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

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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 $IC_{50}$ value of 2.5 μM while did not inhibit butyrylcholinesterase even at 500 μM.


Alzheimer disease is a neurodegenerative disorder that is the most common cause of dementia among the elderly. The neurophathological 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 polyketide–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 iosterreulactones A was not produced in liquid culture media containing glucose 2%, yeast extract 0.2%, polypeptone 0.5%, $MgSO_4$ 0.05%, and $KH_2PO_4$ 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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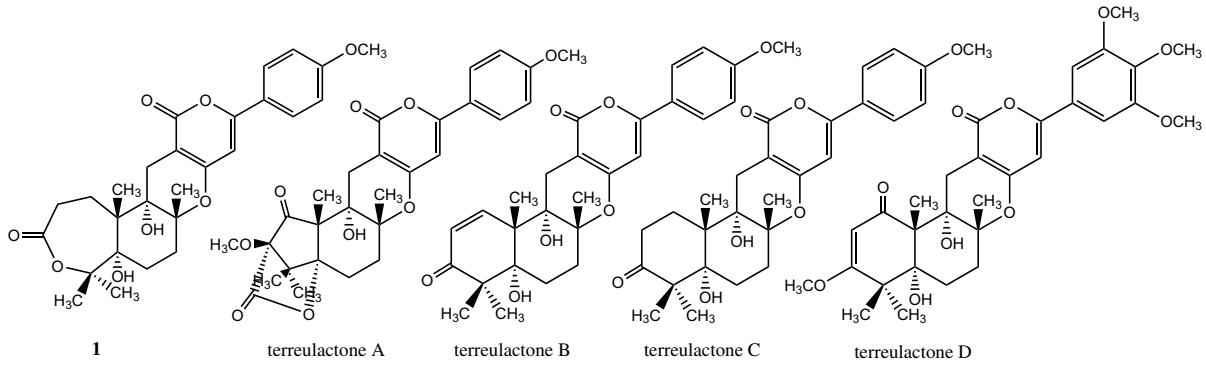


**Figure 1.** The relative structures of isoterreulactone A (**1**) terreulactones A–D.

equal volume of EtOAc three times. EtOAc extract was concentrated in vacuo to dryness. The crude extract was subjected to $SiO_2$ column chromatography followed by stepwise elution with $CHCl_3$–MeOH (100:1, 50:1, 20:1). The active fractions eluted with $CHCl_3$–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 ($20 \times 250$ mm, YMC $C_{18}$) chromatography with a photodiode array detector. The column was eluted with $CH_3CN$–$H_2O$ (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 $C_{28}H_{32}O_8$ on the basis of high resolution FAB-MS [$(M+H)^+$, 485.2177 *m/z* (+0.2 mmu error)] in combination with $^1H$ and $^{13}C$ NMR data. The IR data suggested the presence of a lactone (1696 $cm^{-1}$) and a hydroxyl (3430 $cm^{-1}$) moiety. The $^1H$ and $^{13}C$ 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 $–CH_2–CH_2–$, an isolated methylene, two carboxylic carbons, five $sp^2$ quaternary carbons, five $sp^3$ quaternary carbons, and two exchangeable protons. These spectral data are similar to those of terreulactone A. The major differences were that one more $–CH_2–CH_2–$ group and one exchangeable proton appeared in **1** while the carbonyl carbon, one $sp^3$ 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 ($\delta$ 1.29) to a methylene carbon (C-1, $\delta$ 29.9) of one $–CH_2–CH_2–$ group and three $sp^3$ quaternary carbons [C-13b ($\delta$ 41.9), C-13a ($\delta$ 75.6), and C-5a ($\delta$ 89.4)]. The methylene protons ($H_2$-1, $\delta$ 2.02 and $\delta$ 2.16) of the $–CH_2–CH_2–$ group were in turn long-range coupled to 13b-Me, C-13b, C-5a, and one carboxylic carbon (C-3, $\delta$ 172.2). This spectral data suggested the presence of a $–^{13b}C(CH_3)–^1CH_2–^2CH_2–^3C(=O)–O–$ moiety in the ring A. On the other hand, one exchangeable proton (5a-OH, $\delta$ 4.78) was long-range coupled to C-5a and one $sp^3$ quaternary carbon (C-5, $\delta$ 78.7). The $sp^3$ quaternary carbon of C-5 was correlated with two isolated methyls ($5_\alpha$-Me, $\delta$ 1.34 and $5_\beta$-Me, $\delta$ 1.16). This spectral data

**Table 1.** The $^{13}C$ (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 | $H_\alpha$ 2.02 (m) | C-2, C-3, C-5a, C-13a, C-13b | 8a | 162.8 s | | |
| | | $H_\beta$ 2.16 (m) | C-2, C-3, C-13a, C-13b, 13b-Me | 9 | 96.7 d | 6.74 (s) | C-8a, C-10, C-1′ |
| 2 | 28.7 t | 2.47 (m) | C-1, C-3, C-13b | 10 | 157.1 s | | |
| 3 | 172.2 s | | | 12 | 163.4 s | | |
| 5 | 78.7 s | | | 12a | 96.7 s | | |
| $5_\alpha$-Me | 29.7 q | 1.34 (s) | C-5, C-5a, $5_\beta$-Me | 13 | 25.5 t | 2.50 (s) | C-7a, C-8a, C-12, C-12a, C-13, C-13a |
| $5_\beta$-Me | 29.2 q | 1.16 (s) | C-5, C-5a, $5_\alpha$-Me | 13a | 75.6 s | | |
| 5a | 89.4 s | | | 13a-OH | | 4.64 (s) | C-7a, C-13, C-13a, C-13b |
| 5a-OH | | 4.78 | C-5, C-5a | 13b | 41.9 s | | |
| 6 | 27.8 t | $H_\alpha$ 1.72 (ddd, 14.6, 3.4, 3.7) | C-5a, C-7, C-7a, C-13b | 13b-Me | 23.2 q | 1.29 (s) | C-1, C-5a, C-13a, C-13b |
| | | $H_\beta$ 1.85 (ddd, 14.6, 13.6, 3.0) | C-7, C-7a | 1′ | 123.6 s | | |
| 7 | 27.9 t | $H_\alpha$ 1.45 (ddd, 12.1, 3.7, 3.0) | C-5a, C-7a, C-13a | 2′,6′ | 126.9 d | 7.81 (d, 8.9) | C-10, C-6′, C-4′ |
| | | $H_\beta$ 2.35 (ddd, 13.6, 12.1, 3.4) | C-6, C-7a, 7a-Me | 3′,5′ | 114.6 d | 7.04 (d, 8.9) | C-1′, C-5′, C-4′ |
| 7a | 81.3 s | | | 4′ | 161.1 s | | |
| 7a-Me | 22.8 q | 1.39 (s) | C-7, C-7a, C-13a | 4′-OMe | 55.4 q | 3.81 (s) | C-4′ |

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

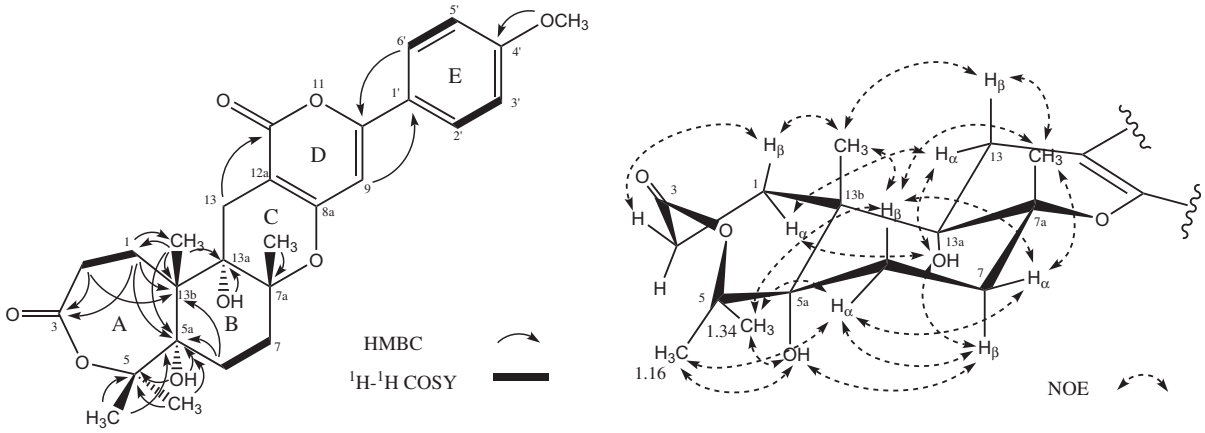


**Figure 2.** Key HMBC and NOE correlations of Isoterreulactone A.

indicated the presence of the free hydroxyl group (5a-OH) at C-5a. The chemical shift ($\delta$ 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 $-^{13b}C(CH_3)-^1CH_2-^2CH_2-^3C(=O)-O-$ moiety to C-5. This was confirmed by HMBC spectral data. The methylene protons ($H_2$-2, $\delta$ 2.47) of the $-^{13b}C(CH_3)-^1CH_2-^2CH_2-^3C(=O)-O-$ 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-$H_\beta$, and 13a-OH. Also, the NOEs effect among 1-$H_\beta$, 13b-Me, 6-$H_\beta$, 13-$H_\beta$, 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 territrems were, however, not detected in this study. Meroterpenoids[15,16] such as pyripyropene and oxalicine, and seven-membered lactone type terpenoids[17] 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 assay[18] 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 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).

**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 |

Isoterreulactone A inhibited acetylcholinesterase in a dose-dependent mode with an $IC_{50}$ (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 ($IC_{50}$ (μM); 0.01) rather than acetylcholinesterase ($IC_{50}$ (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 $2.3 \times 10^{-6}$ and $2.0 \times 10^{-5}$ M, respectively.

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12. Compound **1**: a white powder; UV $\lambda_{max}$ nm ($\varepsilon$) in MeOH: 215 (81,000), 251 (11,000), 330 (12,000); IR (KBr): 3430, 2932, 1696, 1571, 1514, 1258, 1181 $cm^{-1}$; $[\alpha]_D$ +60 (*c* 0.1, $CHCl_3$); HRFAB-MS: *m/z* 485.2177 $(M+H)^+$, $C_{27}H_{32}O_8$ requires 485.2175.
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