TABLE III.—COMPOSITION OF OIL OF CARDAMOM

Peak No.	Constituent	Retention Time[a] (a)	(b)	%, w/w
1	α-Pinene	0.44	0.25	1.9
2	Sabinene	0.71	0.32	4.5
3	Myrcene	0.77	0.32	0.2
4	Limonene	1.00	0.41	14.3
5	Cineole	1.11	0.41	30.7
6	*p*-Cymene	...	0.68	1.9
7	Methyl heptenone	2.05	0.78	0.8
8	Linalool	...	1.00	0.9
9	Linalyl acetate	...	1.12	1.2
10	β-Terpineol	...	1.45	0.8
11	α-Terpineol	...	1.84	3.7
12	α-Terpinyl acetate	...	1.84	28.1
13	Borneol	...	1.95	0.1
14	Neryl acetate	...	1.95	0.3
15	Geraniol	...	2.38	0.7
16	Nerol	...	2.42	1.4
17	Nerolidol	...	5.04	0.3
18	Heptacosane	...	...	0.5
	Unidentified compds.	...	...	7.7

[a] Experimental conditions: (a) temperature, 110°C.; reference standard, limonene; (b) temperature, 170°C.; reference standard, linalool.

oil derived from the Ceylonese cardamom, *E. cardamomum* var. *β-major* Thwaites (17) also were absent in the oil investigated. Therefore, gas chromatographic examination can be applied conveniently to distinguish between the oil of cardamom derived from var. *minuscula* Burkhill and that derived from var. *β-major* Thwaites.

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Potentiation of the Antimicrobial Activity of Bithionol

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Lauricdiethanolamide potentiates the antimicrobial activity of bithionol. This is probably due to the increased solubility of the bithionol in the cellular fluids and to the decreased surface tension which permits a more rapid diffusion of the phenolic compound through the cell wall. This compound also is antagonistic to the phenylmercuric compounds. The antimicrobial activity of bithionol is potentiated by the presence of phenylmercuric ion. This potentiation does not change when the ratio of phenylmercuric ion is changed over a range of 0.1–0.5 parts to 1 part bithionol. Solutions of these compounds were found to be stable when stored at room temperature for 1 year.

THE EMERGENCE of antibiotic resistant microorganisms has brought about a renewed interest in some of the older antimicrobial agents (1–3). The phenolic compounds, the quaternary ammonium compounds and the mercurials have all been reevaluated by different groups in recent years.

The two compounds most worked with are bithionol [2,2′-thiobis(4,6-dichlorophenoxide)] which was first reported by Muth (4) in 1933 and hexachlorophene [2-2′-methylenebis(3,4,6-trichlorophenol)]. These compounds are bacteriostatic agents which are commonly employed in 2% concentrations in soaps and topical solutions (6). These agents, when used over a long period of time, gradually impart a residual activity to the treated area; however, since they are only bacteriostatic in action, they leave much to be desired in the search for a good anti-infective.

Received January 4, 1965, from the Research Division, S. E. Massengill Co., Bristol, Tenn.
Accepted for publication March 17, 1965.

The phenylmercuric compounds are bactericidal in nature, but they do not have any residual activity. Barker (5) reported that some cationic and some anionic agents such as surfactants tend to inactivate these compounds, and thus they cannot be combined with soaps or detergents which would also serve as good mechanical cleansers.

The quaternary ammonium compounds, such as benzalkonium chloride and cetylpyridinium chloride, are inactivated by anionic agents, by serum, and by some of the fats on the skin. These compounds are also incompatible with the phenolics and the phenylmercuric compounds.

This work was undertaken to find, if possible, a nonionic agent which would be compatible with bithionol and the phenylmercuric compounds and also to seek a combination of antimicrobial agents that would give a rapid killing effect and also have a residual activity.

TABLE I.—MINIMUM INHIBITORY CONCENTRATION (IN p.p.m.)

Test Organism	LDA	Bithionol	Phenylmercuric Acetate	Phenylmercuric Nitrate	Bithionol[a] + 2.5 Parts LDA	Phenylmercuric Acetate[b] + 2.5 Parts LDA	Bithionol[a] + 0.1 Part Phenylmercuric Nitrate	Bithionol[a] + 0.1 Part Phenylmercuric Acetate
C. albicans	>1000	100	0.8	1	10	10	3	2
S. aureus	>1000	8	3	2.5	4	30	2	0.8
E. coli	>1000	250	8	10	50	80	4	3
B. subtilis	>1000	10	0.8	1.3	0.8	30	2	2
S. faecalis	>1000	15	3	4	3	40	1	1
S. dysenteriae	>1000	250	10	10	250	100	10	12

[a] Expressed as p.p.m. bithionol. [b] Expressed as p.p.m. phenylmercuric acetate.

TABLE II.—MINIMUM INHIBITOR CONCENTRATIONS OF BITHIONOL (p.p.m.)

Test Organism	Bithionol	PMA[a]	Bithionol + 0.1 Part PMA	Bithionol + 0.2 Part PMA	Bithionol + 0.25 Part PMA	Bithionol + 0.5 Part PMA
C. albicans	100	0.8	2	2	2	2
S. aureus	8	3	0.8	0.8	0.8	0.8

[a] PMA, phenylmercuric acetate.

METHODS AND MATERIAL

The test organisms used in this experiment were *Staphylococcus aureus* (ATCC 9144), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8330), *Bacillus subtilis* (ATCC 6633), *Streptococcus faecalis* (ATCC 10541), and *Shigella dysenteriae* (ATCC 11835). The organisms were incubated in trypticase soy broth (B.B.L.) for 18 hr. at 37°. The cultures were then centrifuged and washed twice with sterile 0.85% saline and finally resuspended in sterile saline. The cultures then were diluted with sterile saline to give 75% light transmission at the 550 mμ wavelength on the Spectronic 20 colorimeter. In all cases exactly 0.1 ml. of this standardized culture was used for the inoculum, and fresh suspensions were prepared daily.

The compounds tested were lauricdiethanolamide, bithionol, phenylmercuric acetate, and phenylmercuric nitrate. The lauricdiethanolamide (LDA) was dissolved in water. The bithionol, phenylmercuric acetate, and phenylmercuric nitrate were dissolved in 25% ethyl alcohol water. All of these solutions were sterilized by filtration. These solutions then were aseptically diluted serially through sterile trypticase soy broth (pH 7.1) which was in 18 × 120 mm. metal cap culture tubes.

The tubes were then inoculated with 0.1 ml. of the test cultures and incubated at 37° for 24 hr. and observed for growth.

RESULTS

The data presented in Table I show that LDA potentiates the action of bithionol while exerting an antagonistic effect on the phenylmercuric salts. This antagonistic effect toward the mercury salts is not unexpected since other nonionics, as well as the cationic and anionic surfactants, precipitate the mercury in phenylmercuric salts. The unexpected activity observed was the potentiating effect of bithionol on the phenylmercuric salts. The results reported here indicate that the LDA potentiation of bithionol is unchanged over a range of 2.0 to 10 parts LDA to 1 part bithionol.

In Table I it can be seen that the addition of one-tenth part phenylmercuric acetate gives a 50-fold increase in the activity of bithionol against *C. albicans* and a tenfold increase in its activity against *S. aureus*. Bithionol is not highly effective against *E. coli* and *S. dysenteriae;* however, the addition of the phenylmercuric acetate or phenylmercuric nitrate increases this activity many times.

The results of this study on the effect of different ratios of bithionol–phenylmercuric acetate are recorded in Table II. It can be seen that by changing the ratio of phenylmercuric acetate from 0.1 part to 0.5 parts to 1 part bithionol, the minimum inhibitory concentration of bithionol is unchanged. These data indicate that there is a potentiation of the bithionol by the phenylmercuric ion, and the concentration is not important over a range of 0.1 to 0.5 parts to 1 part bithionol.

Aqueous solutions of lauricdiethanolamide (LDA) and bithionol which were stored at room temperature retained their activity after 1 year. These solutions were at pH 7.2. Samples stored at 40° for 6 months also retained their antimicrobial activity.

Bithionol and phenylmercuric acetate in ethanol were stable for 1 year at room temperature.

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