CHEMICAL STRUCTURE AND ANTIMICROBIAL ACTIVITY OF BIS-PHENOLS

II. Bactericidal Activity in the Presence of an Anionic Surfactant

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A previous report by Gump and Walter cited the bacteriostatic and fungistatic properties of a series of bis-phenols (1). Numerous other workers have evaluated bis-phenols in general for microbiological activity (2). A review of the literature will indicate that the great majority of published investigations have been concerned with bacteriostatic or fungistatic activity. Several papers have appeared wherein the authors refer to the bactericidal activity of hexachlorophene (G-11®); the size of the inhibition zones on agar plates is measured and taken as criterion of the bactericidal effect (3, 4). Besides our own work (2, 5) with dichlorophene (G-4®) and hexachlorophene, the number of publications dealing with truly bactericidal action are few indeed (6, 7). In addition, bactericidal data have often been erroneously interpreted because of the highly bacteriostatic nature of the bis-phenols coupled with the omission of a neutralizer in the test procedures. Some reports, however, have appeared which depict bactericidal action where adequate precautions against bacteriostatic effects were taken (8).

Several instances have been reported relating to an enhancement phenomenon between anionics and bis-phenols (9, 10). Preliminary work in our laboratories demonstrated that certain anionic surfactants of the alkylaryl sulfonate type will solubilize hexachlorophene and maintain bactericidal action. It was observed that bis-phenols other than hexachlorophene exhibited bactericidal properties when in the presence of an anionic surfactant, and a series of bis-phenols showing bacteriostatic activity, as previously reported (1), was selected and screened for possible bactericidal activity.

Our concern was primarily that of detecting lethal action at low levels of the test compound. It was deemed desirable that the technique employed should measure any kill observed quantitatively, inasmuch as it was anticipated that the magnitude and rate of kill would not be such that extinction

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type procedures could be used, *viz.* the phenol coefficient method. Extinction type tests, due to lack of sensitivity, would not readily detect differences between weakly bactericidal compounds.

Experimental and Discussion

Compound numbers of the bis-phenols employed in this investigation refer to the numbers in a previous publication (1) and are listed in Table 1. Bis-phenols not demonstrating bactericidal action of 95 per cent kill or better are omitted from this report.

Table 1—Names and Numbers of Bis-phenols

Compound No.*	
1	2,2′-methylenebis (3,4,6-trichlorophenol)
2	2,2′-methylenebis (4,5,6-trichlorophenol)
3	2,2′-methylenebis (3,4,5-trichlorophenol)
4	2,2′-methylenebis (4-bromo-3,6-dichlorophenol)
5	2,2′-methylenebis (4-bromo-5,6-dichlorophenol)
6	2,2′-methylenebis (4-chlorophenol)
7	2,2′-methylenebis (4-methylphenol)
8	2,2′-methylenebis (4,6-dichlorophenol)
9	2,2′-methylenebis (5,6-dichlorophenol)
15	2,2′-ethylidenebis (4-chlorophenol)
16	2,2′-ethylidenebis (6-bromo-4-chlorophenol)
19	2,2′-(2,2,2-trichloroethylidene)-bis(6-bromo-4-chlorophenol)
20	5,5′-dichloro-2,2′-stilbenediol
21	3,5,3′,5′-tetrachloro-2,2′-stilbenediol
24	2,2′-benzylidenebis-(4,6-dimethylphenol)
30	2,2′-thiobis (4-chlorophenol)
31	2,2′-thiobis (4-bromophenol)
32	2,2′-thiobis (4,6-dichlorophenol)
33	2,2′-thiobis (6-bromo-4-chlorophenol)
34	2,2′-thiobis (3,4,6-trichlorophenol)
36	2,2′-thiobis (4-chloro-6-isopropyl-3-methylphenol)
37	2,2′-thiobis (4-chloro-3,5-dimethylphenol)
38	2-hydroxy-2-methoxy-5,5′-dichlorodiphenyl sulfide
39	1,1′-methylenebis (2-naphthol)
40	3,3′-methylenebis (2,4,6-trichlorophenol)
41	3,3′-methylenebis (4-bromo-2,6-dichlorophenol)
42	4,4′-methylenebis (2,3,6-trichlorophenol)
43	2,2′-oxybis (tetrachlorophenol)
47	4,4′,6,6′-tetrabromo-o,o′-diphenol
48	salicil (2,2′-dihydroxybenzil)

* Numbers kept uniform with numbering in *J. Soc. Cosmetic Chemists*, **11**, 307 (1960) for future reference purposes.

The basic test procedure for ascertaining bactericidal activity of the compounds was that of inoculating a test system with a known number of organisms followed by enumeration of the survivors after twelve minutes contact time. The test system consisted of 100 ml. distilled water containing 30 μg./ml. sodium dodecylbenzene sulfonate (Ultrawet®-K) (Atlantic) and the specified level of bis-phenol. Aliquots of alcoholic (S.D.A. 30) solutions of the test compounds were added to the anionic-water system,

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but in no case was the final alcohol concentration greater than one per cent. The inoculum consisted of one ml. of a distilled water dilution of a 24-hour A.O.A.C. broth culture of either *Staphylococcus aureus* A.T.C.C. 6538 or *Escherichia coli* A.T.C.C. 11229 (11) added to 100 ml. of the test system. All solutions were equilibrated to a temperature of 20°C. before inoculum addition. One ml. sample aliquots of the inoculated test system were removed at the end of the specified time and placed directly into Astell Roll Tubes (Consolidated) containing 4 ml. antidote agar. The roll tubes were incubated at 34°C. and the survivors counted at the end of 48-hours incubation. Roll tube agar consisted of Dextrose Tryptone Extract Agar (Difco) containing one per cent Tween® 80 (Atlas) fortified with an additional 0.5 per cent agar.

The bis-phenols were initially screened in the surfactant system against *S. aureus* and *E. coli* at levels of 15 μg./ml. and 100 μg./ml. respectively. Percentage kills were calculated and those compounds demonstrating a 95 per cent kill or better were further evaluated at lower levels. All com-

TABLE 2—BIS-PHENOLS DEMONSTRATING BACTERICIDAL ACTIVITY AGAINST *S. aureus* AND *E. coli*

Compound No.	*S. aureus* 15μg 95	15μg 99	10μg 95	10μg 99	5μg 95	5μg 99	1μg 95	1μg 99	*E. coli* 100μg 95	100μg 99	50μg 95	50μg 99	15μg 95	15μg 99	(level) (% kill)
1	*	*	*												
3	*	*	*	*	*	*	*	*	*	*					
4	*	*	*	*	*	*	*								
5	*	*													
6	*	*							*	*	*	*			
7	*	*													
9	*	*	*	*	*	*			*	*	*	*	*	*	
15	*	*							*	*	*	*			
16	*	*	*	*											
19	*	*	*		*										
20	*	*	*	*					*	*	*	*			
21	*	*													
24	*														
30	*	*							*	*	*	*	*	*	
31	*	*							*	*	*	*	*	*	
32	*	*							*	*					
33	*								*	*					
34	*	*							*	*					
36	*	*													
37	*	*													
38	*	*													
39	*								*	*					
40	*	*													
41	*	*													
42	*	*	*	*											
43	*	*	*						*						
47	*								*	*					
48									*	*					
Initial count/ml. of test system	8000		6500		4000		4000		18,000		17,000		17,000		

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pounds for a given dosage level were examined simultaneously so that the activities would be relative within a given dosage level. Results of the bactericidal evaluation may be seen in Table 2. Those compounds demonstrating a 95 per cent kill or better are asterisked at the corresponding dosage level.

The efficacy of Tween 80 as an antidote for hexachlorophene was reported by Lawrence (12). Antidote effectiveness in this investigation was determined by inoculation of roll tubes, with and without the bis-phenols, with a known number of organisms. The level of bis-phenols in the agar was 100 μg./ml. and therefore approximately 5 to 30 times greater than the maximum expected carry-over from the actual test system. No significant differences were observed between tubes with and without bis-phenol, and it was presumed that bacteriostatic effects were eliminated. Each active bis-phenol was examined in this manner and the antidote agar was shown to nullify completely bacteriostatic effects.

It may be noted that the method employed a minimum of extraneous organic material in the test system, due to the inoculum dilution. For this reason the initial numbers of cells is relatively low compared to, for example, the A.O.A.C. methods for evaluating disinfectant type products (11). This factor was purposely taken into account in this evaluation to increase the sensitivity of the method so that differentiation between the compounds would be possible.

The frequent use of bis-phenols as bacteriostatic agents in liquid and bar soaps prompted us to evaluate soap solutions containing the most active bis-phenol (No. 3) and hexachlorophene (No. 1). We employed essentially the same technique as with the synthetic anionic surfactant; however, in this case, the compounds were tested at several ratios of Maxine (Swift) soap to test compound. The level of test compound was held constant at 1 μg./ml. As may be noted in Fig. 1, bactericidal action was observed to decrease as the soap ratio increased. The superior lethal properties of the hexachlorophene isomer (No. 3) to that of hexachlorophene (No. 1) is apparent in Fig. 1. Interest in the reduction of bath water counts by hexachlorophene was previously reported by Ayliffe (8).

We had previously drawn some general conclusions with regard to the relationship between the bacteriostatic properties of the bis-phenols and their chemical structure (1). Although a certain pattern is evident in the case of bactericidal activity, the connection with chemical configuration is less definite.

The specificity of the bis-phenols, i.e., greater potency against gram positive organisms than against gram negative ones, is again noted from the data shown in Table 2. An isomer of G-11, 2,2′-methylenebis-(3,4,5-trichlorophenol) (No. 3), demonstrated the greatest bactericidal activity against *S. aureus*. This same compound was inhibitory at the lowest level

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(0.2 μg./ml.) as was shown previously in the bacteriostatic evaluation. There, it was also the most effective substance against *E. coli;* here, other compounds are superior to it. The other isomer of G-11, 2,2′-methylene-bis(4,5,6-trichlorophenol) (No. 2), is unexpectedly inactive at the test levels; G-11 (No.1) itself falls between the two. 2,2′-Methylenebis(5,6-dichlorophenol) (No. 9), which was a much weaker bacteriostat than G-5® (No. 8) and G-11, is outstanding against both *S. aureus* and *E. coli* in the present series. It is also surprising that a compound with the methylene-linkage in the 4,4′ position, such as 4,4′-methylenebis(2,3,6-

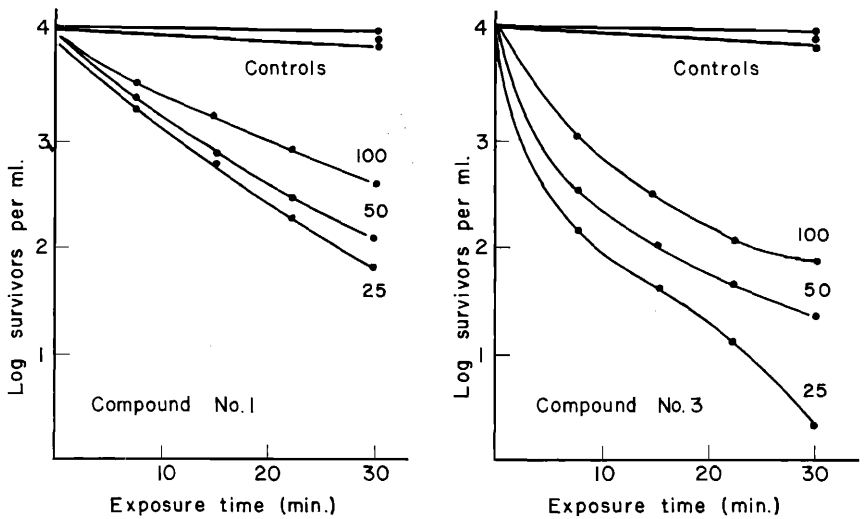

Figure 1.—Bactericidal activity of compounds 1 and 3 against *S. aureus* in 100, 50 and 25 μg./ml. Maxine soap solution at 40°C. Level of compounds is 1.0 μg./ml.

trichlorophenol) (No. 42), is as bactericidal against *S. aureus* as its isomer G-11; bacteriostatically, compound 42 was found to be much inferior to G-11.

In addition to compound 9, the most active compounds against *E. coli* are bis-phenols which have only one halogen substituent in each ring, such as Nos. 6, 15, 20, 30 and 31. It has been shown that the germicidal potency of such bis-phenols against gram negative organisms is greater than that of bis-phenols with four to eight chlorine atoms in the rings when tested by a phenol coefficient type of test (2).

We observed, as we did previously, that no compound without halogen in the phenol rings is active. The thio-linkage appears to lead to somewhat more active compounds than the methylene-bridge. Replacement of the phenols by naphthols results in loss of activity, with the exception of compound 39.

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Summary

A number of bis-phenols were evaluated for bactericidal activity in the presence of a non-lethal level of a synthetic anionic surfactant. Those compounds demonstrating 95 per cent and 99 per cent kill in twelve minutes at 20°C. are reported. Results of the bactericidal evaluation revealed both similarities and differences with the previously reported bacteriostatic properties of bis-phenols. Bactericidal action of several of the bis-phenols was observed to occur at surprisingly low levels against both *S. aureus* and *E. coli.*

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