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The Action of Some Organo-Mercurials and Some Copper Salts on Ringworm Fungi*

By Richard H. Barry,† Walter H. Hartung and Thomas C. Grubb

Comparative studies have been made relating chemical structure to bactericidal activity, particularly in the phenol series (1, 2). In most cases, investigators have observed a close relationship between bactericidal and fungicidal activity.

Copper and its salts possess bactericidal action (3). The successful use of copper salts as fungicides in the control of plant fungi has prompted the study of these compounds as possible effective agents in the treatment of the dermatoses. Copper deposited on the skin by iontophoresis has been used with apparent success in the treatment of "athlete's foot." Copper sulfate is reported to have given good results in the treatment of skin Aspergilliosis (4). Copper and its salts have, however, proved themselves weak fungicides on the basis of laboratory evaluation against pathogenic fungi (5).

Certain aromatic organic acids, particularly salicylic and benzoic, are employed with favorable results in the treatment of mycoses of the hands and feet. Hence, it appeared logical to investigate the fungicidal properties of a combination of the metal with the acids, that is, copper salicylate and benzoate. The effect of copper salicylate, copper benzoate, salicylic acid and benzoic acid in inhibiting spore suspensions of two organisms commonly associated with fungous diseases of the skin is presented in Table I.

Table I.—Fungicidal-Static Activity of Two Organic Copper Salts

Compound	Highest Dilutions of Agents Effective in Inhibiting Growth in 10 Minutes	
	E. interdigitale	*A. gypseum*
Copper benzoate Suspension 1:20	1:40	..
Copper salicylate Suspension 1:100	1:300	..
Benzoic acid Suspension 1:50	1:400	..
Salicylic acid Suspension 1:50	1:700	..
Copper benzoate 1:50 in dioxane	1:300	1:450
Copper salicylate 1:50 in 50% dioxane	1:600	1:800
Benzoic acid 1:50 in dioxane	1:500	1:700
Salicylic acid 1:100 in 50% dioxane	1:1500	1:2500
Dioxane	No inhibition of growth with 15% dioxane	

In the field of organic mercurials there are also indications of a parallelism between bactericidal and fungicidal activity, although there are many exceptions. In the present investigation, the primary object was to compare the chlorophenylmercuric chlorides with the corresponding fluorophenyl derivatives and to observe the effect of position isomerism. In Table II are summarized the activities of six aromatic mercurials toward two strains of pathogenic fungi.

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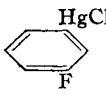
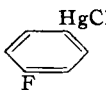
Table II.—Fungicidal-Static Activity of Aromatic Mercurials[a]

Compound 1:1000 Dilution in 75% Acetone	Highest Dilutions (× 10³) of Agents Effective in Inhibiting Growth in 10 Minutes *E. interdigitale*	*A. gypseum*
	15	20
	20	25
	30	35
	25	25
	35	40
 1:1000 in 20% acetone	20	25
$HgCl_2$ 1:1000 in water	15	15
Acetone	No inhibition of growth with 15% acetone	

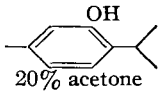
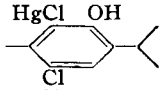
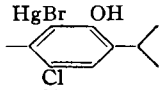
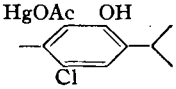
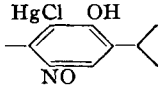
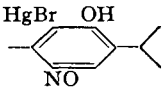
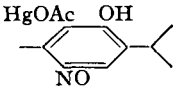
[a] Prepared by Dr. M. F. W. Dunker.

In Table III is summarized the fungicidal activity of three mercurated chlorothymols and nitrosothymols. The effect of replacement of halogen with nitroso group on the activity is shown.

Copper benzoate and copper salicylate were prepared by the reaction of copper sulfate with sodium benzoate and sodium salicylate, respectively. Copper benzoate, ($[C_6H_5COO]_2Cu.2H_2O$), is a blue powder, insoluble in water and alcohol, slightly soluble in acetone, soluble in dioxane. Copper salicylate, ($[C_6H_4OHCOO]_2Cu.4H_2O$), is slightly soluble in water and alcohol, soluble in dioxane. The mercurials tested are very slightly soluble in water, soluble in acetone.

The near insolubility of many organic metallic compounds in water necessitates the testing of stock dilutions of these compounds either as suspensions in water or oils or in solution in some organic solvent. Suspensions of the mercurials listed in Table II were employed by Dunker and Grubb (6) in testing antibacterial activity. It was found in the present investigation that acetone and dioxane, which were employed to prepare the stock dilutions of the compounds, did not exert fungicidal activity in concentrations below 15% in the time interval used (10 minutes). Dioxane has previously been used by investigators at Purdue as a solvent in testing bactericidal activity (7).

Table III.—Fungicidal-Static Activity of Mercurated Thymols[a]

Compound 1:1000 in Acetone	Highest Dilutions (× 10³) of Agents Effective in Inhibiting Growth in 10 Minutes *E. interdigitale*	*A. gypseum*
 20% acetone	3	4
 50% acetone	35	40
 50% acetone	30	45
 75% acetone	25	30
 75% acetone	30	30
 50% acetone	20	25
 75% acetone	15	15
Acetone	No inhibition of growth with 15% acetone	

[a] Prepared by Dr. A. W. Ruddy.

EXPERIMENTAL

Preparation of Stock Dilutions.—Suspensions of the copper salts were made in water. Five-tenths gram of tragacanth per 100 cc. of water was found sufficient to make a stable suspension of the copper

compounds. Stock solutions of the copper salts in dioxane and of the mercurials in acetone were made as indicated in the tables.

Determination of Activity.—The organisms used were *Epidermophyton interdigitale* and *Achorion gypseum*. These organisms are commonly associated with "athlete's foot." (We are indebted to Dr. Adelia McCrea and to Dr. George Reddish for these cultures.) The method of testing employed was essentially that of McCrea (8). The organisms were grown on Sabouraud's dextrose agar in Kolle flasks. After two weeks' incubation at room temperature (22–27° C.), spore suspensions were prepared. One-half cc. of spore suspension was inoculated into 2 cc. of test dilution and gently mixed by tapping the tube. At intervals of ten minutes, one loopful of the mixture was removed and subcultured on Sabouraud's agar slants, which were then incubated in the dark at room temperature for 10 to 14 days. The results are shown in the accompanying tables.

Discussion of Results.—The dilutions shown in the tables are to be considered as fungicidal-static, a term which is intended to mean that the compounds tested in the manner described, exert fungistatic as well as fungicidal activity. It is obvious that some of the compound tested is carried over to the agar slants when subcultures are made, thereby enabling it to exert fungistatic acitivity.

The difference in response to the copper salts of the two fungi tested is not significant. However, it can be seen that *E. interdigitale* was the more resistant. There is noted a difference in activity of the copper salts according to the manner in which the stock preparations were made. Suspensions of the salts were found not as active as were the same compounds dissolved in dioxane. Salicylic acid was found superior to benzoic acid as a fungicide, both in suspension and in solution. Similarly, copper salicylate is more fungicidal than copper benzoate. But both copper salts are inferior to their corresponding acids as fungicides *in vitro*.

The introduction of halogen or hydroxyl on the ring in phenylmercuric halides increases the fungicidal-static activity. The *p*-chloro and *p*-fluoro substituted compounds were found more active than the *o*-chloro and *m*-fluoro substituted isomers, respectively. *p*-Fluorophenyl mercuric chloride was the most active of the series tested. These results parallel the anti-bacterial activity observed with these compounds (6). The fungicidal activity observed with *p*-hydroxyphenylmercuric chloride and mercuric chloride agrees with earlier reports on these compounds (9, 10).

Of the mercurated thymols tested, chlorothymolmercuric chloride was found to be superior to the bromide or acetate. Substitution of a nitroso group for Cl lowers the activity of the thymol. In this respect the nitroso group (NO) is probably similar to the nitro group (NO_2) (2, 11). Chlorothymolmercuric chloride was found to be the most active of all the mercurials tested. *E. interdigitale* was more resistant to the mercurials than *A. gypseum*.

CONCLUSIONS

1. Copper salicylate and copper benzoate were prepared and a study of the fungicidal-static activity against two dermatophytes shows them to be inferior to salicylic and benzoic acids as fungicides.

2. Introduction of halogen or hydroxyl into phenylmercuric chloride increases fungicidal-static activity. Halogen substitution increases the activity in the order ortho, meta, para. *p*-Fluorophenylmercuric chloride was found more active than *p*-chlorophenylmercuric chloride. Anti-bacterial and fungicidal activity were observed to be parallel in the case of the *p*-fluoro and *p*-chloro mercuric halides.

3. The mercurated chlorothymols are good fungicides. The order of increasing activity in this series is acetate, bromide, chloride. Substitution of a nitroso group for halogen lowers the activity of the thymol.

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