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Inhibitory effect on α-glucosidase by *Adhatoda vasica* Nees

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Abstract

Methanolic extracts from the medicinal parts of 40 traditional Chinese herbs were tested in screening experiments for rat intestinal α-glucosidase. The methanolic extract from the leaves of *Adhatoda vasica* Nees (Acanthaceae) showed the highest sucrase inhibitory activity with sucrose as a substrate. Enzyme assay-guided fractionation of this extract afforded vasicine (**1**) and vasicinol (**2**), and the structures of these compounds were elucidated on the basis of MS and NMR analysis. Compounds **1** and **2** showed a high sucrase inhibitory activity, and the IC_{50} values were 125 μM and 250 μM, respectively. Both **1** and **2** were shown to be reversible inhibitors of sucrase. Kinetic data revealed that compounds **1** and **2** inhibited sucrose-hydrolysing activity of rat intestinal α-glucosidase competitively with K_i values of 82 μM and 183 μM, respectively. This is the first report on the mammalian α-glucosidase inhibition of *A. vasica* and the inhibitory effect on sucrase by **1** and **2** from this herb species. These results suggest a use of the extract of *A. vasica* as an antidiabetic agent and show a possibility that compounds **1** and **2** could be an useful treatment for metabolic disorders.

Keywords: α-Glucosidase; Sucrase inhibitor; Postprandial hyperglycemia; *Adhatoda vasica*

1. Introduction

Diabetes is a common metabolic disease characterised by abnormally high plasma glucose levels, leading to major complications, such as diabetic neuropathy, retinopathy and cardiovascular diseases (He & King, 2004; Krentz, Clough, & Byrne, 2007). Epidemiological studies and clinical trials strongly support that control of hyperglycaemia is critical in treatment of not only diabetic patients but also individuals with impaired glucose tolerance (Valeri, Pozzilli, & Leslie, 2004). Mammalian α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) located in the brush-border surface membrane of intestinal cells, is the key enzyme which catalyses the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppressed postprandial hyperglycaemia (Lebovitz, 1997).

Much effort has been expended in the search for effective and safe α-glucosidase inhibitors from natural materials, in order to develop a physiological functional food or lead compounds for use as antidiabetic agents. In a series of our studies on rat intestinal α-glucosidase-inhibiting principles from plant sources, we previously reported that baicalein (5,6,7-trihydroxyflavone) from *Scutellaria baicalensis* and the related 6-hydroxyflavones from *Origanum majorana* were a new class of α-glucosidase inhibitors (Kawabata et al., 2003; Nishioka, Kawabata, & Aoyama, 1998). As a continued study, the various mechanistic and structural development studies have been reported (Gao & Kawabata, 2004, 2005; Gao, Nishioka, Kawabata, & Kasai, 2004). In a recent study, we reported the screening results for sucrase and maltase-inhibitory activities of 50 Chinese herbal extracts and the isolation of maltase inhibitory principles, chebulanin, chebulagic acid and chebulinic acid

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from the fruits of *Terminalia chebula* (Gao, Huang, Xu, & Kawabata, 2007) together with 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and rutin from *Tussilago farfarae* (Gao et al., 2008).

In the course of our ongoing program on identifying α-glucosidase-inhibiting principles from natural medicines in China, we screened 40 additional herbal extracts and found that the aqueous methanolic extract of the leaves of *Adhatoda vasica* Nees showed the highest sucrase inhibitory activity among the tested herbs, although this extract did not show maltase inhibitory activity, in which sucrase and maltase activities designate sucrose- and maltose-hydrolysing activities in crude rat intestinal glucosidase complexes, respectively. *Adhatoda vasica* Nees, indigenous to India, is a number of the Acanthaceae family. It is a well-known plant drug in Ayurvedic and Unani medicine. In China, *A. vasica*, also commonly known as Ya-Zui-Hua, is a popular folk medicine and used for the treatment of cough, asthma and colds, and also as a remedy for rheumatic diseases and traumatic injuries. Since this is the first observation on the inhibitory effect of *A. vasica* on α-glucosidase activity, this finding prompted us to undertake the isolation and structural elucidation of active compounds from this plant species, as well as to study their inhibitory properties and mechanism of action.

2. Materials and methods

2.1. Materials

The 40 species of traditional Chinese herbs were purchased from a local herbal market in Chengdu, People's Republic of China. All voucher specimens are deposited in the Department of Public Health, Hua Xi Medical Center of Sichuan University. All chemicals used were of reagent grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan), unless otherwise stated. All solvents were distilled before use.

2.2. General procedure

NMR spectra were recorded with a Bruker AMX500 instrument (^{1}H, 500 MHz). Electron ionisation (EI) and EI-high resolution (HR) mass spectra were obtained on a JEOL JMS-SX102A instrument (JEOL Ltd., Tokyo, Japan). Optical rotations were measured on a JASCO DIP-370 digital polarimeter (JASCO International Co. Ltd., Tokyo, Japan). Column chromatography was performed with Diaion HP-20 (Mitsubishi Chemical Corp., Tokyo, Japan), Cosmosil 75C_{18}-OPN (Nakarai Tesque, Inc., Kyoto, Japan) and Sephadex LH-20 (Amersham Plc., Little Chalfont, UK). Thin layer chromatography was done with precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm, normal phase). Detection was done by UV lamp (254 nm) or by spraying with 5% H_2SO_4 in ethanol followed by heating.

2.3. Intestinal α-glucosidase inhibitory activity determination

The α-glucosidase inhibitory activity was measured as described previously (Gao & Kawabata, 2005). The crude enzyme solution prepared from rat intestinal acetone powder (Sigma–Aldrich Japan Co., Tokyo, Japan), was used as the small intestinal α-glucosidases, sucrase, maltase and isomaltase, showing specific activities of 0.38 U/ml, 1.85 U/ml and 1.56 U/ml, respectively. The reaction mixture consisted of crude enzyme solution (as sucrase, 0.2 ml; as maltase, 0.05 ml and as isomaltase, 0.2 ml), substrate (sucrose: 56 mM, 0.2 ml; maltose: 3.5 mM, 0.35 ml and isomaltose: 7 mM, 0.2 ml, respectively) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample in 50% aqueous dimethyl sulfoxide (DMSO, 0.1 ml). The final reaction concentrations for sucrose, maltose and isomaltose were 22.4 mM, 2.45 mM and 2.8 mM in the total assay mixture of 0.5 ml, respectively. After incubation for 15 min at 37 °C, the reaction was stopped by adding 0.75 ml of 2 M Tris–HCl buffer (pH 7.0). The reaction mixture was passed through a short column of basic alumina (ICN Alumina B, grade I, ICN Biomedical GmbH, Eschwege, Germany) to remove phenolic compounds, which might interfere with glucose quantification. The amount of liberated glucose was measured by the glucose oxidase method, using a commercial test kit (Glucose B-test Wako, Wako Pure Chem. Co., Osaka, Japan). The concentration of inhibitors required for inhibiting 50% of the α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

2.4. Porcine pancreatic α-amylase inhibitory activity determination

The inhibitory effect of the compounds on α-amylase activity was assessed by the usual method (Gao & Kawabata, 2005). Briefly, starch azure (Sigma; 2.0 mg) used as a substrate was suspended in 0.7 ml of 50 mM Tris–HCl buffer (pH 6.9) containing 10 mM $CaCl_2$, and boiled for 5 min at 100 °C. Then, the starch azure solution was pre-incubated for 10 min at 37 °C. The test samples in 0.2 ml of 50% DMSO, and 0.1 ml of porcine pancreatic α-amylase solution (Sigma, A-6255; 2.0 U/ml; 50 mM Tris–HCl buffer containing 10 mM $CaCl_2$, pH 6.9) were added into each assay. The total volume of 1.0 ml reaction mixture was incubated at 37 °C for 10 min and stopped by adding 0.5 ml of 50% acetic acid. The reaction mixture was then centrifuged at 2000 rpm for 5 min at 4 °C. The absorbance of the supernatant at 595 nm was measured.

2.5. Screening experiment

One gram each of 40 species of dried herbs was extracted with 10 ml of 50% aqueous methanol for 24 h at room temperature. The crude extract was obtained by filtration and was evaporated to dryness with a rotary evaporator under reduced pressure. The dried residue was re-dissolved in

10 ml of 50% dimethyl sulfoxide (DMSO) and 0.1 ml of the test samples were studied for rat intestinal α-glucosidase inhibitory activity, as described in Section 2.3. The preparation concentration of the test samples used for each of assay was determined as 1 g fr wt/10 ml of 50% DMSO, where 1 g fr wt is the dried weight of 50% methanol (10 ml) extract from each of 1 g dried plants materials.

2.6. Extraction and isolation of the inhibitors from A. vasica

The dried leaves of *A. vasica* (50 g) were extracted three times with 50% methanol (2 l in total) for 24 h with continuous stirring at room temperature and then filtered. Evaporation of the solvent under reduced pressure yielded the 50% methanol extract (5.8 g), which was applied to a Diaion HP-20 column (4.2 × 56 cm) with a water–methanol gradient system, to give three fractions. The eluent volume and dry weight of each fraction were as follows: fraction 1, water (1 l, 4.0 g); fraction 2, 50% methanol in water (2 l, 1.3 g); fraction 3, methanol (1 l, 0.20 g).

The active fraction 2 (1.3 g) was then subjected to a Cosmosil 75C_{18}-OPN column (3.6 × 25 cm) with a water–methanol gradient (500 ml) system, to give six fractions: fraction 1 (water, 0.27 g), fraction 2 (10% methanol, 0.25 g), fraction 3 (20% methanol, 0.13 g), fraction 4 (30% methanol, 0.16 g), fraction 5 (50% methanol, 0.13 g) and fraction 6 (methanol, 0.05 g).

The active fraction 1 (0.27 g) was then applied to a Sephadex LH-20 column (3 × 30 cm) with water as an eluent. Every 10 ml of eluate was collected into 30 fractions and tested for sucrase inhibitory activity. Fractions 5–8 showed a high inhibition for this enzyme. Fractions 5 and 6, which showed a similar TLC profile (main spot: R_f 0.25, mobile phase: chloroform/methanol/water = 4:6:1), were combined and further purified by a Sephadex LH-20 column (3 × 30 cm; elution with water) to give 80 mg of vasicine (**1**, 0.16%). Fractions 7 and 8, which showed a similar TLC profile (main spot: R_f 0.18, mobile phase: chloroform/methanol/water = 4:6:1), were combined and further purified by a Sephadex LH-20 column (3 × 30 cm; elution with water) to give 100 mg of vasicinol (**2**, 0.2%).

2.7. Effect of ultrafiltration on sucrase by vasicine (1) and vasicinol (2)

The effect of ultrafiltration on sucrase by vasicine (**1**) and vasicinol (**2**) was done as the reported method (Niwa, Doi, & Osawa, 2003). Briefly, rat intestinal α-glucosidase solution (0.45 ml) was treated with 0.05 ml of H_2O, **1** (10 mM) or **2** (20 mM) in H_2O. A 0.5 ml aliquot of each solution was applied to a USY-1 ultrafilter (10,000 nominal molecular weight limit) (Advance, Dublin, CA). An unfiltered high-molecular weight fraction was re-dissolved with 0.5 ml of 0.1 M phosphate buffer (pH 6.3), and the recovered enzymatic activity of 0.2 ml was measured by adding 56 mM sucrose (0.2 ml) and the same buffer (0.1 ml). The enzyme solutions without ultrafiltration were also provided for measurement.

2.8. Kinetics of enzyme inhibition

For kinetic analyses of sucrase by vasicine (**1**) and vasicinol (**2**), the enzyme and the test compounds (**1**, 125 μM; or **2**, 250 μM] were incubated with increasing concentrations of sucrose. Inhibitory kinetics of **1** and **2** for sucrase was analysed by Lineweaver–Burk plots ($1/s$ vs $1/v$). Competitive inhibition was observed in each case, and therefore, we estimated the K_i value using the following equation (Kimura et al., 2004):

$$K_i = I/(K_p/K_m - 1)$$

where I, K_m and K_p are the inhibitor concentration used, Michaelis constant for substrate, and the apparent K_m in the presence of inhibitor, respectively.

3. Result

3.1. Screening experiments

In the screening experiments for rat intestinal sucrase and maltase inhibitors in traditional Chinese herbal medicines, 40 materials were tested (Table 1). Maltase inhibitory activity of the methanolic extracts of the selected traditional Chinese herbs ranged from 0% to 40%, and sucrase activity of these extracts ranged from 0% to 40%, except for *A. vasica*, and the methanolic extract of the leaves of *A. vasica,* which showed the highest sucrase inhibitory activity (63% inhibition) among the tested herbs (Table 1). Since the methanol extract of *A. vasica* showed potent sucrase inhibitory activity, further fractionation of this extract was carried out to isolate the active principles.

3.2. Extraction and isolation of active compounds vasicine (1) and vasicinol (2)

The dried leaves of *A. vasica* were extracted with 50% methanol at room temperature. At a final reaction concentration of 0.5 mg/ml, the aqueous methanolic extracts inhibited 50% sucrase activity. However, this extract showed only 38% inhibition of maltase even at a higher final reaction concentration of 2 mg/ml. In order to isolate sucrase inhibitors from the leaves of *A. vasica*, the aqueous methanolic extracts were fractionated by monitoring sucrase inhibitory activity against rat small intestinal enzyme (Fig. 1). Firstly, the extract was subjected to Diaion HP-20 column chromatography to yield three fractions. Next, the active fraction 2 (67% inhibition at a final reaction concentration of 0.5 mg/ml) was then separated by Cosmosil 75C_{18}-OPN column chromatography, to give six sub-fractions (based on water–methanol gradients). Finally, sub-fraction 1 (77% inhibition at a final reaction concentration of 0.5 mg/ml) was further purified by

Table 1
Inhibitory effects of 40 species of traditional Chinese herbal materials[a] on α-glucosidase[b]

Scientific name	Parts	Sucrase (%)[c]	Maltase (%)[d]
Rosa rugosa Thunb.	Leaf	11	35
Dalbergia odorifera T. Chen.	Stem	5	17
Adenanthera microsperma Teijsm. & Binn.	Fruit	5	11
Phaseolus calcaratus Roxb.	Seed	30	18
Liquidambar formosana Hance	Fruit	14	5
Euryale ferox Salisb.	Fruit	5	4
Artemisia scoparia Waldst et Kit.	Root	14	35
Patrinia Villosa Juss.	Stem	9	23
Equisetum hyemale L.	Root	0	3
Daemonorops draco Blume	Stem	12	23
Glossogyne tenuifolia Cass.	Stem	8	40
Lycopodium clavatum L.	Stem	3	27
Lobelia chinensis Lour.	Stem	9	18
Typha latifolia L.	Pollen	1	9
Dendrobium moniliforme F. Muell.	Stem	4	20
Nelumbo nucifera Gaertn.	Root	13	25
Lycium chinense Mill.	Stem	8	13
Erythrina variegata L.	Bark	6	15
Santalum alubum L.	Stem	0	2
Curcuma longa L.	Stem	0	17
Drynaria fortunei J. Sm.	Stem	22	32
Pulsatilla chinensis C. Muell.	Root	19	38
Paeonia lactiflora Pall.	Root	3	34
Dioscorea batatas Decne.	Stem	8	10
Poria cocos (Schw.) wolf	Bark	2	2
Coix lacryma-jobi L.	Seed	1	2
Eucommia ulmoides Oliver	Skin	21	21
Alisma orientalis Juzep.	Tuber	3	11
Atractylodes macrocephala Koidz.	Root	12	19
Polygonatum sibiricum Red.	Root	19	37
Pseudostellaria heterophylla Miq.	Root	0	3
Astragalus complanatus R. Br. ex Bunge	Stem	0	1
Cuscuta japonica Choisy	Seed	8	22
Rehmannia glutinosa Steud.	Bark	13	26
Inula cappa DC.	Leaf	26	35
Achyranthes aspra L.	Bark	20	29
Rhodiola rosea L.	Bark	21	18
Ephedra sinica Stapf	Stem	3	29
Erigeron breviscapus Hand.	Flower	32	25
Adhatoda vasica Nees	Leaf	63	38

[a] The herbal materials were purchased from a local herbal market in Chengdu, People's Republic of China. All voucher specimens are deposited in the department of Public Health, Hua Xi Medical Center of Sichuan University. One gram of each of plants materials was extracted with 10 ml of 50% aqueous methanol for 24 h at room temperature. The solvent was evaporated from one part of the extract. The dried residue was redissolved in 10 ml of 50% DMSO and 0.1 ml of the test samples was subjected to each assay.

[b] The crude enzyme solution prepared from rat intestinal acetone powder (Sigma Aldrich Japan Co., Tokyo, Japan), was used as the small intestinal α-glucosidases. Sucrase and maltase showing specific activities of 0.38 U/ml and 1.85 U/ml, respectively. Percentage of inhibition was calculated as: inhibitory activity (%) = $(OD_{control} - OD_{sample})/OD_{control} \times 100$.

[c] 56 mM sucrose as a substrate.

[d] 3.5 mM maltose as a substrate.

Sephadex LH-20 chromatography to yield vasicine (**1**) and vasicinol (**2**) as active principles.

To determine the absolute stereochemistry of compound **1**, we treated 20 mg of **1** with 10% hydrogen chloride in methanol (5 ml) to give the hydrochloride of **1**, which showed an optical rotation of $[\alpha]_D^{25}$ −21° (MeOH; *c* 1.0). Subsequently, 2 ml of 25% ammonia solution was added to 15 mg of the hydrochloride of **1** in 5 ml of methanol, and stirred for 10 min at room temperature. After evaporation, this residue was dissolved in methanol and subjected to reversed-phase column chromatography (20% methanol in water as the eluant) to give vasicine free base, which showed an optical rotation of $[\alpha]_D^{25}$ −125° (MeOH; *c* 1.0). The change in optical rotation of **1** with or without the addition of hydrogen chloride was in accord with previous observations (Joshi, Newton, Lee, Barber, & Pelletier, 1996; Szulzewsky, Hoehne, Johne, & Geroeger, 1976). In addition, the isolates were identified by comparison of their spectral data with those in the literature (Hua, Cheng, Li, & Pei, 2002; Joshi, Bai, Puar, Dubose, & Pelletier, 1994). The structures of compounds **1** and **2** are shown in Fig. 2.

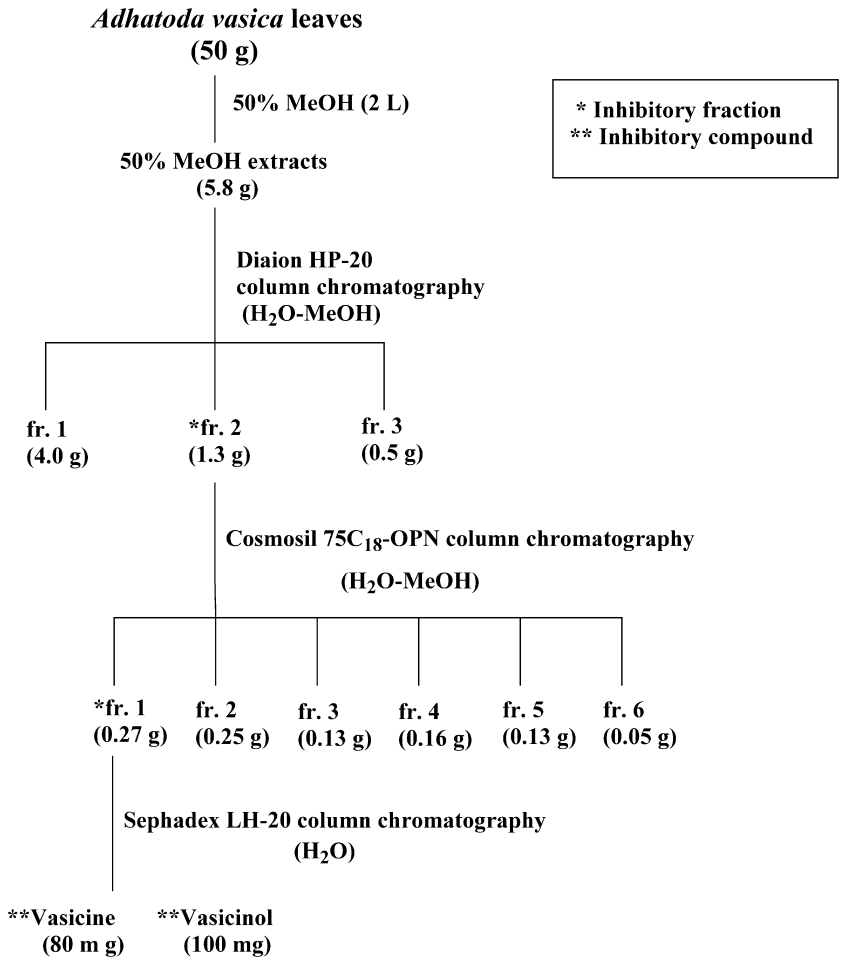

Fig. 1. Isolation scheme of the sucrase inhibitory compounds from the leaves of *Adhatoda vasica* Nees.

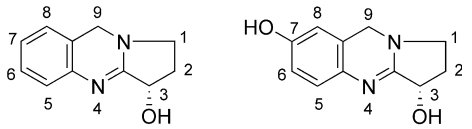

Fig. 2. Chemical structures of vasicine (**1**) and vasicinol (**2**).

3.2.1. Compound (*1*): (−)-vasicine

Off-white powder, $[\alpha]_D^{25}$ −125° (MeOH; *c* 1.0); EI–HRMS, *m*/*z* 188.0936 $[M]^+$ calcd. for $C_{11}H_{12}N_2O$, 188.0951; ^1H NMR δ (D_2O) ppm (*J* in Hz): 1.97 (1H, m, 2-H), 2.51 (1H, m, 2-H), 3.46 (1H, m, 1-H), 3.54 (1H, m, 1-H), 4.57 (2H, m, 9-H), 4.94 (1H, t, *J* = 7.9, 3-H), 6.90 (1H, d, *J* = 7.9, 8-H), 6.97 (1H, d, *J* = 7.6, 5-H), 7.09 (1H, m, 7-H), 7.18 (1H, m, 6-H).

3.2.2. Compound (*2*): (+)-vasicinol

Off-white powder, $[\alpha]_D^{25}$ +31° (MeOH; *c* 1.0); EI–HRMS, *m*/*z* 204.0915 $[M]^+$ calcd. for $C_{11}H_{12}N_2O_2$, 204.0900; ^1H NMR δ (D_2O) ppm (*J* in Hz): 1.96 (1H, m, 2-H), 2.50 (1H, m, 2-H), 3.41 (1H, m, 1-H), 3.50 (1H, m, 1-H), 4.29, 4.34 (each 1H, d, *J* = 16.0, 9-H), 4.95 (1H, t, *J* = 7.9, 3-H), 6.32 (1H, brs, 8-H), 6.54 (1H, d, *J* = 8.6, 6-H), 6.67 (1H, d, *J* = 8.6, 5-H).

3.3. Enzyme inhibitory activity

Vasicine (**1**) and vasicinol (**2**) inhibited sucrase activity in a dose-dependent manner (Fig. 3). The IC_{50} values for compounds **1** and **2** were determined to be 125 μM and 250 μM, respectively.

α-Glucosidase and α-amylase inhibitory activities of compounds **1** and **2** were shown in Fig. 4. At a concentration of 1 mM, the sucrase inhibitory activity of compounds **1** and **2** were 93% and 81%, respectively. However, these compounds showed weak inhibitory activity against maltase, isomaltase and porcine pancreatic α-amylase, from 7% to 34%.

3.4. Effect of ultrafiltration on sucrase by vasicine (*1*) and vasicinol (*2*)

Rat intestinal α-glucosidase solution (0.45 ml) was treated with 0.05 ml of H_2O, **1** (10 mM) or **2** (20 mM) in H_2O and was applied to a USY-1 ultrafilter. An unfiltered high-molecular weight fraction was re-dissolved in 0.5 ml of

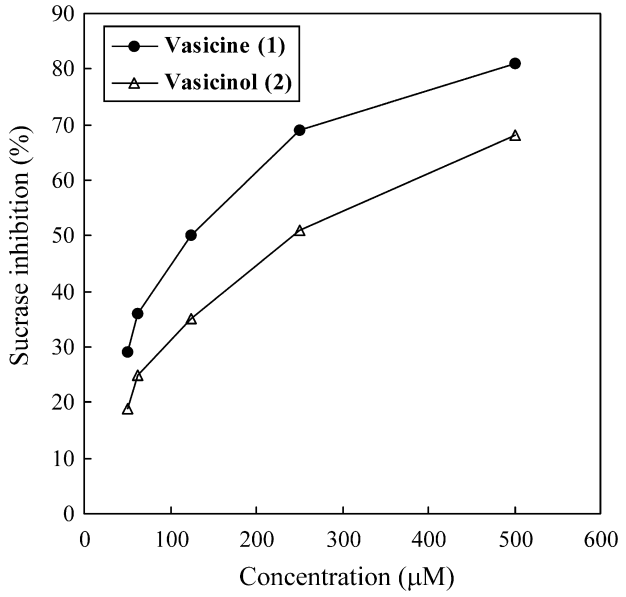

Fig. 3. Dose-dependent inhibition of sucrose by vasicine (**1**) and vasicinol (**2**). IC_{50} values for **1** and **2** were calculated from the dose-dependent inhibition curves.

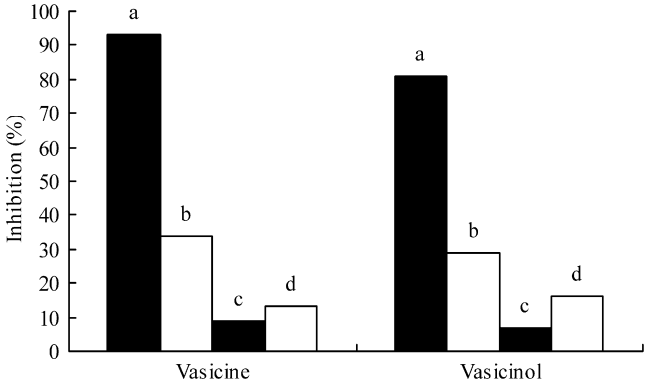

Fig. 4. Selectivity of vasicine (**1**) and vasicinol (**2**) for rat intestinal α-glucosidase and porcine pancreatic α-amylase inhibition at the concentration of 1 mM. (a) Sucrase activity; (b) maltase activity; (c) isomaltase activity; (d) porcine pancreatic α-amylase activity.

0.1 M phosphate buffer (pH 6.3) and the residual enzymatic activity was determined. The optical density (OD) at 490 nm of control, **1** and **2** was 0.556, 0.2235 and 0.2335, respectively (Fig. 5). Before ultrafiltration, $OD_{490\ nm}$ of control, **1** and **2** was 0.505, 0.45 and 0.48, respectively (Fig. 5).

3.5. Kinetics of enzyme inhibition

In the Lineweaver–Burk plot (Fig. 6), the linear regression and extrapolation of data gave a series of lines crossing on the vertical axis, which indicated that vasicine (**1**) and vasicinol (**2**) inhibit the enzyme competitively. K_i values were determined to be 82 μM and 183 μM, respectively.

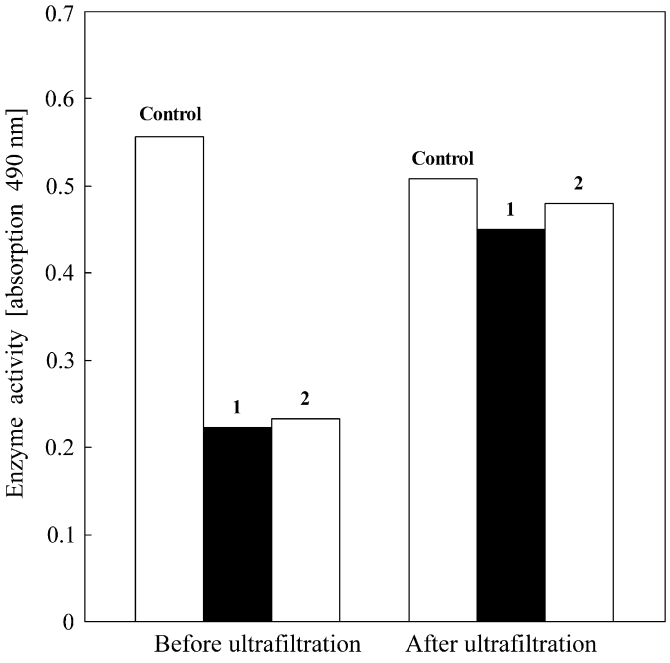

Fig. 5. Recovery of enzymatic activity by elimination of vasicine (**1**) and vasicinol (**2**) by ultrafiltration.

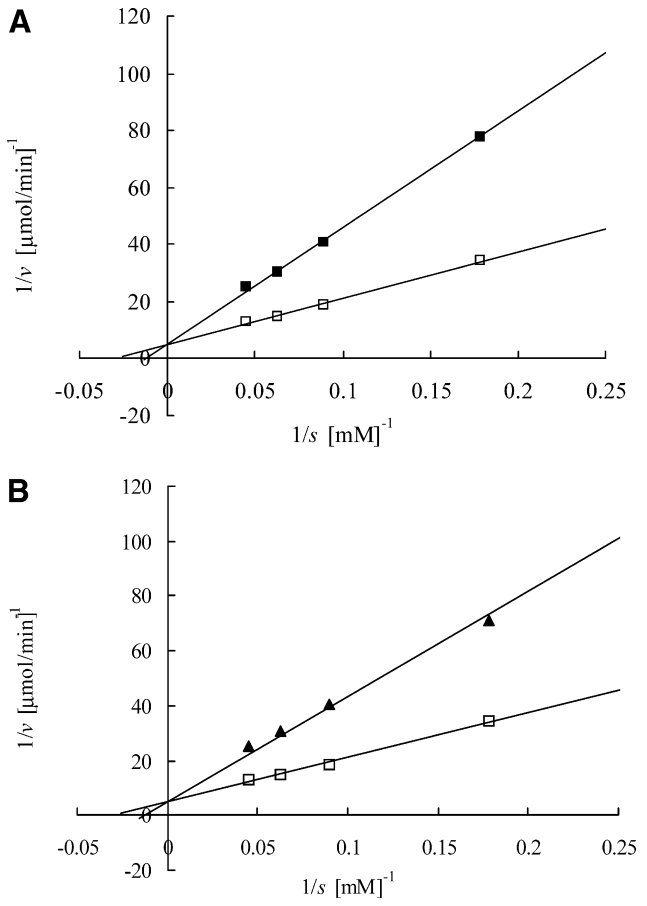

Fig. 6. Lineweaver–Burk plots analysis of inhibition kinetics of rat small intestinal α-glucosidase inhibitory effects by vasicine (A; □, 0 μM; ■, 125 μM) and vasicinol (B; □, 0 μM; ▲, 250 μM).

4. Discussion and conclusion

Postprandial hyperglycaemia has been the potent risk factor for diabetes and various targets, as well as pharmacological agents, have been identified to manage this risk factor (Carroll, Gutierrez, Castro, Tsewang, & Schade, 2003; Johnston et al., 1988; Rendell, 2004). Thus, inhibitors of mammalian intestinal α-glucosidase have become exciting candidates to slow down the digestion of carbohydrates and in turn mitigate postprandial hyperglycaemic excursions. Traditional Chinese medicines have long used plant and herbal extracts as anti-diabetic agents. Therefore, investigation of such agents from traditional medicinal plants has become more important and researchers are competing to find new, effective and safe therapeutic agents for the treatment of diabetes. In the screening experiments of forty species of selected traditional Chinese herbs, the methanolic extract from the leaves of *A. vasica* showed the highest sucrase inhibitory activity. This is the first report of this herb species with regards to its sucrase inhibitory effect, although *A. vasica* has been studied for its pharmacological activities, namely, antitussive activity (Dhuley, 1999), antiinflammatory activity (Chakraborty & Brantner, 2001) and antibacterial activity (Brantner & Chakraborty, 1998). An enzyme assay-guided fractionation of the methanolic extract using column chromatography led to the isolation of vasicine (**1**) and vasicinol (**2**).

A. vasica (Acanthaceae) is known to furnish quinazoline alkaloids, such as vasicine, vasicinone, deoxyvasicinone, vasicinol vasicol, adhavasicinone and some minor compounds in the same series (Claeson, Malmfors, Wikman, & Bruhn, 2000). The biological activities of vasicine (**1**), including bronchodilatory, uterotonic and oxytocic activities have been reported (Claeson et al., 2000). However, **1** has never been tested for intestinal α-glucosidase inhibitory activity so far. This compound inhibited sucrase activity, with an IC_{50} value of 125 μM. On the other hand, **1** has no inhibitory effects ($IC_{50} > 1$ mM) on maltase, isomaltase and α-amylase. Hence, this result indicated that **1** is a selective sucrase inhibitor.

Vasicinol (**2**) had been isolated from many species and the antifertility effect of **2** has been reported (Saxena, Tikku, Atal, & Opender, 1986). Our finding is the first report on the intestinal α-glucosidase inhibitory activity of **2**, which inhibited sucrase activity with an IC_{50} value of 250 μM. However, compound **2** did not show inhibitory effects ($IC_{50} > 1$ mM) on intestinal maltase, isomaltase and α-amylase. From this result, it is revealed that compound **2**, like **1**, is a selective sucrase inhibitor.

Because this is the first observation of the inhibitory effect of compounds **1** and **2** on sucrase, we described the inhibitory mode of action of compounds **1** and **2** against this enzyme. To determine whether **1** and **2** are reversible sucrase inhibitors, we removed the unreacted inhibitor from the enzyme solution by ultrafiltration, and examined whether the activity would be recovered or not. When an enzyme solution containing **1** or **2** was treated with ultrafiltration and then redissolved to the same concentration, the sucrase activity was considerably recovered. From this result, we can conclude that each of **1** and **2** is a reversible inhibitor against sucrase.

To further characterise the inhibitory mechanism of compounds **1** and **2**, we analysed the change in kinetic parameters of sucrase activity in response to different concentrations of sucrose ranging from 5.6 to 22.4 mM (reaction concentration). The double reciprocal Lineweaver–Burk plot demonstrated that both compounds **1** and **2** behave as classical competitive inhibitors. The precise explanation how compounds **1** or **2** interact with the enzyme on a molecular basis is still unknown since the structure of α-glucosidase used for this study has not yet been established; however, this research may offer references and insights for designing and synthesising a new class of α-glucosidase inhibitors with the skeleton of quinazoline alkaloids.

From the results presented here, compounds **1** and **2** did not have comparable activities to the commercial α-glucosidase inhibitor, acarbose (sucrase inhibition, $IC_{50} = 0.8$ μM) (Gao & Kawabata, 2004). However, the observed biological activities in general are in line with the traditional uses of this herb. In present study, the data partially suggest that vasicine (**1**) and vasicinol (**2**) offer a possibility of being developed as successful α-glucosidase inhibitors.

In conclusion, the enzyme-guided fractionation of the extract from the leaves of *A. vasica* led to the isolation of vasicine (**1**) and vasicinol (**2**) as intestinal sucrase inhibitors. This result suggests that *A. vasica* can be physiologically useful for suppressing postprandial hyperglycaemia by ingesting it or its extract in the diet.

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