

## $\alpha$ -Pyrones and Yellow Pigments from the Sponge-Derived Fungus *Paecilomyces lilacinus*

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New  $\alpha$ -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  $\mu\text{g/mL}$ .

**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.<sup>1,2</sup> In addition, some of the bioactive compounds isolated from marine invertebrates were suggested to be produced by fungi associated with them.<sup>3</sup> 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.<sup>4</sup> Polysaccharides constituted of glucose and galactose, and peptidal antibiotics (P168 and leucinostatin A) have been reported from *Paecilomyces lilacinus*.<sup>5–7</sup> 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

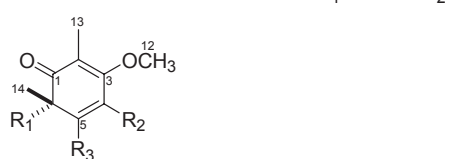
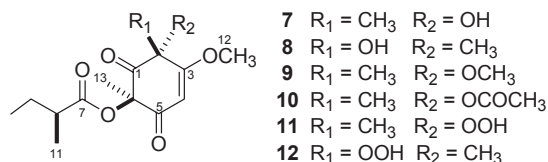
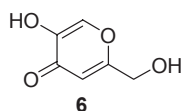
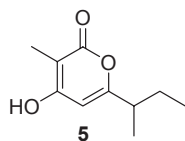
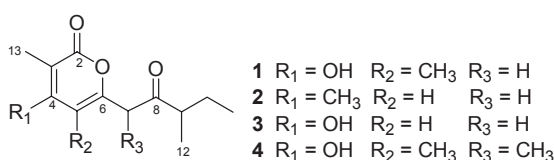
*Petrosia* sp., collected from Jeju Island, South Korea, 2004. The fungal isolate was cultured for 3 weeks at 35 °C in a liquid PMG seawater-based medium (peptone, 0.1%; malt extract, 2%; D-glucose, 2%). The EtOAc extract of the whole broth culture (9 L) was partitioned between 90% aqueous MeOH and *n*-hexane. The aqueous MeOH soluble portions were purified by <sup>1</sup>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<sub>13</sub>H<sub>18</sub>O<sub>4</sub>, was established by HRFABMS ([M+2Na–H]<sup>+</sup>, *m/z* 283.0916,  $\Delta$ –0.6 mmu) indicating the presence of five degrees of unsaturation. The  $\alpha$ -pyrone ring of **1** was characterized by two methyl signals at  $\delta_{\text{H}}$  1.85, (H<sub>3</sub>–13) and 1.84 (H<sub>3</sub>–14), as well as by the diagnostic carbon chemical shifts of C-3 ( $\delta_{\text{C}}$  96.1), C-5 ( $\delta_{\text{C}}$  114.6), C-6 ( $\delta_{\text{C}}$  150.6), C-2 ( $\delta_{\text{C}}$  168.2), and C-4 ( $\delta_{\text{C}}$  175.6) (Table 1). The spectroscopic NMR data of the  $\alpha$ -pyrone ring was in a good agreement with reported values.<sup>8–10</sup> The <sup>1</sup>H NMR spectrum of **1** also showed additional signals of two methyl groups ( $\delta_{\text{H}}$  0.87/H-11, 1.07/H-12), two methylene groups ( $\delta_{\text{H}}$  1.40, 1.70/H-10, 3.58/H-7) and a methine proton ( $\delta_{\text{H}}$  2.64/H-9). The COSY spectrum showed coupling between terminal methyl protons ( $\delta_{\text{H}}$  0.87/H-11) and methylene protons ( $\delta_{\text{H}}$  1.40, 1.70/H-10) which were coupled with a methine proton at  $\delta_{\text{H}}$  2.64 (H-9), which in turn coupled with a secondary methyl group ( $\delta_{\text{H}}$  1.07/H-12) suggesting a presence of *sec*-butyl group in **1**. In the HMBC spectrum, correlation was observed from the methine proton ( $\delta_{\text{H}}$  2.64/H-9) to a ketone carbon at  $\delta_{\text{C}}$  210.0 (C-8). Furthermore, HMBC correlations from H-13 ( $\delta_{\text{H}}$  1.85) to C-2 ( $\delta_{\text{C}}$  168.2), C-3 ( $\delta_{\text{C}}$  96.1), and C-4 ( $\delta_{\text{C}}$  175.6), and from H-14 ( $\delta_{\text{H}}$  1.84) to

<sup>a</sup>These authors equally contributed to this work.

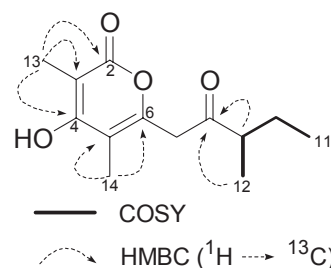
**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data (500 MHz,  $\text{CD}_3\text{OD}$ ) of Compounds **1**, **2**, **13**, and **14**<sup>a</sup>

position	<b>1</b>		<b>2</b>		<b>13</b>		<b>14</b>	
	$\delta_{\text{C}}^b$	$\delta_{\text{H}}$	$\delta_{\text{C}}^b$	$\delta_{\text{H}}$	$\delta_{\text{C}}^b$	$\delta_{\text{H}}$	$\delta_{\text{C}}^b$	$\delta_{\text{H}}$
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)

<sup>a</sup>Multiplicities and coupling constants are in parentheses. <sup>b</sup>Assignments based on HMBC and HSQC spectroscopic data.**Figure 1.** Structures of compounds **1**–**18**.

C-5 ( $\delta_{\text{C}}$  114.6) and C-6 ( $\delta_{\text{C}}$  150.6) supported the assignment of an  $\alpha$ -pyrone ring (Fig. 2). Thus, two partial structures, an  $\alpha$ -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 ( $\delta_{\text{H}}$  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**),<sup>8</sup> ascalipyrene,<sup>9</sup> and micropyrene (**4**).<sup>10</sup> The only difference between **1** and micropyrene (**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  $\text{C}_{13}\text{H}_{18}\text{O}_3$  on the basis of HRESIMS and NMR data. The exact mass of the  $[\text{M}-\text{H}]^-$  ion ( $m/z$  221.1184) matched well with the expected molecular formula  $\text{C}_{13}\text{H}_{17}\text{O}_3$  ( $\Delta +0.1$  mmu), which was 16 amu lesser than **1**. The  $^1\text{H}$  NMR spectrum of **2** was basically the same as that of **1**, except for the presence of an olefinic proton signal at  $\delta_{\text{H}}$  6.03 (H-5) indicating a trisubstituted pyrone moiety. The  $^1\text{H}$  NMR spectrum of **2** exhibited two vinyl methyl groups ( $\delta_{\text{H}}$  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 ( $\delta_{\text{C}}$  110.0) and C-7 ( $\delta_{\text{C}}$

**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.<sup>11</sup> It was recently confirmed that the 2-pyrone isolated from fungi are of polyketide origin and those from marine mollusc are polypropionates.<sup>12</sup>

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*.<sup>8</sup> Kojic acid (**6**) is a common microbial metabolite produced by *Aspergillus*,<sup>13</sup> *Penicillium*,<sup>14</sup> and *Alternaria* spp.<sup>15</sup> Compounds **7–12** were identified as phomaligol A (**7**), phomaligol A<sub>1</sub> (**8**), methylphomaligol A (**9**), acetylphomaligol A (**10**), phomaligol A hydroperoxide (**11**), and phomaligol A<sub>1</sub> 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.<sup>16–19</sup> 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*:  $\delta_{\text{H}}$  2.80, *cis*:  $\delta_{\text{H}}$  3.58) and hydroperoxyl (*trans*:  $\delta_{\text{H}}$  8.60, *cis*:  $\delta_{\text{H}}$  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<sub>15</sub>H<sub>22</sub>O<sub>7</sub>. The exact mass of the [M + Na]<sup>+</sup> ion (*m/z* 337.1266) matched well with the expected formula C<sub>15</sub>H<sub>22</sub>O<sub>7</sub>Na ( $\Delta$ +0.3 mmu). The <sup>1</sup>H NMR spectrum of **13** (Table 1) showed signals for a *sec*-butyl group ( $\delta_{\text{H}}$  0.92/H-10, 1.11/H-11, 1.62/Ha-9, 1.46/Hb-9, 2.40/H-8), two methyl groups ( $\delta_{\text{H}}$  1.81/H-13; 1.32/H-14), and two methoxyl groups ( $\delta_{\text{H}}$  4.08/H-12; 3.82/H-15). Four olefinic carbons ( $\delta_{\text{C}}$  177.8/C-3, 172.3/C-4, C-5, and 115.5/C-2), a ketone carbonyl carbon ( $\delta_{\text{C}}$  202.2/C-1), an ester carbonyl carbon ( $\delta_{\text{C}}$  176.5/C-7), an oxygenated quaternary carbon ( $\delta_{\text{C}}$  83.0/C-6), and two methoxy carbons ( $\delta_{\text{C}}$  59.4/C-12 and 53.3/C-15) were observed in the <sup>13</sup>C NMR data of **13** (Table 1). The remaining six carbon signals were attributed to a *sec*-butyl ( $\delta_{\text{C}}$  11.7/C-10, 16.9/C-11, 28.0/C-9, and 41.9/C-8) and two methyl groups ( $\delta_{\text{C}}$  20.3/C-14, 7.1/C-13). Thus, <sup>1</sup>H and <sup>13</sup>C NMR data suggested that **13** is a cyclohexenone derivative.<sup>19–22</sup> HMBC correlations were observed from H<sub>3</sub>-13 ( $\delta_{\text{H}}$  1.81) to C-2 ( $\delta_{\text{C}}$  115.5), C-1 ( $\delta_{\text{C}}$  202.2), and C-3 ( $\delta_{\text{C}}$  177.8), from H<sub>3</sub>-14 ( $\delta_{\text{H}}$  1.32) to C-1 ( $\delta_{\text{C}}$  202.2) and C-6 ( $\delta_{\text{C}}$  83.0), from H<sub>3</sub>-12 ( $\delta_{\text{H}}$  4.08) to C-3 ( $\delta_{\text{C}}$  177.8) and from H<sub>3</sub>-15 ( $\delta_{\text{H}}$  3.82) to C-5 ( $\delta_{\text{C}}$  172.3), confirming the presence of a cyclohexenone ring in **13** (Fig. 3). Methine proton at  $\delta_{\text{H}}$  2.40 (H-8) and methyl protons at  $\delta_{\text{H}}$  1.11 (H-11) showed HMBC correlation to the carbonyl carbon at  $\delta_{\text{C}}$  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  $\delta_{\text{H}}$  1.32 (H-14) and methoxyl protons at  $\delta_{\text{H}}$  3.82 (OMe-15), supporting the assignment of C-5 ( $\delta_{\text{C}}$  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 [ $\alpha$ ]<sub>D</sub><sup>24</sup> +68 (*c* 0.04 in MeOH). The structures most similar to **13** were wasabidienone B<sub>1</sub> (**15**)<sup>20</sup> and methyl derivative of wasabidienone A (**16**).<sup>21</sup> The obtained <sup>1</sup>H 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<sub>15</sub>H<sub>22</sub>O<sub>7</sub>, the same as **13**. The exact mass of the [M + Na]<sup>+</sup> ion (*m/z* 337.1266) matched well with the expected formula C<sub>15</sub>H<sub>22</sub>O<sub>7</sub>Na ( $\Delta$ +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 [ $\alpha$ ]<sub>D</sub><sup>24</sup> +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**)<sup>19,22</sup> and phomaligin A (**18**)<sup>19</sup> 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**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> +68; **14**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> +213; **15**: [ $\alpha$ ]<sub>D</sub> +118; **16**: [ $\alpha$ ]<sub>D</sub> +142; **17**: [ $\alpha$ ]<sub>D</sub> +90; **18**: [ $\alpha$ ]<sub>D</sub> +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  $\mu\text{g/mL}$ .

Fungal strains of the genus *Paecilomyces* have been reported to contain metabolites of various chemical classes such as xanthenes,<sup>23</sup> chromones,<sup>24</sup> peptides,<sup>25</sup> alkaloids,<sup>26</sup> trichothecanes,<sup>27</sup> polyketides,<sup>28</sup> and anthraquinones.<sup>29</sup> 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.

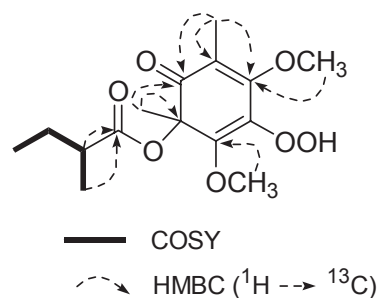


Figure 3. Key COSY and HMBC correlations of compound **13**



## Experimental Section

**General Procedures.** Optical rotations were measured with a Jasco P-1020 polarimeter using a 1 dm path length cell. The  $^1\text{H}$  NMR spectra were recorded at 500 and/or 400 MHz,  $^2\text{D}$  NMR spectra at 500 MHz and  $^{13}\text{C}$  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 ( $\delta_{\text{H}}$  3.30 and  $\delta_{\text{C}}$  49.0 for  $\text{CD}_3\text{OD}$ ,  $\delta_{\text{H}}$  7.28 for  $\text{CDCl}_3$ ). HPLC was performed with a Shodex C18M 10E (preparative,  $250 \times 10$  mm,  $5 \mu\text{m}$ , and  $100 \text{ \AA}$ ) and YMC-Pack CN (preparative,  $250 \times 10$  mm,  $5 \mu\text{m}$ , and  $120 \text{ \AA}$ ) 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  $\text{H}_2\text{O}$ . 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  $\text{H}_2\text{O}$ ), 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  $30^\circ\text{C}$  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  $\text{EtOAc}$  ( $3 \times 9$  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  $\text{CHCl}_3$  to  $\text{EtOAc}$  to MeOH, to yield twenty fractions (500 mL each). These fractions were evaluated for activity by the brine shrimp lethality assay,<sup>30</sup> 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  $\text{CHCl}_3$  to MeOH, to yield nine sub-fractions 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  $\text{CHCl}_3$  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;  $[\alpha]_{\text{D}}^{26} +28^\circ$  (*c* 0.12 in MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; FABMS  $m/z$  239  $[\text{M} + \text{H}]^+$ , 261  $[\text{M} + \text{Na}]^+$ , 283  $[\text{M} + 2\text{Na} - \text{H}]^+$ ; HRFABMS  $m/z$  283.0916  $[\text{M} + 2\text{Na} - \text{H}]^+$  (calc. for  $\text{C}_{13}\text{H}_{17}\text{O}_4\text{Na}_2$ , 283.0922).

**Paecilopyrone B (2):** white solid;  $[\alpha]_{\text{D}}^{26} +32^\circ$  (*c* 0.05 in MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; ESIMS  $m/z$  245  $[\text{M} + \text{Na}]^+$ ; HRESIMS  $m/z$  221.1184  $[\text{M} - \text{H}]^-$  (calc. for  $\text{C}_{13}\text{H}_{17}\text{O}_3$ , 221.1183).

**Phomapyrone B (3):** white solid;  $[\alpha]_{\text{D}}^{26} +40^\circ$  (*c* 0.15 in MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 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);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 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  $[\text{M} - \text{H}]^-$ , 225  $[\text{M} + \text{H}]^+$ , 269  $[\text{M} + 2\text{Na} - \text{H}]^+$ ; HRFABMS  $m/z$  269.0767  $[\text{M} + 2\text{Na} - \text{H}]^+$  (calc. for  $\text{C}_{12}\text{H}_{16}\text{O}_4$ , 269.0766).

**Phomapyrone C (5):** white solid;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 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;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 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;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 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);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 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  $[\text{M} - \text{H}]^-$ , 285  $[\text{M} + \text{H}]^+$ ; HRFABMS  $m/z$  285.1342  $[\text{M} + \text{H}]^+$  (calc. for  $\text{C}_{16}\text{H}_{12}\text{O}_7$ , 285.1338).

**Phomaligol A<sub>1</sub> (8):** yellow oil;  $[\alpha]_{\text{D}}^{28} -36.4^\circ$  (*c* 0.03 in MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  5.61 (1H, s, H-4), 3.89 (3H, s, H-12), 3.58 (1H, br s, OH,  $\text{CDCl}_3$ ), 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;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 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;  $[\alpha]_{\text{D}}^{28} -7.4^\circ$  (*c* 0.05

in MeOH);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 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);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 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  $[\text{M} + \text{Na}]^+$ ; HRFABMS  $m/z$  348.9233 (calc. for  $\text{C}_{16}\text{H}_{22}\text{O}_7\text{Na}$ , 349.1263).

**Phomaligol A hydroperoxide (11):** yellow oil;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 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<sub>1</sub> hydroperoxide (12):** yellow oil;  $[\alpha]_{\text{D}}^{28} -109.2^\circ$  ( $c$  0.02 in MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  8.73 (1H, br s, OH,  $\text{CDCl}_3$ ), 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;  $[\alpha]_{\text{D}}^{24} +68^\circ$  ( $c$  0.04 in MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; FABMS  $m/z$  315  $[\text{M} + \text{H}]^+$ , 337  $[\text{M} + \text{Na}]^+$ , 359  $[\text{M} + 2\text{Na} - \text{H}]^+$ ; HRFABMS  $m/z$  337.1266 (calc. for  $\text{C}_{15}\text{H}_{22}\text{O}_7\text{Na}$ , 337.1263).

**Phomaligol C (14):** yellow oil;  $[\alpha]_{\text{D}}^{24} +213^\circ$  ( $c$  0.08 in MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; FABMS  $m/z$  315  $[\text{M} + \text{H}]^+$ , 337  $[\text{M} + \text{Na}]^+$ , 359  $[\text{M} + 2\text{Na} - \text{H}]^+$ ; HRFABMS  $m/z$  337.1266  $[\text{M} + \text{Na}]^+$  (calc. for  $\text{C}_{15}\text{H}_{22}\text{O}_7\text{Na}$ , 337.1263).

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