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# α-Glucosidase inhibitors from Devil tree (*Alstonia scholaris*)

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**Abstract**

α-Glucosidase inhibitors are used in the treatment of non-insulin-dependent diabetes mellitus. We attempt to isolate α-glucosidase inhibitors from 24 traditional Thai medicinal plant samples. Potent α-glucosidase inhibitory activity was found in aqueous methanol extract of dried Devil tree (*Alstonia scholaris*) leaves. Active principles against α-glucosidase, prepared from rat small intestine acetone powder, were isolated and identified. The structures of these isolated compounds were found to be quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-D-galactopyranoside and (−)-lyoniresinol 3-*O*-β-D-glucopyranoside on the basis of chemical and spectral evidence. The latter exhibited an inhibitory activity against both sucrase and maltase with $IC_{50}$ values of 1.95 and 1.43 mM, respectively, whereas the former inhibited only maltase with $IC_{50}$ values of 1.96 mM. This preliminary observation will provide the basis for further examination of the suitability of *Alstonia scholaris* as a medicinal supplement that contributes toward the treatment and prevention of diabetes.


*Keywords:* *Alstonia scholaris*; α-Glucosidase inhibitors; Diabetes; Thailand

## 1. Introduction

Diabetes mellitus (types 1 and 2) is recognized as a serious global health problem, often resulting in substantial morbidity and mortality. Type 2 diabetes mellitus is a group of disorders characterized by hyperglycemia and associated with microvascular (i.e., retinal, renal, possibly neuropathic), macrovascular (i.e., coronary, peripheral vascular), and neuropathic (i.e., autonomic, peripheral) complications. Unlike type 1 diabetes mellitus, the patients are not absolutely dependent upon insulin for life, even though many of these patients ultimately are treated with insulin. The management of type 2 diabetes mellitus often demands combined regimens, including diet and/or medicines, including sulfonylurea, and biguanide, as well as insulin. Besides the use of multiple approaches, α-glucosidase inhibitors are one of the alternative therapeutic approaches. The inhibition of intestinal α-glucosidases, would delay the digestion and absorption of carbohydrates and consequently suppress postprandial hyperglycemia (Puls, Keup, Krause, Thomas, & Hofmeister, 1977). Furthermore, other benefits of α-glucosidase inhibitors, such as reducing triglycerides (Lebowitz, 1998) and postprandial insulin (Johnston et al., 1994) levels and anti-HIV activity (Bridges, Brennan, Taylor, McPherson, & Tyms, 1994; Fischer et al., 1995; Fischer, Karlsson, Butters, Dwek, & Platt, 1996; Fischer, Karlsson, Dwek, & Platt, 1996) have been reported.

For these reasons, there has been extensive research on α-glucosidase inhibitors during the past forty years. The α-glucosidase inhibitory activity of natural sources, such as plants, foodstuffs and microbes has been studied (Fujita & Yamagami, 2001; Fujita, Yamagami, & Ohshima, 2001, 2003; Matsui et al., 2001; Toda, Kawabata, & Kasai, 2000) and several α-glucosidase inhibitors have been isolated from these sources (Kim, Wang, & Rhee, 2004; Matsuura, Asakawa, Kurimoto, & Mizutani, 2002, 2004; Nishioka, Kawabata, & Aoyama, 1998; Toda et al., 2000).

In the search for potent α-glucosidase inhibitors, we focussed on traditional Thai medicinal plants as the sources of α-glucosidase inhibitors, since they are known to have many pharmaceutical effects and health benefits.

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The 50% aqueous methanol extracts of 24 traditional Thai medicinal plant samples were examined for their inhibitory activities against rat intestinal sucrase and maltase. Among them, Devil tree leaf extract exhibited strong inhibition against enzyme activities. In this paper, we report the isolation and identification of the sucrase/maltase inhibitors from Devil tree leaves.

## 2. Materials and methods

### 2.1. Materials

Thai herbs were obtained from Rajamangala Institute of Technology, Surin campus, Thailand. Rat intestinal acetone powder and porcine pancreatic α-amylase were supplied by Sigma Aldrich Japan Co. (Tokyo, Japan). ICN Alumina B, Akt. I was purchased from ICN Biomedicals GmbH (Eschwege, Germany). All chemicals used were of analytical grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan), unless otherwise stated.

### 2.2. Assay for rat intestinal sucrase inhibitory activity

Rat intestinal sucrase inhibitory activity was determined using the method described previously (Nishioka et al., 1998) with a slight modification. Sucrose (56 mM) in 0.1 M potassium phosphate buffer (pH 7, 0.2 ml) was mixed with test sample in 50% aqueous dimethyl sulfoxide (DMSO, 0.1 ml). After pre-incubating at 37 °C for 5 min, rat intestinal α-glucosidase solution (0.2 ml) prepared from rat intestinal acetone powder was added. The reaction was carried out at 37 °C for 15 min and then was stopped by adding 2M Tris–HCl buffer (pH 6.9, 0.75 ml). The reaction mixture was passed through a basic alumina column ($\phi$ 6 mm × 3.5 mm h) to eliminate phenolic or acidic compounds. The amount of liberated glucose was determined by the glucose oxidase method, using a commercial test kit (Glucose-B Test Kit, Wako Pure Chem. Co., Osaka, Japan).

### 2.3. Assay for rat intestinal maltase inhibitory activity

Rat intestinal maltase inhibitory activity was determined using the method described previously (Toda et al., 2000) with a slight modification. The assay was carried out in the same manner as for the assay for rat intestinal sucrase inhibitory activity except for using 3.5 mM maltose in 0.1M potassium phosphate buffer (pH 7, 0.35 ml) as a substrate and rat intestinal α-glucosidase solution added was 0.05 ml.

### 2.4. Isolation of active principles from Devil tree

Dried leaves of *Alstonia scholaris* (100 g) were cut into small pieces and extracted with 50% aqueous MeOH (1000 ml) at room temperature for 24 h. The evaporated extract was partitioned between EtOAc and $H_2O$. The $H_2O$ layer was chromatographed on Cosmosil 75C18-OPN (Nacalai Tesque, Inc., Kyoto, Japan) with $H_2O$–MeOH gradient elution. The active fraction, eluted with $H_2O$–MeOH (3:2), was subjected to HPLC [column: Inertsil PREP-ODS, 20.0 × 250 mm; mobile phase: $H_2O$–MeOH (13:7); flow rate: 5 ml/min; detection: UV 254 nm] to yield quercetin 3-*O*-β-D-xylopyranosyl(1‴ → 2″)-β-D-galactopyranoside (**1**, 20 mg, $t_R$ 54 min). The other active HPLC eluted fraction was further purified by the same HPLC system except using a more polar mobile phase $H_2O$–MeOH (3:1) to give (−)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**2**, 4 mg, $t_R$80 min) and (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**, 3.4 mg, $t_R$91 min).

Compound **1**: FAB-MS (negative): *m/z* 595 $[M-H]^-$; FAB-HR-MS (negative): *m/z* 595.1302 (calcd. For $C_{26}H_{27}O_{16}$, 595.1300); $^1H$ NMR and $^{13}C$ NMR: see Table 2.

Compound **2**: FAB-MS (negative): *m/z* 581 $[M-H]^-$; FAB-HR-MS (negative): *m/z* 581.2233 (calcd. For $C_{28}H_{37}O_{13}$, 581.2235); $[\alpha]_D$−33.9° (c 0.2, MeOH); $^1H$ NMR and $^{13}C$ NMR: see Table 3.

Table 1
Rat intestinal sucrase and maltase inhibitory activities (%) of traditional Thai medicinal plant extracts

| Name of plants | Sucrase | Maltase |
|---|---|---|
| *Alstonia scholaris* (L.) R. Br. (Devil tree) | | |
| Leaf | 51 | 74 |
| Bark | 9 | 0 |
| *Cratoxylum mangayi* Dyer | | |
| Leaf | 27 | 62 |
| Bark | 0 | 0 |
| *Cratoxylum formosum* Benth. & Hook. F. ex Dyer | | |
| Leaf | 5 | 28 |
| Bark | 5 | 18 |
| *Dillenia indica* L. | | |
| Leaf | 40 | 56 |
| Bark | 8 | 15 |
| *Dolichandrone spathacea* Seem. | | |
| Flower | 7 | 5 |
| Leaf | 0 | 26 |
| Bark | 3 | 10 |
| *Hydnocarpus anthelminthicus* Pierre ex Laness. (Leaf) | 5 | 25 |
| *Ipomoea digitata* L. (Leaf) | 23 | 41 |
| *Lagerstroemia floribunda* Jack | | |
| Leaf | 3 | 4 |
| Bark | 13 | 24 |
| *Mimusops elengi* L. | | |
| Flower | 0 | 0 |
| Leaf | 20 | 41 |
| Fruit | 7 | 24 |
| Bark | 23 | 26 |
| *Mitragyna diversifolia* (Wall. ex G. Don) Havil. (Leaf) | 11 | 32 |
| *Passiflora foetida* L. (Leaf) | 0 | 0 |
| *Sindora siamensis* Teijsm. Ex Miq. | | |
| Leaf | 26 | 30 |
| Bark | 5 | 6 |
| *Suregada multiflora* Baill.(Bark) | 3 | 18 |

Compound **3**: FAB-MS (negative): *m/z* 581 $[M-H]^-$; FAB-HR-MS (negative): *m/z* 595.2250 (calcd. For $C_{28}H_{37}O_{13}$, 581.2235); $[\alpha]_D$ +35.6° (c 0.2, MeOH); $^1H$ NMR and $^{13}C$ NMR: see Table 4.

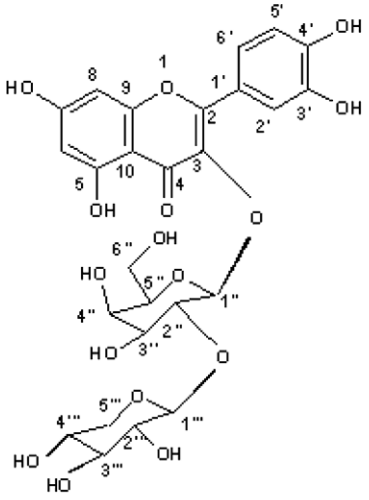
### 2.5. Spectrometric analysis

$^1H$ and $^{13}C$ NMR spectra were recorded with a Bruker AMX 500 spectrometer at 500 and 125 MHz, respectively. FAB-mass spectra were obtained on a JEOL AX-500 spectrometer. Optical rotation was measured with a JASCO DIP-370 digital polarimeter. HPLC was performed with a JASCO 802-SC system.

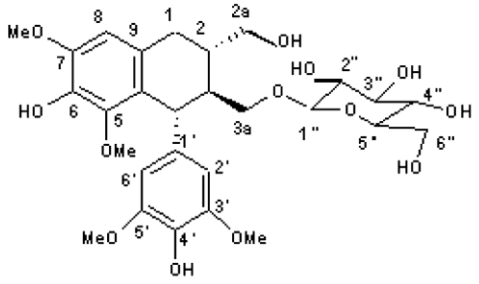
## 3. Results and discussion

Results of screening for sucrase and maltase inhibitory activities are shown in Table 1. The sucrase inhibitory activities ranged from 0% to 51%, with the highest value in *A. scholaris*, leaf (51), followed by *D. indica*, leaf (40) and *C. mangayi*, bark (27). Similarly, maltase inhibitory activity ranged from 0% to 74%, with the highest value in *A. scholaris*, leaf (74), followed by *D. indica*, leaf (56) and *C. mangayi*, bark (62). Among the plants studied, *A. scholaris* exhibited the highest inhibitory activities for both sucrase and maltase. Since Devil tree leaf extract showed potent inhibitory activity, further isolation and purification of the active principles from this plant extract were carried out. The extract was partitioned between $H_2O$ and EtOAc and both fractions were examined for their sucrase and maltase inhibitory activities. The $H_2O$ fraction, which showed higher inhibitory activities (data not shown) was further purified by a series of chromatography techniques to obtain one flavonoid glycoside, quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-D-galactopyranoside (**1**) and one lignan glucoside, (−)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**2**), as the active principles, together with another linan glucoside, (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**) with no inhibitory activity (Fig. 1).

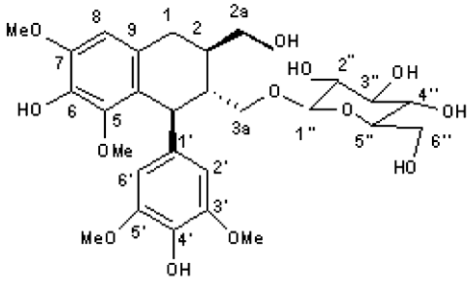
Compound **1** afforded a $[M-H]^-$ ion at *m/z* 595 in the FAB mass spectrum and a high-resolution analysis disclosed its molecular formula to be $C_{26}H_{28}O_{16}$. The $^{13}C$ and $^1H$ NMR data (Table 2) demonstrated that this compound had a quercetin skeleton with the presence of galactopyranose and xylopyranose units. The large coupling constants of the anomeric protons indicated a β-configuration for the xylosyl and galactosyl units (6.6 and 7.6 Hz, respectively). Spectroscopic data agreed well with previously



Quercetin 3-*O*-β-D-xylopyranosyl(1‴→2″)-β-D-galactopyranoside (**1**)



(-)-Lyoniresinol 3a-*O*-β-D-glucopyranoside (**2**)



(-)-Lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**)

Fig. 1. Compounds isolated from Devil tree leaves.

Table 2
$^{13}C$ (DMSO-$d_6$) and $^{1}H$ (MeOH-$d_4$) NMR spectroscopic data of compound **1**

| Position | $\delta$ ($^{13}C$) | $\delta$ ($^{1}H$) | Coupling constant, $J$ (Hz) |
|---|---|---|---|
| 6 | 98.3 | 6.37 | |
| 8 | 79.7 | 6.18 | |
| 2′ | 115.3 | 7.71 | d, 2.1 |
| 5′ | 115.1 | 6.86 | d, 8.5 |
| 6′ | 122.1 | 6.50 | dd, 8.4/ 2.1 |
| 1″ | 98.3 | 5.40 | d, 7.6 |
| 2″ | 76.0 | 4.02 | dd, 9.5/ 7.7 |
| 3″ | 73.6 | 3.72 | dd, 9.46/ 3.41 |
| 4″ | 67.7 | 3.83 | d, 3.4 |
| 5″ | 73.8 | 3.46 | m |
| 6″ | 59.9 | $H_A$ − 3.62 | dd, 11.3/5.9 |
| | | $H_B$ − 3.55 | dd, 11.2/6.3 |
| 1‴ | 104.5 | 4.76 | d, 6.6 |
| 2‴ | 76.0 | 3.37 | m |
| 3‴ | 75.8 | 3.40 | m |
| 4‴ | 69.3 | 3.50 | m |
| 5‴ | 65.5 | $H_A$-3.94 | dd, 11.6/4.9 |
| | | $H_B$-3.24 | dd, 11.6/9.4 |

reported data (Grayer et al., 2002; Larsen, Nielsen, & Sorensen, 1982) for quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-D-galactopyranoside. This compound has been identified in many plant species, including *Armoracia rusticama* (Larsen et al., 1982), *Saxifraga stellaris* (Chevalley, Marston, & Hostettmann, 1998), *Trifolium repens* L. (Hofmann et al., 2000) and *Ocimum lamiifolium* (Grayer et al., 2002). However, there has been no report on its α-glucosidase inhibitory activity.

Compound **2** afforded a $[M-H]^-$ ion at *m*/*z* 581 in the FAB mass spectrum and a high-resolution analysis disclosed its molecular formula to be $C_{28}H_{38}O_{13}$. The $^{1}H$ and $^{13}C$ NMR data of this compound (Table 3) indicated a lignan glucoside structure with two 4-hydroxy-3,5-dimethoxybenzene rings and were in good agreement with those of (−)-lyoniresinol 3-*O*-β-D-glucopyranoside, which was obtained from *Stemmadenia minima* (Achenbach, Lowel, Waibel, Gupta, & Solis, 1992) and *Tabernaemontana cymosa* (Achenbach, Benirschke, & Torrenegra, 1997).

Compound **3** afforded a $[M-H]^-$ ion at *m*/*z* 581 in the FAB mass spectrum and a high-resolution analysis disclosed its molecular formula to be $C_{28}H_{38}O_{13}$. The $^{1}H$ and $^{13}C$ NMR data (Table 4) were quite similar to those of **2** and were in accordance with published data for (+)-lyoniresinol 3-*O*-β-D-glucopyranoside (Achenbach et al., 1997; Dada, Corbani, Manitto, Speranza, & Lunazzi, 1989), corresponding to an enantiomeric glucoside of **2**.

In the rat intestinal sucrase/maltase inhibitory activity assay (Table 5), among these three isolated compounds, compound **2** exhibited the highest inhibitory activities against both sucrase and maltase, with $IC_{50}$ value of 1.95 and 1.43 mM, respectively, whereas, compound **1** exhibited the high inhibitory activity against only maltase, i.e. an $IC_{50}$ value of 1.96 mM. Interestingly, compound **3**, which carries an optical antipode as an aglycone compared to compound **2**, showed far lower inhibition against sucrase

Table 3
$^{13}C$ and $^{1}H$ NMR spectroscopic data of compound **2** (MeOH-$d_4$)

| Position | $\delta$ ($^{13}C$) | $\delta$ ($^{1}H$) | Coupling constant, $J$ (Hz) |
|---|---|---|---|
| 1 | 33.8 | $H_A$, $H_B$ − 2.66 | m |
| 2 | 41.3 | 1.67 | m |
| 2a | 66.2 | 3.61 | d, 5.2 |
| 3 | 46.6 | 2.12 | m |
| 3a | 71.9 | $H_A$ − 3.59 | m |
| | | $H_B$ − 3.88 | m |
| 4 | 43.2 | 4.22 | d, 6.5 |
| 5 | 147.5 | | |
| 6 | 138.9 | | |
| 7 | 148.7 | | |
| 8 | 107.8 | 6.57 | |
| 9 | 130.2 | | |
| 10 | 126.2 | | |
| 1′ | 139.5 | | |
| 2′,6′ | 107.1 | 6.40 | |
| 3′ | 149.0 | | |
| 4′ | 134.6 | | |
| 5′ | 149.0 | | |
| 1″ | 104.3 | 4.12 | d, 7.8 |
| 2″ | 75.1 | 3.19 | m |
| 3″ | 78.2 | 3.30 | m |
| 4″ | 71.6 | 3.28 | m |
| 5″ | 78.0 | 3.14 | m |
| 6″ | 62.7 | $H_A$ − 3.68 | dd, 11.9/5.4 |
| | | $H_B$ − 3.82 | m |
| OMe-5 | 60.1 | 3.31 | |
| OMe-7 | 56.6 | 3.84 | |
| OMe-3′,5′ | 56.9 | 3.74 | |

Table 4
$^{13}C$ (MeOH-$d_4$) and $^{1}H$ NMR (pyridine-$d_5$) spectroscopic data of compound **3**

| Position | $\delta$ ($^{13}C$) | $\delta$ ($^{1}H$) | Coupling constant, $J$ (Hz) |
|---|---|---|---|
| 1 | 33.8 | $H_A$ − 3.08 | dd, 15.0/4.2 |
| | | $H_B$ − 3.18 | dd, 14./11.8 |
| 2 | 40.6 | 2.13 | m |
| 2a | 66.2 | | |
| 3 | 46.7 | 2.73 | m |
| 3a | 71.7 | | |
| 4 | 43.8 | 5.15 | d, 6.0 |
| 5 | 147.6 | | |
| 6 | 138.9 | | |
| 7 | 148.6 | | |
| 8 | 107.8 | 6.73 | |
| 9 | 130.2 | | |
| 10 | 126.4 | | |
| 1′ | 139.4 | | |
| 2′,6′ | 106.9 | 7.05 | |
| 3′ | 149.0 | | |
| 4′ | 134.5 | | |
| 5′ | 149.0 | | |
| 1″ | 104.8 | 4.24 | d, 8.0 |
| 2″ | 75.2 | | |
| 3″ | 78.2 | | |
| 4″ | 71.4 | | |
| 5″ | 78.0 | | |
| 6″ | 62.8 | | |
| OMe-5 | 60.2 | 3.76 | |
| OMe-7 | 56.6 | 3.74 | |
| OMe-3′,5′ | 56.9 | 3.69 | |

Table 5
Rat intestinal sucrase and maltase inhibitory activities of **1**–**3**

| Compound | $IC_{50}$ value | |
|---|---|---|
| | Sucrase (mM) | Maltase (mM) |
| Quercetin 3-*O*-β-D-xylopyranosyl(1‴ → 2″)-β-D-galactopyranoside (**1**) | 17.2 | 1.96 |
| (−)-Lyoniresinol 3a-*O*-β-D-glucopyranoside (**2**) | 1.95 | 1.43 |
| (+)-Lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**) | >10 | >10 |

and maltase. Although, compared with commercial inhibitors, compounds **1** and **2** exhibited lower inhibitory activities against these carbohydrate-hydrolyzing enzymes, it is hoped that these preliminary observations will provide the basis for further examination of the suitability of *Alstonia scholaris* as a medicinal supplement that contributes toward the treatment and prevention of diabetes. Besides, compounds **2** and **3** have been reported to exhibit scavenging activity against DPPH radicals and inhibitory activity against lipid peroxidation (Min et al., 2003), which further increases the potential efficacy of these traditional herbal medicines. However, further studies are warranted for more extensive biological evaluations to elucidate plausible mechanisms of these inhibitions, before bringing them into commercial use.

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