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Antifungal Properties of 2-*n*-Alkyn-1-ols

HERMAN GERSHON *x, GAIL E. ROWE *, ROBERT C. SANTORE *, JOHN R. GILBERTSON ‡, and HERMAN LANGKAMP §

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Abstract □ Fourteen 2-*n*-alkynols (C_3-C_{14}, C_{16}, and C_{18}) were tested against *Aspergillus oryzae*, *Aspergillus niger*, *Trichoderma viride*, and *Myrothecium verrucaria* in Sabouraud dextrose agar at pH 5.6 and 7.0. Toxicity to *Candida albicans*, *Candida tropicalis*, *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. Fungitoxicity was strongly influenced by chain length, slightly by the pH of the medium, and significantly by the presence of beef serum. 2-*n*-Undecyn-1-ol was the most active member of the series, and there was marked synergism between it and ketoconazole.

Keyphrases □ *n*-2-Alkyn-1-ols—*in vitro* antifungal activity, effect of chain length, pH, adsorbents □ Antifungal activity—*n*-alkynols, *in vitro*, effect of chain length, pH, adsorbents □ Structure-activity relationships—*n*-alkynols, antifungal activity, *in vitro* □ Synergism—2-undecyn-1-ol and ketoconazole, *in vitro*, *Candida albicans* and *Candida tropicalis*

In our studies of the effect of structural modification on the fungitoxicity of alkanoic acids (1), 2-alkenoic acids (2), 2-alkynoic acids (3), 2-fluoroalkanoic acids (1), 2-bromoalkanoic acids (2), α,ω-alkanedicarboxylic acids (4), alkoxyacetic acids (5), and *n*-alkanols (6), two major physical factors were observed to influence the activities of the toxicants. These included the partition coefficient and absence or presence of adsorbents such as albumin in the growth medium. Factors that affect partition coefficients of the acids include chain length, pK_a, and pH of the medium (3). The pH of the medium had no significant effect on the activity of the alcohols, and for increased fungitoxicity, a lipophilic substituent at the terminal end of the alkanol is useful (6).

Since the alkynoic acids (3) were considerably more toxic to fungi than the alkanoic acids (1), it was of interest to compare the alkanols (6) with an analogous series of alkynols. It was reported that 1-hydroxy-2-nonyn-4-one, a metabolite from *Ischnoderma benzoinum* (Wahl.) Karst had antifungal activity (7). Also of interest was the observation that alcohol oxidase was irreversibly inhibited by propargyl alcohol and 1,4-butynediol, acetylenic alcohols (8).

The present work is concerned with a systematic evaluation of the homologous series of 2-*n*-alkyn-1-ols of chain lengths C_3-C_{14}, C_{16}, and C_{18} against eight fungi: *Aspergillus oryzae*, *Aspergillus niger*, *Trichoderma viride*, *Myrothecium verrucaria*, *Candida albicans*, *Candida tropicalis*, *Trichophyton mentagrophytes*, and *Mucor mucedo*. Since synergism was demonstrated between ketoconazole, a promising antimycotic agent (9), and amphotericin B and other fungitoxicants (10), it was also desired to determine if there would be any synergism between the most active of the alkynols and ketoconazole.

EXPERIMENTAL SECTION

Some compounds were obtained from commercial sources[1]. Literature methods were used for the preparation of the remaining alkynols: C_{12} (11), C_{14} (12), and C_{16} and C_{18} (13).

The test fungi included *A. oryzae* (ATCC 1101), *A. niger* (ATCC 1004), *T. viride* (ATCC 8678), *M. verrucaria* (ATCC 9095C), *C. albicans* (ATCC 10231), *C. tropicalis* (ATCC 9741), *T. mentagrophytes* (ATCC 9129), and *M. mucedo* (ATCC 7941) (14)[2]. The compounds were tested against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* in Sabouraud dextrose agar[3] at pH 5.6 and 7.0 according to published methods (1). Graded levels of test compound dissolved in dimethyl sulfoxide (Me_2SO) were incorporated into the growth medium which was subsequently inoculated with the respective fungus. The inoculum was one drop of spore suspension. The preparation of spore suspensions of *A. niger*, *T. viride*, *M. verrucaria*, and *A. oryzae* was accomplished by growing the fungi on potato dextrose agar[3] for several weeks at 28°C in flat, wide-mouth bottles until extensive sporulation had occurred. The spores were harvested by adding 5 mL of sterile 0.85% NaCl solution to the bottles together with ~10 sterile beads, 5 mm in diameter. The spores were freed of the mycelia by shaking, and the suspension was transferred to sterile test tubes and counted in a hemocytometer. The spore concentration was adjusted to 6×10^6 spores/mL by diluting with sterile saline. Inoculations were carried out with Pasteur pipets which deliver 40 drops/mL. Incubation took place at 28°C for 5 d.

For *T. mentagrophytes*, *M. mucedo*, *C. albicans*, and *C. tropicalis* previously described methods were employed (15). Graded levels of test compound

[1] The C_3, C_5-C_{11}, and C_{13} 2-alkyn-1-ols were purchased from Farchan Labs, Willoughby, Ohio, and the C_4 alkynol was obtained from Aldrich Chemical Co., Milwaukee, Wis. Ketoconazole is a product of Janssen Pharmaceutica Inc., New Brunswick, N.J.

[2] These authors present evidence that ATCC 7941 is *Mucor cirinelloides*. We will retain the ATCC nomenclature until it is changed by the American Type Culture Collection.

[3] Difco Labs, Detroit, Mich.

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Table I—Antifungal Activity of 2-*n*-Alkyn-1-ols Against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* in Sabouraud Dextrose Agar [a]

$H(CH_2)_nC{\equiv}CCH_2OH$

	Level of Inhibition at pH 5.6 [b]				Antifungal	Level of Inhibition at pH 7.0				Antifungal
n	*A. oryzae*	*A. niger*	*T. viride*	*M. verrucaria*	Spectrum Index [c]	*A. oryzae*	*A. niger*	*T. viride*	*M. verrucaria*	Spectrum Index
0	0	0	0	1	1	0	0	0	1	1
1	1	1	1	1	16	1	1	1	1	16
2	1	1	1	1	16	1	1	1	1	16
3	1	1	1	1	16	1	1	1	1	16
4	2	2	1	2	28	2	2	1	2	28
5	2	2	2	2	32	2	2	2	2	32
6	2	2	2	2	32	2	2	2	2	32
7	3(60)	3(90)	3(40)	3(30)	48	3(90)	3(90)	3(40)	3(30)	48
8	3(50)	3(40)	3(40)	3(30)	48	3(50)	3(40)	3(40)	3(30)	48
9	3(40)	3(100)	3(30)	3(20)	48	3(50)	3(100)	3(30)	3(20)	48
10	0	0	0	3(30)	3	0	0	0	3(20)	3
11	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0

[a] At 28°C for 5 d. [b] Compounds were incorporated in test medium at 10^4, 10^3, and 10^2 μg/mL. Key: (3) inhibition at all levels of compound; (2) inhibition at the two highest levels; (1) inhibition at the highest level only; (0) compound inactive at the highest level tested; numbers in parentheses are MIC values, μg/mL. [c] Antifungal spectrum index = the sum of the numbers of levels of inhibition multiplied by the number of organisms inhibited.

Table II—Antifungal Activity of 2-*n*-Alkyn-1-ols Against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo* in Sabouraud Dextrose Agar [a]

$H(CH_2)_nC{\equiv}CCH_2OH$

	Levels of Inhibition [b]																
	C. albicans				*C. tropicalis*				*T. mentagrophytes*				*M. mucedo*				
	pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0		Antifungal
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	Spectrum
n	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Index [c]
0	0		0		0		0		1	1	1	1	1	1	1	1	16
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	64
2	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	72
3	2	1	2	1	1	1	1	1	2	1	2	1	2	1	2	1	88
4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	128
5	2	2	2	2	2	2	2	2	3(30)	2	3(40)	2	2	2	2	2	136
6	2	2	2	2	2	2	2	2	3(60)	3(70)	3(70)	3(70)	2	2	2	2	144
7	2	2	2	2	2	2	2	2	3(20)	3(30)	3(20)	3(40)	2	2	2	2	144
8	3(20)	3(90)	3(30)	3(90)	3(30)	3(80)	3(30)	3(90)	3(3)	3(20)	3(4)	3(30)	3(20)	3(60)	3(20)	3(40)	192
9	3(40)	2	3(40)	2	3(70)	2	3(90)	2	3(2)	3(30)	3(3)	3(30)	3(6)	3(30)	3(6)	3(50)	176
10	2	2	2	2	2	2	2	2	3(4)	3(40)	3(4)	3(50)	0		0		84
11	0		0		0		0		3(3)	2	3(5)	2	0		0		10
13	0		0		0		0		0		0		0		0		0
15	0		0		0		0		0		0		0		0		0

[a] In the presence and absence of beef serum. *C. albicans*, *C. tropicalis*, and *M. mucedo* were incubated at 37°C for 20 h and *T. mentagrophytes* at 28°C for 5 d. [b] Compounds were incorporated in test medium at 10^4, 10^3, and 10^2 μg/mL. Key: (3) inhibition at all levels of compound; (2) inhibition at the two highest levels; (1) inhibition at the highest level only; (0) compound inactive at the highest level tested; numbers in parentheses are MIC values, μg/mL. [c] Antifungal spectrum index = sum of the number of levels of inhibition multiplied by the number of organisms inhibited.

dissolved in Me_2SO were added to Sabouraud dextrose agar at pH 5.6 and 7.0 alone and supplemented with 10% beef serum[4] and inoculated with the respective fungi. Inocula for *T. mentagrophytes* and *M. mucedo* were prepared as above except that the growth media were Mycocel agar[5] and Sabouraud dextrose agar[3], respectively. One drop of spore suspension was used. The inocula of *C. albicans* and *C. tropicalis* were obtained from 20-h cultures in Sabouraud dextrose broth[3]. The cell counts ranged from 3 to 7.5 × 10^6 cells/mL. The tests with *T. mentagrophytes* were incubated at 28°C for 5 d; those with *C. albicans*, *C. tropicalis*, and *M. mucedo* were incubated for 20 h at 37°C. All tests were carried out in duplicate in "I" plate Petri dishes[6]. Data of growth or no growth were recorded.

The compounds were tested at 10^4, 10^3, and 10^2 μg/mL. Minimal inhibitory concentrations (MIC) were sought in increments of 10 μg/mL for 10–100 μg/mL and in 1-μg/mL increments for 1–10 μg/mL. 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 at the concentrations of 10^4, 10^3, and 10^2 μg/mL (16, 17).

It should be mentioned that serum was used with the second set of four fungi because they are animal pathogens, except that *M. mucedo* is a model for a pathogenic *Mucor* species. These compounds are also being studied as potential chemotherapeutic agents in animal infections.

[4] Miles Labs, Elkhart, Ind.
[5] BBL, Cockeysville, Md.
[6] Falcon; Becton, Dickenson, and Co., Oxnard, Calif.

The effect of ketoconazole on the inhibition of 2-*n*-undecyn-1-ol of *C. albicans* and *C. tropicalis* in Sabouraud dextrose agar at pH 7.0 containing 10% human serum[3] was examined. The MIC values of ketoconazole and the undecynol were codissolved in Me_2SO and added to the agar in increments of 0.1 ranging from 1 to 0.1. The plates were incubated at 37°C for 20 h, and the MIC for the mixture was determined.

RESULTS

The results of the 2-alkyn-1-ols against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* are given in Table I, and those against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo* are in Table II. The study of the effect of ketoconazole on the toxicity of 2-*n*-undecyn-1-ol to *C. albicans* and *C. tropicalis* is shown in Table III.

On comparing the activity of the alkynols at pH 5.6 and 7.0 against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria*, it is apparent from the antifungal spectrum indices (Table I) that the alcohols of 10–12 carbon atom chain length are most toxic. The order of toxicity of the homologous series is: 12 = 11 = 10 > 9 = 8 > 7 > 6 = 5 = 4 > 13 > 3 > 14 = 16 = 18.

The most fungitoxic alkynol against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo* was the C_{11} homologue, and the overall order of toxicity was: 11 > 12 > 10 = 9 > 8 > 7 > 6 > 13 > 5 > 4 > 3 > 14 > 16 = 18 (Table II). The data of Table III indicate that the MIC for undecynol is 120 μg/mL for both *Candida* species in the presence of human serum at pH 7.0. This level of compound is one-third higher than the corresponding MIC values

Table III—Effect of Ketoconazole (K) on the Minimal Inhibitory Concentration of 2-*n*-Undecyn-1-ol (U) Against *C. albicans* and *C. tropicalis*[a]

	C. albicans	*C. tropicalis*
U	120	120
K	50	50
U + K[b]	36 + 15	36 + 15

[a] In Sabouraud dextrose agar containing 10% human serum at pH 7.0 at 37°C for 20 h; MIC values, μg/mL. [b] Combined MIC values of U and K in increments of 1, 0.9, 0.8 . . . 0.1 parts of the mixture. The numbers represent 0.3 of the combined MIC.

using beef serum. The minimal inhibitory concentrations for ketoconazole was 50 μg/mL against both fungi. A solution containing 30% of the MIC of both compounds was completely inhibitory to both organisms.

DISCUSSION

It was demonstrated previously that in a homologous series of normal alcohols the most fungitoxic member was the C_{10} alkanol (6). In the present study, the C_{11} alkynol was the most active against the fungi. Since biological activity in a homologous series of compounds is influenced by the partition coefficient, which in turn is determined by chain length (lipophilicity) and pK_a (hydrophilicity), the longer chain length of the alkynol required for maximal activity can be rationalized as follows. Aliphatic monobasic hydroxylic compounds in water have a pK_a of 15.5, whereas 2-alkyn-1-ols have a pK_a of 13.6 in water (18). Since water solubility increases with decreasing pK_a, it requires a longer chain length to restore the necessary amphiphilicity for optimal biological activity. The pH of the growth medium has a minor effect on the activity of the test compounds. This can be observed best where low levels of toxicant were required for inhibition of the fungus. It is generally consistent in that at the higher pH more compound is needed for inhibition.

The presence of beef serum reduced the activity of the toxicant (Table II). It was most noticeable against organisms which were inhibited by low levels of compound. Although hydrogen bonding may be responsible for this in part, it is also possible that the alkynol reacted by a nucleophilic reaction with the proteins or other components of the serum.

Although it is not firmly established, the mechanism of action of the acetylenic alcohols, once penetration of the spore had taken place, is by the irreversible inhibition of the enzyme alcohol oxidase. This inhibition was demonstrated with the enzyme, *in vitro*, using propargyl alcohol (8). The lower acetylenic alcohols were not fungitoxic because they are too water soluble to penetrate the fungal spore membrane. Ketoconazole was able to potentiate the toxicity of 2-undecyn-1-ol toward the two *Candida* species (Table III). It was reported that ketoconazole inhibited ergosterol biosynthesis. Thus, it is believed to cause functional changes in the membrane, such as permeability (19).

If we compare the fungitoxicities of the most active member of each of the series of alkynoic acids (3), ω-chloroalkanols (6), alkanols (6), and alkynols by comparing the sums of the antifungal spectrum indices for *A. niger*, *T. viride*, and *M. verrucaria* at pH 5.6 and *C. albicans*, *T. mentagrophytes*, and *M. mucedo* at pH 5.6 and 7.0 with and without beef serum, the order of activity is as follows: 2-*n*-undecyn-1-ol (135) > 2-*n*-hexadecynoic acid (117) > 9-*n*-chlorononan-1-ol (111) = 10-*n*-chlorodecan-1-ol (111) > *n*-nonan-1-ol (93).

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