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[1,2]. The broth of strain FO-4259 produced also the other acetylcholinesterase inhibitors, territrems B (**4**) and C (**5**) and cyclopenin. Territrems A (**3**), B, and C were reported as tremorigenic mycotoxins and acetylcholinesterase inhibitors isolated from *Aspergillus terreus*[3~5]. We found that territrems, together with arisugacins, showed highly selective inhibition to acetylcholinesterase compared with butyrylcholinesterase[1,2].

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

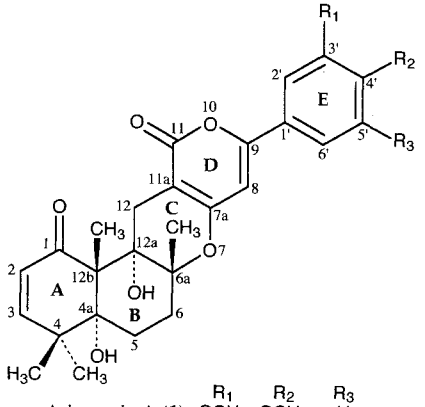
Structure Elucidation of Arisugacin A (1)

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 ^1H and ^{13}C NMR data of **1** and **2** observed in pyridine-d_5 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, $C_{28}H_{32}O_8$, 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**[2,3]. The chemical shifts of the rings A, B, C, and D were quite similar to those of **4** in the ^1H and ^{13}C NMR (Tables 2 and 3). The ^1H-^1H 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 ^1H-^{13}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_3 (δ 3.77) and C-3′ (δ 149.7), and between 4′-OCH_3 (δ 3.76) and C-4′ (δ 152.1) revealed that each methoxy residue connected to respective quaternary carbons. The ^1H-^1H COSY showed the coupling between 5′-H (δ 6.99) and

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



	R_1	R_2	R_3
Arisugacin A (**1**)	OCH_3	OCH_3	H
Arisugacin B (**2**)	H	OCH_3	H
Territrem A (**3**)	$-OCH_2O-$		OCH_3
Territrem B (**4**)	OCH_3	OCH_3	OCH_3
Territrem C (**5**)	OCH_3	OH	OCH_3

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, $CHCl_3$)	+72°	+26°
Molecular formula	$C_{28}H_{32}O_8$	$C_{27}H_{30}O_7$
Molecular weight	496	466
HR FAB-MS (m/z): calcd	497.2176 $(M+H)^+$	467.2070 $(M+H)^+$
found	497.2144 $(M+H)^+$	467.2071 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	217 (25,600), 334 (12,500)	210 (21,900), 252 (12,700), 330 (15,500)
UV $\lambda_{max}^{MeOH-HCl}$ nm (ε)	222 (17,400), 336 (13,000)	210 (15,100), 230 (12,600), 251 (14,500), 322 (15,100)
UV $\lambda_{max}^{MeOH-NaOH}$ nm (ε)	210 (66,800), 335 (12,600)	214 (86,200), 252 (12,700), 331 (14,100)
IR ν_{max} (KBr) cm^{-1}	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	MeOH, EtOH, $CHCl_3$	MeOH, EtOH, $CHCl_3$
insoluble	H_2O, Hexane	H_2O, Hexane

Table 2. The ^1H NMR data of **1**, **2**, and **4**.

Position	**1**	**2**	**4**[6]
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)
4α-Me	1.29 s	1.28 s	1.31 s
4β-Me	1.19 s	1.18 s	1.23 s
4a-OH	7.66 s	7.66 br. s	
5-H_α	1.91 ddd (4.0, 4.3, 14.0),	1.90 m,	1.83~1.92 m
5-H_β	1.97 ddd (3.4, 13.0, 14.0)	1.95 ddd (3.5, 13.0, 14.0)	
6-H_α	2.89 ddd (4.3, 12.0, 13.0)	2.88 ddd (4.4, 12.0, 13.0)	2.82 m
6-H_β	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_α	4.34 d (17.9)	4.32 d (17.6)	4.22 d (17.6)
12-H_β	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_5 signal (8.73 ppm) was used as a reference. The coupling constants (Hz) are in parentheses.

6'-H (δ 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 4β-CH_3 (δ 1.19), 5-H_β (δ 1.97), 6a-CH_3 (δ 1.49), 12-H_β (δ 3.16), and 12b-CH_3 (δ 1.45) suggest that they are all β configuration. The NOEs between 4a-OH (δ 7.66) and 6-H_α (δ 2.89), and between 12-H_α (δ 4.34) and 12a-OH (δ 8.89) suggest that they are all α 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**[7].

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

The molecular formula of **2** was elucidated as $C_{27}H_{30}O_7$ 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}H-^{1}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 (δ 7.03, 2H, J=8.9 Hz and δ 7.88, 2H, J=8.9 Hz) and one methoxy residue (δ 3.69, 3H) were observed in ^{1}H NMR, suggesting the ring is a *p*-methoxyphenyl residue. C-1′ and C-2′(6′) were assigned by the ^{1}H-^{13}C long-range couplings between 8-H (δ 6.74) and C-1′ (δ 124.8), and between 2′(6′)-H (δ 7.88) and C-9 (δ 158.6) in the HMBC experiment. C-4′ was assigned by the long-range couplings between 2′(6′)-H and C-4′ (δ 162.0), and between 3′(5′)-H (δ 7.03) and C-4′. The methoxy residue was shown to be connected to C-4′ by the long-range coupling between 4′-OCH$_3$ (δ 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.

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

Position	1	2	4[6]
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
4α-Me	25.9 q	25.9 q	26.0 q
4β-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_5 signal (150.0 ppm) was used as a reference.

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[8~11]. 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[12]. 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

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

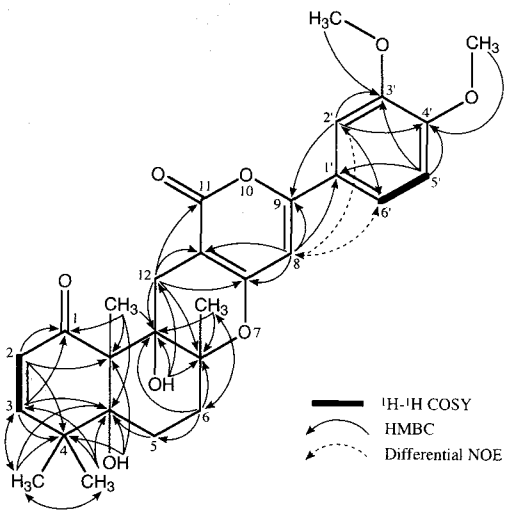


Fig. 3. NOE experiments of **1**.

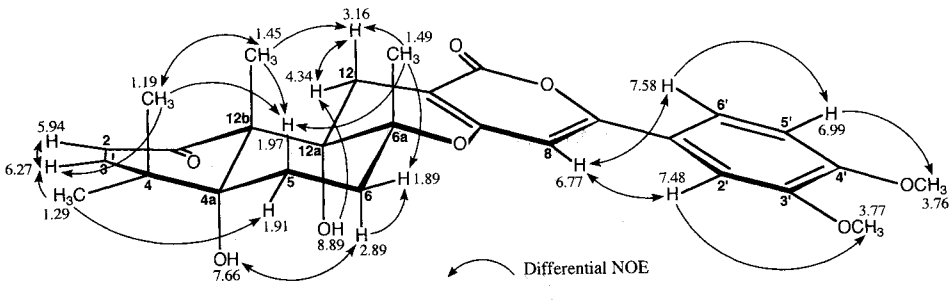
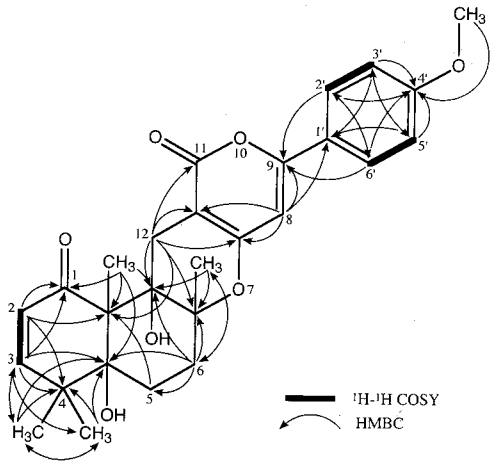


Fig. 4. Structure of **2** elucidated by NMR analysis.



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 territrem C 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 territrem 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_2, the major metabolite of territrem 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: (4a*R*,6a*R*,12a*S*,12b*S*)-4a,6,6a,12,12a,12b-Hexahydro-4a,12a-dihydroxy-4,4,6a,12b-tetramethyl-9-(3,4,5-trimethoxyphenyl)-4*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-1,11(5*H*)-dione, territrem B, $C_{29}H_{34}O_9$. 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