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Inhibitory Activities of the Alkaloids from Coptidis Rhizoma against Aldose Reductase

Hyun Ah Jung, Na Young Yoon, Hyun Ju Bae, Byung-Sun Min[1], and Jae Sue Choi

Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea and [1]College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea

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As part of our ongoing search of natural sources for therapeutic and preventive agents for diabetic complications, the rat lens aldose reductase (RLAR) inhibitory effect of Coptidis Rhizoma (the rhizome of *Coptis chinensis* Franch) was evaluated. Its extract and fractions exhibited broad and moderate RLAR inhibitory activities of 38.9~67.5 μg/mL. In an attempt to identify bioactive components, six quaternary protoberberine-type alkaloids (berberine, palmatine, jateorrhizine, epiberberine, coptisine, and groenlandicine) and one quaternary aporphine-type alkaloid (magnoflorine) were isolated from the most active *n*-BuOH fraction, and the chemical structures therein were elucidated on the basis of spectroscopic evidence and comparison with published data. The anti-diabetic complications capacities of seven *C. chinensis*-derived alkaloids were evaluated *via* RLAR and human recombinant AR (HRAR) inhibitory assays. Although berberine and palmatine were previously reported as prime contributors to AR inhibition, these two major components exhibited no AR inhibitory effects at a higher concentration of 50 μg/ml in the present study. Conversely, epiberberine, coptisine, and groenlandicine exhibited moderate inhibitory effects with IC_{50} values of 100.1, 118.4, 140.1 μM for RLAR and 168.1, 187.3, 154.2 μM for HRAR. The results clearly indicated that the presence of the dioxymethylene group in the D ring and the oxidized form of the dioxymethylene group in the A ring were partly responsible for the AR inhibitory activities of protoberberine-type alkaloids. Therefore, Coptidis Rhizoma, and the alkaloids contained therein, would clearly have beneficial uses in the development of therapeutic and preventive agents for diabetic complications and diabetes mellitus.

Key words: *Coptis chinensis*, Coptidis Rhizoma, Alkaloids, Aldose reductase, Diabetic complications

INTRODUCTION

Aldose reductase (AR) is one of the important enzymes in the polyol pathway that catalyzes the reduction of glucose to sorbitol and thus plays a crucial part in the development of diabetic complications, including retinopathy, neuropathy, nephropathy, and cataracts (Kawanishi et al., 2003; de la Fuente et al., 2003). For these reasons, there are growing interests in AR inhibitors (ARIs) to alleviate the various symptoms of diabetic complications and treat its many related diseases. Although several synthetic ARIs have been proposed, including zopolrestat, epalrestat, and sorbinil, most of them place a limit on their usage, and/or have been withdrawn from clinical trials due to relatively low efficacy, poor pharmacokinetics, and unsatisfactory safety (Kawanishi et al., 2003; Manzanaro et al., 2006). In an attempt to develop potent, safe, and new anti-diabetic complications agents from natural sources (de la Fuente et al., 2003), we began an effort to search for ARIs and evaluate their potentials.

Coptis chinensis Franch, of the Ranunculaceae family, is a perennial, stemless herb that grows throughout China. Coptidis Rhizoma, the rhizome of *C. chinensis*, has commonly been prescribed for treatment of diabetes mellitus in Chinese traditional herbal medicine for a long time due to its blood sugar-lowing properties. It also relaxes blood vessels, lowers fevers, stimulates circulation, and exerts antibacterial, antiviral, and antifungal activity (Huang, 1999). In addition, *Coptis chinensis* are reported to possess anti-inflammatory (Schinella et al., 2002), anti-proliferative (Tse et al., 2006), antioxidant (Schinella et al., 2002;

Correspondence to: Jae Sue Choi, Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea
Tel: 82-51-629-5845, Fax: 82-51-629-5842
E-mail: choijs@pknu.ac.kr

Yokozawa et al., 2005), and anti-hypertensive activities (Ko et al., 2000), as well as hypoglycemic and hypocholesterolemic effects (Yuan et al., 2006).

Coptidis Rhizoma is known to harbor a diversity of alkaloids, including berberine, palmatine, jateorrhizine, epiberberine, and coptisine, which are considered to be its active constituents (Sun et al., 2006). In particular, berberine is the most predominant component and shows various pharmacological and biological effects, including anti-hypertensive (Ko et al., 2000), anti-diabetic (Tang et al., 2006; Huang et al., 2006), anti-inflammatory (Kuo et al., 2004), hypolipidemic (Kong et al., 2004; Doggrell, 2005), and antioxidant effects (Hsieh et al., 2007). Berberine, palmatine, and jateorrhizine have been reported to exhibit hypoglycemic and hypocholesterolemic effects (Yuan et al., 2006). Yokozawa et al. (2005) reported that coptisine, palmatine, magnoflorine, and epiberberine might contribute to the protective effects of Cotidis Rhizoma on oxidative stress, including inhibition of cellular peroxynitrite generation. Jateorrhizine and magnoflorine were also reported to exhibit significant antioxidant and antiradical capacities (Racková et al., 2004; Hung et al., 2007).

Since Coptidis Rhizoma has been used for treatment of diabetes mellitus, and several alkaloids isolated from Coptidis Rhizoma showed preventive and inhibitory activity against several diabetic complications-related symptoms, including antioxidant, hypolipidemic, and hypocholesterolemic effects, the present study on the inhibitory effects of Coptidis Rhizoma on diabetic complications is worthy of promoting this field of research. In particular, our present results seem to be inconsistent with previous research on the AR inhibitory effects of Coptidis Rhizoma-derived alkaloids (Lee, 2002; Nakai et al., 1985), therefore, the objectives of the present work are to re-evaluate the AR inhibitory effects of several alkaloids and reaffirm the structure-activity relationship of Coptidis Rhizoma-derived alkaloids.

MATERIALS AND METHODS

General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were determined using a JEOL JNM ECP-400 spectrometer (JEOL, Japan) at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The EI-MS data was collected on a GCMS QP-5050A spectrometer (Shimadzu, Japan). Column chromatography was conducted using silica (Si) gel 60 (70-230 mesh, Merck, Germany), and MCI CHP20P gel (75-150 μm, Mitsubishi Chemical Co., Tokyo, Japan). TLC was conducted on precoated Merck Kieselgel 60 F_{254} plates (20×20 cm, 0.25 mm).

Chemicals

Nicotinamide adenine dinucleotide phosphate (NADPH), quercetin, and DL-glyceraldehyde dimer were purchased from Sigma Chemical Company (St. Louis, MO, USA). Human recombinant aldose reductase (HRAR, 0.4 unit) was purchased from Wako Chemicals (Osaka, Japan).

Plant materials

The rhizome of *C. chinensis* was purchased from a local retailer and authenticated by Prof. J. H. Lee at Dongguk University, Gyeongbuk Province, Korea. A voucher specimen (No. 20060420) was deposited in the author's laboratory (Prof. J. S. Choi).

Extraction, fractionation, and isolation

The powdered rhizome of *C. chinensis* (10 kg) were refluxed with methanol (MeOH) for 3 h (3×10 L). The total filtrate was then concentrated to dryness *in vacuo* at 40°C, in order to render the MeOH extract (2.2 kg). This extract was suspended in distilled water (H_2O) and then successively partitioned with methylene chloride (CH_2Cl_2), *n*-butanol (BuOH), to yield CH_2Cl_2 (230 g), *n*-BuOH (1.1 kg) fractions, respectively, as well as H_2O residue (840 g). A portion of *n*-BuOH fraction (316 g) was initially chromatographed over a Si gel column using a mixed solvent of EtOAc, MeOH, and H_2O (EtOAc:MeOH:H_2O 21:4:3 → 21:10:3 → EtOAc:MeOH:2% HCl 21:10:3, gradient conditions) to yield 14 subfractions (BF1~ BF18). BF02 (9.3 g) was repeatedly chromatographed over a Si gel column using a mixed solvent of EtOAc, MeOH, and H_2O (EtOAc:MeOH:H_2O 21:4:3) to yield palmatine (3 g). BF03 (21.1 g) was rechrystallized with MeOH, yielding berberine (7 g). The filtrate of BF03 (15 g) was subjected to column chromatography over a Si gel column with a mixture solvent of CH_2Cl_2 and MeOH (CH_2Cl_2:MeOH 5:1 → 0:1, gradient conditions) to yield jateorrhizine (50 mg). BF12 (17.3 g) was chromatographed over a MCI gel column with aqueous MeOH (0% → 100%), followed by recrystallization with MeOH to obtain groenlandicine (60 mg), and coptisine (80 mg). A portion of BF13 (20 g) was chromatographed over a MCI gel column with aqueous MeOH (0% → 100%), followed by recrystallization with MeOH to obtain epiberberine (90 mg). Magnoflorine (20 mg) were isolated from BF14 (13 g) by MCI gel column chromatography with aqueous MeOH (0% → 100%). The chemical structures of isolated alkaloids were elucidated on the basis of spectroscopic evidences and by comparison with published data (Grycová et al., 2007; Lee and Kim, 1997). The NMR data of isolated alkaloids were as follows.

Berberine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.75 (1H, s, H-8), 8.69 (1H, s, H-13), 8.10 (1H, d, J = 8.3 Hz, H-11), 7.98 (1H, d, J = 8.4 Hz, H-12), 7.64 (1H, s, H-1), 6.94 (1H, s,

H-4), 6.09 (2H, s, OCH_2O), 4.91 (2H, t, J = 6.4, H-6), 4.18 (3H, s, 9-OCH_3), 4.09 (3H, s, 10-OCH_3), 3.24 (2H, t, J = 6.4 Hz, H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 151.0 (C-10), 150.8 (C-3), 148.7 (C-2), 144.6 (C-9), 145.2 (C-8), 138.5 (C-13a), 134.0 (C-12a), 130.7 (C-4a), 126.8(C-11), 123.3(C-12), 122.1 (C-8a), 120.7 (C-13b), 120.3 (C-13), 108.2 (C-4), 105.3 (C-1), 102.5 (OCH_2O), 61.3 (C-9, OCH_3), 56.4 (C-10, OCH_3), 56.0 (C-6), 27..0 (C-5).

Magnoflorine

^{1}H-NMR (400 MHz, CD_3OD): δ 6.65 (1H, d, J = 6.5 Hz, H-9), 6.46 (1H, d, J = 6.5 Hz, H-8), 6.45 (1H, s, H-3), 3.78 (3H, s, 10-OCH_3), 3.80 (1H, m, C-6), 3.80 (3H, s, 2-OCH_3), 3.45 (1H, m, H-5), 3.23 (1H, m, H-5), 3.23 (3H, s, N^+CH_3), 3.10 (1H, m, H-4), 2.93 (1H, dd, J = 3.2, 12.3 Hz, H-7), 2.77 (3H, s, N^+CH_3), 2.58 (1H, m, H-4), 2.42 (1H, br t, J = 12.3, 13.6 Hz, H-7); ^{13}C-NMR (100 MHz, CD_3OD): δ 151.9 (C-2), 150.5 (C-10), 149.4 (C-1), 148.4 (C-11), 124.8 (C-7a), 122.4 (C-11b), 122.3 (C-11a), 119.8 (C-6b), 115.9 (C-8), 114.7 (C-3a), 109.4 (C-9), 108.3 (C-3), 69.9 (C-6a), 61.12 (C-5), 55.1 (2-OCH_3), 54.8 (10-OCH_3), 52.7 (N^+CH_3), 42.8 (N^+CH_3), 30.5 (C-7), 23.5 (C-4).

Groenlandicine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.62 (1H, s, H-8), 8.69 (1H, s, H-13), 7.86 (1H, d, J = 8.0 Hz, H-11), 7.82 (1H, d, J = 8.0 Hz, H-12), 7.60 (1H, s, H-1), 6.83 (1H, s, H-4), 6.43 (2H, s, OCH_2O), 4.89 (2H, m, H-6), 3.99 (2-OCH_3), 3.28 (H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 150.8 (C-3), 148.5 (C-2), 147.7 (C-10), 144.4 (C-9), 143.9 (C-8), 138.4 (C-13a), 133.5 (C-12a), 129.0 (C-4a), 121.7 (C-12), 121.0 (C-11), 120.6 (C-13), 118.1 (C-13b), 114.7 (C-4), 112.4 (C-8a), 108.6 (C-1), 104.8 (OCH_2O), 56.2 (C-6), 55.7 (2- OCH_3), 26.4 (C-5).

Jateorrhizine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.71 (1H, s, H-8), 8.74 (1H, s, H-13), 8.08 (1H, d, J = 8.0 Hz, H-11), 7.97 (1H, d, J = 8.0 Hz, H-12), 7.63 (1H, s, H-1), 6.84 (1H, s, H-4), 4.48 (2H, m, H-6), 4.18 (3H, s, 9-OCH_3), 4.09 (3H, s, 10-OCH_3), 4.00 (3H, s, 2-OCH_3), 3.18 (2H, m, H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 150.6 (C-9), 150.5 (C-2), 148.4 (C-3), 145.0(C-8), 144.5 (C-10), 139.1 (C-13a), 134.2 (C-12a), 129.1 (C-4a), 126.8 (C-12), 123.1(C-11), 122.0 (C-13b), 119.7 (C-13), 118.2 (C-8a), 114.7 (C-4), 108.8 (C-1), 61.3 (9-OCH_3), 55.7 (10-OCH_3), 56.4 (2-OCH_3), 56.2 (C-6), 26.4 (C-5).

Coptisine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.71 (1H, s, H-8), 8.71 (1H, s, H-13), 7.87 (1H, d, J = 8.0 Hz, H-11), 7.83 (1H, d, J = 8.0 Hz, H-12), 7.63 (1H, s, H-1), 6.84 (1H, s, H-4), 6.45 (2H, s, OCH_2O), 6.09 (2H, s, OCH_2O), 4.89 (2H, m, H-6), 3.23 (2H, m, H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 151.0 (C-10), 148.8 (C-3), 148.1 (C-2), 144.6 (C-8), 144.1 (C-9), 137.8 (C-13a), 133.2 (C-12a), 130.6 (C-4a), 121.9 (C-12), 121.6 (C-13b), 120.7 (C-13), 120.7 (C-11), 112.5 (C-8a), 108.2 (C-4), 105.3 (C-1), 105.0 (OCH_2O), 102.5 (OCH_2O), 56.0 (C-6), 27.0 (C-5).

Palmatine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.78 (1H, s, H-8), 8.88 (1H, s, H-13), 8.10 (1H, d, J = 8.0 Hz, H-11), 8.00 (1H, d, J = 8.0 Hz, H-12), 7.63 (1H, s, H-1), 7.03 (1H, s, H-4), 4.87 (2H, m, H-6), 4.19 (3H, s, 9-OCH_3), 4.08 (3H, s, 10-OCH_3), 3.97 (3H, s, 2-OCH_3), 3.92 (3H, s, 3-OCH_3), 3.52 (2H, m, H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 152.6 (C-3), 150.7 (C-10), 149.7 (C-2), 145.2(C-8), 144.5 (C-9), 138.6 (C-13a), 134.0 (C-12a), 128.9 (C-4a), 126.8 (C-12), 123.3(C-11), 122.1 (C-13b), 120.1 (C-13), 119.3 (C-8a), 111.0 (C-4), 108.7 (C-1), 61.3 (9-OCH_3), 56.4 (10-OCH_3), 56.1 (2-OCH_3), 55.8 (3-OCH_3), 55.4 (C-6), 26.6 (C-5); LC-ESI-MS/MS m/z 352 $[M]^+$, 337, 322, 308.

Epiberberine

^{1}H-NMR (400 MHz, CD_3OD) : δ 9.70 (1H, s, H-8), 8.81 (1H, s, H-13), 7.88 (1H, d, J = 8.0 Hz, H-11), 7.83 (1H, d, J = 8.0 Hz, H-12), 7.63 (1H, s, H-1), 7.03 (1H, s, H-4), 6.45 (2H, s, OCH_2O), 4.88 (2H, m, H-6), 3.97 (3H, s, 2-OCH_3), 3.92 (3H, s, 3-OCH_3), 3.26 (2H, m, H-5); ^{13}C-NMR (100 MHz, CD_3OD) : δ 152.6 (C-3), 149.7 (C-2), 147.9 (C-10), 144.5 (C-9), 144.1 (C-8), 137.9 (C-13a), 133.3 (C-12a), 128.7 (C-4a), 121.8(C-12), 121.1(C-11), 121.0 (C-13), 119.3 (C-13b), 112.5 (C-8a), 111.0 (C-4), 108.5 (C-1), 104.9 (OCH_2O), 56.2 (C-6), 55.8 (2-OCH_3), 55.5 (3-OCH_3), 26.5 (C-5).

Assay for RLAR inhibitory activity

In these experiments we followed The Guidelines for Care and Use of Laboratory Animals as approved by Pukyong National University. According to the modified method of Hayman and Kinoshita (1965), rat lens homogenate was prepared. Briefly, the lens were removed from the eyes of Sprague-Dawley rats (Samtako BioKorea, Inc.) weighing 250-280 g. The lens are homogenized in sodium phosphate buffer (pH 6.2), which was prepared from sodium phosphate dibasic ($Na_2HPO_4 \cdot H_2O$, 0.66 g) and sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$, 1.27 g) in 100 mL of double distilled water. The supernatant was obtained by centrifugation of the homogenate at 10,000 rpm at 4°C for 20 min and was frozen until use. A crude AR, with a specific activity of 6.5 U/mg, was used in the evaluations for enzyme inhibition. The partially purified material was separated into 1.0 mL aliquots, and

stored at -40°C. Each 1.0 mL cuvette contained equal units of enzyme, 100 mM sodium phosphate buffer (pH 6.2), and 1.6 mM NADPH, both with and without 50 mM of the substrate, DL-glyceraldehyde, and an inhibitor (f.c. 10~100 μg/mL for the extract, and fractions, and 12.5~ 50 μg/mL for the compounds, dissolved in 100% DMSO). The AR activity was determined by measuring the decrease in NADPH absorption at 340 nm over a 4 min period on a Ultrospec®2100pro UV/Visible spectrophotometer with SWIFT II Applications software (Amersham Biosciences, New Jersey, USA). Quercetin, a well known ARI was used as references. The inhibition percentage (%) was calculated as $[1 - (\Delta A\ sample/min - \Delta A\ blank/min)/(\Delta A\ control/min - \Delta A\ blank/min)] \times 100$, where ΔA sample/min represents the reduction of absorbance for 4 min with the test sample and substrate, respectively, and ΔA control/min represents the same, but with 100% DMSO instead of a sample. The 50% inhibition concentrations of the RLAR inhibitory activity were calculated from the log-dose inhibition curve within test concentrations. The IC_{50} values are expressed as the mean ± SEM.

Assay for HRAR inhibitory activity

The recombinant human AR inhibitory activities were examined according to the method of Nishimura et al (1991). The reaction mixture was prepared as follows: 100 μL of 0.15 mM NADPH, 100 μL of 10 mM DL-glyceraldehyde, as a substrate, 5 μL of the recombinant human AR, and various concentrations of the samples (f.c. 25~75 μg/mL, dissolved in 100% DMSO) in a total volume of 1.0 mL of 100 mM sodium phosphate buffer (pH 6.2). The AR activity was determined by measuring the decrease in NADPH absorption at 340 nm over a 1 min period on a Ultrospec®2100pro UV/Visible spectrophotometer with SWIFT II Applications software (Amersham Biosciences). Quercetin, a well known ARI was used as references. The inhibition percentage (%) was calculated similar to the RLAR assay, except that ΔA sample/min represents the reduction of absorbance for 1 min with the test samples and substrate. The 50% inhibition concentrations of the HLAR inhibitory activity were calculated from the log-dose inhibition curve within test concentrations. The IC_{50} values are expressed as the mean ± SEM.

Statistics

All results are presented as mean ± S.E.M. Statistical significance was analyzed via one-way ANOVA and Student's *t*-test (Systat In., Evaston, III., USA).

RESULTS AND DISCUSSION

To evaluate the anti-diabetic complication effects of Coptidis Rhizoma, the RLAR inhibitory activities of the MeOH extract and its CH_2Cl_2, *n*-BuOH, and H_2O fractions were measured using the modified method of Hayman and Kinoshita (1965). As shown in Table I, the extract and its fractions exhibited moderate RLAR inhibitory activities with IC_{50} values of 49.1, 64.6, 38.9, and 67.5 μg/mL respectively, as compared to a positive control, quercetin (IC_{50} 0.4 μg/mL). Lee (2002) previously demonstrated that the MeOH extract, and the CH_2Cl_2, and *n*-BuOH fractions showed significant RLAR inhibitory effects, particularly the CH_2Cl_2 fraction exhibiting RLAR inhibitory effects similar to quercitrin. In the present study, the *n*-BuOH fraction was the most active fraction, followed by the CH_2Cl_2 and H_2O fractions, whereas Lee (2002) stated that the H_2O fraction showed only marginal activity. The TLC analyses of the MeOH extract and its fractions indicated that *n*-BuOH and H_2O fractions also harbored a variety of alkaloids. Thus these polar fractions are expected to exert as good RLAR activity as other fractions. Since the *n*-BuOH fraction exhibited the most RLAR inhibitory effect in the present work, further phytochemical examinations were carried out to obtain seven alkaloids, including six quaternary protoberberine-type alkaloids (berberine, palmatine, jateorrhizine, epiberberine, coptisine, and groenlandicine) and one quaternary aporphine-

Table I. RLAR inhibitory activities of the MeOH extract and its fractions from *C. chinensis*

Sample	Conc. (μg/mL)[a]	Inhibition[b] (%)		IC_{50} (μg/mL)[c] Mean±SEM
Quercetin	5	83.33	85.71	0.39 ± 0.13
	1	67.86	67.86	
	0.2	39.29	35.71	
MeOH	100	81.31	78.69	49.14 ± 3.59
	50	52.68	42.86	
	10	29.46	29.46	
CH_2Cl_2	100	60.66	63.93	64.62 ± 1.91
	50	50.00	45.12	
	10	23.17	30.49	
n-BuOH	50	60.61	57.58	38.87 ± 1.60
	10	27.27	25.76	
H_2O	100	80.33	95.08	67.48 ± 5.07
	50	18.03	24.59	
	10	-1.64	1.64	

[a]Final concentrations of test samples were 10~100 μg/mL for the extract, and fractions, and 1~10 μg/mL for quercetin, which were dissolved in 100% DMSO. [b]The inhibition percentage (%) was calculated as $[1 - (\Delta A\ sample/min - \Delta A\ blank/min)/(\Delta A\ control/min \times \Delta A\ blank/min)] \times 100$, where ΔA sample/min represents the reduction of absorbance for 4 min with the test sample and substrate, respectively, and ΔA control/min represents that with 100% DMSO instead of a sample. The 50% inhibition concentration is expressed as the mean ± SEM of triple experiments.

type alkaloid (magnoflorine) (Fig. 1).

The RLAR inhibitory activities of the seven alkaloids isolated from the most active *n*-BuOH fraction are shown in Table II. Among the tested compounds, berberine and palmatine exhibited no RLAR inhibitory activities within the test concentration of 12.5-50 μg/mL. Magnoflorine and

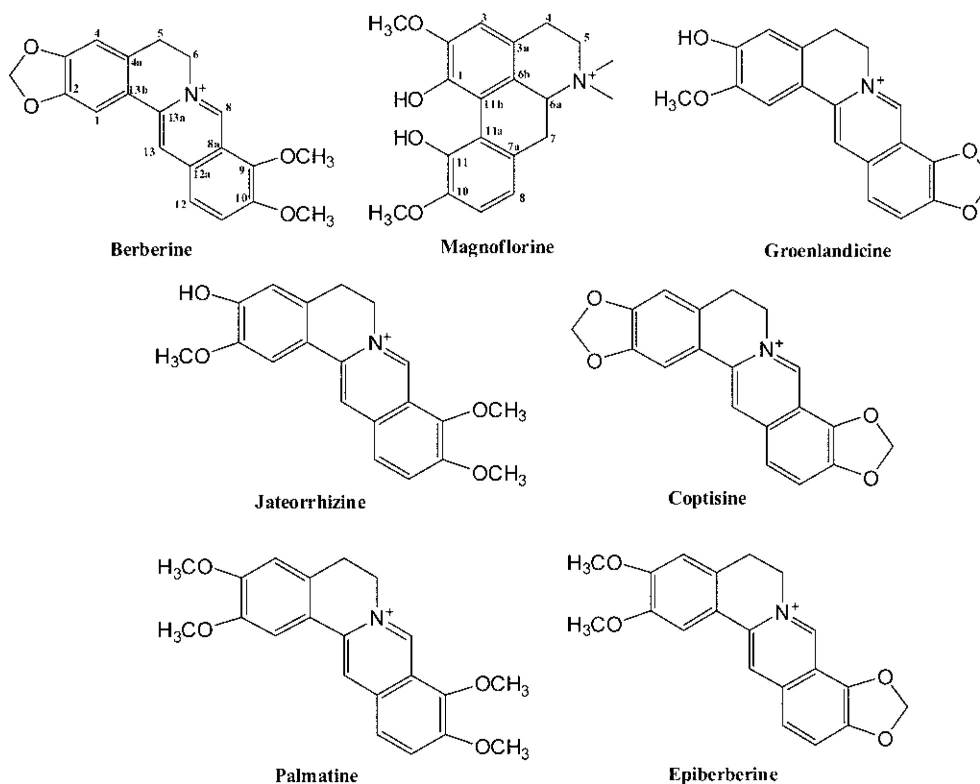

Fig. 1. Structures of alkaloids from *C. chinensis*

Table II. RLAR inhibitory activities of seven alkaloids from *C. chinensis*

	Conc. μg/mL[a]	Inhibition[b] %		IC_{50} (μg/mL)[c] Mean±SEM	IC_{50} (μM)[d] Mean±SEM
Quercetin	5	77.94	76.47	0.57 ±0.20	1.88 ± 0.47
	1	66.18	63.24		
	0.2	33.82	41.18		
Berberine	50			> 50	
Palmatine	50			> 50	
Jateorrhizin	50	14.08	12.68	> 50	
Epiberberine	50	101.59	98.41	33.62 ± 0.30	100.07 ± 0.63
	33.3	50.51	50.11		
	25	12.50	12.50		
Coptisine	50	86.87	85.86	37.87 ± 0.35	118.36 ± 0.78
	33.3	31.31	35.13		
	25	15.28	9.72		
	12.5	4.84	3.23		
Groenlandicine	50	52.38	60.32	45.12 ± 2.96	140.13 ± 6.50
	25	23.61	18.06		
	12.5	10.96	10.96		
Magnoflorine	50	29.85	18.33	> 50	

[a]Final concentrations of test samples were 10~50 μg/mL for the compounds, which were dissolved in 100% DMSO. [b]The inhibition percentage (%) was the same as in Table I. [c,d]The 50% inhibition concentrations (μg/mL and μM, respectively) were calculated from the log dose inhibition curve and expressed as the mean ± SEM of duplicate experiments.

jateorrhizine showed marginal RLAR inhibitory activities with 18% and 13% inhibition at a concentration of 50 μg/mL, respectively. Conversely, epiberberine, coptisine, and groenlandicine showed good inhibitory activities with IC_{50} values of 33.6, 37.9, and 45.2 μg/mL (100.1, 118.4, and 140.1 μM), respectively, in the RLAR assay. As shown in Table III, groenlandicine, epiberberine, and coptisine also exhibited the HRAR inhibitory effects with IC_{50} values of 51.2, 56.5, and 66.7 μg/mL (154.2, 168.1, and 187.3 μM), respectively. The results suggested that the presence of dioxymethylene group in the D ring and the oxidized form of the dioxymethylene group in the A ring were at least partly attributed to the RLAR and HRAR inhibitory activities of protoberberine-type alkaloids. Nakai et al. (1985) and Lee (2002) reported that both berberine and palmatine showed potent RLAR inhibitory effects, with palmatine exhibiting potency greater than berberine, due to the oxidized form of the dioxymethylene group in the A ring. Similar to these previous results, the oxidized form of the dioxymethylene group in the A ring might contribute to the RLAR and HRAR inhibitory effects of the alkaloids in present study, however, the presence of dioxymethylene group in the D ring seems to play a much more crucial role in the AR inhibitory activity: epiberberine, coptisine, groenlandicine vs. berberine, palmatine. The important structural characterization of ARI may be harbored within the polar portion as well as the hydrophobic ring system. The hydrophobic ring systems of the ARIs are tightly bound adjacent to the anion-bind site of AR, and the polar systems are bound in an anion-binding site adjacent to the nicotinamide ring of the coenzyme (El-Kabbani and Podjarny, 2007). Epiberberine and groenlandicine, possessing the dimethylene group in the D ring as the hydrophobic ring system, and methoxyl group in the A ring as the polar systems of an alkaloid unit, may be deemed the reasonable ARIs in the RLAR and HRAR systems. The AR-polyol pathway is expected to be highly implicated in oxidative stress, such as reduction/oxidation of NADPH and glutathione. According to Yokozawa's study (2005) on Cotidis Rhizoma and its alkaloids, coptisine, palmatine, epiberberine, jateorrhizine, and magnoflorine inhibited peroxynitrite- and SIN-1-induced cellular damage, except for berberine. In part, epiberberine, and jateorrhizine could potentially exhibit the RLAR inhibitory effects through inhibition of reactive nitrogen species formation, however, the precise mechanism remains unclear since there are other biological aspects of berberine to consider, including strong radical scavenging, inhibition of lipid peroxidation, LDL oxidation, and nitric oxide generation, as well as chelation of metal ion (Shirwaikar et al., 2006; Hsieh et al. 2007). Moreover, the remarked difference between previous results (Lee, 2002; Nakai et al., 1985) and our present data might be due to the modification of enzyme preparation.

Until now, several kinds of minor compounds have been isolated from the *Coptis* species, including lignans (Cho et al., 2001), neolignans (Yoshikawa et al., 1995), coumarins (Mizuno et al., 1992), acids and phenolics (Yahara et al., 1985). Since coumarins and phenolics have been reported to possess AR inhibitory activity, these two kinds of compounds are likely to play more important roles in AR inhibition than the alkaloids, in spite of limited presence.

In conclusion, Coptidis Rhizoma, and the alkaloids contained within, would clearly have beneficial uses in the development of therapeutic and/or preventive agents for diabetic complications and diabetes mellitus.

Table III. HRAR inhibitory activities of alkaloids from *C. chinensis*

	Conc. μg/mL[a]	Inhibition[b] %		IC_{50} (μg/mL)[c] Mean ± SEM	IC_{50} (μM)[d] Mean ± SEM
Quercetin	0.25	53.85	53.85	0.22 ± 0.00	0.73 ± 0.01
	0.05	30.77	23.08		
Coptisine	75	53.85	61.54	66.67 ± 3.57	187.27 ± 10.03
	50	30.77	38.46		
	25	0.00	7.69		
Epiberberine	75	69.23	76.92	56.48 ± 1.85	168.10 ± 5.51
	50	46.15	46.15		
	25	0.00	7.69		
Groenlandicine	75	76.92	69.23	51.19 ± 2.38	154.19 ± 7.17
	50	53.85	53.85		
	25	23.08	15.38		

[a]Final concentrations of test samples were 10~50 μg/mL for the compounds, which were dissolved in 100% DMSO. [b]The inhibition percentage (%) was the same as in Table I. [c,d]The 50% inhibition concentrations (μg/mL and μM, respectively) were calculated from the log dose inhibition curve and expressed as the mean ± SEM of duplicate experiments.

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