Antioxidative Flavonoids from the Leaves of *Morus alba*

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Nine flavonoids (**1-9**) were isolated from the leaves of *Morus alba* (Moraceae). The structures of compounds were determined to be kaempferol-3-*O*-β-D-glucopyranoside (astragalin, **1**) kaempferol-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside (**2**), quercetin-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside (**3**), quercetin-3-*O*-β-D-glucopyranoside (**4**), kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**5**), quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (rutin, **6**), quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**7**), quercetin-3,7-di-*O*-β-D-glucopyranoside (**8**) and quercetin (**9**) on the basis of spectroscopic and chemical studies. Compounds **7** and **9** exhibited significant radical scavenging effect on 1,1-diphenyl-2-picryl-hydrazyl radical.

Key words : *Morus alba*, Moraceae, DPPH radical, Antioxidative activity

INTRODUCTION

Mulberry (*Morus alba* L.) leaves containing many nutritional components are the best feed for silkworms. It was reported that the extracts from mulberry leaves showed a potent antihyperglycemic activity in diabetic mice (Chen *et al.*, 1989; Chen *et al.*, 1995). N-containing sugars (Asano *et al.*, 1994; Yagi *et al.*, 1976), plant hormone (Takemoto *et al.*, 1967), and moracetin (Naitoh, 1969) have been also isolated from mulberry leaves. Many phenolic compounds have been identified from the root barks of mulberry tree (Nomura and Fukai, 1981). *M. alba* contained rutin (Naitoh, 1968), isoquercitrin (Onogi *et al.*, 1993) and astragalin (Onogi *et al.*, 1993). However, antioxidative activities of flavonoids of *M. alba* have not been reported so far. We isolated nine flavonoids **1-9** from the 85% methanol extract of the leaves of *M. alba*. Compounds **5**, **7** and **8** have been isolated from the leaves of the mulberry tree for the first time. This paper reports the isolation, structure elucidation, and the free radical scavenging property of compounds **1-9**.

MATERIALS AND METHODS

Instrumentation and general techniques

UV spectra were obtained on a Unical UV/VIS spectrophotometer. IR spectra were recorded with a Jasco FT/IR 5300 spectrometer, ^{1}H- and ^{13}C-NMR spectra were run on a Bruker AMX 500 spectrometer. FABMS spectra were measured on a VG 70-VSEQ mass spectrometer with a direct inlet system using PEG600/glycerol as a matrix. TLC and column chromatography was carried out on Merck precoated silica gel F254 plates and Si gel 60 (Merck, 70~230 mesh) or sephadex LH 20 (Sigma, 25~100 μm). All other chemicals and solvent were analytical grade and used without further purification.

Plant materials

Mulberry (*M. alba*) leaves were collected at Suwon, Kyunggi Province, Korea in June, 1997. The voucher specimen is deposited in the herbarium of the Department of National Sericulture and Entomology, NIAST, RDA. The leaves were harvested, cleaned, freeze-dried and ground into fine powder in a mill (Tecator cemotec 1090 sample mill, Hoganas, Sweden). The material that passed through an 80-mesh sieve was retained, sealed in a glass bottle and stored at 4°C until use.

Extraction and isolation

Dry powdered leaves of *M. alba* (2 kg) were extracted with a mixture of water and methanol (15 : 85) in a sonicator (5×5 l, 25°C) for 1 hour and then concentrated under vacuum. The resulting MeOH extract (287 g) was suspended in water (200 ml), and

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was partitioned with CH_2Cl_2 (500 ml×5) and BuOH (400 ml×5), successively, to give CH_2Cl_2 (53 g), BuOH (43 g), and H_2O (166 g) fractions. The BuOH extract (43 g) was subjected to Dianion HP-20 column chromatography with H_2O (3500 ml), 50% EtOH (2000 ml), and EtOH (2000 ml) respectively. The 50% EtOH eluate (18 g) was chromatographed on a silica gel column (55×5 cm) eluting with CH_2Cl_2-MeOH-H_2O [80:10:0, (2500 ml); 60:10:0 (2100 ml); 40:10:1 (1530 ml); 50:15:2 (2100 ml); 70:30:5 (3150 ml); 0:1:0 (3000 ml)] to give 12 fractions (A-L). Fraction C was then subjected to Sephadex LH-20 chromatography with MeOH to obtain two subfractions C_1 and C_2. C_2 was further chromatographed on silica gel to obtain compound **1** (105 mg, 0.005%) and a mixture which was subjected to Sephadex LH-20 column chromatography eluting with H_2O-MeOH (1:1, 250 ml) to give compound **2** (13 mg, 0.001%). Fraction D was subjected to LH-20 chromatography with H_2O-MeOH (3:7, 300 ml) to give a mixture with compounds D-a and D-b which was chromatographed again on a silica gel column eluting with EtOAc-MeOH-H_2O (25:1:1, 1000 ml) to obtain a pure compound **3** (20 mg, 0.001%). Fraction F was subjected to Sephadex LH-20 chromatography with H_2O-MeOH (2:8, 400 ml) and produced 280 mg, a yield of 0.028%, of compound **4**. Fraction G was subjected to Sephadex LH-20 chromatography with gradient H_2O-MeOH (1:0→0:1, each of 350 ml) and produced 30 mg, a yield of 0.002%, of compound **5**. Fraction I was subjected to Sephadex LH-20 chromatography with gradient H_2O-MeOH (1:0→0:1, each of 250 ml) and produced 300 mg, a yield of 0.015%, of compound **6** and residues of mixed compounds I-a and I-b. The other mixed compound I-b was further chromatographyed on a Sephadex LH-20 column eluting with H_2O-MeOH (3:7, 150 ml) and produced 7 mg, a yield of 0.0004%, of compound **7**. Fraction K was subjected to Sephadex LH-20 chromatography with gradient H_2O-MeOH (1:0→0:1, each of 350 ml) and produced 25 mg, a yield of 0.001%, of compound **8**. The EtOH elute (1.51 g) was chromatographed on a Sephadex LH-20 column with gradient H_2O-MeOH (1:0→0:1, each of 450 ml) and produced 30 mg, a yield of 0.002%, of compound **9**.

Compound **1** (astragalin); pale yellow needles, mp. 231~234°C; IR ν_{max}^{KBr} (cm^{-1}): 3439 (OH), 1655 (C=O), 1604, 1498 (C=C), 1062, 1016 (glycosidic C-O); UV (MeOH) λ_{max}: 214, 267, 302 (sh), 349 nm; FABMS (*m/z*) 449 $[MH]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): δ 6.20 (1H, d, *J*=2.0 Hz, H-6), 6.43 (1H, d, *J*=2.0 Hz, H-8), 8.04 (2H, d, *J*=8.8, 2.0 Hz, H-2', 6'), 6.88 (2H, d, *J*=8.8 Hz, H-3', 5'), 5.45 (1H, d, *J*=7.5 Hz, H-1"); ^{13}C-NMR (DMSO-d_6, 500 MHz, ppm): δ 156.2 (C-2), 133.2 (C-3), 177.3 (C-4), 159.9 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.5 (C-9), 103.9 (C-10), 120.8 (C-1'), 1130.8 (C-2'), 115.1 (C-3'), 161.2 (C-4'), 115.1 (C-5'), 130.8 (C-6'), 100.9 (C-1"), 74.2 (C-2"), 76.4 (C-3"), 69.9 (C-4"), 77.4 (C-5"), 60.8 (C-6"). Acid hydrolysis of **1** gave kaempferol and glucose.

Compound **2** (kaempferol-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside); yellow needles, mp 258~263°C, IR ν_{max}^{KBr} (cm^{-1}): 3412 (OH), 1721 (-$COCH_3$), 1655 (C=O), 1609, 1512 (aromatic C=C), 1209 (ester), 1180, 1086 (glycosidic C-O); UV (MeOH) λ_{max}: 208, 266, 302, 350 nm; FABMS (*m/z*) 491 $[MH]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): δ 6.20 (1H, d, *J*=2.0 Hz, H-6), 6.44 (1H, d, *J*=2.0 Hz, H-8), 7.98 (2H, dd, *J*=8.8, 2.0 Hz, H-2', 6'), 6.87 (2H, d, *J*=8.8 Hz, H-3', 5'), 12.56 (brs, 5-OH), 5.35 (1H, d, *J*=7.4 Hz, H-1"), 10.83, 10.13 (each brs, 4', 7-OH) 1.73 (3H, s, -$COCH_3$); ^{13}C-NMR (DMSO-d_6): δ 158.4 (C-2), 134.7 (C-3), 178.4 (C-4), 161.6 (C-5), 99.43 (C-6), 164.9 (C-7), 94.5 (C-8), 157.4 (C-9), 104.7 (C-10), 122.4 (C-1'), 115.7 (C-2'), 144.8 (C-3'), 148.9 (C-4'), 117.0 (C-5'), 122.9 (C-6'), 101.10 (C-1"), 74.0 (C-2"), 76.1 (C-3"), 69.7 (C-4"), 73.8 (C-5"), 62.8 (C-6"), 169.7, 20.1 (-COCH3). Acid hydrolysis of **2** gave kaempferol and glucose.

Compound **3** (quercetin-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside); yellow needles, mp 217~219°C; IR ν_{max}^{KBr} (cm^{-1}): 3310 (OH), 1665 (C=O), 1607 (aromatic), 1086 (C-O); UV (MeOH) λ_{max}: 208, 257, 267(sh), 359 nm; FABMS (*m/z*) 507 $[MH]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): δ 6.20 (1H, s, H-6), 6.40 (1H, s, H-8), 7.49 (1H, s, H-2'), 6.82 (1H, d, *J*=8.8 Hz, H-5'), 7.50 (1H, d, *J*=8.8 Hz, H-6'), 5.31 (1H, d, *J*=7.4 Hz, H-1"), 1.68 (3H, s, -$COCH_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ: 156.3 (C-2), 133.2 (C-3), 177.3 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.4 (C-9), 103.8 (C-10), 121.5 (C-1'), 115.1 (C-2'), 148.4 (C-3'), 144.8 (C-4'), 116.1 (C-5'), 121.0 (C-6'), 100.9 (C-1"), 74.2 (C-2"), 76.4 (C-3"), 69.9 (C-4"), 73.9 (C-5"), 62.8 (C-6"), 169.8, 20.0 (-$COCH_3$). On acid hydrolysis, **3** gave quercetin and glucose.

Compound **4** (quercetin-3-*O*-β-D-glucoside); yellow needles, mp 238~241°C; IR ν_{max}^{KBr} (cm^{-1}): 3310 (OH), 1665 (C=O), 1607, 1507 (aromatic C=C); UV (MeOH) λ_{max}: 208, 257, 267 (sh), 359 nm; FABMS (*m/z*) 465 $[MH]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): δ 6.19 (1H, d, *J*=2.0 Hz, H-6), 6.40 (1H, d, *J*=2.0 Hz, H-8), 7.58 (1H, d, *J*=2.0 Hz, H-2'), 6.84 (1H, d, *J*=8.8 Hz, H-5'), 7.57 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 5.45 (1H, d, *J*=7.4 Hz, H-1"); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm): δ 156.1 (C-2), 133.2 (C-3), 177.4 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.4 (C-8), 156.3 (C-9), 103.9 (C-10), 121.5 (C-1'), 115.1 (C-2'), 148.4 (C-3'), 144.7 (C-4'), 116.1 (C-5'), 121.1 (C-6'), 100.8 (C-1"), 74.0 (C-2"), 76.4 (C-3"), 69.9 (C-4"), 77.5 (C-5"), 60.9 (C-6"). On acid hydrolysis, **4** gave quercetin and glucose.

Compound **5** (kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside); yellow needles, mp 215~220°C; IR ν_{max}^{KBr} (cm^{-1}): 3310 (OH), 1665 (C=O); UV (MeOH) λ_{max}: 208, 266, 349, 359 nm; FABMS (*m/z*)

595 [MH]$^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): δ 6.20 (1H, d, *J*=2.0 Hz, H-6), 6.40 (1H, d, *J*=2.0 Hz, H-8), 7.98 (2H, dd, *J*=8.8, 2.0 Hz, H-2', 6'), 6.86 (2H, dd, *J*=8.8, 2.0 Hz, H-3', 5'), 0.98 (3H, d, *J*=4 Hz, -CH_3 of rhamnose), 4. 56 (1H, s , rha-1), 5.31 (1H, d, *J*=7.5 Hz, glc-1); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm): δ 156.8 (C-2), 133.2 (C-3), 177.3 (C-4), 159.8 (C-5), 98.7 (C-6), 164.1 (C-7), 93.7 (C-8), 156.4 (C-9), 103.9 (C-10), 120.8 (C-1'), 130.8 (C-2'), 115.0 (C-3'), 161.1 (C-4'), 115.0(C-5'), 130.8 (C-6'), 100.7 (C-1''), 74.1 (C-2''), 76.3 (C-3''), 69.9 (C-4''), 75.7 (C-5''), 66.8 (C-6''), 101.3 (C-1'''), 70.5 (C-2'''), 70.3 (C-3'''), 71.8 (C-4'''), 68.2 (C-5'''), 17.7 (C-6'''). On acid hydrolysis, **5** gave kaempferol, rhamnose and glucose.

Compound **6** (quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside); yellow needles, mp 186~188°C; IR ν_{max}^{KBr} (cm^{-1}): 3300 (OH), 1650 (C=O); UV (MeOH) λ_{max}: 259, 359 nm, UV (NaOMe) λ_{max}: 272, 327, 410 nm; FABMS (*m/z*) 611 [MH]$^+$ ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): σ 6.19 (1H, s, H-6), 6.40 (1H, s, H-8), 7.58 (1H, d, *J*=2.0 Hz, H-2'), 6.84 (1H, d, *J*=8.8 Hz, H-5'), 7.57 (1H, dd, *J*=8.2, 2.0 Hz, H-6'), 1.10 (3H, d, *J*=4 Hz, -CH_3 of rhamnose), 4. 56 (1H, s, rha-1), 5.45 (1H, d, *J*=7.4 Hz, glc-1); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ: 158.4 (C-2), 134.7 (C-3), 178.4 (C-4), 161.6 (C-5), 99.4 (C-6), 164.9 (C-7), 94.5 (C-8), 157.4 (C-9), 104.7 (C-10), 122.4 (C-1'), 115.7 (C-2'), 144.8 (C-3'), 148.9 (C-4'), 117.0(C-5'), 122.9 (C-6'), 101.4 (C-1''), 74.6 (C-2''), 76.9 (C-3''), 70.0 (C-4''), 75.9 (C-5''), 68.8 (C-6''), 103.7 (C-1'''), 71.2 (C-2'''), 70.8 (C-3'''), 72.7 (C-4'''), 67.5 (C-5'''), 17.4 (C-6'''). On acid hydrolysis, **6** gave quercetin, rhamnose and glucose.

Compound **7** (quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside); yellow powder, mp 224~228 °C; IR ν_{max}^{KBr} (cm^{-1}): 3310 (OH), 1665 (C=O); UV (MeOH) λ_{max}: 209, 256, 267(sh), 357 nm; FABMS (*m/z*) 627 [MH]$^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): σ 6.18 (1H, s, H-6), 6.39 (1H, s, H-8), 7.54 (1H, d, *J*=2.0 Hz, H-2'), 6.68 (1H, d, *J*=8.8 Hz, H-5'), 7.59 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 5.69 (1H, d, *J*=7.6, Hz, H-1''). 4.60 ppm (1H, d, *J*=7.7 Hz). On acid hydrolysis, **7** gave quercetin and glucose.

Compound **8** (quercetin-3,7-di-*O*-β-D-glucopyranoside); yellow needles; IR ν_{max}^{KBr} (cm^{-1}): 3310 (OH), 1665 (C=O); UV (MeOH) λ_{max}: 208, 256, 356 nm, UV (NaOAc) λ_{max}: 259, 367 nm, UV (NaOAc+H_3BO_4) λ_{max}: 261, 383 nm, UV ($AlCl_3$) λ_{max}: 275, 434 nm, UV ($AlCl_3$+HCl) λ_{max}: 270, 299 (sh) nm; FABMS (*m/z*) 627 [MH]$^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): σ 6.43 (1H, d, *J*=2.0 Hz, H-6), 6.75 (1H, d, *J*=2.0 Hz, H-8), 7.59 (1H, d, *J*=2.0 Hz, H-2'), 6.65 (1H, d, *J*=8.5 Hz, H-5'), 7.58 (1H, dd, *J*=8.5, 2.0 Hz, H-6'), 5.48 (1H, d, *J*=7.3 Hz, H-1''), 5.08 (1H, d, *J*=7.3 Hz, H-1''); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ: 156.0 (C-2), 133.4 (C-3), 177.6 (C-4), 160.7 (C-5), 99.3 (C-6), 162.7 (C-7), 94.4 (C-8), 156.8 (C-9), 105.6 (C-10), 121.4 (C-1'), 115.2 (C-2'), 144.4 (C-3'), 148.8 (C-4'), 116.3 (C-5'), 120.4 (C-6'), 100.7 (C-1''), 74.2 (C-2''), 76.4 (C-3''), 69.9 (C-4''), 77.5 (C-5''), 60.8 (C-6''), 99.7 (C-1'''), 73.0 (C-2'''), 76.4 (C-3'''), 69.6 (C-4'''), 77.2 (C-5'''), 60.6 (C-6'''). On acid hydrolysis, **8** gave quercetin and glucose.

Compound **9** (quercetin); yellow needles, mp 311~313°C (dec.); IR ν_{max}^{KBr} (cm^{-1}): 3350 (OH), 1685 (C=O); UV (MeOH) λ_{max}: 255, 370 nm, UV (MeOH) λ_{max}: 321; FABMS (*m/z*) 303 [MH]$^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm): σ 6.15 (1H, s, H-6), 6.35 (1H, s, H-8), 7.47 (1H, *J*=2.0 Hz, H-2'), 6.81 (1H, d, *J*=8.5 Hz, H-5'), 7.40 (1H, d, *J*=8.5 Hz, H-6'); ^{13}C-NMR (DMSO-d_6, 125 MHz,

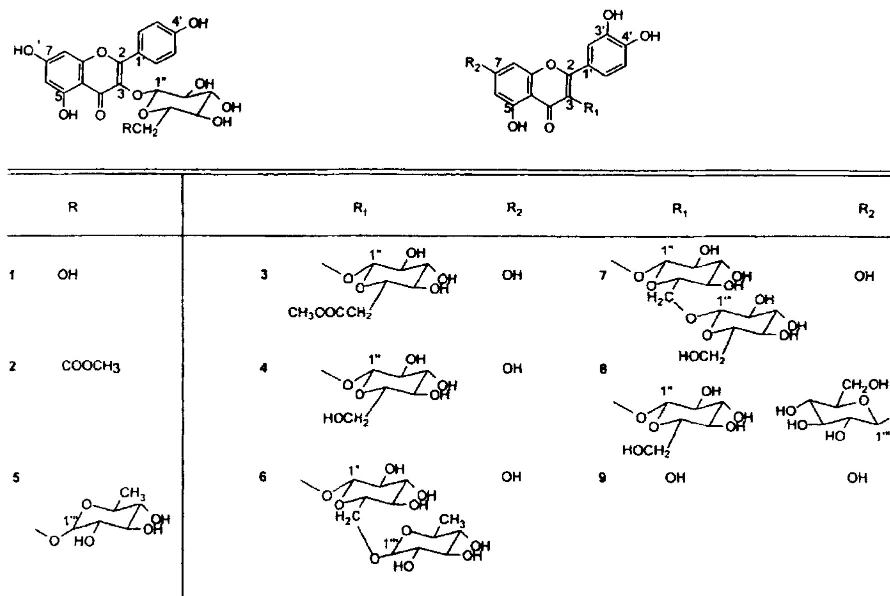

Fig. 1. The structures of compounds **1-9**

ppm): δ 146.9 (C-2), 135.8 (C-3), 175.9 (C-4), 160.8 (C-5), 98.3 (C-6), 164.0 (C-7), 93.5 (C-8), 156.2 (C-9), 103.1 (C-10), 122.1 (C-1'), 115.2 (C-2'), 145.1 (C-3'), 147.7 (C-4'), 115.7 (C-5'), 120.1 (C-6').

Acid hydrolysis of compounds

Three mgs. of each compound was dissolved in 2 ml of 2 N HCl-MeOH (1:1) in a 10 ml round bottomed flask and heated at 100°C for 1 h. To isolate the sugar and aglycons for further analysis, the reaction mixture was evaporated to half of original volumes to remove the MeOH, and then extracted several times with EtOAc by shaking vigorously in a test tube. In each case, the aglycon was fractionated into the EtOAc layer and the sugar into H_2O layer. The aglycone portion was analyzed by analytical HPLC with an ODS column (CH_3CN-H_2O, 1:1, 250×4.60 mm) and the sugar portion by analytical HPLC with a carbohydrate column (CH_3CN-H_2O, 4:1, 300×3.90 mm). The retention times were compared with those of authentic samples.

Testing for antioxidant activity

MeOH solutions (4 ml) of flavonoids at various concentrations (1~14 μg/ml) were added to a solution of DPPH in MeOH (1.5×10^{-4} M, 1 ml) and the reaction mixture were shaken vigorously. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm (Yoshida *et al.*, 1989). And the radical scavenging activity of each flavonoid was expressed by the ratio of the lowering of the absorption of DPPH (%) relative to the absorption (100%) of the DPPH solution in the absence of flavonoids. The mean values were obtained from triplicate experiments.

RESULTS AND DISCUSSION

The investigation of 85% methanol extract of the leaves of *M. alba* yielded nine flavonoids. Compounds **5, 7,** and **8** were reported from *M. alba* for the first time.

The structures of compound **1-4, 6** and **9** were determined by comparison of their mps, UV, and NMR spectral data with those reported in the literature; Compound **1** was determined as astragalin (Kim *et al.*, 1995), compound **2** as kaempferol-3-*O*-(6''-*O*-acetyl)-β-D-glucopyranoside (Do *et al.*, 1992), compound **3** as quercetin-3-*O*-(6''-*O*-acetyl)-β-D-glucopyranoside (Onogi *et al.*, 1993), compound **4** as isoquercitrin (Kang and Woo, 1984), compound **6** as rutin (Shin *et al.*, 1995), and compound **9** as quercetin (Kim *et al.*, 1995), respectively.

The ^{1}H- and ^{13}C- NMR spectra of compound **5** were similar to those of compound **1**, except for the presence of significant signals of rhamnose [^{1}H: 5.33 ppm (1H, brs, H-1), 0.98 ppm (3H, *J*=6.2 Hz H-6); ^{13}C: 101.3 (C-1) and 17.7 ppm (C-6)]. Compared to compound **1**, C-6 signal of compound **5** shifted downfield from 60.8 ppm in compound **1** to 66.8 ppm. It was shown that rhamnose links to C-5 of glucose. From the chemical shifts and the coupling constant of the anomeric proton of rhamnose, the glycosidic linkage of rhamnose is α type. From these data, compound **5** was identified as kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (Takagi *et al.*, 1981). Compound **7** was isolated in the form of yellow powder. ^{1}H- and ^{13}C-NMR spectra were similar to those of compound **4**, isoquercitrin except for the presence of an additional glucose signals [H: 4.60 ppm (1H, d, *J*=7.7 Hz)]. The type of glycosidic linkage was determined to be β from the chemical shift and the coupling constant of the anomeric proton of glucose. In the ^{13}C-NMR spectrum, the C-6 signal shifted downfield compared to isoquercitrin. Thus, the structure of compound **7** was established as quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (Nohara *et al.*, 1982). The *ortho*-dihydroxyl group in the B-ring of compound **8** was confirmed on the basis of band I in the $AlCl_3$ and $AlCl_3$/HCl spectrum as well as the NaOAc/ H_3BO_3 spectrum. ^{1}H NMR spectrum of compound **8** was similar to that isoquercitrin except for the presence of an additional glucose unit [H: 5.08 (d, *J*=7.3) for anomeric proton signal]. This showed that compound **8** was a monoglucoside of isoquercitrin. Comparing with isoquercitrin, H-6 and H-8 signals of compound **8** were shifted downfield to 6.43 ppm and 6.75 ppm, respectively. The glycosidation site was also deduced from ^{13}C-NMR data (Harborne and Mabry, 1982). Only the C-3 and C-7 signals were shifted upfield by 2.4 and 1.3 ppm respectively in comparison with quercetin. These changes confirmed that the quercetin was substituted at C-3 and C-7. The downfield shifts of C-2, C-4, C-6 and C-8 signals also supported this assignment. The

Table I. Radical scavenging effects of compounds **1-9** on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

sample[a]	EC_{50}[b] (μg)
Control (BHA)	9.5
compound **1**	>480.0
compound **2**	>480.0
compound **3**	13.2
compound **4**	17.6
compound **5**	>480.0
compound **6**	15.7
compound **7**	12.9
compound **8**	14.6
compound **9**	7.3

[a]Each compound was examined with four concentration in triplicate.

[b]ED_{50} value represents the concentration of a compound required for 50% decreases of DPPH radicals.

coupling constant (*J*=7.3 Hz) between H-1 glucose and H-2 glucose indicated that the linked sugars at both site were β configuration. The pyranoside form of sugar was established on the basis of ^{13}C-NMR spectral data (Markham *et al.*, 1978). From the above data, compound **8** was determined to be quercetin-3, 7-di-O-β-D-glucopyranoside (Kodama *et al.*, 1990).

Damage caused by the toxic effects of OH · radicals is often decreased by radical scavengers such as phenolic compounds (Cederbaum and Cohen, 1985; Halliwell and Gutteridge, 1984). The flavonoids are also known to act as strong superoxide radicals ($\cdot O_2^-$) scavengers and singlet oxygen ($^1O_2$) quenchers (Husain, 1987). To examine the radical scavenging effects of flavonoides, nine flavonoids isolated from leaves of *Morus alba* L. were tested by the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical. (Table I) The radical scavenging activity of the flavonoids isolated decreases in the order: Compound **9** > Compound **7** > Compound **3** > Compound **8** > Compound **6** > Compound **4**. But compounds **1, 2** and **5** showed scarcely any effect. The compound **9** exhibited higher activity than BHA, positive standard.

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