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Antifungal Properties of *n*-Alkoxyacetic Acids and Their Methyl Esters

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Abstract □ Eleven *n*-alkoxyacetic acids and their methyl esters, in which the alkyl group was C_1–C_9, C_{11}, or C_{13}, were tested against *Aspergillus niger*, *Trichoderma viride*, and *Myrothecium verrucaria* in Sabouraud dextrose agar at pH 4.0 and 5.6. Toxicity to *Candida albicans*, *Trichophyton mentagrophytes*, and *Mucor mucedo* was determined in the same medium at pH 5.6 and 7.0 in the absence and presence of 10% beef serum. The fungitoxicity of the acids was influenced by chain length, pH of the medium, and absence or presence of adsorbents. The toxicity of the esters was influenced primarily by chain length and to a lesser extent by the medium pH and the presence of beef serum. The order of activity of the *n*-alkoxyacetic acids was according to the number of linear atoms in the chain: 11 > 12 > 10 > 9 > 8 > 7 > 6 > 14 > 16. *T. mentagrophytes* was the organism most strongly affected by these compounds, with the esters being slightly more active than the acids. Compared to other fatty acid analogs, the order of fungitoxicity, on a weight basis, was 2-alkynoic acids > 2-alkenoic acids > alkanoic acids > 2-bromoalkanoic acids > 2-fluoroalkanoic acids > *n*-alkoxyacetic acids.

Keyphrases □ *n*-Alkoxyacetic acids and methyl esters—antifungal activity evaluated *in vitro*, effect of chain length, pH, and adsorbents □ Antifungal activity—*n*-alkoxyacetic acids and methyl esters evaluated *in vitro*, effect of chain length, pH, and adsorbents □ Structure–activity relationships—*n*-alkoxyacetic acids and methyl esters, antifungal activity evaluated *in vitro*

As part of a search for fungitoxic agents, the structure–activity relationships of fatty acids were studied (1–6). In addition, compounds were evaluated for potential use against opportunistic fungi, which are frequent invaders of immunosuppressed and debilitated patients (4–10). Such fungi include species of *Candida*, *Aspergillus*, *Mucor*, and *Cryptococcus* (11).

BACKGROUND

Fatty acids possess significant antifungal activity. For systemic use, in spite of their low toxicities, they have been ineffective, possibly because they are metabolized readily by the host through the usual fatty acid pathways. The fungitoxicity of 2-fluoro fatty acids paralleled that of the nonfluorinated fatty acids (1). Since it is believed that 2-fluoro fatty acids do not undergo β-oxidation (12), they possess at least one potential advantage over the nonfluorinated fatty acids for systemic antifungal activity.

Another approach for preparing fatty acids that do not undergo β-oxidation is to replace the β-methylene group with an oxygen (13). In the structure $ROCH_2COOH$, the C–O–C angle is 111° and the corresponding C–C–C angle is 112°. The C–O bond length is 1.43 Å, and the length of the C–C bond is 1.54 Å. Replacement of the CH_2 group by O would not greatly alter the steric configuration of the analog, and it would not be β-oxidizable because of the lack of β-hydrogens.

The major factors influencing the fungitoxicity of fatty acids and derivatives are the partition coefficient and the absence or presence of adsorbents such as albumin in the growth medium. Chain length, pKa of the acid, and pH of the medium are among the factors determining the partition coefficient of the test compound (6). The deactivating effect of the adsorbent can be explained as being due to hydrogen bonding (5).

No systematic antimicrobial studies with 3-oxa acids or esters have been reported. 11-Fluoroundecyloxyacetic acid and several other 3-oxa acids inhibited *Mycobacterium tuberculosis in vitro* at 50 μg/ml (14).

The present study was concerned with the preparation of the straight-chain 3-alkoxyacetic acids and their methyl esters in which the alkyl groups included 1–9, 11, or 13 carbon atoms. Methoxy- and ethoxyacetic acids were purchased[1]. The following acids were prepared by published methods: *n*-propyloxyacetic acid, *n*-butyloxyacetic acid, *n*-pentyloxyacetic acid, *n*-hexyloxyacetic acid, *n*-heptyloxyacetic acid, *n*-octyloxyacetic acid (15), *n*-undecyloxyacetic acid, and *n*-tridecyloxyacetic acid (13).

The following methyl esters were also previously reported: methyl methoxyacetate, methyl ethoxyacetate, methyl *n*-propyloxyacetate (16), methyl *n*-butyloxyacetate (17), and methyl *n*-octyloxyacetate (18). *n*-Nonyloxyacetic acid was prepared by condensing *n*-nonyl alcohol with chloroacetic acid by means of sodium in toluene, and the previously unknown methyl esters were obtained from the acids by heating with methyl alcohol in the presence of a catalytic quantity of thionyl chloride.

The data characterizing the new compounds are in Table I. The purity of all compounds was verified by GLC.

The fungi used were *Aspergillus niger* (ATCC 1004), *Trichoderma viride* (ATCC 8678), *Myrothecium verrucaria* (ATCC 9095C), *Candida albicans* (ATCC 10231), *Trichophyton mentagrophytes* (ATCC 9129), and *Mucor mucedo* (ATCC 7941).

The compounds were screened against *A. niger*, *T. viride*, and *M. verrucaria* in Sabouraud dextrose agar[2] at pH 4.0 and 5.6 according to published methods (1). Graded levels of test compound dissolved in dimethyl sulfoxide were incorporated into the growth medium, which was then inoculated with the respective fungus. The inoculum consisted of

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Difco, Detroit, Mich.

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Table I—*n*-Alkoxyacetic Acids and Methyl Esters (n-$R_1OCH_2COOR_2$)

R_1	R_2	Yield, %	Boiling Point (mm)[a]	$n_D^{25°}$	ν neat, cm^{-1} C=O	ν neat, cm^{-1} -O-	Formula		Analysis, % Calc.	Analysis, % Found
n-C_9H_{19}	H	47	123° (0.3)[b]	—	1725	1142	$C_{11}H_{22}O_3$	C	65.31	65.20
								H	10.96	11.05
n-C_5H_{11}	CH_3	77	42° (0.4)	1.4230	1770	1142	$C_8H_{16}O_3$	C	59.97	60.11
								H	10.07	9.96
n-C_6H_{13}	CH_3	77	52° (0.3)	1.4184	1780	1148	$C_9H_{17}O_3$	C	62.04	62.00
								H	10.41	10.27
n-C_7H_{15}	CH_3	82	62° (0.3)	1.4235	1780	1146	$C_{10}H_{20}O_3$	C	63.79	63.79
								H	10.71	10.86
n-C_9H_{19}	CH_3	82	64° (0.25)	1.4284	1771	1145	$C_{12}H_{24}O_3$	C	66.63	66.65
								H	11.18	11.11
n-$C_{11}H_{23}$	CH_3	80	108° (0.25)	1.4343	1772	1145	$C_{14}H_{28}O_3$	C	68.85	68.55
								H	11.52	11.37
n-$C_{13}H_{27}$	CH_3	57	127° (0.25)	1.4385	1776	1147	$C_{16}H_{32}O_3$	C	70.54	70.35
								H	11.84	11.70

[a] Analytical sample. [b] Mp 32.5–33.5° (hexane).

Table II—Antifungal Activity of *n*-Alkoxyacetic Acids and Their Methyl Esters at pH 4.0 and 5.6 in Sabouraud Dextrose Agar after 5 Days at 28° against *A. niger*, *T. viride*, and *M. verrucaria*

R	Levels of Inhibition at pH 4.0[a] *A. niger*	*T. viride*	*M. verrucaria*	Antifungal Spectrum Index[b]	Levels of Inhibition at pH 5.6 *A. niger*	*T. viride*	*M. verrucaria*	Antifungal Spectrum Index[b]
				$ROCH_2COOH$				
CH_3	0	1	1	4	0	0	0	0
C_2H_5	0	1	1	4	0	0	0	0
n-C_3H_7	0	1	1	4	0	1	1	4
n-C_4H_9	1	1	1	9	0	1	1	4
n-C_5H_{11}	1	1	2	12	1	1	1	9
n-C_6H_{13}	1	2	2	15	1	2	2	15
n-C_7H_{15}	2	3	2	21	1	2	2	15
n-C_8H_{17}	2	3	3	24	2	3	3	24
n-C_9H_{19}	2	3	3	24	2	3	3	24
n-$C_{11}H_{23}$	0	3	3	12	0	3	3	12
n-$C_{13}H_{27}$	0	1	1	4	0	1	1	4
				$ROCH_2COOCH_3$				
CH_3	1	0	1	4	1	0	1	4
C_2H_5	0	0	0	0	0	0	0	0
n-C_3H_7	0	1	1	4	0	1	1	4
n-C_4H_9	0	1	1	4	0	1	1	4
n-C_5H_{11}	11	1	1	9	1	1	1	9
n-C_6H_{13}	1	1	1	9	1	1	1	9
n-C_7H_{15}	1	1	2	12	1	1	2	12
n-C_8H_{17}	1	2	2	15	1	2	2	15
n-C_9H_{19}	1	2	3	18	1	2	3	18
n-$C_{11}H_{23}$	0	0	0	0	0	0	0	0
n-$C_{13}H_{27}$	0	0	0	0	0	0	0	0

[a] Compounds were incorporated in test medium at 10^4, 10^3, and 10^2 μg/ml; 3 = inhibition at all levels of compound, 2 = inhibition at the two highest levels, 1 = inhibition at the highest level only, and 0 = compound inactive at highest level tested. [b] Antifungal spectrum index = the total number of levels of inhibition multiplied by the number of organisms inhibited.

1 drop of spore suspension containing 6×10^6 spores/ml in 0.85% NaCl solution. Incubation took place at 28° for 5 days.

For *T. mentagrophytes*, *C. albicans*, and *M. mucedo*, the methods described previously were used (7). To the Sabouraud dextrose agar at pH 5.6 and 7.0, both with and without 10% beef serum[3], were added graded levels of test compound dissolved in dimethyl sulfoxide. The inocula of *T. mentagrophytes* and *M. mucedo* consisted of 1 drop of spore suspension containing 6×10^6 spores/ml in 0.85% NaCl solution; the inoculum of *C. albicans* was 1 drop of a suspension obtained from a 20-hr culture in Sabouraud dextrose broth[2] incubated at 37°.

The results reported are numbers of levels of compound causing 100% inhibition of the test organisms. The compounds were tested at 10^4, 10^3, and 10^2 μg/ml. All tests were carried out in duplicate in "I" plate petri dishes[4]. The results were weighted by calculating the antifungal spectrum index, which is defined as the sum of the number of levels of complete inhibition multiplied by the number of organisms inhibited within the defined system (19, 20).

The test results against *A. niger*, *T. viride*, and *M. verrucaria* are presented in Table II; those against *C. albicans*, *T. mentagrophytes*, and *M. mucedo* are in Table III.

[3] Miles Laboratories, Kankakee, Ill.
[4] Falcon.

EXPERIMENTAL[5]

***n*-Nonyloxyacetic Acid**—Sodium metal (30.6 g, 1.33 g-atom) was added to 2000 ml of dry toluene and heated to boiling with vigorous stirring. *n*-Nonyl alcohol (95.9 g, 0.66 mole) dissolved in 300 ml of toluene was added dropwise with continued heating and stirring. When all of the alcohol was converted to the alkoxide, as evidenced by cessation of hydrogen evolution, chloroacetic acid (62.6 g, 0.66 mole) dissolved in 500 ml of toluene was added, and the mixture was stirred and heated overnight.

The toluene suspension was extracted several times with water to dissolve the salts, and the combined aqueous extracts were extracted several times with ether to remove occluded toluene and unreacted octanol. The water solution was acidified with 6 *N* HCl and extracted with ether to remove the oxa acid. The ether extract was dried (sodium sul-

[5] Melting points were taken in a Thomas-Hoover apparatus and are uncorrected. GLC was performed on a Varian Aerograph model 1200 gas chromatograph with a flame-ionization detector to which was attached a Varian Aerograph model 20 recorder. The purity of the acids was established by analyzing the silyl esters on a column containing 3% Dexsil 400 on 90–100-mesh Anachrom A (Analabs, New Haven, Conn.). All samples tested microbiologically were at least 95% pure. IR spectra were obtained with a Perkin-Elmer model 221 spectrophotometer, and refractive indexes were taken with an Abbe-3L B & L refractometer.

Table III—Antifungal Activity of *n*-Alkoxyacetic Acids and Their Methyl Esters at pH 5.6 and 7.0 in Sabouraud Dextrose Agar in the Absence and Presence of Beef Serum against *C. albicans*, *T. mentagrophytes*, and *M. mucedo* [a]

R	Levels of Inhibition [b]												Antifungal [c] Spectrum Index
	C. albicans				*T. mentagrophytes*				*M. mucedo*				
	pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0		
	Without Serum	With Serum	Without Serum	With Serum	Without Serum	With Serum	Without Serum	With Serum	Without Serum	With Serum	Without Serum	With Serum	
						$ROCH_2COOH$							
CH_3	0	0	0	0	1	0	0	0	0	0	0	0	1
C_2H_5	0	0	0	0	1	0	0	0	0	0	0	0	1
n-C_3H_7	0	0	0	0	1	1	0	0	1	1	1	1	12
n-C_4H_9	0	0	0	0	2	1	0	0	1	1	1	1	14
n-C_5H_{11}	1	1	0	0	2	2	1	1	2	1	1	1	39
n-C_6H_{13}	1	1	1	1	2	2	1	1	2	2	1	1	48
n-C_7H_{15}	1	1	1	1	3	2	2	1	2	2	2	2	60
n-C_8H_{17}	2	2	1	1	3	3	2	2	2	2	2	2	72
n-C_9H_{19}	2	2	1	1	3	3	2	2	2	2	1	1	66
n-$C_{11}H_{23}$	0	0	0	0	3	3	3	2	0	0	0	0	11
n-$C_{13}H_{27}$	0	0	0	0	3	2	3	2	0	0	0	0	10
						$ROCH_2COOCH_3$							
CH_3	1	1	1	1	1	1	1	1	1	1	1	1	36
C_2H_5	0	0	0	0	1	0	0	0	1	1	1	1	10
n-C_3H_7	0	0	0	0	1	1	1	1	1	1	1	1	16
n-C_4H_9	1	1	1	1	1	1	1	1	1	1	1	1	36
n-C_5H_{11}	1	1	1	1	1	1	1	1	1	1	1	1	36
n-C_6H_{13}	1	1	1	1	1	1	1	1	2	1	1	1	39
n-C_7H_{15}	1	1	1	1	3	2	2	1	1	1	1	1	48
n-C_8H_{17}	1	1	1	1	3	3	3	3	1	1	1	1	60
n-C_9H_{19}	0	0	0	0	3	3	3	2	0	0	0	0	11
n-$C_{11}H_{23}$	0	0	0	0	3	3	3	2	0	0	0	0	11
n-$C_{13}H_{27}$	0	0	0	0	3	3	3	2	0	0	0	0	11

[a] *C. albicans* and *M. mucedo* were incubated at 37° for 20 hr, and *T. mentagrophytes* was incubated at 28° for 5 days. [b] Same as footnote *a* in Table I. [c] Same as footnote *b* in Table II.

fate), and the solvent was removed in a rotary still. The residue then was fractionated under reduced pressure.

Methyl *n*-Nonyloxyacetate—To 20 g (0.099 mole) of *n*-nonyloxyacetic acid dissolved in 200 ml of methyl alcohol was added 2 ml of thionyl chloride. The mixture was heated under reflux overnight. The solvent was removed in a rotary evaporator, and the residue was distilled fractionally under reduced pressure.

RESULTS AND DISCUSSION

The fungitoxicity of compounds can be compared by using the combined antifungal spectrum indexes at pH 4.0 and 5.6. The 2-alkoxyacetic acids (Table II) were most toxic to *A. niger*, *T. viride*, and *M. verrucaria* at chain lengths of seven to 14 atoms. The methyl esters showed their greatest activity at chain lengths of six to 12 atoms. The order of fungitoxicity of the acids was at chain lengths of 12 = 11 > 10 > 9 > 14 > 8 > 7 > 6 = 16 atoms; for the esters, the order was 12 > 11 > 10 > 9 = 8 > 7 = 6. The antifungal activity of the acids was greater in many cases at pH 4.0 than at pH 5.6, and the activity of the esters was unaffected by the medium pH. The fungitoxicity of the acids was considerably greater than that of the esters.

With respect to *C. albicans*, *T. mentagrophytes*, and *M. mucedo*, the most fungitoxic acids were at chain lengths of six to 16 atoms and the most fungitoxic esters were at chain lengths of three to 16 atoms (Table III). When the pH of the medium was raised and beef serum was added, antifungal activity decreased. The same was true for the esters but to a lesser extent. The organism most affected was *T. mentagrophytes*, with the esters being slightly more active than the acids. The order of toxicity of the alkoxyacetic acids to these fungi according to chain length was 11 > 12 > 10 > 9 > 8 > 7 > 6 > 14 > 16 atoms; for the esters, the order was 11 > 10 > 9 > 8 = 7 = 4 > 6 > 12 = 14 = 16 > 5.

Totaling of the antifungal spectrum indexes of the seven most active acids at pH 4.0 and 5.6 for *A. niger*, *T. viride*, and *M. verrucaria* showed that, on a weight basis, the order of fungitoxicity of five types of fatty acids was: 2-alkynoic acids > 2-alkenoic acids > alkanoic acids > 2-bromoalkanoic acids > 2-fluoroalkanoic acids (6). The present results place the alkoxyacetic acids slightly below the activity of the 2-fluoroalkanoic acids. However, as for the 2-fluoroalkanoic acids, the alkoxyacetic acids should possess a longer life within the host and, consequently, reach the parasite in greater concentration than acids susceptible to β-oxidation.

The relative contributions of α-, β-, and ω-oxidative pathways to fatty acid oxidation in rat liver were studied (21). It was reported that α-oxidation was negligible and that β-oxidation of stearic acid was 25 times as rapid as ω-oxidation. For palmitic acid, β-oxidation was even more rapid than for stearic acid.

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