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Novel trichothecanes, paecilomycine A, B, and C, isolated from entomopathogenic fungus, *Paecilomyces tenuipes*

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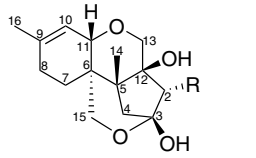
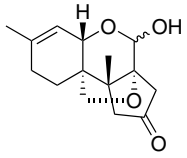
Abstract—Paecilomycine A (**1**), B (**2**), and C (**3**) were isolated from cultivated fruiting bodies of *Paecilomyces tenuipes* (*Isaria japonica*), which is a popular entomopathogenic fungus used in folk medicine and health foods in China, Korea, and Japan. The structures of **1**–**3** were deduced from their spectroscopic data and their absolute configurations were elucidated by preparing their MPA esters. Compound **1** showed activity in the neurotrophic factor biosynthesis in glial cells.

Trichothecanes are a growing class of closely related sesquiterpenoids produced by molds, especially from various species of Fungi imperfecti.[1] Many members of this class display a wide range of biological effects, such as antibacterial, antifungal, insecticidal, and cytostatic properties and phytotoxicity. *Paecilomyces tenuipes* (*Isaria japonica*) is a popular entomopathogenic fungus used in folk medicine and health foods in China, Korea, and Japan. Working up a large-scale cultivation of its fruiting body in barley grain, a series of hitherto unknown minor trichothecanes named paecilomycine A (**1**), B (**2**), and C (**3**) were produced along with conventional trichothecenes and spirotenuipesine A and B.[2] In this paper, we report their isolation, structure elucidation, and biological activity.



Paecilomycine A (**1**) : R = H
B (**2**) : R = OH



Paecilomycine C (**3**)

Our previous report described the cultivation and extraction of *P. tenuipes*. [2] The ethyl acetate soluble fraction (159 g) of the methanol extract was separated by repeated column chromatography on silica gel and ODS to yield three novel trichothecanes **1** (105.4 mg),[3] **2** (3.4 mg),[4] and **3** (8.6 mg).[5]

HREIMS (*m/z* 266.1471 [M^+]), ^1H and ^{13}C NMR spectra (Table 1) indicated that the molecular formula of **1** was $C_{15}H_{22}O_4$. ^1H–^1H COSY revealed that C-7 and C-8 were connected. The methyl protons at C-16 were correlated to three carbons (C-8, -9, and -10), H-11 was correlated to C-6 and C-9, and H-15 was correlated to C-6 and C-7 in the HMBC spectrum, which suggested a substituted cyclohexene ring (Fig. 1A). The methyl protons at C-14 were correlated to three carbons (C-4, -5, and -12), H-13 was correlated to C-2 and C-5, and cross peaks of H-2–C-3 and H-4–C-3 were detected, which implied a substituted cyclopentane ring (Fig. 1B). The correlational peaks of H-11–C-5, H-15–C-5, H-4–C-6, and H-14–C-6 revealed that the partial structures A and B were connected between the C-5–C-6 bond. The two connections through the oxygen atom were deduced by the correlations of H-13–C-11 and H-15–C-3. Therefore, Figure 1C depicts the planar structure of **1** for a trichothecane sesquiterpenoid.

The cross peaks of H-7β–H-14 and H-11–H-14 in the NOESY spectrum indicated that the conformation of

Keywords: *Paecilomyces tenuipes*; Trichothecane; Glial cell; PC-12 cell.
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Table 1. ^{13}C and ^{1}H NMR spectral data of paecilomycine A (**1**) and B (**2**)

	Paecilomycine A (**1**)[a,b]		Paecilomycine B (**2**)[a,c]	
	^{13}C	^{1}H	^{13}C	^{1}H
2α	47.8	2.76 (1H, dd, 15.4, 2.2)	86.7	
β		1.98 (1H, d, 15.4)		3.66 (1H, s)
3	103.3		102.2	
4α	43.1	1.66 (1H, dd, 11.5, 2.2)	38.4	1.68 (1H, d, 12.6)
β		1.99 (1H, d, 11.5)		1.96 (1H, d, 12.6)
5	49.0		46.9	
6	36.9		40.8	
7α	23.4	1.95 (1H, dd, 13.7, 6.3)	27.6	1.65–1.70 (1H, m)
β		1.49–1.55 (1H, m)		1.52 (1H, dt, 13.2, 9.0)
8α	27.8	2.08–2.16 (1H, m)	28.6	1.91–1.98 (2H, m)
β		1.95–2.02 (1H, m)		
9	136.2		137.2	
10	122.1	5.16 (1H, dt, 3.5, 1.5)	123.5	5.23 (1H, m)
11	73.1	3.98 (1H, br s)	76.7	3.94–3.99 (1H, m)
12	76.7		77.7	
13α	71.3	3.83 (1H, d, 11.6)	70.2	4.10 (1H, d, 11.6)
β		3.41 (1H, d, 11.6)		3.31 (1H, d, 11.6)
14	13.3	1.01 (3H, s)	15.2	1.03 (3H, s)
15A	64.3	4.16 (1H, dd, 12.9, 2.1)	66.4	4.33 (1H, d, 12.2)
B		3.71 (1H, d, 12.9)		3.70 (1H, d, 12.2)
16	22.6	1.62 (3H, s)	22.5	1.64 (3H, s)

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Dissolved in $CDCl_3$.
[c] Dissolved in CD_3OD.

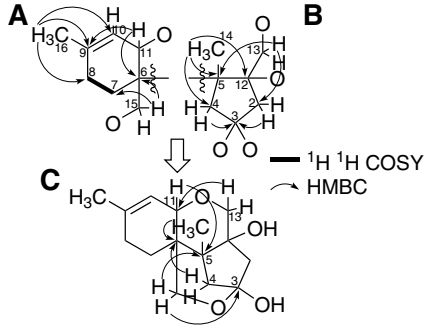


Figure 1. Planar structure of paecilomycine A (**1**).

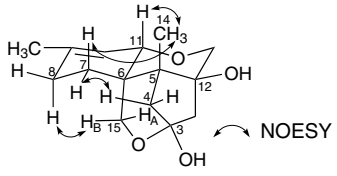


Figure 2. Relative structure of paecilomycine A (**1**).

the two six-membered rings bridged by C-6–C-11 bond was a *trans*-decaline type and the configuration of the methyl group at position 14 was axial. The configurations of stereocenters at C-3 and C-12 were determined by the correlational peaks of H-4α–H-7α and H-8α–H-15B (Fig. 2) and steric constraint. The structural rigidity of the tetracyclo ring in **1** allowed the W-couplings between H-2α–H-4α ($J = 2.2$ Hz) and H-7β–H-15A ($J = 2.1$ Hz) to be observed.

The HREIMS of **2** (*m*/*z* 282.1461 [M^+]) gave a molecular formula, $C_{15}H_{22}O_5$, which differs from that of **1** by an oxygen atom. The ^{1}H NMR spectrum of **2** was nearly identical to that of **1**, although **2** lacked the methylene signals at C-2 (δ_H 2.76 and 1.98), which were replaced by oxymethine proton (δ_H 3.66). Therefore, it was determined that compound **2** had hydroxy group at C-2. The cross peak between H-2 and H-4 in the NOESY spectrum revealed that the hydroxy group at C-2 had α-configuration.

HREIMS (*m*/*z* 264.1361 [M^+]) established that the molecular formula of **3** was $C_{15}H_{20}O_4$. ^{1}H and ^{13}C NMR spectra of **3** (Table 2) suggested that **3** was a mixture of two stereoisomers (**3a** and **3b**) in a ratio of 3:1. Benzoylation of **3** afforded benzoate **4** as a sole product (Scheme 1).[6] Thus, structure elucidation of **4** was initially performed. HREIMS (*m*/*z* 368.1613), ^{1}H, and ^{13}C NMR spectra (Table 2) indicated that the molecular formula of **4** was $C_{22}H_{24}O_5$. ^{1}H–^{1}H COSY revealed that C-7–C-8 and C-10–C-11 were connected. The partial structure A was constructed by the correlations of H-16–C-8, -9, and -10, H-15–C-6, -7, and -11, and H-10–C-6 in HMBC spectrum (Fig. 3A). The partial structure B was deduced by the correlations of H-14–C-4, -5, and -12, H-2–C-3, -5, and -12, H-4–C-3, and H-13–C-12 (Fig. 3B). The correlation between H-13 and the ester carbonyl carbon (δ 165.1) indicated that the benzoyl group was substituted at C-13. The correlational peaks between H-15–C-5, H-14–C-6, and H-15–C-12 revealed that the partial structures A and B were connected, which was used to determine the planar structure of **4** (Fig. 3C).

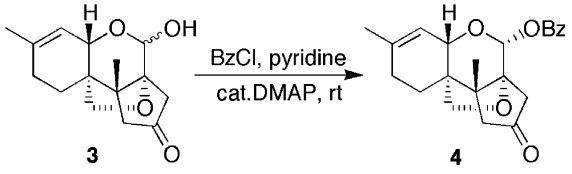
The cross peaks between H-7β–H-14, H-7β–H-11, and H-11–H-14 in the NOESY spectrum indicated that the conformation of the two six-membered rings was the same as that of **1**. The configuration of the stereocenter at C-12 was determined by the cross peaks of H-2β–H-14 (Fig. 4). The proton at C-13 was correlated to H-11 and methyl protons at C-14, suggesting that the configuration of benzoyloxy group at C-13 was α. Thus, the structure of benzoate **4** led to determination of the structure of **3**. Compound **3** was an equilibrium mixture of **3a** and **3b** caused by the hemiacetal moiety at C-13. In NOESY spectrum of **3**, the cross peak of H-13–H-14 for the major isomer **3a** indicated that the configurations of the hydroxy group at C-13 of **3a** and **3b** were α and β, respectively.

To determine the absolute configuration of **1**, the following transformation was conducted (Scheme 2). Tetraol **5** was prepared by treating **1** with osmium tetroxide.[7] Esterification of **5** with (*R*)-α-methoxyphenylacetic acid in the presence of EDCI and DMAP yielded 10-*O*-(*R*)-MPA ester (**6a**).[8] In a similar manner, 10-*O*-(*S*)-MPA ester (**6b**) was afforded.[9] The $\Delta\delta_{RS}$ value of each proton was calculated from the difference in chemical shifts between **6a** and **6b** (Fig. 5). Then the structure of **5** was fitted into the proposed model of α-methoxy-

Table 2. ^{13}C and ^{1}H NMR spectral data of paecilomycine C (**3**) and its benzoate (**4**)[a]

	Major isomer (**3a**)		Minor isomer (**3b**)		Benzoate (**4**)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2α	42.3	2.47 (1H, d, 18.5)	42.6	2.65 (1H, d, 18.2)	41.9	2.52 (1H, d, 18.2)
β		2.36 (1H, d, 18.5)		2.38 (1H, d, 18.2)		2.37 (1H, d, 18.2)
3	213.1		214.1		212.6	
4α	47.0	2.42 (1H, d, 17.9)	47.6	2.37 (1H, d, 17.1)	46.9	2.47 (1H, d, 18.0)
β		1.99 (1H, d, 17.9)		1.95 (1H, d, 17.1)		2.00 (1H, d, 18.0)
5	49.4		49.2		50.4	
6	47.1		47.8		47.5	
7α	23.1	1.51 (1H, ddd, 13.3, 5.7, 1.4)	23.8	1.47 (1H, ddd, 13.6, 5.4, 1.8)	23.2	1.51–1.59 (1H, m)
β		1.66 (1H, ddd, 13.3, 11.6, 6.6)		1.62–1.70 (1H, m)		1.67 (1H, ddd, 14.5, 12.6, 6.5)
8α	28.2	1.83–1.92 (1H, m)	28.0	1.80–1.86 (1H, m)	28.2	1.82–1.94 (1H, m)
β		1.98 (1H, br d, 17.9)		1.90 (1H, d, 17.6)		1.99 (1H, br d, 17.9)
9	135.8		136.3		136.3	
10	121.6	5.33 (1H, br s)	121.5	5.26 (1H, br s)	121.1	5.36 (1H, br s)
11	72.9	4.60 (1H, br s)	69.5	4.76 (1H, br s)	73.9	4.75 (1H, br s)
12	88.9		87.2		87.6	
13	92.7	5.08 (1H, d, 12.8)	95.7	5.18 (1H, d, 3.7)	91.9	6.36 (1H, s)
14	15.4	1.21 (3H, s)	16.7	1.39 (3H, s)	15.9	1.32 (3H, s)
15A	72.3	3.67 (1H, d, 8.1)	71.0	3.60 (1H, d, 8.1)	72.3	3.73 (1H, d, 8.3)
B		4.21 (1H, d, 8.1)		4.19 (1H, d, 8.1)		4.35 (1H, d, 8.3)
16	22.8	1.70 (3H, s)	23.0	1.70 (3H, s)	22.8	1.68 (3H, s)
13OH		3.25 (1H, d, 12.8)		3.03 (1H, d, 3.7)		
PhC*O*					165.1	
Ph-1					129.1	
Ph-2					130.1	7.39–7.44 (2H, m)
Ph-3					128.3	8.05–8.09 (2H, m)
Ph-4					133.5	7.56 (1H, tt, 7.5, 1.3)

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$.



Scheme 1. Conversion of **3** into **4**.

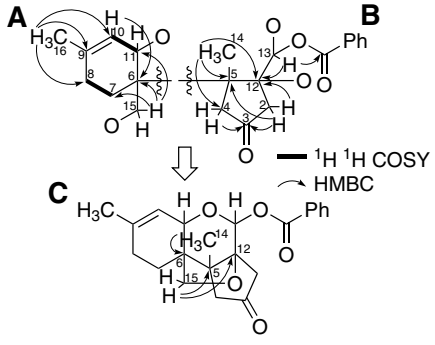


Figure 3. Planar structure of **4**.

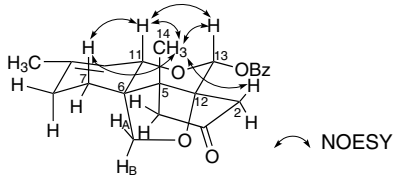
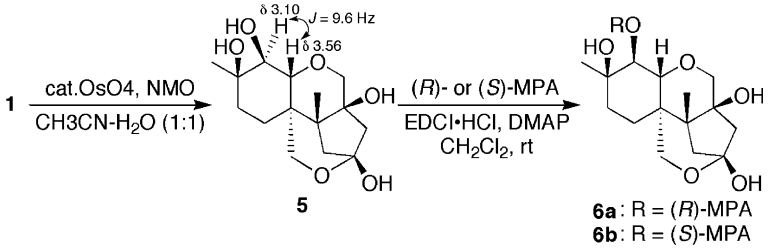


Figure 4. Relative structure of **4**.

phenylacetate[10] in accordance with the sign of $\Delta\delta_{RS}$ and thus the absolute configuration of **1** was determined to be 3*R*, 5*R*, 6*R*, 11*S*, and 12*S*. Since **1**–**3** were supposed to be biosynthesized from a common precursor, the absolute configurations of **2** and **3** were presumed to be the same as that of **1**.

To investigate the biological effects, 1321N1 human astrocytoma cells (glial cell line) were incubated for 2 days with each compound. Then rat pheochromocytoma (PC-12) cells were cultivated for 2 days in the conditioned 1321N1 culture medium, which has been shown to contain neurotrophic factors synthesized in 1321N1 cells and promotes the differentiation of PC-12 cells.[11] The culture medium conditioned with 10 nM of **1** enhanced the extension of neurite outgrowth of PC-12 cells. This result indicated that compound **1** biosynthesized and released neurotrophic factors from 1321N1 cells and the released neurotrophic factors promoted neuronal differentiation of PC-12 cells, like cyathane diterpenoids, scabronine A and G.[12] The potency of **1** to release neurotrophic factors was 1000 times higher than scabronine G, indicating that compound **1** may be a lead compound in drug synthesis for neurodegenerative diseases. However, this effect was not observed with 1 μM or more of **3**. The amount of **2** was insufficient to be tested.

Although many trichothecane-type sesquiterpenoids have been isolated as fungal metabolites, compounds containing a cyclic skeleton like **1**–**3** are scarcely known except sambucoin (**7**)[13] and its derivatives.[14,15] In addition to spirotenuipesines,[2] the isolation of **1**–**3**



Scheme 2. Conversion of **1** into **6a** and **6b**.

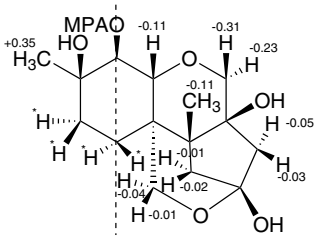
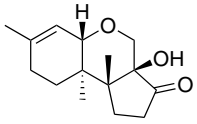


Figure 5. $\Delta\delta_{RS}$ values for (*R*)-MPA ester (**6a**) and (*S*)-MPA ester (**6b**).

suggests that *P. tenuipes* is a rich source for producing various secondary metabolites, especially novel trichothecanes.



Sambucoin (**7**)

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3. Data for **1**: colorless oil; $[\alpha]_D^{25}$ +30.8 (*c* 0.694, $CHCl_3$); EIMS *m/z* 266 $[M]^+$, 236, 218, 203, 179 (base); HREIMS *m/z* 266.1471 (266.1518 calculated for $C_{15}H_{22}O_4$). NMR data are shown in Table 1.
4. Data for **2**: colorless oil; $[\alpha]_D^{25}$ +11.6 (*c* 0.933, CH_3OH); EIMS *m/z* 282 $[M]^+$, 264, 236, 218, 207, 191, 179, 161, 41 (base); HREIMS *m/z* 282.1482 (282.1467 calculated for $C_{15}H_{22}O_5$). NMR data are shown in Table 1.
5. Data for **3**: colorless oil; $[\alpha]_D^{25}$ +97.7 (*c* 0.682, $CHCl_3$); EIMS *m/z* 264 $[M]^+$, 249, 235, 107 (base); HREIMS *m/z* 264.1361 (264.1362 calculated for $C_{15}H_{20}O_4$). NMR data are shown in Table 2.
6. Data for **4**: colorless oil; $[\alpha]_D^{25}$ +91.8 (*c* 0.134, $CHCl_3$); EIMS *m/z* 368 $[M]^+$, 353, 246, 105 (base); HREIMS *m/z* 368.1613 (368.1624 calculated for $C_{22}H_{24}O_5$). NMR data are shown in Table 2.
7. Data for **5**: colorless oil; $[\alpha]_D^{25}$ −11.1 (*c* 0.542, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 4.25 (1H, dd, *J* = 12.3, 2.1 Hz), 3.88 (1H, d, *J* = 12.3 Hz), 3.87 (1H, d, *J* = 11.5 Hz), 3.56 (1H, d, *J* = 9.8 Hz), 3.36 (1H, d, *J* = 11.5 Hz), 3.10 (1H, d, *J* = 9.8 Hz), 2.75 (1H, dd, *J* = 15.4, 2.2 Hz), 2.00 (1H, d, *J* = 11.5 Hz), 1.99 (1H, d, *J* = 15.4 Hz), 1.71–1.80 (1H, m), 1.45–1.66 (3H, m), 1.62 (1H, dd, *J* = 11.5, 2.2 Hz), 1.24 (3H, s), 1.09 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 103.3, 76.5, 76.3, 73.7, 71.1, 71.0, 63.9, 49.8, 47.4, 43.2, 38.9, 32.4, 27.3, 21.7, 13.3; EIMS *m/z* 300 $[M]^+$, 282, 264, 249, 43 (base); HREIMS *m/z* 300.1549 (300.1573 calculated for $C_{15}H_{24}O_6$).
8. Data for **6a**: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) δ 7.42–7.45 (2H, m), 7.28–7.36 (3H, m), 4.83 (1H, s), 4.57 (1H, d, *J* = 10.3 Hz), 4.32 (1H, dd, *J* = 12.4, 2.0 Hz), 3.88 (1H, d, *J* = 12.4 Hz), 3.59 (1H, d, *J* = 10.3 Hz), 3.51 (1H, d, *J* = 11.8 Hz), 3.44 (3H, s), 2.92 (1H, d, *J* = 11.8 Hz), 2.70 (1H, dd, *J* = 15.5, 1.9 Hz), 1.96 (1H, d, *J* = 11.6 Hz), 1.91 (1H, d, *J* = 15.5 Hz), 1.50–1.79 (4H, m), 1.59 (1H, dd, *J* = 11.6, 1.9 Hz), 1.04 (3H, s), 0.97 (3H, s); EIMS *m/z* 448 $[M]^+$, 430, 282, 264, 121 (base); HREIMS *m/z* 448.2101 (448.2097 calculated for $C_{24}H_{32}O_8$).
9. Data for **6b**: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) δ 7.44–7.46 (2H, m), 7.30–7.38 (3H, m), 4.82 (1H, s), 4.56 (1H, d, *J* = 10.2 Hz), 4.36 (1H, dd, *J* = 12.4, 2.1 Hz), 3.89 (1H, d, *J* = 12.4 Hz), 3.74 (1H, d, *J* = 11.8 Hz), 3.70 (1H, d, *J* = 10.2 Hz), 3.42 (3H, s), 3.23 (1H, d, *J* = 11.8 Hz), 2.75 (1H, dd, *J* = 15.6, 2.2 Hz), 1.98 (1H, d, *J* = 11.7 Hz), 1.94 (1H, d, *J* = 15.6 Hz), 1.50–1.78 (4H, m), 1.61 (1H, dd, *J* = 11.7, 2.2 Hz), 1.08 (3H, s), 0.69 (3H, s); EIMS *m/z* 448 $[M]^+$, 430, 282, 264, 249, 121 (base); HREIMS *m/z* 448.2110 (448.2097 calculated for $C_{24}H_{32}O_8$).
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