Arisugacin, a Novel and Selective Inhibitor of Acetylcholinesterase from *Penicillium* sp. FO-4259

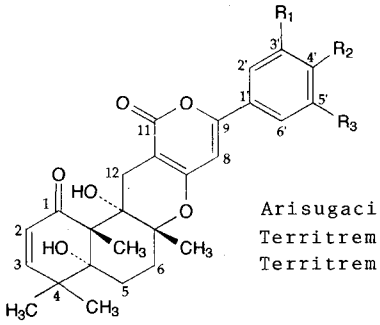
Sir:

In the course of our screening for microbial metabolites that inhibit the activity of acetylcholinesterase (AChE), we isolated a novel and selective inhibitor of AChE, arisugacin, from the culture broth of *Penicillium* sp. FO-4259. Arisugacin was co-produced with known tremorgenic compounds, territrems B and C, which were reported as the metabolites of *Aspergillus terreus*[1,2]. This communication deals with production, isolation, physico-chemical properties and biological activity of arisugacin.

The producing organism was isolated from a soil collected in Minato-Ku, Tokyo, Japan, and was identified as *Penicillium* sp. The slant culture of the producing organism was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 2.0%, Polypepton 0.5%, yeast extract 0.2%, agar 0.1%, KH_2PO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.05% (adjusted to pH 6.0 before sterilization). After incubation at 27°C for 72 hours on a rotary shaker, 1 vol.% of the seed culture was inoculated into one hundred 500-ml Erlenmeyer flasks containing 100 ml each of a producing medium consisting of saccharose 2.0%, glucose 1.0%, corn steep powder 0.5%, meat extract 0.5%, agar 0.1%, KH_2PO_4 0.1% and $CaCO_3$ 0.3% (adjusted to pH 6.0 before sterilization). The fermentation was carried out at 27°C for 192 hours on a rotary shaker (210 rpm). The culture broth (10 liters) was filtered using Celite and the culture filtrate was extracted with ethyl acetate. The mycelium was extracted with methanol and the extract was concentrated *in vacuo* to give an aqueous residue, which was then extracted with ethyl acetate. Both ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The crude extract (1.6 g) was chromatographed on a silica gel column with chloroform-methanol (200:1 ~ 100:1). The active fractions were further purified with preparative HPLC (column: J'sphere ODS-M80, 2.0 × 25.0 cm, YMC Co., Ltd. mobile phase: 45% acetonitrile) to yield a white powder of arisugacin (2.0 mg).

The molecular formula of arisugacin was elucidated as $C_{28}H_{32}O_8$ by HRFAB-MS (*m/z* 497.2144 $(M+H)^+$; calcd for $C_{28}H_{33}O_8$, 497.2176). The other physico-chemical properties of arisugacin were as follows. MP >300°C. $[\alpha]_D^{25}$ +72° (*c* 0.1, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε) 217 (25,600), 334 (12,500). IR ν_{max} (KBr) cm^{-1} 3450, 2362, 1686, 1635, 1560, 1541, 1500, 1473, 1457, 1408, 1269, 1144. ^1H NMR (400 MHz, pyridine-d_5) δ 8.89 (1H, s), 7.66 (1H, s), 7.59 (1H, m), 7.48 (1H, d, *J*=2.0 Hz), 6.99 (1H, d, *J*=8.5 Hz), 6.77 (1H, s), 6.27 (1H, d, *J*=10.2 Hz), 5.94 (1H, d, *J*=10.2 Hz), 4.34 (1H, d, *J*=17.9 Hz), 3.77 (3H, s), 3.76 (3H, s), 3.16 (1H, d, *J*=17.9 Hz), 2.89 (1H, ddd, *J*=4.5, 14.0, 14.0 Hz), 1.97 (1H, ddd, *J*=3.5, 14.0, 14.0 Hz), 1.91 (1H, m), 1.89 (1H, m), 1.49 (3H, s), 1.45 (3H, s), 1.29 (3H, s), 1.19 (3H, s). ^{13}C NMR (100 MHz, pyridine-d_5) 202.2 s, 164.0 s, 163.3 s, 158.7 s, 153.1 d, 152.1 s, 149.7 s, 125.0 s, 124.1 d, 119.2 d, 112.3 d, 109.3 d, 98.0 s, 97.4 d, 81.5 s, 79.5 s, 76.3 s, 56.6 s, 56.01 q, 55.98 q, 42.8 s, 29.5 t, 27.6 t, 26.2 t, 25.9 q, 23.9 q, 23.6 q, 22.1 q.

Fig. 1. Structures of arisugacin and territrems B and C.



Arisugacin R_1=H, R_2=R_3=OCH_3
Territrem B R_1=R_2=R_3=OCH_3
Territrem C R_1=R_3=OCH_3, R_2=OH

Table 1. Inhibitory activities of arisugacin and the related compounds against acetylcholinesterase[a] and butyrylcholinesterase[a].

Compound	IC_{50} (nM)	
	AChE	BChE
Arisugacin	1.0	>18,000
Territrem B	7.6	>20,000
Territrem C	6.8	>26,000
Tacrine[b]	200.0	12.0

[a] Sigma. [b] Research Biochemical Incorporated.

The NMR study of arisugacin revealed its structure as 3'-demethoxy-territrem B (Fig. 1). The details of the structure elucidation will be reported elsewhere.

The inhibitory activities of arisugacin against AChE (from human erythrocytes) and butyrylcholinesterase (BChE, from horse serum) are shown in Table 1. The inhibitory activities of AChE and BChE were measured by the method of OKABE *et al.*[4] with some modification. Territrem B has been reported as an inhibitor of AChE in molluscan neurons[5] and insect head[6]. Tacrine is an inhibitor of AChE for the potential use in the treatment of Alzheimer's disease[7]. The inhibitory activity of arisugacin against AChE was 7.6, 6.8 and 200 times stronger than those of territrems B, C and tacrine, respectively. On the other hand, the inhibitory activities of arisugacin and territrems B and C against BChE were more than 1,500 times weaker than that of tacrine. Since arisugacin is a highly selective inhibitor against AChE, it is expected to be effective in Alzheimer's disease, too.

Acknowledgment

This work was supported in part by a Grant-in-Aid for AKPS from Kitasato Gakuen Corp. We wish to thank Miss Y. NAKAMURA and Mrs. K. KUNIYASU and T. KAWAKUBO for

their technical assistance.

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(Received January 5, 1995)

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