# α-Pyrones and Yellow Pigments from the Sponge-Derived Fungus *Paecilomyces lilacinus*

**Mohamed Elbandy,[†,P,a] Pramod B. Shinde,[†,a] Jongki Hong,[‡] Kyung Sook Bae,[§] Mi Ae Kim,[†] Sang Mong Lee,[#] and Jee H. Jung[†,*]**

[†]*College of Pharmacy, Pusan National University, Busan 609-735, Korea.* [*]*E-mail: jhjung@pusan.ac.kr*
[‡]*College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*
[§]*Korea Research Institute of Chemical Technology, Daejeon 300-716, Korea*
[#]*College of Natural Resources and Life Science, Pusan National University, Busan 609-735, Korea*
[P]*Department of Food Sciences and Technology, Faculty of Environmental Agricultural Sciences, Suez Canal University, Elarish, North Sinai, Egypt*
*Received September 29, 2008, Accepted December 5, 2008*

New α-pyrones (**1** and **2**) and cyclohexenones (**13** and **14**) were isolated along with known analogues (**3**, **5**–**12**) from the ethyl acetate extract of the whole broth of the fungus *Paecilomyces lilacinus*, a strain derived from a marine sponge *Petrosia* sp. Their structures were established by interpretation of 1D and 2D NMR, and FABMS data. It is interesting to isolate cyclohexenone derivatives from the genus *Paecilomyces* (family Trichocomaceae, order Eurotiales), since these cyclohexenones were previously reported only from far distinct genera, *Phoma* and *Alternaria* (family Pleosporaceae, order Pleosporales). Compounds **6**, **7**, and **9** were evaluated for cytotoxicity against a small panel of human solid tumor cell lines. Their cytotoxicity was insignificant upto a concentration of 30 μg/mL.

**Key Words**: Sponge-derived fungus, *Paecilomyces lilacinus*, Pyrones, Cyclohexenones

## Introduction

Marine microorganisms, including marine fungi, represent an underdeveloped and potentially prolific source of structurally diverse secondary metabolites.[1,2] In addition, some of the bioactive compounds isolated from marine invertebrates were suggested to be produced by fungi associated with them.[3] In our search for cytotoxic metabolites from marine microorganisms, the sponge-derived fungi *Paecilomyces lilacinus* (family: Trichocomaceae, order: Eurotiales), isolated from a marine sponge *Petrosia* sp., was investigated. The genus *Paecilomyces* is a cosmopolitan filamentous fungus, and the strain *Paecilomyces lilacinus* has been isolated from a wide range of habitats including cultivated and uncultivated soils, forests, grassland, deserts, and sewage sludge as well as from nematode eggs and occasionally from females of root-knot and cyst nematodes. In addition, it has frequently been isolated from the rhizosphere of many crops. This fungus was also isolated from a marine source, mullet gut.[4] Polysaccharides constituted of glucose and galactose, and peptidal antibiotics (P168 and leucinostatin A) have been reported from *Paecilomyces lilacinus*.[5,6] In this paper, we report the isolation and characterization of four new compounds (**1**, **2**, **13**, and **14**), along with nine known analogues (**3**, **5**-**12**), which represent additional chemical classes of natural products from this fungal genus.

## Results and Discussion

*Paecilomyces lilacinus* was isolated from a marine sponge *Petrosia* sp., collected from Jeju Island, South Korea, 2004. The fungal isolate was cultured for 3 weeks at 35 °C in a liquid PMG seawater-based medium (peptone, 0.1%; malt extract, 2%; D-glucose, 2%). The EtOAc extract of the whole broth culture (9 L) was partitioned between 90% aqueous MeOH and *n*-hexane. The aqueous MeOH soluble portions were purified by $^1$H NMR-monitoring and/or bioassay-directed fractionation (brine shrimp lethality) employing flash column chromatography over silica followed by reversed-phase CN or ODS HPLC to afford compounds **1**-**3** and **5**-**14** (Fig. 1).

Paecilopyrone A (**1**) was obtained as a white solid. Its molecular formula, $C_{13}H_{18}O_4$, was established by HRFABMS ($[M+2Na-H]^-$, *m/z* 283.0916, Δ–0.6 mmu) indicating the presence of five degrees of unsaturation. The α-pyrone ring of **1** was characterized by two methyl signals at $\delta_H$ 1.85, ($H_3$-13) and 1.84 ($H_3$-14), as well as by the diagnostic carbon chemical shifts of C-3 ($\delta_C$ 96.1), C-5 ($\delta_C$ 114.6), C-6 ($\delta_C$ 150.6), C-2 ($\delta_C$ 168.2), and C-4 ($\delta_C$ 175.6) (Table 1). The spectroscopic NMR data of the α-pyrone ring was in a good agreement with reported values.[8-10] The $^1$H NMR spectrum of **1** also showed additional signals of two methyl groups ($\delta_H$ 0.87/H-11, 1.07/H-12), two methylene groups ($\delta_H$ 1.40, 1.70/H-10, 3.58/H-7) and a methine proton ($\delta_H$ 2.64/H-9). The COSY spectrum showed coupling between terminal methyl protons ($\delta_H$ 0.87/H-11) and methylene protons ($\delta_H$ 1.40, 1.70/H-10) which were coupled with a methine proton at $\delta_H$ 2.64 (H-9), which in turn coupled with a secondary methyl group ($\delta_H$ 1.07/H-12) suggesting a presence of *sec*-butyl group in **1**. In the HMBC spectrum, correlation was observed from the methine proton ($\delta_H$ 2.64/H-9) to a ketone carbon at $\delta_C$ 210.0 (C-8). Furthermore, HMBC correlations from H-13 ($\delta_H$ 1.85) to C-2 ($\delta_C$ 168.2), C-3 ($\delta_C$ 96.1), and C-4 ($\delta_C$ 175.6), and from H-14 ($\delta_H$ 1.84) to

[a]These authors equally contributed to this work.

**Table 1.** $^{1}H$ and $^{13}C$ NMR Data (500 MHz, $CD_3OD$) of Compounds **1**, **2**, **13**, and **14**[a]

| position | 1 | | 2 | | 13 | | 14 | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_C$[b] | $\delta_H$ | $\delta_C$[b] | $\delta_H$ | $\delta_C$[b] | $\delta_H$ | $\delta_C$[b] | $\delta_H$ |
| 1 | | | | | 202.2 | | 199.0 | |
| 2 | 168.2 | | 162.0 | | 115.5 | | 114.5 | |
| 3 | 96.1 | | 110.0 | | 177.5 | | 176.0 | |
| 4 | 175.6 | | 157.7 | | 172.3 | | 170.0 | |
| 5 | 114.6 | | 110.0 | 6.03(brs) | 172.3 | | 170.0 | |
| 6 | 150.6 | | 157.7 | | 83.0 | | 82.8 | |
| 7 | 49.0 | 3.58(m) | 49.0 | 3.58(m) | 176.5 | | 176.1 | |
| 8 | 210.0 | | 215.0 | | 41.9 | 2.40(m) | 42.3 | 2.32(m) |
| 9a | 47.0 | 2.64(m) | 46.5 | 2.70(m) | 28.0 | 1.62(m) | 27.6 | 1.62(m) |
| 9b | | | | | | 1.46(m) | | 1.40(m) |
| 10a | 25.7 | 1.70(m) | 25.6 | 1.71(m) | 11.7 | 0.92(t,7.5) | 11.9 | 0.91(t,7.5) |
| 10b | | 1.40(m) | | 1.36(m) | | | | |
| 11 | 10.3 | 0.87(t,7.5) | 11.5 | 0.85(t,7.5) | 16.9 | 1.11(d,6.5) | 16.9 | 1.09(d,7.0) |
| 12 | 15.1 | 1.07(d,7.0) | 15.0 | 1.04(d,7.0) | 59.4 | 4.08(s) | 60.0 | 4.12(s) |
| 13 | 8.0 | 1.85(s) | 7.3 | 2.01(s) | 7.1 | 1.81(s) | 7.0 | 1.81(s) |
| 14 | 8.2 | 1.84(s) | 8.1 | 2.00(s) | 20.3 | 1.32(s) | 20.4 | 1.47(s) |
| 15 | | | | | 53.3 | 3.82(s) | 53.8 | 3.69(s) |

[a]Multiplicities and coupling constants are in parentheses. [b]Assignments based on HMBC and HSQC spectroscopic data.

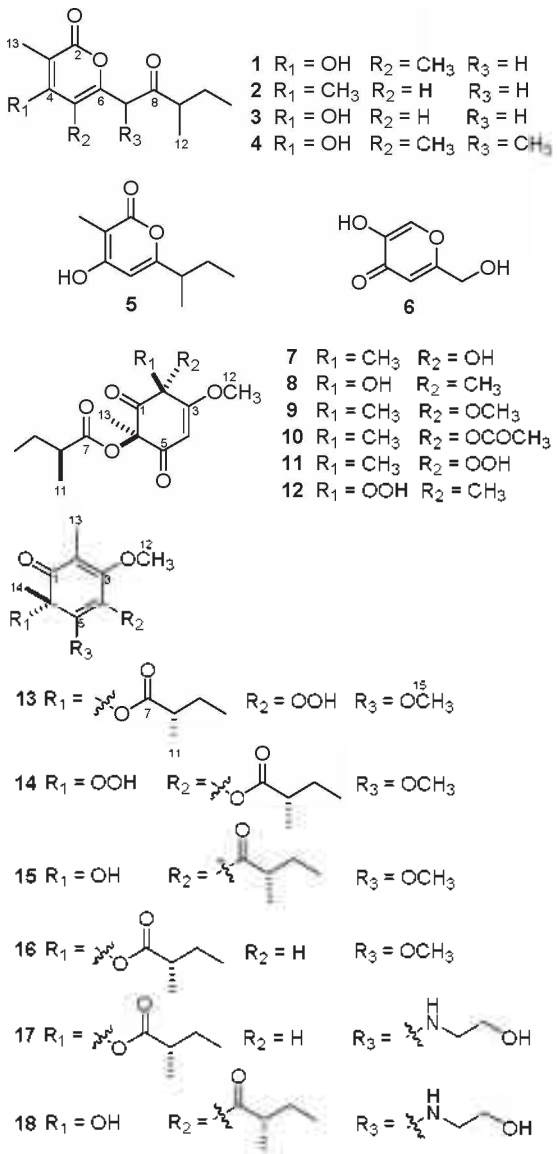


**Figure 1.** Structures of compounds **1**-**18**.

C-5 ($\delta_C$ 114.6) and C-6 ($\delta_C$ 150.6) supported the assignment of an α-pyrone ring (Fig. 2). Thus, two partial structures, an α-pyrone ring and a *sec*-butyl group attached to a ketone carbonyl carbon were established; however, no HMBC correlation was detected for the isolated methylene protons ($\delta_H$ 3.58/H-7). Hence, the isolated methylene group was inserted at C-7 position connecting two partial structures, by comparison of the NMR data with those of phomapyrone B (**3**),[8] ascosalipyrone,[9] and micropyrone (**4**).[10] The only difference between **1** and micropyrone (**4**), isolated from the plant *Helichrysum italicum*, was the absence of the methyl group on C-7 in the linear side chain of **1**. Therefore, the structure of paecilopyrone A (**1**) was established as 4-hydroxy-3,5-dimethyl-6-(3-methyl-2-oxo-pentyl)-pyran-2-one. The stereochemistry at C-9 remains to be determined.

Paecilopyrone B (**2**) was obtained as a white solid. Its molecular formula was established as $C_{13}H_{18}O_3$ on the basis of HRESIMS and NMR data. The exact mass of the $[M-H]^-$ ion (*m/z* 221.1184) matched well with the expected molecular formula $C_{13}H_{17}O_3$ ($\Delta$ +0.1 mmu), which was 16 amu lesser than **1**. The $^{1}H$ NMR spectrum of **2** was basically the same as that of **1**, except for the presence of an olefinic proton signal at $\delta_H$ 6.03 (H-5) indicating a trisubstituted pyrone moiety. The $^{1}H$ NMR spectrum of **2** exhibited two vinyl methyl groups ($\delta_H$ 2.01/H-13 and 2.00/H-14) similar to those of **1**. Position of the olefinic proton at C-5 was unambiguously assigned on the basis of HMBC correlations with C-3 ($\delta_C$ 110.0) and C-7 ($\delta_C$

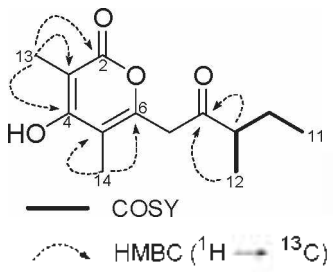


**Figure 2.** Key COSY and HMBC correlations of compound **1**.

49.0). Thus, the chemical structure of paecilopyrone B (**2**) was assigned as 3,4-dimethyl-6-(3-methyl-2-oxo-pentyl)-pyran-2-one. Compounds **1** and **2** may derived from a pentaketide in a similar way as one of the reported compound, ascosalipyrone.[11] It was recently confirmed that the 2-pyrones isolated from fungi are of polyketide origin and those from marine mollusc are polypropionates.[12]

Compounds **3** and **5** were identified on the basis of 1D and 2D NMR and FABMS data as phomapyrones B and C, respectively, which have previously been isolated from the blackleg fungus *Phoma lingam*.[8] Kojic acid (**6**) is a common microbial metabolite produced by *Aspergillus*,[13] *Penicillium*,[14] and *Alternaria* spp.[15] Compounds **7**-**12** were identified as phomaligol A (**7**), phomaligol $A_1$ (**8**), methylphomaligol A (**9**), acetylphomaligol A (**10**), phomaligol A hydroperoxide (**11**), and phomaligol $A_1$ hydroperoxide (**12**), respectively. Compounds **7**, **8**, **11**, and **12** were previously isolated from *Phoma lingam* and *Phoma wasabiae*, while compounds **9** and **10** were reported as synthetic analogues.[16-19] The relative configurations of C-2 and C-6 in compounds **7**-**12** were deduced as either *cis* or *trans* by comparison of chemical shift values of C-2 hydroxyl (*trans*: $\delta_H$ 2.80, *cis*: $\delta_H$ 3.58) and hydroperoxyl (*trans*: $\delta_H$ 8.60, *cis*: $\delta_H$ 8.73) protons and the absolute configuration was deduced by comparison of optical rotation with reported values.

Compound **13** was obtained as a yellow oil. The HRFABMS of **13** supported the molecular formula $C_{15}H_{22}O_7$. The exact mass of the $[M + Na]^+$ ion (*m/z* 337.1266) matched well with the expected formula $C_{15}H_{22}O_7Na$ ($\Delta$+0.3 mmu). The $^1H$ NMR spectrum of **13** (Table 1) showed signals for a *sec*-butyl group ($\delta_H$ 0.92/H-10, 1.11/H-11, 1.62/Ha-9, 1.46/Hb-9, 2.40/H-8), two methyl groups ($\delta_H$ 1.81/H-13; 1.32/H-14), and two methoxyl groups ($\delta_H$ 4.08/H-12; 3.82/H-15). Four olefinic carbons ($\delta_C$ 177.8/C-3, 172.3/C-4, C-5, and 115.5/C-2), a ketone carbonyl carbon ($\delta_C$ 202.2/C-1), an ester carbonyl carbon ($\delta_C$ 176.5/C-7), an oxygenated quaternary carbon ($\delta_C$ 83.0/C-6), and two methoxy carbons ($\delta_C$ 59.4/C-12 and 53.3/C-15) were observed in the $^{13}C$ NMR data of **13** (Table 1). The remaining six carbon signals were attributed to a *sec*-butyl ($\delta_C$ 11.7/C-10, 16.9/C-11, 28.0/C-9, and 41.9/C-8) and two methyl groups ($\delta_C$ 20.3/C-14, 7.1/C-13). Thus, $^1H$ and $^{13}C$ NMR data suggested that **13** is a cyclohexenone derivative.[19-22] HMBC correlations were observed from $H_3$-13 ($\delta_H$ 1.81) to C-2 ($\delta_C$ 115.5), C-1 ($\delta_C$ 202.2), and C-3 ($\delta_C$ 177.8), from $H_3$-14 ($\delta_H$ 1.32) to C-1 ($\delta_C$ 202.2) and C-6 ($\delta_C$ 83.0), from $H_3$-12 ($\delta_H$ 4.08) to C-3 ($\delta_C$ 177.8) and from $H_3$-15 ($\delta_H$ 3.82) to C-5 ($\delta_C$ 172.3), confirming the presence of a cyclohexenone ring in **13** (Fig. 3). Methine proton at $\delta_H$ 2.40 (H-8) and methyl protons at $\delta_H$ 1.11 (H-11) showed HMBC correlation to the carbonyl carbon at $\delta_C$ 176.5 (C-7). In this way the partial structures were established and the number of unsaturation predicted by the molecular formula (i.e. five) was fulfilled. In the NOESY spectrum, a correlation was observed between methyl protons at $\delta_H$ 1.32 (H-14) and methoxyl protons at $\delta_H$ 3.82 (OMe-15), supporting the assignment of C-5 ($\delta$ 172.3). Thus, remaining two groups should be attached at C-4 and C-6. The 2-methylbutanoyl group was supposed to be attached to the C-6 and the hydroperoxyl group was connected to C-4 (*vide infra*). Compound **13** was optically active $[\alpha]_D^{24}$ +68 (*c* 0.04 in MeOH). The structures most similar to **13** were wasabidienone $B_1$ (**15**)[20] and methyl derivative of wasabidienone A (**16**).[21] The obtained $^1H$ NMR data of **13** (Table 1) was identical with that of **16**, except for the absence of olefinic proton signal ($\delta$ 5.47/H-4 in **16**) which was substituted by hydroperoxyl group in **13**.

The HRFABMS of compound **14** suggested the molecular formula to be $C_{15}H_{22}O_7$, the same as **13**. The exact mass of the $[M + Na]^+$ ion (*m/z* 337.1266) matched well with the expected formula $C_{15}H_{22}O_7Na$ ($\Delta$ +0.3 mmu). Comparison of the NMR spectra of both compounds **13** and **14** clearly showed an identical pattern with only slight difference in the chemical shift values (Table 1), indicating that **14** also contain cyclohexenone and 2-methylbutanoyl moieties. Compound **14** also showed positive optical rotation $[\alpha]_D^{24}$ +213 (*c* 0.08 in MeOH), which suggested that compounds **13** and **14** were not configurational isomers at C-6. Hence, the 2-methylbutanoyl group and the hydroperoxyl group were supposed to be switched in **14**. There have been reports on isolation of similar pairs of isomers such as wasabidienone E (**17**)[19,22] and phomaligin A (**18**)[19] from a single specimen. The configuration of the methylbutanoyl moiety is usually *R* in case of reported analogues. Hence, the configuration of compounds **13** and **14** was assumed to be the same as compounds **15**-**18** considering the similar optical rotation with known compounds (**13**: $[\alpha]_D^{24}$+68; **14**: $[\alpha]_D^{24}$ +213; **15**: $[\alpha]_D$ +118; **16**: $[\alpha]_D$ +142; **17**: $[\alpha]_D$ +90; **18**: $[\alpha]_D$ +139). The paucity of the samples did not allow confirming the stereochemistry of **13** and **14**.

Compounds **6**, **7**, and **9** were evaluated for the cytotoxicity against A-549 (human lung cancer), SK-OV-3 (human ovarian cancer), SK-MEL-2 (human skin cancer), XF-498 (human CNS cancer), and HCT-15 (human colon cancer) human solid tumor cell lines and found to be inactive upto a concentration of 30 $\mu$g/mL.

Fungal strains of the genus *Paecilomyces* have been reported to contain metabolites of various chemical classes such as xanthones,[23] chromones,[24] peptides,[25] alkaloids,[26] trichothecanes,[27] polyketides,[28] and anthraquinones.[29] But none of its species was found to produce cyclohexenones so far except in our present study on *Paecilomyces liacinus*. These cyclohexenones have been previously reported only from fungal genera *Phoma* and *Alternaria*, both of which belong to the family Pleosporaceae (order Pleosporales) while the genus *Paecilomyces* belong to the family Trichocomaceae (order Eurotiales). Isolation of these compounds from far distinct genera is interesting in respect of chemotaxonomic relationship among them.

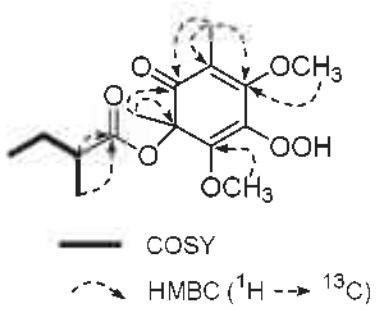


**Figure 3.** Key COSY and HMBC correlations of compound **13**

## Experimental Section

**General Procedures.** Optical rotations were measured with a Jasco P-1020 polarimeter using a 1 dm path length cell. The $^1$H NMR spectra were recorded at 500 and/or 400 MHz, 2D NMR spectra at 500 MHz and $^{13}$C NMR spectra at 100 MHz using Varian INOVA 500 and Varian UNITY 400 spectrometers. FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HRFABMS data were obtained on a JEOL JMS SX-101A spectrometer. LRESIMS data were recorded on an API-2000 LC/MS/MS spectrometer. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks ($\delta_H$ 3.30 and $\delta_C$ 49.0 for $CD_3OD$, $\delta_H$ 7.28 for $CDCl_3$). HPLC was performed with a Shodex C18M 10E (preparative, 250×10 mm, 5 $\mu$m, and 100 Å) and YMC-Pack CN (preparative, 250×10 mm, 5 $\mu$m, and 120 Å) columns using a Shodex RI-101 detector.

**Isolation and Taxonomy of the Fungal Strain.** A marine sponge *Petrosia* sp. was collected by scuba diving from waters around Jeju Island during a collection trip in October, 2004. After sterilization with 70% aq ethanol, the sponge sample was rinsed with sterile $H_2O$. The sterilized sponge was then cut into small pieces and placed on agar plates containing PMG medium: 20 g/L agar, 1 L of water (75% seawater and 25% distilled $H_2O$), penicillin-streptomycin solution (cell culture reagent, 10,000 units/mL, 5 mL/L). Fungal and yeast colonies growing out of the sponge tissue were transferred onto new agar plates containing PMG medium for sporulation. The strain (J04J-1) F-9 was identified as *Paecilomyces lilacinus* on the basis of morphology and 18S rDNA sequence.

**Cultivation.** The fungi preculture was prepared in eight 500 mL Erlenmeyer flasks, each containing 300 mL of in PMG seawater medium (peptone, 0.1%; malt extract, 2% and D-glucose, 2%) by incubation for 7 days at 30 °C and 145 rpm on a rotary shaker liquid medium. These precultures were transferred into a 2 L Erlenmeyer flasks, each containing 1.25 L of PMG seawater medium (8 flasks, each 300 mL) in the same medium and incubated under the same condition for 21 days.

**Extraction and Isolation.** The mass cultivated fungi, whole broth culture was exhaustively extracted with EtOAc (3 × 9 L) to yield a brownish material (17.1 g). The residue was redissolved in 90% aq MeOH and partitioned with *n*-hexane. The 90% aq MeOH fraction (6.5 g) was fractionated by flash column chromatography (Si gel 60, 0.015-0.040 mm, Merck) employing gradient elution from $CHCl_3$ to EtOAc to MeOH, to yield twenty fractions (500 mL each). These fractions were evaluated for activity by the brine shrimp lethality assay,[30] but none of the fraction was active. Fraction EF6 was separated by MPLC (Si gel 60, 0.015–0.040 mm, Merck) employing gradient elution from $CHCl_3$ to MeOH, to yield nine subfractions EF6.1-EF6.9. Compounds **1** (1.4 mg) and **2** (0.7 mg) were obtained by CN HPLC eluting with a solvent system of 30% aq MeOH from subfractions EF6.4 and EF6.8, respectively. Subfraction EF6.3 was subjected to CN HPLC using solvent system of 30% aq MeOH to afford compounds **3** (2.0 mg) and **5** (1.0 mg). Compound **6** (800 mg) was purified from fraction EF8 by normal-phase flash column chromatography (Si gel 60, 0.015-0.040 mm, Merck) using gradient elution from $CHCl_3$ to MeOH. Compounds **7** (10.5 mg), **8** (2.1 mg), and **13** (0.7 mg) were obtained by purification of fraction EF1. ODS HPLC chromatographic separation of the fraction EF2 eluting with a solvent system of 50% aq MeOH yielded compound **14** (0.8 mg). Compound **10** (0.8 mg) was obtained by purification of fraction EF4 by ODS HPLC, eluting with a solvent system of 50% aq MeOH. Fraction EF5 was subjected to ODS HPLC chromatographic separation eluting with a solvent system of 50% aq MeOH to yield compounds **9** (1.5 mg), **11** (0.2 mg), and **12** (0.3 mg).

**Paecilopyrone A (1):** white solid; $[\alpha]_D^{26}$ +28° (*c* 0.12 in MeOH); $^1$H and $^{13}$C NMR data, see Table 1; FABMS *m/z* 239 $[M + H]^-$, 261 $[M + Na]^-$, 283 $[M + 2Na - H]^-$; HRFABMS *m/z* 283.0916 $[M + 2Na - H]^+$ (calc. for $C_{13}H_{17}O_4Na_2$, 283.0922).

**Paecilopyrone B (2):** white solid; $[\alpha]_D^{26}$ +32° (*c* 0.05 in MeOH); $^1$H and $^{13}$C NMR data, see Table 1; ESIMS *m/z* 245 $[M + Na]^-$; HRESIMS *m/z* 221.1184 $[M-H]^-$ (calc. for $C_{13}H_{17}O_3$, 221.1183).

**Phomapyrone B (3):** white solid; $[\alpha]_D^{26}$ +40° (*c* 0.15 in MeOH); $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 5.84 (1H, brs, H-5), 3.50 (2H, overlapped, H-7), 2.64 (1H, dd, $J$ = 7.0, 6.5 Hz, H-9), 1.80 (3H, s, H-13), 1.70 (1H, m, H-10), 1.39 (1H, m, H-10), 1.08 (3H, d, $J$ = 7.0 Hz, H-12), 0.87 (3H, t, $J$ = 7.5 Hz, H-11); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ 211.0 (C-8), 179.7 (C-4), 170.0 (C-1), 155.8 (C-6), 111.0 (C-5), 96.2 (C-3), 49.0 (C-7), 48.0 (C-9), 26.8 (C-10), 16.1 (C-12), 11.5 (C-11), 8.0 (C-13); FABMS *m/z* 223 $[M- H]^-$, 225 $[M + H]^+$, 269 $[M + 2Na-H]^+$; HRFABMS *m/z* 269.0767 $[M + 2Na-H]^-$ (calc. for $C_{12}H_{16}O_4$, 269.0766).

**Phomapyrone C (5):** white solid; $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 5.80 (1H, brs, H-5), 2.40 (1H, m, H-7), 1.80 (3H, s, H-11), 1.70 (1H, m, H-8), 1.39 (1H, m, H-8), 1.20 (3H, d, $J$ = 7.0 Hz, H-10), 0.87 (3H, t, $J$ = 7.5 Hz, H-9).

**Kojic acid (6):** white crystals; $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 7.95 (1H, s, H-2), 6.49 (1H, s, H-5), 4.40 (2H, s, H-7).

**Phomaligol A (7):** yellow oil; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.56 (1H, s, H-4), 3.87 (3H, s, H-12), 2.80 (1H, br s, OH), 2.50 (1H, m, H-8), 1.73 (1H, m, H-9), 1.70 (3H, s, H-14), 1.65 (3H, s, H-13), 1.51 (1H, m, H-9), 1.17 (3H, d, $J$ = 7.0 Hz, H-11), 0.96 (3H, t, $J$ = 7.5 Hz, H-10); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ 202.5 (C-1), 191.7 (C-5), 175.8 (C-7), 173.0 (C-3), 99.9 (C-4), 81.1 (C-6), 73.5 (C-2), 56.8 (C-12), 39.8 (C-8), 26.6 (C-9), 24.1 (C-13), 23.3 (C-14), 16.1 (C-11), 11.3 (C-10); FABMS *m/z* 283 $[M-H]^-$, 285 $[M + H]^+$; HRFABMS *m/z* 285.1342 $[M + H]^-$ (calc. for $C_{16}H_{12}O_5$, 285.1338).

**Phomaligol A$_1$ (8):** yellow oil; $[\alpha]_D^{28}$ −36.4° (*c* 0.03 in MeOH); $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 5.61 (1H, s, H-4), 3.89 (3H, s, H-12), 3.58 (1H, br s, OH, $CDCl_3$), 2.44 (1H, m, H-8), 1.73 (1H, m, H-9), 1.63 (3H, s, H-14), 1.60 (3H, s, H-13), 1.51 (1H, m, H-9), 1.13(3H, d, $J$ = 7.0 Hz, H-11), 0.97 (3H, t, $J$ = 7.5 Hz, H-10).

**Methylphomaligol A (9):** yellow oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 5.53 (1H, s, H-4), 3.86 (6H, s, H-12, H-15), 2.47 (1H, m, H-8), 1.71 (1H, m, H-9), 1.66 (3H, s, H-14), 1.62 (3H, s, H-13), 1.51 (1H, m, H-9), 1.14 (3H, d, $J$ = 7.0 Hz, H-11), 0.95 (3H, t, $J$ = 7.5 Hz, H-10).

**Acetylphomaligol A (10):** yellowish oil; $[\alpha]_D^{28}$ −7.4° (*c* 0.05

in MeOH); $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.60 (1H, s, H-4), 3.88 (3H, s, H-12), 2.43 (1H, m, H-8), 1.88 (3H, s, H-16), 1.65 (1H, m, H-9), 1.63 (3H, s, H-13), 1.55 (3H, s, H-14), 1.46 (1H, m, H-9), 1.13 (3H, d, $J$ = 7.0 Hz, H-11), 0.95 (3H, t, $J$ = 7.5 Hz, H-10); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ 202.6 (C-1), 194.1 (C-5), 176.8 (C-7), 176.5 (C-3), 100.4 (C-4), 83.2 (C-6), 73.6 (C-2), 57.4 (C-12), 41.2 (C-8), 27.7 (C-9), 23.7 (C-13), 21.5 (C-14), 16.6 (C-11), 11.6 (C-10); FABMS *m/z* 349 $[M+Na]^-$; HRFABMS *m/z* 348.9233 (calc. for $C_{16}H_{22}O_7Na$, 349.1263).

**Phomaligol A hydroperoxide (11):** yellow oil; $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 5.72 (1H, s, H-4), 3.90 (3H, s, H-12), 2.42 (1H, m, H-8), 1.62 (1H, m, H-9), 1.55 (3H, s, H-14), 1.48 (3H, s, H-13), 1.45 (1H, m, H-9), 1.10 (3H, d, $J$ = 7.0 Hz, H-11), 0.91 (3H, t, $J$ = 7.5 Hz, H-10).

**Phomaligol $A_1$ hydroperoxide (12):** yellow oil; $[\alpha]_D^{28}$ −109.2° (*c* 0.02 in MeOH); $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 8.73 (1H, br s, OH, $CDCl_3$), 5.69 (1H, s, H-4), 3.90 (3H, s, H-12), 2.51 (1H, m, H-8), 1.71 (1H, m, H-9), 1.64 (3H, s, H-14), 1.62 (3H, s, H-13), 1.50 (1H, m, H-9), 1.18 (3H, d, $J$ = 7.0 Hz, H-11), 0.95 (3H, t, $J$ = 7.5 Hz, H-10).

**Phomaligol B (13):** yellow oil; $[\alpha]_D^{24}$ +68° (*c* 0.04 in MeOH); $^1$H and $^{13}$C NMR data, see Table 1; FABMS *m/z* 315 $[M+H]^+$, 337 $[M+Na]^-$, 359 $[M+2Na-H]^+$; HRFABMS *m/z* 337.1266 (calc. for $C_{15}H_{22}O_7Na$, 337.1263).

**Phomaligol C (14):** yellow oil; $[\alpha]_D^{24}$ +213° (*c* 0.08 in MeOH); $^1$H and $^{13}$C NMR data, see Table 1; FABMS *m/z* 315 $[M+H]^-$, 337 $[M+Na]^+$, 359 $[M+2Na-H]^+$; HRFABMS *m/z* 337.1266 $[M+Na]^-$ (calc. for $C_{15}H_{22}O_7Na$, 337.1263).

**Acknowledgments.** This study was supported by a grant from Pusan National University.

## References

1. Pietra, F. *Nat. Prod. Rep.* **1997**, *14*, 453.
2. Saleem, M.; Ali, M. S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y. S. *Nat. Prod. Rep.* **2007**, *24*, 1142.
3. Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143.
4. Mountfort, D. O.; Rhodes, L. L. *Appl. Environm. Microbiol.* **1991**, *57*, 1963.
5. Sarkar, J. M. *Biotech. Lett.* **1986**, *8*, 769.
6. Isogai, A.; Suzuki, A.; Higashikawa, S.; Kuyama, S.; Tamura, S. *Agric. Biol. Chem.* **1980**, *44*, 3033.
7. Mori, Y.; Tsuboi, M.; Suzuki, M.; Fukushima, K.; Arai, T. *J. Antibiotic.* **1982**, *35*, 543.
8. Pedras, M. S. C.; Morales, V. M.; Taylor, J. L. *Phytochemistry* **1994**, *36*, 1315.
9. Osterhage, C.; Kaminsky, R.; Konig, G. M.; Wright, A. D. *J. Org. Chem.* **2000**, *65*, 6412.
10. Appendino, G.; Ottino, M.; Marquez, N.; Bianchi, F.; Giana, A.; Ballero, M.; Sterner, O.; Fiebich, B. L.; Munoz, E. *J. Nat. Prod.* **2007**, *70*, 608.
11. Seibert, S. F.; Eguereva, E.; Krick, A.; Kehraus, S.; Voloshina, E.; Raabe, G.; Fleischhauer, J.; Leistner, E.; Wiese, M.; Prinz, H.; Alexandrov, K.; Janning, P.; Waldmann, H.; Konig, G. M. *Org. Biomol. Chem.* **2006**, *4*, 2233.
12. Pedras, M. S. C.; Chumala, P. B. *Phytochemistry* **2005**, *66*, 81.
13. Shiomi, K.; Hatae, K.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Kobayashi, S.; Omura, S. *J. Antibiot.* **2002**, *55*, 952.
14. Parrish, F. W.; Wiley, B. J.; Simmons, E. G.; Long, L., Jr. *Appl. Microbiol.* **1966**, *14*, 139.
15. Li, X.; Jeong, J. H.; Lee, K. T.; Pho, J. R.; Choi, H. D.; Kang, J. S.; Son, B. W. *Arch. Pharm. Res.* **2003**, *27*, 532.
16. Pedras, M. S. C.; Morales, V. M.; Taylor, J. L. *Tetrahedron* **1993**, *49*, 8317.
17. Pedras, M. S. C. *Can. J. Chem.* **1996**, *74*, 1597.
18. Soga, O.; Iwamoto, H.; Hata, K.; Maeba, R.; Takuwa, A.; Fujiwara, T.; Hsu, Y. H.; Nakayama, M. *Agric. Biol. Chem.* **1988**, *52*, 865.
19. Pedras, M. S. C.; Taylor, J. L.; Morales, V. M. *Phytochemistry* **1995**, *38*, 1215.
20. Omolo, J. O.; Anke, H.; Chhabra, S.; Sterner, O. *J. Nat. Prod.* **2000**, *63*, 975.
21. Soga, O.; Iwamoto, H.; Takuwa, A.; Nozaki, H.; Kuramoto, J.; Nakayama, M. *Agric. Biol. Chem.* **1987**, *51*, 283.
22. Soga, O.; Iwamoto, H.; Ota, Y.; Odoi, M.; Saito, K.; Takuwa, A.; Nakayama, M. *Chem. Lett.* **1987**, *51*, 815.
23. Wen, L.; Lin, Y. C.; She, Z. G.; Du, D. S.; Chan, W. L.; Zheng, Z. H. *J. Asian Nat. Prod. Res.* **2008**, *10*, 133.
24. Guo, Z.; She, Z.; Shao, C.; Wen, L.; Fan, Z.; Lin, Y. *Mag. Res. Chem.* **2007**, *45*, 777.
25. Isaka, M.; Palasarn, S.; Lapanun, S.; Sriklung, K. *J. Nat. Prod.* **2007**, *70*, 675.
26. Cheng, Y.; Schneider, B.; Riese, U.; Schubert, B.; Li, Z.; Hamburger, M. *J. Nat. Prod.* **2006**, *69*, 436.
27. Kikuchi, H.; Miyagawa, Y.; Nakamura, K.; Sahasi, Y.; Inatomi, S.; Oshima, Y. *Org. Lett.* **2004**, *6*, 4531.
28. Rahbk, L.; Sperry, S.; Piper, J. E.; Crews, P. *J. Nat. Prod.* **1998**, *61*, 1571.
29. Fredenhagen, A.; Hug, P.; Sauter, H.; Peter, H. H. *J. Antibiot.* **1995**, *48*, 199.
30. Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31.