THE ANTI-FUNGAL ACTIVITY OF METAL DERIVATIVES OF 3-PYRIDINETHIOL*

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The role which sulfur and sulfur containing compounds, as well as combinations of these with metals, have played as antifungal agents has been masterfully described by Horsfall (1). The corroboration of his findings on plant pathogens by experiments with these compounds against dermatophytes (2) encouraged further studies on the effect of metal derivatives of sulfur containing compounds on the dermatophytes.

A chemically well defined compound containing zinc and sulfur, ortho-aminobenzene-thiol zinc salt, was shown by Ladd (3) to be highly active against Sclerotinia fructicola. The identical salt plus other metal derivatives were synthesized by Dr. N. Steiger of the Roche Chemical Laboratories (4). Appreciable activity against the dermatophytes was generally observed with the zinc derivative being of particularly marked activity. Although these compounds were non-irritating for the skin and conjunctiva of animals, preliminary studies on the skin of humans showed that 30% of the volunteers responded with blistering eruptions to repeated treatment with a 5% ointment.†

The satisfactory activity of the metal derivatives of aromatic thiol compounds did, however, encourage investigations in this field to find compounds of high anti-fungal activity without skin irritating properties. Through the work of Dr. N. Steiger a new compound, 3-pyridinethiol became available. This compound and its metal derivatives were examined for anti-fungal activity and toxicological effect which included irritation tests in animals and humans. The results of this work are presented in this paper.

MATERIALS AND METHODS

In the in vitro anti-fungal and anti-bacterial experiments the metal derivatives of 3-pyridinethiol which were insoluble were tested as suspensions in the presence of appropriate inert emulsifiers. The soluble hydrochlorides of the free mercaptan and the disulfide of 3-pyridinethiol previously described by British investigators (5) as well as the sodium salt were tested as solutions in water.

The fungistatic activity was determined by the plate test. This consisted of incorporating proper concentrations of the drug into Sabouraud's glucose agar gel and seeding the center of the plate with a small piece of agar mat, mycelial surface down. The plates were incubated at room temperature and a final reading was taken at the end of 21 days.

Among the dermatophytes tested were two strains of T. mentagrophytes and two strains of M. lanosum. Two plant pathogens, Fusarium oxysporum and Botrytis paeoniae were also tested.

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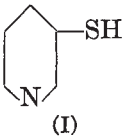
The bacteriostatic spectrum, which was also determined by the plate test, included representative gram positive and gram negative organisms.

EXPERIMENTAL

The chemical constitution of 3-pyridinethiol (I) and its metal derivatives which were used in these experiments, including the previously described disulfide, is given below. They are all derivatives of 3-pyridinethiol:



(I)

Nu-1485/1 :$C_5H_5NS \cdot HCl$-3-pyridinethiol hydrochloride.
Nu-1485/10:C_5H_4NSNa—sodium salt of 3-pyridinethiol.
Nu-1678 :$C_{10}H_8N_2S_2 \cdot 2\ HCl$—di(3-pyridyl)disulfide dihydrochloride.
Nu-1485/6 :$C_{10}H_8N_2S_2Cu$—copper derivative of 3-pyridinethiol.
Nu-1485/8 :$C_{10}H_8N_2S_2Fe$—iron derivative of 3-pyridinethiol.
Nu-1485/7 :$C_{10}H_8N_2S_2Mn$—manganese derivative of 3-pyridinethiol.
Nu-1485/5 :$C_{10}H_8N_2S_2Hg$—mercury derivative of 3-pyridinethiol.
Nu-1485 :$C_{10}H_8N_2S_2Zn$—zinc derivation of 3-pyridinethiol.
Nu-1485/4 :$C_{15}H_{12}N_3S_3Bi$—bismuth derivative of 3-pyridinethiol.

ACTIVITY TOWARDS DERMATOPHYTES

The results of the experiments with the different strains of dermatophytes are combined in Table 1 since there was no essential difference between the strains. This table which is based on the results of the agar plate method shows that the strains of T. mentagrophytes and the strains of M. lanosum had the same relative range of sensitivity although the different metal derivatives were effective on a different level of activity. Three types of activity were encountered: the sodium, copper and bismuth derivatives had only a moderate fungistatic effect; the manganese, mercury and iron derivatives were fairly active; while the zinc derivative appeared as the most active with a fifty-per cent inhibiting concentration of 1:14,000.

The superiority of the zinc derivative, particularly in case of the dermatophytes, was also observed in the dilution method with Sabouraud's broth (6). Under these experimental conditions Nu-1485 inhibited the growth of T. mentagrophytes at 1:2,048,000 while the sodium salt was active at 1:128,000.

It might be mentioned that the zinc derivative of 3-pyridinethiol was of superior activity than its isomers derived from 2- and 4-pyridinethiol respectively. The results of comparative experiments using the plate method as well as the dilution technic demonstrated that the zinc derivative of 3-pyridinethiol was four to seven times more active than the isomers against T. mentagrophytes and M. lanosum.

Another group of experiments using the agar cup method as recommended by the FDC demonstrated that an ointment containing 5% of the zinc derivative of 3-pyridinethiol gave an area of inhibition of 15.20 cm^2 against T. gypseum and 50.26 cm^2 against M. audouini.

ACTIVITY TOWARDS PLANT PATHOGENS

The metal derivatives of 3-pyridinethiol when tested against plant pathogens exhibited considerable activity. The 50% growth inhibiting concentrations obtained in experiments with Fusarium oxysporum and Botrytis paeoniae varied in a range of from 1:20,000 to 1:100,000. F. oxysporum as a rule was more sensitive than B. paeoniae and the range of activity encountered with the various metals followed the order of Mn < Cu < Fe < Hg < Zn.

ACTIVITY TOWARDS BACTERIA

Similar observations were made in the experiments on bacteriostatic activity. The active concentrations of the different metal derivatives were as a rule 1:10,000 against the gram positive and gram negative organisms, hemolytic

TABLE 1

Fungistatic activity of 3-pyridinethiol and its metal derivatives on dermatophytes

Technic: agar plate method.
Observation time: 3 weeks.

COMPOUND Nu-	METAL DERIVATIVE	50% GROWTH INHIBITING CONCENTRATION	
		T. mentagroph.	*M. lanosum*
1485/1	—*	9,300†	9,100
1485/10	Na	3,000	4,000
1678	S-S*	6,000	8,000
1485/6	Cu	3,000	3,500
1485/8	Fe	9,000	21,000
1485/7	Mn	8,000	16,000
1485/5	Hg	10,000	11,000
1485	Zn	14,000	14,000
1485/4	Bi	4,250	4,750

* Hydrochloride.
† The figures representing activity are given as reciprocals.

streptococci, pneumococci and coli-typhoid-shigella group. All compounds exhibited, however, a high activity towards Staphylococcus aureus which was inhibited by a concentration of 1:100,000 and in case of the zinc derivative of 1:500,000. The copper and iron derivatives were on the whole less active than the other metals.

The marked anti-fungal and anti-bacterial activity which particularly characterized the zinc derivative was accompanied by low toxicity and lack of irritation.

TOXICITY OF THE ZINC DERIVATIVE OF 3-PYRIDINETHIOL

In mice, a total dose of 15.0 gm./kg. per os given in three doses of 5.0 gm./kg. on three successive days was found to be lethal. An oral dose of 1.0 gm./kg. on ten successive days was tolerated by eight out of ten animals. The death of the two animals after one and three treatments might have been accidental.

By the subcutaneous route, 0.25 gm./kg. were tolerated by all mice.

IRRITATION TEST WITH 3-PYRIDINETHIOL

Repeated administration of a 5% ointment to the shaved skin of the abdomen of guinea pigs and a single application of the same ointment to the eyes of rabbits resulted in no untoward reactions.

It was interesting to note that the metal free disulfide derived from 3-pyridinethiol produced considerable irritation of the rabbit's eye after conjunctival administration which was similar to that produced by fatty acids, particularly the lower members of the homologous series.

As compared with the disulfides and the fatty acids, the zinc derivative of 3-pyridinethiol appeared experimentally free of irritative properties. Its innocuousness for the skin was later confirmed in clinical trials (7).

SUMMARY

Studies with 3-pyridinethiol and its metal derivatives showed appreciable antimicrobial activity of the soluble but unstable parent substance as well as of the insoluble, stable metal derivatives. The results of studies on bacteriostatic as well as on fungistatic activity showed that the zinc derivative of 3-pyridinethiol possessed marked activity. In addition, this compound was of low toxicity and apparently free of irritative effects.

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