Available online at www.sciencedirect.com
SCIENCE DIRECT
PHYTOCHEMISTRY
Phytochemistry 67 (2006) 1539–1546
www.elsevier.com/locate/phytochem

Bioassay-guided isolation of aldose reductase inhibitors from *Artemisia dracunculus*

Sithes Logendra [a], David M. Ribnicky [a,*], Hui Yang [b], Alexander Poulev [a], Jun Ma [a], Edward J. Kennelly [b], Ilya Raskin [a]

[a] *Biotech Center, Cook College, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901-8520, USA*
[b] *Department of Biological Sciences, Lehman College and The Graduate Center, The City University of New York, 250 Bedford Park Boulevard West, Bronx, NY 10468, USA*

Received 1 November 2005; received in revised form 3 May 2006
Available online 27 June 2006

Abstract

An ethanolic extract of *Artemisia dracunculus* L. having antidiabetic activity was examined as a possible aldose reductase (ALR2) inhibitor, a key enzyme involved in diabetic complications. At 3.75 μg/mL, the total extract inhibited ALR2 activity by 40%, while quercitrin, a known ALR2 inhibitor, inhibited its activity by 54%. Bioactivity guided fractionation and isolation of the compounds that inhibit ALR2 activity was carried out with the total ethanolic extract yielding four bioactive compounds with ALR2 inhibitory activity ranging from 58% to 77% at 3.75 μg/mL. Using LC/MS, ^{1}H NMR, ^{13}C NMR and 2D NMR spectroscopic analyses, the four compounds were identified as 4,5-di-*O*-caffeoylquinic acid, davidigenin, 6-demethoxycapillarisin and 2′,4′-dihydroxy-4-methoxydihydrochalcone. This is the first report on their isolation from *A. dracunculus* and the ALR2 inhibitory activity of 4,5-di-*O*-caffeoylquinic acid, 6-demethoxycapillarisin and 2′,4′-dihydroxy-4-methoxydihydrochalcone. These results suggest a use of the extract of *A. dracunculus* for ameliorating diabetic complications.

Keywords: *Artemisia dracunculus*; Asteraceae; Russian tarragon; Aldose reductase; Aldose reductase inhibitors; Davidigenin; 6-Demethoxycapillarisin; Dihydrochalcones; 2′,4′-Dihydroxy-4-methoxydihydrochalcone; 4,5-Di-*O*-caffeoylquinic acid

1. Introduction

Diabetes is a disease associated with elevated blood glucose levels, leading to major complications such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases. While multiple mechanisms have been proposed for these complications (Koya and King, 1998; Ishii et al., 1996; Friedman, 1999; Brownlee, 2000; Baynes and Thorpe, 1999; Betteridge, 2000; Yabe-Nishimura, 1998), evidence suggests that the polyol pathway is centrally associated with the etiology of these chronic diseases (Miyamoto, 2002; De la Fuente et al., 2003; Gabbay et al., 1966). Aldose reductase (ALR2, alditol, EC 1.1.1.21), a member of the aldoketo reductase superfamily, is the first enzyme of the polyol pathway and catalyzes the conversion of blood glucose into sorbitol in the presence of NADPH (Fig. 1). Under normal glucose conditions, ALR2 functions as a scavenging enzyme for toxic aldehydes in nerve cells (Kawamura et al., 2002) as well as an enzyme that regulates cell growth (Donohue et al., 1994; Laeng et al., 1995). Under euglycemic conditions, ALR2 has low affinity for glucose and converts very little glucose into sorbitol. Under hyperglycemic conditions, however, the blood glucose is increased, leading to high glucose concentrations in tissues that have insulin independent glucose entry such as the vascular endothelial cells of peripheral nerves, kidney, and the retina of the eye. This excess glucose is converted to sorbitol by the ALR2 enzyme which then accumulates in the cells since sorbitol cannot pass through cell membranes. Sorbitol accumulation leads to osmotic

* Corresponding author. Tel.: +1 732 932 8165x226; fax: +1 732 932 6535.
E-mail address: ribnicky@aesop.rutgers.edu (D.M. Ribnicky).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.05.015

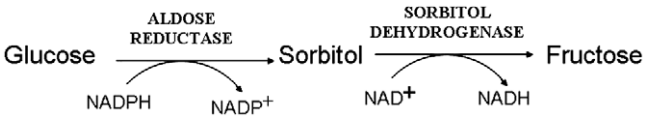

Fig. 1. Polyol pathway.

stress and damage to the cells. In addition, some of the sorbitol is oxidized to fructose by sorbitol dehydrogenase leading to an increase in the ratio of NADH:NAD^+ thereby creating a condition of pseudohypoxia (Mylari et al., 2003; Williamson et al., 1993). Depletion of NADPH by activation of the polyol pathway further increases tissue oxidative stress (Miyamoto, 2002). The osmotic stress, oxidative stress and other stresses such as protein kinase activation and glycation reactions lead to serious vascular and neural disfunction (Mylari et al., 2005; Tilton et al., 1995). Inhibitors of ALR2 have been shown to block this pathway (De la Fuente et al., 2003; Miyamoto, 2002), by binding to the active site of the enzyme (Wilson et al., 1993; Hohman et al., 1998). Many ALR2 inhibitors have been either synthesized (Sarges and Oates, 1993; Constantino et al., 1996; Severi et al., 1996; Lim et al., 2001) or isolated from plants (De la Fuente and Manzanaro, 2003; Benvenuti et al., 1996; Kawanishi et al., 2003). Due to toxicity, potency and efficacy problems encountered in preclinical and clinical trials, however, no ALR2 inhibitors have advanced through the process of clinical development.

Artemisia dracunculus or Russian tarragon is a perennial herb that belongs to the Asteraceae family. Many edible and medicinal uses have been attributed to this species and it is commonly used for flavoring food in many traditional recipes (Phillips and Foy, 1990). The use of *A. dracunculus* as an antiepileptic remedy in Iranian folkloric medicine has been evaluated and the anticonvulsant and sedative effects were related to the presence of monoterpenoids in the essential oils of the aerial parts of the plant (Sayyah et al., 2004). Due to the mild sedative nature of the plant, it has also been used as a sleep aid (Chevallier, 1996). Rats fed with hyperlipidemic diet showed reduction in serum cholesterol and triglycerides, when treated with *A. dracunculus* extract (Yazdanparast and Saee, 1999) and one cultivar of tarragon has been traditionally used as a treatment for diabetes in England (Swanston-Flatt et al., 1991).

Previously, we have shown that the ethanolic extract of *A. dracunculus* has antidiabetic properties (Ribnicky et al., 2005). It lowered blood glucose levels in both chemically induced diabetic mice lacking insulin and in genetically diabetic mice with insulin resistance. The extract also enhanced insulin stimulated glucose uptake and increased the accumulation of IRS-2 (insulin receptor substrate-2) in skeletal muscle cell cultures of obese rats. In an ongoing clinical trial, the extract reduced blood insulin levels in mildly diabetic patients (Ribnicky et al., 2005). This report investigates the ALR2 inhibitory activity of the extract to evaluate its potential for treatment of diabetic complications that are caused by the enhanced activation of the polyol pathway during hyperglycemia. This investigation led to isolation and identification of four new compounds from *A. dracunculus* that inhibit the activity of ALR2, two of which are new to the genus *Artemisia*.

2. Results and discussion

2.1. Isolation of pure compounds with ALR2 inhibitory activity

At 3.75 μg/mL, the ethanolic extract of *A. dracunculus* shoots inhibited the human recombinant ALR2 enzyme activity by 40% while the pure compound, quercitrin, had an inhibition of 54% (Fig. 2a). Quercitrin is a flavonoid that is a well-known inhibitor of the ALR2 enzyme (Matsuda et al., 2002; Lee, 2005) and was used in this study as a positive control. In order to begin the process of identifying the active compounds within the extract, the extract was divided into 10 fractions (based on elution time) by HPLC, which were then tested for ALR2 inhibitory activity. Of the ten fractions collected from the total extract, fractions 5 and 7 showed significantly higher inhibition of ALR2 at 3.75 μg/mL than the other fractions and a similar inhibition as quercitrin (Fig. 2b). These two fractions were selected for further purification.

Fractionation of the active fraction, F5, yielded eight sub-fractions. The aldose reductase inhibition assay revealed two active sub-fractions, F5-6 and F5-7 (Fig. 2c). Sub-fraction 5-7, after another step of purification gave a single pure compound (**1**) which inhibited the enzyme by 78% at 3.75 μg/mL, which is 19% greater inhibition than that caused by quercitrin.

Nine sub-fractions were collected from the purification of the compounds in F7. Of these nine sub-fractions, F7-1, F7-2, F7-3, and F7-9 showed similar or higher inhibitory activity than quercitrin (Fig. 2d) when tested at 3.75 μg/mL. Since F7-2 and F7-9 showed a slightly higher percent inhibition compared to F7-1 and F7-3, these two sub-fractions were selected for additional purification.

Further purification of the active sub-fraction, F7-2, yielded two different compounds as determined by liquid chromatography–mass spectrometry (LC–MS) analysis. These two compounds (**2** and **3**) had similar ALR2 inhibitory activity (Fig. 2e – F7-2/P1 and F7-2/P2) and slightly higher ALR2 inhibitory activity than quercitrin when tested at 3.75 μg/mL. Further purification of the other active sub-fraction, F7-9, of F7, revealed that the sub-fraction consisted of at least three different compounds. When the ALR2 inhibition assay was carried out with these compounds, F7-9/P3 (**4**) at 3.75 μg/mL showed the highest inhibition at 58.2%, almost similar to quercitrin (Fig. 2f). The purified compounds within the active fractions were identified by a combination of LC–MS and nuclear magnetic resonance (NMR) as described in Section 3 and from published data (Zhu et al., 2004; Sharon et al., 1992; Jensen et al., 1977). The other fractions or sub-fractions with

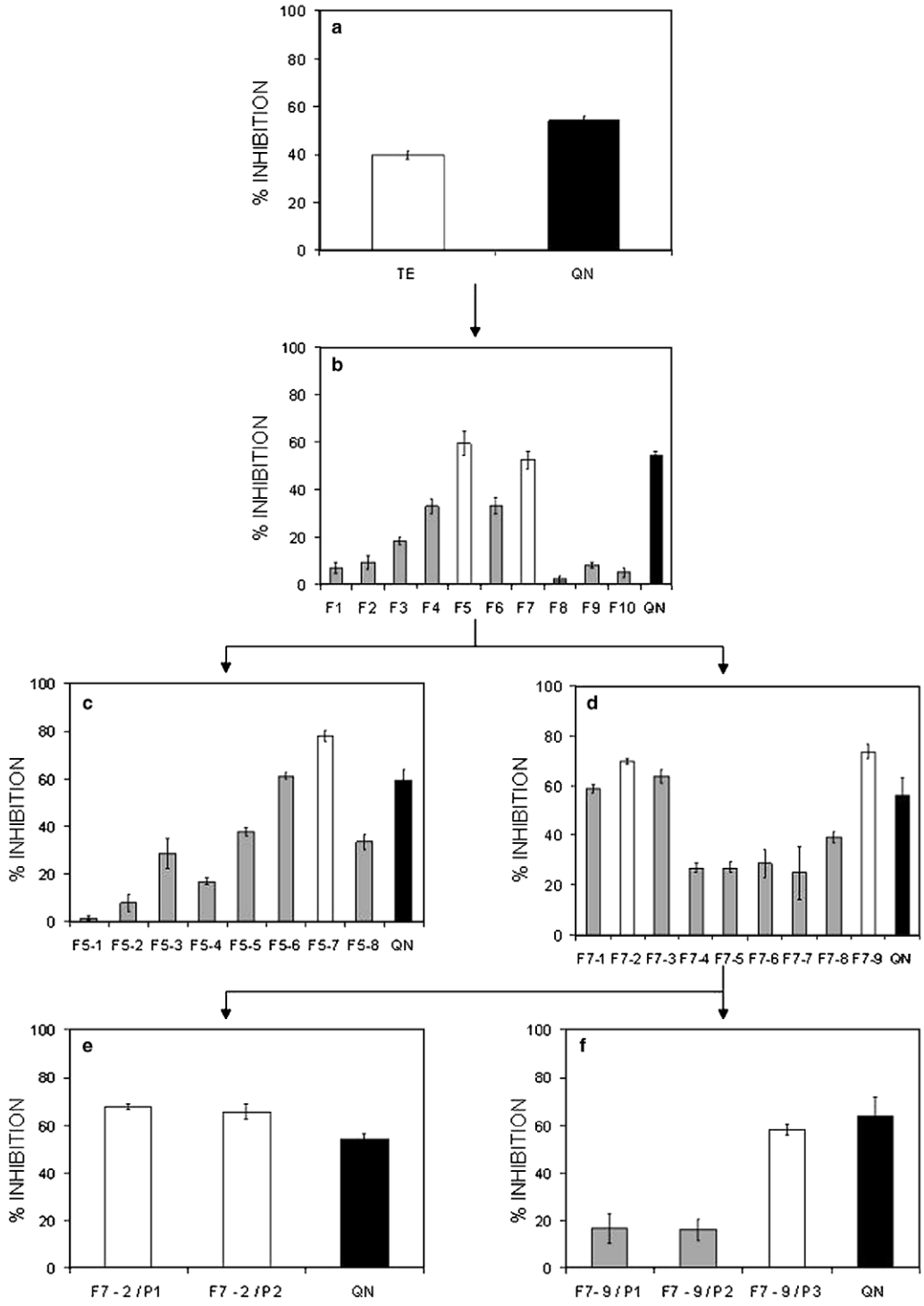

Fig. 2. Activity-guided purification of the ethanolic extract of *A. dracunculus*: TE, total extract; QN, quercitrin; F, fraction; P, peak.

lower ALR2 inhibitory activity were not further characterized, although they may contain compounds that have high activity, but present in low concentration.

2.2. 4,5-Di-O-caffeoylquinic acid (*1*)

This is a well-known compound (Fig. 3(1)) that had been previously isolated from many plant species (Chiang et al., 2004; Islam et al., 2002; Mahmood et al., 1993), including *Artemisia herba-alba Asso*. (Kim et al., 2004). It has also been chemically synthesized by Nagaoka et al. (2001). There are many reports on the biological activities of this compound, including hepatoprotective (Nagaoka et al., 2001), inhibition of HIV replication (Mahmood et al., 1993), analgesic (dos Santos et al., 2005) and antimicrobial (Zhu et al., 2004) activities. However, 4,5-di-*O*-caf-

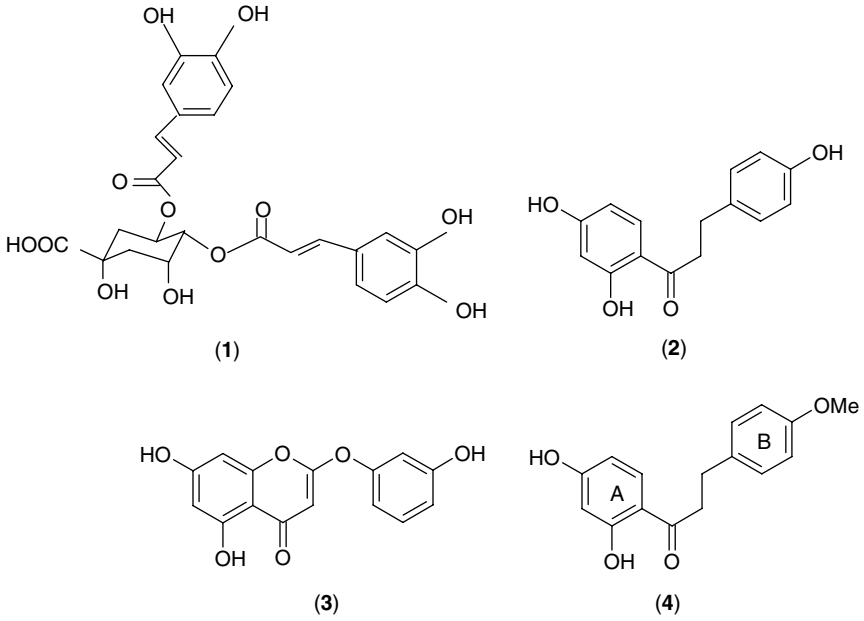

Fig. 3. The structures of compounds **1**–**4** isolated from *A. dracunculus* shoots: (**1**) 4,5-di-*O*-caffeoylquinic acid; (**2**) davidigenin; (**3**) 6-demethoxycapillarisin; (**4**) 2′,4′-dihydroxy-4-methoxydihydrochalcone.

feoylquinic acid (**1**) has never been tested for ALR2 inhibition activity to date. This compound had the highest inhibitory activity against ALR2 compared to the other three compounds isolated from *A. dracunculus* or to the positive control, quercitrin (Fig. 2).

2.3. Davidigenin (*2*)

One of the active compounds in F7-2 was identified as davidigenin (**2**, Fig. 3(2)). Davidigenin (**2**) is a dihydrochalcone that is reported to be a constituent of plants (Madureira et al., 2004; Jensen et al., 1977) and has also been chemically synthesized (Severi et al., 1998). It has been tested for many biological activities. It induces apoptosis in human lung fibroblasts (Liu et al., 2002), and inhibits production of leukotrienes from human polymorphonuclear leucocytes (Homma et al., 2000). Davidigenin (**2**) has also been previously tested for ALR2 inhibitory activity. In one study, the IC_{50} of davidigenin (**2**) for ALR2 enzyme was more than 10 μM whereas a related dihydrochalcone, 2,2′,4′-trihydroxydihydrochalcone, had an IC_{50} of 0.62 μM suggesting that davidigenin (**2**) is not an effective ALR2 inhibitor (Kawanishi et al., 2003). In a different study, (Severi et al., 1998), davidigenin (**2**) had an IC_{50} of 12. 70 μM whereas the other chalcones tested had IC_{50} values ranging from 6.4 to 78.5 μM, inferring that davidigenin (**2**) is a relatively active ALR2 inhibitor. The differences in the ALR2 inhibitory activities between these studies may be related to study design. It has been reported that the presence of either 2′,4′-dihydroxy group or a hydroxyl at position 4′ in the A ring is key for the inhibitory activity of chalcones (Rastelli et al., 2000; Severi et al., 1998; Kawanishi et al., 2003). Our finding is the first report on the isolation of davidigenin (**2**) from the genus *Artemisia*.

2.4. 6-Demethoxycapillarisin (*3*)

The second purified compound in F7-2 was identified as 6-demethoxycapillarisin (**3**, Fig. 3(3)), a naturally occurring 2-phenoxychromone. This compound had been reported as a constituent of some plant species (Leon et al., 2004; Kijjoa et al., 1999; Hashidoko et al., 1991), including *Artemisia capillaris* (Wu et al., 2001). It is interesting to note that a similar compound, capillarisin, isolated from *A. capillaris*, had exhibited inhibitory effect on bovine lens aldose reductase (Okada et al., 1995). 6-Demethoxycapillarisin (**3**) had also been synthesized using the Witting reaction (Takeno et al., 1981). No biological activity has been reported for this compound to date. Here, we report on the isolation and ALR2 inhibitory activity of 6-demethoxycapillarisin (**3**) from *A. dracunculus*.

2.5. 2′,4′-Dihydroxy-4-methoxydihydrochalcone (*4*)

The ^{1}H and ^{13}C NMR spectroscopic data for compound **4** are consistent with previously published data for davidigenin (**2**) with the exception of an additional methyl group (Jensen et al., 1977). HMBC (heteronuclear multiple bond coherence) correlations clearly revealed that the *O*-methyl protons (δ 3.77) are long-range coupled to the C-4 (δ 158.2) in the benzene ring B. This indicates that the *O*-methyl group is attached at the 4 position of benzene ring B (Fig. 3(4)). Therefore, compound **4** was identified as 2′,4′-dihydroxy-4-methoxydihydrochalcone (**4**, Jain and Mehta, 1985). Although there are reports on the chemical synthesis of 2′,4′-dihydroxy-4-methoxydihydrochalcone (**4**, Shinoda and Sato, 1928; Massicot et al., 1954), there is no information on the isolation of this compound from any plant species or on its biological activity. Here we

are reporting for the first time that this compound has been isolated from *A. dracunculus* and that it inhibits ALR2.

In summary, we have isolated four compounds **1**–**4** from the ethanolic extract of *A. dracunculus* that are new to this species and have ALR2 inhibitory activity that is similar to or greater than quercitrin, a well-known ALR2 inhibitor. Three of these four compounds are newly identified ALR2 inhibitors and constituents of *A. dracunculus*. It seems clear from the data that the extract contains additional sub-fractions with significant ALR2 inhibitory activity (Fig. 2). The crude ethanolic extract is only slightly less active than the pure quercitrin positive control, and each of the purified compounds is only similar or slightly more active than quercitrin. It is likely that the active compounds in the extract potentiate each other's effects, since they represent only a small fraction of the entire extract. The potentiating effects of phytochemicals are often observed (Raskin et al., 2002; Lila and Raskin, 2005) but careful *in vivo* studies must be performed to validate them.

3. Experimental

3.1. General

Purification and isolation of compounds were carried out using a preparatory HPLC from Waters consisting of W717 plus auto sampler, W600E multi solvent delivery system, W600 controller, W490E multi wavelength detector and Waters fraction collector. LC/MS system used for analysis includes the Waters (Milford, MA) LC–MS Integrity™ system consisting of a solvent delivery system with a W616 pump and W600S controller, W717plus auto-sampler, W996 PDA detector and Waters TMD Thermabeam™ electron impact (EI) single quadrupole mass detector with fixed ionization energy of 70 eV. Data were collected and analyzed with the Waters Millennium® v. 3.2 software, linked with the 6th edition of the Wiley Registry of Mass Spectral Data, containing 229,119 EI spectra of 200,500 compounds. After the 996 PDA detector, the eluent flow was split into 2 equal flow paths with an adjustable flow splitter, model 600-PO10-06 (Analytical Scientific Instruments, El Sobrante, CA). One of them was to the Thermabeam EI mass detector, and the other to a Varian 1200L (Varian Inc., Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface (ESI), operated in either positive, or negative ionization mode. The electrospray voltage was −4.5 kV, heated capillary temperature was 240 °C, sheath gas air for the negative mode, and electrospray voltage 5 kV and sheath gas nitrogen for the positive ionization mode; mass detector scanning from 110 to 1400 atomic mass units. Data from the Varian 1200L mass detector was collected and compiled using Varian's MS Workstation, v. 6.41, SP2. The ^{1}H, ^{13}C NMR and 2D NMR spectroscopic experiments were recorded using a Bruker Avance AV-300 NMR spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). The 2D experiments ^{1}H–^{1}H COSY (Correlation Spectroscopy), HMBC, and edited-HSQC (heteronuclear single quantum coherence) were acquired using standard Bruker software. All compounds were measured in CD_3OD. For the enzyme assay, a Beckman spectrophotometer (DU Series 600), was used to measure the change in absorbance of NADPH.

3.2. Plant material

The seeds of *A. dracunculus* L. were purchased from Sheffield's Seed Co. Inc. (Locke, New York). The plants were grown in hydroponics and harvested as the total plant material above the root mass. The harvested plants were frozen and stored at −20 °C prior to extraction.

3.3. Extraction

Four kilograms of the shoot material was heated to 80 °C, with EtOH–H_2O (4:1, 12 L) for 2 h. The extraction was continued for an additional 10 h at 20 °C. The extract was then filtered through cheesecloth and evaporated with a rotary evaporator and the final volume was reduced to 1 L. The aqueous extract was freeze-dried for 48 h and the dried extract was homogenized with a mortar and pestle.

3.4. Purification, isolation and identification

3.4.1. High performance liquid chromatography analysis

One gram of the dried extract was dissolved in EtOH–H_2O–CH_3CN (3:2:0.5, 5.5 mL) and purified using a preparatory HPLC. For the initial purification, a Waters 19 × 300 mm symmetry prep, C8, reversed phase column with a particle size of 7 μm was used. The mobile phases consisted of two components: Solvent A (0.5% ACS grade AcOH in double distilled de-ionized H_2O, pH 3–3.5), and Solvent B (CH_3CN). For the initial separation, a gradient run of 5% B in A to 95% B over 35 min was used at a flow rate of 8 mL/min. Ten fractions at 5-min intervals were collected and tested for ALR2 inhibitory activity. Fractions and sub-fractions that showed higher percent inhibition of the enzyme were further purified using different conditions. Fractions 5, 5–7, 7 and 7–9 were purified by altering the gradient conditions, described above, with flow rates ranging from 1–8 mL/min. Fraction 7–2 was purified using an isocratic condition with a mobile phase consisting of 0.5% AcOH in H_2O–MeOH–CH_3CN (5:3:2). Fraction 5–7 was purified using a Phenomenex 10 × 250 mm C-18 reversed phase column with a particle size of 5 μm. The ultra-violet (UV) profiles were monitored at wavelengths of 210 and 290 nm. From 1 g of total extract, 1.1 mg of **1**, 0.7 mg of **2**, 0.45 mg of **3** and 0.6 mg of **4** were isolated.

3.4.2. Liquid chromatography–mass spectrometry analysis

Substances were separated on a Phenomenex® Luna C-8 reversed phase column, size 250 × 4.6 mm, particle size 5 μm, equipped with a Phenomenex® SecurityGuard™

pre-column. The mobile phase consisted of two components: Solvent A (0.5% ACS grade AcOH in double distilled de-ionized H_2O, pH 3–3.5), and Solvent B (CH_3CN). The mobile phase flow was adjusted at 0.5 mL/min, and a gradient of 15% B in A to 95% B over 30 min was used.

3.4.3. 4,5-Di-O-caffeoylquinic acid (*1*)

UV and ESI-MS spectral data were consistent with that reported in the literature (Clifford et al., 2003; Schütz et al., 2004). EI-MS *m/z* (% rel. int.): 182 (19), 163 (15), 149 (12), 136 (51), 123 (100), 110 (75), 94 (44). ^{1}H NMR and ^{13}C NMR data were consistent with previously reported data (Zhu et al., 2004).

3.4.4. Davidigenin (*2*)

UV and EI-MS spectral data were consistent with that reported in the literature (Bohm and Glennie, 1969). (−)ESI-MS *m/z* (% rel. int.): 257 (100) $[M-H]^-$, 151 (6). ^{1}H NMR and ^{13}C NMR data were consistent with previously reported data (Jensen et al., 1977).

3.4.5. 6-Demethoxycapillarisin (*3*)

UV and ESI-MS spectral data were consistent with that reported in the literature (Kijjoa et al., 1999; Hashidoko et al., 1991). EI-MS *m/z* (% rel. int.): 286 (100) $[M^+]$, 269 (2), 257 (8), 229 (3), 194 (4), 153 (35), 134 (19), 121 (5), 106 (8). ^{1}H NMR and ^{13}C NMR data were consistent with previously reported data (Sharon et al., 1992).

3.4.6. 2′,4′-Dihydroxy-4-methoxydihydrochalcone (*4*)

UV λ_{max} (CH_3CN): 215, 276, 312. EI-MS *m/z* (% rel. int.): 272 (46) $[M^+]$, consistent with molecular formula $C_{16}H_{16}O_4$, 253 (15), 166 (9), 151 (100), 137 (4), 120 (23), 107 (20), 95 (7); (−)ESI-MS *m/z* (% rel. int.): 165 (100), 271 (89) $[M-H]^-$, 541 (22) $[2\times M-H]^-$. ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.84 (1H, *d*, $J=8.7$ Hz, H-6′), 7.23 (2H, *d*, $J=8.4$ Hz, H-2 and 6), 6.85 (2H, *d*, $J=8.4$ Hz, H-3 and 5), 6.43 (1H, *dd*, $J=8.7$, 1.8 Hz, H-5′), 6.33 (1H, *d*, $J=1.8$ Hz, H-3′), 3.77 (3H, *s*, OCH_3), 3.28 (2H, *t*, $J=7.7$ Hz, H-α), 2.96 (2H, *t*, $J=7.7$ Hz, H-β); ^{13}C NMR (acetone-d_6, 75 MHz) δ 204.0 (C=O), 164.8 (C-2′), 164.7 (C-4′), 158.2 (C-4), 133.1 (C-1), 132.8 (C-6′), 129.3 (C-2, 6), 113.7 (C-3, 5), 112.9 (C-1′), 107.9 (C-5′), 102.6 (C-3′), 54.5 (OCH_3), 39.4 (C-α), 29.7 (C-β) (Jensen et al., 1977).

3.5. Enzyme assay

Human recombinant ALR2 enzyme was purchased from Wako Chemicals USA Inc. Enzyme activity was measured at each step of purification of the extract, by monitoring the decrease in NADPH absorbance at a wavelength of 340 nm (Nishimura et al., 1991), using a spectrophotometer. One hundred microliters of the reaction mixture contained 100 mM sodium phosphate buffer (pH 6.2), 0.15 mM NADPH, 10 mM DL-glyceraldehyde and 1 mU of human recombinant ALR2 enzyme. The samples were prepared in 10% DMSO and the final concentration of the samples or the positive control quercitrin was 3.75 μg/mL. Other controls with 10% DMSO included a positive control with uninhibited enzyme to ensure activity and a negative control without enzyme to monitor the NADPH degradation. The reaction was initiated by adding the enzyme and the change in NADPH absorbance was monitored over 7 min.

Acknowledgements

The authors thank Teresa Cornwell for assistance with the preparation of figures in this manuscript. This work was supported by the NIH Center for Dietary Supplements Research on Botanicals and Metabolic Syndrome; Grant # 1-P50 AT002776-01, Cook College of Rutgers University, The Agricultural Experiment Station and Phytomedics Inc.

References

Baynes, J.W., Thorpe, S.R., 1999. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 48 (1), 1–9.

Benvenuti, S., Severi, F., Costantino, L., Melegari, M., Mucci, A., 1996. Identification, characterization, and biological activity of chalcone derivatives of *Glycyrrhiza glabra* L.. Rivista Italiana 7 (20), 13–16.

Betteridge, D.J., 2000. What is oxidative stress? Metab. Clin. Exp. 49 (2, Suppl. 1), 3–8.

Brownlee, M., 2000. Negative consequences of glycation. Metab. Clin. Exp. 49 (2, Suppl. 1), 9–13.

Bohm, B.A., Glennie, C.W., 1969. Isolation of 2′,4,4′-trihydroxydihydrochalcone from *Viburnum davidi*. Phytochemistry 8 (5), 905–908.

Chevallier, A., 1996. The encyclopedia of medicinal plants. Dorling Kinderseley Ltd., London, p. 336.

Chiang, Y., Chuang, D., Wang, S., Kuo, Y., Tsai, P., Shyur, L., 2004. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. J. Ethnopharmacol. 95 (2–3), 409–419.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhnert, N., 2003. Hierarchical scheme for LC–MS^n identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900–2911.

Constantino, L., Rastelli, G., Vescovini, K., Cignarella, G., Vianello, P., Del Corso, A., Capiello, M., Mura, U., Barlocco, D., 1996. Synthesis, activity, and molecular modeling of a new series of tricyclic pyridazinones as selective aldose reductase inhibitors. J. Med. Chem. 39, 4396–4405.

De la Fuente, J.A., Manzanaro, S., 2003. Aldose reductase inhibitors from natural sources. Nat. Product Rep. 20 (2), 243–251.

De la Fuente, J.A., Manzanaro, S., Martin, M.J., de Quesada, T.G., Reymundo, I., Luego, S.M., Gago, F., 2003. Synthesis, activity, and moleculer modeling studies of novel human aldose reductase inhibitors based on a marine natural product. J. Med. Chem. 46 (24), 5208–5221.

Donohue, P.J., Alberts, G.F., Hampton, B.S., Winkles, J.A., 1994. A delayed-early gene activated by fibroblast growth factor-1 encodes a protein related to aldose reductase. J. Biol. Chem. 269 (11), 8604–8609.

dos Santos, Michel, D., Gobbo-Neto, L., Albarella, L., de Souza, G.E.P., Lopes, N.P., 2005. Analgesic activity of di-caffeoylquinic acids from roots of *Lychnophora ericoides* (*Arnica da serra*). J. Ethnopharmacol. 96 (3), 545–549.

Friedman, E.A., 1999. Advanced glycosylated end products and hyperglycemia in the pathogenesis of diabetic complications. Diabetes Care 22 (Suppl. 2B), 65–71.

Gabbay, K.H., Merola, L.O., Field, R.A., 1966. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. Science 51 (707), 209–210.

Hashidoko, Y., Tahara, S., Mizutani, J., 1991. 2-Phenoxychromones and a structurally related flavone from leaves of *Rosa rugosa*. Phytochemistry 30 (11), 3837–3838.

Hohman, T.C., El-Kabbani, O., Malamas, M.S., Lai, K., Putilina, T., McGowan, M.H., Wane, Y.Q., Carper, D.A., 1998. Probing the inhibitor-binding site of aldose reductase with site-directed mutagenesis. Eur. J. Biochem. 256, 310–316.

Homma, M., Minami, M., Taniguchi, C., Oka, K., Morita, S., Niitsuma, T., Hayashi, T., 2000. Inhibitory effects of lignans and flavonoids in Saiboku-To, a herbal medicine for bronchial asthma, on the release of leukotrienes from human polymorphonuclear leukocytes. Planta Med. 66 (1), 88–91.

Ishii, H., Jirousek, M.R., Koya, D., Takagi, C., Xia, P., Clermont, A., Bursell, S.E., Kern, T.S., Ballas, L.M., Heath, W.F., Stramm, L.E., Feener, E.P., King, G.L., 1996. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. Science 272 (5262), 728–731.

Islam, Md.S., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., Yamakawa, O., 2002. Identification and characterization of foliar polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) genotypes. J. Agric. Food Chem. 50 (13), 3718–3722.

Jain, A.C., Mehta, A., 1985. A new synthesis of homoisoflavanones (3-benzyl-4-chromanones). Ind. J. Chem. 24B (11), 1170–1171.

Jensen, S.R., Nielsen, B.J., Norn, V., 1977. Dihydrochalcones from *Viburnum davidii* and *V. lantanoides*. Phytochemistry 16, 2036–2038.

Kawanishi, K., Ueda, H., Moriyasu, M., 2003. Aldose reductase inhibitors from the nature. Curr. Med. Chem. 10, 1353–1374.

Kawamura, M., Eisenhofer, G., Kopin, I.J., Kador, P.F., Lee, Y.S., Fujisawa, S., Sato, S., 2002. Aldose reductase: an aldehyde scavenging enzyme in the intraneuronal metabolism of norepinephrine in human sympathetic ganglia. Auton. Neurosci. 96 (2), 131–139.

Kijjoa, A., Vieira, L.M., Pereira, J.A., Silva, A.M.S., Herz, W., 1999. Further constituents of *Achillea ageratum*. Phytochemistry 51 (4), 555–558.

Kim, T., Ito, H., Hatano, T., Taniguchi, S., Khennouf, S., Yoshida, T., 2004. Chemical constituents of *Artemisia herba-alba Asso*. Nat. Med. (Tokyo, Japan) 58 (4), 165.

Koya, D., King, G.L., 1998. Protein kinase C activation and the development of diabetic complications. Diabetes 47 (6), 859–866.

Laeng, P., Bouillon, P., Taupenot, L., Labourdette, G., 1995. Long-term induction of an aldose reductase protein by basic fibroblast growth factor in rat astrocytes *in vitro*. Electrophoresis 16 (7), 1240–1250.

Lee, H., 2005. Cuminaldehyde: aldose reductase and glucosidase inhibitor derived from *Cuminum cyminum* L. seeds. J. Agric. Food Chem. 53 (7), 2446–2450.

Leon, L., Maldonado, E., Cruz, A., Ortega, A., 2004. Tenuiflorins A-C: new 2-phenoxychromones from the leaves of *Mimosa tenuiflora*. Planta Med. 70 (6), 536–539.

Lila, M.A., Raskin, I., 2005. Health related interactions of phytochemicals. J. Food Sci. 70, 20–27.

Lim, S.S., Jung, S.H., Ji, J., Shin, K.H., Keum, S.R., 2001. Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. J. Pharm. Pharmacol. 53 (5), 653–668.

Liu, Z., Tanaka, S., Horigome, H., Hirano, T., Oka, K., 2002. Induction of apoptosis in human lung fibroblasts and peripheral lymphocytes *in vitro* by shosaiko-to derived phenolic metabolites. Biol. Pharm. Bull. 25, 37–41.

Madureira, A.M., Molnar, A., Abreu, P.M., Molnar, J., Ferreira, M.U., 2004. A new sesquiterpene-coumarin ether and a new abietane diterpene and their effects as inhibitors of P-glycoprotein. Planta Med. 70 (9), 828–833.

Mahmood, N., Moore, P.S., De Tommasi, N., De Simone, F., Colman, S., Hay, A.J., Pizza, C., 1993. Inhibition of HIV infection by caffeoylquinic acid derivatives. Antiviral Chem. Chemother. 4 (4), 235–240.

Massicot, J., Mentzer, C., Pillon, D., 1954. The reduction of flavones to flavanones. Compt. Rend. 238, 111–112.

Matsuda, H., Morikawa, T., Yoshikawa, M., 2002. Antidiabetogenic constituents from several natural medicines. Pure Appl. Chem. 74 (7), 1301–1308.

Miyamoto, S., 2002. Molecular modeling and structure-based drug discovery studies of aldose reductase inhibitors. Chem-Bio. Inform. J. 2 (3), 74–85.

Mylari, B.L., Armento, S.J., Beebe, D.A., Conn, E.L., Coutcher, J.B., Dina, M.S., O'Gorman, M.T., Linhares, M.C., Martin, W.H., Oates, P.J., Tess, D.A., Withbroe, G.J., Zembrowski, W.J., 2003. A highly selective, non-hydantoin, non-carboxylic acid inhibitor of aldose reductase with potent oral activity in diabetic rat models: 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2-H-pyridazin-3-one. J. Med. Chem. 46 (12), 2283–2286.

Mylari, B.L., Armento, S.J., Beebe, D.A., Conn, E.L., Coutcher, J.B., Dina, M.S., O'Gorman, M.T., Linhares, M.C., Martin, W.H., Oates, P.J., Tess, D.A., Withbroe, G.J., Zembrowski, W.J., 2005. A novel series of non-carboxylic acid, non-hydantoin inhibitors of aldose reductase with potent oral activity in diabetic rat models: 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2H-pyridazin-3-one and congeners. J. Med. Chem. 48, 6326–6339.

Nagaoka, T., Banskota, A.H., Xiong, Q., Tezuka, Y., Kadota, S., 2001. Synthesis and antihepatotoxic and antiproliferative activities of di- and tri-*O*-caffeoylquinic acid derivatives. Wakan Iyakugaku Zasshi 18 (5), 183–190.

Nishimura, C., Yamaoka, T., Mizutani, M., Yamashita, K., Akera, T., Tanimoto, T., 1991. Purification and characterization of recombinant aldose reductase expressed in baculovirus system. Biochim. Biophys. Acta, 1078–1171.

Okada, Y., Miyauchi, N., Suzuki, K., Kobayashi, T., Tsutsui, C., Mayuzumi, K., Nishibe, S., Okuyama, T., 1995. Search for naturally occurring substances to prevent the complications of diabetes. II. Inhibitory effect of coumarin and flavonoid derivatives on bovine lens aldose reductase and rabbit platelet aggregation. Chem. Pharm. Bull. 43 (8), 1385–1387.

Phillips, R., Foy, N., 1990. Herbs. Pan Books Ltd., London, pp. 171–178.

Raskin, I., Ribnicky, D.M., Komarnytsky, S., Ilić, N., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D.A., Ripoll, C., Yakoby, N., O'Neal, J.M., Cornwell, T., Pastor, I., Fridlender, B., 2002. Plants and human health in the twenty-first century. Trends Biotechnol. 20 (12), 522–531.

Rastelli, G., Antolini, L., Benvenuti, S., Costantino, L., 2000. Structural bases for the inhibition of aldose reductase by phenolic compounds. Bioorg. Med. Chem. 8 (5), 1151–1158.

Ribnicky, D.M., Poulev, A., Logendra, S., Cefalu, W.T., Wang, Z., Raskin, I., 2005. The development of an extract of *Artemisia dracunculus* for decreasing the insulin resistance associated with diabetes, from concept to clinic. In: Gordon Research Conference on Agricultural Sciences "Adding More Value to Production Agriculture", February 13–18, 2005, Ventura, CA, USA.

Sarges, R., Oates, P.J., 1993. Aldose reductase inhibitors: recent developments. Prog. Drug Res. 40, 99–161.

Sayyah, M., Nadjafnia, L., Kamalinejad, M., 2004. Anticonvulsant activity and chemical composition of *Artemisia dracunculus* L. essential oil. J. Ethnopharmacol. 94 (2-3), 283–287.

Severi, F., Benvenuti, S., Costantino, L., Vampa, G., Melegari, M., Antolini, L., 1998. Synthesis and activity of a new series of chalcones as aldose reductase inhibitors. Eur. J. Med. Chem. 33, 859–866.

Severi, F., Costantino, L., Benvenuti, S., Vampa, G., Mucci, A., 1996. Synthesis and description of chalcone-like compounds, inhibitors of aldose reductase. Med. Chem. Res. 6 (2), 128–136.

Sharon, A., Ghirlando, R., Gressel, J., 1992. Isolation, purification, and identification of 2-(*p*-hydroxyphenoxy)-5,7-dihydroxychromone: a fungal-induced phytoalexin from *Cassia obtusifolia*. Plant Phys. 98, 303–308.

Shinoda, J., Sato, S., 1928. Synthesis of polyhydroxychalcones and flavanones. 1. Synthesis of 2′,4′-dyhydrochalcone; 2′,4′-dihydroxy-4-methoxyhydrochalcone and isosakuranetin. Tokushima Higher Tech. School, Yakugaku Zasshi. 48, pp. 791–801.

Schütz, K., Kammerer, D., Carle, R., Schieber, A., 2004. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MS^n. J. Agric. Food Chem. 52, 4090–4096.

Swanston-Flatt, S.K., Flatt, P.R., Day, C., Bailey, C.J., 1991. Traditional dietary adjuncts for the treatment of *Diabetes Mellitus*. Proc. Nutr. Soc. 50, 641–651.

Takeno, H., Hashimoto, M., Koma, Y., Horiai, H., Kikuchi, H., 1981. Synthesis of demethoxycapillarisin, a naturally occurring 2-phenoxychromone, and related compounds. J. Chem. Soc., Chem. Commun. (10), 474–475.

Tilton, R.G., Chang, K., Nyengaard, J.R., Van den Enden, M., Ido, Y., Williamson, J.R., 1995. Inhibition of sorbitol dehydrogenase. Effects on vascular and neural dysfunction in streptozocin-induced diabetic rats. Diabetes 44 (2), 234–242.

Wilson, D.K., Tarle, I., Petrash, J.M., Quiocho, F.A., 1993. Refined 1.8 A structure of human aldose reductase complexed with the potent inhibitor zopolrestst. Proc. Natl. Acad. Sci. USA 90, 9847–9851.

Williamson, J.R., Chang, K., Frangos, M., Hasan, K.S., Ido, Y., Kawamura, T., Nyengaard, J.R., Van den Enden, M., Kilo, C., Tilton, R.G., 1993. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42 (6), 801–813.

Wu, T., Tsang, Z., Wu, P., Lin, F., Li, C., Teng, C., Lee, K., 2001. New constituents and antiplatelet aggregation and anti-HIV principles of *Artemisia capillaris*. Bioorg. Med. Chem. 9 (1), 77–83.

Yabe-Nishimura, C., 1998. Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. Pharmacol. Rev. 50 (1), 21–33.

Yazdanparast, R., Saee, A., 1999. Effects of aqueous tarragon, *Artemisia dracunculus*, extract on lipid and cogulatory parameters in rats. Biomed. Lett. 59, 137–141.

Zhu, X., Zhang, H., Lo, R., 2004. Phenolic compounds from the leaf extract of Artichoke (*Cynara scolymus* L.) and their antimicrobial activities. J. Agric. Food Chem. 52 (24), 7272–7278.