A set of interesting sequoiatones stereoisomers from a wetland soil-derived fungus *Talaromyces flavus*

Tian-Yu Suna, Jian Zoua, Guo-Dong Chena,*, Dan Hua, Bin Wub, Xing-Zhong Liub, Xin-Sheng Yaoa, Hao Gaona,*,

Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, China

Received 7 May 2016; revised 22 June 2016; accepted 27 June 2016

**KEY WORDS**
- Wetland soil-derived fungus
- *Talaromyces flavus*
- Stereoisomers
- Sequoiatone
- Electronic circular dichroism

**Abstract** Four interesting sequoiatones stereoisomers (1–4) were isolated from a wetland soil-derived fungus *Talaromyces flavus* by chiral HPLC. On the basis of comprehensive NMR and mass analyses, their planar structures were elucidated as the same as that of sequoiatone B. Among them, 1 and 3 (or 2 and 4) were a pair of enantiomers, and 1 and 2 (or 3 and 4) were a pair of stereoisomers with epimerization at C-12, which indicated that sequoiatone-type metabolites exist as enantiomers rather than as optically pure compounds in some strains. With the quantum chemical ECD calculations, the absolute configurations of C-8 in 1–4 were determined, which is the first report to establish the absolute configuration of C-8 in sequoiatones. However, the absolute configurations of C-12 in sequoiatones are still unsolved.

© 2016 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Sequoiatones, a kind of fungal metabolites, possess an octahydrocyclopenta[c]pyran skeleton with a long conjugated system \[ \text{C}_{14} \], which is similar to the azaphilones monascorubrin and monocillium \[ \text{C}_{16} \]. To date, only few sequoiatones, such as sequoiatones \( A-F \), were isolated from fungi \( \text{Aspergillus parasiticus} \) and \( \text{Penicillium sp.} \). Their structural diversity depend on diversiform ring systems (dicyclic or tricyclic systems), the position of CH\(_3\) in the molecular formula of sequoiatones \( 1 \), and the hydroxylation at C-18.

In the course of our search for bioactive secondary metabolites from wetland fungi \( \text{Talaromyces sp.} \), the chemical investigation of metabolites from \( \text{Talaromyces flavus} \) (AHK073) was carried out, which led to the isolation of four stereoisomers (1–4) (Fig. 1) of sequoiatones with chiral HPLC (Fig. 2), whose planar structures were the same as that of sequoiatone B. According to the analyses of NMR, optical rotation, and electronic circular dichroism (ECD) data, 1 and 3 (or 2 and 4) were a pair of enantiomers, and 1 and 2 (or 3 and 4) were a pair of stereoisomers with epimerization at C-12. In addition, the absolute configurations of C-8 in 1–4 were determined by quantum chemical ECD calculations. Detail of the isolation and structural elucidations of compounds 1–4 are presented herein.

2. Results and discussion

Compound 1 was obtained as a yellow oil. The quasi-molecular ion at \( m/z \) 375.2177 [M+H]\(^+\) by HR-ESI-MS indicated the molecular formula of 1 was \( \text{C}_{22}\text{H}_{30}\text{O}_{3} \) with 8 degrees of unsaturation. The \( ^{13}\text{C} \) NMR spectrum combined with the DEPT-135 spectrum showed 22 signals (Table 1), assigned to seven sp\(^2\) quaternary carbons [including one ketone carbonyl (\( \delta_C \) 208.0), one ester carbonyl (\( \delta_C \) 165.4), and five olefinic carbons (\( \delta_C \) 168.7, 163.9, 152.7, 135.4 and 106.7)], three sp\(^3\) methine carbons (\( \delta_C \) 141.9, 113.2 and 106.6), one sp\(^3\) oxygenated quaternary carbon (\( \delta_C \) 77.7), one sp\(^3\) methine carbon (\( \delta_C \) 47.4), five sp\(^3\) methylene carbons (\( \delta_C \) 34.0, 31.7, 29.4, 27.4 and 22.6), and five methyl carbons (\( \delta_C \) 50.9, 27.3, 20.3, 17.4 and 14.1). In the \( ^{1}H \) NMR spectrum of 1 (Table 1), the characteristic signals of one exchangeable proton [\( \delta_H \) 7.54 (1H, s)], three aromatic or olefinic protons [\( \delta_H \) 7.40 (1H, s), 7.21 (1H, s) and 6.96 (1H, s)], one sp\(^3\) methine proton [\( \delta_H \) 2.67 (1H, m)], and five methyl protons [\( \delta_H \) 3.84 (3H, s), 2.26 (3H, s), 1.53 (3H, s), 1.14 (3H, d, J=6.9 Hz) and 0.86 (3H, t, J=6.8 Hz)] were observed. The non-exchangeable proton resonances were associated with the directly attached carbon atoms in the HSQC experiment (Table 1). The analysis of the \( ^{1}H–H \) COSY experiment and the coupling values of the protons indicated the presence of one subunit (C-22–C-12–C-13–C-1–C-15–C-16–C-17–C-18) as shown in Fig. 3. Combined with the above deduced subunit and molecular formula, the HMBC correlations from H-18 to C-16/C-17, from H-19 to C-1, from H-20 to C-4/C-5, from H-21 to C-7/C-8/C-9, from H-22 to C-11/C-12/C-13, from H-4 to C-5/C-7/C-20, from H-6 to C-3/C-5/C-7/C-10, from H-10 to C-2/C-8/C-9/C-11, from 8-OH to C-7/C-8, and the NOESY correlations between H-19 and H-4/H-10, revealed the planer structure of 1 (Fig. 3).

The assignments of all proton and carbon resonances are provided in Table 1. Furthermore, the key NOESY correlation between H-19 and H-10 also suggested the Z configuration of the trisubstituted olefin (C-9–C-10) in 1.

Compounds 2–4 were also obtained as yellow oils. Based on the analysis of HR-ESI-MS data, the molecular formulas of 2–4 were the same as 1. Furthermore, the 1D and 2D NMR data revealed that 2–4 had the same planer structure as 1, which were stereoisomers of 1. In addition, the irradiation of H3-19 causing enhancements of H-10 in the selective 1D NOESY experiment of 2–4 revealed the Z configurations of the trisubstituted olefin (C-9–C-10) in 2–4, which were the same as 1 (see Supplementary Information Figs. S14, S21 and S27).

In the structures of 1–4, there are two stereogenic centers (C-8 and C-12) in the skeleton, which are isolated by three carbons. Detailed analyses of the 1D NMR data of 1–4 presented that the NMR data of 1 and 3 are identical except for the exchangeable proton (Table 1). The similar observation was in NMR data of 2 and 4, and there is only 0.01 ppm offset at H-12. Two sets of NMR data with little offset (0.01/0.1 ppm) at few protons/carbon (except for exchangeable protons) can be recognized as identical \(^{11}\). For the identical NMR data and the similar absolute values of optical rotations but in opposite directions of 1 and 3 (or 2 and 4), 1 and 3 (or 2 and 4) should be a pair of enantiomers.

Between 1 and 2 (or 3 and 4), there were several noticeable offsets at C-10 (0.2 ppm), H-12 (0.02 ppm), and C-22 (0.3 ppm)/H-22 (0.02 ppm) (Table 1), which resulted from the epimerization at C-8 or C-12. In ECD experiments, the ECD curves of 1 and 2 were similar with each other, which were opposite to those of 3 and 4. To investigate the effect of different configurations of C-8 and C-12 to ECD of 1–4, the ECD calculations were carried out. Since the flexible side chain at C-12 has negligible effect on the ECD of sequoiatones \( \text{C}_{12} \), the ECD curves of \( \text{C}_{12} \)–\( \text{C}_{12} \) were apparently controlled by the absolute configuration of C-8. The experimental data of 1 and 2 were similar to the predicted ECD curve of \( \text{C}_{12} \)–\( \text{C}_{12} \), which suggested that the absolute configurations of C-8 in 1 and 2 were \( \text{R} \). The experimental data of 3 and 4 were similar to the predicted ECD curve of \( \text{C}_{12} \)–\( \text{C}_{12} \) (Fig. 5), which suggested that the absolute configurations of C-8 in 3 and 4 were \( \text{S} \). Therefore, the epimerization between 1 and 2 (or 3 and 4) was at C-12. However, the configurations of C-12 were not determined.
3. Conclusions

Up to now, only few sequoiatones have been reported. Except the configuration of sequoiatone A had been established by X-ray crystallography\(^1\), the absolute configurations of C-8 and C-12 in other sequoiatones were not unambiguously investigated. In our investigation, four stereoisomers of sequoiatones were isolated by chiral HPLC, whose planar structures were the same as that of sequoiatone B\(^1\). Among them, 1 and 3 (or 2 and 4) are a pair of enantiomers, and 1 and 2 (or 3 and 4) are a pair of stereoisomers with epimerization at C-12, which indicated that sequoiatone-type metabolites exist as enantiomers rather than as optically pure compounds in some strains. Based on the analyses of experimental and quantum chemical ECD, it was found that the absolute configuration of C-8 in sequoiatones can be determined by ECD, which is the first time to discuss the absolute configuration of C-8 in sequoiatones. The absolute configurations of C-12 in sequoiatones are still unsolved.

![Figure 2](image-url)  
**Figure 2** HPLC analysis of 1–4 (A: the analysis of 1–4 on routine ODS HPLC; B: the analysis of 1–4 on chiral HPLC).

![Figure 3](image-url)  
**Figure 3** Key \(^1\)H–\(^1\)H COSY, HMBC, and NOESY correlations of 1.

| Table 1 \(^1\)H NMR (400 MHz) and \(^13\)C NMR (100 MHz) spectral data of 1–4 (δ in ppm, J in Hz, CDCl\(_3\)). |
|---|---|---|---|---|
| No. | 1 | 2 | 3 | 4 |
| δ\(_C\) | δ\(_H\)^a | δ\(_C\) | δ\(_H\)^a | δ\(_C\) | δ\(_H\)^a | δ\(_C\) | δ\(_H\)^a |
| 1 | 165.4 | 165.4 | 165.4 | 165.4 | 165.4 | 165.4 |
| 2 | 106.7 | 106.7 | 106.7 | 106.7 | 106.7 | 106.7 |
| 3 | 152.7 | 152.8 | 152.7 | 152.8 | 152.7 | 152.8 |
| 4 | 106.6 | 6.96, s | 106.6 | 6.96, s | 106.6 | 6.96, s | 106.6 | 6.96, s |
| 5 | 163.9 | | 163.9 | | 163.9 | | 163.9 | |
| 6 | 141.9 | 7.40, s | 141.9 | 7.41, s | 141.9 | 7.40, s | 141.9 | 7.41, s |
| 7 | 135.4 | | 135.4 | | 135.4 | | 135.4 | |
| 8 | 77.7 | | 77.6 | | 77.7 | | 77.6 | |
| 9 | 168.7 | 168.6 | | 168.7 | | 168.6 | |
| 10 | 113.2 | 7.21, s | 113.4 | 7.21, s | 113.2 | 7.21, s | 113.4 | 7.21, s |
| 11 | 208.0 | | 208.0 | | 208.0 | | 208.0 | |
| 12 | 47.4 | 2.67 | 47.3 | 2.69 | 47.4 | 2.67 | 47.3 | 2.69 |
| 13 | 34.0 | 1.68, Ha; 1.38, Hb | 34.0 | 1.69, Ha; 1.40, Hb | 34.0 | 1.68, Ha; 1.39, Hb | 34.0 | 1.69, Ha; 1.39, Hb |
| 14 | 27.4 | 1.28^b | 27.4 | 1.28^b | 27.4 | 1.28^b | 27.4 | 1.28^b |
| 15 | 29.4 | 1.28^b | 29.4 | 1.28^b | 29.4 | 1.28^b | 29.4 | 1.28^b |
| 16 | 31.7 | 1.28^b | 31.7 | 1.28^b | 31.7 | 1.28^b | 31.7 | 1.28^b |
| 17 | 22.6 | 1.25 | 22.6 | 1.25 | 22.6 | 1.25 | 22.6 | 1.25 |
| 18 | 14.1 | 0.86, t (6.8) | 14.1 | 0.86, t (6.8) | 14.1 | 0.86, t (6.8) | 14.1 | 0.86, t (6.8) |
| 19 | 50.9 | 3.84, s | 50.9 | 3.84, s | 50.9 | 3.84, s | 50.9 | 3.84, s |
| 20 | 20.3 | 2.26, s | 20.4 | 2.26, s | 20.3 | 2.26, s | 20.4 | 2.26, s |
| 21 | 27.3 | 1.53, s | 27.3 | 1.53, s | 27.3 | 1.53, s | 27.3 | 1.53, s |
| 22 | 17.4 | 1.14, d (6.9) | 17.1 | 1.12, d (6.8) | 17.4 | 1.14, d (6.9) | 17.1 | 1.12, d (6.8) |
| OH-8 | 7.54, s | 7.58, br s | 7.53, s | 7.57, br s | 7.54, s | 7.58, br s | 7.53, s | 7.57, br s |

^aIndiscernible signals owing to overlapping or having complex multiplicity are reported without designating multiplicity.

^bThe assignments in each column may be interchanged.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P1020 digital polarimeter (Jasco International Co., Ltd., Tokyo, Japan). UV data were recorded using a JASCO V-550 UV/vis spectrometer (Jasco International Co., Ltd., Tokyo, Japan). IR data were recorded on a JASCO FT/IR-480 plus spectrometer (Jasco International Co., Ltd., Tokyo, Japan). ECD spectrum was recorded on a JASCO J-810 spectrophotometer using MeOH as the solvent (Jasco International Co., Ltd., Tokyo, Japan). The ESI-MS spectra were performed on a Finnigan LCQ Advantage MAX mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). The HR-ESI-MS spectra were obtained on a Micromass Q-TOF mass spectrometer (Waters Corporation, Milford, MA, USA). The NMR spectra were measured with Bruker AV-400 spectrometer (Bruker BioSpin Group, Faellanden, Switzerland) using solvent signals (CDCl3: δH 7.26/δC 77.0) as internal standard. The analytical HPLC was performed on a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 diode array detector (DAD), an Ultimate 3000 Column Compartment, and an Ultimate 3000 autosampler (Thermo Fisher Scientific, Inc., Waltham, MA, USA) using a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 μm) (Phenomenex Inc., Los Angeles, CA, USA). The semi-preparative HPLC was performed on a Shimadzu LC-6-AD Liquid Chromatography with an SPD-20A Detector using a Phenomenex Gemini C18 column (250 mm × 10.0 mm, 5 μm) (Phenomenex Inc., Los Angeles, CA, USA). The chiral HPLC was performed on a Shimadzu LC-6-AD Liquid Chromatography with an SPD-20A Detector using a Phenomenex Lux Cellulose-2 chiral column (250 mm × 4.6 mm, 3 μm) (Phenomenex Inc., Los Angeles, CA, USA). The medium pressure liquid chromatography (MPLC) was performed on ODS (60–80 μm, YMC Co., Ltd., Tokyo, Japan) and equipped with a dual pump gradient system, a UV preparative detector, and a Dr Flash II fraction collector system (Lisui E-Tech Co., Ltd., Shanghai, China).

4.2. Fungus material

The strain AHK07-3 was isolated from the soil, collected at the wetland of Ahongkou, Sinkiang Province, China. The strain was identified as Talaromyces flavus based on the morphological characters and gene sequence analysis. The ribosomal internal transcribed spacer (ITS) and the 5.8S rRNA gene sequences (ITS1-5.8S-ITS2) of the strain have been deposited at GenBank as KX011167. The fungus was cultured on slants of potato dextrose agar at 25°C for 5 days. Agar plugs were used to inoculate four Erlenmeyer flasks (250 mL), each containing 100 mL of potato dextrose broth. Four flasks of the inoculated media were incubated at 25°C on a rotary shaker at 200 rpm for 5 days to prepare the seed culture. Fermentation was carried out in 20 Erlenmeyer flasks (500 mL), each containing 70 g of rice. Distilled H2O (105 mL) was added to each flask, and the rice was soaked overnight before autoclaving at 120 °C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 50 days.

4.3. Extraction and isolation

The culture was extracted three times with EtOAc, and the organic solvent was removed under reduced pressure to yield crude extract (19.7 g). The crude extract was dissolved in 90% v/v aqueous MeOH (400 mL) and partitioned against the same volume of cyclohexane to afford a cyclohexane fraction (C, 4.6 g) and an aqueous MeOH fraction (W, 14.7 g). The aqueous MeOH fraction

---

Figure 4 The calculated ECD spectra of (8S,12R)-5, (8R,12S)-5, (8S,12S)-5, and (8R,12R)-5 (band width σ=0.3 eV).

Figure 5 The experimental ECD spectra of 1–4 and calculated ECD spectra of (8S,12R)-5 and (8R,12R)-5 (UV correction = 0 nm, band width σ=0.3 eV).
A set of interesting sequoatones stereoisomers from a wetland soil-derived fungus Talaromyces flavus

4.4. ECD calculation

Before molecular initial 3D structures were generated with CORINA version 3.4, the molecules of (8S, 12R)-5, (8R, 12S)-5, (8S, 12S)-5, and (8R, 12R)-5 were converted into SMILES codes, respectively. Conformer databases were generated in CONFLEX version 7.0 using the MMFF94s force-field, with an energy window for acceptable conformers (ewindow) of 5 kcal/mol above the ground state, a maximum number of conformations per molecule (maxconfs) of 100, and an RMSD cutoff (rmsd) of 0.5 Å. Then each conformer of the acceptable conformers was optimized with HF/6-31G(d) method in Gaussian09. Further optimization at the APFD/6-31G(d) level led the dihedral angles to be got. After that, thirteen lowest energy conformers of (8S, 12R)-5 and (8R, 12S)-5 were found out (see Supplementary Information Table S5 and Fig. S1). Fourteen lowest energy conformers of (8S, 12S)-5 and (8R, 12R)-5 were found out (see supporting information Table S6 and Fig. S2). The optimized conformers were taken for the ECD calculations, which were performed with Gaussian09 (APFD/6-311++G(2d,p)). The solvent effect was taken into account by the polarizable-conductor calculation model (IEFPCM, methanol as the solvent). Comparisons of the experimental and calculated spectra were done with the software SpecDis. It was also used to apply a UV shift to the ECD spectra, Talaromyces broadening of the excitations, and Boltzmann weighting of the spectra.

Acknowledgments

This work was financially supported by grants from the National Natural Science Foundation of China (Nos. 81422054 and 81172945), the Guangdong Natural Science Funds for Distinguished Young Scholar (2013050014287), Guangdong Special Support Program (2014TQ01R420), and Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (Hao Gao, 2014).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2016.07.005.

References


