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Glycosidase inhibitory phenolic compounds from the seed of *Psoralea corylifolia*

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ABSTRACT

The seeds of *Psoralea corylifolia* were extracted into five different polar solvents: chloroform, 50% ethanol in water, ethanol, methanol and water. All extracts were evaluated for glycosidase inhibitory activity. The chloroform extract (CE) showed the lowest IC_{50} values against α-glucosidase (82.9 μg/ml) and α-mannosidase (132 μg/ml). Chromatography of CE yielded nine phenolic compounds which were identified as isovabachalcone (**1**), 4′-*O*-methylbavachalcone (**2**), isobavachromene (**3**), corylifolin (**4**), bavachinin (**5**), psoralidin (**6**), neobavaisoflavone (**7**), corylifol A (**8**), and bakuchiol (**9**). All isolated compounds, apart from compound **5**, possessed α-glucosidase inhibitory activities. Among them, compounds **6**–**8** exhibited potent inhibition with IC_{50}s of 13.7, 27.7 and 11.3 μM, respectively. Furthermore, compounds **2** and **6** showed α-mannosidase inhibitory activity. Mechanistic analysis of their inhibition modes against α-glucosidase showed that compounds (**6** and **7**) were noncompetitive, whereas compound **8** was mixed. Furthermore, the most active glycosidase inhibitors (**2**, **6**–**8**) were proven to be present in the native seed in high quantities by an HPLC chromatogram.

1. Introduction

Screening of glycosidase inhibitors derived from edible plant sources is becoming increasingly popular. Among the most common classes of glycosidases targeted for inhibition for therapeutic purposes are α-glucosidases (EC 3.2.1.20) and α-mannosidases (EC 3.2.1.24) both of which are exo-acting enzymes that play essential roles in carbohydrate quality control. In general glycosidase inhibitors can be used in the treatment of numerous diseases, including diabetes mellitus type II (van de Laar et al., 2005; Robinson et al., 1991), cancer (Fernandes, Sagman, Auger, Demetrio, & Dennism, 1991), and HIV (Ogawa, Maruyama, Odagiri, Yuasa, & Hashimoto, 2001). At the biochemical level, the first effect can be linked to the role of glycosidases in sugar metabolism: by retarding the cleavage of complex carbohydrates, postprandial glucose absorption *in vivo* can be attenuated, thus regulating blood sugar levels (Maki et al., 2007). The latter effects can be traced to the crucial role played by glycans in cell signalling, recognition and adhesion (Zhao et al., 2008). This is because glycosidases are deeply involved in the biosynthesis and processing of oligosaccharide chains of N-linked glycoproteins [including 14 monosaccharide residues (Glc3Man9GlcNAc2) (Kornfeld & Kornfeld, 1985)] in endoplasmic reticulum (ER). Inhibition of α-glucosidase and α-mannosidase thus has a profound effect on glycan structure, which in turn has contingent effects on the maturation, transport, secretion, and function of glycoproteins. The end result is alteration of cell–cell or cell–virus recognition processes (Asano, 2003; Dwek, Butters, Platt, & Zitzmann, 2002; Jacob, 1995). In this way, glycosidase inhibition may retard cancer growth because the spread of cancer, as well as the structural changes of cell surface glycoconjugates within neoplasmic cells, is proliferated by glycosidases in the sera and interstitial fluid around the tumour (Bernacki, Niedbala, & Korytnyk, 1985).

Psoralea corylifolia L. is renowned as a polyphenol-rich plant which has been used in traditional Chinese medicine. This species belongs to the family of legumes, the seeds of which have been used throughout history to cure premature ejaculation, spermatorrhea, backache, and pollakiuria (Chang & But, 1986). Its main bioactive constituents are flavonoids, isobavachalcone, neobavaisoflavone, and bavachinin (Bhalla, Nayak, & Dev, 1968; Kotiyal & Sharma, 1992), as well as coumarins and bakuchiol (Kubo, Dohi, Odani, Tanaka, & Iwamura, 1989). Many of these exhibit antibacterial (Yin, Fan, Wang, Lei, & Yue, 2004), antifungal (Ragendra, Anandi, Balasubramanian, & Pugalendi, 2004), antitumor (Bapat et al., 2005) and antioxidative activities (Jiangning, Xinchu, Hou,

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Qinghua, & Kaishun, 2005). However, to the best of our knowledge, there is no report of the seeds of *P. corylifolia* eliciting glycosidase inhibition and it is with this strongly in mind that we set about this work. In this study, we isolated nine phenolic compounds from the seeds of *P. corylifolia* and their structures were identified using spectroscopic methods. The isolated compounds were evaluated separately for their inhibitory activities against α-glucosidase and α-mannosidase. Their inhibition mechanisms were ascertained using Lineweaver–Burk and Dixon plots. We also assessed the relative abundance of these extracts within the native seed using an HPLC chromatogram profile.

2. Materials and methods

2.1. Chemicals and materials

Organic solvents used for isolation were of first grade and the stock solution and buffers were prepared with milli Q water. Methanol, acetonitrile and acetic acid for HPLC were purchased as of analytical grade from J.T. Baker (Phillipsburg, NJ, USA). Column chromatography was carried out using silica gel (230–400 mesh, Merck), RP-18 (ODS-A, 12 nm, S-150 mM, YMC), and Sephadex LH-20 (Amersham Biosciences). For biochemical assays, α-glucosidase (EC 3.2.1.20, from Baker's Yeast), *p*-nitrophenyl-α-D-glucopyranoside, α-mannosidase (EC 3.2.1.24, from jack beans) and *p*-nitrophenyl-α-D-mannopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *P. corylifolia* [imported from Myanmar, as permitted by Korea Food and Drug Administration (KFDA)] (approximately 2.8 kg) was purchased from a market.

2.2. Instruments

UV spectra were measured on a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, USA). ^{1}H and ^{13}C NMR, as well as 2D NMR data, were obtained on a Bruker AM 500 (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz) spectrometer (Bruker, Karlsruhe, Germany) in CD_3OD, DMSO-d_6, $CDCl_3$, and acetone-d_6 with TMS as internal standard. EIMS, HREIMS were obtained on a JEOL JMS-700 mass spectrometer (JEOL, Tokyo, Japan). Qualitative analyses were made using a Perkin–Elmer HPLC S200 (Perkin–Elmer, Bridgeport, USA). LC/MS was measured in a 3200 Q Trap LC/MS/MS System (Applied Biosystems, Lincoln, USA). Reagent grade chemicals were purchased from Sigma Chemical Co. (St. Louis, USA).

2.3. Preparation of extracts

First, seeds were washed with clean sterile water. Then 10 g of seed were extracted (with shaking) into 100 ml of solvent at 30 °C for 1 week. Five different solvent extraction systems were used: chloroform, ethanol, 50% ethanol, methanol and distilled water (H_2O).

2.4. Glycosidase inhibitory activity

α-Glucosidase (EC 3.2.1.20) and α-mannosidase (EC 3.2.1.24) inhibitory activities (Kato et al., 2005) were calculated using literature experimental procedures with some modifications. α-Glucosidase and α-mannosidase activities were determined using the appropriate substrate (*p*-nitrophenyl-α-D-glucopyranoside or *p*-nitrophenyl-α-D-mannopyranoside, respectively) at the optimum pH of each enzyme. The reaction was stopped by adding 2 M NaOH. The released *p*-nitrophenol was measured spectrometrically at 405 nm. The inhibitory effects of the tested compounds were expressed as the concentrations that inhibited 50% of the enzyme activity (IC_{50}). Kinetic parameters were determined using the Lineweaver–Burk double-reciprocal-plot method and Dixon plot method at increasing concentrations of substrates and inhibitors.

2.5. HPLC analysis

Quantification of the relative abundance of the compounds assayed in this manuscript within the crude seed extract was carried out by HPLC (Perkin–Elmer 200 series, Perkin–Elmer Co., Bridgeport, USA) using a SPHERI-5 RP-18 column (4.6 × 250 mm, 5 μM, Perkin–Elmer, USA). Absorbances were measured at 310 nm. About 20 μl of 100 mg/ml of crude seed extract were loaded onto the column. The initial mobile phase consisted of 20% solvent B (acetonitrile) in solvent A [0.1% acetic acid in water (A)]. This was linearly increased to 60% B over a period of 1 h at a constant flow rate of 1 ml/min. The column was then held at 60% B for 10 min.

2.6. Extraction and isolation

The seeds of *P. corylifolia* were extracted in separate flasks (10 g dry seeds each) with 0.1 l of chloroform, ethanol, 50% ethanol in water, methanol or distilled water at room temperature for 1 week to examine the enzymatic inhibitory activities against glycosidase as a function of solvent used (Table 1). The chloroform extract was determined as the target extract for the isolation of α-glucosidase inhibitors and α-mannosidase as it gave the strongest inhibition for both enzymes. The dried seeds (2.8 kg) of *P. corylifolia* were macerated and extracted with chloroform (5 l × 4) for one week at room temperature, and then filtered and the clarified solvent was evaporated under reduced pressure to afford the chloroform extract (386.4 g). This extract was fractionated by column chromatography on silica gel (10 × 75 cm, 230–400 mesh, 950 g), eluting with *n*-hexane–EtOAc with gradual increment of the EtOAc content (90:10 → 80:20 → 70:30 → 60:40 → 50:50 → 40:60 → 20:80 → 0:100, each 2000 ml) to give 11 fractions (A–K). Fractions D (6.0 g) and E (8.0 g) were grouped together and rechromatographed over a silica gel column (4 × 60 cm, 230 × 400 mesh, 250 g) using a *n*-hexane–EtOAc gradient (15:1 → 1:1). This was then purified by a second flash silica gel chromatography step (3.0 × 50 cm, 230–400 mesh, 150 g) using a *n*-hexane–acetone gradient [15:1 (500 ml), 12:1 (350 ml), 8:1 (350 ml), 5:1 (350 ml), 3:1 (350 ml), 1:1 (350 ml)] to yield compounds **8** (35 mg) and **9** (53 mg). Fraction G (20.4 g) was separated by column chromatography (4.5 × 70 cm, 300 g) on silica gel (230 × 400 mesh) with a *n*-hexane–EtOAc mixture of increasing polarity (12:1 → 1:1) to afford 37 subfractions. Subfractions 25–30 were subjected to further column chromatography (3.0 × 50 cm, 150 g) on silica gel (230–400 mesh), eluting with a *n*-hexane–acetone gradient (12:1 → 3:1) to yield compounds **2** (19 mg) and **5** (27 mg). Fraction H (24.1 g) was applied to a silica gel column (4.5 × 70 cm, 230–400 mesh, 300 g) and chromatographed using a *n*-hexane–acetone gradient (20:1 → 2:1) to afford 50 subfractions. Subfractions 25–28 (2.5 g) were rechromatographed on a silica gel column (2 × 6 cm, 230–400 mesh, 40 g) eluting with *n*-hexane–acetone (14:1 → 4:1) to yield compounds **3** (15 mg) and **6** (24 mg). Subfractions 32–36 (1.4 g) were rechromatographed on a silica gel column (2.5 × 15 cm, 230–400 mesh, 80 g), using *n*-hexane–acetone (10:1 → 1:1), to give 20 fractions. Fractions 13–17 were chromatographed on Sephadex LH-20 column (1.5 × 25 cm, 40 g) to afford compounds **4** (18 mg) and **7** (21 mg). Fraction I (4.6 g) was resubjected to silica gel column chromatography (2.5 × 50 cm, 230–400 mesh, 110 g), eluting with a $CHCl_3$–acetone gradient (16:1 → 4:1) to give 25 subfractions. Subfractions 17–21 from this column were evaporated and recrystallized from acetone to give compound **1** (18 mg).

Activity-guided fractionation of the chloroform extract gave nine phenolic compounds (**1–9**) which were purified over silica gel, Sephadex LH-20 and octadecyl-functionalized silica gel as