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Potential Antifungal Benzhydrazides

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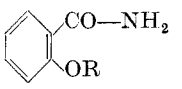
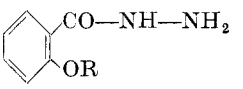
The close biological and taxonomic relationships between mycobacteria and the fungi belonging to the order *Actinomycetales* have frequently led to the testing of antifungal compounds for tuberculostatic[1] and antileprosy activity,[2] and *vice versa*.[2] A growth-inhibiting effect can be exerted on mycobacteria as well as on many fungi by substances endowed with chelating properties towards heavy metals; thus, isonicotinhydrazide displays antifungal activity towards *Trichophyton mentagrophytes*, and some of its hydrazones show this effect to an even greater degree.[3] This suggested a search for antifungal compounds in the group of hydrazides, especially among 2-alkoxybenzhydrazides and 2-hydroxybenzhydrazides, whose molecular structures are closely related to the 2-alkoxybenzamides (I), several members of which group are known to be strongly antifungal.[4]

The hydrazides investigated fall into three categories: *o*-alkoxybenzhydrazides (II), 2-hydroxy-4-alkoxybenzhydrazides (III), and 2-hydroxy-5-alkoxybenzhydrazides (IV). The intermediates used for the preparation of hydrazides of the first category were alkyl 2-alkoxybenzoates, obtained either by monoalkylation of



(I)



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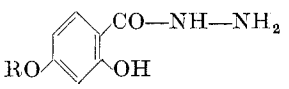
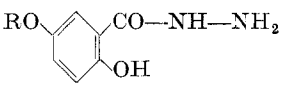


(III)



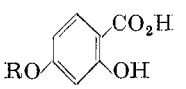
(IV)

methyl salicylate with the appropriate alkyl iodides in the presence of potassium hydroxide, or by dialkylation of salicylic acid itself with the appropriate alkyl iodides in the presence of potassium hydroxide or, better still, silver oxide. It should be

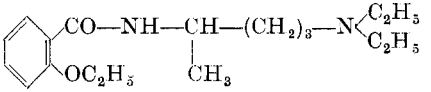
mentioned that in the hydrazinolysis of these esters, the formation of a diaroylhydrazine was observed in one instance.

The 2-hydroxy-4-alkoxybenzhydrazides, listed in Table III, were prepared by hydrazinolysis of methyl 2-hydroxy-4-alkoxybenzoates (Table II), these being obtained by esterification of 2-hydroxy-4-alkoxybenzoic acids (V). These acids were readily prepared by monoalkylation of β-resorcylic acid according to a procedure already reported,[5] and are listed in Table I. The hydrazides of the third category, listed in Table IV, were synthesized by hydrazinolysis of methyl 2-hydroxy-5-alkoxybenzoates; the corresponding 2-hydroxy-5-alkoxybenzoic acids (Table V) were prepared by monoalkylation of gentisic acid.

Determinations of antifungal properties, performed on a strain of *Candida albicans*, showed several 2-hydroxy-4-alkoxy- and 2-hydroxy-5-alkoxybenzhydrazides to be notably active, although to a lesser degree than nystatin. Among the substances tested for tuberculostatic properties (determined *in vitro* on *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}RvD$), 2-hydroxy-4-pentoxybenzhydrazide displayed the greatest activity, although it was more than one hundred times lower than that of isonicotinhydrazide. Various compounds related to the structures discussed in this work, including halogenated 2-ethoxybenzanilides, *N*,*N*-dibutyl-2-ethoxybenzamide, and 1-diethylamino-4-(2-ethoxybenzamido)pentane (VI) were also prepared, but showed no noteworthy antifungal or tuberculostatic activity; this was also the case with 2-(4-chlorobenzyloxy)- and 2-(3,4-dichlorobenzyloxy)-benzamide.



(V)



(VI)

Experimental

Propyl o-*propoxybenzoate.* To a solution of salicylic acid (5·5 g) and propyl iodide (20 g) in anhydrous benzene (10 ml), silver oxide (14 g) was added cautiously in small portions with stirring, and the mixture refluxed for 2 h. After cooling, the silver iodide formed was filtered off, washed with benzene, and the benzene liquor dried ($CaCl_2$ anhyd.). The residue from

evaporation of the solvent was fractionated *in vacuo*, giving a colourless oil (5 g), b.p. 155–156°/15 mm, n_D^{21} 1·5080.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70·2; H, 8·1. Found: C, 70·0; H, 8·1.

The following esters were prepared in the same way:

Isopropyl o-*isopropoxybenzoate*, colourless oil, b.p. 147–149°/15 mm, n_D^{23} 1·5015.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70·2; H, 8·1. Found: C, 70·1; H, 8·0.

Isobutyl o-*isobutoxybenzoate*, b.p. 154–155°/13 mm, n_D^{21} 1·5060.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71·9; H, 8·8. Found: C, 71·6; H, 8·5.

Methyl o-*isopropoxybenzoate*. To a solution of potassium hydroxide (6·5 g) in ethanol (20 ml), methyl salicylate (17·8 g), and then isopropyl iodide (20 g), were added with stirring; the mixture was refluxed for 2 h, the solvent distilled off, the residue treated with water (50 ml), and the reaction product taken up in benzene. The benzene solution was washed with water, dried (Na_2SO_4 anhyd.), the solvent removed, and the residue fractionated *in vacuo*. Yield: 14 g of a colourless oil, b.p. 125°/13 mm, n_D^{21} 1·5209.

o-*Ethoxybenzhydrazide*. A solution of ethyl *o*-ethoxybenzoate (5 g) and 95 per cent hydrazine hydrate (1·2 g) in ethanol (10 ml) was refluxed for 3 h; the residue from evaporation of the solvent was recrystallized from cyclohexane, giving silky colourless needles (3·3 g), m.p. 64°.

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 59·9; H, 6·7; N, 15·5. Found: C, 60·0; H, 6·7; N, 15·6.

o-*Propoxybenzhydrazide* crystallized from cyclohexane in colourless prisms, m.p. 53°.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61·7; H, 7·2; N, 14·4. Found: C, 61·6; H, 7·1; N, 14·6.

o-*Isopropoxybenzhydrazide*. The reaction of methyl *o*-isopropoxybenzoate (2 g) with hydrazine hydrate (1 g) in ethanol (10 ml) furnished o-*isopropoxybenzhydrazide*, which crystallized from cyclohexane in colourless leaflets (1 g), m.p. 65°, along with N,N′-*di*(o-*isopropoxybenzoyl*)*hydrazine*, insoluble in cyclohexane and crystallizing from ethanol in colourless prisms (0·34 g), m.p. 150°.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61·7; H, 7·2; N, 14·4. Found: C, 61·6; H, 7·1; N, 14·3.

Anal. Calcd. for $C_{20}H_{24}N_2O_4$: C, 69·3; H, 6·9; N, 8·0. Found: C, 69·1; H, 6·8; N, 8·3.

Table I. 2-Hydroxy-4-alkoxybenzoic acids (V)

R	Formula	m.p., °C	Anal.			
			Calcd.		Found	
			C	H	C	H
C_3H_7	$C_{10}H_{12}O_4$	164	61·2	6·1	61·1	5·9
i-C_3H_7	$C_{10}H_{12}O_4$	142	61·2	6·1	61·4	6·2
C_4H_9	$C_{11}H_{14}O_4$	139	62·8	6·7	62·4	6·9
C_5H_{11}	$C_{12}H_{16}O_4$	121	64·4	7·1	64·2	6·9
C_8H_{17}	$C_{15}H_{22}O_4$	108	67·6	8·3	67·7	8·3
Benzyl	$C_{14}H_{12}O_4$	188	68·8	4·9	68·8	5·0

Table II. Methyl 2-hydroxy-4-alkoxybenzoates

R	Formula	b.p., °C		n_D
C_3H_7	$C_{11}H_{14}O_4$	156–157/14 mm	[22]	1·5452
i-C_3H_7	$C_{11}H_{14}O_4$	156–157/15 mm	[19]	1·5514
C_4H_9	$C_{12}H_{16}O_4$	174–175/14 mm	[24]	1·5380
C_5H_{11}	$C_{13}H_{18}O_4$	184–185/14 mm	[25]	1·5406
C_8H_{17}	$C_{16}H_{24}O_4$	226–228/15 mm	[26]	1·5100
Benzyl	$C_{15}H_{14}O_4$	234–238/15 mm	[28]	1·6089

Table III. 2-Hydroxy-4-alkoxybenzhydrazides (III)

R	Formula	m.p., °C	Anal.	
			Calcd. N	Found N
C_3H_7	$C_{10}H_{14}N_2O_3$	158	13·3	13·2
i-C_3H_7	$C_{10}H_{14}N_2O_3$	182	13·3	13·1
C_4H_9	$C_{11}H_{16}N_2O_3$	142	12·5	12·3
C_5H_{11}	$C_{12}H_{18}N_2O_3$	148	11·7	11·7
Benzyl	$C_{14}H_{14}N_2O_3$	192	10·8	10·9

Table IV. 2-Hydroxy-5-alkoxybenzhydrazides (IV)

R	Formula	m.p., °C	Anal. Calcd. N	Anal. Found N
H	$C_7H_8N_2O_3$	220	16·6	16·3
C_2H_5	$C_9H_{12}N_2O_3$	140	14·2	14·0
C_3H_7	$C_{10}H_{14}N_2O_3$	130	13·3	13·4
C_4H_9	$C_{11}H_{16}N_2O_3$	117	12·5	12·4
i-C_4H_9	$C_{11}H_{16}N_2O_3$	106	12·5	12·6
C_5H_{11}	$C_{12}H_{18}N_2O_3$	117	11·7	11·7
i-C_5H_{11}	$C_{12}H_{18}N_2O_3$	122	11·7	11·4
C_6H_{13}	$C_{13}H_{20}N_2O_3$	116	11·1	11·0

Table V. 2-Hydroxy-5-alkoxybenzoic acids

R	Formula	m.p., °C	Anal. Calcd. C	Anal. Calcd. H	Anal. Found C	Anal. Found H
C_3H_7	$C_{10}H_{12}O_4$	112	61·2	6·1	61·2	6·0
i-C_3H_7	$C_{10}H_{12}O_4$	117	61·2	6·1	61·5	6·0
C_4H_9	$C_{11}H_{14}O_4$	91	62·8	6·7	62·6	6·7
i-C_4H_9	$C_{11}H_{14}O_4$	118	62·8	6·7	62·7	6·5
C_6H_{13}	$C_{13}H_{18}O_4$	95	65·5	7·6	65·4	7·5

N,N-*Dibutyl-2-ethoxybenzamide.* To an ice-cooled solution of dibutylamine (6 g) in anhydrous pyridine (10 ml), *o*-ethoxybenzoyl chloride (6 g) was added dropwise with stirring, and the mixture left for 1 h at room temperature. After treatment with dilute hydrochloric acid, the product was taken up in ether and fractionated *in vacuo*. Yield: 7·5 g of a pale yellow oil, b.p. 204°/15 mm, n_D^{20} 1·5119.

Anal. Calcd. for $C_{17}H_{27}NO_2$: C, 73·5; H, 9·8; N, 5·1. Found: C, 73·6; H, 9·6; N, 5·0.

1-Diethylamino-4-(2-ethoxybenzamido)pentane (VI). Similarly prepared, using 1-diethylamino-4-aminopentane (4 g) and

o-ethoxybenzoyl chloride (4·5 g), this amide was obtained as a pale yellow viscous oil (5·5 g), b.p. 235°/13 mm, n_D^{22} 1·5202.

Anal. Calcd. for $C_{18}H_{30}N_2O_2$: N, 9·1. Found: N, 9·0.

2-(4-Chlorobenzoxy)benzamide. Prepared from salicylamide and *p*-chlorobenzyl chloride in the presence of ethanolic sodium hydroxide, this amide crystallized from ethanol as shiny colourless prisms, m.p. 154° (yield, 90 per cent).

Anal. Calcd. for $C_{14}H_{12}ClNO_2$: C, 64·2; H, 4·5; N, 5·3. Found: C, 64·0; H, 4·7; N, 5·2.

2-(3,4-Dichlorobenzoxy)benzamide. This amide, prepared from 3,4-dichlorobenzyl chloride, crystallized from ethanol as silky colourless needles, m.p. 166°.

Anal. Calcd. for $C_{14}H_{11}Cl_2NO_2$: C, 56·7; H, 3·7; N, 4·7. Found: C, 56·5; H, 3·8; N, 4·4.

Cinnamaldehyde 5-isoamyloxy-2-hydroxybenzoylhydrazone. Prepared by refluxing cinnamaldehyde (15 g) with the corresponding hydrazide (24 g) for 2 h, this compound (67 per cent yield) crystallized from ethanol in pale yellow leaflets, m.p. 190°.

Anal. Calcd. for $C_{21}H_{24}N_2O_3$: N, 8·0. Found: N, 7·8.

Cinnamaldehyde 5-ethoxy-2-hydroxybenzoylhydrazone. Crystallized from ethanol in silky needles, m.p. 221°.

Anal. Calcd. for $C_{18}H_{18}N_2O_3$: N, 9·0. Found: N, 8·8.

Determination of Antifungal and Tuberculostatic Activity

The antifungal activity was determined on a pathogenic strain of *Candida albicans* recently isolated from a patient and maintained at 37° on Sabouraud's glucose–agar medium, to which penicillin (50 Oxford units per ml) and streptomycin (50 μg per ml) had been added. The substances to be tested were suspended in this medium and maintained at 37°, and the minimum dose necessary for inhibition of the growth of the fungus was determined.

o-Ethoxybenzhydrazide and *o*-isopropoxybenzhydrazide were active at a concentration of 500–1000 μg per ml. The compounds listed in Tables III and IV were active at concentrations of 125 to 250 μg per ml. As control, the sensitivity of the fungus towards nystatin was measured in the same conditions, and found to be between 7·8 and 15 μg per ml.

The tuberculostatic activity was determined on Dubos medium, the inoculation being made with 10 μg of bacteria per 5 ml of culture medium. The substances to be tested were dissolved in diethylene glycol and the cultures kept for 12 days. The inhibition of growth was measured by opacimetry by means of an electrophotometer.

Summary. A number of 2-alkoxybenzhydrazides, 2-hydroxy-4- and 2-hydroxy-5-alkoxybenzhydrazides, and some related compounds, have been prepared and tested for antifungal and tuberculostatic activity. Both the 2-hydroxy-4- and the 2-hydroxy-5-alkoxybenzhydrazides were notably active against *Candida albicans*.

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