

Isoterreulactone A, a novel meroterpenoid with anti-acetylcholinesterase activity produced by *Aspergillus terreus*

Ick-Dong Yoo,^a Kyung-Mi Cho,^{a,b} Chong-Kil Lee^b and Won-Gon Kim^{a,*}

^aKorea Research Institute of Bioscience and Biotechnology, PO Box 115, Yusong, Taejeon 305-600, Korea

^bDepartment of Pharmacy, Chung-Buk National University, Cheong-Ju 361-763, Korea

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Abstract—A new seven-membered lactone type meroterpenoid, isoterreulactone A, was isolated from the solid state fermentation of *Aspergillus terreus* and its structure was established by various spectral analysis. Isoterreulactone A inhibited acetylcholinesterase with an IC₅₀ value of 2.5 μM while did not inhibit butyrylcholinesterase even at 500 μM.

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Alzheimer disease is a neurodegenerative disorder that is the most common cause of dementia among the elderly. The neuropathological evidences have demonstrated that cholinergic functions declined in the basal forebrain and cortex in senile dementia of the Alzheimer type.^{1,2} Accordingly, enhancement of cholinergic neurotransmission have been considered as one potential therapeutic approach against Alzheimer disease. One treatment strategy to enhance cholinergic functions is the use of acetylcholinesterase (AChE, EC 3.1.1.7) inhibitors to increase the amount of acetylcholine present in the synapses between cholinergic neurons.^{3,4} Acetylcholinesterase inhibitors like tacrine, one of the most extensively evaluated acetylcholinesterase inhibitors, have been shown to significantly improve cognitive function in Alzheimer's disease.^{5,6} Tacrine, however, has been known to cause hepatotoxic side effects by also inhibiting butyrylcholinesterase (BuChE, EC 3.1.1.8), which is found in plasma.⁷ In this respect, an inhibitor selective for acetylcholinesterase has attracted particular attention for treatment of the Alzheimer-type dementia. In the course of our screening for selective inhibitors of acetylcholinesterase from microbial metabolites, we previously isolated terreulactones A–D^{8–10} and quinolactacins A1 and A2.¹¹ Terreulactones A–D are meroterpenoid type compounds that have mixed polyke-

tide–terpenoid structures. Especially, terreulactone A is a sesquiterpene lactone type meroterpenoid incorporating an uniquely fused lactone skeleton in its sesquiterpene moiety.

Further investigation on polar metabolites of *Aspergillus terreus* Fb000501, which is the producer of terreulactones A–D, has resulted in isolation of new seven-membered lactone type meroterpenoid named isoterreulactone A (**1**)¹² (Fig. 1). We report here the isolation, physico-chemical properties, structure determination, and biological activities of **1**.

Fermentation of *A. terreus* Fb000501 was carried out in solid state of moistured wheat-bran because isoterreulactones A was not produced in liquid culture media containing glucose 2%, yeast extract 0.2%, polypeptone 0.5%, MgSO₄ 0.05%, and KH₂PO₄ 0.1% (pH 5.7 before sterilization). A piece of strain Fb000501 was inoculated from a mature plate culture into 500 mL baffled Erlenmeyer flasks each containing 100 mL of a sterile seed medium with the above composition. After incubation at 28 °C for 3 days on a rotary shaker (150 rpm), 5 mL of the seed culture was transferred to 500 mL Erlenmeyer flasks containing 90 g of moistured wheat-bran. The fermentation was carried out at 28 °C for 10 days under a stationary condition. The solid-state fermented whole medium (1.8 kg) was extracted with 80% acetone and the extract was concentrated in vacuo to an aqueous solution, which was then extracted with an

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* Corresponding author. Tel.: +82 42 860 4298; fax: +82 42 860 4595; e-mail: wgkim@kribb.re.kr

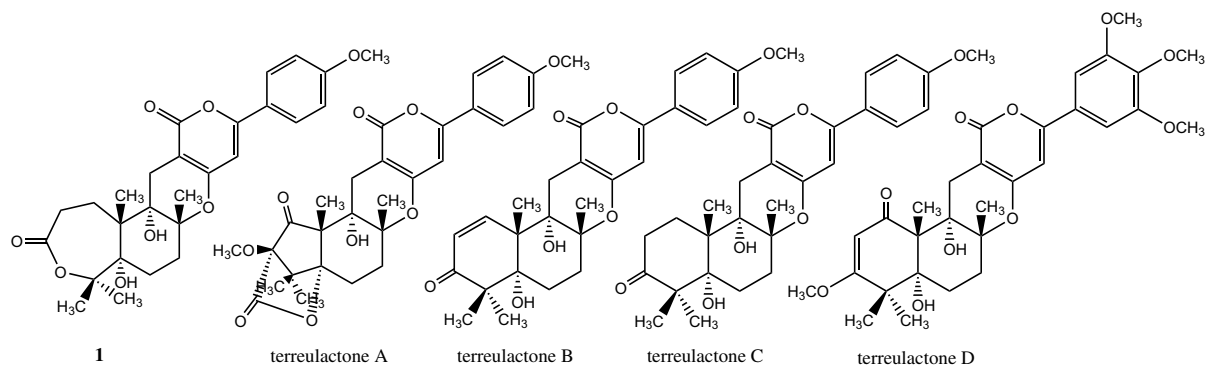


Figure 1. The relative structures of isoterreulactone A (**1**) terreulactones A–D.

equal volume of EtOAc three times. EtOAc extract was concentrated in vacuo to dryness. The crude extract was subjected to SiO₂ column chromatography followed by stepwise elution with CHCl₃–MeOH (100:1, 50:1, 20:1). The active fractions eluted with CHCl₃–MeOH (20:1) were pooled and concentrated in vacuo to give an oily residue. The residue was applied again to a Sephadex LH-20 and then eluted with MeOH. The active fraction dissolved in MeOH was further purified by reverse phase HPLC column (20 × 250 mm, YMC C₁₈) chromatography with a photodiode array detector. The column was eluted with CH₃CN–H₂O (55:45) at a flow rate of 8 mL/min to afford isoterreulactone A (85 mg) at a retention time of 11.9 min as a white powder.

The molecular formula of **1** was determined to be C₂₈H₃₂O₈ on the basis of high resolution FAB-MS [(M+H)⁺, 485.2177 *m/z* (+0.2 mmu error)] in combination with ¹H and ¹³C NMR data. The IR data suggested the presence of a lactone (1696 cm^{−1}) and a hydroxyl (3430 cm^{−1}) moiety. The ¹H and ¹³C NMR data (Table 1) with DEPT, ¹H–¹H COSY, and HMQC data suggested the presence of an 1,4-disubstituted benzene ring, an olefinic methine, four isolated methyls, one methoxy,

two –CH₂–CH₂–, an isolated methylene, two carboxylic carbons, five sp² quaternary carbons, five sp³ quaternary carbons, and two exchangeable protons. These spectral data are similar to those of terreulactone A. The major differences were that one more –CH₂–CH₂– group and one exchangeable proton appeared in **1** while the carbonyl carbon, one sp³ quaternary carbon, and one methoxy of terreulactone A disappeared. This data suggested that the structure of the ring A of terreulactone A was changed in **1**. The structure of the ring A was determined by HMBC spectral data. Long-range couplings were observed from the methyl protons of 13b-Me (δ 1.29) to a methylene carbon (C-1, δ 29.9) of one –CH₂–CH₂– group and three sp³ quaternary carbons [C-13b (δ 41.9), C-13a (δ 75.6), and C-5a (δ 89.4)]. The methylene protons (H₂-1, δ 2.02 and δ 2.16) of the –CH₂–CH₂– group were in turn long-range coupled to 13b-Me, C-13b, C-5a, and one carboxylic carbon (C-3, δ 172.2). This spectral data suggested the presence of a ^{−13b}C(CH₃)–¹CH₂–²CH₂–³C(=O)–O– moiety in the ring A. On the other hand, one exchangeable proton (5a-OH, δ 4.78) was long-range coupled to C-5a and one sp³ quaternary carbon (C-5, δ 78.7). The sp³ quaternary carbon of C-5 was correlated with two isolated methyls (5_α-Me, δ 1.34 and 5_β-Me, δ 1.16). This spectral data

Table 1. The ¹³C (125 MHz) and ¹H (600 MHz) NMR spectral data of **1** in DMSO-*d*₆

C	DEPT	δH (J, Hz)	HMBC	C	DEPT	δH (J, Hz)	HMBC
1	29.9 t	H _α 2.02 (m) H _β 2.16 (m)	C-2, C-3, C-5a, C-13a, C-13b	8a	162.8 s		
2	28.7 t	2.47 (m)	C-2, C-3, C-13a, C-13b, 13b-Me	9	96.7 d	6.74 (s)	C-8a, C-10, C-1'
3	172.2 s		C-1, C-3, C-13b	10	157.1 s		
5	78.7 s			12	163.4 s		
5 _α -Me	29.7 q	1.34 (s)	C-5, C-5a, 5 _β -Me	12a	96.7 s		
5 _β -Me	29.2 q	1.16 (s)	C-5, C-5a, 5 _α -Me	13	25.5 t	2.50 (s)	C-7a, C-8a, C-12, C-12a, C-13, C-13a
5a	89.4 s			13a	75.6 s		
5a-OH		4.78	C-5, C-5a	13a-OH		4.64 (s)	C-7a, C-13, C-13a, C-13b
6	27.8 t	H _α 1.72 (ddd, 14.6, 3.4, 3.7) H _β 1.85 (ddd, 14.6, 13.6, 3.0) H _α 1.45 (ddd, 12.1, 3.7, 3.0) H _β 2.35 (ddd, 13.6, 12.1, 3.4)	C-5a, C-7, C-7a, C-13b	13b	41.9 s		
7	27.9 t		C-7, C-7a	13b-Me	23.2 q	1.29 (s)	C-1, C-5a, C-13a, C-13b
7a	81.3 s		C-5a, C-7a, C-13a	1'	123.6 s		
7a-Me	22.8 q	1.39 (s)	C-7, C-7a, C-13a	2',6'	126.9 d	7.81 (d, 8.9)	C-10, C-6', C-4'
				3',5'	114.6 d	7.04 (d, 8.9)	C-1', C-5', C-4'
				4'	161.1 s		
				4'-OMe	55.4 q	3.81 (s)	C-4'

The assignments were aided by ¹H–¹H COSY, DEPT, NOESY, HMQC, and HMBC.

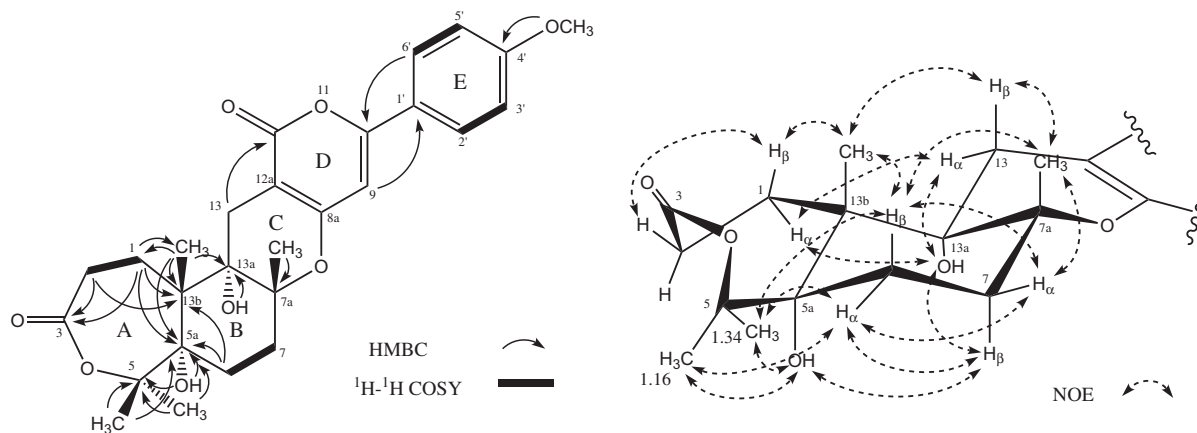


Figure 2. Key HMBC and NOE correlations of Isoterreulactone A.

indicated the presence of the free hydroxyl group (5a-OH) at C-5a. The chemical shift (δ 78.7) of C-5 and unsaturation degree of **1** requiring the existence of one ring in the ring A suggested that the presence of a seven-membered lactone in the ring A through the connection of the carboxylic oxygen of the $-^{13}\text{bC}(\text{CH}_3)-^1\text{CH}_2-^2\text{CH}_2-^3\text{C}(=\text{O})-\text{O}-$ moiety to C-5. This was confirmed by HMBC spectral data. The methylene protons (H_2-2 , δ 2.47) of the $-^{13}\text{bC}(\text{CH}_3)-^1\text{CH}_2-^2\text{CH}_2-^3\text{C}(=\text{O})-\text{O}-$ moiety were long-range coupled to C-5 as well as C-13b. The remaining rings B, C, D, and E were also confirmed by HMBC data. The relative stereochemistry was determined by NOESY spectral data. The NOEs effect were observed among 5a-OH, 7- H_β , and 13a-OH. Also, the NOEs effect among 1- H_β , 13b-Me, 6- H_β , 13- H_β , and 7a-Me were observed. Thus, the relative stereochemistry of C-5a, C-7a, C-13a, and C-13b were determined to be S^* , R^* , S^* , and S^* , respectively (Fig. 2).

Isoterreulactone A is a new meroterpenoid incorporating a seven-membered lactone skeleton in its molecule. Since some derivatives of arisugacin **C**¹³ and territrem B,¹⁴ terreulactones C and D, respectively, were also detected in the same culture, isoterreulactone A seems to be biogenetically related to arisugacin isolated from *Penicillium* sp. Interestingly, arisugacins and territremes were, however, not detected in this study. Meroterpenoids^{15,16} such as pyripyropene and oxalicine, and seven-membered lactone type terpenoids¹⁷ such as andilesins, anditomin, fumigatonin, and obacunol have been isolated from fungi.

The inhibitory activity of isoterreulactone A against acetylcholinesterase was examined according to Ellman's coupled enzyme assay¹⁸ with some modifications as follows; 0.08 units AChE dissolved in 0.1 M potassium phosphate buffer (pH 7.4) and purified compounds dissolved in methanol were added to each well of a 96-well plate. Then, acetylthiocholine iodide and 5,5'-dithiobis(2-nitrobenzoic acid) dissolved in 0.1 M potassium phosphate buffer (pH 7.4) were added to final 20 and 30 μM , respectively, to each well. The reaction was carried out at room temperature for 5 min and the initial rate of the enzyme was analyzed by measuring the

Table 2. Inhibitory activities of isoterreulactone A and terreulactones A–D against acetylcholinesterase and butyrylcholinesterase

	IC ₅₀ (μM)	
	AChE	BuChE
1	2.5	>500
Terreulactone A	0.23	>200
Terreulactone B	0.09	>200
Terreulactone C	0.06	>200
Terreulactone D	0.42	>200
Tacrine	0.09	0.01

formation of 5-thio-2-nitrobenzoate, yellow anion, at 412 nm of UV wavelength. The inhibitory activities against BuChE were measured as described above for AChE by using 0.16 unit BuChE and 20 μM butyrylthiocholine iodide instead of AChE and acetylthiocholine iodide for enzyme and substrate, respectively (Table 2).

Isoterreulactone A inhibited acetylcholinesterase in a dose-dependent mode with an IC₅₀ (μM) value of 2.5. Anti-acetylcholinesterase activity of isoterreulactone A was 10 times weaker than that (0.23 μM) of terreulactone A, which suggested the important role of the ring A in acetylcholinesterase inhibitory activity. Isoterreulactone A, however, did not inhibit butyrylcholinesterase even at 500 μM . Therefore, isoterreulactones A showed more than 250 times potent inhibitory activity against AChE compared with that against BuChE while tacrine, as a positive control, had a low selectivity with a stronger inhibitory activity on butyrylcholinesterase (IC₅₀ (μM); 0.01) rather than acetylcholinesterase (IC₅₀ (μM); 0.09) in this assay system. By Lineweaver–Burk plot analysis, isoterreulactone A exhibited noncompetitive inhibition with acetylcholine and its K_i and K_m values for acetylcholinesterase were 2.3×10^{-6} and 2.0×10^{-5} M, respectively.

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12. Compound **1**: a white powder; UV $\lambda_{\max}$ nm (ϵ) in MeOH: 215 (81,000), 251 (11,000), 330 (12,000); IR (KBr): 3430, 2932, 1696, 1571, 1514, 1258, 1181 cm^{-1} ; $[\alpha]_{\text{D}}^{20}$ +60 (c 0.1, CHCl_3); HRFAB-MS: m/z 485.2177 ($\text{M}+\text{H}$)⁺, $\text{C}_{27}\text{H}_{32}\text{O}_8$ requires 485.2175.
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