Secondary metabolites of *Xylaria* sp., an endophytic fungus from *Taxus mairei*

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**ABSTRACT**

One new metabolite 3,7-dimethyl-9-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol, together with nine known compounds, were isolated from the strain *Xylaria* sp., an endophytic fungus of *Taxus mairei*. Their structures were deduced from 1D and 2D NMR data. In vitro cytotoxicity and antibacterial activity of these compounds were evaluated. Some of them exhibited substantial activity.

1. Introduction

Paclitaxel was first isolated from the bark of the pacific yew tree in 1970s (Ketchum et al. 1999). Taxol (paclitaxel) appears to be one of the most promising antineoplastic agents of the last decade, with demonstrated activity in advanced and refractory ovarian, breast, lung, and head and neck cancers (Rowinsky et al. 1993). The discovery of paclitaxel (taxol) as a potent anticancer drug, initially isolated from *Taxus brevifolia*, has spurred several groups all...
over the world to conduct research work on other Taxus species, to isolate potentially more effective paclitaxel derivatives or as starting materials for semisynthesis (Sun et al. 2015).

Endophytes are a rich source of natural products displaying a broad spectrum of biological activities (Tan & Zou 2001; Strobel 2003; Strobel et al. 2004). After an initial report of the production of paclitaxel from an endophyte of the North-west Pacific yew (Stierle et al. 1993), isolation of anticancer agents from fungal endophytes has gained increased interest. Taxus chinensis var. mairei is a valuable plant that belongs to the Taxaceae family (Wu et al. 2015). During our screening for bioactive endophytic fungus from this medicinal plant, one strain (Xylaria sp.) was fermented according to its cytotoxic activity. Xylaria have received attention due to their potential as a source of novel and bioactive secondary metabolites (Huang et al. 2014; Rivera-Chávez et al. 2015; Sawadsitang et al. 2015). Isolation of the chemical constituents of this strain led to the discovery of one new and nine known compounds. This article illustrated the isolation, structure elucidation of the new compound and the bioactivities of these compounds.

2. Results and discussion

The fermentation extract of the strain was purified on various columns and yielded ten compounds 1–10 (Figure 1). Their structures were elucidated as 3,7-dimethyl-9-(-2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol (5), along with nine known compounds (3R,6E,10S)-2,6,10-trimethyl-3-hydroxydodeca-6,11-diene-2,10-diol (1) (Macías et al. 2009), euryfuran (2) (Gulavita et al. 1992), (SR*,5aR*,9aR*)-4,5,5a,6,7,8,9,9a-Octahydro-6,6,9a-trimethylnaphtho[1,2-c]furan-5-ol (3) (Baba et al. 1994), danilol (4) (Echeverri et al. 1997), methyl isodrimeninol (6) (Matsuda et al. 2002), (-)-drimenin (7) (Rukachaisirikul et al. 2010),
rugulosin A (8) (Yamazaki et al. 2010), chrysophanol (9) (Zhou et al. 2006) and nalgiovensin (10) (Banville & Brassard 1976). The structures of these known compounds were confirmed by extensive NMR spectroscopic methods experiments and by comparison of their NMR data with those reported for authentic samples.

Compound 5 was obtained as white powder. The molecular formula C_{18}H_{32}O_{3} was deduced from ESI-MS (m/z: 297.1 [M + H]+) and 13C NMR. The 13C NMR spectra data of compound 5 were similar to those of (3R, 6E, 10S)-2, 6, 10-trimethyl-3-hydroxydodeca-6, 11-diene-2, 10-diol (1), except that there were three more carbon signals in compound 5, including two methyls C-17 (δc 25.7), C-18 (δc 27.6) and one quaternary carbon which was linking two methyls together according to the 1H-13C long-range correlations of the protons at δH 1.13 (H-17) with the carbons at δc 27.6 (C-18) and δc 105.4 (C-1), and the protons at δH 1.18 (H-18) with the carbons at δc 25.7 (C-17) and δc 105.4 (C-1). By comparing compound 5’s MS data with known Compound 1, we determined 5 to be 3,7-dimethyl-9-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol. Compound 5 exhibited strong inhibition against three pathogenic bacterial strains. It may become a leading compound in the treatment of these pathogens.

3. Experimental

3.1. General experimental procedures

The 1H (600 MHz), 13C (125 MHz) and 2D NMR spectra were recorded in C_{3}D_{6}O on a Bruker DRX2500 instrument using TMS as an internal reference. ESI-MS was acquired using Finnigan LCQ-Advantage.

4. Fungal material

The strain was preserved at School of Life Sciences, Xiamen University. A BLAST search result showed that the internal transcribed spaces sequence of this fungus was highly homologous (99% similarity) to that of Xylaria sp. JK50 (accession number: JX624289.1), indicating that the fungus belongs to this genus.

4.1. Culture conditions, extraction and isolation

The strain was grown on PDA plates (10 L) at 28 °C for 15 days. The cultured agar was extracted with EtOAc–MeOH–AcOH (80:15:5, v/v/v, 10 L) at room temperature overnight for three times. The organic filtrations were concentrated under vacuum and the remaining aqueous solution was extracted with EtOAc to afford EtOAc extract (3.81 g).

The crude extract (3.81 g) was subjected to C-18 silica gel (170 g) flash chromatography eluted with gradient elution using methanol and water (0, 30, 50, 70, 100% v/v), 2 L for each gradient. Five fractions (Fr I–VI) were collected.

Fraction I (363 mg) was first chromatographed with Sephadex LH-20 (140 g) and eluted with methanol. Two fractions (Fr I.a–b) were collected according to the TLC results. Fr I.a was purified by normal-phase silica gel eluted with petroleum ether–acetone (15:1, 20:1, v/v) to give compound 1 (13 mg).

Fr I.b was subjected to C-18 silica gel (30 g) eluted with gradient elution using acetone and water (5, 10, 20%, v/v) to give an impure fraction (30 mg) containing Compound 2. Final
purification of Compound 2 (5 mg) was acquired by normal-phase silica gel eluted with a stepwise petroleum ether–chloroform (4:1, 3:1, 1:1, v/v).

Fraction II (150 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give two subfractions (Fr IIa–b). Fr IIa (20 mg) was further purified by column chromatography on silica gel [eluted with petroleum ether–ethyl acetate (80:1, 60:1, 50:1, v/v)] to yield Compound 3 (3 mg). Fr IIb (36 mg) was subjected to column chromatography on silica gel [eluted with petroleum ether–acetone (100:1, 80:1, v/v)] to yield Compound 4 (2 mg).

Fraction III (150 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give three subfractions (Fr IIIa–c). Fr IIIa (14 mg) was purified by silica gel eluted with petroleum ether–acetone (50:1, 30:1, 1:1, v/v) to give Compound 5 (2 mg). Fr IIId (70 mg) was subjected to column chromatography on silica gel [eluted with petroleum ether–ethyl acetate (400:1, 80:1, v/v)] to yield Compound 6 (2 mg).

Fraction IV (325 mg) was first chromatographed with Sephadex LH-20 (140 g) and eluted with methanol. Subfraction (Fr IVa) was subjected to C-18 silica gel (30 g) eluted with gradient elution using methanol and water (50, 60, 66%, v/v) to give Compound 7 (3 mg).

Fraction V (241 mg) separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give subfraction (Fr Va). The recrystallisation of subfraction Fr Va to give Compound 8 (85 mg).

Fraction VI (110 mg) was first chromatographed with Sephadex LH-20 (140 g) and eluted with methanol to give two subfractions (Vla–b). Fr Vla (40 mg) was subjected to C-18 silica gel (30 g) eluted with a stepwise methanol (60, 70%, v/v) to give Compound 9 (10 mg). Fr VIb (5 mg) was further purified by normal-phase silica gel eluted with petroleum ether–chloroform (5:1, 3:1, 2:1, v/v) to give Compound 10 (2 mg).

4.2. Structure and identification

Compound 5, white powder. ESI-MS m/z: 297.1 [M + H]+, 1D and 2D NMR (600 Hz, CD3OD) (Table S1).

4.3. Biological assay

Hundred microlitre of medium (test microorganism concentration:106 cfu) and 100 μL of compound solution (50 μg/mL final concentration) were incubated at 30 °C for 18 h. Growth was evaluated by the percentage decrease in the OD at 560 nm in comparison to that of the negative control with the formula: [OD (negative control) – OD (sample)]/OD (negative control). Escherichia coli ATCC 25922, Bacillus subtilis ATCC 9372, Bacillus pumilus ATCC 7061 and Staphylococcus aureus ATCC 25923 were inoculated in LB medium; Aspergillus niger16888 and Candida albicans As 2.538 were inoculated in potato dextrose (PD) medium.

Compound 5 exhibited strong inhibition against three strains, B. subtilis ATCC 9372 48.1%, B. pumilus 7061 31.6% and S. aureus ATCC 25923 47.1%. Compound 10 exhibited broad inhibition against following strains, C. albicans As 2.538 46.0%, A. niger16888 40.0%, S. aureus ATCC 25923 42.1%, B. subtilis ATCC 9372 36.8%, B. pumilus ATCC 7061 47.1% and E. coli ATCC 25922 41.2%.

The cytotoxic activities of the compounds were measured by MTT method (Solis et al. 1993) in HeLa cell line. Compound 10 exhibited prominent bioactivity of 94.1% inhibition at concentration of 10 μg/mL.
5. Conclusion

Ten compounds were isolated from the strain *Xylaria* sp., an endophytic fungus of *Taxus mairei*. One new metabolite 3,7-dimethyl-9-((2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol was obtained. Their cytotoxicity and antibacterial activity were tested. Compound 5 and 10 exhibited significant bioactivities.

Disclosure statement

No potential conflict of interest was reported by the authors.

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