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Effects of alkyl parabens on plant pathogenic fungi

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A B S T R A C T

Alkyl parabens are used as antimicrobial preservatives in cosmetics, food, and pharmaceutical products. However, the mode of action of these chemicals has not been assessed thoroughly. In this study, we determined the effects of alkyl parabens on plant pathogenic fungi. All the fungi tested, were susceptible to parabens. The effect of linear alkyl parabens on plant pathogenic fungi was related to the length of the alkyl chain. In addition, the antifungal activity was correlated with the paraben-induced inhibition of oxygen consumption. The antifungal activity of linear alkyl parabens likely originates, at least in part, from their ability to inhibit the membrane respiratory chain, especially mitochondrial complex II. Additionally, we determined that some alkyl parabens inhibit *Alternaria brassicicola* infection of cabbage.

Alkyl parabens (*p*-hydroxybenzoic acid alkyl esters) are common preservatives used in cosmetics due to their properties such as, broad antimicrobial spectrum, relatively low toxicity, good chemical stability, and non-volatility although they have been reported to have potential health risks for humans.[1] Methyl and propyl parabens are mostly used as cosmetic preservatives, and as the number of carbon in the paraben alkyl chain increases, it enhances their antimicrobial activity.[2] Sapra et al. recently tested the antimicrobial potential of alkyl parabens on five microorganisms.[3] They reported that decyl paraben was effective against *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*, while propyl and butyl parabens showed the highest antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*, respectively.[3] Thus, the effect of alkyl parabens vary according to the microorganism treated with it. Short-chain alkyl parabens (ethyl and butyl) were reported to disrupt the function of mechanosensitive channels and induce potassium efflux in *E. coli*; however, the underlying mechanism of the antifungal activity of alkyl parabens is poorly understood.[4a,b,5]

In our previous study, we evaluated the antifungal properties of alkyl gallates and their mode of action.[6] The length of the alkyl chain was linked to their antifungal activity, and nonyl gallate was the most potent antifungal agent. In addition, linear alkyl gallates inhibited mitochondrial complex II activity depending on their chain length, which was consistent with their antifungal activity. Because of a structural similarity between gallates and parabens (Fig. 1), we hypothesized that alkyl parabens may also inhibit the activity of mitochondrial complex II. Therefore, we synthesized alkyl parabens and their derivatives, investigated their antifungal properties and mode of action against plant pathogenic fungi, such as, *Fusarium solani*, *Colletotrichum acutatum*, *Colletotrichum dematium*, and *Alternaria brassicicola*. These fungi are known to cause serious damage to economically important crops and fruits across the world.

The chemicals were prepared as reported in earlier studies, with slight modifications.[6] Substituted benzoic acids and alkanols dissolved in tetrahydrofuran were stirred at room temperature in the presence of *N*,*N*′-dicyclohexylcarbodiimide. The reaction mixture was quenched with distilled water, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried and concentrated under reduced pressure. The resulting oil was purified by a column chromatography. The structures of the alkyl parabens and their derivatives have been shown in Figure 2. Log*P* values were calculated by ChemBioDraw Ultra software (MA, USA). *C. acutatum* (CAB03) was received from Dr. Nakaune.[7] *A. brassicicola* (MAFF No. 726527, 726705, 237450 and 305011)

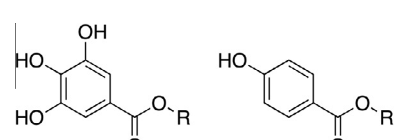

Figure 1. Chemical structures of alkyl gallate (left) and alkyl paraben (right).

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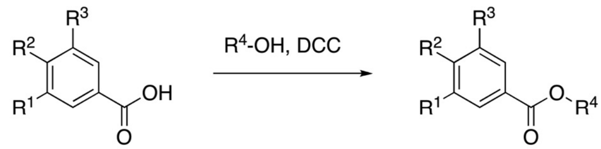

	R^1	R^2	R^3	R^4	log *P*
1	H	OH	H	$-CH_3$	1.46
2	H	OH	H	$-CH_2CH_3$	1.8
3	H	OH	H	$-(CH_2)_2CH_3$	2.29
4	H	OH	H	$-(CH_2)_3CH_3$	2.71
5	H	OH	H	$-(CH_2)_4CH_3$	3.12
6	H	OH	H	$-(CH_2)_6CH_3$	3.96
7	H	OH	H	$-(CH_2)_8CH_3$	4.79
8	H	OH	H	$-C(CH_3)_3$	2.34
9	H	OH	H	$-CH_2C(CH_3)_3$	3.23
10	H	OH	H	$-(CH_2)_2C(CH_3)_3$	3.51
11	OH	OH	OH	$-(CH_2)_8CH_3$	4.01
12	OH	OH	OH	$-(CH_2)_2C(CH_3)_3$	2.73
13	H	H	H	$-(CH_2)_2C(CH_3)_3$	3.9
14	OH	H	H	$-(CH_2)_2C(CH_3)_3$	3.51
15	OH	H	OH	$-(CH_2)_2C(CH_3)_3$	3.12

Figure 2. Chemical structures and log *P* values of parabens.

was purchased from NIAS Genebank (Tsukuba city, Japan). The other fungi were received from Prof. Natsuaki (Tokyo Univ. of Agric.).

We estimated the role of alkyl parabens against plant pathogenic fungi (Tables 1 and 2). Antifungal activities were determined by measuring the minimum inhibitory concentration (MIC).[6] Methyl, ethyl, propyl, butyl, and pentyl parabens were not effective against all the fungal strains tested in this study. As for gallates,[6] an increase in the chain length of linear alkyl parabens led to enhanced antifungal activity. Among the linear alkyl parabens tested (**1**–**7**), nonyl paraben (**7**), which was less active than nonyl gallate (**11**), had the highest antifungal activity. Consistent with the antifungal activity of alkyl gallates,[6] branched alkyl parabens showed stronger activity than linear alkyl parabens with similar log *P* values. Compounds **10** and **12**, which have 4-hydroxyl group, showed the increased antifungal activities in comparison with the compounds **13** and **15**, which do not have 4-hydroxyl group, respectively. This result suggests that the introduction of 4-hydroxyl group is important for these derivatives to show the antifungal activities. In addition, all *A. brassicicola* strains were more sensitive to branched alkyl parabens than the other fungal strains tested (Table 2). Notably, 3,3-dimethylbutyl paraben (**10**) had the highest antifungal activity against *A. brassicicola*.[8] Interestingly, only monohydroxy benzoates (**10** and **14**) showed higher antifungal activity against *A. brassicicola* than against the other fungi. In addition, some alkyl parabens and gallates (**8**, **10**, **11**, **12** and **14**) were effective against benomyl-resistant *C. acutatum* (CAB03). Though benomyl is a fungicide used worldwide, the rapid development of resistant strains has limited its use. These results suggest the possibility of using alkyl parabens as antifungal agents.

It has been proposed that the mode of antimicrobial action of alkyl parabens depends on their ability to disrupt the native membrane due to their hydrophobicity.[9] Though mechanosensitive channels are one of the targets of short-chain alkyl parabens (methyl to butyl), the target of longer-chain alkyl parabens has not been investigated. As previously described, the structurally similar linear alkyl gallates inhibit the mitochondrial complex II activity. In addition, such inhibitory activity against complex II was correlated with the antifungal property of linear alkyl gallates.[6] These findings helped to determine the effects of alkyl parabens on oxygen consumption and activity of complex II in fungi.[10,11] The inhibitory action on oxygen consumption was enhanced by increasing the chain length of linear alkyl parabens in *A. brassicicola* and *F. solani* (Fig. 3A and B). Especially, nonyl paraben (**7**) showed the highest activity consistent with the antifungal activities of linear alkyl parabens. Branched alkyl paraben (**10**) also inhibited the oxygen consumption, although its inhibition was slightly weaker than that of the nonyl parabens (**7**). In addition, nonyl and 3,3-dimethylbutyl parabens (**7** and **10**, respectively)

Table 1
MIC values (μM) of alkyl parabens and derivatives against plant pathogenic fungi

	F. solani	*C. dematium*	*C. acutatum*	*C. acutatum* (CAB03)
1	>50	>50	>50	>50
2	>50	>50	>50	>50
3	>50	>50	>50	>50
4	>50	>50	>50	>50
5	>50	>50	>50	>50
6	50	50	50	>50
7	50	50	25	50
8	25	50	25	25
9	>50	>50	50	50
10	25	25	25	25
11	6.25	12.5	6.25	12.5
12	12.5	25	12.5	12.5
13	>50	>50	>50	>50
14	25	6.25	25	25
15	50	50	>50	>50
Benomyl	3.13*	25*	12.5*	50
Boscalid	3.13	nd	nd	nd

nd: not determined.
* The values of the previously described.[6]