Antifungal Activity of Thiosemicarbazones

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The antifungal activity of several substituted thiosemicarbazones was studied, and a direct relation was found between antifungal activity and reaction of the thiosemicarbazones with copper ions. N^2-Methyl derivatives do not form complexes with copper ions and have little antifungal activity, while the other derivatives for which complex formation is possible have an activity similar to that of the unsubstituted thiosemicarbazones. 9-Undecenal thiosemicarbazone was found to be active against *Aspergillus niger* in a metal-deficient medium, but its effect can be neutralized by the addition of equivalent amounts of copper acetate.

IT HAS BEEN suggested that the antimicrobial activity of thiosemicarbazones is due to their ability to form complexes with copper ions (1, 2), and consequently, the poor activity of the oxygen analogs (semicarbazones) has been attributed to their lesser ability to form such complexes (3). By the same reasoning and in view of the relationship oxygen–sulfur–selenium, compounds of the latter would be expected to be more effective than the first two against organisms and, in fact, selenoureas were found to be 10 to 100 times more active than thioureas, and selenosemicarbazones to have sufficient antimicrobial properties to warrant further research (3). The authors will report on the activity of selenosemicarbazones in a future publication.

The antifungal activity of a large number of thiosemicarbazones and their copper complexes has been described earlier (4). Two fungi were used in that work, and it has been found that the copper complexes were generally inactive while the free thiosemicarbazones were quite active, many at 100 p.p.m. and one, 9-undecenal thiosemicarbazone, at 10 p.p.m. The antimicrobial properties of a series of thiosemicarbazones from saturated and unsaturated aliphatic aldehydes were reported by Manowitz and Walter (5), who found the highest activity amongst the unsaturated compounds with chain lengths C_{10}–C_{12}. More recently, these authors reported on the activity of methyl *n*-alkyl thiosemicarbazones (6), maximum activity being reached with the 2-dodecanone derivative.

The authors have prepared various mono and dimethyl thiosemicarbazones in order to study their reaction with copper (7), and the relation between complex formation and antifungal activity. The latter is the subject of this paper. In another part of the present work, the effect of adding trace amounts of metals to a metal-deficient medium inoculated with *Aspergillus niger* and containing various concentrations of a thiosemicarbazone was studied.

EXPERIMENTAL

Materials and Methods.—Thiosemicarbazone derivatives were prepared in this laboratory and are described elsewhere (7). The test organisms used were *Chaetomium globosum*, strain USDA 1042.4, and *A. niger*, strain USDA 215-5373.16. Tests were carried out by the tube dilution method described earlier (4). Results are summarized in Table I.

The effect of adding trace amounts of metals to an inoculated metal-deficient medium was studied in the presence of 9-undecenal thiosemicarbazone. The medium was prepared as described by Anderson and Swaby (8). The method consisted of adding various concentrations of the thiosemicarbazone and copper acetate to the inoculated medium and, after a 7-day incubation period at 30°, the amount of growth was determined by weighing the mycelial mat. All experiments were done in triplicate, and the concentrations used as well as the average weights of the mycelium obtained are recorded in Table II.

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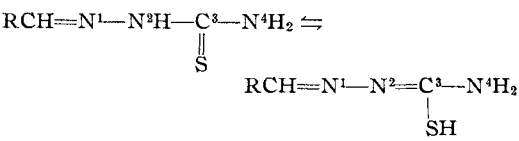
RESULTS AND DISCUSSION

During the course of our investigation on the structure of thiosemicarbazone–copper complexes (7), it has been found that the presence of the N^2 hydrogen atom was essential for the formation of a complex. Indeed, replacement of this hydrogen atom by a methyl group resulted in complete inability to form complexes. This can be explained by a consideration of the following tautomeric equilibrium which takes place upon dissolution of a thiosemicarbazone.



In the cases of the N^2 methyl derivatives, this equilibrium does not take place. That the N^2 hydrogen atom was involved in the tautomerism, instead of one of the hydrogen atoms at the 4 position, was also demonstrated by the fact that replacement of both N^4 hydrogen atoms by methyl groups resulted in derivatives that had retained their ability to form complexes with copper.

It can be seen from the data in Table I that the derivatives which do not form complexes (N^2-methyl derivatives) are relatively inactive, or at least that their activity has been reduced considerably. Biological tests were not carried out at concentrations above 1000 p.p.m. The activities of derivatives which were still capable of forming complexes were found to be comparable to that of the original compounds. These findings suggest that the antifungal activity of thiosemicarbazones is closely related to their ability to form complexes.

It should be pointed out that the copper complexes of thiosemicarbazones were found to be inactive (4), suggesting that the mode of action of these compounds was different from that of dithizone and 8-hydroxyquinoline. It was found by Anderson and Swaby (8) that these two compounds were not active in a copper-deficient medium, and Albert (9) found that these compounds became fungistatic when copper was supplied.

TABLE I.—RELATION BETWEEN POSSIBILITY OF COMPLEX FORMATION AND ANTIFUNGAL ACTIVITY OF METHYL THIOSEMICARBAZONES

Methyl Thiosemicarbazones	Complex Formation	Antifungal[a] Activity, p.p.m.	Antifungal Activity[a] of Original Thiosemicarbazone, p.p.m.
Heptanal-2-methyl	No	1000	100
Octanal-2-methyl	No	1000	100
Octanal-4-methyl	Yes	10	100
Nonanal-2-methyl	No	Inactive	100
Decanal-2-methyl	No	Inactive	100
Undecanal-2-methyl	No	Inactive	100
9-Undecenal-2-methyl	No	Inactive	10
Benzaldehyde-2-methyl	No	Inactive	100
Benzaldehyde-4-methyl	Yes	100	100
Benzaldehyde-2,4-dimethyl	No	Inactive	100
Benzaldehyde-4,4-dimethyl	Yes	100	100
p-Chlorobenzaldehyde-2,4-dimethyl	No	Inactive	100
p-Chlorobenzaldehyde-4,4-dimethyl	Yes	100	100
Acetone-*S*-benzyl	No	Inactive	1000

[a] Antifungal activity against *A. niger* and *C. globosum* was the same.

TABLE II.—EFFECT OF COPPER ACETATE ON ACTIVITY OF 9-UNDECENAL THIOSEMICARBAZONE

Concn. of 9-Undecenal Thiosemicarbazone, p.p.m.	Concn. of Copper Acetate Added, p.p.m.	Wt. of Mycelium of *A. niger* after 7-Day Incubation, mg.
0	0	19
0	10	17
5	0	5
10	0	0
10	10	17
100	0	0
100	10	0
100	100	11
1000	0	0
1000	1000	19

The effect of the presence of metals on the antifungal activity of thiosemicarbazones is demonstrated further by the results shown in Table II.

It can readily be seen from Table II that the thiosemicarbazone prevented the growth of the organism, at concentrations of 10, 100, and 1000 p.p.m. in a metal-deficient medium. Additions of copper acetate in equivalent amounts appeared to neutralize the action of the thiosemicarbazone, and normal growth of the organism was obtained. Similar experiments using iron, manganese, and zinc showed these metals to have no effect. It had also been noticed (7), during the course of our work on the thiosemicarbazone–copper complexes, that thiosemicarbazones exhibit a definite selectivity toward copper.

The assumption that the activity of thiosemicarbazones is closely related to their ability to form complexes with metals is further supported by results obtained on other types of derivatives of thiosemicarbazones, *viz.*, the condensation products of thiosemicarbazones and cyanuric chloride described in an earlier publication (10). A number of cyanuric chloride derivatives have been described, in which addition of the cyanuric chloride molecule enhances the antifungal activity of the original compounds (11–14), and it was expected that the products obtained with thiosemicarbazones would possess a greater activity than either starting material. However, it was found that the condensation products had no activity at a concentration of 1000 p.p.m., and this was related to the fact that addition of cyanuric chloride took place on the N^2 atom, as was shown by chemical means and infrared spectroscopy (10).

SUMMARY AND CONCLUSION

The antifungal action of *N*-methyl thiosemicarbazones was studied and a relation was found between ability to form complexes with copper and antifungal activity. N^2-methyl derivatives do not form complexes with copper and are relatively inactive, while the other methyl derivatives had an activity similar to that of the unmethylated thiosemicarbazones.

9-Undecenal thiosemicarbazone prevents the growth of *A. niger* in a metal-deficient medium, but addition of copper acetate in equimolecular amounts neutralizes the effect of the thiosemicarbazone, and normal growth of the organism can be observed.

These findings show a definite correlation between antifungal activity and copper complex formation. It is realized that other factors are also involved since the activity is restricted, to a certain degree, to the thiosemicarbazones made from aliphatic aldehydes with a chain length of C_7 to C_{11}, and particularly, from unsaturated aldehydes; this likely has to do with lipophilic properties and permeation through the cell wall.

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