Antibacterial Synergism Between the Halogenated Biphenols and the Halogenated Aromatic Anilides and Carbanilides

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Numerous antibacterial agents currently are being used in toilet soaps to degerm the human skin and by so doing, among other things, inhibit the development of objectionable perspiration odor. For the most part, these chemicals have been members of a series of halogenated biphenols of which hexachlorophene (2,2'-methylenebis(3,4,6-trichlorophenol)) (Gump, 1941) and bithionol (2,2'-thiobis(4,6-dichlorophenol)) (Shumard et al., 1953) are prominent examples. More recently however, other agents such as TCC (3,4,4'-trichlorocarbanilide) (Roman et al., 1957) and the halogenated salicylanilides, represented by Anobial (2-hydroxy-5-chlorobenzoic acid 3',4'-dichloroanilide) (Firmenich and Cie., 1954), have also been utilized as bacteriostats in various brands of toilet soaps. All of these are highly active in soap, inhibiting Staphylococcus aureus, a common skin organism, in concentrations as low as 0.2 to 0.5 ppm, \textit{in vitro}.

A variety of other antibacterial agents have at one time or another been proposed for use or used in soap. These, to mention a few, were TMTD (tetrathiomethylthiouramdisulfide) (Vinson, 1954), phenols (Hartung, 1941), mercury compounds (Schoeller and Schrath, 1910), sulfur (Hojka, 1938), sulfonamides (SICF, 1947) and even antibiotics (American Cyanamid Company, 1956). None of these has had a great deal of success owing to toxicity factors, disagreeable odors, or discolorations in the soap bar, although some may have exhibited antibacterial properties.

With the synthesis of the halogenated bisphenol, hexachlorophene, by Gump (1941) there became available an effective soap-active antibacterial agent. Traub et al. (1944), using the serial basin handwashing technique, established that a reduction of the bacterial flora of the skin of more than 90 per cent was obtained through the use of hexachlorophene soap. This finding has been widely confirmed by other investigators. The accomplishment of a low bacterial skin flora was a great aid to surgeons and to sanitary inspectors in the prevention of the transfer of pathogenic organisms through hand contact (Seastone, 1947; Udinsky, 1945).

In 1953, Gould and Bosniak, of the Massachusetts Institute of Technology, reported a potentiating action of hexachlorophene by the use of a relatively inactive unhalogenated bisphenol. These workers, using enzyme inhibition experiments measuring oxygen uptake as the criterion of biological activity, concluded that both hexachlorophene and the unhalogenated bisphenol competed for the same enzyme system of the bacterial cell. In combination with hexachlorophene, therefore, the bisphenol exerted a sparing action, leaving the more highly active hexachlorophene to act more effectively on other cell enzymes. This effect was described as greater than the combined additive effect of the individual agents, or a synergistic effect. Bacterial inhibition tests by the authors with combinations of hexachlorophene and the unhalogenated bisphenol did not establish synergistic activity between the two

<table>
<thead>
<tr>
<th>Bacteriostats</th>
<th>Media</th>
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<tbody>
<tr>
<td>A</td>
<td>2-Hydroxy-5-chlorobenzoic acid 3',4'-dichloroanilide (Anobial)*</td>
</tr>
<tr>
<td>AA</td>
<td>2-Hydroxy-5-chlorobenzoic acid 4'-chloroanilide</td>
</tr>
<tr>
<td>B</td>
<td>2,2'-Thiobis(4,6-dichlorophenol) (bithionol)</td>
</tr>
<tr>
<td>G-4†</td>
<td>2,2'-Methylenebis(4-chlorophenol) (dichlorophene)</td>
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<tr>
<td>G-5†</td>
<td>2,2'-Methylenebis(4,6-dichlorophenol) (tetra-chlorophene)</td>
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<tr>
<td>G-11†</td>
<td>2,2'-Methylenebis(3,4,6-trichlorophenol) (hexachlorophene)</td>
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<tr>
<td>G-11-S†</td>
<td>2,2'-Thiobis(3,4,6-trichlorophenol)</td>
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<tr>
<td>S-1‡</td>
<td>2,2'-Thiobis(4-chloro-6-methylphenol)</td>
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<tr>
<td>T§</td>
<td>3,4,4'-Trichlorocarbanilide</td>
</tr>
<tr>
<td>TA §</td>
<td>3,3',4'-Trichlorocarbanilide</td>
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</table>

1. Neutral white toilet soap base composed of 20 per cent sodium coconut oil soap and 80 per cent sodium tallow soap
2. Nonionic syndet Energetic W-100* (ethoxylated nonylphenol)
3. Anionic syndet (sodium dodecylbenzene sulfonate)

* Registered trademark of Firmenich and Company, New York, New York.
† Registered trademarks of the Sindar Corp., New York, New York.
‡ Registered trademark of Norda Inc., New York, New York.
§ Monsanto Chemical Co., St. Louis, Missouri.
¶ Registered trademark of Armour and Company, Chicago, Illinois.

1 Presented at the 59th General Meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 10 to 14, 1959.
agents in inhibiting growth of the bacterial cell by either in vitro or in vivo (handwashing tests) methods.

The concept of synergistic antibacterial activity between the halogenated bisphenols and highly active agents such as the antibiotics, however, has been demonstrated by several workers. Thus Florestano et al. (1956) claim potentiated activity for combinations of bithionol and tyrothricin. Craige and Kleckner (1949) have found synergism to exist for combinations of dichlorophene and tyrothricin. Synergism among the antibiotics themselves has been established by a number of investigators (Gunnison et al., 1955; Manten, 1956) and such potentiated activity is today universally utilized to achieve excellent therapeutic results. Synergistic biocidal properties for combinations of substituted carbanilide complexes have also been shown (Cuckler et al., 1955).

It is the purpose of this paper to describe the existence of synergistic antibacterial activity between the halogenated bisphenols and the halogenated aromatic anilides and carbanilides.

**Experimental Methods**

In pursuing this investigation, hexachlorophene and other halogenated bisphenols were combined with various chemicals of greater antibacterial activity than the unhalogenated bisphenols, and the additive, antagonistic, or synergistic properties of the combinations determined. After preliminary screening in the presence of soap, it was decided also to employ the combinations in both aqueous suspension and in other types of carrying agents, such as nonionic and anionic syndets. This would determine whether synergistic activity was demonstrated by the chemicals per se as in aqueous suspension, or whether the synergism was in some way potentiated by the vehicle in which the agents were carried. Some of the bacteriostats selected for testing, and the media in which they were employed, are given in table 1.

**Antibacterial test procedure.** The individual chemicals and 50:50 ratios of combinations of these chemicals were incorporated into soap, nonionic, and anionic detergents at a level of 1.0 per cent. With water as the carrier, suspensions of the agents were prepared at concentrations of 1 ppm.

The soap consisted of a neutral white toilet soap base made from 20 per cent sodium coconut oil soap and 80 per cent sodium tallow soap, as is typical for fine toilet bars. The nonionic syndet employed was an ethoxylated nonylphenol (Energetic W-100). Sodium dodecylbenzene sulfonate constituted the non-soap anionic syndet.

Aliquot amounts of both the individual bacteriostats and their combinations in the various media were thoroughly dispersed into measured amounts of liquid nutrient agar at concentrations of 0.1, 0.2, and 0.4 ppm. Previous screening of these agents had indicated that inhibition of growth of the test organism S. aureus strain FDA no. 209 occurred with the most active of these agents within this concentration range.

Plates then were poured, allowed to solidify, and streaked with a 24-hr broth culture of S. aureus. After incubation for 24 hr at 37 C, the amount of growth appearing was rated heavy, light, and none. The results represented graphically in figures 1 through 8 are the average result of three separate tests.

**Results and Discussion**

The term synergism as used in this paper requires that the combination of two chemicals must have a greater inhibitory effect on the test organism than either agent acting alone at the same total weight concentration.

3,4,4'-Trichlorocarbanilide (T) and 3,3',4-trichlorocarbanilide (TA) both show synergistic action when combined with hexachlorophene (G-11), the sulfur analog of hexachlorophene (G-11-S), tetrachlorophene (G-5), bithionol (B), and 2,2'-thiobis(4-chloro,6-methylphenol) (S-1) (figures 1 to 4).

2-Hydroxy-5-chlorobenzoic acid 3',4'-dichloroanilide (A) and 2-hydroxy-5-chlorobenzoic acid 4'-chloroanilide (AA) both cooperate synergistically with hexachlorophene (G-11), the sulfur analog of hexachlorophene (G-11-S), tetrachlorophene (G-5), and bithionol (B) (figures 5 to 8).

Synergism was not exhibited between either 3,4,4'-trichlorocarbanilide (T) or 3,3',4-trichlorocarbanilide (TA) with dichlorophene (G-4) (figures 1 to 4). Similarly, no synergism was found to exist for either 2-hydroxy-5-chlorobenzoic acid 3',4'-dichloroanilide (A) or 2-hydroxy-5-chlorobenzoic acid 4'-chloroanilide (AA) with dichlorophene (G-4) (figures 5 to 8), or with 2,2'-thiobis(4-chloro-6-methylphenol) (S-1) (figures 5 to 8). This indicates that synergism is a highly specific property, and that slight changes in molecular structure.

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* Figures 1 to 8. Inhibition of Staphylococcus aureus on agar plates. Figures 1 and 5, bacteriostats introduced in water suspension. Figures 2 and 6, bacteriostats introduced in soap suspension. Figures 3 and 7, bacteriostats in nonionic detergent solution. Figures 4 and 8, bacteriostats introduced in synthetic anionic detergent suspension.

* Code for figures 1 to 8: A = 2-Hydroxy-5-chlorobenzoic acid 3',4'-dichloroanilide. (Anobial*); AA = 2-Hydroxy-5-chlorobenzoic acid 4'-chloroanilide; B = 2,2'-Thiobis(4,6-dichlorophenol) (bithionol); G-4* = 2,2'-Methylenbis(4-chlorophenol) (dichlorophene); G-5* = 2,2'-Methylenbis(4,6-dichlorophenol) (tetrachlorophene); G-11* = 2,2'-Methylenbis(3,4,6-trichlorophenol) (hexachlorophene); G-11-S* = 2,2'-Thiobis(3,4,6-trichlorophenol); S-1* = 2,2'-Thiobis(4-chloro-6-methylphenol); T = 3,4,4'-Trichlorocarbanilide; TA = 3,3',4'-Trichlorocarbanilide; and UB = p,p'-Isopropylidenediphenol (unhalogenated bisphenol).

* Registered trademark (see table 1).
markedly alter this activity. The well known “lock and key” theory of molecular specificity might apply in describing this effect.

The agar streak method of analysis used in these tests is regarded as superior to the zone of inhibition methods generally employed for testing antibacterial activity in vitro. The zone method relies on migration of agent through the agar medium and resultant zones are thus dependent on the solubility of the chemical, as well as its antibacterial activity. Small differences in zone size as measured in millimeters cannot always be distinguished or effectively reproduced. The agar streak method, on the other hand, does not depend on migration since both agent and organism are in direct contact, assuming a uniform mixture. Differences in amount of growth on plates as a measure of the antibacterial potency of the agent are readily distinguished and can be conveniently rated as 1+, 2+, 3+, and so forth (light, moderate, and heavy) without counting actual bacterial colonies. This test has also been found to be highly reproducible.

Similar synergistic activity has been found, in vivo, for the combinations covered in this paper. This work is as yet unpublished.

SUMMARY

It has been demonstrated that some of the halogenated bisphenols show antibacterial synergy when combined with some halogenated aromatic anilides and carbanilides by tests in vitro. This synergism was found to exist for the chemicals, per se, and to be independent of the carrier used, whether water, soap, nonionic or anionic (other than soap) synthetic detergent. The most active synergy was found for the higher halogenated bisphenols, that is, the hexachloro- and tetrachlorocompounds, with the trichlorocarbanilides and the chlorinated salicylanilides.

REFERENCES


