, and it was from the empirical yet systematic investigations of one such class of chrysoidine dye-stuff that knowledge of the activity of sulphanilamide arose. The growing evidence that the antibacterial action of sulphanil drugs was due to competition with an enzymic metabolic process suggested to Fildes [1940] a new approach to the problem. This was the design of antibacterial agents by the synthesis of compounds with a close spatial resemblance to substances vital to, or closely associated with, bacterial growth and reproduction. These growth substances include the water-soluble vitamins of the animal world, which now appear to be essential to the life of all growing cells. The method has been recently used in a sufficiently diverse series of independent investigations to confirm the principle [McIlwain, 1943].

Other compounds which exert a pronounced physiological activity in minute concentration are the animal hormones related to cyclopentanophenanthrene; for example, oestradiol, when injected into an ovariectomized rat of 50 g. body weight in a dose of $0.2 \mu\text{g.}$, is capable of doubling the weight of the uterus; with a blood volume of 2 ml., this represents an activity at a concentration of 1 in 10^7 . These compounds are not known to be hormones for the vegetable kingdom, but they are widely distributed in nature and are probably constituents of all growing cells, being found in all plants and bacteria [Aschheim & Hohlweg, 1933], as well as in all animals. It seems to us that these compounds can readily be incorporated into the living cell, and that such molecules constitute suitable material which, while not necessarily competitive with known essential systems, is capable of modification to contain known antibacterial groupings. Synthetic work in the cyclopentanophenanthrene series is difficult, and it is fortunate that the work of Dodds, Golberg, Lawson & Robinson [1939] provided, in the diethylstilboestrols, simple substances with space configurations similar to those of the naturally occurring

compounds, and with their intense physiological activity.

We have prepared and tested a number of compounds in this series for antibacterial properties and have found some of them very powerfully bactericidal. As with the oestrogenic properties of compounds in this series, the antibacterial activities vary considerably with the structure of the molecule, though these variations are not altogether analogous. The compounds tested are set out in Table 1 and fall into seven main classes:

- I. Deoxystilboestrol and its dihydro-derivative (1 and 2).
- II. $\alpha:\alpha'$ -Diethylstilbene, intermediates in its preparation and some derivatives (3-7).
- III. Deoxydiethylstilboestrol, intermediates and derivatives (8-14).
- IV. Diethylstilboestrol and derivatives (15-20).
- V. Amino-, cyano-, amidino- and nitro-derivatives (21-29).
- VI. Naturally occurring steroids (31 and 32).
- VII. Penicillic acid and citrinin (33 and 34).

I-VI are poorly soluble in water and in broth; therefore saturated solutions in broth were first prepared by making use of the solubility of the compounds in 90% (v/v) ethanol, in which a 10% solution can be prepared. Small volumes of this solution, containing a known amount of drug, were added to measured volumes of nutrient broth and the mixture autoclaved. When a volume of 0.1 ml. was added to 100 ml. of broth, the material remained in solution, or in a very finely divided suspension, at 37° , and from the starting solution or suspension a series of concentrations of drug was made by pipetting 5 ml. into a suitable quantity of sterile broth. This series of drug broths was then inoculated with a suspension of the strains of *Staphylococcus* or *Streptococcus* used in these experiments. The inocula were adjusted to give a final count of 1000-2000 organisms/ml. of drug broth and the actual numbers present were determined by a plate count in each case.

Figures are given in Table 1 for the minimal concentration of drug which just prevents visible growth of the organism after 48 hr. incubation at 37° . Subcultures from tubes containing the minimal inhibitory concentration showed that the action of

Table 1. *Bactericidal activity of the compounds tested against strains of Staphylococcus and Streptococcus*

		<i>Staphylococcus</i>	<i>Streptococcus</i>
1. 4-OH.C ₆ H ₄ .CH=CH.C ₆ H ₅	Hewitt <i>et al.</i> [1912]	1: 50,000	1: 50,000
2. 4-OH.C ₆ H ₄ .CH ₂ .CH ₂ .C ₆ H ₅	Stoermer & Kippe [1903]*	1: 40,000	1: 20,000
3. C ₆ H ₅ .CO.CHEt.C ₆ H ₅	Janssen [1889]	1: 200,000	1: 200,000
4. C ₆ H ₅ .C(OH)Et.CHEt.C ₆ H ₅		1: 30,000	1: 30,000
5. C ₆ H ₅ .CEt=CEt.C ₆ H ₅	Kharasch & Kleiman [1943]*	1: 10,000	1: 60,000
6. C ₆ H ₅ .CHEt.CHEt.C ₆ H ₅	Kharasch & Kleiman [1943]*	Inactive	Inactive
7. C ₆ H ₅ .C(OH)Et.C(OH)Et.C ₆ H ₅	Ciamician & Silber [1914]	Slight bacteriostatic activity	Inactive
8. 4-MeO.C ₆ H ₄ .CO.CHEt.C ₆ H ₅	Dodds <i>et al.</i> [1939]	Inactive	Inactive
9. 4-MeO.C ₆ H ₄ .C(OH)Et.CHEt.C ₆ H ₅	Dodds <i>et al.</i> [1939]	Inactive	Inactive
10. 4-MeO.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₅		Inactive	Inactive
11. 4-OH.C ₆ H ₄ .CEt=CEt.C ₆ H ₅	Dodds <i>et al.</i> [1939]*	1: 640,000	1: 2,000,000
12. 4-OH.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₅		1: 200,000	1: 800,000
13. 4-BzO.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₅		Inactive	Inactive
14. 4-HSO ₃ O.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₅		1: 3,000	1: 640,000
15. 4-OH.C ₆ H ₄ .CEt=CEt.C ₆ H ₄ .OMe-4'		1: 10,000	1: 20,000
16. 4-OH.C ₆ H ₄ .CEt=CEt.C ₆ H ₄ .OH-4' (h.m.†)	Dodds <i>et al.</i> [1939]	1: 100,000	1: 400,000
17. 4-OH.C ₆ H ₄ .CEt=CEt.C ₆ H ₄ .OH-4' (l.m.†)	Dodds <i>et al.</i> [1939]	1: 100,000	1: 400,000
18. 4-OH.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₄ .OH-4' (h.m.)	Dodds <i>et al.</i> [1939]	1: 100,000	1: 300,000
19. 4-OH.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₄ .OH-4' (l.m.)	Dodds <i>et al.</i> [1939]	1: 80,000	1: 400,000
20. 4-AcO.C ₆ H ₄ .CHEt.CHEt.C ₆ H ₄ .OAc-4' (l.m.)	Dodds <i>et al.</i> [1939]	1: 50,000	1: 100,000
21. C ₆ H ₅ .CO.CHEt.C ₆ H ₄ .NH ₂ -4		1: 8,000	1: 8,000
22. C ₆ H ₅ .C(OH)Et.CHEt.C ₆ H ₄ .NH ₂ -4		1: 5,000	1: 10,000
23. C ₆ H ₅ .CEt=CEt.C ₆ H ₄ .NH ₂ -4		1: 32,000	1: 128,000
24. C ₆ H ₅ .CEt=CEt.C ₆ H ₄ .NHSO ₂ .C ₆ H ₄ .NH ₂ -4		1: 40,000	1: 40,000
25. 4-MeO.C ₆ H ₄ .CH=CH.C ₆ H ₄ .NO ₂ -4'	Hewitt <i>et al.</i> [1912]	1: 10,000	1: 10,000
26. 4-OH.C ₆ H ₄ .CH=CH.C ₆ H ₄ .NO ₂ -4'	Cullinane [1923]	1: 20,000	1: 20,000
27. 4-OH.C ₆ H ₄ .CH=CH.C ₆ H ₄ .NH ₂ -4'		Inactive	Slight activity
28. 4-OH.C ₆ H ₄ .CH=CH.C ₆ H ₄ .CN-4'		Inactive	Slight activity
29. 4-OH.C ₆ H ₄ .CH=CH.C ₆ H ₄ .C $\begin{smallmatrix} \text{NH} \\ \text{NH}_2 \end{smallmatrix}$ -4'		1: 30,000	1: 30,000
30. 4-OH.C ₆ H ₄ .CO.CHOH.CH ₃	Auwers & Noll [1938]	1: 600	1: 600
31. Oestrone		1: 33,000	1: 33,000
32. Oestradiol		1: 15,000	1: 40,000
33. Penicillic acid	Oxford [1942]	1: 1,500	—
34. Citrinin	Oxford [1942]	1: 15,000	—

* Revised method of preparation given.

† h.m. = high-melting.

‡ l.m. = low-melting.

the drugs was usually bactericidal, but bacteriostasis was also shown in some cases at lower concentrations by the appearance of delayed growth 24–48 hr. after inoculation.

When a compound is stated in Table 1 to be inactive, it indicates that a saturated solution obtained by filtering broth suspension containing the drug in a concentration of 1/100,000 failed to prevent the growth of a small number of organisms.

The antibacterial activities of deoxydiethylstilboestrol and its dihydro-derivative were tested against a number of gram-negative organisms, but both compounds were generally inactive in the highest concentrations obtainable.

So that our results may be comparative we have included in the series estimates of penicillic acid and citrinin; with our strain of *Staphylococcus* penicillic acid is bactericidal at a concentration of 1:1500 and citrinin at 1:15,000, so that our most active specimen, 4-OH.C₆H₄.CEt=CEt.C₆H₅ (11), is some 40 times as active as the latter.

Deoxydiethylstilboestrol is soluble in soap solutions and its germicidal action may be compared with soap solutions of the chlorinated phenols. The

results obtained in a Rideal-Walker type test, where in addition to *Eberthella typhosa*, strains of *Streptococcus*, *Staphylococcus*, and *Pseudomonas aeruginosa* (*Ps. pyocyanea*) were examined, are given in Table 2. The minimal concentration required to kill gives the concentration of disinfectant in which life

Table 2. *Comparative bactericidal properties of phenol, chlorinated phenols and deoxydiethylstilboestrol*

Material*	Minimal concentration required to kill			
	<i>Streptococcus pyogenes</i> (P 90)	<i>Staphylococcus aureus</i> (133)	<i>Ps. aeruginosa</i> (1)	<i>Eberthella typhosa</i> (Rawlings)
1% deoxydiethylstilboestrol	1:1600	1: 5	1:10	1: 2.2
1% <i>p</i> -chloro- <i>m</i> -xylenol	1:1600	1: 5	1:10	1:27
1% <i>p</i> -chloro- <i>m</i> -cresol	1: 800	1: 2.5	1:10	1:20
5% phenol	1: 120	1:80	1:70	1:90

* The compounds were dissolved in 5% sodium oleate solution.

survives for $2\frac{1}{2}$ and 5 min. but not thereafter. Deoxydiethylstilboestrol is as effective a disinfectant as chloroxylenol against gram-positive organisms but is less effective against gram-negative types.

Some of the compounds were tested in mice infected with virulent strains of staphylococci and streptococci, the drugs being administered by mouth in three doses, immediately, 5 hr., and 24 hr. after infection. The dose was 1–10 mg./g. of mouse, and no protection was afforded by any of the compounds tried, with the possible exception of

which showed very slight activity in this respect.

DISCUSSION

The comparison of the bactericidal activities presented in the table shows that the two most active compounds are deoxydiethylstilboestrol (11) and its dihydro-derivative (12). The factors which appear to effect the activity of the compounds tested are: (a) the carbon skeleton; (b) the substituent groups; (c) the degree of unsaturation of the central bridge.

The preferred spatial configuration of the diethylstilbene type as compared with the unalkylated stilbene type is shown by a comparison of

and

and

(The ratios here and in subsequent comparisons refer to the minimal bactericidal dilutions (Table 1) of these compounds against *Staphylococcus*.)

The effect of the hydroxy group in the 4-position is shown by the pairs:

and

and

This evidence is of interest in that bactericidal activity and oestrogenic activity both require the presence of the 4-hydroxy group.

However, analogy with oestrogenic activity ends here, since the reduction of the centre double-bond

diminishes, not enhances, the antibacterial activity, e.g.

and

and

and

and the introduction of a hydroxyl into the second ring diminishes the activity:

and

and

The two forms of diethylstilboestrol (16 and 17) have similar antibacterial activities, whereas the normal form is about 14 times as potent an oestrogen as ψ -stilboestrol. Thus while both molecules may be readily accepted by cells, both may prove bactericidal while only one is capable of fulfilling the oestrogenic function to a marked degree. (This is interesting if indirect evidence that the lack of oestrogenic activity of ψ -stilboestrol is not due to any 'hindrance' effect of the cell membrane.) Furthermore, high-melting hexoestrol (18) is very much more active oestrogenically than its lower melting stereoisomer (19), but their antibacterial activities are very close (1:100,000 and 1:80,000 respectively).

Conversion of the 4-hydroxyl to methoxyl destroys the activity completely, as shown by the inactivity of 4-MeO $\cdot$ $\text{C}_6\text{H}_4 \cdot \text{CHEt} \cdot \text{CHEt} \cdot \text{C}_6\text{H}_5$ (10); if there are two substituent hydroxyls, conversion of one of them to methoxyl greatly reduces the activity:

and

cf. also

and

The influence of hydroxy groups in the centre of the molecule is interesting.

which, in its enolic form, is

is bactericidal at 1:200,000;

which contains a hydroxyl but no double bond, at 1:30,000; the analogous hydrocarbons

and

are bactericidal at 1:10,000, and inactive, respectively. The provision of a second hydroxyl in the centre, cf.

lowers the activity.

In the 4-amino series the effect is not quite the same. Here compounds containing the hydroxyl in the centre are less active than the parent substance, though the activity is still greater in presence of a double bond. Thus

is active at 1:8000 and

at 1:5000. However, the compound

is active at 1:32,000, an increase possibly to be ascribed to the amino group here freed from any interference by the hydroxyl.

Since in the diethylstilboestrol series the maximal oestrogenic activity appears to be associated with compounds containing two hydroxyls in the 4:4'-positions, it seemed possible that some group present in the 4'-position might enhance the activity of 4-hydroxystilbene. Accordingly, a series of 4-hydroxystilbenes were prepared with the groups

(27, 28, 29 and 26 respectively) in the 4'-position. However, the last two depressed the activity slightly, whilst the first two practically destroyed it.

CHEMICAL METHODS

4-Hydroxydibenzyl (2). This compound was prepared by a more convenient method than that given in the literature [Stoermer & Kippe, 1903; Freund & Remse, 1890; Späth, 1913]. 4-Methoxydeoxybenzoin (7 g.) was refluxed with amalgamated zinc (30 g.), conc. HCl (100 ml.) and water (100 ml.) for 5 hr., fresh HCl being added each hour. The hot liquid was decanted from the zinc, cooled, the solid product collected, washed with water and crystallized from ethanol. 4-Methoxydibenzyl was obtained in colourless plates, m.p. 61–62°, as in the literature. For hydrolysis this ether (6.3 g.) was dissolved in an ethereal solution of

methyl magnesium iodide (prepared from 0.8 g. of magnesium, and 4.0 ml. of methyl iodide in 10 ml. of ether). The mixture was evaporated to dryness and heated in an oil-bath until frothing started (c. 180°); as this decreased the temperature was gradually raised to 200°. After 3 hr. the mixture was decomposed with ice and HCl and the colourless insoluble product filtered, washed and crystallized (as the sodium salt) from hot aqueous 2N NaOH. The regenerated 4-hydroxydibenzyl crystallized from carbon tetrachloride in colourless plates (4 g.), m.p. 100–101° as in the literature.

α : β -Diethyl- α -hydroxydibenzyl (4). α -Ethyldeoxybenzoin (14.0 g.) was dissolved in ether (50 ml.) and gradually treated with a solution of ethyl magnesium bromide (from 2 g. of magnesium and 7 ml. of ethyl bromide in 30 ml. ether). The addition completed, the mixture was heated on a water-bath for 10 min., cooled, and the product decomposed with ice and HCl. The ethereal layer was separated, dried, the solvent removed and the residue distilled *in vacuo*, b.p. 185–195° at 18 mm. (13 g.). On redistillation, α : β -diethyl- α -hydroxydibenzyl was obtained as a colourless viscous oil, b.p. 182–186° at 14 mm. (Found: C, 85.0; H, 8.6%. $\text{C}_{18}\text{H}_{22}\text{O}$ requires C, 85.0; H, 8.7%.)

α : β -Diethylstilbene (5). α : β -Diethyl- α -hydroxydibenzyl was dehydrated with phosphorus trichloride [Dodds *et al.* 1939] giving α : β -diethylstilbene as a colourless mobile oil, b.p. 170° at 15 mm. (Found: C, 91.2; H, 8.4%. Calc. for $\text{C}_{18}\text{H}_{20}$, C, 91.5; H, 8.5%.) This is in agreement with Ramart-Lucas & Anagnostopoulos [1928] and also with Kharasch & Kleiman [1943], but not with Rising & Zee [1928] or Carlisle & Crowfoot [1941], who describe it as a crystalline solid.

α : β -Diethylstilbenyl (6). Diethylstilbene (5.6 g.) was shaken in an atmosphere of hydrogen with platinum oxide (0.5 g.) and acetone (50 ml.). The solution was filtered, evaporated, and the residue (4.5 g.; m.p. 74–76°) recrystallized from methanol. α : β -Diethylstilbenyl was obtained in long needles, m.p. 83–84°. (Found: C, 90.7; H, 9.1%. Calc. for $\text{C}_{18}\text{H}_{22}$, C, 90.7; H, 9.3%.) This melting-point is much lower than values recorded in the literature. Moritz & Wolfenstein [1899] give 88°; Späth [1913] gives 92–93°.

γ : δ -Dihydroxy- γ : δ -diphenylhexane (7). Propiophenone (6 g.) in wet ether (100 ml.) was treated with aluminium amalgam (prepared from 6 g. aluminium powder and 9 g. mercuric chloride in 300 ml. ethanol) and, when the reaction had subsided, the mixture was refluxed on a water-bath for 45 min. The ether was evaporated and the residue crystallized repeatedly from light petroleum (b.p. 40–60°) yielding a white solid, m.p. 135–136° (Ciamician & Silber [1914] give 138–139°).

4-Methoxy- α : β -diethylstilbenyl (10). 4-Methoxy- α : β -diethylstilbene (20 g.) was shaken in acetone (100 ml.) with palladium charcoal catalyst (prepared by reducing 0.2 g. palladium chloride in presence of 3.8 g. charcoal) in hydrogen at 1 atm. pressure. After filtration the solution was evaporated and the product crystallized from ethanol; it had m.p. 89–90° (19 g.). (Found: C, 84.6; H, 8.8%. $\text{C}_{19}\text{H}_{24}\text{O}$ requires C, 85.0; H, 9.0%.)

4-Methoxy- α : β -diethylstilbene. δ -Phenyl- γ -anisylhexan- γ -ol, without purification, distilled at ordinary pressure with elimination of water, at 325–330°. The product, crystallized from ethanol, had m.p. 79–80°. (Found: C, 85.5; H, 8.2%. $\text{C}_{19}\text{H}_{22}\text{O}$ requires C, 85.7; H, 8.3%); the yield was quantitative.

4-Hydroxy- α : β -diethylstilbene (11). To a solution of methylmagnesium iodide (prepared from 5 g. of magnesium and

12 ml. of methyl iodide in 200 ml. of dry ether) was added 4-methoxydiethylstilbene (20 g.), and after evaporation of the ether the mixture was heated in an oil-bath, first at 170° until frothing subsided, and then at 200° for 30 min. After decomposition with water and HCl, the product was extracted with carbon tetrachloride and the solution concentrated to crystallize; m.p. 125–127°. (Found: C, 84.9; H, 8.0%. Calc. for $C_{18}H_{20}O$, C, 85.7; H, 8.0%.)

4-Hydroxy- α : β -diethylidibenzyl (12). To a solution of methylmagnesium iodide (prepared from 7.5 g. of magnesium and 18 ml. of methyl iodide in 300 ml. of ether) was added 4-methoxydiethylidibenzyl (30 g.) and, after evaporation of the ether, the mixture was heated in an oil-bath, first to 160° and then, when the reaction had subsided, to 195° for 30 min. After decomposition with water and acidification with HCl, the precipitate was crystallized from carbon tetrachloride; m.p. 139–140° (15 g.). (Found: C, 85.1; H, 8.7%. $C_{18}H_{20}O$ requires C, 85.0; H, 8.7%.) The benzoate (13), prepared by the Schotten-Baumann method or by the action of benzoyl chloride on a pyridine solution of the phenol, crystallized from methanol in long prisms, m.p. 110°. (Found: C, 83.8; H, 7.1%. $C_{26}H_{28}O$ requires C, 83.8; H, 7.3%.)

The *sulphonate* (14) was obtained as its pyridine salt by the action of chlorosulphonic acid (1.5 ml.) on a pyridine solution of the parent phenol (1.0 g.). As it was somewhat unstable to heat, it was purified by dissolution in cold chloroform and precipitation with ligroin. Colourless plates, m.p. 195–196°. (Found: C, 66.3; H, 6.4; S, 7.8; N, 3.5%. $C_{23}H_{27}O_4NS$ requires C, 66.8; H, 6.5; S, 7.7; N, 3.4%.)

Diethylstilboestrol monomethyl ether (15) was obtained as a by-product in the production of diethylstilboestrol by hydrolysis of the dimethyl ether. It crystallized from ethanol; m.p. 101–102°. (Found: C, 80.4; H, 7.7%. $C_{19}H_{22}O_2$ requires C, 80.8; H, 7.8%.)

4-Nitro- α -ethyldeoxybenzoin. 4-Nitrodeoxybenzoin [Petrov-Kritschenko, 1892; Womack, Campbell & Dodds, 1938] (35 g.), was added to a hot solution of sodium (3.9 g.) in absolute ethanol (800 ml.), ethyl iodide (39 ml.) was added as quickly as possible to the resulting solution, and the whole refluxed until the solution was neutral (2–3 hr.). During this time the colour changed from deep purple to yellow. After pouring into water the solution was extracted with benzene, the benzene layer washed twice with water, dried over calcium chloride, and concentrated to c. 100 ml. This solution was treated with an equal volume of light petroleum (b.p. 60–80°), when a crystalline solid separated (31 g., m.p. 75–78°). Recrystallized from ethanol (charcoal), 4-nitro- α -ethyldeoxybenzoin formed almost colourless prisms, m.p. 78–80°. (Found: C, 71.2; H, 5.9; N, 5.4%. $C_{16}H_{15}O_3N$ requires C, 71.4; H, 5.6; N, 5.2%.)

4-Amino- α -ethyldeoxybenzoin (21). 4-Nitro- α -ethyldeoxybenzoin (8 g.), iron filings (24 g.) and water (8 ml.) were ground into a paste, transferred to a flask together with xylene (8 ml.) and 1% $FeCl_3$ solution (12 ml.) and heated on a vigorously boiling water-bath for 3 hr., with continuous stirring. After 1½ hr., more xylene (4 ml.) was added. The mixture was allowed to cool somewhat, treated with benzene (100 ml.) and the iron oxide filtered off and washed thoroughly with a further quantity of warm benzene (100 ml.). The combined benzene layers were separated from the aqueous portion and exhaustively extracted with *N* HCl. By this means coloured resinous impurities remained in the benzene solution whilst, on making alkaline, the HCl deposited an oil which rapidly set to an almost colourless solid. This was collected, dried (6.6 g.) and

crystallized (charcoal) from cyclohexane, from which 4-amino- α -ethyldeoxybenzoin separated in sprays of needles, m.p. 128–129°. (Found: C, 80.5; H, 7.2; N, 6.0%. $C_{16}H_{17}ON$ requires C, 80.3; H, 7.1; N, 5.9%.)

4-Amino- α : β -diethyl- β -hydroxydibenzyl (22). A solution of 4-amino- α -ethyldeoxybenzoin (5.0 g.) in benzene (80 ml.) was gradually run into an ice-cooled solution of ethylmagnesium bromide (from 2 g. magnesium, 30 ml. ether and 7 ml. ethyl bromide, the ether being subsequently removed) in benzene (30 ml.). A thick yellow precipitate formed immediately. After standing overnight at room temperature the mixture was refluxed on a water-bath for 5 min., cooled and the magnesium complex decomposed with ice and ammonium chloride. A small amount of insoluble material was filtered off and the benzene layer separated, washed once with water and then extracted with HCl. The acid solution was made alkaline with ammonia, the resulting oil taken up in ether and the ethereal solution dried and evaporated. The crude residue crystallized on cooling and was purified by repeated recrystallization from a mixture of carbon tetrachloride and cyclohexane. Finally 4-amino- α : β -diethyl- β -hydroxydibenzyl was obtained in colourless needles, m.p. 91–92°. (Found: C, 80.1; H, 8.3%. $C_{18}H_{23}ON$ requires C, 80.2; H, 8.6%.)

4-Amino- α : β -diethylstilbene (23). A solution of 4-amino- α : β -diethyl- β -hydroxydibenzyl (10 g.) in glacial acetic acid (30 ml.) was saturated with hydrogen chloride at room temperature. After 72 hr. it was diluted with conc. HCl (30 ml.) and after a further 72 hr. the thick, rather discoloured precipitate (8.7 g., m.p. 220–230°) was recrystallized (charcoal) from ethanol diluted with 4 vol. of 0.5 *N* HCl. 4-Amino- α : β -diethylstilbene hydrochloride was obtained in colourless needles, m.p. 254–255°. (Found: C, 75.2; H, 7.5%. $C_{18}H_{22}NCl$ requires C, 75.1; H, 7.7%.) The parent base regenerated in the usual fashion, crystallized from ligroin (b.p. 40–60°) in sprays of needles, m.p. 96–97°. (Found: C, 86.3; H, 8.4. $C_{18}H_{21}N$ requires C, 86.1; H, 8.4%.)

4-Sulphanilamido- α : β -diethylstilbene (24). Treatment of 4-amino- α : β -diethylstilbene in pyridine solution with 4-acetamidobenzenesulphonyl chloride gave 4-[4'-acetamidobenzenesulphonamido]- α : β -diethylstilbene. This crystallized readily from methanol in colourless needles, m.p. 207–208°. (Found: C, 69.5; H, 6.6. $C_{26}H_{28}O_3N_2S$ requires C, 69.6; H, 6.3%.)

On hydrolysis with HCl in ethanol, 4-sulphanilamido- α : β -diethylstilbene was readily obtained. It crystallized from methanol or 95% ethanol in colourless needles, m.p. 180–182°. (Found: C, 70.9; H, 6.4; N, 7.15%. $C_{24}H_{26}O_2N_2S$ requires C, 70.9; H, 6.4; N, 6.9%.)

4-Hydroxy-4'-aminostilbene (27). A solution of 4-hydroxy-4'-nitrostilbene (1.0 g.) in ethanol (15 ml.) and concentrated ammonia (d. 0.880; 16 ml.) was gradually added to a boiling solution of $FeSO_4$ (11.0 g.) in water (75 ml.) and ethanol (150 ml.). The addition completed (1 hr.), the mixture was boiled for 1 hr. After cooling, the precipitated hydroxide was dissolved in dilute HCl, the yellow insoluble portion filtered off and crystallized from hot aqueous 2 *N* NaOH. The crystalline sodium salt was collected and the parent phenol regenerated by treatment with dilute acetic acid. The crude 4-hydroxy-4'-aminostilbene (0.75 g.) was a buff-coloured powder; it was sparingly soluble in most common organic solvents and could not be obtained quite pure. It crystallized from benzyl cyanide in bundles of yellowish plates, m.p. 270–271° (decomp.). (Found: C, 80.1; H, 6.2; N, 7.0%. $C_{14}H_{13}ON$ requires C, 79.6; H, 6.1; N, 6.6%.)

4-Hydroxy-4'-cyanostilbene (28). 4-Cyanophenylacetic acid [Jaeger & Robinson, 1941] (2.0 g.), 4-hydroxybenzaldehyde (1.0 g.) and piperidine (1 ml.) were heated at 140° for 1½ hr. The semi-liquid product was mixed with 50% ethanol, when a yellowish solid was rapidly deposited. This was collected, washed with fresh 50% ethanol, crystallized twice from glacial acetic acid and finally from 70% ethanol: 4-hydroxy-4'-cyanostilbene was obtained in faintly coloured prisms, m.p. 221–223°. (Found: C, 81.3; H, 5.0; N, 6.4%. $C_{15}H_{11}NO$ requires C, 81.4; H, 5.0; N, 6.3%.)

4-Hydroxy-4'-amidinostilbene hydrochloride (29). This was prepared from 4-hydroxy-4'-cyanostilbene according to the general procedure of Ashley, Barber, Ewins, Newbery & Self [1942]. 4-Hydroxy-4'-amidinostilbene hydrochloride crystallized from hot water in long yellowish needles, m.p. 316–317° (decomp.). (Found: C, 65.6; H, 5.4; N, 9.9; Cl, 12.5%. $C_{15}H_{10}ON_3Cl$ requires C, 65.6; H, 5.5; N, 10.2; Cl, 12.8%.)

SUMMARY

1. A series of compounds of the stilboestrol type has been prepared and tested *in vitro* for bactericidal activity against staphylococci and

streptococci. Several have been found to possess exceptionally high activity, the most active being

which is some 40 times as active as the mould metabolic product citrinin, and as active as chloroxylenol under the conditions employed.

2. A comparison of the bactericidal and oestrogenic activities of these compounds shows that the two actions are not necessarily concurrent, though both probably depend on the ready acceptance by the cell of molecules of the cyclopentanophenanthrene and stilboestrol types.

3. *In vivo* the compounds tested showed little or no activity, possibly on account of their very low solubility.

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Growth-promoting Substances from Bacteriological Filter Pads

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During an investigation of the nutritional requirements of acid-fast saprophytes [Edson & Hunter, 1943], synthetic media were employed, solutions of possible growth stimulants being added aseptically to a basal salt mixture. The methods of sterilization were selected according to the nature and stability of the chemical compounds involved. It soon be-

came evident that the results varied with the mode of preparation and filtration of the test substances. It was shown that solutions, both in water and in saline, were able to extract materials from filter pads in a concentration sufficiently high to stimulate the growth of acid-fast organisms, so that false positive results were sometimes recorded.