# Sesquiterpenoids from the Root Bark of *Acanthopanax leucorrhizus* and Their Biological Activities

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Three furoeremophilane-type sesquiterpenoids, including one new, 1$\alpha$-acetoxy-6$\beta$-(benzoyloxy)-10$\beta$-hydroxy-9-oxofuroeremophilane (**1**), and two known, 1$\beta$,6$\beta$-diacetoxy-9-oxofuroeremophilane (**2**) and (6$\alpha$)-furoeremophilan-14,6-olide (**3**), were isolated from the root bark of *Acanthopanax leucorrhizus* from China. Their structures were elucidated on the basis of comprehensive spectroscopic analyses, including IR, HR-ESI-MS, 1D- and 2D-NMR experiments. A preliminary bioassay revealed that compound **1** exhibits weak cytotoxicities against the human tumor cell lines MCF-7 and SMMC-7721 with the $IC_{50}$ values of $75.12 \pm 1.69$ and $168.36 \pm 2.01$ μg/ml, respectively. Compound **1** and **2** showed moderate activities against *Escherichia coli* with the *MIC* values of 32 and 64 μg/ml, respectively.

**Introduction.** – The *Acanthopanax* genus of the Araliaceae family (37 species (excluding varieties) around the world), is widely distributed in Korea, Japan, China, and the far-eastern regions of Russia. About 26 species and 18 varieties grow in mainland China [1][2]. The roots and stem barks of these plants have been used for a long time as a tonic and sedative [3][4], as well as for the treatment of rheumatism, diabetes, bronchitis, hypertension, stress and ischemic heart disease, and gastric ulcer [5][6]. *Acanthopanax leucorrhizus* HARMS is an endemic medicinal plant growing abundantly in Gansu province of China. Its stem barks possess biological features such as removing wind-dampness, smoothening energy channel, and strengthening bones and muscles actions, and they have been used for a long time to treat rheumatism, numbness, contracture, quadribblelegia, hemiplegia, traumatic injury, edema, and itchy skin [7]. However, to the best of our knowledge, few phytochemical and bioactivity informations have been reported on this plant except for the chemical composition, antimicrobial, antioxidant, and cytotoxic properties of the essential oil [8]. As a part of our ongoing search for bioactive constituents from this plant, further investigation of the EtOH extract led to the isolation of three furoeremophilane-type sesquiterpenoids, **1**–**3**, from the root bark of *A. leucorrhizus*. Herein, we report the isolation, structure elucidation, and cytotoxic and antimicrobial activities of three metabolites, **1**–**3** (*Fig. 1*).

**Results and Discussion.** – Compound **1** was obtained as colorless crystals. The molecular formula was determined as $C_{24}H_{26}O_7$ by the molecular-ion peak at *m*/*z* 425.1587 ($[M-H]^-$; calc. 425.1600) in the negative-ion mode HR-ESI-MS, implying twelve degrees of unsaturation. The IR and UV spectra exhibited absorptions at 1681

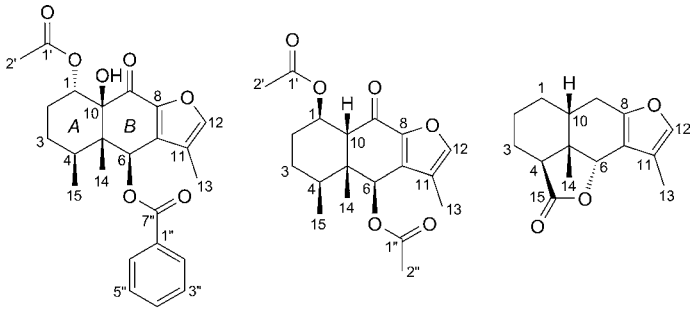

Fig. 1. *Structures of compounds* **1**–**3**

and 1593 $cm^{-1}$, and $\lambda_{max}$ 281 nm (log $\varepsilon$ 4.12), respectively, suggesting the presence of a typical 9-oxofuranoeremophilane skeleton [9]. In addition, the IR spectrum also evidenced the presence of OH (3418 $cm^{-1}$), unsaturated ester (1721 $cm^{-1}$), (1604 $cm^{-1}$), and Ph groups (1624 and 1450 $cm^{-1}$), and conjugated C=C bonds. The $^1$H- and $^{13}$C-NMR spectra (*Table 1*) indicated that compound **1** possesses three tertiary Me groups ($\delta$(H) 1.91 (*s*, Me(13)), 1.05 (*s*, Me(14)), and 0.85 (*d*, $J = 6.8$, Me(15)); $\delta$(C) 8.4 (*q*, Me(13)), 15.8 (*q*, Me(14)), and 15.2 (*q*, Me(15))), one furan ring ($\delta$(H) 7.28 (*s*, H–C(12)); $\delta$(C) 138.7 (*s*, C(7)), 146.3 (*s*, C(8)), 120.8 (*s*, C(11)), and 146.5 (*d*, C(12))), one AcO group ($\delta$(H) 2.19 (*s*, Me(2′)); $\delta$(C) 170.6 (*s*, C(1′)), and 20.8 (*q*, Me (2′))), one *O*-benzoyl group ($\delta$(H) 7.95 (*dd*, $J = 8.2$, 1.0, H–C(2″), H–C(6″)), 7.54–7.61 (*m*, H–C(4″)), and 7.41–7.46 (*m*, H–C(3″), H–C(5″)); $\delta$(C) 130.5 (*s*, C(1″)), 129.1 (*d*, C (2″), C(6″)), 128.3 (*d*, C(3″), C(5″)), 132.4 (*d*, C(4″)); and 165.2 (*s*, C(7″))), two cyclic $CH_2$ groups ($\delta$(H) 1.64–1.67 (*m*, $H_\alpha$–C(2)) and 2.21–2.29 (*m*, $H_\beta$–C(2)), 2.32–2.38 (*m*, $H_\alpha$–C(3)) and 1.43–1.52 (*m*, $H_\beta$–C(3)), $\delta$(C) 21.1 (*t*, C(2)) and 23.8 (*t*, C(3))), one cyclic CH group ($\delta$(H) 1.68–1.74 (*m*, H–C(4)), $\delta$(C) 31.8 (*d*, C(4))), two O-bearing CH groups ($\delta$(H) 4.88 (br. *s*, H–C(1)) and 6.97 (*s*, H–C(6)); $\delta$(C) 74.6 (*d*, C(1)) and 66.9 (*d*, C(6))), one tertiary OH group ($\delta$(H) 4.18 (*s*, HO–C(10)), $\delta$(C) 79.2 (*s*, C(10))), one quaternary C-atom ($\delta$(C) 51.0 (*s*, C(5))), and an allylic CO group ($\delta$(C) 185.9 (*s*, C(9)); *Table 1*). From these spectral characteristics, compound **1** was considered as a 9-oxofuranoeremophilane derivative endowed with one OH, one AcO, and one BzO group [10].

The locations of the substituents in compound **1** were established by its HMBC spectrum (*Fig. 2*). Two long-range correlations of H–C(6) ($\delta$(H) 6.97) with C(7″) ($\delta$(C) 165.2), and of H–C(1) ($\delta$(H) 4.88) with C(1′) ($\delta$(C) 170.6) suggested that the AcO and BzO groups were at C(1) and C(6), respectively. The correlations of Me(15) ($\delta$(H) 0.85) with C(3) ($\delta$(C) 23.8), C(4) ($\delta$(C) 31.8), and C(5) ($\delta$(C) 51.0), of Me(14) ($\delta$(H) 1.05) with C(4), C(5), C(6) ($\delta$(C) 66.9), and C(10) ($\delta$(C) 79.2) indicated that the Me(15) and Me(14) were at C(4) and C(5), respectively. The correlations of Me(13) ($\delta$(H) 1.91) with C(7) ($\delta$(C) 138.7), C(11) ($\delta$(C) 120.8), and of C(12) ($\delta$(C) 146.5), H–C(12) ($\delta$(H) 7.28) with C(11) and C(8) ($\delta$(C) 146.3) indicated that the two C=C bonds are located between C(7) and C(8), and C(11) and C(12). The $^{13}$C-NMR spectra also revealed that **1** contained three $sp^3$ C-atoms connected to O-atoms ($\delta$(C) 74.6 (*d*), 66.9 (*d*) and 79.2 (*s*)), indicating that the OH group was located at C(10). Based on

Table 1. *$^1$H- and $^{13}$C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of Compound* **1**. $\delta$ in ppm, $J$ in Hz. Atom numbering as indicated in *Fig. 1*.

| Position | $\delta$(H) | $\delta$(C) | Position | $\delta$(H) | $\delta$(C) |
|---|---|---|---|---|---|
| 1 | 4.88 (br. *s*) | 74.6 (*d*) | 14 | 1.05 (*s*) | 15.8 (*q*) |
| 2 | 1.64–1.67 (*m*), 2.21–2.29 (*m*) | 21.1 (*t*) | 15 | 0.85 (*d*, $J$ = 6.8) | 15.2 (*q*) |
| 3 | 2.32–2.38 (*m*), 1.43–1.52 (*m*) | 23.8 (*t*) | OH | 4.18 (*s*) | |
| 4 | 1.68–1.74 (*m*) | 31.8 (*d*) | 1′ | | 170.6 (*s*) |
| 5 | | 51.0 (*s*) | 2′ | 2.19 (*s*) | 20.8 (*q*) |
| 6 | 6.97 (*s*) | 66.9 (*d*) | 1″ | | 130.5 (*s*) |
| 7 | | 138.7 (*s*) | 2″ | 7.95 (*dd*, $J$ = 8.2, 1.0) | 129.1 (*d*) |
| 8 | | 146.3 (*s*) | 3″ | 7.41–7.46 (*m*) | 128.3 (*d*) |
| 9 | | 185.9 (*s*) | 4″ | 7.54–7.61 (*m*) | 132.4 (*d*) |
| 10 | | 79.2 (*s*) | 5″ | 7.41–7.46 (*m*) | 128.3 (*d*) |
| 11 | | 120.8 (*s*) | 6″ | 7.95 (*dd*, $J$ = 8.2, 1.0) | 129.1 (*d*) |
| 12 | 7.28 (*s*) | 146.5 (*d*) | 7″ | | 165.2 (*s*) |
| 13 | 1.91 (*s*) | 8.4 (*q*) | | | |

biosynthetic considerations, Me(14) and Me(15) are likely to be $\beta$-oriented. The $\beta$-orientation of the OH group at C(10) and the *cis*-fused *A*/*B* ring junction in **1** were confirmed by the observation of a relatively low-field shift of Me(14) ($\delta$(H) 1.05) due to its *quasi*-1,3-diaxial interaction with HO–C(10). Similarly, the $\beta$-orientation of the BzO group at C(6) was also deduced from the observation of a relatively high-field shift of Me(15) ($\delta$(H) 0.85) due to its *quasi*-1,3-diaxial interaction with H–C(6) ($\delta$(H) 6.97). A broad *singlet* of 4.88 ppm implied that H-C(1) is $\beta$-oriented. The substituents at C(6) and C(10) in **1** were further deduced as $\beta$-oriented, from a positive *Cotton* effect at 280 nm ($\Delta\varepsilon_{280\,nm}$ + 3.8) and a negative *Cotton* effect at 240 nm ($\Delta\varepsilon_{240\,nm}$ − 12.5) in the CD spectrum, which was similar to that of a reference reported in [11]. Furthermore, the relative configuration of **1** including the *cis*-fused *A*/*B* ring system was confirmed by the observed spatial ROESY correlations $H_\beta$–C(1)/HO–C(10), $H_\beta$–C(1)/Me(14), $H_\alpha$–C(6)/$H_\alpha$–C(4), as well as $H_\alpha$–C(6)/Me(15) [12][13] (*Fig. 2*). All $^1$H- and $^{13}$C-NMR signals were completely assigned by the interpretation of the DEPT, HMBC and

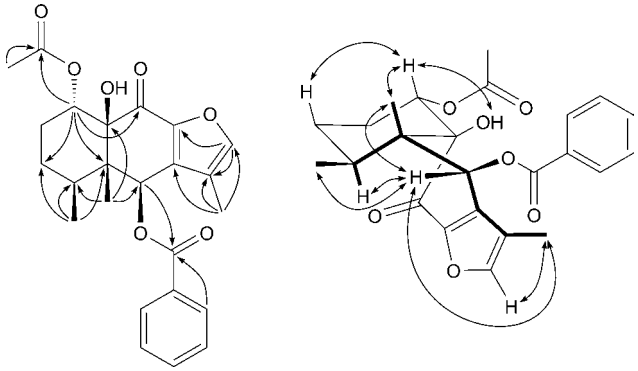


Fig. 2. *Key HMBCs* (→) *and ROESY* (↔) *correlations of compound* **1**

ROESY spectra. On the basis of the above evidences, compound **1** was established as 1$\alpha$-acetoxy-6$\beta$-(benzoyloxy)-10$\beta$-hydroxy-9-oxofuroeremophilane.

The two known sesquiterpenoids were identified as 1$\beta$,6$\beta$-diacetoxy-9-oxofuroeremophilane (**2**) and (6$\alpha$)-furoeremophilan-14,6-olide (**3**) by comparison with the reported spectral data [14–16].

The isolated compounds **1**–**3** were evaluated for their cytotoxic and antimicrobial activities. As compiled in *Table 2*, compound **1** exhibited a weak cytotoxic effect only against MCF-7 and SMMC-7721 cells with the $IC_{50}$ values of $75.12 \pm 1.69$ and $168.36 \pm 2.01$ μg/ml, respectively, while it was inactive against HO-8910 and K-562 cell lines ($IC_{50} > 200$ μg/ml). Compounds **2** and **3** were inactive against all tested cells lines ($IC_{50} > 200$ μg/ml). Compound **1** and **2** showed moderate activities against *Escherichia coli* with *MIC* values of 32 and 64 μg/ml, respectively, while they were inactive against the other three strains ($MIC \geq 128$ μg/ml). Compound **3** was inactive against all tested strains ($MIC > 128$ μg/ml).

Table 2. *Cytotoxic and Antimicrobial Activities of Compounds* **1**–**3**[a])[b])

| Compound | $IC_{50}$ [μg/ml] | | | | *MIC* [μg/ml] | | | |
|---|---|---|---|---|---|---|---|---|
| | MCF-7 | SMMC-7721 | HO-8910 | A549 | *S. aureus* | *E. coli* | *B. subtilis* | *C. albicans* |
| **1** | $75.12 \pm 1.69$ | $168.36 \pm 2.01$ | > 200 | > 200 | 128 | 32 | > 128 | > 128 |
| **2** | > 200 | > 200 | > 200 | > 200 | > 128 | 64 | > 128 | > 128 |
| **3** | > 200 | > 200 | > 200 | > 200 | > 128 | > 128 | > 128 | > 128 |

[a]) Cell lines used: MCF-7, human breast adenocarcinoma; SMMC-7721, human hepatoma; HO-8910, human ovarian carcinoma; A549, human lung carcinoma. [b]) Microorganisms used: *S. aureus*, *Staphylococcus aureus* ATCC 25923; *E. coli, Escherichia coli* ATCC 25922; *B. subtilis*, *Bacillus subtilis* ATCC 10907; *C. albicans*, *Candida albicans* ATCC 10239.

**Conclusions.** – One new furoeremophilane-type sesquiterpenoid, **1**, and two known metabolites, **2** and **3**, were isolated from the EtOH extract of the root bark of *A. leucorrhizus*. In addition, it is noteworthy that furanoeremophilanes have already been found in the genera *Senecio* and *Ligularia* [13][16][17], while they have been isolated for the first time from the *Acanthopanax* species. Upon evaluation of the cytotoxic and antimicrobial activities of compounds **1**–**3**, **1** exhibited relatively weak cytotoxicity against MCF-7 and SMMC-7721 cells, and **1** and **2** showed moderate activities against *Escherichia coli*. These results might afford some reference data for future systematic phytochemical and pharmacological screenings.

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## Experimental Part

*General.* TLC: Silica-gel $GF_{254}$ plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): silica gel *H* ($SiO_2$; 200–300 mesh, 300–400 mesh; *Qingdao Haiyang Chemical Plant*) and *Sephadex LH-20* gel (25–100 μm; *Pharmacia*). M.p.: *X-4* Digital micro-melting point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 341* digital polarimeter. CD Spectra: *JASCO J-715*

spectropolarimeter. UV Spectra: *Shimadzu UV-2401* spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Perkin-Elmer 1700* spectrometer; KBr pellets; $\tilde{\nu}$ in $cm^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *Bruker AMX-400* spectrometer; at 400 ($^1H$) or 100 ($^{13}C$) MHz; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, $J$ in Hz; 2D spectra recorded with standard pulse programs and acquisition parameters. ESI-MS: *Bruker APEX II* high-resolution (HR) mass spectrometer; in $m/z$ (rel. %).

*Plant Material.* The plant material was collected from Tianshui, Gansu Province, in September 2011 and identified by Prof. *Xiao-Qiang Guo* (College of Life Science of Longdong University, P. R. China). A voucher specimen (No. 20090912021) was deposited with the Herbarium of College of Life Science, Longdong University, P. R. China. The stem bark was washed and dried in the shade. Then, the dried plants were powdered by using a grinder.

*Extraction and Isolation.* The air-dried and pulverized stem bark of *A. leucorrhizus* (4.2 kg) was extracted three times with 95% EtOH (10 l × 7 d, each time) at r.t., and then the extracts were combined and concentrated under reduced pressure at 60° to yield 291 g of a brown viscous residue. The EtOH extract was suspended in $H_2O$ and partitioned successively with hexane, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$-soluble extract (85 g) was subjected to CC ($SiO_2$; $CHCl_3$/acetone from 10:1 to 0:5 (*v*/*v*)), the eluted fractions were examined by anal. TLC, and similar fractions were pooled together to give ten main fractions ($A_1$–$A_{10}$). *Fr.* $A_4$ (5.5 g) was separated by CC ($SiO_2$; $CHCl_3$/MeOH 8:1 → 1:8) to give two subtractions ($A_{41}$ and $A_{42}$). *Subfr.* $A_{41}$ was submitted to CC (*Sephadex LH-20*; acetone) to afford compound **1** (8 mg). *Subfr.* $A_{42}$ was purified by CC ($SiO_2$; $CHCl_3$/MeOH 5:1) to furnish compound **2** (12 mg). *Fr.* $A_5$ was purified by CC ($SiO_2$; $CHCl_3$/MeOH 4:1; and *Sephadex LH-20*; acetone) to yield compound **3** (14 mg).

*1α-Acetoxy-6β-(benzoyloxy)-10β-hydroxy-9-oxofuroeremophilane* (= *(4*S*,4a*S*,5*S*,8*S*,8a*S*)-8-(Acetyloxy)-4,4a,5,6,7,8,8a,9-octahydro-8a-hydroxy-3,4a,5-trimethyl-9-oxo-naphtho[2,3-*b*]furan-4-yl Benzoate*; **1**). Colorless crystals (9 mg). M.p. 158–160°. $[\alpha]_D^{25} = +31.4$ ($c = 0.26$, EtOH). UV (EtOH): 206 (4.36), 281 (4.12). IR (KBr): 3418, 1721, 1681, 1624, 1604, 1593, 1465, 1450, 1383, 1275, 1116, 717. CD ($c = 0.003$, MeOH): +2.1 (320), +3.8 (280), 0 (268), −12.5 (240). $^1H$- and $^{13}C$-NMR: see *Table 1*. ESI-MS: 425 ($[M-H]^-$). HR-ESI-MS: 425.1587 ($[M-H]^-$, $C_{24}H_{26}O_7^-$; calc. 425.1600).

*1β,6β-Diacetoxy-9-oxofuroeremophilane* (= *(4*S*,4a*R*,5*S*,8*R*,8a*R*)-4,4a,5,6,7,8,8a,9-Octahydro-3,4a,5-trimethyl-9-oxonaphtho[2,3-*b*]furan-4,8-diyl Diacetate*; **2**). Colorless crystals (12 mg). M.p. 203–205°. $[\alpha]_D^{25} = -42.6$ ($c = 0.08$, EtOH). UV (EtOH): 280 (4.12). IR (KBr): 3018, 1736, 1686, 1631, 1542, 1379, 1268, 1152. $^1H$-NMR ($CDCl_3$, 400 MHz): 7.37 (*s*, H–C(12)); 6.37 (*s*, $H_\alpha$–C(6)); 5.29 (*ddd*, $J = 5.6$, 10.2, 11.2, $H_\alpha$–C(1)); 2.71 (*d*, $J = 10.2$, $H_\beta$–C(10)); 2.19 (*s*, Me(2′)); 2.18 (*s*, Me(2″)); 2.11–2.15 (*m*, $H_\alpha$–C(2)); 1.91 (*s*, Me(13)); 1.85–1.89 (*m*, $H_\alpha$–C(4)); 1.47–1.51 (*m*, $H_\alpha$–C(3)); 1.43–1.46 (*m*, $H_\beta$–C(2)); 1.40–1.45 (*m*, $H_\beta$-C(3)); 0.95 (*s*, Me(14)); 0.89–1.93 (*m*, Me(15)). $^{13}C$-NMR ($CDCl_3$, 100 MHz): 184.5 (*s*, C(9)); 171.4 (*s*, C(1′)); 170.5 (*s*, C(1″)); 147.6 (*s*, C(8)); 145.1 (*d*, C(12)); 133.2 (*s*, C(7)); 121.1 (*s*, C(11)); 75.4 (*d*, C(1)); 67.7 (*d*, C(6)); 56.8 (*d*, C( 10)); 51.6 (*s*, C(5)); 41.3 (*d*, C(4)); 31.2 (*t*, C(2)); 29.5 (*t*, C(3)); 21.8 (*q*, C(15)); 21.5 (*q*, C(14)); 17.6 (*q*, C(13)); 8.91 (*q*, C(2′)); 8.84 (*q*, C(2″)). EI-MS: 348 (34), 305 (27), 246 (55), 228 (18), 138 (72), 43 (100).

*(6α)-Furoeremophilan-14,6-olide* (= *(2a*S*,5a*R*,9b*R*,9c*S*)-2a,3,4,5,5a,6,9b,9c-Octahydro-9,9c-dimethyl-2*H*-naphtho[2,3-*b*:*4,5*-b′c′]difuran*; **3**). White plates. M.p. 168–170°. $[\alpha]_D^{25} = -54.2$ ($c = 0.12$, EtOH). UV (EtOH): 210.5 (3.86), 224.2 (4.12). IR (KBr): 1763, 1687, 1472, 1446, 1268, 1056. $^1H$-NMR ($CDCl_3$, 400 MHz): 7.11 (*s*, H–C(12)); 6.01 (*s*, $H_\beta$–C(6)); 2.78 (br. *d*, $H_\beta$–C(9)); 2.45 (br. *d*, $H_\alpha$–C(9)); 2.26 (*dd*, $J = 3.2$, 11.8, $H_\alpha$–C(4)); 1.95–2.01 (*m*, $H_\beta$–C(10)); 1.89–1.95 (*m*, $H_\beta$–C(3)); 1.84 (*s*, Me(13)); 1.79–1.83 (*m*, $H_\alpha$–C(1)); 1.76–1.79 (*m*, $H_\alpha$–C(2)); 1.45–1.49 (*m*, $H_\alpha$–C(3)); 1.42–1.46 (*m*, $H_\beta$–C(2)); 1.36–1.39 (*m*, $H_\beta$–C(1)); 1.07 (*s*, Me(14)). $^{13}C$-NMR ($CDCl_3$, 100 MHz): 177.5 (*s*, C(15)); 149.9 (*s*, C(8)); 139.1 (*d*, C(12)); 121.2 (*s*, C(7)); 115.6 (*s*, C(11)); 81.7 (*d*, C(6)); 41.6 (*s*, C(5)); 41.3 (*d*, C(4)); 36.8 (*d*, C(10)); 26.1 (*t*, C(9)); 23.5 (*t*, C(3)); 21.1 (*t*, C(1)); 19.5 (*q*, C(14)); 19.2 (*t*, C(2)); 8.9 (*q*, C(13).

*Cytotoxicity Assay.* The cytotoxicities against human breast adenocarcinoma MCF-7, hepatoma SMMC-7721, lung carcinoma A549, and ovarian carcinoma HO-8910 cell lines were determined by using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay in 96-well microplates as described in [18]. Cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of the compounds for 48 h. Subsequently, 10 µl of MTT (5 mg/ml in phosphated-buffered saline) was added to each well, and the plate was incubated at 37° under

5% $CO_2$ for 4 h. The absorbance of the soln. was measured at 570 nm on a plate reader. The cytotoxicities were expressed as $IC_{50}$ values [μg/ml], which reduced the viable cell number by 50%. The $IC_{50}$ values were determined by regression analyses and expressed as means ± SD of three independent replicates.

*Antimicrobial Assay.* The antimicrobial activity against four microorganisms, including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 10907, and *Candida albicans* ATCC 10239, were evaluated by the microdilution method [19], and expressed as *MIC* (minimum inhibitory concentration, [μg/ml]) value. Test strains were cultured in 96-well microtiter plate at a density of $5 \times 10^5$ cfu/ml. Serial double dilutions of the compounds were prepared in a 96-well microtiter plate and ranged from 0.5 to 128 μg/ml. Testing of these isolates, incubated at 37° for 24 h for bacteria and at 30° for 48 h for yeasts, was performed in three replicates.

## REFERENCES

[1] Delectis Florae Reipublicae Popularis Sinicae, Agendae Academiae Sinicae Edits, 'Flora Reipublicae Popularis Sinicae', Science Press, Beijing, 1980, 54, p. 86.
[2] N. Ni, X. Q. Liu, *Chin. Tradit. Herb. Drugs* **2006**, *37*, 1895.
[3] S. Nisibe, H. Kinoshita, H. Takeda, G. Okano, *Chem. Pharm. Bull.* **1990**, *38*, 1763.
[4] T. Fujikawa, A. Yamaguchi, I. Morita, H. Takeda, S. Nishibe, *Biol. Pharm. Bull.* **1996**, *19*, 1227.
[5] S. Lee, J. Ji, K. H. Shin, B.-K. Kim, *Planta Med.* **2002**, *68*, 939.
[6] S.-Y. Park, S.-Y. Chang, C.-S. Yook, T. Nohara, *J. Nat. Prod.* **2000**, *63*, 1630.
[7] D. H. Zhao, K. Q. Fang, F. Ma, B. H. Sheng, P. H. Pan, *Northwest Pharm. J.* **1987**, *2*, 15.
[8] H. Hu, X. Zheng, H. Hu, *Environ. Toxicol. Pharmacol.* **2012**, *34*, 618.
[9] D. L. Cheng, J. K. Niu, L. Yang, J. K. Cheng, *Acta Bot. Sin.* **1994**, *36*, 401.
[10] Q. Wang, Q. Mu, M. Shibano, S. L. Morris-Natschke, K.-H. Lee, D.-F. Chen, *J. Nat. Prod.* **2007**, *70*, 1259.
[11] L. P. Zhang, L. P. Ma, P. Yang, D. L. Cheng, *Chem. J. Chin. Univ.* **2006**, *27*, 1286.
[12] S. Y. Shi, Y. Zhao, Y. P. Zhang, K. L. Huang, S. Q. Liu, *Chem. J. Chin. Univ.* **2008**, *29*, 941.
[13] J.-Q. Gu, Y. Wang, S. G. Franzblau, G. Montenegro, B. N. Timmermann, *J. Nat. Prod.* **2004**, *67*, 1483.
[14] D. L. Cheng, J. J. Gao, L. Yang, *Chem. J. Chin. Univ.* **1992**, *13*, 781.
[15] J. A. Marco, J. F. Sanz, A. Garcia-Sarrión, A. Rustaiyan, *Phytochemistry* **1991**, *30*, 2325.
[16] Y. Moriyana, T. Tsuyuki, T. Takahashi, H. Koyama, *Phytochemistry* **1974**, *13*, 288.
[17] S. Huang, J.-F. Zhang, J. Huang, X.-L. Zhou, L.-H. Shan, C.-J. Wang, J. Weng, *Chin. J. Org. Chem.* **2013**, *33*, 1337.
[18] J.-M. Jin, Y.-J. Zhang, C.-R. Yang, *J. Nat. Prod.* **2004**, *67*, 5.
[19] J. Qu, C. Xie, H. Guo, W. Yu, H. Lou, *Phytochemistry* **2007**, *68*, 1767.

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