

# Antiphotooxidative Activity of Protoberberines Derived from *Coptis japonica* Makino in the Chlorophyll-Sensitized Photooxidation of Oil

Jin Pyo Kim,<sup>†</sup> Mun Yhung Jung,\* Jong-Pyung Kim,<sup>‡</sup> and Sung Yeol Kim<sup>†</sup>

Department of Food Science and Technology, Chungnam National University, Taejeon, Republic of Korea, Department of Food Science and Technology, Woosuk University, Samrea-Up, Wanju-Kun, Jeonbuk Province 565-701, Republic of Korea, and Korea Research Institute of Bioscience and Biotechnology, Taejeon, Republic of Korea

Antiphotooxidative components were isolated from the methanolic extract of *Coptis japonica* Makino by liquid–liquid partitioning fractionation, subsequent column chromatography on Sephadex LH-20 and silica gel, and preparative silica gel TLC. The isolated compounds were identified as coptisine, jatrorrhizine, berberine, and magnoflorine by a combination of spectroscopic studies using UV–visible, IR, mass-spectrometry, and NMR. Coptisine, jatrorrhizine, and berberine isolated from *Coptis japonica* Makino showed strong antiphotooxidative activity in the chlorophyll-sensitized photooxidation of linoleic acid. However, these compounds did not show either inhibitory activity against lipid peroxidation in rat liver microsomes nor DPPH radical scavenging activity, indicating that their antiphotooxidative activity was not due to the radical chain reaction breaking ability but due to singlet oxygen quenching activity. Commercially available authentic protoberberines (berberine chloride and palmatine chloride) also showed strong antioxidative activity in the chlorophyll-sensitized photooxidation of linoleic acid. The antiphotooxidative activities of the berberine chloride and palmatine chloride were significantly higher than that of ascorbyl palmitate in the chlorophyll-sensitized photooxidation of linoleic acid. These results clearly showed for the first time the antiphotooxidative properties of protoberberines in chlorophyll-sensitized photooxidation of oil.

**Keywords:** *Coptis japonica* Makino; photooxidation; berberine; coptisine; jatrorrhizine; magnoflorine; chlorophylls; singlet oxygen

## INTRODUCTION

Oils, amino acids, proteins, vitamins (ascorbic acid, retinyl palmitate, ergosterol, carotenoids, tocopherols), cholesterol, limonene, and conjugated terpenes in various types of foods are very susceptible to photooxidation, especially when photosensitizers such as chlorophylls and riboflavin are present in the systems (Koryck-Dahl and Richardson, 1978; King and Min, 1998; Jung et al., 1998a,b). Photooxidation occurs through a Type I or II reaction pathway. Type I photosensitized reaction involves the formation of superoxide anion and other radicals due to the transfer of hydrogen atoms or electrons by interaction of triplet sensitizer with molecular oxygen or other components. The type II process involves the generation of singlet oxygen by energy transfer from an excited triplet sensitizer to a triplet oxygen. The photochemical processes in the food system are dependent on the types and concentration of sensitizers and substances in the system.

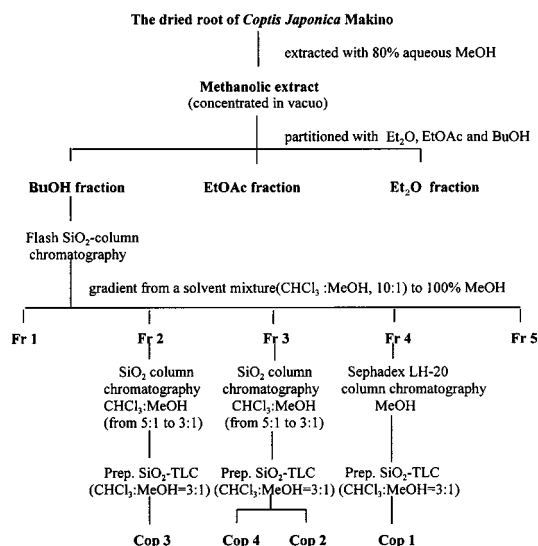
Chlorophylls, myoglobin derivatives, and riboflavin are reportedly efficient photochemical sensitizers for the formation of both singlet oxygen and/or superoxide anion radicals present in various foods (Berliner et al.,

1994; Koryck-Dahl and Richardson, 1978; Whang and Peng, 1988a,b). The foods containing these sensitizers deteriorate easily under light-illuminated conditions. Effective radical scavengers such as BHA and BHT do not possess antioxidative properties in the photosensitized oxidation of oils due to their lack of ability in scavenging singlet oxygen (Clements et al., 1973; Carlsson et al., 1976; Chan, 1977). There are few antioxidants that can be used for the protection of foods from the photosensitized oxidation. These antioxidants are ascorbic acid, ascorbyl palmitate, carotenoids, and tocopherols (Jung et al., 1991; Jung and Min, 1991; Lee and Min, 1991; Jung et al., 1995; Lee et al., 1997; Jung et al., 1998a,b). Thus, the need for novel antioxidants for effective reduction of photosensitized oxidation of food components is obvious, and academia and industry continue to look for novel natural antiphotooxidants. Previously, Jung et al. (1999) screened the methanolic extracts of 47 plant species for their antioxidative activity in the photosensitized oxidation of oils. These authors found that the methanolic extract of *Coptis japonica* Makino showed the strongest antioxidative activity in both methylene blue-sensitized and chlorophyll-sensitized photooxidation of linoleic acid. The authors obtained three fractions (diethyl ether fraction, ethyl acetate fraction, and butanol fraction) by liquid–liquid partition extraction. Among these three fractions, the butanol extract showed highest antioxidative activity in both chlorophyll-sensitized and methylene blue-sensitized photooxidation of linoleic acid.

\* To whom correspondence should be addressed at Woosuk University. Tel.: 82-652-290-1438. Fax: 82-652-291-9312. E-mail: munjung@core.woosuk.ac.kr.

<sup>†</sup> Chungnam National University.

<sup>‡</sup> Korea Institute of Bioscience and Biotechnology.



**Figure 1.** Purification scheme for antiphototoxidative components from the dried root of *Coptis japonica* Makino.

The objective of this research was to isolate and identify the antiphototoxidative components in the butanol fraction of methanolic extract of *Coptis japonica* Makino against the chlorophyll sensitized photooxidation of linoleic acid.

## MATERIALS AND METHODS

**Materials.** The dried root of *Coptis japonica* Makino was purchased from a local Oriental herbal store in Seoul, Korea. Ascorbic acid, chlorophyll *b*, berberine chloride, palmatine chloride, BHA, BHT, DPPH (1,1-diphenyl-2-picrylhydrazyl), and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO).

**Extraction.** The dried root of *Coptis japonica* Makino was first ground, and the ground root (5 kg) was extracted twice with 22.5 L of 80% aqueous methanol for 3 days at 25 °C. The extracted solution was filtered through filter paper to obtain the particle-free extract.

**Fractionation by Liquid-Liquid Partitioning.** Distilled and demineralized water was added to the methanolic extract of *Coptis japonica* Makino. Methanol in the extracted solution was removed using a rotary vacuum evaporator at 30 °C. The concentrated extract was subjected to successive organic solvent extraction with diethyl ether (Et<sub>2</sub>O), ethyl acetate (EtOAc), and butanol (BuOH).

**Isolation of Antiphototoxidative Components.** The active components were isolated from the butanol fraction by a flash silica gel column chromatography. The eluting solvent used was a solvent mixture of CHCl<sub>3</sub> and MeOH (10:1) initially, and the eluting solvent was gradually changed to 100% methanol. The obtained fractions were designated as Fr 1–Fr 6. Based on the antiphototoxidative activity of the fractions, the active components from Fr 2–Fr 4 were further purified by Sephadex LH-20 column chromatography or silica gel column chromatography, followed by preparative silica gel thin-layer chromatography. The fractionation and isolation procedures are summarized in Figure 1.

**Identification of the Active Components.** The identification of the components was carried out by UV-visible, IR, MS and NMR spectroscopy. The UV-visible spectra (550–190 nm) were obtained with a Shimadzu UV-260 spectrometer after the isolated components were dissolved in methanol. The IR spectrum was obtained with an Equinox 55 FT-IR spectrometer (Bruker) after the preparation of a KBr disk with isolated components. For mass spectrometry, a Hewlett-Packard model HP 5989A EI-MS system was used. A Varian UNITY spectrometer (300 MHz) was used to obtain the <sup>1</sup>H NMR spectrum.

**Determination of Antiphototoxidative Activity.** Sample solutions containing 0.15 M linoleic acid and chlorophyll *b* (6 μg/mL) in methanol were prepared, and 5 mL of the prepared sample solution was, in duplicate or triplicate, transferred into a 30 mL-capacity serum bottle. The prepared sample bottles were stored in a light storage box for 5 h as described previously (Jung et al., 1995). The light intensity of the sample level was 5500 lx. To minimize the possible radical chain reaction, the light storage box was placed in a 4 °C working cooler. The temperature within the light box was 7 ± 1 °C during light storage. The linoleic acid oxidation was determined by measuring peroxides according to the AOCS Official Method Cd 8-53 (AOCS, 1990).

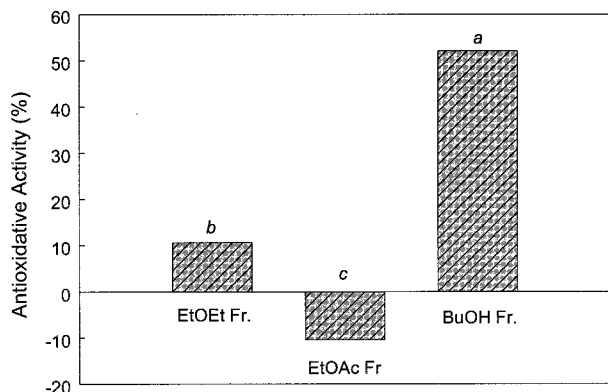
**Rat Liver Microsomal Lipid Peroxidation Inhibitory Activity.** Lipid peroxidation inhibitory activity in rat liver microsomes was evaluated by the thiobarbituric acid method (Ohakawa et al., 1979) with minor modifications. Rat liver microsomes were prepared according to the method of Hogeboom (1965), with some modifications, and suspended in 100 mM Tris-HCl buffer (pH 7.4). Reaction was initiated by the addition of 100 μM FeSO<sub>4</sub>·7H<sub>2</sub>O (0.1 mL) into a mixture of Tris-HCl buffer (0.7 mL), 0.2 mL ascorbic acid (50 μL), 0.5 μg protein mL<sup>-1</sup> microsomal suspension (40 μL) and 10 μL of sample. The reaction mixture was incubated at 37 °C for 30 min. After incubation, the reaction was stopped by addition of 0.25 mL of a TCA (3M)-HCl (2N) 1:1 mixture and then centrifuged at 3500g for 10 min. 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 min. The lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm. Lipid peroxidation inhibitory activity was calculated as follows:  $\{1 - (T - B)/(C - B)\} \times 100$  (%), in which T, C, and B are absorbances at 532 nm of the sample treatment, the control (without samples), and the zero time control, respectively. The IC<sub>50</sub> value is the concentration (μg/mL) of compounds required for 50% inhibition of microsomal lipid peroxidation.

**DPPH Free Radical Scavenging Activity.** Four milliliters of various concentrations of each component of authentic berberine chloride, palmatine chloride, BHA, or BHT in methanol were added to a methanol solution of 1 mL of 0.15 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) in test tubes. The mixture was shaken vigorously and left to stand for 30 min at room temperature. The absorbance of the solution was then measured at 517 nm with a spectrophotometer (Shimadzu UV 160 A, Shimadzu). The IC<sub>50</sub> values are the amounts (μg) of compounds required for a 50% decrease of the DPPH radical.

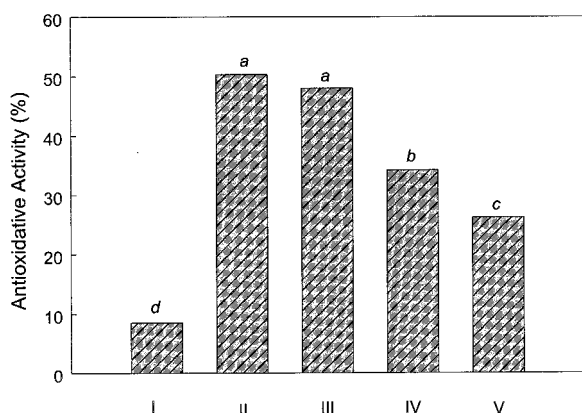
**Statistical Analysis.** Statistical analysis was accomplished using an SAS method (SAS Institute, Inc., 1991). Duncan's multiple-range test was used to ascertain the treatment effects on the rat liver microsomal lipid peroxidation, the DPPH free radical scavenging activity, or the chlorophyll-sensitized photooxidation of linoleic acid.

## RESULTS AND DISCUSSION

**Antiphototoxidative Activity of Butanol Fraction.** The methanolic extracts of *Coptis japonica* Makino were successively fractionated into three fractions by liquid-liquid partitioning extraction with diethyl ether, ethyl acetate, and butanol. The antioxidative activities of these isolated fractions were studied in the chlorophyll-sensitized photooxidation of linoleic acid (Figure 2). After 5 h of fluorescent light illumination, the peroxide values of the control (no added antioxidative components) was 53.2 meq/kg oil. However, treatment with the butanol fraction and the diethyl ether fraction decreased the formation of peroxide during light illumination. The result in Figure 2 shows that the antiphototoxidative activity of the butanol fraction (0.05%, w/v, based on the total solution volume) was especially high, showing 52.1% prevention of photooxidation of linoleic acid in the chlorophyll-sensitized photooxidation



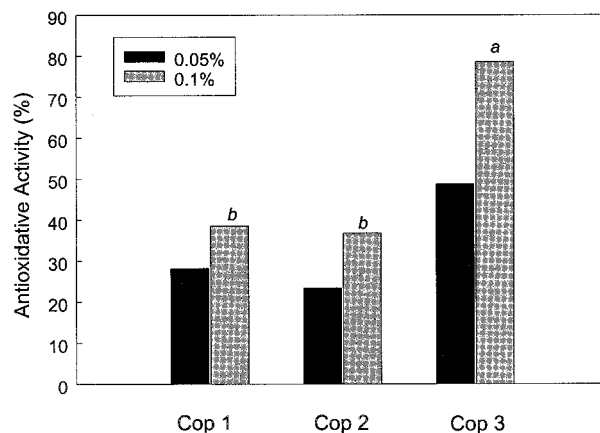
**Figure 2.** Effects of the 0.2% diethyl ether fraction (Et<sub>2</sub>O fr.), ethyl acetate fraction (EtOAc fr.), or butanol fraction (BuOH fr.) of the methanolic extract of *Coptis japonica* Makino on the chlorophyll *b*-sensitized photooxidation of linoleic acid in methanol under fluorescent light illumination (5500 lx) for 5 h at  $7 \pm 1$  °C. The chlorophyll concentration in this experimental system was  $6 \mu\text{g/mL}$ . The antioxidative activities with the same italicized letter shown on the top of the bar were not significantly different at  $\alpha = 0.01$ .



**Figure 3.** Effects of the 0.2% fractions (Fr I, II, III, IV, and V) obtained from the butanol fraction of the methanolic extract *Coptis japonica* Makino on the chlorophyll *b*-sensitized photooxidation of linoleic acid in methanol during 5 h of fluorescent light illumination (5500 lx) at  $7 \pm 1$  °C. The chlorophyll concentration in this experimental system was  $6 \mu\text{g/mL}$ . The antioxidative activities with the same italicized letter shown on the top of the bar were not significantly different at  $\alpha = 0.01$ .

of linoleic acid during 5 h of fluorescent light illumination. The diethyl ether (Et<sub>2</sub>O) fraction (0.05%, w/v) showed slight antioxidative activity (10.6% inhibition). The ethyl acetate (EtOAc) fraction did not show antioxidant activity but showed prooxidant activity in the chlorophyll-sensitized photooxidation of linoleic acid. These results consistent with previous data in that the butanol fraction of the methanolic extract of *Coptis japonica* Makino showed especially strong antiphotooxidative activity (Jung et al., 1999).

**Isolation of Active Components from the Butanol Fraction.** The butanol fraction was fractionated into five fractions (Fr I–Fr V) by flash silica gel column chromatography. The antioxidative activities of these isolated fractions (0.05%, w/v, based on total solution volume) were studied in the chlorophyll-sensitized photooxidation of linoleic acid (Figure 3). The peroxide value of the control (no addition of any fraction) was 60.6 meq/kg oil after 5 h of fluorescent light illumination. Fr I and Fr V inhibited 8.5% and 26.5% of chlorophyll-sensitized photooxidation of linoleic acid, respec-



**Figure 4.** Effects of 0.05 and 0.1% Cop 1, Cop 2, Cop 3, and Cop 4 on the chlorophyll *b*-sensitized photooxidation of linoleic acid in methanol during 5 h of fluorescent light illumination (5500 lx) at  $7 \pm 1$  °C. The chlorophyll concentration in this experimental system was  $6 \mu\text{g/mL}$ . The antioxidative activities with the same italicized letter shown on the top of the bar were not significantly different at  $\alpha = 0.01$ .

tively, showing relatively low antiphotooxidative activity. Three fractions, Fr II, Fr III, and Fr IV, however, showed strong antiphotooxidative activity, resulting in 50.3, 48.0, and 34.2% inhibition of linoleic acid oxidation, respectively. Cop 1 was isolated from Fr IV through Sephadex-LH20 column chromatography and preparative silica gel TLC. Cop 2 and Cop 4 were isolated from Fr III by silica gel column chromatography and preparative silica gel TLC. Cop 3 was fractionated from Fr I by silica gel column chromatography and preparative silica gel TLC. The antiphotooxidative activities of the isolated Cop 1, Cop 2, and Cop 3 are shown in Figure 4. We could not collect enough Cop 4 to study its antiphotooxidative activity since it was a minor component in the butanol fraction. Thus, the antiphotooxidative activity of Cop 4 could not be measured. The peroxide value of the control after 5 h of fluorescent light illumination was 58.4 meq/kg oil. All the tested components (Cop 1, Cop 2, and Cop 3) showed antiphotooxidative activity in the chlorophyll-sensitized photooxidation of linoleic acid. Cop 3 had especially strong antiphotooxidative activity, showing 78.6% inhibition of linoleic acid oxidation at the 0.1% concentration.

**Identification of Cop 1, Cop 2, Cop 3, and Cop 4.** The physicochemical properties of isolated Cop 1, Cop 2, Cop 3, and Cop 4 are summarized in Table 1. The IR spectrum showed that all these compounds contained  $-\text{OH}$  ( $3400 \text{ cm}^{-1}$ ), aromatic  $\text{C}=\text{C}$  ( $1560, 1410 \text{ cm}^{-1}$ ), and amine ( $1030 \text{ cm}^{-1}$ ) groups in their molecular structure, indicating aromatic alkaloids. The EI-MS shows that molecular weights of the Cop 1, Cop 2, Cop 3, and Cop 4 were 320, 339, 337, and 342, respectively. Based on the IR and MS analysis, the molecular formulas for Cop 1, 2, 3, and 4 were postulated as  $[\text{C}_{19}\text{H}_{14}\text{NO}_4]^+$ ,  $[\text{C}_{20}\text{H}_{20}\text{NO}_4]^+$ ,  $[\text{C}_{20}\text{H}_{18}\text{NO}_4]^+$ , and  $[\text{C}_{20}\text{H}_{24}\text{NO}_4]^+$ , respectively. The UV spectra of Cops 1, 2, and 3 were similar to each other, but the UV spectrum of Cop 4 was very different from those of the others.

The <sup>1</sup>H NMR spectrum (300 MHz, CD<sub>3</sub>OD) of Cop 1 showed 10 signals due to aromatic methines (6.96, 7.67, 7.87, 7.89, 8.64, and 9.73 ppm) and methylenes (3.25, 4.80, 6.11, and 6.46 ppm (Table 2). On the basis of the results from the obtained physical and chemical properties, mass spectrum and NMR spectrum, Cop 1 was positively identified as coptisine.



**Table 1. Physicochemical Properties of Cop 1, 2, 3, and 4**

	Cop 1	Cop 2	Cop 3	Cop 4
appearance	yellowish needles	reddish-yellow needles	yellow needles	white
EIMS ( $m/z$ )	320 ( $M^+$ )	339 ( $M^+$ )	337 ( $M^+$ )	342 ( $M^+$ )
UV $\lambda_{max}$ nm (log $\epsilon$ )	215 (4.54)	224 (5.08)	228 (4.99)	228 (4.00)
in MeOH	264 (4.43)	264 (5.01)	264 (4.92)	272 (3.40)
	357 (4.43)	349 (4.99)	347 (4.89)	318 (3.36)
	360 (3.71)	390 (4.57)	428 (4.24)	
IR ( $cm^{-1}$ )	3441, 2923	3433, 2923	3423, 2923	3433, 2924
	1560, 1412	2852, 2392	2362, 1637	1645, 1561
	1035	2343, 1560	1561, 1508	1409, 1407
		1419, 1023	1385, 1278	
			1232, 1105	
			1037	
molecular formula	$[C_{19}H_{14}NO_4]^+$	$[C_{20}H_{20}NO_4]^+$	$[C_{20}H_{18}NO_4]^+$	$[C_{20}H_{24}NO_4]^+$

**Table 2.  $^1H$  NMR Spectral Data for Cop 1, 2, 3, and 4**

carbon no.	Cop 1, $\delta_H$	Cop 2, $\delta_H$	Cop 3, $\delta_H$	Cop 4, $\delta_H$
1	7.67 (1H, s)	7.33 (1H, s)	7.66 (1H, s)	
3				6.59 (1H, s)
4	6.96 (1H, s)	6.5 (1H, s)	6.96 (1H, s)	2.75 (1H, dd, 4.2, 17.6)
4a				3.21 (1H, br ddd, 5.7, 17.6, 12.3)
5	3.25 (1H, t, 6.3)	3.07 (2H, t, 6.5)	3.25 (2H, t, 6.3)	3.58 (1H, dd, 5.8, 12.3)
6				3.43 (1H, ddd, 4.7, 12.3)
7	N.D.	4.79 (2H, t, 6.5)	4.92 (2H, t, 6.3)	4.05 (1H, br dd, 3.2, 13.6)
8				2.60 (1H, br t, 12.3, 13.6)
9	9.73 (1H, s)	9.5 (1H, s)	9.77 (1H, s)	3.05 (1H, dd, 3.2, 12.3)
11	7.89 (1H, d, 2.3)	7.96 (1H, d, 9.3)	8.12 (1H, d, 9.3)	6.56 (1H, d, 15.6)
12	7.87 (1H, d, 2.3)	7.85 (1H, d, 9.3)	7.99 (1H, d, 9.3)	6.64 (1H, d, 15.6)
13	8.64 (1H, s)	8.44 (1H, s)	8.71 (1H, s)	
2a-OCH <sub>2</sub> O	6.11 (2H, s)		6.11 (2H, s)	
9a-OCH <sub>2</sub> O	6.46 (2H, s)			
2-OMe		3.89 (3H, s)		3.79 (3H, s)
9-OMe		4.06 (3H, s)	4.11 (3H, s)	
10-OMe		4.16 (3H, s)	4.20 (3H, s)	3.81 (3H, s)
CH <sub>3</sub>				2.89 (3H, s)
CH <sub>3</sub>				3.31 (3H, s)

The  $^1H$  NMR signals for Cop 2 (Table 2) represents six aromatic methines, including four singlet methines (6.50, 7.33, 8.44, and 9.50 ppm) and ortho-coupled methines (7.85 and 7.96 ppm,  $J = 9.3$  Hz), two vicinal methylenes (3.07 and 4.79 ppm,  $J = 6.5$  Hz), and three aromatic methoxyl groups (3.89, 4.06, and 4.16 ppm). By comparing its physical and chemical properties, the MS, and the NMR spectral data in the references, Cop 2 was positively identified as jatrorrhizine.

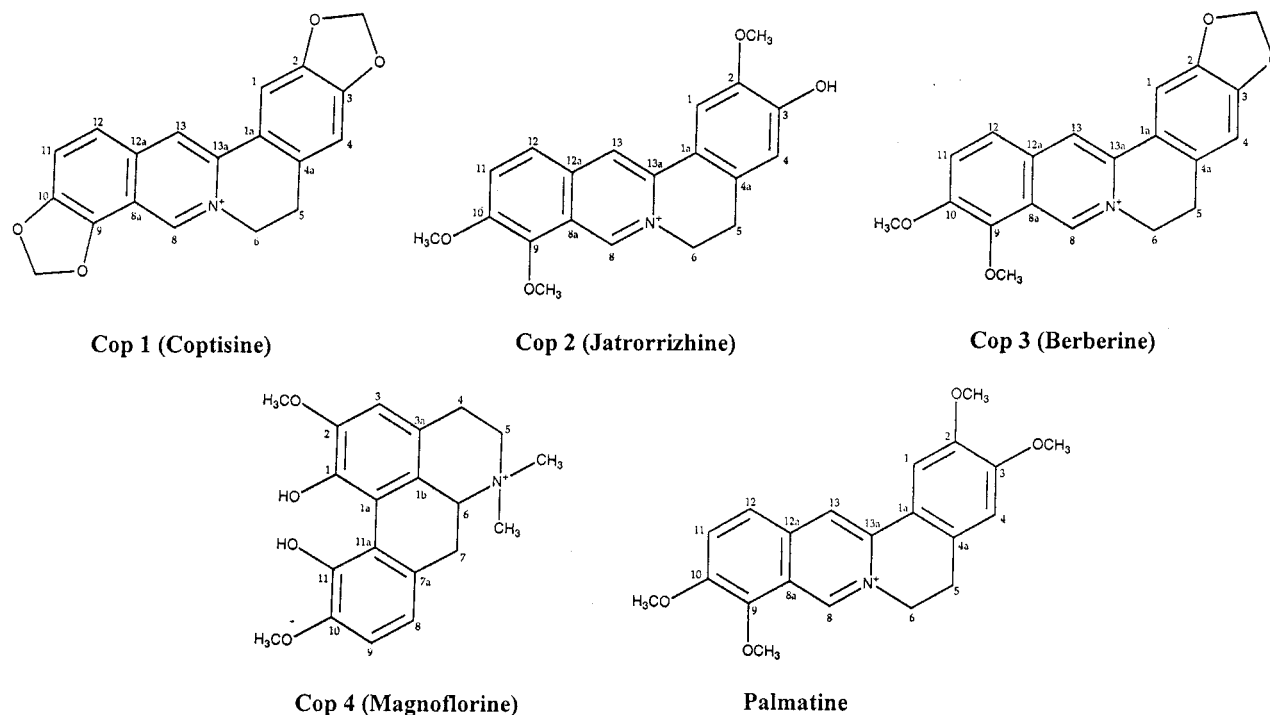
The  $^1H$  NMR spectrum of Cop 3 showed characteristic signals for six aromatic methines (6.96, 7.66, 7.99, 8.12, 8.70, and 9.77 ppm), two methylenes (7.99 and 8.12,  $J = 6.3$  Hz), and two aromatic methoxyl groups (4.11 and 4.2 ppm) (Table 2). With the results obtained from the physical and chemical properties, the mass spectrum, and the NMR spectrum, Cop 3 was positively identified as berberine.

The  $^1H$  NMR spectrum for Cop 4 showed signals due to methines and methylenes (2.60, 2.75, 3.05, 3.21, 3.43, 3.58, 4.05, 6.56, 6.59, and 6.64 ppm), methoxyl groups (3.79 and 3.81 ppm), and a methyl group (2.89 and 3.31 ppm) (Table 2), which were in good agreement with that of magnoflorine in the references. With the physical and chemical properties and the MS and NMR data, Cop 4 was positively identified as magnoflorine. The chemical structures of these identified components are shown in Figure 5.

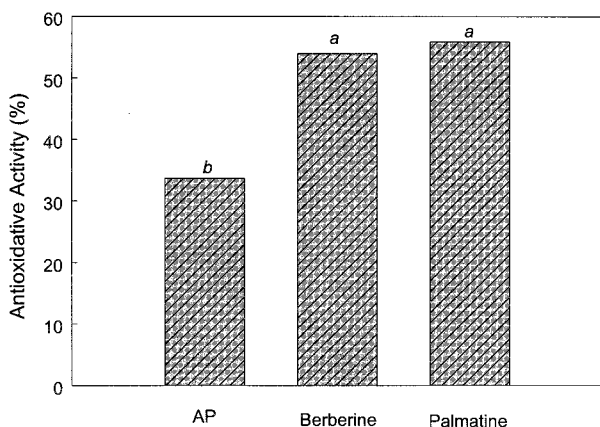
**Antiphotooxidative Activity of Authentic Protoberberines.** Since the protoberberines isolated from *Coptis japonica* Makino showed strong antiphotooxidative activity in chlorophyll-sensitized photooxidation of linoleic acid, we tested the antioxidative activity of

two commercially available authentic protoberberines (berberine chloride and palmatine chloride) in the chlorophyll-sensitized photooxidation of linoleic acid. The positive control used was ascorbyl palmitate, which was a known singlet oxygen quencher (Lee et al., 1997). The peroxide value of the control after 5 h of fluorescent light illumination was 56.5 meq/kg oil. Figure 6 showed that the authentic berberine and palmatine possess strong antiphotooxidative activity, showing 54.0 and 55.9% inhibition of the linoleic acid photooxidation after 5 h storage under fluorescent light. The antiphotooxidative activities of berberine chloride and palmatine chloride were not significantly different from each other ( $P > 0.01$ ). The antiphotooxidative activities of these protoberberines were significantly higher than ascorbyl palmitate ( $P < 0.01$ ). Their antioxidative activities were approximately 1.6 times higher than ascorbyl palmitate at the same molar concentration in the chlorophyll-sensitized photooxidation of linoleic acid.

**Postulated Mechanism for the Inhibitory Activity of Protoberberines.** Chlorophylls are very effective for the generation of singlet oxygen in the presence of light (Koryck-Dahl and Richardson, 1978; Whang and Peng, 1988a). Thus, the linoleic acid oxidation seemed to be, at least initially, due to singlet oxygen formed in the presence of chlorophyll and light. However, at the later stage of oxidation, the oxidized linoleic acid could initiate a radical chain reaction. Thus, the preventive mechanism of the isolated compounds involved in this experimental system might be due to either singlet oxygen quenching or the free radical scavenging ability of the compounds. To explore the preventive mechanism



**Figure 5.** Chemical structures of coptisine (Cop 1), jatrorrhizine (Cop 2), berberine (Cop 3), magnoflorine (Cop 4), and palmatine.



**Figure 6.** Effects of  $1.6 \times 10^{-3}$  M authentic protoberberines (berberine chloride and palmatine chloride) on the chlorophyll *b*-sensitized photooxidation of linoleic acid in methanol during 5 h of fluorescent light illumination (5500 lx) at  $7 \pm 1$  °C. The chlorophyll *b* concentration in this experimental system was  $6 \mu\text{g/mL}$ . The antioxidative activities with the same italicized letter shown on the top of the bar were not significantly different at  $\alpha = 0.01$ .

**Table 3. Inhibitory Activity of Cop 1, Cop 2, and Cop 3 against Rat Liver Microsomal Lipid Peroxidation**

sample	IC <sub>50</sub> ( $\mu\text{g/mL}$ )	sample	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
$\alpha$ -tocopherol	2.9	Cop 2	25.2
Cop 1	22.3	Cop 3	69.0

of these isolated compounds in the chlorophyll-sensitized photooxidation of linoleic acid, we analyzed the inhibitory activity of Cop 1, Cop 2, and Cop 3 on the rat microsomal lipid peroxidation. The IC<sub>50</sub> of  $\alpha$ -tocopherol, Cop 1, Cop 2, and Cop 3 were 2.9, 22.3, 25.2, and 69.0  $\mu\text{g/mL}$ , respectively (Table 3). This result clearly indicated that Cop 1, Cop 2, and Cop 3 possessed very weak radical chain reaction breaking activity. We also analyzed the DPPH radical scavenging activity of the commercially available protoberberines (berberine chlo-

**Table 4. Scavenging Effects of Commercially Available Protoberberines (Berberine Chloride and Palmatine Chloride) on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical**

sample	IC <sub>50</sub> ( $\mu\text{g}$ )	sample	IC <sub>50</sub> ( $\mu\text{g}$ )
BHA	4.5	berberine chloride	> 150
BHT	13.2	palmatine chloride	> 150

ride and palmatine chloride) along with well-known radical scavengers of BHA and BHT (Table 4). The BHA and BHT showed strong DPPH radical scavenging activity. However, neither of the protoberberines (berberine chloride and palmatine chloride) showed DPPH radical scavenging activity. This result indicated that these components did not possess the ability for breaking the radical chain reaction. The results also suggested that the preventive activity of the isolated compounds in the chlorophyll-sensitized photooxidation of linoleic acid was mainly due to their singlet oxygen quenching activity. However, to positively verify this postulation, further confirmatory research is required.

**Conclusion.** Berberine, jatrorrhizine, and magnoflorine isolated from the root of *Coptis japonica* Makino have strong antiphototoxidative activity in the chlorophyll-sensitized photooxidation of linoleic acid. Palmatine, which has a similar chemical structure to these compounds, also showed strong antiphototoxidative activity. That is, their antiphototoxidative properties were closely related to their basic alkaloid structure. The antiphototoxidative activity of these compounds seemed to be mainly due to their singlet oxygen quenching ability. The antiphototoxidative properties of these protoberberines have never been previously reported in the literature. However, for the utilization of these protoberberines as antiphototoxidative additives in real food systems, further information on their effectiveness, availability, cost, toxicity, and effects on physical properties in individual food items should be garnered.

## LITERATURE CITED

- AOCS. *Official Methods and Recommendations of the American Oil Chemists' Society*, 4th ed.; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 1990.
- Berliner, L. J.; Bradley, D. G.; Meinheltz, D.; Min, D. B.; Ogata, T. The detection of riboflavin-photosensitized singlet oxygen formation in milk by electron spin resonance. In *Supramolecular Structure and Function*; Pifat, G., Ed.; Balaban Publishers: Croatia, 1994; pp 67–77.
- Carlsson, D. J.; Suprunchuk, T.; Siles, D. M. Photooxidation of unsaturated oils: Effects of singlet oxygen quenchers. *J. Am. Oil Chem. Soc.* **1976**, *53*, 656–660.
- Chan, H. W. S. Photosensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways. *J. Am. Oil Chem. Soc.* **1977**, *54*, 100–104.
- Clements, A. H.; Van Den Engh, R. H.; Frost, D. J.; Hoogenhout, K. Participation of singlet oxygen in photosensitized oxidation of 1,4-dienoic systems and photooxidation of soybean oil. *J. Am. Oil Chem. Soc.* **1973**, *50*, 325–330.
- Hogeboom, G. H. General methods for the isolation of liver cell components: Fraction of cell components of animal tissues. *Methods Enzymol.* **1965**, *1*, 16–19.
- Jung, M. Y.; Choe, E.; Min, D. B. Alpha-, gamma- and delta-tocopherol effects on chlorophyll photosensitized oxidation of soybean oil. *J. Food Sci.* **1991**, *56*, 807–810 and 815.
- Jung, M. Y.; Kim, J. P.; Kim, S. Y. Methanolic extract of *Coptis japonica* Makino reduces the chlorophyll-sensitized photooxidation of oils. *Food Chem.* **1999**, *67*, 261–268.
- Jung, M. Y.; Kim, S. K.; Kim, S. Y. Riboflavin-sensitized photooxidation of ascorbic acid: kinetics and amino acid effects. *Food Chem.* **1995**, *53*, 397–403.
- Jung, M. Y.; Lee, K. H.; Kim, S. Y. Retinyl palmitate isomers in skim milk during light storage as affected by ascorbic acid. *J. Food Sci.* **1998a**, *63*, 597–600.
- Jung, M. Y.; Min, D. B. Effects of quenching mechanisms of carotenoids on the photosensitized oxidation of soybean oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 563–568.
- Jung, M. Y.; Yoon, S. H.; Lee, H. O.; Min, D. B. Singlet oxygen and ascorbic acid effects on the dimethyl disulfide and off-flavor formation in skim milk exposed to light. *J. Food Sci.* **1998b**, *63*, 408–412.
- King, J. M.; Min, D. B. Riboflavin-photosensitized singlet oxygen oxidation of vitamin D. *J. Food Sci.* **1998**, *63*, 31–34.
- Koryck-Dahl, M. B.; Richardson, T. Activated oxygen species and oxidation of food constituents. In *Critical Reviews in Food Science and Nutrition*; CRC Press: Boca Raton, FL, 1978; pp 209–241.
- Lee, K. H.; Jung, M. Y.; Kim, S. Y. Quenching mechanism and kinetics of ascorbyl palmitate for the reduction of the photosensitized oxidation of oils. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1053–1057.
- Lee, S. W.; Min, D. B. Effects, quenching mechanisms, and kinetics of nickel chelates in singlet oxygen oxidation of soybean oil. *J. Agric. Food Chem.* **1991**, *39*, 642–646.
- Ohakawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxidations in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351–358.
- Statistical Analysis System User's Guide*, Version 6.03; SAS Institute, Inc.: Cary, NC, 1991.
- Whang, K.; Peng, I. C. Detection of singlet oxygen generation by chlorophyll using electron paramagnetic resonance spectroscopy. *J. Food Sci.* **1988a**, *53*, 1918–1919.
- Whang, K.; Peng, I. C. Electron paramagnetic resonance studies of the effectiveness of myoglobin and its derivative as photosensitizers in singlet oxygen generation. *J. Food Sci.* **1988b**, *53*, 1863–1865.

Received for review August 23, 1999. Accepted January 7, 2000. We express our gratitude to the Korean Ministry of Agriculture and Forestry for their financial support. This research was also supported in part by Woosuk University Research Fund Program.

JF9909297