Two New Diterpenoids from *Plectranthus nummularius* Briq.

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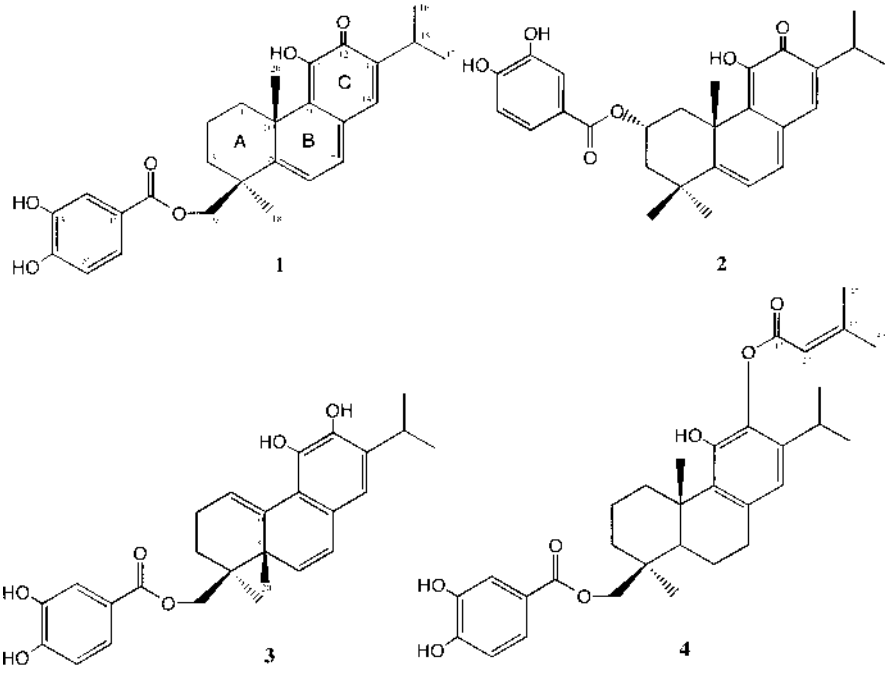
Two new antioxidative diterpenoids, plectranthol A (3)[19-*O*-(3,4-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-*abeo*-abieta-1(10),6,8,11,13-tetraene] and plectranthol B (4)[12-*O*-(3-methyl-2-butenoyl)-19-*O*-(3, 4-dihydroxybenzoyl)-11-hydroxyabieta- 8,11,13-triene] along with two known diterpenoids, 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.

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

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 reports[2] 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 parvifloron F (**2**) from *Plectranthus nummularius* Briq. and their antioxidative activity.

Fresh leaves of *Plectranthus nummularius* were extracted with acetone and the extract was partitioned between AcOEt and H_2O. 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 $C_{27}H_{30}O_6$ ($[M+H]^+$; *m/z* 451.2096) by the high-resolution (HR)-FAB-MS spectrum. Its ^{13}C-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}=1.8, J_{AX}=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 ^{1}H-NMR spectrum suggested that **3** was the abietane type diterpenoid with 3,4-dihydroxybenzoyl moiety. When the ^{1}H-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=9.8 Hz) and δ 5.92 (1H, d, J=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 ^{1}H, ^{1}H-correlation spectroscopy (COSY) and homo-nuclear Hartman–Harn spectroscopy (HOHAHA) spectra showed the



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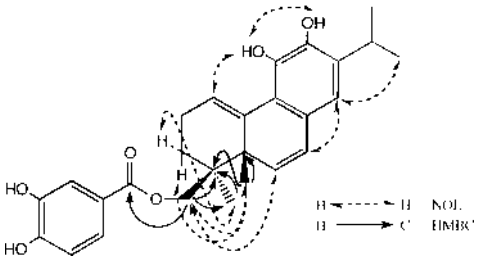

Fig. 1. Significant NOE and HMBC Correlations of Compound **3**

coupled olefinic proton at δ 6.13 to be part of the =CH–CH_2–CH_2– 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 (δ_H 1.04, δ_C 20.0) and the AB methylene group of C-19 (δ_H 4.36, 4.26, δ_C 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 methylene 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*-abieta-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 *Salvia*[4] and *Pygmaeopremna*.[5]

Compound **4** isolated as a brownish amorphous powder, was shown to have the molecular formula $C_{32}H_{40}O_7$ ([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 ^{13}C-NMR spectrum showed six methyl groups, four double bonds with two olefinic protons and one carbonyl carbon (excepting 3,4-dihydroxybenzoyl moiety). This suggested that **4** was an abietane type diterpenoid bearing one more acyl 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.[6] In the HMBC spectrum, the olefinic proton signal at δ 6.00 (1H, s, 2″-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 ^{13}C-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 ^{1}H,^{1}H-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-triene. This is also a new diterpenoid, named plectranthol B.

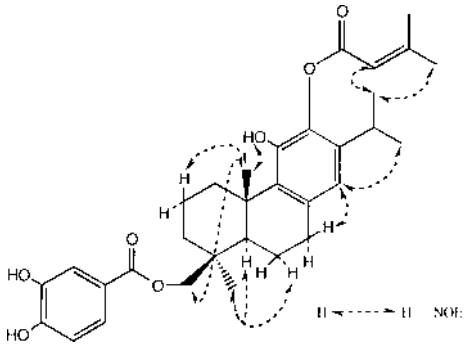

Fig. 2. Significant NOE Correlations of Compound **4**

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

Sample	EC_{50} (mM)
Compound **1**	0.086
2	0.131
3	0.073
4	0.099
α-Tocopherol	0.134

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.

Experimental

General Optical rotations were measured at 25 °C on a JASCO DIP-1000 digital polarimeter. ^{1}H-, ^{13}C-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. ESR spectra were recorded on a JEOL JES-RE1X spectrometer. TLC was performed on silica gel 60 F_{254} and RP-18 F_{254}S (Merck) and detection was carried out by spraying vanillin–H_2SO_4 reagent followed by heating. Column chromatography was carried out on silica gel (Silica gel 60, Merck), Sephadex LH-20 (Pharmacia) and Lobar LiChropep RP-18 (Merck).

Plant Material Leaves of *Plectranthus nummularius* Briq. were collected in the botanical garden herbary 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 H_2O 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–H_2O (1 : 1—4 : 1) to afford plectranthol A (**3**, 23.8 mg). The fourth fraction was chromatographed on Sephadex LH-20 ($CHCl_3$–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*-abieta-1(10),6,8,11,13-tetraene]: Brownish oil, $[\alpha]_D^{25}$

−154.2° (c=0.22, MeOH). FAB-MS m/z: 451 $[M+H]^+$, HR-FAB-MS m/z: Calcd for $C_{27}H_{31}O_6$: 451.2121. Found: 451.2096. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 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=7.0 Hz H-16, 17), 1.08 (3H, s, H-20), 1.04 (3H, s, H-18). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 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-8), 123.7 (C-5′), 122.4 (C-1′), 116.6 (C-2′), 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).

Plectranthol B (**4**) [12-*O*-(3-Methyl-2-butenoyl)-19-*O*-(3, 4-dihydroxybenzoyl)-11-hydroxyabieta-8,11,13-triene]: Brownish amorphous powder, $[\alpha]_D^{25}$ −20.6° (c=0.20, MeOH). FAB-MS m/z: 537 $[M+H]^+$, HR-FAB-MS m/z: Calcd for $C_{32}H_{41}O_7$: 537.2852. Found: 537.2887. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.60 (1H, d, J=1.8 Hz, H-2′), 7.54 (1H, dd, J=1.8, 8.5 Hz, H-6′), 6.84 (1H, d, J=8.5 Hz, H-5′), 6.54 (1H, s, H-14), 6.00 (1H, s, H-2″), 4.50, 4.22 (each 1H, d, J=11 Hz, H-19), 3.15 (1H, m, H-1β), 2.24 (3H, s, H-5″), 2.02 (3H, s, H-4″), 1.35 (3H, s, H-20), 1.15, 1.16 (each 3H, d, J=7.0 Hz H-16, 17), 1.12 (3H, s, H-18). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 167.3 (C=O), 165.1 (C-1″), 162.3 (C-3″), 148.9 (C-4′), 145.8 (C-11), 143.4 (C-3′), 137.9 (C-12), 135.0 (C-8), 134.4 (C-13), 133.8 (C-9), 123.6 (C-22), 122.6 (C-1′), 118.4 (C-14), 116.5 (C-2′), 114.7 (C-5′), 114.2 (C-2″), 68.4 (C-19), 53.5 (C-5), 39.3 (C-10), 37.8 (C-4), 36.4 (C-3), 36.2 (C-1), 33.2 (C-7), 28.0 (C-18), 27.8 (C-5″), 27.5 (C-15), 23.0 (C-16), 23.0 (C-17), 20.8 (C-4″), 20.5 (C-20), 19.7 (C-6), 19.4 (C-2).

Measurement of DPPH Radical Scavenging Activity[9] 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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