Antibacterial Activity of Dipropofol and Related Compounds

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Phenolic compounds, in general, exhibit antioxidant and antibacterial activities. We studied antimicrobial activity of the phenolic antioxidants, propofol (2,6-diisopropylphenol), tocopherol, eugenol, butylated hydroxyanisole (BHA), and several of their dimer compounds. Dipropofol (dimer of 2,6-diisopropylphenol) showed strong antibacterial activity against gram-positive strains including methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant Enterococci (VRE), while propofol and other monomeric and dimeric phenols having methyl or tert-butyl groups showed no remarkable activity. The results indicated that the dimeric structure of 2,6-diisopropylphenol moiety may play an important role in the antibacterial activity.

Key words dipropofol; antimicrobial activity; vancomycin resistant Enterococci (VRE); antioxidant; methicillin resistant Staphylococcus aureus (MRSA)

During the past decade, nosocomial infections caused by methicillin-resistant Staphylococcus aureus (MRSA) in hospitals have become a serious clinical problem.2) A glycopeptide antibiotic, vancomycin, has been used for the treatment of infections due to MRSA. Enterococci, which have some a part of the normal faecal flora of humans and animals have generally emerged as important nosocomial pathogens. Acquired resistance to vancomycin and teicoplanin in enterococci is due to the replacement of the cell wall component D-Ala–d-Ala replaced by the depsipeptide D-Ala–D-lactate. This substitution leads to the formation of modified peptidoglycan precursors, for which glycopeptides exhibit 1000-fold lower binding affinities.3) In most clinical isolates of enterococci, resistance is due to acquisition for plasmids that are often transferable to other gram-positive bacteria by conjugation.4) Recently, MRSA strain which has vanA gene with high resistance to vancomycin (minimum inhibitory concentration (MIC) > 128 μg/ml), has been reported in clinical isolates.5) Therefore, a new anti-MRSA antibiotic, which differs from vancomycin in its mode of action, is clinically of interest.

In the course of our screening system for new anti-MRSA and anti-vancomycin resistant Enterococci (VRE) antibiotics from some phenolic compounds, we investigated the antibacterial activity of propofol (2,6-diisopropylphenol), tocopherol, eugenol, butylated hydroxyanisole (BHA), and several of their dimer compounds. Dipropofol, which is an oxidative compound of propofol, showed inhibitory activity against gram-positive bacteria. The present study deals with antibacterial activities of propofol, dipropofol and related phenols.

MATERIALS AND METHODS

Chemicals 2,6-Di-tert-butylphenol, 2,6-dimethylphenol, and 2,6-diisopropylphenol (propofol) were purchased from Sigma-Aldrich Japan K.K. (Tokyo). BHA and eugenol were purchased from Tokyo Kasei Kogyo Co. (Tokyo). α-Tocopherol was purchased from E. Merck (Darmstadt, Germany). Muller-Hinton broth and agar were purchased from Nissui Pharmaceutical Co. (Tokyo).

Synthesis of Dipropofol Propofol (1 g) was dissolved in CHCl3 (10 ml) and stirred with CuCl(OH) · tetramethylethylenediamin (TMEDA) (16 mg) for 5 h at room temperature.6) The reaction product was extracted with AcOEt and then crystallized from hexane to give dipropofol (590 mg, 95%) as a white solid. FAB-MS m/z: 355 (M + H) + . 1H-NMR (500 MHz, CDCl3, δ ppm): 1.32 (24H, d, J = 6.7 Hz, 4 × CH3), 3.20 (4H, m, 4 × CH2), 7.20 (4H, s, 4 × H-Ar). HR-FAB-MS: 355.2640 (Calcd for C24H35O2: 355.2637). mp 108 °C.

Synthesis of DiBHA BHA (10 g) was dissolved in pyridine (10 g) and heated with FeSO4 (100 mg) and 31% H2O2 (20 g × 3) at 60 °C for 3 h. The reaction product was extracted with AcOEt and distilled by steam distillation. The residue was recrystallized from ethanol to give DiBHA (3.52 g, 35%) as a white solid. FAB-MS m/z: 359 (M + H) + . 1H-NMR (500 MHz, CDCl3, δ ppm): 1.43 (18H, s, 2 × tert-butyl), 3.77 (6H, s, 2 × OCH3), 6.60 (2H, s, H-4, 4'), 6.96 (2H, s, H-6, 6'). mp 223 °C.

Synthesis of Di(2,6-di-tert-butylphenol) 2,6-Di-tert-butylphenol (500 mg) was dissolved in CH3Cl2 (10 ml) and stirred with CuCl(OH) · tetramethylethylenediamin (TMEDA) (16 mg) for 2 h at room temperature. The reaction product was extracted with AcOEt and then heated with Na2SO4 (2 g) for 2 h. Then the precipitate was collected on a filter and dried with ether and recrystallized from hexane to give di(2,6-di-tert-butylphenol) (481 mg, 96%) as a white solid. FAB-MS m/z: 411 (M + H) + . 1H-NMR (500 MHz, CDCl3, δ ppm): 1.47 (36H, s, 4 × tert-butyl), 5.23 (2H, s, 2 × OH), 7.36 (4H, s, 4 × H-Ar). mp 112 °C.

Synthesis of Di(2,6-dimethylphenol) 2,6-Dimethylphenol (500 mg) was dissolved in CH3Cl2 (10 ml) and mixed with CuCl(OH) · TMEDA (16 mg) at room temperature at 5 h. The reaction product was extracted with AcOEt and evaporated. The reactant was dissolved in ethanol, Na2SO4 (2 g) was added and the mixture was heated for 2 h. Then the precipitate was collected on a filter and recrystallized from hexane to give di(2,6-dimethylphenol) (462 mg, 93%) as a white solid. FAB-MS m/z: 243 (M + H) + . 1H-NMR (500 MHz, CDCl3, δ ppm): 1.32 (18H, s, 2 × tert-butyl), 3.12 (6H, s, 2 × OCH3), 6.70 (2H, s, H-4, 4'), 6.76 (2H, s, H-6, 6'). mp 223 °C.
CDCl₃, δ, ppm): 2.10 (12H, s, 4×CH₃), 6.41 (2H, s, 2×-OH), 7.22 (4H, s, 4×H-Ar). mp 118 °C.³

**Synthesis of Dieugenol** Eugenol (10 g) was dissolved in pyridine (10 g) and mixed with FeSO₄ (100 mg) and 31% H₂O₂ (20 g×3) at 60 °C for 24 h. The reaction product was extracted with AcOEt and distilled by steam distillation. The residue was recrystallized from ethanol to give dieugenol (3.56 g, 35.6%) as a white solid. FAB-MS m/z: 327 (M+H)+.

1H-NMR (500 MHz, CDCl₃, δ, ppm): 3.33 (4H, d, 3.56 Hz, J= 17.1 Hz), 5.06 (4H, m, J= 9.8 Hz, 2×H-7, 7'), 3.82 (6H, s, 2×OCH₃), 6.74 (2H, s, H-6, 6'), 6.69 (2H, s, H-2, 2'), 6.74 (2H, s, H-6, 6'). mp 93 °C.⁹

**Antibacterial Susceptibility** The minimum inhibitory concentrations (MICs) of test compounds were determined by an agar double dilution method. An overnight bacterial culture was diluted 100-fold (to approximately 10⁶ cfu/ml) and the suspension was inoculated on Muller-Hinton agar plates prepared with each agar. The MICs were determined after overnight culture at 37 °C.¹⁰

**RESULTS AND DISCUSSION**

It is well known that phenolic compounds exhibit anti oxidant and antimicrobial activities. Propofol (2,6-disopropyl phenol), a rapid, short-acting general anesthetic that is widely employed in the induction and maintenance of anesthesia, has antioxidant activity,¹¹ but its antimicrobial activity is unknown. We therefore studied the antibacterial activities of propofol and other phenolic compounds having antioxidant activity (Fig. 1), in the phenolic compounds we tested, only dipropofol showed antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Table 1).

Antimicrobial activities (MICs) of dipropofol were compared with those of vancomycin against standard bacteria. The results are shown in Table 2. The antibacterial spectrum of dipropofol was recognized against gram-positive bacteria but not against gram-negative as vancomycin.

Next, we studied the antibacterial activity of dipropofol against the antibiotic resistant strains MRSA and VRE (Table 3). Dipropofol has strong activity against VRE (MIC...
In general, phenol (at high concentration) acts as a protoplasm toxin to destroy the cell wall system and to precipitate protein in cells, whereas at low concentration it inhibits the multiplication of enzymes \textit{in vitro}. However, phenol and some phenolic compounds such as cresol, thymol, and epigallocatechin gallate, have no antimicrobial activity against MRSA and VRE at low concentration (≤100 μg/ml) (data not shown).\textsuperscript{12,13} In our investigations of the structure–activity relationships of some phenolic antioxidants, dipropofol showed strong antibacterial activity against gram-positive strains including MRSA and VRE, while propofol and other monomeric and dimeric phenols having methyl or \textit{tert}-butyl groups showed no remarkable activity.\textsuperscript{14} The results indicated that the dimeric structure of 2,6-diisopropylphenol moiety may play an important role of the antibacterial activity. We concluded that the mode of action of dipropofol was mediated by inhibition of protein synthesis or an amino acid incorporation system.\textsuperscript{14} However, interaction of the hydroxyl group, isopropyl group, and dimeric skeleton in the expression of antibacterial activity is not clear. Based on the evidence, it was suggested that dipropofol seems to be a useful compound for a hospital infection such as Linezolid, because of its strong antibacterial activity against VRE and MRSA. Mechanistic studies are in progress.

\textbf{Acknowledgments} We thank Kazue Kunikane, Naomi Huse and Aki Ogata for helpful assistance. This work was supported in part by a research grant from the KANPO Science Foundation, Tokyo, Japan, the Ministry of Education, Culture, Sports, Science and Technology, Japan and the New Energy and Industrial Technology Development Organization of Japan.

\textbf{REFERENCES AND NOTES}

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\begin{table}[h]
\centering
\caption{Antibacterial Activities of Dipropofol and Vancomycin against MRSA and VRE}
\begin{tabular}{|l|c|c|}
\hline
Organism & Dipropofol & Vancomycin \\
\hline
MRSA N315 IR94 & 2 & 0.5 \\
MRSA N315 IR94-HR-1 & 2 & 1 \\
MRSA 70 & 2 & 0.5 \\
\textit{E. faecalis} (vanA) & 4 & >128 \\
\textit{E. faecium} (vanA) & 2 & >128 \\
\textit{E. faecalis} (vanB) & 4 & 32 \\
\textit{E. faecium} (vanB) & 4 & >128 \\
\textit{E. gallinarum} (vanC) & 4 & 16 \\
\textit{E. casseliflavus} (vanC) & 4 & 32 \\
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