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Lipid Peroxidation Inhibitory Activity of Some Constituents Isolated from the Stem Bark of *Eucalyptus globulus*

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Twelve compounds with lipid peroxidation inhibitory activity were isolated from the stem bark of *E. globulus*. Their structures were assigned as a new aromatic monoterpene (**1**) and eleven known compounds, pinoresinol (**2**), vomifoliol (**3**), 3,4,5-trimethoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucopyranoside (**4**), methyl gallate (**5**), rhamnazin (**6**), rhamnetin (**7**), eriodictyol (**8**), quercetin (**9**), taxifolin (**10**), engelitin (**11**), and catechin (**12**) on the basis of UV, mass, and NMR spectroscopic analyses. These compounds except vomifoliol significantly inhibited lipid peroxidation in rat liver microsome.

Key words: *Eucalyptus globulus*, Myrtaceae, α,α-Dimethyl-4-hydroxymethylbenzyl alcohol, Lipid peroxidation inhibitory activity

INTRODUCTION

Eucalyptus globulus Labill (Myrtaceae) is established in plantations in many countries, mainly for pulp and paper production. As a consequence, there is potential for development of secondary industries to recover not only the essential oils as a by product, but also other potentially useful compounds available from the leaves and bark. Previous phytochemical investigations on this species resulted in the isolation of 10-aromadendranol, eucalyptone, euglobals I-VII, 16,18-tritriacontanedione, macrocarpals, and several terpenoids (Ghisalberti, 1996; Kozuka *et al.*, 1982a; Kozuka *et al.*, 1982b; Nishizawa *et al.*, 1992; Osawa *et al.*, 1995; Santos *et al.*, 1997; Yamakoshi *et al.*, 1992). In the search for antioxidative substances from the stem bark of *E. globulus* that showed strong lipid peroxidation inhibitory activity, twelve constituents **1-12** were isolated. This paper reports their isolation, structural elucidation and lipid peroxidation inhibitory activity.

MATERIALS AND METHODS

General

UV and IR spectra were recorded on a Shimadzu UV-260 spectrophotometer and a Laser Precision Analect RFX65S FT-IR spectrometer, respectively. NMR spectra were obtained using Varian UNITY 300 and UNITY 500 NMR spectrometers in CD_3OD and in a mixture of CD_3OD and $CDCl_3$ for **6**, **8**, and **9** with TMS as an internal standard. Chemical shifts are given in δ from TMS. FABMS and HRFABMS were measured using *m*-nitrobenzyl alcohol matrix with polyethylene glycol as internal standard on a JEOL JMS-HX 110/100A spectrometer. EIMS and HREIMS spectra were taken on a JEOL JMS-SX 102A spectrometer operating at 70 eV.

Plant material

The stem bark of *Eucalyptus globulus*, 8 years old trees grown at the George's tree farm in Australia, was provided by Hansol Institute of Science and Technology. Fresh stem bark was dried in a dark, well-ventilated place. The voucher specimen is deposited in the Antibiotics Research Laboratory of KRIBB.

Extraction and isolation

The dried stem bark of *E. globulus* (6 kg) was extracted twice with 80% aq. MeOH at room temperature for 2 days. The MeOH extract was filtered and concentrated under reduced pressure. The liquid residue was partitioned between *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and water. The $CHCl_3$ and EtOAc extracts of them showed strong antioxidative activity in monitoring with lipid peroxidation inhibitory activity. The $CHCl_3$ layer (7.2 g) was concentrated *in vacuo* and chromato-

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graphed twice on each SiO_2 flash column eluted with a mixture of *n*-hexane and EtOAc, and a mixture of $CHCl_3$ and MeOH. In result, two active fractions were obtained. Each fraction was further purified by Sephadex LH-20 column chromatography, followed by ODS TLC to afford **2** (9.6 mg) and **6** (1.1 mg), respectively. SiO_2 column chromatography of EtOAc extract (71.2 g) gave two active fractions (Frs. 1 and 2). Fr. 1 was subjected to a column of Sephadex LH-20 and Fr. 2 was rechromatographed on SiO_2 column to give each two active fractions (Frs. 1a, 1b, 2a, and 2b). **1** (1 mg) was isolated together with **3** (13 mg) from Fr. 1a by preparative SiO_2 TLC developed with $CHCl_3$/MeOH (10:1) and HPLC (ODS column, 13% aq. CH_3CN). Fr. 1b was further purified with preparative SiO_2 TLC and ODS TLC to give **5** (12 mg), **7** (21 mg), and **8** (2.4 mg). Compounds **9** (2.2 mg), **10** (12 mg), **11** (52 mg), and **12** (1.5 g) were obtained from Fr. 2a through Sephadex LH-20 column chromatography, preparative SiO_2 TLC, and ODS TLC. Fr. 2b was chromatographed on open columns of Sephadex LH-20 eluted with MeOH and ODS eluted with 50% aq. MeOH to give **4** (30 mg).

Aromatic monoterpene [1]

Oily compound, UV (MeOH): λ_{max} (log ε) 210 (3.72), 217 (3.71, sh), 235 (3.18, sh), 258 (2.73), 277 (2.78) nm, IR (KBr) ν_{max} 3433, 2926, 1608, 1366, 1070 cm^{-1}, ^{1}H-NMR (CD_3OD) 7.45 (2H, d, *J*=8.2 Hz, H-2,6), 7.29 (2H, d, *J*=8.2 Hz, H-3,5), 4.57 (2H, s , 4-CH_2), 1.51 (6H, s, α-Me), ^{13}C-NMR (CD_3OD) 150.0 (C-1), 140.7 (C-4), 127.8 (C-3,5), 125.6 (C-2,6), 72.9 (C-α), 65.0 (4-CH_2), 32.0 (α-Me), HREIMS: *m/z* 166.0984 $[M]^+$ ($C_{10}H_{14}O_2$ requires 166.0994).

Other compounds **2**~**12** were identified by the comparison with the spectral data of the authentic samples in literatures.

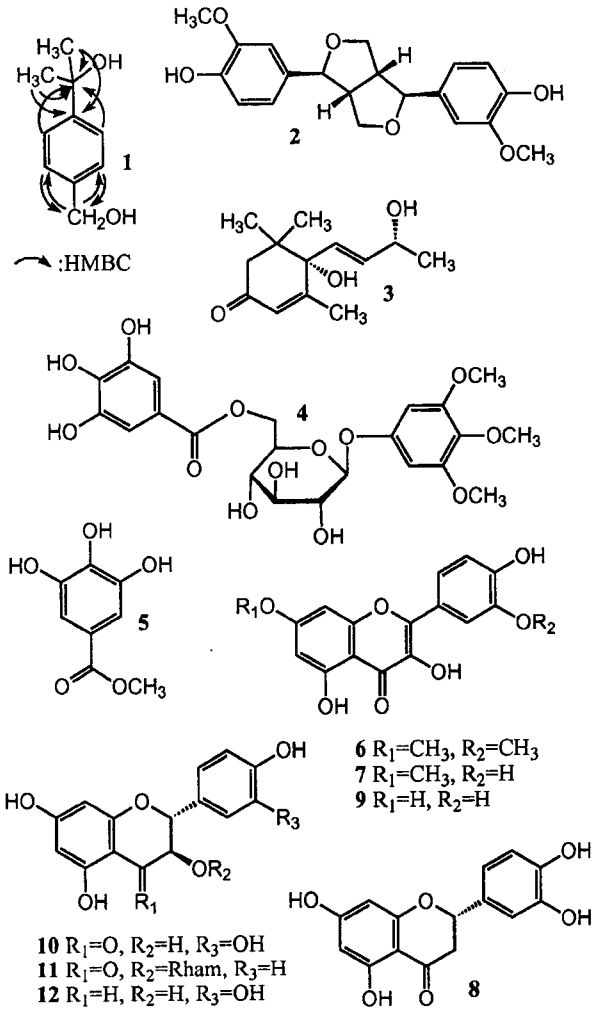

Fig. 1. Compounds isolated from the stem bark of *E. globulus*.

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity in rat liver microsomes was evaluated by the thiobarbituric acid method (Ohkawa *et al.*, 1979) with minor modifications. The rat liver microsomes were prepared according to the method of Hogeboom (Hogeboom, 1965) with some modifications and finally suspended in 100 mM Tris-HCl buffer (pH 7.4). The reaction was initiated by the addition of 100 μM $FeSO_4 \cdot H_2O$ (0.1 ml) into a mixture of a Tris-HCl buffer (0.7 ml), 0.2 mM ascorbic acid (50 μl), a 0.5 μg protein/ml microsomal suspension (40 μl), and 10 μl of the sample solution. The reaction mixture was incubated at 37°C for 30 minutes. After incubation, the reaction was stopped by the addition of 0.25 ml of a TCA (3M)-HCl (2N) 1:1 mixture and then centrifuged at 3500 g for 10 minutes. The reaction supernatant (1 ml) was mixed with 0.67% (w/v) thiobarbituric acid (0.25 ml) and then heated in boiling water for 10 minutes. The lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm. Lipid peroxidation inhibitory activity was calculated as follows: [1-(T-B)/(C-B)] × 100(%), in which T, C, and B are the absorbance values at 532 nm of the sample treatment, the control (without the sample), and the zero time control, respectively. The value of IC_{50} means the concentration (μg/ml) of the compounds required for a 50% inhibition of the microsomal lipid peroxidation.

RESULTS AND DISCUSSION

The high resolution EI-mass spectrometry of **1** obtained as oily compound showed its molecular composition to be $C_{10}H_{14}O_2$ (found *m/z* 166.0984 M^+, calculated 166.0994). Other fragments at *m/z* 151 $[M\text{-}Me]^+$, 91

[benzyl group], and 77 [phenyl group] were note worthy. The IR spectrum revealed the bands due to hydroxyl group (3433 cm^{-1}), aromatic C=C stretching vibration (1600 cm^{-1}), and C-O stretching vibration (1070 cm^{-1}). The ^1H NMR spectrum of **1** indicated the presence of two methyls at δ 1.51 (6H, s, α-CH_3), a methylene at δ 4.57 (2H, s, 4-CH_2) and *p*-disubstituted benzene [7.29 (2H, d, *J*=8.2Hz, H-3,5), 7.45 (2H, d, *J*=8.2Hz, H-2,6)]. The ten carbon signals observed in the ^{13}C-NMR spectrum were characterized by a DEPT experiment, which suggested that **1** had two methyls at δ 32.0 (α-CH_3), an oxygenated methylene at δ 65.0 (4-CH_2), an oxygenated quaternary carbon at δ 72.9 (C-α), four aromatic methines at δ 125.6 (C-2, 6) and 127.8 (C-3, 5) and two sp^2 quaternary carbons, at δ 140.7 (C-4) and 150.0 (C-1). In combination of the above physicochemical properties and NMR spectral data, the structure of **1** was unambiguously established to be α,α-dimethyl-4-hydroxyme-thylbenzyl alcohol. That was also supported by HMBC data showing the long range correlations from the methylene protons at δ 4.57 to C-3, 4, and 5 and from the methyl protons at δ 1.51 to C-α (δ 72.9) and C-1 (δ 150.0). Several aromatic monoterpenes structurally related to **1** have been isolated as oily constituents of *Lavandula gibsonii, Eupatorium tashiroi, Citrus reticulata, Citrus aurantifolia*, and so on (Patwardhan and Gupta, 1983; Piovetti *et al.*, 1980; Wu *et al.*, 1985). In spite of the simplest structure, as far as we know, compound **2** was isolated for the first time.

Compounds **2, 6, 7, 8, 9, 10,** and **12** were identified as pinoresinol, rhamnazin, rhamnetin, eriodictyol, quercetin, taxifolin, and catechin, respectively, by UV, mass, and ^1H-NMR spectra. In addition, the attached posi-tions of methoxyl groups for flavonoids **6** and **7** were determined with UV shift pattern using the diagnostic reagents of NaOMe, $AlCl_3$/HCl, and NaOAc/H_3BO_3. **5** required additional ^{13}C-NMR spectra for identification. The structures of **3**, **4**, and **11** were assigned as vomifoliol, 3,4,5-trimethoxyphenol **1**-*O*-β-D-(6'-*O*-galloyl)glucopyranoside, and engelitin, respectively, by ^1H-NMR, ^{13}C-NMR, COSY, DEPT, and HMBC spectra. The spectral data of these compounds were in good agreement with those in literatures (Herderich *et al.*, 1992; Ishimaru *et al.*, 1987; Nonaka *et al.*, 1982; Okuyama *et al.*, 1995). All compounds except **3** were reported for the first time from *Eucalyptus globulus*.

For the purpose of evaluating the antioxidative activity of compounds **1-12**, lipid peroxidation inhibitory activity were investigated. Taxifolin, rhamnetin, rhamnazin, quercetin, and eriodictyol significantly inhibited the lipid peroxidation induced by non-enzymic Fe(II)-ascorbic acid system in rat liver microsomes, with IC_{50} values of 0.08, 0.1, 0.3, 0.3, and 0.6 μg/ml, respectively. The inhibition was shown to proceed in a dose-dependent fashion. These compounds showed higher activity than vitamin E (IC_{50},1.0 μg/ml) which was used as the control. Also, catechin and methyl gallate exhibited comparable activity with that of vitamin E, while 3,4,5-trime-thoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucopyranoside, pinoresinol, and new compound, aromatic monoterpene showed moderate inhibitory activity against lipid peroxidation with IC_{50} values of 7.0, 7.9, and 10.2 μg/ml, respectively, as shown in Table I. Vomifoliol did not exhibit this activity up to the concentration of 30 μg/ml.

Table I. Inhibitory activity of compounds **1-12** against lipid peroxidation in rat liver microsomes

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**
IC_{50} (μg/ml)	10.2	7.9	>30	7.0	1.1	0.3	0.1
Compounds	**8**	**9**	**10**	**11**	**12**	Vitamin E	
IC_{50} (μg/ml)	0.6	0.3	0.08	3.3	1.1	1.0	

REFERENCES

Ghisalberti, E. L., Bioactive acylphloroglucinol derivatives from *Eucalyptus* species. *Phytochemistry*, 41, 7-22 (1996).

Herderich, M., Feser, W. and Schreier, P., Vomifoliol 9-*O*-β-D-glucopyranosyl-4-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranoside: a trisaccharide glycoside from apple fruit. *Phytochemistry*, 31, 895-897 (1992).

Hogeboom, G. H., General methods for the isolation of liver cell components: Fraction of cell components of animal tissues. *Meth. Enzymol.*, 1, 16-19 (1965).

Ishimaru, K., Nonaka, G. and Nishioka, I., Phenolic glucoside gallates from *Quercus mongolica* and *Q. acutissima*. *Phytochemistry*, 26, 1147-1152 (1987).

Kozuka, M., Sawada, T., Kasahara, F., Mizuta, E., Amano, T., Komiya, T. and Goto, M., The granulation-inhibiting principles from *Eucalyptus globulus* LABILL. II. the strutures of euglobal -Ia$_1$, -Ia$_2$, -Ib, -Ic, -IIa, -IIb and -IIc. *Chem. Pharm. Bull.*, 30, 1952-1963 (1982a).

Kozuka, M., Sawada, T., Mizuta, E., Kasahara, F., Amano, T., Komiya, T. and Goto, M., The granulation-inhibiting principles from *Eucalyptus globulus* LABILL. III. the structures of euglobal-III, -IVb and -VII. *Chem. Pharm. Bull.*, 30, 1964 - 1973 (1982b).

Nishizawa, M., Emura, M., Kan, Y., Yamada, H., Ogawa, K. and Hamanaka, N., Macrocarpals: HIV-RTase inhibitors of *Eucalyptus globulus*. *Tetrahedron Lett.*, 33, 2983-2986 (1992).

Nonaka, G., Nishimura, H. and Nishioka, I., Tannins and related compounds. IV. seven new phenol glucoside gallates from *Quercus stenophylla* MAKINO. *Chem. Pharm. Bull.*, 30, 2061-2067 (1982).

Ohkawa, H., Ohishi, N. and Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95, 351-358 (1979).

Okuyama, E., Suzumura, K. and Yamazaki, M., Pharma-

cologically active components of Todopon Puok (*Fagraea racemosa*), a medicinal plant from Borneo. *Chem. Pharm. Bull.*, 43, 2200-2204 (1995).

Osawa, K., Yasuda, H., Morita, H., Takeya, K. and Itokawa, H., Eucalyptone from *Eucalyptus globulus*. *Phytochemistry*, 40, 183-184 (1995).

Patwardhan, S. A. and Gupta, A. S., Aromatic monoterpenes from *Lavandula gibsonii*. *Phytochemistry*, 22, 2080-2081 (1983).

Piovetti, L., Combaut, G. and Diara, A., Monoterpenes et sesquiterpenes oxygenes de *Cupressus dupreziana*. *Phytochemistry*, 19, 2117-2120 (1980).

Santos, G. G., Alves, J. C. N., Rodilla, J. M. L., Duarte, A. P., Lithgow, A. M. and Urones, J. G., Terpenoids and other constituents of *Eucalyptus globulus*. *Phytochemistry*, 44, 1309-1312 (1997).

Wu, T.-S., Niwa, M., Furukawa, H. and Kuoh, C.-S., Eupatriol, a new monoterpene from *Eupatorium tashiroi* HAYATA. *Chem. Pharm. Bull.*, 33, 4005-4006 (1985).

Yamakoshi, Y., Murata, M., Shimizu, A. and Homma, S., Isolation and characterization of macrocarpals B-G antibacterial compounds from *Eucalyptus macrocarpa*. *Biosci. Biotech. Biochem.*, 56, 1570-1576 (1992).