Bioorganic & Medicinal Chemistry Letters 18 (2008) 4956–4958

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Phenylethyl cinnamides: A new series of α-glucosidase inhibitors from the leaves of *Aegle marmelos*

Preecha Phuwapraisirisan [a,*], Thanchanok Puksasook [a,b], Jonkolnee Jong-aramruang [c], Udom Kokpol [a]

[a] *Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand*
[b] *Program of Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[c] *Department of Chemistry, Faculty of Science, Burapha University, Chonburi 20231, Thailand*

ARTICLE INFO

Article history:
Received 26 June 2008
Revised 8 August 2008
Accepted 11 August 2008
Available online 14 August 2008

Keywords:
Phenylethyl cinnamide
Aegle marmelos
Anhydromarmeline
Aegelinosides A
Aegelinosides B
α-Glucosidase
Diabetes mellitus

ABSTRACT

A series of phenylethyl cinnamides, which included new compounds named anhydromarmeline (**1**), aegelinosides A (**7**) and B (**8**), were isolated from *Aegle marmelos* leaves as α-glucosidase inhibitors. The structures of new compounds were characterized by spectroscopic data and chemical degradation. Of compounds isolated, anhydroaegeline (**2**) revealed the most potent inhibitory effect against α-glucosidase with IC_{50} value of 35.8 μM. The present result also supports ethnopharmacological use of *A. marmelos* as a remedy for diabetes mellitus.

Type 2 diabetes mellitus affects approximately 215 million people worldwide. It is currently clear that aggressive control of hyperglycemia in patients with type 2 diabetes can attenuate the development of chronic complications such as retinopathy and nephropathy.[1] To date, therapy for type 2 diabetes relies mainly on several approaches intended to suppress the hyperglycemia, which include reducing gut glucose absorption.

Therefore inhibition of α-glucosidase, an enzyme catalyzing the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates, is a method of choice to control elevated glucose level in blood. Although novel generations of α-glucosidase inhibitors have been consistently synthesized, those having multiple actions are greatly required. Recent investigations have reported the exceptional actions of α-glucosidase inhibitors from natural sources.[2] Prominent examples included aegeline, a hydroxyl amide alkaloid from *Aegle marmelos* leaves, which suppressed both blood glucose and plasma triglyceride levels.[3]

Aegle marmelos is typically known as 'bael' in India. It belongs to the family of Rutaceae, which is widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus. Despite several reports of its antihyperglycemic activity,[4] the active principles have not been identified. In this Letter, we describe the isolation, characterization and glucosidase inhibitory effect of phenylethyl cinnamides; three of which named anhydromarmeline (**1**), aegelinosides A (**7**) and B (**8**), are newly discovered (see Fig. 1).

The air-dried leaves of *A. marmelos*, collected in Nakornpatom in April 2006, were extracted with CH_2Cl_2 and 7:3 MeOH-H_2O using

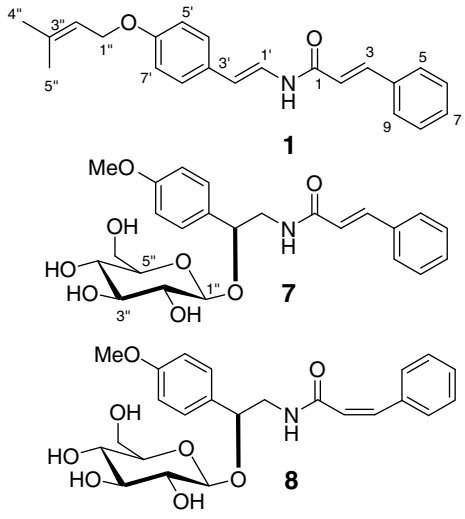
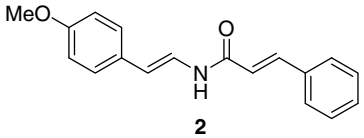


Figure 1. New metabolites isolated from *A. marmelos*.

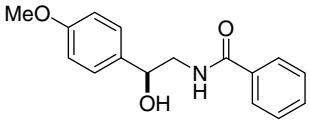
* Corresponding author. Tel.: +662 218 7624; fax: +662 218 7598.
E-mail address: preecha.p@chula.ac.th (P. Phuwapraisirisan).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.024

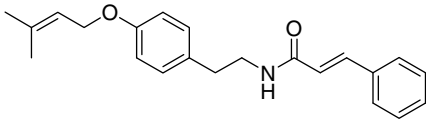
Soxhlet extractor. The CH_2Cl_2 extract was dissolved in 4:1 MeOH–H_2O and partitioned with hexane. The methanolic layer was chromatographed on silica gel using stepwise MeOH–CH_2Cl_2 (0:1, 5:95, 10:90, 20:80 and 50:50), yielding five major fractions. The combined fractions 1 and 2 were further purified on Sephadex LH-20 (1:9 MeOH–CH_2Cl_2) and silica gel (5:95 MeOH–CH_2Cl_2) to afford anhydromarmeline[5] (**1**, 14 mg), anhydroaegeline (**2**, 50 mg), and (−)-tembamide (**3**, 8 mg). In addition, dehydromarmeline (**4**, 11 mg), (−)-aegeline (**5**, 65 mg), and (−)-*O*-methylether aegeline (**6**, 20 mg) were also obtained from fraction 3, on purification using silica gel (10:90 MeOH–CH_2Cl_2). The 7:3 MeOH–H_2O extract was loaded onto Diaion HP-20 and excessively eluted with H_2O, MeOH and acetone. The MeOH fraction was separated by VCC (stepwise 5:95, 10:90, 15:85, 50:50, 70:30 and 100:0 MeOH–CH_2Cl_2) The combined fractions eluted with 15:85 and 50:50 MeOH–CH_2Cl_2 were subsequently purified by Sephadex LH-20 (3:7 MeOH–CH_2Cl_2) followed by RPHPLC (ODS, 65:35 MeOH–H_2O, UV 254 nm) to furnished aegelinosides A (**7**, 20 mg, t_R 32.4 min) and B (**8**, 10 mg, t_R 27.1 min).



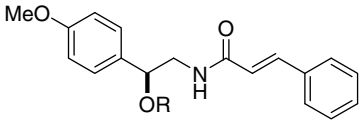
2



3



4



5 R = H

6 R = Me

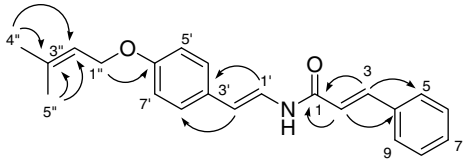


Figure 2. Selected HMBC correlations of **1**.

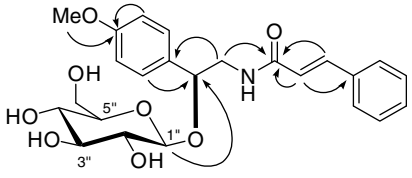


Figure 3. Selected HMBC correlations of **7**.

Table 1
^1H and ^{13}C NMR data for aegelinosides A (**7**, CD_3OD) and B (**8**, acetone-d_6)

Position	Aegelinoside A (**7**)		Aegelinoside B (**8**)	
	^{13}C	^1H	^{13}C	^1H
1	166.5		166.4	
2	120.5	6.65, d, 15.6	124.1	6.05, d, 12.8
3	140.5	7.50, d, 15.6	136.4	6.68, d, 12.8
4	135.4		135.9	
5, 9	127.5	7.57, m	129.8	7.68, m
6, 8	128.1	7.35, m	128.0	7.31, m
7	129.3	7.35, m	129.4	7.31, m
1′	46.0	3.57, d, 6.4	45.4	3.56, m
				3.38, m
2′	77.0	5.00, t, 6.4	77.9	4.89, dd, 7.6, 4.4
3′	130.0		131.3	
4′, 8′	128.0	7.35, d, 8.8	128.2	7.37, d, 8.6
5′, 7′	113.7	6.92, d, 8.8	113.5	6.89, d, 8.6
6′	159.6		159.4	
1″	99.5	4.12, d, 7.2	100.5	4.18, d, 7.2
2″	73.7	3.28, m	73.7	3.24, m
3″	76.3	3.23, m	76.8	3.27, m
4″	70.4	3.26, m	70.5	3.32, m
5″	76.6	3.09, m	76.3	3.18, m
6″	61.5	3.87, dd, 11.6, 2.0	62.0	3.65, m
		3.67, dd, 11.6. 5.6		3.84, m
OMe	54.1	3.78, s	54.4	3.78, s

Anhydromarmeline (**1**)[6] was obtained as yellow needle. The molecular formula was established as $C_{22}H_{23}NO_2$ by HRESIMS. The ^1H NMR spectrum displayed most signals in aromatic region (6.1–7.7), in addition to the upfield resonances that are ascribable to oxygenated prenyl moiety [δ_H 5.49 (m, 1H), 4.50 (d, J = 6.8 Hz), 1.80 and 1.74 (s, each 3H)].[7] The ^{13}C NMR showed 22 signals, five of which were quarternary carbons which included resonance of

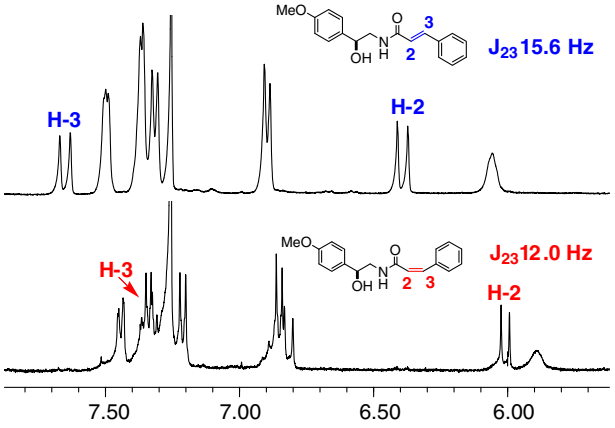


Figure 4. Partial ^1H NMR spectra of aglycones **5** (top) and **8a** (bottom) obtained from hydrolysis of **7** and **8**, respectively.

Table 2
α-Glucosidase inhibitory effect of isolated compounds

Compound	% Inhibition at various concentration (μg/mL)			
	10.0	5.0	2.5	1.25
1	30.1 ± 1.32	13.2 ± 0.25	8.1 ± 0.6	1.3 ± 0.05
2	56.5 ± 0.64	25.2 ± 0.91	7.5 ± 0.87	1.7 ± 0.11
3	13.5 ± 0.68	11.4 ± 0.60	7.8 ± 0.44	1.5 ± 0.13
4	19.7 ± 1.59	15.9 ± 1.05	NI	NI
5	36.0 ± 0.34	20.9 ± 1.40	11.2 ± 1.11	5.3 ± 0.77
6	12.6 ± 1.05	NI	NI	NI
7	17.6 ± 1.07	11.6 ± 0.51	6.1 ± 0.54	4.6 ± 0.99
8	8.8 ± 0.39	3.4 ± 0.59	NI	NI

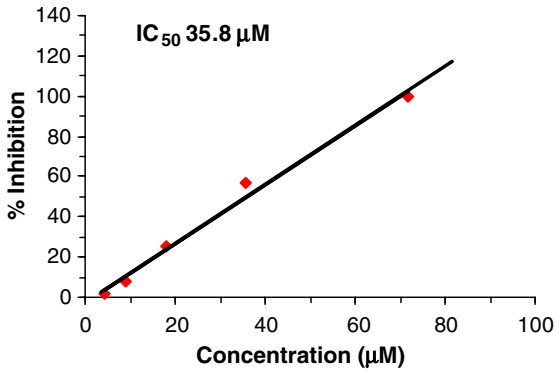


Figure 5. Inhibitory effect of anhydroaegeline (**2**) against α-glucosidase on hydrolysis of *p*-nitrophenyl α-D-glucopyranoside.

amide (δ_C 162.7). Interpretation of 2D NMR resulted in the construction of two separated aromatic systems, which were connected through amide linkage. A monosubstituted benzene [δ_H 7.53 (2H) and 7.38 (3H)] was connected to *trans*-olefinic protons [δ_H 7.75 (d, J = 15.2 Hz) and 6.44 (d, J = 15.2 Hz)], which were in turn linked to amide carbon based on HMBC correlations from H-2 and H-3 to C-1. The other aromatic motif was assigned to a *para*-substituted benzene [δ_H 7.28 (d, J = 8.4 Hz, 2H) and 6.86 (d, J = 8.4 Hz, 2H)], which was accommodated by the oxygenated prenyl (Me_2C=$CHCH_2O$–) and ethyleneamine (–CH=CH-NH–) moieties. A large coupling constant (14.4 Hz) of olefinic protons (H-1″ and H-2″) pointed out that they were *E*-oriented. Therefore the gross structure of anhydromarmeline (**1**) was depicted (see Fig. 2).

Aegelinoside A (**7**)[8] was isolated from 7:3 MeOH–H_2O extract and displayed $[M+Na]^+$ ion in HRESIMS at *m*/*z* 482.1781 that led to molecular formula of $C_{24}H_{29}NO_8$. The ^{1}H NMR spectrum (Table 1) of **7** in CD_3OD showed signals of aromatic and olefinic protons in range of 6.6–7.6 (11H) and oxygenated methylenes and methines (δ_H 3.0–5.2, 10H). The ^{13}C NMR spectrum displayed 24 signals, which included resonance of amide (δ_C 166.5). The resonances of δ_H 7.57 (m, 2H), 7.50 (d, J = 15.6 Hz, 1H), 7.35 (m, 3H), and 6.65 (d, J = 15.6 Hz, 1H) were ascribable to *trans*-cinnamide based on COSY and HMBC data. The signals at δ_H 7.35 (d, J = 8.8 Hz, 2H) and 6.92 (d, J = 8.8 Hz, 2H) were assigned to *p*-disubstituted benzene which was accommodated by methoxy group (δ_H 3.78 and δ_C 54.1) at C-6′ and oxygenated ethyl amine moiety (–OCH-CH_2-NH–) at C-3′. The HMBC cross peaks (Fig. 3) observed for H-2, H-3, and H-1′ to C-1 indicated that these two separated aromatic systems were linked through amide bond. The remaining oxygenated methylenes and methines were assigned to β-D-glucose residue, which was attached to C-2′ based on HMBC correlation from H-1″ (4.12, d, J = 7.2 Hz) to C-2′. Therefore overall structure of **7** was accomplished. The absolute configuration of C-2′ was determined by chemical degradation. Hydrolysis of **7** in 1 M HCl under reflux yielded D-glucose and (−)-aegeline; the latter of which was identical in all respects, particularly optical rotation ($[\alpha]^{26}_D$ −27.6), to *R*-aegeline (lit.$[\alpha]^{25}_D$ −35.9)[9].

Aegelinoside B (**8**)[10] was isomeric to **7** as evidenced by a molecular formula of $C_{24}H_{29}NO_8$. Although direct comparison of their ^{1}H and ^{13}C NMR spectra could not be made since they were recorded in different solvents, **8** revealed signals essentially identical to those of **7**. Significant difference observed by us was slightly upfield olefinic protons H-2 (6.05, d, J = 12.8 Hz) and H-3 (6.68, d, J = 12.8 Hz). A relative small coupling constant (J_{23} = 12.8 Hz) indicated that Δ^2 in **8** was *cis*-oriented instead of *trans*-oriented in **7**. The gross structure of **8** was subsequently confirmed by 2D NMR data. The absolute configuration of C-2′ was also deduced by chemical degradation. Acid hydrolysis of **8** afforded D-glucose and corresponding hydrolysate named aegeline B (**8a**, Fig. 4), whose minus sign of specific rotation ($[\alpha]^{26}_D$ −20.6) was reminiscent to that of a 2′*R*-phenylethyl cinnamide.[11]

Compounds **1**, **7** and **8** displayed slightly weak inhibition (30.1, 17.6 and 8.8%, respectively) against α-glucosidase, even at concentration of 10 µg/mL (see Table 2).[12] Of compounds isolated, anhydroaegeline (**2**) turned out to be the most potent inhibitor possessing IC_{50} value of 35.8 µM (Fig. 5).

In summary, we have isolated a variety of phenylethyl cinnamides, which included anhydromarmeline (**1**), aegelinosides A (**7**) and B (**8**), as a novel type of α-glucosidase inhibitors. Although phenylethyl cinnamides have been commonly encountered in certain genus such as *Aegle*[13] and *Hibiscus*,[14] the presence of *cis*-cinnamide moiety in **8** is extraordinarily rare in Nature. Recently Narender et al. have demonstrated that aegeline (**5**), the related congener of **7** and **8,** could serve as a potential remedy for diabetes mellitus by suppressing blood glucose and plasma triglyceride levels. Therefore they are likely to deserve further development discovering new antidiabetes drugs having dual functions.

Acknowledgments

This work was financially supported by Thailand Research Fund (MRG5180158) and the 90th Anniversary of Chulalongkorn University Fund (F-31-GS-ES13/28).

References and notes

1. Moller, D. E. *Nature* **2001**, *414*, 821.
2. For comprehensive reviews of natural α-glucosidase inhibitors, see: (a) Melo, E. B.; Gomes, A. S.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277; (b) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265.
3. Narender, T.; Shweta, S.; Tiwari, P.; Reddy, K. P.; Khaliq, T.; Prathipati, P.; Puri, A.; Srivastava, A. K.; Chander, R.; Agarwal, S. C.; Raj, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1808.
4. For recent reports, see (a) Kesari, A. N.; Gupta, R. K.; Singh, S. K.; Diwakar, S.; Watal, G. *J. Ethnopharmacol.* **2006**, *107*, 374; (b) Panda, S.; Kar, A. *Phytother. Res.* **2006**, *20*, 1103; (c) Upadhya, S.; Shanbhag, K. K.; Suneetha, G.; Naidu, M. B.; Upadhya, S. *Indian J. Physiol. Pharmacol.* **2004**, *48*, 476.
5. Although several phenylethyl cinnamides have been isolated from *A. marmelos*, many of which have not been given the IUPAC or trivial names, for the sage of communication in this Letter, we adopted terms 'marmeline' for phenylethyl cinnamides having 6′-phenyloxyl and 'aegeline' for those having 6′-methoxyl.
6. Anhydromarmeline (**1**): UV (MeOH) λ_{max} (log ε) 277 (4.81), 333 (4.46); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (3H, s, H-5″), 1.80 (3H, s, H-4″), 4.50 (2H, d, J = 6.8 Hz, H-1″), 5.49 (1H, m, H-3″), 6.14 (1H, d, J = 14.4 Hz, H-3′), 6.44 (1H, d, J = 15.2 Hz, H-2), 6.86 (2H, d, J = 8.4 Hz, H-5′ and H-7′), 7.28 (2H, d, J = 8.4 Hz, H-4′ and H-8′), 7.38 (3H, m, H-6, H-7 and H-8), 7.53 (3H, m, H-5, H-9 and H-1′), 7.75 (1H, d, J = 15.2 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (C-5″), 25.8 (C-4″), 65.4 (C-1″), 113.2 (C-3′), 115.0 (C-5′ and C-7′), 119.7 (C-2), 119.9 (C-2″), 126.7 (C-4′ and C-8′), 128.0 (C-5 and C-9), 129.1 (C-6 and C-8), 130.1 (C-7), 132.4 (C-4), 137.5 (C-1′), 138.3 (C-3″), 143.8 (C-3), 158.1 (C-6′), 162.7 (C-1); HRESIMS *m*/*z* $[M+Na]^+$ 356.1623 (calcd for $C_{24}H_{29}NO_8Na$, 356.1626).
7. Phuwapraisirisan, P.; Udomchotphruet, S.; Surapinit, S.; Tip-pyang, S. *Nat. Prod. Res.* **2006**, *20*, 1332.
8. Aegelinoside A (**7**): $[\alpha]^{25}_D$ −26.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 274 (4.73); ^{1}H and ^{13}C NMR (see Table 1); HRESIMS *m*/*z* $[M+Na]^+$ 482.1781 (calcd for $C_{24}H_{29}NO_8Na$, 482.1791).
9. Kamal, A.; Shaik, A. A.; Sandbhor, M.; Malik, M. S. *Tetrahedron Asymmetry* **2004**, *15*, 3939.
10. Aegelinoside B (**8**): $[\alpha]^{25}_D$ −33.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.43); ^{1}H and ^{13}C NMR (see Table 1); HRESIMS *m*/*z* $[M+Na]^+$ 482.1786 (calcd for $C_{24}H_{29}NO_8Na$, 482.1791).
11. Optically pure phenylethyl amides having only one chiral secondary alcohol revealed different signs of specific rotation; (−) for *R* and (+) for *S* isomers. For certain instances, see Ref. 9.
12. The inhibitory effects of isolated compounds were validated using previous protocol with slight modification. For detailed experiment, see: Schäfer, A.; Högger, P. *Diabetes Res. Clin. Pract.* **2007**, *77*, 41.
13. (a) Govindachari, T. R.; Premila, M. S. *Phytochemistry* **1983**, *22*, 755; (b) Sharma, B. R.; Rattan, R. K.; Sharma, P. *Phytochemistry* **1981**, *20*, 2606; (c) Manandhar, M. D.; Shoeb, A.; Kapil, R. S.; Popli, S. P. *Phytochemistry* **1978**, *17*, 1814; (d) Basu, D.; Sen, R. *Phytochemistry* **1974**, *13*, 2329; (e) Shoeb, A.; Kapil, R. S.; Popli, S. P. *Phytochemistry* **1973**, *12*, 2071.
14. Seca, A. M. L.; Silva, A. M. S.; Silvestre, A. J. D.; Cavaleiro, J. A. S.; Domingues, F. M. J.; Pascoal-Neto, C. *Phytochemistry* **2001**, *58*, 1219.