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Diarylheptanoids and phenylphenalenones from *Musa itinerans* fruits

Fang Liu [a,1], Yu Zhang [a,1], Qian-Yun Sun [b], Fu-Mei Yang [b], Wei Gu [a], Jun Yang [a], Hong-Mei Niu [a], Yue-Hu Wang [a,*], Chun-Lin Long [a,c,d,*]

[a] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China
[b] Key Laboratory of Chemistry for Natural Products, Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, People's Republic of China
[c] College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, People's Republic of China
[d] Yunnan Agricultural University, Kunming 650201, People's Republic of China

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ABSTRACT

Two diarylheptanoids, musaitinerins A and B, one heterodimeric phenylphenalenone musaitinerone and four known phenylphenalenones, identified as 4-hydroxy-2-methoxy-9-phenyl-1*H*-phenalen-1-one, musanolone E, hydroxyanigorufone and irenolone were isolated from the fruits of *Musa itinerans* Cheesm. Their structures were elucidated using spectroscopic analyses. The antimicrobial activity of these compounds was evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*; the cytotoxic activity of these compounds was also evaluated against human erythromyeloblastoid leukemia (K562) and human alveolar carcinoma epithelial (A549) cell lines, respectively. Musaitinerone and musanolone E exhibited weak effects against the A549 cell line, as compared with adriamycin. However, these two compounds did not exhibit any growth inhibition against K562 cells, *S. aureus*, *E. coli* or *C. albicans*. The other compounds were inactive against all of the tested cell lines and microorganisms, even at concentrations as high as 50 μM.

Introduction

The genus *Musa* (Musaceae) encompasses approximately 30 species, and these are mainly distributed in Southeast Asia with 11 species in China (Wu and Kress, 2000). Certain species, such as *Musa basjoo* Sieb. & Zucc., *Musa balbisiana* Colla, *Musa*× *paradisiaca* Linn. and *Musa itinerans* Cheesm, are used as traditional Chinese herbs (Editorial Board of Zhonghua Bencao, 1999). The major chemical constituents of *Musa* include phenylphenalenones and their respective dimers; these compounds have a variety of biological activities, such as antifungal (Kamo et al., 2001; Luis et al., 1995; Otálvaro et al., 2007), α-glucosidase inhibitory (Zhang and Kang, 2010) and cancer chemopreventive properties (Jang et al., 2002). *M. itinerans*, which is synonymous to *Musa wilsonii* Tutch., is mainly distributed in southern China and has been used to treat malaria (Liu and Li, 2000; Liu et al., 2002). However, its chemical constituents have not yet been reported. With an ongoing interest in natural anticancer agents (Zhao et al., 2010; Tang et al., 2011; Su et al., 2013; Yang et al., 2013) and cytotoxic phenylphenalenones (Dong et al., 2011), a phytochemical analysis of the fruits of *M. itinerans*, was carried out. Thus led to isolation of two new diarylheptanoids (**1** and **2**), one new heterodimeric phenylphenalenone (**3**) and four known phenylphenalenones (**4**–**7**). This report describes the structural characterization of the new compounds and the bioassay results obtained for all isolates.

Results and discussion

A methanol (MeOH) extract of *M. itinerans* fruits was suspended in water (H_2O) and partitioned with ethyl acetate (EtOAc). Repeated column chromatography of the EtOAc-soluble portion, which was performed on silica gel, C_{18} silica gel and Sephadex LH-20, respectively, was followed by preparative thin-layer chromatography (TLC) and semipreparative high-performance liquid chromatography (HPLC) to yield compounds **1** to **7** (Fig. 1).

Compound **1** was obtained as a white amorphous solid, and its molecular formula, $C_{41}H_{44}O_{12}$, was assigned based on high-resolution electrospray ionization mass spectroscopy (HRESIMS) data, which indicated 20 degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxyl (3428 cm^{-1}), conjugated

* Corresponding authors. Address: Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China. Tel.: +86 10 68930381; fax: +86 0871 65223318.
E-mail addresses: wangyuehu@mail.kib.ac.cn (Y.-H. Wang), long@mail.kib.ac.cn (C.-L. Long).
[1] These authors contributed equally to this work.

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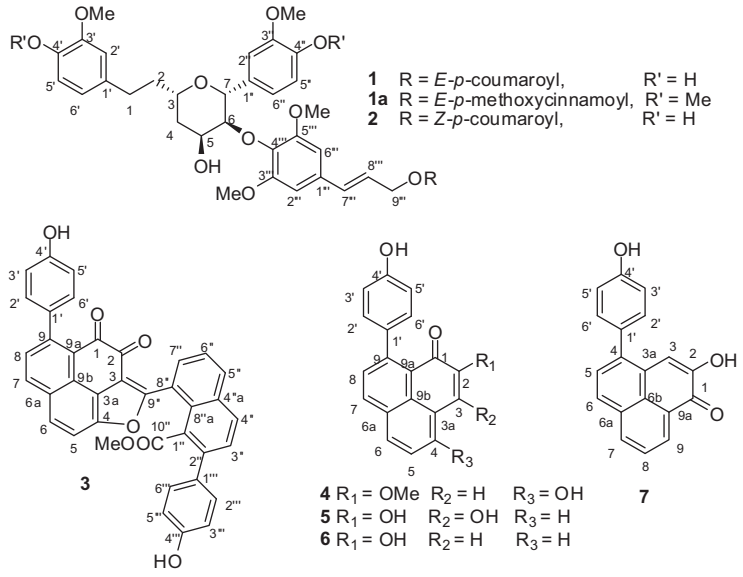

Fig. 1. Structures of compounds **1**–**7** and **1a**.

carboxyl (1706 and 1632 cm^{-1}) and aromatic (1605, 1585, 1516 and 1453 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra for this compound displayed signals indicative of four substituted benzene rings, two *E*-double bonds [δ_H 7.64 (d, J = 15.9 Hz), 6.64 (d, J = 15.9 Hz), 6.38 (dt, J = 15.9 and 6.1 Hz) and 6.38 (d, J = 15.9 Hz)], one ester carbonyl group (δ_C 167.2) and four *O*-methyl groups (Table 1).

Several connections, such as the linkages from C-1 to C-7 and from C-7‴ to C-9‴, were elucidated based on ^1H–^1H correlation spectroscopy (COSY) (Fig. 2). Furthermore, three fragments, namely a substituted diarylheptanoid, a sinapyl alcohol and an *E*-*p*-coumaroyl group, were deduced through ^1H–^1H COSY, heteronuclear multiple bond correlation (HMBC) and rotational frame nuclear Overhauser effect spectroscopy (ROESY) experiments (Fig. 2), respectively. The *E*-*p*-coumaroyl group was connected to the 9‴-OH of the sinapyl alcohol moiety by examining HMBC correlations between H_2-9‴ and C-9‴′. Furthermore, the same group was also linked to the diarylheptanoid moiety through a C-6-O-C-4‴ bonding pattern by analyzing the HMBC correlation between H-6 and C-4‴. This linkage was also indicated by the relatively low-field chemical shifts of H-6 (δ_H 4.26) and C-6 (δ_C 82.4) compared with the analogous chemical shifts (δ_H 3.51 and δ_C 73.6) of the known compound, (3*S*,5*S*,6*S*,7*R*)-5,6-dihydroxy-1,7-bis(4-hydroxyphenyl)-4″-de-*O*-methylcentrolobine (Ali et al., 2001).

The relative configuration of **1** was deduced from analysis of the coupling constants in its ^1H NMR spectrum (Table 1) and ROESY correlations (Fig. 3). If the pyran ring is in a chair conformation, H-7 and H-6 should have a *trans*-diaxial relationship, which is indicated by the $J_{6,7}$ = 9.5 Hz coupling constant. H-7 was arbitrarily assigned to have a β-orientation, which would make H-6 α-oriented. The $J_{5,6}$ = 2.4 Hz value indicated that H-5 and H-6 were in a *cis* relationship, with an H-5 equatorial- and α-orientation. Two protons [δ_H 1.90 (br d, J = 12.1 Hz) and 1.56 (br t, J = 12.1 Hz)] were linked to C-4; the former should be equatorial, and the latter should be axial. In the ROESY spectrum of **1**, the correlations between H-4α and H-6 indicated that these two protons were co-facial. The ROESY correlations between H-4α and H_2-2 indicated that H-4α and H-3 were in a *trans* relationship, which was also indicated by the $J_{3,4\alpha}$ = 12.1 Hz. Therefore, H-3 was assigned as β-oriented. Other ROESY correlations, such as that between H-3 and H-7, as well as that observed between 5-OH and H-7, further confirmed the deduction above. Thus, the relative configuration of **1** was deduced and was given the common name musaitinerin A.

To determine its absolute configuration, its 4′,4″,4‴′-tri-*O*-methyl derivative **1a** (Fig. 1) and Mosher esters of **1a** were synthesised. However, the $\Delta\delta_H$ ($\delta_S - \delta_R$) values contradicted Mosher's rule (Supplementary Data), which might be due to steric compression of H-3 and H-7 to the MTPA group (Ohtani et al., 1991). In addition, the calculated and experimental electron circular dichroism (ECD) curves were not self-consistent (data not shown). The absolute configuration of **1** remains unclear.

Musaitinerin B (**2**) was obtained as a white amorphous solid, and its molecular formula, $C_{41}H_{44}O_{12}$, was assigned based on the HRESIMS data, which indicated 20 degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 2) of **2** were similar to those of **1**. Analysis of the 2D NMR spectra (Supplementary Data) of **2** indicated that it was also composed of three fragments: a substituted diarylheptanoid, a sinapyl alcohol and a *Z*-*p*-coumaroyl group. According to the 2D NMR correlations (Supplementary Data), the linkage patterns and the relative configuration were analogous to those deduced for **1**. Thus, the structure of musaitinerin B (**2**) was determined, as shown in Fig. 1.

Musaitinerone (**3**) was obtained as an orange amorphous solid, and its molecular formula, $C_{38}H_{22}O_7$, was assigned based on the HRESIMS data (m/z 613.1267 $[M+Na]^+$), which indicated 28 degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 2) displayed signals that indicated two *para*-disubstituted benzene rings [δ_H 7.36 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 8.5 Hz), 6.92 (2H, d, J = 8.5 Hz) and 6.87 (2H, d, J = 8.5 Hz)], one *O*-methyl group [δ_H 2.87 (3H, s); δ_C 51.8] and three carbonyl groups (δ_C 183.1, 175.6 and 169.9).

Phenylphenalenones, such as compounds **4** to **7**, are the major constituents of *Musa* plants. These compounds usually possess a C_{19} molecular skeleton, with approximately 14 degrees of unsaturation. Comparison of the collected data to the aforementioned characteristics indicated that compound **3** may be tentatively considered a phenylphenalenone dimer. Based on the ^1H–^1H COSY, HMBC and ROESY correlations (Fig. 2) for **3**, fragments A (left moiety, shown in blue) and B (right moiety, shown in black) were elucidated. Specifically, the key HMBC correlations such as 4′-OH to C-5′, H-6′ to C-9, H-8 to C-1′ and C-9a, H-7 to C-9b, H-6 to C-4 and H-5 to C-3a supported the presence of fragment A. Although the HMBC spectrum of **3** showed no long-range correlations for C-1, C-2 and C-3, this fragment should consist of a phenylphenalenone skeleton based on a comparison of its NMR spectroscopic data with those of known compounds **4** to **7**. Fragment B was