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Arisugacins C and D, Novel Acetylcholinesterase Inhibitors and Their Related Novel Metabolites Produced by *Penicillium* sp. FO-4259-11

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The mutant of *Penicillium* sp. FO-4259, an arisugacins A and B producing strain, was found to produce a series of metabolites, designated arisugacins C, D, E, F, G and H, which were structurally related to arisugacins A and B. These compounds were isolated from the culture broth and the physico-chemical and biological properties were examined. The IC_{50} values of arisugacins C and D against acetylcholinesterase (AChE) were 2.5 μM and 3.5 μM, respectively. However arisugacins E, F, G and H did not inhibit AChE at 100 μM. Though they showed only weak or no activity against AChE compared with arisugacins A and B, they may be useful for the study of the structure-activity relationship.

In the course of screening for selective acetylcholinesterase (AChE) inhibitors of microbial origin, we have previously discovered arisugacins A and B (**1** and **2**, Fig. 1), which were isolated from the cultured broth of *Penicillium* sp. FO-4259[1~4] together with the structurally related known compounds, territrems B and C[5,6] (**9** and **10**, Fig. 1). These compounds showed strong selective inhibition against AChE. To obtain a large amount of arisugacin A, the parent strain FO-4259 was treated with UV-light to yield a mutant strain FO-4259-11 as a high producer of arisugacins. Further isolation study from the culture broth of the mutant strain led to the discovery of arisugacins C, D, E, F, G and H (**3**~**8**, Fig. 1).

In this paper, fermentation, isolation, physico-chemical properties, structure elucidation and biological properties of **3**~**8** are described.

Material and Methods

General

NMR spectra were obtained with Varian Unity 400 spectrometer using $CDCl_3$ as a solvent. NOE experiments were carried out by NOESY (for **3**, **4**, and **6**~**8**) and differential NOE experiments (for **5**). Mass spectrometry was conducted on a JEOL JMS-AX500 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.

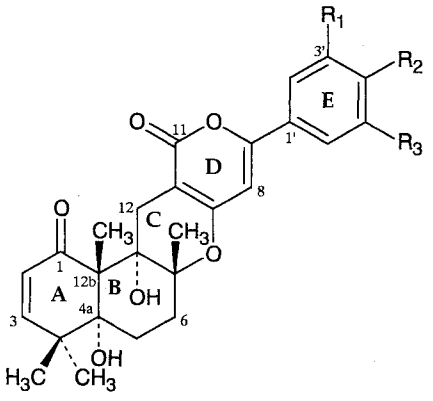
Microorganism

The mutant strain *Penicillium* sp. FO-4259-11 was selected from UV-light treatment of the spores suspension of the parent *Penicillium* sp. FO-4259 according to the established method[7]. The mutant strain was grown on YpSs agar slants containing soluble starch 1.5%, yeast extract 0.4%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% and agar 2.0% (adjusted to pH 6.0 before sterilization). The slants were incubated at 27°C for 8 days. The spores were collected, suspended in 50%-glycerol, and stored at −80°C.

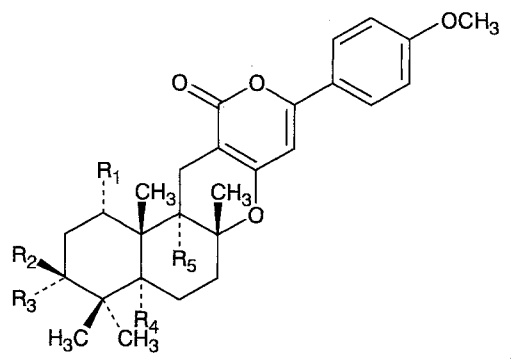
Culture and Medium Condition

A 0.1 ml of the aqueous spore suspension (5×10^5 cell/ml) was inoculated into four 500-ml Erlenmeyer flasks each containing 100 ml of a seed medium consisting of glucose 2.0%, yeast extract 0.2%, agar 0.1%, K_2HPO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.05% (adjusted to pH 6.0 before sterilization). After incubation at 27°C for 3 days on a rotary shaker, 200 ml of the seed culture was transferred to

Fig. 1. Structures of arisugacins A~H (**1**~**8**) and territrems A~C.

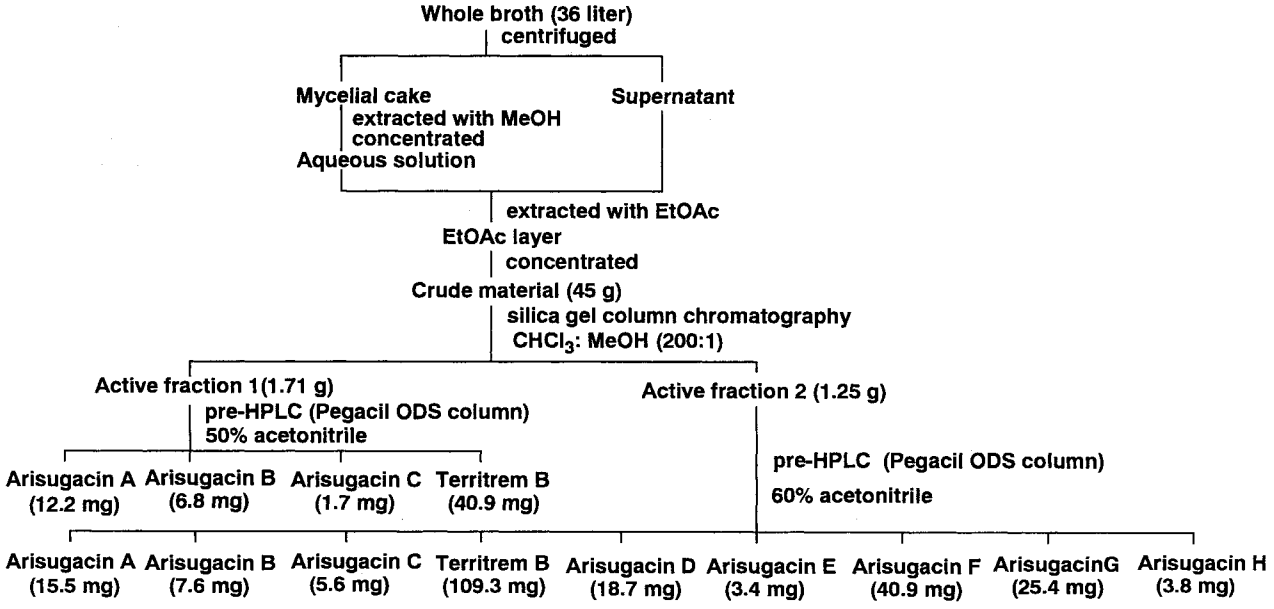


	R_1	R_2	R_3
Arisugacin A (**1**)	OCH_3	OCH_3	H
Arisugacin B (**2**)	H	OCH_3	H
Territrem A	$-OCH_2O-$		OCH_3
Territrem B (**9**)	OCH_3	OCH_3	OCH_3
Territrem C (**10**)	OCH_3	OH	OCH_3



	R_1	R_2	R_3	R_4	R_5
Arisugacin C (**3**)	H	=O		OH	H
Arisugacin D (**4**)	H	H	$OCOCH_3$	OH	OH
Arisugacin E (**5**)	H	H	OH	OH	H
Arisugacin F (**6**)	H	OH	H	H	H
Arisugacin G (**7**)	H	=O		H	H
Arisugacin H (**8**)	OH	H	$OCOCH_3$	OH	OH

Fig. 2. Isolation procedures for arisugacins.



each two 30-liter jar fermenters containing 20 liters of the producing medium consisting of sucrose 2.0%, glucose 1.0%, soybean meal 0.5%, meat extract 0.5%, agar 0.1%, K_2HPO_4 0.1% and $CaCO_3$ 0.3% (adjusted to pH 6.0 before sterilization). The fermentation was carried out at 27°C for 13 days with agitation at 150 rpm and aeration of 5 liters per minute.

Determination of Cholinesterase Activities

The AChE (from human erythrocytes) and butyryl-

Table 1. Physico-chemical properties of arisugacins C-H.

	Arisugacin C	Arisugacin D
Appearance	Yellowish white powder	Yellowish white powder
MP	128°C	>300°C
$[\alpha]_D^{23}$ (c 0.1, $CHCl_3$)	+120°	+32°
Molecular formula	$C_{27}H_{32}O_6$	$C_{29}H_{36}O_8$
Molecular weight	452	512
HR FAB-MS (m/z): calcd	453.2277 $(M+H)^+$	513.2488 $(M+H)^+$
found	453.2283 $(M+H)^+$	513.2496 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	206 (28,500), 253 (14,900), 330 (19,100)	208 (63,500), 250 (19,000), 330 (23,300)
UV $\lambda_{max}^{MeOH-HCl}$ nm (ε)	206 (30,600), 253 (15,100), 331 (19,900)	208 (44,200), 250 (16,900), 331 (18,200)
UV $\lambda_{max}^{MeOH-NaOH}$ nm (ε)	214 (112,300), 253 (19,600), 330 (21,100)	251 (16,300), 331 (18,200)
IR ν_{max} (KBr) cm^{-1}	3440, 2929, 1701, 1637, 1570, 1514, 1406, 1259, 1205, 1180, 1128, 1022	3448, 2927, 2320, 1684, 1635, 1572, 1512, 1458, 1259, 1180, 1120
Solubility: soluble	MeOH, EtOH, $CHCl_3$	MeOH, EtOH, $CHCl_3$
insoluble	H_2O, Hexane	H_2O, Hexane

	Arisugacin E	Arisugacin F
Appearance	Yellowish white powder	Yellowish white powder
MP	>300°C	261°C
$[\alpha]_D^{23}$ (c 0.1, $CHCl_3$)	+132°	+ 66°
Molecular formula	$C_{27}H_{34}O_6$	$C_{27}H_{34}O_5$
Molecular weight	454	438
HR FAB-MS (m/z): calcd	455.2434 $(M+H)^+$	439.2485 $(M+H)^+$
found	455.2458 $(M+H)^+$	439.2473 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	204 (89,000), 251 (17,000), 330 (17,700)	242 (22,200), 319 (24,700)
UV $\lambda_{max}^{MeOH-HCl}$ nm (ε)	204 (69,000), 251 (19,500), 330 (19,100)	205 (34,500), 235 (25,800), 314 (26,400)
UV $\lambda_{max}^{MeOH-NaOH}$ nm (ε)	217 (93,400), 249 (19,700), 330 (18,500)	252 (23,400), 330 (26,000)
IR ν_{max} (KBr) cm^{-1}	3435, 2924, 2360, 1635, 1568, 1514, 1259, 1173, 1113, 1026	3442, 2941, 2360, 1689, 1637, 1570, 1514, 1404, 1257, 1182, 1124, 1045
Solubility: soluble	MeOH, EtOH, $CHCl_3$	MeOH, EtOH, $CHCl_3$
insoluble	H_2O, Hexane	H_2O, Hexane

	Arisugacin G	Arisugacin H
Appearance	Yellowish white powder	White powder
MP	151°C	146°C
$[\alpha]_D^{23}$ (c 0.1, $CHCl_3$)	+118°	+ 44°
Molecular formula	$C_{27}H_{32}O_5$	$C_{29}H_{36}O_9$
Molecular weight	436	528
HR FAB-MS (m/z): calcd	437.2328 $(M+H)^+$	551.2257 $(M+Na)^+$
found	437.2355 $(M+H)^+$	551.2255 $(M+Na)^+$
UV λ_{max}^{MeOH} nm (ε)	204 (86,500), 250 (13,800), 330 (13,300)	219 (26,900), 252 (14,700), 331 (15,200)
UV $\lambda_{max}^{MeOH-HCl}$ nm (ε)	250 (16,500), 331 (14,700)	206 (51,500), 247 (14,600), 331 (12,900)
UV $\lambda_{max}^{MeOH-NaOH}$ nm (ε)	252 (14,800), 330 (14,000)	224 (40,900), 251 (17,100), 330 (15,900)
IR ν_{max} (KBr) cm^{-1}	2947, 1701, 1637, 1608, 1574, 1516, 1404, 1255, 1184, 1120, 1034	3398, 2929, 1680, 1635, 1572, 1514, 1408, 1259, 1209, 1182, 1117, 1028
Solubility: soluble	MeOH, EtOH, $CHCl_3$	MeOH, EtOH, $CHCl_3$
insoluble	H_2O, Hexane	H_2O, Hexane

Table 2. The ^{1}H and ^{13}C NMR data of arisugacins C-H[a].

Position	Arisugacin C (3) ^{13}C	Arisugacin C (3) ^{1}H[b]	Arisugacin D (4) ^{13}C	Arisugacin D (4) ^{1}H[b]	Arisugacin E (5) ^{13}C	Arisugacin E (5) ^{1}H[b]
1	32.7 t	2.04 ddd (8.0, 9.0, 13.0), 1.74 ddd (4.4, 7.2, 13.0)	21.5 t	2.37 ddd (4.0, 14.0, 14.3), 1.38 ddd (2.5, 4.3, 14.3)	26.3 t	1.33 m
2	33.6 t	2.56 ddd (4.4, 8.0, 16.0), 2.60 ddd (7.2, 9.0, 16.0)	22.7 t	1.84 m, 2.15 m	24.8 t	1.71 m, 2.12 m
3	215.5 s		79.0 d	4.89 dd (2.8, 2.8)	77.7 d	3.62 dd (2.9, 2.9)
3-OAc (C=O)			168.6 s			
3-OAc (CH_3)			21.3 q	2.13 s		
4	52.9 s		41.9 s		41.0 s	
4α-Me	21.4 q	1.15 s	22.8 q	1.02 s	23.7 q	1.18 s
4β-Me	23.4 q	1.23 s	24.5 q	1.13 s	23.8 q	0.99 s
4a	79.1 s		81.5 s		77.2 s	
4a-OH		1.48 s		4.48 s		
5	25.8 t	1.65 ddd (3.4, 4.3, 15.4), 1.96 ddd (3.8, 14.4, 15.4)	25.1 t	1.83 m	25.6 t	1.74 m, 2.12 m
6	33.5 t	2.17 ddd (4.3, 12.6, 14.4), 1.92 ddd (3.4, 3.8, 12.6)	29.1 t	2.44 m, 1.73 ddd (3.2, 3.2, 13.0)	33.3 t	2.27 ddd (6.6, 12.4, 12.4), 1.79 ddd (3.5, 3.5, 12.4)
6a	79.8 s		81.5 s		80.5 s	
6a-Me	20.4 q	1.31 s	24.8 q	1.44 s	21.2 q	1.27 s
7a	163.5 s		163.3 s		163.9 s	
8	96.7 d	6.26 s	96.9 d	6.34 s	97.0 d	6.27 s
9	158.4 s		158.2 s		158.1 s	
11	164.6 s		165.1 s		164.9 s	
11a	98.4 s		97.8 s		98.7 s	
12	17.2 t	2.44 dd (4.7, 17.2), 2.29 dd (13.9, 17.2)	25.9 t	2.46 d (16.6), 2.65 dd (2.7, 16.6)	17.2 t	2.41 dd (4.8, 16.6), 2.21 dd (13.3, 16.6)
12a	43.5 d	2.46 dd (4.7, 13.9)	76.5 s		42.8 d	2.65 dd (4.8, 13.3)
12a-OH				6.76 d (2.7)		
12b	40.7 s		43.3 s		41.1 d	
12b-Me	18.6 q	1.16 s	21.2 q	1.19 s	18.7 q	1.05 s
1'	124.0 s		124.2 s		124.2 s	
2'	127.0 d	7.73 d (9.0)	127.0 d	7.73 d (9.0)	127.0 d	7.73 d (8.9)
3'	114.2 d	6.93 d (9.0)	114.2 d	6.93 d (9.0)	114.2 d	6.93 d (8.9)
4'	161.5 s		161.4 s		161.4 s	
4'-OMe	55.4 q	3.85 s	55.4 q	3.84 s	55.4 q	3.85 s
5'	114.2 d	6.93 d (9.0)	114.2 d	6.93 d (9.0)	114.2 d	6.93 d (8.9)
6'	127.0 d	7.73 d (9.0)	127.0 d	7.73 d (9.0)	127.0 d	7.73 d (8.9)

Position	Arisugacin F (6) ^{13}C	Arisugacin F (6) ^{1}H[b]	Arisugacin G (7) ^{13}C	Arisugacin G (7) ^{1}H[b]	Arisugacin H (8) ^{13}C	Arisugacin H (8) ^{1}H[b]
1	37.5 t	1.11 ddd (4.0, 13.0, 13.0), 1.81 m	37.9 t	1.54 m, 2.06 ddd (3.7, 7.5, 13.3)	73.0 d	4.24 dd (2.4, 3.6)
2	27.2 t	1.70 m, 1.62 m	33.8 t	2.46 ddd (3.7, 7.2, 16.2), 2.60 ddd (7.5, 10.8, 16.2)	29.4 t	2.16 ddd (2.2, 2.4, 16.5), 2.39 ddd (3.6, 3.8, 16.5)
3	78.5 d	3.24 dd (4.8, 11.6)	216.0 s		77.2 d	4.90 dd (2.2, 3.8)
3-OAc (C=O)					169.7 s	
3-OAc (CH_3)					21.4 q	2.10 s
4	38.8 s		47.3 s		42.2 s	
4α-Me	28.1 q	1.03 s	26.6 q	1.14 s	22.9 q	1.10 s
4β-Me	15.5 q	0.81 s	21.3 q	1.07 s	25.2 q	1.13 s
4a	55.0 d	1.00 dd (2.0, 12.1)	54.7 d	1.56 m	81.3 s	
5	19.4 t	1.81 m, 1.44 dddd (3.0, 12.1, 13.5, 13.5)	20.6 t	1.75 m, 1.57 m	25.4 t	1.85 m
6	40.4 t	1.67 m, 2.12 ddd (3.0, 3.0, 12.4)	39.8 t	1.73 m, 2.16 ddd (3.2, 3.2, 12.6)	29.0 t	2.42 m, 1.81 m
6a	80.5 s		80.2 s		80.6 s	
6a-Me	20.7 q	1.25 s	20.5 q	1.31 s	24.6 q	1.45 s
7a	163.5 s		163.5 s		163.3 s	
8	96.7 d	6.25 s	96.6 d	6.26 s	96.6 d	6.34 s
9	158.3 s		158.5 s		158.7 s	
11	164.7 s		164.5 s		164.9 s	
11a	98.4 s		98.2 s		96.9 s	
12	17.2 t	2.51 dd (4.8, 16.9), 2.22 dd (12.9, 16.9)	17.3 t	2.55 dd (4.3, 16.6), 2.29 dd (12.9, 16.6)	26.0 t	2.68 d (17.0), 2.85 d (17.0)
12a	51.6 d	1.49 dd (4.8, 12.9)	51.0 d	1.58 m	79.3 s	
12b	36.9 s		36.7 s		44.5 s	
12b-Me	15.1 q	0.91 s	14.7 q	1.04 s	22.2 q	1.07 s
1'	124.0 s		124.0 s		124.0 s	
2'	127.0 d	7.73 d (8.8)	127.0 d	7.73 d (9.0)	127.1 d	7.74 d (9.0)
3'	114.2 d	6.93 d (8.8)	114.2 d	6.94 d (9.0)	114.2 d	6.94 d (9.0)
4'	161.5 s		161.5 s		161.5 s	
4'-OMe	55.4 q	3.85 s	55.4 q	3.85 s	55.4 q	3.85 s
5'	114.2 d	6.93 d (8.8)	114.2 d	6.94 d (9.0)	114.2 d	6.94 d (9.0)
6'	127.0 d	7.73 d (8.8)	127.0 d	7.73 d (9.0)	127.1 d	7.74 d (9.0)

a: The spectra were obtained with Varian Unity 400 spectrometer. The $CDCl_3$ signals (7.26 ppm of ^{1}H and 77.0 ppm of ^{13}C) were used as references.

b: The coupling constants (Hz) are in parentheses.

cholinesterase (BuChE, from horse serum) inhibitory activities of **3**~**8** were measured according to the method described previously[2].

Results and Discussion

Purification and Isolation

The isolation procedure of the arisugacins C, D, E, F, G and H from the culture broth of *Penicillium* sp. FO-4259-11 is schematically shown in Fig. 2. The cultured broth (36 liters) was centrifuged and the supernatant was extracted with ethyl acetate. The mycelial methanol 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 (45 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH (200:1). The active fractions were further purified by HPLC using a Pegasil ODS column (i.d. 20×250 mm; Senshu Scientific Co., Ltd.; mobile phase, 50% or 60% CH_3CN) to yield yellowish white powder of **3** (7.3 mg), **4** (18.7 mg), **5** (3.4 mg), **6** (40.9 mg), and **7** (25.4 mg), and white powder of **8** (3.8 mg) (Fig. 2).

Physico-chemical Properties

The physico-chemical properties of **3**~**8** are summarized in Table 1. They showed UV absorption maxima about 250 nm and 330 nm, and the spectra were similar to those of **2**[3]. The IR spectra of **3**~**8** also resembled the spectrum of **2**. Therefore the structures of **3**~**8** were considered to be similar to **2**.

Chemical shifts in the ^1H and ^{13}C NMR of **3**~**8** are shown in Table 2. The HMQC experiments revealed the bonding of each proton and carbon.

Structure Elucidation

Arisugacin C (**3**)

The molecular formula of **3** was deduced as $C_{27}H_{32}O_6$ by HR-FAB-MS. In the DEPT spectra, **3** showed five methyl, five methylene, six methine, and eleven quaternary carbon signals. The chemical shifts of the rings C, D, and E (except C-12 and C-12a) were quite similar to those of **2**[3] in the ^1H and ^{13}C NMR. The ^1H-^1H COSY and HMBC experiments of **3** revealed that these rings were the same as **2** as shown in Fig. 3. The ^1H-^{13}C long-range couplings were observed from 12-H_2 (δ 2.29 and δ 2.44) to C-7a (δ 163.5), C-11 (δ 164.6), and C-11a (δ 98.4), which indicated that the methylene connected with C-11a. The long-range couplings between 12-H_2 and C-12a (δ 43.5), and between 12a-H (δ 2.46) and C-11a revealed that the carbon at δ 43.5 (C-12a) was connected to C-12. Hence C-12a was a methine in **3** instead of a quaternary hydroxy carbon as in **2**.

Two units of $>C(CH_3)CH_2CH_2-$ and their connection to C-12a was defined by the ^1H-^1H COSY and HMBC

Fig. 3. Structure elucidation of arisugacin C (**3**) by NMR analysis.

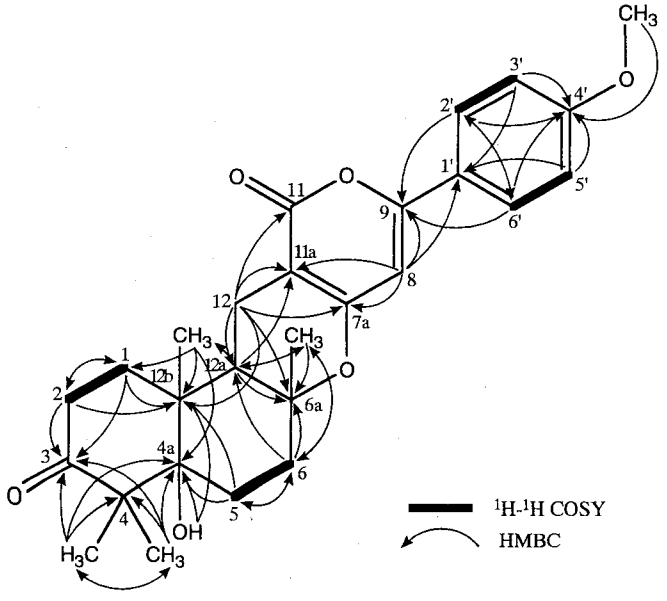
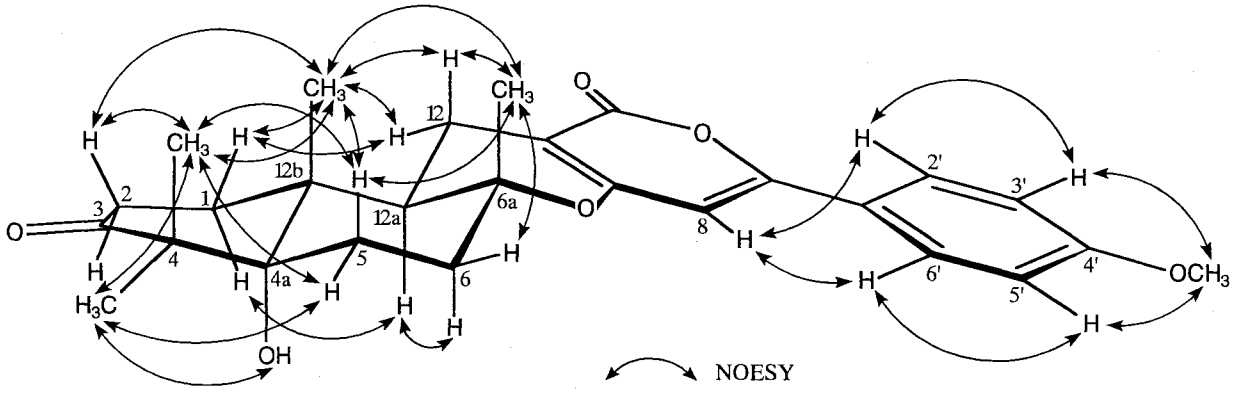


Fig. 4. NOESY experiments of arisugacin C (**3**).



experiments. Another unit of $-COC(CH_3)_2C(OH)<$ was also revealed by the HMBC. Those three units were shown to form rings A and B by the long-range couplings between 1-H_2 (δ 1.74 and δ 2.04) and C-3 (δ 215.5), 2-H_2 (δ 2.56 and δ 2.60) and C-3, 4a-OH (δ 1.48) and C-12b (δ 40.7), 5-H_2 (δ 1.65 and δ 1.96) and C-4a (δ 79.1), 5-H_2 and C-12b, and 12b-CH_3 (δ 1.16) and C-4a (Fig. 3). Thus the planar structure of **3** was elucidated. Though **3** has a ketone carbonyl at ring A as do **1** and **2**, the carbonyl carbon of **3** is not C-1 but C-3.

The relative configuration of **3** was examined by the NOESY experiment as shown in Fig. 4. The NOEs among 4β-CH_3 (δ 1.23), 5-H_β (δ 1.96), 6a-CH_3 (δ 1.31), 12-H_β (δ 2.29), and 12b-CH_3 (δ 1.16) suggested that they were all of the β configuration. The NOEs between 4α-CH_3 (δ 1.15) and 4a-OH (δ 1.48), 1-H_α (δ 2.04) and 12a-H (δ 2.46), and 6-H_α (δ 2.17) and 12a-H suggested that they were all of the α configuration. Thus the *trans*-4a-*transoid*-4a,6a-*trans*-6a configuration of **3** was elucidated (Fig. 1), which is the same as that of **1** and **2**[3].

Arisugacins D, E, F, G and H (**4**~**8**)

The molecular formula of **4** was deduced as $C_{29}H_{36}O_8$ by HR-FAB-MS. The chemical shifts of the rings B, C, D, and E of **4** resembled those of **2**[3] in the ^1H and ^{13}C NMR, and the ^1H-^1H COSY and HMBC experiments revealed that these rings are the same as that of **2**. The remaining atoms were elucidated as $-CH_2CH_2CH(OAc)C(CH_3)_2-$ by the NMR experiments. This substructure was assigned to C-1, 2, 3, and 4 by the long-range couplings between 1-H_2 (δ 1.38 and δ 2.37) and C-12b (δ 43.3), 2-H_2 (δ 1.84 and δ 2.15) and C-12b, 12b-CH_3 (δ 1.19) and C-1 (δ 21.5), 3-H (δ 4.89) and C-4a (δ 81.5), 4α-CH_3 (δ 1.02) and C-4a, and 4β-CH_3 (δ 1.13) and C-4a (Fig. 5). The relative configuration of **4** was shown to be the same as that of **1**~**3** by the NOESY experiment (Fig. 6). The coupling constants ($J_{2,3}$=2.8, 2.8 Hz) and the NOESY data indicated that 3-H was of the β configuration. Thus the structure of **4** was shown to be 3α-acetoxy-3-deoxo-12a-hydroxy-**3**.

The structure of **5** was elucidated by the comparison of its spectral data and those of **3**. Compound **5** had two more hydrogens than **3**. A carbonyl carbon (C-3) of **3** was replaced by a hydroxyl methine (δ_C 77.7, δ_H 3.62) as observed in the NMR spectra of **5**. Therefore **5** was suggested to be 3-deoxo-3-hydroxy-**3**, and the ^1H-^1H COSY and HMBC experiments supported the structure (Fig. 5). The coupling constants ($J_{2,3}$=2.9, 2.9 Hz) and the differential NOE experiments indicated that the 3-H was of the β configuration (Fig. 6).

Compound **6** had one less oxygen than **5**. The ^1H-^1H COSY and HMBC experiments of **6** suggested the planar structure of **6** as 4a-deoxy-**5**. The coupling constants of the 3-H of **6** ($J_{2,3}$=4.8, 11.6 Hz) were much larger than those of **4** and **5** indicating an α configuration for this hydrogen. The NOESY experiment supported this configuration.

Compound **7** had one less oxygen than **3**. The ^1H-^1H COSY and HMBC experiments of **7** revealed that **7** was 4a-deoxy-**3**.

Compound **8** had one more oxygen than **4**. The ^1H-^1H COSY and HMBC experiments of **8** suggested the planar structure of **8** as 1-hydroxy-**4**. The coupling constants ($J_{1,2}$=2.4, 3.6 Hz, $J_{3,4}$=2.2, 3.8 Hz) and the NOESY data indicated that the 1- and 3- Hs were both of the β configuration.

Fig. 5. Structure elucidation of arisugacins D~H (**4**~**8**) by NMR analysis.

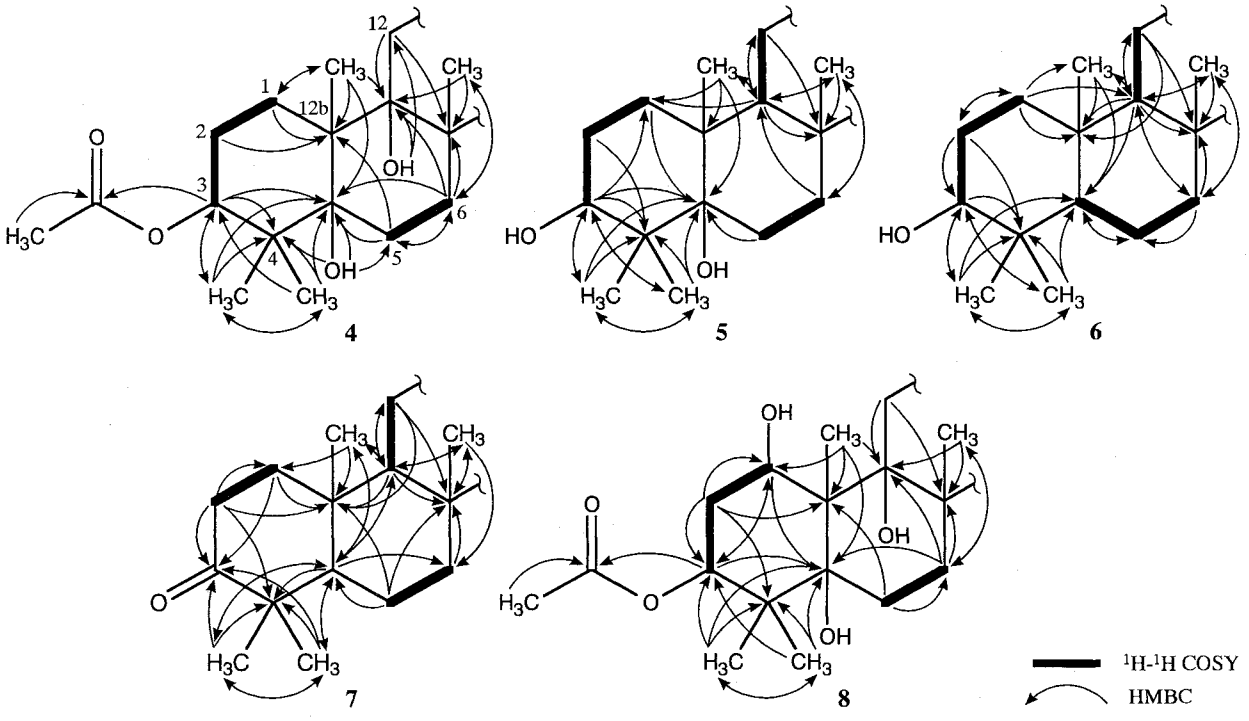


Fig. 6. NOE experiments of arisugacins D~H (**4**~**8**).

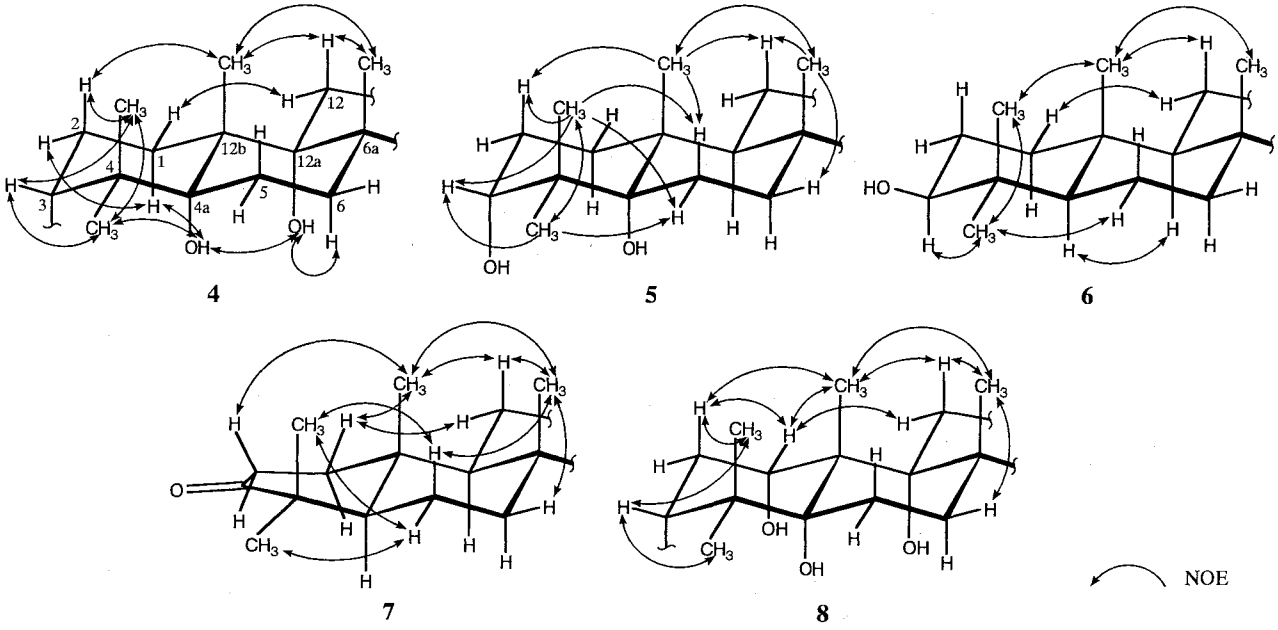


Table 3. Inhibitory activities of arisugacins A~H and territrems B and C against AChE and BuChE.

Compound	IC_{50} (μM)	
	AChE	BuChE
Arisugacin A	**0.001**	**>21.0**
Arisugacin B	**0.0258**	**>516.0**
Arisugacin C	**2.5**	**>30.0**
Arisugacin D	**3.5**	**>30.0**
Arisugacin E	**>100.0**	**>30.0**
Arisugacin F	**>100.0**	**>30.0**
Arisugacin G	**>100.0**	**>30.0**
Arisugacin H	**>100.0**	**>30.0**
Territrem B	**0.0076**	**>20.0**
Territrem C	**0.0068**	**>26.0**

Biological Activities

The IC_{50} values of **3**~**8** against AChE and BuChE are shown in Table 3. Though **3** and **4** inhibited AChE selectively with the IC_{50} values of 2.5 μM and 3.5 μM, respectively, the inhibitory activities of **3** and **4** were about one hundred times weaker than that of **2**. Furthermore, **5**~**8** did not inhibit AChE at 100 μM. However, they may be useful for the structure-activity relationship and biosynthetic studies of the arisugacins.

The structures of **1**, **2**, territrem B (**9**), and territrem C (**10**) differ only in the substituents on their aromatic moieties (Fig. 1). On the other hand, **3**~**8** differs structure from **2** in rings A and B. Recently, PENG[8] reported that catalytic hydrogenation of territrem B with H_2 over Pd/C gave 2,3-dihydroterritrem B, and its inhibition against AChE was 10 times weaker than that of territrem B. The inhibitory activities of **3** (arisugacin C) and **4** (arisugacin D) against AChE were 97 and 136 times weaker than that of **2** (arisugacin B), which also suggested the importance of the enone moiety. In addition, **7** (4a-deoxyarisugacin C) and **5** (3-deoxo-3-hydroxyarisugacin C) lost AChE inhibitory activity. Therefore the 4a-OH and ketone moiety of rings A may be important for AChE inhibition. Detailed studies on biological activities of arisugacins are in progress.

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