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Potentiation of the activity of β-lactam antibiotics by farnesol and its derivatives

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ABSTRACT

Farnesol, a sesquiterpene alcohol, potentiates the activity of β-lactam antibiotics against antibiotic-resistant bacteria. We document that farnesol and two synthetic derivatives (compounds **2** and **6**) have poor antibacterial activities of their own, but they potentiate the activities of ampicillin and oxacillin against *Staphylococcus aureus* strains (including methicillin-resistant *S. aureus*). These compounds attenuate the rate of growth of bacteria, which has to be taken into account in assessment of the potentiation effect.

Farnesol (**1**), a sesquiterpene, has been shown to exhibit a measurable antimicrobial activity against certain bacteria[1–6] and it inhibits biofilm formation of *Staphylococcus* spp.[7,8] Furthermore, it has been reported to potentiate the activity of β-lactam antibiotics.[3,6] The mechanisms of action of these activities are not well understood. These activities are not unanticipated as farnesol is a chemical constituent of farnesyl pyrophosphate, which is ultimately converted to undecaprenyl pyrophosphate, the key bacterial lipid carrier. Lipid II (Scheme 1A), the precursor of the peptidoglycan, the major constituent of the cell wall, contains undecaprenyl pyrophosphate. All prenyl-containing metabolites, including undecaprenol, are present in limited quantity in bacteria, hence the biosynthetic pathways that utilize them are potential targets of antibiotics.

The report of potentiation of β-lactam activity by farnesol intrigued us.[3,6] We wondered since farnesol, a substrate for several enzymes, exhibited some antibacterial activity, whether structural variants of farnesol could in principle be even better, as they would potentially inhibit enzymes that would bind them as mimetics of farnesol and its phosphorylated derivatives. As an effort to explore this possibility, seven derivatives of *E,E*-farnesol were synthetically prepared (Scheme 1B). First, farnesyl amine (**2**) was prepared by a literature method,[9] as shown in Scheme 1C. Briefly, the hydroxyl group was converted to the corresponding bromo derivative, which was substituted with an amine by the use of lithium bis (trimethylsilyl)amide. Subsequently, both the alcohol in farnesol and amine in compound **2** were further derivatized with various phosphoryl or thiophosphoryl chlorides to yield compounds **4**, **5**, **7**, and **8** using a general literature procedure.[10] The synthesis of compounds **3** and **6** are given as representative examples in Scheme 1D.[11] The overall synthesis of thiophosphate derivatives was similar to that of farnesylphosphate using a base-labile cyanoethyl group as the protective group. The insertion of sulfur was accomplished by a literature method using sulfur powder.[12,13] Among them, compounds **2** and **6** (discussed below) showed improved MIC values compared to the parental farnesol. These two were selected for the study of potentiation with ampicillin (AMP) and oxacillin (OXA), both penicillins.

Three methicillin-resistant *Staphylococcus aureus* (MRSA) strains (NRS100, NRS123 and NRS70) and one methicillin-susceptible *S. aureus* (MSSA) strain (ATCC29213) were used to determine susceptibility to farnesol and its derivatives. The MIC values were examined by the micro-dilution method using cation-adjusted Mueller-Hinton II medium (Difco, USA) supplemented with 1% Tween-80 (CAMHB-T), as described by Kuroda and colleagues.[3] The concentration range was from 2 to 2048 μg/mL for all compounds. The MIC value of the parental farnesol was decidedly poor at 2048 μg/mL, consistent with the data that had been reported.[3] Compounds **2** and **6** (among the synthetic derivatives) exhibited improved yet modest MICs. The MIC values of Compound **2** were 256 μg/mL for strains NRS100 and NRS70, and 512 μg/mL for

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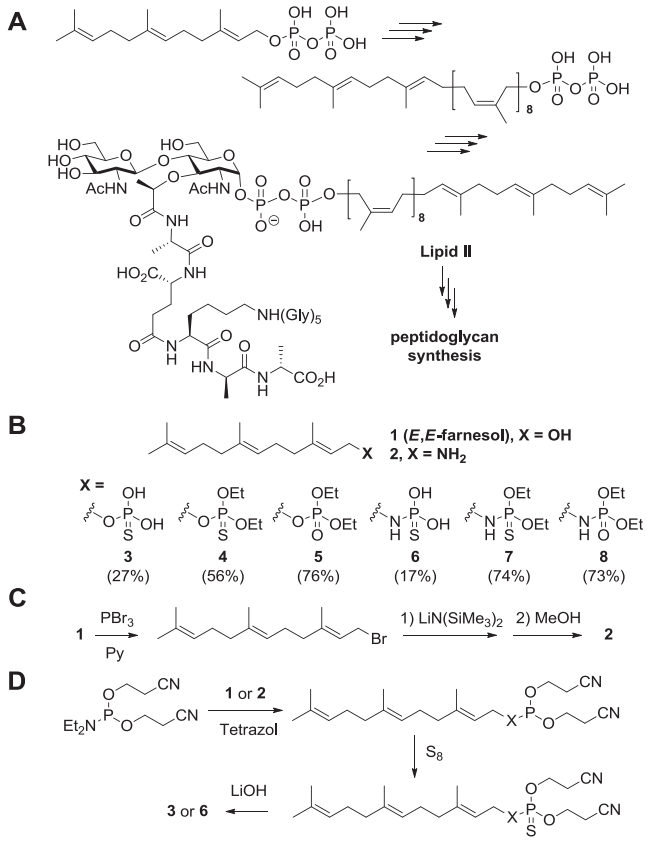

Scheme 1. (A) Lipid II, anchored to the membrane by the undecaprenyl moiety, is the precursor of peptidoglycan. (B) Chemical structures of farnesyl derivatives used in this study. Overall synthetic yields are in parentheses. (C, D) Syntheses of farnesyl amine (**2**) and thiophosphoryl derivative.

strains ATCC29213 and NRS123, whereas compound **6** exhibited MIC of 512 μg/mL to all strains. The MIC values of farnesol compounds could not be determined without Tween-80 since they are immiscible with the media. Tween-80 at 1% of concentration did not affect the MICs of β-lactam antibiotics.

In order to investigate potentiation of β-lactam antibiotics—we used ampicillin (AMP) and oxacillin (OXA)—by farnesol and its derivative, the MIC determination was performed using the Etest (bioMereux, USA) on CAMHB-T agar plates containing farnesol derivatives at half-MIC concentrations: farnesol, 1000 μg/mL for all strains; compound **6**, 256 μg/mL for all strains; compound **2**, 128 μg/mL for NRS100 and NRS70 and 256 μg/mL for ATCC29213 and NRS123. The results are given in Table 1.

The potentiation of AMP and OXA by farnesol was different from what Kuroda *et al.* described.[3] They reported dramatic improvement in susceptibilities to AMP and OXA by farnesol. MIC values of AMP and OXA were reported decreased by 24- and >64-fold for NRS100 and 256- and 96-fold for NRS70, respectively. In our hands, the improvement in susceptibility by farnesol was modest, with MICs for AMP and OXA attenuated by 4- and >32-fold for NRS100, and a mere 4- and 2.7-fold for NRS70, respectively. Farnesol reduced MICs of AMP and OXA as much as 4- and 12-fold for NRS123 as well. We attributed the difference between our determinations and those of Kuroda *et al.* by our observation of slow growth of all strains, especially NRS70, on CAMHB-T agar plates containing 1000 μg/mL of farnesol. The reported large effect of farnesol on susceptibility to β-lactams might be the result of not taking into account the slow growth of bacteria in the presence of farnesol. That is to say that the MIC values determined after the 24-hour incubation in the presence of farnesol were similar to those reported by Kuroda and colleagues (Figure 1). However, the MIC values were significantly larger against the MRSA strains when read after a 48-hour incubation period (Figure 1). Chemical stability of compounds **2** and **6** was checked by LC-MS.[14] A total of 88 (±1)% of compound **2** remains after 48-hour incubation in the growth medium. However, compound **6** was not detected beyond 24-hour of incubation. Compound **6** is a prodrug of compound **2**.

Compound **2** produced larger improvement in susceptibility of strain NRS123 to both β-lactams—12-fold for AMP and 32-fold for OXA—compared to the case of farnesol. As for compound **6**, it exhibited the largest effect with OXA against strains NRS100 (>128-fold) and NRS70 (32-fold); compared to that of farnesol (>32-fold for NRS100 and 2.7-fold for NRS70). When concentration of farnesol was reduced further (256 and 512 μg/mL) and then strains NRS100 and NRS70 were challenged by AMP and OXA, we saw no effect on bacterial growth. The MIC values for the β-lactams under these conditions were the same as those in the absence of farnesol. Compounds **2** and **6** exhibited similar effects on β-lactam susceptibility as the parental farnesol, except they were larger. The MIC drop or potentiation by farnesol and the synthetic derivatives on AMP and OXA against the strains NRS100 and NRS70 was due to attenuation of the rate of the growth.

The potential for synergism between OXA and farnesol (or its derivatives) was checked in the checkerboard assay with measurements of the fractional inhibitory concentration index (FICI).[15–17] We could not observe any synergy between the β-lactams and farnesol or its derivatives with $0.53 \leq \text{FICI} \leq 1.13$ (Fig. 2). Farnesol may best be characterized as a potentiator of β-lactam antibiotics, rather than a synergistic effector.

Table 1
Effect of farnesol and its derivatives on β-lactam susceptibility.[a,b,c]

S. aureus strain	SCC*mec* type	MIC (μg/mL) of ampicillin				MIC (μg/mL) of oxacillin			
		None	Farnesol	**2**[d]	**6**	None	Farnesol	**2**[d]	**6**
ATCC29213		0.5	0.5 (1)	0.2 (2.5)	0.5 (1)	0.4	0.5 (0.8)	0.5 (0.8)	0.4 (1)
NRS100 (COL)	I	16	4 (**4**)[e]	8 (2)	3 (**5.3**)[e]	>256	8 (**>32**)[e]	>256 (–)	2 (**>128**)[e]
NRS70 (N315)	II	16	4 (**4**)[e]	16 (1)	6 (2.7)[e]	32	12 (2.7)[e]	32 (1)	1 (**32**)[e]
NRS123 (MW2)	IV	12	3 (**4**)[e]	1 (**12**)[e]	8 (1.5)	24	2 (**12**)[e]	0.75 (**32**)[e]	16 (1.5)

[a]MIC was determined by Etest on CAMHB-T agar without/with half-MIC of farnesol or its derivatives: 1000 μg/mL of farnesol; 256 μg/mL of **2** and **6**.
[b]Fold ratios of increased susceptibility are in parentheses.
[c]Entry with fold increases of 4 or higher are in bold.
[d]Compound **2** was used at 128 μg/mL for NRS70 (N315) and NRS100 (COL).
[e]The bacterial growth got slower at the half-MICs of farnesol and its derivatives; highlighted. The MIC determination was performed in triplicate; each experiment exhibited similar results.