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The synthesis of some potential antibacterial agents

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The preparation of 37 potential antibacterial agents is described. Most of them possess the structure R—X—R′ where R and R′ are substituted phenyl groups and X is a short bridging group; 25 different examples of X have been studied. Minimum inhibitory concentrations against *Staphylococcus aureus* and against *Escherichia coli* are recorded.

OUR interest in new antibacterial agents lay primarily in their potential use in soap tablets for personal or domestic use. Such an objective imposes considerable restriction on the types of compound which can be considered since many bactericides are inactivated by soap over a range of relative concentrations. There are other limitations imposed by the need to avoid toxic effects like irritation, sensitisation or photosensitisation, on human skin and to avoid interference with the physical properties of the tablet, but neither of these broad aspects is considered here.

Many bactericides, particularly cationic bactericides such as the quaternary ammonium compounds, are bacteriologically incompatible with soap over almost the whole range of relative concentrations. Others, such as the halogenated cresols and xylenols, may be compatible if the soap concentration is of the same order as, or less than their own, so that they can be successfully solubilised in formulations that include relatively small amounts of soap (as in some household disinfectants) but are inactivated by the higher ratios of soap: bactericide met with in a soap tablet containing perhaps 1% of "bactericide". The nature of the soap-phenol, or in the more general case surfactant-disinfectant interaction has been studied in a few instances, notably by Alexander and by Berry, but although the picture has been much clarified it is still incompletely understood (Agar & Alexander, 1949; Alexander & Tomlinson, 1949; Bean & Berry, 1948, 1950, 1951, 1953; Berry, 1952; Berry & Bean, 1950; Berry & Briggs, 1956; Berry, Cook & Wills, 1956. See also Brudney, 1956; Cook, 1960). A measure of the interaction is given by the Soap Inactivation Coefficient (S.I.C.) which has been defined (Hurst, Stuttard & Woodroffe, 1960) as the concentration of a disinfectant in soap solution divided by its concentration in an equally potent soap-free solution. Thus an SIC of 1 shows that the compound is unaffected by the presence of soap, while a value greater or less than one shows inhibition and potentiation respectively. It may be noted that the S.I.C. value of any substance is affected by the soap concentration employed and a 10% solution, approximating to that in the lather formed in washing the hands, is normally used as a standard. Furthermore, where disinfection of the skin is the objective, the possession of a high S.I.C. value does not necessarily preclude the use of a potential disinfectant in a formulation involving soap. Provided that the disinfectant has adequate affinity for ("substantivity" to) the skin it will remain on the skin surface after rinsing

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and continue to exert an antibacterial effect. Hexachlorophane, for example, despite having an S.I.C. of about 200, is an effective disinfectant and has been widely used both in a preparation for pre-operative scrubbing of surgeons' hands and in a number of toilet soap tablets.

Many of the bactericides which are known to be effective in soap tablets have broadly similar structures. Thus hexachlorophane [G11; di(3,5,6-trichloro-2-hydroxyphenyl)methane], bithionol [Actamer; di-(3,5-dichloro-2-hydroxy)phenyl sulphide], salicylanilides such as 3,5,3′,4′-tetrachloro- (TCS), 5,3′,4′-trichloro- (Anobial) and 3,5,4′-tribromo-salicylanilide (TBS), and TCC (3,4,4′-trichlorocarbanilide) possess, in common, two benzene rings linked by a short bridging group. The benzene rings are all substituted by halogen and with the exception of TCC also contain at least one hydroxyl group adjacent to the bridge. There are, of course, others such as TMTD (tetramethylthiuram disulphide) and the mercurials, which are of totally different chemical structure. Nevertheless, it seemed to us that the examination of further examples of compounds consisting of substituted benzene rings linked by short bridges might well bring to light some new antibacterial agents, particularly as Jerchel & Oberheiden (1955) in their review of disinfectants of this type have shown that activity is not restricted to the bridging groups cited above.

Thirty-seven compounds have been examined, exemplifying 25 different bridges. For convenience of discussion of both synthesis and bacteriological properties these have been classified (see Table 1) into five groups.

(1) SULPHONAMIDE BRIDGE COMPOUNDS

Compounds (I) and (II) were prepared by condensation of 3,4-dichlorobenzenesulphonyl chloride with 4-chloro- and 3,4-dichloro-aniline respectively. Alkylation of (II) proceeded smoothly to give compounds (III), (IV) and (V). The dibromosulphanilide (VI) was prepared by bromination of sulphanilide itself, which was obtained in moderate yield from aniline and sulphuryl chloride. Attempts to prepare halogenated sulphanilides directly from halogenated anilines failed. Parnall (1960) has recently described a modified procedure which is successful in this respect, but in view of his report of apparent lack of activity in this series we have not attempted to prepare further examples.

(2) UREA AND THIOUREA BRIDGE COMPOUNDS

The urea derivatives (VII–X) were prepared from *p*-chlorophenyl isocyanate and various amines. The thiourea derivatives (XI–XXI) were obtained similarly from the corresponding isothiocyanate or (in the case of symmetrical compounds) from the anilines and carbon disulphide.

(3) AMIDE BRIDGE COMPOUNDS

The hydrazine derivative (XXIV) was prepared from ethyl 2,4-dichlorobenzoate and hydrazine hydrate, and the remaining amides (XXII, XXIII, XXV–XXVIII) via acid chlorides. Compound (XXVII) melts over 100° higher than the melting-point recorded in the literature (Beaver,

Roman & Stoffel, 1957). Since our material gives satisfactory elementary analysis it appears likely that there is a typographical error.

(4) OTHER BRIDGES

The substituted benzoic, carbonic and oxalic esters (XXIX–XXXI) were prepared by standard methods. Alkylation of 2,4,5-trichlorophenol with dichlorodiethyl ether gave the ether (XXXII).

(5) HETEROCYCLIC COMPOUNDS

The benzimidazoles (XXXIII and XXXIV) were prepared from 4-chloro-*o*-phenylene diamine. Treatment of cyanuric chloride with 3,4-dichloro-aniline gave the dianilinotriazine (XXXV), which with diethanolamine gave (XXXVI). The phthalimide (XXXVII) was synthesised from tetrachlorophthalic anhydride and 3,4-dichloroaniline, via the phthalamic acid, which readily underwent ring closure.

Experimental

PREPARATION OF COMPOUNDS

3,4,4′-*Trichlorobenzenesulphonanilide* (I). To a solution of *p*-chloroaniline (5 g) in dry pyridine (10 ml) was added 3,4-dichlorobenzenesulphonyl chloride (10 g). After 15 min the mixture was cooled, poured into water and acidified with concentrated hydrochloric acid. The product was filtered, washed with water and dried *in vacuo* (13·0 g, 98%, m.p. 126–8°). Recrystallisation from aqueous ethanol afforded the *sulphonanilide*, m.p. 131–3°. Found: C, 42·8; H, 2·4; Cl, 31·6; N, 4·2; S, 9·5. $C_{12}H_8Cl_3NO_2S$ requires C, 42·9; H, 2·1; Cl, 31·9; N, 4·3; S, 9·5%.

3,4,3′,4′-*Tetrachlorobenzenesulphonanilide* (II). In a similar manner 3,4-dichloroaniline (16 g), pyridine (25 ml) and 3,4-dichlorobenzenesulphonyl chloride (25 g) gave 3,4,3′,4′-*tetrachlorobenzenesulphonanilide*, which separated from aqueous ethanol in colourless needles, m.p. 140–2°. Found: C, 38·6; H, 1·9; Cl, 37·8. $C_{12}H_7Cl_4NO_2S$ requires C, 38·8; H, 1·9; Cl, 38·2%.

N-*Methyl*-3,4,3′,4′-*tetrachlorobenzenesulphonanilide* (III). To a solution of the above sulphonanilide (20 g) in methanol (60 ml) and 10% aqueous sodium hydroxide solution (30 ml) at 25° was added dropwise with stirring dimethyl sulphate (10·2 g). When the addition was complete the temperature was raised to 40° for 1 hr and the solution cooled and filtered. N-*Methyl*-3,4,3′,4′-*tetrachlorobenzenesulphonanilide* (16·0 g) separated from ethanol in colourless needles. Found: C, 40·8; H, 2·7; Cl, 37·0. $C_{13}H_9Cl_4NO_2S$ requires C, 40·5; H, 2·4; Cl, 36·8%.

N-*n-Butyl*-3,4,3′,4′-*tetrachlorobenzenesulphonanilide* (IV). To the tetrachlorobenzenesulphonanilide (15 g) and butyl bromide (15 g) in methanol (60 ml) was added 2N aqueous sodium hydroxide (20 ml) and the mixture boiled under reflux for 4 hr. The N-*butyl derivative* separated out on cooling. Recrystallisation from methanol afforded colourless needles, m.p. 83–4°. Found: C, 45·2; H, 3·9. $C_{16}H_{15}Cl_4NO_2S$ requires C, 45·0 H, 3·5%.

N-*n*-*Dodecyl*-3,4,3′,4′-*tetrachlorobenzenesulphonanilide* (V). Prepared in a similar manner to the butyl derivative, the N-*dodecyl analogue* separated from methanol in colourless needles, m.p. 83–4°. Found: C, 53·2; H, 5·8. $C_{24}H_{31}Cl_4NO_2S$ requires C, 53·4; H, 5·8%.

4,4′-*Dibromosulphanilide* (VI). Prepared by the method of Wohl & Koch (1910) 4,4′-dibromosulphanilide had m.p. 122–3° (lit. 124–5°).

Urea and thiourea derivatives (VII–XXI). With the exception of (XI), (XVII) and (XX) (see below) all compounds in this class were prepared by one of the following methods (see Table 2).

Method A. To a solution of the amine (0·1M) in dry benzene (100 ml) was added dropwise with stirring 0·1M (0·2M for diamines) of the appropriate isocyanate or isothiocyanate in the same solvent (100 ml). The mixture was then boiled under reflux for 15 min, cooled, and the product filtered and recrystallised from a suitable solvent.

Method B. (Thiocarbanilides only.) The carbon disulphide-iodine method described by Fry (1913).

Thiocarbanilide (XI) was prepared by the method of Vogel (1948) and its 4,4′-diethoxy derivative (XVII) by that of von Braun & Beschke (1906). 3,4-Dichlorophenylthiourea (XX) was made by the method described (Kurzer, 1951) for the 2-chloro-analogue.

2,4,3′,4′-*Tetrachlorobenzanilide* (XXII). A mixture of 3,4-dichloroaniline (17 g) and 2,4-dichlorobenzoyl chloride (10·5 g) was heated on a steam-bath for 1½ hr. The cooled residue was crushed, extracted with several portions of hot 0·1N HCl and finally with water. Recrystallisation from aqueous ethanol gave 2,4,3′,4′-*tetrachlorobenzanilide* in colourless needles, m.p. 155–6°. Found: C, 46·8; H, 2·1; Cl, 42·1. $C_{13}H_7Cl_4NO$ requires C, 46·6; H, 2·1; Cl, 42·3%.

2′-*Amino*-2,4-*dichlorobenzanilide* (XXIII). To a solution of *o*-phenylene diamine (20 g) in chloroform (250 ml) was added dropwise with stirring a solution of 2,4-dichlorobenzoyl chloride (20 g) in chloroform (70 ml). The mixture was heated under reflux for a further hour, cooled and filtered, and the product washed well with water. The residue (19·2 g, m.p. 164–172°) on recrystallisation from benzene formed colourless plates, m.p. 175–9°. Found: C, 55·8; H, 3·8; Cl, 25·2. $C_{13}H_{10}Cl_2N_2O$ requires C, 55·5; H, 3·6; Cl, 25·2%.

N,N′-*Di*(2,4-*dichlorobenzoyl*)*hydrazine* (XXIV). To hydrazine hydrate (40 g of 80%) in ethanol (100 ml) was added ethyl 2,4-dichlorobenzoate (50 g) in ethanol (100 ml) and the solution boiled under reflux for 30 min. After removal of solvent under reduced pressure the residue was recrystallised from ethanol to give N,N′-*di*(2,4-*dichlorobenzoyl*)*hydrazine* (31 g), m.p. 264–265°. Found: Cl, 37·3; N, 7·2. $C_{14}H_8Cl_4N_2O_2$ requires Cl, 37·5; N, 7·4%.

N,N′-*Di*(3,4-*dichlorophenyl*)*oxamide* (XXV). To a solution of 3,4-dichloroaniline (30 g) in dry benzene (200 ml) was added dropwise with stirring oxalyl chloride (7 g) in dry benzene (50 ml). Stirring was continued for 30 min after the addition was complete. Water (700 ml) was then added and the N,N′-*di*(3,4-*dichlorophenyl*)*oxamide* (15.9 g, m.p.

222–6°) filtered off and washed with water. Recrystallisation from benzene raised the m.p. to 226–8°. Found: C, 44·2; H, 2·3. Calculated for $C_{14}H_8Cl_4N_2O_2$, C, 44·4; H, 2·1%. (Beaver & others, 1957, quote m.p. 228·2–229·1°).

N-(3,4-*Dichlorophenyl*)-2,4,5-*trichlorophenoxyacetamide* (XXVI). A mixture of 2,4,5-trichlorophenoxyacetic acid (9·8 g) and thionyl chloride (35 ml) was boiled under reflux for 30 min. Dry benzene (35 ml) was then added and the solution evaporated under reduced pressure. A further portion (20 ml) of benzene was added and similarly removed. To the residual acid chloride in dry benzene (35 ml) was added dropwise a solution of 3,4-dichloroaniline (15 g) in 100 ml of benzene. After completion of the addition, the mixture was poured into excess of dilute HCl and filtered. The N-(3,4-*dichlorophenyl*)-2,4,5-*trichlorophenoxyacetamide* (15 g) had m.p. 202–7°, raised to 217–220° after recrystallisation from benzene and from ethanol. Found: N, 3·6. $C_{14}H_8Cl_5NO_2$ requires N, 3·5%.

N,N′-*Di*(3,4-*dichlorophenyl*)*fumaramide* (XXVII). The use of fumaryl chloride (7 g) in place of oxalyl chloride (7 g) in the preparation of (XXV) led to N,N′-*di*(3,4-*dichlorophenyl*)*fumaramide* (14·1 g), m.p. 337–340°. Found: C, 47·5; H, 2·7; Cl, 35·0. Calculated for $C_{16}H_{10}Cl_4N_2O_2$: C, 47·5; H, 2·5; Cl, 35·1%. (Beaver & others, 1957, record m.p. 227–9°).

2,4,3′,4′-*Tetrachlorocinnamanilide* (XXVIII). To a solution of 3,4-dichloroaniline (7·8 g) and pyridine (5 g) in dry benzene (70 ml) was added dropwise 3,4-dichlorocinnamoyl chloride (7·5 g) in dry benzene (100 ml). After a further 30 min at room temperature the solid was filtered off, washed with water and recrystallised from ethanol to give 2,4,3′,4′-*tetrachlorocinnamanilide* in colourless needles, m.p. 205°. Found: C, 49·9; H, 2·65. $C_{15}H_9Cl_4NO$ requires C, 49·9; H, 2·5%.

2,4,5-*Trichlorophenyl* 3,4-*dichlorobenzoate* (XXIX). To a solution of 2,4,5-trichlorophenol (10 g) in benzene (100 ml) and pyridine (10 ml) was added 3,4-dichlorobenzoyl chloride (10·5 g). After 30 min at room temperature the mixture was diluted with N HCl (200 ml), extracted with ether and the extract washed successively with 0·5N aqueous sodium carbonate and with water. Evaporation of the dried ether extract and recrystallisation from ethanol gave 2,4,5-*trichlorophenyl* 3,4-*dichlorobenzoate*, m.p. 142–3°. Found: C, 42·4; H, 1·5. $C_{13}H_5Cl_5O_2$ requires C, 42·1; H, 1·4%.

Di(4-*chlorophenyl*) *carbonate* (XXX). To a solution of *p*-chlorophenol (16 g) in a mixture of dry acetone (50 ml) and pyridine (25 ml) was added dropwise a solution of phosgene in toluene (38·5 g of 12½%). After 2½ hr at room temperature, the mixture was diluted with water, extracted with ether and the ether extract washed with 5% aqueous sodium hydroxide solution. Evaporation of the ether, followed by recrystallisation from ethanol, gave di(*p*-chlorophenyl) carbonate (6·4 g), m.p. 149–151°; British Patent 753,766 (1956), m.p. 149°.

Di(4-*chlorophenyl*) *oxalate* (XXXI). To a solution of *p*-chlorophenol (15 g) in dry benzene (100 ml) and pyridine (20 g) was added dropwise

with stirring a solution of oxalyl chloride (6·4 g) in dry benzene (25 ml) the temperature being kept below 30°. After a further 30 min at 20–30°, the mixture was diluted with ether (100 ml) and water (200 ml) and the *di*(4-*chlorophenyl*) *oxalate* (8·7 g) collected by filtration. A further quantity (0·8 g) was obtained by evaporation of the solvent. An analytical sample separated from benzene in colourless needles, m.p. 186–7°. Found: C, 54·3; H, 2·4; Cl, 22·5. $C_{14}H_8Cl_2O_4$ requires C, 54·1; H, 2·6; Cl, 22·8%.

Di[2-(2,4,5-*trichlorophenoxy*)*ethyl*] *ether* (XXXII). A mixture of 2,4,5-trichlorophenol (54·4 g), di(2-chloroethyl) ether (20 g), sodium hydroxide (11·2 g) and 50% (v/v) aqueous ethanol (60 ml) was boiled under reflux for 24 hr. Dilution of the cold solution with water precipitated *di*[2-(2,4,5-*trichlorophenoxyethyl*)] *ether* (46·3 g), which on recrystallisation from methanol had m.p. 92–3°. Found: C, 41·5; H, 2·55. $C_{16}H_{12}Cl_6O_3$ requires C, 31·3; H, 2·6%.

5-*Chloro*-2-*mercaptobenzimidazole* (XXXIII). The use of 4-chloro-*o*-phenylene diamine in the method described (Allan & Deacon, 1950) for 2-mercaptobenzimidazole gave the 5-*chloro-derivative*, m.p. 305° (decomp.). Knobloch, Winkelmann & Rintelen (1958) quote m.p. 290–292°.

5-*Chloro*-2-(2,4-*dichlorophenyl*)*benzimidazole* (XXXIV). To a solution of 4-chloro-*o*-phenylene diamine (6 g) and copper acetate (16 g) in 50% aqueous methanol (200 ml) was added 2,4-dichlorobenzaldehyde (8·4 g) in methanol (75 ml). The mixture was boiled under reflux for 30 min, cooled and filtered. The copper salt was suspended in 50% (v/v) aqueous ethanol at 50°, treated with hydrogen sulphide, filtered and the residue washed well with hot ethanol. 5-*Chloro*-2-(2,4-*dichlorophenyl*)*benzimidazole* (4·4 g) separated from the combined filtrates on cooling and had m.p. 184–5° after recrystallisation from aqueous ethanol. Found: C, 52·1; H, 2·6; Cl, 35·7; N, 9·7. Calculated for $C_{13}H_7Cl_3N_2$: C, 52·4; H, 2·35; Cl, 35·8; N, 9·4%. Subba Row & Ratnam (1958) found m.p. 184°.

2-*Chloro*-4,6-*di*(3,4-*dichloroanilino*)-1,3,5-*triazine* (XXXV). To ice-cold water (400 ml) was added dropwise with stirring a solution of cyanuric chloride (27·6 g) in acetone (120 ml), followed by a solution of 3,4-dichloroaniline (49 g) in acetone (150 ml), the temperature being maintained at 0–8°. A solution of sodium bicarbonate (25·2 g) in water (200 ml) was then added and the reaction temperature raised to 45–50° for 2 hr. The mixture was then cooled and the 2-*chloro*-4,6-*di*(3,4-*dichloroanilino*)-1,3,5-*triazine* (61·6 g) collected by filtration, washed well with water and dried *in vacuo* at 50–60°. A portion recrystallised from aqueous acetone had m.p. 243–4°. Found: Cl, 40·8. $C_{15}H_8Cl_5N_5$ requires Cl, 40·8%.

4,6-*Di*(3,4-*dichloroanilino*)-2-[*di*(2-*hydroxyethyl*)*amino*]-1,3,5-*triazine* (XXXVI). A mixture of the above chlorotriazine (9 g) and diethanolamine (9 g) was heated to 100° for 2 hr, cooled and diluted with water. The precipitated 4,6-*di*(3,4-*dichloroanilino*)-2-[*di*(2-*hydroxyethyl*)*amino*]-1,3,5-*triazine* (9·3 g) had m.p. 179–185°, raised to 188–190° after recrystallisation from ethanol. Found: Cl, 28·3. $C_{19}H_{18}Cl_4N_6O_2$ requires Cl, 28·2%.

3,4,5,6-*Tetrachloro*-N-(3,4-*dichlorophenyl*)*phthalimide* (XXXVII). To a solution of 3,4,5,6-tetrachlorophthalic anhydride (14·3 g) in dry benzene (300 ml) was added 3,4-dichloroaniline (10 g) in the same solvent (50 ml). The mixture was boiled under reflux for 15 min, cooled and filtered. The product (21·8 g), presumably 2-carboxy-3,4,5,6,3′,4′-hexachlorobenzanilide, showed a change of crystal form at 183° and melted at 290–292°. Attempted crystallisation from ethanol converted the material to 3,4,5,6-*tetrachloro*-N-(3,4-*dichlorophenyl*)*phthalimide*, a sample of which, after recrystallisation from dimethyl formamide followed by sublimation at 260–270°/15 mm, formed pale yellow needles, m.p. 296–298°. Found: Cl, 49·9; N, 3·4. $C_{14}H_3Cl_6NO_2$ requires Cl, 49·5; N, 3·3%.

SCREENING TESTS

Determination of minimum bacteriostatic concentrations and of soap inactivation coefficients was carried out as described by Hurst, Stuttard & Woodroffe (1960).

Results and discussion

(1) SULPHONAMIDE BRIDGE COMPOUNDS

The sulphonanilides (I and II) showed moderate activity against *Staphylococcus aureus* but much less against *Escherichia coli* (see Table 1). Activity was lost on alkylation (III, IV, V). It is now generally accepted that, as postulated by Woods (1940) for sulphanilamide itself, many sulphanilamide derivatives act by interfering with the utilisation of *p*-aminobenzoic acid (PABA). Bell & Roblin (1942) showed that for such compounds activity could be related to the pK_a (optimum activity at pK_a of about 6·5) and that in general the ionised form of the compound was more active than the un-ionised. The peak in activity has been accounted for in terms of the negative character of the sulphonyl group (Bell & Roblin, 1942) and in terms of penetration of the cell-wall by an un-ionised molecule, which subsequently ionises within the cell (Cowles, 1942; Brueckner, 1943; Klotz, 1944). By contrast, sulphanilanilides (i.e., *N*-phenylsulphanilamides) do not appear to act by interference with PABA utilisation, at any rate against Gram-positive organisms. Schmidt & Sesler (1946) (see also Goetchius & Lawrence, 1945) showed that the activity of the 3′,5′-dibromo-derivative and of several related compounds against a pneumococcus and against a β-haemolytic streptococcus was not antagonised by PABA, although that against Gram-negative bacteria (Friendlander's bacillus, *E. coli*) was antagonised. Evidence on the mechanism of action of the corresponding compounds without the *p*-amino-group (i.e., benzenesulphonanilides) is lacking but it would seem likely from our examples that the ionised form is again much more active than the un-ionised, since alkylation of the nitrogen atom (which precludes ionisation) destroys activity. The sulphanilide (VI) showed weak but similar activity against *Staph. aureus* and *E. coli*, in contrast to the sulphonanilides (I and II) which, like the conventional sulphonamides, showed much greater activity against the former than the latter.

TABLE 1. COMPOUNDS OF THE TYPE R—X—R′

Compound No.	R	X	R′	Minimum inhibitory concentration (μg/ml) against *Staph. aureus*	Minimum inhibitory concentration (μg/ml) against *E. coli*
Sulphonamide bridges—					
I	3,4-Dichlorophenyl	—SO_2·NH—	4-Chlorophenyl	6	100
II	3,4-Dichlorophenyl	—SO_2·NH—	3,4-Dichlorophenyl	12	>100
III	3,4-Dichlorophenyl	—SO_2·N(CH_3)—	3,4-Dichlorophenyl	>100	>100
IV	3,4-Dichlorophenyl	—SO_2·N(*n*—C_4H_9)—	3,4-Dichlorophenyl	>100	>100
V	3,4-Dichlorophenyl	—SO_2·N(*n*—$C_{12}H_{25}$)—	3,4-Dichlorophenyl	>100	>100
VI	4-Bromophenyl	—NH·SO_2·NH—	4-Bromophenyl	50	50
Urea and thiourea bridges—					
VII	4,Chlorophenyl	—NH·CO·NH—	4-Chlorophenyl	100	100
VIII	4-Chlorophenyl	—NH·CO·NH—	n-Dodecyl	50	50
IX	4-Chlorophenyl	—NH·CO·NH[CH_2]$_6$·NH·CO·NH—	4-Chlorophenyl	100	100
X	4-Chlorophenyl	—NH·CO·NH·CH_2(CHOH)·CH_2·NH·CO·NH—	4-Chlorophenyl	>100	>100
XI	Phenyl	—NH·CS·NH—	Phenyl	100	50
XII	3-Chlorophenyl	—NH·CS·NH—	3-Chlorophenyl	6	25
XIII	4-Chlorophenyl	—NH·CS·NH—	Phenyl	12	50
XIV	4-Chlorophenyl	—NH·CS·NH—	4-Chlorophenyl	3	100
XV	3,4-Dichlorophenyl	—NH·CS·NH—	4-Chlorophenyl	0·1	50
XVI	3,4-Dichlorophenyl	—NH·CS·NH—	3,4-Dichlorophenyl	0·1	100
XVII	4-Ethoxyphenyl	—NH·CS·NH—	4-Ethoxyphenyl	100	100
XVIII	4-Chloro-2-methoxyphenyl	—NH·CS·NH—	4-Chloro-2-methoxyphenyl	100	50
XIX	4-Chlorophenyl	—NH·CS·NH—	4-Carboxyphenyl	50	50
XX	3,4-Dichlorophenyl	—NH·CS·NH—	H	3	25
XXI	4-Chlorophenyl	—NH·CS·NH·CH_2·CH_2·NH·CS·NH—	4-Chlorophenyl	25	50
Amide bridges—					
XXII	2,4-Dichlorophenyl	—CO·NH—	3,4-Dichlorophenyl	50	100
XXIII	2,4-Dichlorophenyl	—CO·NH—	2-Aminophenyl	50	50
XXIV	2,4-Dichlorophenyl	—CO·NH·NH·CO—	2,4-Dichlorophenyl	50	50
XXV	2,4-Dichlorophenyl	—NH·CO·CO·NH—	3,4-Dichlorophenyl	100	100
XXVI	2,4,5-Trichlorophenyl	—O·CH_2·CO·NH—	3,4-Dichlorophenyl	100	100
XXVII	3,4-Dichlorophenyl	—NH·CO·CH:CH·CO·NH— (*trans*)	3,4-Dichlorophenyl	12	50
XXVIII	2,4-Dichlorophenyl	—CH:CH·CO·NH— (*trans*)	3,4-Dichlorophenyl	>100	>200
Other bridges—					
XXIX	3,4-Dichlorophenyl	—CO·O—	2,4,5-Trichlorophenyl	3	>100
XXX	4-Chlorophenyl	—O·CO·O—	4-Chlorophenyl	50	100
XXXI	4-Chlorophenyl	—O·CO·CO·O—	4-Chlorophenyl	50	25
XXXII	2,4,5-Trichlorophenyl	—O·CH_2·CH_2·O·CH_2·CH_2·O—	2,4,5-Trichlorophenyl	>100	>100
Heterocyclic compounds—					
XXXIII	5-Chloro-2-mercaptobenzimidazole			50	50
XXXIV	5-Chloro-2-(2,4-dichlorophenyl)benzimidazole			100	50
XXXV	2-Chloro-4,6-di(3,4-dichloroanilino)-1,3,5-triazine			50	50
XXXVI	4,6-Di(3,4-dichloranilino)-2-[di(2-hydroxyethyl)amino]-1,3,5-triazine			100	100
XXXVII	3,4,5,6-Tetrachloro-*N*-(3,4-dichlorophenyl)-phthalimide			25	100

TABLE 2. PREPARATION OF UREA AND THIOUREA DERIVATIVES

Compound No.	Method	Recrystallisation solvent	Found (%)					Formula	Required (%)					m.p. (°C)	Lit. m.p. (°C)
			C	H	Cl	N	S		C	H	Cl	N	S		
VII	A	Acetone	—	—	—	—	—	$C_{13}H_{10}Cl_3N_3O$	—	—	—	—	—	308–10	310(*a*)
VIII ..	A	Benzene	67·6	8·8	—	—	—	$C_{19}H_{31}ClN_2O$	67·4	9·2	—	—	—	126–7	—
IX	A	Dimethyl formamide	—	—	16·9	—	—	$C_{20}H_{28}Cl_2N_4O_3$	—	—	16·8	—	—	249–50	—
X	A*	Acetic acid	51·3	4·8	17·6	—	—	$C_{17}H_{18}Cl_2N_4O_3$	51·4	4·5	17·9	—	—	237–8	—
XI	See text	Aqueous ethanol	—	—	—	—	—	$C_{13}H_{15}N_3S$	—	—	—	—	—	152–3	154(*b*)
XII	B	Benzene	—	—	23·9	9·7	10·9	$C_{13}H_{10}Cl_2N_2S$	—	—	23·9	9·4	10·8	120–2	121–2(*c*)
XIII ..	A	Ethanol	—	—	—	—	—	$C_{13}H_{11}ClN_2S$	—	—	—	—	—	152–3	152(*d*)
XIV ..	B	Benzene	—	—	—	9·6	10·7	$C_{13}H_{10}Cl_2N_2S$	—	—	—	9·4	10·8	175–6	176(*e*)
XV	A	Benzene	47·1	2·8	—	—	—	$C_{13}H_9Cl_3N_2S$	47·1	2·7	—	—	—	154–5	154·2–154·9(*f*)
XVI ..	B	Benzene	—	—	—	7·3	8·7	$C_{13}H_8Cl_4N_2S$	—	—	—	7·6	8·7	160–1	162·6–163·5(*f*); 144(*e*)
XVII ..	See text	Ethanol	—	—	—	8·6	10·3	$C_{17}H_{20}N_2O_2S$	—	—	—	8·9	10·1	169–70	170(*g*)
XVIII ..	B	Benzene	—	—	—	—	—	$C_{15}H_{14}Cl_2N_2O_2S$	—	—	—	—	—	162–3	152–5(*h*)
XIX.. ..	A	Ethanol	54·8	3·4	11·4	8·95	10·5	$C_{14}H_{11}ClN_2O_2S$	54·8	3·6	11·6	9·1	10·4	200 (dec.)	—
XX	See text	Acetic acid	—	—	—	—	—	$C_7H_8Cl_2N_2S$	—	—	—	—	—	203–5	203–4(*i*); 164(*e*)
XXI ..	A	Acetic acid	—	—	—	14·1	—	$C_{16}H_{16}Cl_2N_4S$	—	—	—	—	—	192–4	—

* Temperature kept below 40° during reaction. (*a*) Chattaway & Orton (1901). (*b*) Vogel (1948). (*c*) Fry (1913). (*d*) Kjellin (1903). (*e*) Dyson, George & Hunter (1926). (*f*) Beaver, Roman & Stoffel (1957). (*g*) Braun & Beschke (1906). (*h*) Herold (1882). (*i*) Douglass & Dains (1934).

TABLE 3. SOAP INACTIVATION CONSTANTS (S.I.C.) AGAINST *Staph. aureus* OF SOME OF THE COMPOUNDS LISTED IN TABLE 1

Compound	Concentration in soap (μg/ml)	S.I.C.
I	2000	1·7
VI	500	1·6
XV	62·5	1·5
XVI	31·2	1·2
XIX	500	1·0

(2) UREA AND THIOUREA BRIDGE COMPOUNDS

TCC (3,4,4′-trichlorocarbanilide) is known to be a bacteriostat, but when this work was begun not many analogues appeared to have been studied. However, fairly soon afterwards Beaver & others (1957) recorded the synthesis of 205 urea, thiourea and related derivatives and showed that peak activity occurred in the 3,4,3′- and 3,4,4′-trichlorocarbanilides, both of which inhibit *Micrococcus pyogenes* var. *aureus* at 1 in 3×10^7. It is of interest that the thio-analogues, such as "Thio-TCC" (XV), show a similar order of activity, although they are unlikely to have a comparable utility owing to their relatively poor stability in mildly alkaline media such as soap. Peak activity seems to be in the compounds with a 3,4-dichlorophenyl group, not only in the carbanilides and thiocarbanilides, but also in the related isothiocyanates, dibenzylthioureas and benzyl dithiocarbamate esters (McKay & others, 1959). It is somewhat surprising that, although optimum activity in the ureas and thioureas occurs in compounds with a suitably halogenated pair of aromatic rings, significant activity can occur in compounds in which one ring is absent (XX) or replaced by a long aliphatic chain (VIII). The coupling of two urea or thiourea groups by various chains (IX, X, XXI) did not lead to enhanced activity.

(3) AMIDE BRIDGES

The only amide showing appreciable activity was the fumaramide (XXVII), which inhibited *Staph. aureus* at 12·5 μg/ml.

(4) OTHER BRIDGES

Of these, notable activity was shown only by a chlorinated phenyl benzoate (XXIX).

(5) HETEROCYCLIC COMPOUNDS

No appreciable activity was observed within this group. High activity has previously been recorded (Jerchel, Fischer & Kracht, 1952) for a phenolic benzimidazole related to XXXIV. Some anilino-triazine analogues of XXXV and XXXVI have been claimed as fungicides (Wolf, 1955).

The soap inactivation coefficients of some of the more active compounds are shown in Table 3.

Some further examples of the more promising types of compound are being studied.

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