CCA-363

547.254.9

Original Scientific Paper

Antifungal Activity and Inhibitory Effect on Urease of Some Organo-Mercuric Compounds

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Received February 11, 1965

A number of aliphatic and aromatic organo-mercuric compounds of the general formula R—Hg—X, in which R represents an organic radical and X an anionic radical, were prepared. The antifungal and inhibitory effect of the tested compounds was compared by applying the auxanographic diffusion method and by measuring the degree of inhibition of urease. It was found that the antifungal activity depends mainly on the organic radical R and that the anionic radical has no effect on fungitoxicity.

INTRODUCTION

It is known that compounds of the general formula R—Hg—X, in which R represents an organic radical and X an anionic radical of an inorganic or organic acid, exhibit a fungitoxic and/or fungistatic activity. Although recently fungicides with a far better therapeutic index have been discovered, organo-mercuric compounds nevertheless are still extensively used as fungicides in the treatment of seeds of cereals and industrial plants.

We synthesized a number of aliphatic and aromatic organo-mercuric compounds with different organic radicals and anionic groups; these compounds are presented in Table I. Our aim was to compare the antifungal and inhibitory effect of mercurials and to verify the conclusions of G. Gassner[1] that fungicidal activity depends only on the organic radical R and that the anionic radical has no effect on fungitoxicity. Therefore we chose two approaches to the testing of the organo mercuric compounds. The auxanographic diffusion method[2] proved useful for comparing the relative activities of these compounds. In order to compare biological activity the inhibition of urease was tested. Urease was chosen as a representative of enzymes which contain sulfhydryl groups.

MATERIALS AND METHODS

Synthesis of organo-mercuric compounds

The synthesis of the phenyl mercuric salts started with phenyl mercuric acetate[3] or phenyl mercuric hydroxide[4] which was subjected to reaction with a corresponding acid or its alkali metal salt in aqueous or alcoholic solution. Methoxy and cetoxy ethyl mercuric acetates were prepared from mercuric oxide and the corresponding alcohol in acetic acid by introducing a stream of ethylene[5]. Ethyl mercuric chloride was synthesized by way of the Grignard compound[6]. *Bis*-(phenyl mercuric) cyanoguanidine was prepared from phenyl mercuric acetate and the

potassium salt of cyanoguanidine in alcoholic solution and *bis*-(ethyl mercuric) cyanoguanidine was prepared in the same way, starting from ethyl mercuric chloride and the potassium salt of cyanoguanidine[7]. All tested compounds were purified to analytical grade.

Estimation of fungitoxicity

The experiments were carried out with *Aspergillus niger*. Experimental conditions were described earlier[8]. For purpose of testing, clear solutions of the corresponding organo-mercuric compound were prepared in concentrations of 10^{-3} *M* to 10^{-5} *M*. In the case of water insoluble compounds (cetoxy ethyl mercuric acetate, phenyl mercuric salicylate) water suspensions were prepared with the aid of an non-ionic emulgator. By a separate experiment we made sure that the emulgator has no influence on the fungitoxicity. The auxanographic diffusion method was carried out in a Petri-dish of 10 cm diameter, in a monolayer of agar with a hole in the middle of the nutritive medium, in which a fungicidal compound has been introduced.* The width of the zone in which no visible growth occurred was measured. Estimation of the average diameter of the inhibition zone was made by measuring in five different directions. Each experiment was repeated three times for every concentration. The results are presented in Fig. 1.

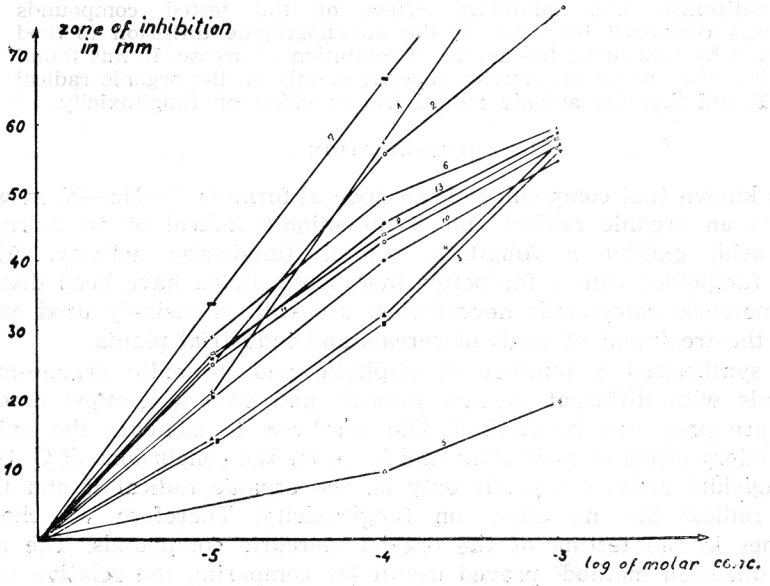

Fig. 1. Inhibitory effect of mercurials on growth of *Aspergillus niger*. Numbers of curves correspond to numbers of compounds in Table I.

Inhibition of urease activity

The urease was isolated from soybean flour (*Soia hispida*) by using Sumner's method[9]. The flour was obtained from sprouting soybean under conditions considered optimal. Estimation of urease activity was performed in *M*/50 phosphate buffer (*p*H 7.0) as described by Sumner[10]. Conway's microdiffusion method was used for the determination of ammonia formed from urea used as a substrate[11]. Nitrogen was determined in the crude urease preparation by Kjeldahl's method. The reaction system for the assay of enzymatic activity contained 1 ml of urease (0.0523 units/ml),

* We assumed that the rate of diffusion of water-soluble organo-mercuric compounds is of the same order of magnitude, because we applied the same molar concentrations of tested compounds which have similar chemical structure.

TABLE I

No	Compound	Formula
1.	Methyl mercuric bromide	CH_3HgBr
2.	Ethyl mercuric chloride	CH_3CH_2HgCl
3.	Metoxy ethyl mercuric acetate	$CH_3OCH_2CH_2HgOAc$
4.	Metoxy ethyl mercuric chloride	$CH_3OCH_2CH_2HgCl$
5.	Cetoxy ethyl mercuric acetate	$C_{16}H_{33}OCH_2CH_2HgOAc$
6.	*Bis*-(Ethyl mercuric) cyanoguanidine	$(C_2H_5Hg)_2—C_2N_4H_2$
7.	*Bis*-(Phenyl mercuric) cyanoguanidine	$(C_6H_5Hg)_2—C_2N_4H_2$
8.	Phenyl mercuric acetate	C_6H_5HgOAc
9.	Phenyl mercuric benzoate	$C_6H_5HgOCOC_6H_5$
10.	Phenyl mercuric chloride	C_6H_5HgCl
11.	Phenyl mercuric crotonate	$C_6H_5HgC_4H_5O_2$
12.	Phenyl mercuric hydroxyde	C_6H_6HgOH
13.	Phenyl mercuric lactate	$C_6H_5HgOCOC_2H_5O$
14.	Phenyl mercuric salicylate	$C_6H_5HgOCOC_6H_5O$
15.	*Bis*-(Phenyl mercuric) adipate	$(C_6H_5Hg)_2C_6H_8O_4$
16.	*Bis*-(Phenyl mercuric) fumarate	$(C_6H_5Hg)_2C_4H_2O_4$
17.	*Bis*-(Phenyl mercuric) maleinate	$(C_6H_5Hg)_2C_4H_2O_4$

1 ml of *M*/50 phosphate buffer (pH 7.0) and 1 ml 3% urea. The mixture was incubated 30 min. at 25°C in absence or presence of graded concentrations of each mercurial, then the activity of the enzyme solution thus treated was estimated. In each enzymatic experiment, the effect of the mercurials on the activity was expressed in terms of per cent inhibition (H) calculated as follows:

$$H = (1 — A/A_0) \cdot 100 \text{ (per cent)}$$

in which A and A_0 represent relative activities of enzyme solutions with and without addition of a mercurial, respectively[12]. Results are presented in Fig. 2.

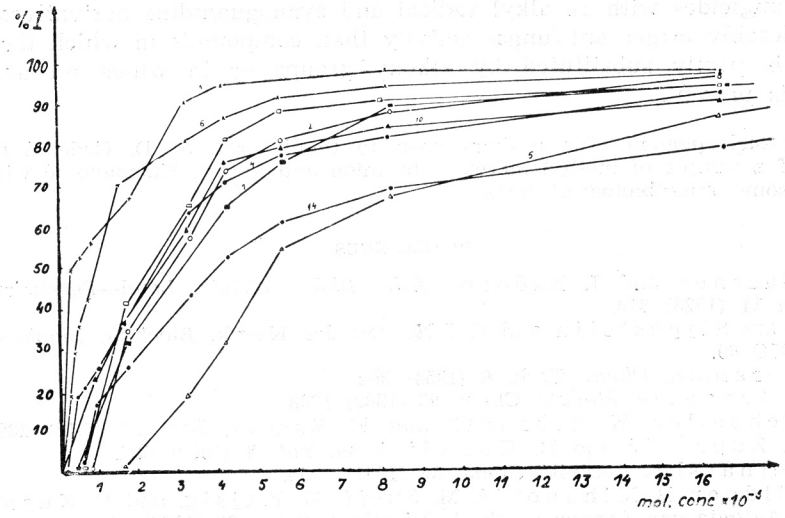

Fig. 2. Inhibitory effect of mercurials on activity of urease
Numbers of curves correspond to numbers of compounds in Table I.

RESULTS AND DISCUSSION

As can be seen from Fig. 1, the strongest fungicidal effect can be attributed to *bis*-(phenyl mercuric)-cyanoguanidine(7) and methyl mercuric bromide(1) followed immediately by ethyl mercuric chloride(2). The weakest action is that of cetoxy-ethyl mercuric acetate(5). The remaining compounds, phenyl mercuric acetate(8), salicylate(14), benzoate(9), lactate(13) and hydroxide(12) had a nearly identical effect, as presented on the graph. All other tested phenyl mercuric salts [*e.g.* fumarate(16), maleinate(17), crotonate(11) and adipate(15)] fall into the same range; their curves are identical with those of the above mentioned phenyl mercuric salts, and therefore they are not presented on the graph. It is interesting to see that methoxy ethyl mercuric acetate and chloride have nearly the same activity.

As can be seen from Fig. 2, methyl mercuric bromide has the greatest slope and exhibits the strongest inhibitive action. Cetoxy ethyl mercuric acetate has the smallest inhibition, which is in perfect accordance with the results obtained by the auxanographic method. Next accordance to the effect is bis-(ethyl mercuric) cyanoguanidine. The rest show an equal inhibitory effect. Phenyl mercuric salicylate is in a way an exception; because of its insolubility in water, suspensions with emulgator should have been prepared.

The results of our experiments lead to the following conclusions:

1. If we compare phenyl mercuric compounds having different anionic radicals *i.e.* phenyl-mercuric acetate, chloride, benzoate, salicylate etc., we see that in both tests they show an equal effect and that the slopes of the inhibition curves very often correspond. Accordingly, Gassner's statements that the anionic radical has no effect on fungitoxicity is verified.

2. Methoxy ethyl mercuric acetate and chloride have the same activity, which is a further proof of the conclusion that the anionic radical has no influence on fungitoxicity.

3. Fungicides with an alkyl radical and cyanoguanidine derivatives show a considerably larger antifungal activity than compounds in which the alkyl radical is partly substituted by alkoxy groups or in which an aromatic radical is present.

Acknowledgement. The authors wish to thank prof. dr D. Grdenić for the supply of a sample of methyl mercuric bromide and Mr. V. Kuzmanović who performed some microbiological tests.

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IZVOD

Antifungalna aktivnost i inhibitorsko djelovanje na ureazu nekih organo-živinih spojeva

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Sintelizirani su brojni alifatski i aromatski organoživini spojevi opće formule R—Hg—X, gdje je R organski radikal, a X anionski ostatak neke kiseline. Uspoređivano je antifungalno i inhibitorno djelovanje ovih spojeva primjenom auksanografske difuzione metode i mjerenjem stupnja inhibicije enzima ureaze. Ustanovljeno je da stupanj fungitoksičnosti u velikoj mjeri ovisi o organskom radikalu R, dok anionski ostatak nema utjecaj na aktivnost spoja.

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Primljeno 11. veljače 1965.