

**Arisugacins A and B, Novel and Selective Acetylcholinesterase  
Inhibitors from *Penicillium* sp. FO-4259**

**II. Structure Elucidation**

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The structures of new acetylcholinesterase inhibitors, arisugacins A and B, were elucidated by NMR study. Arisugacins have a (4a*R*,6a*R*,12a*S*,12b*S*)-4a,6,6a,12,12a,12b-hexahydro-4a,12a-dihydroxy-4,4,6a,12b-tetramethyl-4*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-1,11(5*H*)-dione moiety in common and 3,4-dimethoxyphenyl or 4-methoxyphenyl residues are attached to C-9 of the moiety.

In the course of screening for selective inhibitors of acetylcholinesterase, we have found new compounds, arisugacins A and B (**1** and **2**, Fig. 1), from the cultured broth of *Penicillium* sp. FO-4259<sup>1,2)</sup>. The broth of strain FO-4259 produced also the other acetylcholinesterase inhibitors, territrem B (**4**) and C (**5**) and cyclopinin. Territrem A (**3**), B, and C were reported as tremorigenic mycotoxins and acetylcholinesterase inhibitors isolated from *Aspergillus terreus*<sup>3~5)</sup>. We found that territrem, together with arisugacins, showed highly selective inhibition to acetylcholinesterase compared with butyrylcholinesterase<sup>1,2)</sup>.

As the physico-chemical properties of **1** and **2** were similar to territrem, their structures were presumed to be related to territrem. In this paper, the physico-chemical properties and the structure elucidation of **1** and **2** are described.

Structure Elucidation of Arisugacin A (**1**)

The physico-chemical properties of **1** and **2** are summarized in Table 1. Both were obtained as white powders and showed positive color reaction with sulfuric acid. The <sup>1</sup>H and <sup>13</sup>C NMR data of **1** and **2** observed in pyridine-*d*<sub>5</sub> are shown in Tables 2 and 3. The HMQC experiments revealed the connectivity of each proton and carbon.

HR FAB-MS of **1** revealed its molecular formula, C<sub>28</sub>H<sub>32</sub>O<sub>8</sub>, as shown in Table 1. Compound **1** showed six methyl, three methylene, six methine, and thirteen quaternary carbon signals in the DEPT spectra. The UV spectrum of **1** resembled **4**<sup>2,3)</sup>. The chemical shifts of the rings A, B, C, and D were quite similar to those of **4** in

the <sup>1</sup>H and <sup>13</sup>C NMR (Tables 2 and 3). The <sup>1</sup>H-<sup>1</sup>H COSY and HMBC experiments of **1** revealed that these rings were the same as **4** (Fig. 2).

The remaining two methoxy, three aromatic methine, and three aromatic quaternary carbons were assigned as follows. The <sup>1</sup>H-<sup>13</sup>C long-range couplings between 8-H (δ 6.77) and C-1' (δ 125.0), and between 2'-H (δ 7.48) and C-9 (δ 158.7) revealed the alignment of C-9-C-1'-C-2'. The long-range couplings between 3'-OCH<sub>3</sub> (δ 3.77) and C-3' (δ 149.7), and between 4'-OCH<sub>3</sub> (δ 3.76) and C-4' (δ 152.1) revealed that each methoxy residue connected to respective quaternary carbons. The <sup>1</sup>H-<sup>1</sup>H COSY showed the coupling between 5'-H (δ 6.99) and

Fig. 1. Structures of arisugacins A (**1**) and B (**2**) and territrem A~C (**3**~**5**).

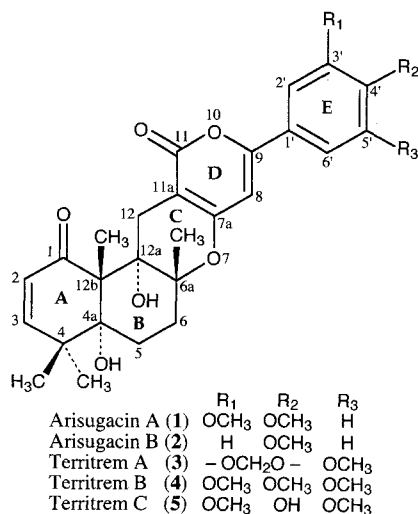


Table 1. Physico-chemical properties of 1 and 2.

	1	2
Appearance	White powder	White powder
MP	>300°C	>300°C
$[\alpha]_D^{23}$ (c 0.1, CHCl <sub>3</sub> )	+72°	+26°
Molecular formula	C <sub>28</sub> H <sub>32</sub> O <sub>8</sub>	C <sub>27</sub> H <sub>30</sub> O <sub>7</sub>
Molecular weight	496	466
HR FAB-MS ( <i>m/z</i> ): calcd	497.2176 (M+H) <sup>+</sup>	467.2070 (M+H) <sup>+</sup>
found	497.2144 (M+H) <sup>+</sup>	467.2071 (M+H) <sup>+</sup>
UV $\lambda_{\max}^{\text{MeOH}}$ nm ( $\epsilon$ )	217 (25,600), 334 (12,500)	210 (21,900), 252 (12,700), 330 (15,500)
UV $\lambda_{\max}^{\text{MeOH-HCl}}$ nm ( $\epsilon$ )	222 (17,400), 336 (13,000)	210 (15,100), 230 (12,600), 251 (14,500), 322 (15,100)
UV $\lambda_{\max}^{\text{MeOH-NaOH}}$ nm ( $\epsilon$ )	210 (66,800), 335 (12,600)	214 (86,200), 252 (12,700), 331 (14,100)
IR $\nu_{\max}$ (KBr) cm <sup>-1</sup>	3450, 2362, 1686, 1635, 1560, 1541, 1500, 1473, 1457, 1408, 1269, 1144	3508, 3369, 1705, 1674, 1635, 1514, 1402, 1254, 1209, 1174, 1119, 1024
Solubility:	soluble insoluble	MeOH, EtOH, CHCl <sub>3</sub> H <sub>2</sub> O, Hexane

Table 2. The <sup>1</sup>H NMR data of 1, 2, and 4.

Position	1	2	4 <sup>6)</sup>
2-H	5.94 d (10.2)	5.94 d (10.2)	5.92 d (10.0)
3-H	6.27 d (10.2)	6.27 d (10.2)	6.31 d (10.0)
4 $\alpha$ -Me	1.29 s	1.28 s	1.31 s
4 $\beta$ -Me	1.19 s	1.18 s	1.23 s
4a-OH	7.66 s	7.66 br. s	
5-H $\alpha$	1.91 ddd (4.0, 4.3, 14.0),	1.90 m,	1.83~1.92 m
5-H $\beta$	1.97 ddd (3.4, 13.0, 14.0)	1.95 ddd (3.5, 13.0, 14.0)	
6-H $\alpha$	2.89 ddd (4.3, 12.0, 13.0)	2.88 ddd (4.4, 12.0, 13.0)	2.82 m
6-H $\beta$	1.89 m	1.88 m	1.92 m
6a-Me	1.49 s	1.48 s	1.52 s
8-H	6.77 s	6.74 s	6.80 s
12-H $\alpha$	4.34 d (17.9)	4.32 d (17.6)	4.22 d (17.6)
12-H $\beta$	3.16 d (17.9)	3.14 d (17.6)	3.15 d (17.6)
12a-OH	8.89 s	8.90 br. s	
12b-Me	1.45 s	1.44 s	1.49 s
2'-H	7.48 d (2.0)	7.88 d (8.9)	7.31 s
3'-H		7.03 d (8.9)	
3'-OMe	3.77 s		3.83 s
4'-OMe	3.76 s	3.69 s	3.93 s
5'-H	6.99 d (8.5)	7.03 d (8.9)	
5'-OMe			3.83 s
6'-H	7.58 m	7.88 d (8.9)	7.31 s

The pyridine-*d*<sub>5</sub> signal (8.73 ppm) was used as a reference. The coupling constants (Hz) are in parentheses.

6'-H ( $\delta$  7.58). The above results indicated that the remainder was 3,4-dimethoxyphenyl or 3,6-dimethoxyphenyl residue. The differential NOE experiments (Fig. 3) revealed NOEs between 8-H and 2'-H and between 8-H and 6'-H, suggesting a 3,4-dimethoxyphenyl residue. Therefore, the planar structure of 1 was elucidated as 4a,6,6a,12,12a,12b-hexahydro-4a,12a-dihydroxy-4,4,6a,12b-tetramethyl-9-(3,4-dimethoxyphenyl)-4*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-1,11(5*H*)-dione.

The relative configuration of 1 was examined by the differential NOE experiments. As shown in Fig. 3, the NOEs among 4 $\beta$ -CH<sub>3</sub> ( $\delta$  1.19), 5-H $\beta$  ( $\delta$  1.97), 6a-CH<sub>3</sub> ( $\delta$

1.49), 12-H $\beta$  ( $\delta$  3.16), and 12b-CH<sub>3</sub> ( $\delta$  1.45) suggest that they are all  $\beta$  configuration. The NOEs between 4a-OH ( $\delta$  7.66) and 6-H $\alpha$  ( $\delta$  2.89), and between 12-H $\alpha$  ( $\delta$  4.34) and 12a-OH ( $\delta$  8.89) suggest that they are all  $\alpha$  configuration. Thus the relative configuration of 1 was elucidated as 4a*R*,6a*R*,12a*S*,12b*S* (Fig. 1), which is the same as 4<sup>7)</sup>.

#### Structure Elucidation of Arisugacin B (2)

The molecular formula of 2 was elucidated as C<sub>27</sub>H<sub>30</sub>O<sub>7</sub> by HR FAB-MS, suggesting that 2 was demethoxy-1. The NMR chemical shifts (Tables 2 and 3) of the rings A, B, C, and D resembled those of 1. The

$^1\text{H}$ - $^1\text{H}$  COSY and HMBC experiments of **2** revealed that these rings are the same as **1** (Fig. 4). As for ring E, two doublet methines ( $\delta$  7.03, 2H,  $J=8.9$  Hz and  $\delta$  7.88, 2H,  $J=8.9$  Hz) and one methoxy residue ( $\delta$  3.69, 3H) were observed in  $^1\text{H}$  NMR, suggesting the ring is a *p*-methoxyphenyl residue. C-1' and C-2'(6') were assigned by the  $^1\text{H}$ - $^{13}\text{C}$  long-range couplings between 8-H ( $\delta$  6.74) and C-1' ( $\delta$  124.8), and between 2'(6')-H ( $\delta$  7.88) and C-9 ( $\delta$  158.6) in the HMBC experiment. C-4' was assigned by the long-range couplings between 2'(6')-H and C-4' ( $\delta$  162.0), and between 3'(5')-H ( $\delta$  7.03) and C-4'. The methoxy residue was shown to be connected to C-4' by the long-range coupling between 4'-OCH<sub>3</sub> ( $\delta$  3.69) and

C-4'. Thus the planar structure of **2** was elucidated as 3'-demethoxy-**1**. The relative configuration of **2** was suggested to be the same as **1** by the similarity of their chemical shifts and coupling constants.

Microbial products that have naphtho(2,1-*b*)pyrano-(3,4-*e*)pyran moiety are not common. Only territrems and pyripyropenes have been reported as far as we know. Pyripyropenes were isolated from the culture broth of *Aspergillus fumigatus* by our group and showed potent inhibition against acyl-CoA:cholesterol acyltransferase<sup>8-11</sup>. Though skeletons of arisugacins and pyripyropenes are similar, arisugacins did not inhibit acyl-CoA:cholesterol acyltransferase and pyripyropenes did not inhibit acetylcholinesterase (data not shown). Their structure-activity relationship is interesting and now under study. The skeleton of pyripyropenes is biosynthesized from a sesquiterpene, a diketide, and a nicotinic acid<sup>12</sup>). Arisugacins are suggested to be synthesized from a sesquiterpene, a diketide, and a benzoic acid from their structural similarity to pyripyropenes. Therefore, arisugacins may be members of the meroterpenoid compounds that have mixed

Table 3. The  $^{13}\text{C}$  NMR data of **1**, **2**, and **4**.

Position	<b>1</b>	<b>2</b>	<b>4</b> <sup>6)</sup>
C-1	202.2 s	202.2 s	202.2 s
C-2	124.1 d	124.3 d	124.3 d
C-3	153.1 d	153.1 d	153.2 d
C-4	42.8 s	42.8 s	42.9 s
4 $\alpha$ -Me	25.9 q	25.9 q	26.0 q
4 $\beta$ -Me	23.9 q	23.9 q	24.1 q
C-4a	79.5 s	79.5 s	79.6 s
C-5	26.2 t	26.2 t	26.3 t
C-6	29.5 t	29.5 t	29.6 t
C-6a	81.5 s	81.5 s	81.6 s
6a-Me	23.6 q	23.6 q	23.8 q
C-7a	163.3 s	163.3 s	163.2 s
C-8	97.4 d	97.1 d	98.5 d
C-9	158.7 s	158.6 s	158.4 s
C-11	164.0 s	164.0 s	163.9 s
C-11a	98.0 s	98.0 s	98.5 s
C-12	27.6 t	27.6 t	27.7 t
C-12a	76.3 s	76.3 s	76.3 s
C-12b	56.6 s	56.6 s	56.6 s
12b-Me	22.1 q	22.1 q	22.2 q
C-1'	125.0 s	124.8 s	127.7 s
C-2'	109.3 d	127.4 d	103.8 d
C-3'	149.7 s	114.9 d	154.4 s
3'-OMe	56.0 q		56.5 q
C-4'	152.1 s	162.0 s	141.4 s
4'-OMe	56.0 q	55.5 q	60.9 q
C-5'	112.3 d	114.9 d	154.4 s
5'-OMe			56.5 q
C-6'	119.2 d	127.4 d	103.8 d

The pyridine-*d*<sub>5</sub> signal (150.0 ppm) was used as a reference.

Fig. 2. Structure of **1** elucidated by NMR analysis.

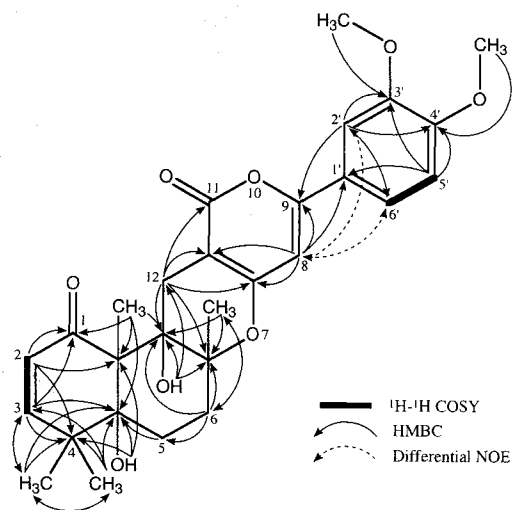


Fig. 3. NOE experiments of **1**.

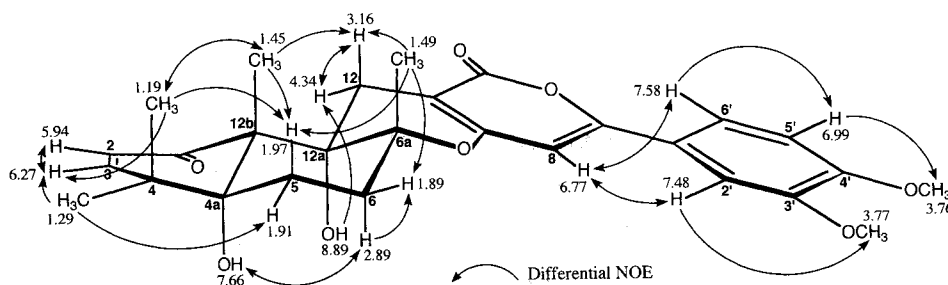
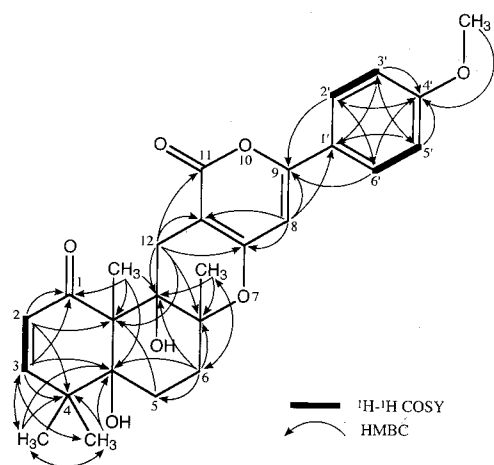


Fig. 4. Structure of 2 elucidated by NMR analysis.



polyketide-terpenoid structures.

### Experimental

Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. Optical rotation was recorded on a JASCO model DIP-181 polarimeter. Melting point was measured with a Yanaco micro melting point apparatus MP-S3. NMR spectra were obtained with a Valian Unity 400 spectrometer (400 MHz) using pyridine- $d_5$  as a solvent.

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### References

- 1) ŌMURA, S.; F. KUNO, K. OTOGURO, T. SUNAZUKA, K. SHIOMI, R. MASUMA & Y. IWAI: Arisugacin, a novel and selective inhibitor of acetylcholinesterase from *Penicillium*

- sp. FO-4259. *J. Antibiotics* 48: 745~746, 1995
- 2) KUNO, F.; K. OTOGURO, K. SHIOMI, Y. IWAI & S. ŌMURA: Arisugacins A and B, novel and selective acetylcholinesterase inhibitors from *Penicillium* sp. FO-4259. I. Screening, taxonomy, fermentation, isolation and biological activity. *J. Antibiotics* 49: 742~747, 1996
- 3) LING, K. H.; C.-K. YANG & F.-T. PENG: Territrems, tremorgenic mycotoxins of *Aspergillus terreus*. *Appl. Environ. Microbiol.* 37: 355~357, 1979
- 4) LING, K. H.; H.-H. LIOU, C.-M. YANG & C.-K. YANG: Isolation, chemical structure, acute toxicity, and some physicochemical properties of territrems from *Aspergillus terreus*. *Appl. Environ. Microbiol.* 47: 98~100, 1984
- 5) LING, K. H.; F.-C. PENG, B. J. CHEN, Y. WANG & G. H. LEE: Isolation, physicochemical properties and toxicities of territrems A' and B'. *Kor. J. Pharmacogn.* 17: 153~160, 1986
- 6) LEE, S.-S.; F.-C. PENG, C.-M. CHIOU & K. H. LING: NMR assignments of territrems A, B, and C and the structure of MB<sub>2</sub>, the major metabolite of territrems B by rat liver microsomal fraction. *J. Nat. Prod.* 55: 251~255, 1992
- 7) HSEU, T. H.; C. K. YANG, K. H. LING, C. J. WANG & C. P. TANG: (4aR,6aR,12aS,12bS)-4a,6,6a,12,12a,12b-Hexahydro-4a,12a-dihydroxy-4,4,6a,12b-tetramethyl-9-(3,4,5-trimethoxyphenyl)-4H,11H-naphtho[2,1-b]pyrano[3,4-e]pyran-1,11(5H)-dione, territrems B, C<sub>29</sub>H<sub>34</sub>O<sub>9</sub>. *Cryst. Struct. Commun.* 11: 199~206, 1982
- 8) ŌMURA, S.; H. TOMODA, Y. K. KIM & H. NISHIDA: Pyripyropenes, highly potent inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. *J. Antibiotics* 46: 1168~1169, 1993
- 9) TOMODA, H.; Y. K. KIM, H. NISHIDA, R. MASUMA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. I. Production, isolation, and biological properties. *J. Antibiotics* 47: 148~153, 1994
- 10) KIM, Y. K.; H. TOMODA, H. NISHIDA, T. SUNAZUKA, R. OBATA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. II. Structure elucidation of pyripyropenes A, B, C and D. *J. Antibiotics* 47: 154~162, 1994
- 11) TOMODA, H.; N. TABATA, D.-J. YANG, H. TAKAYANAGI, H. NISHIDA, S. ŌMURA & T. TAKESAKO: Pyripyropenes, novel ACAT inhibitors produced by *Aspergillus fumigatus*. III. Structure elucidation of pyripyropenes E to I. *J. Antibiotics* 48: 495~503, 1995
- 12) TOMODA, H.; N. TABATA, Y. NAKATA, H. NISHIDA, T. KANEKO, R. OBATA, T. SUNAZUKA & S. ŌMURA: Biosynthesis of pyripyropene A. *J. Org. Chem.* 61: 882~886, 1996