Anti-α-glucosidase Alkaloids from the Root Bark of *Zanthoxylum rhetsa*

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ABSTRACT

The hexane and ethyl acetate extracts from the root bark of *Zanthoxylum rhetsa* were investigated. Eight known alkaloids, dihydroavicine (**1**), dihydronitidine (**2**), paraensidimerine C (**3**), *N*-methylflindersine (**4**), 8-acetonyldihydroavicine (**5**), paraensidimerine A (**6**), 8-acetonyldihydronitidine (**7**), and skimmianine (**8**) were isolated. Among them, alkaloids **3**-**6** were obtained for the first time from this plant. Compound **5** exhibited the highest α-glucosidase inhibitory activity against maltase and sucrase with IC_{50} values of 1.72 and 0.45 mM, respectively. Subsequent investigation on mechanism underlying inhibitory effect of **5** indicated that it blocked maltase and sucrase function by mixed inhibition through competitive and noncompetitive manners. The SAR information obtained from the series of benzophenanthridine alkaloids showed that the presence of the methylenedioxy moiety on C-2 and C-3 and acetonyl group of compound **5** are essential for α-glucosidase inhibitory activity.

Keywords: *Anti-α-glucosidase, Alkaloids, Zanthoxylum rhetsa*

INTRODUCTION

Diabetes mellitus (DM), a multifactorial disease affecting over 300 million people in both developed and developing countries, is characterized by hyperglycemia resulting from an absolute or relative of insulin secretion, low insulin sensitivity, or both (Funke & Melzig., 2006). This disease still is a major cause of microvascular pathologies, especially in the eye, kidney, and peripheral nerve (Oyedemi *et al*., 2009). One effective strategy to manage DM is to decrease postpandrial hyperglycemia by retarding the glucose absorbtion. This is achieved by the inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase in the digestive organs (Kumar *et al.*, 2013). Currently, α-glucosidase inhibitors are becoming a new class of anti-diabetic drugs. Several α-glucosidase inhibitors were obtained from natural sources such as acarbose and voglibose and have been used effectively in the clinical treatment of DM (Van de Laar *et al*., 2009).

Article history:
Received 18 June 2019; Received in revised from 19 October 2020;
Accepted 03 December 2020; Available online 21 June 2021

All of them contain sugar moieties and their synthesis requires the complicated procedures. Moreover, clinically they have been associated with serious side effects to gastrointestinal symptoms (Zaklos *et al.*, 2015). Therefore, the discovery of highly effective α-glucosidase inhibitors without side reactions from natural sources will be very helpful in the development of new anti-diabetic drugs.

Zanthoxylum rhetsa belonging to Rutaceae family, is a medium-sized tree that grows in the tropical counties such as Bangaladesh, India, Myanmar, Malaysia and Thailand. For Thailand, the plant was largely distributed in Northern provinces and also found in the Western area, especially in Phetchaburi province. The fruits and stem bark of this plant are used traditionally as an astringent, a stimulant, as well as heart troubles, asthma, toothache and bronchitis (Van-Valkenburg *et al.*, 2001). Essential oils from fruit shows significant local anesthetic and anti-inflammatory activities (Pai *et al.*, 2002). A number of constituents isolated from *Z. rhetsa* include alkaloids, 8-methoxy-*N*-methylfindersine (Ruangrungsi *et al.*, 1981), dihydrochelerythrin (Ahsan *et al.*, 2001), dihydroavicine (Joshi *et al.*, 1991), dictamine (Chatterjee *et al.*, 1959), zanthobungeanine (Gopinath *et al.*, 1959), chelerythrine (Banerjee *et al.*, 1989), rutaecarpine, rhetsine, rhetine, chelerythrine and hydroxyevodiamine from stem bark (Ahsan *et al.*, 2014), arborine and dictamine from fruits (Hanaoka *et al.*, 1987) and rutaecarpine from seeds (Rong *et al.*, 1994). In addition, reports have identified the dimeric alkaloids, including chelerybulgarine, simulanoquinoline, 2'-episimulanoquinoline, 2,11-didemethoxyvepridimerine B and rhetsidimerine, in the root bark of *Z. rhetsa* (Nissanka *et al.*, 2001).

We report herein the isolation and evaluation for α-glucosidase inhibitory activity of four known benzophenanthridine alkaloids; dihydroavicine (**1**), dihydronitidine (**2**), 8-acetonyldihydroavicine (**5**), and 8-acetonyldihydronitidine (**7**), three quinolinone alkaloids; *N*-methylflindersine (**4**), paraensidimerine C (**3**), and paraensidimerin A (**6**), and one furoquinoline alkaloid; skimmianine (**8**). To the best of our knowledge, this is the first report on the isolation of **3**-**6** from *Z. rhetsa* and the evaluation of α-glucosidase inhibitory activity of some benzophenanthridine-type and *bis*-quinolinone type alkaloids. Additionally, compound **5** was reported for the first time in this analysis with respect to its kinetics in the α-glucosidase inhibitory mechanism.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

^{1}H- and ^{13}C-NMR spectra were carried out using a Bruker AVANCE 300 FT-NMR spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). Electrospray (ES) mass spectra were obtained using a Finnigan Polaris Q mass spectrometer. The $[\alpha]_D$ values were determined with a Bellingham & Stanly ADP440 polarimeter. IR spectra

were recorded on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. Melting points were determined on a Griffin melting point apparatus and are uncorrected. Column chromatography was carried out using Merck silica gel 60 (<0.063 mm). The progress of the fractions combining were monitored using a TLC sheet pre-coated with UV fluorescent Merck silica gel 60 F_{254} and were visualized under UV light and by spraying with an anisaldehyde–H_2SO_4 reagent followed by heating. Alkaloid fractions were detected with dropping of Dragendorff's reagent.

PLANT MATERIAL

The *Z. rhetsa* root was collected from Phetchaburi, Thailand, in August, 2016. The plant was identified in comparison with an authentic herbarium specimen of this species at The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Chatuchak, Bangkok, Thailand. A voucher specimen (PCS-Z-002) was deposited in the Research Unit of Natural Products Chemistry, Division of Chemistry, Faculty of Science and Technology, Phetchaburi Rajabhat University. The root bark was separated and cleaned before being air-dried and ground into a coarse powder, yielding 3.5 kg.

EXTRACTION AND ISOLATION

The powdered root bark (3.5 kg) was extracted with hexane, EtOAc and MeOH by maceration at room temperature. The macerates were concentrated under reduced pressure to yield crude hexane (43.61 g), EtOAc (239.11 g) and MeOH (527.53 g) extracts. All extracts were screened for α-glucosidase inhibitory activity, with the hexane and EtOAc extracts exhibiting the moderate to high activity. From Dragendorff's test, the crude hexane and EtOAc extracts possess the highest amount of alkaloids, therefore an extraction of the alkaloids was then performed using the modified method of Yang *et al* (2012). The alkaloid mixture was obtained from hexane (20.79 g) and EtOAc (26.33 g) extracts in 1.37 (PCS-Hex) and 4.22 g (PCS-EtOAc), respectively. The extract PCS-Hex (1.05 g) was subjected to column chromatography using a gradient system of hexane, hexane-acetone, acetone, acetone-methanol, and methanol with increasing amounts of the more polar solvent. The following compounds were isolated: dihidroavicine (**1**, 27.0 mg) from the fraction eluting with 2% v/v acetone in hexane; dihydronitidine (**2**, 18.3 mg) from the fraction eluting with 4% v/v acetone in hexane and paraensidimerine C (**3**, 53.2 mg) from the fraction eluting with 10% v/v acetone in hexane.

The extract PCS-EtOAc (3.04 g) was also isolated using the same purified procedure of the hexane extract. Five alkaloids were obtained including *N*-methylflindersine (**4**, 73.8 mg) from eluting with 4% v/v acetone in hexane, 8-acetonyldihydroavicine (**5**, 31.1 mg) from eluting with 6 % v/v acetone in hexane, paraensidimerine A (**6**, 21.5 mg) from eluting with 10 % v/v acetone in hexane, 8-

acetonyldihydronitidine (**7**, 125.6 mg) from eluting with 14 % v/v acetone in hexane and skimmianine (**8**, 44.0 mg) from eluting with 18% v/v acetone in hexane.

Dihydroavicine (**1**, 27.0 mg): white solid; m.p. 180-183 °C (lit. m.p. 212-213 °C; Joshi *et al.*, 1991); R_f = 0.61 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2916, 1637, 1473, 1464, 1379, 1321, 1188, 1043, 879, 719; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 2.56 (3H, s, NC*H*$_3$), 4.07 2H, s, H-8), 5.97 (2H, s, C2-OC*H*$_2$O-C3), 6.03 (2H, s, C12-OC*H*$_2$O-C13), 6.75 (1H, s, H-1), 7.09 (1H, s, H-11), 7.26 (1H, s, H-4), 7.46 (1H, d, J= 8.5Hz, H-10), 7.60 (1H, d, J= 8.5Hz, H-9), 7.61 (1H, s, H-14); ESI-MS: *m/z* (%) 332 $[M-H]^-$(100).

Dihydronitidine (**2**, 18.3 mg): white solid; m.p. 169-172 °C (lit. 192-195 °C; Moura *et al*., 1997); R_f = 0.55 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2983, 1622, 1500, 1458, 1388, 1321, 1116, 750; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 2.60 (3H, s, NC*H*$_3$), 3.95 (3H, s, 2-OC*H*$_3$), 4.00 (3H, s, 3-OC*H*$_3$), 4.10 (2H, s, H-8), 6.05 (2H, s, C12-OC*H*$_2$O-C13), 6.80 (1H, s, H-1), 7.12 (1H, s, H-4), 7.31 (1H, s, H-11), 7.50 (1H, d, J= 8.5 Hz, H-10), 7.66 (1H, s, H-14), 7.70 (1H, d, J= 8.5 Hz, H-9); ESI-MS: *m/z* (%) 348 $[M-H]^-$(100).

Paraensidimerine C (**3**, 53.2 mg): white solid; m.p. 283-285 ºC (lit. m.p. 280-281 ºC; Jurd *et al.*, 2007); R_f = 0.45 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2978, 2941, 2362, 1626, 1593, 1464, 1390, 1315, 1176, 1114, 1101, 881, 767; Optically inactive (c 0.081, $CHCl_3$; lit. Optically inactive; Jurd *et al*., 1982); ^1H NMR ($CDCl_3$, 300 MHz): δ_H 1.33 (3H, s, 6-C*H*$_3$), 1.46 (1H, dd, J= 4.3, 14.6 Hz, H-19$_{ax}$), 1.50 (1H, m, H-16$_{eq}$), 1.64 (1H, dd, J= 3.4, 12.4 Hz, H-6a), 1.72 (3H, s, 15-C*H*$_3$), 1.89 (3H, s, 6-C*H*$_3$), 2.17 (1H, dd, J= 3.4, 13.5 Hz, H-16$_{ax}$), 2.66 (1H, ddd, 2.5, 3.4, 12.4 Hz, H-7), 3.27 (1H, dd, J= 3.4, 12.4 Hz, H-16a), 3.64 (3H, s, 18-NC*H*$_3$), 3.70 (3H, s, 9-NC*H*$_3$), 3.88 (1H, dd, 4.3, 14.6 Hz, H-19$_{eq}$), 7.18 (1H, m, H-3), 7.22 (1H, m, H-12), 7.26 (1H, m, H-10), 7.28 (1H, m, H-1), 7.50 (1H, m, H-11), 7.52 (1H, *br* t, J= 6.9 Hz, H-2), 7.94 (1H, *br* t, J= 8.4 Hz, H-13), 7.95 (1H, *br* t, J= 8.4 Hz, H-4); ESI-MS: *m/z* (%) 483 $[M+H]^+$ (59).

N-Methylflindersine (**4**, 73.8 mg): white solid; m.p. 78-80 °C (lit. 83-85 °C; Chen *et al*., 2007); R_f = 0.42 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2978, 1631, 1593, 1504, 1464, 1388, 1315, 1176, 1101, 1012, 767; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 1.51 (6H, s, 2xC*H*$_3$), 3.69 (3H, s, NC*H*$_3$), 5.53 (1H, d, J= 9.9 Hz, H-2'), 6.75 (1H, d, J= 9.9 Hz, H-1'), 7.23 (1H, dd, J= 7.1, 7.9 Hz, H-6), 7.31 (1H, d, J= 8.5, H-8), 7.54 (1H, ddd, J= 1.3, 7.1, 8.5 Hz, H-7), 7.96 (1H, dd, J= 1.3, 7.9 Hz, H-5); ESI-MS: *m/z* (%) 505 $[2M+Na]^+$(55).

8-Acetonyldihydroavicine (**5**, 31.1 mg): white solid; m.p. 187-189 °C (lit. 184-185 °C; Nissanka *et al*., 2001); R_f = 0.41 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2947, 2360, 1676, 1637, 1452, 1379, 1043, 879; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 1.96 (3H, s, CH_2COC*H*$_3$), 2.30 (1H, dd, J= 6.0, 15.9 Hz, H-8), 2.61 (3H, s, NC*H*$_3$), 2.66 (1H, dd, J= 8.7, 15.9 Hz, C*H*$_2$COCH$_3$), 4.52 (1H, dd, J= 6.0, 8.7 Hz, C*H*$_2$COCH$_3$), 5.98 (2H, d, J= 1.5 Hz, C2-OC*H*$_2$O-C3), 6.04 (2H, d, J= 1.5 Hz, C12-OC*H*$_2$O-C13), 6.81 (1H, s, H-1),

7.11 (1H, s, H-4), 7.29 (1H, s, H-14), 7.47 (1H, d, J= 8.7 Hz, H-10), 7.54 (1H, s, H-11), 7.62 (1H, d, J= 8.7 Hz, H-9); ESI-MS: *m/z* (%) 390 $[M+H]^+$ (100).

Paraensidimerine A (**6**, 21.5 mg): white solid; m.p. 308-312 ºC (lit. m.p. 311-312 ºC; Jurd *et al*., 1982); R_f = 0.37 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2998, 2964, 2838, 1653, 1492, 1466, 1443, 1364, 1190, 1076, 1038, 757; Optically inactive (c 0.058, $CHCl_3$; lit. Optically inactive, Jurd *et al*., 1982); ^1H NMR ($CDCl_3$, 300 MHz): δ_H 1.55 (3H, s, 6-C*H*$_3$), 1.73 (1H, m, H-19$_{eq}$), 1.78 (1H, m, H-16$_{ax}$), 1.86 (6H, s, 6-C*H*$_3$ and 15-C*H*$_3$), 2.22 (1H, *br* dd, J= 3.3, 6.3 Hz, H-6a), 2.24 (1H, m, H-19$_{ax}$), 3.03 (1H, dt, J= 6.0, 12.6 Hz, H-16a), 3.19 (1H, dd, J= 5.1, 11.2 Hz, H-16$_{eq}$), 3.62 (3H, s, 18-NC*H*$_3$), 3.68 (3H, s, 9-NC*H*$_3$), 7.24 (1H, m, H-12), 7.25 (3H, m, H-1, H-3 and H-10), 7.52 (1H, dd, J= 1.1, 6.9 Hz, H-2), 7.53 (1H, m, H-11), 7.97 (1H, dd, J= 1.0, 8.0 Hz, H-4), 8.07 (1H, dd, J=1.0, 8.0 Hz, H-13); ESI-MS: *m/z* (%) 505 $[M+Na]^+$ (100).

8-Acetonyldihydronitidine (**7**, 125.6 mg): colorless crystal; m.p. 164-166 °C (lit. Nissanka *et al*., 2001; 165-167 °C); R_f = 0.35 (hexane:acetone,70:30); IR (KBr) cm^{-1}: 1710, 1608, 1525, 1496, 1464, 1352, 1244, 1147, 1033, 860, 848, 802, 767; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 1.97 (3H, s, CH_2COC*H*$_3$), 2.32 (1H, dd, J= 5.6, 15.7 Hz, C*H*$_2$COCH$_3$), 2.61 (3H, s, NC*H*$_3$), 2.69 (1H, dd, J= 8.6, 15.7 Hz, C*H*$_2$COCH$_3$), 3.94 (3H, s, 3-OC*H*$_3$), 3.99 (3H, s, 2-OC*H*$_3$), 4.55 (1H, dd, J= 5.6, 8.6 Hz, H-8), 6.03 (2H, d, J= 1.2 Hz, C12-OC*H*$_2$O-C13), 6.85 (1H, s, H-1), 7.12 (1H, s, H-14), 7.32 (1H, d, J= 8.5 Hz, H-10), 7.51 (1H, s, H-4), 7.70 (1H, d, J= 8.5 Hz, H-9); ESI-MS: *m/z* (%) 428 $[M+Na]^+$ (100).

Skimmianine (**8**, 44.0 mg): white solid; m.p. 178-179 °C (lit. 140 °C; Moura *et al*., 1997; 179-181 °C; Ratheesh *et al*., 2013); R_f = 0.30 (hexane:acetone, 70:30); IR (KBr) cm^{-1}: 2960, 2362, 1608, 1527, 1496, 1465, 1396, 1352, 1244, 1147, 1041, 850, 773; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 4.03 (3H, s, 7-OC*H*$_3$), 4.12 (3H, s, 8-OC*H*$_3$), 4.42 (3H, s, 4-OC*H*$_3$), 7.03 (1H, d, J= 2.7 Hz, H-3'), 7.23 (1H, d, J= 9.4 Hz, H-6), 7.58(1H, d, J= 2.7 Hz, H-2'), 8.01 (1H, d, J= 9.4 Hz, H-5); ESI-MS: *m/z* (%) 260 $[M+H]^+$ (30), 541 $[2M+Na]^+$ (100).

α-GLUCOSIDASE INHIBITORY ACTIVITY

The inhibitory activity of the isolated alkaloids against α-glucosidase from rat intestines (as maltase and sucrase) was assessed based on *p*-nitrophenoxide colorimetric method (Barham & Trinder., 1972) at the Department of Chemistry, Faculty of Science, Chulalongkorn University. Maltase and sucrase were obtained from rat intestinal acetone powder (Sigma, St. Louis). Briefly, 1.0 g of the powder was homogenized with 30 mL of 0.9% w/v NaCl solution. The aliquot containing both maltase and sucrase was obtained by centrifugation (12,000 g) for 30 min. The test alkaloid (1 mg/mL, 10 μL) was pre-incubated with crude enzyme solution (as maltase, 20 μL; as sucrase, 20 μL, respectively) at 37 ºC for 10 min. The solution of substrates

in 0.1 M phosphate buffer (pH 6.9) including maltose 0.58 mM (20 μL) and sucrose 20 mM (20 μL) were therefore added to the reaction mixture and further incubated at 37 ^{o}C for 40 min. The mixture was then heated in an oven at 80 ^{o}C for 15 min to quench the reaction. The concentration of glucose released from the reaction mixture was monitored by the glucose oxidase method using a commercial glucose assay kit (SU-GLLQ2, Human). The inhibition of all tested alkaloids were reported as percentage inhibition by $[(A_0 - A_1)/A_0] \times 100$, where A_1 and A_0 are the absorbance with and without the alkaloids, respectively. The IC_{50} value was determined from a plot of percentage inhibition against alkaloid concentration and standard drug acarbose was used as a positive control (Rattanangkool *et al.*, 2013).

KINETIC STUDY OF α-GLUCOSIDASE INHIBITION

The selected highly active alkaloids were evaluated for their kinetics in inhibiting α-glucosidase activity. The type of inhibition of the active compound against α-glucosidase was determined by analyzing enzyme kinetic data using the aforementioned reactions. The activities of maltase and sucrase were maintained at 0.45 and 0.09 U/mg protein, respectively, in the presence of a various concentrations of the inhibitors at 0 to 2.57 mM containing a series of substrate solutions including maltose ranging from 1.0 to 10.0 mM and sucrose ranging from 5.0 to 100 mM. A series of V_{max} and K_m values were obtained from Y intercepts and calculated by slope x V_{max}, respectively (Rattanangkool *et al.*, 2013).

RESULTS AND DISCUSSION

The structures of isolated natural known alkaloids were identified mainly by mass and NMR spectroscopic methods (1D- and 2D-NMR) and by comparison of their NMR data and physical properties with those reported in the literature. Thus, alkaloids **1**-**8** were determined to be: dihydroavicine (**1**, Joshi *et al.*, 1991), dihydronitidine (**2**, Hanaoka *et al.*, 1987), paraensidimerine C (**3**, Jurd *et al.*, 1982; Chen *et al.*, 2007), *N*-methylflindersine (**4**, Rong *et al.*, 1994), 8-acetonyldihydroavicine (**5**, Nissanka *et al.*, 2001), paraensidimerine A (**6**, Jurd *et al.*, 1982), 8-acetonyldihydronitidine (**7,** Nissanka *et al.*, 2001), and skimmianine (**8,** Ratheesh *et al.*, 2013), respectively (Figure 1). Interestingly, a search of the relevant literature revealed that benzophenanthidine alkaloid **5** and quinolinone alkaloids **3**-**4** and **6** were obtained for the first time from this plant and some of them were reported as a potent cytotoxic agents (Sudta *et al.*, 2016).

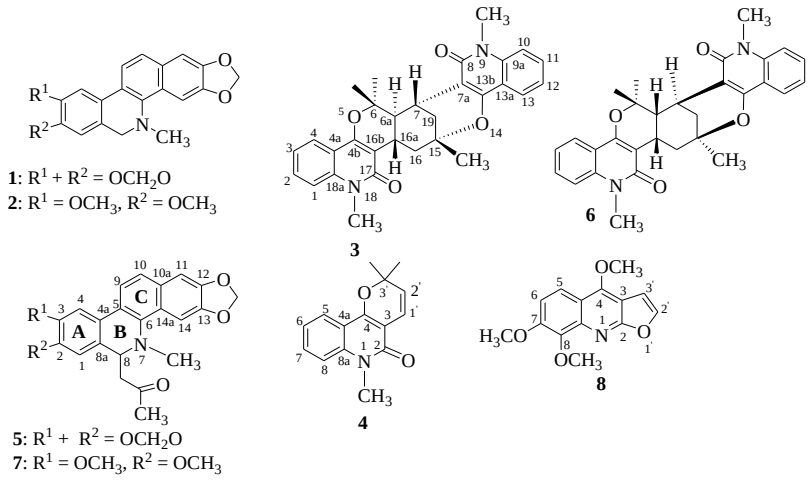

Figure 1: Structures of the isolated alkaloids **1**-**8**.

α-GLUCOSIDASE INHIBITORY ACTIVITY OF THE ISOLATED ALKALOIDS (**1**-**8**)

All known isolated alkaloids were subjected to evaluation for their *α*-glucosidase inhibitory effect using enzymes from the small intestine of rats (maltase and sucrase). The commercial antidiabetic drug acarbose was used as the standard compound, and the IC_{50} values are reported in Table 1. The benzophenanthridine alkaloids **1**-**2**, **5** and **7** displayed inhibitory effects in the range of 0.45-10.08 mM. Notably, the presence of acetonyl moiety at C-8 in **5** and **7** exhibited an *α*-glucosidase inhibitory activity higher than that of parents **1** and **2**, respectively, implying that the acetonyl moiety is more crucial for □-glucosidase inhibitory activity. Moreover, the decrease in activity of **2** and **7** when compared with **1** and **5** indicates that the benzophenanthridine core structure substituted with one extra methylenedioxy group at C2-C3 is more important for this kind of activity than having two methoxyl groups. Among quinolinone alkaloids, compound **4** exhibited an *α*-glucosidase inhibitory activity with the IC_{50} values of 3.40 and 2.44 mM for inhibition of maltase and sucrase, respectively. Its dimer **3** displayed poor activity whereas dimer **6** was not active, suggesting that the free pyran moiety in **4** is required for this activity, and the fusion of such pyran with cyclohexane in **3** and **6** attenuated the activity. Furoquinoline alkaloid skimmianine (**8**) showed no *α*-glucosidase inhibitory effect. The observed results indicated that alkaloid **5** was the most potent for inhibiting both maltase and sucrase.

Table 1. α-Glucosidase inhibitory effect of alkaloids **1**-**8**

Compound	IC_{50}[a] (mM)		Compound	IC_{50}[a] (mM)	
	Maltase[b]	Sucrase[b]		Maltase[b]	Sucrase[b]
1	3.07	1.53	**6**	NI[c]	NI[c]
2	10.08	4.13	**7**	2.67	1.87
3	26.12	6.12	**8**	NI[c]	NI[c]
4	3.40	2.44	Acarbose	0.02	0.05
5	1.72	0.45			

[a] The data shown as mean of duplicate experiments.

[b] α-Glucosidase was obtained from rat small intestine.

[c] NI, no inhibition, inhibitory effect less than 30% at a 10 mg/mL.

THE MECHANISM UNDERLYING INHIBITORY EFFECT OF COMPOUND 5 AGAINST RAT INTESTINAL α-GLUCOSIDASE

The highly active alkaloid **5** was determined further with respect to how it interacted with rat intestinal maltase and sucrase, and the inhibition mode of **5** was analyzed by a kinetic study. The Lineweaver-Burk plot of **5** against maltase (Figure 2a) showed a series of straight lines; all of which intersected in the second quadrant. Kinetic analysis showed that V_{max} decreased with elevated K_m in the presence of increasing concentrations of **5**. This behavior suggested that compound **5** inhibited maltase in a mixed-type manner comprising two different pathways, competitive and noncompetitive. The result observed was elaborated by a simultaneous formation of enzyme-inhibitor (EI) and enzyme-substrate-inhibitor (ESI) complexes in competitive and noncompetitive manners, respectively.

We further investigated the pathway in which **5** was preferentially preceded by determining dissociation constants of EI (K_i) and ESI (K'_i) complexes (Table 2). Apparently, the secondary plots (Figures 2b and 2c) demonstrated K_i and K'_i values of 6.9 and 31.3 mM, respectively, thus indicating that **5** was predominantly bound to maltase (EI) rather than the formed ESI complex. The inhibitory mechanism of **5** against sucrase (Figure 3) was also examined using the above methodology. Apparently, **5** inhibited sucrase via both competitive and noncompetitive manners (mixed-type inhibition) and all kinetic factors are summarized in Table 2.

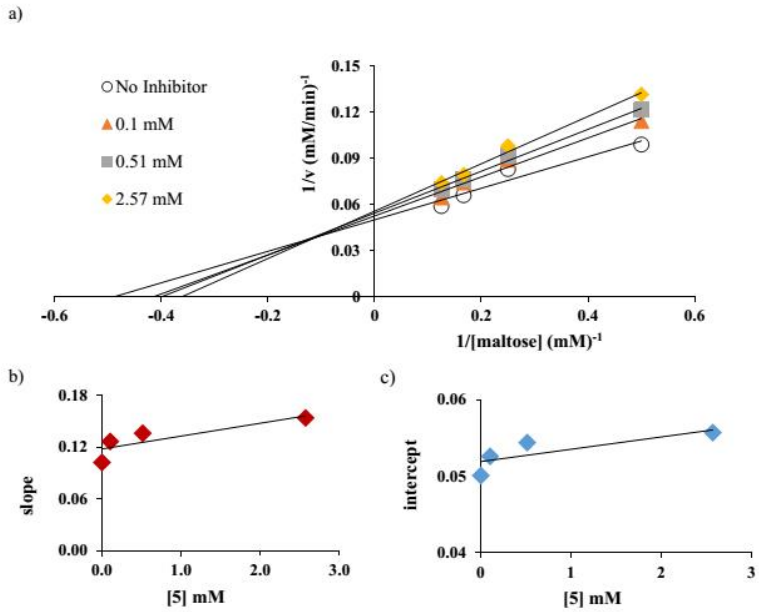

Figure 2: Lineweaver-Burk plots for inhibitory activity of **5** against rat intestinal maltase (a). Secondary replots of slope versus [**5**] (b) and (c) intercept versus [**5**] from a primary Lineweaver-Burk plot for the determination of K_i and K'_i, respectively.

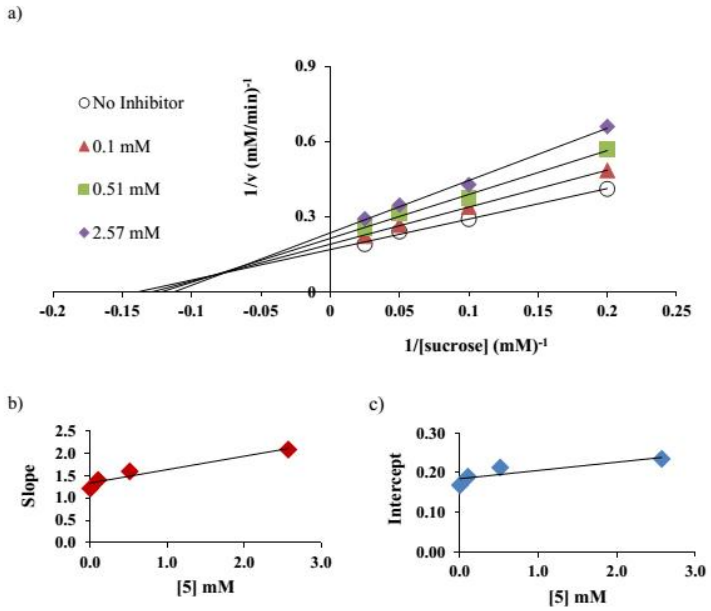

Figure 3: Lineweaver-Burk plots for inhibitory activity of **5** against rat intestinal sucrase (a). Secondary replots of slope versus [**5**] (b) and intercept versus [**5**] (c) from a primary Lineweaver-Burk plot for the determination of K_i and K'_i, respectively.

Table 2. Kinetic data of α-Glucosidase inhibition of alkaloids **5**

Maltase			Sucrase		
K_i (mM)	K'_i (mM)	Inhibition type	K_i (mM)	K'_i (mM)	Inhibition type
6.9	31.3	Mixed-inhibition	4.0	8.1	Mixed-inhibition

CONCLUSION

In summary, eight known alkaloids, dihydroavicine (**1**), dihydronitidine (**2**), paraensidimerine C (**3**), *N*-methylflindersine (**4**), 8-acetonyldihydroavicine (**5**), paraensidimerine A (**6**), 8-acetonyldihydronitidine (**7**), and skimmianine (**8**) were isolated from the root bark of *Z. rhetsa*. Compounds **3**-**6** were isolated for the first time from this plant. Of the isolated alkaloids, benzophenantridine alkaloid **5** showed the most potent α-glucosidase inhibitory activity. In addition, the mechanism underlying the inhibitory effect of **5** against maltase and sucrase was proved to be a mixed-type inhibition. From some of the knowledge regarding structure-activity relationship (SAR) of a series of benzophenanthridine-type alkaloids (**1**-**2**, **5**, and **7**) the presence of methylenedioxy group on C-2 and C-3 and acetonyl moiety at C-8 of compound **5** are crucial for α-glucosidase inhibitory activity. Taken together, the insight from the present study may benefit the rationale for seeking potentially new antidiabetic drugs without sugar moiety from the structural modification of benzophenanthridine-type alkaloids.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Dr. Apichart Susamrarn for the mass spectra, Assoc. Prof. Dr. Sunit Suksamrarn for the NMR data, as well as Assoc. Prof. Dr. Preecha Phuwapraisirisan for the α-glucosidase inhibitory activity evaluation. We are indebted to the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation for the determination of the herbarium voucher and the Department of Chemistry, Faculty of Science, Silpakorn University, for the optical rotation data.

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