Antifungal Properties of 3-*n*-Alkyn-1-ols and Synergism with 2-*n*-Alkyn-1-ols and Ketoconazole

HERMAN GERSHON*[x], JULIE A. JEROME*, AND KENNETH F. MCELWAIN[‡]

Received June 11, 1984, from * *Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, NY 14853* and ‡ *6321 Devon Street, Madison, OH 44058.* Accepted for publication December 18, 1984.

Abstract □ Twelve 3-*n*-alkyn-1-ols (C_4–C_{12}, 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 pH of the medium, and significantly but not strongly by the presence of beef serum. 3-*n*-Decyn-1-ol, 3-*n*-undecyn-1-ol, and 3-*n*-dodecyn-1-ol were the most active members of the series. Synergism toward *C. albicans* and *C. tropicalis* was observed between 3-*n*-undecyn-1-ol and ketoconazole, and a mixture of 3-*n*-undecyn-1-ol, 2-*n*-undecyn-1-ol, and ketoconazole in Sabouraud dextrose agar at pH 7.0 in the presence of 10% human serum.

In a recent paper[1] we reported the antifungal activity of the homologous series 2-*n*-alkyn-1-ols. The most active member was 2-*n*-undecyn-1-ol which was more fungitoxic than the most active members of the comparable series of fatty acids and alcohols we studied previously: *n*-alkanoic acids,[2] 2-*n*-alkenoic acids,[3] 2-*n*-alkynoic acids,[4] 2-*n*-fluoroalkanoic acids,[2] 2-*n*-bromoalkanoic acids,[3] α,ω-*n*-alkanedicarboxylic acids,[5] ω-*n*-alkoxyacetic acids,[6] *n*-alkanols,[7] α,ω-*n*-alkanediols,[7] ω-chloro-*n*-alkanols.[7] Two important physical factors were observed to influence the toxicities of these compounds to fungi: partition coefficients and absence or presence of adsorbent such as albumin from the growth medium. Chain length, pK_a, and pH of the medium influence the partition coefficient. The pH of the medium had no strong effect on the activity of the alcohols, but beef serum did cause reduced activity.[1,7] A chlorine atom at the terminal end of the alcohol increased the fungitoxicity significantly.[7]

Because the 2-*n*-alkyn-1-ols demonstrated good antifungal activity against eight fungi (*Aspergillus oryzae*, *Aspergillus niger*, *Trichoderma viride*, *Myrothecium verrucaria*, *Candida albicans*, *Candida tropicalis*, *Trichophyton mentagrophytes*, and *Mucor mucedo*) and showed significant synergism with ketoconazole, a frequently used antimycotic,[8] it was of interest to our program to determine the effect on antifungal activity of moving the triple bond from the 2-position to the 3-position of the alkynol.

It was reported that propargyl alcohol, a 2-alkyn-1-ol, inhibited alcohol oxidase by being oxidized to the aldehyde which then reacted irreversibly with the enzyme by Michael addition. The presence of glutathione almost completely prevented the inhibition by binding the aldehyde.[9]

The present work relates to a systematic study of the fungitoxicity of the homologous series of 3-*n*-alkyn-1-ols of chain lengths C_4–C_{12}, C_{14}, C_{16}, and C_{18} to the same eight fungi. Synergism between one of the most active 3-alkynols and 2-*n*-undecyn-1-ol[1] was sought as well as between ketoconazole and that 3-alkynol alone and with the mixture of 2- and 3-alkynols.

Experimental Section

Melting points were taken in a Thomas-Hoover apparatus and are uncorrected. GC was performed on a Varian Aerograph model 1200 gas chromatograph with a flame-ionization detector to which was attached a Hitachi model QPD 53 recorder. Compound purity was established with a column 1.5 m (5 ft.) × 0.3 cm (⅛ in.) o.d., packed with 3% Dexsil 400 on 90–100-mesh Anachrom A (Analabs, New Haven, CT). Nitrogen was used as the carrier gas. GC–MS results were obtained with a Hewlett-Packard 5985 GC–MS system using helium as the carrier gas; the column employed was the same as described above. IR spectra were obtained with a Perkin-Elmer model 221 spectrophotometer.

All of the 3-alkyn-1-ols were purchased from Farchan Labs, Willoughy, OH, and the ketoconazole was a gift from Janssen Pharmaceutical, New Brunswick, NJ, generously supplied by Mr. George Heinze.

3-*n*-Hexadecyn-1-ol, not previously reported, was prepared according to the method described by Knight and Diamond.[10] The Grignard reagent of 1-tetradecyne was treated with ethylene oxide and subsequently hydrolyzed with cold 20% sulfuric acid. The product was isolated in 48% yield, and the analytical sample was crystallized from hexane, mp 42°C. IR (neat):3185, 2905, 2835, 1473, 1095, and 715 cm^{-1}; MS: *m/z* 238.

Anal.—Calc. for $C_{16}H_{30}O$: C, 80.61; H, 12.68; O, 6.71. Found: C, 80.64; H, 12.37; O, 6.76.

The fungi employed included *A. oryzae* (ATCC 1101), *A. niger* (ATCC 1004), *T. viride* (ATCC 8678), *M. verrucaria* (ATCC 9095 C), *C. albicans* (ATCC 10231), *C. tropicalis* (ATCC 9741), *T. mentagrophytes* (ATCC 9129), and *M. mucedo* (ATCC 7941).[11] Testing of the compounds was carried out in Sabouraud dextrose agar (Difco Labs, Detroit, MI) at pH 5.6 and 7.0 according to published methods.[1] Graded levels of test compound in dimethyl sulfoxide (Me_2SO) were incorporated into the growth medium which was then inoculated with the respective fungus. The inoculum was one drop of spore suspension containing 6×10^6 spores/mL in sterile saline,[1] and incubation took place at 28°C for 5 d.

Previously described methods for testing and inocula preparation were also used for *T. mentagrophytes*, *M. mucedo*, *C. albicans*, and *C. tropicalis*.[1] Graded levels of test compound dissolved in Me_2SO were added to Sabouraud dextrose agar at pH 5.6 and 7.0 alone and supplemented with 10% beef serum (Miles Labs, Elkhart, IN) and inoculated with the respective fungi. The tests with *T. mentagrophytes* were incubated at 28°C for 5 d and those with *C. albicans*, *C. tropicalis*, and *M. mucedo*, for 20 h at 37°C. All tests were carried out in duplicate in "I" plate petri dishes (Falcon, Becton Dickinson, Oxnard, CA). The inocula for *T. mentagrophytes* and *M. mucedo* were one drop of spore suspension containing 6×10^6 spores/mL in sterile saline, and the inocula for *C. albicans* and *C. tropicalis* were one drop of a 20-h culture in Sabouraud dextrose broth. Data of growth or no growth were recorded.

0022-3549/85/0500-0556$01.00/0

The compounds were tested at 10^4, 10^3, and 10^2 μg/mL, and if fungitoxicity was observed at 100 μg/mL, minimal inhibitory concentrations (MIC) were sought in increments of 10 μg/mL between 100 and 10 μg/mL and 1 μg/mL between 10 and 1 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 μl/mL.[12,13] Since these compounds are also being studied as potential chemotherapeutic agents in animal infections, tests with *T. mentagrophytes*, *C. albicans*, *C. tropicalis*, and *M. mucedo* were carried out in the presence of beef serum.

The effect of ketoconazole on the inhibition of 3-*n*-undecyn-1-ol of *C. albicans* and *C. tropicalis* in Sabouraud dextrose agar at pH 7.0 containing 10% human serum (Miles Labs, Elkhart, IN) was examined. Synergism was sought between 3-*n*-undecyn-1-ol and 2-*n*-undecyn-1-ol[1] and between the mixture of the two alkynols and ketoconazole. Levels of ketoconazole and 3-*n*-undecyn-1-ol to yield MICs were codissolved in Me_2SO and added to the agar in increments of 0.1, ranging from 1 to 0.1. The mixture of 3-*n*-undecyn-1-ol and 2-*n*-undecyn-1-ol was treated similarly, as was the mixture of ketoconazole and the combined undecynols. The plates were incubated at 37°C for 20 h, and the MICs for the mixtures were determined.

To determine whether the observations of Nichols and Cromartie[9] on the inhibition of alcohol oxidase by 2-alkynols apply to the present studies, MICs of 2-*n*-undecyn-1-ol and 3-*n*-undecyn-1-ol were added to Sabouraud dextrose agar along with 50-fold excess molar quantities of glutathione. The mixtures were incubated at 37°C for 20 h with inocula of *C. albicans* and *C. tropicalis*, respectively.

Results

The antifungal results of the 3-*n*-alkyn-1-ols against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* are presented 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 combinations of 2-*n*-undecynol, 3-*n*-undecynol, and ketoconazole on toxicity to *C. albicans* and *C. tropicalis* is shown in Table III.

The data of Table I indicate that the alkynols of chain length 10–12 carbon atoms are the most toxic to *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* with 3-*n*-dodecyn-1-ol being the most fungitoxic. The order of toxicity of the homologous series is 12 > 11 > 10 > 9 = 8 > 7 > 6 > 5 > 14 > 4 = 16 = 18. The most toxic alkynols against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo* were also the 10–12 carbon homologues, and the order of toxicity is 10 > 11 = 12 > 9 > 8 > 7 > 6 > 5 > 4 > 14 > 4 > 16 = 18 (Table II).

The data in Table III show that the inhibitory concentrations of 3-*n*-undecynol are 150 and 200 μg/mL against *C. albicans* and *C. tropicalis*, respectively, whereas the MICs for ketoconazole were 50 μg/mL for each of the two fungi. The medium containing 30% of the MICs of 3-*n*-undecynol and ketoconazole completely inhibited *C. albicans*, and the medium containing 20% of the MICs of the two compounds caused complete inhibition of *C. tropicalis*. The medium containing 50% of the MICs of the mixture of 2-*n*-undecynol and 3-*n*-undecynol caused complete inhibition of both *Candida* species. When the medium contained the two alkynols and ketoconazole, complete inhibition was observed at 20% of the MICs of the compounds in the mixture for both species. For *C. albicans*, the inhibitory mixture contained 2-*n*-undecynol (24 μg/mL), 3-*n*-undecynol (30 μg/mL), and ketoconazole (10 μg/mL), and for *C. tropicalis* the inhibitory mixture was composed of 2-*n*-undecynol (24 μg/mL), 3-*n*-undecynol (40 μg/mL), and ketoconazole (10 μg/mL).

The addition of overwhelming concentrations of glutathione to the growth medium containing MICs of 2-*n*-undecyn-1-ol and 3-*n*-undecyn-1-ol had no effect on the inhibition of the two *Candida* species.

Discussion

According to antifungal spectrum index, 3-*n*-dodecyn-1-ol is the most active compound against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* in this study, and 3-*n*-decyn-1-ol is most active against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo*. On comparing the 3-alkynols with the 2-alkynols,[1] it can be seen that the 2-alkynols are somewhat more fungitoxic than the 3-analogues. The effect of pH on the toxicity of the 3-alkynols is modest and is apparent only at low concentrations of compound, whereas the effect of beef serum on fungitoxicity is more marked.

For synergism studies, the 2- and 3-undecynols were selected. If a common intermediate would be involved in the mechanism of action, it might be thus detected. It was considered that an allene could be formed from the 2- and 3-alkynols and possess a common structure derived from both acetylenic alcohols. This type of reaction was first reported by Morisaki and Bloch,[14] in which 3-decynoyl CoA was isomerized to the 2,3-allene that bound irreversibly to β-hydroxydecanoylthioester dehydrase. This concept was extended by Wood and Lee[15] who claimed that 2-hexadecynoyl CoA was similarly isomerized to the 2,3-allene. The IR spectra of the test samples of the 2- and 3-

Table I—Antifungal Activity of 3-*n*-Alkyn-1-ols at pH 5.6 and 7.0 against *A. oryzae*, *A. niger*, *T. viride*, and *M. verrucaria* in Sabouraud Dextrose Agar at 28°C for 5 d

$H(CH_2)_nC{\equiv}CCH_2CH_2OH$

n	Level of Inhibition at pH 5.6[a]				Level of Inhibition at pH 7.0				Antifungal Spectrum Index[b]
	A. oryzae	*A. niger*	*T. viride*	*M. verrucaria*	*A. oryzae*	*A. niger*	*T. viride*	*M. verrucaria*	
0	0	0	0	0	0	0	0	0	0
1	0	0	1	1	0	0	1	1	8
2	1	1	1	1	1	1	1	1	32
3	1	1	2	2	1	1	2	2	48
4	2	2	2	2	2	2	2	2	64
5	2	2	2	2	2	2	2	2	64
6	2	2	3 (90)	3 (70)	2	2	3 (60)	3 (70)	80
7	3 (80)	2	3 (100)	3 (20)	3 (80)	2	3 (90)	3 (20)	88
8	3 (70)	3 (90)	3 (40)	3 (40)	3 (70)	3 (90)	3 (30)	3 (20)	96
10	0	0	0	3 (5)	0	0	0	3 (6)	6
12	0	0	0	0	0	0	0	0	0
14	0	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. 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 = MIC (μg/mL). [b] Antifungal spectrum index equals the sum of the numbers of levels of inhibition multiplied by the number of organisms inhibited. The index was identical at each pH level for each compound.

Table II—Antifungal Activity of 3-*n*-Alkyn-1-ols at pH 5.6 and 7.0 Against *C. albicans*, *C. tropicalis*, *T. mentagrophytes*, and *M. mucedo* in Sabouraud Dextrose Agar in the Absence and Presence of Beef Serum[a]

$H(CH_2)_nC{\equiv}CCH_2CH_2OH$

n	Level of Inhibition at pH 5.6[b]								Level of Inhibition at pH 7.0								Antifungal Spectrum Index[c]
	C. albicans		*C. tropicalis*		*T. mentagrophytes*		*M. mucedo*		*C. albicans*		*C. tropicalis*		*T. mentagrophytes*		*M. mucedo*		
	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	– Serum	+ Serum	
0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	2	2	8
1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	1	1	36
2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	96
3	2	2	1	1	2	2	2	2	1	2	1	1	2	2	2	2	112
4	2	2	2	2	2	2	3 (50)	3 (60)	2	2	2	2	2	2	3 (90)	3 (100)	144
5	2	2	2	2	3 (70)	2	3 (80)	3 (70)	2	2	2		3 (100)	2	3 (80)	2 (70)	152
6	3 (60)	3 (90)	3 (80)	3 (100)	3 (30)	3 (50)	3 (50)	3 (50)	3 (60)	3 (90)	3 (80)	3 (100)	3 (30)	3 (70)	3 (50)	3 (50)	192
7	3 (30)	3 (40)	3 (40)	2	3 (20)	3 (30)	3 (90)	3 (100)	3 (30)	3 (40)	3 (30)	2	3 (20)	3 (30)	3 (90)	3 (100)	184
8	3 (20)	3 (70)	3 (40)	2	3 (50)	3 (50)	3 (70)	3 (60)	3 (20)	3 (70)	3 (30)	2	3 (50)	3 (50)	3 (70)	3 (80)	184
10	0	0	0	0	3 (4)	3 (40)	1	1	0	0	0	0	3 (4)	3 (40)	1	1	32
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] *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 = MIC (μg/mL). [c] Antifungal spectrum index equals the sum of the number of levels of inhibition multiplied by the number of organisms inhibited. The index was identical at each pH level for each compound.

Table III—Minimum Inhibitory Concentrations of 2-*n*-Undecynol, 3-*n*-Undecynol, and Ketoconazole and Combinations of These Compounds Against *C. albicans* and *C. tropicalis*[a]

Compound	Minimum Inhibitory Conc., μg/mL	
	C. albicans	*C. tropicalis*
2-*n*-Undecynol[b]	120	120
3-*n*-Undecynol	150	200
Ketoconazole[b]	50	50
2-*n*-Undecynol + ketoconazole[b,c]	36 + 15 (0.3)[d]	36 + 15 (0.3)
3-*n*-Undecynol + ketoconazole[c]	45 + 15 (0.3)	40 + 10 (0.2)
2-*n*-Undecynol + 3-*n*-undecynol[c]	60 + 75 (0.5)	60 + 100 (0.5)
2-*n*-Undecynol + 3-*n*-undecynol + ketoconazole[c]	24 + 30 + 10 (0.2)	24 + 40 + 10 (0.2)

[a] Tests were carried out in Sabouraud dextrose agar containing 10% human serum at pH 7.0 at 37°C for 20 h. [b] Data taken from ref. 1. [c] Mixtures of compounds at appropriate concentrations to yield MICs of each when added to the agar medium. Increments of 1, 0.9, 0.8, . . ., 0.1 parts of the mixture were tested. [d] The numbers in parentheses represent the part of the mixture causing complete inhibition.

undecynols showed that there were no allenic contaminants present by the absence of peaks in the *1900–2000* cm^{-1} region.

The data of Table III show that the combined MICs of 3-*n*-undecyn-1-ol and ketoconazole formed a synergistic mixture and inhibited the *C. albicans* at the 30% level of the mixture. This was similar to the results with 2-*n*-undecyn-1-ol and ketoconazole.[1] For *C. tropicalis*, the inhibitory level was 20% of the mixture, whereas for the comparable mixture with 2-*n*-undecyn-1-ol the inhibitory level was at 30% of the mixture.[1]

Although 2-*n*-undecyn-1-ol and 3-*n*-undecyn-1-ol inhibited the two *Candida* species at different levels of compound, it was of interest to examine the effect of the mixture of MICs of these compounds on the two test organisms. Both species of *Candida* were inhibited at the 50% level of the mixture. When the inhibitor mixture of the combination of MICs of the 2- and 3-alkynols were tested together with ketoconazole, complete inhibition was observed at the 20% level of the mixture. This indicated synergism between the mixture of alkynols and ketoconazole.

Although it was shown that glutathione could protect alcohol oxidase from irreversible inhibition by propargyl alcohol in vitro,[9] *C. albicans* and *C. tropicalis* were not protected by glutathione against the toxicity of 2-*n*-undecyn-1-ol or 3-*n*-undecyn-1-ol in our tests. This suggests that the mechanism of fungitoxicity of the alkynols may not be that related in the work of Nichols and Cromartie.[9]

References and Notes

1. Gershon, H.; Rowe, G. E.; Santore, R. C.; Gilbertson, J. R.; Langkamp, H. *J. Pharm. Sci.* **1984,** *73,* 1840.
2. Gershon, H.; Parmegiani, R. *J. Med. Chem.* **1967,** *10,* 186–188.
3. Gershon, H.; McNeil, M. W.; Parmegiani, R.; Godfrey, P. K.; Baricko, J. M. *Antimicrob. Agents Chemother.* **1973,** *4,* 435–438.
4. Gershon, H.; Shanks, L. *Can. J. Microbiol.* **1978,** *24,* 593–597.
5. Gershon, H.; Shanks, L. *Can. J. Microbiol.* **1976,** *22,* 1198–1201.
6. Gershon, H.; Shanks, L.; DeAngelis, A. *J. Pharm. Sci.* **1979,** *68,* 82–84.
7. Gershon, H.; Shanks, L. *J. Pharm. Sci.* **1980,** *69,* 381–384.
8. Heeres, J.; Van den Bossche, H. in "Reports in Medicinal Chemistry," vol. 17; Hess, H.-J., Ed.; Academic Press: New York, 1982; pp 139–150.
9. Nichols, C. S.; Cromartie, T. H. *Biochem. Biophys. Res. Commun.* **1980,** *97,* 216–221.
10. Knight, J. A.; Diamond, J. H. *J. Org. Chem.* **1959,** *24,* 400–403.
11. Hammill, T. M.; Secor, D. L.; Thompson, B. E. *Mycologia* **1983,** *75,* 311–315. These authors presented evidence that ATCC 7941 is *Mucor cirinelloides.* We will retain the ATCC nomenclature until it is changed by the American Type Culture Collection.
12. Gershon, H.; Parmegiani, R. *Appl. Microbiol.* **1962,** *10,* 348–353.
13. Gershon, H.; Parmegiani, R.; Nickerson, W. J. *Appl. Microbiol.* **1962,** *10,* 556–560.
14. Morisaki, M.; Bloch, K. *Biochemistry* **1972,** *11,* 309–314.
15. Wood, R.; Lee, T. *J. Biol. Chem.* **1981,** *256,* 12,379–12,386.