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A Novel Carbon Skeletal Trichothecane, Tenuipesine A, Isolated from an Entomopathogenic Fungus, *Paecilomyces tenuipes*

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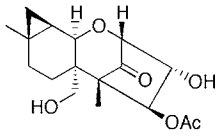
ABSTRACT



Tenuipesine A (**1**)

Tenuipesine A (1), a novel trichothecane with an unprecedented carbon-migrated skeleton that embodies of a cyclopropane ring, was isolated from cultivated fruiting bodies of *Paecilomyces tenuipes* (*Isaria japonica*), a popular entomopathogenic fungi employed in folk medicine and health foods in China, Korea, and Japan. The structure was determined on the basis of two-dimensional NMR data. Its stereochemistry was elucidated by spectroscopic data and the chemical transformation of the coexisting trichothecene, 4β-acetoxy-12,13-epoxytrichothec-9-ene-3α,15-diol (2).

A group of entomopathogenic fungi that form fruiting bodies are known as caterpillar fungi. Some of these fungi are used to treat various diseases in traditional and folk remedies in Asian countries such as China, Korea and Japan.[1] *Paecilomyces tenuipes*, also called *Isaria japonica*, is a popular entomopathogenic fungi and often used as folk medicine and health food. Although its chemical components and its pharmacological activity have attracted the attention of phytochemists and pharmacologists, few scientific reports have been presented.[2] Now, we have cultivated the fruiting body of this fungus on a large scale and investigated their secondary metabolites.[3,4] In this paper, we describe the isolation of a novel trichothecane, tenuipesine A (**1**), where C-13 migrated to the C-9 double bond present in common trichothecenes.[5]

The cultured fruiting body of *P. tenuipes* (6.8 kg) was extracted three times with MeOH at room temperature, and

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Table 1. ^{13}C and ^{1}H NMR Spectral Data of Tenuipesine A (**1**)[a]

position	^{13}C	^{1}H
2	78.9	3.87 (1H, d, J = 4.9 Hz)
3	73.2	4.00 (1H, td, J = 4.9, 3.4 Hz)
4	81.7	5.91 (1H, d, J = 3.4 Hz)
5	58.0	
6	51.4	
7α	19.4	1.28 (1H, ddt, J = 13.9, 5.6, 2.1 Hz)
b		0.94 (1H, td, J = 13.9, 5,5 Hz)
8α	25.8	1.49 (1H, td, J = 13.9, 5.6 Hz)
β		1.76 (1H, ddd, J = 13.9, 5.5, 2.1 Hz)
9	15.1	
10	21.1	1.12 (1H, td, J = 9.0, 4.9 Hz)
11	67.6	4.66 (1H, dd, J = 9.0, 2.1 Hz)
12	211.1	
13α	12.5	0.44 (1H, dd, J = 9.0, 4.9 Hz)
β		0.76 (1H, t, J = 4.9 Hz)
14	7.7	0.97 (3H, s)
15	63.3	3.89 (1H, dd, J = 12.2, 8.0 Hz)
		3.63 (1H, dd, J = 12.2, 4.1 Hz)
16	26.3	1.09 (3H, s)
$COCH_3$	172.4	
$COCH_3$	20.8	2.12 (3H, s)
3-OH		3.01 (1H, d, J = 4.9 Hz)
15-OH		2.76 (1H, dd, J = 8.0, 4.1 Hz)

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.

the MeOH extract was partitioned between ethyl acetate and water. The ethyl acetate solubles (159 g) were separated by column chromatography over silica gel and ODS repeatedly to yield tenuipesine A (**1**) (3 mg, 0.00004%).

The molecular formula of tenuipesine A (**1**), $C_{17}H_{24}O_6$, was established by HREIMS (*m*/*z* 324.1577). The ^{13}C NMR spectrum (Table 1) revealed 17 carbon signals due to two carbonyls, three quaternary carbons, five methines, four methylenes, and three methyls. Among them, the four methines (δ 81.7, 78.9, 73.2, and 67.6) and one methylene (δ 63.3) were ascribed to those bearing an oxygen atom. ^{1}H and ^{13}C NMR signals, δ_H 2.12 and δ_C 172.4 and 20.8, indicated the presence of an acetyl group.

The $^{1}H-^{1}H$ COSY spectrum revealed the connectivities of three partial structures **a** (C-11−C-10−C-13), **b** (C-2 to C-4), and **c** (C-7−C-8) as shown in Figure 1. HMBC correlations were observed for H_3-16 to C-8, C-9, C-10, and C-13, indicating that a methyl group was on the cyclopropane ring (Figure 1A). HMBC correlations for H_3-14 to C-4, C-5, C-6, and C-12; H-2 to C-12; and H_3-4 to acetyl carbonyl

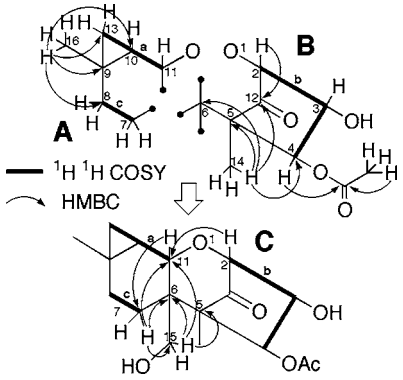


Figure 1. Selected two-dimensional NMR correlations for tenuipesine A (**1**).

carbon indicated a cyclopentanone moiety (Figure 1B). Furthermore, HMBC correlations for H-2 to C-11 and C-12; H_2-7 to C-6, C-11, and C-15; H-11 to C-7; and H_2-15 to C-5, C-6, and C-11 suggested that the planar structure for **1** was a kind of trichothecane where C-13 migrated to the C-9 double bond (Figure 1C).

Regarding stereochemistry of tenuipesine A (**1**), the cross-peaks observed between H-4−H-11 and H-2−H-3 in the NOESY spectrum demonstrated that compound **1** had identical relative configurations at C-2, C-3, C-4, C-5, C-6, and C-11 to those of the common trichothecanes such as the coexisting trichothecene, 4β-acetoxy-12,13-epoxytrichothec-9-ene-3α,15-diol (**2**).[3] β-Relative configuration of the cyclopropane ring was indicated by the NOE peaks of H-7β−H-13β, H-8β−H-13β, H-10−H-11, and H-10−H-13α.A plausible biogenetic pathway for tenuipesine A (**1**)

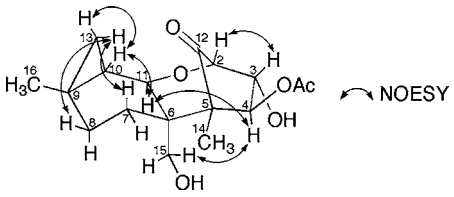
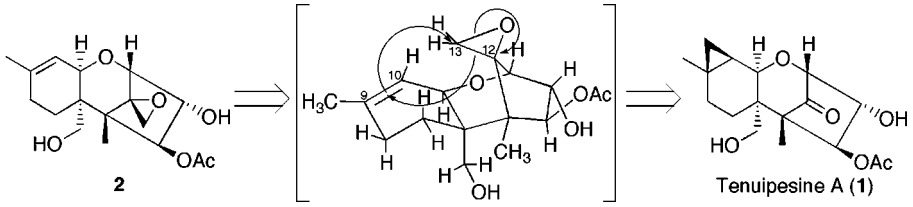


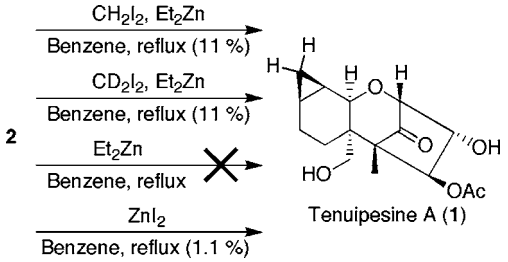
Figure 2. Relative structure of tenuipesine A (**1**).

is proposed in Scheme 1. The spatially close relationship of the C-13 epoxide ring and the C-9 double bond in the

Scheme 1. Plausible Biogenetic Pathway for Tenuipesine A (**1**).



Scheme 2. Transformation of **2** into Tenuipesine A (**1**).



trichothecene **2** may allow a nucleophilic reaction between them, which leads to the production of tenuipesine A (**1**).

Transformation of **2** using diethylzinc and diiodomethane afforded tenuipesine A (**1**) in a very low yield (11%) (Scheme 2), and the absolute configuration of tenuipesine A (**1**) is thus determined to be the same as that of **2**. No incorporation of deuteron into **1** was given by the use of diiodomethane-d_2 in this reaction, demonstrating that a C-13 methylene carbon in **1** was transferred from an epoxide ring in **2**. The use of only diethylzinc did not lead to reaction, and the reaction of **2** with zinc iodide afforded **1** in 1.1% yield (Scheme 2). From these results, the reaction may be catalyzed by Lewis acids such as zinc iodide, which coordinate oxygen atom of epoxide ring. The reaction supports the plausible biogenetic pathway for **1** in Scheme 1.

To the best of our knowledge, compound **1** is the first example of trichothecane in which C-13 migrated to the C-9 double bond to produce a cyclopropane ring. *P. tenuipes* may be a promising source for producing new types of trichothecanes that bear chemical and biological novelity.

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Supporting Information Available: Experimental procedure and NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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