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45. Kiichiro Tanaka, Issei Iwai, Yasuo Yura, and Kazuo Tomita : Studies on Acetylenic Compounds. XII.*1 Antifungal Activity of Some Acetylenic Mercury Compounds.

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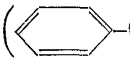
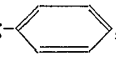
In the preceding paper,[1] some synthetic acetylenic compounds related to capillin (-CO-C≡C-C≡C-CH_3) were shown to have high antifungal activity. Subsequently, Iwai, *et al.*[2] prepared some acetylenic mercury compounds having the following general formulae :

R-C≡C-Hg-C≡C-R (Series A) R-C≡C-Hg-, R-C≡C-Hg-Cl (Series B)

The present paper deals with some results of investigations on the antimicrobial activity of these compounds.

The data given in Tables II and III, which show antimicrobial spectra of these compounds, illustrate the fact that in both series all compounds containing halogen in their structures are inactive but that most of the other compounds are as highly active as phenylmercury acetate, the most common mercurial used as agricultural chemicals.

Up to date, many organomercury compounds have been studied regarding their antimicrobial activity, some of which are used in practice as disinfectant in medicine and agriculture. Most of them have the general formula R-Hg-X containing the negatively charged residue X. On the other hand, alkylated mercury has not been used, because of toxicity due to high vapor pressure.[3]

From the data obtained in the experiments (Table IV), in which the antifungal activity of the compounds in vapor state was compared with that of phenylmercury acetate and ethylmercury chloride, it is clear that the vapor action of the compounds in series A and probably those in series B is relatively lower than that of phenylmercury acetate and ethylmercury chloride, suggesting that the toxicity of the former would be far less than that of alkylmercury.

Further, the data show all active compounds tested are effective against both fungi and bacteria with the exception of $(C_4H_9-C\equiv C-)_2Hg$ which is inactive against *Candida albicans* and *Escherichia coli*.

On the other hand, it has been already shown that some acetylenic compounds not containing mercury*3 are effective only against fungi and not against bacteria.[1] These observations suggest that the mercury in the structure of compounds in series A and B would be more essential for their activity than the acetylenic linkage.

Table I. Culture Conditions for Test Microörganisms

Strain	Medium	Temp. (°C)	Incubation period (day)
Trichophyton asteroides	Sabouraud's glucose-agar	27	5
Piricularia oryzae	Potato-sucrose-agar	27	5
Candida albicans	Sabouraud's glucose-agar	30	2
Escherichia coli	Bouillon-agar	37	1
Bacillus subtilis	〃	37	1

*1 Part XI : Y. Okajima : Yakugaku Zasshi, **80**, 322(1960).

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*3 Compounds represented by the general formula R_1-CO-$(C\equiv C)_n$-R_2 (n=1 or 2) in which R_1 is phenyl or naphthyl and R_2 is hydrogen, methyl, or ethyl group.

1) K. Tanaka, I. Iwai, Y. Okajima, T. Konotsune : Antibiotics & Chemotherapy, **9**, 151(1959).
2) I. Iwai, Y. Yura : Ann. Rept. Takamine Lab., **10**, 30(1958).
3) W. H. Hill : Can. J. Public Health, **34**, 158(1943).

Table II. Antimicrobial Activity of Series A Compounds

General formula $(R\text{-}C{\equiv}C)_2Hg$

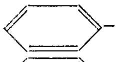
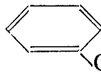
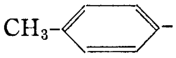
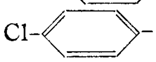
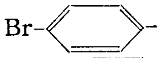
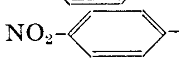
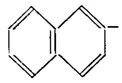
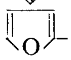
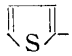
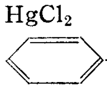
R	Min. concn. required for complete inhibition of growth (γ/cc.)				
	T. asteroides	*P. oryzae*	*C. albicans*	*E. coli*	*B. subtilis*
C_4H_9-	0.8	0.8	>50	>50	0.8
	0.4	0.2	0.8	3	3
-CO-	0.8	0.8	0.8	1.6	0.8
-CH(OH)-	6	6	>50	3	3
-CO- OH	>50	—	>50	—	—
	50	>50	>50	>50	>50
	>50	>50	>50	>50	>50
	>50	>50	>50	>50	>50
	>25	>25	>13	—	—
	>50	>50	>50	>50	>50
	0.2	0.8	0.4	1.6	1.6
	0.2	0.8	0.4	3	3
	0.8	0.8	1.6	0.8	1.6
$HgCl_2$	6	25	13	1.6	1.6
-Hg-OAc	0.8	0.4	0.4	1.6	0.8

Table III. Antimicrobial Activity of Series B Compounds

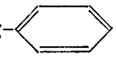
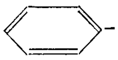
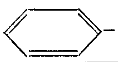
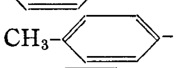
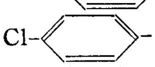
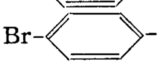
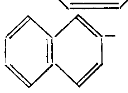
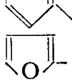
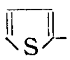
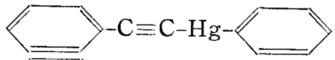
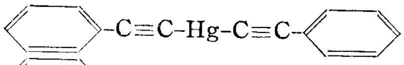
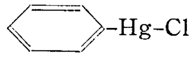
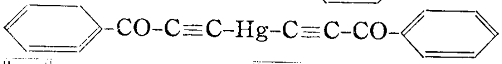
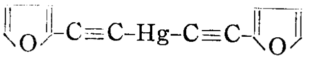
R	Min. concn. required for complete inhibition of growth (γ/cc.)				
	T. asteroides	*P. oryzae*	*C. albicans*	*E. coli*	*B. subtilis*
R-C≡C-Hg-					
C_4H_9-	0.8	0.4	1.6	1.6	0.8
	0.8	0.4	0.8	1.6	0.8
-CO-	0.8	0.8	0.8	1.6	0.8
-CH(OH)-	0.8	0.8	0.8	3	1.6
R-C≡C-HgCl					
	50	>25	>25	13	13
	25	>50	>50	13	13
	>50	>50	>50	50	50
	>50	>50	>50	>50	>50
	25	>50	>50	25	13
	>50	>50	>50	>50	>50
	50	>50	>50	13	13

Table IV. Antifungal Activity of Some Compounds in Vapor State

Compound	$H(\%)$	Compound	$H(\%)$
C_4H_9-C≡C-Hg-C≡C-C_4H_9	33		78
	2		97
	60	C_2H_5-Hg-Cl	119
	10		

Test organism : *Cochliobolus miyabeanus* H : Degree of inhibition

Experimental

Antimicrobial Spectra of the Compounds in Series A and B—The tests were performed by the agar-streak dilution technique using *Trichophyton asteroides*, *Piricularia oryzae*, *Candida albicans*, *Escherichia coli*, and *Bacillus subtilis*, as test microörganisms.

Each compound was dissolved in Me_2CO or pyridine in adequate concentration. In all cases, the final concentration of Me_2CO and pyridine was less than 0.5 and 0.25%, respectively.

The culture conditions for the test microörganisms are shown in Table I. The antimicrobial activity of each compound is expressed by the minimum concentration required for complete inhibition of the test organism after the incubation period.

Antifungal Activity of Some Compounds in Vapor State—A small piece of mycelium of *Cochliobolus miyabeanus* was inoculated on potato-sucrose-agar plate, using a ground-glass covered petri dish, 10 cm. in diameter and 3 cm. in height. After 2 days of subculture, each dish was inverted to turn over the agar plate, as illustrated in Fig. 1.

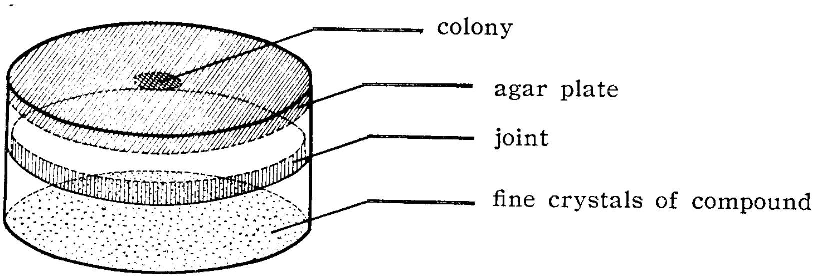


Fig. 1. Petri Dish used for the Test of Antifungal Activity of Compounds in Vapor State

Fine crystals (100 mg.) of each compound to be tested were scattered on the bottom of the dish and sealed. Each petri dish was incubated for further 24 hr. at 30°. The degree of inhibition of each compound in vapor state, H, was calculated from the following equation :

$$H(\%) = 1 - \frac{D^t - D^0}{D^t_c - D^0_c} \times 100$$

where D^0 and D^t are diameters of colony before and after incubation, respectively, and D^0_c and D^t_c are those of the control.

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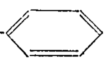
Summary

Antimicrobial activity of two series of acetylenic mercury compounds was tested, using three kinds of fungi and two kinds of bacteria as test organisms. In both series, the compounds represented by the general formulae $(R-C{\equiv}C-)_2Hg$ and R-C≡C-Hg- had high activity against both fungi and bacteria. Their antifungal activity in vapor state was also examined.

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