THE PREPARATION AND EVALUATION OF SOME PHENOLIC ETHERS AS ANTIFUNGAL AGENTS

By L. V. Coates, D. J. Drain, F. June Macrae and K. Tattersall

From Smith & Nephew Research Limited, Hunsdon Laboratories, Ware, Herts.

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A series of phenolic ethers, derivatives of hydroxybenzoic acids, benzaldehydes and acylophenones have been prepared and tested *in vitro* for antifungal action. The highest activity was found in those compounds having an amyloxy group *ortho* to a carbonyl radical. Data are presented on the *in vivo* antifungal activity and toxicity of 2-*n*-amyloxybenzamide and 2-*n*-amyloxyacetophenone.

Routine screening of compounds for fungistatic activity had previously shown 2-*n*-amyloxybenzamide to be the most active of a series of salicylamide derivatives[1]. This work has been extended to cover corresponding *meta* and *para* alkoxybenzamides which have been shown to be considerably less active than the *ortho* derivatives described in our earlier paper. In view of the high *in vitro* fungistatic activity of the 2-alkoxybenzamides, a series of related 2-alkoxy compounds, in which the amide group was replaced by other carbonyl radicals, was prepared and many of these also showed pronounced fungistatic properties. 2-*n*-Amyloxyacetophenone was the most promising of the compounds examined, and together with 2-*n*-amyloxybenzamide has been subjected to more detailed biological examination.

Experimental

Chemical

The new compounds are listed in Table I.

Unless otherwise stated, the alkyl ethers were prepared from the appropriate phenol by the following general method. The phenol (1 mole) was reacted with an alkyl bromide (1 mole) in boiling ethanolic sodium ethoxide (1 mole). After the mixture had been refluxed for up to 24 hours, the ethanol was removed by distillation to give a residue which was washed with 2N sodium hydroxide. The alkyl ether was collected by filtration or ether extraction and purified by either re-crystallisation or distillation. In the alkylation of methyl hydroxybenzoates, methanol was used as solvent in this reaction, in order to eliminate the possibility of ester interchange.

Alkoxybenzoic acids. Methyl alkoxybenzoates were hydrolysed with 10 per cent ethanolic potassium hydroxide to give alkoxybenzoic acids. 2-*n*-Amylmercaptobenzoic acid was similarly prepared from phenyl 2-*n*-amylmercaptobenzoate.

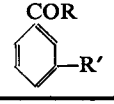
3-*Alkoxy and* 4-*alkoxybenzamides.* The appropriate alkoxybenzoic acid was treated with thionyl chloride (2 equivalents) to form the acid chloride. A solution of the crude acid chloride in benzene was poured into an excess of aqueous ammonia solution and the amide was collected by filtration.

2-*n-Amylmercaptobenzamide* was prepared in the same way from 2-*n*-amylmercaptobenzoic acid.

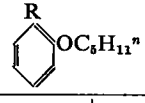
2-n-*Amyloxybenzhydroxamic acid.* Methyl 2-*n*-amyloxybenzoate (22·2 g.) was added to a solution of hydroxylamine prepared from hydroxylamine hydrochloride (14 g.) and potassium hydroxide (16·8 g.) in

TABLE I

NEW COMPOUNDS



R	R′	Physical form	Melting point or boiling point, ° C.	Empirical formula	Required per cent			Found per cent		
					C	H	N	C	H	N
NH_2	2-$SC_5H_{11}^n$	Needles (*a*)	103	$C_{12}H_{17}NOS$	64·5	7·7	6·3	64·4	7·7	6·1
NH_2	3-$OC_5H_{11}^n$	Prisms (*b*)	117	$C_{12}H_{17}NO_2$	69·5	8·3	6·8	69·9	8·2	6·6
NH_2	3-$OC_6H_{13}^n$	Plates (*b*)	115	$C_{13}H_{19}NO_2$	70·5	8·6	6·3	70·7	8·5	6·1
NH_2	4-$OC_5H_{11}^n$	Needles (*b*)	158	$C_{12}H_{17}NO_2$	69·5	8·3	6·8	69·4	8·2	
NH_2	4-$OC_6H_{13}^n$	Prisms (*b*)	154	$C_{13}H_{19}NO_2$	70·5	8·6	6·3	70·7	8·4	6·2
OH	2-$OC_5H_{11}^n$	Prisms (*a*)	31–32	$C_{12}H_{16}O_2$	69·2	7·8		68·9	7·8	6·6
OH	2-$SC_5H_{11}^n$	Microcrystalline (*b*)	105	$C_{12}H_{16}O_2S$	64·3	7·2		64·5	7·3	
OMe	2-OBu^n	Liquid	118/17 mm.	$C_{12}H_{16}O_2$	69·2	7·7		69·4	7·8	
OMe	2-$OC_5H_{11}^n$	Liquid	110°/0·6 mm.	$C_{12}H_{16}O_2$	70·2	8·2		70·2	8·3	
OMe	3-$OC_5H_{11}^n$	Liquid	86/0·01 mm.	$C_{13}H_{18}O_2$	70·2	8·2		70·2	8·4	
OMe	3-$OC_6H_{13}^n$	Liquid	116/0·05 mm.	$C_{14}H_{20}O_2$	71·1	8·5		71·2	8·3	
OMe	4-$OC_6H_{13}^n$	Prisms (*a*)	22°	$C_{14}H_{20}O_2$	71·1	8·5		71·3	8·7	



R	Physical form	Melting point or boiling point, ° C.	Empirical formula	Required per cent			Found per cent		
				C	H	N	C	H	N
CONHOH	Matted needles (*c*)	87–88	$C_{12}H_{17}NO_2$	64·6	7·7	6·3	64·6	7·8	6·3
$CONHNH_2$	Needles (*d*)	32–33	$C_{12}H_{18}N_2O_2$	64·8	8·2	12·6	64·8	8·2	12·8
$CONHN{=}CHC_6H_5O$ (*f*)	Needles (*b*)	134–135	$C_{19}H_{21}N_2O_3$	69·9	6·8	8·6	70·0	6·9	8·5
CN	Liquid	126/1·0 mm.	$C_{12}H_{15}NO$	76·1	8·0	7·4	76·1	8·1	7·0
$CSNH_2$	Prisms (*b*)	80–81	$C_{12}H_{17}NOS$	64·5	7·7	6·3	64·2	7·8	6·3
$CH{=}NNHCONH_2$	Prisms (*b*)	182	$C_{13}H_{19}N_3O_2$	62·6	7·7	16·9	62·5	7·6	17·0
$CH{=}CHCO_2H$	Prisms (*e*)	98·5	$C_{14}H_{18}O_2$	71·8	7·7		71·7	7·7	
$CH{=}CHCONH_2$	Needles (*a*)	127	$C_{14}H_{19}NO_2$	72·1	8·2	6·0	71·8	8·1	5·9

Analyses by Drs. Weiler and Strauss and C. S. McRoe of these laboratories. M.ps. uncorrected.
(*a*) Recrystallised from light petroleum. (*b*) Recrystallised from ethanol.
(*c*) Recrystallised from aqueous ethanol. (*d*) Recrystallised from benzene/light petroleum.
(*e*) Recrystallised from ethyl acetate. (*f*) CHC_6H_5O = salicylidene.

R	R′	Boiling point °C.	Empirical formula	Required per cent		Found per cent	
				C	H	C	H
H	$C_6H_{13}^n$	108/0·1 mm.	$C_{13}H_{18}O_2$	75·7	8·8	75·7	8·8
Me	Bu^n	158°/19 mm.	$C_{12}H_{16}O_2$	75·0	8·4	74·9	8·7
Me	$C_5H_{11}^n$	170/17 mm.	$C_{13}H_{18}O_2$	75·7	8·8	75·9	8·7
Me	$C_6H_{13}^n$	106/0·2 mm.	$C_{14}H_{20}O_2$	76·3	9·2	76·1	9·2
Et	Bu^n	155/12 mm.	$C_{13}H_{18}O_2$	75·7	8·8	75·8	8·8
Et	$C_5H_{11}^n$	159/10 mm.	$C_{14}H_{20}O_2$	76·3	9·2	76·0	8·9
Et	$C_6H_{13}^n$	177/12 mm.	$C_{15}H_{22}O_2$	76·9	9·5	77·1	9·3

methanol (115 ml.) and left at room temperature for 12 hours. The solution was neutralised with acetic acid, and diluted with water (150 ml.) whereupon the product crystallised and was collected by filtration.

2-n-*Amyloxybenzhydrazide.* Methyl 2-*n*-amyloxybenzoate (10 g.) was heated under reflux with hydrazine hydrate (50 ml.) in ethanol (40 ml.) for 5 hours. The hydrazide was distilled, b.p. 163 to 164°/0·7 mm. and on cooling, solidified. The salicylidene derivative was formed from 2-*n*-amyloxybenzhydrazide and salicylaldehyde by the standard procedure.

2-n-*Amyloxybenzonitrile.* 2-*n*-Amyloxybenzamide (21·1 g.) was mixed with phosphorus pentoxide (14·2 g.) in dry xylene (250 ml.) and refluxed for 1 hour. The reaction mixture was then distilled, giving crude 2-*n*-amyloxybenzonitrile b.p. 178°/12 mm.

2-n-*Amyloxybenzthioamide.* 2-*n*-Amyloxybenzonitrile (20 g.) was dissolved in saturated ethanolic ammonium sulphide (120 ml.) and heated in a sealed tube at 100° for 5 hours. After evaporation of the solvent some solid separated and was collected and recrystallised to give 2-*n*-amyloxybenzthioamide. An attempt to prepare this compound by treating 2-*n*-amyloxybenzamide with phosphorus pentasulphide gave only 2-*n*-amyloxybenzonitrile in poor yield.

2-n-*Amyloxybenzaldehydesemicarbazone* was prepared from 2-*n*-amyloxybenzaldehyde and semicarbazide by the standard procedure.

2-n-*Amyloxycinnamic acid and* 2-n-*amyloxycinnamamide.* 2-*n*-Amyloxybenzaldehyde (19·2 g.) malonic acid (10·4 g.) and pyridine (15·8 g.) were mixed and heated at 100° for 4 hours. The mixture was poured into water and the oil extracted with chloroform. The chloroform solution was dried and evaporated, giving a residue of 2-*n*-amyloxycinnamic acid, which was purified by crystallisation. 2-*n*-Amyloxycinnamamide was prepared through the acid chloride by standard procedures.

Microbiological

Fungistatic test. Antifungal activity was assayed as previously described[1].

Fungicidal test. This is a modification, not previously reported, of the method of Golden and Oster[2].

1 sq. cm. plaques of Oxoid Membrane filter were inoculated from a culture of *Trichophyton mentagrophytes* and placed upon a plate of malt agar. After incubation at 28° for 10 days, the plaques covered with mycelium were removed from the agar, and placed in a solution of the fungicide in 25 per cent v/v acetone, 25 per cent v/v ethanol and 50 per cent distilled water. After an exposure of 1 hour the plaques were gently rinsed in 30 per cent aqueous acetone for 5 minutes and transferred to 20 ml. of Sabouraud broth for 1 hour. Finally, the plaques were placed mycelium downwards onto 10 per cent serum malt agar and incubated for 14 days at 28°.

In vivo *tests.* It is not possible to simulate human dermatomycoses in small laboratory animals. The method finally adopted was based upon that used by Frey[3].

Albino guinea pigs of approximately 250 g. were shaved over the flanks and the skin gently abraded by a scalpel blade without excessive bleeding. A suspension of 5×10^6/ml. spores of *T. mentagrophytes* in 50 per cent serum broth was then rubbed into the abraded area.

The infection normally follows a pattern; inflammation and scaling of the skin can be observed after 3 days, which increases until between the 4th and 10th day there is marked induration and exudation. The lesion then becomes heavily encrusted and spontaneous healing begins after 20 to 30 days. Baldness may persist for 50 days after infection.

Treatment was commenced 5 days after infection and continued for 8 days, assessment of infection was made visually at frequent intervals and where possible by microscopic and cultural examination. The period required for the regeneration of hair was taken as a further criterion in the assessment of a cure.

Toxicity

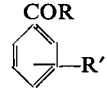
Acute toxicity was determined in albino mice using oral and intraperitoneal routes.

Irritation and sensitisation effects were examined by a modification of the method by Halpern[4], which depends on the increased capillary permeability caused by a local sensitivity reaction. 0·2 ml. of a 5 per cent ethanolic solution of the test compound was rubbed into the shaved backs of female albino guinea pigs once daily for ten days. Fourteen days after the last application the animals were given a shock dose of the compound (in a 20 per cent Tween 80) and injected intravenously with a solution of azovan blue. The appearance of a blue patch on the skin indicates a sensitivity reaction. Finally the animals were killed and the effect of the test compound on the isolated uterus was measured. A contraction of the uterus demonstrates a sensitivity reaction.

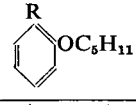
Patch tests, using 0·5 ml. of alcoholic gel covered by an occlusive dressing, were made on 93 human volunteers.

TABLE II

FUNGISTATIC ACTIVITIES

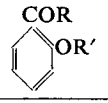


R	R′	Minimum inhibitory concentration, μg./ml.						
		Tm*	Tt	Tc	Tr	Ef	Ma	Index
NH_2	2-$OC_5H_{11}^n$ (7)	6	3	3	3	1·5	1·5	3
NH_2	2-$SC_5H_{11}^n$	>50	>50	>50	>50	>50	>50	>50
NH_2	2-$OC_8H_{13}^n$ (7)	12·5	3	3	3	3	1·5	4·3
NH_2	3-$OC_5H_{11}^n$	>50	25	25	25	25	12·5	>27
NH_2	3-$OC_6H_{12}^n$	>50	>50	>50	>50	>50	>50	>50
NH_2	4-$OC_5H_{11}^n$	>50	>50	>50	>50	>50	>50	>50
NH_2	4-$OC_8H_{13}^n$	>50	>50	>50	>50	>50	>50	>50
OH	2-O Bu^n (8)	>50	>50	>50	>50	>50	>50	>50
OH	2-$OC_5H_{11}^n$	>50	50	25	50	25	25	>38
OH	2-$SC_5H_{11}^n$	>50	>50	>50	>50	>50	>50	>50
OH	3-$OC_5H_{11}^n$ (8)	50	25	25	25	25	6	26
OH	3-$OC_6H_{13}^n$ (9)	>50	25	25	>50	25	25	>33
OH	4-$OC_5H_{11}^n$ (10)	50	25	25	>50	50	25	>38
OH	4-$OC_8H_{13}^n$ (10)	>50	50	50	>50	50	50	>50
OMe	2-O Bu^n	50	25	25	25	50	25	33
OMe	2-$OC_5H_{11}^n$	50	25	25	12·5	25	12·5	25
OMe	3-$OC_5H_{11}^n$	>50	25	25	25	50	25	>33
OMe	3-$OC_6H_{11}^n$	>50	>50	>50	>50	50	50	>50
OMe	4-$OC_6H_{11}^n$ (11)	>50	>50	50	25	>50	25	>42
OMe	4-$OC_8H_{13}^n$	>50	>50	>50	>50	>50	>50	>50



R	Tm	Tt	Tc	Tr	Ef	Ma	Index
CONHOH	50	25	12·5	6	12·5	12·5	20
$CONHNH_2$	>50	>50	>50	>50	>50	>50	>50
$CONHN{=}CHC_6H_5O$†	>50	>50	>50	>50	>50	>50	>50
CN	50	50	50	50	>50	25	>46
$CSNH_2$	25	12·5	6	6	6	1·5	9·5
$CH{=}NNHCONH_2$	>50	12·5	12·5	25	25	6	>22
$CH{=}CHCO_2H$	50	25	25	50	25	12·5	23
$CH{=}CHCONH_2$	>50	>50	>50	>50	>50	>50	>50

• *Key*—Tm = *T. mentagrophytes*; Tt = *T. tonsurans*; Tc = *T. concentricum*; Tr = *T. rubrum*; Ef = *E. floccosum*; Ma = *M. audouini*.
† CHC_6H_5O = salicylidene.



R	R′	Tm	Tt	Tc	Tr	Ef	Ma	Index
H	H	>50	>50	>50	>50	>50	>50	>50
H	Bu^n (12)	50	25	25	50	12·5	12·5	29
H	$C_5H_{11}{}^n$ (13)	25	9·3	12·5	12·5	12·5	9·3	13·5
H	$C_6H_{13}{}^n$	25	12·5	25	25	25	25	23
Me	H	>50	>50	>50	>50	>50	>50	>50
Me	Bu^n	25	25	25	25	12·5	6	20
Me	$C_5H_{11}{}^n$	18·5	18·5	9·3	18·5	9·3	4·6	13·1
Me	$C_6H_{13}{}^n$	50	25	12·5	12·5	12·5	6	20
Et	H	>50	>50	>50	>50	>50	>50	>50
Et	Bu^n	50	25	25	25	12·5	6	24
Et	$C_5H_{11}{}^n$	50	25	6	50	6	3	23
Et	$C_6H_{13}{}^n$	>50	>50	>50	>50	>50	>50	>50

Results

Microbiological

Fungistatic tests. The minimum inhibitory concentration of 40 compounds against six representative dermatophytes are recorded in Table II.

Antagonism. 2-*n*-Amyloxyacetophenone and 2-*n*-amyloxybenzamide have been screened in the presence of 10 per cent whole blood, 10 per cent serum, 8 per cent keratin hydrolysate, 2·5 per cent by weight of hair, 0·1 per cent soap, 0·02 per cent of sodium lauryl sulphate, 0·025 per cent of an artificial sebum mixture[5], and 0·01 per cent *p*-aminobenzoic acid. 10 per cent serum halved the activity of 2-*n*-amyloxyacetophenone and reduced

TABLE III
FUNGICIDAL ACTIVITY

Compound	Exposure period	Concentration per cent	
		1·0	0·1
2-*n*-Amyloxybenzamide	1 hr.	–	–
2-*n*-Amyloxyacetophenone ..	1 hr.	–	–
Salicylanilide	1 hr.	–	–
Control-solvent only..	1 hr.	+ Growth after 7 days incubation	

– No growth
+ Growth
} on subculture medium at 14 days

the activity of 2-*n*-amyloxybenzamide to one third. Sodium lauryl sulphate halved the activity of 2-*n*-amyloxybenzamide but had no effect on 2-*n*-amyloxyacetophenone. The remaining substances tested did not significantly affect the minimum inhibitory concentrations obtained for the two compounds.

Fungicidal tests. Results of fungicidal tests are given in Table III.

In vivo *tests.* *In vivo* test results are shown in Table IV.

Experiment 1 shows the effect of 2-*n*-amyloxybenzamide and salicylic acid, used as a keratolytic agent, singly or in combination, using a water-in-oil emulsion base. Experiment 2 shows the effect of 2-*n*-amyloxybenzamide, 2-*n*-amyloxyacetophenone and salicylic acid in different

TABLE IV

IN VIVO FUNGICIDAL ACTIVITY USING GUINEA PIGS

Group	No. of animals	Treatment	No. cured at 18 days	Time taken for hair regrowth
Exper. 1 1	5	3 per cent AB* in water in oil emulsion base	None	>47 days
2	5	3 per cent AB, 2 per cent SA in water in oil emulsion base	4	37 days
3	5	2 per cent SA, in water in oil emulsion base	2	47 days
4	5	Controls base only	None	47 days
Exper. 2 1	5	2 per cent AA, 2 per cent AB and 1 per cent SA in alcoholic gel	3	45 days
2	5	2 per cent AA and 1 per cent SA in alcoholic gel	1	>45 days
3	5	2 per cent AB and 1 per cent SA in alcoholic gel	1 and 1 improved	>45 days
4	5	2 per cent AA and 2 per cent AB in alcoholic gel	3	>45 days
5	5	2 per cent AA in alcoholic gel	1 improved	45 days
6	5	Controls gel base only in alcoholic gel	1 improved	>45 days

Exper. 3			Progress of cure at day				Animals culture positive at day				Hair regrowth
			10	14	17	21	10	14	17	21	
1	6	2 per cent AA, 2 per cent AB, 1 per cent SA in alcoholic gel base	22	16	5	0	1	0	1	Insufficient material	36 days
2	6	2 per cent AA, 2 per cent AB, 1 per cent SA in water in oil emulsion	7	12	5	1	2	0	1		>36 days
3	6	2 per cent AA, 2 per cent AB, 10 per cent U in Carbopol 934 gel	21	9	2	1	0	1	1		>36 days
4	6	2 per cent AA, 2 per cent AB, 1 per cent SA in P.E.G. base	7	6	3	0	2	0	1		36 days
5	6	0·5 per cent DM(DE)B in P.E.G. base	17	17	7	2	6	1	4		<36 days
6	6	Untreated	24	13	6	2	3	4	4		>36 days

* *Key*—AA = 2-*n*-amyloxyacetophenone; AB = 2-*n*-amyloxybenzamide; U = urea; SA = Salicylic acid and DM(DE)B = 2-dimethylamino-6-(β-diethylaminoethoxy)benzthiazole.
P.E.G. = Polyethylene glycol.

combinations in an alcoholic gel base. Experiment 3 records the results obtained using the two active ingredients incorporated with a keratolytic in a series of different bases.

In this last experiment a scoring system was used and cultures of skin scales taken to determine the presence or absence of fungi. This was found necessary when it became clear that a single assessment of "cure"

at a fixed time during the test gave an incomplete picture of the efficacy of a preparation. A parallel experience is cited by Frey[3]. The scoring system involved a visual estimate of the state of infection, the observer being unaware of the nature of the treatment. Values of 0, 1, 2, 3 and 4 were used to indicate increasing severity of infection, i.e., 0 indicates complete cure and 4, a heavy infection. The figures in Table IV, Experiment 3, represent the total score for the group. The number of positive cultures, and the time for hair re-growth gives additional information concerning the value of the treatment.

Toxicity

Acute Toxicity in mice. Oral LD50 2-*n*-amyloxybenzamide 4·7 g./kg.[6], 2-*n*-amyloxyacetophenone 6·0 g./kg. Intraperitoneal LD50 0·9 g./kg. and 0·6 g./kg., respectively.

Irritation and sensitisation effects. With 2-*n*-amyloxybenzamide and 2-*n*-amyloxyacetophenone no reactions were recorded when each compound was tested in 20 guinea pigs using the Halpern method. Similarly no reactions were recorded by the isolated uterus technique, from which it is concluded that neither substance is likely to prove a sensitising agent. With human volunteers no reactions were recorded in a patch test to a mixture of 2-*n*-amyloxybenzamide and 2-*n*-amyloxyacetophenone, applied in an alcoholic gel base. Samples of a product containing 2 per cent 2-*n*-amyloxybenzamide, 2 per cent 2-*n*-amyloxyacetophenone and 1 per cent salicylic acid have been used clinically in 60 patients and have given no adverse reactions.

Discussion

It has been previously shown[1] that in a series of 2-alkoxybenzamides peak activity occurred with the amyloxy and hexyloxy compounds and in the work now reported, alkoxy substitution was confined to these groups and the effects of alteration of the nature and position of the other substituent have been examined. To study the effect of alteration in relative position of the two substituents, a group of 3-, and 4-alkoxybenzamides were examined and shown to be of very low activity compared with the 2-isomers. Replacement of the amide group by methoxycarbonyl caused a reduction in activity but here also the 2-isomers were the most active, with highest activity occurring in the amyloxy compound. The corresponding carboxylic acids were of low activity and showed no clear relationship between structure and activity.

In view of previous reports[14] that the replacement of oxygen by sulphur increases antifungal activity, 2-*n*-amylmercaptobenzamide and 2-*n*-amyloxybenzthioamide were examined. The replacement of ether oxygen by sulphur led to complete loss of activity, and similar replacement of the carbonyl oxygen, to a slight loss of activity, although this latter compound was the most active of all those reported in this paper. 2-*n*-Amyloxybenzhydrazide and its salicylidene derivatives were inactive, but the corresponding hydroxamic acid retained some activity, although of a reduced order. Urbanski and co-workers[15,16] have recently reported on the use of

salicylhydroxamic acid, orally, in the clinical treatment of dermatophyte infections, and on the powerful *in vitro* fungistatic effect of a series of halogenated phenoxyacethydroxamic acids.

The replacement of the amide group of 2-*n*-amyloxybenzamide by other carbonyl containing radicals has yielded interesting results. 2-Alkoxybenzaldehydes, acetophenones and propiophenones all showed pronounced activity, and in this group of compounds peak activity was again found with the amyloxy-derivatives. 2-*n*-Amyloxybenzaldehyde and 2-*n*-amyloxyacetophenone were the most active of this group, and the ketone was selected for further more detailed study in view of its greater stability.

It seems that within the limited class of compounds studied, the necessary structural requirements for antifungal activity are a benzene ring bearing an *n*-amyloxy-group *ortho* to a carbonyl radical, although exceptions to this rule were noted (2-*n*-amyloxybenzoic acid and -benzhydrazide).

The fungicidal test results (Table III) show 2-*n*-amyloxybenzamide, 2-*n*-amyloxyacetophenone and salicylanilide to be fungicidal after an exposure of 1 hour to concentrations of 0·1 per cent in water : ethanol : acetone mixture. We previously reported[1] 2-*n*-amyloxybenzamide to be fungicidal only after 24 hours exposure to 0·1 per cent concentration of the compound in Dubos broth. The difference between these two results is probably due to the sparingly soluble nature of 2-*n*-amyloxybenzamide in aqueous media.

All available methods for the assessment of antifungal activity in experimental animals suffer from certain disadvantages, such as the difference between animal and human skin, the differences in pathogenesis, and the spontaneous regression which occurs with dermatophyte infections in animals. Calves[17,18] and guinea pigs[3,19] have been suggested as suitable experimental animals. Guinea pigs were chosen because of availability and the regular and reproducible nature of the infection produced in such animals, although the lesions differ in many important respects from those occurring in man.

Experiment 1 (Table IV) showed 2-*n*-amyloxybenzamide to be inactive when used alone in a water-in-oil emulsion base, but when salicylic acid was added as a keratolytic a marked effect was shown. Salicylic acid alone produced some curative action but was considerably less effective than the mixture.

The discovery of the antifungal effect of 2-*n*-amyloxyacetophenone, an oil, together with its powerful solvent properties for 2-*n*-amyloxybenzamide led us to investigate the effect of combinations of these two substances with and without salicylic acid in an alcoholic gel base. Experiment 2 (Table IV) showed these to be an effective combination.

Bushby and Stewart[20] emphasised the importance of the pharmaceutical base in which antifungal agents are administered. Experiment 3 was designed to show the effect of different bases on the antifungal effect of a standard mixture of the two active substances combined with a keratolytic. Experience with earlier tests led us to the conclusion that therapeutic effect should not be assessed at one time only, for the infection shows

three phases: an initial inflammatory phase, then a period when isolation of the fungus is readily accomplished, which is followed by regression of infection and hair regrowth. In this last test, therefore, the state of infection was visually assessed at various times after infection, and in addition, scrapings from the lesions were examined both microscopically and by culture for the presence of fungi. The results show that the duration of the inflammatory phase was markedly shortened by water-in-oil emulsion, and polyethylene glycol based preparations of 2-*n*-amyloxybenzamide and 2-*n*-amyloxyacetophenone. The same ingredients in an alcoholic gel base, although showing little effect on the inflammatory phase, nevertheless considerably reduced the number of positive cultures, and the time necessary for hair regrowth.

These results, together with the low toxicity of the active ingredients and their lack of sensitising or irritant properties, suggested that suitable formulations of these compounds might be of value in the treatment of human dermatophyte infections.

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After Mr. Tattersall presented the paper there was a DISCUSSION.