The genus *Plectranthus* (Labiatae) consists of some 350 species, distributed from Africa through to Asia and Australia. Several species of them are used as a folk medicine for skin irritations, antiseptics, vermicide, and nausea.1) There are some reports2) about the occurrence of unique diterpenoids in several *Plectranthus* species and moderate antibacterial activity. This paper deals with the isolation and structure determination of two new diterpenoids, 19-O-(3,4-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-abeo-abieta-1(10),6,8,11,13-tetraene (3) and 12-O-(3-methyl-2-butenoyl)-19-O-(3,4-dihydroxybenzoyl)-11-hydroxyabieta-8,11,13-triene (4), along with two known diterpenoids, parvifloron E (1) and F (2) from *Plectranthus nummularius* Briq. Antioxidative activities of the compounds were measured by the α,α-diphenyl-β-picrylhydrazyl (DPPH) method.

Key words *Plectranthus nummularius*; Labiatae; abietane type diterpenoid; antioxidative activity

Two new antioxidative diterpenoids, plecrranthol A (3)[19-O-(3,4-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-abeo-abieta-1(10),6,8,11,13-tetraene] and plecrranthol B (4)[12-O-(3-methyl-2-butenoyl)-19-O-(3,4-dihydroxybenzoyl)-11-hydroxyabieta-8,11,13-triene] along with two known diterpenoids, parvifloron E (1) and F (2) were isolated from the leaves of *Plectranthus nummularius* Briq. Antioxidative activities of the compounds were measured by the α,α-diphenyl-β-picrylhydrazyl (DPPH) method.

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Fresh leaves of *Plectranthus nummularius* were extracted with acetone and the extract was partitioned between AcOEt and H2O. The AcOEt layer was chromatographed on silica gel, Sephadex LH-20 and Lobar RP-18 column repeatedly, to give compounds 1, 2, 3 and 4. Compounds 1 and 2 were identified as parvifloron E and parvifloron F by comparison of the spectral data with published values.3) Compound 3 was isolated as brownish oil, and was shown to have the molecular formula C27H30O6 ([M+H]+; m/z 451.2096) by the high-resolution (HR)-FAB-MS spectrum. Its 13C-NMR spectrum showed 27 carbon signals, which were partially similar to those in 1 having the abietane nucleus. The appearance of an ABX system of aromatic protons at δ 6.91—7.63 (J_{AB} 5 1.8, J_{AX} 5 8.5 Hz) and existence of an isopropyl group (two doublet methyl protons at δ 1.21, 1.23 and one septet methine proton at δ 3.22) in the 1H-NMR spectrum suggested that 3 was the abietane type diterpenoid with 3,4-dihydroxybenzoyl moiety. When the 1H-NMR spectral data of 3 were compared with those of 1, 3 had the signal of four hydroxyl protons and seven olefinic protons. The appearance of two correlated olefinic protons at δ 6.23 (1H, d, J 5 9.8 Hz) and δ 5.92 (1H, d, J 5 9.8 Hz) suggested that the double bond was located at the 6 (7) position in partial structure of the B ring and an olefinic proton at δ 6.44 (1H, s) indicated that the C ring had a penta-substituted aromatic ring by the nuclear Overhauser effect spectroscopy (NOESY) experiment as shown in Fig. 1. The remaining A ring, which had an olefinic proton at δ 6.13 (1H, br s), two methyls, δ 1.08 (3H, s), δ 1.04 (3H, s) and the non-equivalent methylene protons at δ 4.36, 4.26 (each 1H, d, J=11 Hz). The 1H, 1H-correlation spectroscopy (COSY) and homo-nuclear Hartman–Harn spectroscopy (HOHAHA) spectra showed the
coupled olefinic proton at δ 6.13 to be part of the =CH–CH₂–CH= spin system. The important correlations of long-range H–C coupling were determined by the hetero-nuclear multiple bond connectivity (HMBC) spectrum (Fig. 1), indicating that two methyl protons at δ 1.08 and 1.04 were located at the vicinal position since the long-range coupling was observed between their methyl proton signals and quaternary carbon signals at δ 43.0 and 36.7. Additionally, long-range coupling to each other was also observed between one methyl group of C-18 (δH 1.04, δC 20.0) and the AB methylene group of C-19 (δH 4.36, 4.26, δC 70.8). This suggested that C-18 and C-19 were located at the geminal position. These facts indicated that C-20 methyl group migrated from C-10 to C-5 position in normal abietane type diterpenoid.

The 3, 4-dihydroxybenzoyl moiety was determined to be attached at the C-19 position by the correlation between the signal of H-19 methane protons and carbonyl signal at δ 167.4 in the HMBC spectrum. The stereochemistry of 3 was confirmed by the NOESY experiment. It was apparent that the C-20 methyl group was β-orientation since NOE correlations were observed between H-20 methyl proton and H-3β, H-19 protons. In conclusion, the structure of 3 was determined to be 19-O-(3, 4-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-abeo-abietane-1(10),6,8,11,13-tetraene. This is a new diterpenoid, named plectranthol A. Migrated 20(10→5)-abeo-abietane derivatives have been isolated previously from several species of Salvia⁵ and Pygmaeopremna.⁶

Compound 4 isolated as a brownish amorphous powder, was shown to have the molecular formula C₃₂H₴₀O₇ ([M+H]+; m/z 537.2887) by HR-FAB-MS. It was assumed that 4 was also the abietane type diterpenoid with 19-O-3,4-dihydroxybenzoyl moiety when the NMR data of 4 were compared with those of 1 and 3. The 13C-NMR spectrum showed six methyl groups, four double bonds with two olefinic protons and one carbonyl proton (excepting 3,4-dihydroxybenzoyl moiety). This suggested that 4 was an abietane type diterpenoid bearing one more acetyl moiety. The existence of olefinic proton at δ 6.54 (1H, s) and related aromatic carbons indicated that the C ring was a penta-substituted aromatic ring. Additionally, a deshielded proton at δ 3.15 due to H-1β indicated existence of the hydroxyl group at C-11.⁷ In the HMBC spectrum, the olefinic proton signal at δ 6.00 (1H, s, 2α-H) showed the long-range correlation between carbonyl carbon at δ 165.1, quaternary carbon at δ 162.3 and two methyl carbons at δ 27.8, δ 20.8. From this fact, the remaining acyl group was identified as 2-methyl-3-butenoyl (senecioyl) moiety.⁷ The 13C-NMR spectrum data of 4 showed that the senecioyl group was attached to hydroxyl group of C-12 since the C-12 carbon signal was shifted upfield and the C-11 carbon signal was downfield shifted compared with those data of 3 determined by the HMBC spectrum. The assignment of abietane nucleus of 4 was confirmed by the analyses of 1H, 1H-COSY and NOESY spectra as shown in Fig. 2. Thus, compound 4 was 12-O-(3-methyl-2-butenoyl)-19-O-(3,4-dihydroxybenzoyl)-11-hydroxyabieta-8,11,13-tetraene. This is also a new diterpenoid, named plectranthol B.

The radical scavenging activity was determined using the stable radical, α,α-diphenyl-β-picrylhydrazyl (DPPH)⁸ by ESR measurements. As shown in Table 1, compounds 1—4 scavenged the DPPH radical more than α-tocopherol.

### Table 1. Radical Scavenging Effect of Diterpenoids from Plectranthus nummularius on DPPH Radical

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.086</td>
</tr>
<tr>
<td>2</td>
<td>0.131</td>
</tr>
<tr>
<td>3</td>
<td>0.073</td>
</tr>
<tr>
<td>4</td>
<td>0.099</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.134</td>
</tr>
</tbody>
</table>

**Experimental**

**General** Optical rotations were measured at 25 °C on a JASCO DIP-1000 digital polarimeter. 1H-, 13C-NMR spectra were recorded on a JEOL A-500 FT-NMR spectrometer and the chemical shifts were expressed on the δ (ppm) scale with the TMS as internal standard. FAB-MS and high resolution FAB-MS were measured on a JEOL JMS-700 spectrometer. TLC was performed on silica gel 60 F254 (Merck) and detection was carried out by spraying vanillin–H₂SO₄ reagent followed by heating. Column chromatography was carried out on silica gel (Silica gel 60, Merck), Sephadex LH-20 (Pharmacia) and Lobar LiChroprep RP-18 (Merck).

**Plant Material** Leaves of Plectranthus nummularius Bieb., were collected in the botanical garden herbarium of Kyoritsu College of Pharmacy, Saitama, and voucher specimens have been deposited at the herbarium of this College.

**Extraction and Isolation** Fresh leaves of the plant (1.5 kg) were extracted with acetone (2×5 l) for 24 h at room temperature. The acetone extract was concentrated under reduced pressure and the residue (30.1 g) was partitioned with H₂O and AcOEt. The AcOEt layer was concentrated in vacuo to give a residue (8.8 g) and the residue was subjected to silica gel column chromatography eluting with hexane–AcOEt–MeOH (1:0:0—0:1:1) to obtain 8 fractions. The third fraction was subjected to Lobar RP-8 column chromatography eluting with MeCN–H₂O (1:1—4:1) to afford plectranthol A (3, 23.8 mg). The fourth fraction was chromatographed on Sephadex LH-20 (CHCl₃–MeOH, 1:1) and Lobar RP-8 repeatedly, to give parvifloron E (1, 64.8 mg), parvifloron F (2, 54.2 mg) and plectranthol B (4, 15.5 mg).

Plectranthol A (3) [19-O-(3,4-Dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-abeo-abietane-1(10),6,8,11,13-tetraene]: Brownish oil, [α]₂⁰

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Fig. 1. Significant NOE and HMBC Correlations of Compound 3

Fig. 2. Significant NOE Correlations of Compound 4
Calcd for C$_{32}$H$_{41}$O$_7$: 537.2852. Found: 537.2887. 1H-NMR (500 MHz, CDCl$_3$): 7.63 (1H, d, $J=1.8$ Hz, H-2’), 7.55 (1H, dd, $J=1.8$, 8.5 Hz, H-6’), 6.91 (1H, d, $J=8.5$ Hz, H-5’), 6.44 (1H, s, H-14), 6.23 (1H, d, $J=9.8$ Hz, H-7), 6.13 (1H, br s, H-1), 5.92 (1H, d, $J=9.8$ Hz, H-6), 4.36, 4.26 (each 1H, d, $J=11$ Hz, H-19), 3.22 (1H, qui, H-15), 1.23, 1.21 (each 3H, d, $J=6.8$ Hz, H-16, 17), 1.12 (3H, s, H-18), 1H-NMR (500 MHz, CDCl$_3$): 167.4 (C=O), 149.1 (C-4’), 143.4 (C-3’), 141.6 (C-11), 140.4 (C-12), 137.7 (C-10), 133.4 (C-13), 131.6 (C-6), 125.6 (C-7), 125.0 (C-1), 124.7 (C-22), 122.6 (C-16), 122.4 (C-1’), 116.4 (C-9), 116.0 (C-14), 115.0 (C-5’), 70.8 (C-19), 43.0 (C-5), 36.7 (C-4), 27.4 (C-2), 27.0 (C-15), 22.7 (C-3), 22.6 (C-16), 22.2 (C-17), 21.6 (C-20), 20.0 (C-18).

Acknowledgement

We are grateful to Mrs. J. Hada for providing HR-FAB-MS data.

References


Measurement of DPPH Radical Scavenging Activity

A solution of test compound in ethanol (500 μl) was added to an ethanol solution of DPPH radical (1×10$^{-3}$M, 500 μl). After mixing for 30 s on the vortex mixer, the resulting solution was placed in a flat cell. Sweeping for the ESR spectrum was started 5 min after addition of the sample solution. The scavenging activity was expressed in terms of EC$_{50}$, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical.

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