Four New Cuparene-Type Sesquiterpenes from *Flammulina velutipes*

by **Ya-Qi Wang**[a]), **Li Bao**[b]), **Xiao-Li Yang**[b]), **Huan-Qin Dai**[c]), **Hui Guo**[c]), **Xin-Sheng Yao***[a]), **Li-Xin Zhang**[c]), and **Hong-Wei Liu***[b])

[a]) Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou 510632, P. R. China (phone/fax: +86-20-85225849; e-mail: tyaoxs@jnu.edu.cn)

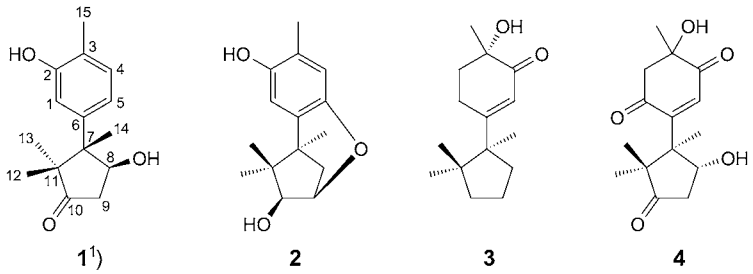
[b]) State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 9 Beiertiao, Zhongguancun, Haidian District, Beijing 100190, P. R. China (phone/fax: +86-10-62566577; e-mail: liuhw@im.ac.cn)

[c]) CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, No. 9 Beiertiao, Zhongguancun, Haidian District, Beijing 100090, P. R. China

Flamvelutpenoids A – D (**1** – **4**), four new cuparene-type sesquiterpenes, were isolated from the solid culture of *Flammulina velutipes*. Their structures were elucidated by NMR experiments. The absolute configurations of **1** and **2** were assigned *via* the circular dichroism data of the $[Rh_2(OCOCF_3)_4]$ complex, whereas that of C(3) of **3** was determined by applying the octant rule for the α,β-unsaturated ketone moiety. Compounds **1** – **4** showed weak antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and methicillin-resistant *Staphylococcus aureus* with *MIC* values larger than 100 μM.

Introduction. – The fungus *Flammulina velutipes* (CURTIS) SINGER, belonging to the family Tricolomataceae (Hymenomycetes, Basidiomycota), is an edible mushroom often consumed in China and Japan. Previous chemical investigations of the fruiting body and mycelial culture of this fungus have revealed bioactive compounds with various chemical structures and interesting medicinal properties, including polysaccharides with immunomodulatory activity [1], proteins with antiviral and immunomodulatory activity [2], lectins with antitumor activity [3], sterols [4], monoterpenetriols [5], and sesquiterpenes with antimicrobial activity [6][7]. To further exploit the potential of *F. velutipes* in producing bioactive natural products, the solid culture of *F. velutipes* was chemically investigated. Herein, we report the isolation and characterization of the four new cuparene-type sesquiterpenes **1** – **4** from *F. velutipes* (*Fig. 1*). Their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and methicillin-resistant *Staphylococcus aureus* was also evaluated.

Results and Discussion. – Compound **1** was isolated as colorless needles. Its molecular formula, $C_{15}H_{20}O_3$ (six degrees of unsaturation), was deduced from HR-ESI-MS and NMR data. Analysis of the ^{1}H- and ^{13}C-NMR (*Table 1*) and HSQC data **1** revealed the presence of four tertiary Me groups at δ(H) 0.61 (*s*, Me(12)), 1.12 (*s*, Me(13)), 1.26 (*s*, Me(14)), and 2.19 (*s*, Me(15)), a CH_2 group at δ(H) 2.38 (*dd*, $J = 8.6$, 19.3 Hz, 1 H–C(9)) and 2.85 (*dd*, $J = 9.2$, 19.3 Hz, 1 H–C(9)), an oxymethine unit at δ(H) 5.22 (*t*, $J = 8.85$ Hz, H–C(8)), a trisubstituted benzene ring at δ(H) 6.89 (*d*,



1[1]) **2** **3** **4**

Fig. 1. *Compounds* **1**–**4**, *isolated from* Flammulina velutipes

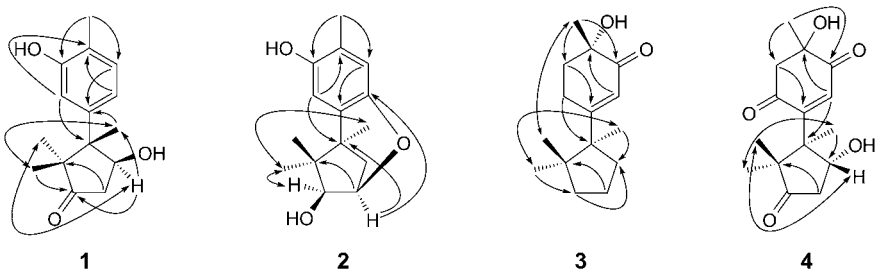
Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[2]). δ in ppm, J in Hz.

	1[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	6.91 (*s*)	114.8	6.50 (*s*)	113.5
C(2)	–	156.3	–	147.4
C(3)	–	123.6	–	123.9
H–C(4)	7.06 (*d*, J = 7.9)	131.4	6.51 (*s*)	118.1
H–C(5)	6.89 (*d*, J = 7.9)	119.3	–	146.1
C(6)	–	142.3	–	131.3
C(7)	–	53.6	–	51.8
H–C(8) or CH_2(8)	5.22 (*t*, J = 8.9)	69.5	2.12 (*dd*, J = 3.1, 12.6), 1.80 (*d*, J = 12.6)	36.7
CH_2(9) or H–C(9)	2.38 (*dd*, J = 8.6, 19.3), 2.85 (*dd*, J = 9.2, 19.3)	30.8	4.33 (*d*, J = 3.5)	83.2
C(10) or H–C(10)	–	228.9	3.92 (br. *s*)	87.3
C(11)	–	56.4	–	46.6
Me(12)	0.61 (*s*)	23.0	0.76 (*s*)	25.0
Me(13)	1.12 (*s*)	18.7	0.92 (*s*)	19.4
Me(14)	1.26 (*s*)	17.8	1.24 (*s*)	18.5
Me(15)	2.19 (*s*)	15.7	2.16 (*s*)	16.2

[a]) In CD_3OD at 600 and 150 MHz, resp. [b]) In $CDCl_3$ at 500 and 125 MHz, resp.

J = 7.9 Hz, H–C(5)), 6.91 (*s*, H–C(1)), and 7.06 (*d*, J = 7.9 Hz, H–C(4)) and δ(C) 114.8 (C(1)), 156.3 (C(2)), 123.6 (C(3)), 131.4 (C(4)), 119.3 (C(5)) and 142.3 (C(6)), and a C=O group at δ(C) 228.9 (C(10)). These data accounted for all of the ^{1}H- and ^{13}C-NMR resonances and required **1** to be a bicyclic sesquiterpene derivative. The NMR data of **1** were unambiguously assigned by HSQC and HMBC experiments. The HMBC cross-peaks H–C(1) and H–C(4)/C(7) and H–C(8)/C(6) supported the linkage of the aromatic ring with the cyclopentanone moiety (*Fig. 2*). In the NOESY plot, the correlations Me(14)/Me(12) and Me(13)/H–C(8) (*Fig. 2*) indicated that Me(12), Me(14), and OH–C(8) are all *cis* oriented. The absolute configuration at C(8) of **1** was determined on the basis of the circular dichroism (CD) of an *in situ* formed complex

[1]) Trivial atom numbering; for systematic names, see *Exper. Part*.



1 **2** **3** **4**

Fig. 2. *Key HMBC* (H → C) *and NOESY* (H ↔ H) *features of compounds* **1**–**4**

with $[Rh_2(OCOCF_3)_4]$, with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **1** in CH_2Cl_2, a metal complex was generated as an auxiliary chromophore. It has been demonstrated that the sign of the *E* band (at *ca.* 350 nm) can be used to correlate the absolute configuration of a secondary alcohol by applying the bulkiness rule [8]. In this case, the Rh-complex of **1** displayed a positive *E* band, correlating with an (8*S*) absolute configuration (*Fig. 3*). Combining with the relative configuration established by the NOE data, the absolute configuration of compound **1** (flamvelutpenoid A) was deduced as (7*S*,8*S*), as shown in *Fig. 1*.

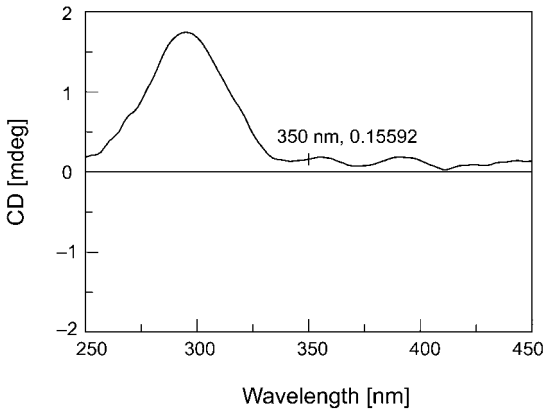


Fig. 3. *CD Spectrum of the Rh-complex of* **1** *with the inherent CD spectrum subtracted*

Compound **2** was obtained as colorless needles. The molecular formula $C_{15}H_{20}O_3$ (six degrees of unsaturation) of **2** was determined by the NMR and HR-ESI-MS data. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**. The ^{13}C-NMR spectrum of **2** (*Table 1*) showed signals for 15 C-atoms, including six aromatic ones, four Me, one CH_2, and two oxymethine groups, and two quaternary C-atoms. The ^{1}H- and ^{13}C-NMR spectra displayed resonances for one aromatic Me group at δ(H) 2.16 (*s*) and δ(C) 16.2 (Me(15)), three quaternary Me groups at δ(H) 0.76 (*s*) and δ(C) 25.0 (Me(12)), δ(H) 0.92 (*s*) and δ(C) 19.4 (Me(13)), and δ(H) 1.24 (*s*)and δ(C) 18.5 (Me(14)), two aromatic H-atoms at δ(H) 6.50 (*s*, H–C(1)) (δ(C) 113.5) and 6.51 (*s*, H–C(4)) (δ(C)

118.1), two oxymethine H-atoms at δ(H) 4.33 (*d*, $J = 3.5$ Hz, H–C(9)) and (δ(C) 83.2) and δ(H) 3.92 (br. *s*, H–C(10)) (δ(C) 87.3), and one CH_2 group at δ(H) 1.80 (*d*, $J =$ 12.6 Hz) and 2.12 (*dd*, $J = 3.1$, 12.6 Hz, CH_2(8)) (δ(C) 36.7). Since **2** has six degrees of unsaturation, it must contain two more (saturated) rings apart from the established benzene ring. Detailed analysis of its HMBC spectrum suggested the presence of the same cuparene skeleton as in **1** (*Fig. 2*). The ether bridge between C(5) and C(9) was supported by the HMBC cross-peak H–C(9)/C(5). The attachment of the OH groups at C(10) and C(2) was determined by the HMBCs Me(12) and Me(13)/C(7), C(10), and C(11), and Me(15)/C(2), C(3), and C(4). The smaller coupling constant observed between H–C(9) and H–C(10) together with the NOE correlations H–C(10)/Me(13) and Me(14) indicated that H–C(9), H–C(10), Me(13), and Me(14) were on the same face of the molecule (*Fig. 2*). The absolute configuration at C(10) was determined by forming the complex with $[Rh_2(OCOCF_3)_4]$ and applying the bulkiness rule as described in the case of **1**. Here, the Rh-complex of **2** also displayed a positive *E* band at 350 nm, correlating with an absolute configuration (*S*) at C(10). Combining with the relative configuration established by NOE data, the absolute structure of **2** was identified as (7*S*,9*R*,10*S*). Thus, the structure of **2** was assigned and designated as flamvelutpenoid B.

Compound **3** was assigned the molecular formula $C_{15}H_{24}O_2$ (four degrees of unsaturation) on the basis of HR-TOF-ESI-MS and NMR data (*Table 2*). The ^{1}H-NMR spectrum of **3** showed signals due to four Me groups at δ(H) 0.82 (*s*, Me(12)), 1.08 (*s*, Me(13)), 1.10 (*s*, Me(14)), and 1.31 (*s*, Me(15)), an olefinic H-atom at δ(H) 6.00 (*s*, H–C(5)), and five CH_2 groups. Its ^{13}C-NMR spectrum together with HSQC data indicated fifteen C-atoms, including four Me groups, five CH_2 groups, three quaternary

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3** *and* **4**[2]). δ in ppm, J in Hz.

	3[a])		**4**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1) or C(1)	2.62–2.68 (*m*), 2.35–2.43 (*m*)	27.8	–	198.6
CH_2(2)	2.10–2.15 (*m*), 1.91–1.96 (*m*)	37.1	3.16 (*d*, $J = 13.6$), 3.08 (*d*, $J = 13.6$)	55.0
C(3)	–	73.1	–	75.2
C(4)	–	203.7	–	201.1
H–C(5)	6.00 (*s*)	123.0	6.83 (*s*)	137.2
C(6)	–	173.5	–	157.2
C(7)	–	53.5	–	53.9
CH_2(8) or H–C(8)	2.17–2.25 (*m*), 1.49–1.55 (*m*)	36.8	4.95 (*dd*, $J = 8.8$, 9.8)	69.3
CH_2(9)	1.74–1.77 (*m*), 1.66–1.72 (*m*)	19.6	2.78 (*dd*, $J = 8.5$, 19.3), 2.43 (*dd*, $J = 10.1$, 19.3)	42.5
CH_2(10) or C(10)	1.68–1.74 (*m*), 1.51–1.56 (*m*)	41.0	–	219.1
C(11)	–	45.1	–	55.1
Me(12)	0.82 (*s*)	26.8	0.84 (*s*)	23.3
Me(13)	1.08 (*s*)	25.1	1.21 (*s*)	20.5
Me(14)	1.10 (*s*)	23.1	1.23 (*s*)	15.3
Me(15)	1.31 (*s*)	24.6	1.43 (*s*)	25.5

[a]) In $CDCl_3$ at 500 and 125 MHz, resp. [b]) In $CDCl_3$ at 600 and 150 MHz, resp.

C-atoms with one bearing an O-atom at δ(C) 73.1, two olefinic C-atoms at δ(C) 123.0 and 173.5, and a C=O group at δ(C) 203.7. These data accounted for all the ^{1}H- and ^{13}C-NMR resonances and required **3** to be a bicyclic compound. Further analysis of the 2D-NMR spectra (HSQC and HMBC) revealed the constitutional formula of **3** (*Fig. 2*). In the CD spectrum of **3**, the negative *Cotton* effect at 335 nm (exciton coupling of the n–π* transition of an α,β-unsaturated ketone moiety of a cyclohexanone) confirmed the (3*S*) configuration of **3** according to the octant rule [9][10]. Considering the same biosynthetic origin of **1**–**3**, the absolute configuration at C(7) was assumed to be (*S*). Thus, the structure of **3** was determined and named as flamvelutpenoid C.

The molecular formula $C_{15}H_{20}O_5$ (six degrees of unsaturation) of **4** was assigned from HR-TOF-ESI-MS and NMR data (*Table 2*). The ^{13}C-NMR spectrum of **4** revealed signals for four Me groups, two CH_2 groups, and one oxymethine group at δ(C) 69.3, three quaternary C-atoms with one bearing an O-atom at δ(C) 75.2, two olefinic C-atoms at δ(C) 137.2 and 157.2, and three C=O groups at δ(C) 198.6, 201.1, and 219.1. The ^{1}H-NMR spectrum of **4** showed signals for four Me groups at δ(H) 0.84 (*s*, Me(12)), 1.21 (*s*, Me(13)), 1.23 (*s*, Me(14)), 1.43 (*s*, Me(15)), one oxymethine group at δ(H) 4.95 (*dd*, $J = 8.8, 9.8$ Hz), and an olefinic H-atom at δ 6.83 (*s*, H–C(5)), and two CH_2 groups. These NMR data were quite similar with those of enokipodin D [7], except for the absence of the trisubstituted C=C bond and for the presence of one additional CH_2 group and a quaternary C-atom bearing an O-atom, which is consistent with the observed molecular-mass difference of 18 amu. Analysis of the 2D-NMR spectra (HSQC and HMBC) of **4** established its constitution (*Fig. 2*). The relative configuration of **4** was assigned on the basis of NOESY data. Correlations of Me(13) with Me(14), and of H–C(8) with Me(12) determined the *cis* relationship of Me–C(7) and OH–C(8) at C(7) and C(8) in the cyclopentanone moiety (*Fig. 2*). The absolute configuration at C(7) was assumed to be (*S*) based on biosynthetic considerations, and thus the (8*R*) configuration can be deduced. The configuration at C(3) remains unsolved at present. From the above analysis, the structure of **4** was assigned and named as flamvelutpenoid D.

Flamvelutpenoids A–D (**1**–**4**) were evaluated for their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and methicillin-resistant *Staphylococcus aureus*. Compounds **1**–**4** showed weak antibacterial activity against the above bacteria with MIC_{90} values larger than 100 μM.

This work was supported by the *National Key Basic Research Project of China* (2009CB522300) and *MOST* (2007DFB31620), *NSFC* (31000036 and 21072219), and grants from the *Chinese Academy of Sciences* (KSCX2-YW-G-074-04).

Experimental Part

General. Column chromatography (CC): *LH-20* (*Amersham Biosciences*) and *ODS* (*Lobar*, 40–63 μm; *Merck*). TLC: silica gel *60 F_{254}*; detection by spraying with 10% H_2SO_4 soln. and heating. Prep. HPLC: *Agilent-1200* HPLC system with an *ODS* column (*RP-8*, 250 × 10 mm; *YMC Pak*, 5 μm; detector UV), flow rate 2.5 ml min^{-1}; t_R in min. Optical rotations: *Perkin–Elmer-241* polarimeter. CD Spectra: *Jasco-J-815* spectropolarimeter; MeOH as solvent; λ_{max} ($\Delta\varepsilon$). IR Spectra: *Nicolet-Magna-IR-750* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian-Mercury-500* and *Varian-Mercury-600*

spectrometers; solvent signals as references ($CDCl_3$: δ(H) 7.26 and δ(C) 77.7; CD_3OD: δ(H) 3.30 and δ(C) 49.9); δ in ppm, J in Hz; HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, resp. ESI-MS: *Bruker-Esquire-3000plus* spectrometer; in *m*/*z*. HR-ESI-MS: *Bruker-APEX-III-7.0 T* spectrometer; in *m*/*z*.

Fungal Material and Cultivation Condition. The strain of *Flammulina velutipes* used in this work is kept in the culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing (access No. CGMCC5.786). The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25° for 10 d. Agar plugs were inoculated in a 500 ml *Erlenmeyer* flask containing 120 ml of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract; the final pH of the media was adjusted to 6.5) before sterilization, and incubated at 25° on a rotary shaker at 170 rpm for one week. A large scale fermentation was carried out in seventeen 500 ml *Fernbach* flasks each containing 80 g of rice and 120 ml of dist. H_2O. Each flask was inoculated with 5.0 ml of the culture medium and incubated at 25° for 40 d.

Extraction and Isolation. The fermented rice substrate was extracted with AcOEt by exhaustive maceration (3×1 l), and the org. solvent was evaporated to afford the crude extract (25 g). The AcOEt extract was subjected to CC (SiO_2, CH_2Cl_2/acetone gradient elution). *Fraction E3* (1 g; eluted with CH_2Cl_2/acetone 100:0) was further separated by CC (*ODS*, (20 → 100% MeOH/H_2O): *Frs. E3.1 – E3.8*. *Fr. E3.2* (20 mg) was purified by reversed-phase HPLC (MeOH/H_2O 4:6): **1** (2 mg; t_R 25.4). *Fr. E3.4* (60 mg) was separated by reversed-phase HPLC (MeOH/H_2O 55:45): **2** (6 mg; t_R 20.2) and **3** (4 mg; t_R 30.6). Compound **4** (1.8 mg; t_R 25.1) was obtained by reversed-phase HPLC (MeOH/H_2O 75:25) purification from *Fr. E3.5* (20 mg).

Flamvelutpenoid A (= *(3S,4S)-4-Hydroxy-3-(3-hydroxy-4-methylphenyl)-2,2,3-trimethylcyclopentanone*; **1**): Colorless needles. $[\alpha]_D^{25} = -46.4$ ($c = 0.16$, MeOH). UV (MeOH): 200 (3.6). IR (neat): 3368, 2975, 2925, 1689, 1586, 1415, 1206, 1135, 1065. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS (pos.): 249.1483 ($C_{15}H_{21}O_3^+$; calc. 249.1485).

Flamvelutpenoid B (= *(2R,3S,5S)-2,3,4,5-Tetrahydro-4,4,5,8-tetramethyl-2,5-methano-1-benzoxepin-3,7-diol*; **2**): Colorless needles. $[\alpha]_D^{25} = +16.6$ ($c = 0.5$, MeOH). UV (MeOH): 200 (4.2), 230 (3.2), 300 (3.0). IR (neat): 3382, 2971, 2875, 1497, 1455, 1415, 1180, 1036, 1006. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS (neg.): 247.1376 ($C_{15}H_{19}O_3^-$; calc. 247.1340).

Flamvelutpenoid C (= *(6S)-6-Hydroxy-6-methyl-3-[(1S)-1,2,2-trimethylcyclopentyl]cyclohex-2-en-1-one*; **3**): Purple oil. $[\alpha]_D^{25} = -68$ ($c = 0.2$, MeOH). CD ($c = 0.13 \cdot 10^{-3}$ M, MeOH): 246 (−5.9), 335 (−1.6). UV (MeOH): 240 (3.8). IR (neat): 3484, 2964, 2877, 1671, 1605, 1461, 1375, 1149, 987. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS (pos.): 237.1834 ($C_{15}H_{25}O_2^+$; calc. 237.1849).

Flamvelutpenoid D (= *5-Hydroxy-2-[(1S,5R)-5-hydroxy-1,2,2-trimethyl-3-oxocyclopentyl]-5-methylcyclohex-2-ene-1,4-dione*; **4**): Yellow needles. $[\alpha]_D^{25} = -98.2$ ($c = 0.17$, MeOH). UV (MeOH): 245 (3.9). IR (neat): 3427, 2974, 2934, 1732, 1690, 1379, 1258, 1127, 1071, 972. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS (pos.): 281.1380 ($C_{15}H_{21}O_5^+$; calc. 281.1384).

Absolute Configuration of **1** *and* **2**. According to a published procedure [8], a sample of **1** or **2** (each 0.5 mg) was dissolved in a dry soln. of $[Rh_2(OCOCF_3)_4]$ (1.5 mg) in CH_2Cl_2 (200 µl) and was subjected to CD measurements at a concentration of 2.5 mg/ml. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary conditions were reached (*ca.* 10 min after mixing). The inherent CD was subtracted. The observed sign of the *E* band at *ca.* 350 nm in the induced CD spectrum was correlated to the absolute configuration of the secondary alcohol moiety.

Antimicrobial Bioassay. Antimicrobial bioassays were conducted in triplicate by following the *National Center for Clinical Laboratory Standards* (*NCCLS*) recommendations. *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051), and methicillin-resistant *Staphylococcus aureus* (*MRSA*, clinical isolates, Beijing Chao-yang Hospital) were obtained from the *China General Microbial Culture Collection* (*CGMCC*). *E. coli*, *MRSA*, and *B. subtilis* were grown in an agar plate by using a LB medium consisting of 0.5% yeast extract, 1% peptone, 0.5% NaCl, and 2% agar in deionized H_2O. The *A. fumigatus* strain was grown in the PDA medium. The assay was carried out in a flat-bottom 96-well microtiter plate, according to the method described in *Fiedler* and co-workers' report [11], with some modifications. A soln. of tbe compound in DMSO (2 µl) was transferred to each well. *E. coli*, *MRSA*, and *B. subtilis* diluted by LB medium (without agar) with the final concentration of $1 \cdot 10^5$ CFU/ml were added to medium (100 µl) and incubated at 28° for 24 h, and then the *OD* value was determined at

595 nm with a *Perkin–Elmer-EnVision*™ multilabel plate reader *2103*. *MIC* is defined as the lowest concentration of compound that results in inhibition of visible bacterial growth (no turbidity) compared with the positive control. Positive control drugs were used, *i.e.*, vancomycin for the *MRSA* assay with a *MIC* of 1 μg/ml, vancomycin for the *B. subtilis* assay with a *MIC* of 1 μg/ml, ciprofloxacin for the *E. coli* assay with a *MIC* of 0.25 μg/ml.

REFERENCES

[1] S. P. Wasser, A. L. Weis, *Crit. Rev. Immunol.* **1999**, *19*, 65.
[2] P.-H. Wang, C.-I. Hsu, S.-C. Tang, Y.-L. Huang, J.-Y. Lin, J.-L. Ko, *J. Agric. Food Chem.* **2004**, *52*, 2721.
[3] H. Wang, T. B. Ng, V. E. C. Ooi, *Mycol. Res.* **1998**, *102*, 897.
[4] Y. Yaoita, K. Amemiya, H. Ohnuma, K. Furumura, A. Masaki, T. Matsuki, M. Kikuchi, *Chem. Pharm. Bull.* **1998**, *46*, 944.
[5] Y. Hirai, M. Ikeda, T. Murayama, T. Ohata, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1364.
[6] N. K. Ishikawa, K. Yamaji, S. Tahara, Y. Fukushi, K. Takahashi, *Phytochemistry* **2000**, *54*, 777.
[7] N. K. Ishikawa, Y. Fukushi, K. Yamaji, S. Tahara, K. Takahashi, *J. Nat. Prod*. **2001**, *64*, 932.
[8] J. Frelek, W. J. Szczepek, *Tetrahedron: Asymmetry* **1999**, *10*, 1507.
[9] W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, C. Djerassi, *J. Am. Chem. Soc.* **1961**, *83*, 4013.
[10] C. Djerassi, R. Records, E. Bunnenberg, K. Mislow, A. Moscowitz, *J. Am. Chem. Soc.* **1962**, *84*, 870.
[11] J. Riedlinger, A. Reicke, H. Zähner, B. Krismer, A. T. Bull, L. A. Maldonado, A. C. Ward, M. Goodfellow, B. Bister, D. Bischoff, R. D. Süssmuth, H.-P. Fiedler, *J. Antibiot.* **2004**, *57*, 271.

Received July 14, 2011