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# Antifungal activities of triterpenoids from the roots of *Astilbe myriantha Diels*

Qiu-Yan Song, Wei-Yan Qi, Zheng-Ming Li, Jie Zhao, Jian-Jun Chen *, Kun Gao *

*State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China*

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ABSTRACT

During a search for plant extracts that possess activity against the fungi that cause diseases in plants, a 95% ethanol extract from the roots of *Astilbe myriantha Diels* showed inhibitory activity against *Colletotrichum gloeosporioides*. Bioassay-guided fractionation of the 95% ethanol extract led to the isolation of seven triterpenoids (1–7). Their structures were elucidated by spectroscopy and by a comparison with literature data. Among these compounds, 3β,6β,24-trihydroxyurs-12-en-27-oic acid (7) was the most active inhibitor of *C. gloeosporioides*, with >68.0% inhibition at 100 mg/ml after 72 h. Moreover, compound 7 displayed broad-spectrum inhibitory activity against *Rhizoctonia solani, Fusarium oxysporum f.* sp. Niveum, Fusarium oxysporum f. sp. Cubens, C. gloeosporioides, Penicillium citrinum, Colletotrichum lagenarium, and *Penicillium digitatum*, with EC50 values ranging from 13.9 to 34.0 μg/ml. The potential of compound 7 as a fungicide is therefore promising. In addition, compound 1 was found to be a new triterpenoid, 3β,6β-dihydroxyurs-12-en-7α,27α-olide, which possesses six rings.

## 1. Introduction

Currently, a substantial reduction in the yield of crops, due to destruction by insects and infection by disease-causing fungi, bacteria, and viruses, is a major problem in both developing and developed countries. Additionally, the growth of fungi and bacteria in foods may lead to spoilage, to the formation of toxins, and to the deterioration of food products (Fletcher et al., 2006). Numerous synthetic chemical agents have been applied to control plant diseases. However, they may leave toxic residues in treated plants (Barnard, Padgitt, & Uri, 1997). There is, therefore, a growing interest in the development of new types of effective and nontoxic antifungal agents from natural sources (Kubo, Lunde, & Kubo, 1995). Research into more effective antifungal agents, especially natural agents derived from various species of edible and medicinal plants, has been more prominent in the last decade (Burt, 2004).

The genus *Astilbe*, consisting of 25 species and belonging to the family *Saxifragaceae*, is distributed mainly in Asia and North America (Pan, 1995). Several species of this genus have been reported to possess various biological activities, such as cytotoxicity (Cai et al., 2009), apoptosis-induction (Sun, Zheng, & Tu, 2006), anti-inflammatory effects (Moon et al., 2005), healing of burn wounds (Kimura, Sumiyoshi, & Sakanaka, 2007), and protein tyrosine phosphatase 1B inhibition (Na et al., 2006). The characteristic components of these species are triterpenoids, flavonoids, steroids, and coumarin (Zheng, Zhao, Wu, & Wu, 2002). In some species of the genus of *Astilbe*, triterpenoids with a C-27 carboxylic acid functionality are reported to be the major biologically active compounds present (Kimura et al., 2007; Moon et al., 2005). The diversity of this bioactivity has resulted in significant attention being paid to this class of triterpenoid. One particular member of the *Astilbe* genus, *Astilbe myriantha Diels*, is commonly used folk medicine (its roots have been used to treat headache, common cold, and hemicrania) (Huang, Ding, & Zhao, 2000) and as a food supplement in China. When we began this study, there were no previous reports of either the inhibitory activity against plant pathogens of extracts from the roots of *A. myriantha Diels* or of the chemical constituents present therein.

In the present paper, seven triterpenoids with a C-27 carboxylic acid functional group were obtained by bioassay-guided. Among those triterpenoids, the new compound 1 was isolated and its structure was elucidated by detailed spectroscopic analysis. Additionally, compound 7 is the most active against *Colletotrichum gloeosporioides* with >68.0% inhibition and demonstrates broad-spectrum inhibitory activity against all of the tested pathogenic fungi with EC50 values ranging from 13.9 to 34.0 μg/ml.

## 2. Materials and methods

### 2.1. Pathogens

The pathogens, *Rhizoctonia solani*, *Fusarium oxysporum f.* sp. Niveum, *Fusarium oxysporum f.* sp. Cubens, *C. gloeosporioides*, *Penicillium citrinum*, *Colletotrichum lagenarium*, and *Penicillium digitatum*, used in the bioassays were identified by Ru-Qian Pan, at the Key Lab of Natural Pesticide and Chemical Biology with the

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* Corresponding authors. Tel.: +86 0931 8912592; fax: +86 931 8912582.
*E-mail addresses:* chenjj@lzu.edu.cn. (J.-J. Chen), npchem@lzu.edu.cn (K. Gao).

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Ministry of Education, South China Agricultural University, Guangzhou, China.

### 2.2. Plant material

Roots of *A. myriantha Diels* were collected from Guangxi Province, China, in the summer of 2008. The plant was identified by Xi-Cang Yang, a professor at Lanzhou Chinese Medical College, China. A voucher (No. 08–05) specimen was deposited with the Natural Organic Academy of Lanzhou University.

### 2.3. General

Column chromatography: silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China). TLC: silica gel $GF_{254}$ plates (10–40 μm; Qingdao Marine Chemical Factory, China). Sephadex LH-20 (Amersham Pharmacia Biotech). M.p.: X-4 melting-points (Beijing TECH Instrument Co. Ltd., P. R. China). IR Spectra: Nicolet FT-IR-360 spectrometer. $^1H$ and $^{13}C$ NMR Spectra: Bruker AM-400BB (400 MHz) spectrometer; recorded in $CDCl_3$ and MeOD; $\delta$ in ppm relative to TMS; *J* values in Hz. X-ray crystallography: Bruker Smart CCD diffractometer using graphic-monochromated Mo Kα radiation. EI-MS: VG ZAB-HS instrument; at 70 eV; in *m/z*. HRESIMS: Bruker APEX-II mass spectrometer; in *m/z*.

### 2.4. Extraction and isolation

The air-dried and powdered roots of *A. myriantha Diels* (6.5 kg) were extracted three times (each for 7 days) with 95% EtOH at room temperature. The solvent was evaporated under reduced pressure, leaving an extract (1110 g) that was suspended in hot $H_2O$ (60 °C, 2000 ml). This suspension was extracted successively with petroleum ether (PE), EtOAc, and n-BuOH. According to thin layer chromatography (TLC) analysis, the composition of the PE-soluble fraction was the same as that of the EtOAc-soluble fraction, so the two could be mixed together. Furthermore, the TLC analysis revealed that the remaining $H_2O$ extract contained no compounds. The main active components were identified when testing the inhibitory activity of the EtOAc-soluble fraction and of the n-BuOH-soluble fraction against *C. gloeosporioides*. In this bioassay-guided fractionation, the EtOAc-soluble fraction proved to be more active than the n-BuOH-soluble fraction.

**Table 1**
$^1H$ NMR, $^{13}C$ NMR (DEPT) data[a] of compound **1**[b].

| No. | **1** | |
|---|---|---|
| | $^1H$ | $^{13}C$ |
| 1 | | 39.9 ($CH_2$) |
| 2 | | 27.5 ($CH_2$) |
| 3 | 3.22 t (7.6) | 78.8 (CH) |
| 4 | | 39.4 (C) |
| 5 | | 50.1 (CH) |
| 6 | 4.57 br s | 68.3 (CH) |
| 7 | 4.27 d (2.8) | 83.2 (CH) |
| 8 | | 43.5 (C) |
| 9 | | 50.3 (CH) |
| 10 | | 36.2 (C) |
| 11 | 5.57 q (2.8, 4.4) | 21.8 ($CH_2$) |
| 12 | | 130.4 (CH) |
| 13 | | 131.6 (C) |
| 14 | | 56.4 (C) |
| 15 | | 23.4 ($CH_2$) |
| 16 | | 28.0 ($CH_2$) |
| 17 | | 33.9 (C) |
| 18 | | 60.4 (CH) |
| 19 | | 36.6 (CH) |
| 20 | | 39.6 (CH) |
| 21 | | 31.2 ($CH_2$) |
| 22 | | 41.4 ($CH_2$) |
| 23 | 1.09 s | 27.5 ($CH_3$) |
| 24 | 1.10 s | 17.8 ($CH_3$) |
| 25 | 1.31 s | 18.3 ($CH_3$) |
| 26 | 1.22 s | 13.3 ($CH_3$) |
| 27 | | 177.2 (C) |
| 28 | 0.84 s | 29.0 ($CH_3$) |
| 29 | 0.77 d (6.4) | 18.2 ($CH_3$) |
| 30 | 0.97 d (6.0) | 21.3 ($CH_3$) |

[a] H NMR (400 MHz), $^{13}C$ NMR and DEPT (100 MHz), TMS, $\delta$ ppm, *J* in Hz.

[b] Measured in $CDCl_3$.

The EtOAc-soluble fraction (146 g) was subjected to silica gel column chromatography (200–300 mesh, 1200 g), eluting with a gradient of PE – Acetone (50:1, 20:1, 10:1, 5:1, 2:1, and then 0:1). Seven fractions (Fr.1–7) were collected and subjected to TLC (Thin layer chromatography) analysis. Further purification of Fr.3 (0.85 g) by column chromatography ($S_iO_2$), eluting with PE-Acetone (20:1), yielded compound 2 (20 mg) and compound 4 (7 mg). Fr.4 (1.013 g) was subjected further column chromatography to give compound 3 (35 mg) by eluting with PE-Acetone (20:1). Compound 1 (11 mg) and compound 5 (6 mg) were obtained by further purification of Fr.5 by recrystallization and the use of Sephadex LH-20 (CHCl3-CH3OH; 1:1). Fr.6 was separated by preparative TLC, eluting with CHCl3-EtOAc (20:1,×3), yielding compound 6 (8 mg). Fr.7 was further purified by silica gel column chromatography, eluting with PE-Acetone (1:1), to obtain compound 7 (426 mg).

### 2.5. Characteristic data of compound

3β,6β-dihydroxyurs-12-en-7α,27α-olide (**1**): White crystals, m.p. 218–220 °C. IR: νKBr max 3444, 2850, 2360, 2330, 1741, 1618, 1384, 1021 $cm^{-1}$. MS (EI) (70 eV): *m/z* 470 (443, $M^+$), 455 (106), 388 (138), 255 (603), 105 (2828), 43 (10000). $^1H$ NMR, $^{13}C$ NMR, and DEPT data see Table 1.

### 2.6. Determination of inhibitory activities against hyphal growth of pathogenic fungi

Inhibitory activity against the hyphal growth of pathogenic fungi was determined using a growth rate method (Mu, 1994). The sample was dissolved in Acetone-$H_2O$ (1:1 by volume) to give a test solution of the required concentration. One milliliter of this solution was thoroughly mixed with 24 ml of PDA molten agar medium, in a measuring cylinder. The mixture was then distributed equally into 3 petri dishes (6 cm diameter) to produce a toxic culture media. Acetone-$H_2O$ (1:1 by volume), without any sample, was processed similarly to produce the control culture media. Discs of hyphae were cut from the edge of a colony, which had been incubated in advance with a 0.4 cm bearer. These were placed in the middle of the surface of the culture medium, with the hyphae downwards. The discs, regression equations, $EC_{50}$, and 95% confidence intervals were calculated by the least-squares method.

## 3. Results and discussion

### 3.1. Phytochemical investigation of the active components

The organic extract of the roots of *A. myriantha Diels* was repeatedly separated by column chromatography (silica gel and Sephadex LH-20), preparative TLC, and recrystallization to yield a new compound (**1**) and 6 previously known compounds (**2**–**7**) (Fig. 1).

Compound **1** was obtained as colourless crystals. The $^{13}C$ NMR and DEPT spectra, together with a molecular ion peak in the HRESIMS of compound **1** at *m/z* 471.3456($[M + H]^+$ (calc. for $C_{30}H_{46}O_4$, which is 471.3469), suggests a molecular formula of $C_{30}H_{46}O_4$. The