

Antifungal Activity of Selected Benzimidazole Compounds

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The comparative antifungal activity of four benzimidazole compounds was determined against 11 fungi.

A recent report by Kilgore and White (1) describes a degradation product of a compound known to have strong antifungal activity—1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester [F-1991 (E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) or "Benlate"]. The degradation product was identified as 2-benzimidazole carbamic acid, methyl ester; limited tests showed that it has an antifungal activity similar to that of Benlate. Thus, Kilgore and White concluded that this breakdown product may actually be the active component of Benlate (1).

During the past year, tests conducted in our laboratory of 11 different pathogenic fungi (both plant and animal pathogens) may support the belief that this degradation product might be the active component of Benlate. In a series of tests on antifungal compounds, we have been examining a compound designated as CTR-6669. This compound is known to be 2-benzimidazole carbamic acid, methyl ester, which is the same compound identified by Kilgore and White as the degradation product of Benlate. We have also examined the antifungal activity of two closely related benzimidazole compounds, parabendazole and thiabendazole. The structures of the four compounds used in this study are shown in Fig. 1. Benlate, CTR-6669, and parabendazole are closely related benzimidazole carbamates, with CTR-6669 having the simplest structure; Benlate contains the butylcarbamoyl group in the 1 position of the ring, whereas parabendazole has a butyl group in the 5 (6) position. Thiabendazole, on the other hand, is not a carbamate but instead contains the 4'-thiazolyl ring.

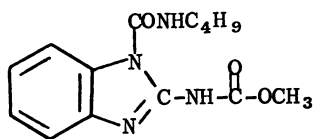
The minimal inhibitory concentration (MIC) values are determined by a tube dilution method similar to that described by Robinson et al. (2). Cultures for inoculation were grown in Sabouraud dextrose broth for 72 hr, collected by centrifugation, and ground to a fine suspension with a tissue homogenizer. Samples were then streaked onto

Sabouraud dextrose agar slants that contained the test material. The test compounds were added to sterile distilled water containing 0.003% of Tween 80 and sonically treated to produce a uniform suspension. The sample was then added to Sabouraud dextrose agar held at 50 C. It solidified almost immediately after slants were poured, and no apparent settling of the test suspension occurred. The samples were examined for fungistatic activity 1 to 2 weeks after growth occurred on control slants. Control slants were prepared identically to the test slants minus the test compound. The pH of the medium was 5.7 with and without the test compound. The MIC was designated as the concentration of the test compound on which the test organisms would no longer grow. Animal pathogens were incubated at 37 C, and plant pathogens were incubated at room temperature (22 C \pm 2 C).

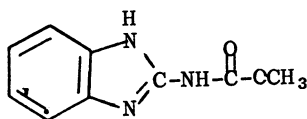
The results presented in Table 1 show that CTR-6669 and Benlate have the same MIC values for the concentrations tested with the exception of the values obtained for *Botrytis cinerea*. In this case, the difference is not extremely great. The two additional compounds that were tested also show similar activities, with thiabendazole showing the closest similarity. The greatest difference between thiabendazole and the two compounds, CTR-6669 and Benlate, is the relative ineffectiveness of thiabendazole for the strain of *Aspergillus fumigatus* that was tested. Parabendazole showed the least fungistatic activity of the four benzimidazoles examined. The MIC values obtained for thiabendazole compare favorably with those presented in the literature with the exception of the low activity against *Alternaria solani* and *A. fumigatus* (2-4).

The suggestion that the degradation product (2-benzimidazole carbamic acid, methyl ester) of Benlate, which is identical to CTR-6669 tested in our laboratory, may actually be the active component of Benlate (1) is supported by the results of this study. These results are not defini-

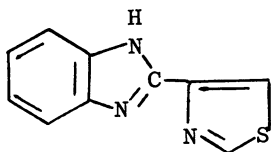
tive since concentrations lower than 1 $\mu\text{g/ml}$ were not tested. Such tests are in progress in conjunction with an overall screening program for a large number of fungistatic compounds. However, a report on the findings described here appear timely in that they support the work of Kilgore and White (1).



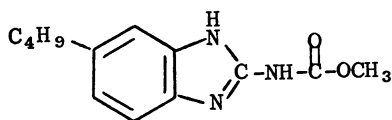
I



II



III



IV

FIG. 1. Structures of the four benzimidazole compounds used in the antifungal tests. (I) Benlate; (II) CTR-6669; (III) thiabendazole; (IV) parabendazole.

TABLE 1. Antifungal activity of four benzimidazole compounds

Organism	Minimal inhibitory concn ($\mu\text{g/ml}$)			
	Benlate	CTR-6669	Parabendazole	Thiabendazole
<i>Alternaria solani</i> SR 70-1	>100	>100	>100	>100
<i>Aspergillus fumigatus</i> SR 70-3	<1	<1	>100	>100
<i>A. niger</i> SR 70-4	<1	<1	<1	<1
<i>Botrytis cinerea</i> SR 70-5	<1	40	<1	<1
<i>Candida albicans</i> SR 70-6	>100	>100	>100	>100
<i>Cryptococcus neoformans</i> SR 70-8	10	10	<1	20
<i>Epidermophyton floccosum</i> SR 70-9	<1	<1	<1	<1
<i>Fusarium roseum</i> SR 70-10	<1	<1	>100	<1
<i>Microsporium canis</i> SR 70-14	<1	<1	>200	<1
<i>Rhizopus stolonifer</i> SR 70-20	>100	>100	>100	>100
<i>Trichophyton mentagrophytes</i> SR 70-22	<1	<1	>100	<1

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