Novel Abietane Diterpenoids and Aromatic Compounds from *Cladonia rangiferina* and Their Antimicrobial Activity against Antibiotics Resistant Bacteria

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In the course of our research program aimed at the discovery of biologically active compounds from fungi,1,2 we have initiated the chemical study of the Japanese lichen, *Cladonia rangiferina* (L.) Web. (Cladoniaceae). The lichen, *C. rangiferina*, is widely distributed in southern Japan and which grows on the ground and rocks from high mountains to low lands.3) An earlier chemical constituent study of this lichen resulted in no report of the isolation and the biological activity. In our investigation, two new abietane diterpenoids, called hanagokenols A (1), and B (2), along with 15 known compounds including abietane,3—5) labdane,6—8) isopimara5) diterpenoids, monocyclic aromatic compound,9,10) depside,12,13) and dibenzofuran 14,15) were isolated from *C. rangiferina*. We describe here the isolation, purification, and structural elucidation of the unique abietane diterpenes, 1 and 2, primarily by extensive NMR experiments, and the antimicrobial activities of all the isolated compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE). *C. rangiferina* was milled and exhaustively extracted with AcOEt at room temperature for 8 weeks. The AcOEt extract was fractionated into seven fractions by column chromatography (silica gel), followed by repeated separations of their seven portions by chromatography over silica gel. Reversed-phase silica gel furnished hanagokenols A (1), and B (2), obtuahydrine (3),3) sugiol (4),4) 5,6-dehydrodugsiol (5),4) montbretol (6),5) cis-communic acid (7),6) imbricatol acid (8),7) 15-acetyl-imbricatolic acid (9),8) junicedric acid (10),8) 7α-hydroxy-sandaracopimaric acid (11),9) β-resorcylic acid (12),10) atranol (13),11) barbitic acid (14),12) homosekikaic acid (15),13) didymic acid (16),14) and condidamic acid (17).15)

Hanagokenol A (1), [α]D 25 +185.9°, was obtained as an amorphous solid and was considered to have the molecular formula of C20H32O5 based on the high-resolution electron ion mass spectrum (HR-ESI-MS) of the molecular ion at m/z 314.1864. The IR spectrum of 1 showed absorption bands at 3370 (OH), 1690 (C=O), and 1610 (aromatic) cm⁻¹. The presence of an aromatic ring was supported by the UV data (λmax 217, 236 and 288 nm). The 20 carbon signals observed in the 13C-NMR spectrum and distortionless enhancement by polarization transfer (DEPT) experiment (Table 1) revealed the presence of a ketone at δ 195.7 (s); a benzene ring at δ 111.3 (d), 124.0 (s), 127.3 (d), 134.8 (s), 155.3 (s), and 161.5 (s); two oxygenated carbons at δ 77.1 (d), and 84.1 (t), which requires that 1 should contain four rings. The 1H-NMR spec-

### Table 1. 1H- and 13C-NMR Assignments for Compounds 1 and 2

<table>
<thead>
<tr>
<th>Positions</th>
<th>1</th>
<th>2</th>
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<tr>
<td>1α</td>
<td>1.26 (dd, 13.2, 13.2, 4.4)</td>
<td>38.4 (s)</td>
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<tr>
<td>1β</td>
<td>1.98 (dd, 13.2, 3.0, 3.0)</td>
<td>1.98 (m)</td>
</tr>
<tr>
<td>2α</td>
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<tr>
<td>2β</td>
<td>1.50 (m)</td>
<td>1.86 (m)</td>
</tr>
<tr>
<td>3α</td>
<td>1.26 (dd, 13.5, 3.3, 3.3)</td>
<td>35.1 (t)</td>
</tr>
<tr>
<td>3β</td>
<td>1.57 (dd, 13.5, 3.3, 3.3)</td>
<td>1.80 (m)</td>
</tr>
<tr>
<td>4</td>
<td>40.5 (s)</td>
<td>42.7 (s)</td>
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<tr>
<td>5</td>
<td>2.00 (d, 14.3)</td>
<td>58.8 (d)</td>
</tr>
<tr>
<td>6</td>
<td>4.67 (d, 14.3)</td>
<td>77.1 (d)</td>
</tr>
<tr>
<td>7</td>
<td>195.7 (s)</td>
<td>173.3 (s)</td>
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<td>8</td>
<td>124.0 (s)</td>
<td>123.9 (s)</td>
</tr>
<tr>
<td>9</td>
<td>155.3 (s)</td>
<td>143.8 (s)</td>
</tr>
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<td>10</td>
<td>38.6 (s)</td>
<td>38.8 (s)</td>
</tr>
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<td>11</td>
<td>7.06 (s)</td>
<td>111.3 (d)</td>
</tr>
<tr>
<td>12</td>
<td>161.5 (s)</td>
<td>154.7 (s)</td>
</tr>
<tr>
<td>13</td>
<td>134.8 (s)</td>
<td>132.8 (s)</td>
</tr>
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<td>14</td>
<td>8.40 (s)</td>
<td>127.3 (d)</td>
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<td>15</td>
<td>3.60 (sept, 6.9)</td>
<td>27.7 (d)</td>
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<tr>
<td>16</td>
<td>1.35 (d, 6.9)</td>
<td>23.0 (q)</td>
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<td>17</td>
<td>1.36 (d, 6.9)</td>
<td>23.0 (q)</td>
</tr>
<tr>
<td>18a</td>
<td>3.46 (d, 6.9)</td>
<td>84.1 (t)</td>
</tr>
<tr>
<td>18b</td>
<td>3.76 (d, 7.0)</td>
<td>4.03 (d, 8.0)</td>
</tr>
<tr>
<td>19</td>
<td>1.09 (s)</td>
<td>18.6 (q)</td>
</tr>
<tr>
<td>20</td>
<td>1.20 (s)</td>
<td>22.3 (q)</td>
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</table>

Measurements were performed in CD3OD at 600 MHz for 1H- and 125 Hz for 13C-NMR. 13C multiplicities were established by DEPT pulse sequences.

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trum of 1 showed two tertiary methyl signals at δ 1.09, and 1.20, an isopropyl group at δ 1.35 and 1.36 (each d, J = 6.9 Hz), 3.60 (sept, J = 6.9 Hz) and two aromatic protons at δ 7.06 (s), and 8.40 (s), AX type signals at δ 2.00 (d, J = 14.3 Hz) and 4.67 (d, J = 14.3 Hz), and AB type signals at δ 3.46 (d, J = 6.9 Hz) and 3.76 (d, J = 6.9 Hz). These data suggested that 1 was an abietane-type diterpene, as compounds 3—6. The gross structure of 1 was determined by analysis of the NMR data including heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments. The HMBC spectrum (Fig. 1) of 1 showed long-range correlation from the AX type protons (δ 4.67, 2.00), which was assigned to H-6 and H-5, respectively, to the ketone at δ 195.7 assigned to C-7, and then from the H-18b (δ 3.76), which was correlated with the oxygenated carbon at δ 84.1 by HMQC spectrum, to C-5 (δ 56.8) and C-6 (δ 77.1), indicating that 1 had an oxolane formed between C-6 and C-18 in sugiol (4). The stereochemistry of 1 was deduced by ROESY experiment (Fig. 2) and also coupling constant. The α-substituted group at C-6 could be assigned from the NOEs between H-6 (δ 4.67) and H-120 (δ 1.29), and between H-5 (δ 2.00) and H-18a (δ 3.46), and the observed large coupling constant (J = 14.3 Hz) between H-5 and H-6. Thus, from the above findings and the biosynthesis considerations, 1 was shown to be 12-hydroxy-7-oxo-6α,18-epoxy-8,11,13-abietatrien and termed hanagokenol A.

Hanagokenol B (2), [α] D 25 −14.4°, was obtained as an amorphous solid and considered to have the molecular formula of C20H26O5 based on the HR-EI-MS of the molecular ion at m/z 346.1768 [M]+, which suggested the presence of eight degrees of unsaturation. The IR spectrum of 2 showed absorption bands at 3370 (OH), 1760, 1705 (C = O), 1610 and 1590 (aromatic) cm −1. The presence of an aromatic ring was supported by the UV data (λmax 233 and 287 nm). The 20 carbon signals observed in the 13C-NMR spectrum (Table 1) and DEPT experiment showed the presence of two carbonyl signals. Its NMR data (Table 1) showed an isopropyl group at δ 1.23, 1.24 (each d, J = 6.9 Hz).
Hz), and 3.15 (sept, J=6.9 Hz), an oxygenated methylene at δ 3.92, 4.03 (each d, J=8.0 Hz), in addition to the signals at δ 6.96 (s), and 7.34 (s) attributed to a 1,2,4,5-tetrasubstituted benzene ring. The above spectral data suggested 2 to be a secoabietane diterpene.15,16 The gross structure of 2 was determined by analysis of the NMR data, including HMQC, HMBC, and ROESY experiments. The HMBC experiment (Fig. 1) of 2 showed the long-range correlations from H-11 (δ 6.96) to C-12, and C-13; from H-14 (δ 7.34) to C-7 (δ 177.3), and C-12; and from H-15 (δ 3.15) to C-12, C-13 and C-14. Thus, the aromatic moiety of 2 was deduced to be 1-carboxy-4-hydroxy-5-isopropyl-2-substituted benzene. Additional HMBC correlations from H$_2$-19 (δ 1.30) to C-3, C-4, C-5, and C-18 (δ 81.8); from H$_2$-20 (δ 1.61) to C-1, C-5, C-9, and C-10; from H-5 (δ 2.35) to C-6 (δ 177.3); from H-11 (δ 6.96) to C-10; and from H$_2$-18b (δ 4.03) to C-4, C-5, C-6, and C-19 established the γ-lactone ring between C-6/C-18 in 6,7-seco-12-hydroxydehydroabietinol.15 Furthermore, NOEs (Fig. 2) between H$_2$-19 (δ 1.30) and H-2β (δ 1.86), between H$_2$-19 and H-3β (δ 1.80), and between H$_2$-19 and H-20 (δ 1.61), confirmed the α-orientations of H-5 and H$_2$-18. From the above findings and the biosynthesis considerations, the structure of hanagokenol B was established to be that shown as 2.

The isolation of diterpenoids, for example, labdane and isopimarane diterpenes18,19 from lichen is very rare. To the best of our knowledge, this is the first report of abietane diterpenes (1—6) from lichen. Also, the abietane anhydride derivative, obtuanhydride (3) was obtained for the second time as a natural product. Seventeen compounds isolated from Cladonia rangiferina were evaluated for their antimicrobial activity by the disk-diffusion test for MRSA and VRE.20 Among those tested, compounds 1 and 2 showed mild activity. Depside derivatives, (14, 15) and benzofuran derivatives, (16, 17) showed good correlation activities against MRSA and VRE strains (Table 2).

### Experimental

#### General Experimental Procedures

Optical rotations were taken on a JASCO DIP-1400 digital polarimeter; IR spectra were measured on a JASCO FT/IR-5300 instrument and UV spectra were recorded with a Shimadzu UV-6000 spectrophotometer. NMR spectra were recorded on a Varian Unity 600 spectrometer. The chemical shifts are given in δ (ppm) in CD$_3$N or CDCl$_3$, solution, using tetramethylsilane (TMS) as an internal standard. NMR experiments included 1H–1H COSY, HMQC, HMBC, and ROESY. Coupling constants (J values) are given in hertz (Hz). HR-EI-MS were measured on a JEOL JMS-700 MS station. Kieselgel 60 (230—400 mesh, Merck) was used for column chromatography, and silica gel 60F-254 (Merck) for TLC. HPLC was carried out on a JASCO-PU 1580 instrument using a COSMOSIL C18 P-MS (4.6×150 mm and 20×250 mm) column.

#### Lichen Material

Cladonia rangiferina (L.) Wsin. from Naka-cho, Tokushima, was collected in March, 2004 and identified by Akinori Kawamata, a chief researcher from Ehime Prefectural Science Museum. A voucher specimen (TB 3101) has been deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

#### Extraction and Isolation

**C. rangiferina (280 g)** was exhaustively extracted with AcOEt at room temperature for 8 weeks. The AcOEt extract was evaporated under a vacuum to yield a brown residue (10.4 g), which was subjected to silica gel column chromatography with hexane–AcOEt–MeOH (1: 9: 0—10: 3) to afford fractions 1—7. Fraction 2 (0.1 g) was purified by preparative HPLC (78—100% MeOH, flow rate 8 ml/min) to yield cis-communic acid (7, 38.6 mg). Fraction 3 (0.65 g) was passed through silica gel with hexane–AcOEt (1: 1—1: 5) and purified by preparative HPLC (73—100% MeOH, flow rate 8 ml/min) to afford 5,6-dehydrosugiol (4, 5.0 mg), norlinal (6, 8.8 mg), acetylimbricatoloic acid (9, 7.9 mg), and β-resorcylic acid (12, 11.5 mg). Fraction 4 (0.51 g) was passed through silica gel with hexane–AcOEt (3: 7—10: 1) and purified by preparative HPLC (70—100% MeOH, flow rate 8 ml/min) to yield obtuanhydride (3, 3.8 mg), sugiol (4, 18.7 mg), atranol (13, 9.1 mg), barbic acid (14, 12.9 mg), homosekikaic acid (15, 78.0 mg), didymic acid (16, 12.9 mg), and condidydic acid (17, 11.5 mg). Fraction 5 (0.39 g) was purified by preparative HPLC (70% MeOH, flow rate 8 ml/min) to afford imbricatoloic acid (8, 5.2 mg), and juniceric acid (10, 4.6 mg). Fraction 6 (0.43 g) was passed through silica gel with hexane–AcOEt (7: 3—10: 10) and purified by preparative HPLC (70—100% MeOH, flow rate 8 ml/min) to afford hanagokenol A (1, 2.8 mg), B (2, 2.9 mg), and 7α-hydroxy-sandaracopimaric acid (11, 2.3 mg). Hanagokenol A (1): An amorphous solid; [α]$_D^{25}$ +185.9° (c=0.18, MeOH); FT-IR (dry film) cm$^{-1}$: 3370, 1690, 1610; UV $\lambda_{max}$ (MeOH) nm (log e): 217 (4.90), 236 (4.57), 288 (4.57); 1H- and 13C-NMR see Table 1; HR-EI-MS m/z: 314.1864 (Calcd for C$_{19}$H$_{20}$O$_3$: 314.1882).

Hanagokenol B (2): An amorphous solid; [α]$_D^{25}$ +14.4° (c=0.29, MeOH); FT-IR (dry film) cm$^{-1}$: 3370, 1760, 1705, 1509, 1270, 1030; UV $\lambda_{max}$ (MeOH) nm (log e): 212 (4.11), 233 (4.17), 287 (4.00); 1H- and 13C-NMR see Table 1; HR-EI-MS m/z: 346.1768 (Calcd for C$_{20}$H$_{26}$O$_5$: 346.1781).

### References


### Table 2. Antibacterial Activities against Staphylococcus aureus COL (MRSA) and E. fucum (Van A) (VRE) of the Compounds Isolated from Cladonia rangiferina

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Staphylococcus aureus COL (MRSA)</th>
<th>E. fucum (Van A) (VRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanagokenol A (1)</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Hanagokenol B (2)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Obtuanhydride (3)</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td>Sugiol (4)</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>5,6-Dehydrosugiol (5)</td>
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<td>–</td>
</tr>
<tr>
<td>Monobretol (6)</td>
<td>13</td>
<td>±</td>
</tr>
<tr>
<td>cis-Communic acid (7)</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Imbricatoloic acid (8)</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>15-Acetyl-imbricatoloic acid (9)</td>
<td>±</td>
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<td>Junecedric acid (10)</td>
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<td>–</td>
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<tr>
<td>7α-Hydroxysandaracopimaric acid (11)</td>
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<td>10</td>
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<tr>
<td>β-Resorcylic acid (12)</td>
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<tr>
<td>Arranol (13)</td>
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<tr>
<td>Barbatic acid (14)</td>
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<td>Didymic acid (16)</td>
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<td>Condidydic acid (17)</td>
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<td>EM$^a$</td>
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<td>–</td>
</tr>
<tr>
<td>TC$^c$</td>
<td>–</td>
<td>8</td>
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</table>

$^a$ 100 µg/disk (mm). $^b$ EM, erythromycin (100 µg/ml) as reference for MRSA strain. $^c$ TC, tetracycline (100 µg/ml) as reference for VRE strain.