Natural Product Sciences
13(1) : 90-96 (2007)

Polyketides from a Sponge-Derived Fungus, *Aspergillus versicolor*

Yoon Mi Lee[1], Tayyab A. Mansoor[1], Jongki Hong[2], Chong-O. Lee[3], Kyung Sook Bae[4], and Jee H. Jung[1,*]

[1]College of Pharmacy, Pusan National University, Busan 609-735, Korea.
[2]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea.
[3]Korea Research Institute of Chemical Technology, Daejon 305-343, Korea.
[4]Korea Research Institute of Bioscience and Biotechnology, Daejon 305-333, Korea.

Abstract – Bioactivity guided fractionation of the cultured filtrates of *Aspergillus versicolor*, which was derived from a marine sponge *Petrosia* sp., yielded three polyketides: decumbenones A (**1**), B (**2**), and versiol (**3**). These compounds were identified on the basis of 1D and 2D NMR spectroscopic and MS analysis. The absolute configuration was defined by the modified Mosher's method. The isolated compounds were tested for cytotoxicity against a panel of five human solid tumor cell lines and antibacterial activity against twenty clinically isolated methicillin-resistant strains. This is the first report on the isolation of these compounds from a marine source.
Keywords – sponge-derived fungi, *Aspergillus versicolor*, polyketide, decumbenones A and B, versiol, *Petrosia* sp.

Introduction

Since marine microorganisms grow in an unique and extreme habitat, they may have the capability to produce unique metabolites. Marine microorganisms such as fungi inhabit virtually any environment in the water, and exhibit greatest diversity. Especially marine sponges are known to host plentiful and diverse microorganisms such as fungi, bacteria, cyanobacteria, and microalga. To date, more than 122 metabolites from over 30 sponge-derived fungal strains have been described (unpublished data). Even though the nature of these associations between sponges and fungi is not yet fully understood, it has been repeatedly shown that sponge-associated fungi are an interesting source of new bioactive natural products (Holler *et al.*, 2000; Renner *et al.*, 2000).

Fungi of the genus *Aspergillus* were previously isolated from marine sources such as sponge. Chlorinated polyketides were reported from a cultured fungus *Aspergillus* sp. which was derived from the marine sponge *Jaspis* cf. *coriacea* (Abrell *et al.*, 1996). Sponge-derived *Aspergillus niger* was reported to produce diverse metabolites such as asperic acid, hexylitaconic acid, malformin C, pyrophen, and asperazine (Varoglu and Crews, 2000; Hiort *et al.*, 2004; Ye *et al.*, 2005; Belofsky *et al.*, 1998).

Recently, a strain of *Aspergillus versicolor*, isolated from the sponge *Xestospongia exiqua*, was reported to contain new chromone derivatives such as aspergillitine, aspergiones (Lin *et al.*, 2003), and hydropyranoindeno-derivatives, namely aspergillones 1, 2, 3, and 12-acetyl-aspergillon (Lin *et al.*, 2001a; Lin *et al.*, 2001b).

In the course of our search for bioactive secondary metabolites, the bioassay guided fractionation of the sponge-derived fungus *Aspergillus versicolor* led to the isolation of decumbenones A (**1**), B (**2**), and versiol (**3**).

*Author for correspondence
Fax: +82-51-513-6754; E-mail: jhjung@pusan.ac.kr.

Experimental

General experimental procedures – The ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Unity Inova 400 MHz and 500 MHz spectrometers. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). LRFABMS data were obtained on a JEOL JMS SX-102A spectrometer. HPLC was performed with a C8-5E Shodex packed column (250 × 10 mm, 5 mm, 100 Å).

Animal material – The sponge was collected by hand using SCUBA (20 m depth) in 2004 off the coast of Jeju Island, Korea. The collected sample was frozen immediately. This specimen was identified as *Petrosia* sp. and the morphology of the sponge specimen was described elsewhere (Lim, 2000).

Fungal strain – The fungal strain was isolated from a marine sponge *Petrosia* sp. Following a rinse with sterile sea water, small pieces (1 × 1 mm^3) of the surface and

inner tissue of the sponge were homogenized and then inoculated on malt extract agar (MEA) petri dishes. The sterilized MEA medium (prepared with 75% sea water) contained glucose (20 g/L), malt extract (20 g/L), agar (20 g/L), peptone (1 g/L), and antibiotics (10,000 units/mL penicillin and 10,000 μg/mL streptomycin, 5 mL/L). Emerging fungal colonies were transferred to the same media in a petri dish and incubated at 25 °C for 10 - 14 days to allow colony development (Tsukamoto *et al.*, 2004; Hyde *et al.*, 1987). The pure fungal strain, designated as PF10M, was identified as *Aspergillus versicolor* by morphological and biochemical analysis.

Extraction and isolation – Production fermentation was performed in 250 mL malt media in 500 mL Erlenmeyer flasks for subculture. For the massive culture, 250 mL of subculture was transferred into a 2 L Erlenmeyer flask each containing 20 g/L malt media, and fermentation was carried out on a rotary shaker (32 °C, 150 rpm, 21 days). The cultured fungus (3 L) was extracted with 6 L of EtOAc, to afford the EtOAc extract (2.0 g, LD_{50} 32 μg/mL) (Meyer *et al.*, 1982), which was partitioned between *n*-hexane (0.7 g, LD_{50} 51 μg/mL) and 90% MeOH (1.27 g, LD_{50} 0.4 μg/mL). The 90% MeOH layer was subjected to a stepped-gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) eluting with 50% - 100% MeOH to afford 13 fractions. Fraction 3, one of the bioactive fractions (LD_{50} 32 μg/mL), was subjected to a reversed-phase HPLC (C8 Shodex Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 55% MeOH to afford 8 fractions. Compound **1** (2.4 mg) was obtained by purification of subfraction 3.

Second fermentation was performed in 250 mL malt media in 500 mL Erlenmeyer flasks for subculture. For the massive culture, 250 mL of subculture was transferred into 2 L Erlenmeyer flasks each containing 20 g/L malt media, and fermentation was carried out on a rotary shaker (32 °C, 150 rpm, 21 days). The cultured fungus (8 L) was extracted with 16 L of EtOAc, to afford the EtOAc extract (3.8 g, LD_{50} 48 μg/mL), which was partitioned between *n*-hexane (1.7 g, LD_{50} 100 μg/mL) and 90% MeOH (2.0 g, LD_{50} 32 μg/mL). The 90% MeOH layer was subjected to a stepped-gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) eluting with 40% - 100% MeOH to afford 15 fractions. Fraction 3, one of the bioactive fractions (LD_{50} 0.4 μg/mL), was subjected to a reversed-phase HPLC (C8 Shodex Pack ODS, 250 × 10 mm, 5 mm, 120 Å) eluting with 45% MeOH to afford 10 fractions. Compounds **2** and **3** were obtained by purification of fraction 8, employing the same HPLC conditions.

Evaluation of cytotoxicity – Cytotoxicity assay against five human tumor cell lines was performed at Korea Research Institute of Chemical Technology. Employed cell lines were A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; and HCT15, human colon cancer.

Evaluation of antibacterial activity – Antibacterial assay was performed at Korea Research Institute of Chemical Technology. Mueller Hinton Agar plates were impregnated with 17 serial dilutions of the sample and standards (meropenem and imipenem) to make a final concentration of 25 μg/mL to 0.002 μg/mL. The strains were inoculated into Fleisch extract broth (containing 10% horse serum depending on strains), and incubated for 18 h at 37 °C. The cultured strains were inoculated onto the Mueller Hinton agar plates with 104 CFU per spot population by automatic inoculater (Dynatech, U.S.A.). The MIC was measured after 18 h of incubation.

MTPA esterification of compounds 1 and 2 – Compound **1** (1.0 mg, 2.7 μmol) was treated with (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (4 μL) in dry pyridine (50 μL) to yield (*R*)-and (*S*)-MTPA esters, respectively. After keeping at room temperature for 24 h, the reaction mixtures were evaporated to dryness under vacuum. The products were dissolved in MeOH and purified by reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 80% MeOH. Compound **2** was treated in the same manner to yield (*S*)-and (*R*)-MTPA esters.

Decumbenone A (1) – yellow oil; $[\alpha]^{24}_{D}$ +54° (*c* 0.18, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz), δ 5.91 (1H, d, *J* = 10.0 Hz, H-11), 5.60 (1H, br s, H-9), 5.33 (1H, d, *J* = 10.0 Hz, H-12), 4.21 (1H, br s, H-6), 3.80 (2H, m, H-1), 3.05 (1H, m, H-2a), 2.90 (1H, d, *J* = 3.5 Hz, H-5), 2.85 (1H, m, H-2b), 2.50 (1H, br s, H-8), 1.85 (1H, m, H-7a), 1.45 (3H, s, H_3-15), 1.25 (1H, t, *J* = 12.0 Hz, H-7b), 1.08 (3H, s, H_3-14), 1.02 (3H, d, *J* = 6.5 Hz, H_3-16); ^{13}C-NMR (CD_3OD, 75 MHz) δ 215.0 (C, C-3), 133.7 (CH, C-9), 133.0 (CH, C-12), 131.5 (C, C-10), 128.3 (CH, C-11), 73.8 (C, C-13), 66.1 (CH, C-6), 58.8 (C, C-4), 58.0 (CH_2, C-1), 44.0 (CH_2, C-2), 42.2 (CH, C-5), 39.6 (CH_2, C-7), 25.7 (CH, C-8), 25.3 (CH_3, C-14), 20.4 (CH_3, C-16), 13.6 (CH_3, C-15); LRFABMS *m/z* 263 $[M - H_2O + H]^+$.

(*S*)-MTPA ester of 1 colorless oil; ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.91 (1H, d, *J* = 10.0 Hz, H-11), 5.57 (1H, br s, H-9), 5.32 (1H, d, *J* = 10.0 Hz, H-12), 4.15 (1H, br s, H-6), 3.55 (2H, m, H-1), 3.07(1H, m, H-2a), 2.91 (1H, s, H-5), 2.90 (1H, m, H-2b), 2.51 (1H, br s, H-8), 1.83 (1H, m, H-7a), 1.45 (3H, s, H_3-15), 1.22 (1H, t, *J* = 12.5 Hz, H-

7b), 1.04 (3H, s, H_3-14), 1.01 (3H, d, J = 7.5 Hz, H_3-16).

(*R*)-MTPA ester of 1 colorless oil; ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.91 (1H, d, J = 10.0 Hz, H-11), 5.58 (1H, s, H-9), 5.31 (1H, d, J = 10.0 Hz, H-12), 4.18 (1H, br s, H-6), 3.54 (2H, m, H-1), 3.05 (1H, m, H-2a), 2.90 (1H, s, H-5), 2.89 (1H, m, H-2b), 2.52 (1H, br s, H-8), 1.86 (1H, m, H-7a), 1.41 (3H, s, H_3-15), 1.23 (1H, t, J = 12 Hz, H-7b), 1.02 (3H, d, J = 7.5 Hz, H_3-16), 0.96 (3H, s, H_3-14).

Decumbenone B (2) – yellow oil; $[\alpha]^{24}_D$ +8° (*c* 0.12, MeOH); ^{1}H-NMR data and ^{13}C-NMR data, see Table 1; LRFABMS *m/z* 265 $[M-H_2O+H]^+$.); ^{1}H-NMR data and ^{13}C-NMR data, see Table 1; LRFABMS *m/z* 265 $[M-H_2O+H]^+$.

(*S*)-MTPA ester of 2 colorless oil; ^{1}H-NMR (CD_3OD, 500 MHz) data, see Table 1.

(*R*)-MTPA ester of 2 colorless oil; ^{1}H-NMR (CD_3OD, 500 MHz) data, see Table 1.

Versiol (3) yellow oil; $[\alpha]^{24}_D$ –58° (*c* 0.12, MeOH); ^{1}H-NMR data ($CDCl_3$, 500 MHz) δ 6.21 (1H, d, J = 9.5, H-11), 5.72 (1H, brs, H-9), 5.44 (1H, d, J = 9.5, H-12), 4.12 (1H, ddd, J = 11.8, 8.9, H-1a), 3.98 (1H, ddd, J = 12.0, 11.8, 3.4, H-1b), 3.96 (1H, m, H-6), 3.32 (1H, m, H-5), 2.86 (1H, ddd, J = 15.0, 12.1, 8.9, H-2a), 2.62 (1H, m, H-8), 2.36 (1H, dd, J = 15.0, 3.4, H-2b), 1.95 (1H, m, H-7a), 1.29 (1H, m, H-7b), 1.28 (3H, s, H-14), 1.12 (3H, s, H-15), 1.04 (3H, d, J = 7.0, H-16)

Results and Discussion

The fungal strain was isolated from a marine sponge *Petrosia* sp. The pure fungus strain was identified as *Aspergillus versicolor* by morphological and biochemical analysis. The fungal culture (3 L) was extracted with 6 L of EtOAc to afford the EtOAc extract (2.0 g). This EtOAc extract was partitioned between *n*-hexane (0.7 g) and aqueous MeOH (1.27 g). The 90% MeOH extract was more toxic to brine shrimp larvae (LD_{50} 0.4 μg/mL) than *n*-hexane layer (LD_{50} 51 μg/mL). The aqueous MeOH layer was subjected to stepped-gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) and followed by repeated reversed-phase HPLC separation of afford 8 fractions. The HPLC separation and purification of less active fraction 3 (LD_{50} 32 μg/mL) yielded compound **1**.

Second culture of the fungus (8 L) was extracted with EtOAc (LD_{50} 48 μg/mL) and partitioned between *n*-hexane (LD_{50} 100 μg/mL) and 90% MeOH (LD_{50} 32 μg/mL). The 90% MeOH layer was subjected to a stepped-gradient MPLC to afford 15 fractions. Fraction 3, one of the bioactive fractions (LD_{50} 0.4 μg/mL), was subjected to a reversed-phase HPLC to afford 10 fractions. Compounds **2** and **3** were obtained by purification of fraction 8 (Fig. 1). Other class of compounds are considered to be responsible for the activity of 90% MeOH layer and

Table 1. NMR data of compound **2** and its MTPA esters [a]

Position	δ_H [b]	δ_C [c]	(*S*)-MTPA ester [b]	(*R*)-MTPA ester [b]
1	3.76 (t, 3.5)	57.1	3.515 (t, 3.0)	3.529 (t, 3.0)
2	3.05 (m)	44.5	3.158 (m)	2.158 (m)
	2.80 (m)		2.880 (m)	2.880 (m)
3		215.0		
4		57.2		
5	1.83 (br s)	46.0	1.790 (br s)	1.778 (br s)
6	4.10 (s)	66.9	4.020 (s)	4.058 (s)
7	1.70 (ddd, 13.5, 5.0, 4.0)	43.0	1.675 (d, 12.5)	1.686 (d, 13.0)
	1.15 (ddd, 13.5, 13.0, 2.5)		1.140 (dd, 13, 13.5)	1.140 (dd, 13.0, 12.5)
8	1.90 (br s)	26.7	1.887 (br s)	1.884 (br s)
9	1.80 (d, 12.0)	42.0	1.812 (d, 12.0)	1.799 (d, 12.0)
	0.70 (q, 12.5)		0.714 (q, 12.5)	0.703 (q, 12.0)
10	2.38 (t, 10.5)	31.5	2.361 (t, 10.0)	2.363 (t, 10.0)
11	5.40 (dd, 10.5, 2.5)	130.6	5.373 (dd, 10.0, 2.5)	5.366 (dd, 10.0, 2.5)
12	5.30 (dd, 10.5, 2.5)	133.0	5.270 (dd, 10.0, 2.5)	5.269 (dd, 10.0, 2.5)
13		73.4		
14	1.05 (s)	26.4	0.980 (s)	0.897 (s)
15	1.50 (s)	12.9	1.480 (s)	1.474 (s)
16	0.85 (d, 7.0)	21.4	0.884 (d, 7.5)	0.882 (d, 7.5)

[a] Multiplicities and coupling constants are in parentheses.
[b] Measured at 500 MHz (CD_3OD)
[c] Measured at 100 MHz (CD_3OD)

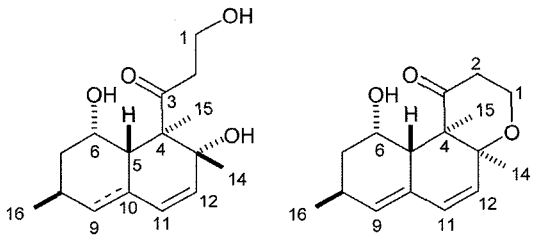


Fig. 1. Chemical structures of compounds **1-3**.

active fractions. Purification of these compounds are in progress.

Compound **1** was isolated as a light yellow oil. On the basis of LRFABMS (*m/z* 263, $[M-H_2O+H]^+$) and ^{13}C-NMR data, its molecular formula was found to be $C_{16}H_{24}O_4$. The ^{13}C-NMR spectrum of **1** showed the signals of three methyls, three methylenes, six methines, and four quaternary carbons. Three olefinic protons at δ 5.33 (H-12), 5.60 (H-9), and 5.91(H-11), and two oxygenated protons at δ 3.80 (H-1) and 4.21 (H-6) were observed in the ^{1}H-NMR spectrum. The COSY spectrum revealed two sets of correlations for two separate partial structures, one from H-5 through H-12 and another between H-1 and H-2. The key HMBC correlations from H-2 to C-3, from H_3-15 to C-3, 4, 5, and C-13, from H_3-14 to C-4, 12, and C-13, and from H-5 to C-9, 10, and C-11, connected partial structures as a bicyclic polyketide derivative. All the protonated carbons were assigned by HSQC spectrum. Chemical structure deduced on the basis of these data, was identical to decumbenone A. Decumbenone A was previously isolated from *Penicillium decumbenons* (Fujii *et al.*, 2002). It is the first report on its isolation from a marine source.

Compound **2** was obtained as a light yellow oil. Its molecular formula was assigned as $C_{16}H_{26}O_4$ by combined ^{13}C-NMR and FABMS (*m/z* 265, $[M-H_2O+H]^+$) spectroscopic analysis. The NMR data of **2** was almost identical to those of **1**, except for the olefinic region. In the ^{1}H-NMR spectrum of **2** only two olefinic proton signals at δ 5.27 (H-12) and 5.37 (H-11) were observed, which were attached to C-12 (δ 133.0) and C-11 (δ 130.6), respectively. In addition, two methylene carbon signals for C-9 (δ 42.0) and C-10 (δ 31.5) were also observed, suggesting the structure of **2** as a 9,10-dihydro derivative of **1**, which was previously reported from *Penicillium decumbenons* (Fujii *et al.*, 2002). The COSY, HMBC, and HSQC spectral data of **2** were in agreement with the reported data of decumbenone B.

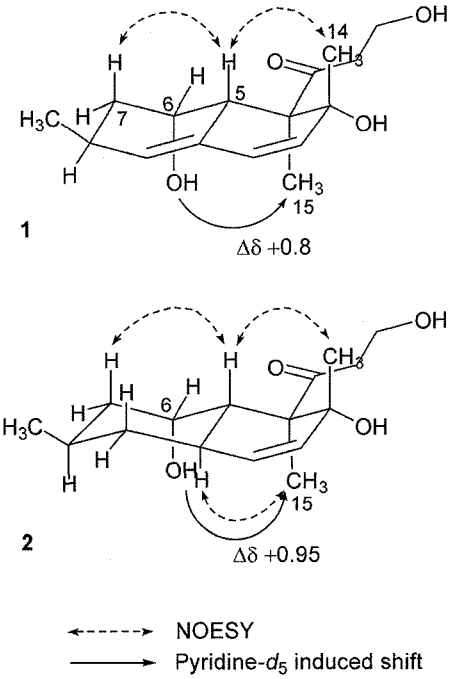
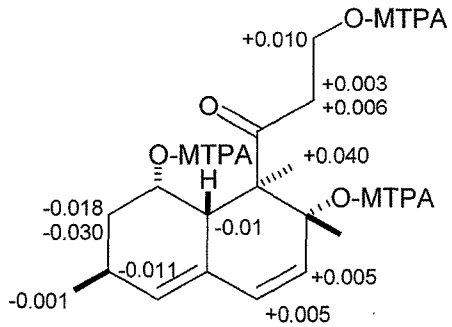


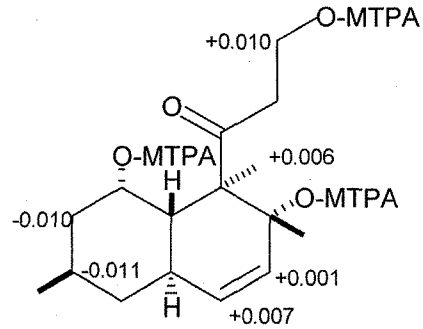
Fig. 2. NOESY correlations and pyridine-d_5 induced shift of compounds **1** and **2**.

The stereochemistry of **1** and **2** was defined by NOESY, pyridine-d_5 induced effect (Demarco *et al.*, 1968), and modified Mosher's method (Dale *et al.*, 1973). In the NOESY experiments for **1**, the methine proton H-5 showed correlations to the methyl protons H_3-14 and the methine proton H-7 (Fig. 2). In the pyridine-d_5 induced effect, only H_3-15 methyl signal was deshielded (Δδ + 0.8), which suggests that H_3-15 is on the same side as 6-OH group. Also in the pyridine-d_5 induced effect of **2**, only H_3-15 methyl signal was deshielded (Fig. 2). The absolute configuration at C-6 was assigned as *S* by Mosher's method. The (*S*) and (*R*)-MTPA esters were prepared and $\Delta\delta$ ($\delta_S-\delta_R$) values for all assignable protons were observed (Fig. 3). Although chemical shift differences between the two esters gave complicated results due to the triple esterification of the hydroxyl functions at C-1, C-6, and C-13. Since OH-1 group is away from the ring skeleton, only OH-6 and OH-13 MTPA effects were considered. Accordingly, the absolute stereochemistry of **2** was defined as (4*R*, 5*S*, 6*S*, 8*R*, 10*R*, 13*R*).

Compound **3** was obtained as a light yellow oil. Its ^{1}H-NMR data was similar to those of **1** and **2**, characterizing its nature also as a polyketide derivative. Compound **3** was identified as versiol by comparison of its spectral data with those of reported (see experiment section). It is previously isolated from *Aspergillus versicolor* (Fukuyama *et al.*, 1978), as an antifungal agent from *Spororamia*



1



2

Fig. 3. δ ($\delta s - \delta_R$) values for the MTPA esters of compounds **1** and **2**.

affinis, and from a soil sample of *Penicillum striatisporum* (Michael *et al*, 2005). Hence, it is the first report on its isolation from a marine source. Versiol showed opposite optical rotation ($[\alpha]^{24}_D - 58°$), to those of decumbenones A and B ($[\alpha]^{24}_D + 54°$ and $[\alpha]^{24}_D + 8°$), respectly, maybe due to the difference in the stereochemistry at C-13 (Fig. 1).

Compounds **1**, **2**, and **3** were previously isolated from terrestrial fungi *Penicillium decumbens* (Fujii *et al.*, 2002). In the same study, decumbenone A (**1**) inhibited the melanizator in *Magnaporthe grisea*, the rice blast pathogen. Whereas decumbenone B (**2**) was inactive. This inhibitory activity was attributed to the diene group present in **1** (Fujii *et al.*, 2002).

In the present study, compounds **1** and **2** were evaluated for cytotoxicity against a panel of five human solid tumor cell lines (A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; and HCT15, human colon cancer). They were practically inactive ($ED_{50} > 30$ μg/mL). Antibacterial activity against clinically isolated methicillin-resistant strains. The isolated compounds did not show significant activity (Table 2).

Polyketides constitude a large class of natural products grouped together on purely biosynthesis grounds. Their

Table 2. Antibacterial assay data of compounds **1** and **2** [a]

bacterial strains	decumbenone A (**1**)	decumbenone B (**2**)	meropenem	imipenem
Streptococcus Pyogenes 308A	100	100	0.013	0.004
Streptococcus Pyogenes 77A	100	100	0.004	0.004
Streptococcus faecium MD 8b	> 100	> 100	6.250	0.781
Streptococcus aureus SG511	> 100	> 100	0.098	0.013
Streptococcus aureus 285	100	> 100	0.195	0.013
Streptococcus aureus 503	> 100	> 100	0.049	0.007
Escherichia coli 078	> 100	> 100	0.025	0.195
Escherichia coli DC 0	> 100	> 100	0.025	0.095
Escherichia coli DC 2	> 100	100	0.025	0.391
Escherichia coli TEM	> 100	100	0.025	0.195
Escherichia coli 1507E	> 100	> 100	0.025	0.098
Pseudomonas aeruginosa 9027	> 100	100	0.195	0.391
Pseudomonas aeruginosa 1592E	100	100	0.195	0.781
Pseudomonas aeruginosa 1771	50	100	0.391	0.781
Pseudomonas aeruginosa 1771M	100	100	0.049	0.195
Salmonella typhimurium	> 100	> 100	0.049	0.781
Klebsiella oxytoca 1082E	> 100	> 100	0.049	0.391
Klebsiella aerogenes 1522E	> 100	> 100	0.049	0.195
Enterobacter cloacae P99	> 100	100	0.049	0.195
Enterobacter cloacae 1321E	> 100	100	0.025	0.195

[a] Data expressed in MIC values (μg/mL)

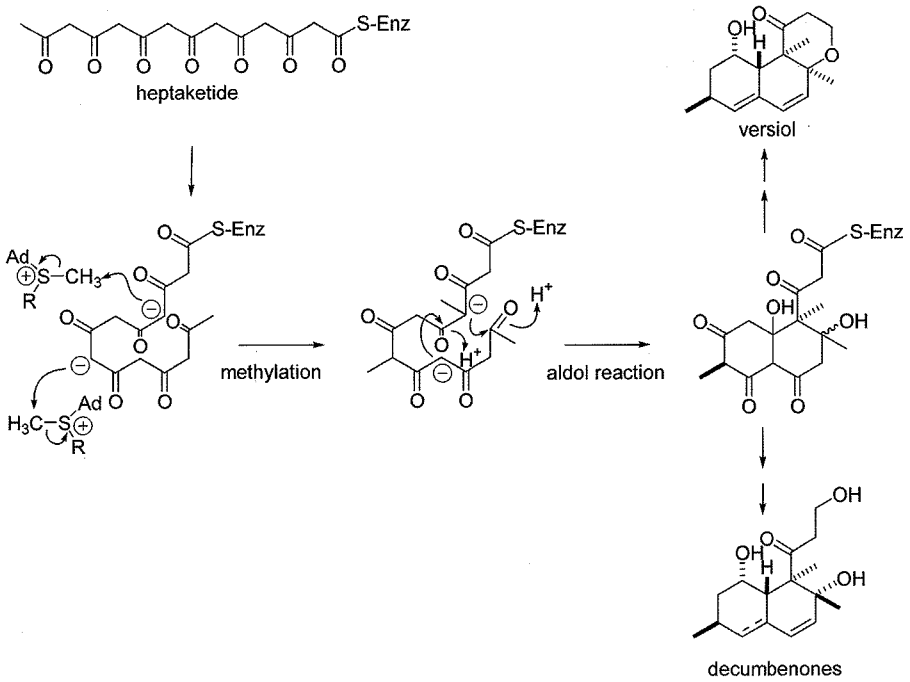


Fig. 4. Proposed biogenesis of decumbenone A, B, and versiol derivatives.

dcompounds (decumbenone A, B, and versiol) can be explained as being derived from C_{14} poly-β-keto chains, and the ester side-chain is derived as a separate unit form two acetates with a methyl from methionine, again with C-methylation proceeding reduction process.

The poly-β-keto ester is very reactive, and there are various possibilities for undergoing intramolecular aldol reaction. It could be envisaged as a cyclization product from a poly-β-keto ester, requiring a variety of production processes and formation of ring by aldol condenstation near the carboxyl terminal. Methyl branching such as H_3-14 and H_3-15 are rare in polyketide-derived metabolites. They may be incoporated by methylation acetate-derived structures found in species of *Penicillium* and *Aspergillus* fungi were formed by folding of a polyketide to form the carbon skeleton (Paul, 2004) (Fig. 4).

Acknowledgement

This work was supported by a grant from the Pusan National University, Korea.

References

Abrell, L.M., Borgeson, B., and Crews, P., Chloro polyketides from the cultured fungus (*Aspergillus*) separated from a marine sponge. *Tetrahedron Lett.*, **37**(14), 2331-2334 (1996).

Belofsky, G.N., Jensen, P.R., Enner, M.K., and Fenical, W., New cytotxic sesquiterpenoid nitrobenzoyl esters from a marine isolate of the fungus *Aspergillus versicolor*. *Tetrahedron*, **54**, 1715-1724 (1998).

Dale, J.A. and Mosher, H.S., Nuclear magnetic resonance enantiomer reagents : Configurational correlations *via* nuclear magnetic resonance chemical shifts of diastereomeric mandelate, *O*-methylmandelate, and α-methoxy-α-trifluorometylphenylacetate (MTPA) esters. *J. Am. Chem. Soc.*, **95**(2), 512-519 (1973).

Demarco, P.V., Farkas, E., Doddrell, D., Mylari, L.B., and Wenkert, E., Pyridine-induced solvent shifts in the nuclear magnetic resonance spactra of hydroxylic compounds. *J. Am. Chem. Soc.*, **90**(20), 5480-5486 (1968).

Fujii, Y., Asahara, M., Ichinoe, M., and Nakajima, H., Fungal melanin inhibitor and related compounds from *Penicillium decumbens*. *Phytochemistry*, **60**, 703-708 (2002).

Fukuyama, K., Katsube, Y.K., Hamasaki, T., and Hatsuda, Y., Struture and absolute configuration of versiol, a metabolite from *Aspergillus versicolor*. *J. Chem. Soc. Perkin Trans.*, **2**, 683-686 (1978).

Hiort, J., Maksimenka, K., Reichert, M., Ottstadt, S.P., Lin, W.H., Wray, V., Steube, K., Schauma, K., Weber, H., Proksch, P., Ebel, R., Muller, W. E. G, and Bringmann, G, New natural products from the sponge-derived fungus *Aspergillus niger*. *J. Nat. Prod.*, **67**, 1532-1543 (2004).

Holler, U., Wright, A.D., Matthee, G.F., Konig, G.M., Draeger, S., Aust, H.J., and Schulz, B., Fungi from marine sponges: diversity, biological activity and secondary metabolites. *Mycol. Res.*, **104**(11), 1354-1365 (2000).

Hyde, K.D., Farrant, C.A., and Jones, E.B.G, Isolation and culture of marine fungi. *Botanica Marina*, **30**, 291-303 (1987).

Lim, Y.J., New Cytotoxic Polyacetylenes from the Marine Sponge, *Petrosia* sp., master degree thesis, Pusan National University, 25-26 (2000).

Lin, H.W., Fu, Z.H., Li, J., and Proksche, P., Novel chromone derivatives from marine fungus *Aspergillus versicolor* isolated from the sponge *Xestospongia exigua*. *Chinese Chem. Lett.*, **12**(3), 235-238 (2001a).

Lin, H.W., Fu, Z.H., Li, J., and Proksche, P., Four novel hydropyranoindeno-derivatives from marine fungus *Aspergillus versicolor*. *Chinese Chem. Lett.*, **12**(5), 435-438 (2001b).

Lin, H.W., Brauers, G., Ebel, R., Wary, V., Berg, A., and Proksch, P., Novel chromone derivatives from the fungus *Aspergillus versicolor* isolated from the marine sponge *Xestospongia exigua*. *J. Nat. Prod.*, **66**, 57-61 (2003).

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L., Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Med.*, **45**, 31-34 (1982).

Michael, S., Robert, J.C., Ernest, L., Shaun, T., Jennifer, H.G., and Calbistrin, E., Two other new metabolites from an Australian isolate of *Penicillium striatisporum*. *J. Nat. Prod.*, **68**, 581-584 (2005).

Paul, M.D., Medicinal Natural Product-A Biosynthetic Approach, *Willey* 2nd, 2002, pp. 35-63.

Renner, M.K., Jensen, P.R., and William, F.P., Mangicols: Structures and biosynthesis of a new class of sesterterpene polyols from a marine fungus of the genus *Fusarium*. *J. Org. Chem.*, **65**, 4843-4852 (2000).

Tsukamoto, S., Miura, S., Yamashita, Y., and Ohta, T., Aspermytin A. : A new neurotrophic polyketide isolated from a marine- derived fungus of the genus *Aspergillus*. *Bioorg. Med. Chem. Lett.*, **14**, 417-420 (2004).

Varoglu, M. and Crews, P., Biosynthetically diverse compounds from a saltwater culture of sponge-derived *Aspergillus niger*. *J. Nat. Prod.*, **63**, 41-43 (2000).

Ye, Y.H., Zhu, H.L., Song, T.C., Liu, J.Y., and Tan, R.X., Structural revision of aspernigrin A, reisolated from *Cladosporium herbarum* IFG-E002. *J. Nat. Prod.*, **68**, 1106-1108 (2005).

(Accepted February 20, 2007)