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New Meroterpenoids from *Aspergillus terreus* with Inhibition of Cyclooxygenase-2 Expression

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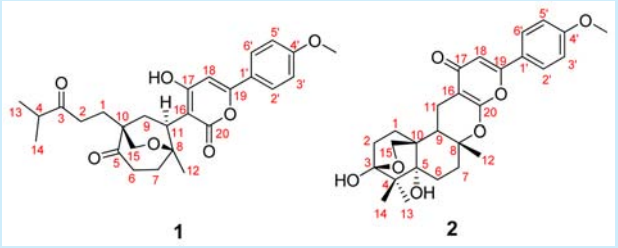
Supporting Information

ABSTRACT: Two novel meroterpenoids, yaminterritrems A (**1**) and B (**2**), were isolated from *Aspergillus terreus* collected from hot spring zones in Yang-Ming Mountain, Taiwan, and cultured at 40 °C. The structures of **1** and **2** were elucidated by NMR, MS spectral and X-ray crystallographic analyses. The biosynthetic route for **1** and **2** involving the conversion of the sesquiterpene with phenyl-α-pyrone is proposed. Besides, **2** exhibited a dose-dependent inhibitory effect on COX-2 expression in LPS-stimulated RAW264.7 macrophages.



Meroterpenoids, a characteristic type of fungal metabolites, merge polyketide−terpenoid structures. Some of them have been reported as an inhibitor selective for acetylcholinesterase,[1] which can decrease the amount of acetylcholine present in the synapses between cholinergic neurons.[2] In our previous studies on indigenous thermophilic fungi, a large group of compounds from thermophilic *Aspergillus terreus* (Trichocomaceae) have been identified as sources of biofunctional chemical components.[3] Our ongoing study on chemical investigations of the indigenous fungi are beginning to afford two novel meroterpenoids, yaminterritrems A (**1**) and B (**2**), in which the former possesses an unusual seven-member ring and the latter has a naphtho[2,1-*b*]pyrano-[3,2-*e*]pyran moiety.

We report, herein, the isolation and structure elucidation of compounds **1** and **2**. The fungus, *A. terreus* (Stain No. C9408-3), collected from a hot spring zone in Yang-Ming Mountain, Taipei, was cultured at 40 °C for 7 days on potato dextrose agar (PDA) plates (400 plates) and then were extracted with ethyl acetate. The ethyl acetate extract (3.28 g, ASP-EA) was fractionated using a Sephadex LH-20 column eluted with MeOH to yield 20 fractions. Fraction 6 (ASP-EA-f6) was further separated by column chromatography on Sephadex LH-20 with MeOH and purified by RP-HPLC (Sunfire C18, 250 mm × 4.5 mm, 1.0 mL/min, CH_3CN−H_2O, 60:40) to give compound **1** (1.7 mg, t_R 5.24 min). There were some particles precipitated in Fraction 3. Then the precipitated particles were further separated by silica gel column and eluted with $CHCl_3$ to 30:1 $CHCl_3$−MeOH to yield compound **2** (28 mg).

Yaminterritrem A (**1**) was obtained as a yellowish oil, and its molecular formula was determined to be $C_{27}H_{32}O_7$ by HRESIMS on the $[M + Na]^+$ (*m*/*z* 491.2043, calcd 491.2046 for $C_{27}H_{32}O_7Na$). The IR spectrum showed the presence of hydroxyl at 3406 cm^{-1} and ester/lactone carbonyl at 1692 cm^{-1}. The ^{13}C NMR and DEPT spectra of **1** (Table 1) exhibited the presence of 27 carbon resonances, containing three carbonyl carbons (δ_C 215.4, 212.2, and 165.8), five quaternary aromatic carbons, four aromatic methines, one olefinic methine, one oxygen-bearing quaternary carbon (δ_C 84.7), one oxymethylene (δ_C 74.0), one quaternary carbon (δ_C 60.4), two methines, five methylenes, one methoxyl, and three methyls.

The ^1H NMR spectrum of **1** (Table 1) revealed the presence of a 1,4-disubstituted phenolic moiety (δ_H 7.78, dd, J = 8.9, 2.0 Hz; 7.05, dd, J = 8.9, 2.0 Hz), an olefinic methine (δ_H 6.55, s), one oxymethylene (δ_H 3.96, J = 8.7 Hz; 3.89, J = 8.7 Hz), one methoxyl at δ_H 3.88, and three methyls (δ_H 1.38, s; 1.06, d, J = 7.0 Hz; 1.03, d, J = 7.0 Hz).

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Table 1. NMR Spectroscopic Data of 1 and 2

	1			2[b]		
no.		δ_H (J in Hz)[a]	δ_C[b]		δ_H (J in Hz)[c]	δ_C[d]
1	a	1.95 m	20.9	a	2.07 dd (9.2, 5.2)	28.9
	b	1.81 m		b	1.84 dd (9.2, 9.2)	
2	a	2.77 m	36.0	a	2.32 ddd (9.2, 9.2, 5.2)	29.6
	b	2.67 m		b	1.92 dd (9.2, 9.2)	
3			215.4			98.3
4		2.61 sep (6.8)	40.3			46.5
5			212.2			74.9
6	a	2.68 m	34.7	a	2.13 dd (11.6, 2.4)	28.4
	b	2.41 m		b	1.69 dt (11.6, 2.4)	
7	a	1.94 m	38.4	a	2.22 m	33.5
	b	1.89 m		b	1.91 d (9.6)	
8			84.7			84.0
9	a	2.78 m	19.3		2.20 m	42.2
	b	2.47 m				
10			60.5			40.5
11		2.77 m	46.7	a	2.64 m	16.3
				b	2.20 m	
12		1.38 s	20.9		1.28 s	19.9
13		1.03 d (7.0)	17.1		1.10 s	19.6
14		1.06 d (7.0)	17.1		1.14 s	21.3
15	a	3.96 d (8.7)	74.0	a	4.04 d (7.6)	66.8
	b	3.89 d (8.7)		b	3.98 d (7.6)	
16			100.9			99.6
17			166.8			180.8
18		6.55 s	95.9		6.55 s	107.5
19			158.8			159.7
20			165.8			162.8
1′			123.2			123.4
2′, 6′		7.78 dd (8.9, 2.0)	126.6		7.65 dd (7.2, 2.5)	127.4
3′, 5′		7.05 dd (8.9, 2.0)	114.0		6.93 dd (7.2, 2.5)	114.5
4′			162.0			162.1
OMe		3.88 s	54.4		3.83 s	55.6

[a]Measured at 400 MHz in methanol-d_4. [b]Measured at 100 MHz in methanol-d_4. [c]Measured at 500 MHz in $CDCl_3$. [d]Measured at 125 MHz in $CDCl_3$.

The gross structure of **1** was elucidated by analysis of 2D-NMR data including the ^{1}H−^{1}H COSY, ROESY, HSQC, and HMBC spectra in CD_3OD. Four proton sequences, H_3-13−H-4−H_3-14, H_2-2−H_2-1, H_2-9−H-11, and H_2-6−H_2-7 were disclosed by the ^{1}H−^{1}H COSY spectrum of **1**. The HMBC experiment used to connect the above substructures was found to show the following correlations: H_2-1 to C-10; H_2-6 to C-5; H_2-7 to C-8 and C-11; H_2-9 to C-8, C-11, and C-16; H-11 to C-17 and C-20; H_3-13 and H_3-14 to C-3 and C-4; H_2-15 to C-5, C-8, C-9, and C-10. The key connectivity between C-15 and C-8 through an ether bridge was revealed by an HMBC correlation for H-15a (δ_H 3.96) to C-8. The presence of an α-pyrone ($\alpha,\beta,\gamma,\delta$-unsaturated δ-lactone) moiety was indicated by HMBC cross-peaks of H-18 (δ_H 6.55) to C-19 (δ_C 158.8), C-16 (δ_C 100.9), and C-1′ (δ_C 123.2). HMBC correlations for H-2′/H-6′ (δ_H 7.78) to C-19 (δ_C 158.8) revealed the connectivity of C-1′ to C-19. A methoxy group (δ_H 3.88) was positioned at C-4′ (δ_C 162.0) based on the HMBC cross-peak between them. Thus, the gross structure of yaminterritrem A was deduced as **1** (Figure 1).

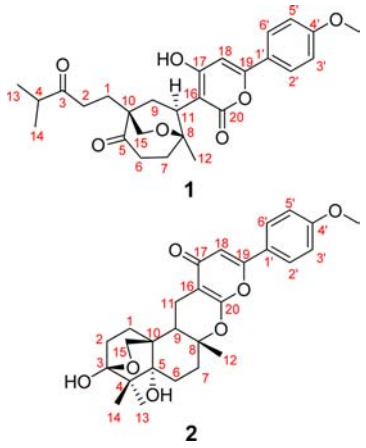

Figure 1. Structures of yaminterritrems A (**1**) and B (**2**).

The relative stereochemistry of **1** was determined by ROESY spectrum. The key ROESY correlation of H-11/H-7a suggested that H-11 and H-7a maintained a pseudo-1,3 diaxial conformation, so that H-11 should be at the opposite end of the fused ether bridge and H-11 should be in the same α orientation as Me-12 (Figure 2 and Supporting Information Figure S7). Moreover, the ROESY cross-peaks between H-15b/H-9a, and H-15a/H-6a could also be observed. Besides, due to the free rotation of the C1−C15 bond, the cross-peaks between H-15b/H-1a and H-1b can be observed.

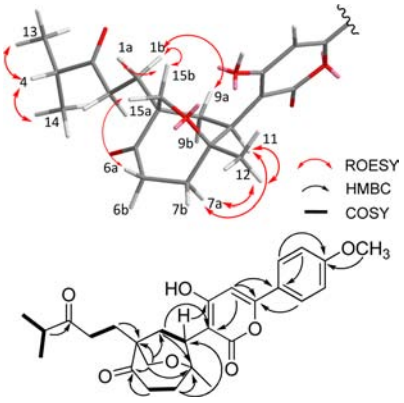

Figure 2. Selected ^{1}H−^{1}H COSY, HMBC, and ROESY correlations for compound **1**.

Yaminterritrem B (**2**) was obtained as colorless powders, and its molecular formula was determined to be $C_{27}H_{32}O_7$ by HRESIMS on the $[M + H]^+$ (m/z 469.2203, calcd 469.2226 for $C_{27}H_{33}O_7$), implying 12 degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **2** (Table 1) exhibited the presence of 27 carbon resonances, containing one carbonyl carbon (δ_C 180.8), five quaternary aromatic carbons, four aromatic methines, one olefinic methine, three oxygen-bearing quaternary carbons (δ_C 98.3, 84.0, 74.9), one oxymethylene (δ_C 66.8), one quaternary carbon (δ_C 46.5), one methine, six methylenes, one methoxyl, and three methyls. The ^{1}H NMR spectrum of **2** (Table 1) revealed the presence of the same 1,4-disubstituted phenolic moiety (δ_H 7.65, dd, J = 7.2, 2.5 Hz; 6.93, dd, J = 7.2, 2.5 Hz) and an olefinic methine (δ_H 6.55, s) as in the case of compound **1**. And, there are one oxymethylene (δ_H 4.04, J = 7.6 Hz; 3.98, d, J = 7.6 Hz), one methoxyl (δ_H 3.83), and three singlet methyls (δ_H 1.28, 1.14, and 1.10).

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The gross structure of **2** was elucidated by analysis of 2D-NMR data including the ^{1}H−^{1}H COSY, ROESY, HSQC, and HMBC spectra in chloroform-*d*. Three proton sequences, H_2-1−H_2-2, H_2-6−H_2-7, H-9−H_2-11, were disclosed by the ^{1}H−^{1}H COSY spectrum of **2**. The HMBC experiment used to connect the above substructures was found to show the following correlations: H_2-2 to C-3 and C-4; H_2-1 to C-2, C-3, C-10, and C-15; H_2-6 to C-5, C-7, C-8, and C-10; H_2-7 to C-5, C-6, C-8, C-9, and C-12; H-9 to C-11, C-12, and C-16; H_2-11 to C-16 and C-20; H_3-13 and H_3-14 to C-3 and C-4; H_2-15 to C-1, C-3, C-9, and C-10. Key connectivity of C-15 (δ_C 66.8) and C-3 (δ_C 98.3) through an oxygen atom was revealed by an HMBC correlation for H-15 (δ_H 4.04) to C-3. The presence of a pyran-4-one moiety was indicated by HMBC cross-peaks of H-18 (δ_H 6.55) to C-19 (δ_C 159.7) and C-16 (δ_C 99.6). The *p*-methoxyphenyl group at C-19 was established based on the HMBC cross-peaks for the signal at δ_H 3.88 to C-4′ (δ_C 162.0) and H-2′/H-6′ (δ_H 7.78) to C-19 (δ_C 159.7). Thus, the gross structure of yaminterritrem B was deduced as **2** (Figure 1).

The NOESY correlations of **2** between H-11b/H-15b, Me-12/H-15b, and Me-14/H-15b suggested that Me-12 should be the same orientation as CH_2-15 (Supporting Information Figure S15). The absolute configuration of **2** was established by a single-crystal X-ray diffraction analysis using Cu Kα radiation (Figure 3 and Supporting Information Tables S1−S5). The

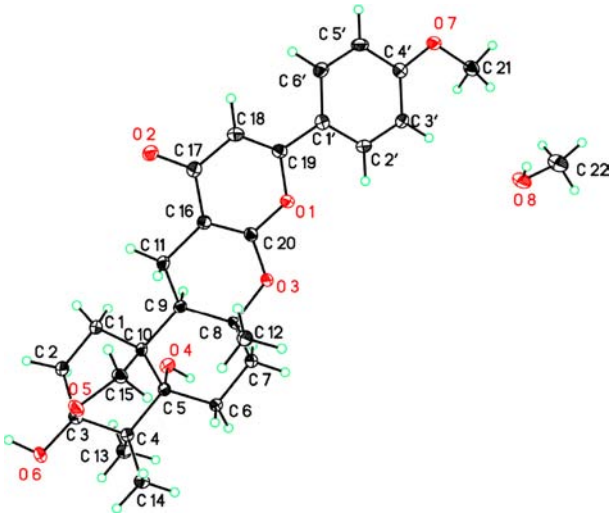
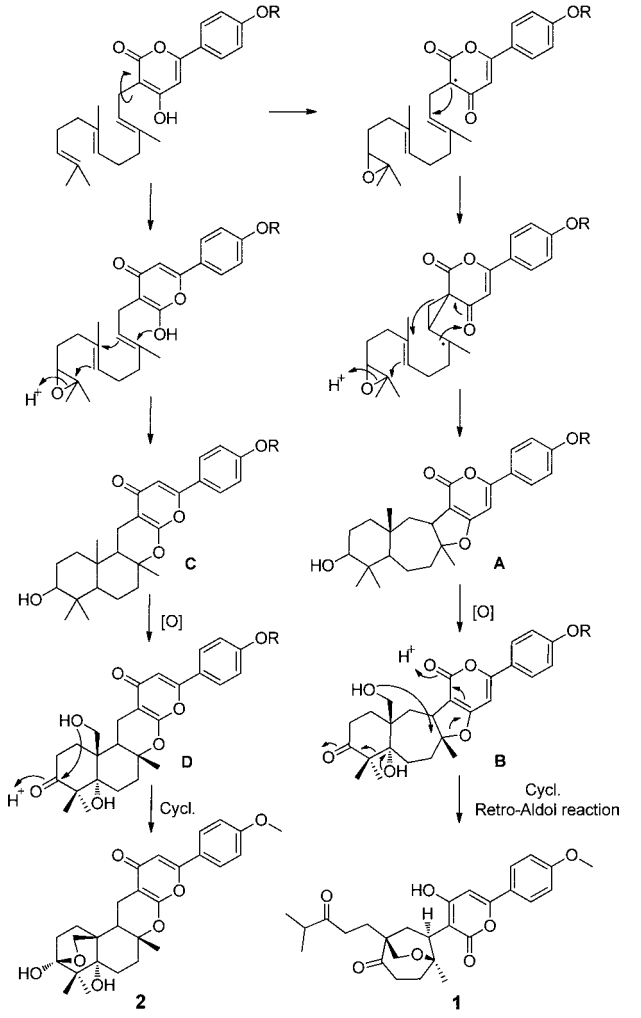


Figure 3. X-ray crystallographic structure of **2** by a single-crystal X-ray diffraction analysis using Cu Kα radiation.

result demonstrated that the chiral centers in **2** were 3*S*, 5*R*, 8*R*, and 10*R*. Thus, the structure of **2** was assigned as and named yaminterritrem B.

A biosynthetic origin of yaminterritrems A (**1**) and B (**2**) was proposed as shown in Scheme 1. Both compounds seem to be biogenetically related to some meroterpenoids, such as arisugacin isolated *Penicillum* sp.,[1c] terreulactone A and territrems from *A. terreus*,[4] and pyripyropene from *A. fumigatus*.[5] Yaminterritrem B (**2**) might be derived from the stereospecific cyclization of the sesquiterpene with a phenyl-α-pyrone moiety through proton-initiated carbocation formation. Yaminterritrem A (**1**) might be produced from the same precursor of compound **2** though intermediates **A** and **B**. The biosynthesis pathway of compound **1** might involve the formation of an unusual cycloheptane moiety of the key intermediate **B** and the cleavage of the C4−C5 bond in the ring

Scheme 1. Plausible Biosynthetic Pathway for 1 and 2



A of the terpenoid unit of the intermediate **B** to produce **1** via retro-Aldol reaction.

Epidemiological studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the risk of developing Alzheimer Disease (AD).[6] Conventional NSAIDs inhibit both isoforms of cyclooxygenase (COX) playing an important role in conversion of arachidonic acid to various prostaglandins (PG). Cyclooxygenase-2 (COX-2) is an inducible isozyme, which promotes cellular proliferation, angiogenesis, cancer invasiveness, and antiapoptosis.[7] LPS-stimulated RAW264.7 macrophages were treated by yaminterritrem B (**2**) to investigate its anti-inflammation effect. The results indicated that **2** could reduce the LPS-induced COX-2 expression in protein and RNA levels with the EC_{50} value at 18.3 μM (Figure 4). Dexamethasone (DEX) and NS398 were used as possible controls (Figure S19).

In summary, we discovered two novel meroterpenoids with unusual fused-ring skeleton by an ether bridge linkage from the thermophilic *A. terreus*, determined the absolute stereochemistry of compound **2** with a single-crystal X-ray diffraction study, and demonstrated the inhibition of **2** on the COX-2 expression.

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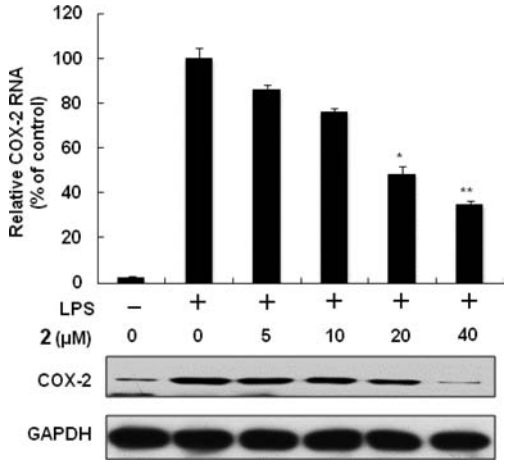

Figure 4. Inhibition effect on COX-2 expression of **2** in LPS-stimulated RAW264.7 macrophages. Cells were seeded in a 24-well plate, treated with **2** at 0, 5, 10, 20, or 40 μM, and then incubated with 1 μg/mL LPS for 24 h. The RNA and protein levels of COX-2 were determined by RT-qPCR or Western blotting, respectively. Data were presented as the mean ± SD from at least triplicate observations. Asterisks indicated a significant difference compared with untreated RAW264.7 macrophages. *P < 0.05; **P < 0.01.

■ ASSOCIATED CONTENT

Supporting Information

Extraction and isolation, bioassay method, 1D and 2D selective NMR, HRESI-MS, IR and UV spectra of **1** and **2**, and the X-ray crystallographic analytical method and data for **2**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Peng, F. C. *J. Nat. Prod.* **1995**, *58*, 857−862. (b) Yoo, I. D.; Cho, K. M.; Lee, C. K.; Kim, W. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 353−356. (c) Omura, S.; Kuno, F.; Otoguro, K.; Sunazuka, T.; Shiomi, K.; Masuma, R.; Iwai, Y. *J. Antibiot.* **1995**, *48*, 745−746.

(2) Kim, W. G.; Cho, K. M.; Lee, C. K.; Yoo, I. D. *J. Antibiot.* **2003**, *56*, 351−357.

(3) Liao, W.-Y.; Shen, C.-N.; Lin, L.-H.; Yang, Y.-L.; Han, H.-Y.; Chen, J.-W.; Kuo, S.-C.; Wu, S.-H.; Liaw, C.-C. *J. Nat. Prod.* **2012**, *75*, 630−635.

(4) Ling, K. H.; Liou, H. H.; Yang, C. M.; Yang, C. K. *Appl. Environ. Microbiol.* **1984**, *47*, 98−100.

(5) Omura, S.; Tomoda, H.; Kim, Y. K.; Nishida, H. *J. Antibiot.* **1993**, *46*, 1168−1169.

(6) (a) Breitner, J. C. S.; Welsh, K. A.; Helms, M. J.; Gaskell, P. C.; Gau, B. A.; Roses, A. D.; Pericak-Vance, M. A.; Saunders, A. M. *Neurobiol. Aging* **1995**, *16*, 523−530. (b) Liang, X.; Wang, Q.; Hand, T.; Wu, L.; Breyer, R. M.; Montine, T. J.; Andreasson, K. *J. Neurosci.* **2005**, *25*, 10180−10187.

(7) (a) Gee, J.; Lee, I. L.; Grossman, H. B.; Sabichi, A. L. *Urol. Oncol.* **2008**, *26*, 641−645. (b) Ghosh, N.; Chaki, R.; Mandal, V.; Mandal, S. C. *Pharmacol. Rep.* **2010**, *62*, 233−244.

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