Isolation of a Potent Anti-MRSA Sesquiterpenoid Quinone from *Ulmus davidiana* var. *japonica*

Dong-Yun SHIN,^a Hwa-Soon KIM,^a Kyung-Hoon MIN,^a Soon-Sil HYUN,^a Soon-Ai KIM,^a Hoon HUH,^a Eung-Chil CHOI,^a Young Hae CHOI,^a Jinwoong KIM,^a Seong-Hak CHOI,^b Won-Bae KIM,^b and Young-Ger SUH^{*,a}

College of Pharmacy, Seoul National University,^a San 56-1, Shinrim-Dong, Kwanak-Gu, Seoul 151-742, Korea and Research Laboratories, Dong-A Pharmaceutical Company,^b 47-5, Sangal-Ri, Kiheung-Up, Yongin-Si, Kyunggi-Do, 449-900, Korea. Received May 29, 2000; accepted July 21, 2000

A highly potent anti-MRSA sesquiterpenoid has been isolated from *Ulmus davidiana* var. *japonica*, which has been traditionally used to treat infectious diseases in Korea. This naturally occurring antibiotic was identified as mansonone F (1). This compound has been found to be highly active specifically against MRSA and showed an MIC range of 0.39–3.13 µg/ml which is comparable to that of vancomycin.

Key words anti-MRSA sesquiterpenoid; *Ulmus davidiana* var. *japonica*; mansonone F

During the last two decades, the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has had an enormous impact on infection control policies.¹⁾ Few, if any classes of glycopeptides, have played a greater role in the severe infection or allergy to β -lactam-derived antibiotics than vancomycin analogues.²⁾ However, even though they are widely used, the structural complexity and toxic side effects of these antibiotics have prompted increased efforts to find and investigate new and effective antibiotics.³⁾

In continuation of our extensive studies on a new anti-MRSA natural alternative to vancomycin, we have recently evaluated the anti-MRSA activities of the components isolated from the root bark of *Ulmus davidiana* var. *japonica*, which has traditionally been used to treat infectious diseases in Korea.⁴⁾ We herewith report the isolation of an important antibacterial component specifically against MRSA strains from the plant.

Experimental

Extraction and Isolation The root bark of *U. davidiana* Planch. var. *japonica* NAKAI were collected in Korea in July 1996 and identified by one (H.H.) of the authors. The dried root bark (2 kg) of this plant was extracted three times with MeOH (8 l) under reflux. The MeOH extract (166 g) in water suspension was partitioned successively with diethyl ether and *n*-BuOH. The residue (17 g) from the ether fraction was chromatographed on a silica gel column using a mixture of *n*-hexane and ethyl acetate (2:3) to afford seven fractions. The fourth fraction (3 g), which showed the highest antibacterial activity (MIC 25 µg/ml) against MRSA, was further chromatographed on a silica gel column using a mixture of CHCl₃ and MeOH (50:1) to give the pure antibacterial component (18 mg) as a characteristic dark purple solid.

Antibacterial Activity Assays The antibacterial activity tests of the extract and mansonone F from this species were carried out according to the National Committee for Clinical Laboratory Standards (NCCLS) procedure⁵⁾ and MICs were determined by a two-fold serial agar dilution method. The media used for preculture and MIC determination were Mueller–Hinton broth (Difco Laboratories, Detroit, MI, U.S.A.) and Mueller–Hinton agar (Difco), respectively. Overnight cultures were diluted with sterile saline solution to the final bacterial density of 10⁷ cfu/ml and each bacterial suspension was inoculated with a replicator (Microplanter, Sakuma, Tokyo, Japan) onto a series of Mueller–Hinton agar plates containing each antibacterial agent. The MIC was defined as the lowest concentration of an antibacterial agent that inhibited the development of visible bacterial growth after incubation for 18 h at 37 °C.

Results and Discussion

The fourth fraction among the diethyl ether-soluble portion of an aqueous MeOH extract of *U. davidiana* var. *japonica* bark showed outstanding antibacterial activity against Gram-positive bacterial strains compared to the other fractions. An active compound (1) was isolated from the fourth fraction using silica gel column chromatography. Its IR spectrum exhibited absorption bands at 1685 cm⁻¹ (C=O), 1600 (olefinic C=C) and 1580 (aromatic C=C). The EI-mass spectrum of 1 showed a characteristic peak of *ortho*-naphthoquinone due to [M+2]⁺ at *m/z* 242. The ¹H-NMR spectrum suggested the compound to be mansonone F (1), showing signals due to three methyl groups (3H each, s at δ 1.92, 2.06, and 2.65). In addition to these signals, a singlet aromatic proton of H-2 (1H, s at δ 7.02) and two doublet aromatic protons of H-4 (1H, d, *J*=8.4 Hz at δ 7.41) and H-5 (1H, d, *J*=8.4 Hz at δ 7.35) were observed. Based on these data together with the ¹³C-NMR spectrum and mp (219–222 °C), the active compound was identified as 1 by comparison of its spectral data with those reported.^{6,7)}

Among the constituents isolated from this plant, 1 was known to be a phytoalexin which is accumulated in the heartwood of the genus *Ulmus* in response to infections of plant pathogens.^{8–10)} This fact indicates the probability of the antibacterial activity of 1. As expected, a component which has shown exceptionally potent antibacterial activity was identified as 1 of a known sesquiterpenoid quinone. This compound was selected after initial antibacterial activity tests of all the isolated constituents against the Gram-positive and Gram-negative bacterial strains. The other components ex-

1

Fig. 1. Chemical Structure of Mansonone F (1)

* To whom correspondence should be addressed.

Table 1. Antibacterial Activities of **1** against Gram-Positive and Gram-Negative Bacterial Strains^{a)}

Standard strains	MIC ($\mu\text{g/ml}$)
<i>Staphylococcus aureus</i> SG 511	1.56
<i>S. aureus</i> Giorgio	1.56
<i>S. aureus</i> 209 P	1.56
<i>S. aureus</i> 285	0.78
<i>S. aureus</i> 503	0.78
<i>S. aureus</i> Smith	0.78
<i>Micrococcus luteus</i> ATCC 9341	0.2
<i>Bacillus subtilis</i> ATCC 6633	0.78
<i>Escherichia coli</i> TEM 02	>50
<i>Pseudomonas aeruginosa</i> ATCC 6027	>50
<i>Klebsiella pneumoniae</i> ATCC 10031	>50
<i>Proteus mirabilis</i> ATCC 14273	>50
<i>Enterobacter cloacae</i> 1321 E	>50
<i>Citrobacter freundii</i> ATCC 9090	>50
<i>Serratia marcescens</i> 6093	>50

a) Only seven representative MICs among the 29 MICs of mansonone F against the Gram-negative strains tested were presented.

Table 2. Antibacterial Activities of **1** against 19 MRSAs^{a)}

Antibacterial agents	MIC ($\mu\text{g/ml}$)		
	Range	MIC ₅₀	MIC ₉₀
Mansonone F	0.39—3.13	1.56	1.56
Vancomycin	0.39—1.56	0.78	0.78

a) The clinical isolates were obtained from Seoul National University Hospital in Seoul, Korea.¹¹⁾

cept **1** showed low or no activity against all the bacterial strains tested.

Table 1 summarizes the minimal inhibitory concentrations (MICs) of **1** against eight Gram-positive bacterial strains and 29 Gram-negative bacterial strains which are routinely used for evaluation of the efficacy of potential antibacterial substances. Mansonone F specifically showed potent activity against all the Gram-positive bacterial strains tested. How-

ever, it showed relatively low or no activity against the Gram-negative bacterial strains.

Because **1** showed excellent activity against all of six *Staphylococcus aureus* strains, its effectiveness against MRSA was also intensively evaluated. Its MICs against 19 MRSA strains were examined. The ranges of MIC, MIC₅₀ and MIC₉₀ for **1** and vancomycin as a positive control are listed in Table 2. All the strains of MRSA were susceptible to mansonone F and vancomycin. In particular, the antibacterial activity of mansonone F against MRSA was comparable to that of vancomycin.

This structurally simple and unique tricyclic *ortho*-naphthoquinone obviously offers a novel and promising lead as a new anti-MRSA drug prospect.

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