

## New Lactone-Containing Metabolites from a Marine-Derived Bacterium of the Genus *Streptomyces*

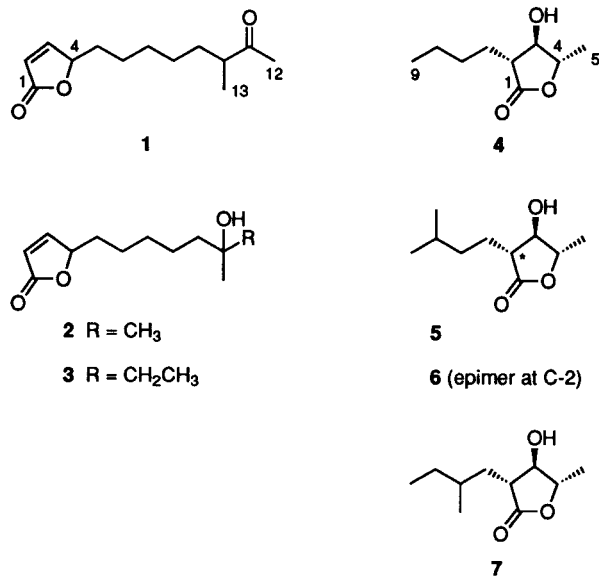
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Six novel metabolites containing a lactone moiety as a common structural feature, along with the previously described (–)-blastmycinolactol, have been isolated from the cultivation broth of a bacterium of the genus *Streptomyces* isolated from marine sediment. On the basis of the results of combined spectroscopic analysis, the structures of the new compounds have been determined as butenolides and 3-hydroxy- $\gamma$ -butyrolactones.

Microorganisms derived from marine environments are widely recognized as an emerging source of novel natural products.<sup>1–3</sup> In recent years, numerous metabolites possessing uncommon structures and potent bioactivity have been isolated from strains of bacteria and fungi collected from diverse marine environments, such as animals, plants, and sediments.<sup>4</sup> During the course of our search for biologically active substances from marine-derived actinomycetes, we isolated a strain of the genus *Streptomyces* from shallow-water sediment rich with decaying organic materials collected from Tongyoung Bay, Korea.<sup>5</sup> The organic extract from the culture broth of this strain exhibited significant antifungal activity (in paper disk method, inhibition zone 19 mm at the concentration of 50  $\mu$ g/disk) against *Candida albicans*. We describe herein the structure determination of six new lactone-containing metabolites along with the previously reported (–)-blastmycinolactol.<sup>6–8</sup> Compounds **1–3** are butenolides possessing branched side chains, while **4–7** are 3-hydroxy- $\gamma$ -butyrolactones, structurally related to the antifungal agents of the antimycin class.<sup>9</sup>



The strain M02750 was fermented in a 3 L Fernbach flask for 10 days. The culture broth was filtered through a

membrane and subjected to HP 20 adsorption chromatography. The fractions eluted with MeOH and acetone were combined and repeatedly solvent/solvent partitioned. The moderately polar fractions were separated by LH 20 gel filtration chromatography followed by reversed-phase HPLC to yield MKN-003A to -003C (**1–3**) and MKN-004A to -004D (**4–7**) as pure metabolites.

MKN-003A (**1**) was isolated as a colorless gum which analyzed for C<sub>13</sub>H<sub>20</sub>O<sub>3</sub> on the basis of its combined HREIMS and <sup>13</sup>C NMR features. The <sup>13</sup>C NMR data of this compound contained signals of quaternary carbons at  $\delta$  212.8 and 173.1 (Table 1). Coupled with the absorption bands at 1710 and 1755 cm<sup>-1</sup> in the IR spectrum, these were readily interpreted as being due to a ketone and an ester, respectively. The <sup>13</sup>C NMR data also displayed signals of olefinic carbons at  $\delta$  156.2 (CH) and 121.4 (CH). Consideration of the molecular formula revealed that **1** had an additional degree of unsaturation, hence a cyclic framework.

A combination of <sup>1</sup>H COSY and <sup>1</sup>H TOCSY data revealed that all of the protons except for an isolated methyl proton at  $\delta$  2.11 (3H, s) were spin-coupled throughout the entire molecule. The chemical shift of this isolated methyl group indicated its direct attachment to a carbonyl group. This interpretation was consistent with the gradient HSQC and gradient HMBC data in which carbon–proton correlations assigned a methyl ketone group at the terminus of molecule as well as its connectivity to a methine ( $\delta_{\text{H}}$  2.47,  $\delta_{\text{C}}$  47.0). On the other hand, long-range correlations of the ester carbon at  $\delta$  173.1 with the olefinic protons at  $\delta$  7.43 and 6.08, combined with the proton spin couplings of these with a methine at  $\delta$  5.01 in the <sup>1</sup>H COSY data, revealed the presence of a  $\gamma$ -oxygenated  $\alpha,\beta$ -unsaturated ester functionality. Although it was not directly evidenced by the gradient HMBC experiment, consideration of the molecular formula as well as the small coupling constant between the olefinic protons ( $J_{2,3} = 5.7$  Hz) indicated the presence of an oxygen bridge between the ester and methine carbon, thereby forming a five-membered ring. Thus, the structure of MKN-003A (**1**) was determined as a metabolite consisting of a butenolide moiety and a branched chain. A literature study revealed that butenolides are frequently incorporated into the natural products of terrestrial plants, microorganisms, and marine animals.<sup>4,10,11</sup>

The molecular formula of MKN-003B (**2**) was deduced as C<sub>12</sub>H<sub>20</sub>O<sub>3</sub> by combined HRFABMS and <sup>13</sup>C NMR analysis. The spectral data of this compound were highly compatible with those obtained for **1**. Preliminary exami-

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**Table 1.** Carbon NMR Assignments of Compounds **1–7** in CDCl<sub>3</sub>

position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>
1	173.1 (s)	173.1 (s)	173.1 (s)	175.8 (s)	176.4 (s)	177.0 (s)	176.3 (s)
2	121.4 (d)	121.6 (d)	121.4 (d)	48.6 (d)	48.8 (d)	43.9 (d)	46.4 (d)
3	156.2 (d)	156.2 (d)	156.3 (d)	79.1 (d)	78.9 (d)	73.8 (d)	79.8 (d)
4	83.2 (d)	83.3 (d)	83.3 (d)	80.0 (d)	80.1 (d)	82.3 (d)	79.9 (d)
5	32.9 (t)	33.1 (t)	33.0 (t)	18.2 (q)	18.2 (q)	18.1 (q)	18.3 (q)
6	24.7 (t)	25.0 (t)	24.9 (t)	29.2 (t)	26.3 (t)	21.2 (t)	36.2 (t)
7	29.2 (t)	29.8 (t)	29.8 (t)	28.9 (t)	35.7 (t)	36.8 (t)	31.7 (d)
8	26.8 (t)	24.1 (t)	23.5 (t)	22.6 (t)	28.1 (d)	28.1 (d)	28.8 (t)
9	32.5 (t)	43.7 (t)	41.0 (t)	13.8 (q)	22.4 (q)	22.5 (q)	11.0 (q)
10	47.0 (d)	70.9 (s)	72.7 (s)		22.4 (q)	22.4 (q)	19.2 (q)
11	212.8 (s)	29.3 (q)	34.1 (t)				
12	27.9 (q)	29.2 (q)	8.1 (q)				
13	16.2 (q)		26.3 (q)				

nation of the NMR data showed that **2** had the same butenolide moiety as **1**. In addition to the loss of a carbon signal in the <sup>13</sup>C NMR spectra of **2**, however, the signal of the ketone carbon of **1** was replaced by that of a quaternary carbon at  $\delta$  70.9. A corresponding difference was also observed in the <sup>1</sup>H NMR spectrum in which the signal of the methyl protons at  $\delta$  2.11 was shifted upfield at  $\delta$  1.21. With this information, detailed interpretation of 2D NMR data defined the structure of MKN-003B (**2**) as a butenolide containing a dimethyl carbinol group at the terminus of the side chain.

MKN-003C (**3**) was assigned the molecular formula C<sub>13</sub>H<sub>22</sub>O<sub>3</sub> by HRFABMS and <sup>13</sup>C NMR spectrometry. The spectral data of this compound were very similar to those of **2**. In particular, the <sup>1</sup>H and <sup>13</sup>C NMR spectra of this compound, except for the signals corresponding to an upfield methylene, were almost identical to those of **2**. This upfield methylene was placed at C-11 on the basis of the results of <sup>1</sup>H COSY and gradient HMBC experiments. Thus, the structure of MKN-003C (**3**) was defined as a butenolide containing a branched side chain.

In addition to the butenolides **1–3**, the strain M02750 produced metabolites of another structural class. MKN-004A (**4**) was isolated as a colorless gum which analyzed for C<sub>9</sub>H<sub>16</sub>O<sub>3</sub> on the basis of HRCIMS and <sup>13</sup>C NMR features. On the basis of the results of combined 2D NMR data, the structure of **4** was proved to be 2-butyl-3-hydroxy-4-methyl- $\gamma$ -butyrolactone. A literature study revealed that compound **4** was indeed (–)-blastmycinolactol, reported as a metabolite of the antimycin-producing *Streptomyces* spp.<sup>6</sup> Comparison of the spectral data including the specific rotation of **4** showed good agreement with those reported previously.<sup>7,8</sup> Since these are frequently found in bioactive natural products, 3-hydroxy- $\gamma$ -butyrolactone and similar ring systems have received considerable synthetic attention.<sup>11–14</sup>

The molecular formula of the related metabolite MKN-004B (**5**) was deduced as C<sub>10</sub>H<sub>18</sub>O<sub>3</sub> by combined HRFABMS and <sup>13</sup>C NMR analysis. Except for the appearance of signals corresponding to an additional methyl group, the NMR data of this compound were very similar to those obtained for **4**. The newly incorporated methyl group was determined to be attached at C-8, hence forming a terminal isopropyl group, on the basis of the <sup>1</sup>H COSY and gradient HMBC data. A ROESY experiment showed strong cross-peaks at H-2/H-4 and H-3/H-5. Thus, coupled with a comparison of the specific rotation ( $[\alpha]_D$  –10.5° and –10.1° for **4** and **5**, respectively), the absolute configurations at the asymmetric carbon centers were assigned as 2*R*, 3*R*, and 4*S*, identical to (–)-blastmycinolactol.<sup>7,8</sup>

MKN-004C (**6**) was obtained as a colorless gum which analyzed for the same molecular formula, C<sub>10</sub>H<sub>18</sub>O<sub>3</sub>, as compound **5** by HRFABMS and <sup>13</sup>C NMR spectrometry.

Detailed interpretation of the <sup>1</sup>H COSY, gradient HSQC, and gradient HMBC data showed that **5** and **6** had identical planar structures. However, chemical shifts of C-2, C-3, and C-6 in the <sup>13</sup>C NMR data of **6** were significantly shifted upfield (Table 1). In the <sup>1</sup>H NMR data, differences of about 0.3 ppm were observed for the chemical shifts of H-3 and H-4. These compounds also differed considerably in the specific rotation;  $[\alpha]_D$  –10.1° and 1.1° for **5** and **6**, respectively. A ROESY experiment of **6** displayed cross-peaks between H-5 and both H-3 and H-4, suggesting the *cis* orientation of these on the lactone ring. The <sup>13</sup>C NMR measurement of synthetic blastmycinolactol analogues showed that the chemical shifts of C-2–C-6 were significantly influenced by the configurations at the asymmetric carbon centers, thereby indicative of the stereochemistry of the 3-hydroxy- $\gamma$ -butyrolactone system.<sup>12,13</sup> Comparison of the <sup>13</sup>C NMR data of **6** with those of synthetic compounds revealed that the chemical shifts of C-1–C-6 were consistent with those of 2-epiblastmycinolactol. Thus, the structure of MKN-004C (**6**) was defined as the C-2 epimer of MKN-004B (**5**).

The molecular formula of MKN-004D (**7**) was established as C<sub>13</sub>H<sub>22</sub>O<sub>3</sub> by HRFABMS and <sup>13</sup>C NMR spectrometry. Combined 2D NMR experiments defined the structure of **7** as a derivative of **5** possessing an additional methyl group at C-7. The absolute configurations at the asymmetric centers were assigned as 2*R*, 3*R*, 4*S*, identical to **4** and **5**, on the basis of the ROESY cross-peaks at H-2/H-4 and H-3/H-5 as well as comparison of the specific rotation ( $[\alpha]_D$  –8.3° for **7**) with other metabolites.

Both the crude extract of M02750 and LH 20 chromatographic fractions containing compounds **1–7** showed remarkable antifungal activity against *C. albicans*. However, the same measurement using pure metabolites revealed that the bioactivity was attributable to neither the butenolides nor butyrolactones. A plausible explanation is the presence of antimycins or related antifungal agents since literature studies revealed that (–)-blastmycinolactol was a hydration product of the potent antifungal agent antimycin A<sub>3</sub> (=blastmycin) and both metabolites frequently occurred together.<sup>6–8</sup> However, careful examination of the <sup>1</sup>H NMR spectra did not indicate these metabolites to be in the crude extract or its chromatographic fractions. Furthermore, production of several novel 3-hydroxy- $\gamma$ -butyrolactones and butenolides distinguished the strain M02750 from the previously described antimycin-producing *Streptomyces* spp. and suggested the presence of another class of antifungal substances which remained to be identified.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO digital polarimeter using a 5 cm

cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. NMR spectra were recorded in CDCl<sub>3</sub> solutions containing Me<sub>4</sub>Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were provided by the Mass Spectrometry Facility (compounds **1** and **4**), Department of Chemistry, University of California, Riverside, and Korea Basic Science Institute (**2**, **3**, **5**–**7**), Taejeon, Korea. GC analysis was performed on a Hewlett-Packard HP5890-II plus chromatograph equipped with FID as detector. All solvents used were spectral grade or were distilled from glass prior to use.

**Collection and Taxonomic Identification.** The bacterial strain M02750 was isolated from underwater sediment (depth 25 m) rich with decaying organic materials collected off the coast of Tongyoung Bay, Korea, in December 1995. The strain was maintained on a yeast extract–malt extract agar containing 70% natural seawater. The colony morphology of the strain M02750 grown on ISP-5 agar at 30 °C for 3 days was round, regular, entire, and matt type olive green vegetative, having a white aerial mycelium with a short chain of conidia sporopore. The strain was Gram positive and immobile. The temperature for optimum growth was at 30 °C, and the strain did not grow at 55 and 10 °C. A scanning electron microscope picture of the strain showed strangled chains tangled with numerous wrinkled and convex spores.

For the identification of this strain, its biochemical properties were examined according to *Bergey's Manual of Determinative Bacteriology*, and the physiological characteristics including the utilization of carbon sources were examined following the method developed by Pridham and Gottlieb.<sup>15,16</sup> Casein, gelatin, and starch were degraded by M02750. However, this strain could not utilize D-glucose, D-xylose, L-arabinose, and cellulose. From an analysis of cell wall materials, only the meso form of diaminopimelic acid was found and a cellular sugar analysis with TLC showed the presence of only arabinose.

For the fatty acid composition, the mycelium of M02750 (dry weight 0.1 g) was extracted with Folch solution (CHCl<sub>3</sub>/MeOH, 2:1, 3 × 2 mL) and saponified. Fatty acid methyl esters were prepared with 5% HCl in MeOH at 80 °C for 1 h, extracted with *n*-hexane, concentrated, and analyzed with a gas chromatograph equipped with an Omegawax-320 capillary column (0.32 mm × 30 m, Supelco). Cellular fatty acid profile revealed anteisopentadecanoic acid (a-C15:0, 22.8%) and palmitic acid (C16:0, 30.8%) as the major fatty acids. On the basis of these biochemical and physiological characteristics, the strain M02750 was assigned to the genus *Streptomyces*. The strain is currently on deposit in the Microbial Collection, KORDI, under the curatorship of K.W.C.

**Fermentation.** The slant culture of M02750 was inoculated in a 500 mL Erlenmeyer flask containing 100 mL of SYP medium (starch 1%, yeast extract 0.4%, peptone 0.2%) in 50% aged seawater, adjusted at pH 8.0 by 1 M Tris buffer, and incubated at 27 °C for 4 days on a rotary shaker (150 rpm). Fermentation was carried out by transfer of 5 mL of the seed culture to a 3 L Fernbach flask containing 0.8 L of the same medium and incubation for 10 days under similar conditions.

**Isolation and Purification.** The combined fermentation broth (80 L) was filtered using a membrane (pore size 0.45 μm) in a tangential filter system. The filtered broth was subjected to HP 20 adsorption chromatography sequentially using H<sub>2</sub>O, 50% aqueous MeOH, 50% aqueous acetone, MeOH, and finally acetone. The fractions eluted with MeOH and acetone were combined, evaporated to dryness in vacuo (5.92 g), and partitioned between H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub>. Then the latter phase was re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (850 mg) was separated by LH 20 gel filtration chromatography eluting with a mixture of hexane, CH<sub>2</sub>Cl<sub>2</sub>, and MeOH (50:45:5, v/v).

Fraction 17 (5 mL each fraction) was dried and purified by reversed-phase HPLC (YMC ODS-A column, 40% aqueous MeOH) to yield 36.0 mg of MKN-003A (**1**). Fractions 20–39 were combined and separated by HPLC (40% aqueous MeOH)

to yield 6.3 and 32.4 mg of MKN-003B (**2**) and -003C (**3**), respectively. Fractions 40–57 were combined and separated by HPLC (40% aqueous MeOH) to afford in the order of elution MKN-004A (**4**), -004C (**6**), -004D (**7**), and -004B (**5**). Final purification was accomplished by HPLC (50% aqueous MeOH) to afford 3.1, 27.5, 4.9, and 3.9 mg of **4**–**7**, respectively.

**MKN-003A (1):** [α]<sub>D</sub><sup>25</sup> 18.4° (c 0.18, MeOH); UV λ<sub>max</sub> (MeOH) 206 nm (log ε 3.53); IR ν<sub>max</sub> 2930, 2860, 1755, 1710, 1600, 1360, 1165 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.43 (1H, dd, *J* = 5.7, 1.5 Hz, H-3), 6.08 (1H, dd, *J* = 5.7, 2.0 Hz, H-2), 5.01 (1H, dddd, *J* = 7.3, 5.4, 2.0, 1.5 Hz, H-4), 2.47 (1H, tq, *J* = 7.4, 6.8 Hz, H-10), 2.11 (3H, s, H-12), 1.75 (1H, dddd, *J* = 13.7, 9.8, 5.9, 5.4 Hz, H-5), 1.63 (1H, m, H-9), 1.60 (1H, m, H-5), 1.41 (2H, m, H-6), 1.31 (3H, m, H-7(2H), H-9), 1.24 (2H, m, H-8), 1.06 (3H, d, *J* = 6.8 Hz, H-13); <sup>13</sup>C NMR data, see Table 1; HMBC correlations H-2/C-1, C-3, C-4; H-3/C-1, C-2, C-4; H-4/C-2, C-3, C-5, C-6; H-5/C-4, C-6; H-6/C-7; H-9/C-10, C-11, C-13; H-10/C-8, C-9, C-11, C-13; H-12/C-10, C-11; H-13/C-10, C-11; HREIMS *m/z* 224.1417 [M]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>20</sub>O<sub>3</sub>, *m/z* 224.1412).

**MKN-003B (2):** [α]<sub>D</sub><sup>25</sup> 19.4° (c 0.42, MeOH); UV λ<sub>max</sub> (MeOH) 208 nm (log ε 3.61); IR ν<sub>max</sub> 3450, 2970, 2930, 2870, 1755, 1600, 1455, 1165 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.44 (1H, dd, *J* = 5.7, 1.5 Hz, H-3), 6.11 (1H, dd, *J* = 5.7, 2.0 Hz, H-2), 5.04 (1H, dddd, *J* = 7.3, 5.9, 2.0, 1.5 Hz, H-4), 1.77 (1H, m, H-5), 1.67 (1H, m, H-5), 1.48 (2H, m, H-6), 1.45 (2H, m, H-9), 1.36 (4H, m, H-7, H-8), 1.21 (6H, s, H-11, H-12); <sup>13</sup>C NMR data, see Table 1; HRFABMS *m/z* 235.1311 [M + Na]<sup>+</sup> (calcd for C<sub>12</sub>H<sub>20</sub>O<sub>3</sub>Na, *m/z* 235.1310).

**MKN-003C (3):** [α]<sub>D</sub><sup>25</sup> 20.3° (c 0.16, MeOH); UV λ<sub>max</sub> (MeOH) 206 nm (log ε 3.58); IR ν<sub>max</sub> 3450, 2970, 2935, 1750, 1600, 1460, 1375, 1165 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.44 (1H, dd, *J* = 5.9, 1.5 Hz, H-3), 6.08 (1H, dd, *J* = 5.9, 2.0 Hz, H-2), 5.02 (1H, dddd, *J* = 7.3, 5.4, 2.0, 1.5 Hz, H-4), 1.75 (1H, m, H-5), 1.63 (1H, m, H-5), 1.45 (2H, q, *J* = 7.3 Hz, H-11), 1.41 (2H, m, H-6), 1.38 (2H, m, H-9), 1.33 (2H, m, H-7), 1.31 (2H, m, H-8), 1.10 (3H, s, H-13), 0.86 (3H, t, *J* = 7.3 Hz, H-12); <sup>13</sup>C NMR data, see Table 1; HRFABMS *m/z* 249.1469 [M + Na]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>Na, *m/z* 249.1467).

**MKN-004A (4):** [α]<sub>D</sub><sup>25</sup> -10.5° (c 0.18, MeOH), lit. -5.3° (c 7.8, MeOH),<sup>6</sup> -18° (c 1.6, MeOH);<sup>7</sup> IR ν<sub>max</sub> 3400, 2960, 1750, 1460, 1385, 1195, 1060 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.20 (1H, dq, *J* = 6.8, 6.4 Hz, H-4), 3.84 (1H, m, H-3), 2.55 (1H, dt, *J* = 5.9, 8.8 Hz, H-2), 2.11 (1H, br d, *J* = 4.8 Hz, 3-OH), 1.87 (1H, m, H-6), 1.61 (1H, m, H-6), 1.49 (2H, m, H-7), 1.45 (3H, d, *J* = 6.4 Hz, H-5), 1.38 (2H, hex, *J* = 7.3 Hz, H-8), 0.93 (3H, t, *J* = 7.3 Hz, H-9); <sup>13</sup>C NMR data, see Table 1; HRCIMS *m/z* 172.1329 [M - H<sub>2</sub>O + NH<sub>4</sub>]<sup>+</sup> (calcd for C<sub>9</sub>H<sub>18</sub>NO<sub>2</sub>, *m/z* 172.1337).

**MKN-004B (5):** [α]<sub>D</sub><sup>25</sup> -10.1° (c 0.24, MeOH); IR ν<sub>max</sub> 3400, 2960, 1745, 1470, 1385, 1055 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.20 (1H, dq, *J* = 7.3, 5.9 Hz, H-4), 3.83 (1H, dd, *J* = 8.3, 7.3 Hz, H-3), 2.53 (1H, dt, *J* = 5.4, 8.3 Hz, H-2), 1.85 (1H, ddt, *J* = 13.7, 11.7, 5.4 Hz, H-6), 1.59 (1H, m, H-6), 1.57 (1H, m, H-8), 1.44 (3H, d, *J* = 5.9 Hz, H-5), 1.39 (1H, m, H-7), 1.33 (1H, m, H-7), 0.90 (6H, d, *J* = 6.8 Hz, H-9, H-10); <sup>13</sup>C NMR data, see Table 1; HRFABMS *m/z* 209.1154 [M + Na]<sup>+</sup> (calcd for C<sub>10</sub>H<sub>18</sub>O<sub>3</sub>Na, *m/z* 209.1154).

**MKN-004C (6):** [α]<sub>D</sub><sup>25</sup> 1.1° (c 0.42, MeOH); IR ν<sub>max</sub> 3400, 2960, 2875, 1750, 1470, 1195, 1055 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.52 (1H, q, *J* = 6.3 Hz, H-4), 4.20 (1H, d, *J* = 5.4 Hz, H-3), 2.58 (1H, dt, *J* = 9.8, 5.4 Hz, H-2), 1.84 (1H, m, H-6), 1.63 (1H, m, H-6), 1.58 (1H, m, H-8), 1.36 (1H, m, H-7), 1.35 (3H, d, *J* = 6.3 Hz, H-5), 1.30 (1H, m, H-7), 0.93 (3H, d, *J* = 6.8 Hz, H-9), 0.92 (3H, d, *J* = 6.4 Hz, H-9); <sup>13</sup>C NMR data, see Table 1; HRFABMS *m/z* 209.1155 [M + Na]<sup>+</sup> (calcd for C<sub>10</sub>H<sub>18</sub>O<sub>3</sub>Na, *m/z* 209.1154).

**MKN-004D (7):** [α]<sub>D</sub><sup>25</sup> -8.3° (c 0.25, MeOH); IR ν<sub>max</sub> 3400, 2970, 1750, 1465, 1375, 1060 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.21 (1H, dq, *J* = 6.4, 6.4 Hz, H-4), 3.80 (1H, dd, *J* = 8.3, 6.4 Hz, H-3), 2.62 (1H, dt, *J* = 7.3, 8.3 Hz, H-2), 1.86 (1H, ddd, *J* = 14.2, 7.3, 6.8 Hz, H-6), 1.77 (1H, m, H-7), 1.45 (3H, d, *J* = 6.4 Hz, H-5), 1.44 (1H, m, H-8), 1.38 (1H, m, 6), 1.19 (1H, dp, *J* = 13.7, 7.3 Hz, H-8), 0.93 (3H, d, *J* = 6.8 Hz, H-10), 0.90 (3H, t, *J* = 7.3 Hz, H-9); <sup>13</sup>C NMR data, see Table 1; HRFABMS *m/z* 209.1156 [M + Na]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>Na, *m/z* 209.1154).

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