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New Sesquicillins, Insecticidal Antibiotics Produced by *Albophoma* sp. FKI-1778

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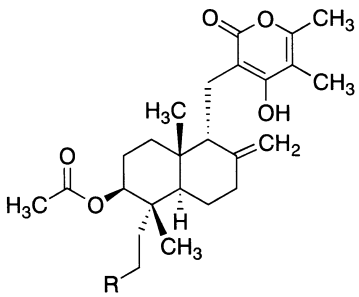
Abstract Four new antibiotics, sesquicillins B to E were isolated from the culture broth of *Albophoma* sp. FKI-1778 together with known sesquicillin (sesquicillin A in this paper). The structures of sesquicillins were elucidated by spectroscopic studies including various NMR experiments. All sesquicillins have a common pyrano-diterpene skeleton. Sesquicillins showed moderate inhibitory activity against the growth of *Artemia salina* (brine shrimps) and Jurkat cells.

Keywords sesquicillins, insecticidal, antibiotic, fungal metabolites, *Albophoma* sp., pyrano-diterpene skeleton

Introduction

In the course of our screening program for insecticidal antibiotics using *Artemia salina* (brine shrimps) as a test organism, we have reported several new antibiotics of microbial origin [1～3]. Our continuous efforts rewarded us the discovery of five structurally related antibiotics, which were isolated from the cultured broth of *Albophoma* sp. FKI-1778. One was identified as sesquicillin (**1**) (Fig. 1), previously reported to show a variety of biological activities such as antihypertensive, bronchoapasmolytic, anti-inflammatory, laxative activities, anti-cancer, glucocorticoid mediated signal transduction inhibitor and G1 phase arrest [4～7]. But four others were found to be new compounds. Therefore, known sesquicillin was renamed sesquicillin A, and new ones were designated



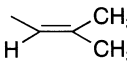
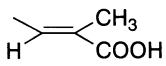
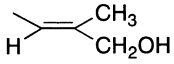
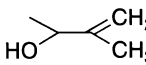
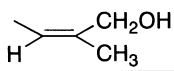
Sesquicillin	R
A (**1**) (Sesquicillin)	
B (**2**)	
C (**3**)	
D (**4**)	
E (**5**)	

Fig. 1 Structures of sesquicillins.

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sesquicillins B (**2**) to E (**5**) (Fig. 1). In this paper, the taxonomy of the producing fungus, fermentation, isolation, structure elucidation and biological properties of sesquicillins are described.

Materials and Methods

General

NMR spectra were recorded on a Varian Inova 600 spectrometer ($^{2-3}J_{CH}$=8 Hz in HMBC). Chemical shifts are shown in δ values (ppm) relative to methanol-d_4 at 3.31 ppm for ^{1}H NMR and at 49.0 ppm for ^{13}C NMR. FAB mass spectrometry was conducted on a JEOL JMS-AX505H spectrometer. UV and IR spectra were measured with a Beckman DU640 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. Optical rotations were recorded on a JASCO model DIP-181 polarimeter.

Taxonomic Studies of the Producing Fungus

The fungal strain FKI-1778 was isolated from a soil sample collected at Amamiooshima Island, Kagoshima Prefecture, Japan. Morphological observations were done under the microscopes (Olympus Vanox-S AH-2 and Olympus SZH10). Color hues were described according to Color Harmony Manual, 4th Ed [8]. For the taxonomic studies of the fungus, potato dextrose agar (PDA, Difco), cornmeal agar (CMA), malt extract agar (MEA), Miura's medium (LCA) were used.

Fermentation

For production of sesquicillins, a seed medium contained glucose 2.0%, yeast extract (Oriental Yeast Co.) 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton (Daigo Nutritive Chemicals) 0.5%, KH_3PO_4 0.1% and agar 0.1%. The pH was adjusted to 6.0 prior to sterilization. The production medium was composed of glycerol 3.0%, oat meal 2.0%, dry yeast 1.0%, KH_2PO_4 1.0%, Na_2HPO_4 1.0% and $MgSO_4 \cdot 7H_2O$ 0.05%. The pH was not adjusted prior to sterilization. A stock culture of strain *Albophoma* sp. FKI-1778 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of the seed medium and incubated on a rotary shaker at 27°C for 2 days. The main culture was initiated by transferring 1 ml of the seed culture into a 500-ml Erlenmeyer flask containing 100 ml of the production medium, and the fermentation was carried out at 27°C with a rotary shaker at 210 rpm.

Quantitative Analysis of Sesquicillins by HPLC

The amount of sesquicillins was measured by analytical HPLC (HP1100, Hewlett-Packard). Samples dissolved in methanol were analyzed under the following conditions: column, Symmetry C18/3.5 μm (2.1×150 mm, Waters); mobile phase, a 20-minute linear gradient from 30% CH_3CN/0.05% H_3PO_4 to 70% CH_3CN/0.05% H_3PO_4; flow rate, 0.2 ml/minute; detection, UV at 240 nm. Compounds **1** through **5** eluted at retention times of 12.4, 4.9, 5.6, 6.1 and 6.9 minutes, respectively.

Insecticidal Activity

Insecticidal activity was assayed by a microtiter-plate assay using brine shrimps, *A. salina* (Pfizer Consumer Inc) as reported previously [1]. Briefly, about 10 nauplii larvae hatched from eggs of brine shrimps were incubated in a well of 96-well microplates at room temperature with test samples. After 48 hours, the motilities were assessed visually in comparison with controls (no samples).

Antimicrobial Activity

Antimicrobial activity against 14 species of microorganisms was measured by a paper disk method. Media for microorganisms are as follows: GAM agar (Nissui Seiyaku Co.) for *Bacteroides fragilis*; Waksman agar for *Mycobacterium smegmatis*; Bacto PPLO agar (Difco) supplemented with horse serum 15%, glucose 0.1%, phenol red (5 mg/ml) 0.2% and agar 1.5% for *Acholeplasma laidlawii*; nutrient agar for *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Xanthomonas oryzae*; a medium composed of glucose 1.0%, yeast extract 0.5%, and agar 0.8% for *Pyricularia oryzae*, *Aspergillus niger*, *Mucor racemosus*, *Candida albicans* and *Saccharomyces cerevisiae*. A paper disk (i.d. 6 mm, Advantec) containing 10 μg of a sample was placed on the agar plate. Bacteria, except *X. oryzae*, were incubated at 37°C for 24 hours. Yeasts and *X. oryzae* were incubated at 27°C for 24 hours. Fungi were incubated at 27°C for 48 hours. Antimicrobial activity was expressed as diameter (mm) of inhibitory zone.

Cytotoxic Activity

Jurkat cells, from a human T cell leukemia, were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified chamber at 37°C containing 5% CO_2. Cell growth was measured by the colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay [9]. Jurkat cells (5×10^4 cells in 50 μl per well) in 96-well microplates and 50 μl of various concentrations of sesquicillins were added to each well and incubated for 24 hours at 37°C. MTT (10 μl of 5.5 mg/ml stock solution, SIGMA) and 90 μl of cell lysate

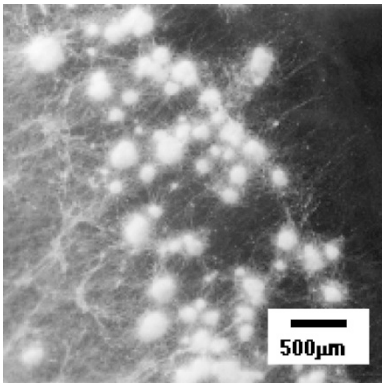

Fig. 2 Photomicrograph of pycnidia of strain FKI-1778.

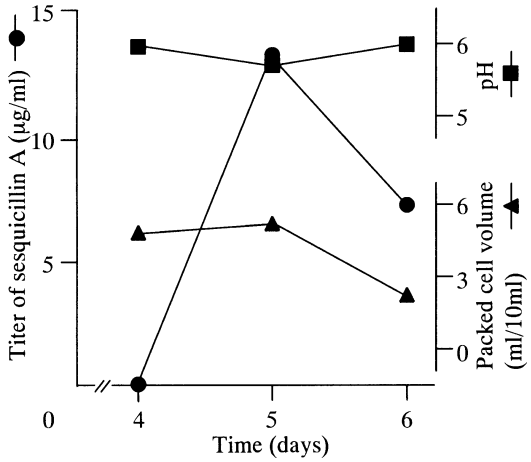

Fig. 3 A typical time course of sesquicillin production by *Albophoma* sp. FKI-1778.

solution (40% N, N-dimethylformamide, 20% sodium dodecyl sulfate, 2% CH_3COOH, 0.03% HCl) was added and the plate was shaken for 1 hour. The optical density of each well was measured at 540 nm using a microtiter-plate reader (Elx 808, Bio-Tek Instruments, Inc.).

Results

Taxonomy of the Producing Fungus

Colonies on PDA were 72~74 mm in diameter after 14 days at 25°C, floccose, corrugate, white (a) in color with moderately pycnidia formation; reverse was cream (1 1/2 ca). Colonies on CMA were 70~71 mm in diameter after 14 days at 25°C, floccose, white (a) to cream (1 1/2 ca) with moderately pycnidia formation. Colonies on MEA were 72~73 mm in diameter after 14 days at 25°C, floccose, pulvinate in the center and, white (a) to cream (1 1/2 ca) in color with poor pycnidia formation; reverse was light ivory (2 ca). Colonies on LcA were 50~60 mm in diameter after 14 days at 25°C, floccose, white (a) with moderately pycnidia formation. Pycnidia were pure white, epibiotic, globose to subglobose, often gregarious, and 100~300 μm in size. Pycnidial wall was thin, fleshy and soft, and prosenchymatous (Fig. 2). Conidiophores were arising from inner hyphae of pycnidium, producing conidia holoblastically. Conidia were unicellular, hyaline, globose, and 1.6~2.2 μm in size.

From the above characteristics, strain FKI-1778 was considered to belong to the genus *Albophoma* [10] and named it *Albophoma* sp. FKI-1778. The strain was deposited to the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology Japan, as FERM BP-08668.

Fermentation

A typical time course of the sesquicillin production is shown in Fig. 3. Sesquicillin A was not detected in the culture until day 4, but the concentration reached a maximal level (13 μg/ml) on day five.

Isolation

The isolation procedure for sesquicillins is summarized in Fig. 4. The 168-hour old culture broth (6 liters) was centrifuged to obtain the supernatant which was extracted with an equal volume of ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a dark brown oil (814 mg). After dissolving with MeOH, the soluble fraction was applied to centrifugal partition chromatography (Sanki Engineering Ltd.). The lower phase of the solvent was introduced by ascending method. The active fractions were eluted with the upper phase to give two fractions (Fr. 1 and 2) and concentrated under reduced pressure. Fr. 1 (90.3 mg) containing **2** through **5** was subjected to preparative HPLC. Compounds **2** through **5** eluted at retention times of 21.8, 25.3, 28.7 and 37.2 minutes, respectively. Each collected fraction was concentrated to dryness to give pure **2** (4.4 mg), **3** (18.6 mg), **4** (2.5 mg) and **5** (3.9 mg) as a pale yellow powder. Fr. 2 (70.4 mg) containing **1** was subjected to preparative HPLC. Compound **1** eluted at retention time of 57 minutes. The collected fraction was concentrated to dryness to give pure **1** (4.6 mg), as a pale yellow powder.

Structure Elucidation

The physico-chemical properties of **1** to **5** are summarized in Table 1. The molecular formulas of **2** to **5** were revealed to be $C_{29}H_{40}O_7$, $C_{29}H_{42}O_6$, $C_{29}H_{42}O_6$ and $C_{29}H_{42}O_6$ by HR-

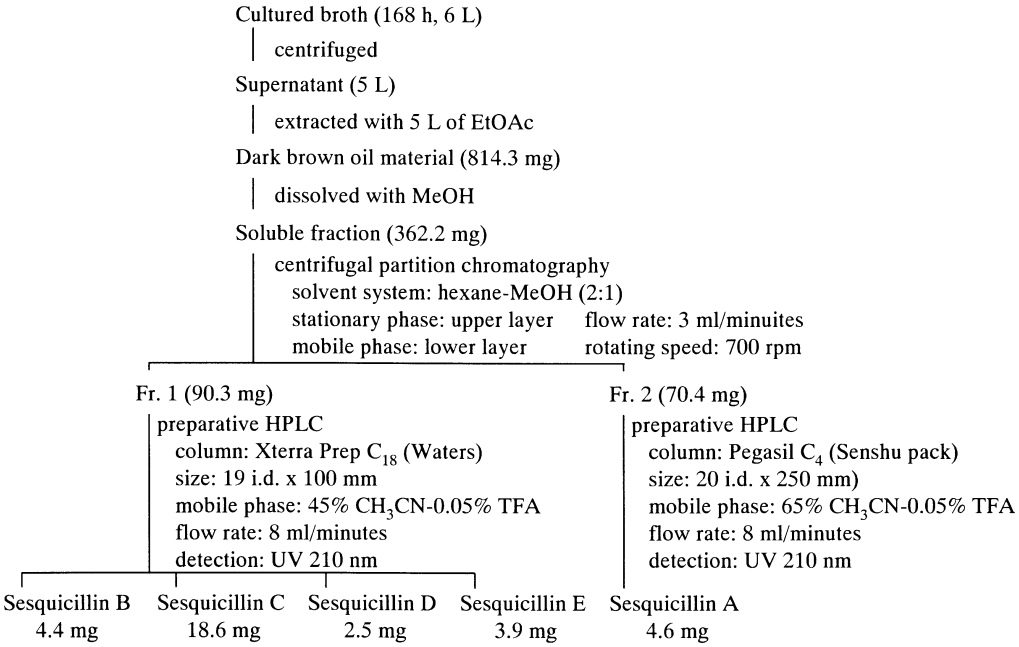

Fig. 4 Purification procedure of sesquicillins from the culture broth of *Albophoma* sp. FKI-1778.

Table 1 Physico-chemical properties of **1** to **5**

	1	**2**	**3**	**4**	**5**
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder
Molecular weight	470	500	486	486	486
Molecular formula	$C_{29}H_{42}O_5$	$C_{29}H_{40}O_7$	$C_{29}H_{42}O_6$	$C_{29}H_{42}O_6$	$C_{29}H_{42}O_6$
HRFAB-MS					
calcd	470.3032 $[M]^+$	523.2672 $(M+Na)^+$	509.2879 $(M+Na)^+$	509.2879 $(M+Na)^+$	509.2879 $(M+Na)^+$
found	470.3055 $[M]^+$	523.2705 $(M+Na)^+$	509.2899 $(M+Na)^+$	509.2857 $(M+Na)^+$	509.2885 $(M+Na)^+$
UV λ_{max} nm (ε) in MeOH	290 (10,900)	294 (9,300)	297 (6,100)	293 (7,800)	295 (6,500)
IR ϖ_{max} cm^{-1} (KBr)	3425, 2930, 1735, 1606, 1670,1560, 1450, 1385, 1240, 1115, 1075, 1030	3430, 2960, 1687, 1564, 1450, 1390, 1259, 1097, 1036, 806	3430, 2938, 2360, 1725, 1673, 1563, 1448, 1388, 1247, 1146, 1028, 887	3430, 2940, 2327, 1678, 1558, 1461, 1390, 1254, 1081, 1037, 808	3430, 2933, 2358, 1677, 1563, 1450, 1388, 1257, 1197, 1085, 1033, 806
$[\alpha]_D$ (23°C)	−18.0° (*c* 0.1, MeOH)	−27.4° (*c* 0.1, MeOH)	−59.0° (*c* 0.1, MeOH)	−27.3° (*c* 0.1, MeOH)	−34.9° (*c* 0.1, MeOH)
Solubility					
soluble	$CHCl_3$, MeOH, DMSO	$CHCl_3$, MeOH, DMSO	$CHCl_3$, MeOH, DMSO	$CHCl_3$, MeOH, DMSO	$CHCl_3$, MeOH, DMSO
insoluble	Hexane, H_2O	Hexane, H_2O	Hexane, H_2O	Hexane, H_2O	Hexane, H_2O

FAB-MS, respectively. Similarity in physico-chemical properties strongly suggested that they are structurally related.

MS data and ^1H and ^{13}C NMR analysis identified **1** as sesquicillin A, which was previously isolated from a fungus. Generally, the structures of **2** to **5** were elucidated by extensive NMR experiments in CD_3OD. The assignment of the ^1H and ^{13}C NMR signals was facilitated by ^1H-^1H COSY and HMQC experiments (Table 2). The carbon skeleton and the relative stereochemistry were demonstrated by HMBC and NOE experiments (pertinent HMBC and NOESY correlations are shown in Fig. 5). A

Table 2 ^{1}H NMR chemical shifts of **1** to **5**

	1[a]			**2**[b]			**3**[b]			**4**[b]			**5**[b]		
1	Ha	1.96	1H, m	Ha	1.89	1H, m	Ha	1.85	1H, m	Ha	1.87	1H, br.d, (13.2)	Ha	1.82	1H, m
	Hb	1.29	1H, m	Hb	1.35	1H, m	Hb	1.34	1H, m	Hb	1.34	1H, m	Hb	1.34	1H, m
2	Ha	1.75	1H, m	Ha	1.84	1H, m	Ha	1.37	1H, m	Ha	1.82	1H, m	Ha	1.87	1H, m
	Hb	1.71	1H, m	Hb	1.73	1H, m	Hb	1.71	1H, m	Hb	1.72	1H, m	Hb	1.71	1H, m
3		4.81	1H, dd (5.9, 10.1)		4.87	1H, m		4.87	1H, dd, (4.5, 11.4)		4.76	1H, m		4.86	1H, dd, (4.2, 11.4)
5		1.68	1H, dd, (2.5, 12.0)		1.84	1H, br.d, (15.2)		1.85	1H, dd, (2.4, 15.6)		1.77	1H, br.d, (12.6)		1.82	1H, m
6	Ha	1.56	1H, m	Ha	1.56	1H, m	Ha	1.56	1H, m	Ha	1.57		Ha	1.56	1H, m
	Hb	1.39	1H, dddd, (5.0, 12.0, 12.0, 14.0)	Hb	1.35	1H, m	Hb	1.36	1H, m	Hb	1.37		Hb	1.36	1H, m
7	Ha	2.41	1H, ddd, (5.0, 12.0, 14.0)	Ha	2.47	1H, dt, (6.0, 14.0)	Ha	2.46	1H, dt, (5.4, 13.8)	Ha	2.44	1H, dt, (5.8, 14.0)	Ha	2.46	1H, dt, (4.8, 13.2)
	Hb	2.22	1H, m	Hb	2.10	1H, br.d, (14.0)	Hb	2.06	1H, br.d, (13.8)	Hb	2.08	1H, br.d, (13.2)	Hb	2.09	1H, br.d, (3.2)
9		1.94	1H, m		2.20	1H, m		2.20	1H, m		2.17	1H, dd, (3.6, 12.6)		2.19	1H, m
11	Ha	2.75	1H, dd, (3.2, 14.0)	Ha	2.78	1H, t, (12.6)	Ha	2.77	1H, t, (12.6)	Ha	2.77	1H, t, (12.0)	Ha	2.77	1H, dd, (12.0, 12.6)
	Hb	2.50	1H, dd, (10.6, 14.0)	Hb	2.61	1H, dd, (4.2, 12.6)	Hb	2.80	1H, dd, (4.8, 12.6)	Hb	2.59	1H, dd, (3.6, 12.0)	Hb	2.59	1H, dd, (4.2, 12.6)
12	Ha	4.65	1H, m	Ha	4.52	1H, m	Ha	4.51	1H, m	Ha	4.51	1H, m	Ha	4.51	1H, m
	Hb	4.45	1H, m	Hb	4.25	1H, m	Hb	4.23	1H, m	Hb	4.23	1H, m	Hb	4.23	1H, m
13		0.95	1H, s		1.02	1H, s		1.01	1H, s		1.00	1H, s		1.01	1H, s
14		0.86	1H, s		0.93	1H, s		0.91	1H, s		0.91	1H, s		0.91	1H, s
15	Ha	1.27	1H, m	Ha	1.42	1H, m	Ha	1.38	1H, m	Ha	1.37	1H, m	Ha	1.33	1H, m
	Hb	1.18	1H, m	Hb	1.26	1H, m	Hb	1.22	1H, m	Hb	1.13	1H, m	Hb	1.19	1H, m
16	Ha	1.97	1H, m	Ha	2.22	1H, m	Ha	2.09	1H, m	Ha	1.55	1H, m	Ha	2.10	1H, m
	Hb	1.86	1H, m	Hb	2.18	1H, m	Hb	2.07	1H, m	Hb	1.49	1H, m	Hb	2.08	1H, m
17		5.03	1H, t, (7.0)		6.73	1H, t, (7.3)		5.36	1H, t, (7.2)		3.87	1H, t, (6.0)		5.23	1H, t, (7.2)
19		1.66	1H, s					3.91	2H, s		1.69	1H, s		1.76	1H, s
20		1.58	1H, s		1.82	1H, s		1.56	1H, s	Ha	4.89	1H, m		4.07	1H, dd, (12.0, 16.2)
										Hb	4.83	1H, m			
6′		1.90	1H, s		1.93	1H, s		1.93	1H, s		1.93	1H, s		1.93	1H, s
7′		2.18	1H, s		2.21	1H, s		0.21	1H, s		2.20	1H, s		2.21	1H, s
3-Ac															
		2.03	1H, s		2.05	1H, s		2.03	1H, s		2.02	1H, s		2.03	1H, s

[a] Data from ref. [6]. Spectra taken at 500 MHz. Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm. *J* values are given in Hz in parentheses.

[b] Spectra taken at 600 MHz. Chemical shifts are shown with reference to CD_3OD as 3.31 ppm. *J* values are given in Hz in parentheses.

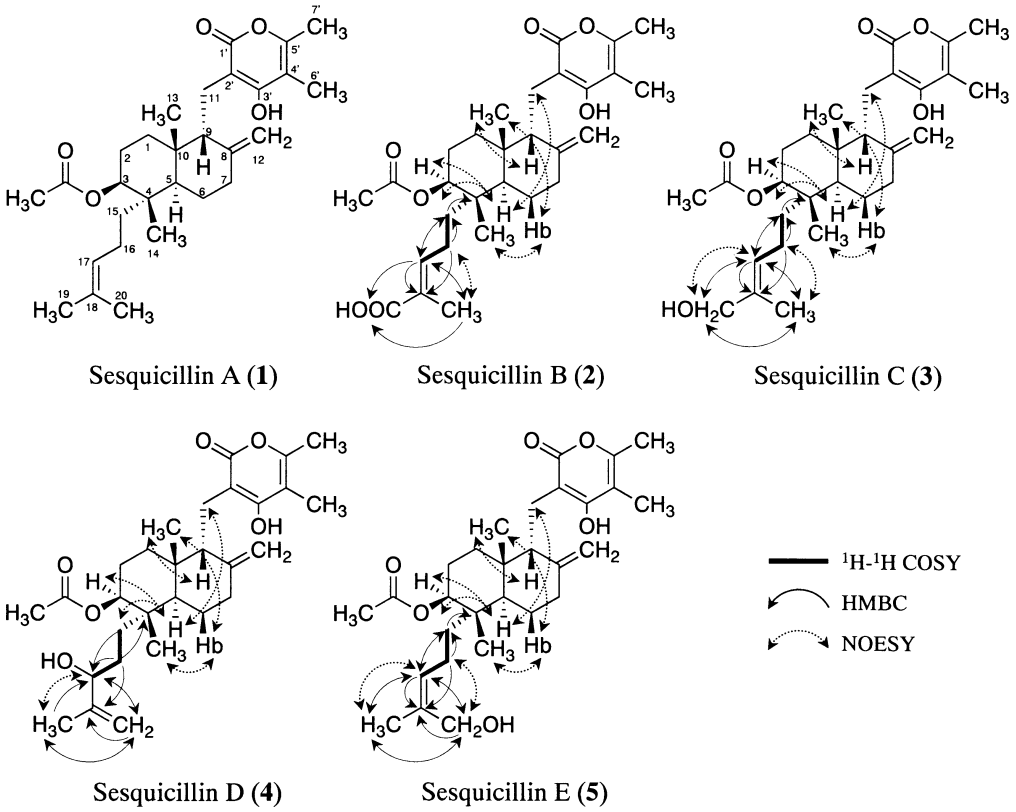

Fig. 5 ^{1}H-^{1}H COSY, HMBC and NOESY experiments for sesquicillins.

comparison of ^{1}H and ^{13}C NMR data of **2** to **5** with those of **1** indicated the presence of pyrano-diterpenoid moiety, in common.

Structure of **2**: The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) resembled those of **1** except for the proton and carbon signals of C-17, C-18, C-19 and C-20. The ^{13}C-^{1}H long-range couplings of ^{2}J and ^{3}J in the HMBC experiments are shown in Fig. 5, giving the following results. The cross peaks from 16-H^{2} (δ 2.18, 2.22) to sp^{2} quaternary carbon C-18 (δ 128.7), from 17-H (δ 6.73) to C-18, carboxy carbon C-19 (δ 171.6) and methyl carbon C-20 (δ 12.3) and from 20-H3 (δ 1.82) to sp^{2} methine carbon C-17 (δ 143.7) and C-19 indicated that **2** has a carboxy group instead of the methyl group (C-19) of **1**. The NOESY spectrum of **2** had the cross peaks between 16-H_2 and 20-H_3, but not between 17-H_2 and 20-H_3, indicating that the double bond between C-17 and C-18 is the *E* isomer.

Structure of **3**: The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) also resembled those of **1** except for the proton and carbon signals of C-19. In the HMBC experiments (Fig. 5), the cross peaks from oxymethylene proton 19-H_2 (δ 3.91) to sp^{2} methine carbon C-17 (δ 126.8), sp^{2} quaternary carbon C-18 (δ 135.9) and methyl carbon C-20 (δ 17.5) indicated that **3** has a hydroxymethyl residue at C-19 instead of the methyl group of **1**. The NOESY spectrum of **3** had the cross peaks between 16-H_2 and 20-H_3 and between 17-H and 19-H_2, indicating that the double bond between C-17 and C-18 is the *E* isomer.

Structure of **4**: The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) also resembled those of **1** except for the proton and carbon signals of C-17, C-18, C-19 and C-20. The ^{13}C-^{1}H long-range couplings of ^{2}J and ^{3}J in the HMBC experiments are also shown in Fig. 5, giving the following results. The cross peaks from the methylene proton 16-H_2 (δ 1.49, 1.55) to oxymethine carbon C-17 (δ 77.8) and sp^{2} quaternary carbon C-18 (δ 144.8), from oxymethine proton 17-H (δ 3.87) to methylene carbon C-16 (δ 29.3) and exo-methylene carbon C-20 (δ 111.9), from methyl proton 19-H_3 (δ 1.69) to C-17 and C-18 and from exo-methylene proton 20-H_2 (δ 4.83, 4.89) to C-17, C-18 and C-19, indicated that the 3-methyl-but-3-en-2-ol residue is connected to C-16.

Structure of **5**: The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) also resembled those of **3** except for the proton and carbon signals of C-16, C-19 and C-20. From these data, the planner structure was presumed to be the same as **3**. In the HMBC experiments, the cross peaks from

Table 3 ^{13}C NMR chemical shifts of **1** to **5**

Position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	33.8 t	35.0 t	35.0 t	35.0 t	35.0 t
2	24.0 t	25.1 t	25.1 t	25.1 t	25.1 t
3	76.2 d	77.3 d	77.5 d	77.7 d	77.5 d
4	40.0 s	41.3 s	41.2 s	41.5 s	41.2 s
5	39.3 d	40.4 d	40.4 d	40.6 d	40.4 d
6	22.6 t	23.8 t	23.8 t	23.8 t	23.8 t
7	30.9 t	32.1 t	32.1 t	32.1 t	32.1 t
8	149.0 s	149.7 s	149.9 s	150.0 s	149.9 s
9	56.5 d	56.2 d	56.2 d	56.2 d	56.2 d
10	37.6 s	38.6 s	38.6 s	38.6 s	38.6 s
11	22.2 t	22.6 t	22.4 t	22.5 t	22.4 t
12	111.0 t	110.6 t	110.6 t	110.6 t	110.6 t
13	22.8 q	23.6 q	23.6 q	23.7 q	23.6 q
14	18.0 q	18.6 q	18.7 q	18.8 q	18.7 q
15	37.8 t	37.2 t	38.5 t	35.1 t	38.5 t
16	21.8 t	23.5 t	22.5 t	29.3 t	21.8 t
17	124.4 d	143.7 d	126.8 d	77.8 d	127.5 d
18	131.4 s	128.7 s	135.9 s	148.8 s	134.4 s
19	25.7 q	171.6 s	69.0 t	17.5 q	20.3 q
20	17.5 q	12.3 q	13.6 q	111.9 t	60.0 t
1′	165.0 s	168.1 s	168.1 s	168.4 s	168.1 s
2′	103.0 s	104.0 s	104.0 s	104.1 s	104.0 s
3′	164.3 s	167.7 s	167.7 s	167.9 s	167.7 s
4′	106.0 s	108.7 s	108.7 s	108.7 s	108.7 s
5′	155.7 s	156.9 s	156.9 s	157.0 s	156.9 s
6′	9.9 q	10.4 q	10.4 q	10.4 q	10.4 q
7′	17.2 q	17.2 q	17.2 q	17.2 q	17.2 q
3-Ac	170.7 s	172.6 s	172.6 s	172.7 s	172.6 s
	21.2 q	21.1 q	21.1 q	21.1 q	21.1 q

[a] Data from ref. [6]. Spectra taken at 125 MHz. Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm.
[b] Spectra taken at 150 MHz. Chemical shifts are shown with reference to CD_3OD as 49.0 ppm.

oxymethylene proton 20-H_2 (δ 4.06, 4.09) to sp^2 methine carbon C-17 (δ 127.5), sp^2 quaternary carbon C-18 (δ 134.4) and methyl carbon C-19 (δ 20.3) indicated that **5** has an hydroxymethyl residue instead of the methyl group C-20 of **1**. The NOESY spectrum of **5** had the cross peaks between 16-H_2 and 20-H_2 and between 17-H and 19-H_3, indicating that the double bond between C-17 and C-18 is Z isomer.

Biological Activities

Insecticidal activity

Insecticidal activity of sesquicillins was studied against *A. salina*. Minimum growth inhibitory concentrations (MIC) are summarized in Table 4. Compound **1** showed the most potent with the MIC value of 6.25 μg/ml, followed by **3** and **5** with 100 μg/ml. However, **2** and **4** showed very weak inhibition at 200 μg/ml.

Table 4 Insecticidal and cytotoxic activities of **1** to **5**

Sesquicillin	Insecticidal activity[a] MIC (μg/ml)	Cytotoxic activity[b] IC_{50} (μM)
A (**1**)	6.25	34.0
B (**2**)	>200	>100
C (**3**)	100	38.3
D (**4**)	>200	>100
E (**5**)	100	>100

[a] Growth inhibition against *A. salina*.
[b] Growth inhibition against Jurkat cells.

Antimicrobial Activity

Compounds **1** to **5** showed no antimicrobial activity against 14 microorganisms as described above at 10 μg/6 mm disk.

Cytotoxic Activity

Cytotoxicity of **1** to **5** to Jurkat cells are summarized in Table 4. Compounds **1** and **3** showed cytotoxic with the IC_{50} value of 34.0 and 38.3 μg/ml, respectively, while **2**, **4** and **5** showed very weak cytotoxic activity at 100 μg/ml.

Discussion

Five sesquicillin-related compounds including new ones **2** to **5** were isolated from the culture broth of *Albophoma* sp. FKI-1778. They have a common pyrano-diterpene moiety and a different isoprenoid side chain (Fig. 1). Sesquicillin A was reported to be produced by *Sesquicillium* and *Acremonium*. As described in this paper, however, the compound was also produced by a different genus *Albophoma*, which was originally reported as a new genus producing indoloditerpenes terpendoles, inhibiters of acyl-CoA: cholesterol acyltransferase [11,12]. It might be interesting to test whether these sesquicillin A-producing fungi produce structurally related terpendoles. In fact, we found that *Albophoma* sp. FKI-1778 produced terpendole C under the culture condition described above (data not shown). The detail about the relation between the fungi and the products will be reported.

Regarding the biosynthetic route, it was speculated that sescuicillin A (**1**) is first biosynthesized *via* coupling of a diterpene part (decalin) and a polyketide-derived pyranone part [13], then oxidized to give **3**, **4** and **5** and finally to

give **2**.

Regarding the biological activities of sesquicillins, a similar order of potency was observed in insecticidal and cytotoxic activities (Table 4); **1** is the most potent, followed by **3** and **5**, and **2** and **4** show very weak activities. These results suggested that hydrophobic residues of the terminal isoprene moiety (**R** in Fig. 1) are responsible for the biological activities.

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