Structure and Anti-Acetylcholinesterase Activity of 4α-(Hydroxymethyl)-4α-demethylterritrem B

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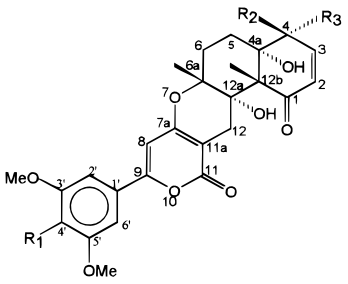
The structure of a metabolite produced on incubation of territrem B (**1**) with rat liver microsomes has been established to be 4α-(hydroxymethyl)-4α-demethylterritrem B (5). Compound **5** was a potent inhibitor of electric eel acetylcholinesterase (AChE) (E.C. 3.1.1.7).

Territrems A, B, and C are secondary metabolites, isolated from $CHCl_3$ extracts of cultures of *Aspergillus terreus* 23-1.[1−3] These compounds induced whole body tremor when injected ip into rats or mice.[4] It was demonstrated previously that the site of tremorgenic action in animals injected with territrem B (**1**) was the peripheral motor nerve ending and that functional integrity was necessary for the tremorgenic activity.[4] It was indicated that **1** might potentiate the release of acetylcholine in the presynaptic area of the peripheral motor nerve ending.[4] However, it has also been demonstrated that **1** is 20 times more potent than neostigmine in inhibiting AChE activity in human red blood cells.[5] On investigation of the relationship between the structures and inhibition of AChE activity, five derivatives of **1** were obtained, and their inhibitory potencies on electric eel AChE were tested.[6] It was concluded that substitution on the aromatic ring of **1** has little effect on anti-AChE activity.[5] However, the enone and pyrone moieties of **1** seem to play important roles in AChE inhibitory activity.[6]

Previous work had shown that incubation of **1** with rat liver microsomes produced four metabolites designated MB_1−MB_4. The reaction was NADPH dependent and enhanced by pretreatment of the rats with phenobarbital.[7] MB_2 (**2**) is a major metabolite that arose from hydroxylation of the pro-S methyl group at C4 of **1**. MB_4 (**3**) was identical to territrem C.[7] MB_1 (**4**) was shown to be the 4′-demethyl analogue of **2** and the major metabolite obtained from incubation of territrem C with rat liver microsome.[7] The structure of MB_3 (**5**) was not determined previously. A large-scale incubation experiment has provided sufficient **5** for elucidation of its structure and inhibitory effect on eel AChE. The results are described in this paper.

The ^{1}H- and ^{13}C-NMR spectra of **5** were similar to those of **1** except that the signals assigned to the 4α-methyl of the latter were absent and had been replaced by an AB pattern at δ_H 3.27, 4.08 (J = 10.8 Hz) and by a triplet at δ_c 69.44. The HRFABMS showed an $[M + 1]^+$ at m/z 543.2224 consistent with a hydroxylated derivative of **1** having molecular formula $C_{29}H_{34}O_{10}$. The hetero-COSY spectrum revealed the relationship between the signals of a hydroxymethyl group. This information indicates that **5** is the C-4 epimer of **2** (Figure 1).

The inhibitory activity of **1** and **5** on electric eel AChE was evaluated by the colorimetric method.[9] The IC_{50} of 1 on eel AChE was 2.6×10^{-7} M, and the IC_{50} of **5** was 4.23×10^{-10} M, indicating that **5** is 68 times more potent than **1**. The data (Table 2) showed that **5** is the most potent inhibitor of eel AChE (of **1** and its derivatives tested).



	R_1	R_2	R_3
1	CH_3O	CH_3	CH_3
2	CH_3O	CH_2OH	CH_3
3	OH	CH_3	CH_3
4	OH	CH_2OH	CH_3
5	CH_3O	CH_3	CH_2OH

Figure 1. The structure of territrem derivatives.

Experimental Section

General Experimental Conditions. The melting point was measured on a hot-stage melting point apparatus (Shimatzu Seisakusho Ltd.) and was uncorrected. The mass spectrum was recorded using a JEOL JMS-HX 110 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 400 spectrometer using solvent peaks as the reference standard.

Extraction and Isolation of 1. Compound **1** was isolated from rice culture of *Aspergillus terreus* 23−1 according to previously published procedures.[1−3] ^{1}H- and ^{13}C-NMR data; see Table 1.

Preparation of 5. Male Wistar rats, weighing 200−250 g, were fed freely with a phenobarbital−H_2O solution (sodium salt, Wako Pure Chemical Industries, Ltd., 1 g/L H_2O) for two weeks and sacrificed on the 15th day. The preparation of the S_9 fraction from the rat livers followed the method of Maron and Ames.[8] The solution contained 20 μL S_9 (4 mg/mL protein), 0.1 M NADP (20 μL) (Sigma), 0.4 M KCl−1.65 M $MgCl_2$ solution (10 μL), 0.1 M glucose-6-phosphate solution salt (5 μL) (Sigma), 0.1 M sodium phosphate buffer (250 μL), pH 7.4, and distilled H_2O to make the final volume to 500 μL. After the solution was preincubated at 37 °C for 30 min, 4 μL of **1** (1 mg/mL MeOH) was added. An

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Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data ($CDCl_3$, δ) for 1 and 5

	1		5	
position	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1		204.47 s		203.88 s
2	5.81 (d, J = 10.0)	123.30 d	6.08 (d, J = 10.0)	126.68 d
3	6.29 (d, J = 10.0)	153.41 d	6.51 (d, J = 10.0)	151.39 d
4		42.59 s		46.53 s
4a		79.06 s		79.69 s
5		25.75 t		26.19 t
5α, 5β	1.85−1.89 (m)		1.85−2.09 (m)	
6		28.45 t		28.27 t
6α	1.79−1.81 (m)		1.79−1.85 (m)	
6β	2.40−2.47 (m)		2.44−2.52 (m)	
6a		79.97 s		82.66 t
7a		162.83 s		162.83 s
8	6.30 (s)	97.56 d	6.35 (s)	97.46 d
9		158.47 s		158.72 s
11		164.31 s		164.26 s
11a		97.27 s		96.99 s
12α	3.41 (d, J = 18.0)		3.41 (d, J = 17.8)	
12β	2.84 (d, J = 18.0)		2.83 (d, J = 17.8)	
12		27.86 t		27.79 t
12a		76.10 s		77.32 s
12b		56.29 s		55.81 s
4α-Me	1.24 (s)	23.83 q		
4α-CH_2			3.27, 4.08 (d, J = 10.8)	69.44 t
4β-Me	1.15 (s)	25.44 q	1.23 (s)	19.33 q
6a-Me	1.49 (s)	23.80 q	1.51 (s)	24.00 q
12b-Me	1.43 (s)	21.79 q	1.43 (s)	21.65 q
1′		126.72 s		126.68 s
2′,6′	6.96 (s)	102.77 d	6.98 (s)	102.83 d
3′,5′		153.44 s		153.50 s
4′		140.31 s		140.43 s
3′,5′-OMe	3.87 (s)	56.21 q	3.89 (s)	56.33 q
4′-OM4	3.86 (s)	60.94 q	3.88 (s)	60.98 q

Table 2. Inhibition of Electric Eel Acetylcholinesterase by Territrem B (**1**) Derivatives

compound	in vitro I_{50} (M)[a]
1	2.60×10^{-7}
2	7.9×10^{-7}[a]
5	4.23×10^{-10}
BW284C51[b]	1.0×10^{-8}

[a] I_{50} values were calculated by probit analysis from responses obtained from eight doses of inhibitor, each differing by an order of magnitude. See Chen and Ling.[5] [b] BW281C51: 1,5-bis[4-(allyldimethylammonio)phenyl]pentan-3-one dibromide, see Chen.[11]

additional 60-min incubation was carried out at 37 °C by shaking (100 oscillations/min). The reaction was stopped by adding 1 mL MeOH. The reaction mixture was centrifuged at 15 000 rpm, and then 100 μL of the supernatant was taken for HPLC analysis. For isolation of large quantities of the product, the amounts of the above-described reaction mixture were scaled up to 200-fold and divided into 14 Erlenmeyer flasks. To each flask (250 mL), 32 mg of **1** (1 mg/mL MeOH) was added. Compound **5** (0.32 mg) was separated via preparative TLC [C_6H_6−EtOAc−HOAc−HOAc (6:3:1)] and finally purified by ODS HPLC [MeCN−H_2O (6:4)].

4α-(Hydroxymethyl)-4α-demethylterritrem B (5): mp 240−242 °C (from $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 331 (1.1), 218 (3.2); ^{1}H- and ^{13}C-NMR data; see Table 1, FABMS m/z $[M + 1]^+$ 543 (92), 524 (9), 509 (14), 359 (5), 291 (44), 237 (14), 214 (24), 195 (68); HRFABMS m/z $[M + 1]^+$ 543.2224 (calcd for $C_{29}H_{34}O_{10}$, 542.2152).

Assay of Acetylcholinesterase. The AChE activity was determined by the method of Ellman *et al.*[9] Typically, an aliquot of 20−40 μL of the working enzyme solution or of the inhibited specimen was added to 1 mL of the assay system containing 4.8×10^{-4} M acetylthiocholine and 3.2×10^{-4} M DTNB in a 0.1 M phosphate buffer, pH, 8.0. The initial rate of substrate hydrolysis was determined at 412 nm at room temperature using a Beckman spectrophotometer. The activity of AChE was calculated according to Gordon *et al.*[10]

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References and Notes

(1) Ling, K. H.; Yang, C. K.; Peng, F. C. *Appl. Environ. Microbiol.* **1979**, *17*, 355−357.
(2) Ling, K. H.; Liou, H. H.; Yang, C. M.; Yang, C. K. *Appl. Environ. Microbiol.* **1984**, *47*, 98−100.
(3) Yang, C. K. Studies on territrems from *Aspergillus terreus*: isolation, assay methods, molecular structure. Ph.D. Thesis, Institute of Biochemistry, College of Medicine, National Taiwan University, Republic of China, 1981; pp 16−39.
(4) Ling, K. H.; Liou, H. H.; Fu, T. C.; Kuo, L.; Tasi, M. C.; Lin, M. Y. Mechansim of action of the territrem, tremorgenic mycotoxin isolated from *Aspergillus terreus*. In *Mycotoxins and Phycotoxins*, Steyn, P.S., Velggaar, R., Eds. 6th International IUPAC Symposium on Mycotoxins and Phcotoxins. Elsevier: Amsterdam, 1985; pp 387−298.
(5) Chen, J. W.; Ling, K. H. *Territrem, an Inhibitor of Acetylcholinesterase* ROC−Japan seminar on mycotoxins, National Taiwan University, Taipei, 1991, Sep 22−24, p 24.
(6) Peng F. C. *J. Nat. Prods.* **1995**, *58*, 857−862.
(7) Ling, K. H.; Chiou, C. M.; Tseng, Y. L. *Drug. Metab. Dispos.* **1991**, *19*, 587.
(8) Maron, D. M.; Ames, B. *Mutat. Res.* **1983**, *113*, 171−205.
(9) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *77*, 88−95.
(10) Gordon, A.; Carpenter, D. E.; Wilson, I. B. *Mol. Pharmacol.* **1978**, 14, 266−270.
(11) Chen, J. W. Inhibition mechanism of acetylcholinesterase by territrem. Ph.D. Thesis, Institute of Biochemistry, College of Medicine, National Taiwan University, Republic of China, 1991; p 17.

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