Determination of Absolute Stereochemistries of Arisugacin F and Territrem B, Novel Acetylcholinesterase Inhibitors

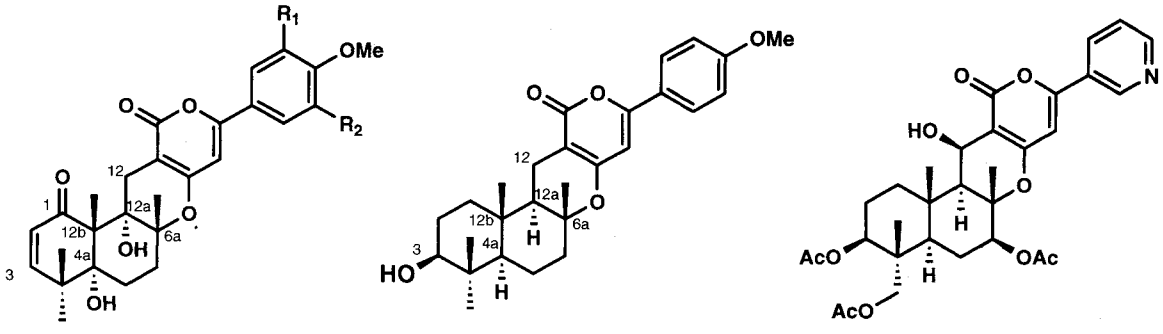
Sir:

Recently we disclosed the isolation, biological properties, and structures of arisugacin A (**1**) and B (**2**), novel polyoxygenated metabolites of *Penicillium* sp. FO-4259[1~4] which strongly inhibited acetylcholinesterase (AchE) selectively, together with the structurally related known compound, territrem B (**3**) (Fig. 1)[5,6]. Moreover, the mutant of the parent strain FO-4259 produced arisugacin F (**4**)[7]. The relative stereochemistries of **1**, **2**, and **4** have been elucidated by differential NOE experiments, and that of **3** by X-ray crystallographic analysis[8]. Interestingly, structures **1**~**4** resemble the pyripyropene A (**5**), which strongly inhibited acyl-CoA : cholesterol acyltransferase (ACAT), the enzyme that catalyzed intracellular esterification of cholesterol, and was isolated from *Asperigillus fumigatus* FO-1289 in our group[9~12]. The first asymmetric total synthesis of pyripyropene A has been achieved *via* a convergent and efficient strategy[13].

As a prelude to total synthesis, we describe here the determination of the complete absolute stereochemistries of arisugacin F (**4**) and 2, 3-dihydro-1α-ol territrem B (**9**), derived stereoselectively from territrem B (**3**), *via* the Kakisawa-Kashman modification[14] of the Mosher NMR method[15].

The (*S*) and (*R*) MTPA esters (**6** and **7**) were prepared by a treatment of **4** with (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) acid in the presence of DCC and DMAP (CH_2Cl_2, room temperature, 99% yield, 88% yield, respectively) (Scheme 1). The ^{1}H NMR spectra of **6** and **7** could be completely assigned *via* selective ^{1}H decoupling. The calculated $\Delta\delta$ values ($\Delta\delta=\delta S-\delta R$) are indicated in Fig. 2. **4** revealed negative $\Delta\delta$ values for the upside half of the molecule, especially that $\Delta\delta$ value at 2β-H showed large negative value ($\Delta\delta=-0.10$) and $\Delta\delta$ value at 4α-Me showed a large positive value ($\Delta\delta=+0.09$), so the absolute configuration of **4** is determined as 3-(*S*), 4a-(*S*), 6a-(*R*), 12a-(*R*), and 12b-(*S*) (Fig. 2). Analytical data of **6**: Rf=0.50 (silica gel, hexane : EtOAc=1 : 1), mp 98~99°C, $[\alpha]_D^{23}=+59.3°$ (*c* 0.41, $CHCl_3$), IR (KBr) ν cm^{-1}: 1709 (pyrone), 1743, 1639, 839, 814 (arom.), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (3H, s, 4β-Me), 0.92 (3H, s, 12bβ-Me), 0.95 (3H, s, 4α-Me), 1.11 (1H, dd, *J*=12.0, 2.0 Hz, 4aα-H), 1.20 (1H, m, 1α-H), 1.25 (3H, s, 6aβ-Me), 1.44 (1H, m, 5β-H), 1.52 (1H, dd, *J*=12.9, 4.9 Hz, 12aα-H), 1.69 (2H, m, 2β, 6α-H), 1.80 (1H, m, 5α-H), 1.84 (2H, m, 1β, 2α-H), 2.13 (1H, ddd, *J*=12.5, 3.0, 3.0 Hz, 6β-H), 2.23 (1H, dd, *J*=17.0, 12.9 Hz, 12β-H), 2.50 (1H, dd, *J*=17.0, 4.9 Hz, 12α-H), 3.85 (3H, s, 4′-OMe), 4.72 (1H, dd, *J*=11.8, 4.2 Hz, 3α-H), 6.25 (1H, s, 8-H), 6.93 (2H, d, *J*=8.8 Hz, 3′, 5′-H), 7.73 (2H, *J*=8.8 Hz, 2′, 6′-H), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 164.6 (C-11), 163.5 (C-7a), 161.5 (C-4′), 158.5 (C-9), 127.0 (C-2′, 6′), 124.0 (C-1′), 114.2 (C-3′, 5′), 98.3 (C-11a), 96.7 (C-8), 83.5 (C-3), 80.3 (C-6a), 55.4 (4′-OMe), 55.0 (C-4a), 51.5 (C-12a), 40.2 (C-6), 37.8 (C-4), 37.0 (C-1), 36.7 (C-12b), 28.2 (4α-Me),

Fig. 1. Structure of arisugacins A~B (**1**~**2**), territrem B (**3**), arisugacin F (**4**), and pyripyropene A (**5**).



1 ; R_1=H, R_2=OMe: Arisugacin A
2 ; R_1=H, R_2=H: Arisugacin B
3 ; R_1=R_2=OMe: Territrem B

4; Arisugacin F

5; Pyripyropene A

Scheme 1. Synthesis of (−)/(+) MTPA ester **6** and **7**.

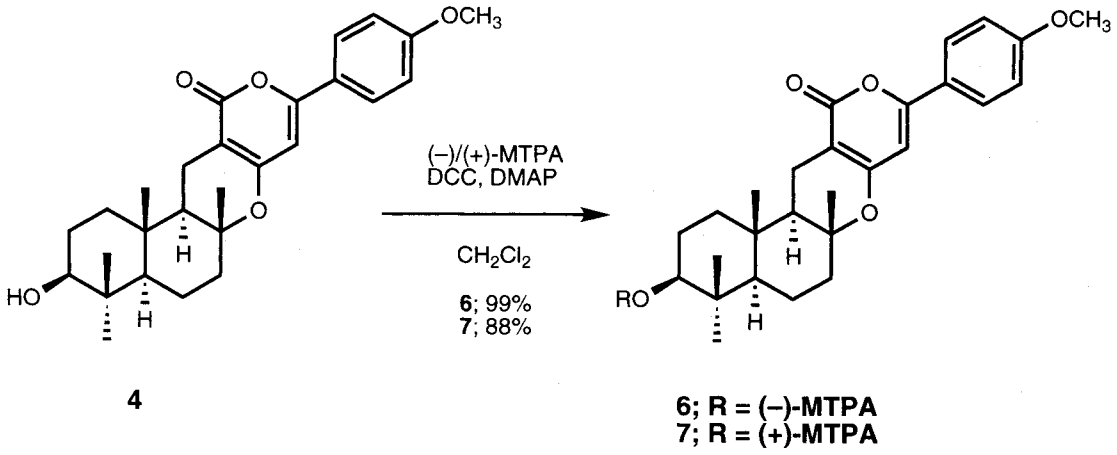
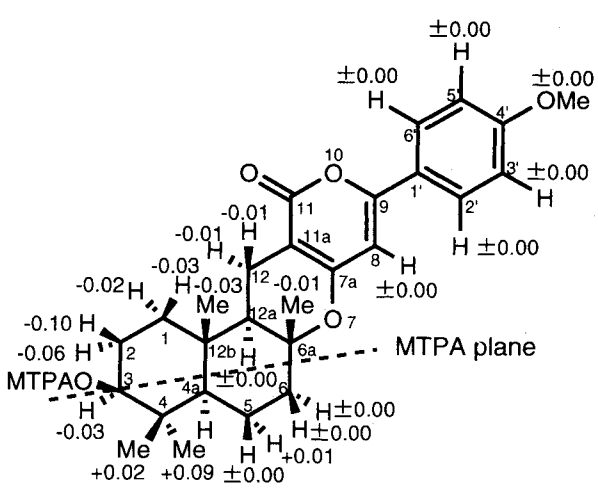
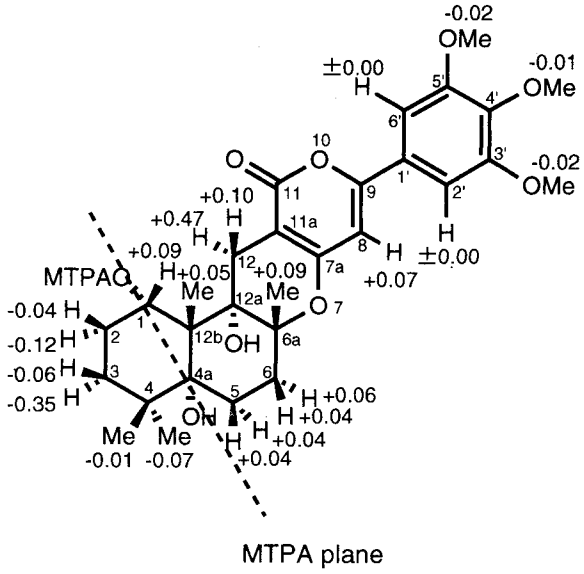


Fig. 2. $\Delta\delta$ values of MTPA esters derived arisugacin F (**4**) and 2, 3-dihydro-1α-ol territrem B (**9**).



MTPA ester of arisugacin F **4**



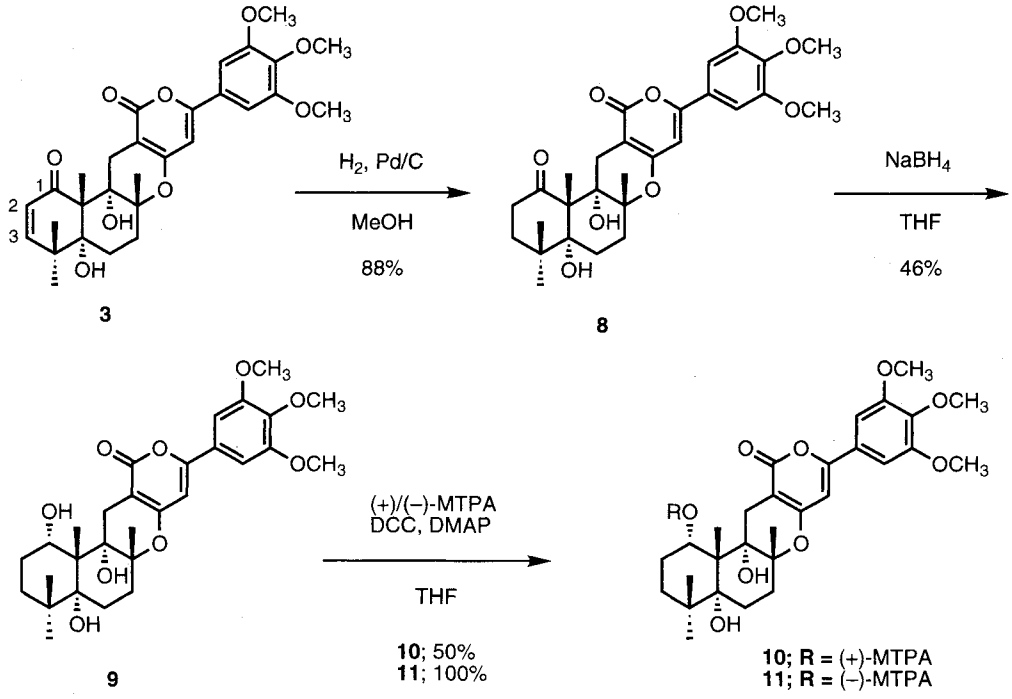
MTPA ester of 2,3-dihydro-1α-ol territrem B **9**

23.1 (C-2), 20.7 (6aβ-Me), 19.2 (C-5), 17.2 (C-12), 16.5 (4β-Me), 15.1 (12bβ-Me).

HRFABMS *m/z*: 654.2807 $[M]^+$, Calcd for $C_{37}H_{41}O_7F_3$: 654.2804 [M].

Analytical data of **7**: Rf=0.57 (silica gel, hexane: EtOAc=1:1), mp 93~94°C, $[\alpha]_D^{22}=+68.1°$ (*c* 0.34, $CHCl_3$), IR (KBr) ν cm^{-1}: 1707 (pyrone), 1745, 1639, 837, 810 (arom.), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.83 (3H, s, 4β-Me), 0.86 (3H, s, 4α-Me), 0.95 (3H, s, 12bβ-Me), 1.11 (1H, dd, *J*=12.0, 2.0 Hz, 4aα-H), 1.22 (1H, m, 1α-H), 1.26 (3H, s, 6aβ-Me), 1.44 (1H, m, 5β-H), 1.52 (1H, dd, *J*=12.9, 4.9 Hz, 12aα-H), 1.69 (1H, m, 6α-H), 1.79 (2H, m, 2β, 5α-H), 1.87 (1H, m, 1β-H), 1.90 (1H, m, 2α-H), 2.13 (1H, ddd, *J*=12.3, 3.0, 3.0 Hz, 6β-H), 2.24 (1H, dd, *J*=17.0, 12.9 Hz, 12β-H), 2.51 (1H, dd, *J*=17.0, 4.9 Hz, 12α-H), 3.85 (3H, s, 4′-OMe), 4.75 (1H, dd, *J*=12.0, 4.4 Hz, 3α-H), 6.25 (1H, s, 8-H), 6.93 (2H, d, *J*=8.9 Hz, 3′, 5′-H), 7.73 (2H, d, *J*=8.9 Hz, 2′, 6′-H), ^{13}C-NMR (100.6

Scheme 2. Synthesis of (+)/(−) MTPA ester **10** and **11**.



MHz, $CDCl_3$) δ: 164.6 (C-11), 163.5 (C-7a), 161.5 (C-4′), 158.5 (C-9), 127.0 (C-2′, 6′), 124.0 (C-1′), 114.2 (C-3′, 5′), 98.3 (C-11a), 96.7 (C-8), 83.3 (C-3), 80.3 (C-6a), 55.4 (4′-OMe), 55.0 (C-4a), 51.5 (C-12a), 40.2 (C-6), 37.9 (C-4), 37.1 (C-1), 36.7 (C-12b), 27.8 (4α-Me), 23.4 (C-2), 20.7 (6aβ-Me), 19.2 (C-5), 17.3 (C-12), 16.3 (4β-Me), 15.1 (12bβ-Me).

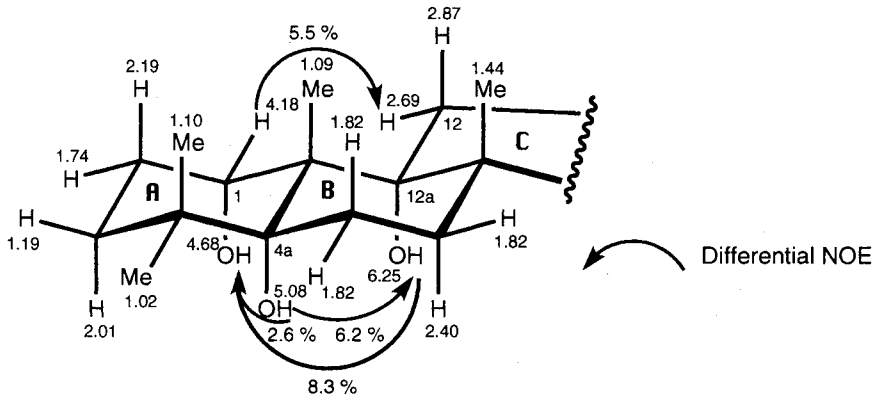
HRFABMS *m/z*: 654.2793 $[M]^+$, Calcd for $C_{37}H_{41}O_7F_3$: 654.2804 [M].

On the other hand, the alcohol **9** was derived from **3**, followed by (*S*) and (*R*) MTPA esterification similarly (Scheme 2). Ketone **8** was prepared from **3** by hydrogenation of the 2, 3-olefin (H_2, Pd/C, MeOH, 88% yield). The structure of **8** was identified by comparison of the ^{1}H-NMR spectrum to that reported in the literature[16]. **8** was then reduced stereoselectively to the alcohol **9** ($NaBH_4$, THF, 46% yield), and the structure was established by analyzing the ^{1}H-NMR spectrum and NOE experiments (Fig. 3), which show the 1α-hydroxyl group at δ 4.68 and 1β-proton at δ 4.18. **9** was furthermore converted to the (*R*) and (*S*) MTPA esters (**10** and **11**) [(*R*) or (*S*)-MTPA, DCC, DMAP, CH_2Cl_2, 50% yield, 100% yield, respectively].

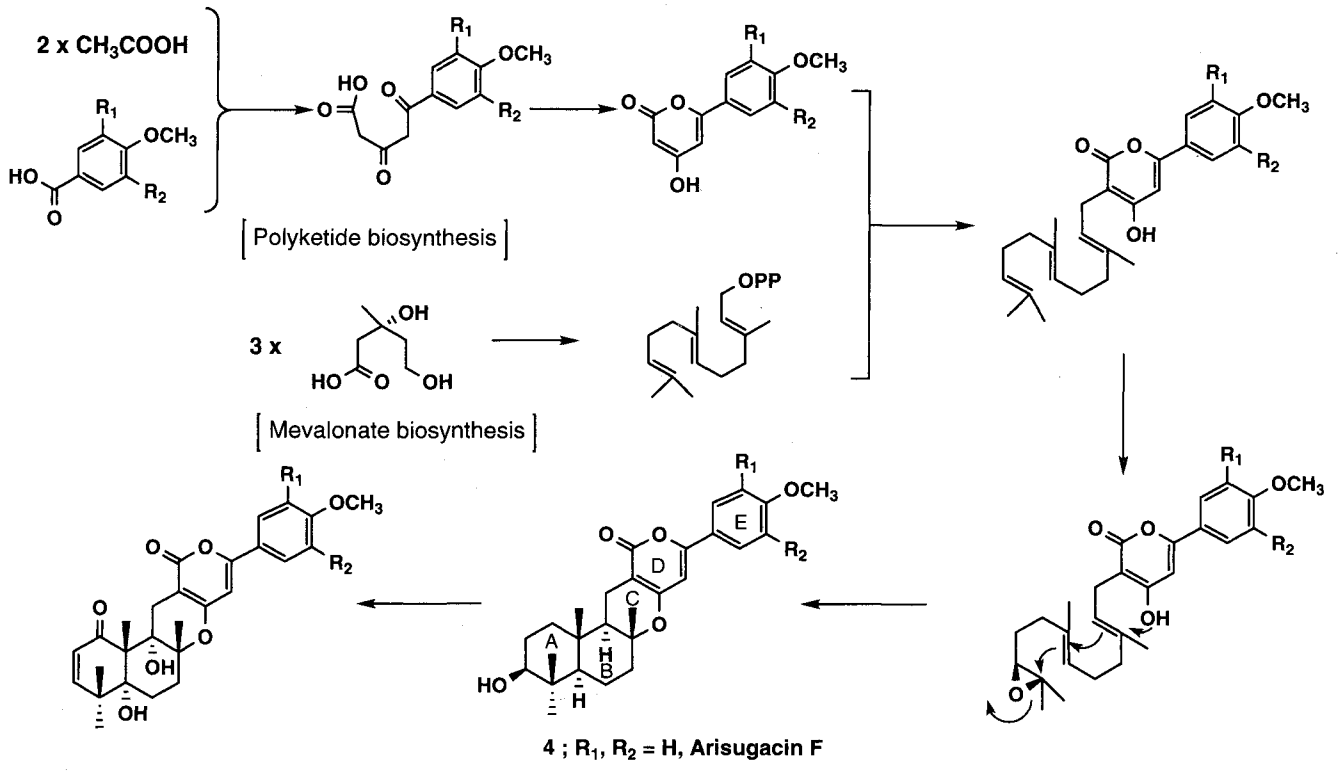
Figure 2 shows the $\Delta\delta$ values of MTPA esters **10** and **11**. Since the values having opposite signs are arranged on the left and right-hand sides of the MTPA plane, the absolute configuration of **9** is determined as 1-(*S*), 4a-(*R*), 6a-(*R*), 12a-(*S*), and 12b-(*S*). The absolute stereochemistries of **9** is the same as that of arisugacin F (**4**). Analytical data of **10**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.02 (3H, s, 4α-Me), 1.07 (3H, s, 4β-Me), 1.10 (1H, m, 3β-H), 1.13 (3H, s, 12bβ-Me), 1.33 (3H, s, 6aβ-Me), 1.70 (1H, m, 6β-H), 1.80 (2H, m, 5-H_2), 1.85 (1H, d, J=18.0 Hz, 12α-H), 1.99 (1H, m, 2α-H), 2.20 (1H, m, 3α-H), 2.26 (1H, m, 2β-H), 2.38 (1H, m, 6α-H), 2.72 (1H, d, J=18.0 Hz, 12β-H), 2.80 (1H, s, 12aα-OH), 3.91 (3H, s, 4′-OMe), 3.94 (6H, s, 3′, 5′-OMe), 5.11 (1H, d, J=3.0 Hz, 1β-H), 5.71 (1H, s, 4aα-OH), 6.30 (1H, s, 8-H), 7.02 (2H, s, 2′, 6′-H), ^{13}C-NMR (100.6 MHz, $CDCl_3$): 164.1 (C-11), 162.6 (C-7a), 158.2 (C-9), 153.6 (C-3′, 5′), 140.4 (C-4′), 126.7 (C-1′), 102.8 (C-2′, 6′), 97.6 (C-8), 96.4 (C-11a), 81.3 (C-6a), 78.3 (C-12a), 78.0 (C-4a), 77.9 (C-1), 61.0 (4′-OMe), 56.4 (3′, 5′-OMe), 44.4 (C-12b), 39.0 (C-4), 30.7 (C-3), 29.2 (C-6), 27.5 (4α-Me), 26.2 (C-12), 25.8 (C-5), 24.6 (4β-Me), 24.4 (6aβ-Me), 23.3 (C-2), 22.6 (12bβ-Me).

Analytical data of **11**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.95 (3H, s, 4α-Me), 1.04 (1H, m, 3β-H), 1.06 (3H, s, 4β-Me), 1.18 (3H, s, 12bβ-Me), 1.42 (3H, s, 6aβ-Me), 1.74 (1H, m, 6β-H), 1.84 (2H, m, 5-H_2), 1.85 (1H, m, 3α-H),

Fig. 3. NOE experiments of **9**.



Scheme 3. Proposed biosynthetic pathway of arisugacin F (**4**), A (**1**), and territrem B (**3**).



1.87 (1H, m, 2α-H), 2.22 (1H, m, 2β-H), 2.32 (1H, d, J=17.5 Hz, 12α-H), 2.44 (1H, m, 6α-H), 2.82 (1H, d, J=17.5 Hz, 12β-H), 3.90 (3H, s, 4′-OMe), 3.92 (6H, s, 3′, 5′-OMe), 4.20 (1H, s, 12aα-OH), 5.11 (1H, s, 4aα-OH), 5.20 (1H, d, J=3.0 Hz, 1β-H), 6.37 (1H, s, 8-H), 7.02 (2H, s, 2′, 6′-OMe), ^{13}C-NMR (100.6 MHz, $CDCl_3$): 165.1 (C-11), 162.9 (C-7a), 158.2 (C-9), 153.5 (C-3′, 5′), 140.3 (C-4′), 126.8 (C-1′), 102.8 (C-2′, 6′), 97.8 (C-8), 96.8 (C-11a), 81.4 (C-6a), 78.6 (C-4a), 78.1 (C-12a), 77.6 (C-1), 61.0 (4′-OMe), 56.3 (3′, 5′-OMe), 45.6 (C-12b), 38.8 (C-4), 30.3 (C-3), 29.3 (C-6), 27.4 (4α-Me), 26.1 (C-12), 25.6 (C-5), 24.8 (6a-Me), 24.5 (4β-Me), 23.1 (C-2), 22.6 (12bβ-Me).

In view of the common biosynthetic origin of the

arisugacins and territrems, we presume that congeners A (**1**), and B (**2**) share the relative and absolute stereochemistry of **3** and **4** as well.

We reported the biosynthetic origin of pyripyropene A (**5**)[17]; (1) a pyridino-α-pyrone moiety is produced *via* condensation of a primer nicotinic acid with two acetate (2) an all-*trans* farnesyl pyrophosphate is produced *via* the mevalonate pathway, (3) the two parts are linked, and cyclized to form the core skeleton, and (4) three acetyl residues from acetate are introduced into the skeleton to yield pyripyropene A (**5**). On the other hand, the benzene ring of territrem B is biosynthesized from shikimate, and the nonaromatic moiety from mevalonate[18]. Based on these results, we proposed a biosynthetic pathway of arisugacin F (**4**), A (**1**), and territrem B (**3**) as shown in Scheme 3; (1) a benzene-α-pyrone moiety is produced *via* condensation of a benzoic acid from shikimate with two acetate, (2) an all-*trans* farnesyl pyrophosphate is produced *via* the mevalonate pathway, (3) the two parts are linked, epoxidized, and polyene cyclized to form the core skeleton **4**, and (4) oxidized to yield arisugacin A (**1**), B (**2**), and territrem B (**3**) (Scheme 3).

Studies directed toward the total synthesis of the arisugacins will be reported in due course.

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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) KUNO, F.; K. SHIOMI, K. OTOGURO, T. SUNAZUKA & S. ŌMURA: Arisugacins A and B, novel and selective acetylcholinesterase inhibitors from *Penicillium* sp. FO-4259. II. Structure elucidation. J. Antibiotics 49: 748~751, 1996
4) OTOGURO, K.; F. KUNO & S. ŌMURA: Arisugacins, selective acetylcholinesterase inhibitors of microbial origin. Pharmacol. Ther. 76: 45~54, 1997
5) LING, K. H.; C.-K. YANG & F.-T. PENG: Territrems, tremorgenic mycotoxins of *Aspergillus terreus*. Appl. Environ. Microbiol. 37: 355~357, 1979
6) LING, K. H.; H.-H. LION, 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
7) OTOGURO, K.; K. SHIOMI, Y. YAMAGUCHI, N. ARAI, T. SUNAZUKA, R. MASUMA, Y. IWAI & S. ŌMURA: Arisugacins C and D, novel acetylcholinesterase inhibitors and their related novel metabolites produced by *Penicillium* sp. FO-4259-11. J. Antibiotics 53: 50~57, 2000
8) 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
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) 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
11) 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
12) TOMODA, H.; H. NISHIDA, Y. K. KIM, R. OBATA, T. SUNAZUKA, S. ŌMURA, J. BORDNER, M. GUADLLANA, P. G. DORMER & A. B. SMITH III: Relative and absolute stereochemistry of pyripyropene A, a potent, bioavailable inhibitor of acyl-CoA: cholesterol acyltransferase

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(ACAT). J. Am. Chem. Soc. 116: 12097~12098, 1994

13) NAGAMITSU, T.; T. SUNAZUKA, R. OBATA, H. TOMODA, H. TANAKA, Y. HARIGAYA & S. ŌMURA: Total synthesis of (+)-pyripyropene A. A potent, orally bioavailable inhibitor of acyl-CoA: cholesterol acyltransferase. J. Org. Chem. 60: 8126~8127, 1995

14) OHTANI, I.; T. KUSUMI, Y. KASHMAN & H. KAKISAWA: A new aspect of the high-field NMR application of Mosher's method. The absolute configuration of marine triterpene sipholenol-A. J. Org. Chem. 56: 1296~1298, 1991

15) DAKE, J. A.; D. L. DULL & H. S. MOSHER: α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. J. Org. Chem. 34: 2543~2549, 1969

16) PENG, F.-C.: Acetylcholinesterase inhibition by territrem B derivatives. J. Natural Products 58: 857~862, 1995

17) 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, 1994

18) LING, K. H. & Y. W. PENG: Biosynthesis of territrems by *Aspergillus terreus*. Appl. Environ. Microbiol. 54: 585~587, 1988