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NOTES

Leucomentin-5 and -6, Two New Leucomentin Derivatives from the Mushroom *Paxillus panuoides*

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Free radicals cause peroxidative disintegration of cells and organellar membranes implicated in various pathological processes and especially involved in the pathogenesis of diseases such as myocardial and cerebral ischemia, atherosclerosis, diabetes, rheumatoid arthritis, and cancer-initiation and aging process[1~3]. Thus, many free radical scavengers having the potential as protective agents against these diseases have been discovered from microbial metabolites[4~6].

In our continuing investigation for biologically active constituents, especially free radical scavengers[7~9], from basidiomycetes, we have isolated four *p*-terphenyl compounds from the methanolic extract of the fruit body of *Paxillus panuoides*, which was previously reported to produce diphenyl benzoquinones, flavomentins and spiromentins[10,11]. Two of them, identified as leucomentin-2 and -4, were previously isolated from *Paxillus atrotomentosus*[12] and the others were determined to be new leucomentin derivatives. In this paper, we describe the isolation, structure elucidation and biological activity of these compounds.

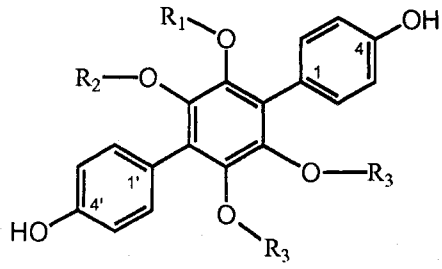
The mushroom *Paxillus panuoides* was collected at Sokri mountain in Chungbuk Province, Korea and identified by the staff of the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the Hongo's method[13]. After drying in a dark and well-ventilated place, the fruit body of *P. panuoides* (690 g, fresh weight) was extracted twice with methanol for isolation of active principles. The methanolic extract was concentrated *in vacuo* and the residue was partitioned twice between ethyl acetate and water. The compounds **1**~**4** were obtained from the EtOAc-soluble fraction by monitoring with lipid peroxidation inhibitory activity in combination with analytical TLC. After concentration of the solvent layer, the concentrate was chromatographed on silica gel eluting with $CHCl_3$-MeOH (30:1~5:1) to give two active fractions. One of the fractions was purified by Sephadex LH-20 column chromatography eluting with MeOH, followed by reverse-phase (ODS) open column chromatography with 50% aq. MeOH to afford compound **1** (110 mg). The other fraction was further purified by column chromatographies on Sephadex LH-20 eluting with $CHCl_3$-MeOH (1:1) and silica gel eluting with $CHCl_3$-MeOH (20:1). Finally, reverse-phase HPLC (column, MAXSIL 5μ C18 i.d. 21.2× 250 mm; solvent, 70% aq. MeOH; flow rate, 6 ml/minute) gave pure compounds **2** (r.t. 18 minutes, 30 mg), **3** (r. t. 16 minutes, 4 mg) and **4** (r.t. 23 minutes, 3 mg).

Compounds **1** and **2** were identified as leucomentin-2 and leucomentin-4, respectively, by comparison with the literature[12] and on the basis of various spectroscopic analysis. Compounds **3** and **4** were determined to be new leucomentin analogs through spectroscopic analysis and by the comparison with NMR spectra of the known leucomentins. The physico-chemical properties of compounds **3** and **4** are as follows.

3: Gray powder; $[\alpha]_D$ +64° (*c* 0.22, MeOH); UV λ_{max} (MeOH) nm (log ε) 258 (4.19), 220 (sh), 209 (4.58); IR ν_{max} (KBr) 3464, 2925, 2855, 1748, 1640, 1613, 1525, 1450, 1273, 1130, 850 cm^{-1}; HRFAB-MS *m/z* 721.1898 $(M+Na)^+$ calcd. for $C_{38}H_{34}O_{13}$ (Δ+0.1 mmu); ^1H NMR ($CDCl_3$, ppm) 7.20 (2H, d, *J*=8.6 Hz, H-2, 6 or 2′, 6′), 7.19 (2H, d, *J*=8.6 Hz, H-2, 6 or 2′, 6′), 6.84 (2H, d, *J*=8.6 Hz, H-3, 5 or H-3′, 5′), 6.82 (2H, d, *J*=8.6 Hz, H-3, 5 or H-3′, 5′), 5.95~5.77 (6H, H-6, 7), 3.95 (3H, H-8), 2.89 (3H, m, H-9), 1.97 (3H, s, H-12), 1.36~1.33 (9H, H-10).

4: Gray powder; $[\alpha]_D$ +55° (*c* 0.33, MeOH); UV λ_{max} (MeOH) nm (log ε) 266 (4.52), 220 (sh), 210 (4.65); IR ν_{max} (KBr) 3435, 2970, 2929, 1748, 1638, 1613, 1525, 1447, 1413, 1271, 1133, 850 cm^{-1}; HRFAB-MS *m/z* 773.2219 $(M+Na)^+$ calcd. for $C_{42}H_{38}O_{13}$ (Δ+0.9 mmu); ^1H NMR ($CDCl_3$, ppm) 7.20 (4H, d, *J*=8.4 Hz, H-2, 6, 2′, 6′), 7.15 (1H, br. t, *J*=14.9, 11.3 Hz, H-14), 6.81 (2H, d, *J*=8.4 Hz, H-3, 5 or H-3′, 5′), 6.80 (2H, d, *J*=8.4 Hz, H-3, 5 or H-3′, 5′), 6.55 (1H, t, *J*=11.3 Hz, H-13), 6.07 (1H, dq, *J*=14.9, 6.6 Hz, H-15), 5.93~5.73 (6H, H-6, 7), 5.49 (1H, d, *J*=11.3 Hz, H-12), 3.93 (3H, br. d, *J*=6.4 Hz, H-8), 2.87 (3H, m, H-9), 1.84 (3H, d, *J*=6.6 Hz, H-16), 1.34 (9H, d,

Fig. 1. Structures of compounds **1**~**4**.



Leucomentin-2 (**1**) $R_1, R_2 = H$

$R_3 = CO^{5}-CH^{6}=\overset{Z}{C}H^{7}-CH^{8}(O)-CH^{9}-CH^{10}-CH_3$

Leucomentin-4 (**2**) $R_1, R_2, R_3 =$ CO—CH=CH—CH(O)—CH—CH$_3$

Leucomentin-5 (**3**) $R_1 = CO^{11}-CH_3^{12}$

$R_2, R_3 =$ CO—CH=CH—CH(O)—CH—CH$_3$

Leucomentin-6 (**4**) $R_1 = CO^{11}-CH^{12}=\overset{Z}{C}H^{13}-CH^{14}=\overset{E}{C}H^{15}-CH^{16}-CH_3$

$R_2, R_3 =$ CO—CH=CH—CH(O)—CH—CH$_3$

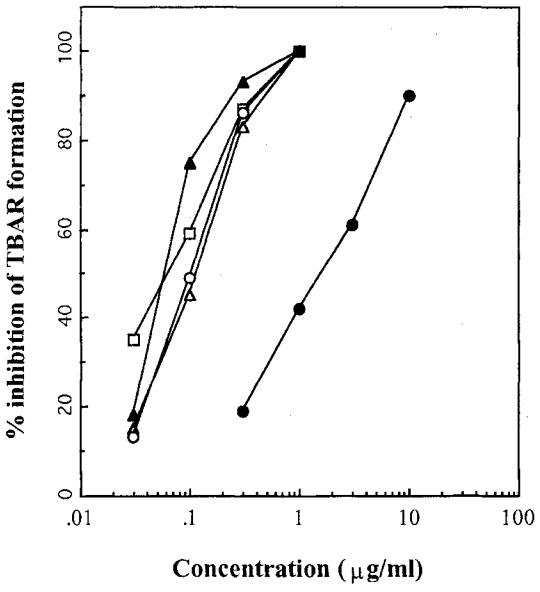
J=5.0 Hz, H-10).

The molecular formula of compound **3**, named leucomentin-5, was established as $C_{38}H_{34}O_{13}$ by high-resolution FAB mass spectroscopy. The IR absorptions at 3464, 1748 and 1130 cm^{-1} suggested the presence of hydroxyl, carbonyl and C–O groups, respectively. The UV spectrum of **3** was very similar to those of compounds **1** and **2**, showing UV maxima at 258, 220 (sh.) and 209 nm. The ^1H NMR spectrum in $CDCl_3$ revealed signals attributable to eight aromatic protons from two 1,4-disubstituted benzenes, six olefinic protons at 5.95~5.77 ppm, three methines at 3.95 and 2.89 ppm, one singlet methyl at 1.97 ppm from an acetyl group and three doublet methyls at 1.36~1.33 ppm. Also, ^1H NMR splitting pattern revealed the partial structure of 4,5-epoxy-2-hexenoic acid. The partial structure and ^1H NMR spectrum of **3** were in good agreement with those of leucomentin-4[12], suggesting that **3** was a leucomentin analog. However, compound **3** was distinguishable from leucomentin-4 by integration of ^1H NMR peaks and the presence of an acetyl group. Leucomentin-4 was composed of four 4,5-epoxy-2-hexenoic acid side chains on *p*-terphenyl skeleton, while the ^1H NMR spectrum of **3** showed the presence of three hexanoic acid units and an acetyl group. This suggested that one 4,5-epoxy-2-hexenoyloxyl group in leucomentin-4 was replaced by an acetoxyl group in compound **3** and this was supported by the mass peak at *m/z* 721 $(M+Na)^+$. Thus, the structure of leucomentin-5 was assigned as a new *p*-terphenyl compound related to the leucomentins, as shown in Fig. 1. The geometries of the double bonds were assigned as *cis* on the basis of the vicinal coupling constants of J=11.6 Hz. Also the relative configurations of the oxiranes were determined to be *trans* by the small vicinal coupling constants of about 2.0 Hz.

Compound **4** (leucomentin-6) was closely related to **3** in its physico-chemical properties and NMR spectra, suggesting that **4** was also a leucomentin analog. The molecular formula was established as $C_{42}H_{38}O_{13}$ by high resolution FAB-mass spectrometry. The IR absorptions at 3435 and 1748 cm^{-1} suggested the presence of hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum in $CDCl_3$ revealed the presence of two 1,4-disubstituted benzenes (7.20, 7.15, 6.81, 6.80 ppm) from the *p*-terphenyl moiety and six olefinic protons (5.93~5.73 ppm), three methines at 3.93 and 2.87 ppm, and three doublet methyls (1.34 ppm) from 4,5-epoxy-2-hexenoic acid. These peaks were in common with other leucomentins. In addition, four olefinic protons at 7.15, 6.55, 6.07 and 5.49 ppm and one

Fig. 2. Lipid peroxidation inhibitory activity of compounds **1**~**4** in rat liver microsomes.

1 (□), **2** (△), **3** (O), **4** (▲), vitamin E (●).



doublet methyl protons at 1.84 ppm were observed and assigned as part of a 2,4-hexadienoic ester group by ^{1}H-^{1}H COSY experiment. These partial structures suggested that the structure of leucomentin-6 was very similar to leucomentin-4, the difference being that a 4,5-epoxy-2-hexenoyloxyl group in leucomentin-4 was replaced by a 2,4-hexadienoyloxyl group, as shown in Fig. 1. The deduced structure was supported by a FAB-mass peak at *m/z* 773 $(M+Na)^+$. The geometries of the double bonds in the 4,5-epoxy-2-hexenoyloxyl groups were assigned as *cis* based upon the vicinal coupling constants of J=11.6 Hz and the olefin geometries in the 2,4-hexadienoyloxyl group were assigned as 2*Z* and 4*E* by the coupling constants of J=11.3 and 14.9 Hz, respectively. Also the relative configurations of the oxiranes were established to be *trans* by the coupling constants of about J=2.0 Hz.

Free radical scavenging activity was evaluated by the inhibitory activity of compounds **1**~**4** against lipid peroxidation in rat liver microsomes according to the method of YAGI *et al.*[14]. Compounds **1**~**4** showed strong lipid peroxidation inhibitory activities with IC_{50} values of 0.06, 0.11, 0.11 and 0.06 μg/ml, respectively, in a dose-dependent fashion. Compounds **1** and **4** were twenty-five times as active as vitamin E (IC_{50} 1.5 μg/ml) which was used as a control, as shown in Fig. 2.

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