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Antimicrobial activities of some *N*-alkylmaleimides

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SUMMARY

A variety of *N*-alkylmaleimides have been prepared and screened for antimicrobial activity using various fungi, yeasts and bacteria. *N*-Methyl, *N*-octyl and *N*-decylmaleimide showed antifungal activities. All test compounds showed antibacterial activity.

INTRODUCTION

Many studies on the bactericidal and fungicidal actions of *N*-ethyl-maleimide and its related derivatives have been reported [2,6,8]. However, antimicrobial activities of *N*-(higher-alkylated)-maleimides have not been known in detail. We have reported that *N*-(dialkylated-phenyl)-maleimides show good antimicrobial activities [3]. In the present study *N*-alkylmaleimides including *N*-octyl, *N*-lauryl and *N*-myristyl derivatives, were prepared, and examined for antifungal and antibacterial activities of those compounds.

MATERIALS AND METHODS

Materials. All of *N*-(higher-alkylated)-maleimides were prepared by the method according to Cava et al. [1] and Kanaoka et al. [5] and identified by ¹H-NMR and IR.

Antimicrobial activity test. The following fungi were used. *Aspergillus niger* FERM S-1; *Penicillium citrinum* FERM S-5; *Rhizopus stolonifer* FERM S-7; *Cladosporium cladosporioides* FERM S-8; *Aureobasidium pullulans* FERM S-9; *Geotrichum candidum* IFO 4598.

The following yeasts were used. *Rhodotorula rubra* ATCC 4054; *Saccharomyces cerevisiae* ATCC 9804.

The following bacteria were used. *Bacillus subtilis* ATCC 6051; *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 11775; *Pseudomonas aeruginosa* ATCC 10145.

Test method [4,7]. All test chemicals were dissolved in dimethyl formamide (DMF) and were subjected to the following assay. Agar (10 ml) containing test chemicals (5–100 ppm) were placed in a dish having a diameter of 50 mm and solidified. Beef serum (10%) was used for bacteria, and potato soup was used for fungi and yeasts. Spots of microorganisms were inoculated on this agar. The agar was kept at 28°C for 5 days for fungi and yeast, and at 37°C for 24 h for bacteria, respectively. The increase of the sizes of microorganism colonies was observed with the naked eye. Minimum inhibitory concentration (MIC) was determined according to references [4,7].

RESULTS AND DISCUSSION

Antimicrobial activities of *N*-substituted maleimide derivatives are known [2,6,8]. However, the studies on their actions against fungi, yeasts and bacteria in industrial fields have not been reported. *N*-Alkylmaleimides, were prepared and screened for antimicrobial activity against various fungi, yeasts and bacteria. All test chemicals were dissolved in DMF and were subjected to the test. Using the plate dilution method, all of the synthesized chemicals were tested for inhibitory activities against fungi and yeasts in nutrient potato soup agar, and against bacteria nutrient beef serum agar. DMF alone had no antimicrobial activity on the cells under the experimental conditions used in this study. *N*-Methylmaleimide (Tokyo Kasei Chemical Co. Ltd., Tokyo, Japan) was used as a reference compound. Table 1 shows the minimum inhibitory concentration (MIC) of *N*-alkylmaleimides against fungi and yeasts. *N*-Octyl and *N*-decylmaleimides

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TABLE 1

Minimum inhibitory concentration (MIC) of maleimides (1-5) against fungi and yeast

R	MIC (ppm)							
	A.n.	P.c.	C.c.	R.s.	A.p.	G.c.	R.r.	S.c.
1. methyl	20	10	5	20	5	20	20	10
2. <i>n</i> -octyl	20	5	5	> 100	20	50	10	5
3. <i>n</i> -decyl	100	50	5	> 50	50	> 50	50	50
4. <i>n</i> -docecyl	≥ 100	≥ 100				ND ^a		
5. <i>n</i> -myristyl	≥ 100	≥ 100				ND ^a		

Fungi: A.n., *Aspergillus niger*; P.c., *Penicillium citrinum*; C.c., *Cladosporium cladosporioides*; R.c., *Rhizopus stolonifer*; A.p., *Aureobasidium pullulans*; G.c., *Geotrichum candidum*.

Yeasts: R.r., *Rhodotorula rubra*; S.c., *Saccharomyces cerevisiae*.

^a Not done.

TABLE 2

Minimum inhibitory concentration (MIC) of maleimides [1-5] against bacteria

R	MIC (ppm)							
	B.s.		S.a.		E.c.		P.a.	
	8 h ^a	24 h ^a	8 h	24 h	8 h	24 h	8 h	24 h
1. methyl	10-20	> 100	50-100	> 100	10-20	50-100	20-30	40-50
2. <i>n</i> -octyl	20-30	> 100	10-20	40	50-100	> 100	20-30	> 100
3. <i>n</i> -decyl	< 10	30-40	< 10	20-30	100	> 100	20-30	> 100
4. <i>n</i> -dodecyl	< 10	< 10	< 10	10-20	> 100	> 100	> 100	> 100
5. <i>n</i> -myristyl	< 10	< 10	< 10	20-30	> 100	> 100	> 100	> 100

Bacteria: B.s., *Bacillus subtilis* (Gram-positive bacteria); S.a., *Staphylococcus aureus* (Gram-positive bacteria); E.c., *Escherichia coli* (Gram-negative bacteria); P.c., *Pseudomonas aeruginosa* (Gram-negative bacteria).

^a Time of incubation (37 °C).

exhibited antifungal activity against fungi and yeasts at relatively low concentrations. On the other hand *N*-dodecyl and *N*-myristylmaleimides were inactive against the tested microorganisms under the present conditions. These results show that antifungal activity may be decreased in proportion to the increase of alkyl chain length. Interestingly we have found that all of tested chemicals showed antibacterial activity against Gram-positive bacteria at relatively low concentrations as shown in Table 2. From the above mentioned results, we have found that *N*-octyl and *N*-decylmaleimides have excellent antimicrobial activity, and all of the tested *N*-alkylmaleimides can be used for industrial preservatives.

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