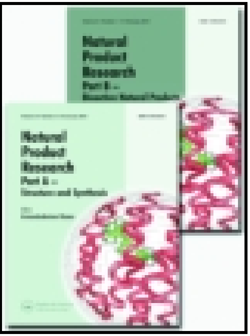

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A new megastigmane sulphoglycoside and polyphenolic constituents from pericarps of *Garcinia mangostana*

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ABSTRACT
A megastigmane sulphoglycoside together with three phenolic compounds were isolated from the water-soluble fraction of the pericarps of *Garcinia mangostana*. The structure of the new compound was determined as 4-*O*-sulpho-*β*-D-glucopyranosyl abscisate (**1**) by spectroscopic data. Proanthocyanidin A2 (**2**) showed potent α-glucosidase inhibitory and DPPH scavenging activities with IC_{50} values of 3.46 and 11.6 µM, respectively.

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Garcinia mangostana; megastigmane sulphoglycoside; proanthocyanidin A2; α-glucosidase; DPPH

1. Introduction

The plants in the genus *Garcinia* (Guttiferae) are well known to be rich in biologically active compounds including xanthones, benzophenones, proanthocyanidins and biphenyls (Mungmee et al. 2013; Feng et al. 2014; Fouotsa et al. 2014; Jiang et al. 2014; Anu Aravind et al. 2015). Mangosteen (*Garcinia mangostana* Linn.) is a plant that is widely cultivated in Vietnam and other Southeast Asian countries. The pericarp of this plant has been used as a remedy in traditional medicine for the treatment of diarrhoea, inflammation, skin infections and wounds (Vo 2012). Previous studies have shown that it contains high amounts of polyphenolic constituents, in which xanthones have been most frequently reported (Obolskiy et al.

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Figure 1. The chemical structures of compounds **1**–**4**.

2009; Dharmaratne et al. 2013; Morelli et al. 2015; Zhou et al. 2015). Mangosteen pericarp has exhibited a variety of interesting biological activities including antibacterial, antifungal, anti-inflammatory, antimalarial and antidiabetic (Pedraza-Chaverri et al. 2008; Obolskiy et al. 2009; Dharmaratne et al. 2013). α-Glucosidase, a membrane-bound enzyme at the epithelium of the small intestine, hydrolyses starch oligosaccharides to monosaccharides. The inhibitors of this enzyme delay carbohydrate digestion and thus cause a reduction in the rate of glucose absorption and lower the postprandial hyperglycaemia. Therefore, the inhibition of α-glucosidase plays a major role in preventing the rise of the postprandial glucose level in diabetics (Kumar et al. 2011).

In our search for natural inhibitors of α-glucosidase, we found that the methanol extract of mangosteen pericarp showed a potent inhibitory effect against α-glucosidase. The water-soluble fraction of the methanol extract of mangosteen pericarp, which strongly inhibited α-glucosidase, was investigated for its chemical constituents. Four compounds (Figure 1) were isolated and elucidated as a new megastigmane sulphoglycoside (**1**) and three known compounds: proanthocyanidin A2 (**2**) (Cai et al. 1991), (-)-epicatechin (**3**) (Lou et al. 1999) and 2*R*,3*R*-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(glucosyloxymethyl)-7-methoxy-benzofuran-5-propanol (**4**) (Baderschneider & Winterhalter 2001). All compounds were tested for their α-glucosidase inhibitory and DPPH scavenging activities. The present paper describes the isolation, structure elucidation and biological characterisation of the isolated compounds.

2. Results and discussion

Compound **1** was obtained as a colourless solid. Its HRESIMS showed the peak at *m/z* 507.1526 $[M + H]^+$ corresponding to the molecular formula $C_{21}H_{31}O_{12}S$ (Calcd 507.1536). The ^1H NMR spectrum of **1** showed signals for a *trans* double bond at δ_H 6.23 (1H, d, $J = 16.0$ Hz, H-5), 7.70 (1H, d, $J = 16.0$ Hz, H-4), two olefinic proton singlets at δ_H 5.73 (1H, s, H-2), 5.84 (1H, s, H-3′),

Table 1. α-Glucosidase inhibitory and DPPH scavenging activities[a] of compounds 1–4 isolated from pericarps of *G. mangostana*.

Compounds	α-Glucosidase	DPPH
1	not active	247.8 ± 13.9
2	3.46 ± 0.68	11.6 ± 0.78
3	740.3 ± 47.2	25.5 ± 1.8
4	172.4 ± 8.4	182.9 ± 7.3
Acarbose	1550.3 ± 77.5	–
Ascorbic acid	–	210.5 ± 9.6

[a]IC_{50} values in μM are means ± SD from three independent experiments.

four tertiary methyl groups at δ_H 1.98 (3H, s, H-6), 1.83 (3H, s, H-7′), 0.97 (3H, s, H-8′) and 0.92 (3H, s, H-9′). In addition, a sugar moiety was recognised by signals of a *β*-anomeric proton at δ_H 5.43 (1H, d, *J* = 8.5 Hz, H-1″). The ^{13}C-NMR and DEPT spectra of **1** indicated the presence of four methyl, two methylene, nine methine and six quaternary carbon groups. The HMBC correlations showed the couplings from H-2 (δ_H 5.73) to the carboxylic C-1 (δ_C 166.3) and C-3 (δ_C 153.7) and from H-3′ (δ_H 5.84) to the ketone C-4′ (δ_C 200.9) and C-2′ (δ_C 165.7) indicating the presence of two *α,β*-unsaturated carbonyl systems. The sugar moiety attached to the carboxylic group was shown by the coupling from the anomeric proton (δ_H 5.43) to C-1 (δ_C 166.3). These data were very similar to those of the megastigmane *β*-D-glucopyranosyl abscisate except for the difference in ^{13}C chemical shift of C-4″ (Hirai et al. 2000). The NOESY correlation between H-2 and H-6 and the upfield shift of H-4 and downfield C-4 (Ferreres et al. 1996) confirmed the *cis* configuration of the C-2–C-3 double bond. Acid hydrolysis of **1** allowed identification of the aglycone as (*S*)-abscisic acid by its positive optical rotation (Hirai et al. 2000). TLC and optical rotation analysis of the aqueous solution in comparison with standard sugars confirmed the presence of D-glucose. Addition of $BaCl_2$ to the aqueous solution resulted in a formation of white precipitate indicating the presence of sulphate group. This result agreed with the mass spectral data. The downfield shift of C-4″ resonance (δ_C 77.0 instead of 71.1) suggested the sulphate group is located at C-4″ (Shitamoto et al. 2011). Thus, compound **1** was elucidated to be 4-*O*-sulpho-*β*-D-glucopyranosyl abscisate, a new megastigmane sulphoglycoside.

All isolated compounds were tested for their α-glucosidase inhibitory activity. Most of the isolated compounds are phenolic and may possess antioxidant activity, thus the compounds were evaluated for their DPPH scavenging capacity. As shown in Table 1, proanthocyanidin A2 (**2**) was most active with the IC_{50} values of 3.46 and 11.6 μM for α-glucosidase inhibitory and DPPH scavenging activities, respectively. The known antioxidant compound (-)-epicatechin (**3**) and the benzofuran glycoside (**4**) also showed moderated α-glucosidase inhibitory effect. The new megastigmane sulphoglycoside (**1**) exhibited significant DPPH scavenging capacity in comparison with ascorbic acid. A previous study reported that the prenylated xanthones in the pericarps of mangosteen potently inhibited α-glucosidase (Ryu et al. 2011). In the present work, proanthocyanidin A2, a polar compound isolated from the water-soluble fraction of mangosteen pericarps showed potent α-glucosidase inhibitory activity in comparison with the antidiabetic drug acarbose. This result coincided with the previous study assuming that proanthocyanidin components in the pericarps of mangosteen are potent inhibitors of α-amylase, another enzyme involving in the metabolism of carbohydrates and hyperglycaemia (Loo & Huang 2007). Although previous study showed that total proanthocyanidins from *Cinnamomum cassia* strongly inhibited α-glucosidase (Kang et al. 2014), the present paper

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is the first report for the α-glucosidase inhibitory activity of proanthocyanidin A2. A lot of studies have shown that the prenylated xanthones in mangosteen are potent inhibitors of α-glucosidase. However, these compounds are mostly soluble in organic solvents of moderate polarity. Our result showed that the water-soluble fraction of mangosteen pericarp contains attractive α-glucosidase inhibitors. Thus, these data reinforce the health benefit of mangosteen as an alternative medicine to help lower postprandial glucose absorption.

3. Experimental

3.1. General procedures

Optical rotation values were recorded on a JASCO P-2000 digital polarimeter (JASCO, Tokyo, Japan). The UV spectra were recorded on a JASCO V-630 spectrophotometer (JASCO, Tokyo, Japan). The IR spectrum was obtained from a Tensor 37 FT-IR spectrometer (Bruker, Ettlingen, Germany). NMR experiments were carried out on a Bruker AM500 FT-NMR spectrometer (Bruker, Rheinstetten, Germany) using tetramethylsilane (TMS) as internal standard. The HR-ESI-MS were recorded on an FT-ICR mass spectrometer (Bruker Dal-tonics, Bremen, Germany).

3.2. Plant material

The fruits of *G. mangostana* were purchased from markets in Hanoi in June, 2011 and identified by Prof Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The voucher specimens (BK-10) were deposited at School of Chemical Engineering, Hanoi University of Science and Technology. The fruit pericarps were collected, air dried and powdered.

3.3. Extraction and isolation

The air-dried and powdered materials (2.0 kg) were extracted with methanol (4 L × 3 times) at room temperature for 24 h. The combined extracts were concentrated under vacuum to obtain a crude residue (152.0 g, 81% α-glucosidase inhibition at 100 μg/mL), which was then resuspended in water (1 L), and extracted by chloroform (1 L × 3 times). The organic layers were combined and concentrated to give 48.5 g of chloroform residue. The water layer (87% α-glucosidase inhibition at 100 μg/mL) was passed through a dianion HP-20 column and washed with 1.5 L water, then eluted with 50 and 100% methanol (1 L each). The eluant from 100% methanol was concentrated and chromatographed on a RP-18 column using methanol–water (1:4 v/v) as the mobile phase to afford five fractions of FA1–5. Compound **2** (60.3 mg) was purified from FA2 by using a silica gel column eluted by chloroform:methanol:water (45:10:1 v/v). Fraction FA3 was passed through a C-18 reverse-phase column using methanol:water (1:2 v/v) as the mobile phase to obtain compounds **3** (98.5 mg) and **4** (8.0 mg). FA5 was chromatographed on a silica gel column eluted with ethyl acetate:methanol:water (60:10:1 v/v) to give **1** (6.1 mg).

3.3.1. *4-O-sulpho-β-*d-glucopyranosyl abscisate (1)

Colourless solid. $[\alpha]_D^{25}$ = +45.3 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε) 270 (3.75) nm. ^{1}H NMR (500 MHz, CD_3OD): δ 0.92 (3H, s, H-9′), 0.97 (3H, s, H-8′), 1.83 (3H, s, H-7′), 1.98 (3H, s, H-6), 2.10

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(1H, d, $J = 17.0$ Hz, H-5′a), 2.44 (1H, d, $J = 17.0$ Hz, H-5′b), 3.64 (1H, t, $J = 9.0$ Hz, H-3″), 3.44 (1H, m, H-5″), 3.36 (1H, t, $J = 9.0$ Hz, H-2″), 3.77 (1H, dd, $J = 2.0$, 12.0 Hz, H-6″a), 3.67 (1H, m, H-6″b), 4.09 (1H, t, $J = 9.0$ Hz, H-4″), 5.43 (1H, d, $J = 8.5$ Hz, H-1″), 5.73 (1H, s, H-2), 5.84 (1H, s, H-3′), 6.23 (1H, d, $J = 16.0$ Hz, H-5), 7.70 (1H, d, $J = 16.0$ Hz, H-4). ^{13}C NMR (125 MHz, CD_3OD): δ 166.3 (C-1), 118.0 (C-2), 153.7 (C-3), 129.3 (C-4), 139.2 (C-5), 21.3 (C-6), 80.6 (C-1′), 165.7 (C-2′), 127.6 (C-3′), 200.9 (C-4′), 50.6 (C-5′), 42.8 (C-6′), 19.5 (C-7′), 23.5 (C-8′), 24.6 (C-9′), 95.0 (C-1″), 73.9 (C-2″), 76.7 (C-3″), 77.0 (C-4″), 77.1 (C-5″), 62.1 (C-6″). HR-ESI-MS *m/z*: 507.1526 $[M + H]^+$ (Calcd 507.1536 for $C_{21}H_{31}O_{12}S$).

3.3.2. Acid hydrolysis of 1

Compound **1** (2 mg) was heated in 1 N HCl (1 mL) at 80 °C for 2 h, then the solution was cooled and extracted with ethyl acetate (1 mL × 3). The organic and aqueous layers were concentrated to 1 mL and their optical rotations were checked. (*S*)-abscisic acid was obtained from the organic layer, $[\alpha]_D^{25} = +107.6$ (*c* 0.03, MeOH). The sugar product in the aqueous layer was identified as D-glucose by silica gel TLC (Rf 0.55 developed with acetone–methanol–water 100:10:1 (v/v) and was sprayed with a 10% sulphuric acid solution containing 2% vanillin), and by the positive value of optical rotation ($[\alpha]_D^{25} = +3.2$ (*c* 0.03, H_2O) in comparison with D-glucose standard. The addition of $BaCl_2$ to the aqueous solution produced a white precipitate indicating the presence of SO_4^{2-} anions.

3.4. Assay for α-glucosidase inhibition

The α-glucosidase (G0660–750UN, Sigma) enzyme inhibition assay was performed according to a previously described method (Luyen et al. 2013). The sample solution (2 μL dissolved in DMSO) and 0.5 U/mL α-glucosidase (40 μL) were mixed in 120 μL of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (40 μL) was added and the solution was incubated at 37 °C for 30 min. The absorbance of released 4-nitrophenol was measured at 405 nm using a microplate reader (Molecular Devices, CA). The IC_{50} value was graphically measured using a plot of the per cent inhibition vs. log of the concentration of the test compound.

3.5. DPPH radical scavenging activity

The antioxidant activity of the isolated compounds was evaluated by its scavenging capacity of the DPPH radical. Briefly, the tested samples (10 μL) at various concentrations were mixed with 150 μM DPPH solution (190 μL) in 96 well plates. The plate was incubated in the dark at room temperature for 30 min. Then the absorbance of the reaction mixture was measured at 520 nm on a microplate reader. The IC_{50} value is defined as the concentration of a sample required to scavenge 50% of the DPPH radical.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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