Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin-Resistant *Staphylococcus aureus*

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The antibacterial activity of the extracts prepared from 181 species (75 families) of tropical and subtropical plants was screened against various types of pathogenic bacteria. Among the 505 extracts tested, 53 of them inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). The active extracts obtained from barks of *Shorea hemsleyana* and roots of *Cyphostemma bainessi* were separated to their components, some of which greatly reduced the viable cell number of MRSA. These active compounds were all identified as stilbene derivatives. Hemsleyanol D, one of the stilbene tetramer isolated from *S. hemsleyana*, was the most effective compound and had MIC of 2 μg/ml.

Key words —— antibacterial activity, MRSA, plant extract, stilbene derivative

INTRODUCTION

Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganism, animals, and plants. One of such resources is folk medicines and systematic screening of them may result in the discovery of novel effective compounds. We have intensively searched for new effective medicines among natural products, especially in folk medicines around the world. In a survey of bioactive compounds from plant sources, we recently found the active extracts and compounds from western North American plants effective for human immunodeficiency virus-1 reverse transcriptase.[1–3)]

In the course of screening for bacteriostatically or bactericidally active compounds against pathogenic bacteria from tropical and subtropical plants, we here report very active stilbene derivatives isolated from *Shorea hemsleyana* (Dipterocarpaceae) and *Cyphostemma bainessi* (Vitaceae). These natural stilbenes exhibited remarkable inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) as comparable to that of vancomycin.

MATERIALS AND METHODS

Bacterial Strain and Cell Growth —— The cells of *Staphylococcus aureus*, IFO1677 (MRSA, MIC > 200 μg/ml), SU02 (MRSA, MIC > 120 μg/ml), SU03 (MRSA, MIC > 200 μg/ml), and IFO12732 (MSSA), were grown in Tryptic Soy broth (Difco) at 37°C. The two strains of MRSA (SU02 and SU03) were clinically isolated. The screening of antibacterial activity was carried out by a partially modified liquid microdilution method[4)] in the presence of the plant extracts (final concentration of 0.33 or 0.17 mg/ml). The MICs were determined by the liquid microdilution method,[4)] using serially diluted (two-fold) plant extracts.

Plant Materials and Preparation of Extracts —— Tropical or subtropical plant samples were collected or purchased in western U.S.A., Mexico, Guatemala, Honduras, El Salvador, Japan, Indonesia, *etc*. in both Summer of 1997 and 1998. They were identified by J. and H. Murata and F. A Lang. The voucher specimens were deposited in the Herbarium of the Botanical Gardens, Faculty of Science, University of Tokyo. The plant samples were first divided into stems, leaves, fruits, underground parts, *etc*., each of which was extracted with acetone, MeOH, and/or 70% aq. MeOH in that order at room temperature for 2 weeks. Evaporation of the solvents *in vacuo* gave acetone, MeOH, and 70% aq. MeOH extracts, which were used for the plant bioassay. The fresh underground parts of *C. bainesii* cultivated in Ja-

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pan, and the barks part of *S. hemsleyana* collected in Indonesia were extracted with acetone or MeOH as described previously.[5–9)]

RESULTS AND DISCUSSION

To search for an antimicrobial material in the natural sources, in total 505 extract samples obtained from 181 species (75 families) of tropical and subtropical plants were screened by the modified microdilution method. Among the samples tested, 272 samples had an effect on many pathogenic Gram-positive and Gram-negative bacteria including MRSA, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *S. epidermidis*, *Bacillus licheniformis* and vancomysin-resistant *Enterococcus*. No remarkable activity was observed against enterohemorrhagic *Escherichia coli* S180 (O157 : H7), which was clinically isolated from a patient who suffered diarrhea during a mass outbreak in Osaka, Japan, in 1996. Among those extracts, in particulars, 53 of them strongly inhibited the growth of MRSA.

To confirm the antimicrobial activity, we then screened the active fraction and constituents against MRSA as follows. The plant extracts of G254 and G231, which correspond to a MeOH extract of *C. bainessi* and an acetone extract of bark of *S. hemsleyana*, respectively, were further analyzed because these extracts had stronger inhibitory activity for the growth of MRSA after incubation for 24 hr. To identify the active material, the ethyl acetate soluble portion of G254 was further separated into 14 fractions as described,[5)] and four of them, tentatively named CB-1 to CB-4,[5–8)] had the same level of inhibitory activity against the growth of MRSA (data not shown). They were identified as stilbenes, resveratrol, genetin E, parthenocissin A, and ε-viniferin, respectively (Fig. 1).[9)]

The extract of G231was also separated into 23 fractions and were tentatively named SH-1 to SH-23.[5–8)] Among of them, SH-1, SH-13, SH-14, SH-17, and SH-21 showed stronger inhibitory activity against the growth of MRSA, and these were also identified as stilbenes as shown in Fig. 2.[5–8)]

The MIC of the active compounds was determined (Table 1). The most effective compound against MRSA was G231/SH-14 (hemsleyanol D) and its MIC was 2 μg/ml and that of G254/CB-2 (= genetin E) was 4 μg/ml. The same level of MIC was observed when clinically isolated strains of MRSA (MIC ≥ 200 μg/ml) were tested by the same method.

Many types of stilbene polymers found in the

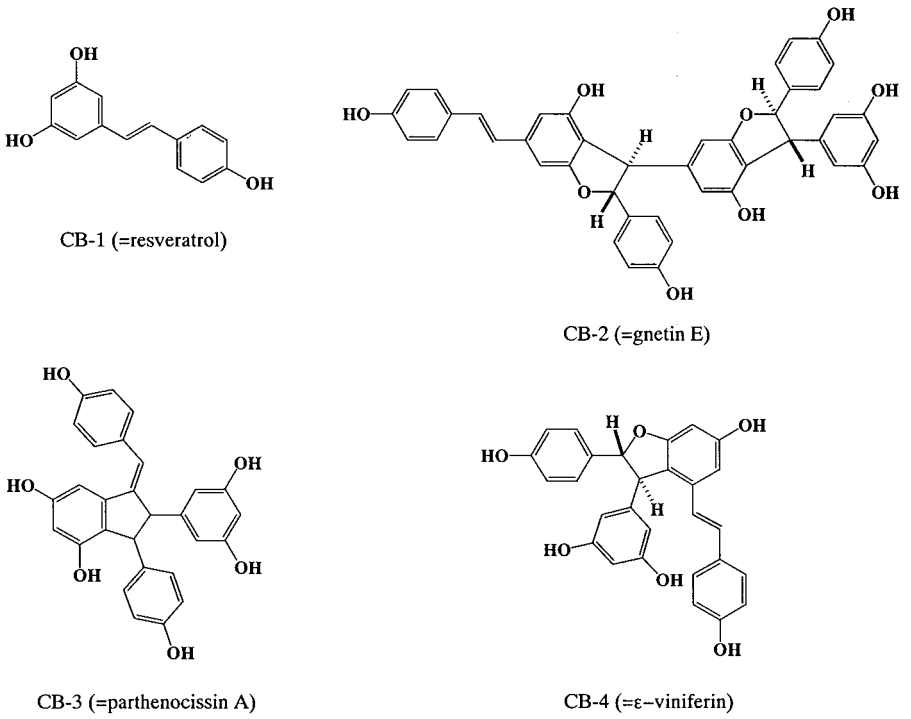

Fig. 1. Structures of Antibacterial Compounds Isolated from *C. baiaessi*

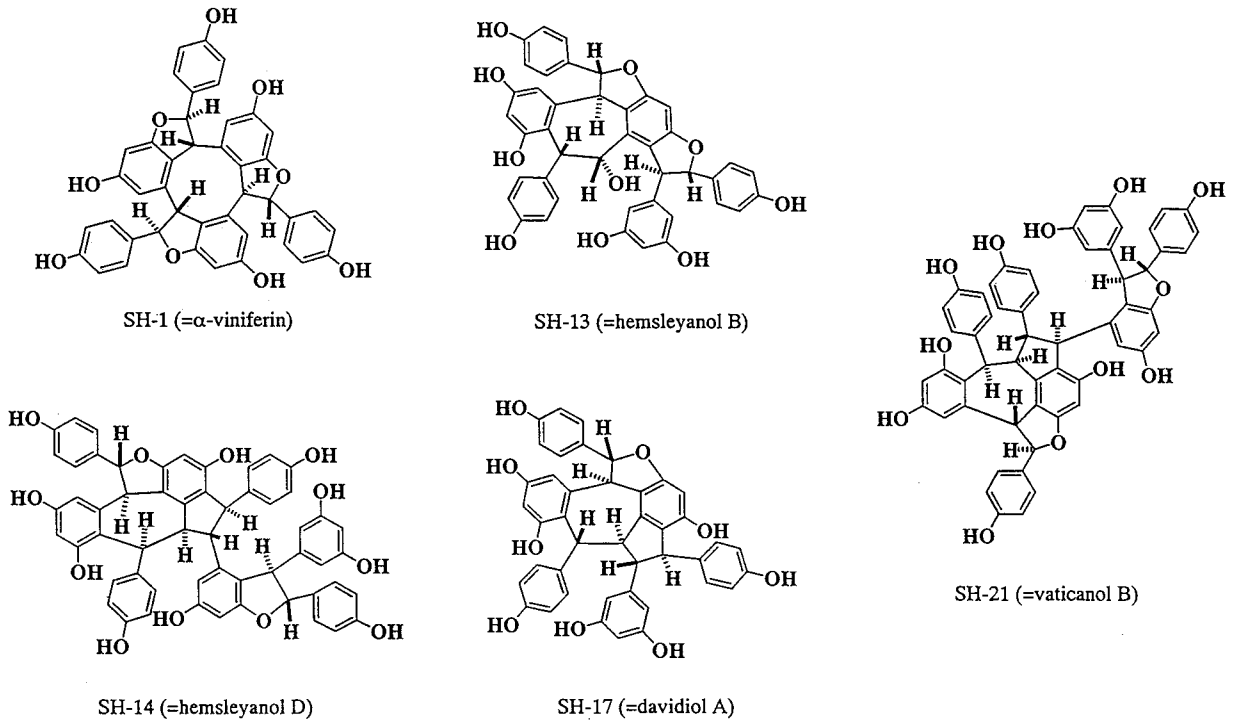

Fig. 2. Structures of Antibacterial Compounds Isolated from *S. hemsleyana*

Table 1. MIC of Active Fractions Obtained from Plants

	MIC (μg/ml)			
Fractions	IFO1677	SU02	SU03	IFO12372
G231/SH-1	8	16	16	ND*
G231/SH-13	16	8	16	ND
G231/SH-14	2	2	2	2
G231/SH-17	16	32	16	ND
G231/SH-21	8	8	4	2
G254/CB-1	>200	>128	>128	ND
G254/CB-2	4	4	8	8
G254/CB-3	32	32	16	ND
G254/CB-4	16	32	32	ND
Vancomycin	1	1	1	1
Methicillin	>200	>128	>128	2

*: Not determined

crude extracts of *S. hemsleyana* and *C. bainesii* showed strong anti-MRSA activity as shown in this work. The relationship between structure and activity in stilbene derivatives is now under investigation. The precise mechanism of action of the stilbene derivatives on MRSA is unclear; one possibility is that they will target the membrane because of their hydrophobicity and will destroy the membrane structure resulting in burst of the cell. Tsuchiya *et al*. have recently reported interesting results that tellimagrandin I extracted from rose red restored the effectiveness of β-lactams such as oxacillin on MRSA.[10,11] To contrast, stilbene derivatives themselves were effective against MRSA without any other antibiotics. We are now investigating further the function of these compounds against MRSA. We succeeded in identification of these compounds, which may have therapeutic value as antibacterial agents against MRSA.

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