Isolation and Identification of a New Tetrahydro-β-carboline Diketopiperazine from the Dominant Endophytic Fungus of Ancient Tree Dali Tea (Postprint)

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Abstract

To investigate the chemical constituents of the dominant endophytic fungus *Diaporthe tectonicgena* from ancient tree *Camellia taliensis*, the rice solid-state fermentation extract of this strain was separated and purified using silica gel, macroporous adsorption resin Diaion HP20, Sephadex LH-20, and other column chromatography methods, and the obtained compounds were structurally identified through spectroscopic analyses including HRMS, ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, and COSY. The results showed that: (1) Four compounds were isolated from the rice solid-state fermentation extract of this strain, among which new compound 1 was identified as a tetrahydro-β-carboline diketopiperazine alkaloid and named tectonicgenazine A. (2) Three known compounds were identified as trans-cyclo-(D-tryptophanyl-Ltyrosyl) (2), 1H-indole-3-carboxylic acid-2,3-dihydroxypropyl ester (3), and N-hydroxyethyl-2-acetylpyrrole (4), among which compound 3 was isolated from nature for the first time.

Full Text

Isolation and Identification of a New Tetrahydro-β-carboline Diketopiperazine from a Predominant Endophytic Fungus in Ancient Camellia taliensis

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Abstract

To investigate the chemical constituents of *Diaporthe tectonigena*, a predominant endophytic fungus isolated from the ancient tea tree *Camellia taliensis*, the rice solid-state fermentation extract was isolated and purified using silica gel, Diaion HP20, and Sephadex LH-20 column chromatography. Chemical structures were elucidated through extensive spectroscopic analyses including ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, COSY, and HR-MS. Four compounds were isolated from the rice solid-state fermentation extract, including one new tetrahydro-β-carboline diketopiperazine alkaloid named tectonicgenazine A (1). Three known compounds were identified as trans-cyclo-(D-tryptophanyl-L-tyrosyl) (2), 1H-indole-3-carboxylic acid-2,3-dihydroxypropyl ester (3), and N-hydroxyethyl-2-acetylpyrrole (4). Notably, compound 3 was isolated from nature for the first time.

Keywords: *Camellia taliensis*, endophytic fungus, *Diaporthe*, solid-state fermentation, diketopiperazine

Introduction

Yunnan Province harbors diverse wild tea tree resources and is recognized globally as the origin and center of tea plant domestication. *Camellia taliensis* is widely distributed across western and southwestern Yunnan, representing the most extensively distributed, abundant, and adaptable wild tea tree population in the province’ s germplasm resources. As an important wild relative of cultivated tea, *C. taliensis* possesses primitive evolutionary characteristics, strong stress resistance, and unique biochemical components, making it an invaluable genetic resource for tea variety improvement. The “Jinxiuchazu” ancient tea tree (Xiangzhuqing No. 1) in Xiaowan Town, Fengqing County, Yunnan, is a premium *C. taliensis* resource considered the oldest and largest cultivated ancient tea tree in existence. Its perennial vigorous growth suggests a well-developed microecosystem that plays a crucial role in enhancing environmental adaptability. Previous studies have revealed that endophytic fungal communities from healthy branches and leaves of “Jinxiuchazu” exhibit high species richness, diversity, and evenness, with most demonstrating strong antagonistic activity against phytopathogenic fungi. *Diaporthe tectonigena* (family Diaporthaceae) was identified as one of the predominant endophytic fungal species in the branches of this ancient tea tree.

The genus *Diaporthe* is widely distributed globally and has yielded numerous structurally novel secondary metabolites, including polyketides, alkaloids, terpenoids, and anthraquinones with significant antitumor, antimicrobial, and anti-hyperlipidemic activities. Additionally, *Diaporthe* species are utilized in pattern formation on *Aquilaria sinensis* wood. *Diaporthe tectonigena* was first isolated and identified as a new species from teak (*Tectona grandis*) in northern Thailand in 2017, and subsequently reported from soybean (*Glycine max*) and *Camellia sinensis* var. *sinensis* from Yunnan. However, research on this endophytic fungus has primarily focused on taxonomic identification, with no reports on its secondary metabolites. This study employed silica gel, Diaion HP20, and Sephadex LH-20 column chromatography combined with MS and NMR spectroscopic techniques to isolate and identify secondary metabolites from the rice solid-state fermentation extract of the predominant endophytic fungus *D. tectonigena* from healthy branches of "Jinxiuchazu," providing a foundation for further exploration and utilization of bioactive natural products from this strain.

Materials and Methods

1.1 Experimental Materials

The endophytic fungal strain was isolated from healthy, asymptomatic young branches of the ancient *Camellia taliensis* tree (Xiangzhuqing No. 1) in Fengqing County, Yunnan Province. The strain was identified as *Diaporthe tectonigena* through morphological and molecular biological characterization and stored at -80 °C in the laboratory.

1.2 Reagents and Instruments

Methanol, chloroform, and ethyl acetate (all chemical grade) were purchased from Chinese suppliers. Chromatographic materials included Diaion HP20 (Mitsubishi Chemical Corporation), silica gel (200-300 mesh, Qingdao Marine Chemical Co., Ltd.), and Sephadex LH-20 (GE Healthcare, USA). Instrumentation comprised Bruker DRX-400 and DRX-500 NMR spectrometers, API QSTAR Pulsar-1 mass spectrometer, Agilent G6230 TOF mass spectrometer, biological safety cabinet, autoclave, programmable climate chamber, analytical balance, ultrapure water system, water bath, and EYELA N-1001 rotary evaporator.

1.3 Experimental Methods

The *Diaporthe tectonigena* strain was activated on PDA medium, then transferred to PDB medium (100 mL) and cultured on a rotary shaker at 27 °C and 140 rpm for 7 days. Five milliliters of this seed culture were inoculated into 250 mL Erlenmeyer flasks containing rice solid medium (30 g rice, 45 mL distilled water per flask) and incubated statically at 27 °C for 40 days (total 16 kg rice). The fermented rice was extracted with ethyl acetate (5×) under ultrasonication, and the combined filtrates were concentrated to yield 297.82 g of ethyl acetate extract. The residue was subsequently extracted with methanol (3×) to afford 2029.14 g of methanol extract.

The ethyl acetate extract was subjected to silica gel column chromatography (63 cm × 9.5 cm) with gradient elution using petroleum ether:ethyl acetate (80:1$→1 : 1)*andchloroform* : *methanol*(80 : 1→$0:1). TLC analysis yielded fractions Fr.1-Fr.16. Fraction Fr.15 (19.87 g) was further purified on Sephadex LH-20 (50 cm × 8 cm) with 100% methanol to give three subfractions (Fr.15-1-Fr.15-3). Subfraction Fr.15-2 (15.25 g) was chromatographed on silica gel (50 cm × 4.8 cm) using chloroform:methanol:water (60:1:0$→$8:2:0.2) to afford eight fractions (Fr.15-2-1-Fr.15-2-8). Compound 1 (9.0 mg) was obtained from Fr.15-2-2 (2.28 g) by Sephadex LH-20 chromatography (45 cm × 4 cm) with 100% methanol. Compounds 2 (11.0 mg) and 3 (13.0 mg) were isolated from fraction Fr.15-4 (6.90 g) using Sephadex LH-20 (55 cm × 4 cm) with 100% methanol.

The methanol extract was fractionated on Diaion HP20 (60 cm × 7.5 cm) with water washing followed by stepwise elution with 40%, 60%, 80%, and 100% methanol to give four fractions (Fr.1-Fr.4). Fraction Fr.1 was purified on Sephadex LH-20 with 100% methanol to yield three subfractions (Fr.1-1-Fr.1-3). Subfraction Fr.1-2 (9.39 g) was chromatographed on silica gel (50 cm × 4 cm) using chloroform:methanol (30:1, 20:1) and 100% methanol to afford compound 4 (5.0 mg).

Results and Analysis

Four compounds were isolated from the rice solid-state fermentation extract of *Diaporthe tectonigena* and identified through 1D, 2D NMR, and HRMS analyses. Compound 1 was determined to be a new tetrahydro-β-carboline diketopiperazine, while compounds 2-4 were identified as trans-cyclo-(D-tryptophanyl-L-tyrosyl) (2), 1H-indole-3-carboxylic acid-2,3-dihydroxypropyl ester (3), and N-hydroxyethyl-2-acetylpyrrole (4).

Compound 1 was obtained as an amorphous white powder. ESI$^+$-MS showed a quasi-molecular ion peak at m/z 382 [M+Na]$^+$, and HR-ESI$^+$-MS displayed a quasi-molecular ion peak at m/z 382.1527 [M+Na]$^+$ (calcd. 382.1526). ^{13}C NMR and DEPT analyses revealed 22 carbon signals: one primary carbon (CH_3), two secondary carbons (CH_2), twelve tertiary carbons (CH), and seven quaternary carbons, suggesting a molecular formula of $C_{22}H_{21}N_3O_2$ with a molecular weight of 359 and 14 degrees of unsaturation.

The ^{1}H NMR spectrum indicated a 1,2-disubstituted benzene ring [δ 7.16 (1H, d, J = 7.8 Hz, H-12), δ 6.95 (1H, t, J = 7.0 Hz, H-13), δ 7.08 (1H, m, H-14), δ 7.25 (1H, d, J = 8.1 Hz, H-15)] and a monosubstituted benzene with A_2B_2 coupling system [δ 7.08 (5H, m, H-18,19,20,21,22)]. Three methine proton signals [δ 5.74 (1H, dd, J = 7.0, 3.8 Hz, H-3), δ 4.45 (1H, t, J = 3.8 Hz, H-6), δ 4.11 (1H, dd, J = 11.8, 4.1 Hz, H-9)], two methylene proton signals [δ 2.70 (2H, dd, J = 14.8, 4.2 Hz, H-10), δ 2.97 (2H, dd, J = 13.9, 4.9 Hz, H-16)], and one methyl proton signal attached to a tertiary carbon [δ 1.48 (3H, d, J = 6.9 Hz, H-1')] were observed.

The ^{13}C NMR spectrum displayed, in addition to the two benzene ring carbon signals, two carbonyl quaternary carbons [δ 164.5 (C-5), δ 167.9 (C-8)], two olefinic quaternary carbons [δ 132.5 (C-2), δ 105.2 (C-11)], three tertiary carbons [δ 45.8 (C-3), δ 56.1 (C-6), δ 52.2 (C-9)], two secondary carbons [δ 25.9 (C-10), δ 39.6 (C-16)], and one methyl carbon at δC 17.5 (C-1'). HSQC and ^{1}H-^{1}H COSY analyses enabled unambiguous assignment of proton and carbon signals (Table 1), confirming the presence of one 1,2-disubstituted and one monosubstituted benzene ring. Correlations observed between δH 4.11/δC 52.2 (CH-9) and δH 2.70/δC 25.9 (CH$_2$-10), δH 4.45/δC 56.1 (CH-6) and δH 2.97/δC 39.6 (CH$_2$-16), and δH 5.74/δC 45.8 (CH-3) and δH 1.48/δC 17.5 (CH$_3$-1') suggested a diketopiperazine skeleton.

The HMBC spectrum of compound 1 showed key correlations between δH 1.48 (H-1) and δC 132.5 (C-2), δH 5.74 (H-3) and δC 132.5 (C-2), δC 164.5 (C-5), δC 52.2 (C-9), and δC 105.2 (C-11), δH 4.45 (H-6) and δC 164.5 (C-5) and δC 167.9 (C-8), δH 4.11 (H-9) and δC 45.8 (C-3), δC 164.5 (C-5), δC 167.9 (C-8), and δC 105.2 (C-11), and δH 2.70 (H-10) and δC 132.5 (C-2), δC 105.2 (C-11), and δC 126.0 (C-11a) [Figure 2: see original paper]. These data confirmed the tetrahydro-β-carboline diketopiperazine structural unit. HMBC correlations between δH 2.97 (H-16) and δC 56.1 (C-6), δC 134.9 (C-17), and δC 130.0 (C-18, C-22) indicated that the monosubstituted benzene was connected to the diketopiperazine skeleton at C-6 via the methylene at position 16. The ROESY spectrum revealed NOE correlations between δH 5.74 (H-3)/δH 4.11 (H-9)/δH 2.97 (H-16) and δH 1.48 (CH$_3$-1')/δH 4.45 (H-6) [Figure 2: see original paper], establishing that H-3, H-9, and H-16 reside on the same face of the diketopiperazine ring, while CH$_3$-1 and H-6 are on the opposite face. This defined the relative configurations of H-3, H-6, and H-9 as 3α, 6β, and 9α, respectively. A SciFinder database search confirmed compound 1 as a new compound, designated tectonicgenazine A, with the chemical structure shown in Figure 1 [Figure 1: see original paper].

Conclusion and Discussion

This study isolated and identified one new tetrahydro-β-carboline diketopiperazine alkaloid, tectonicgenazine A (1), and three known compounds —trans-cyclo-(D-tryptophanyl-L-tyrosyl) (2), 1H-indole-3-carboxylic acid-2,3-dihydroxypropyl ester (3), and N-hydroxyethyl-2-acetylpyrrole (4)—from the rice solid-state fermentation extract of the predominant endophytic fungus *Diaporthe tectonigena* isolated from healthy branches of the ancient *Camellia taliensis* "Jinxiuchazu." All four compounds were obtained from this fungal strain for the first time.

Compound 2 was first isolated from *Microbispora rosea* subsp. *aerata* in 2013 and later reported from the ethyl acetate extract of endophytic *Diaporthe sp.*

from *Tylophora floribunda*. Compound 3 represents a new natural product isolated from nature for the first time, though it was previously reported in 1967 as a synthetic intermediate for indole glyceride derivatives. Compound 4 was first isolated from *Sauropus spatulifolius* in 2013, and had earlier been documented as an intermediate in the synthesis of marine pyrroles. Tetrahydro-β-carboline diketopiperazines are specialized indole alkaloids widely distributed in nature with diverse bioactivities including antitumor and antimicrobial effects. The β-carboline alkaloids have become the most intensively studied class of carboline alkaloids due to their abundance and relatively simple structures, while the diketopiperazine moiety serves as an important pharmacophore in medicinal chemistry. Whether the new compound tectonicgenazine A (1) possesses similar biological activities requires further investigation, and additional unknown diketopiperazine analogues from *Diaporthe tectonigena* warrant deeper exploration.

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