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The antifungal activity of some thiocyanatoaniline derivatives

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A series of derivatives of *p*-thiocyanatoaniline has been synthesised and tested *in vitro* for activity against dermatophytes; some structure-activity relationships are discussed. One of the more highly active compounds, 2,6-dichloro-4-thiocyanatoaniline, showed some activity against experimental dermatophyte infections when applied topically, was of low toxicity to animals, and did not sensitise them. In a clinical trial the compound also showed some therapeutic activity but caused skin sensitisation.

THE fungicidal activity of a variety of organic sulphur compounds is well known but only a few such investigations of aromatic thiocyanates have been reported (Wilcoxon & McCallan, 1935; Walker, 1937; Walker, Morell & Foster, 1937; Morel, 1946; Davies & Sexton, 1946; Muncie & Morofski, 1949). More recently Landis, Kley & Ercoli (1951) examined a number of oxyalkyl thiocyanate derivatives against several dermatophytes *in vitro* and Pohloudek-Fabini & Weuffen (1960; 1964) have recorded the marked fungistatic activity of a variety of aryl thiocyanates. We have therefore prepared a series of simple derivatives of *p*-thiocyanatoaniline and have tested them against a range of dermatophytes *in vitro*. Because of its higher activity 2,6-dichloro-4-thiocyanatoaniline was further examined *in vitro* and *in vivo*.

Experimental

4-Thiocyanatoanilines were readily prepared by the method of Kaufmann & Oehring (1926). Some were brominated in position 2 and/or position 6 as described by Dienske (1927). Acetyl derivatives were obtained by known methods (Dienske, 1927; Smith & Orton, 1908). Benzoylation was accomplished with benzoyl chloride in pyridine, and chloroacetyl and dichloroacetyl derivatives were prepared by using the acid chloride in benzene. Melting points are given in Table 1. Satisfactory analyses were obtained for all novel compounds.

BIOLOGICAL METHODS

The fungistatic activity of acetone solutions of the compounds was determined *in vitro* by agar-dilution tests using Sabouraud agar. Plates were surface-inoculated using a multi-point inoculator (Hale & Inkley, 1965) with 0·01 ml of suspensions of fungal spores (prepared from 7–21 days old agar slopes with 2 ml of Sabouraud broth). Some compounds were also tested in Sabouraud liquid medium using twofold serial dilutions in 2 ml amounts. The inoculum was 0·02 ml of a spore suspension prepared as above. Fungistatic tests on compounds formulated in oil-in-water creams (Polawax 15%, liquid paraffin 25% and distilled water to 100%) were made by the diffusion method of Burlingame & Reddish (1939).

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TABLE 1. THE ANTIFUNGAL ACTIVITY OF *p*-THIOCYANATOANILINE AND SOME HALOGENATED DERIVATIVES

Compound No.	R	R′	X	m.p.	Minimum inhibitory concentration (μg/ml) after 7 days at 26°		
					Trichophyton sp.*	*Microsporon* sp.†	*Epidermophyton floccosum* WB2
1	H	H	–	57°	50	12·5–50	50
2	H	H	2-Cl	67°	3·0–12·5	3·0	3·0
3	H	H	2-Br	82°	3·0–12·5	1·0	3·0
4	H	H	3-Cl	75°	12·5	3·0–12·5	3·0
5	H	H	3-I	85–86°	12·5–50	12·5	12·5
6	Me	Me	2-SCN	94·5–95·5°	3·0	1·0	1·0
7	Me	Me	3-Cl	72–73°	3·0	3·0	3·0
8	Pr	H	2-Cl	40–41°	3·0	3·0	0·3
9	Ac	H	2-Cl	141–142°	12·5	3·0	3·0
10	Ac	H	2-Br	144–145°	3·0–12·5	3·0	3·0
11	Ac	H	3-I	194°	12·5–50	12·5	12·5
12	$ClCH_2·CO$	H	2-Cl	105–107°	0·3–1·0	0·3	0·3
13	$Cl_2CH·CO$	H	2-Cl	93–94·5°	1·0–3·0	3·0	1·0
14	H	H	3-Cl,2-Me	112–113°	12·5	3·0	3·0
15	H	H	2,6-Cl_2	96·5–97°‡	0·1–1·0	0·3	0·3
16	H	H	2-Br,6-Cl	93–94°	0·1–3·0	0·3	0·3
17	H	H	2,6-Br_2	123°	0·3–1·0	0·3	1·0
18	H	H	2-Br,3-Me,6-Br	104–105°	1·0	0·3	1·0
19	H	H	2,3-Cl_2	141–142°	3·0	1·0	1·0
20	H	H	2,5-Cl_2	118–119°	3·0	1·0	1·0
21	H	H	3,5-Cl_2	166–167°	3·0	1·0	1·0
22	Me	Me	2,3-Cl_2	62–62·5°	3·0	1·0	1·0
23	Me	H	2,5-Cl_2	94°	3·0	1·0	1·0
24	Me	Me	3,5-Cl_2	165–166°	12·5–50	12·5	50
25	Ac	Ac	2,6-Cl_2	95°	12·5	12·5	12·5
26	Ac	H	2,6-Cl_2	161–162°	50	12·5	12·5
27	$ClCH_2·CO$	H	2,6-Cl_2	158–159°	12·5	12·5	12·5
28	$Cl_2CH·CO$	H	2,6-Cl_2	191°	3·0	3·0	3·0
29	Ph·CO	Ph·CO	2,6-Cl_2	176–177°	50	12·5	50
30	Ph·CO	H	2,6-Cl_2	142°	50	12·5	50
31	$Cl_2CH·CO$	Me	2,5-Cl_2	103°	3·0–12·5	12·5	12·5
32	Ac	H	3,5-Cl_2	156–157°	12·5	1·0–3·0	1·0
33	H	H	2,6-Me_2	87–88°	50	50	200
34	Me	Me	–	72–73°	50	50	50

* *Trichophyton* sp. = *T. mentagrophytes* A280, *T. interdigitale* NCTC 175, *T. verrucosum* NCTC 168 *T. tonsurans* WB1, *T. rubrum* WB2.

† *Microsporon* sp. = *M. audouini* NCTC D617, *M. canis* Arkland 834E.

‡ On treatment with aqueous alcoholic sodium hydroxide solution, di(4-amino-3,5-dichlorophenyl) disulphide, m.pt 174°, was obtained. Lempert, Beke & Borovansky (1956) give m.pt 171·5–172·5°.

Fungicidal activity was determined using the Coates, Drain, Macrae & Tattersall (1959) modification of the method of Golden & Oster (1947). In tests with formulated compounds the mycelium-covered membranes were embedded in the creams for 1 or 2 hr, washed and plated.

In vivo tests were made in guinea-pigs infected with *T. mentagrophytes* (A280) using the method of Martin (1959). Commencing on the day after infection, the formulated compounds were applied once daily for 7 days, one animal being used for each preparation. Other guinea-pigs were infected with *M. canis* and after 5 days, when this infection was established, topical treatment with formulated compound was given once daily for 6 days.

Results and discussion

The fungistatic activity of the compounds is shown in Table 1. Our results confirm reports of the activity of *p*-thiocyanatoaniline, Compound 1 (Ortel & Weuffen, 1959; Zsolnai, 1962). The introduction of a halogen atom into the ring (Compounds 2–5) enhanced activity and this was further

increased by the introduction of a second halogen atom (Compounds 15–17 and 19–21). The insertion of a second thiocyanato-group, a "pseudo-halogen" (Compound 6), was also highly effective in increasing fungistatic activity. There are indications that halogen substitution in position 2 or 6 (Compounds 15–17) is more effective than substitution in other positions. Halogenation may enhance the lipid solubility of *p*-thiocyanatoaniline and so facilitate penetration into the dermatophytes. However, Pohloudek-Fabini & Weuffen (1964) found that although halogenation increased the antifungal activity of Schiff's bases of thiocyanatoaniline, there was no definite relationship between the activity of the compounds and their solubility in organic solvents or water. Acylation of, or substitution in, the amino-group generally diminished activity, and alkyl substitution in the ring did not enhance it (e.g. compare Compounds 1 and 33).

It has been suggested that the thiocyanato-group reacts with sulphydryl groups of fungal enzymes, because the antifungal activity of several organic thiocyanates is antagonised by cysteine (Zsolnai, 1962). However, bacteria also possess essential sulphydryl-containing enzymes, yet concentrations of less than 50 μg/ml of the thiocyanates that we have examined did not inhibit the growth of a range of pathogenic bacteria. Further work is needed to elucidate the mode of action of these relatively specific antifungal compounds.

The fungistatic activity of four of the most active compounds was much greater than that of a number of agents used in the treatment of dermatophyte infections (Table 2). When the activity of the formulated thiocyanates was compared with that of aqueous suspensions in diffusion tests, 2,6-dichloro-4-thiocyanatoaniline (Compound 15) was satisfactorily released from the cream and was studied further. In fungistatic tests in Sabouraud broth, it showed little decrease in activity in the presence of

TABLE 2. THE ACTIVITY IN LIQUID MEDIA OF SOME AROMATIC THIOCYANATES COMPARED WITH OTHER ANTIFUNGAL AGENTS

Compound		Minimum inhibitory concentration μg/ml after 7 days at 26°							
		T. mentagrophytes A280	*T. tonsurans* WB1	*T. interdigitale* NCTC 175	*T. rubrum* WB2	*T. verrucosum* NCTC 168	*M. audouini* NCTC D617	*M. canis* Arkland, 834E	*Epid. floccosum* WB2
15	2,6-Dichloro-4-thiocyanatoaniline	1·56	1·56	0·39	0·39	0·19	0·78	0·78	0·78
17	2,6-Dibromo-4-thiocyanatoaniline	1·56	3·1	0·39	0·39	0·39	0·78	0·78	0·39
16	2-Bromo-6-chloro-4-thiocyanatoaniline	0·78	1·56	0·39	0·39	0·39	0·39	1·56	0·39
12	α,2-Dichloro-4-thiocyanatoacetanilide	1·56	1·56	0·78	0·39	0·39	0·19	0·78	0·39
	3-(*p*-Bromophenoxy) propyl thiocyanate*	6·25	6·25	3·1	3·1	3·1	6·25	3·1	6·25
	Undecenoic acid B.P.	12·5	12·5	6·25	12·5	12·5	12·5	12·5	6·25
	Griseofulvin B.P.	12·5	3·1	12·5	1·56	1·56	0·39	1·56	1·56
	Dichlorophen B.Vet.C.	6·25	3·1	1·56	6·25	3·1	3·1	6·25	3·1
	N-Butyl-4-chloro-2-hydroxybenzamide†	6·25	6·25	6·25	1·56	3·1	3·1	3·1	6·25

* Landis, Kley & Ercoli (1951).
† This was tested as a 10% tincture also containing 1% salicyclic acid (Korger & Nesemann, 1960).

10% ox serum but there was an eightfold loss in activity when the concentration of serum was increased to 50%. A 0·5% aqueous suspension or a 1% formulation of the compound was not fungicidal to *T. mentagrophytes* or *M. canis* after 2 hr contact.

Although the lower alkyl thiocyanates are highly poisonous to mammals due, perhaps, to the liberation of cyanide (see Negherbon, 1959) aromatic thiocyanates are much less toxic. Compound 15 was not found to be acutely toxic or irritant to laboratory animals. It is a white solid without unpleasant odour or lachrimatory properties. Dr. B. Lessel (private communication) determined that the acute LD50 of this compound for albino mice was about 250 mg/kg by the oral route and 1,300 mg/kg when injected subcutaneously. When applied as a 2% oil-in-water cream it was not irritant to the skin of rabbits and did not cause sensitisation reactions in guinea-pigs. Further experiments were therefore made *in vivo*.

In guinea-pigs infected with *T. mentagrophytes* the topical application of Compound 15 as a 2·0% cream, the highest concentration tested, reduced the lesions but this was less effective than similar application of griseofulvin 1·0%. Orally or subcutaneoulsy administered Compound 15, at 200 mg/kg daily for 6 days, failed to reduce the extent of lesions; griseofulvin at 60 mg/kg had a significant effect. Topical application of Compound 15 did not completely eliminate the *M. canis* infection but the extent of fluorescence under Wood's light was reduced, particularly with a 2·0% cream. The reduction in the extent of fluorescence was greater with the topical application of a 0·1% cream than with one containing 2% 3-(*p*-bromophenoxy)propyl thiocyanate and was similar to that of a glycol solution containing 1% griseofulvin. Although highly inhibitory to dermatophytes *in vitro* the topical administration of Compound 15 is thus only moderately effective in reducing experimental infections. It is well known that many compounds which are highly active *in vitro* show disappointing activity when used for topical medication. Probably, as Campbell (1964) has recently re-emphasised, this is partly due to the difficult problem of the penetration of keratin.

Unfortunately this compound can cause sensitisation in man. In a limited clinical trial of a stable cream formulation containing 0·1% of Compound 15 the preparation showed some therapeutic effect but skin reactions appeared in 6% of patients. Positive patch tests to this thiocyanate were obtained in several of them (Dr. P. T. Main, private communication). This result exemplifies the inadequacy of animal tests to detect compounds which can cause skin sensitisation in man (see Carter & Griffith, 1965). We are unaware of previous reports of organic thiocyanates acting as skin sensitisers.

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References

Burlingame, E. M. & Reddish, G. F. (1939). *J. Lab. clin. Med.*, **24**, 765–772.
Campbell, A. H. (1964). In *Experimental Chemotherapy*, Vol. 3, editors Schnitzer, R. J. & Hawking, F., p. 467, London: Academic Press.
Carter, R. O. & Griffith, J. F. (1965). *Toxic. appl. Pharmac.*, **7**, Suppl. 2, 60–73.
Coates, L. V., Drain, D. J., Macrae, F. J. & Tattersall, K. (1959). *J. Pharm. Pharmac.*, **11**, 240*T*-249*T*.
Davies, W. H. & Sexton, W. A. (1946). *Biochem. J.*, **40**, 331–334.
Dienske, J. W. (1927). *Recl. Trav. chim. Pays-Bas Belg.*, **46**, 154–157.
Golden, M. J. & Oster, K. A. (1947). *J. Am. pharm. Ass., Sci. Edn*, **36**, 359–362.
Hale, L. J. & Inkley, G. W. (1965). *Lab. Pract.*, **14**, 452–453.
Kaufmann, H. P. & Oehring, W. (1926). *Ber. dt. chem. Ges.*, **59**, 187–104.
Korger, V. G. & Nesemann, G. (1960). *Arzneimittel-Forsch.*, **10**, 194–109.
Landis, L., Kley, D. & Ercoli, N. (1951). *J. Am. pharm. Ass., Sci. Edn*, **40**, 321–325.
Lempert, K., Beke, D. & Borovansky, A. (1956). *Magy. kém. Foly.*, **62**, 373–377.
Martin, A. R. (1959). *J. invest. Derm.*, **32**, 525–528.
Morel, G. (1946). *Revue Vitic.*, **92**, 7, 210–213.
Muncie, J. H. & Morofski, W. F. (1949). *Am. Potato J.*, **26**, 287–291.
Negherbon, W. O. (1959). *Handbook of Toxicology*, Vol. 3, pp. 570–574. Philadelphia: W. B. Saunders.
Ortel, S. & Weuffen, W. (1959). *Pharmazie*, **14**, 204–214.
Pohloudek-Fabini, R. & Weuffen, W. (1960). *Arch. Pharm., Berl.*, **293**, 333–342.
Pohloudek-Fabini, R. & Weuffen, W. (1964). *Ibid.*, 554–565.
Smith, A. G. & Orton, K. J. P. (1908). *J. chem. Soc.*, **93**, 1242–1250.
Walker, J. C. (1937). *Phytopathology*, **27**, 142.
Walker, J. C., Morell, S. & Foster, H. H. (1937). *Am. J. Bot.*, **24**, 536–541.
Wilcoxon, F. & McCallan, S. E. A. (1935). *Contr. Boyce Thomson Inst. Pl. Res.*, **7**, 333–339.
Zsolnai, T. (1962). *Biochem. Pharmac.*, **11**, 271–297.