รายงานวิจัยฉบับสมบูรณ์

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โดย

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กันยายน 2563

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สารบัญ

	หน้า
Acknowledgements	ก
บทคัดย่อ	ข
Abstract	ค
Part 1: Chemical constituents of *Moringa oleifera* L.	1
Introduction	2
Experimental	3
Results and Discussion	6
References	12
Part 2: Chemical constituents of *Gnaphalium polycarpon* Persoon	16
Introduction	17
Experimental	17
Results and Discussion	20
References	24
Part 3: Chemical constituents of *Scleropyrum pentandrum*	26
Introduction	27
Experimental	27
Results and Discussion	32
References	45
Part 4: Chemical constituents of *Ruellia tuberosa* L.	48
Introduction	49
Experimental	49
Results and Discussion	52
References	58
Part 5: Chemical Constituents of *Grammatphyllum speciosum* Blume	61
Introduction	62
Experimental	62
Results and Discussion	69
References	78

Part 6: Chemical constituents of Mitragyna rotundidolia (Roxb.) Kuntz 80
Introduction 81
Experimental 81
Results and Discussion 84
References 90
Part 7: Chemical constituents of Bruguiera gymnorrhiza (L.) Svigny 92
Introduction 93
Experimental 93
Results and Discussion 96
References 105
Part 8: Chemical constituents of *Micromelum minutum* (G.Forst.) Wright & Arn. 108
Introduction 109
Experimental 109
Results and Discussion 112
References 119
Part 9: Chemical constituents of *Gloriosa superba* L. 124
Introduction 125
Experimental 125
Results and Discussion 128
References 134
Part 10: Chemical constituents of *Desmdium heterocarpon* (L.) DC. 136
Introduction 137
Experimental 137
Results and Discussion 139
References 145

Research Outputs 147
Appendix 1: Unusual glycosides of pyrrole alkaloid and 4'-hydroxyphenylethanamide from leaves of *Moringa oleifera* 149
Appendix 2: Appendix 2: 3-Hydroxydihydrobenzofuran glucosides from *Gnaphalium polycaulon* 150

Appendix 3: Unpredented furan-2-carbonyl *C*-glycosides and phenolic diglycosides from *Scleropyrum pentandrum* 151

Appendix 4: Phenylethanoid and flavones glycosides from *Ruellia tuberosa* L. 152

Appendix 5: Glucopyranosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid and (*R*)-eucomic acid, and an aromatic glucoside from the pseudobulbs of *Grammatophyllum speciosum* 153

Appendix 6: New *trans*- and *cis*-*p*-coumaroyl flavonol tetraglycosides from the leaves of *Mitragyna rotundifolia* 154

Appendix 7: 5-Methyl ether flavone glucosides from the leaves of *Bruguiera gymnorrhiza* 155

Appendix 8: Glucosides of phenylpropanoic acid derivatives and coumarins from *Micromelum minutum* 156

Appendix 9: Colchicinoid glucosides from seedless pods of Thai origin Gloriosa superba 157

Appendix 10: 2-Carboxy-dihydrostilbene and flavan glycosides from *Desmodium heterocarpon* 158

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ระยะเวลาโครงการ : 3 ปี

จากโครงการวิจัย เป็นการพิสูจน์หาสารต้านอนุมูลอิสระ ต้านมะเร็ง และออกฤทธิ์สมานแผล จากพืชที่มีสรรพคุณกล่าวอ้างตามสังคมไทย 10 ชนิด ได้ทำการสกัดแยกสารจากมะรุม (*Moringa oleifera*) ชิวคัก (*Gnaphalium polycerpo*n) ขี้หนอน (*Scleropyrum pentandrum*) ต้อยติ่ง (*Ruellia tuberosa*) ว่านเพชรหึง (*Grammatophyllum speciosum*) กระทุ่มหมู (*Mitragyna rotuntifolia*) โกงกางหัวสุม (*Bruguiera gymnorrhiza*) สมัด (*Micromelum minutum*) ดองดึง (*Gloriosa superba*) และ ขางคันนา (*Desmodium heterocarpon*) โดยนำมาสกัดแยก เพื่อหาสารองค์ประกอบบริสุทธิ์ที่มีความเป็นขั้วสูง ใช้เทคนิคทางโครมาโตรกราฟี และกรรมวิธีหลายชนิด รวมถึงพยายามพัฒนาเทคนิคการแยกสารที่มีความเป็นขั้วสูง หลังจากที่แยกสารสำคัญบริสุทธิ์ได้แล้ว สารทุกตัวจะนำมาตรวจสอบเพื่อหาโครงสร้างทางเคมี โดยวิธีทางสเปกโตรสโกปี หรือวิธีการอื่น ๆ ที่จำเป็น จนกระทั่งหาอธิบายโครงสร้างได้สมบูรณ์ สารสำคัญจากต้น หนอน (*Scleropyrum pentandrum*) ต้อยติ่ง (*Ruellia tuberosa*) พบว่ามีฤทธิ์ต้นอนุมูลอิสระ

คำสำคัญ : สมุนไพรไทย; อนุมูลอิสระ; Phenolic glycosides;

Abstract

Project Code : DBG5480007

Project Title : Investigation for antioxidant, cytotoxic and wound healing substances from plants as claimed by Thai society

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Project Peroid : 3 years

This project was investigation and structure elucidation of the chemical constituents from ten Thai medicinal plants including *Moringa oleifera*, *Gnaphalium polycerpo*n, *Scleropyrum pentandrum*, *Ruellia tuberose*, *Grammatophyllum speciosum*, *Mitragyna rotuntifolia*, *Bruguiera gymnorrhiza*, *Micromelum minutum*, *Gloriosa superb* and *Desmodium heterocarpon*) by various chromatographed methods. The structure determinations were based on the results from spectroscopic evidences. The constituents from two plants, *Scleropyrum pentandrum* and *Ruellia tuberosa showed the antioxidant activities.*

Keywords: Thai medicinal plants; Antioxidant activity; Phenolic glycosides

Part 1

Chemical Constituents of *Moringa oleifera* Lam.

Chemical Constituents of *Moringa oleifera* Lam.

Introduction

Moringa oleifera Lam. (Moringaceae; Thai name: Ma-Rum) is an ornamental plant native to tropical and subtropical areas, and commonly cultivated in all parts of Thailand as a vegetable for cooking purposes. The leaves and pods are used in Thai traditional medicine to decrease blood pressure, as well as for antipyretic agents and antidote for detoxification of poisons. This plant is known to have an excellent nutritional value, as well as various medicinal usages for treatment of different ailments in traditional medical systems (Fahey, 2005). The phytochemical compositions, medicinal use, pharmacological properties and pharmaceutical applications have been reviewed by Anwar et al. (2007) and Aney et al. (2009). This plant has been extensively studied for its constituents and biological activities such as antitumor, anti-inflammatory, antimicrobial, antioxidant and antihypertensive activities, and is well known to contain carbamate and thiocarbamate glycosides (Eilert et al., 1981; Faizi et al., 1992, 1994a,b, 1995; Guevara et al., 1999; Francis et al., 2004; Nikkon et al., 2009; Sreelatha and Padma, 2009; Cheenpracha et al., 2010; Oluduro et al., 2010). However, there is no report on the constituents from the plant source of Thai origin. In Thailand, this plant is quite popular as a daily food supplement. Ma-Rum products have been sold in various forms such as leaf powder in capsules, spray-dried plant extracts in capsules, or herbal tea from leaves or seeds. This paper describes the isolation and identification of 11 polar compounds, including nitrogen-containing phenolic glycosides (**1**–**5**) (see Fig. 1), benzyl glycosides (**6, 7**),

flavonol glycosides (**8, 9**), a nucleoside (**10**) and an amino acid (**11**) from leaves of this plant. The usage of this plant as a food supplement is also discussed.

Experimental

General Procedures: NMR spectra were recorded in CD_3OD using a JEOL JNM α-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. FTIR spectra were recorded on a universal attenuated total reflectance attached (UATR) to a Perkin–Elmer Spectrum One spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 lm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 _ 250 mm i.d., Vertisep™ AQS) with a Jasco UV-970 detector at 220 nm. The flow rate was 8 ml/min. The spraying reagent used for TLC was 10% H2SO4 in H_2O-EtOH (1:1, v/v).

Plant Material: Leaves of *M. oleifera* Lam. were collected in February 2010 from Khon Kaen province, Thailand, with plant identification done by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0064) is in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation: Dried leaves of M. oleifera (2.2 kg) were extracted with MeOH (each 20 L for 24 h, 3 times) at room temperature. After removal of the

solvent, the residue (632.1 g) was partitioned with Et_2O and H_2O (each 1.0 L, 3 times). The aqueous soluble fraction (409.5 g) was subjected to a Diaion HP-20 column, and successfully eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (90.7 g) was subjected to silica gel cc using solvent systems EtOAc–MeOH (9:1, 4.0 L), EtOAc–MeOH–H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 6.0 L) and EtOAc–MeOH–H_2O (6:4:1, 12.0 L), respectively, to provide seven fractions. Fraction **1** (1.9 g) was applied to a RP-18 column using a gradient solvent system H_2O-MeOH (90:10→20:80, v/v) to give five fractions. Fraction 1–3 was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (85:15, v/v) to afford compounds **1** (11.2 mg) and **5** (8.8 mg). Fraction 2 (14.3 g) was subjected to a RP-18 column using a gradient solvent system, H_2O–MeOH (90:10→20:80, v/v) to provide fifteen fractions. Fraction 2–6 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (90:10, v/v) to provide compound **6** (357.7 mg). Fraction 2–5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compound **2** (154.8 mg). Fraction **3** (22.2 g) was applied to a RP-18 column using a gradient solvent system H_2O–MeOH (90:10→20:80, v/v) to afford twelve fractions. Fraction 3–1 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to give compound **3** (171.9 mg). Fraction 3–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to yield compound 7 (313.0 mg). Fraction 4 (20.6 g) was separated on a RP-18 column using a solvent system H_2O–MeOH (90:10→20:80, v/v) to give seven fractions. Fraction 4–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **4** (786.6 mg) and **10** (104.3 mg). Fraction 4–5 was purified by preparative HPLC-ODS

with solvent system H_2O–MeCN (80:20) to obtain compounds **8** (756.4 mg) and **9** (190.6 mg). Finally, Fraction 6 (4.9 g) was similarly subjected to RP-18 cc using a solvent system H_2O–MeOH (90:10→20:80, v/v) to afford five fractions. Faction 6– 2 was purified by preparative HPLC-ODS with solvent system H2O–MeCN (90:10, v/v) to give compound **11** (244.5 mg).

Pyrrolemarumine 4"-O-α-L-rhamnopyranoside (2) : Amorphous powder; $[\alpha]_D$ 2763.2 (MeOH, c 0.49); IR (UATR)$_{Vmax}$ 3361, 2934, 1646, 1610, 1509, 1371, 1231, 1060, 1021, 983 cm_1; for 1H and 13C NMR (DMSO-d6): spectroscopic data, see Table 1; negative HR-APCI-TOFMS, m/z: 412.1163 $[M+Cl]^-$ (calcd for $C_{19}H_{23}ClNO_7$, 412.1169).

Acid hydrolysis of pyrrolemarumine 4"-*O*-α-L-rhamnopyranoside (2): A solution of pyrrolemarumine 4"-*O*-α-L-rhamnopyranoside (*ca* 20 mg) in 1,4-dioxane (0.5 ml) and 2 N HCl (4.5 ml) was heated at 80 °C for 4 h. After cooling, H2O (5 ml) was added and neutralized with 2 N KOH. The mixture was extracted with EtOAc (30 ml x 2) and the combined organic parts were concentrated in *vacuo* to provide pyrrolemarumine (2a, 7.2 mg). Its structure was assigned by NMR spectroscopic analyses.

Pyrrolemarumine (2a): Amorphous powder; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 1; positive HR-APCI-TOFMS, m/z: 232.0971 [M+H]+ (calcd for $C_{13}H_{14}NO_3$, 232.0968).

Marumoside A (3): Amorphous powder, $[\alpha]_D^{28}$ 94.8 (MeOH, *c* 1.00); IR (UATR)$_{Vmax}$ 3351, 2933, 1667, 1610, 1510, 1383, 1230, 1063, 1021, 983 cm^{-1}; for ^1H

and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 2**;** negative HR-ESI-TOFMS, m/z: 332.0916 $[M+Cl]^-$ (calcd for $C_{14}H_{19}ClNO_6$, 332.0906).

Marumoside B (4): Amorphous powder, $[\alpha]_D^{28}$ 65.2 (MeOH, *c* 0.42); IR $(UATR)_{Vmax}$ 3355, 2920, 1668, 1610, 1509, 1376, 1229, 1070, 1022, 983 cm^{-1}; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 2; negative HR-ESI-TOFMS, m/z: 494.1448 $[M+Cl]^-$ (calcd for $C_{20}H_{29}ClNO_{11}$, 494.1434).

Methyl 4-(α-L-rhamnopyranosyloxy)benzylcarbamate (5): Amorphous powder, $[\alpha]_D^{27}$ 123.0 (MeOH, c 0.46); IR $(UATR)_{Vmax}$ 3337, 2933, 1701, 1533, 1509, 1263, 1229, 1063, 1020, 983 cm^{-1}; 1H NMR (DMSO-d6): d 7.62 (1H, t, J = 6.1 Hz, N–H), 7.17 (2H, d, J = 8.6 Hz, H-2, 6), 6.97 (2H, d, J = 8.6 Hz, H-3, 5), 5.32 (1H, d, J = 1.6 Hz, H-1"), 4.10 (2H, d, J = 6.1 Hz, H-7), 3.80 (1H, br s, H-200), 3.62 (1H, dd, J = 9.7, 1.8 Hz, H-3"), 3.53 (3H, s, OCH_3), 3.45 (1H, m, H-5"), 3.26 (1H, dd, J = 9.7, 9.6 Hz, H-4"), 1.08 (3H, d, J = 6.2 Hz, H-6"); 13C NMR (DMSO-d_6): d 157.3 (C-10), 155.4 (C-4), 133.6 (C-1), 128.7 (C-2, 6), 116.7 (C-3, 5), 98.9 (C-100), 72.2 (C-4"), 70.9 (C-2"), 70.6 (C-3"), 69.8 (C-5"), 51.8 (OCH_3), 43.7 (C-7), 18.3 (C-6"); negative HR-ESI-TOFMS, m/z: 362.1018 $[M+Cl]^-$ (calcd for $C_{15}H_{21}ClNO_7$, 362.1012).

Results and discussion

The methanolic extract of the leaves of M. oleifera was suspendedin H2O and partitioned with Et_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to afford 11 compounds. Seven compounds were identified as niazirin (4-(α-L-rhamnopyranosyloxy)phenylacetonitrile, **1**) (Faizi et al., 1994a,b),

benzyl β-D-glucopyranoside (**6**) (Kanchanapoom et al., 2001a), benzyl β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**7**), kaempferol 3-*O*-β-D-glucopyranoside (**8**) (Noiarsa et al., 2007), quercetin 3-O-b-D-glucopyranoside (**9**), adenosine (**10**) (Kanchanapoom et al., 2001b) and L-tryptophan (**11**) (Kanchanapoom et al., 2007) by comparison of physical data with literature values and from spectroscopic evidence.

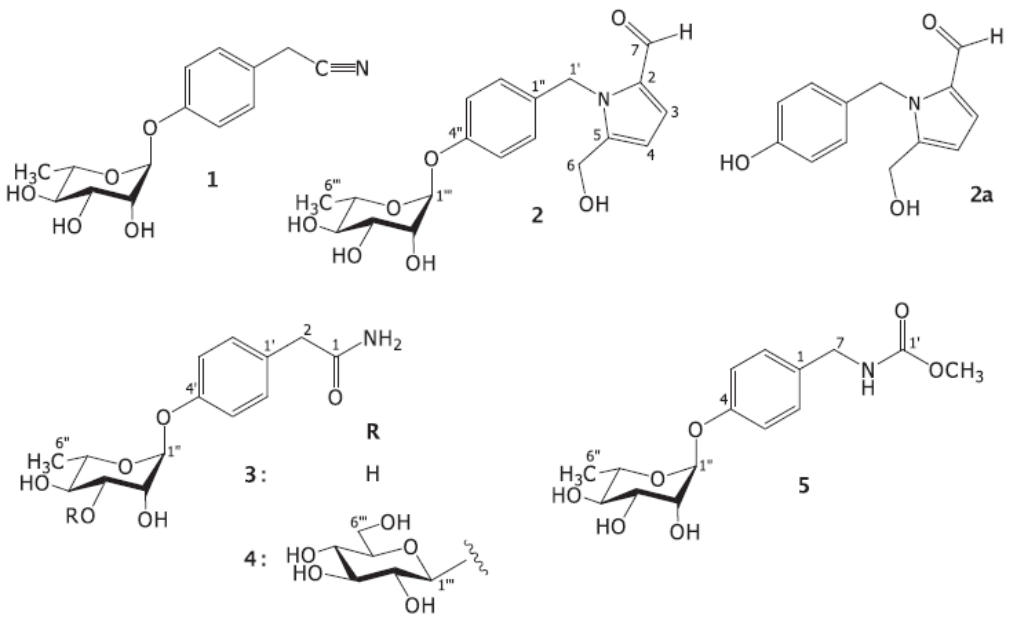

Fig. 1 Structures of isolated compounds **1-5**

Compound **2** was isolated as an amorphous powder. Its molecular formula was determined to be $C19H_{23}NO_7$ by high-resolution atmospheric pressure chemical ionization time-of-flight mass spectrometric analysis (HR-APCI-TOFMS). The IR spectrum displayed the strong absorption bands of hydroxyl groups at 3361 cm_1 and a carbonyl group at 1646 cm^{-1}. Analysis of the 1H NMR spectrum indicated the

presence of an one sugar unit, suggested to be α-L-rhamnose from the chemical shifts of its anomeric proton at δ_H 5.32 (1H, br s) and a secondary methyl group at δ_H 1.08 (3H, d, J = 6.2 Hz). Two doublet protons at δ_H 7.04 and 6.28, which appeared as an AB system (d, J = 3.9 Hz) were characteristic of a nitrogen-containing heterocyclic ring, consistent with a 2,5-disubstituted pyrrole ring (Chin et al., 2003). The chemical shift at δ_H 9.45 (1H, s) was assigned for an aldehyde group. The remaining resonances were the singlet signal of four equivalent protons at δ_H 6.95 and the signals of two methylene groups at δ_H 5.56 (2H, s) and 4.42 (2H, s). Analysis of the 13C-NMR spectroscopic data showed that the sugar unit was an α-L-rhamnopyranosyl moiety from the set of the chemical shifts at δ_C 98.9, 70.8, 70.6, 72.2, 69.9 and 18.3. In addition, the resonances of 1,4-disubstituted aromatic ring were observed from the chemical shifts of one oxy-aryl carbon at dC 155.6, four aryl methines at δ_C 116.9, 127.9 (each 2C), and one non-protonated carbon at δ_C 131.8. The ^{13}C NMR spectrum also showed signals of an aldehyde group at dC 179.9, two methylenes at δ_C 47.5 and 55.4, one of which was oxygenated (δ_C 55.4). The chemical shifts of two methines at δ_C 110.3 and 124.6 together with two quaternary carbons at δ_C 132.1 and 114.3 belonged to the 2,5-disubstituted pyrrole ring (Chin et al., 2003). The complete structure of compound 2 was deduced by the results from HMQC and HMBC spectroscopic methods. The aldehyde group at δ_H 9.45 (δ_C 179.9) and the methylene group at δ_H 4.42 (δ_C 55.4) were attached to C-2 and C-5, respectively, of the pyrrole ring, based on the HMBC data (Fig. 2). The downfield singlet methylene protons at δ_H 5.56, corresponding to the methylene carbon at δ_C 47.5, showed the HMBC correlations to C-2 (δ_C 132.1) and C-5 (δ_C 144.3) of the pyrrole ring and C-1" (δ_C

131.8) and C-2", 6" (δ_C 127.9) of the aromatic ring, indicating that this carbon was located between these two ring systems. The sugar moiety was attached to C-4" (δ_C 155.6) of the aromatic ring by the HMBC correlation from H-10" (δ_H 5.32) to C-4". Therefore, the structure of compound **2** was elucidated as shown. However, it is quite unusual that the ^{1}H NMR spectrum showed the equivalent of four protons of the aromatic ring system instead of the AA'BB' aromatic ring system. This compound was hydrolyzed by HCl to yield a new aglycone **2a**, namely pyrrolemarumine. From the 1H NMR spectrum of **2a**, the splitting patterns of protons for the aromatic ring were in agreement with those of 1,4-disubstituted aromatic ring (Table 1), providing confirmation of the structure of compound **2**. Consequently, compound **2** was pyrrolemarumine 4"-*O*-α-L-rhamnopyranoside.

Table 1
NMR spectroscopic data of compounds **2** and **2a** (DMSO-d_6).

Position	**2**		**2a**	
	Carbon	Proton	Carbon	Proton
Aglycone				
2	132.1		132.1	
3	124.6	7.04 (1H, d, J = 3.9 Hz)	124.0	7.02 (1H, d, J = 4.0 Hz)
4	110.3	6.28 (1H, d, J = 3.9 Hz)	110.2	6.25 (1H, d, J = 4.0 Hz)
5	144.3		144.3	
6	55.4	4.42 (2H, s)	55.4	4.41 (2H, s)
7	179.9	9.45 (1H, s)	179.8	9.47 (1H, s)
1′	47.5	5.56 (2H, s)	47.5	5.50 (2H, s)
1″	131.8		128.6	
2″,6″	127.9	6.95 (4H, s)	128.0	6.83 (2H, d, J = 8.5 Hz)
3″,5″	116.9		115.6	6.67 (2H, d, J = 8.5 Hz)
4″	155.6		156.9	
Sugar moiety				
1‴	98.9	5.32 (1H, br s)		
2‴	70.8	3.80 (1H, br s)		
3‴	70.6	3.63 (1H, br d, J = 9.0 Hz)		
4‴	72.2	3.28 (1H, dd, J = 9.0, 8.9 Hz)		
5‴	69.9	3.42 (1H, m)		
6‴	18.3	1.08 (3H, d, J = 6.2 Hz)		

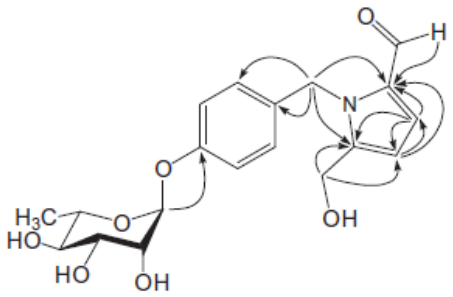

Fig. 2 HMBC correlations of compound **2**

Compound **3** was obtained as an amorphous powder and determined to be $C_{14}H_{19}NO_6$ by high resolution electrospray time of-flight mass spectrometric analysis

(HR-ESI-TOFMS). Its IR spectrum exhibited characteristic absorption bands at 3351 and 2933 cm^{-1} for hydroxyl groups and a primary amide, respectively in addition to the band at 1667 cm^{-1} for the carbonyl group of an amide (Pretsch et al., 2009). The 1H NMR spectral data indicated the presence of an 1,4-disubstituted aromatic ring from the chemical shifts at δ_H 7.17 and 6.94 (each 2H, d, J = 8.2 Hz), two protons of an amide group at δ_H 7.46, 6.86 (each 1H, br s), two methylene protons at δ_H 3.30 (2H, s) along with the signals a-Lrhamnopyranosyl moiety. The 13C NMR spectrum showed signals of an 1,4-disubstituted aromatic ring and α-L-rhamnopyranosyl unit, closely related to those of compounds **1** and **2**. The resonance at δ_C 172.8 was assigned as a carbonyl carbon of the amide group. The chemical shift at δ_C 41.5 was identified to the intermediate methylene carbon between the aromatic ring and the amide group. The structure of compound **3** was supported by the HMBC correlations between (i) NH_2 (δ_H 7.46 and 6.86) and C-2 (δ_C 41.5); (ii) H_2-2 (δ_H 3.30) and C-1' (δ_C 129.9), C-2', 6' (δ_C 130.2); and (iii) H-100 (δ_H 5.32) and C-4' (δ_C 154.8). Therefore, compound **3** was elucidated as 4'-hydroxyphenylethanamide-α-L-rhamnopyranoside, namely marumoside A.

Compound **4** was isolated as an amorphous powder and determined to be $C_{14}H_{19}NO_6$ by high resolution electrospray time-of flight mass spectrometric analysis (HR-ESI-TOFMS). The ^{1}H and ^{13}C NMR spectra were very similar to those of compound **3**, except for a set of additional signals arising from the β-D-glucopyranosyl moiety in **4**. The additional unit was assigned to be located at C-3" of the rhamnopyranosyl moiety since the downfield of this carbon atom to δ_C 81.4 together with the upfield shifts of C-2 and C-4 to δ_C 69.3 and 71.1, respectively, as

compared to compound **3**. Moreover, the HMBC spectrum provided further confirmation of the structure with the correlations between H-10" (δ_H 4.48) and C-3" as shown in Fig. 3. Consequently, this compound was identified as the 3"-*O*-β-D-glucopyranosyl-derivative of compound **3**, namely marumoside B.

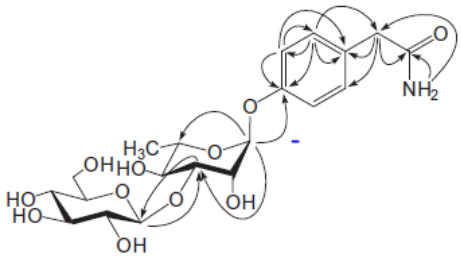

Fig. 3 HMBC correlations of compound **4**.

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Part 2

Chemical Constituents of *Gnaphalium polycaulon* Persoon

Chemical Constituents of *Gnaphalium polycaulon* Persoon

Introduction

Gnaphalium polycaulon PERSOON is an annual widespread weed in tropical and subtropical Africa, Asia, Australia and America.[1)] In Thailand, it is believed that this species was brought into cultivation by the Chinese and commonly grown around cultivated fields, especially in Damnoen Saduak district, Ratchaburi Province. The aerial parts are available in cool season from November to January and used as a flavor ingredient in foods for carminative purpose during the Chinese New Year celebrations by Chinese descendants. The phytochemical investigation of this plant has not been carried out. However, diterpenoids and flavonoids have been isolated from other *Gnaphalium* species.[2—5)] This paper describes the isolation and identification of four polar compounds, including a new 3-hydroxydihydrobenzofuran glucoside (**2**) and three known compounds: a 3-hydroxydihydrobenzofuran glucoside (**1**), an alkyl glucoside (**3**) and a nucleoside (**4**) (Fig.1), from the aqueous soluble fraction of the aerial parts of this plant in addition to determination of the absolute configurations of two 3-hydroxydihydrobenzofuran glucosides (**1**, **2**).

Experimental

General Procedure: Melting point was determined with a Stuart Scientific SMP3 apparatus and was uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in CD_3OD using a Bruker AV-400 spectrometer. Fourier Transform (FT)-IR spectra were obtained on a universal attenuated total reflectance attached (UATR) to a Perkin-Elmer Spectrum One spectrometer. The MS data was recorded on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-

1020 digital polarimeter. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd., Japan), silica gel 60 (70—230 mesh, Merck, Germany), and RP-18 (50m m,YMC, U.S.A.) were used. HPLC (Jasco PU-980 pump) was carried out on an octadecyl silica (ODS) column (21.2 x 250 mm i.d., VertisepTM AQS) with a Jasco UV-970 detector at 220 nm. The flow rate was 8 ml/min. The spraying reagent used for TLC was 10% H2SO4 in 50% EtOH.

Plant Material: The aerial parts of *G. polycaulon* (Asteraceae) PERSOON were collected from Damnoen Saduak district, Ratchaburi province, in December 2009 Thailand. The identification was done by Mr. Nopporn Nontapa, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TKPSKKU-0065) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation: The aerial parts of *G. polycaulon* (1.9 kg) were extracted with MeOH (3 times, each 12.0 l, 24 h) at room temperature. After removal of the solvent *in vacuo*, the residue (308.4 g) was partitioned with Et_2O and H_2O (each 1.0 l, 3 times). The aqueous soluble fraction (173.6 g) was subjected to a Diaion HP-20 column, and successively eluted with H_2O, MeOH and acetone. The fraction eluted with MeOH (11.0 g) was subjected to a column of silica gel using solvent systems EtOAc–MeOH (9 : 1, 2.0 l), EtOAc–MeOH–H2O (40 : 10 : 1, 2.0 l), EtOAc–MeOH–H2O (70 : 30 : 3, 4.0 l) and EtOAc–MeOH–H2O (6 : 4 : 1, 5.5 l), respectively, to provide four fractions, monitored by TLC. Fraction 1 (4.5 g) was applied to a RP-18 column using a gradient solvent system 10—80% aqueous MeOH to give eight fractions. Fraction 1-1 was purified by preparative HPLC ODS using solvent system 10% aqueous MeCN to afford compound **4** (122.0 mg). Fraction 1-4

was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to provide compound **3** (36.7 mg). Fraction 1-5 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to provide compound **1** (83.6 mg). Finally, fraction 1-6 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to afford compound **2** (34.2 mg).

Gnaphaliol 3-*O*-β -D-Glucopyranoside (1): Amorphous powder; mp 129 °C (dec.); $[\alpha]_D$ -43.8 (*c*_0.75, MeOH); IR (UATR) ν max 3475, 3333, 2934, 2880, 1660, 1603, 1490, 1436, 1357, 1262, 1069, 1026 cm^{-1}; ^1H- and ^{13}C-NMR (CD_3OD): see Table 1; negative HR-APCI-TOF-MS, *m*/*z*: 431.1114 $[M+Cl]^-$ (Calcd for $C_{19}H_{24}ClO_9$, 431.1114).

Gnaphaliol (1a): Amorphous powder; $[\alpha]_D$ -1.03 (*c*_0.64, MeOH); IR (UATR) ν max 3353, 2920, 1663, 1607, 1590, 1490, 1433, 1360, 1261, 1065, 1002 cm^{-1}; ^1H- and ^{13}C-NMR (CD_3OD): see Table 1; positive HR-ESI-TOFMS, *m*/*z*: 257.0784 $[M+Na]^+$ (Calcd for $C_{13}H_{14}NaO_4$, 257.0784).

Gnaphaliol 9-*O*-β-D-Glucopyranoside (2): Amorphous powder; $[\alpha]_D$ -11.2 (*c*_1.5, MeOH); IR (UATR) ν max 3349, 2926, 2880, 1659, 1607, 1587, 1489, 1437, 1360, 1263, 1070, 1036 cm^{-1}; ^1H- and ^{13}C-NMR(CD_3OD): see Table 1; negative HR-APCI-TOF-MS, *m*/*z*: 431.1099 $[M+Cl]^-$ (Calcd for $C_{19}H_{24}ClO_9$, 431.1114).

Enzymatic Hydrolysis of 1 and 2 and Determination of the Absolute Configuration of Glucose: Compound **1** (10 mg) in 1,4-dioxane (0.5 ml) was added a solution of crude hesperidinase (80 mg) in H_2O (3 ml) and stirred at 37 °C for 48 h. The reaction was extracted with EtOAc, and the organic part was concentrated to provide gnaphaliol (**1a**) (6.2 mg). The structure of compound **1a** was identified by

1H- and 13C-NMR spectroscopic analysis. The aqueous layer was partitioned with *n*-BuOH, and the organic part was concentrated and analyzed with an optical rotation detector (JASCO OR-2090*plus*) with a Polyamine II column (YMC, 70% aqueous MeCN, 1 ml/min). It showed a peak for D-glucose at the retention time of 7.4 min. By the same method, compound **2** provided **1a** (8.4 mg) and D-glucose from the HPLC analysis.

Results and discussion

The methanolic extract of the aerial part of *G. polycaulon* was suspended in H2O and extracted with Et2O. The aqueous layer was applied to a column of Diaion HP-20, with H_2O, MeOH and Me_2CO as eluants, successively. The portion eluted with MeOH was separated by a combination of chromatographic procedures to provide four compounds. Two were identified as the known compounds (*Z*)-3-hexenyl *O*-β-D-glucopyranoside (**3**)[6] and adenosine (**4**),[7] by comparison of physical data with values reported in literatures and from spectroscopic evidence.

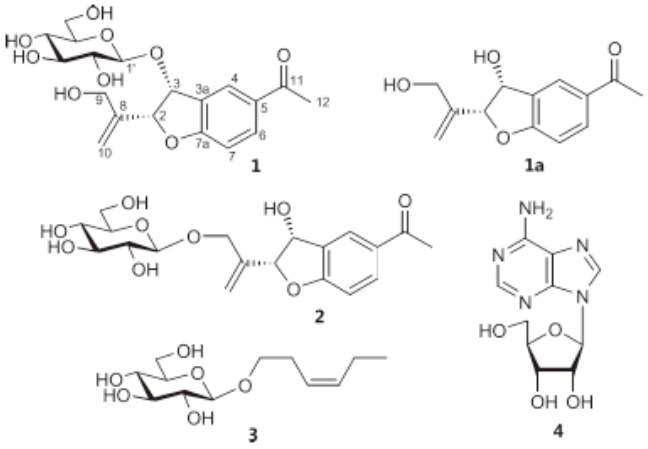

Fig. 1 Structures of compounds **1, 1a, 2-4**

Compound **1** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{24}O_9$ by high resolution atmospheric pressure chemical ionization time-offlight mass spectrometric analysis (HR-APCI-TOF-MS). The 1H-NMR spectrum displayed the presence of the signals of a typical ABX aromatic ring system at d H 8.32 (d, *J* = 2.0 Hz), 7.96 (dd, *J* = 8.6, 2.2 Hz) and 6.92 (d, *J* = 8.6 Hz), an AB type of methylene signals at δ_H 4.26, 4.16 (each d, *J* = 13.9 Hz), one singlet acetyl group at d H 2.59, two heterocyclic protonsat d H 5.43 and 5.28 (each d, *J*_6.9 Hz), two geminal olefinic protons at d H 5.42 and 5.39 (each s), as well as one anomeric proton at δ_H 4.58 (d, *J* = 7.8 Hz). The ^{13}C-NMR spectrum showed the signals of a β-glucopyranosyl unit in addition to 13 carbon signals for the aglycone moiety. The structure of compound **1** was assigned by the results from 1H–1H-correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond ccorrelation spectroscopy (HMBC) (Fig. 2) spectroscopic methods. This compound has a 3-hydroxydihydrobenzofuran as a core structure having a (1-hydroxylmethyl)vinyl and an acetylgroups located at C-2 and C-5, respectively, and the sugar moiety was assigned to be connected at C-3. The physical and spectroscopic data of compound **1** were identical to those of 1-{(2*R**,3*S**)-3-(β -D-glucopyranosyloxy)-2,3-dihydro-2-[1-(hydroxymethyl)vinyl]-1-benzofuran-5-yl}-ethanone, previously reported from *Leontopodium alpinum*.[8] The vicinal coupling constant of two protons at δ_H 5.28 (H-2) and 5.43 (H-3) with *J* = 6.9 Hz indicated that the functional groups on the five membered ring were *cis*.[9] However the determination of the absolute configurations at C-2 and C-3 positions has not been investigated. Since the attachment of a sugar moiety at C-3 position, the absolute configuration in this position could be determined by the application of β-D-

glycosylation shift-trend rule on carbon chemical shifts of secondary hydroxyl group (a -carbon) and the upfield shift difference values [$\Delta\delta_C$: δ_C (alcoholic glucoside) – δ_C (alcohol)] of their neighboring carbon atoms (two β-carbons) in the aglycone part.[10—12] The glycosylation shifts mainly depended on the chirality of the aglycone alcohols. The rotation around the glycosidic bond was rather restricted and caused unequal glycosylation shifts of these neighboring carbon atoms. Therefore, compound **1** was enzymatically hydrolyzed with crude hesperidinase to provide a new aglycone identified as 1-(3-hydroxy-2-(1-hydroxyprop-2-en-2-yl)-2,3- dihydrobenzofuran-5-yl)ethanone with the trivial name gnaphaliol (**1a**), together with D-glucose, which was determined by HPLC analysis. From the ^{13}C-NMR spectroscopic data, the upfield shift differences [$\Delta\delta_C$: δ_C (**1**)– δ_C (**1a**)] of C-2 and C-3a were calculated to be -0.9 and -2.1 ppm (Table 1), respectively. Generally, the upfield shift differences of the b -carbon *anti* to the pyranose ring oxygen was larger than that of the b -carbon *syn* to the oxygen.[11—13] Thus, the conformation around glucosidic linkage of this compound could be illustrated as shown in Fig. 3 and led to conclude the absolute configuration of C-3 position to be *R*. Besides the absolute configuration of C-2 position was assigned to be *R*. Consequently, compound **1** was gnaphaliol 3-*O*-β -D-glucopyranoside.

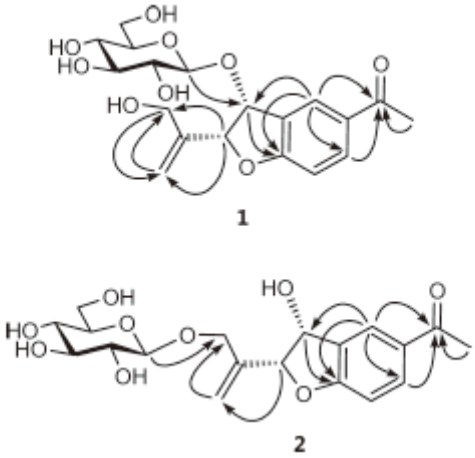

Fig. 2 HMBC correlations of compounds **1** and **2**

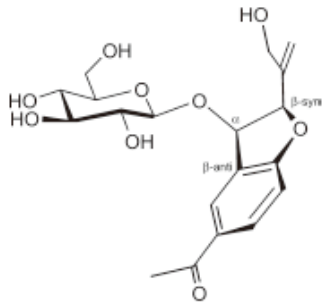

Fig. 3 Conformation around Glucosidic Linkage of Compound **1**

Compound **2** was isolated as an amorphous powder, and its molecular formula was determined to be $C_{19}H_{24}O_9$ by HRAPCI- TOF-MS. The NMR spectroscopic data were closely related to those of compound **1**. In particular, the chemical shifts of the core structure were in agreement with those of **1a**. The significant difference was the downfield shift of C-9, together with the upfield shift of C-8 indicating that the β –D-glucopyranosylunit was connected to C-9 position. The assignment was confirmed by HMBC correlations (Fig. 2). This compound should possess the same aglycone part due to the co-occurrence in the same plant species. Enzymatic hydrolysis with crude

hesperidinase provided **1a**, and the stereochemistry of glucose was identified to be D-form. Therefore, the structure of compound **2** was elucidated to be gnaphaliol 9-*O*-β -D-glucopyranoside.

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compounds **1**, **2** and Their Aglycone **1a** (400 MHz for ^{1}H Data and 100 MHz for ^{13}C Data, Recorded in CD_3OD)

Position	**1**		**2**		**1a**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	89.2 (−0.9)[a]	5.28 (1H, d, 6.9)	90.1	5.22 (1H, d, 5.9)	90.1	5.20 (1H, d, 6.3)
3	81.2 (+8.8)[a]	5.43 (1H, d, 6.9)	72.3	5.30 (1H, d, 5.9)	72.4	5.27 (1H, d, 6.3)
3a	129.1 (−2.1)[a]		131.3		131.2	
4	131.0	8.32 (1H, d, 2.0)	128.5	8.09 (1H, d, 1.9)	128.5	8.09 (1H, d, 2.0)
5	132.0		132.3		132.2	
6	133.0	7.96 (1H, dd, 8.6, 2.0)	133.2	7.99 (1H, dd, 8.5, 1.9)	133.2	7.99 (1H, dd, 8.5, 2.0)
7	111.0	6.92 (1H, d, 8.6)	111.1	6.97 (1H, d, 1.9)	111.1	6.97 (1H, d, 8.5)
7a	165.4		165.3		165.3	
8	144.6		140.8		144.3	
9	63.5	4.26 (1H, d, 13.9) 4.16 (1H, d, 13.9)	71.9	4.54 (1H, d, 12.4) 4.35 (1H, d, 12.4)	63.9	4.25 (1H, d, 13.5) 4.20 (1H, d, 13.5)
10	114.5	5.42 (1H, s) 5.39 (1H, s)	115.9	5.46 (1H, s) 5.47 (1H, s)	113.9	5.39 (2H, br s)
11	199.7		199.2		199.1	
12	26.6	2.59 (3H, s)	26.5	2.57 (3H, s)	26.5	2.57 (3H, s)
1′	105.4	4.58 (1H, d, 7.8)	103.7	4.38 (1H, d, 7.8)		
2′	75.1	3.17 (1H, dd, 9.0, 7.8)	75.0	3.25 (1H, dd, 8.9, 7.8)		
3′	78.1	3.37 (1H, dd, 9.1, 9.0)	78.1	3.31 (1H)[b]		
4′	71.3	3.29 (1H, dd, 9.0, 9.0)	71.6	3.31 (1H)[b]		
5′	78.0	3.43 (1H, m)	78.0	3.38 (1H, m)		
6′	62.7	4.00 (1H, dd, 11.7, 2.1) 3.76 (1H, dd, 11.7, 5.8)	62.8	3.89 (1H, dd, 11.9, 1.3) 3.67 (1H, dd, 11.9, 5.4)		

a) $\Delta\delta_C$: δ_C (**1**) − δ_C (**1a**). *b*) Chemical shifts were assigned by HMQC.

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Part 3

Chemical Constituents of *Scleropyrum pentandrum*

Chemical Constituents of *Scleropyrum pentandrum*

Introduction

Scleropyrum pentandrum (Dennst.) Mabb. [Santalaceae; Thai name: Khi-Non; syn. S. wallichianum (Wight & Arn.) Arn.] is a small tree, commonly found in the evergreen dipterocarp forests in North-eastern Thailand (Macklin and Parnell, 2005). In previous phytochemical investigation, nonpolar compounds such as acetylenic acids, fatty acids and sterol derivatives have been reported (Wang et al., 1992; Suksamrarn et al., 2005). The present paper describes the isolation and structure elucidation of 15 polar compounds, including five unprecedented furan-2-carbonyl C-glycosides (1–5, Fig. 1), two new phenolic glycosides (7–8, Fig. 1) in addition to five flavone C-glycosides (9–13), a phenolic glycoside (6), a nucleoside (14) and an amino acid (15) from the aqueous soluble fraction of leaves and twigs of this plant. Also, the isolated compounds 1–13 were evaluated for their antioxidant activity using DPPH and ORAC assays.

Experimental

General Procedure: NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for 1H NMR and 100 MHz for 13C NMR) spectrometers, whereas MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. CD spectra were recorded on a Jasco J-815 spectropolarimeter. For column chromatography (CC), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 lm, YMC) were used. HPLC (Jasco PU-980 pump) was

carried out on an ODS column (21.2 x 250 mm i.d., Vertisep™ AQS) with a Jasco MD-2010 detector at 220 nm. The flow rate was 8 ml/min. The spraying reagent used for TLC was 10% H2SO4 in H_2O–EtOH (1:1, v/v).

Plant Material: Dried leaves and twigs of S. pentandrum (Dennst.) Mabb. Were collected in April 2010 from Khon Kaen Province, Thailand. The plant was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0067) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation: Dried powder of leaves and twigs of *S. pentandrum* (2.4 kg) was extracted with MeOH at room temperature (each 20 L for 24 h, 3 times). The combined MeOH extracts were concentrated in *vacuo* to dryness. The resulting residue (374.4 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (235.9 g) was applied to a Diaion HP-20 column, and eluted with H_2O, H_2O–MeOH (1:1, v/v), MeOH and acetone, successively. The fraction eluted with H_2O–MeOH (1:1, v/v) (38.3 g) was subjected to silica gel CC using solvent systems EtOAc–MeOH (9:1, 4.0 L), EtOAc–MeOH-H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 8.0 L) and EtOAc–MeOH–H_2O (6:4:1, 6.0 L), respectively, to obtain six fractions. Fraction 1 (1.3 g) was applied to a RP-18 column using a gradient solvent system, H2O–MeOH (90:10→20:80, v/v) to provide seven fractions. Fraction 1–6 was purified by preparative HPLC-ODS using solvent system H2O–MeCN (80:20, v/v) to afford compound **10** (113.1 mg). Fraction **2** (4.0 g) was subjected to a RP-18 column using solvent system, H_2O–MeOH (90:10→20:80, v/v)

to provide seven fractions. Fraction 2–1 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to provide compounds **1** (149.7 mg) and **2** (32.0 mg). Fraction 4 (12.4 g) was applied to a RP-18 column using solvent system, H_2O–MeOH (90:10→20:80, v/v) to afford thirteen fractions. Fraction 4–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to give compounds **3** (81.8 mg), 7 (74.2 mg) and **8** (59.6 mg). Fraction 4–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **6** (264.0 mg) and **14** (15.1 mg). Compound **11** (24.3 mg) was crystallized from fraction 4–7. Fraction 4–8 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compound **12** (106.5 mg). Fraction 4–9 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to yield compound **13** (191.2 mg). Finally, fraction 5 (5.0 g) was similarly subjected to a RP-18 colum using, H2O–MeOH (90:10→20:80, v/v) to afford eleven fractions. Faction 5–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to provide compound **15** (15.0 mg). Fraction 5–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **4** (21.7 mg) and **5** (22.7 mg). Fraction 5–4 was purified by preparative HPLC-ODS with solvent system H2O–MeCN (85:15, v/v) to give compounds **9** (23.0 mg) and **12** (47.4 mg).

Scleropentaside A (1): White amorphous powder; $[\alpha]_D$ -28.8 (MeOH, *c* 1.00); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, m/z: 293.0432 $[M+Cl]^-$ (calcd for C11H14ClO7, 293.0434).

Reduction of scleropentaside A (1): To a solution of ketone 1 (45 mg) in EtOH (5 mL) was added solid $NaBH_4$ (40 mg) in small portion at 0 _C, and the reaction was further stirred at room temperature for an hour. The mixture was quenched with 5% NH4Cl aq. (5 mL) and evaporated to dryness in *vacuo* to afford a mixture of compounds **1a** and **1b** which was applied to a Diaion HP-20 column, and eluted with H_2O and MeOH, successively. The obtained solid from MeOH part was purified by preparative HPLC-ODS using solvent system H2O–MeCN (95:5, v/v) to afford compounds **1a** (14.0 mg) and **1b** (17.6 mg).

Reduced form of scleropentaside (1a): White amorphous powder; $[\alpha]_D$ +18.4 (MeOH, c 1.32); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, m/z: 295.0584 $[M+Cl]^-$ (calcd for $C_{11}H_{16}ClO_7$, 295.0590).

Reduced form of scleropentaside (1b): White amorphous powder; $[\alpha]_D$ –32.9 (MeOH, *c* 1.47); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, m/z: 295.0580 $[M+Cl]^-$ (calcd for $C_{11}H_{16}ClO_7$, 295.0590).

Scleropentaside B (2): White amorphous powder; $[\alpha]_D$ –36.9 (MeOH, *c* 1.20); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 2; negative HR-ESI-TOFMS, m/z: 439.1008 $[M+Cl]^-$ (calcd for $C_{17}H_{24}ClO_{11}$, 439.1013).

Scleropentaside C (3): White amorphous powder; $[\alpha]_D$ –39.8 (MeOH, *c* 1.00); for ^1H NMR (DMSO-d_6) spectroscopic data, see: Table 2; negative HRESI- TOFMS, m/z: 425.0849 $[M+Cl]^-$ (calcd for $C_{16}H_{22}ClO_{11}$, 425.0856).

Scleropentaside D (4): White amorphous powder; $[\alpha]_D$ –42.0 (MeOH, *c* 0.19); CD (MeOH) $\Delta\varepsilon$ (nm) +9.1 (238), -2.7 (274), and -1.1 (312); for ^1H and ^{13}C NMR

(DMSO-d_6) spectroscopic data, see: Table 3; negative HR-ESI-TOFMS, m/z: 559.0720 $[M–H]^-$ (calcd for $C_{25}H_{19}O_{15}$, 559.0729).

Scleropentaside E (5): White amorphous powder; $[\alpha]_D$ +5.0 (MeOH, *c* 1.07); CD (MeOH) Δε (nm) +8.0 (237), -1.9 (269), +0.7 (292), and -0.6 (315); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 3; negative HR-ESI-TOFMS, m/z: 711.0843 [M-H]– (calcd for $C_{32}H_{23}O_{19}$, 711.0839).

4-Hydroxy-3-methoxybenzyl alcohol 4-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (7): Yellow amorphous powder; $[\alpha]_D$ –56.9 (MeOH, *c* 1.02); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 4; negative HR-ESI TOFMS, m/z: 483.1287 $[M+Cl]^-$ (calcd for $C_{19}H_{28}ClO_{12}$, 483.1275).

2,6-Dimethoxy-p-hydroquinone 1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (8): White amorphous powder; $[\alpha]_D$ -57.0 (MeOH, *c* 1.05); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 5; negative HR-ESI-TOFMS, m/z: 499.1223 $[M+Cl]^-$ (calcd for $C_{19}H_{28}ClO_{13}$, 494.1224).

Determination of the absolute configurations of sugars: Monosaccharide subunits of scleropentasides B, C (**2, 3**) and compounds **7, 8** were obtained by acid hydrolysis. Each sample (*ca* 10 mg) in 2 N HCl-dioxane (1:1, 4 ml) was heated at 80 °C for 5 h, except for compounds **7, 8** whose reaction conditions included was heating for 15 h. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous layer was neutralized with 2 N KOH, concentrated to dryness providing the sugar fraction. Each of these was dissolved in H_2O (2 mL) and partitioned with n-BuOH (2 ml). Each organic part was concentrated in *vacuo* to a

provide residue, which was analysed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep™ sugar LMP column, 7.8 x 300 mm i.d.; mobile phase water; flow rate 0.4 ml/min; temperature 80 °C) and comparison of their retention times and optical rotations with authentic samples.

Hydrolysis of scleropentasides B (**2**) gave peaks corresponding to L-rhamnose at 22.5 min (positive optical rotation) and scleropentaside A (**1**) at 32.5 min (negative optical rotation).

Hydrolysis of scleropentasides C (**3**) gave peaks of D-xylose (positive optical rotation) at 20.5 min and scleropentaside A (**1**) at 32.5 min (negative optical rotation).

Hydrolysis of compound **7** gave peaks of D-glucose and D-xylose at 19.0 min and 20.8 min, respectively (both positive optical rotations).

Hydrolysis of compound **8** gave peaks of D-glucose and D-xylose at 19.0 min and 20.5 min, respectively (both positive optical rotations).

Results and discussion

The methanolic extract of the leaves and twigs of S. pentandrum was partitioned with Et2O. The aqueous soluble fraction was separated by combination of chromatographic methods to provide 15 compounds. Eight compounds were identified as potalioside B (**6**) (Li et al., 2005), luteolin 6-*C*-β-D-glucopyranoside (isoorientin, **9**) (Kumarasamy et al., 2004), apigenin 8- *C*-β-D-glucopyranoside (vitexin, **10**) (Eldahshan et al., 2008), apigenin 6,8-di-*C*-β-D-glucopyranoside (vicenin-2, **11**), apigenin 6-C-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranoside

(isoschaftoside, **12**) (Xie et al., 2003), apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-xylopyranoside (**13**), (Fiorentino et al., 2008), adenosine (**14**) (Kanchanapoom et al., 2001) and L-tryptophan (*C*-β-) (Kanchanapoom et al., 2007) by comparison of physical data with literature values and from spectroscopic evidence.

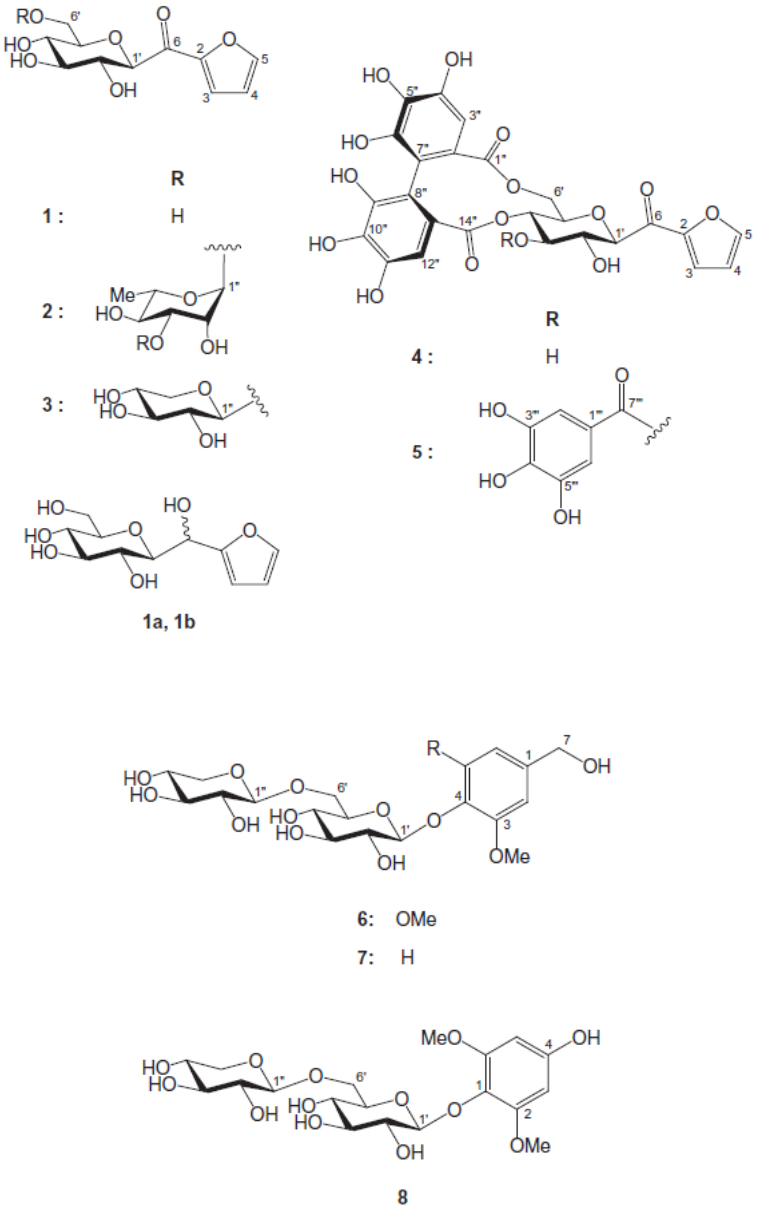

Fig. 1 Structures of isolated compounds **1–8**

Compound **1** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{11}H_{14}O_7$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The 1H NMR spectrum (Table 1) indicated the presence of a 2-furanyl group from the chemical shifts at δ_H 8.03 (d, J = 1.6 Hz), 7.60 (d, J = 3.6 Hz), and 6.73 (dd, J = 3.6, 1.6 Hz), and the resonances from δ_H 3.0 to 4.4 were suggested to be the signals of one sugar moiety. Analysis of the ^{13}C NMR spectroscopic data showed that this compound contained a furan-2-carbonyl part from a set of the chemical shifts at δ_C 151.5, 121.4, 112.8 and 148.8, and one carbonyl group at δ_C 184.6 (Pouchert and Behnke, 1993). The remaining six carbons at δ_C 79.7, 71.7, 78.1, 70.1, 81.7 and 61.3 belonging to the sugar part could be identified as a *C*-β-glucopyranosyl unit by comparing the chemical shifts with those of compounds **9**–**13**, and this was confirmed by the detailed analysis of COSY, HMQC (Fig. 2) along with the results from the splitting pattern of each axial protons in the sugar moiety (Table 1). A HMBC experiment provided the structure assignment (Fig. 2) from the correlations between (i) H-1' and C-2 and C-6, (ii) H-2' and C-6, and (iii) H-3 and C-6. Consequently, the structure of this compound was elucidated as furan-2-carbonyl *C*-β-glucopyranoside, namely scleropentaside A. However the presence of a *C*-glycoside connecting with the carbonyl group was unusual from a natural source. In order to confirm the structure, the carbonyl group of compound **1** was reduced to a hydroxyl group, affording two hydroxyl forms (**1a** and **1b**). The chemical shifts from analysis of ^{1}H and ^{13}C NMR spectra around the secondary alcohol in **1a** and **1b** significantly changed as compared to those of **1** (Table 1). Also, the partial connectivities of the structures 1a and 1b were observed from C-6 to C-6' by COSY and HMQC spectral analysis. HMBC experiments of both forms displayed significant

correlations from H-6 to C-2, C-3, C-1' and C-2', providing strong evidence of the structure of scleropentaside A (**1**).

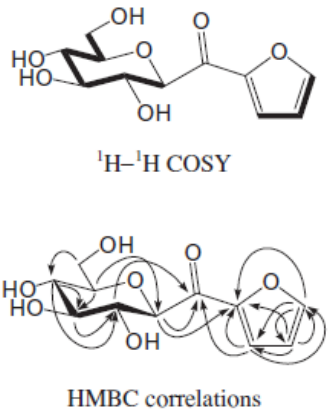

Fig. 2 COSY and HMBC correlations of scleropentaside A (**1**)

Table 1
NMR spectroscopic data of scleropentasides A (**1**) and its reduced forms (**1a** and **1b**) (DMSO-d_6).

Position	1		1a		1b	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		151.5		154.6		156.7
3	7.60 (1H, d, *J* = 3.6 Hz)	121.4	6.34 (1H, d, *J* = 3.1 Hz)	107.6	6.36 (1H, s)	106.5
4	6.73 (1H, dd, *J* = 3.6, 1.6 Hz)	112.8	6.38 (1H, dd, *J* = 3.1, 1.8 Hz)	110.3	6.36 (1H, s)	110.3
5	8.03 (1H, d, *J* = 1.6 Hz)	148.8	7.54 (1H, d, *J* = 1.8 Hz)	141.4	7.52 (1H, s)	141.2
6		184.6	4.77 (1H, d, *J* = 2.8 Hz)	66.5	4.75 (1H, br s)	64.2
1′	4.30 (1H, d, *J* = 9.5 Hz)	79.7	3.37 (1H, dd, *J* = 9.7, 2.8 Hz)	81.5	3.29 (1H, br d, *J* = 9.5 Hz)	79.7
2′	3.47 (1H, dd, *J* = 9.5, 9.1 Hz)	71.7	2.91 (1H, dd, *J* = 9.7, 8.7 Hz)	71.2	3.34 (1H, dd, *J* = 9.5, 8.3 Hz)	69.3
3′	3.31 (1H, dd, *J* = 9.1, 8.4 Hz)	78.1	3.15 (1H, dd, *J* = 8.7, 8.7 Hz)	78.3	3.19 (1H, dd, *J* = 8.5, 8.3 Hz)	78.2
4′	3.15 (1H, dd, *J* = 9.1, 8.4 Hz)	70.1	2.93 (1H, dd, *J* = 9.5, 8.7 Hz)	70.5	3.13 (1H, dd, *J* = 9.2, 8.5 Hz)	69.5
5′	3.27 (1H, m)	81.7	3.07 (1H, m)	80.9	3.09 (1H, m)	80.1
6′	3.68 (1H, dd, *J* = 11.8, 1.2 Hz) 3.45 (1H, dd, *J* = 11.8, 6.3 Hz)	61.3	3.65 (1H, br d, *J* = 11.2 Hz) 3.40 (1H, dd, *J* = 11.2, 3.5 Hz)	62.0	3.61 (1H, br d, *J* = 11.2 Hz) 3.45 (1H)[a]	60.8

[a] Chemical shifts were assigned from COSY and HMQC spectra.

Compound **2** was obtained as an amorphous powder and its molecular formula was determined to be $C_{17}H_{24}O_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra were related to those of compound **1**, except for a set of additional signals of α-L-rhamnopyranosyl unit deduced from the chemical shifts of its anomeric proton at δ_H 4.53 (1H, d, J = 1.5

Hz) and a secondary methyl protons at δ_H 1.09 (3H, d, J = 5.2 Hz). In the ^{13}C NMR spectrum, a set of the carbon signals for this sugar unit at δ_C 100.7, 70.5, 70.7, 72.0, 68.4 and 18.0 was observed. This additional moiety was assigned to be located at C-6' of the glucopyranosyl moiety due to the downfield shift of this carbon atom to δ_H 67.0 as compared to compound 1. A HMBC spectrum provided strong confirmation of the structure from the three bond correlation from H-1" (δ_H 4.53, d, J = 1.5 Hz) to C-6'. In addition, acid hydrolysis yielded scleropentaside A (**1**) and L-rhamnose, which were identified by HPLC analysis using the optical rotation detector. Accordingly, this compound was characterized as furan-2-carbonyl C-(6"-*O*-α-L-rhamnopyranosyl)-β-glucopyranoside, namely scleropentaside B.

Compound **3** was isolated as an amorphous powder. The molecular formula was identified as $C_{16}H_{22}O_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The NMR spectra were very similar to those of compounds **1** and **2**, indicating that this compound was a functional group analogue of both compounds. From the 13C NMR spectrum, a set of the chemical shifts of β-D-xylopyranosyl unit at δ_C 104.0, 73.4, 76.7, 69.7 and 68.8 were found instead of those of α-L-rhamnopyranosyl unit in compound **2**. This sugar moiety was also assigned to be attached to C-6' of a β-D-glucopyranosyl moiety, since the appearance of this carbon atom at δ_C 65.8. The structure was also confirmed by HMBC spectrum, in which correlation were found between H-1" (δ_H 4.12, d, J = 7.5 Hz) and C-6'. Acid hydrolysis liberated scleropentaside A (1) and D-xylose. Therefore, the structure of compound **3** was identified as furan-2-carbonyl *C*-(6"-O-β-D-xylopyranosyl)-β-glucopyranoside, namely scleropentaside C.

Table 2
NMR spectroscopic data of scleropentasides B (**2**) and C (**3**) (DMSO-d_6).

Position	2		3	
	δ_H	δ_C	δ_H	δ_C
2		151.1		151.4
3	7.59 (1H, d, *J* = 3.6 Hz)	121.8	7.61 (1H, d, *J* = 3.5 Hz)	121.7
4	6.73 (1H, dd, *J* = 3.6, 1.7 Hz)	112.8	6.72 (1H, dd, *J* = 3.5, 1.6 Hz)	112.9
5	8.03 (1H, d, *J* = 1.7 Hz)	148.6	8.02 (1H, d, *J* = 1.6 Hz)	148.8
6		184.4		184.5
1′	4.26 (1H, d, *J* = 9.6 Hz)	79.8	4.29 (1H, d, *J* = 9.5 Hz)	79.7
2′	3.42 (1H)[a]	71.7	3.43 (1H, dd, *J* = 9.5, 9.2 Hz)	71.6
3′	3.28 (1H, dd, *J* = 8.8, 8.3 Hz)	77.8	3.30 (1H, dd, *J* = 9.2, 9.0 Hz)	77.9
4′	3.12 (1H, dd, *J* = 9.2, 8.3 Hz)	70.0	3.16 (1H, dd, *J* = 9.1, 9.0 Hz)	69.9
5′	3.42 (1H, m)	80.1	3.45 (1H, m)	80.1
6′	3.82 (1H, br d, *J* = 9.9 Hz) 3.45 (1H)[a]	67.0	3.66 (1H, dd, *J* = 11.2, 5.3 Hz) 2.98 (1H, br d, *J* = 11.2 Hz)	65.8
1″	4.53 (1H, d, *J* = 1.5 Hz)	100.7	4.12 (1H, d, *J* = 7.5 Hz)	104.0
2″	3.58 (1H, dd, *J* = 3.2, 1.5 Hz)	70.5	2.95 (1H)[a]	73.4
3″	3.42 (1H)[a]	70.7	3.06 (1H, dd, *J* = 8.7, 8.5 Hz)	76.7
4″	3.16 (1H, dd, *J* = 9.2, 9.2 Hz)	72.0	3.26 (1H, m)	69.7
5″	3.37 (1H, m)	68.4	3.96 (1H, br d, *J* = 10.6 Hz) 3.56 (1H, dd, *J* = 10.6, 5.2 Hz)	68.8
6″	1.09 (3H, d, *J* = 6.2 Hz)	18.0		

[a] Chemical shifts were assigned from COSY and HMQC spectra.

Compound **4** was isolated as an amorphous powder. Its molecular formula was identified as $C_{25}H_{20}O_{15}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data (Table 3) suggested the presence of furan-2-carbonyl *C*-β-glucopyranoside as a core structure. In addition, the signals of the hexahydroxydiphenoyl (HHDP) group were observed in the NMR spectra (Okuda et al., 1983). The structure assignment of compound 4 was based on the results from COSY, HMQC and HMBC spectroscopic methods. COSY and HMQC were used to determine protons for observing the splitting pattern of each sugar protons (Table 3), confirming that the sugar part of this compound was a β-glucopyranosyl moiety. Analysis of the HMBC spectrum provided assignment of the HHDP group to C-4' and C-6' of the glucopyranosyl part from the three bond correlations between H-40 (δ_H 4.61, dd, J = 9.6, 9.2 Hz) and C-14" (δ_C 167.2) as well as H2-6' (δ_H 4.96, dd, J = 12.9, 5.7 Hz and δ_H 3.70, br d, J = 12.9 Hz) and C-1" (δ_C 168.2) as illustrated in Fig. 3. The absolute configuration of the

hexahydroxydiphenoyl group was established as S configuration from the CD spectrum, which showed extreme values for Δε (nm) +9.1 (238), -2.7 (274), and -1.1 (312), relating to the data of (*S*)-configurated compounds (Okuda et al., 1983). Therefore, the structure of compound 4 was elucidated to be furan-2-carbonyl *C*-[4,6-di-*O*-(*S*)-hexahydroxydiphenoyl]-β-glucopyranoside, namely scleropentaside D.

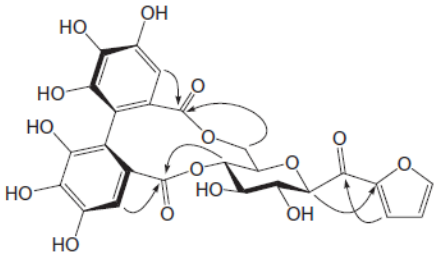

Fig. 3 Significant HMBC correlations of scleropentaside D (**4**)

Compound **5** was obtained as an amorphous powder and the molecular formula was identified to be $C_{32}H_{24}O_{19}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The NMR spectroscopic data were very similar to those of compound **4**, except for a set of signals arising from a galloyl group at δ_H 6.89 (2H, s) in the 1H NMR spectrum and δ_C 119.2, 109.0(2C), 145.3(2C), 138.5, 165.7 in the 13C NMR spectrum in compound **5**. This galloyl unit was suggested to be located at C-30 of the glucopyranosyl moiety with the noted downfield shift of this carbon atom to δ_C 75.9 together with the upfield shifts of C-2' and C-4' to δ_C 69.3 and 69.9, respectively, as compared to **4**. The complete assignment was confirmed by COSY, HMQC and HMBC spectroscopic methods, in

which long range correlations were observed from the HMBC spectrum between (i) H-3' (δ_H 5.35, dd, J = 9.5, 9.4 Hz) and C-7''' (δ_C 165.7), (ii) H-4' (δ_H 4.78, dd, J = 9.9, 9.9 Hz) and C-14'' (δ_C 167.0), and iii) H2-6' (δ_H 5.06, dd, J = 13.4, 6.2 Hz and δ_H 3.78, br d, J = 13.4 Hz) and C-1'' (δ_C 168.2) as shown in Fig. 4. The biphenyl configuration was assigned to be S by its CD spectrum, which established extreme values for Δε (nm) +8.0 (237), -1.9 (269), +0.7 (292), and -0.6 (315) as compared to compound **4**. Consequently, compound **5** was identified to be 3'-*O*-galloyl-derivative of compound **4**, namely scleropentaside E.

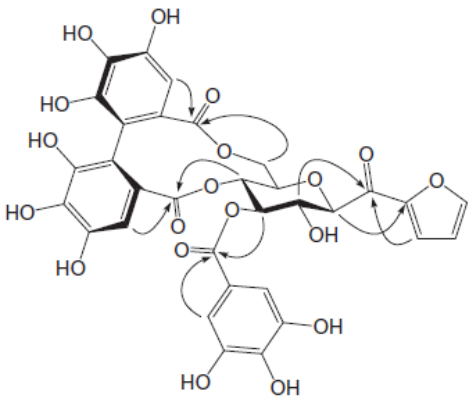

Fig. 4 Significant HMBC correlations of scleropentaside E (**5**)

Table 3
NMR spectroscopic data of scleropentasides D (**4**) and E (**5**) (DMSO-d_6).

Position	4		5	
	δ_H	δ_C	δ_H	δ_C
2		151.5		151.4
3	7.62 (1H, d, *J* = 3.6 Hz)	121.5	7.71 (1H, d, *J* = 3.6 Hz)	122.1
4	6.78 (1H, dd, *J* = 3.6, 1.6 Hz)	112.9	6.81 (1H, dd, *J* = 3.6, 1.5 Hz)	113.0
5	8.08 (1H, d, *J* = 1.6 Hz)	149.1	8.13 (1H, d, *J* = 1.5 Hz)	149.5
6		183.4		182.5
1′	4.53 (1H, d, *J* = 9.2 Hz)	79.7	4.79 (1H, d, *J* = 9.5 Hz)	79.0
2′	3.63 (1H, dd, *J* = 9.2, 8.8 Hz)	71.8	3.94 (1H, dd, *J* = 9.5, 9.3 Hz)	69.3
3′	3.57 (1H, dd, *J* = 9.2, 8.8 Hz)	75.5	5.35 (1H, dd, *J* = 9.5, 9.4 Hz)	75.9
4′	4.61 (1H, dd, *J* = 9.6, 9.2 Hz)	71.7	4.78 (1H, dd, *J* = 9.9, 9.9 Hz)	69.9
5′	4.01 (1H, m)	75.8	4.31 (1H, m)	75.6
6′	4.96 (1H, dd, *J* = 12.9, 5.7 Hz) 3.70 (1H, br d, *J* = 12.9 Hz)	63.0	5.06 (1H, dd, *J* = 13.4, 6.2 Hz) 3.78 (1H, br d, *J* = 13.4 Hz)	62.6
1″		168.0		167.6
2″		124.7		123.9
3″	6.34 (1H, s)	105.5	6.39 (1H, s)	105.7
4″		144.1		144.3
5″		135.1		135.3
6″		144.9		144.8
7″		115.4		115.4
8″		115.8		115.3
9″		145.0		144.8
10″		135.4		135.4
11″		144.1		144.3
12″	6.34 (1H, s)	106.2	6.27 (1H, s)	105.5
13″		124.5		124.4
14″		167.2		167.0
1‴				119.2
2‴,6″			6.89 (2H, s)	109.0
3‴,5″				145.3
4‴				138.5
7‴				165.7

Compound **7** was isolated as an amorphous powder. Its molecular formula was determined to be $C_19H_{28}O_{12}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis of the 1H NMR spectrum suggested the presence of 1,2,4-trisubstituted aromatic ring system from the chemical shifts at δ_H 7.07 (1H, d, J = 8.3 Hz), 6.93 (1H, br s) and 6.80 (1H, br d, J = 8.3 Hz), one singlet methylene proton signal at δ_H 4.42 (2H, s) and one methoxyl group at δ_H 3.75 (3H, s) in addition to two anomeric resonances at δ_H 4.83 (1H, d, J = 7.0 Hz) and 4.14 (1H, d, J = 7.5 Hz). The ^{13}C NMR spectrum showed 19 carbon signals, of which eight were assignable to two oxy-aryl carbons at δ_C 145.3, 148.8; three aryl-methines at δ_C 111.2,

115.6, 119.0; one aryl quarternary carbon at δ_C 136.5; one hydroxymethyl at δ_C 62.9; and one methoxyl group at δ_C 55.7 for the aglycone moiety. The remaining 11 carbons belonging to the sugar part could be identified as β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl unit by comparing the chemical shifts with those of potalioside B (**6**). The monosaccharide subunits were confirmed to be D-xylose and D-glucose by acid hydrolysis and identified by HPLC analysis using optical rotation detector. All protonated carbons were assigned by the result from HMQC spectrum. The structure of this compound was related to compound **6**, differing by the absence of one methoxyl group from the aromatic ring, and confirmed by the HMBC experiment. Significant correlations were observed between (i) H-7 (δ_H 4.42), and C-2 (δ_C 111.2), C-6 (δ_C 119.0); (ii) H-6 (δ_H 6.80) and C-4 (δ_C 145.3); (iii) MeO-3 (δ_H 3.75) and C-3 (δ_C 148.8); H-1' (δ_H 4.83) and C-4 (δ_C 145.3); and H-1" (δ_H 4.14); and C-6' (δ_C 68.2) as illustrated in Fig. 5. On the basis of these evidence, the structure of compound 7 was identified to be 4-hydroxy-3-methoxybenzyl alcohol 4-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

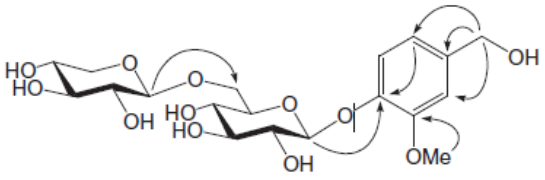

Fig. 5 Significant HMBC correlations of compound **7**

Table 4
NMR spectroscopic data of compound **7** (DMSO-d_6).

Position	δ_H	δ_C
1		136.5
2	6.93 (1H, br s)	111.2
3		148.8
4		145.3
5	7.07 (1H, d, J = 8.3 Hz)	115.6
6	6.80 (1H, br d, J = 8.3 Hz)	119.0
7	4.42 (2H, s)	62.9
MeO-3	3.75 (3H, s)	55.7
1′	4.83 (1H, d, J = 7.0 Hz)	100.3
2′	3.25 (1H)[a]	73.3
3′	3.25 (1H)[a]	76.8
4′	3.16 (1H, dd, J = 9.2, 8.3 Hz)	69.7
5′	3.47 (1H, m)	76.0
6′	3.90 (1H, br d, J = 11.0 Hz)	68.2
	3.57 (1H, dd, J = 11.0, 6.1 Hz)	
1″	4.14 (1H, d, J = 7.5 Hz)	103.8
2″	2.94 (1H, dd, J = 8.8, 7.5 Hz)	73.6
3″	3.05 (1H, dd, J = 8.8, 8.7 Hz)	76.5
4″	3.25 (1H)[a]	69.7
5″	3.64 (1H, dd, J = 11.2, 5.0 Hz)	65.7
	2.89 (1H, br d, J = 11.2 Hz)	

[a] Chemical shifts assigned from COSY and HMQC spectra.

Compound **8** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{28}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data indicated the presence of a tetrasubstituted symmetrical aromatic ring, two equivalent methoxyl groups for the aglycone moiety together with a β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl unit for the sugar part, as compared to compounds **6** and **7**. Acid hydrolysis provided D-xylose and D-glucose. The chemical shifts of the core structure were in agreement with those of 2,6-dimethoxy-p-hydroquinone 1-*O*-β-D-glucopyranoside (Otsuka et al., 1989). However, the additional signals of the xylopyranosyl unit were observed. The positions of two methoxyl groups were confirmed to be located at C- 2 and C-6 by an NOE difference

experiment, in which irradiation of the anomeric resonance of the glucopyranosyl unit at δ_H 4.62 gave an NOE enhancement of the methoxy signal at δ_H 3.75. Therefore, the structure of this compound was determined to be 2,6-dimethoxy-p-hydroquinone 1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl unit.

Table 5
NMR spectroscopic data of compound **8** (DMSO-d_6).

Position	δ_H	δ_C
1		127.4
2, 6		153.3
3, 5	6.05 (2H, s)	93.8
4		154.0
MeO-2,6	3.75 (6H, s)	56.2
1′	4.62 (1H, d, *J* = 7.0 Hz)	103.4
2′	3.21 (1H)[a]	74.1
3′	3.24 (1H)[a]	76.4
4′	3.16 (1H)[a]	69.8
5′	3.40 (1H, m)	76.0
6′	3.84 (1H, br d, *J* = 11.2 Hz)	68.2
	3.52 (1H, dd, *J* = 11.2, 4.5 Hz)	
1″	4.08 (1H, d, *J* = 7.5 Hz)	103.6
2″	2.90 (1H, dd, *J* = 8.7, 7.5 Hz)	73.4
3″	3.04 (1H, dd, *J* = 8.7, 8.6 Hz)	76.3
4″	3.25 (1H)[a]	69.6
5″	3.64 (1H, dd, *J* = 11.2, 5.2 Hz)	65.5
	2.95 (1H, br d, *J* = 11.2 Hz)	

[a] Chemical shifts assigned from COSY and HMQC spectra.

In this study, the isolated compounds **1–13** were evaluated for their radical scavenging activities using both DPPH and ORAC assays (Table 6) (Rangkadilok et al., 2007; Disadee et al., 2011). Previous studies reported that furan derivatives showed radical scavenging activity and cytoprotective effects (Nishio et al., 2008). The furan-2-carbonyl derivatives, scleropentasides A–C (**1–3**), bearing with mono- or disaccharides moiety displayed relative scavenging activity in the same range of Trolox, using ORAC assay, while in DPPH assay they were inactive. However when furan-2-carbonyl part connects with sugar moieties esterified by HHDP group

(scleropentaside D, **4**) and/or galloyl group (scleropentaside E, **5**), they were more potent than positive controls in both assays. These results were consistent with the previous report that polyphenols related compounds have been known to have antioxidant properties (Okuda and Ito, 2011). HHDP and galloyl groups were an important contributor towards the radical scavenging activities of scleropentasides D–E (**4–5**). It has been known that the antioxidant activities of phenolic natural products are predominantly due to their redox properties (Kumarasamy et al., 2004). Therefore, phenolic glycosides having no free hydroxyl group on phenyl ring (**6–7**) were inactive, whereas the other one having free hydroxyl group (**8**) was about 3 folds more potent than Trolox. In addition flavone C-glycosides (**9–13**) were also exhibited the most active in the ORAC assay with 2–8 folds more potent than Trolox.

Table 6
Radical scavenging activities of isolated compounds **1–13**.

Compounds	DPPH assay (SC_{50}, μM)[a,c]	ORAC (ROO·, unit)[b]
1	318 ± 2	0.3
2	I (43%)	0.6
3	I (6%)	0.2
4	17.5 ± 5.4	1.5
5	5.9 ± 0.4	1.5
6	I (4%)	0.2
7	I (21%)	0.5
8	I (48%)	3.0
9	21.9 ± 5.6	2.2
10	160 ± 2	4.9
11	134 ± 6	7.7
12	I (33%)	6.5
13	137 ± 4	6.6
Ascorbic acid	21.2 ± 1.4	ND
Trolox	ND	1

[a] SC_{50} is half-maximal scavenging concentration.
[b] 1 ORAC unit equals the net protection of fluorescein produced by 1 μM Trolox.
[c] I is inactive and numbers in parentheses indicate the percentage of scavenging.

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Part 4

Chemical Constituents of *Ruellia tuberosa* L.

Chemical Constituents of *Ruellia tuberosa* L.

Introduction

Ruellia tuberosa L. (Acanthaceae, Thai name: Toi-ting) is a perennial herb widely distributed in tropical areas of Asian countries. It has been externally used in Thai tradition medicine as an anti-inflammatory, an antiseptic as well as an antidote for detoxification of poisons. Previous phytochemical studies of this plant revealed the presence of steroids, terpenoids, long-chain aliphatic compounds and flavonoids [1–6]. In pharmacological studies, the aerial part extracts showed antioxidant, antinociceptive, and anti-inflammatory activities [7–9]. This present study deals with the isolation and structure determination of polar constituents including a new phenylethanoid (8) and two new flavone glycosides (11, 12) together with nine known compounds. Also, their antioxidant properties using DPPH and ORAC assays were evaluated.

Experimental

General Procedure: ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AV-400 and a Brucker AVANCE III ultrashield 300 MHz spectrometers. Mass spectra were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 lm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on ODS column (21.2 x 250 mm i.d., VertisepTM AQS, 8

mL/min) with a Jasco MD-2010 detector at 220 nm. The spraying reagent used for TLC was 10% H2SO4 in 50% EtOH.

Plant material: The aerial portions of Ruellia tuberosa L. were collected in March 2010, Bangkok, Thailand. The identification of the plant was done by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0069) is on file in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation: The air-dried aerial portions of R. tuberosa (3.2 kg) were macerated with MeOH three times (12 L for each extraction) at room temperature. The MeOH extract was concentrated in *vacuo* to dryness. This residue (318.1 g) was suspended in H2O, and partitioned with Et2O (each 1.0 L, 3 times). The aqueous soluble fraction (255.0 g) was subjected to a Diaion HP-20 column, and eluted with H_2O, MeOH and $(CH_3)_2CO$, successively. The fraction eluted with MeOH (41.5 g) was subjected to a silica gel column using solvent systems EtOAc–MeOH (9:1, 5.0 L), EtOAc–MeOH–H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 5.0 L) and EtOAc–MeOH–H_2O (6:4:1, 10.0 L), respectively, to afford seven fractions. Fraction 3 (6.6 g) was applied to a RP-18 column using a gradient solvent system H_2O–MeOH (90:10 → 20:80, v/v) to provide eight fractions. Fraction 3–4 (2.4 g) was purified by preparative HPLC-ODS using solvent system H2O–MeCN (80:20, v/v) to give compounds **1** (792.3 mg), **2** (171.8 mg), **3** (19.4 mg) and **4** (17.7 mg). Fraction 5 (9.7 g) was subjected to a RP-18 column using solvent system H2O–MeOH (90:10 → 20:80, v/v) to provide eleven fractions. Fraction 5–6 (3.4 g) was purified by preparative HPLCODS with solvent system H_2O–MeCN (80:20, v/v) to provide

compounds **5** (332.5 mg), **6** (32.6 mg), **7** (27.4 mg) and **8** (66.2 mg). Fraction 6 (5.8 g) was applied to a RP-18 column using solvent system H_2O–MeOH (90:10 → 20:80, v/v) to afford six fractions. Fraction 6–2 (2.1 g) was purified by preparative HPLC-ODS with solvent system H2O–MeCN (85:15, v/v) to obtain compounds **9** (87.8 mg), **10** (59.9 mg), **11** (89.0 mg) and **12** (90.1 mg).

Isocassifolioside (8): Yellow amorphous powder; $[\alpha]_D$ -54.5 (MeOH, *c* 1.05); IR spectrum: V_{max} = 3386, 2936, 1693, 1604, 1278, 1040 cm^{-1}; negative HRMS (APCI-TOF): $[M-H]^-$, found 769.2545. $C_{35}H_{45}O_{19}$ requires 769.2561. ^1H and ^{13}C NMR data: see Table 1.

Hispidulin 7-*O*-α-L-rhamnopyranosyl-(1'"→2")-*O*-β-D-glucuronopyranoside (11): Yellow amorphous powder; [a]D -44.2 (DMSO, *c* 1.00); IR spectrum: V_{max} = 3266, 2926, 1604, 1458, 1351, 1286, 1023 cm^{-1}; negative HRMS (APCI-TOF): $[M-H]^-$, found 621.1444. $C_{28}H_{29}O_{16}$ requires 621.1461. ^1H and ^{13}C NMR data: see Table 2.

Pectolinaringenin 7-*O*-α-L-rhamnopyranosyl-(1'"→2")-*O*-β-D-glucuronopyranoside (12): Yellow amorphous powder; [a]D -69.4 (DMSO, c 1.03); IR spectrum: V_{max} = 3320, 2926, 1603, 1459, 1354, 1250, 1022 cm-1; negative HRMS (APCI-TOF): $[M-H]^-$, found 635.1604. $C_{29}H_{31}O_{16}$ requires 635.1617. ^1H and ^{13}C NMR data: see Table 2.

Determination of the absolute configurations of sugars: Monosaccharide subunits of new compounds (**8, 11** and **12**) were obtained by acid hydrolysis. Each sample (ca. 5 mg) in 2 N HCl-dioxane (6:1, 3.5 ml) was heated at 80 °C for 6 h. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous

layer was concentrated to dryness providing the sugar fraction. Each of these was dissolved in H_2O (1 mL) and analysed by HPLC (Jasco OR-2090 plus chiral detector; column A: VertisepTM sugar LMP or column B: VertisepTM OA, 7.8 x 300 mm i.d.; mobile phase A: water or B: 0.003 M H_2SO_4 aq.; flow rate 0.4 ml/min; temperature 40 or 80 °C) and comparison of their retention times and optical rotations with authentic samples.

Hydrolysis of compound **8** gave peaks corresponding to D-glucose at 19.0 min and L-rhamnose at 23.0 min (both positive optical rotation) (column A, mobile phase A, 80 °C).

Hydrolysis of compound **11** gave peaks of D-glucuronic acid at 12.6 min and L-rhamnose at 15.2 min (both positive optical rotations) (column B, mobile phase B, 40 °C).

Hydrolysis of compound **12** gave peaks of D-glucuronic acid at 12.6 min and L-rhamnose at 15.2 min (both positive optical rotations) (column B, mobile phase B, 40 °C).

Results and discussion

The methanolic extract of the aerial part of R. tuberosa was suspended in H2O and extracted with Et_2O. The aqueous layer was applied to a column of Diaion HP-20, with H_2O, MeOH and Me_2CO as eluants, successively. The portion eluted with MeOH was separated by a combination of chromatographic procedures to provide 12 compounds (Fig. 1). Nine were identified as known compounds, including verbascoside (**1**), isoverbascoside (**2**), nuomioside (**3**), isonuomioside (**4**),

forsythoside B (**5**), paucifloside (**6**), cassifolioside (**7**), hispidulin 7-*O*-β-D-glucuronopyranoside (6-*O*-methyl-scutellarein, **9**) and comanthoside B (pectolinaringenin 7-*O*-β-D-glucuronopyranoside, **10**) on the basis of spectroscopic evidence and comparison of the physical data with reported values [10–16].

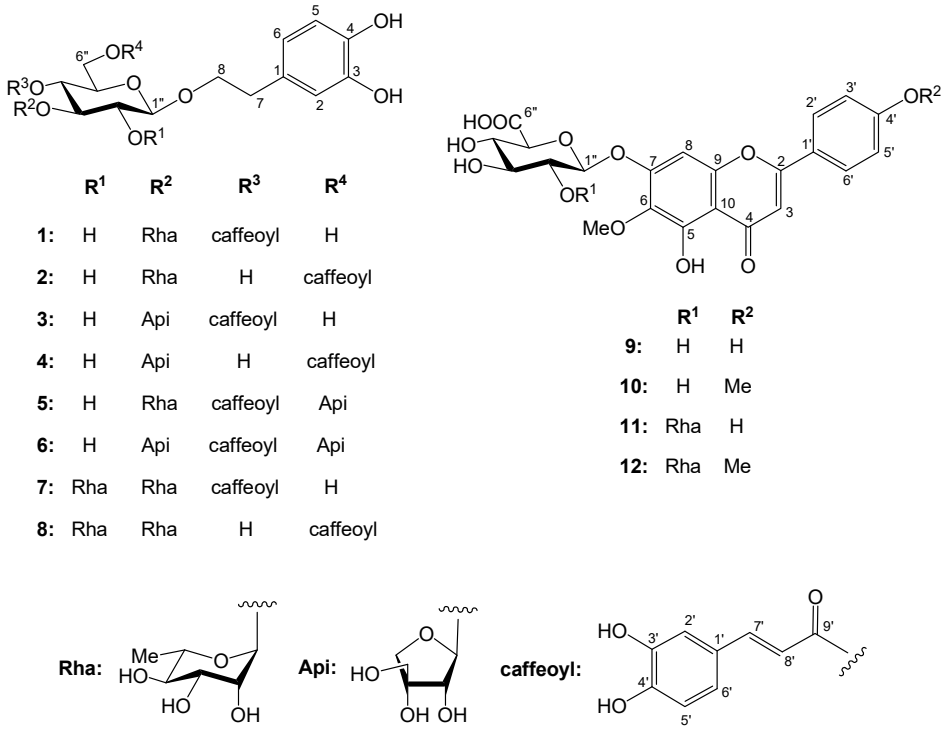

Fig. 1 Structures of compounds **1-12**

Compound **8** was isolated as a yellow amorphous powder, and its molecular formula was determined as $C_{35}H_{46}O_{19}$ by high-resolution atmospheric pressure chemical ionization time-of-flight (HR-APCI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) indicated that compound **8** is an analogue of compounds **1–7** with three sugar moieties. The chemical shifts were

related to those of isoverbascoside (**2**) except for a set of additional signals arising from an α-L-rhamnopyranosyl moiety, deduced from the chemical shifts of the carbon signals at δ_C 102.9, 72.3, 72.3, 73.6, 70.1 and 18.0. This sugar moiety was suggested to be attached at C-2" of the glucopyranosyl moiety due to the downfield shift of this carbon atom at δ_C 80.1 as compared to isoverbascoside (**2**). The assignment was confirmed by means of COSY, HMQC and HMBC. In the HMBC spectrum, the significant correlations were observed from H-1" (δ_H 4.43) to C-8 (δ_C 72.3), H-1"" (δ_H 4.98) to C-2" (δ_C 80.1), H-1"' (δ_H 4.93) to C-3" (δ_C 87.2), and H-6" (δ_H 4.50 and 4.34) to C-9' (δ_C 169.1) as illustrated in Fig. 2. In addition, acid hydrolysis provided D-glucose and L-rhamnose, identified by HPLC analysis using the optical rotation detector. Consequently, the structure was determined as shown. Since compound 8 was an isomer of crassifolioside (**7**), different on the location of the caffeoyl moiety at C-6" instead of C-4", therefore the name isocrassifolioside was proposed for this compound.

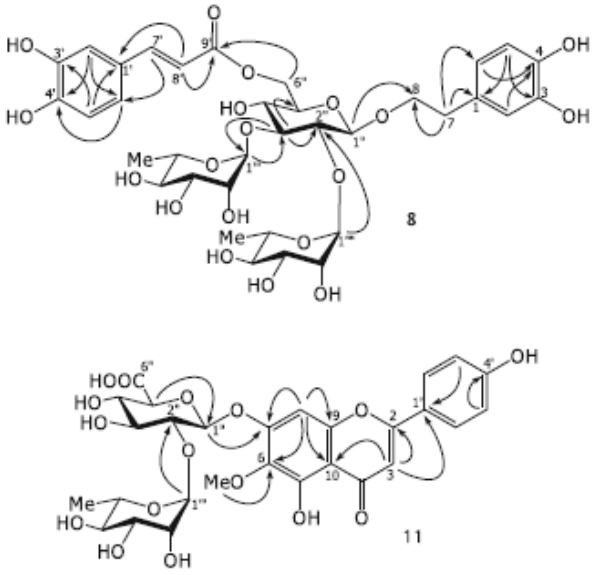

Fig. 2 HMBC correlations of compounds **8** and **11**

Table 1 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of compound **8** (MeOH-d_4)

Position	δ_C	δ_H
Aglycone		
1	131.2	
2	117.1	6.67 (1H, d, 1.9)
3	146.1	
4	144.7	
5	116.5	6.63 (1H, d, 8.1)
6	121.3	6.53 (1H, dd, 8.1, 1.9)
7	36.7	2.79 (2H, m)
8	72.3	3.95 (1H, m)
		3.70 (1H, m)
Caffoyl moiety		
1'	127.7	
2'	115.2	7.03 (1H, d, 1.9)
3'	146.7	
4'	149.5	
5'	116.6	6.77 (1H, d, 8.2)
6'	123.2	6.87 (1H, dd, 8.2, 1.9)
7'	147.2	7.55 (1H, d, 15.9)
8'	114.9	6.28 (1H, d, 15.9)
9'	169.1	
Glc		
1"	102.8	4.43 (1H, d, 7.7)
2"	80.1	3.46 (1H, dd, 8.9, 7.7)
3"	87.2	3.61 (1H, dd, 8.9, 8.9)
4"	70.7	3.44 (1H)[a]
5"	75.2	3.53 (1H)[a]
6"	64.5	4.50 (1H, dd, 11.7, 1.7)
		4.34 (1H, dd, 11.7, 5.9)
Rha-I		
1'"	103.7	4.93 (1H, brs)
2'"	72.4	3.89 (1H)[a]
3'"	72.3	3.71 (1H)[a]
4'"	73.9	3.42 (1H)[a]
5'"	70.7	3.98 (1H)[a]
6'"	17.8	1.19 (3H, d, 6.0)
Rha-II		
1""	102.9	4.98 (1H, brs)
2""	72.3	3.89 (1H)[a]
3'"	72.3	3.71 (1H)[a]
4""	73.6	3.42 (1H)[a]
5""	70.1	3.98 (1H)[a]
6""	18.0	1.21 (3H, d, 6.0)

[a] Signal unclear due to overlapping

Compound **11** had the molecular formula $C_{28}H_{30}O_{16}$ based on HR-APCI-TOF mass spectrometric analysis. The 1H- and 13C-NMR spectroscopic data (Table 2) were similar to those of hispidulin 7-*O*-β-D-glucuronopyranoside (**9**) except for the

presence of an α-L-rhamnopyranosyl unit. This sugar moiety was assigned to be attached at C-2" of the β-D-glucuronopyranosyl because the chemical shifts of C-1" and C-2" were significantly changed to 97.6 and 76.3 ppm, respectively, relative to compound **9**. Moreover, HMBC correlation was observed between H-1"' (δ_H 5.23) and C-2" (δ_C 76.3), as shown in Fig. 2. The absolute configuration of two sugar units were identified as D-glucuronic acid and L-rhamnose by HPLC analysis. Consequently, the structure of this compound was elucidated to be hispidulin 7-*O*-α-L-rhamnopyranosyl-(1"' → 2")-*O*-β-D-glucuronopyranoside.

The molecular formula of compound **12** was determined as $C_{29}H_{32}O_{16}$ by HR-APCI-TOF mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data (Table 2) revealed that this compound had the same aglycone as pectolinaringenin 7-*O*-β-D-glucuronopyranoside (**10**), while the sugar moieties were identical to those of compound **11**. Accordingly, this compound was determined as pectolinaringenin 7-*O*-α-L-rhamnopyranosyl-(1"' → 2")-*O*-β-D-glucuronopyranoside.

In this study, the isolated compounds **1–12** were evaluated for their radical scavenging activities using both DPPH and ORAC assays (Table 3) [17]. In DPPH assay, phenylethanoid glycosides (**1–8**) displayed relative scavenging activity in same range of the positive control, ascorbic acid, while flavonoid glycosides (**9–12**) were inactive. In the ORAC assay, the unit values of all isolated compounds were in the range of 2–6 folds more potent than the positive control, Trolox. The predominantly antioxidant activity of phenylethanoid glycosides (**1–8**) and was due to the presence of a caffeoyl and free two hydroxyl groups on phenyl ring. Position of a caffeoyl

group locating on C-4" or C-6" of sugar moiety seemed to be no effect on the activity. In addition, flavonoid glycosides having free hydroxyl group on phenyl ring (**9, 11**) were active about 2 folds more potent than methoxy analogues (**10, 12**).

Table 2 ^{1}H and ^{13}C NMR spectroscopic data of compounds **11** and **12** (DMSO-d_6)

No.	**11**		**12**	
	δ_C	δ_H	δ_C	δ_H
2	164.5		163.9	
3	102.3	6.65 (1H, s)	103.3	6.88 (1H, s)
4	182.2		182.3	
5	152.0		152.1	
6	132.9		133.0	
7	155.9		156.0	
8	94.1	6.86 (1H, s)	94.5	7.02 (1H, s)
9	152.5		152.5	
10	105.8		105.9	
1'	120.3		122.7	
2',6'	128.3	7.76 (2H, d, 8.5)	128.4	8.01 (2H, d, 8.9)
3',5'	116.1	6.78 (2H, d, 8.5)	114.7	7.09 (2H, d, 8.9)
4'	162.2		162.5	
6-OMe	60.4	3.78 (3H, s)	60.3	3.78 (3H, s)
4'-OMe			55.6	3.83 (3H, s)
5-OH		13.0 (1H, brs)		12.9 (1H, brs)
GlcA				
1"	97.6	5.33 (1H, d, 7.3)	98.0	5.33 (1H, d, 7.6)
2"	76.3	1H	76.1	3.61 (1H, dd, 8.8, 7.6)
3"	77.7	1H	77.7	3.54 (1H, dd, 9.3, 8.8)
4"	72.1	3.27 (1H, dd, 9.5, 9.0)	72.0	3.26 (1H, dd, 9.4, 9.3)
5"	73.9	1H	73.9	3.67 (1H, d, 10.0)
6"	172.7		172.2	
Rha				
1'"	100.2	5.23 (1H, brs)	100.1	5.27 (1H, brs)
2'"	70.6	3.70 (1H)	70.5	3.72 (1H)
3'"	70.5	3.33 (1H, dd, 9.6, 2.9)	70.5	3.38 (1H, dd, 9.4, 3.2)
4'"	72.0	3.16 (1H, dd, 9.6, 9.2)	72.0	3.18 (1H, dd, 9.4, 9.3)
5'"	68.7	3.70 (1H, m)	68.6	3.72 (1H, m)
6'"	18.1	1.08 (3H, d, 6.0)	18.1	1.08 (3H, d, 6.1)

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Part 5

Chemical Constituents of *Grammatophyllum speciosum* Blume

Chemical Constituents of *Grammatophyllum speciosum* Blume

Introcuction

Grammatophyllum speciosum Blume (Orchidaceae; Thai name: Waan-Phet-Cha-hueng) is the largest orchid of the world, distributed in the tropical rainforests of Thailand, Malaysia, Indonesia, and the Philippines. In Thai traditional medicine, the decoction made from pseudobulbs is used to treat sore throats and bronchitis, while the pseudobulb paste is applied externally to relieve pain from scorpion stings. Phytochemical investigation has not been reported for this species. However, the phytochemical compositions and pharmacological properties of orchids have been reviewed.[1] As part of our continuing studies on Thai medicinal plants, we investigated the polar constituents from this plant. This paper describes the isolation and structure determination of 10 polar compounds, including five glucosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid (**1-3**) and of (*R*)-eucomic acid (**4**, **5**), four phenolic glycosides (**6-9**), a flavone C-glucoside (**10**), and a nucleotide (**11**) from the pseudobulbs of this plant. Also, the structures of previously isolated glucosyloxybenzyl eucomate derivatives are discussed.

Experimental

General experimental procedures: NMR spectra were recorded in DMSO-d_6 using a Bruker AV-300 spectrometer. MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50

μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (20 × 250 mm i.d., YMC) with a Jasco RI-2031 refractive index detector. The flow rate was 6 mL/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant material: The fresh pseudobulbs of *Grammatophyllum speciosum* Blume were collected in March 2011 from the Herbal Paradise Garden, Chulabhorn Research Institute. The identification of the plant was confirmed by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0068) has been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation: The fresh pseudobulbs of *G. speciosum* (11.6 kg) were extracted with MeOH (30 L for 2 days, × 3) at room temperature. The MeOH extract was concentrated *in vacuo* to dryness. This residue (215.8 g) was suspended in H_2O (500 mL), and partitioned with Et_2O (3 × 1.0 L). The water soluble fraction (189.4 g) was subjected to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (35.4 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 7.0 L), EtOAc-MeOH-H_2O (40:10:1, 6.0 L), EtOAc-MeOH-H_2O (70:30:3, 5.0 L) and EtOAc-MeOH-H_2O (6:4:1, 9.0 L), respectively, to provide six fractions (A to F). Fraction B (2.9 g) was applied to a RP-18 column using a gradient solvent system H_2O-MeOH (9:1 → 1:4, v/v) to provide five fractions. Fractions B-1 and B-2 were combined and purified by preparative HPLC-ODS using solvent system H_2O-MeCN (9:1, v/v) to afford compounds **8** (1.3 g) and **9** (18.5 mg). Fraction C (1.7 g) was subjected to a RP-18

column using solvent system H_2O-MeOH (9:1 → 1:4, v/v) to provide six fractions. Fraction C-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (9:1, v/v) to yield compound **6** (34.8 mg). Fraction D (19.2 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (9:1 → 1:4, v/v) to afford ten fractions. Fraction D-1 was separated by preparative HPLC-ODS with solvent system H_2O-MeCN (9:1, v/v) to give compounds **1** (368.5 mg), **2** (343.4 mg) and fraction D-1-1. Fraction D-1-1 was further purified by preparative HPLC-Polyamine II with solvent system H_2O-MeCN (1:9, v/v) to provide compounds **7** (11.3 mg). Fraction D-4 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (4:1, v/v) to afford compound **10** (133.0 mg). Fraction D-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (4:1, v/v) to provide compound **5** (105.6 mg). Fractions D-7 and D-8 were combined and purified by preparative HPLC-ODS with solvent system H_2O-MeCN (7:3, v/v) to afford compound **3** (722.9 mg). Finally, fraction E (4.6 g) was similarly applied to a RP-18 column using H_2O-MeOH (9:1 → 1:4, v/v) to obtain seven fractions. Faction E-1 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (9:1, v/v) to provide compound **4** (320.9 mg). Fraction E-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (9:1, v/v) to afford compound **2** (316.0 mg). Fraction E-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (9:1, v/v) to give compound **1** (107.9 mg).

Cronupapine (1): Amorphous powder; $[\alpha]_D^{27}$–50.0 (MeOH, *c* 0.48); IR (UATR) ν_{max} 3364, 2920, 1727, 1583, 1512, 1400, 1230, 1075, 1043 cm^{-1}; ^1H NMR (DMSO-d_6 and MeOH-d_4): Table 1; ^{13}C-NMR (DMSO-d_6 and MeOH-d_4): Table 2; Significant HMBC correlations: (i) H-3 and C-1,2,4,5, (ii) H-5 and C-1,2,3,6,7, (iii)

H-7' and C-1,1',2',6', (iv) H-1" and C-4'; Negative HR-APCI-TOFMS, *m/z*: 491.1554 $[M - H]^-$ (calcd for $C_{24}H_{27}O_{11}$, 491.1559).

Grammatophylloside A (2): Amorphous powder; $[\alpha]_D^{26}$ –32.4 (MeOH, *c* 0.84); IR (UATR) ν_{max} 3369, 2920, 1726, 1599, 1513, 1400, 1342, 1226, 1072, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C-NMR (DMSO-d_6): Table 2; Negative HR-APCI-TOFMS, *m/z*: 491.1554 $[M - H]^-$ (calcd for $C_{24}H_{27}O_{11}$, 491.1559).

Grammatophylloside B (3): Amorphous powder; $[\alpha]_D^{26}$ –52.2 (MeOH, *c* 0.61); IR (UATR) ν_{max} 3328, 2920, 1724, 1610, 1513, 1230, 1068, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C-NMR (DMSO-d_6): Table 2; Negative HR-APCI-TOFMS, *m/z*: 795.2285 $[M + Cl]^-$ (calcd for $C_{37}H_{44}Cl^{35}O_{17}$, 795.2273).

Grammatophylloside C (4): Amorphous powder; $[\alpha]_D^{27}$ –28.0 (MeOH, *c* 0.79); IR (UATR) ν_{max} 3327, 2913, 1717, 1590, 1513, 1344, 1228, 1174, 1071, 1043 cm^{-1}; ^1H NMR (DMSO-d_6 and MeOH-d_4): Table 1; ^{13}C-NMR (DMSO-d_6 and MeOH-d_4): Table 2; Significant HMBC correlations: (i) H-3 and C-1,2,4,5, (ii) H-5 and C-1,2,3,6,7, (iii) H-7' and C-4,1'",2'",6'", (iv) H-1"" and C-4'"; H- Negative HR-APCI-TOFMS, *m/z*: 507.1494 $[M - H]^-$ (calcd for $C_{24}H_{27}O_{12}$, 507.1508).

Vandateroside II (5): Amorphous powder; $[\alpha]_D^{26}$ –30.6 (DMSO, *c* 0.70); IR (UATR) ν_{max} 3320, 2931, 1723, 1614, 1515, 1357, 1232, 1068, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C-NMR (DMSO-d_6): Table 2; Negative HR-APCI-TOFMS, *m/z*: 811.2235 $[M + Cl]^-$ (calcd for $C_{37}H_{44}Cl^{35}O_{18}$, 811.2222).

Alkaline hydrolysis of compounds 1-5: Compound **1** (60 mg, 0.12 mmol) was treated with sodium hydroxide solution (5 mL, 7% aq.) and stirred at room temperature for 6 h. The reaction mixture was neutralized with hydrochloric acid

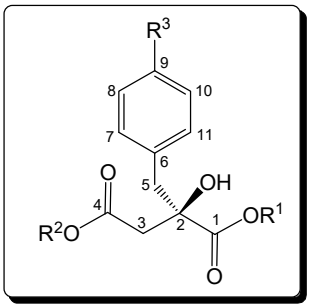
solution (5% aq.) and extracted with EtOAc. The organic layer was separated and the solvent was evaporated *in vacuo* to give (*R*)-benzylmalic acid **1a** (12.0 mg, 45%). By the same method, compounds **2** (60 mg, 0.12 mmol) and **3** (100 mg, 0.13 mmol) provided **1a** (23.4 mg, 87% and 24.5 mg, 83%, respectively). Compounds **4** (95 mg, 0.19 mmol) and **5** (60 mg, 0.08 mmol) yielded **4a** (17.9 mg, 40% and 4.5 mg, 24%, respectively). The structures of **1a** and **4a** were identified by 1D and 2D NMR spectroscopic analysis together with comparison of the optical rotation data with literature values.

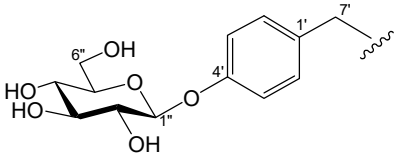
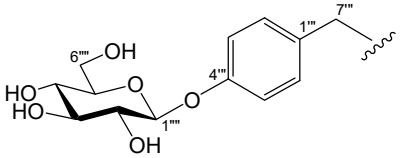
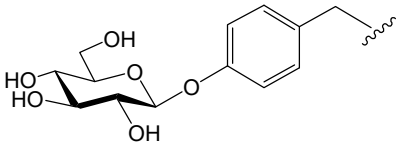
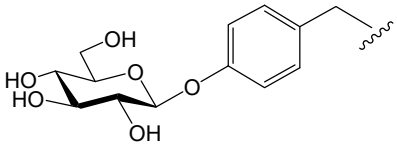
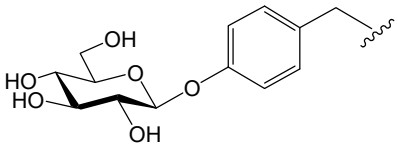
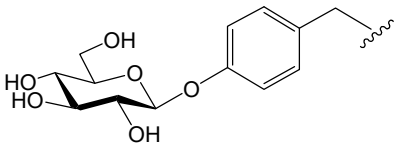
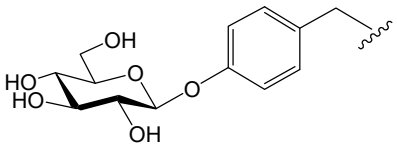
(*R*)-Benzylmalic acid (1a): Amorphous powder; IR (UATR) ν_{max} 3446, 2927, 1716, 1496, 1390, 1207, 1115, 1022 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.19-7.28 (5H, *m*, Ar-H), 2.92 (1H, *d*, *J* = 13.4 Hz, H-5a), 2.86 (1H, *d*, *J* = 13.4 Hz, H-5b), 2.72 (1H, *d*, *J* = 15.8 Hz, H-3a), 2.38 (1H, *d*, *J* = 15.8 Hz, H-3b), or ^{1}H-NMR (MeOH-d_4): δ 7.20-7.26 (5H, *m*, Ar-H), 3.04 (1H, *d*, *J* = 13.4 Hz, H-5a), 2.97 (1H, *d*, *J* = 13.4 Hz, H-3a), 2.94 (1H, *d*, *J* = 15.8 Hz, H-5b), 2.59 (1H, *d*, *J* = 15.8 Hz, H-3b); ^{13}C-NMR (DMSO-d_6): δ 175.6 (C-1), 171.6 (C-4), 136.2 (C-6), 130.6 (C-7, 11), 127.8 (C-8, 10), 126.5 (C-9), 74.7 (C-2), 44.4 (C-5), 42.8 (C-3), or ^{13}C-NMR (MeOH-d_4): δ 177.6 (C-1), 174.1 (C-4), 136.9 (C-6), 131.6 (C-7, 11), 129.0 (C-8, 10), 127.8 (C-9), 76.7 (C-2), 46.1 (C-5), 43.8 (C-3); Negative HR-APCI-TOFMS, *m/z*: 223.0605 $[M - H]^-$ (calcd for $C_{11}H_{11}O_5$, 223.0612).

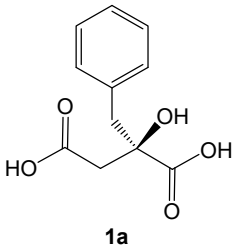
(*R*)-Eucomic acid (4a): Amorphous powder; IR (UATR) ν_{max} 3460, 3030, 2607, 1731, 1515, 1370, 1192, 1135, 1070 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.20 (1H, *s*, 9-OH), 6.99 (2H, *d*, J = 8.4 Hz, H-7,11), 6.63 (2H, d, J = 8.4 Hz, H-8,10), 2.80 (1H, *d*, *J* = 13.4 Hz, H-5a), 2.74 (1H, *d*, *J* = 13.4 Hz, H-5b), 2.70 (1H, *d*, *J* = 15.8 Hz, H-3a),

2.35 (1H, *d*, *J* = 15.8 Hz, H-3b), or ^{1}H-NMR (MeOH-d_4): δ 7.06 (2H, *d*, J = 8.5 Hz, H-7,11), 6.68 (2H, d, J = 8.5 Hz, H-8,10), 2.95 (1H, *d*, *J* = 13.9 Hz, H-5a), 2.94 (1H, *d*, *J* = 16.0 Hz, H-3a), 2.85 (1H, *d*, *J* = 13.9 Hz, H-5b), 2.56 (1H, *d*, *J* = 16.0 Hz, H-3b); ^{13}C-NMR (DMSO-d_6): δ 175.7 (C-1), 171.7 (C-4), 156.0 (C-9), 131.4 (C-7, 11), 126.2 (C-6), 114.6 (C-8, 10), 74.9 (C-2), 43.7 (C-5), 42.6 (C-3), or ^{13}C-NMR (MeOH-d_4): δ 177.7 (C-1), 174.2 (C-4), 157.4 (C-9), 132.6 (C-7, 11), 127.6 (C-6), 115.8 (C-8, 10), 76.8 (C-2), 45.4 (C-5), 43.6 (C-3); Negative HR-APCI-TOFMS, *m/z*: 239.0556 $[M - H]^-$ (calcd for $C_{11}H_{11}O_6$, 239.0561).

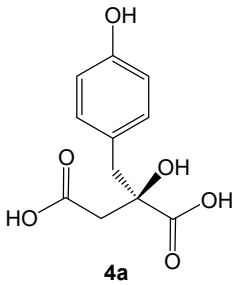
Grammatophylloside D (9): Amorphous powder; $[\alpha]_D^{26}$ −32.9 (MeOH, *c* 0.49); IR (UATR) ν_{max} 3336, 2919, 1698, 1597, 1456, 1304, 1115, 1170, 1071, 1021 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6): Table 3; Negative HR-APCI-TOFMS, *m/z*: 363.0835 $[M + Cl]^-$ (calcd for $C_{15}H_{20}Cl^{35}O_8$, 363.0841).



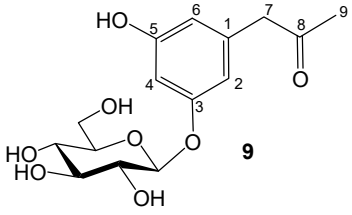
	R^1	R^2	R^3
1 :		H	H
2 :	H		H
3 :			H
4 :	H		OH
5 :			OH



1a



4a



9

Results and discussion

The methanolic extract of the fresh pseudobulbs of *G. speciosum* was partitioned between Et_2O and H_2O. The aqueous soluble fraction was separated by a combination of chromatographic methods to provide 10 compounds.

Compound **1** was isolated as an amorphous powder, and its molecular formula was determined to be $C_{24}H_{28}O_{11}$ by negative HR-APCI-TOF mass spectrometric analysis. The NMR spectroscopic and the physical data of compound **1** were coincident with those of cronupapine, first isolated from *Cronura papirio*.[2] The structure was confirmed by the respective HMBC correlations. However, the absolute configuration at the C-2 position was not identified. Thus, this compound was hydrolyzed in alkaline condition (see Experimental section) to afford 2-benzylmalic acid (**1a**), as a white powder, which was identified by ^1H and ^{13}C NMR spectroscopic analysis, displaying an optical rotation $[\alpha]_D^{27}$ −14.9 (H_2O, *c* 0.48). Since the optical rotation value reported for (*S*)-2-benzylmalic acid showed $[\alpha]_D^{23}$ +15.55 (H_2O, *c* 2.9)[3] and $[\alpha]_D^{29}$ +13.7 (H_2O, *c* 1.0),[4] the absolute configuration of C-2 position was concluded to be *R*.

Compound **2** was obtained as an amorphous powder, and its molecular formula was established as $C_{24}H_{28}O_{11}$ by negative HR-APCI-TOF mass spectrometric analysis. Inspection of the NMR spectroscopic data indicated that this compound contained a 2-benzylmaloyl moiety from the chemical shifts of five overlapping proton signals at δ_H 7.13 (2H) and 7.20 (3H) together with two sets of AB-type methylene protons at δ_H 2.41 and 2.71 (each *d*, J = 14.4 Hz) and δ_H 2.83 and 2.89 (each *d*, J = 13.7 Hz). These proton signals were in agreement with the relevant

carbon signals of a mono-substituted aromatic ring at δ_C 125.6 (1C), 127.3 (2C) and 130.6 (2C); and two methylene carbons at δ_C 43.7 and 44.5. The remaining signals belonging to this part were two carboxyl carbons at δ_C 170.6 and 176.8, an oxygenated quaternary carbon at δ_C 75.1, and a quaternary carbon of an aromatic ring at δ_C 138.4. In addition, the signals of the glucopyranosyloxybenzyl moiety were present as compared to **1**, supported by the proton signals of a 1,4-disubstituted aromatic ring at δ_H 7.30 and 7.00 (each 2H, *d*, J = 8.5 Hz) and an anomeric proton at δ_H 4.86 (1H, *d*, J = 7.3 Hz). This compound was an isomer of **1**, differing by the location of the glucopyranosyloxybenzyl moiety as connected through C-4 instead of C-1. The conclusion was corroborated by the results of the HMBC experiments (Fig. 2), i.e. correlations between H-5 and C-1, C-2 and C-3, and between H-7''' and C-4. The absolute configuration of C-2 was identified to be *R* by alkaline hydrolysis (see Section 4), to obtain (*R*)-2-benzylmalic acid (**1a**), $[\alpha]_D^{27}$ −18.4 (H_2O, *c* 0.57). Therefore, the structure of **2** was elucidated as 4-(4'''-β-glucopyranosyloxybenzyl)-(*R*)-2-benzylmalate, namely grammatophylloside A.

Compound **3** was isolated as an amorphous powder. The molecular formula was identified as $C_{37}H_{44}O_{17}$ by negative HR-APCI-TOF mass spectrometric analysis. The NMR spectra were closely related to those of compounds **1** and **2**, except for the observation of the additional signals for a second glucopyranosyloxybenzyl moiety. The two glucopyranosyloxybenzyl moieties were assigned to be connected through the two ester carbonyl groups based on HMBC correlations from the overlapping signals for H-7' and H-7''' (δ_H 4.94) to C-1 (δ_C, 173.6) and C-4 (δ_C 169.8), respectively. Consequently, this compound was identified to be 1,4-bis(4',4'''-β-glucopyranosyloxybenzyl)-(*R*)-2-benzylmalate, namely grammatophylloside B.

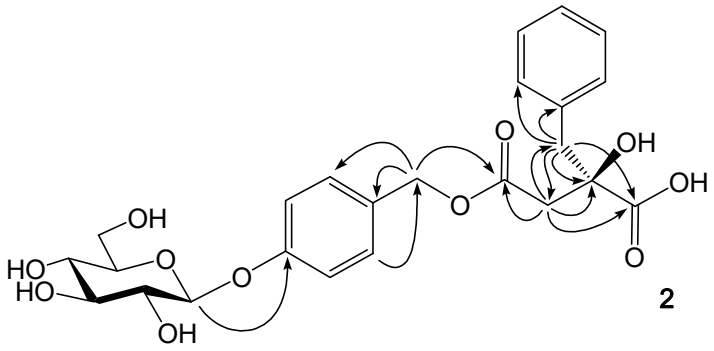


Figure 2. Significant HMBC correlations of grammatophylloside A (**2**)

Compound **4** was isolated as an amorphous powder. Its molecular formula was established as $C_{24}H_{28}O_{12}$ by negative HR-APCI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra indicated that this compound was an analogue of grammatophylloside A (**2**), differing by the presence of one additional hydroxyl function at C-9 of the 2-benzylmaloyl moiety. Consequently, the ^{1}H NMR spectrum showed a set of resonances corresponding to an AA'BB' aromatic ring system, comprising signals at δ_H 6.59 and 6.97 (each 2H, *d*, J = 8.3 Hz) instead of the signals of the mono-substituted aromatic ring systems of **1** and **2**. Assignment of all signals was based on the results of HMQC and HMBC experiments. Since the signals of two AB-type methylene protons of H_2-5 (δ_H 2.71 and 2.76) and one AB-type methylene proton of H-3 at δ_H 2.68 were overlapped in DMSO-d_6, another set of NMR data was acquired in MeOH-d_4 to provide resolved signals (Tables 1 and 2). In the HMBC spectrum, significant correlations were found from δ_H 2.78 (H-5) to δ_C 180.6 (C-1), 77.6 (C-2), 44.3 (C-3), 129.0 (C-6), 132.6 (C-7/11), and δ_H 5.03 (H-7''') to δ_C 177.0 (C-4), indicating that the glucopyranosyloxybenzyl moiety was connected through C-

4. Alkaline hydrolysis of **4** provided (*R*)-eucomic acid (**4a**) as a white powder, identified by spectroscopic data and an optical rotation value $[\alpha]_D^{26}$ −10.1 (MeOH, *c* 1.79), lit $[\alpha]_D^{28}$ −12.9 (MeOH, *c* 0.50).[5] Therefore, the structure of this new natural product was elucidated as shown, namely grammatophylloside C. Interestingly, the same structure had previously been assigned to vandateroside I, isolated from *Vanda teres*[6], but the spectroscopic data obtained in the present study clearly was not identical to the reported NMR data, especially for the (*R*)-eucomoyl part. The structure of vandateroside I was elucidated based on HMBC correlations, in particular from H_2-5 (δ_H 2.77) to C-1 (δ_C 171.6) and from H-7' (δ_H 4.98) to C-4 (δ_C 173.7).[6] However, as stated above the signals of H_2-5 overlapped with one proton signal of H-3 at δ_H 2.74 when measured in DMSO-d_6, leading to ambiguity in assigning C-1 and C-4. Even though vandateroside I was not isolated in the present study, our results strongly suggest that its structure should be revised.

Compound **5** was obtained as an amorphous powder, and its molecular formula was established as $C_{37}H_{44}O_{18}$ by negative HR-APCI-TOF mass spectrometric analysis. The spectroscopic and physical data were identical to those of vandateroside II.[6] However, previously the absolute configuration at C-2 was established as *R* only based on comparison of its negative optical rotation with that of (*R*)-eucomic acid. To provide stronger evidence, compound **5** was subjected to alkaline hydrolysis to give a white amorphous residue. This residue was identified to be (*R*)-eucomic acid (**4a**) with an optical rotation value $[\alpha]_D^{26}$ −15.1 (MeOH, *c* 0.45). Accordingly, the absolute configuration at C-2 position was unambiguously assigned to be *R*.

Table 1. [1]H-NMR spectroscopic data of compounds **1-5** (300 MHz)

Position	**1**[a]	**1**[b]	**2**[a]	**3**[a]
3	2.13 (1H, *d*, J = 15.6 Hz) 2.46 (1H, *d*, J = 15.6 Hz)	2.45 (1H, *d*, J = 16.1 Hz) 2.81 (1H, *d*, J = 16.1 Hz)	2.41 (1H, *d*, J = 14.1 Hz) 2.71 (1H, *d*, J = 14.1 Hz)	2.49 (1H, *d*, J = 15.6 Hz) 2.86 (1H, *d*, J = 15.6 Hz)
5	2.78 (1H, *d*, J = 13.3 Hz) 2.86 (1H, *d*, J = 13.3 Hz)	2.90 (1H, *d*, J = 13.5 Hz) 2.96 (1H, *d*, J = 13.5 Hz)	2.83 (1H, *d*, J = 13.7 Hz) 2.89 (1H, *d*, J = 13.7 Hz)	2.87 (1H, *d*, J = 13.4 Hz) 2.93 (1H, *d*, J = 13.4 Hz)
7/11 8/10 9	7.06-7.12 (2H, m)[c] 7.15-7.21 (3H, m)[c]	7.08-7.12 (2H, m)[c] 7.16-7.20 (3H, m)[c]	7.10-7.16 (2H, m)[c] 7.16-7.22 (3H, m)[c]	7.08-7.12 (2H, m)[c] 7.17-7.21 (3H, m)[c]
2'/6'	7.19 (2H, *d*, J = 8.5 Hz)	7.25 (2H, *d*, J = 8.6 Hz)		7.24 (2H, *d*, J = 8.7 Hz)[d]
3'/5'	6.99 (2H, *d*, J = 8.5 Hz)	7.07 (2H, *d*, J = 8.6 Hz)		6.99 (2H, *d*, J = 8.7 Hz)[e]
7'	4.91 (2H, *s*)	5.00 (2H, *s*)		4.94 (2H, *s*)
Glc-1"	4.87 (1H, *d*, J = 7.1 Hz)	4.93 (1H, *d*, J = 7.1 Hz)		4.85 (1H, *d*, J = 6.9 Hz)[f]
2" 3" 4" 5" 6"	3.13-3.23 (1H, m)[c] 3.23-3.33 (1H, m)[c] 3.13-3.23 (1H, m)[c] 3.31-3.37 (1H, m)[c] 3.49 (1H, *dd*, J = 11.3, 5.0 Hz) 3.69 (1H, *br d*, J = 11.3 Hz)	3.42-3.50 (1H, m)[c] 3.40-3.48 (2H, m)[c] 3.47-3.52 (1H, m)[c] 3.72 (1H, *dd*, J = 11.9, 4.6 Hz) 3.90 (1H, *dd*, J = 11.9, 1.4 Hz)		3.20-3.28 (2H, m)[c] 3.11-3.18 (1H, m)[c] 3.28-3.34 (1H, m)[c] 3.41-3.48 (1H, m)[c] 3.63-3.70 (1H, m)[c]
2'"/6'"			7.30 (2H, *d*, J = 8.5 Hz)	7.20 (2H, *d*, J = 8.7 Hz)[d]
3'"/5'"			7.00 (2H, *d*, J = 8.5 Hz)	6.98 (2H, *d*, J = 8.7 Hz)[e]
7'"			4.97 (2H, *s*)	4.94 (2H, *s*)
Glc-1""			4.86 (1H, *d*, J = 7.3 Hz)	4.87 (1H, *d*, J = 6.9 Hz)[f]
2"" 3"" 4"" 5"" 6""			3.25-3.33 (1H, m)[c] 3.20-3.28 (1H, m)[c] 3.14-3.22 (1H, m)[c] 3.32-3.37 (1H, m)[c] 3.48-3.52 (1H, m)[c] 3.68 (1H, *br d*, J = 11.5 Hz)	3.20-3.28 (2H, m)[c] 3.11-3.18 (1H, m)[c] 3.28-3.34 (1H, m)[c] 3.41-3.48 (1H, m)[c] 3.63-3.70 (1H, m)[c]

Table 1. (continued)

Position	4[a]	4[b]	5[a]
3	2.38 (1H, *d*, J = 14.2 Hz)	2.59 (1H, *d*, J = 15.6 Hz)	2.48 (1H, *d*, J = 15.6 Hz)
	2.68 (1H, *d*, J = 14.2 Hz)	3.00 (1H, *d*, J = 15.6 Hz)	2.88 (1H, *d*, J = 15.6 Hz)
5	2.71 (1H, *d*, J = 13.7 Hz)	2.78 (1H, *d*, J = 13.5 Hz)	2.77 (1H, *d*, J = 13.6 Hz)
	2.76 (1H, *d*, J = 13.7 Hz)	2.95 (1H, *d*, J = 13.5 Hz)	2.83 (1H, *d*, J = 13.6 Hz)
7/11	6.97 (2H, *d*, J = 8.3 Hz)	7.09 (2H, *d*, J = 8.3 Hz)	6.92 (2H, *d*, J = 8.4 Hz)
8/10	6.59 (2H, *d*, J = 8.3 Hz)	6.68 (2H, *d*, J = 8.3 Hz)	6.61 (2H, *d*, J = 8.4 Hz)
9	-	-	-
2'/6'			7.26 (2H, *d*, J = 8.7 Hz)[d]
3'/5'			7.01 (2H, *d*, J = 8.7 Hz)[e]
7'			4.97 (2H, *s*)
Glc-1"			4.85 (1H, *d*, J = 6.9 Hz)[f]
2"			3.21-3.29 (2H, m)[c]
3"			
4"			3.14-3.23 (1H, m)[c]
5"			3.30-3.38 (1H, m)[c]
6"			3.42-3.50 (1H, m)[c]
			3.66-3.74 (1H, m)[c]
2'"/6'"	7.29 (2H, *d*, J = 8.6 Hz)	7.28 (2H, *d*, J = 8.4 Hz)	7.22 (2H, *d*, J = 8.7 Hz)[d]
3'"/5'"	7.01 (2H, *d*, J = 8.6 Hz)	7.06 (2H, *d*, J = 8.4 Hz)	7.00 (2H, *d*, J = 8.7 Hz)[e]
7'"	4.94 (2H, *s*)	5.03 (2H, *s*)	4.97 (2H, *s*)
Glc-1""	4.85 (1H, *d*, J = 7.3 Hz)	4.93 (1H, *d*, J = 6.9 Hz)	4.85 (1H, *d*, J = 6.9 Hz)[f]
2""	3.25-3.32 (1H, m)[c]	3.47-3.53 (1H, m)[c]	3.21-3.29 (2H, m)[c]
3""	3.20-3.28 (1H, m)[c]	3.40-3.48 (2H, m)[c]	
4""	3.18-3.25 (1H, m)[c]		3.14-3.23 (1H, m)[c]
5""	3.30-3.36 (1H, m)[c]	3.44-3.52 (1H, m)[c]	3.30-3.38 (1H, m)[c]
6""	3.41-3.47 (1H, m)[c]	3.69-3.76 (1H, m)[c]	3.42-3.50 (1H, m)[c]
	3.69 (1H, *br d*, J = 10.8 Hz)	3.88 (1H, *br d*, J = 11.9)	3.68-3.74 (1H, m)[c]

[a] Measured in DMSO-d_6.
[b] Measured in MeOH-d_4.
[c] Chemical shifts was assigned by the results from COSY and HMQC.
[d-f] Assignments with the same superscript may be reversed.

Table 2. ^{13}C-NMR spectroscopic data of compounds **1-5** (75 MHz)

Position	1[a]	1[b]	2[a]	3[a]	4[a]	4[b]	5[a]
1	174.9	176.7	176.8	173.6	177.0	180.6	173.6
2	76.0	77.8	75.1	75.6	75.2	77.6	75.5
3	44.1	45.7	43.7	43.0	43.5	44.3	42.6
4	174.9	178.7	170.6	169.8	170.7	173.2	169.6
5	45.0	46.0	44.5	44.7	43.8	45.5	43.8
6	136.7	137.2	138.4	135.7	128.4	129.0	125.7
7/11	130.3	131.5	130.6	130.6	131.4	132.6	131.4
8/10	127.7	129.0	127.3	128.0	114.3	115.6	114.7
9	126.2	127.7	125.6	126.8	155.5	156.8	156.1
1'	129.4	131.0		129.2			129.1
2'/6'	129.4	131.1		129.9			129.6
3'/5'	116.1	117.6		116.3			116.2
4'	157.1	159.0		157.4			157.3
7'	65.2	67.6		66.2[c]			65.9
Glc-1"	100.4	102.2		100.6			100.4
2"	73.3	74.8		73.4			73.3
3"	77.1	78.1		77.1			77.0
4"	69.7	71.3		69.9			69.8
5"	76.7	77.9		76.7			76.6
6"	60.7	62.4		60.9			60.7
1'"			129.8	129.4	129.9	131.3	129.2
2'"/6'"			129.4	129.9	129.4	130.7	129.6
3'"/5'"			116.1	116.3	116.1	117.6	116.2
4'"			157.1	157.4	157.1	158.8	157.3
7'"			64.7	65.6[c]	64.6	66.8	65.4
Glc-1""			100.4	100.6	100.4	102.0	100.4
2""			73.2	73.4	73.3	74.7	73.3
3""			77.1	77.1	77.1	78.0	77.0
4""			69.7	69.9	69.7	71.2	69.8
5""			76.6	76.7	76.7	77.7	76.6
6""			60.7	60.9	60.7	62.3	60.7

[a] Measured in DMSO-d_6.
[b] Measured in MeOH-d_4.
[c] Assignments with the same superscript may be reversed.

Compound **9** was isolated as an amorphous powder. Its molecular formula was established as $C_{15}H_{20}O_8$ by negative HR-APCI-TOF mass spectrometric analysis. The ^{1}H NMR spectrum indicated the presence of a 1,3,5-trisubstituted aromatic ring with signals at δ_H 6.24, 6.30 and 6.33 (each 1H, *br s*), one singlet methylene at δ_H 3.58 (2H), one acetyl group at δ_H 2.08 (3H) as well as one anomeric proton at δ_H 4.77 (1H, *d*, J = 7.5 Hz) for the β-glucopyranosyl unit. In the ^{13}C NMR spectrum, 15 carbon signals were observed, of which nine were assignable to two oxy-aryl carbons at δ_C 158.3 and 158.6, three aryl-methines at δ_C 101.9, 108.6, and 110.3, one non-protonated aryl carbon at δ_C 136.7, one methylene at δ_C 50.0, and two carbon signals of an acetyl group at δ_C 206.1 and 29.4 for the aglycone moiety. The complete assignments were established by analyzing the 2D NMR spectra including HMQC and HMBC. In the HMBC spectrum, the correlations were found between i) δ_H 3.58 (H-7) and δ_C 136.7 (C-1), 108.6 (C-2), 206.1 (C-8), and 29.4 (C-9); ii) δ_H 4.77 (H-1' Glc) and δ_C 158.6 (C-3); and iii) δ_H 9.45 (5-OH) and δ_C 158.3 (C-5), 101.9 (C-4) and 110.3 (C-6), as illustrated in Figure 3. Consequently, the structure of compound **9** was elucidated as 1-(3,5-dihydroxyphenyl)propan-2-one 3-*O*-β-glucopyranoside, namely grammatophylloside D.

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of compound **9** (measured in DMSO-d_6)

Position	δ_H (300 MHz)	δ_C (75 MHz)
1		136.7
2	6.30 (1H, *br s*)	108.6
3		158.6
4	6.33 (1H, *br s*)	101.9
5		158.3
6	6.24 (1H, *br s*)	110.3
7	3.58 (2H, *s*)	50.0
8		206.1
9	2.08 (3H, *s*)	29.4
5-OH	9.45 (1H, *br s*)	
Glc-1'	4.77 (1H, *d*, *J* = 7.5 Hz)	100.4
2'	3.22-3.28 (1H, m)[a]	73.3
3'	3.12-3.17 (1H, m)[a]	77.0
4'	3.22-3.28 (1H, m)[a]	69.6
5'	3.17-3.22 (1H, m)[a]	76.7
6'	3.47 (1H, *dd*, *J* = 11.5, 5.9 Hz) 3.68 (1H, *dd*, *J* = 11.5, 3.4 Hz)	60.6

[a] Chemical shifts was assigned by the results from COSY and HMQC.

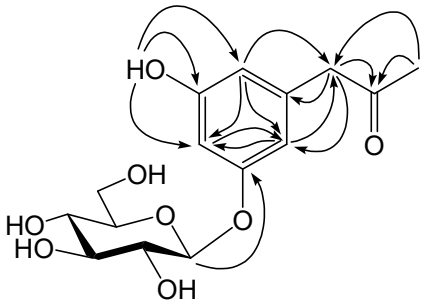


Figure 3. Significant HMBC correlations of grammatophylloside D (**9**)

The remaining known compounds were identified as gastodin (**6**),[7] vanilloloside (**7**),[8] orcinol glucoside (**8**),[9] and isovitexin (**10**)[10] by physical data and spectroscopic evidence.

The present study reported five glucosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid (**1-3**) and of (*R*)-eucomic acid (**4**, **5**), four phenolic glycosides (**6**-**9**), and a flavone C-glucoside (**10**) from the polar fraction of the Thai orchid *Grammatophyllum speciosum*. Cronupapine (**1**), grammatophyllosides A-C (**2-4**), vandateroside II (**5**) were the glucopyranosyloxybenzyl moiety forming with (*R*)-2-benzylmalic acid or (*R*)-eucomic acid through ester bonds, which were quite specific type of compounds to obtain from the orchid family.[2,6,11] These specific compounds provided further confirmation of the typical profile of secondary metabolites found in this family, and might be useful for further chemotaxonomic studies.

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Part 6

Chemical Constituents of *Mitragyna rotundifolia* (Roxb.) Kuntze

Chemical Constituents of *Mitragyna rotundifolia* (Roxb.) Kuntze

Introduction

Mitragyna rotundifolia (Roxb.) Kuntze belongs to the family Rubiaceae (Thai name: Kra-Thum-Mu). This species is commonly found in the dipterocarp forests in North-eastern Thailand. The leaves have been used in Thai traditional medicine for the treatment of diarrhea. Previous phytochemical investigation of this plant reported the isolation of several compounds such as alkaloids, triterpenoids, flavonoids and phenolics (Shellard and Phillipson, 1964; Shellard et al., 1971; Houghton and Shellard, 1974; Kang and Hao, 2006; Kang and Li, 2009; Kang et al., 2006, 2007, 2010). In pharmacological studies, the antioxidant phenolic compounds of this plant and hepatoprotective effect from plant extracts have been reported (Kang et al., 2010; Gong et al., 2012). This present work deals with the isolation and structure elucidation of ten polar compounds from the water soluble fraction of leaves including two new flavonol tetraglycosides and eight known compounds.

Experimental

General Procedures: NMR spectra were recorded in MeOH-d_4 using a Bruker AV-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometers. The MS data was obtained on a Bruker Micro TOF-LC mass spectrometer. UV spectra were recorded on a Shimadzu UV-1700 spectrophotometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump)

was carried out on an ODS column (21.2 x 250 mm i.d., Vertisep^{TM} AQS) with a Jasco MD-2010 detector at 220 nm. The flow rates were 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant Material: The leaves of *Mitragyna rotundifolia* (Roxb.) Kuntze were collected on June 2012 from the Herbal Paradise Garden, Chulabhorn Research Institute, Bangkok, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0070) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation: The collected leaves from The Herbal Paradise Garden (June 2012, 2.8 kg) were extracted with MeOH at room temperature (each 30 L for 48 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (420.4 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (345.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (139.4 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 20.0 L), EtOAc-MeOH-H_2O (40:10:1, 12.0 L), EtOAc-MeOH-H_2O (70:30:3, 2.0 L) and EtOAc-MeOH-H_2O (6:4:1, 2.0 L), respectively to obtain seven fractions (A to G). Fraction A (7.6 g from 46.5 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide seven sub-fractions. Sub-fraction A-3 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (85:15, v/v) to yield compound **5** (650.3 mg). Fraction B (10.5.0 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v)

to provide seven sub-fractions. Sub-fraction B-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to provide compounds **6** (110.3 mg) and **7** (242.7 mg). Fraction C (6.3 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to afford twelve sub-fractions. Sub-fraction C-9 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (70:30, v/v) to obtain compound **8** (332.4 mg). Fraction D (6.2 g) was similarly subjected to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to afford eight sub-fractions. Sub-faction D-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to provide compounds **4** (37.7 mg) and **10** (20.0 mg). Fraction E (10.0 g) was applied to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to afford ten sub-fractions. Sub-faction E-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to provide compound **9** (28.4 mg). Finally, fraction F (6.9 g from 40.32 g) was similarly subjected to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to provide ten sub-fractions. Sub-faction F-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to give compound **3** (35.3 mg). Sub-fraction F-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (78:22, v/v) to afford compounds **1** (76.9 mg) and **2** (34.7 mg).

Krathummuoside A (1): Yellow amorphous powder; $[\alpha]_D^{30}$ −135.1 (MeOH, *c* 1.35); UV λ_{max} (EtOH, *c* 1.34 x 10^{-5} M) 314.4, 266.2, 257.4 nm; IR spectrum: ν_{max} = 3367, 2931, 1643, 1602, 1268, 1167, 1059, 1024 cm^{-1}; ^{1}H-NMR (MeOH-d_4): Table 1; ^{13}C-NMR (MeOH-d_4): Table 2; Negative HRESITOFMS, *m/z*: 1047.2962 $[M-H]^-$ (calcd for $C_{48}H_{55}O_{26}$, 1047.2987).

Krathummuoside B (2): Yellow amorphous powder; $[\alpha]_D^{30}$ −88.6 (MeOH, *c* 1.37); UV λ_{max} (EtOH, *c* 1.01 x 10^{-5} M) 315.0, 257.5 nm; IR spectrum: ν_{max} = 3468, 2916, 1709, 1610, 1280, 1149, 1050, 1022 cm^{-1}; ^{1}H-NMR (MeOH-d_4): Table 1; ^{13}C-NMR (MeOH-d_4): Table 2; Negative HRESITOFMS, *m/z*: 1047.3017 $[M-H]^-$ (calcd for $C_{48}H_{55}O_{26}$, 1047.2987).

Determination of the absolute configurations of sugars: Monosaccharide subunits of krathummuosides A (**1**) and B (**2**) were obtained by acid hydrolysis. Each compound (*ca* 3 mg) was dissolved in 2 N HCl-dioxane (6:1, 3.5 ml) and heated at 80º for 5 hours. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous layer was concentrated to dryness affording the sugar fraction. Each of these was dissolved with H_2O (1 ml), analyzed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep^{TM} sugar LMP, 7.8 x 300 mm i.d.; mobile phase: H_2O; flow rate 0.4 ml/min; temperature: 80º C) and comparison with their retention times and optical rotations with authentic samples. Both samples gave peaks corresponding to D-glucose at 19.3 min and L-rhamnose 23.1 min with positive optical rotations.

Results and discussion

The methanolic extract of the leaves of *M. rotundifolia* was partitioned with Et_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to obtain two new flavonol glycosides, krathummuoside A (**1**) and krathummuoside B (**2**) (Figure 1) in addition to eight known compounds. The known compounds were elucidated as quercetin 3-*O*-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**3**) (Kite

et al., 2007), rutin (**4**), (−)-*epi*-catechin (**5**), 3,4,5-trimethoxyphenyl β-D-glucopyranoside (**6**) (Kanchanapoom, 2007), (6*S*, 9*R*)-roseoside (**7**) (Yamano and Ito, 2005), 3-*O*-β-D-glucopyranosyl quinovic acid 28-*O*-β-D-glucopyranosyl ester (**8**) (Zhao et al., 1995), (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**9**) (Achenbach et al., 1992), (+)-syringaresinol-4-*O*-β-D-glucopyranoside (**10**) (Kobayashi et al., 1985). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

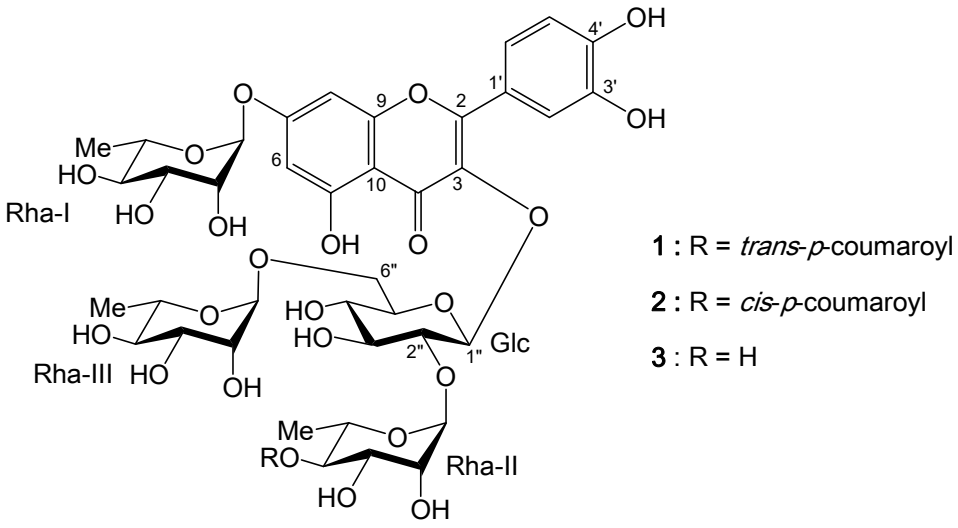

Figure 1 Flavonol tetraglycosides **1-3**

Krathummuoside A (**1**), $[\alpha]_D^{30}$ −135.1, was isolated as a yellow amorphous powder. Its molecular formula was determined to be $C_{48}H_{56}O_{26}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H-NMR spectrum revealed the presence of a pair of *meta*-coupled aromatic protons at δ_H 6.44 (br s) and 6.65 (br s) and a set of ABX aromatic ring system at δ_H 6.89 and 7.62 (each br d, J = 8.4 Hz), and 7.76 (br s) for the aglycone part, four anomeric protons of sugar

moieties at δ_H 4.51 (br s), 5.30 (br s), 5.55 (br s), and 5.59 (d, *J* = 7.3 Hz) in addition to a set of AA'BB' aromatic ring at δ_H 6.71 and 7.26 (each 2H, d, *J* = 8.3 Hz) and two *trans* olefin protons at 6.22 and 7.46 (each d, *J* = 16.0 Hz) assignable for *trans*-*p*-coumaroyl moiety. Analysis of 1D- and 2D-NMR spectroscopic data indicated that this compound is an acylated compound of quercetin 3-*O*-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**3**). The significant difference from **3** was the appearance of an additional set of signals arising from a *trans*-*p*-coumaroyl moiety. This acylated unit was assigned to be connected at C-4 of 2"-*O*-Rha-II since H-4 proton of this position was appeared downfield at δ_H 5.03 along with the downfield shift of this carbon atom, C-4 (δ_C 75.5) and upfield shifts of C-3 (δ_C 70.3) and C-5 (δ_C 67.8) by comparing the chemical shifts with those of compound **3** (see Table 2). Besides the long-range connectivity from HMBC spectrum (Figure 2) provided the further confirmation of the structure. In addition, acid hydrolysis yielded D-glucose and L-rhamnose, identify by HPLC analysis using the optical rotation detector. Thus, **1** was identified to be quercetin 3-*O*-(*4*-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside.

Table 1 ^{1}H-NMR (400 MHz, MeOH-d_4) spectroscopic data of krathummuosides A (**1**) and B (**2**)

Position	**1**	**2**
Aglycone		
6	6.44 (1H, br s)	6.47 (1H, d, J = 1.8 Hz)
8	6.65 (1H, br s)	6.71 (1H, d, J = 1.8 Hz)
2'	7.76 (1H, br s)	7.63 (1H, br s)
5'	6.89 (1H, d, J = 8.4 Hz)	6.88 (1H, d, J = 8.4 Hz)
6'	7.62 (1H, br d, J = 8.4 Hz)	7.63 (1H, br d, J = 8.4 Hz)
Glc		
1	5.59 (1H, d, J = 7.3 Hz)	5.58 (1H, d, J = 7.3 Hz)
2	3.72 (1H, dd, J = 8.4, 8.0 Hz)	3.67 (1H, dd, J = 9.0, 8.6 Hz)
3	3.66 (1H)[a]	3.58 (1H)[a]
4	3.85 (1H)[a]	3.82 (1H)[a]
5	3.35 (1H, m)	3.35 (1H, m)
6	4.19 (1H, dd, J = 12.0, 2.1 Hz)	4.14 (1H, dd, J = 10.0, 1.8 Hz)
	3.44 (1H)[a]	3.38 (1H)[a]
Rha-I		
1	5.55 (1H, br s)	5.55 (1H, br s)
2	4.06 (1H, br s)	4.02 (1H, br s)
3	3.85 (1H)[a]	3.83 (1H)[a]
4	3.36 (1H, dd, *J* = 9.4, 7.6 Hz)	3.44 (1H)[a]
5	3.62 (1H, m)	3.66 (1H, m)
6	1.22 (3H, d, J = 5.9 Hz)	1.27 (3H, d, J = 6.0 Hz)
Rha-II		
1	5.30 (1H, brs)	5.27 (1H, brs)
2	4.14 (1H, br s)	4.08 (1H)[a]
3	3.50 (1H)[a]	3.45 (1H)[a]
4	5.03 (1H, dd, J = 9.6, 9.4 Hz)	4.98 (1H, dd, J = 10.0, 9.8 Hz)
5	4.45 (1H, m)	4.30 (1H, m)
6	0.94 (3H, d, J = 6.1 Hz)	0.90 (3H, d, J = 6.0 Hz)
Rha-III		
1	4.51 (1H, br s)	4.50 (1H, br s)
2	3.67 (1H, br s)	3.60 (1H, br s)
3	3.52 (1H)[a]	3.48 (1H)[a]
4	3.25 (1H, dd, J = 9.4, 9.4 Hz)	3.22 (1H, dd, J = 9.6, 9.4 Hz)
5	3.48 (1H, m)	3.44 (1H, m)
6	1.06 (3H, d, J = 6.0 Hz)	1.07 (3H, d, J = 6.1 Hz)
Coumaroyl		
2, 6	7.26 (2H, d, J = 8.3 Hz)	7.62 (2H, d, J = 8.7 Hz)
3, 5	6.71 (2H, d, J = 8.3 Hz)	6.68 (2H, d, J = 8.7 Hz)
7	7.46 (1H, d, J = 16.0 Hz)	6.78 (1H, d, J = 12.8 Hz)
8	6.22 (1H, d, J = 16.0 Hz)	5.73 (1H, d, J = 12.8 Hz)

[a] Chemical shifts were assigned by HMQC.

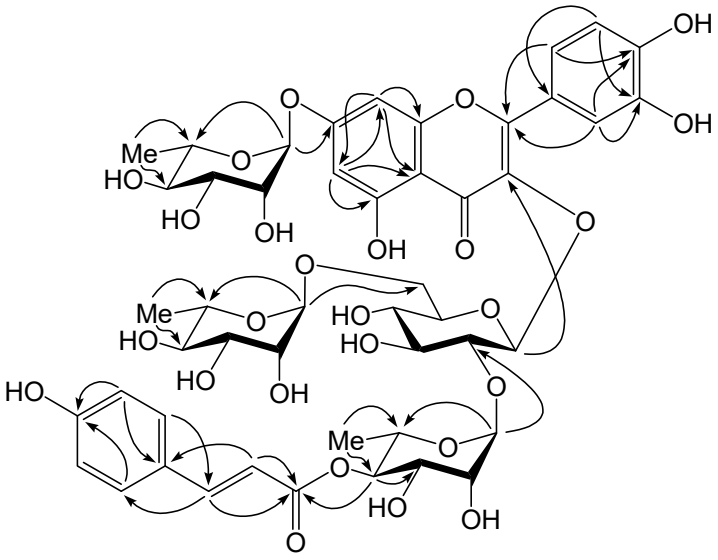

Figure 2 HMBC correlations of krathummuoside A (**1**)

Krathummuoside B (**2**), $[\alpha]_D^{30}$ −88.6, was obtained as a yellow amorphous powder. The molecular formula was the same as **1** by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H- and ^{13}C-NMR spectroscopic data were very similar to those of **1**. The only significant difference was the coupling constants of the olefin protons at δ_H 5.73 and 6.78 with J = 12.8 Hz. Therefore, the structure of compound **2** was determined to be the *cis* isomer of **1**.

In conclusion, the present study reported ten water soluble compounds including five flavonoids (**1-5**), a phenolic glucoside (**6**), a megastigmane glucoside (**7**), a triterpenoid glucoside (**8**) and two lignan glucosides (**9**, **10**). The result was closely related to those of the previous studies performed on this species. However, the occurrence of two new polar flavonol tetraglycoside (**1**, **2**) bearing *p*-coumaroyl unit in *tran*- and *cis*-forms attached to the sugar part was the first report from this genus. This finding might be useful for further chemotaxonomic studies of this genus.

Table 2 ^{13}C-NMR (100 MHz, MeOH-d_4) spectroscopic data of krathummuosides A (**1**), B (**2**) and **3**

Position	1	2	3
Aglycone			
2	159.5	159.6	159.4
3	134.8	134.8	134.7
4	179.1	179.3	179.3
5	162.6	162.9	162.8
6	100.3	100.4	100.4
7	163.2	163.4	163.3
8	95.8	95.7	95.6
9	157.7	158.0	157.9
10	107.3	107.5	107.5
1'	123.1	123.2	123.2
2'	117.6	117.5	117.4
3'	145.7	146.0	145.9
4'	149.7	149.8	149.7
5'	116.1	116.1	116.1
6'	123.5	123.6	123.6
Glc			
1	100.9	100.7	100.4
2	79.9	80.2	80.0
3	78.6	78.8	78.8
4	71.5	71.8	71.7
5	76.7	77.0	77.7
6	68.3	68.3	68.2
Rha-I			
1	99.7	99.9	99.8
2	71.9	72.1	71.9
3	71.9	72.1	72.0
4	73.5	73.6	73.6
5	71.1	71.2	71.2
6	18.0	18.1	18.1
Rha-II			
1	102.3	102.5	102.6
2	71.5	71.7	72.2
3	70.3	70.4	72.3
4	75.5	75.2	74.0
5	67.8	67.9	69.9
6	17.3	17.4	17.5
Rha-III			
1	102.1	102.2	102.1
2	72.1	72.2	72.0
3	72.3	72.5	72.2
4	73.7	73.8	73.8
5	69.6	69.7	69.6
6	17.8	17.8	17.8
Coumaroyl			
1	127.0	127.5	
2, 6	131.0	133.7	
3, 5	116.7	115.7	
4	160.9	160.0	
7	146.5	145.2	
8	115.2	116.1	
9	169.0	168.0	

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Part 7

Chemical Constituents from *Bruguiera gymnorrhiza* (L.) Savigny

Chemical Constituents from *Bruguiera gymnorrhiza* (L.) Savigny

Introduction

Bruguiera gymnorrhiza (L.) Savigny (Thai name: Phanka-Hua-Sum) belongs to the family Rhizophoraceae, commonly found in the mangrove forests of Thailand. In Thailand, the flowers and fruits are used as vegetable for cooking purposes. Members of this genus are known to contain several types of compounds such as tropane derivatives (Loader and Russell, 1969), diterpenes (Han et al., 2004), flavonoids (Li et al., 2010), triterpenes (Homhual et al., 2006), aromatic compounds (Bao et al., 2007; Han et al., 2005; 2007; Yi et al., 2013) and macrocyclic polydisulfide (Sun and Guo, 2004); however there are a few reports on the phytochemical study from the polar compounds of this genus. The present paper describes the isolation and structure elucidation of 11 compounds from the aqueous soluble fraction of leaves including six 5-methyl ether flavones (**1-6**), of which two are new (**5**, **6**), four flavonol glycosides (**7-10**) and an aryl-tetralin lignan rhamnoside (**11**).

Experimental Section

General Procedure: NMR spectra were recorded in DMSO-D_6 using a Bruker AV-300 (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) spectrometer. The MS data was obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-

980 pump) was carried out on an ODS column (20.0 x 250 mm i.d., Vertisep^{TM} UPS) with a Jasco UV-970 detector at 220 nm. The flow rates were 6 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant material: The leaves of *Bruguiera gymnorrhiza* (L.) Savigny were collected on June 2013 from Tambon *NongSano, Amphoe Mueang,* Trat Province, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0072) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation: The collected leaves (3.5 kg) were extracted with MeOH at room temperature (each 15 L for 24 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (599.5 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 5 times). The aqueous soluble fraction (411.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (103.3 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 8.0 L), EtOAc-MeOH-H_2O (40:10:1, 12.0 L), EtOAc-MeOH-H_2O (70:30:3, 1.0 L) and EtOAc-MeOH-H_2O (6:4:1, 9.0 L), respectively to provide five fractions. Fraction I (7.7 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide 12 sub-fractions. Sub-fraction I-3 was purified by preparative HPLC-ODS using solvent system 15% aqueous MeCN to obtain compound **11** (24.0 mg). Sub-fraction I-5 was purified by preparative HPLC-ODS with solvent system 17% aqueous MeCN to give compounds **1** (9.6 mg), **7** (13.1 mg), **8** (110.4 mg) and **9** (6.7 mg). Sub-fraction I-7 was purified by preparative HPLC-ODS with solvent system 23% aqueous MeCN

to obtain compound **2** (20.0 mg). Fraction II (4.8 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction II-4 was purified by preparative HPLC-ODS with solvent system 17% aqueous MeCN to provide compounds **4** (7.0 mg) and **6** (9.0 mg). Fraction III (4.1 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to afford seven sub-fractions. Sub-fraction III-3 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to obtain compounds **3** (8.0 mg) and **5** (4.8 mg). Finally, fraction IV (8.3 g) was similarly subjected to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to provide nine sub-fractions. Sub-faction IV-4 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to yield compound **10** (8.0 mg).

7,4',5'-Trihydroxy-5,3'-dimethoxyflavone 7-*O*-β-D-glucopyranoside (5): Amorphous powder; $[\alpha]_D^{27}$ –44.73 (MeOH, *c* 0.30); ^{1}H-NMR (DMSO-D_6): δ 7.09 (1H, br s, H-2'), 7.07 (1H, br s, H-6'), 6.88 (1H, d, *J* = 1.9 Hz, H-8), 6.59 (1H, d, *J* = 1.9 Hz, H-6), 6.59 (1H, s, H-3), 5.09 (1H, d, *J* = 7.2 Hz, H- 1" Glc), 3.85 (3H, s, 3'-OMe), 3.82 (3H, s, 5-OMe); ^{13}C-NMR (DMSO-D_6): Table 1; Negative HRESITOFMS, *m/z*: 491.1200 $[M-H]^-$ (calcd for $C_{23}H_{23}O_{12}$, 491.1195).

7,4'-Dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside (6): Amorphous powder; $[\alpha]_D^{27}$ –46.57 (MeOH, *c* 0.30); ^{1}H-NMR (DMSO-D_6): δ 7.87 (2H, *d*, J = 8.8 Hz, H-2',6'), 6.91 (1H, d, *J* = 8.8 Hz, H-3',5'), 6.89 (1H, br s, H-8), 6.59 (1H, s, H-6), 6.59 (1H, s, H-3), 5.10 (1H, d, *J* = 7.1 Hz, H- 1" Glc), 3.83 (3H, s, 5-OMe); ^{13}C-NMR (DMSO-D_6): Table 1; Positive HRESITOFMS, *m/z*: 447.1297 $[M+H]^+$ (calcd for $C_{22}H_{23}O_{10}$, 447.1286).

Aryl-tetralin lignan rhamnoside (11): Amorphous powder; $[\alpha]_D^{27}$ –33.72 (MeOH, *c* 0.70); CD (MeOH, *c* 4.4 x 10^{-4} M) Δε (nm) –3.45 (250), –2.51 (272), +3.57 (288); ^{1}H and ^{13}C NMR (DMSO-D_6): Table 2; Negative HRESITOFMS, *m/z*: 601.2042 $[M-Cl]^-$ (calcd for $C_{28}H_{38}Cl_1O_{12}$, 601.2057).

Determination of the absolute configurations of sugars: Each compound (*ca* 5 mg) was dissolved in 2 N HCl-dioxane (6:1, 3.5 ml) and heated at 80º for 5 hours. After cooling, the reaction was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 2N KOH and concentrated to dryness affording the sugar fraction. This part was dissolved with H_2O (1 ml), analyzed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep^{TM} sugar LMP, 7.8 x 300 mm i.d.; mobile phase: H_2O; flow rate 0.4 ml/min; temperature: 80º C) and comparison with their retention times and optical rotations with authentic samples. Hydrolysis of compounds **5** and **6** gave peaks corresponding to D-glucose at 19.2 min with positive optical rotation and hydrolysis of lignan **11** gave peaks of L-rhamnose at 23.0 min with positive optical rotation.

Results and discussion

The methanolic extract of the leaves of *B. gymnorrhiza* was evaporated to dryness and partitioned with Et_2O and H_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to yield 11 compounds. Eight compounds were identified as 7,3',4',5'-tetrahydroxy-5-methoxyflavone (**1**) (Ye et al., 2008), 7,4',5'-trihydroxy-5,5'-dimethoxyflavone (**2**, gramrione) (Raihan, 1994; Feng et al., 2008), luteolin 5-methyl ether 7-*O*-β-D-glucopyranoside (**3**) (Williams and Harborne, 1975), 7,4'-dihydroxy-5,3'-dimethoxyflavone 7-*O*-β-D-glucopyranoside (**4**)

(Ozawa et al., 1995), quercetin 3-*O*-β-D-glucopyranoside (**7**), rutin (**8**), kaempferol 3-*O*-rutinoside (**9**) and myricetin 3-*O*-rutinoside (**10**) (Lu and Foo, 2003) by comparison of physical data with literature values and from spectroscopic evidence.

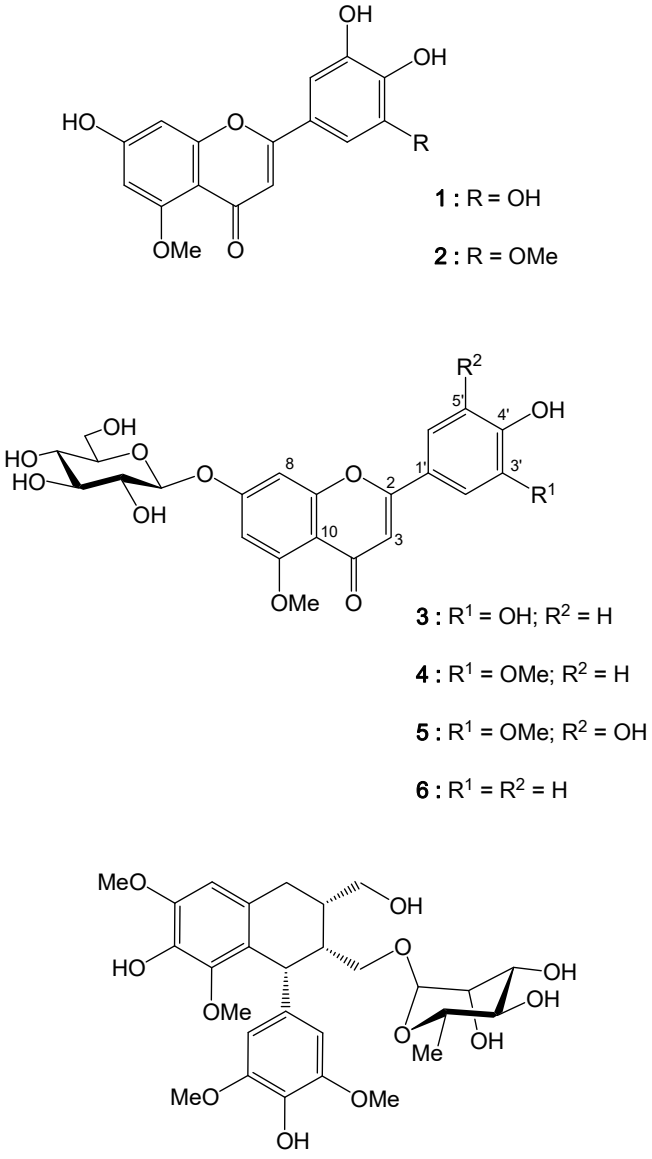

Figure 1 Structures of compounds **1-6** and **11**

Compound **5** was obtained as an amorphous powder. The molecular formula was determined to be $C_{23}H_{24}O_{12}$ by high resolution electrospray time-of-flight (HR-

ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectra revealed the presence of two asymmetrical *tetra*-substituted aromatic rings deduced from two sets of the chemical shifts at δ_H 6.59 and 6.88 (each 1H, d, J = 1.9 Hz), and at δ_H 7.07 and 7.09 (each 1H, br s), two methoxyl groups at δ_C 56.1 with δ_H 3.82 (3H, s) and δ_C 56.2 with δ_H 3.85 (3H, s), a carbonyl carbon atom at δ_C 175.7, in addition to one β-D-glucopyranosyl unit from a set of carbon signals at δ_C 100.0, 77.4, 76.6, 73.2, 69.8 and 60.7 with an anomeric proton at δ_H 5.09 (1H, d, J = 7.2 Hz). The chemical shifts of this compound were related to those of gramrione (**2**) except for a set of additional signals of a β-D-glucopyranosyl unit. This sugar part was suggested to be located at C-7 since the chemical shifts of this carbon and neighboring atoms were significantly changed in comparison with **2** (Table 1). In the ^{1}H NMR spectrum (measured in DMSO-D_6), a broad highly-deshield chelated signal, generally assigned as a hydroxyl group at C-5, was not observed, indicating the presence of a methoxyl group on this position. The second methoxyl group was suggested to be located at C-3' of the B-ring because the splitting pattern of two protons was appeared as *meta*-coupling, characteristic for an asymmetrical *tetra*-substituted aromatic ring. This assumption was supported by an application of 2D-NMR spectroscopy, including COSY, HMQC and HMBC experiments. In the HMBC spectrum (Figure 2), only the signal at δ_H 6.88 showed a correlation to C-9 (δ_C 158.7), this proton could be assigned as H-8. Since this proton together with the anomeric signal at δ_H 5.09 showed correlations to C-7 (δ_C 161.4), the sugar moiety was confirmed to be linked to the hydroxyl group at this carbon atom. Two methoxyl groups were also confirmed to be

linked to C-5 and C-3'. From these data, the structure of **5** was elucidated to be 7,4',5'-trihydroxy-5,3'-dimethoxyflavone 7-*O*-β-D-glucopyranoside**.**

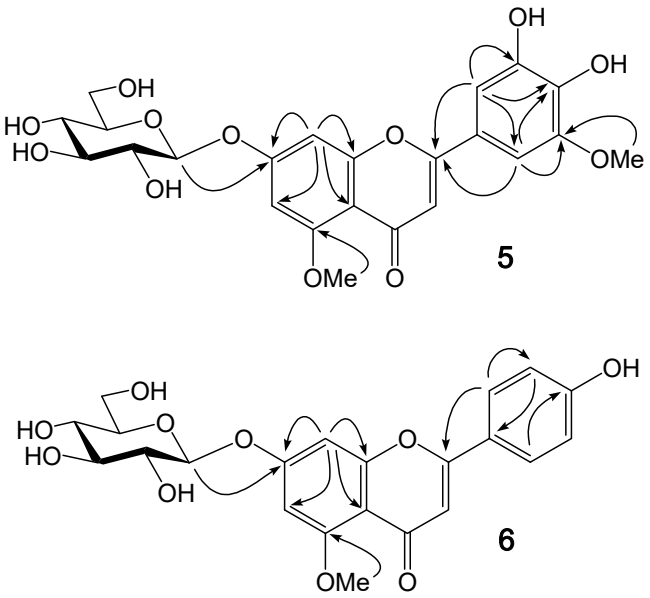

Figure 2 Significant HMBC correlations of compounds **5**

Compound **6** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{22}H_{22}O_{10}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data indicated that this compound is a 5-methyl ether flavone glucoside derivative, as compared to compounds **1-5**. The significant difference was the chemical shifts of the B-ring, which showed the presence of the AA'BB' type aromatic ring system [δ_H 6.91 and 7.87 (each 2H, d, J = 8.8 Hz)]. Thus, this compound was suggested to be 7,4'-dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside. This assumption was established by 2D-NMR spectra. In the HMBC spectrum, the correlations were observed for the A-ring in the same manner as compound **5** (Figure 1), confirming the

location of a sugar unit at C-7, and a methoxyl group at C-5. Interestingly, the same structure had previously been reported from the isolated compound of *Aquilalia sinensis* (Qi et al., 2009), but the spectroscopic data obtained in the present work clearly was not identical to the reported data, especially for the A-ring part. However the structure revision of the compound reported as 7,4'-dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside (Qi et al., 2009) was already proposed to that of 5,4'-dihydroxy-7-methoxyflavone 5-*O*-β-D-glucopyranoside (Veithch and Grayer, 2011). Consequently, this compound is new.

Table 1 ^{13}C NMR Spectroscopic data of compounds **1-6** (75 MHz, DMSO-d_6)

Position	**1**	**2**	**3**	**4**	**5**	**6**
Aglycone						
2	160.2	160.0	160.2	160.3	160.2	160.2
3	106.0	106.4	106.2	106.5	106.6	106.1
4	175.6	175.7	175.6	175.9	175.7	175.9
5	160.6	160.6	160.5	160.3	160.4	160.6
6	96.5	96.5	97.2	97.3	97.2	97.2
7	162.6	162.5	161.4	161.5	161.4	161.4
8	95.0	95.2	96.0	96.3	96.1	96.1
9	159.0	159.1	158.7	158.8	158.7	158.7
10	107.1	107.1	108.9	108.9	108.9	108.9
1'	120.7	120.8	121.8	121.7	120.6	121.3
2'	105.0	101.8	113.0	109.8	101.9	127.9
3'	146.3	148.6	145.7	150.2	148.6	115.9
4'	137.0	137.8	149.1	148.1	137.9	160.6
5'	146.3	145.9	115.9	115.8	146.0	115.9
6'	105.0	107.0	118.3	119.8	107.1	127.9
5-OMe	55.9	55.8	56.1	56.0	56.1	56.1
3'-OMe		56.2		56.1	56.2	
Glc						
1			99.9	100.1	100.0	99.9
2			73.2	73.3	73.2	73.2
3			77.3	77.4	77.4	77.3
4			69.7	69.9	69.8	69.8
5			76.6	76.7	76.6	76.6
6			60.7	60.8	60.7	60.7

Compound **11** was isolated as a yellow amorphous powder. The molecular formula was determined to be $C_{28}H_{38}O_{12}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra revealed the presence of one sugar unit, identified to be an α-L-rhamnopyranosyl moiety from the chemical shifts of its anomeric proton at δ_H 4.54 (1H, br s) and a secondary methyl group at δ_H 1.17 (3H, d, J = 6.0 Hz) together with the set of the chemical shifts at δ_C 100.6, 70.6, 70.9, 68.9 and 18.0. The ^{13}C NMR spectral data (Table 2) showed the signals of two aromatic rings (a *penta*- and a symmetrical 1,3,4,5-*tetra*-substituted aromatic rings), three methylenes (δ_C 32.5, 64.0 and 67.5), three methines (δ_C 39.0, 41.3 and 44.7) in addition to four methoxyl groups [δ_C 55.7, 56.2 (2C) and 58.7] for the aglycone moiety. The spectral data of this part was very characteristic of an aryl-tetralin type lignan, suggested to be a lignan rhamnoside. The assignment was supported by HMBC spectrum (Figure 3), in which the significant correlations were observed between (i), H-1 to C-9, (ii) H-4 to C-2, C-3, C-9, C-10, C-3a and C-1', (iii) H-8 to C-1 and (iv) H-1" Rha to C-3a, as shown in figure 3. The ^{1}H and ^{13}C NMR spectroscopic data were coincident with those of (±) lyoniresinol 3a-*O*-α-L-rhamnopyranoside (Kaneda et al., 1990). Since this compound was pure and exhibited a negative optical rotation value of $[\alpha]_D^{27}$ –33.72 (MeOH, *c* 0.70), the stereochemistry should be different. Therefore, the configurations of this compound must be clarified. The assignment of the relative stereochemistry was established by the coupling constants and difference NOE experiments (Figure 4). The results from the difference NOE correlations between (i) H-3(δ_H 1.92) and H-4 (δ_H 4.12), (ii) H-4

and H-2', 6' (δ_H 6.26) and (iii) H-3 and H-2', 6' together with the coupling constant of H-4 with J = 5.9 Hz indicated that the relation of H-4 and H-3 was an axial-equatorial orientation. H-2 (δ_H 1.50) was assigned to be in an axial orientation due to the large coupling constant with H_{ax}-1 (δ_H 2.50, J = 11.9 Hz). Also, H-2 displayed the NOE enhancements to H-3 and H-4. Thus, the orientation of H-2, H-3 and H-4 was determined as axial, equatorial and axial, respectively. The absolute configuration was established from the CD spectrum, which showed extreme values for Δε (nm) –3.45 (250), –2.51 (272), +3.57 (288), relating to the aryl substituted at C-4 of 4*R* and opposite to the reports for 4*S* compounds (Sakakibara et al., 1974; Ohashi et al., 1994; Yang et a., 2005; Wangteeraprasert and Likhitwitayawuid, 2009). Therefore, the absolute configuration of the chiral positions was concluded to be 2*S*, 3*R* and 4*R*. On the basis of these evidences, the structure was elucidated as shown. The structure of this compound was firstly reported from *Cotoneaster racemiflora* with a trivial name racemiside (Khan et al., 2009). It displayed an optical rotation value of $[\alpha]_D^{20}$ +4.8 (MeOH, *c* 6.2 x 10^{-4}), opposite to that of compound **11**. Although the sign of the specific rotation was different, it was insufficient to conclude compound **11** as a new lignan rhamnoside. Therefore, the result from this study provided the more physical and spectroscopic data of racemiside.

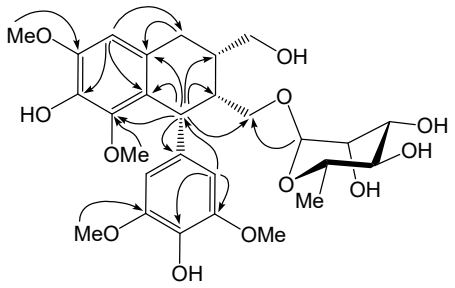

Figure 3 Significant HMBC correlations

Table 2 ^{1}H and ^{13}C NMR Spectroscopic data of lignan **11** (75 MHz, DMSO-d_6)

Position	Proton	Carbon
Aglycone		
1	2.50 ($1H_{ax}$, dd, J = 15.0, 11.9 Hz)	32.5
	2.65 ($1H_{eq}$, dd, J = 15.0, 4.2 Hz)	
2	1.50 (1H, m)	39.0
2a	3.32 (1H)[a]	64.0
	3.48 (1H)[a]	
3	1.92 (1H, m)	44.7
3a	3.22 (1H)[a]	67.5
	3.50 (1H)[a]	
4	4.12 (1H, d, J = 5.9 Hz)	41.3
5		146.4
6		137.3
7		147.0
8	6.55 (1H, s)	106.7
9		128.4
10		124.8
1'		137.7
2', 6'	6.26 (2H, s)	105.9
3', 5'		147.6
4'		133.5
5-OMe	3.25 (3H, s)	58.7
7-OMe	3.76 (3H, s)	55.7
3', 5'-OMe	3.47 (6H, s)	56.1
Rha		
1	4.54 (1H, br s)	100.6
2	3.69 (1H)[a]	70.6
3	3.50 (1H)[a]	70.9
4	3.19 (1H)[a]	71.9
5	3.58 (1H, m)	68.9
6	1.17 (1H, d, J = 6.0 Hz)	18.0

[a] Chemical shifts were assigned by COSY and HMQC.

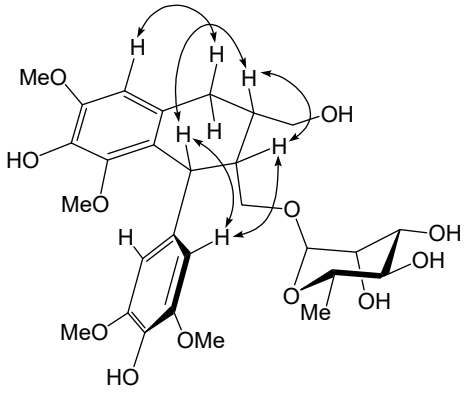

Figure 4 Significant difference NOE correlations of compound

The absolute configuration of the sugar moiety of three compounds **5**, **6** and **11** was determined by acid hydrolysis to provide D-glucose from compounds **5** and **6**; and L-rhamnose from compound **11**, identify by HPLC analysis using the optical rotation detector.

The present study isolated 11 secondary metabolites from the water soluble fraction including 5-methyl ether flavones (**1-6**), flavonol glycosides (**7-10**) and an aryl-tetralin lignan rhamnoside (**11**) of the leaves of *B. gymnorrhiza*. The appearance of 5-methyl ether flavones (**1, 2**) was related to the previous investigations of the same species (Raihan, 1994; Feng et al., 2008; Ye et al., 2008). Compounds **3-6** were the first 5-methyl ether flavones isolated in glucosidic form of this plant. Flavonol glycosides (**7-10**) are commonly reported from the plant kingdom. The occurrence of an aryl- tetralin lignan rhamnoside (**11**) was quite rare to discover from plant source, especially having the negative optical rotation value. These constituents might be useful for further chemotaxonomic studies of this genus.

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Part 8

Chemical Constituents from *Micromelum minutum* (G.Forst.) Wight & Arn.

Chemical Constituents from *Micromelum minutum* (G.Forst.) Wight & Arn.

Introduction

Micromelum minutum (G.Forst.) Wight & Arn. (Thai name: Hat-Sa-Kun), a member of the family Rutaceae, is a shrub native in tropical regions of Southeast Asia. This species is well known to contain coumarins, phenylpropanoic acid derivatives, polyoxygenated flavonoids and carbazole alkaloids (Tantivatana et al., 1983; Tantishaiyakul et al., 1986; Sohrab et al., 1999, 2004; Ito et al., 2000; Nakahara et al.,2002; Rahmani et al., 1993, 1994, 2003). It is used in Thai traditional medicine for anti-fever purposes. In the course of our continuing studies on Thai medicinal plants, we report the isolation and identification of four new polar compounds, including three glucosides of phenylpropanoic acid derivative (**1**, **2** and **4**) and a coumarin glucoside (**10**) from the water soluble fraction of the aerial parts of this plant in addition to 15 known compounds.

Experimental Section

General Procedure: 1D and 2D NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. The MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. UV spectra were recorded on a Shimadzu UV-1700 spectrophotometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco

PU-980 pump) was carried out on an ODS column (21.2 x 250 mm i.d., Vertisep^{TM} AQS) with a Jasco UV-970 detector at 220 nm. The flow rates were 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant material: The aerial portions of *Micromelum minutum* (G. Forst.) Wight & Arn. were collected on November 2011 from Baan Muang Waan, Khon Kaen, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0074) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation: The aerial portions (2.0 kg) were extracted with MeOH at room temperature (each 10 L for 48 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (337.0 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 5 times). The aqueous soluble fraction (197.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (80.5 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 4.0 L), EtOAc-MeOH-H_2O (40:10:1, 12.0 L), EtOAc-MeOH-H_2O (70:30:3, 8.0 L) and EtOAc-MeOH-H_2O (6:4:1, 10.0 L), respectively to obtain eight fractions (A to H). Fraction D (10.0 g) was applied to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide 15 sub-fractions. Sub-fraction D-2 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (93:7, v/v) to afford compounds **10** (12.0 mg), **12** (15.4 mg), **13** (8.0 mg) and **14** (5.2 mg). Sub-fraction D-4 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (90:10, v/v) to provide compounds **5**

(144.1 mg) and **6** (21.2 mg). Sub-fraction D-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (83:17, v/v) to give compounds **8** (830.5 mg) and **19** (140.4 mg). Sub-fraction D-6 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to provide compounds **9** (18.2 mg), **11** (68.6 mg) and **15** (41.9 mg). Sub-fraction D-8 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to yield compounds **16** (14.6 mg) and **17** (32.6 mg). Sub-fraction D-11 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to provide compound **18** (176.6 mg). Fraction E (10.8 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to afford nine sub-fractions. Sub-fraction E-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (83:17, v/v) to provide compound **2** (5.7 mg). Sub-fraction E-6 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to give compounds **3** (192.9 mg) and **4** (43.7 mg). Finally, fraction F (10.3 g) was similarly subjected to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to provide ten sub-fractions. Sub-faction F-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to give compound **1** (142.4 mg). Sub-fraction F-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to afford compound **7** (22.6 mg).

Micromelumoside A (1): Amorphous powder; $[\alpha]_D^{25}$ –40.4 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m/z*: 487.1462 $[M–H]^-$ (calcd for $C_{21}H_{27}O_{13}$, 487.1457).

Micromelumoside B (2): Amorphous powder; $[\alpha]_D^{25}$ –0.2 (MeOH, *c* 0.40); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m/z*: 537.1373 $[M+Cl]^-$ (calcd for $C_{22}H_{30}Cl_1O_{13}$, 537.1380)

Micromelumoside C (4): Amorphous powder; $[\alpha]_D^{25}$ +72.8 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m/z*: 467.1220 $[M+Cl]^-$ (calcd for $C_{21}H_{26}Cl_1O_{10}$, 473.1218).

Micromelumoside D (10): Amorphous powder; $[\alpha]_D^{25}$ −55.1 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 2; Negative HRESITOFMS, *m/z*: 477.1170 $[M+Cl]^-$ (calcd for $C_{20}H_{26}Cl_1O_{11}$, 477.1169).

Results and discussion

The methanolic extract of the aerial parts of *Micromelum minutum* was partitioned with low polar solvent. The aqueous soluble fraction was separated by combination of chromatographic methods to obtain three new phenylpropanoic acid derivatives (**1**, **2**, and **4**), and a new coumarin glucoside (**10**) (Fig. 1) in addition to 15 known compounds.

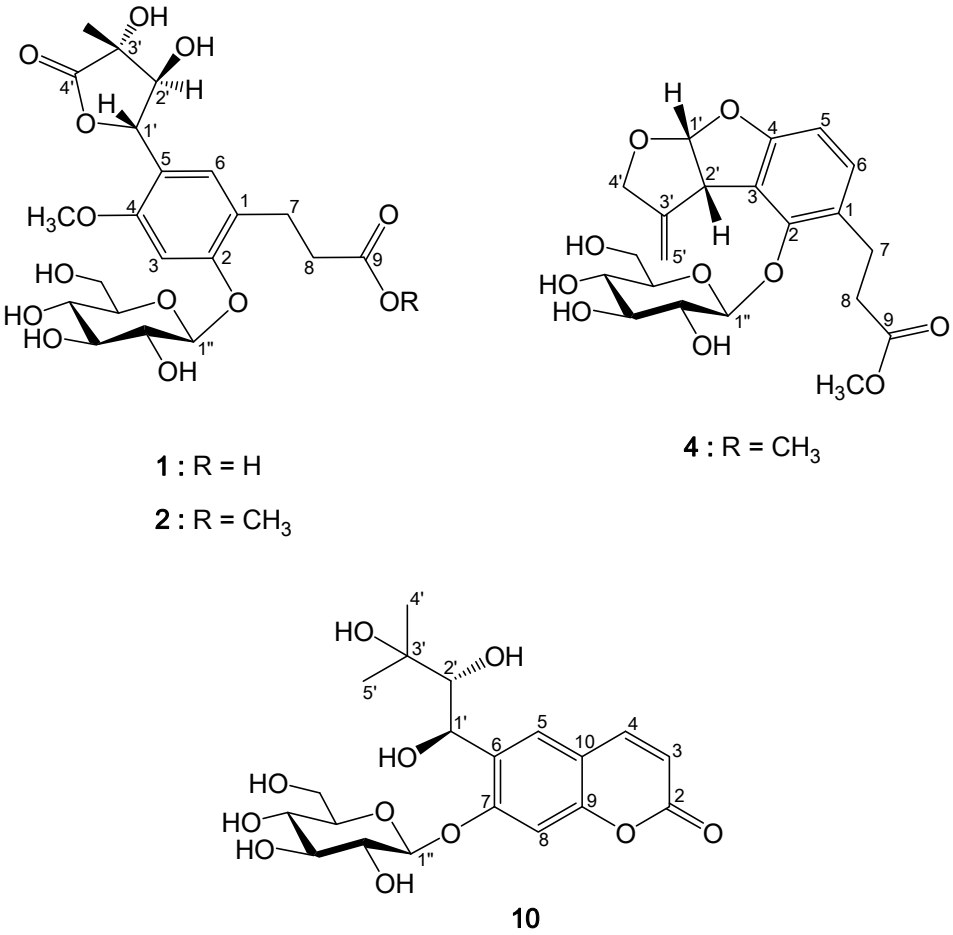

Fig. 1 Structures of compounds **1, 2, 4** and **10**

Micromelumoside A (**1**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{21}H_{28}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{13}C NMR spectrum revealed the presence of an aromatic ring, two methylenes (δ_C 24.8 and 34.2) and a carboxyl group (δ_C 174.2), corresponded to the phenylpropanoic acid part in addition to a methoxyl group (δ_C 55.7) and a β-D-glucopyranosyl unit (δ_C 100.9, 76.9, 76.7, 73.3, 70.2 and 61.0). The remaining five carbon signals (δ_C 177.8, 78.6, 77.4, 75.1 and 17.9) were assigned to be an oxidized cyclic lactone unit, which was similar to that of hydramicromelins A-D, isolated compounds from *M. integerrimum*

(He at al., 2001; Phakhodee et al., 2014). The appearance of two proton at δ_H 6.82 and 7.09 as two singlet signals from the ^{1}H NMR spectrum, corresponding to the aromatic methine carbons at δ_C 99.8 and 129.5, respectively, suggested that this compound was a 1,2,4,5-tetrasubstituted aromatic ring system. The structure was deduced by the results from 2D-NMR spectroscopic methods. This compound has a phenylpropanoic acid as a core structure. The sugar moiety was located at C-2 (δ_C 156.7) since the significant HMBC correlations (Fig. 2) were found from H_2-7 (δ_H 2.75, m) to C-1 (δ_C 121.3), C-2 (δ_C 156.7), and from H-1" (δ_H 4.86, d, J = 7.2 Hz) of the glucopyranosyl moiety to C-2. The methoxyl group was assigned to be linked at C-4 (δ_C 157.3) based on the correlations from its singlet proton signal (δ_H 3.74) to C-4 in the HMBC spectrum, and from the cross peak correlation between the methoxyl group (δ_H 3.74) and H-3 (δ_H 6.82) in the NOESY spectrum. The connection of 5-carbon lactone unit at C-5 (δ_C 117.1) was supported by the HMBC experiment, in which the correlations were observed from H-1' (δ_H 5.07) to C-4 (δ_C 157.3), C-5 (δ_C 117.1) and C-6 (δ_C 117.1). Thus, this compound was confirmed to have a 1,2,4,5-tertrasubstituted aromatic ring. The relative stereochemistry of a 5-memberred lactone unit was determined by the coupling constant together with the NOESY experiment. Two vicinal protons H-1' (δ_H 5.07) and H-2' (δ_H 4.30) showed the coupling constant 8.3 Hz, indicating *trans* configuration at C-1' and C-2'. The NOESY correlation of H-1' with H_3-5' indicated that these protons were located in the same plane. Also the NOESY correlations were found between H-6 and H-1', and H-6 and H-2', therefore, the conformation of this compound could be illustrated as shown in Fig. 3 and led to suggest the configurations of C-1', C-2', C-3' positions to be *S*, *S* and *R*, respectively. Besides, these results were correlated with those reports for synthetic

hydramicromelin B (Huo et al., 2008). Consequently, this compound was identified as shown.

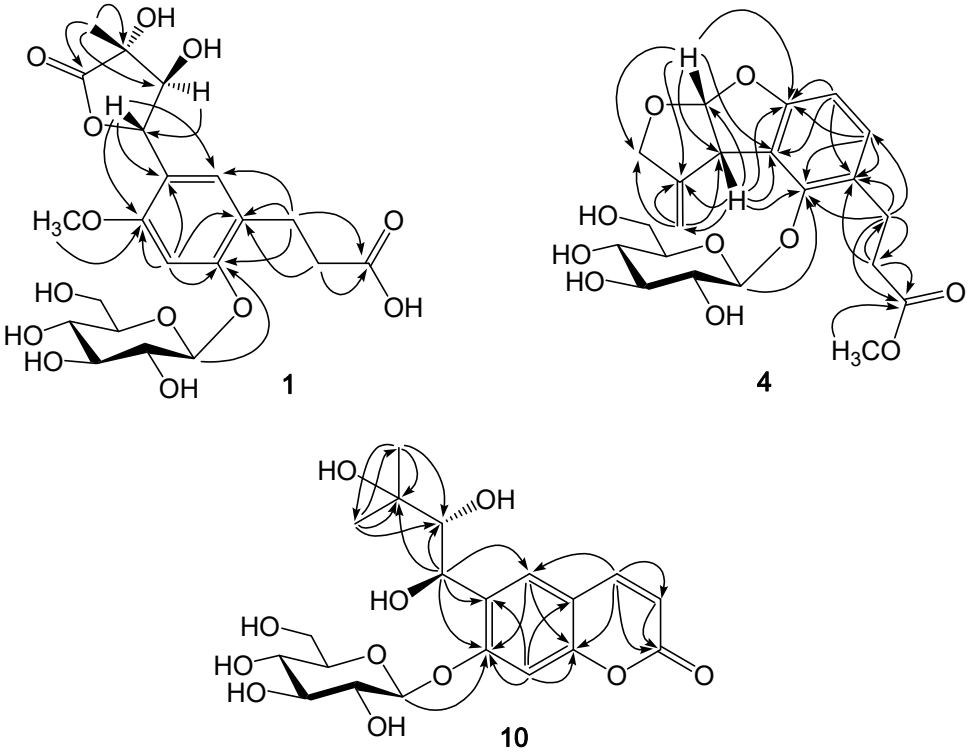

Fig. 2 HMBC correlations of compounds

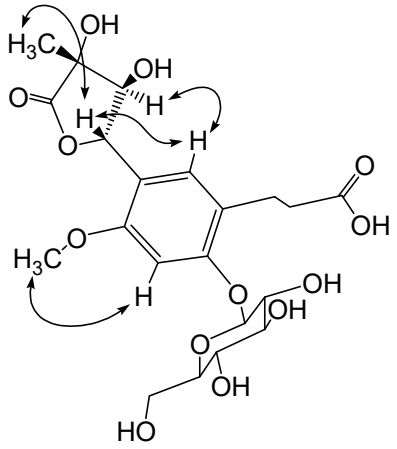

Fig. 3 NOESY correlations of compound

Table 1
NMR spectroscopic data of compounds **1**–**4** (in DMSO-d_6).

Position	1		2		4		3
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_C
Aglycone							
1		121.3		120.9		126.7	127.0
2		156.7		156.8		152.3	152.2
3	2.46 (2H, m)	99.8	2.56 (2H, m)	99.8	2.52 (2H, m)	122.0	121.9
4	2.75 (2H, m)	157.3	2.79 (2H, m)	157.5	2.98 (2H, m)	157.7	157.5
5	7.09 (1H, s)	117.1	7.08 (1H, s)	117.2	6.98 (1H, d, J = 8.2 Hz)	105.9	105.7
6		129.5		129.7	6.53 (1H, d, J = 8.2 Hz)	130.1	129.9
7		24.8		24.8		23.8	23.7
8	6.82 (1H, s)	34.2	6.85 (1H, s)	33.3		34.4	34.8
9		174.2		173.1		173.1	174.2
1′	5.07 (1H, d, J = 8.3 Hz)	77.4	5.07 (1H, d, J = 8.1 Hz)	77.4	6.28 (1H, d, J = 5.8 Hz)	111.9	111.9
2′	4.30 (1H, d, J = 8.3 Hz)	78.6	4.31 (1H, d, J = 8.1 Hz)	78.5	5.01 (1H, br d, J = 5.0 Hz)	49.8	49.8
3		75.1		75.2		146.4	146.4
4′		177.8		177.8	4.16 (1H, dd, J = 12.5, 1.5 Hz) 4.34 (1H, d, J = 12.5 Hz)	70.0	70.0
5′	1.23 (3H, s)	17.9	1.25 (3H, s)	17.9	5.04 (1H, br s) 5.55 (1H, br s)	108.3	108.3
CH_3O— —$COOCH_3$	3.74 (3H, s)	55.7	3.76 (3H, s) 3.56 (3H, s)	55.7 51.3	3.54 (3H, s)	51.3	
Glc							
1″	4.86 (1H, d, J = 7.2 Hz)	100.9	4.85 (1H, d, J = 7.4 Hz)	101.1	4.51 (1H, d, J = 7.3 Hz)	105.8	105.8
2″	3.27 (1H)[a]	73.3	3.28 (1H)[a]	73.4	3.23 (1H)[a]	74.2	74.2
3″	3.36 (1H)[a]	76.9	3.37 (1H)[a]	76.9	3.21 (1H)[a]	76.5	76.5
4″	3.11 (1H)[a]	70.2	3.12 (1H)[a]	70.2	3.14 (1H)[a]	70.3	70.2
5″	3.27 (1H)[a]	76.7	3.26 (1H)[a]	76.9	3.23 (1H)[a]	77.0	77.0
6″	3.41 (1H)[a]	61.0	3.41 (1H)[a]	61.0	3.45 (1H)[a]	61.4	61.4
	3.71 (1H)[a]		3.71 (1H)[a]		3.72 (1H, br d, J = 11.1 Hz)		

[a] Chemical shifts were assigned by COSY and HMQC.

Micromelumoside B (**2**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{22}H_{30}O_{13}$ by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of **1**, except for the additional signal of one more methoxyl group in both spectra (δ_H 3.56 and δ_C 51.3). This functional group was formed to be the methyl ester of **1** since the chemical shift of the carboxyl group was changed to δ_C 173.1 as compared to **1** (Table 1). The structure was confirmed by the long range correlation from this methoxyl group to the carboxyl group. Therefore, the structure was elucidated as shown.

Micromelumoside C (**4**) was isolated as an amorphous powder. The molecular formula was determined to be $C_{21}H_{26}O_{10}$ by HR-ESI-TOF mass spectrometric analysis. This compound was a phenylpropanoic acid derivative containing a bicyclic phenyl moiety, which was related to part of 3,4-dihydro-1,2-*seco*-microminutinin 9-*O*-β-D-glucopyranside (**3**), isolated compound from *M. falcatum* (Kamperdick et al., 1999). In addition this compound displayed the signal of one methoxyl group (δ_H 3.54 and δ_C 51.3). This group was assigned to form an ester linkage to the carboxyl group (δ_C 173.1). The structure was confirmed by HBMC experiment as shown in Fig. 2. Based on this evidence, compound **4** was identified to be a methyl ester of **3**.

Micromelumoside D (**10**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{20}H_{26}O_{11}$ by its HR-ESI-TOF mass spectrometric analysis. The NMR spectroscopic data of compound **10** was related to those of (*S*)-peucedanol 7-*O*-β-D-glucopyranoside (**8**) and (*R*)-peucedanol 7-*O*-β-D-glucopyranoside (**9**) (Table 2). This compound has one oxygen atom more than both compounds, indicating the presence of one more hydroxyl group. This additional group was assigned to be located at C-1' due to the downfield shift of this carbon atom to δ_C 68.0. The structure was confirmed by HMBC experiment, in which the correlation was found from H-1' (δ_H 5.07) to C-5 (δ_C 127.9), C-6 (δ_C 130.8), C-7 (δ_C 158.7), C-2' (δ_C 78.0) and C-3' (δ_C 73.4), as shown in Fig. 2. The absolute configuration at C-2' was assigned to be *R* due to the chemical shifts of two methyls C-4' and C-5' were similar to those of **9**, rather than *S* as shown in **8** (Table 2). The appearance of the coupling constant of two protons H-1' and H-2' with J = 8.5 Hz in the ^{1}H NMR spectrum was in agreement with the reported data for *R*-configuration of

C-1' position (Liu et al., 1995a, 1995b; Kozawa et al., 1982). Therefore, compound **10** was identified to be (1'*R*, 2'*R*)-1'-hydroxy-peucedanol 7-*O*-β-D-glucopyranoside.

Table 2
NMR spectroscopic data of compounds **8**-**10** (in DMSO-d_6)

Position	10		8	9
	δ_H	δ_C	δ_C	δ_C
Aglycone				
2		160.5	160.6	160.9
3	6.30 (1H, d, J=9.5 Hz)	113.1	113.1	112.9
4	8.02 (1H, d, J=9.5 Hz)	144.7	144.5	144.5
5	7.72 (1H, s)	127.9	130.0	130.0
6		130.8	127.1	127.2
7		158.7	158.7	158.3
8	7.06 (1H, s)	102.4	102.1	102.0
9		153.9	153.6	153.6
10		113.1	112.9	112.6
1′	5.07 (1H, d, J=8.5 Hz)	68.0	31.5	31.9
2′	3.43 (1H)	78.0	77.6	76.8
3′		73.4	72.1	72.4
4′	1.19 (3H, s)	24.4	25.0	23.2
5′	1.13 (3H, s)	27.7	26.0	27.3
Glc				
1"	4.88 (1H, d, J=7.2 Hz)	101.4	101.0	100.8
2"	3.30 (1H)[a]	73.3	73.5	73.4
3"	3.28 (1H)[a]	76.5	76.5	76.1
4"	3.17 (1H)[a]	69.8	69.8	69.8
5"	3.32 (1H)[a]	77.3	77.3	77.2
6"	3.41 (1H)[a]	60.8	60.9	60.8
	3.73 (1H)[a]			

[a] Chemical shifts were assigned by COSY and HMQC.

The known compounds were identified to be 3,4-dihydro-1,2-*seco*-microminutinin 9-*O*-β-D-glucopyranside (**3**) (Kamperdick et al., 1999), umbelliferone-7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**5**) (Xu et al., 2012), scopolin (**6**) (Tsukamoto et al., 1985), haloperoside (**7**) (Yuldashev et al., 1980), (*S*)-peucedanol 7-*O*-β-D-glucopyranoside (**8**) (Kitajima et al., 1998b), (*R*)-peucedanol 7-*O*-β-D-glucopyranoside (**9**) (Ikeshiro et al., 1994), decuroside V (**11**)

(Asahara etal., 1984), 4-hydroxy-2,6-dimethoxyphenyl 4-*O*-β-D-glucopyranoside (**12**) (Otsuka et al., 1989), vanilloloside (**13**) (Ida et al., 1994), 3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (**14**) (Kitajima et al., 1998a), (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**15**) (Achenbach et al., 1992), (+)-syringaresinol 4-*O*-β-D-glucopyranoside (**16**) (Kobayashi et al., 1985), kaempferol 3-*O*-sophoroside (**17**), kaempferol 3-*O*-rutinoside (**18**) (Agrawal and Bansal, 1989), and citroside B (**19**) (Umehara et al., 1988). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

The present work isolated 19 secondary metabolites from the water soluble fraction including phenylpropanoic acid derivatives (**1-4**), coumarin glycosides (**5-11**), simple phenolic glucosides (**12-14**), lignan glucosides (**15**, **16**), flavonol glycosides (**17**, **18**) and a megastigmane glucoside (**19**) from *M. minutum.* Phenylpropanoic acid derivatives and coumarins were expected to isolate from this plant since these compound-types were considered as chemotaxonomic markers on this genus. The results from this work provided more information data of the typical profile of the polar secondary metabolites isolated from *Micromelum* genus.

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Part 9

Chemical Constituents from *Gloriosa superba* L.

Chemical Constituents from *Gloriosa superba* L.

Introduction

Gloriosa superba L. (Thai name: Dong-Dueng), a member of the family Colchicaceae, is an herbaceous climber native of tropical Africa, and commonly growing in tropical regions of Asia. In Thailand, this species is described as a toxic plant since there was report on several cases of apparent poisoning related to consumption of its tubers. This plant is well known to contain colchicinoid derivatives such as colchicine, colchicoside, and lumicolchicine (Dunuwille et al., 1968; Chaudhuri and Thakur, 1993; Joshi et al., 2010). Furthermore, the medicinal uses, biological activities and toxicological investigations have been reviewed (Jana and Shekhawat, 2013). This present study deals with the isolation and determination of the chemical constituents from seedless pods and seeds, including three new colchicinoid glucoside (**2-4**), four known colchicinoids (**1**, **5-7**), and three flavonoids (**8-10**).

Experimental Section

General Procedure: NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer. MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, silica gel 60 (70–230 mesh, Merck), RP-18 (50 μm, YMC), and Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.) were used. Preparative HPLC was carried out on an ODS column (250 X 20 mm i.d., YMC) with a Jasco RI-2031 refractive index detector. The flow rate was 6 ml/min.

Plant material: *Gloriosa superba* L. was cultivated at Ban Na-Dok-Mai (16.487216 N, 102.615195 E), Tambon Nong-Bua, Amphoe Ban-Fang, Khon Kaen province in April 2013 and collected the fresh fruits in November 2013. The plant was identified by one of us (T. Kanchanapoom). A voucher specimen (TK-PSKKU-0075) was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation: The fresh fruits of *G. superba* were collected and then divided into two parts, seedless pods and seeds. The seedless pods (254.7 g) were extracted three times with MeOH, and concentrated to dryness. This residue (61.5 g) was suspended in H_2O and partitioned with EtOAc. The EtOAc layer were combined and dried under reduced pressure to give a brownish powder (14.7 g), then re-suspended in 90% aqueous MeOH and defatted with Hexane, successively. The 90% aqueous MeOH part (7.7 g) was chromatographed on a column of silica gel using solvent systems EtOAc–MeOH (19:1, 2.0 L), EtOAc–MeOH (9:1, 3.0 L), EtOAc–MeOH (4:1, 3.0 L), EtOAc–MeOH (7:3, 2.0 L), and EtOAc–MeOH (1:1, 3.0 L), respectively to obtain seven fractions (A_3 to G_3). Fraction E_3 was provided compound **10** (553.9 mg) by crystallization. Fraction F_3 was purified by preparative HPLC-ODS with 25% aqueous MeCN to give compounds **5** (281.4 mg) and **6** (614.9 mg). The aqueous fraction (45.5 g) was also applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The portion eluted with methanol (3.5 g) was repeatedly chromatographed on a column of silica gel using solvent systems EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH–H_2O (40:10:1, 5.0 L), EtOAc–

MeOH–H_2O (70:30:3, 2.0 L) and EtOAc–MeOH–H_2O (6:4:1, 2.0 L), respectively to provide four fractions (A_4 to D_4). Fraction B_4 was purified by preparative HPLC with 15% aqueous MeCN to obtain compound **4** (80.9 mg). Fraction C_4 was further purified by preparative HPLC-ODS with 15% aqueous MeCN to give compounds **1** (365.9 mg), **2** (28.5 mg), and **3** (40.9 mg).

Seeds (372.0 g) were similarly extracted three times with MeOH. The MeOH extract was concentrated *in vacuo* to give a brownish powder (43.6 g). This residue was suspended in H_2O, and partitioned three times with EtOAc. The EtOAc soluble fraction (7.0 g) was applied to a column of silica gel using solvent systems EtOAc–MeOH (19:1, 2.0 L), EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH (4:1, 4.0 L), EtOAc–MeOH (7:3, 3.0 L), and EtOAc–MeOH (1:1, 3.0 L), respectively to obtain seven fractions (A_1 to G_1). Fraction A_1 was provided compound **8** (190.0 mg) by crystallization. Fraction F_1 (1.92 g) was purified by preparative HPLC-ODS with solvent system 25% aqueous MeCN to give compounds **5** (812.6 mg), **6** (8.0 mg), **7** (23.4 mg), and **9** (10.2 mg). The remaining aqueous fraction (36.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (2.0 g) was subjected to a column of silica gel using solvent systems EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH–H_2O (40:10:1, 3.0 L), EtOAc–MeOH–H_2O (70:30:3, 2.0 L) and EtOAc–MeOH–H_2O (6:4:1, 2.0 L), respectively to provide five fractions (A_2 to E_2). Fraction B_2 (1.1 g) was purified by preparative HPLC-ODS with solvent system 28% aqueous MeCN to afford compound **5** (74.9 mg). Finally, fraction C_2 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to provide compound **1** (8.0 mg).

Dongduengoside A (2): Amorphous powder, $[\alpha]_D^{24}$ −109.2 (MeOH, *c* 0.72); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m/z*: 534.1962 ($C_{26}H_{32}NO_{11}$ required 534.1970).

Dongduengoside B (3): Amorphous powder, $[\alpha]_D^{24}$ −88.7 (MeOH, *c* 0.85); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m/z*: 548.2135 ($C_{27}H_{34}NO_{11}$ required 548.2126).

Dongduengoside C (4): Amorphous powder, $[\alpha]_D^{24}$ +72.0 (MeOH, *c* 1.00); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m/z*: 548.2111 ($C_{27}H_{34}NO_{11}$ required 548.2126).

Results and discussion

The methanolic extracts of seedless pods and seeds were processed individually by a combination of chromatographic procedures to provide ten compounds (**1-10**). From seedless pods, three new colchicinoid glucosides (**2-4**) were identified. Seven known compounds were identified as colchicine (**5**), 2-demethylcolchicine (**6**), 3-demethylcolchicine (**7**) (Hufford Hufford et al., 1979), colchicoside (**1**) (Yoshida et al., 1988), *epi*-catechin (**8**) (Agrawal et al., 1989), quercetin 3-*O*-β-D-glucopyranoside (**9**) and luteolin 7-*O*-β-D-glucopyranoside (**10**) (Agrawal and Bansal, 1989) by comparison of physical data with literature values and from spectroscopic evidence.

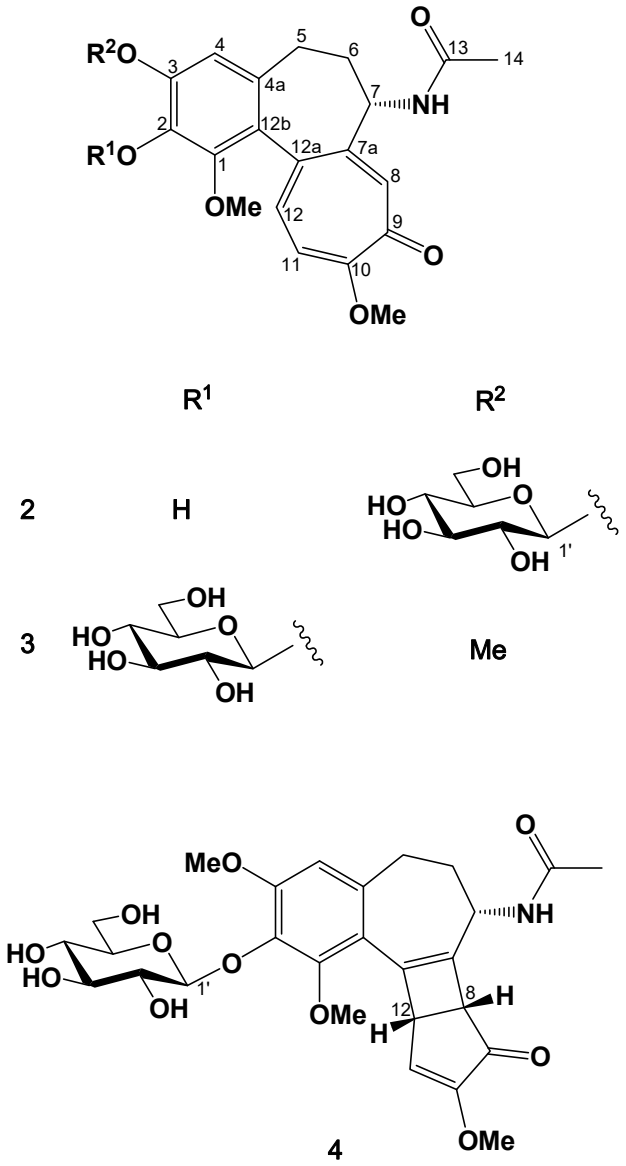

Figure 1 Structures of compounds **2-4**

Dongduengoside A (**2**) was isolated as an amorphous powder. The molecular formula was determined to be $C_{26}H_{31}NO_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectra revealed the presence of two methoxyl groups and one β-D-glucopyranosyl unit in addition to the signals of a colchicinoid skeleton as compared to colchicoside (**1**). The spectroscopic data were related to those of colchicoside (**1**) except for lacking

one methoxyl signal, which established the presence of a hydroxyl group. The locations of these functional groups were assigned by the results of 2D-NMR experiments. All protonated carbons were assigned by HSQC experiment. An NOESY correlation between the anomeric proton signal at δ_H 4.71 (*d*, J = 7.2) and H-4 at δ_H 6.80 (*s*), as well as the HMBC correlations from both protons to C-3 (δ_C 146.0) were observed from the spectra, indicating that the glucopyranosyl unit was placed at C-3 position. Two methoxyl groups at δ_H 3.50 with δ_C 60.1 and δ_H 3.86 with δ_C 56.1 were ascribable to be 1-OMe and 10-OMe, from the HMBC correlations to C-1 (δ_C 145.1) and C-10 (δ_C 163.5), respectively (Figure 2). Thus, the remaining hydroxyl group could be deduced to be linked to C-2 position (δ_C 139.0). Consequently, the structure of compound **5** was elucidated to be 2-demethylcolchicoside or 2,3-didemethylcolchicine 3-*O*-β-D-glucopyranoside.

Dongduengoside B (**3**) was obtained as an amorphous powder and its molecular formula was determined to be $C_{27}H_{33}NO_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data were closely related to those of colchicoside (**1**). The significant differences were the chemical shifts of A-ring, suggesting the interchange of the functional groups. The complete assignments were clearly established by NOESY, HSQC and HMBC experiments. In the HMBC spectrum, the correlation was found from the anomeric proton at δ_H 4.97 (*d*, J = 7.3) to C-2 (δ_C 137.7), indicating the position of the sugar unit to this carbon atom. Also, three methoxyl groups at δ_H 3.44 with δ_C 60.8, δ_H 3.85 with δ_C 56.4, and δ_H 3.85 with δ_C 56.1 were assignable to be 1-OMe, 3-OMe and 10-OMe, respectively, by the observation of HMBC correlations as

illustration in Figure 2. Moreover, the NOESY spectrum showed the significant correlations from the anomeric proton to the 1-OMe, and from H-4 (δ_H 6.75) to 3-OMe, confirming the position of the sugar unit. Therefore, the structure of this compound was identified as 2-demethylcolchicine 2-*O*-β-D-glucopyranoside.

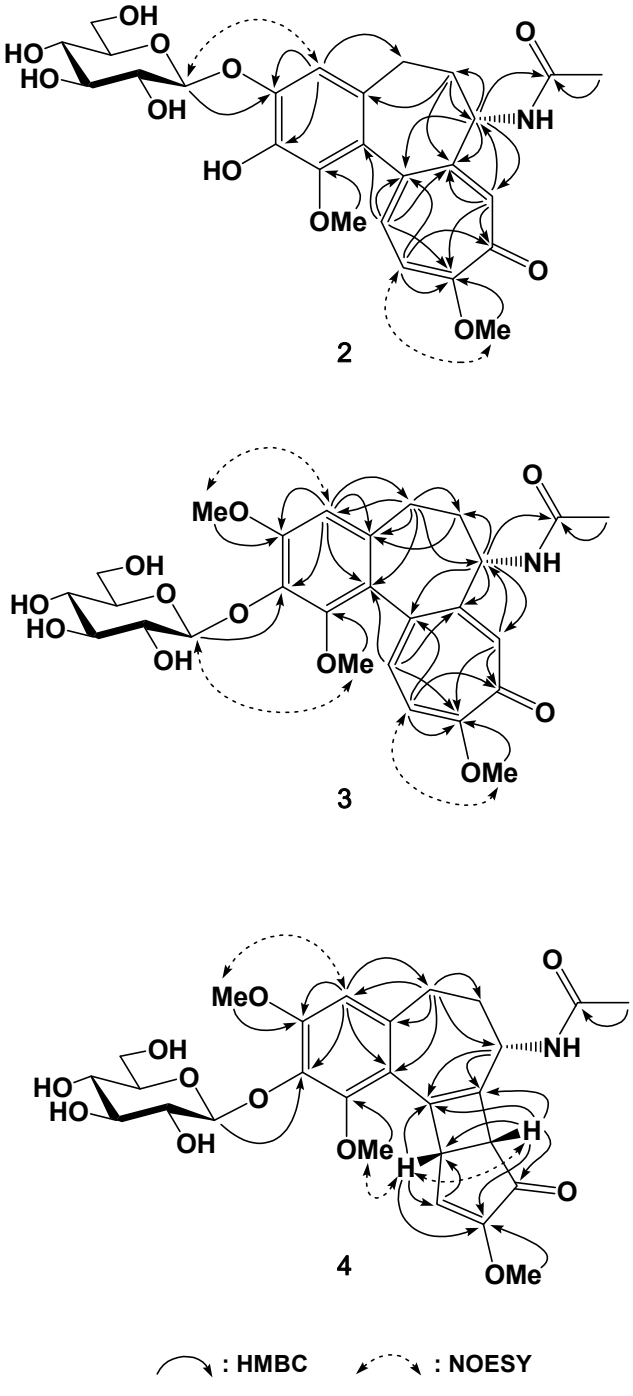

Figure 2 HMBC and NOESY correlations of compounds **2-4**

Table 1 ^{1}H-NMR spectroscopic data of compounds **1-4** (400 MHz, DMSO-d_6)

Position	**1**	**2**	**3**	**4**
4	6.84 (1H, *s*)	6.80 (1H, *s*)	6.75 (1H, *s*)	6.67 (1H, *s*)
5	2.12-2.22 (1H)	2.09-2.18 (1H)	2.14-2.23 (1H)	2.64-2.72 (2H)
	2.52-2.58 (1H)	2.40-2.48 (1H)	2.54-2.60 (1H)	
6	1.73-1.82 (1H)	1.73-1.80 (1H)	1.73-1.80 (1H)	1.70-1.78 (2H)
	1.95-2.05 (1H)	1.94-2.02 (1H)	1.98-2.08 (1H)	
7	4.26-4.36 (1H)	4.27-4.35 (1H)	4.29-4.38 (1H)	4.71 (1H, *dd*, J = 6.9, 1.9 Hz)
8	7.13 (1H, s)	7.11 (1H, s)	7.13 (1H, s)	3.41-3.44 (1H)
11	7.02 (1H, *d*, J = 11.1 Hz)	7.01 (1H, *d*, J = 11.2 Hz)	7.00 (1H, *d*, J = 11.0 Hz)	6.58 (1H, *d*, J = 3.1 Hz)
12	7.11 (1H, *d*, J = 11.1 Hz)	7.09 (1H, *d*, J = 11.2 Hz)	7.11 (1H, *d*, J = 11.0 Hz)	4.01 (1H, *d*, J = 3.1, 2.9 Hz)
14	1.83 (3H, *s*)	1.83 (3H, *s*)	1.83 (3H, *s*)	1.77 (3H, *s*)
1-OMe	3.52 (3H, *s*)	3.50 (3H, *s*)	3.44 (3H, *s*)	3.87 (3H, *s*)
2-OMe	3.83 (3H, *s*)			
3-OMe			3.79 (3H, *s*)	3.75 (3H, *s*)
10-OMe	3.87 (3H, *s*)	3.86 (3H, *s*)	3.85 (3H, *s*)	3.58 (3H, *s*)
NH	8.59 (1H, *d*, J = 7.4 Hz)	8.57 (1H, *d*, J = 7.4 Hz)	8.58 (1H, *d*, J = 7.2 Hz)	4.30 (1H, br s)
Glc-1'	4.92 (1H, *d*, J = 7.3 Hz)	4.71 (1H, *d*, J = 7.2 Hz)	4.97 (1H, *d*, J = 7.3 Hz)	5.04 (1H, *d*, J = 7.3 Hz)
2'	3.25-3.36 (1H)	3.30-3.36 (1H)	3.19-3.27 (1H)	3.18-3.24 (1H)
3'	3.25-3.36 (1H)	3.27 (1H, *dd*, J = 9.3, 8.5 Hz)	3.19-3.27 (1H)	3.14-3.21 (1H)
4'	3.17 (1H, *dd*, J = 9.3, 8.6 Hz)	3.16 (1H, *dd*, J = 9.3, 8.4 Hz)	3.16 (1H, *dd*, J = 9.4, 8.0 Hz)	3.08-3.15 (1H)
5'	3.33-3.40 (1H)	3.32-3.40 (1H)	3.02-3.10 (1H)	3.02-3.10 (1H)
6'	3.66-3.75 (1H)	3.68-3.74 (1H)	3.58-3.63 (1H)	3.36-3.43 (1H)
	3.80-3.87 (1H)	3.78-3.83 (1H)	3.83-3.88 (1H)	3.55-3.62 (1H)

Dongduengoside C (**4**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{27}H_{33}NO_{11}$ by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra showed the presence of three methoxyl groups, one β-D-glucopyranosyl unit in addition to the typical signals for colchicine lumi-derivatives as the core structure (Potešilová et al., 1985; Meksuriyen et al., 1988). The chemical shifts of this compound were related to those of β-lumicolchicine (Meksuriyen et al., 1988) except for a set of additional signals of a β-D-glucopyranosyl unit instead of one methoxyl group. The assignment of the structure was deduced by the results from 2D-NMR spectroscopic methods including NOESY, HSQC and HMBC experiments. The sugar unit was assigned to be located at C-2 (δ_C

136.2) since the HMBC correlation was found from the anomeric proton at δ_H 5.04 (*d*, J = 7.3) to this carbon atom. Three methoxyl groups at δ_H 3.87 with δ_C 61.5, δ_H 3.75 with δ_C 56.3, and δ_H 3.58 with δ_C 56.3 was assigned to be linked at C-1 (δ_C 151.1), C-3 (δ_C 152.3) and C-10 (δ_C 157.4) based on the correlations from their singlet proton signals to C-1, C-3 and C-10, respectively. Furthermore, the NOESY spectrum showed the cross peaks between 1-OMe (δ_H 3.87) and H-12 (δ_H 4.01), and between H-8 (δ_H 3.41-3.44) and H-12 (δ_H 4.01) in Figure 2, supporting the characteristic of β-lumi-derivative skeleton of this compound (Meksuriyen et al., 1988). Also, the NOESY correlation was observed between H-4 (δ_H 6.67) and 3-OMe. Therefore, the structure was elucidated as 2-demethyl-β-lumicolchicine 2-*O*-β-D-glucopyranoside.

In conclusion, the present work reported ten compounds including seven colchicinoids (**1**-**7**) and three flavonoids (**8**-**10**) from Thai plant source. Colchicine (**5**), 2-demethylcolchicine (**6**), 3-demethylcolchicine (**7**) and colchicoside (**1**) were expected to isolate from seeds of this species as previously reported from the literatures (Chaudhuri and Thakur, 1993; Joshi et al., 2010). The new finding of colchicinoids from seedless pods was surprising since three compounds were new, as well as colchicoside (**1**) and colchicine (**5**) contents from this part were high. It seems, therefore that seedless pods from Thai origin *G. superba* could be applied and useful for a source for production of colchicine and colchicoside.

Table 2 ^{13}C NMR spectroscopic data of compounds **1-4** (100 MHz, DMSO-d_6)

Position	1	2	3	4
1	150.5	145.1	150.6	151.1
2	141.2	139.0	137.7	136.2
3	150.9	146.0	152.8	152.3
4	111.1	111.5	108.5	110.3
4a	134.0	128.7	134.5	139.7
5	29.3	28.9	29.2	31.3
6	35.7	36.1	35.7	31.8
7	51.4	51.3	51.4	48.4
7a	151.0	151.1	151.0	138.6
8	130.4	130.4	130.4	51.1
9	178.0	178.1	178.1	197.8
10	163.6	163.5	163.6	157.4
11	112.2	112.2	112.3	127.5
12	134.6	134.5	134.6	42.2
12a	135.2	135.6	135.3	143.4
12b	126.6	127.2	125.9	118.2
13	168.7	168.6	168.7	168.4
14	22.5	22.5	22.5	22.8
1-OMe	61.0	60.1	60.8	61.5
2-OMe	60.9			
3-OMe			56.4	56.3
10-OMe	56.1	56.1	56.1	56.3
Glc -1'	100.5	102.2	103.1	101.9
2'	73.4	73.4	74.1	74.2
3'	76.9	76.0	76.5	76.5
4'	69.9	70.0	70.0	70.0
5'	77.3	77.3	77.2	77.2
6'	60.8	60.8	60.9	60.9

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Part 10

Chemical Constituents from *Desmodium heterocarpon* (L.) DC.

Chemical Constituents from *Desmodium heterocarpon* (L.) DC.

Introduction

Desmodium heterocarpon (L.) DC., a member of the family Fabaceae, is commonly found in the paddy field during the raining season in North-eastern Thailand. It is used in Thai traditional medicine for anti-fever and anthelmintic purposes. Previous phytochemical investigation of this plant reported the isolation of several compounds such as triterpenoids, steroids and flavonoids (Huang et al., 2010). In the biological properties, the antioxidant activities of phenolic components from plant extract have been reported (Tsai et al., 2011). This present study describes the isolation and structure identification of seven glycosides including a new 2-carboxy-dihydrostilbene derivative glucoside (**1**) and a new flavan diglycoide (**3**) from the water soluble fraction of the aerial portion of this plant in addition to five known compounds (**2**, **4-7**).

Experimental Section

General Procedure: NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer. MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. CD spectrum was recorded on a Jasco J-810 spectropolarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 x 250 mm i.d., Vertisep^{TM} AQS) with a Jasco MD-2010 detector

at 220 nm. The flow rates were 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant material: The aerial portion of *Desmodium heterocarpon* (L.) DC was collected from Ban Na-Dok-Mai, Tambon Nong-Bua, Amphoe Ban-Phang, Khon Kaen Province in November 2011. A voucher specimen (TK-PSKKU-0076) was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation: The air dried aerial portion of *D. heterocarpon* (2.5 kg) were extracted three times with MeOH, and concentrated to dryness. The residue (258.2 g) was suspended in H_2O and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (213.9 g) was applied to a Diaion HP-20 column, and eluted with H_2O, and MeOH, successively. The fraction eluted with MeOH (76.0 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 8.0 L), EtOAc-MeOH-H_2O (40:10:1, 10.0 L), EtOAc-MeOH-H_2O (70:30:3, 8.0 L) and EtOAc-MeOH-H_2O (6:4:1, 6.0 L), respectively to obtain seven fractions (A to G). Fraction B (6.8 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide seven sub-fractions. Sub-fraction B-2 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (90:10, v/v) to provide compound **4** (30.5 mg). Sub-fraction B-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to yield compound **5** (50.3 mg). Sub-fraction B-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (82:18, v/v) to provide compound **3** (110.3 mg). Fraction D (13.5 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide

seven sub-fractions. Sub-fraction D-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to provide compound **6** (16.8 mg). Sub-fraction D-7 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (80:20, v/v) to afford compound **7** (50.2 mg). Fraction F (5.9 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to give eight sub-fractions. Sub-fraction F-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (89:11, v/v) to obtain compound **1** (27.1 mg). Sub-fraction F-4 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to give compound **2** (70.0 mg).

2-Carboxy-3,5,4'-trihydroxydihydrostilbene 3-*O*-β-D-glucopyranoside (1): Amorphous powder, $[\alpha]_D^{24}$ –35.9 (DMSO, *c* 0.44); ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): Table 1; Negative HR-FAB-MS, *m/z*: 435.1307 ($C_{21}H_{23}O_{10}$ required 435.1297).

(2S)-5,7,4'-trihydroxyflavan 7-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (3): Amorphous powder, $[\alpha]_D^{24}$ –56.2 (MeOH, *c* 0.47); CD (MeOH, *c* 4.4 x 10^{-4} M) Δε (nm) -3.45 (250), -2.51 (272), +3.57 (288); ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): Table 2; Negative HR-FAB-MS, *m/z*: 551.1778 ($C_{26}H_{31}O_{13}$ required 551.1770).

Results and discussion

The methanolic extract of the aerial portions of *D. heterocarpon* was partitioned between Et_2O and H_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to provide a new glucoside of dihydrostilbenic acid (**1**)

and a new flavan diglycoside (**3**) (Fig. 1) along with five known compounds. The known compounds were identified to be lunularic acid 4'-*O*-β-D-glucopyranoside (**2**) (Fang et al., 2012), vitexin (**4**), isovitexin (**5**) (Agrawal and Bansal, 1989), (−)-*epi*-catechin 3-*O*-β-D-glucopyranoside (**6**) (Morimoto et al., 1985), and (−)-*epi*-afzelachin 3-*O*-β-D-glucopyranoside (**7**) (Morimoto et al., 1988). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

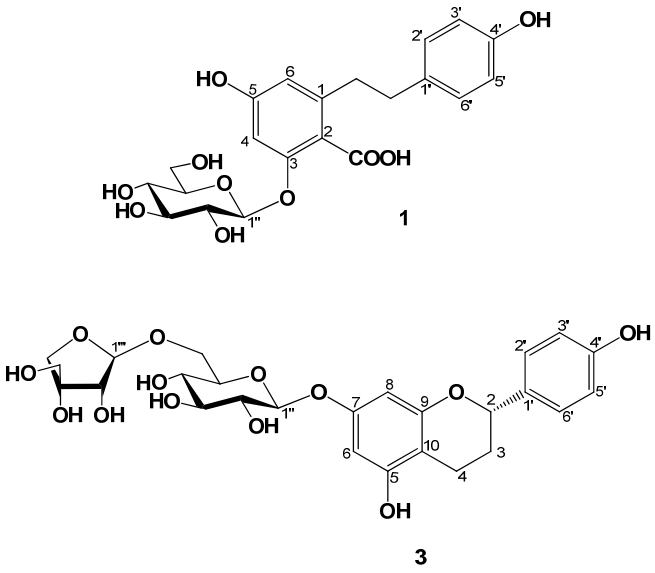

Figure 1 Structures of compounds **1** and **3**

Compound **1**, $[\alpha]_D^{24}$ −35.9, was isolated as an amorphous powder. The molecular formula was determined to be $C_{21}H_{24}O_{10}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H NMR spectrum revealed the presence of a pair of *meta*-coupled aromatic protons at δ_H 6.12 and 6.16 (each 1H, d, J = 2.5 Hz), a set of AA'BB' aromatic ring at δ_H 6.64 and 7.05 (each 2H, d, *J* = 8.4 Hz), two methylenes at δ_H 2.63 (2H, dd, J = 8.2, 8.1 Hz) and 3.18 (2H, m)

in addition to an anomeric proton signal at δ_H 5.59 (1H, d, *J* = 7.3 Hz). The ^{13}C NMR spectrum showed that this compound contained two aromatic ring systems, two methylene carbons, one carboxyl group for the aglycone moiety, as well as one β-D-glucopyranosyl moiety. The NMR spectral data (Table 1) indicated that this compound possessed the same core structure (2-carboxy-dihydrostilbene), as compared to lunularic acid 4'-*O*-β-D-glucopyranoside (**2**). This compound has one oxygen atom more than compound **2**, suggesting the presence of an additional hydroxyl group on the aromatic ring. The complete assignment was based on the analyzing results from 2D-NMR spectroscopic methods including COSY, NOESY, HMQC and HMBC. In the HMBC spectrum (Figure 2), the significant correlations were found from methylene protons at δ_H 3.18 (H_2-α) to an aryl methine carbon at δ_C 108.2, and two non-protonated aryl carbons at δ_C 112.2 and 147.0, ascribable to C-6, C-2 and C-1, respectively. The chemical shift of a methine carbon at δ_C 101.3 was assigned to locate at C-4 position of the aromatic ring due to the coupling constant between δ_H 6.12 (H-6) and 6.16 (H-4) with J = 2.5 Hz, and was also confirmed by the HMBC correlation from δ_H 6.12 (H-6) to this carbon atom. The oxy-aryl carbon at δ_C 159.0 was placed at C-3 position since the HMBC correlation was observed only from δ_H 6.16 (H-4). The appearance of the HMBC correlations from δ_H 6.12 (H-6) and 6.16 (H-4) to the oxy-aryl carbon at δ_C 166.2 indicated that this carbon atom was C-5 position. The remaining oxy-aryl carbon at δ_C 155.4 was belonged to C-4' position of the other aromatic ring, supporting by the HMBC correlations from δ_H 7.05 (H-2',6') and 6.64 (H-3',5'). Finally, the sugar unit was assigned to be linked at C-3 position, deduced from the HMBC correlation from the anomeric proton at δ_H 4.77 (H-1") to C-

3 (δ_C 159.0). Moreover, the NOESY cross peak were found between H-1" and H-4. Therefore, the structure of this compound was elucidated to be 2-carboxy-3,5,4'-trihydroxydihydrostilbene 3-*O*-β-D-glucopyranoside.

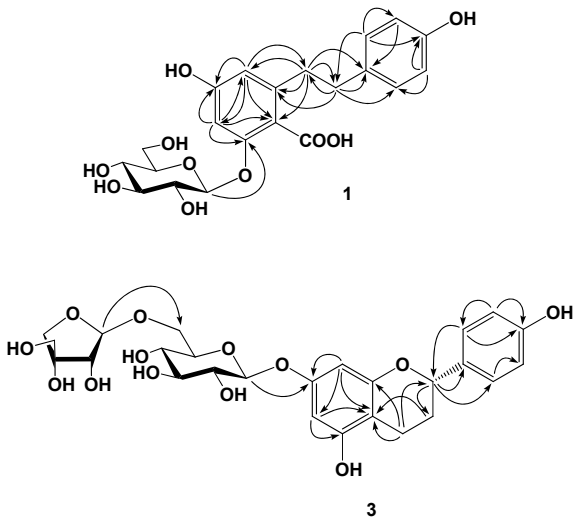

Figure 2 HMBC correlations of compounds **1** and **3**

Compound **3**, $[\alpha]_D^{24}$ −56.2, was isolated as an amorphous powder. The molecular formula was determined to be $C_{26}H_{32}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H NMR spectrum revealed the presence of two sugar units from the anomeric signals at δ_H 4.70 (1H, d, J = 7.6 Hz) and 4.80 (1H, d, J = 3.1 Hz), a pair of *meta*-coupled aromatic protons at δ_H 5.96 and 6.8 (each 1H, d, J = 2.5 Hz), and a set of AA'BB' aromatic ring at δ_H 6.74 and 7.19 (each 2H, d, *J* = 8.4 Hz) in addition to a broad double signal at δ_H 4.86 (1H, J = 9.3 Hz) and multiplets at δ_H 1.88 (1H), 2.03 (1H) and 2.53 (2H). In the ^{13}C NMR spectrum, 11 carbon signals belonging to the sugar part could be identified to be a β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit by comparing chemical shifts with the reported data (Kanchanapoom et al., 2011). The remaining 15 carbon atoms

were consistant with a flavonoid skeleton. The partial connectivities of C-2 to C-3 and C-4 were deduced from COSY and HMQC analysis, indicating that the aglycone of this compound was a flavan (Sauvain et al., 1994). In the HMBC spectrum, the correlation from the anomeric proton at δ_H 4.70 (H-1" Glc) to C-7 (dC 156.7) indicated the location of the sugar moiety to this carbon atom. Besides, the NOSEY spectrum provide the further confirmation of the sugar unit to C-7 position from the cross peak correlation of H-1" Glc with δ_H 5.96 (H-6) and 6.08 (H-8). The absolute configuration at C-2 position was established to be *S* from the CD spectrum, which showed extreme values for Δε (nm) −3.57 (277), relating to the data of 2*S*-configurated compounds (Baba et al., 1986; Yang et al., 2010; Zhong et al., 2013). Consequently, the structure of this compound was identified as (2*S*)-5,7,4'-trihydroxyflavan 7-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

Table 1 NMR spectroscopic data of compounds **1** and **2** (^{1}H NMR, 400 MHz and ^{13}C NMR, 100 MHz)

Position	1		2
	Proton	**Carbon**	**Carbon**
1		147.0	145.0
2		112.2	118.2
3		159.0	163.8
4	6.16 (1H, d, J = 2.5 Hz)	101.3	114.5
5		166.2	130.1
6	6.12 (1H, d, J = 2.5 Hz)	108.2	119.5
1'		133.4	136.4
2', 6'	7.05 (2H, d, J = 8.4 Hz)	129.5	129.2
3', 5'	6.64 (2H, d, J = 8.4 Hz)	115.1	116.0
4'		155.4	155.5
α	3.18 (2H, m)	38.2	37.9
β	2.63 (2H, dd, J = 8.2, 8.1 Hz)	37.3	37.3
COOH		173.1	173.0
Glc-1"	4.77 (1H, d, J = 7.7 Hz)	100.1	100.7
2"	3.13-3.20 (1H)	73.5	73.3
3"	3.30-3.38 (1H)	76.9	76.7
4"	3.13-3.20 (1H)	70.0	69.8
5"	3.21-3.29 (1H)	77.2	77.0
6"	3.40-3.50 (1H)	61.0	60.8
	3.66 (1H, br d, J = 11.5 Hz)		

Table 2 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compound **2**

Position	Proton	Carbon
2	4,86 (1H, br d, J = 9.3 Hz)	76.7
3	1.88 (1H, m)	19.1
	2.03 (1H, m)	
4	2.53 (2H, m)	28.6
5		156.3
6	5.96 (1H, d, J = 2.3 Hz)	95.4
7		156.7
8	6.08 (1H, d, J = 2.3 Hz)	96.3
9		156.1
10		103.4
1'		132.0
2', 6'	7.19 (2H, d, J = 8.4 Hz)	127.6
3', 5'	6.74 (2H, d, J = 8.4 Hz)	115.1
4'		157.0
Glc-1"	4.70 (1H, d, J = 7.6 Hz)	100.5
2"	3.09-3.16 (1H)	73.2
3"	3.23 (1H, dd, J = 8.8, 8.6 Hz)	76.5
4"	3.05-3.13 (1H)	69.7
5"	3.32-3.42 (1H)	75.4
6"	3.40-3.50 (1H)	67.4
	3.77-3.84 (1H)	
Api-1"'	4.80 (1H, d, J = 3.1 Hz)	109.3
2"'	3.72 (1H, d, J = 3.1 Hz)	76.0
3"'		78.8
4"'	3.54 (1H, d, J = 9.4 Hz)	73.4
	3.83 (1H, d. J = 9.4 Hz)	
5"'	3.20-3.32 (2H)	63.3

The present work reported seven compounds from the water soluble part including two 2-carboxy-dihydrostilbene derivatives (**1, 2**) and five flavonoids (**3-7**) from the aerial portion of *D. heterocarpon*. The occurrence of 2-carboxy-dihydrostilbene derivatives from angiosperm was quite interesting since this compound-type was mainly reported from the lower plants such as bryophytes and ferns (Hashimoto et al., 1988; Fang et al., 2012; Asakawa et al., 2013). This new finding adds the information data to the plant kingdom and might be useful for further chemotaxonomic studies of this genus.

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Research Outputs

Publication in International Journals

1. Sahakitpichan, P., Mahidol, C., Disadee, W., Ruchirawat, S., Kanchanapoom, T. Unusual glycosides of pyrrole alkaloid and 4'-hydroxyphenylethanamide from leaves of *Moringa oleifera*. Phytochemistry 72 791-795 (2011).

2. Sahakitpichan, P., Disadee, W., Ruchirawat, S., Kanchanapoom, T. 3-Hydroxydihydrobenzofuran glucosides from *Gnaphalium polycaulon*. Chemical and Pharmaceutical Bulletin 59, 1160-1162 (2011).

3. Disadee, W., Mahidol, C., Sahakitpichan, P., Sitthimonchai, S., Ruchirawat, S., Kanchanapoom, T. Unpredented furan-2-carbonyl *C*-glycosides and phenolic diglycosides from *Scleropyrum pentandrum*. Phytochemistry 74, 115-122 (2012).

4. Phakeovilay, C., Disadee, W., Sahakitpichan, P., Sitthimonchai, S., Kittakoop, P., Ruchirawat, S., Kanchanapoom, T. Phenylethanoid and flavones glycosides from *Ruellia tuberosa* L. Journal of Natural Medicines 67, 228-233 (2013).

5. Sahakitpichan, P., Mahidol, C., Disadee, W., Chimnoi, N., P., Ruchirawat, S., Kanchanapoom, T. Glucopyranosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid and (*R*)-eucomic acid, and an aromatic glucoside from the pseudobulbs of *Grammatophyllum speciosum*. Tetrahedron 69, 1031-1037 (2013).

6. Sahakitpichan, P., Chadmuk, P Disadee, W., Ruchirawat, S., Kanchanapoom, T. New *trans*- and *cis*-*p*-coumaroyl flavonol tetraglycosides from the leaves of *Mitragyna rotundifolia*. Phytochemistry Letters 8, 65-68 (2014).

7. Panyadee, A., Sahakitpichan, P., Ruchirawat, S., Kanchanapoom, T. 5-Methyl ether flavone glucosides from the leaves of *Bruguiera gymnorrhiza*. Phytochemistry Letters 11, 215-219 (2015).

8. Sahakitpicham, P., Tanpatanan, W., Chimnoi, N., Ruchirawat, S., Kanchanapoom, T. Glucosides of phenylpropanoic acid derivatives and coumarins from *Micromelum minutum*. Phytochemistry Letters 14, 12-16 (2015).

9. Sahakitpichan, P., Chimnoi, N., Namsa-aid, A., Panyadee, A., Ruchirawat, S., Kanchanapoom, T. Colchicinoid glucosides from seedless pods of Thai origin *Gloriosa superba*. Phytochemistry Letters 16, 299-302 (2016).

10. Sahakitpichan, P., Chadmuk, P., Chimnoi, N., Namsa-aid, A., Ruchirawat, S., Kanchanapoom, T. 2-Carboxy-dihydrostilbene and flavan glycosides from *Desmodium heterocarpon*. Phytochemistry Letters 19, 94-97 (2017)

Appendix 1:

Unusual glycosides of pyrrole alkaloid and 4'-hydroxyphenylethanamide from leaves of *Moringa oleifera*

Appendix 2:

3-Hydroxydihydrobenzofuran glucosides from *Gnaphalium polycaulon*

Appendix 3:

Unpredented furan-2-carbonyl *C*-glycosides and phenolic diglycosides from *Scleropyrum pentandrum*

Appendix 4:

Phenylethanoid and flavones glycosides from *Ruellia tuberosa* L.

Appendix 5:

Glucopyranosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid and (*R*)-eucomic acid, and an aromatic glucoside from the pseudobulbs of *Grammatophyllum speciosum*

Appendix 6:

New *trans*- and *cis*-*p*-coumaroyl flavonol tetraglycosides from the leaves of *Mitragyna rotundifolia*

Appendix 7:

5-Methyl ether flavone glucosides from the leaves of *Bruguiera gymnorrhiza*

Appendix 8:

Glucosides of phenylpropanoic acid derivatives and coumarins from *Micromelum minutum*

Appendix 9:

Colchicinoid glucosides from seedless pods of Thai origin *Gloriosa superb*

Appendix 10:

2-Carboxy-dihydrostilbene and flavan glycosides from *Desmodium heterocarpon*

Phytochemistry Letters 11 (2015) 215–219

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5-Methyl ether flavone glucosides from the leaves of *Bruguiera gymnorrhiza*

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ABSTRACT

Two new 5-methyl ether flavone glucosides (7,4′,5′-trihydroxy-5,3′-dimethoxyflavone 7-*O*-β-D-glucopyranoside and 7,4′-dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside) were isolated from the leaves of Thai mangrove *Bruguiera gymnorrhiza* together with 7,3′,4′,5′-tetrahydroxy-5-methoxyflavone, 7,4′,5′-trihydroxy-5,3′-dimethoxyflavone, luteolin 5-methyl ether 7-*O*-β-D-glucopyranoside, 7,4′-dihydroxy-5,3′-dimethoxyflavone 7-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside, rutin, kaempferol 3-*O*-rutinoside, myricetin 3-*O*-rutinoside and an aryl-tetralin lignan rhamnoside. The structure of a lignan rhamnoside was found to be related to racemiside, an isolated compound from *Cotoneaster racemiflora*, and also discussed. Structure determinations were based on analyses of physical and spectroscopic data including 1D- and 2D-NMR.

1. Introduction

Bruguiera gymnorrhiza (L.) Savigny (Thai name: Phanka-Hua-Sum) belongs to the family Rhizophoraceae, commonly found in the mangrove forests of Thailand. In Thailand, the flowers and fruits are used as vegetable for cooking purposes. Members of this genus are known to contain several types of compounds such as tropane derivatives (Loader and Russell, 1969), diterpenes (Han et al., 2004), flavonoids (Li et al., 2010), triterpenes (Homhual et al., 2006), aromatic compounds (Bao et al., 2007; Han et al., 2005, 2007; Yi et al., 2013) and macrocyclic polydisulfide (Sun and Guo, 2004); however there are a few reports on the phytochemical study from the polar compounds of this genus. The present paper describes the isolation and structure elucidation of 11 compounds from the aqueous soluble fraction of leaves including six 5-methyl ether flavones (**1–6**), of which two are new (**5** and **6**), four flavonol glycosides (**7–10**) and an aryl-tetralin lignan rhamnoside (**11**).

2. Results and discussion

The methanolic extract of the leaves of *B. gymnorrhiza* was evaporated to dryness and partitioned with Et_2O and H_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to yield 11 compounds. Eight compounds were identified as 7,3′,4′,5′-tetrahydroxy-5-methoxyflavone (**1**) (Ye et al., 2008), 7,4′,5′-trihydroxy-5,5′-dimethoxyflavone (**2**, gramrione) (Raihan, 1994; Feng et al., 2008), luteolin 5-methyl ether 7-*O*-β-D-glucopyranoside (**3**) (Williams and Harborne, 1975), 7,4′-dihydroxy-5,3′-dimethoxyflavone 7-*O*-β-D-glucopyranoside (**4**) (Ozawa et al., 1995), quercetin 3-*O*-β-D-glucopyranoside (**7**), rutin (**8**), kaempferol 3-*O*-rutinoside (**9**) and myricetin 3-*O*-rutinoside (**10**) (Lu and Foo, 2003) by comparison of physical data with literature values and from spectroscopic evidence.

Compound **5** was obtained as an amorphous powder. The molecular formula was determined to be $C_{23}H_{24}O_{12}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^1H and ^{13}C NMR spectra revealed the presence of two asymmetrical *tetra*-substituted aromatic rings deduced from two sets of the chemical shifts at δ_H 6.59 and 6.88 (each 1H, d, J = 1.9 Hz), and at δ_H 7.07 and 7.09 (each 1H, br s), two methoxyl groups at δ_C 56.1 with δ_H 3.82 (3H, s) and δ_C 56.2 with δ_H 3.85 (3H, s), a carbonyl carbon atom at δ_C 175.7, in addition to one

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β-D-glucopyranosyl unit from a set of carbon signals at δ_C 100.0, 77.4, 76.6, 73.2, 69.8 and 60.7 with an anomeric proton at δ_H 5.09 (1H, d, J = 7.2 Hz). The chemical shifts of this compound were related to those of gramrione (**2**) except for a set of additional signals of a β-D-glucopyranosyl unit. This sugar part was suggested to be located at C-7 since the chemical shifts of this carbon and neighboring atoms were significantly changed in comparison with **2** (Table 1). In the ^{1}H NMR spectrum (measured in DMSO-D_6), a broad highly deshielded chelated signal, generally assigned as a hydroxyl group at C-5, was not observed, indicating the presence of a methoxyl group on this position. The second methoxyl group was suggested to be located at C-3′ of the B-ring because the splitting pattern of two protons was appeared as *meta*-coupling, characteristic for an asymmetrical *tetra*-substituted aromatic ring. This assumption was supported by an application of 2D-NMR spectroscopy, including COSY, HMQC and HMBC experiments. In the HMBC spectrum (Fig. 2), only the signal at δ_H 6.88 showed a correlation to C-9 (δ_C 158.7), this proton could be assigned as H-8. Since this proton together with the anomeric signal at δ_H 5.09 showed correlations to C-7 (δ_C 161.4), the sugar moiety was confirmed to be linked to the hydroxyl group at this carbon atom. Two methoxyl groups were also confirmed to be linked to C-5 and C-3′. From these data, the structure of **5** was elucidated to be 7,4′,5′-trihydroxy-5,3′-dimethoxyflavone 7-*O*-β-D-glucopyranoside.

Compound **6** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{22}H_{22}O_{10}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data indicated that this compound is a 5-methyl ether flavone glucoside derivative, as compared to compounds **1–5**. The significant difference was the chemical shifts of the B-ring, which showed the presence of the AA′BB′ type aromatic ring system [δ_H 6.91 and 7.87 (each 2H, d, J = 8.8 Hz)]. Thus, this compound was suggested to be 7,4′-dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside. This assumption was established by 2D-NMR spectra. In the HMBC spectrum, the correlations were observed for the A-ring in the same manner as compound **5** (Fig. 1), confirming the location of a sugar unit at C-7, and a methoxyl group at C-5. Interestingly,

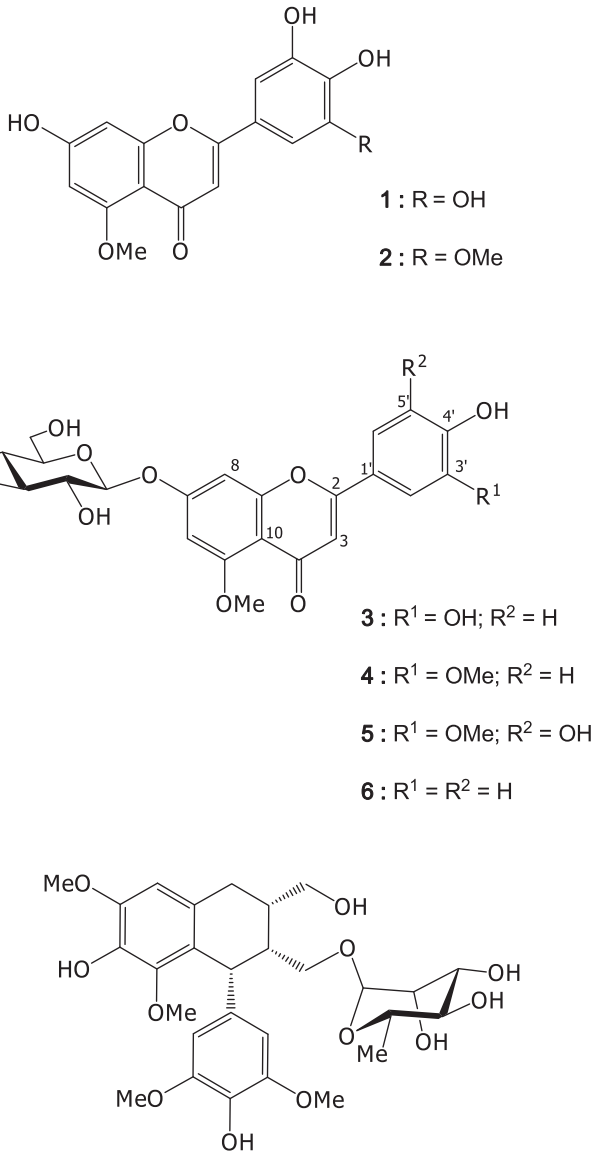

Fig. 1. Structures of compounds **1–6** and **11**.

Table 1
^{13}C NMR spectroscopic data of compounds **1–6** (75 MHz, DMSO-D_6).

Position	1	2	3	4	5	6
Aglycone						
2	160.2	160.0	160.2	160.3	160.2	160.2
3	106.0	106.4	106.2	106.5	106.6	106.1
4	175.6	175.7	175.6	175.9	175.7	175.9
5	160.6	160.6	160.5	160.3	160.4	160.6
6	96.5	96.5	97.2	97.3	97.2	97.2
7	162.6	162.5	161.4	161.5	161.4	161.4
8	95.0	95.2	96.0	96.3	96.1	96.1
9	159.0	159.1	158.7	158.8	158.7	158.7
10	107.1	107.1	108.9	108.9	108.9	108.9
1′	120.7	120.8	121.8	121.7	120.6	121.3
2′	105.0	101.8	113.0	109.8	101.9	127.9
3′	146.3	148.6	145.7	150.2	148.6	115.9
4′	137.0	137.8	149.1	148.1	137.9	160.6
5′	146.3	145.9	115.9	115.8	146.0	115.9
6′	105.0	107.0	118.3	119.8	107.1	127.9
5-OMe	55.9	55.8	56.1	56.0	56.1	56.1
3′-OMe		56.2		56.1	56.2	
Glc						
1			99.9	100.1	100.0	99.9
2			73.2	73.3	73.2	73.2
3			77.3	77.4	77.4	77.3
4			69.7	69.9	69.8	69.8
5			76.6	76.7	76.6	76.6
6			60.7	60.8	60.7	60.7

the same structure had previously been reported from the isolated compound of *Aquilalia sinensis* (Qi et al., 2009), but the spectroscopic data obtained in the present work clearly was not identical to the reported data, especially for the A-ring part. However the structure revision of the compound reported as 7,4′-dihydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside (Qi et al., 2009) was already proposed to that of 5,4′-dihydroxy-7-methoxyflavone 5-*O*-β-D-glucopyranoside (Veitch and Grayer, 2011). Consequently, this compound is new.

Compound **11** was isolated as a yellow amorphous powder. The molecular formula was determined to be $C_{28}H_{38}O_{12}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra revealed the presence of one sugar unit, identified to be an α-L-rhamnopyranosyl moiety from the chemical shifts of its anomeric proton at δ_H 4.54 (1H, br s) and a secondary methyl group at δ_H 1.17 (3H, d, J = 6.0 Hz) together with the set of the chemical shifts at δ_C 100.6, 70.6, 70.9, 68.9 and 18.0. The ^{13}C NMR spectral data (Table 2) showed the signals of two aromatic rings (a *penta*- and a symmetrical 1,3,4,5-*tetra*-substituted aromatic rings), three methylenes (δ_C 32.5, 64.0 and 67.5), three methines (δ_C 39.0, 41.3 and 44.7) in addition to four

Table 2
^{1}H and ^{13}C NMR spectroscopic data of lignan **11** (75 MHz, DMSO-D_6).

Position	Proton	Carbon
Aglycone		
1	2.50 (1H$_{ax}$, dd, J = 15.0, 11.9 Hz)	32.5
	2.65 (1H$_{eq}$, dd, J = 15.0, 4.2 Hz)	
2	1.50 (1H, m)	39.0
2a	3.32 (1H)[a]	64.0
	3.48 (1H)[a]	
3	1.92 (1H, m)	44.7
3a	3.22 (1H)[a]	67.5
	3.50 (1H)[a]	
4	4.12 (1H, d, J = 5.9 Hz)	41.3
5		146.4
6		137.3
7		147.0
8	6.55 (1H, s)	106.7
9		128.4
10		124.8
1′		137.7
2′,6′	6.26 (2H, s)	105.9
3′,5′		147.6
4′		133.5
5-OMe	3.25 (3H, s)	58.7
7-OMe	3.76 (3H, s)	55.7
3′,5′-OMe	3.47 (6H, s)	56.1
Rha		
1	4.54 (1H, br s)	100.6
2	3.69 (1H)[a]	70.6
3	3.50 (1H)[a]	70.9
4	3.19 (1H)[a]	71.9
5	3.58 (1H, m)	68.9
6	1.17 (1H, d, J = 6.0 Hz)	18.0

[a] Chemical shifts were assigned by COSY and HMQC.

methoxyl groups [δ_C 55.7, 56.2 (2C) and 58.7] for the aglycone moiety. The spectral data of this part was very characteristic of an aryl-tetralin type lignan, suggested to be a lignan rhamnoside. The assignment was supported by HMBC spectrum (Fig. 3), in which the significant correlations were observed between (i), H-1 to C-9, (ii) H-4 to C-2, C-3, C-9, C-10, C-3a and C-1′, (iii) H-8 to C-1 and (iv) H-1″ Rha to C-3a, as shown in Fig. 3. The ^{1}H and ^{13}C NMR spectroscopic data were coincident with those of (±) lyoniresinol 3a-*O*-α-L-rhamnopyranoside (Kaneda et al., 1990). Since this

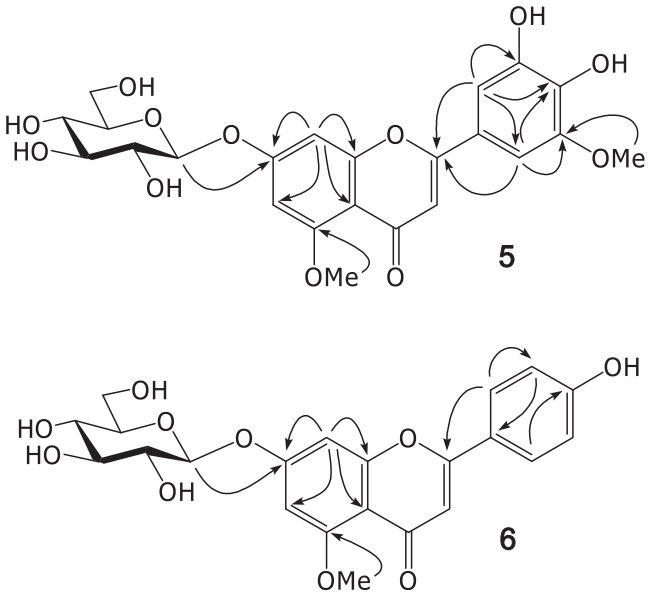

Fig. 2. Significant HMBC correlations of compounds **5** and **6**.

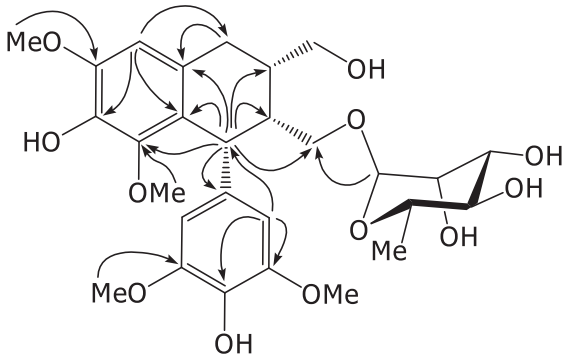

Fig. 3. Significant HMBC correlations of compound **11**.

compound was pure and exhibited a negative optical rotation value of $[\alpha]_D^{27}$ −33.72 (MeOH, *c* 0.70), the stereochemistry should be different. Therefore, the configurations of this compound must be clarified. The assignment of the relative stereochemistry was established by the coupling constants and difference NOE experiments (Fig. 4). The results from the difference NOE correlations between (i) H-3(δ_H 1.92) and H-4 (δ_H 4.12), (ii) H-4 and H-2′,6′ (δ_H 6.26) and (iii) H-3 and H-2′, 6′ together with the coupling constant of H-4 with J = 5.9 Hz indicated that the relation of H-4 and H-3 was an axial-equatorial orientation. H-2 (δ_H 1.50) was assigned to be in an axial orientation due to the large coupling constant with H$_{ax}$-1 (δ_H 2.50, J = 11.9 Hz). Also, H-2 displayed the NOE enhancements to H-3 and H-4. Thus, the orientation of H-2, H-3 and H-4 was determined as axial, equatorial and axial, respectively. The absolute configuration was established from the CD spectrum, which showed extreme values for $\Delta\varepsilon$ (nm) −3.45 (250), −2.51 (272), +3.57 (288), relating to the aryl substituted at C-4 of 4*R* and opposite to the reports for 4*S* compounds (Sakakibara et al., 1974; Ohashi et al., 1994; Yang et al., 2005; Wangteeraprasert and Likhitwitayawuid, 2009). Therefore, the absolute configuration of the chiral positions was concluded to be 2*S*, 3*R* and 4*R*. On the basis of these evidences, the structure was elucidated as shown. The structure of this compound was firstly reported from *Cotoneaster racemiflora* with a trivial name racemiside (Khan et al., 2009). It displayed an optical rotation value of $[\alpha]_D^{20}$ +4.8 (MeOH, *c* 6.2 × 10^{-4}), opposite to that of compound **11**. Although the sign of the specific rotation was different, it was insufficient to conclude compound **11** as a new lignan rhamnoside. Therefore, the result from this study provided the more physical and spectroscopic data of racemiside.

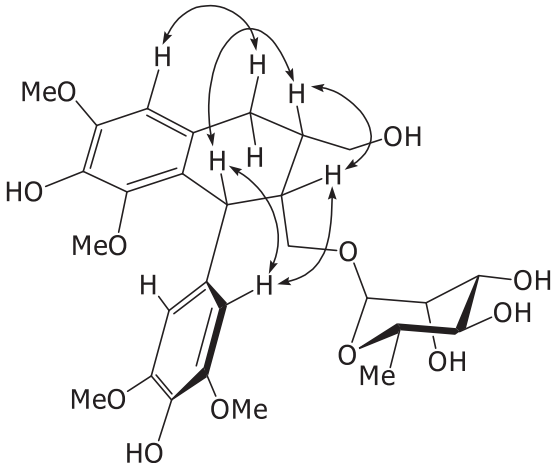

Fig. 4. Significant difference NOE correlations of compound **11**.

The absolute configuration of the sugar moiety of three compounds **5**, **6** and **11** was determined by acid hydrolysis to provide D-glucose from compounds **5** and **6**; and L-rhamnose from compound **11**, identify by HPLC analysis using the optical rotation detector (see Section 3).

The present study isolated 11 secondary metabolites from the water soluble fraction including 5-methyl ether flavones (**1–6**), flavonol glycosides (**7–10**) and an aryl-tetralin lignan rhamnoside (**11**) of the leaves of *B. gymnorrhiza*. The appearance of 5-methyl ether flavones (**1**, **2**) was related to the previous investigations of the same species (Raihan, 1994; Feng et al., 2008; Ye et al., 2008). Compounds **3–6** were the first 5-methyl ether flavones isolated in glucosidic form of this plant. Flavonol glycosides (**7–10**) are commonly reported from the plant kingdom. The occurrence of an aryl-tetralin lignan rhamnoside (**11**) was quite rare to discover from plant source, especially having the negative optical rotation value. These constituents might be useful for further chemotaxonomic studies of this genus.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in DMSO-D_6 using a Bruker AV-300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) spectrometer. The MS data was obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (20.0 mm × 250 mm i.d., Vertisep^{TM} UPS) with a Jasco UV-970 detector at 220 nm. The flow rates were 6 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O–EtOH (1:1, v/v).

3.2. Plant material

The leaves of *B. gymnorrhiza* (L.) Savigny were collected on June 2013 from Tambon NongSano, Amphoe Mueang, Trat Province, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0072) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3. Extraction and isolation

The collected leaves (3.5 kg) were extracted with MeOH at room temperature (each 15 L for 24 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (599.5 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 5 times). The aqueous soluble fraction (411.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (103.3 g) was subjected to silica gel cc using solvent systems EtOAc–MeOH (9:1, 8.0 L), EtOAc–MeOH–H_2O (40:10:1, 12.0 L), EtOAc–MeOH–H_2O (70:30:3, 1.0 L) and EtOAc–MeOH–H_2O (6:4:1, 9.0 L), respectively to provide five fractions. Fraction I (7.7 g) was applies to a RP-18 column using a gradient solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to provide 12 sub-fractions. Sub-fraction I-3 was purified by preparative HPLC-ODS using solvent system 15% aqueous MeCN to obtain compound **11** (24.0 mg). Sub-fraction I-5 was purified by preparative HPLC-ODS with solvent system 17% aqueous MeCN to give compounds **1** (9.6 mg), **7** (13.1 mg), **8** (110.4 mg) and **9** (6.7 mg). Sub-fraction I-7 was purified by preparative HPLC-ODS with solvent system 23% aqueous MeCN to obtain compound **2** (20.0 mg). Fraction II (4.8 g) was separated on a RP-18 column using solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction II-4 was purified by preparative HPLC-ODS with solvent system 17% aqueous MeCN to provide compounds **4** (7.0 mg) and **6** (9.0 mg). Fraction III (4.1 g) was applied to a RP-18 column using solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to afford seven sub-fractions. Sub-fraction III-3 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to obtain compounds **3** (8.0 mg) and **5** (4.8 mg). Finally, fraction IV (8.3 g) was similarly subjected to a RP-18 column using, H_2O–MeOH (90:10 → 20:80, v/v) to provide nine sub-fractions. Sub-faction IV-4 was purified by preparative HPLC-ODS with solvent system 15% aqueous MeCN to yield compound **10** (8.0 mg).

3.4. 7,4′,5′-Trihydroxy-5,3′-dimethoxyflavone 7-O-β-D-glucopyranoside (*5*)

Amorphous powder; $[\alpha]_D^{27}$ −44.73 (MeOH, *c* 0.30); ^{1}H NMR (DMSO-D_6): δ 7.09 (1H, br s, H-2′), 7.07 (1H, br s, H-6′), 6.88 (1H, d, *J* = 1.9 Hz, H-8), 6.59 (1H, d, *J* = 1.9 Hz, H-6), 6.59 (1H, s, H-3), 5.09 (1H, d, *J* = 7.2 Hz, H-1″ Glc), 3.85 (3H, s, 3′-OMe), 3.82 (3H, s, 5-OMe); ^{13}C NMR (DMSO-D_6): Table 1; Negative HRESITOFMS, *m*/*z*: 491.1200 $[M-H]^-$ (calcd for $C_{23}H_{23}O_{12}$, 491.1195).

3.5. 7,4′-Dihydroxy-5-methoxyflavone 7-O-β-D-glucopyranoside (*6*)

Amorphous powder; $[\alpha]_D^{27}$ −46.57 (MeOH, *c* 0.30); ^{1}H NMR (DMSO-D_6): δ 7.87 (2H, *d*, J = 8.8 Hz, H-2′,6′), 6.91 (1H, d, *J* = 8.8 Hz, H-3′,5′), 6.89 (1H, br s, H-8), 6.59 (1H, s, H-6), 6.59 (1H, s, H-3), 5.10 (1H, d, *J* = 7.1 Hz, H-1″ Glc), 3.83 (3H, s, 5-OMe); ^{13}C NMR (DMSO-D_6): Table 1; Positive HRESITOFMS, *m*/*z*: 447.1297 $[M+H]^+$ (calcd for $C_{22}H_{23}O_{10}$, 447.1286).

3.6. Aryl-tetralin lignan rhamnoside (*11*)

Amorphous powder; $[\alpha]_D^{27}$ −33.72 (MeOH, *c* 0.70); CD (MeOH, *c* 4.4×10^{-4} M) $\Delta\varepsilon$ (nm) −3.45 (250), −2.51 (272), +3.57 (288); ^{1}H and ^{13}C NMR (DMSO-D_6): Table 2; Negative HRESITOFMS, *m*/*z*: 601.2042 $[M-Cl]^-$ (calcd for $C_{28}H_{38}Cl_1O_{12}$, 601.2057).

3.7. Determination of the absolute configurations of sugars

Each compound (*ca* 5 mg) was dissolved in 2 N HCl–dioxane (6:1, 3.5 ml) and heated at 80° for 5 h. After cooling, the reaction was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 2 N KOH and concentrated to dryness affording the sugar fraction. This part was dissolved with H_2O (1 ml), analyzed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep^{TM} sugar LMP, 7.8 mm × 300 mm i.d.; mobile phase: H_2O; flow rate 0.4 ml/min; temperature: 80 °C) and comparison with their retention times and optical rotations with authentic samples. Hydrolysis of compounds **5** and **6** gave peaks corresponding to D-glucose at 19.2 min with positive optical rotation and hydrolysis of lignan **11** gave peaks of L-rhamnose at 23.0 min with positive optical rotation.

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Short communication

2-Carboxy-dihydrostilbene and flavan glycosides from *Desmodium heterocarpon*

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ABSTRACT

A new 2-carboxy-dihydrostilbene glucoside, 2-carboxy-3,5,4′-trihydroxydihydrostilbene 3-*O*-β-D-glucopyranoside (**1**) and a new flavan diglycoside, (2*S*)-5,7,4′-trihydroxyflavan 7-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (**3**) were isolated from the aerial portion of Thai origin *Desmodium heterocarpon* together with five known compounds, lunularic acid 4′-*O*-β-D-glucopyranoside (**2**), vitexin (**4**), isovitexin (**5**), (−)-*epi*-catechin 3-*O*-β-D-glucopyranoside (**6**), and (−)-*epi*-afzelachin 3-*O*-β-D-glucopyranoside (**7**). The structure determinations were based on physical data and spectroscopic evidence including 1D- and 2D-NMR experiments. The presence of 2-carboxy-dihydrostilbene derivatives from this species added the information data of this compound-type to the plant kingdom.

1. Introduction

Desmodium heterocarpon (L.) DC., a member of the family Fabaceae, is commonly found in the paddy field during the raining season in North-eastern Thailand. Previous phytochemical investigation of this plant reported the isolation of several compounds such as triterpenoids, steroids and flavonoids (Huang et al., 2010). In the biological properties, the antioxidant activities of phenolic components from plant extract have been reported (Tsai et al., 2011). This present study describes the isolation and structure identification of seven glycosides including a new 2-carboxy-dihydrostilbene derivative glucoside (**1**) and a new flavan diglycoside (**3**) from the water soluble fraction of the aerial portion of this plant in addition to five known compounds (**2**, **4–7**).

2. Results and discussion

The methanolic extract of the aerial portions of *D. heterocarpon* was partitioned between Et_2O and H_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to provide a new glucoside of dihydrostilbenic acid (**1**) and a new flavan diglycoside (**3**) (Fig. 1) along with five known compounds. The known compounds were identified to be lunularic acid 4′-*O*-β-D-glucopyranoside (**2**) (Fang et al., 2012), vitexin (**4**), isovitexin (**5**) (Agrawal and Bansal, 1989), (−)-*epi*-catechin 3-*O*-β-D-glucopyranoside (**6**) (Morimoto et al., 1985), and (−)-*epi*-afzelachin 3-*O*-β-D-glucopyranoside (**7**) (Morimoto et al., 1988). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

Compound **1** was isolated as an amorphous powder. The molecular formula was determined to be $C_{21}H_{24}O_{10}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H NMR spectrum revealed the presence of a pair of *meta*-coupled aromatic protons at δ_H 6.12 and 6.16 (each 1H, d, J = 2.5 Hz), a set of AA'BB' aromatic ring at δ_H 6.64 and 7.05 (each 2H, d, J = 8.4 Hz), two methylenes at δ_H 2.63 (2H, dd, J = 8.2, 8.1 Hz) and 3.18 (2H, m) in addition to an anomeric proton signal at δ_H 4.77 (1H, d, J = 7.7 Hz). The ^{13}C NMR spectrum showed that this compound contained two aromatic ring systems, two methylene carbons, one carboxyl group for the aglycone moiety, as well as one β-D-glucopyranosyl moiety. The NMR spectral data (Table 1) indicated that the structure of this compound was related to lunularic acid 4′-*O*-β-D-glucopyranoside (**2**). This compound has one oxygen atom more than compound **2**, suggesting the presence of an additional hydroxyl group on the aromatic ring. The structure assignment was based on the analyzing results from 2D-NMR spectroscopic methods including COSY, NOESY, HMQC and HMBC.

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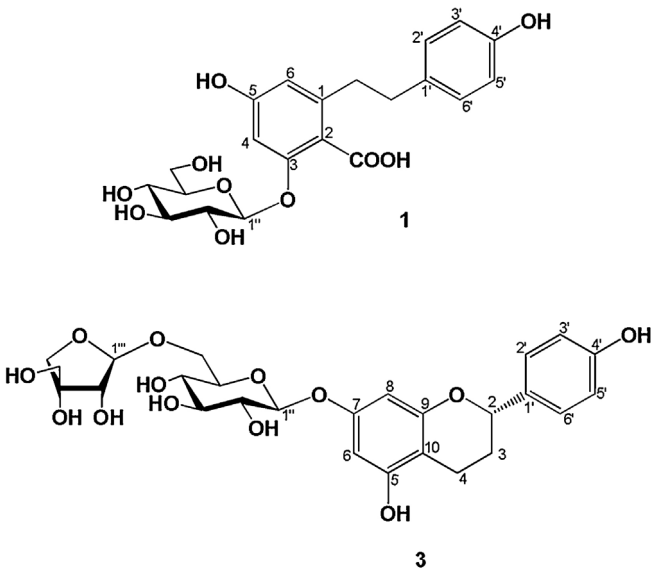

Fig. 1. Structures of compounds **1** and **3**.

In the HMBC spectrum (Fig. 2), the significant correlations were found from methylene protons at δ_H 3.18 (H_2-α) to an aryl methine carbon at δ_C 108.2, and two non-protonated aryl carbons at δ_C 112.2 and 147.0, ascribable to C-6, C-2 and C-1, respectively. The chemical shift of a methine carbon at δ_C 101.3 was assigned to locate at C-4 position of the aromatic ring due to the coupling constant between δ_H 6.12 (H-6) and 6.16 (H-4) with J = 2.5 Hz, and was also confirmed by the HMBC correlation from δ_H 6.12 (H-6) to this carbon atom. The oxy-aryl carbon at δ_C 159.0 was placed at C-3 position since the HMBC correlation was observed only from δ_H 6.16 (H-4). The appearance of the HMBC correlations from δ_H 6.12 (H-6) and 6.16 (H-4) to the oxy-aryl carbon at δ_C 166.2 indicated that this carbon atom was C-5 position. The remaining oxy-aryl carbon at δ_C 155.4 was belonged to C-4′ position of the other aromatic ring, supporting by the HMBC correlations from δ_H 7.05 (H-2′,6′) and 6.64 (H-3′,5′). The sugar unit was assigned to be linked at C-3 position, deduced from the HMBC correlation from the anomeric proton at δ_H 4.77 (H-1″) to C-3 (δ_C 159.0). Also the NOESY correlation was only observed between H-1″ and H-4 (Fig. 2), confirming the sugar unit to C-3 position. Consequently, the structure of this compound was elucidated to be 2-carboxy-3,5,4′-trihydroxydihydrostilbene 3-*O*-β-D-glucopyranoside.

Compound **3** was isolated as an amorphous powder. The molecular formula was determined to be $C_{26}H_{32}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H NMR spectrum revealed the presence of two sugar units from the anomeric signals at δ_H 4.70 (1H, d, J = 7.6 Hz) and 4.80 (1H, d, J = 3.1 Hz), a pair of *meta*-coupled aromatic protons at δ_H 5.96 and 6.8 (each 1H, d, J = 2.5 Hz), and a set of AA'BB' aromatic ring at δ_H 6.74 and 7.19 (each 2H, d, J = 8.4 Hz) in addition to a broad double signal at δ_H 4.86 (1H, J = 9.3 Hz) and multiplets at δ_H 1.88 (1H), 2.03 (1H) and 2.53 (2H). In the ^{13}C NMR spectrum, 11 carbon signals belonging to the sugar part could be identified to be a β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit by comparing chemical shifts with the reported data (Kanchanapoom et al., 2002). The remaining 15 carbon atoms were consistant with a flavonoid skeleton. The partial connectivities of C-2 to C-3 and C-4 were deduced from COSY and HMQC analysis, indicating that the aglycone of this compound was a flavan (Sauvain et al., 1994). In the HMBC spectrum, the correlation from the anomeric proton at δ_H 4.70 (H-1″ Glc) to C-7 (δ_C 156.7) indicated the location of the sugar moiety to this carbon atom. Besides, the NOESY spectrum provided further confirmation of the sugar unit to C-7 position from the cross peak correlation of H-1″ Glc with δ_H 5.96 (H-6) and 6.08 (H-8) as shown in Fig. 2. The absolute configuration at C-2 position was established to be *S* from the CD spectrum, which showed extreme values for Δε (nm) −1.63 (272), relating to the data of 2*S*-configurated compounds (Baba et al., 1986; Antus et al., 2001; Yang et al., 2010; Zhong et al., 2013). Consequently, the structure of this compound was identified as (2*S*)-5,7,4′-trihydroxyflavan 7-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

Table 1
NMR spectroscopic data of compounds **1** and **2** (^{1}H NMR, 400 MHz and ^{13}C NMR, 100 MHz).

Position	1	1	2
	Proton	Carbon	Carbon
1		147.0	145.0
2		112.2	118.2
3		159.0	163.8
4	6.16 (1H, d, J = 2.5 Hz)	101.3	114.5
5		166.2	130.1
6	6.12 (1H, d, J = 2.5 Hz)	108.2	119.5
1′		133.4	136.4
2′, 6′	7.05 (2H, d, J = 8.4 Hz)	129.5	129.2
3′, 5′	6.64 (2H, d, J = 8.4 Hz)	115.1	116.0
4′		155.4	155.5
α	3.18 (2H, m)	38.2	37.9
β	2.63 (2H, dd, J = 8.2, 8.1 Hz)	37.3	37.3
COOH		173.1	173.0
Glc-1”	4.77 (1H, d, J = 7.7 Hz)	100.1	100.7
2”	3.13-3.20 (1H)	73.5	73.3
3”	3.30-3.38 (1H)	76.9	76.7
4”	3.13-3.20 (1H)	70.0	69.8
5”	3.21-3.29 (1H)	77.2	77.0
6”	3.40-3.50 (1H)	61.0	60.8
	3.66 (1H, br d, J = 11.5 Hz)		

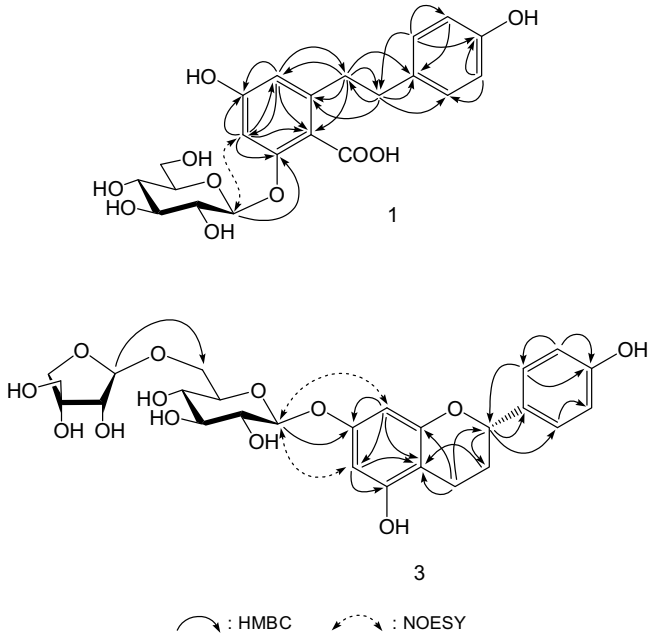

Fig. 2. HMBC and NOESYcorrelations of compounds **1** and **3**.

Table 2
^{1}H and ^{13}C NMR spectroscopic data of compound **3**. (^{1}H NMR, 400 MHz and ^{13}C NMR, 100 MHz).

Position	Proton	Carbon
2	4,86 (1H, br d, *J* = 9.3 Hz)	76.7
3	1.88 (1H, m)	19.1
	2.03 (1H, m)	
4	2.53 (2H, m)	28.6
5		156.3
6	5.96 (1H, d, *J* = 2.3 Hz)	95.4
7		156.7
8	6.08 (1H, d, *J* = 2.3 Hz)	96.3
9		156.1
10		103.4
1′		132.0
2′, 6′	7.19 (2H, d, *J* = 8.4 Hz)	127.6
3′, 5′	6.74 (2H, d, *J* = 8.4 Hz)	115.1
4′		157.0
Glc-1”	4.70 (1H, d, *J* = 7.6 Hz)	100.5
2”	3.09-3.16 (1H)	73.2
3”	3.23 (1H, dd, *J* = 8.8, 8.6 Hz)	76.5
4”	3.05-3.13 (1H)	69.7
5”	3.32-3.42 (1H)	75.4
6”	3.40-3.50 (1H)	67.4
	3.77-3.84 (1H)	
Api-1‴	4.80 (1H, d, *J* = 3.1 Hz)	109.3
2‴	3.72 (1H, d, *J* = 3.1 Hz)	76.0
3‴		78.8
4‴	3.54 (1H, d, *J* = 9.4 Hz)	73.4
	3.83 (1H, d. *J* = 9.4 Hz)	
5‴	3.20-3.32 (2H)	63.3

The present work reported seven compounds from the water soluble part including two 2-carboxy-dihydrostilbene derivatives (**1, 2**) and five flavonoids (**3-7**) from the aerial portion of *D. heterocarpon*. The occurrence of 2-carboxy-dihydrostilbene derivatives from angiosperm was quite interesting since this compound-type was mainly reported from the lower plants such as bryophytes and ferns (Hashimoto et al., 1988; Fang et al., 2012; Asakawa et al., 2013). This new finding adds the information data to the plant kingdom and might be useful for further chemotaxonomic studies of this genus.

3. Experimental

3.1. General procedures

NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. Mass spectrometry values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. Circular dichroism spectrum was recorded on a Jasco J-815 spectropolarimeter. For column chromatography (100 × 500 mm i.d., silica gel 60 70 – 230 mesh, Merck), Diaion HP-20 (1.2 kg, Mitsubishi Chemical Industries Co. Ltd.), and RP-18 (55 × 350 mm i.d., 50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 × 250 mm i.d., Vertisep™ AQS) with a Jasco MD-2010 detector at 220 nm. The flow rates were 8 mL/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

3.2. Plant material

The aerial portion of *Desmodium heterocarpon* (L.) DC was collected from Ban Na-Dok-Mai, Tambon Nong-Bua, Amphoe Ban-Phang, Khon Kaen Province in November 2011. A voucher specimen (TK-PSKKU-0076) was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3. Extraction and isolation

The air dried aerial portion of *D. heterocarpon* (2.5 kg) were extracted three times with MeOH, and concentrated to dryness. The residue (258.2 g) was suspended in H_2O and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (213.9 g) was applied to a Diaion HP-20 column, and eluted with H_2O, and MeOH, successively. The fraction eluted with MeOH (76.0 g) was subjected to silica gel cc using solvent systems EtOAc-MeOH (9:1, 8.0 L), EtOAc-MeOH-H_2O (40:10:1, 10.0 L), EtOAc-MeOH-H_2O (70:30:3, 8.0 L) and EtOAc-MeOH-H_2O (6:4:1, 6.0 L), respectively to obtain seven fractions (A to G).

Fraction B (6.8 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction B-2 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (90:10, v/v) to provide compound **4** (30.5 mg). Sub-fraction B-3 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to yield compound **5** (50.3 mg). Sub-fraction B-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (82:18, v/v) to provide compound **3** (110.3 mg).

Fraction D (13.5 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction D-5 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to provide compound **6** (16.8 mg). Sub-fraction D-7 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (80:20, v/v) to afford compound **7** (50.2 mg).

Fraction F (5.9 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to give eight sub-fractions. Sub-fraction F-2 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (89:11, v/v) to obtain compound **1** (27.1 mg). Sub-fraction F-4 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (85:15, v/v) to give compound **2** (70.0 mg).

3.4. 2-Carboxy-3,5,4′-trihydroxydihydrostilbene 3-O-β-D-glucopyranoside (*1*)

Amorphous powder, $[\alpha]_D^{24}$ −35.9 (DMSO, *c* 0.44); ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): Table 1; Negative HR-FAB-MS, *m/z*: 435.1307 ($C_{21}H_{23}O_{10}$ required 435.1297).

3.5. (2S)-5,7,4′-trihydroxyflavan 7-O-β-D-apiofuranosyl-(1 → 6)-O-β-D-glucopyranoside (*3*)

Amorphous powder, $[\alpha]_D^{24}$ −56.2 (MeOH, *c* 0.47); CD (EtOH, *c* 3.5×10^{-2}) Δε (nm) −5.28 (226), 0 (243), −1.63 (272); ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): Table 2; Negative HR-FAB-MS, *m/z*: 551.1778 ($C_{26}H_{31}O_{13}$ required 551.1770).

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Colchicinoid glucosides from seedless pods of Thai origin *Gloriosa superba*

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ABSTRACT

Three new colchicinoid glucosides, dongduengosides A-C were isolated from seedless pods of Thai origin *Gloriosa superba* together with colchicoside, colchicine, 2-demethylcolchicine and luteolin 7-*O*-β-D-glucopyranoside. In addition, colchicine, 2-demethylcolchicine, 3-methylcolchicine, colchicoside, *epi*-catechin and quercetin 3-*O*-β-D-glucopyranoside were identified from seeds. The structure determinations were based on physical data and spectroscopic evidence including 1D- and 2D-experiments. This study showed that the different part of seedless pods and seeds provided the major difference in compounds.

1. Introduction

Gloriosa superba L. (Thai name: Dong-Dueng), a member of the family Colchicaceae, is an herbaceous climber native of tropical Africa, and commonly growing in tropical regions of Asia. In Thailand, this species is described as a toxic plant since there was report on several cases of apparent poisoning related to consumption of its tubers. This plant is well known to contain colchicinoid derivatives such as colchicine, colchicoside, and lumicolchicine (Dunuwille et al., 1968; Chaudhuri and Thakur, 1993; Joshi et al., 2010). Furthermore, the medicinal uses, biological activities and toxicological investigations have been reviewed (Jana and Shekhawat, 2013). This present study deals with the isolation and determination of the chemical constituents from seedless pods and seeds, including three new colchicinoid glucoside (**2–4**), four known colchicinoids (**1**, **5–7**), and three flavonoids (**8–10**).

2. Results and discussion

The methanolic extracts of seedless pods and seeds were processed individually by a combination of chromatographic procedures to provide ten compounds (**1-10**). From seedless pods, three new colchicinoid glucosides (**2–4**) were identified (Fig. 1). Seven known compounds were identified as colchicine (**5**), 2-demethylcolchicine (**6**), 3-demethylcolchicine (**7**) (Hufford Hufford et al., 1979), colchicoside (**1**) (Yoshida et al., 1988), *epi*-catechin (**8**) (Agrawal et al., 1989), quercetin 3-*O*-β-D-glucopyranoside (**9**) and luteolin 7-*O*-β-D-glucopyranoside (**10**) (Agrawal and Bansal, 1989) by comparison of physical data with literature values and from spectroscopic evidence.

Dongduengoside A (**2**) was isolated as an amorphous powder. The molecular formula was determined to be $C_{26}H_{31}NO_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectra revealed the presence of two methoxyl groups and one β-D-glucopyranosyl unit in addition to the signals of a colchicinoid skeleton as compared to colchicoside (**1**). The spectroscopic data were related to those of colchicoside (**1**) except for lacking one methoxyl signal, which established the presence of a hydroxyl group. The locations of these functional groups were assigned by the results of 2D-NMR experiments. All protonated carbons were assigned by HSQC experiment. An NOESY correlation between the anomeric proton signal at δ_H 4.71 (d, J = 7.2) and H-4 at δ_H 6.80 (*s*), as well as the

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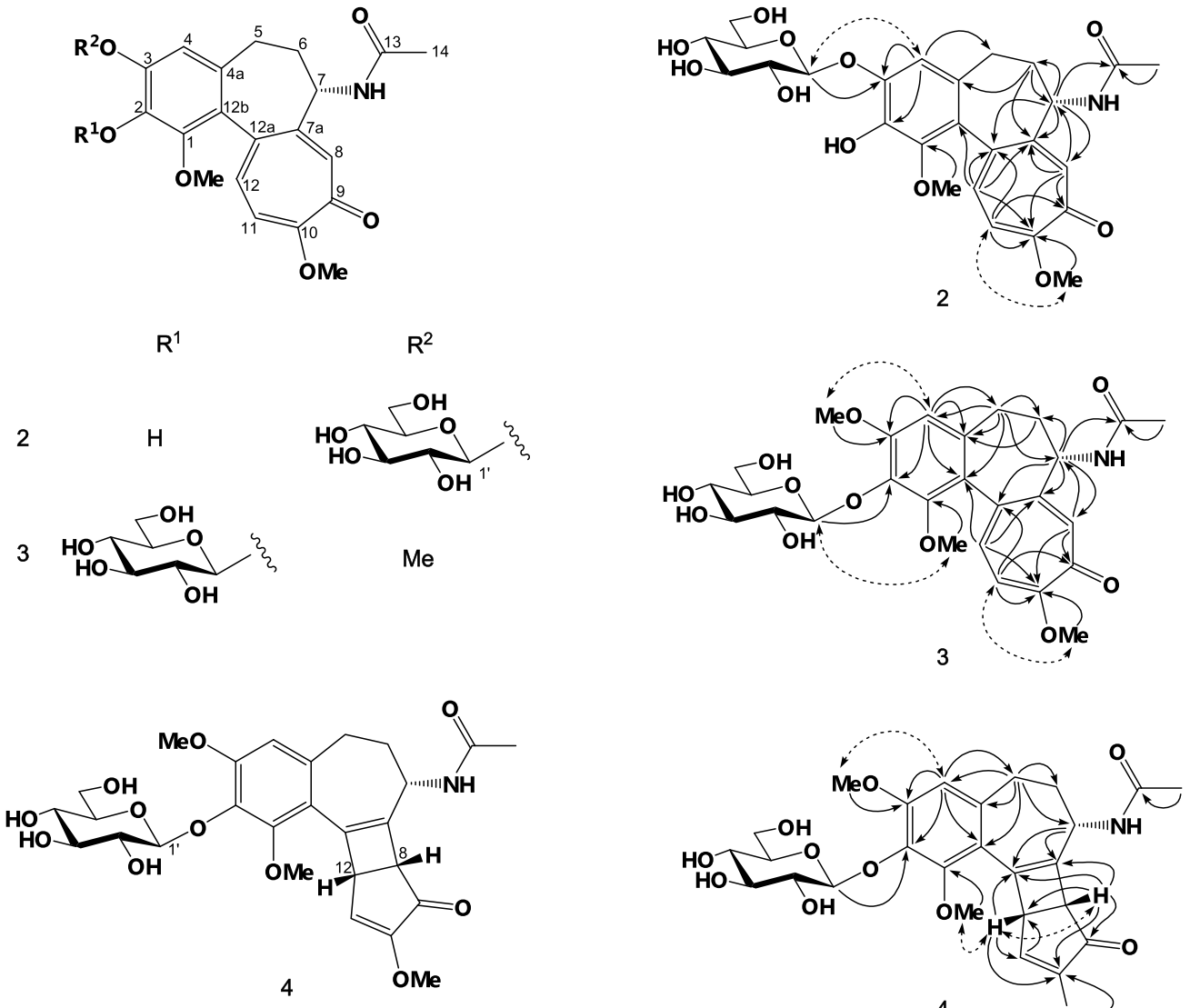

Fig. 1. Structures of compounds **2–4**.

Fig. 2. HMBC and NOESY correlations of compounds **2–4**.

HMBC correlations from both protons to C-3 (δ_C 146.0) were observed from the spectra, indicating that the glucopyranosyl unit was placed at C-3 position. Two methoxyl groups at δ_H 3.50 with δ_C 60.1 and δ_H 3.86 with δ_C 56.1 were ascribable to be 1-OMe and 10-OMe, from the HMBC correlations to C-1 (δ_C 145.1) and C-10 (δ_C 163.5), respectively (Fig. 2). Thus, the remaining hydroxyl group could be deduced to be linked to C-2 position (δ_C 139.0). Consequently, the structure of compound **5** was elucidated to be 2-demethylcolchicoside or 2,3-didemethylcolchicine 3-*O*-β-D-glucopyranoside.

Dongduengoside B (**3**) was obtained as an amorphous powder and its molecular formula was determined to be $C_{27}H_{33}NO_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data were closely related to those of colchicoside (**1**). The significant differences were the chemical shifts of A-ring, suggesting the interchange of the functional groups. The complete assignments were clearly established by NOESY, HSQC and HMBC experiments. In the HMBC spectrum, the correlation was found from the anomeric proton at δ_H 4.97 (d, J = 7.3) to C-2 (δ_C 137.7), indicating the position of the sugar unit to this carbon atom. Also, three methoxyl groups at δ_H 3.44 with δ_C 60.8, δ_H 3.85 with δ_C 56.4, and δ_H 3.85 with δ_C 56.1 were assignable to be 1-OMe, 3-OMe and 10-OMe, respectively, by the observation of HMBC correlations as illustration in Fig. 2. Moreover, the NOESY spectrum showed the significant correlations from the anomeric proton to the 1-OMe, and from H-4 (δ_H 6.75) to 3-OMe, confirming the position of the sugar unit. Therefore, the structure of this compound was identified as 2-demethylcolchicine 2-*O*-β-D-glucopyranoside.

Dongduengoside C (**4**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{27}H_{33}NO_{11}$ by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra showed the presence of three methoxyl groups, one β-D-glucopyranosyl unit in addition to the typical signals for colchicine lumi-derivatives as the core structure (Potešilová et al., 1985; Meksuriyen et al., 1988). The chemical shifts of this compound were related to those of β-lumicolchicine (Meksuriyen et al., 1988) except for a set of additional signals of a β-D-glucopyranosyl unit instead of one methoxyl group. The assignment of the structure was deduced by the results from 2D-NMR spectroscopic methods including NOESY, HSQC and HMBC experiments. The sugar unit was assigned to be located at C-2 (δ_C 136.2) since the HMBC

correlation was found from the anomeric proton at δ_H 5.04 (d, J = 7.3) to this carbon atom. Three methoxyl groups at δ_H 3.87 with δ_C 61.5, δ_H 3.75 with δ_C 56.3, and δ_H 3.58 with δ_C 56.3 were assigned to be linked at C-1 (δ_C 151.1), C-3 (δ_C 152.3) and C-10 (δ_C 157.4) based on the correlations from their singlet proton signals to C-1, C-3 and C-10, respectively. Furthermore, the NOESY spectrum showed the cross peaks between 1-OMe (δ_H 3.87) and H-12 (δ_H 4.01), and between H-8 (δ_H 3.41–3.44) and H-12 (δ_H 4.01) in Fig. 2, supporting the characteristic of β-lumi-derivative skeleton of this compound (Meksuriyen et al., 1988). Also, the NOESY correlation was observed between H-4 (δ_H 6.67) and 3-OMe. Therefore, the structure was elucidated as 2-demethyl-β-lumicolchicine 2-*O*-β-D-glucopyranoside.

In conclusion, the present work reported ten compounds including seven colchicinoids (**1–7**) and three flavonoids (**8**–**10**) from Thai plant source. Colchicine (**5**), 2-demethylcolchicine (**6**), 3-demethylcolchicine (**7**) and colchicoside (**1**) were expected to isolate from seeds of this species as previously reported from the literatures (Chaudhuri and Thakur, 1993; Joshi et al., 2010). The new finding of colchicinoids from seedless pods was surprising since three compounds were new, as well as colchicoside (**1**) and colchicine (**5**) contents from this part were high. It seems, therefore that seedless pods from Thai origin *G. superba* could be applied and useful for a source for production of colchicine and colchicoside.

3. Experimental

3.1. General procedures

NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, silica gel 60 (70 – 230 mesh, Merck), RP-18 (50 μm, YMC), and Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.) were used. Preparative HPLC was carried out on an ODS column (250 × 20 mm i.d., YMC) with a Jasco RI-2031 refractive index detector. The flow rate was 6 mL/min.

3.1.1. Plant material

Gloriosa superba L. was cultivated at Ban Na-Dok-Mai (16.487216 N, 102.615195 E), Tambon Nong-Bua, Amphoe Ban-Fang, Khon Kaen province in April 2013 and collected the fresh fruits in November 2013. The plant was identified by one of us (T. Kanchanapoom). A voucher specimen (TK-PSKKU-0075) was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.1.2. Extraction and isolation

The fresh fruits of *G. superba* were collected and then divided into two parts, seedless pods and seeds. The seedless pods (254.7 g) were extracted three times with MeOH, and concentrated to dryness. This residue (61.5 g) was suspended in H_2O and partitioned with EtOAc. The EtOAc layer was combined and dried under reduced pressure to give a brownish powder (14.7 g), then re-suspended in 90% aqueous MeOH and defatted with Hexane, successively. The 90% aqueous MeOH part (7.7 g) was chromatographed on a column of silica gel using solvent systems EtOAc–MeOH (19:1, 2.0 L), EtOAc–MeOH (9:1, 3.0 L), EtOAc–MeOH (4:1, 3.0 L), EtOAc–MeOH (7:3, 2.0 L), and EtOAc–MeOH (1:1, 3.0 L), respectively to obtain seven fractions (A_3 to G_3). Fraction E_3 was provided compound **10** (553.9 mg) by crystallization. Fraction F_3 was purified by preparative HPLC–ODS with 25% aqueous MeCN to give compounds **5** (281.4 mg) and **6** (614.9 mg). The aqueous fraction (45.5 g) was also applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The portion eluted with methanol (3.5 g) was repeatedly chromatographed on a column of silica gel using solvent systems EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH–H_2O (40:10:1, 5.0 L), EtOAc–MeOH–H_2O (70:30:3, 2.0 L) and EtOAc–MeOH–H_2O (6:4:1, 2.0 L), respectively to provide four fractions (A_4 to D_4). Fraction B_4 was purified by preparative HPLC with 15% aqueous MeCN to obtain compound **4** (80.9 mg). Fraction C_4 was further purified by preparative HPLC–ODS with 15% aqueous MeCN to give compounds **1** (365.9 mg), **2** (28.5 mg), and **3** (40.9 mg).

Seeds (372.0 g) were similarly extracted three times with MeOH. The MeOH extract was concentrated *in vacuo* to give a brownish powder (43.6 g). This residue was suspended in H_2O, and partitioned three times with EtOAc. The EtOAc soluble fraction (7.0 g) was applied to a column of silica gel using solvent systems

Table 1
^{1}H NMR spectroscopic data of compounds **1–4** (400 MHz, DMSO-d_6).

Position	1	2	3	4
4	6.84 (1H, *s*)	6.80 (1H, *s*)	6.75 (1H, *s*)	6.67 (1H, *s*)
5	2.12–2.22 (1H)	2.09–2.18 (1H)	2.14–2.23 (1H)	2.64–2.72 (2H)
	2.52–2.58 (1H)	2.40–2.48 (1H)	2.54–2.60 (1H)	
6	1.73–1.82 (1H)	1.73–1.80 (1H)	1.73–1.80 (1H)	1.70–1.78 (2H)
	1.95–2.05 (1H)	1.94–2.02 (1H)	1.98–2.08 (1H)	
7	4.26–4.36 (1H)	4.27–4.35 (1H)	4.29–4.38 (1H)	4.71 (1H, *dd*, J = 6.9, 1.9 Hz)
8	7.13 (1H, s)	7.11 (1H, s)	7.13 (1H, s)	3.41–3.44 (1H)
11	7.02 (1H, *d*, J = 11.1 Hz)	7.01 (1H, *d*, J = 11.2 Hz)	7.00 (1H, *d*, J = 11.0 Hz)	6.58 (1H, *d*, J = 3.1 Hz)
12	7.11 (1H, *d*, J = 11.1 Hz)	7.09 (1H, *d*, J = 11.2 Hz)	7.11 (1H, *d*, J = 11.0 Hz)	4.01 (1H, *d*, J = 3.1, 2.9 Hz)
14	1.83 (3H, *s*)	1.83 (3H, *s*)	1.83 (3H, *s*)	1.77 (3H, *s*)
1-OMe	3.52 (3H, *s*)	3.50 (3H, *s*)	3.44 (3H, *s*)	3.87 (3H, *s*)
2-OMe	3.83 (3H, *s*)			
3-OMe			3.79 (3H, *s*)	3.75 (3H, *s*)
10-OMe	3.87 (3H, *s*)	3.86 (3H, *s*)	3.85 (3H, *s*)	3.58 (3H, *s*)
NH	8.59 (1H, *d*, J = 7.4 Hz)	8.57 (1H, *d*, J = 7.4 Hz)	8.58 (1H, *d*, J = 7.2 Hz)	4.30 (1H, br s)
Glc-1′	4.92 (1H, *d*, J = 7.3 Hz)	4.71 (1H, *d*, J = 7.2 Hz)	4.97 (1H, *d*, J = 7.3 Hz)	5.04 (1H, *d*, J = 7.3 Hz)
2′	3.25–3.36 (1H)	3.30–3.36 (1H)	3.19–3.27 (1H)	3.18–3.24 (1H)
3′	3.25–3.36 (1H)	3.27 (1H, *dd*, J = 9.3, 8.5 Hz)	3.19–3.27 (1H)	3.14–3.21 (1H)
4′	3.17 (1H, *dd*, J = 9.3, 8.6 Hz)	3.16 (1H, *dd*, J = 9.3, 8.4 Hz)	3.16 (1H, *dd*, J = 9.4, 8.0 Hz)	3.08–3.15 (1H)
5′	3.33–3.40 (1H)	3.32–3.40 (1H)	3.02–3.10 (1H)	3.02–3.10 (1H)
6′	3.66–3.75 (1H)	3.68–3.74 (1H)	3.58–3.63 (1H)	3.36–3.43 (1H)
	3.80–3.87 (1H)	3.78–3.83 (1H)	3.83–3.88 (1H)	3.55–3.62 (1H)

Table 2
^{13}C NMR spectroscopic data of compounds **1–4** (100 MHz, DMSO-d_6).

Position	1	2	3	4
1	150.5	145.1	150.6	151.1
2	141.2	139.0	137.7	136.2
3	150.9	146.0	152.8	152.3
4	111.1	111.5	108.5	110.3
4a	134.0	128.7	134.5	139.7
5	29.3	28.9	29.2	31.3
6	35.7	36.1	35.7	31.8
7	51.4	51.3	51.4	48.4
7a	151.0	151.1	151.0	138.6
8	130.4	130.4	130.4	51.1
9	178.0	178.1	178.1	197.8
10	163.6	163.5	163.6	157.4
11	112.2	112.2	112.3	127.5
12	134.6	134.5	134.6	42.2
12a	135.2	135.6	135.3	143.4
12b	126.6	127.2	125.9	118.2
13	168.7	168.6	168.7	168.4
14	22.5	22.5	22.5	22.8
1-OMe	61.0	60.1	60.8	61.5
2-OMe	60.9			
3-OMe			56.4	56.3
10-OMe	56.1	56.1	56.1	56.3
Glc −1′	100.5	102.2	103.1	101.9
2′	73.4	73.4	74.1	74.2
3′	76.9	76.0	76.5	76.5
4′	69.9	70.0	70.0	70.0
5′	77.3	77.3	77.2	77.2
6′	60.8	60.8	60.9	60.9

EtOAc–MeOH (19:1, 2.0 L), EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH (4:1, 4.0 L), EtOAc–MeOH (7:3, 3.0 L), and EtOAc–MeOH (1:1, 3.0 L), respectively to obtain seven fractions (A_1 to G_1). Fraction A_1 was provided compound **8** (190.0 mg) by crystallization. Fraction F_1 (1.92 g) was purified by preparative HPLC–ODS with solvent system 25% aqueous MeCN to give compounds **5** (812.6 mg), **6** (8.0 mg), **7** (23.4 mg), and **9** (10.2 mg). The remaining aqueous fraction (36.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (2.0 g) was subjected to a column of silica gel using solvent systems EtOAc–MeOH (9:1, 2.0 L), EtOAc–MeOH–H_2O (40:10:1, 3.0 L), EtOAc–MeOH–H_2O (70:30:3, 2.0 L) and EtOAc–MeOH–H_2O (6:4:1, 2.0 L), respectively to provide five fractions (A_2 to E_2). Fraction B_2 (1.1 g) was purified by preparative HPLC–ODS with solvent system 28% aqueous MeCN to afford compound **5** (74.9 mg). Finally, fraction C_2 was purified by preparative HPLC–ODS with solvent system 15% aqueous MeCN to provide compound **1** (8.0 mg).

3.2. Dongduengoside A (*2*)

Amorphous powder, $[\alpha]_D^{24}$ −109.2 (MeOH, *c* 0.72); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m*/*z*: 534.1962 ($C_{26}H_{32}NO_{11}$ required 534.1970).

3.3. Dongduengoside B (*3*)

Amorphous powder, $[\alpha]_D^{24}$ −88.7 (MeOH, *c* 0.85); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m*/*z*: 548.2135 ($C_{27}H_{34}NO_{11}$ required 548.2126).

3.4. Dongduengoside C (*4*)

Amorphous powder, $[\alpha]_D^{24}$ + 72.0 (MeOH, *c* 1.00); ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; Positive HR-FAB-MS, *m*/*z*: 548.2111 ($C_{27}H_{34}NO_{11}$ required 548.2126).

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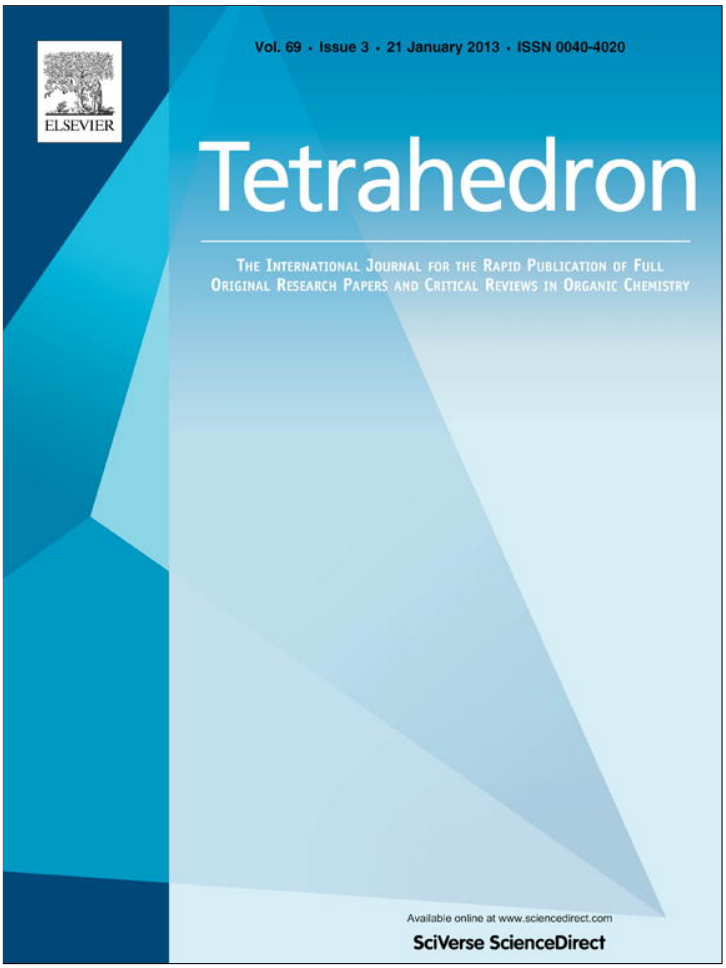

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Glucopyranosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid and (*R*)-eucomic acid, and an aromatic glucoside from the pseudobulbs of *Grammatophyllum speciosum*

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ABSTRACT

Three new glucosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid and of (*R*)-eucomic acid, grammatophyllosides A–C, and a new phenolic glucoside, grammatophylloside D, were isolated from the pseudobulbs of *Grammatophyllum speciosum* along with cronupapine, vandateroside II, gastodin, vanilloloside, orcinol glucoside, and isovitexin. The structure elucidations of these compounds were based on analyses of physical and spectroscopic data, as well as chemical evidence.

1. Introduction

Grammatophyllum speciosum Blume (Orchidaceae; Thai name: Waan-Phet-Cha-hueng) is the largest orchid of the world, distributed in the tropical rainforests of Thailand, Malaysia, Indonesia, and the Philippines. In Thai traditional medicine, the decoction made from pseudobulbs is used to treat sore throats and bronchitis, while the pseudobulb paste is applied externally to relieve pain from scorpion stings. Phytochemical investigation has not been reported for this species. However, the phytochemical compositions and pharmacological properties of orchids have been reviewed.[1] As part of our continuing studies on Thai medicinal plants, we investigated the polar constituents from this plant. This paper describes the isolation and structure determination of 10 polar compounds, including 5 glucosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid (**1**–**3**) and of (*R*)-eucomic acid (**4**, **5**), 4 phenolic glycosides (**6**–**9**), a flavone *C*-glucoside (**10**), and a nucleotide (**11**) from the pseudobulbs of this plant. Also, the structures of previously isolated glucosyloxybenzyl eucomate derivatives are discussed (Fig. 1).

2. Results and discussion

The methanolic extract of the fresh pseudobulbs of *G. speciosum* was partitioned between Et_2O and H_2O. The aqueous soluble fraction was separated by a combination of chromatographic methods to provide 10 compounds.

Compound **1** was isolated as an amorphous powder, and its molecular formula was determined to be $C_{24}H_{28}O_{11}$ by negative HR-APCI-TOF mass spectrometric analysis. The NMR spectroscopic and the physical data of compound **1** were coincident with those of cronupapine, first isolated from *Cronura papirio*.[2] The structure was confirmed by the respective HMBC correlations. However, the absolute configuration at the C-2 position was not identified. Thus, this compound was hydrolyzed in alkaline condition (see Experimental section) to afford 2-benzylmalic acid (**1a**), as a white powder, which was identified by ^1H and ^{13}C NMR spectroscopic analysis, displaying an optical rotation $[\alpha]_D^{27}$ −14.9 (*c* 0.48, H_2O). Since the optical rotation value reported for (*S*)-2-benzylmalic acid

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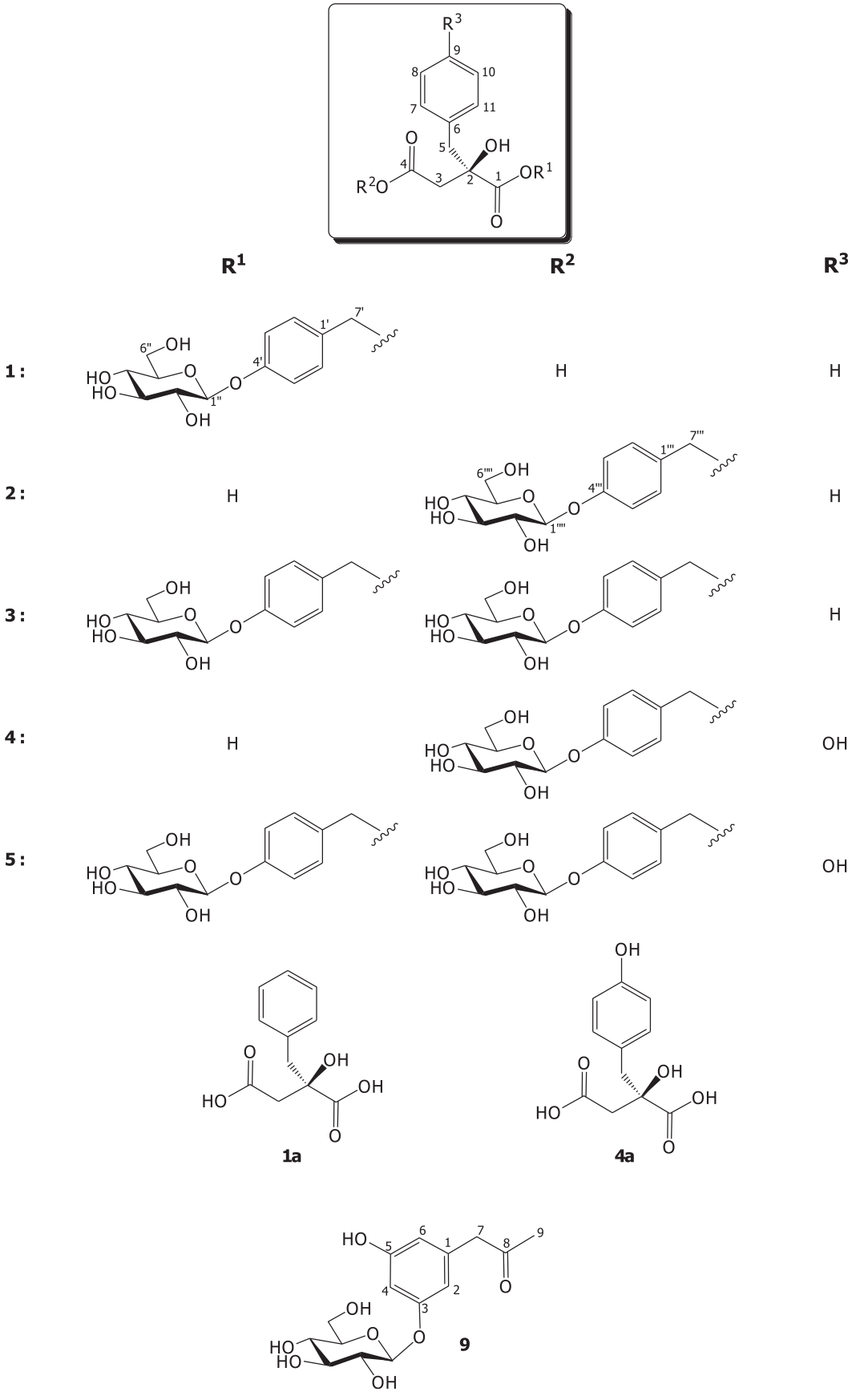

Fig. 1. Structures of compounds **1**–**5**, **9**, **1a**, and **4a**.

showed $[\alpha]_D^{23}$ +15.55 (*c* 2.9, H_2O)[3] and $[\alpha]_D^{29}$ +13.7 (*c* 1.0, H_2O),[4] the absolute configuration of C-2 position was concluded to be *R*.

Compound **2** was obtained as an amorphous powder, and its molecular formula was established as $C_{24}H_{28}O_{11}$ by negative HR-APCI-TOF mass spectrometric analysis. Inspection of the NMR spectroscopic data indicated that this compound contained a 2-benzylmaloyl moiety from the chemical shifts of five overlapping proton signals at δ_H 7.13 (2H) and 7.20 (3H) together with two sets of AB-type methylene protons at δ_H 2.41 and 2.71 (each d, *J*=14.4 Hz) and δ_H 2.83 and 2.89 (each d, *J*=13.7 Hz). These proton signals were in agreement with the relevant carbon signals of a mono-substituted aromatic ring at δ_C 125.6 (1C), 127.3 (2C), and 130.6 (2C); and two methylene carbons at δ_C 43.7 and 44.5. The remaining signals belonging to this part were two carboxyl carbons at δ_C 170.6 and 176.8, an oxygenated quaternary carbon at δ_C 75.1, and a quaternary carbon of an aromatic ring at δ_C 138.4. In addition, the signals of the glucopyranosyloxybenzyl moiety were presented as compared to **1**, supported by the proton signals of a 1,4-disubstituted aromatic ring at δ_H 7.30 and 7.00 (each 2H, d, *J*=8.5 Hz) and an anomeric proton at δ_H 4.86 (1H, d, *J*=7.3 Hz). This compound was an isomer of **1**, differing by the location of the glucopyranosyloxybenzyl moiety as connected through C-4 instead of C-1. The conclusion was corroborated by the results of the HMBC experiments (Fig. 2), for example, correlations between H-5 and C-1, C-2 and C-3, and between H-7‴ and C-4. The absolute configuration of C-2 was identified to be *R* by alkaline hydrolysis (see Section 4), to obtain (*R*)-2-benzylmalic acid (**1a**), $[\alpha]_D^{27}$ −18.4 (*c* 0.57, H_2O). Therefore, the structure of **2** was elucidated as 4-(4‴-β-glucopyranosyloxybenzyl)-(*R*)-2-benzylmalate, namely grammatophylloside A.

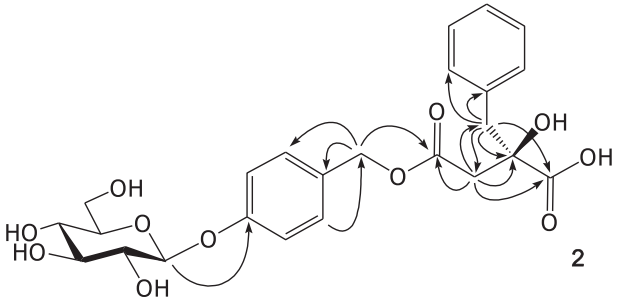

Fig. 2. Significant HMBC correlations of grammatophylloside A (**2**).

Compound **3** was isolated as an amorphous powder. The molecular formula was identified as $C_{37}H_{44}O_{17}$ by negative HR-APCI-TOF mass spectrometric analysis. The NMR spectra were closely related to those of compounds **1** and **2**, except for the observation of the additional signals for a second glucopyranosyloxybenzyl moiety. The two glucopyranosyloxybenzyl moieties were assigned to be connected through the two ester carbonyl groups based on HMBC correlations from the overlapping signals for H-7′ and H-7‴ (δ_H 4.94) to C-1 (δ_C, 173.6) and C-4 (δ_C 169.8), respectively. Consequently, this compound was identified to be 1,4-bis(4′,4‴-β-glucopyranosyloxybenzyl)-(*R*)-2-benzylmalate, namely grammatophylloside B.

Compound **4** was isolated as an amorphous powder. Its molecular formula was established as $C_{24}H_{28}O_{12}$ by negative HR-APCI-TOF mass spectrometric analysis. The ^1H and ^{13}C NMR spectra indicated that this compound was an analogue of grammatophylloside A (**2**), differing by the presence of one additional hydroxyl function at C-9 of the 2-benzylmaloyl moiety. Consequently, the ^1H NMR spectrum showed a set of resonances corresponding to an AA′BB′ aromatic ring system, comprising signals at δ_H 6.59 and 6.97 (each 2H, d, *J*=8.3 Hz) instead of the signals of the mono-substituted aromatic ring systems of **1** and **2**. Assignment of all signals was based on the results of HMQC and HMBC experiments. Since the signals of two AB-type methylene protons of H_2-5 (δ_H 2.71 and 2.76) and one AB-type methylene proton of H-3 at δ_H 2.68 were overlapped in DMSO-d_6, another set of NMR data was acquired in MeOH-d_4 to provide resolved signals (Tables 1 and 2). In the HMBC spectrum, significant correlations were found from δ_H 2.78 (H-5) to δ_C 180.6 (C-1), 77.6 (C-2), 44.3 (C-3), 129.0 (C-6), 132.6 (C-7/11), and δ_H 5.03 (H-7‴) to δ_C 177.0 (C-4), indicating that the glucopyranosyloxybenzyl moiety was connected through C-4. Alkaline hydrolysis of **4** provided (*R*)-eucomic acid (**4a**) as a white powder, identified by spectroscopic data and an optical rotation value $[\alpha]_D^{26}$ −10.1 (*c* 1.79, MeOH), lit. $[\alpha]_D^{28}$ −12.9 (*c* 0.50, MeOH).[5] Therefore, the structure of this new natural product was elucidated as shown, namely grammatophylloside C. Interestingly, the same structure had previously been assigned to vandateroside I, isolated from *Vanda teres*,[6] but the spectroscopic data obtained in the present study clearly was not identical to the reported NMR data, especially for the (*R*)-eucomoyl part. The structure of vandateroside I was elucidated based on HMBC correlations, in particular from H_2-5 (δ_H 2.77) to C-1 (δ_C 171.6) and from H-7‴ (δ_H 4.98) to C-4 (δ_C 173.7).[6] However, as stated above the signals of H_2-5 overlapped with one proton signal of H-3 at δ_H 2.74 when measured in DMSO-d_6, leading to ambiguity in assigning C-1 and C-4. Even though vandateroside I was not isolated in the present study, our results strongly suggest that its structure should be revised.

Compound **5** was obtained as an amorphous powder, and its molecular formula was established as $C_{37}H_{44}O_{18}$ by negative HR-APCI-TOF mass spectrometric analysis. The spectroscopic and physical data were identical to those of vandateroside II.[6] However, previously the absolute configuration at C-2 was established as *R* only based on comparison of its negative optical rotation with that of (*R*)-eucomic acid. To provide stronger evidence, compound **5** was subjected to alkaline hydrolysis to give a white amorphous residue. This residue was identified to be (*R*)-eucomic acid (**4a**) with an optical rotation value $[\alpha]_D^{26}$ −15.1 (*c* 0.45, MeOH). Accordingly, the absolute configuration at C-2 position was unambiguously assigned to be *R*.

Compound **9** was isolated as an amorphous powder. Its molecular formula was established as $C_{15}H_{20}O_8$ by negative HR-APCI-TOF mass spectrometric analysis. The ^1H NMR spectrum indicated the presence of a 1,3,5-trisubstituted aromatic ring with signals at δ_H 6.24, 6.30 and 6.33 (each 1H, br s), one singlet methylene at δ_H 3.58 (2H), one acetyl group at δ_H 2.08 (3H) as well as one anomeric proton at δ_H 4.77 (1H, d, *J*=7.5 Hz) for the β-glucopyranosyl unit. In the ^{13}C NMR spectrum, 15 carbon signals were observed, of which 9 were assignable to two oxy-aryl carbons at δ_C 158.3 and 158.6, 3 aryl-methines at δ_C 101.9, 108.6, and 110.3, 1 non-protonated aryl carbon at δ_C 136.7, 1 methylene at δ_C 50.0, and 2 carbon signals of an acetyl group at δ_C 206.1 and 29.4 for the aglycone moiety. The complete assignments were established by analyzing the 2D NMR spectra including HMQC and HMBC. In the HMBC spectrum, the correlations were found between (i) δ_H 3.58 (H-7) and δ_C 136.7 (C-1), 108.6 (C-2), 206.1 (C-8), and 29.4 (C-9); (ii) δ_H 4.77 (H-1′ Glc) and δ_C 158.6 (C-3); and (iii) δ_H 9.45 (5-OH) and δ_C 158.3 (C-5), 101.9 (C-4), and 110.3 (C-6), as illustrated in Fig. 3. Consequently, the structure of compound **9** was elucidated as 1-(3,5-dihydroxyphenyl)propan-2-one 3-*O*-β-glucopyranoside, namely grammatophylloside D.

The remaining known compounds were identified as gastodin (**6**),[7] vanilloloside (**7**),[8] orcinol glucoside (**8**),[9] and isovitexin (**10**)[10] by physical data and spectroscopic evidence.

3. Conclusions

The present study reported five glucosyloxybenzyl derivatives of (*R*)-2-benzylmalic acid (**1**–**3**) and of (*R*)-eucomic acid (**4**, **5**), four

Table 1
H NMR spectroscopic data of compounds **1**–**5** (300 MHz)

Position	**1**[a]	**1**[b]	**2**[a]	**3**[a]
3	2.13 (1H, d, *J*=15.6 Hz)	2.45 (1H, d, *J*=16.1 Hz)	2.41 (1H, d, *J*=14.1 Hz)	2.49 (1H, d, *J*=15.6 Hz)
	2.46 (1H, d, *J*=15.6 Hz)	2.81 (1H, d, *J*=16.1 Hz)	2.71 (1H, d, *J*=14.1 Hz)	2.86 (1H, d, *J*=15.6 Hz)
5	2.78 (1H, d, *J*=13.3 Hz)	2.90 (1H, d, *J*=13.5 Hz)	2.83 (1H, d, *J*=13.7 Hz)	2.87 (1H, d, *J*=13.4 Hz)
	2.86 (1H, d, *J*=13.3 Hz)	2.96 (1H, d, *J*=13.5 Hz)	2.89 (1H, d, *J*=13.7 Hz)	2.93 (1H, d, *J*=13.4 Hz)
7/11	7.06–7.12 (2H, m)[c]	7.08–7.12 (2H, m)[c]	7.10–7.16 (2H, m)[c]	7.08–7.12 (2H, m)[c]
8/10				
	7.15–7.21 (3H, m)[c]	7.16–7.20 (3H, m)[c]	7.16–7.22 (3H, m)[c]	7.17–7.21 (3H, m)[c]
9				
2′/6′	7.19 (2H, d, *J*=8.5 Hz)	7.25 (2H, d, *J*=8.6 Hz)		7.24 (2H, d, *J*=8.7 Hz)[d]
3′/5′	6.99 (2H, d, *J*=8.5 Hz)	7.07 (2H, d, *J*=8.6 Hz)		6.99 (2H, d, *J*=8.7 Hz)[d]
7′	4.91 (2H, s)	5.00 (2H, s)		4.94 (2H, s)
Glc-1″	4.87 (1H, d, *J*=7.1 Hz)	4.93 (1H, d, *J*=7.1 Hz)		4.85 (1H, d, *J*=6.9 Hz)[d]
2″	3.13–3.23 (1H, m)[c]	3.42–3.50 (1H, m)[c]		
				3.20–3.28 (2H, m)[c]
3″	3.23–3.33 (1H, m)[c]			
		3.40–3.48 (2H, m)[c]		
4″	3.13–3.23 (1H, m)[c]			3.11–3.18 (1H, m)[c]
5″	3.31–3.37 (1H, m)[c]	3.47–3.52 (1H, m)[c]		3.28–3.34 (1H, m)[c]
6″	3.49 (1H, dd, *J*=11.3, 5.0 Hz)	3.72 (1H, dd, *J*=11.9, 4.6 Hz)		3.41–3.48 (1H, m)[c]
	3.69 (1H, br d, *J*=11.3 Hz)	3.90 (1H, dd, *J*=11.9, 1.4 Hz)		3.63–3.70 (1H, m)[c]
2‴/6‴			7.30 (2H, d, *J*=8.5 Hz)	7.20 (2H, d, *J*=8.7 Hz)[d]
3‴/5‴			7.00 (2H, d, *J*=8.5 Hz)	6.98 (2H, d, *J*=8.7 Hz)[d]
7‴			4.97 (2H, s)	4.94 (2H, s)
Glc-1⁗			4.86 (1H, d, *J*=7.3 Hz)	4.87 (1H, d, *J*=6.9 Hz)[d]
2⁗			3.25–3.33 (1H, m)[c]	
				3.20–3.28 (2H, m)[c]
3⁗			3.20–3.28 (1H, m)[c]	
4⁗			3.14–3.22 (1H, m)[c]	3.11–3.18 (1H, m)[c]
5⁗			3.32–3.37 (1H, m)[c]	3.28–3.34 (1H, m)[c]
6⁗			3.48–3.52 (1H, m)[c]	3.41–3.48 (1H, m)[c]
			3.68 (1H, br d, *J*=11.5 Hz)	3.63–3.70 (1H, m)[c]

Position	**4**[a]	**4**[b]	**5**[a]
3	2.38 (1H, d, *J*=14.2 Hz)	2.59 (1H, d, *J*=15.6 Hz)	2.48 (1H, d, *J*=15.6 Hz)
	2.68 (1H, d, *J*=14.2 Hz)	3.00 (1H, d, *J*=15.6 Hz)	2.88 (1H, d, *J*=15.6 Hz)
5	2.71 (1H, d, *J*=13.7 Hz)	2.78 (1H, d, *J*=13.5 Hz)	2.77 (1H, d, *J*=13.6 Hz)
	2.76 (1H, d, *J*=13.7 Hz)	2.95 (1H, d, *J*=13.5 Hz)	2.83 (1H, d, *J*=13.6 Hz)
7/11	6.97 (2H, d, *J*=8.3 Hz)	7.09 (2H, d, *J*=8.3 Hz)	6.92 (2H, d, *J*=8.4 Hz)
8/10	6.59 (2H, d, *J*=8.3 Hz)	6.68 (2H, d, *J*=8.3 Hz)	6.61 (2H, d, *J*=8.4 Hz)
9	—	—	—
2′/6′			7.26 (2H, d, *J*=8.7 Hz)[d]
3′/5′			7.01 (2H, d, *J*=8.7 Hz)[d]
7′			4.97 (2H, s)
Glc-1″			4.85 (1H, d, *J*=6.9 Hz)[d]
2″			
			3.21–3.29 (2H, m)[c]
3″			
4″			3.14–3.23 (1H, m)[c]
5″			3.30–3.38 (1H, m)[c]
6″			3.42–3.50 (1H, m)[c]
			3.66–3.74 (1H, m)[c]
2‴/6‴	7.29 (2H, d, *J*=8.6 Hz)	7.28 (2H, d, *J*=8.4 Hz)	7.22 (2H, d, *J*=8.7 Hz)[d]
3‴/5‴	7.01 (2H, d, *J*=8.6 Hz)	7.06 (2H, d, *J*=8.4 Hz)	7.00 (2H, d, *J*=8.7 Hz)[d]
7‴	4.94 (2H, s)	5.03 (2H, s)	4.97 (2H, s)
Glc-1⁗	4.85 (1H, d, *J*=7.3 Hz)	4.93 (1H, d, *J*=6.9 Hz)	4.85 (1H, d, *J*=6.9 Hz)[d]
2⁗	3.25–3.32 (1H, m)[c]	3.47–3.53 (1H, m)[c]	
			3.21–3.29 (2H, m)[c]
3⁗	3.20–3.28 (1H, m)[c]		
		3.40–3.48 (2H, m)[c]	
4⁗	3.18–3.25 (1H, m)[c]		3.14–3.23 (1H, m)[c]
5⁗	3.30–3.36 (1H, m)[c]	3.44–3.52 (1H, m)[c]	3.30–3.38 (1H, m)[c]

Table 1 (*continued*)

Position	4[a]	4[b]	5[a]
6‴″	3.41–3.47 (1H, m)[c] 3.69 (1H, br d, *J*=10.8 Hz)	3.69–3.76 (1H, m)[c] 3.88 (1H, br d, *J*=11.9)	3.42–3.50 (1H, m)[c] 3.68–3.74 (1H, m)[c]

[a] Measured in DMSO-d_6.
[b] Measured in MeOH-d_4.
[c] Chemical shifts was assigned by the results from COSY and HMQC.
[d] Assignments with the same superscript may be reversed.

Table 2
^{13}C NMR spectroscopic data of compounds **1**–**5** (75 MHz)

Position	1[a]	1[b]	2[a]	3[a]	4[a]	4[b]	5[a]
1	174.9	176.7	176.8	173.6	177.0	180.6	173.6
2	76.0	77.8	75.1	75.6	75.2	77.6	75.5
3	44.1	45.7	43.7	43.0	43.5	44.3	42.6
4	174.9	178.7	170.6	169.8	170.7	173.2	169.6
5	45.0	46.0	44.5	44.7	43.8	45.5	43.8
6	136.7	137.2	138.4	135.7	128.4	129.0	125.7
7/11	130.3	131.5	130.6	130.6	131.4	132.6	131.4
8/10	127.7	129.0	127.3	128.0	114.3	115.6	114.7
9	126.2	127.7	125.6	126.8	155.5	156.8	156.1
1′	129.4	131.0		129.2			129.1
2′/6′	129.4	131.1		129.9			129.6
3′/5′	116.1	117.6		116.3			116.2
4′	157.1	159.0		157.4			157.3
7′	65.2	67.6		66.2[c]			65.9
Glc-1″	100.4	102.2		100.6			100.4
2″	73.3	74.8		73.4			73.3
3″	77.1	78.1		77.1			77.0
4″	69.7	71.3		69.9			69.8
5″	76.7	77.9		76.7			76.6
6″	60.7	62.4		60.9			60.7
1‴			129.8	129.4	129.9	131.3	129.2
2‴/6‴			129.4	129.9	129.4	130.7	129.6
3‴/5‴			116.1	116.3	116.1	117.6	116.2
4‴			157.1	157.4	157.1	158.8	157.3
7‴			64.7	65.6[c]	64.6	66.8	65.4
Glc-1⁗			100.4	100.6	100.4	102.0	100.4
2⁗			73.2	73.4	73.3	74.7	73.3
3⁗			77.1	77.1	77.1	78.0	77.0
4⁗			69.7	69.9	69.7	71.2	69.8
5⁗			76.6	76.7	76.7	77.7	76.6
6⁗			60.7	60.9	60.7	62.3	60.7

[a] Measured in DMSO-d_6.
[b] Measured in MeOH-d_4.
[c] Assignments with the same superscript may be reversed.

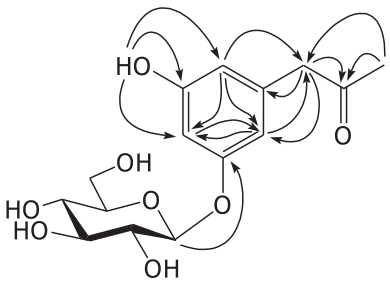

Fig. 3. Significant HMBC correlations of grammatophylloside D (**9**).

phenolic glycosides (**6**–**9**), and a flavone *C*-glucoside (**10**) from the polar fraction of the Thai orchid *G. speciosum*. Cronupapine (**1**), grammatophyllosides A–C (**2**–**4**), vandateroside II (**5**) were the glucopyranosyloxybenzyl moiety forming with (*R*)-2-benzylmalic acid or (*R*)-eucomic acid through ester bonds, which were quite specific type of compounds to obtain from the orchid family.[2,6,11] These specific compounds provided further confirmation of the typical profile of secondary metabolites found in this family, and might be useful for further chemotaxonomic studies.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded in DMSO-d_6 using a Bruker AV-300 spectrometer. MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (20×250 mm i.d., YMC) with a Jasco RI-2031 refractive index detector. The flow rate was 6 mL/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O/EtOH (1:1, v/v).

4.2. Plant material

The fresh pseudobulbs of *G. speciosum* Blume were collected in March 2011 from the Herbal Paradise Garden, Chulabhorn Research Institute. The identification of the plant was confirmed by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0068) has been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

4.3. Extraction and isolation

The fresh pseudobulbs of *G. speciosum* (11.6 kg) were extracted with MeOH (30 L for 2 days, ×3) at room temperature. The MeOH extract was concentrated in vacuo to dryness. This residue (215.8 g) was suspended in H_2O (500 mL), and partitioned with Et_2O (3×1.0 L). The water-soluble fraction (189.4 g) was subjected to a Diaion HP-20 column, and eluted with H_2O, MeOH, and acetone, successively. The fraction eluted with MeOH (35.4 g) was subjected to silica gel cc using solvent systems EtOAc/MeOH (9:1, 7.0 L), EtOAc/MeOH/H_2O (40:10:1, 6.0 L), EtOAc/MeOH/H_2O (70:30:3, 5.0 L), and EtOAc/MeOH/H_2O (6:4:1, 9.0 L), respectively, to provide six fractions (A to F). Fraction B (2.9 g) was applied to an RP-18 column using a gradient solvent system H_2O/MeOH (9:1→1:4, v/v) to provide five fractions. Fractions B-1 and B-2 were combined and purified by preparative HPLC-ODS using solvent system H_2O/MeCN (9:1, v/v) to afford compounds **8** (1.3 g) and **9** (18.5 mg). Fraction C (1.7 g) was subjected to an RP-18 column using solvent system H_2O/MeOH (9:1→1:4, v/v) to provide six fractions. Fraction C-2 was purified by preparative HPLC-ODS with solvent system H_2O/MeCN (9:1, v/v) to yield compound **6** (34.8 mg). Fraction D (19.2 g) was applied to an RP-18 column using solvent system, H_2O/MeOH (9:1→1:4, v/v) to afford 10 fractions. Fraction D-1 was separated by preparative HPLC-ODS with solvent system H_2O/MeCN (9:1, v/v) to give compounds **1** (368.5 mg), **2** (343.4 mg), and fraction D-1-1. Fraction D-1-1 was further purified by preparative HPLC-Polyamine II with solvent system H_2O/MeCN (1:9, v/v) to provide compound **7** (11.3 mg). Fraction D-4 was purified by preparative HPLC-ODS with solvent system H_2O/MeCN (4:1, v/v) to afford compound **10** (133.0 mg). Fraction D-5 was purified by

preparative HPLC-ODS with solvent system H_2O/MeCN (4:1, v/v) to provide compound **5** (105.6 mg). Fractions D-7 and D-8 were combined and purified by preparative HPLC-ODS with solvent system H_2O/MeCN (7:3, v/v) to afford compound **3** (722.9 mg). Finally, fraction E (4.6 g) was similarly applied to an RP-18 column using H_2O/MeOH (9:1→1:4, v/v) to obtain seven fractions. Faction E-1 was purified by preparative HPLC-ODS with solvent system H_2O/MeCN (9:1, v/v) to provide compound **4** (320.9 mg). Fraction E-2 was purified by preparative HPLC-ODS with solvent system H_2O/MeCN (9:1, v/v) to afford compound **2** (316.0 mg). Fraction E-3 was purified by preparative HPLC-ODS with solvent system H_2O/MeCN (9:1, v/v) to give compound **1** (107.9 mg).

*4.3.1. Cronupapine (**1**).* Amorphous powder; $[\alpha]_D^{27}$ −50.0 (*c* 0.48, MeOH); IR (UATR) ν_{max} 3364, 2920, 1727, 1583, 1512, 1400, 1230, 1075, 1043 cm^{-1}; ^1H NMR (DMSO-d_6 and MeOH-d_4): Table 1; ^{13}C NMR (DMSO-d_6 and MeOH-d_4): Table 2; significant HMBC correlations: (i) H-3 and C-1,2,4,5, (ii) H-5 and C-1,2,3,6,7, (iii) H-7′ and C-1,1′,2′,6′, (iv) H-1″ and C-4′; negative HR-APCI-TOFMS, *m*/*z*: 491.1554 $[M-H]^-$ (calcd for $C_{24}H_{27}O_{11}$, 491.1559).

*4.3.2. Grammatophylloside A (**2**).* Amorphous powder; $[\alpha]_D^{26}$ −32.4 (*c* 0.84, MeOH); IR (UATR) ν_{max} 3369, 2920, 1726, 1599, 1513, 1400, 1342, 1226, 1072, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; negative HR-APCI-TOFMS, *m*/*z*: 491.1554 $[M-H]^-$ (calcd for $C_{24}H_{27}O_{11}$, 491.1559).

*4.3.3. Grammatophylloside B (**3**).* Amorphous powder; $[\alpha]_D^{26}$ −52.2 (*c* 0.61, MeOH); IR (UATR) ν_{max} 3328, 2920, 1724, 1610, 1513, 1230, 1068, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; negative HR-APCI-TOFMS, *m*/*z*: 795.2285 $[M+Cl]^-$ (calcd for $C_{37}H_{44}Cl^{35}O_{17}$, 795.2273).

*4.3.4. Grammatophylloside C (**4**).* Amorphous powder; $[\alpha]_D^{27}$ −28.0 (*c* 0.79, MeOH); IR (UATR) ν_{max} 3327, 2913, 1717, 1590, 1513, 1344, 1228, 1174, 1071, 1043 cm^{-1}; ^1H NMR (DMSO-d_6 and MeOH-d_4): Table 1; ^{13}C NMR (DMSO-d_6 and MeOH-d_4): Table 2; significant HMBC correlations: (i) H-3 and C-1,2,4,5, (ii) H-5 and C-1,2,3,6,7, (iii) H-7‴ and C-4,1‴,2‴,6‴, (iv) H-1⁗ and C-4‴; negative HR-APCI-TOFMS, *m*/*z*: 507.1494 $[M-H]^-$ (calcd for $C_{24}H_{27}O_{12}$, 507.1508).

*4.3.5. Vandateroside II (**5**).* Amorphous powder; $[\alpha]_D^{26}$ −30.6 (*c* 0.70, DMSO); IR (UATR) ν_{max} 3320, 2931, 1723, 1614, 1515, 1357, 1232, 1068, 1043 cm^{-1}; ^1H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2; negative HR-APCI-TOFMS, *m*/*z*: 811.2235 $[M+Cl]^-$ (calcd for $C_{37}H_{44}Cl^{35}O_{18}$, 811.2222).

4.4. Alkaline hydrolysis of compounds 1–5

Compound **1** (60 mg, 0.12 mmol) was treated with sodium hydroxide solution (5 mL, 7% aq) and stirred at room temperature for 6 h. The reaction mixture was neutralized with hydrochloric acid solution (5% aq) and extracted with EtOAc. The organic layer was separated and the solvent was evaporated in vacuo to give (*R*)-benzylmalic acid **1a** (12.0 mg, 45%). By the same method, compounds **2** (60 mg, 0.12 mmol) and **3** (100 mg, 0.13 mmol) provided **1a** (23.4 mg, 87% and 24.5 mg, 83%, respectively). Compounds **4** (95 mg, 0.19 mmol) and **5** (60 mg, 0.08 mmol) yielded **4a** (17.9 mg, 40% and 4.5 mg, 24%, respectively). The structures of **1a** and **4a** were identified by 1D and 2D NMR spectroscopic analysis together with comparison of the optical rotation data with the literature values.

*4.4.1. (R)-Benzylmalic acid (**1a**).* Amorphous powder; IR (UATR) ν_{max} 3446, 2927, 1716, 1496, 1390, 1207, 1115, 1022 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.19–7.28 (5H, m, Ar–H), 2.92 (1H, d, *J*=13.4 Hz, H-5a), 2.86 (1H, d, *J*=13.4 Hz, H-5b), 2.72 (1H, d, *J*=15.8 Hz, H-3a), 2.38 (1H, d, *J*=15.8 Hz, H-3b), or ^1H NMR (MeOH-d_4): δ 7.20–7.26 (5H, m, Ar–H), 3.04 (1H, d, *J*=13.4 Hz, H-5a), 2.97 (1H, d, *J*=13.4 Hz, H-3a), 2.94 (1H, d, *J*=15.8 Hz, H-5b), 2.59 (1H, d, *J*=15.8 Hz, H-3b); ^{13}C NMR (DMSO-d_6): δ 175.6 (C-1), 171.6 (C-4), 136.2 (C-6), 130.6 (C-7, 11), 127.8 (C-8, 10), 126.5 (C-9), 74.7 (C-2), 44.4 (C-5), 42.8 (C-3), or ^{13}C NMR (MeOH-d_4): δ 177.6 (C-1), 174.1 (C-4), 136.9 (C-6), 131.6 (C-7, 11), 129.0 (C-8, 10), 127.8 (C-9), 76.7 (C-2), 46.1 (C-5), 43.8 (C-3); negative HR-APCI-TOFMS, *m*/*z*: 223.0605 $[M-H]^-$ (calcd for $C_{11}H_{11}O_5$, 223.0612).

*4.4.2. (R)-Eucomic acid (**4a**).* Amorphous powder; IR (UATR) ν_{max} 3460, 3030, 2607, 1731, 1515, 1370, 1192, 1135, 1070 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.20 (1H, s, 9-OH), 6.99 (2H, d, *J*=8.4 Hz, H-7,11), 6.63 (2H, d, *J*=8.4 Hz, H-8,10), 2.80 (1H, d, *J*=13.4 Hz, H-5a), 2.74 (1H, d, *J*=13.4 Hz, H-5b), 2.70 (1H, d, *J*=15.8 Hz, H-3a), 2.35 (1H, d, *J*=15.8 Hz, H-3b), or ^1H NMR (MeOH-d_4): δ 7.06 (2H, d, *J*=8.5 Hz, H-7,11), 6.68 (2H, d, *J*=8.5 Hz, H-8,10), 2.95 (1H, d, *J*=13.9 Hz, H-5a), 2.94 (1H, d, *J*=16.0 Hz, H-3a), 2.85 (1H, d, *J*=13.9 Hz, H-5b), 2.56 (1H, d, *J*=16.0 Hz, H-3b); ^{13}C NMR (DMSO-d_6): δ 175.7 (C-1), 171.7 (C-4), 156.0 (C-9), 131.4 (C-7, 11), 126.2 (C-6), 114.6 (C-8, 10), 74.9 (C-2), 43.7 (C-5), 42.6 (C-3), or ^{13}C NMR (MeOH-d_4): δ 177.7 (C-1), 174.2 (C-4), 157.4 (C-9), 132.6 (C-7, 11), 127.6 (C-6), 115.8 (C-8, 10), 76.8 (C-2), 45.4 (C-5), 43.6 (C-3); negative HR-APCI-TOFMS, *m*/*z*: 239.0556 $[M-H]^-$ (calcd for $C_{11}H_{11}O_6$, 239.0561).

*4.4.3. Grammatophylloside D (**9**).* Amorphous powder; $[\alpha]_D^{26}$ −32.9 (*c* 0.49, MeOH); IR (UATR) ν_{max} 3336, 2919, 1698, 1597, 1456, 1304, 1115, 1170, 1071, 1021 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6): Table 3; negative HR-APCI-TOFMS, *m*/*z*: 363.0835 $[M+Cl]^-$ (calcd for $C_{15}H_{20}Cl^{35}O_8$, 363.0841).

Table 3
^1H and ^{13}C NMR spectroscopic data of compound **9** (measured in DMSO-d_6)

Position	δ_H (300 MHz)	δ_C (75 MHz)
1		136.7
2	6.30 (1H, br s)	108.6
3		158.6
4	6.33 (1H, br s)	101.9
5		158.3
6	6.24 (1H, br s)	110.3
7	3.58 (2H, s)	50.0
8		206.1
9	2.08 (3H, s)	29.4
5-OH	9.45 (1H, br s)	
Glc-1′	4.77 (1H, d, *J*=7.5 Hz)	100.4
2′	3.22–3.28 (1H, m)[a]	73.3
3′	3.12–3.17 (1H, m)[a]	77.0
4′	3.22–3.28 (1H, m)[a]	69.6
5′	3.17–3.22 (1H, m)[a]	76.7
6′	3.47 (1H, dd, *J*=11.5, 5.9 Hz)	60.6
	3.68 (1H, dd, *J*=11.5, 3.4 Hz)	

[a] Chemical shifts was assigned by the results from COSY and HMQC.

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Short communication

Glucosides of phenylpropanoic acid derivatives and coumarins from *Micromelum minutum*

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ABSTRACT

Three new glucosides of phenylpropanoic acid derivatives (micromelumosides A–C) and a new coumarin glucoside (micromelumoside D) were isolated from the aerial portions of *Micromelum minutum* in addition to 15 known compounds, 3,4-dihydro-1,2-secomicrominutinin 9-*O*-β-D-glucopyranside, umbelliferone-7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, scopolin, haploperoside A, (*S*)-peucedanol 7-*O*-β-D-glucopyranoside, (*R*)-peucedanol 7-*O*-β-D-glucopyranoside, decuroside V, 4-hydroxy-2,6-dimethoxyphenyl 4-*O*-β-D-glucopyranoside, vanilloloside, 3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside, (+)-syringaresinol 4-*O*-β-D-glucopyranoside, kaempferol 3-*O*-sophoroside, kaempferol 3-*O*-rutinoside, and citroside B. The structure elucidation of these compounds was based on analyses of spectroscopic data including 1D and 2D NMR.

1. Introduction

Micromelum minutum (G.Forst.) Wight & Arn. (Thai name: Hat-Sa-Kun), a member of the family Rutaceae, is a shrub native in tropical regions of Southeast Asia. This species is well known to contain coumarins, phenylpropanoic acid derivatives, polyoxygenated flavonoids and carbazole alkaloids (Tantivatana et al., 1983; Tantishaiyakul et al., 1986; Sohrab et al., 1999, 2004; Ito et al., 2000; Nakahara et al., 2002; Rahmani et al., 1993, 1994, 2003 Tantishaiyakul et al., 1986; Sohrab et al., 1999, 2004; Ito et al., 2000; Nakahara et al., 2002; Rahmani et al., 1993, 1994, 2003). It is used in Thai traditional medicine for anti-fever purposes. In the course of our continuing studies on Thai medicinal plants, we report the isolation and identification of four new polar compounds, including three glucosides of phenylpropanoic acid derivative (**1**,**2** and **4**) and a coumarin glucoside (**10**) from the water soluble fraction of the aerial parts of this plant in addition to 15 known compounds.

2. Results and discussion

The methanolic extract of the aerial parts of *M. minutum* was partitioned with low polar solvent. The aqueous soluble fraction was separated by combination of chromatographic methods to obtain three new phenylpropanoic acid derivatives (**1**, **2**, and **4**), and a new coumarin glucoside (**10**) (Fig. 1) in addition to 15 known compounds.

Micromelumoside A (**1**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{21}H_{28}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{13}C NMR spectrum revealed the presence of an aromatic ring, two methylenes (δ_C 24.8 and 34.2) and a carboxyl group (δ_C 174.2), corresponded to the phenylpropanoic acid part in addition to a methoxyl group (δ_C 55.7) and a β-D-glucopyranosyl unit (δ_C 100.9, 76.9, 76.7, 73.3, 70.2 and 61.0). The remaining five carbon signals (δ_C 177.8, 78.6, 77.4, 75.1 and 17.9) were assigned to be an oxidized cyclic lactone unit, which was similar to that of hydramicromelins A–D, isolated compounds from *Micromelum integerrimum* (He et al., 2001; Phakhodee et al., 2014). The appearance of two proton at δ_H 6.82 and 7.09 as two singlet signals from the ^1H NMR spectrum, corresponding to the aromatic methine carbons at δ_C 99.8 and 129.5, respectively, suggested that this compound was a 1,2,4,5-tetrasubstituted aromatic ring system. The structure was deduced by the results from 2D-NMR spectroscopic methods. This compound has a phenylpropanoic acid as a core structure. The sugar moiety was located at C-2 (δ_C

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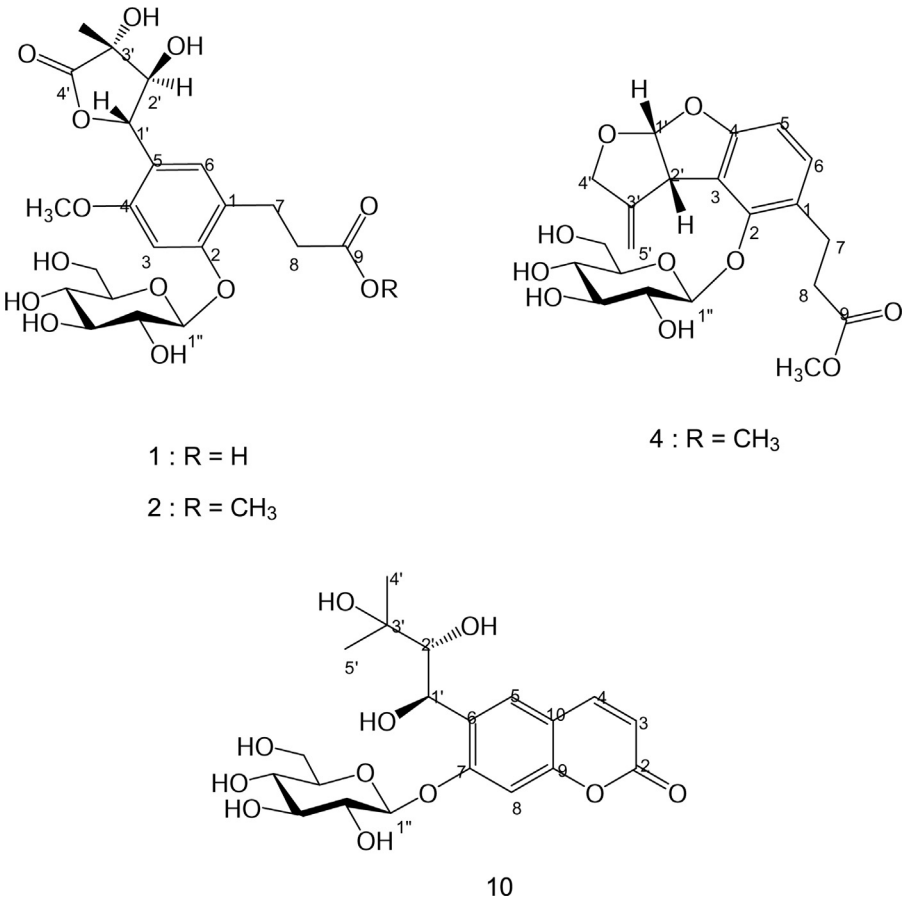

Fig. 1. Structures of compounds **1, 2, 4** and **10**.

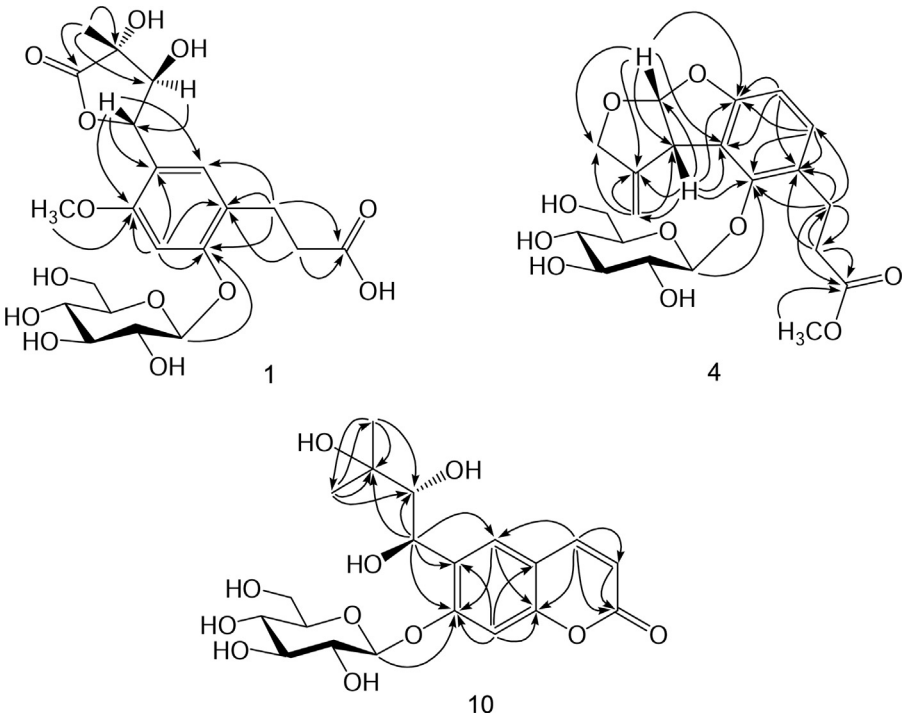

Fig. 2. HMBC correlations of compounds **1, 4** and **10**.

156.7) since the significant HMBC correlations (Fig. 2) were found from H_2-7 (δ_H 2.75, m) to C-1 (δ_C 121.3), C-2 (δ_C 156.7), and from H-1″ (δ_H 4.86, d, J = 7.2 Hz) of the glucopyranosyl moiety to C-2. The methoxyl group was assigned to be linked at C-4 (δ_C 157.3) based on the correlations from its singlet proton signal (δ_H 3.74) to C-4 in the HMBC spectrum, and from the cross peak correlation between the methoxyl group (δ_H 3.74) and H-3 (δ_H 6.82) in the NOESY spectrum. The connection of 5-carbon lactone unit at C-5 (δ_C 117.1) was supported by the HMBC experiment, in which the correlations were observed from H-1′ (δ_H 5.07) to C-4 (δ_C 157.3), C-5 (δ_C 117.1) and C-6 (δ_C 117.1). Thus, this compound was confirmed to have a 1,2,4,5-tertrasubstituted aromatic ring. The relative stereochemistry of a 5-memberred lactone unit was determined by the coupling constant together with the NOESY experiment. Two vicinal protons H-1′ (δ_H 5.07) and H-2′ (δ_H 4.30) showed the coupling constant 8.3 Hz, indicating *trans* configuration at C-1′ and C-2′. The NOESY correlation of H-1′ with H_3-5′ indicated that these protons were located in the same plane. Also the NOESY correlations were found between H-6 and H-1′, and H-6 and H-2′, therefore, the conformation of this compound could be illustrated as shown in Fig. 3 and led to suggest the configurations of C-1′, C-2′, C-3′ positions to be *S*, *S* and *R*, respectively. Besides, these results were correlated with those reports for synthetic hydramicromelin B (Huo et al., 2008). Consequently, this compound was identified as shown.

Micromelumoside B (**2**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{22}H_{30}O_{13}$ by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of **1**, except for the additional signal of one more methoxyl group in both spectra (δ_H 3.56 and δ_C 51.3). This functional group was formed to be the methyl ester of **1** since the chemical shift of the carboxyl group was changed to δ_C 173.1 as compared to **1** (Table 1). The structure was confirmed by the long range correlation from this methoxyl group to the carboxyl group. Therefore, the structure was elucidated as shown.

Micromelumoside C (**4**) was isolated as an amorphous powder. The molecular formula was determined to be $C_{21}H_{26}O_{10}$ by HR-ESI-TOF mass spectrometric analysis. This compound was a phenylpropanoic acid derivative containing a bicyclic phenyl moiety, which was related to part of 3,4-dihydro-1,2-*seco*-microminutinin 9-*O*-β-D-glucopyranside (**3**), isolated compound from *M. falcatum* (Kamperdick et al., 1999). In addition this compound displayed the signal of one methoxyl group (δ_H 3.54 and δ_C 51.3). This group was assigned to form an ester linkage to the carboxyl group (δ_C 173.1). The structure was confirmed by HBMC experiment as shown in Fig. 2. Based on this evidence, compound **4** was identified to be a methyl ester of **3**.

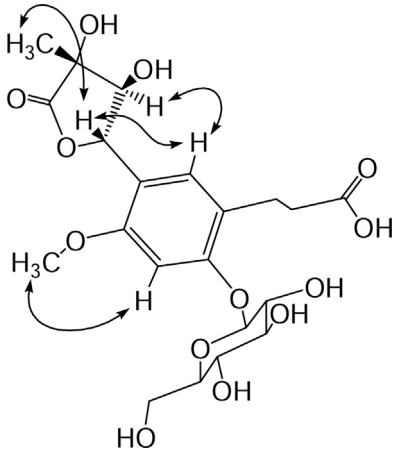

Fig. 3. NOESY correlations of compound **1**.

Micromelumoside D (**10**) was obtained as an amorphous powder. The molecular formula was identified to be $C_{20}H_{26}O_{11}$ by its HR-ESI-TOF mass spectrometric analysis. The NMR spectroscopic data of compound **10** was related to those of (*S*)-peucedanol 7-*O*-β-D-glucopyranoside (**8**) and (*R*)-peucedanol 7-*O*-β-D-glucopyranoside (**9**) (Table 2). This compound has one oxygen atom more than both compounds, indicating the presence of one more hydroxyl group. This additional group was assigned to be located at C-1′ due to the downfield shift of this carbon atom to δ_C 68.0. The structure was confirmed by HMBC experiment, in which the correlation was found from H-1′ (δ_H 5.07) to C-5 (δ_C 127.9), C-6 (δ_C 130.8), C-7 (δ_C 158.7), C-2′ (δ_C 78.0) and C-3′ (δ_C 73.4), as shown in Fig. 2. The absolute configuration at C-2′ was assigned to be *R* due to the chemical shifts of two methyls C-4′ and C-5′ were similar to those of **9**, rather than *S* as shown in **8** (Table 2). The appearance of the coupling constant of two protons H-1′ and H-2′ with J = 8.5 Hz in the ^{1}H NMR spectrum was in agreement with the reported data for *R*-configuration of C-1′ position (Lui et al., 1995a, b; Kozawa et al., 1982). Therefore, compound **10** was identified to be (1′*R*, 2′*R*)-1′-hydroxy-peucedanol 7-*O*-β-D-glucopyranoside.

The known compounds were identified to be 3,4-dihydro-1,2-*seco*-microminutinin 9-*O*-β-D-glucopyranside (**3**) (Kamperdick et al., 1999), umbelliferone-7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**5**) (Xu et al., 2012), scopolin (**6**) (Tsukamoto et al., 1985), haloperoside (**7**) (Yuldashev et al., 1980), (*S*)-peucedanol 7-*O*-β-D-glucopyranoside (**8**) (Kitajima et al., 1998b), (*R*)-peucedanol 7-*O*-β-D-glucopyranoside (**9**) (Ikeshiro et al., 1994), decuroside V (**11**) (Asahara et al., 1984), 4-hydroxy-2,6-dimethoxyphenyl 4-*O*-β-D-glucopyranoside (**12**) (Otsuka et al., 1989), vanilloloside (**13**) (Ida et al., 1994), 3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (**14**) (Kitajima et al., 1998a), (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**15**) (Achenbach et al., 1992), (+)-syringaresinol 4-*O*-β-D-glucopyranoside (**16**) (Kobayashi et al., 1985), kaempferol 3-*O*-sophoroside (**17**), kaempferol 3-*O*-rutinoside (**18**) (Agrawal and Bansal, 1989), and citroside B (**19**) (Umehara et al., 1988). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

The present work isolated 19 secondary metabolites from the water soluble fraction including phenylpropanoic acid derivatives (**1**–**4**), coumarin glycosides (**5**–**11**), simple phenolic glucosides (**12**–**14**), lignan glucosides (**15**, **16**), flavonol glycosides (**17**, **18**) and a megastigmane glucoside (**19**) from *M. minutum*. Phenylpropanoic acid derivatives and coumarins were expected to isolate from this plant since these compound-types were considered as chemotaxonomic markers on this genus. The results from this work provided more information data of the typical profile of the polar secondary metabolites isolated from *Micromelum* genus.

3. Experimental

3.1. General experimental procedures

1D and 2D NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. The MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. UV spectra were recorded on a Shimadzu UV-1700 spectrophotometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 × 250 mm i.d., Vertisep™ AQS) with a Jasco UV-970 detector at 220 nm. The flow rates were 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O–EtOH (1:1, v/v).

Table 1
NMR spectroscopic data of compounds **1**–**4** (in DMSO-d_6).

Position	1		2		4		3
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_C
Aglycone							
1		121.3		120.9		126.7	127.0
2		156.7		156.8		152.3	152.2
3	2.46 (2H, m)	99.8	2.56 (2H, m)	99.8	2.52 (2H, m)	122.0	121.9
4	2.75 (2H, m)	157.3	2.79 (2H, m)	157.5	2.98 (2H, m)	157.7	157.5
5	7.09 (1H, s)	117.1	7.08 (1H, s)	117.2	6.98 (1H, d, J = 8.2 Hz)	105.9	105.7
6		129.5		129.7	6.53 (1H, d, J = 8.2 Hz)	130.1	129.9
7		24.8		24.8		23.8	23.7
8	6.82 (1H, s)	34.2	6.85 (1H, s)	33.3		34.4	34.8
9		174.2		173.1		173.1	174.2
1′	5.07 (1H, d, J = 8.3 Hz)	77.4	5.07 (1H, d, J = 8.1 Hz)	77.4	6.28 (1H, d, J = 5.8 Hz)	111.9	111.9
2′	4.30 (1H, d, J = 8.3 Hz)	78.6	4.31 (1H, d, J = 8.1 Hz)	78.5	5.01 (1H, br d, J = 5.0 Hz)	49.8	49.8
3		75.1		75.2		146.4	146.4
4′		177.8		177.8	4.16 (1H, dd, J = 12.5, 1.5 Hz) 4.34 (1H, d, J = 12.5 Hz)	70.0	70.0
5′	1.23 (3H, s)	17.9	1.25 (3H, s)	17.9	5.04 (1H, br s) 5.55 (1H, br s)	108.3	108.3
CH_3O—	3.74 (3H, s)	55.7	3.76 (3H, s)	55.7			
—$COOCH_3$			3.56 (3H, s)	51.3	3.54 (3H, s)	51.3	
Glc							
1”	4.86 (1H, d, J = 7.2 Hz)	100.9	4.85 (1H, d, J = 7.4 Hz)	101.1	4.51 (1H, d, J = 7.3 Hz)	105.8	105.8
2”	3.27 (1H)[a]	73.3	3.28 (1H)[a]	73.4	3.23 (1H)[a]	74.2	74.2
3”	3.36 (1H)[a]	76.9	3.37 (1H)[a]	76.9	3.21 (1H)[a]	76.5	76.5
4”	3.11 (1H)[a]	70.2	3.12 (1H)[a]	70.2	3.14 (1H)[a]	70.3	70.2
5”	3.27 (1H)[a]	76.7	3.26 (1H)[a]	76.9	3.23 (1H)[a]	77.0	77.0
6”	3.41 (1H)[a] 3.71 (1H)[a]	61.0	3.41 (1H)[a] 3.71 (1H)[a]	61.0	3.45 (1H)[a] 3.72 (1H, br d, J = 11.1 Hz)	61.4	61.4

[a] Chemical shifts were assigned by COSY and HMQC.

Table 2
NMR spectroscopic data of compounds **8-10** (in DMSO-d_6)

Position	10		8	9
	δ_H	δ_C	δ_C	δ_C
Aglycone				
2		160.5	160.6	160.9
3	6.30 (1H, d, J = 9.5 Hz)	113.1	113.1	112.9
4	8.02 (1H, d, J = 9.5 Hz)	144.7	144.5	144.5
5	7.72 (1H, s)	127.9	130.0	130.0
6		130.8	127.1	127.2
7		158.7	158.7	158.3
8	7.06 (1H, s)	102.4	102.1	102.0
9		153.9	153.6	153.6
10		113.1	112.9	112.6
1′	5.07 (1H, d, J = 8.5 Hz)	68.0	31.5	31.9
2′	3.43 (1H)	78.0	77.6	76.8
3′		73.4	72.1	72.4
4′	1.19 (3H, s)	24.4	25.0	23.2
5′	1.13 (3H, s)	27.7	26.0	27.3
Glc				
1”	4.88 (1H, d, J = 7.2 Hz)	101.4	101.0	100.8
2”	3.30 (1H)[a]	73.3	73.5	73.4
3”	3.28 (1H)[a]	76.5	76.5	76.1
4”	3.17 (1H)[a]	69.8	69.8	69.8
5”	3.32 (1H)[a]	77.3	77.3	77.2
6”	3.41 (1H)[a] 3.73 (1H)[a]	60.8	60.9	60.8

[a] Chemical shifts were assigned by COSY and HMQC.

3.2. Plant material

The aerial portions (leaves and stems) of *M. minutum* (G.Forst.) Wight & Arn. were collected on November 2011 from Baan Muang Waan, Khon Kaen, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0074) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3. Extraction and isolation

The aerial portions (2.0 kg) were extracted with MeOH at room temperature (each 10 L for 48 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (337.0 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 5 times). The aqueous soluble fraction (197.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (80.5 g) was subjected to silica gel cc using solvent systems EtOAc–MeOH (9:1, 4.0 L), EtOAc–MeOH–H_2O (40:10:1, 12.0 L), EtOAc–MeOH–H_2O (70:30:3, 8.0 L) and EtOAc–MeOH–H_2O (6:4:1, 10.0 L), respectively to obtain eight fractions (A–H). Fraction D (10.0 g) was applied to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide 15 sub-fractions. Sub-fraction D-2 was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (93:7, v/v) to afford compounds **10** (12.0 mg), **12** (15.4 mg), **13** (8.0 mg) and **14** (5.2 mg). Sub-fraction D-4 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to provide compounds **5** (144.1 mg) and **6** (21.2 mg). Sub-fraction D-5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (83:17, v/v) to give compounds **8** (830.5 mg) and **19** (140.4 mg). Sub-fraction D-6 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compounds **9** (18.2 mg), **11** (68.6 mg) and **15** (41.9 mg). Sub-fraction D-8 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (80:20, v/v) to yield compounds **16** (14.6 mg) and **17** (32.6 mg). Sub-fraction D-11 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (80:20, v/v) to provide compound **18** (176.6 mg). Fraction E (10.8 g) was applied to a RP-18 column using

solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to afford nine sub-fractions. Sub-fraction E-2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (83:17, v/v) to provide compound **2** (5.7 mg). Sub-fraction E-6 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to give compounds **3** (192.9 mg) and **4** (43.7 mg). Finally, fraction F (10.3 g) was similarly subjected to a RP-18 column using, H_2O–MeOH (90:10 → 20:80, v/v) to provide ten sub-fractions. Sub-faction F-3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to give compound **1** (142.4 mg). Sub-fraction F-5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to afford compound **7** (22.6 mg).

*3.3.1. Micromelumoside A (**1**)*

Amorphous powder; $[\alpha]_D^{25}$ – 40.4 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m*/*z*: 487.1462 $[M-H]^-$ (calcd for $C_{21}H_{27}O_{13}$, 487.1457).

*3.3.2. Micromelumoside B (**2**)*

Amorphous powder; $[\alpha]_D^{25}$ – 0.2 (MeOH, *c* 0.40); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m*/*z*: 537.1373 $[M+Cl]^-$ (calcd for $C_{22}H_{30}Cl_1O_{13}$, 537.1380).

*3.3.3. Micromelumoside C (**4**)*

Amorphous powder; $[\alpha]_D^{25}$ + 72.8 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 1; Negative HRESITOFMS, *m*/*z*: 467.1220 $[M+Cl]^-$ (calcd for $C_{21}H_{26}Cl_1O_{10}$, 473.1218).

*3.3.4. Micromelumoside D (**10**)*

Amorphous powder; $[\alpha]_D^{25}$ – 55.1 (MeOH, *c* 1.00); ^1H and ^{13}C NMR (DMSO-d_6): Table 2; Negative HRESITOFMS, *m*/*z*: 477.1170 $[M+Cl]^-$ (calcd for $C_{20}H_{26}Cl_1O_{11}$, 477.1169).

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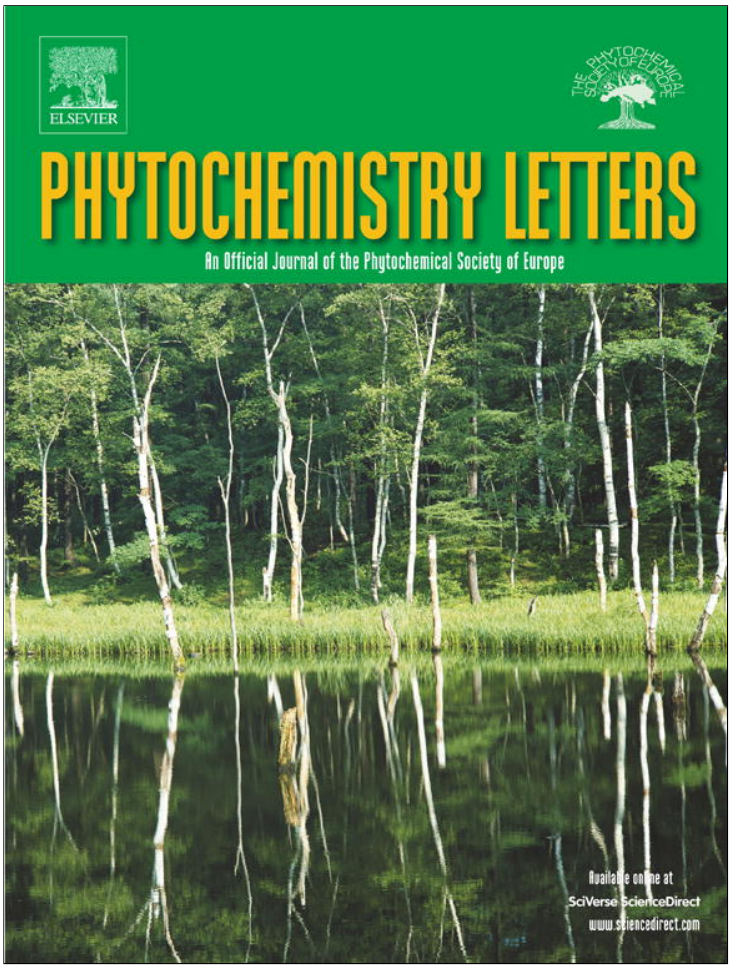

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New *trans*- and *cis*-*p*-coumaroyl flavonol tetraglycosides from the leaves of *Mitragyna rotundifolia*

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ABSTRACT

Two new flavonol tetraglycosides, quercetin 3-*O*-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (krathummuoside A) and quercetin 3-*O*-(4-*O*-*cis*-*p*-coumaroyl)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (krathummuoside B) were isolated from the leaves of *Mitragyna rotundifolia* in addition to eight known compounds, quercetin 3-*O*-α-L-rhamnopuranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, rutin, (−)-*epi*-catechin, 3,4,5-trimethoxyphenyl β-D-glucopyranoside, (6*S*, 9*R*)-roseoside, 3-*O*-β-D-glucopyranosyl quinovic acid 28-*O*-β-D-glucopyranosyl ester, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside, and (+)-syringaresinol-4-*O*-β-D-glucopyranoside. The structure elucidation of these compounds was based on analyses of spectroscopic data including 1D- and 2D-NMR.

1. Introduction

Mitragyna rotundifolia (Roxb.) Kuntze belongs to the family Rubiaceae (Thai name: Kra-Thum-Mu). This species is commonly found in the dipterocarp forests in North-eastern Thailand. The leaves have been used in Thai traditional medicine for the treatment of diarrhea. Previous phytochemical investigation of this plant reported the isolation of several compounds such as alkaloids, triterpenoids, flavonoids and phenolics (Shellard and Phillipson, 1964; Shellard et al., 1971; Houghton and Shellard, 1974; Kang and Hao, 2006; Kang and Li, 2009; Kang et al., 2006, 2007, 2010). In pharmacological studies, the antioxidant phenolic compounds of this plant and hepatoprotective effect from plant extracts have been reported (Kang et al., 2010; Gong et al., 2012). This present work deals with the isolation and structure elucidation of ten polar compounds from the water soluble fraction of leaves including two new flavonol tetraglycosides and eight known compounds.

2. Results and discussion

The methanolic extract of the leaves of *M. rotundifolia* was partitioned with Et_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to obtain two new flavonol glycosides, krathummuoside A (**1**) and krathummuoside B (**2**) (Fig. 1) in addition to eight known compounds. The known compounds were elucidated as quercetin 3-*O*-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**3**) (Kite et al., 2007), rutin (**4**), (−)-*epi*-catechin (**5**), 3,4,5-trimethoxyphenyl β-D-glucopyranoside (**6**) (Kanchanapoom, 2007), (6*S*, 9*R*)-roseoside (**7**) (Yamano and Ito, 2005), 3-*O*-β-D-glucopyranosyl quinovic acid 28-*O*-β-D-glucopyranosyl ester (**8**) (Zhao et al., 1995), (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**9**) (Achenbach et al., 1992), (+)-syringaresinol-4-*O*-β-D-glucopyranoside (**10**) (Kobayashi et al., 1985). All known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

Krathummuoside A (**1**), $[\alpha]_D^{30}$ −135.1, was isolated as a yellow amorphous powder. Its molecular formula was determined to be $C_{48}H_{56}O_{26}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^1H NMR spectrum revealed the presence of a pair of *meta*-coupled aromatic protons at δ_H 6.44 (br s) and 6.65 (br s) and a set of ABX aromatic ring system at

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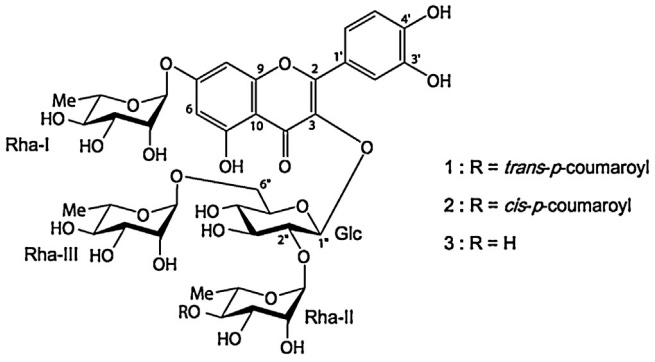

Fig. 1. Flavonol tetraglycosides **1**–**3**.

δ_H 6.89 and 7.62 (each br d, J = 8.4 Hz), and 7.76 (br s) for the aglycone part, four anomeric protons of sugar moieties at δ_H 4.51 (br s), 5.30 (br s), 5.55 (br s), and 5.59 (d, J = 7.3 Hz) in addition to a set of AA′BB′ aromatic ring at δ_H 6.71 and 7.26 (each 2H, d, J = 8.3 Hz) and two *trans* olefin protons at 6.22 and 7.46 (each d, J = 16.0 Hz) assignable for *trans*-*p*-coumaroyl moiety. Analysis of 1D- and 2D-NMR spectroscopic data indicated that this compound is an acylated compound of quercetin 3-*O*-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**3**). The significant difference from **3** was the appearance of an additional set of signals arising from a *trans*-*p*-coumaroyl moiety. This acylated unit was assigned to be connected at C-4 of 2″-*O*-Rha-II since H-4 proton of this position was appeared downfield at δ_H 5.03 along with the downfield shift of this carbon atom, C-4 (δ_C 75.5) and upfield shifts of C-3 (δ_C 70.3) and C-5 (δ_C 67.8) by comparing the chemical shifts with those of compound **3** (see Table 2). Besides the long-range connectivity from HMBC spectrum (Fig. 2) provided the further confirmation of the structure. In addition, acid hydrolysis yielded D-glucose and L-rhamnose, identify by HPLC analysis using the optical rotation detector. Thus, **1** was identified to be quercetin 3-*O*-(*4*-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside.

Krathummuoside B (**2**), $[\alpha]_D^{30}$ −88.6, was obtained as a yellow amorphous powder. The molecular formula was the same as **1** by its HR-ESI-TOF mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of **1**. The only significant difference was the coupling constants of the olefin protons at δ_H 5.73 and 6.78 with J = 12.8 Hz. Therefore, the structure of compound **2** was determined to be the *cis* isomer of **1**.

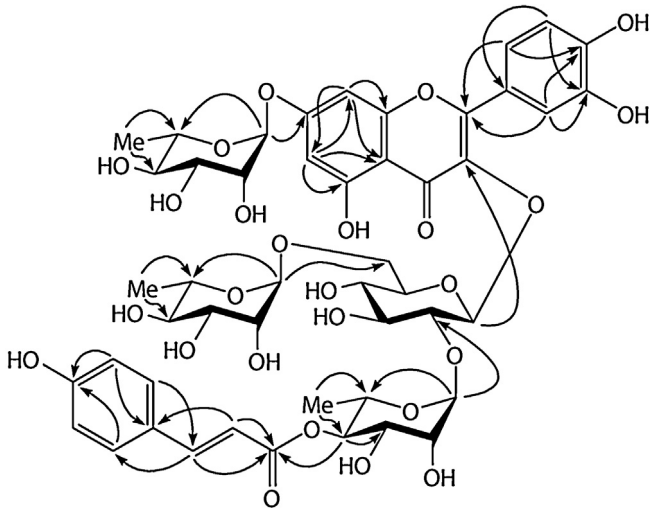

Fig. 2. HMBC correlations of krathummuoside A (**1**).

In conclusion, the present study reported ten water soluble compounds including five flavonoids (**1**–**5**), a phenolic glucoside (**6**), a megastigmane glucoside (**7**), a triterpenoid glucoside (**8**) and two lignan glucosides (**9**, **10**). The result was closely related to those of the previous studies performed on this species. However, the occurrence of two new polar flavonol tetraglycoside (**1**, **2**) bearing *p*-coumaroyl unit in *tran*- and *cis*-forms attached to the sugar part was the first report from this genus. This finding might be useful for further chemotaxonomic studies of this genus.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in MeOH-d_4 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers. The MS data was obtained on a Bruker Micro TOF-LC mass spectrometer. UV spectra were recorded on a Shimadzu UV-1700 spectrophotometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 mm × 250 mm i.d., Vertisep^{TM} AQS) with a Jasco MD-2010 detector at 220 nm. The flow rates were 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O–EtOH (1:1, v/v).

3.2. Plant material

The leaves of *M. rotundifolia* (Roxb.) Kuntze were collected on June 2012 from the Herbal Paradise Garden, Chulabhorn Research Institute, Bangkok, Thailand. Plant specimen was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. Voucher specimens (TK-PSKKU-0070) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3. Extraction and isolation

The collected leaves from The Herbal Paradise Garden (June 2012, 2.8 kg) were extracted with MeOH at room temperature (each 30 L for 48 h, 3 times). The MeOH extract was concentrated *in vacuo* to dryness. The residue (420.4 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (345.2 g) was applied to a Diaion HP-20 column, and eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (139.4 g) was subjected to silica gel cc using solvent systems EtOAc–MeOH (9:1, 20.0 L), EtOAc–MeOH–H_2O (40:10:1, 12.0 L), EtOAc–MeOH–H_2O (70:30:3, 2.0 L) and EtOAc–MeOH–H_2O (6:4:1, 2.0 L), respectively to obtain seven fractions (A to G). Fraction A (7.6 g from 46.5 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction A-3 was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (85:15, v/v) to yield compound **5** (650.3 mg). Fraction B (10.5.0 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide seven sub-fractions. Sub-fraction B-2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compounds **6** (110.3 mg) and **7** (242.7 mg). Fraction C (6.3 g) was applied to a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to afford twelve sub-fractions. Sub-fraction C-9 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (70:30, v/v) to obtain

Table 1
^{1}H NMR (400 MHz, MeOH-d_4) spectroscopic data of krathummuosides A (**1**) and B (**2**).

Position	1	2
Aglycone		
6	6.44 (1H, br s)	6.47 (1H, d, *J* = 1.8 Hz)
8	6.65 (1H, br s)	6.71 (1H, d, *J* = 1.8 Hz)
2′	7.76 (1H, br s)	7.63 (1H, br s)
5′	6.89 (1H, d, *J* = 8.4 Hz)	6.88 (1H, d, *J* = 8.4 Hz)
6′	7.62 (1H, br d, *J* = 8.4 Hz)	7.63 (1H, br d, *J* = 8.4 Hz)
Glc		
1	5.59 (1H, d, *J* = 7.3 Hz)	5.58 (1H, d, *J* = 7.3 Hz)
2	3.72 (1H, dd, *J* = 8.4, 8.0 Hz)	3.67 (1H, dd, *J* = 9.0, 8.6 Hz)
3	3.66 (1H)[a]	3.58 (1H)[a]
4	3.85 (1H)[a]	3.82 (1H)[a]
5	3.35 (1H, m)	3.35 (1H, m)
6	4.19 (1H, dd, *J* = 12.0, 2.1 Hz)	4.14 (1H, dd, *J* = 10.0, 1.8 Hz)
	3.44 (1H)[a]	3.38 (1H)[a]
Rha-I		
1	5.55 (1H, br s)	5.55 (1H, br s)
2	4.06 (1H, br s)	4.02 (1H, br s)
3	3.85 (1H)[a]	3.83 (1H)[a]
4	3.36 (1H, dd, *J* = 9.4, 7.6 Hz)	3.44 (1H)[a]
5	3.62 (1H, m)	3.66 (1H, m)
6	1.22 (3H, d, *J* = 5.9 Hz)	1.27 (3H, d, *J* = 6.0 Hz)
Rha-II		
1	5.30 (1H, brs)	5.27 (1H, brs)
2	4.14 (1H, br s)	4.08 (1H)[a]
3	3.50 (1H)[a]	3.45 (1H)[a]
4	5.03 (1H, dd, *J* = 9.6, 9.4 Hz)	4.98 (1H, dd, *J* = 10.0, 9.8 Hz)
5	4.45 (1H, m)	4.30 (1H, m)
6	0.94 (3H, d, *J* = 6.1 Hz)	0.90 (3H, d, *J* = 6.0 Hz)
Rha-III		
1	4.51 (1H, br s)	4.50 (1H, br s)
2	3.67 (1H, br s)	3.60 (1H, br s)
3	3.52 (1H)[a]	3.48 (1H)[a]
4	3.25 (1H, dd, *J* = 9.4, 9.4 Hz)	3.22 (1H, dd, *J* = 9.6, 9.4 Hz)
5	3.48 (1H, m)	3.44 (1H, m)
6	1.06 (3H, d, *J* = 6.0 Hz)	1.07 (3H, d, *J* = 6.1 Hz)
Coumaroyl		
2, 6	7.26 (2H, d, *J* = 8.3 Hz)	7.62 (2H, d, *J* = 8.7 Hz)
3, 5	6.71 (2H, d, *J* = 8.3 Hz)	6.68 (2H, d, *J* = 8.7 Hz)
7	7.46 (1H, d, *J* = 16.0 Hz)	6.78 (1H, d, *J* = 12.8 Hz)
8	6.22 (1H, d, *J* = 16.0 Hz)	5.73 (1H, d, *J* = 12.8 Hz)

[a] Chemical shifts were assigned by HMQC.

compound **8** (332.4 mg). Fraction D (6.2 g) was similarly subjected to a RP-18 column using, H_2O-MeOH (90:10 → 20:80, v/v) to afford eight sub-fractions. Sub-faction D-3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (80:20, v/v) to provide compounds **4** (37.7 mg) and **10** (20.0 mg). Fraction E (10.0 g) was applied to a RP-18 column using, H_2O–MeOH (90:10 → 20:80, v/v) to afford ten sub-fractions. Sub-faction E-3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compound **9** (28.4 mg). Finally, fraction F (6.9 g from 40.32 g) was similarly subjected to a RP-18 column using, H_2O–MeOH (90:10 → 20:80, v/v) to provide ten sub-fractions. Sub-faction F-2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to give compound **3** (35.3 mg). Sub-fraction F-5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (78:22, v/v) to afford compounds **1** (76.9 mg) and **2** (34.7 mg).

3.3.1. Krathummuoside A (*1*)

Yellow amorphous powder; $[\alpha]_D^{30}$ −135.1 (MeOH, *c* 1.35); UV λ_{max} (EtOH, *c* 1.34 × 10^{-5} M) 314.4, 266.2, 257.4 nm; IR spectrum: ν_{max} = 3367, 2931, 1643, 1602, 1268, 1167, 1059, 1024 cm^{-1}; ^{1}H NMR (MeOH-d_4): Table 1; ^{13}C NMR (MeOH-d_4): Table 2; Negative HRESITOFMS, *m/z*: 1047.2962 $[M-H]^-$ (calcd. for $C_{48}H_{55}O_{26}$, 1047.2987).

Table 2
^{13}C NMR (100 MHz, MeOH-d_4) spectroscopic data of krathummuosides A (**1**), B (**2**) and **3**.

Position	1	2	3
Aglycone			
2	159.5	159.6	159.4
3	134.8	134.8	134.7
4	179.1	179.3	179.3
5	162.6	162.9	162.8
6	100.3	100.4	100.4
7	163.2	163.4	163.3
8	95.8	95.7	95.6
9	157.7	158.0	157.9
10	107.3	107.5	107.5
1′	123.1	123.2	123.2
2′	117.6	117.5	117.4
3′	145.7	146.0	145.9
4′	149.7	149.8	149.7
5′	116.1	116.1	116.1
6′	123.5	123.6	123.6
Glc			
1	100.9	100.7	100.4
2	79.9	80.2	80.0
3	78.6	78.8	78.8
4	71.5	71.8	71.7
5	76.7	77.0	77.7
6	68.3	68.3	68.2
Rha-I			
1	99.7	99.9	99.8
2	71.9	72.1	71.9
3	71.9	72.1	72.0
4	73.5	73.6	73.6
5	71.1	71.2	71.2
6	18.0	18.1	18.1
Rha-II			
1	102.3	102.5	102.6
2	71.5	71.7	72.2
3	70.3	70.4	72.3
4	75.5	75.2	74.0
5	67.8	67.9	69.9
6	17.3	17.4	17.5
Rha-III			
1	102.1	102.2	102.1
2	72.1	72.2	72.0
3	72.3	72.5	72.2
4	73.7	73.8	73.8
5	69.6	69.7	69.6
6	17.8	17.8	17.8
Coumaroyl			
1	127.0	127.5	
2, 6	131.0	133.7	
3, 5	116.7	115.7	
4	160.9	160.0	
7	146.5	145.2	
8	115.2	116.1	
9	169.0	168.0	

3.3.2. Krathummuoside B (*2*)

Yellow amorphous powder; $[\alpha]_D^{30}$ −88.6 (MeOH, *c* 1.37); UV λ_{max} (EtOH, *c* 1.01 × 10^{-5} M) 315.0, 257.5 nm; IR spectrum: ν_{max} = 3468, 2916, 1709, 1610, 1280, 1149, 1050, 1022 cm^{-1}; ^{1}H NMR (MeOH-d_4): Table 1; ^{13}C NMR (MeOH-d_4): Table 2; Negative HRESITOFMS, *m/z*: 1047.3017 $[M-H]^-$ (calcd. for $C_{48}H_{55}O_{26}$, 1047.2987).

3.3.3. Determination of the absolute configurations of sugars

Monosaccharide subunits of krathummuosides A (**1**) and B (**2**) were obtained by acid hydrolysis. Each compound (*ca* 3 mg) was dissolved in 2 N HCl-dioxane (6:1, 3.5 ml) and heated at 80° for 5 h. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous layer was concentrated to dryness

affording the sugar fraction. Each of these was dissolved with H_2O (1 ml), analyzed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep^{TM} sugar LMP, 7.8 mm × 300 mm i.d.; mobile phase: H_2O; flow rate 0.4 ml/min; temperature: 80 °C) and comparison with their retention times and optical rotations with authentic samples. Both samples gave peaks corresponding to D-glucose at 19.3 min and L-rhamnose 23.1 min with positive optical rotations.

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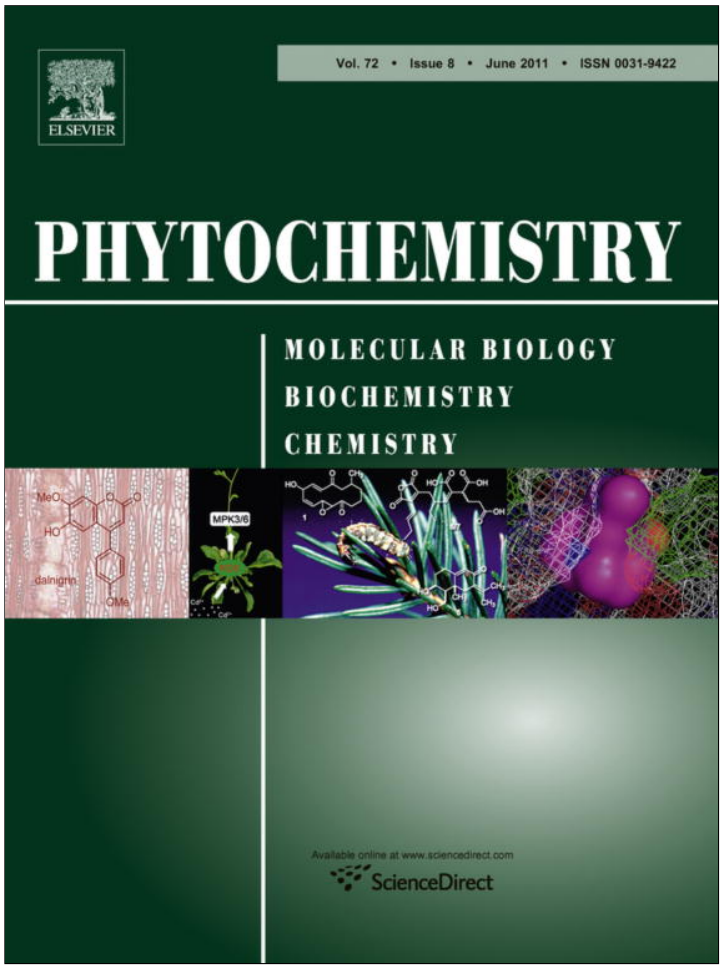

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Unusual glycosides of pyrrole alkaloid and 4′-hydroxyphenylethanamide from leaves of *Moringa oleifera*

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ABSTRACT

Glycosides of pyrrole alkaloid (pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside) and 4′-hydroxyphenylethanamide (marumosides A and B) were isolated from leaves of *Moringa oleifera* along with eight known compounds; niazirin, methyl 4-(α-L-rhamnopyranosyloxy)benzylcarbamate, benzyl β-D-glucopyranoside, benzyl β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside, kaempferol 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside, adenosine and L-tryptophan. Structure elucidations were based on analyses of chemical and spectroscopic data including 1D- and 2D-NMR.

1. Introduction

Moringa oleifera Lam. (Moringaceae; Thai name: Ma-Rum) is an ornamental plant native to tropical and subtropical areas, and commonly cultivated in all parts of Thailand as a vegetable for cooking purposes. The leaves and pods are used in Thai traditional medicine to decrease blood pressure, as well as for antipyretic agents and antidote for detoxification of poisons. This plant is known to have an excellent nutritional value, as well as various medicinal usages for treatment of different ailments in traditional medical systems (Fahey, 2005). The phytochemical compositions, medicinal use, pharmacological properties and pharmaceutical applications have been reviewed by Anwar et al. (2007) and Aney et al. (2009). This plant has been extensively studied for its constituents and biological activities such as antitumor, anti-inflammatory, antimicrobial, antioxidant and antihypertensive activities, and is well known to contain carbamate and thiocarbamate glycosides (Eilert et al., 1981; Faizi et al., 1992, 1994a,b, 1995; Guevara et al., 1999; Francis et al., 2004; Nikkon et al., 2009; Sreelatha and Padma, 2009; Cheenpracha et al., 2010; Oluduro et al., 2010). However, there is no report on the constituents from the plant source of Thai origin. In Thailand, this plant is quite popular as a daily food supplement. Ma-Rum products have been sold in various forms such as leaf powder in capsules, spray-dried plant extracts in capsules, or herbal tea from leaves or seeds. This paper describes the isolation and identification of 11 polar compounds, including nitrogen-containing phenolic glycosides (**1**–**5**) (see Fig. 1), benzyl glycosides (**6**, **7**), flavonol glycosides (**8**, **9**), a nucleoside (**10**) and an amino acid (**11**) from leaves of this plant. The usage of this plant as a food supplement is also discussed.

2. Results and discussion

The methanolic extract of the leaves of *M. oleifera* was suspended in H_2O and partitioned with Et_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to afford 11 compounds. Seven compounds were identified as niazirin (4-(α-L-rhamnopyranosyloxy)phenylacetonitrile, **1**) (Faizi et al., 1994a,b), benzyl β-D-glucopyranoside (**6**) (Kanchanapoom et al., 2001a), benzyl β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**7**), kaempferol 3-*O*-β-D-glucopyranoside (**8**) (Noiarsa et al., 2007), quercetin 3-*O*-β-D-glucopyranoside (**9**), adenosine (**10**) (Kanchanapoom et al., 2001b) and L-tryptophan (**11**)

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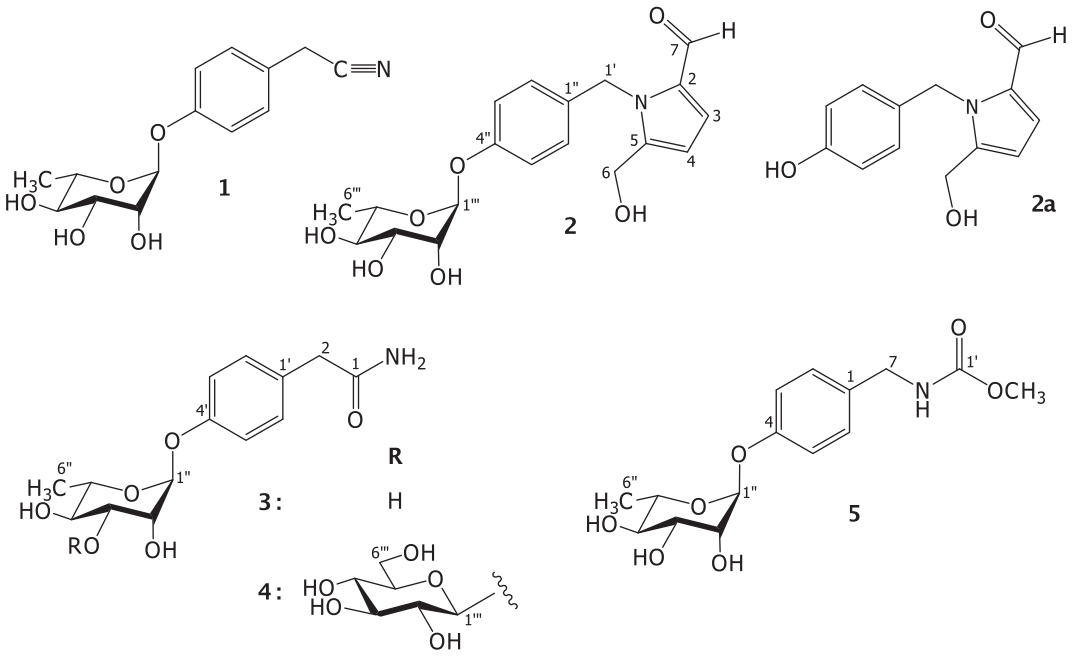

Fig. 1. Structures of isolated compounds **1**–**5**.

(Kanchanapoom et al., 2007) by comparison of physical data with literature values and from spectroscopic evidence.

Compound **2** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{23}NO_7$ by high-resolution atmospheric pressure chemical ionization time-of-flight mass spectrometric analysis (HR-APCI-TOFMS). The IR spectrum displayed the strong absorption bands of hydroxyl groups at 3361 cm^{-1} and a carbonyl group at 1646 cm^{-1}. Analysis of the ^1H NMR spectrum indicated the presence of an one sugar unit, suggested to be α-L-rhamnose from the chemical shifts of its anomeric proton at δ_H 5.32 (1H, *br s*) and a secondary methyl group at δ_H 1.08 (3H, *d*, *J* = 6.2 Hz). Two doublet protons at δ_H 7.04 and 6.28, which appeared as an AB system (*d*, *J* = 3.9 Hz) were characteristic of a nitrogen-containing heterocyclic ring, consistent with a 2,5-disubstituted pyrrole ring (Chin et al., 2003). The chemical shift at δ_H 9.45 (1H, *s*) was assigned for an aldehyde group. The remaining resonances were the singlet signal of four equivalent protons at δ_H 6.95 and the signals of two methylene groups at δ_H 5.56 (2H, *s*) and 4.42 (2H, *s*). Analysis of the ^{13}C-NMR spectroscopic data showed that the sugar unit was an α-L-rhamnopyranosyl moiety from the set of the chemical shifts at δ_C 98.9, 70.8, 70.6, 72.2, 69.9 and 18.3. In addition, the resonances of 1,4-disubstituted aromatic ring were observed from the chemical shifts of one oxy-aryl carbon at δ_C 155.6, four aryl methines at δ_C 116.9, 127.9 (each 2C), and one non-protonated carbon at δ_C 131.8. The ^{13}C NMR spectrum also showed signals of an aldehyde group at δ_C 179.9, two methylenes at δ_C 47.5 and 55.4, one of which was oxygenated (δ_C 55.4). The chemical shifts of two methines at δ_C 110.3 and 124.6 together with two quaternary carbons at δ_C 132.1 and 114.3 belonged to the 2,5-disubstituted pyrrole ring (Chin et al., 2003). The complete structure of compound **2** was deduced by the results from HMQC and HMBC spectroscopic methods. The aldehyde group at δ_H 9.45 (δ_C 179.9) and the methylene group at δ_H 4.42 (δ_C 55.4) were attached to C-2 and C-5, respectively, of the pyrrole ring, based on the HMBC data (Fig. 2). The downfield singlet methylene protons at δ_H 5.56, corresponding to the methylene carbon at δ_C 47.5, showed the HMBC correlations to C-2 (δ_C 132.1) and C-5 (δ_C 144.3) of the pyrrole ring and C-1″ (δ_C 131.8) and C-2″, 6″ (δ_C 127.9) of the aromatic ring, indicating that this carbon was located

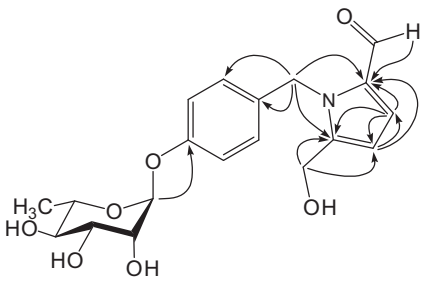

Fig. 2. HMBC correlations of compound **2**.

between these two ring systems. The sugar moiety was attached to C-4″ (δ_C 155.6) of the aromatic ring by the HMBC correlation from H-1‴ (δ_H 5.32) to C-4″. Therefore, the structure of compound **2** was elucidated as shown. However, it is quite unusual that the ^1H NMR spectrum showed the equivalent of four protons of the aromatic ring system instead of the AA′BB′ aromatic ring system. This compound was hydrolyzed by HCl to yield a new aglycone **2a**, namely pyrrolemarumine. From the ^1H NMR spectrum of **2a**, the splitting patterns of protons for the aromatic ring were in agreement with those of 1,4-disubstituted aromatic ring (Table 1), providing confirmation of the structure of compound **2**. Consequently, compound **2** was pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside.

Compound **3** was obtained as an amorphous powder and determined to be $C_{14}H_{19}NO_6$ by high resolution electrospray time-of-flight mass spectrometric analysis (HR-ESI-TOFMS). Its IR spectrum exhibited characteristic absorption bands at 3351 and 2933 cm^{-1} for hydroxyl groups and a primary amide, respectively in addition to the band at 1667 cm^{-1} for the carbonyl group of an amide (Pretsch et al., 2009). The ^1H NMR spectral data indicated the presence of an 1,4-disubstituted aromatic ring from the chemical shifts at δ_H 7.17 and 6.94 (each 2H, *d*, *J* = 8.2 Hz), two protons of an amide group at δ_H 7.46, 6.86 (each 1H, *br s*), two methylene protons at δ_H 3.30 (2H, *s*) along with the signals α-L-rhamnopyranosyl moiety. The ^{13}C NMR spectrum showed signals of an 1,4-disubstituted aromatic ring and α-L-rhamnopyranosyl

Table 1
NMR spectroscopic data of compounds **2** and **2a** (DMSO-d_6).

Position	2		2a	
	Carbon	Proton	Carbon	Proton
Aglycone				
2	132.1		132.1	
3	124.6	7.04 (1H, d, J = 3.9 Hz)	124.0	7.02 (1H, d, J = 4.0 Hz)
4	110.3	6.28 (1H, d, J = 3.9 Hz)	110.2	6.25 (1H, d, J = 4.0 Hz)
5	144.3		144.3	
6	55.4	4.42 (2H, s)	55.4	4.41 (2H, s)
7	179.9	9.45 (1H, s)	179.8	9.47 (1H, s)
1′	47.5	5.56 (2H, s)	47.5	5.50 (2H, s)
1″	131.8		128.6	
2″,6″	127.9	6.95 (4H, s)	128.0	6.83 (2H, d, J = 8.5 Hz)
3″,5″	116.9		115.6	6.67 (2H, d, J = 8.5 Hz)
4″	155.6		156.9	
Sugar moiety				
1‴	98.9	5.32 (1H, br s)		
2‴	70.8	3.80 (1H, br s)		
3‴	70.6	3.63 (1H, br d, J = 9.0 Hz)		
4‴	72.2	3.28 (1H, dd, J = 9.0, 8.9 Hz)		
5‴	69.9	3.42 (1H, m)		
6‴	18.3	1.08 (3H, d, J = 6.2 Hz)		

unit, closely related to those of compounds **1** and **2**. The resonance at δ_C 172.8 was assigned as a carbonyl carbon of the amide group. The chemical shift at δ_C 41.5 was identified to the intermediate methylene carbon between the aromatic ring and the amide group. The structure of compound **3** was supported by the HMBC correlations between (i) NH_2 (δ_H 7.46 and 6.86) and C-2 (δ_C 41.5); (ii) H_2-2 (δ_H 3.30) and C-1′ (δ_C 129.9), C-2′, 6′ (δ_C 130.2); and (iii) H-1″ (δ_H 5.32) and C-4′ (δ_C 154.8). Therefore, compound **3** was elucidated as 4′-hydroxyphenylethanamide-α-L-rhamnopyranoside, namely marumoside A.

Compound **4** was isolated as an amorphous powder and determined to be $C_{14}H_{19}NO_6$ by high resolution electrospray time-of-flight mass spectrometric analysis (HR-ESI-TOFMS). The ^1H and ^{13}C NMR spectra were very similar to those of compound **3**, except for a set of additional signals arising from the β-D-glucopyranosyl moiety in **4**. The additional unit was assigned to be located at C-3″ of the rhamnopyranosyl moiety since the downfield of this carbon atom to δ_C 81.4 together with the upfield shifts of C-2 and C-4 to δ_C 69.3 and 71.1, respectively, as compared to compound **3**. Moreover, the HMBC spectrum provided further confirmation of the structure with the correlations between H-1‴ (δ_H 4.48) and C-3″ as shown in Fig. 3. Consequently, this compound was identified as the 3″-*O*-β-D-glucopyranosyl-derivative of compound **3**, namely marumoside B.

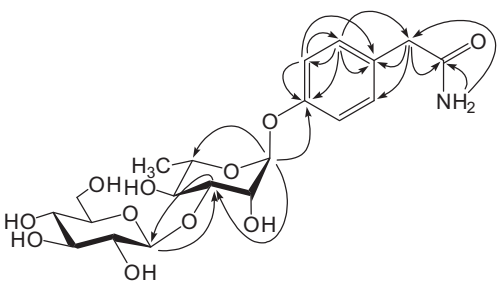

Fig. 3. HMBC correlations of compound **4**.

Compound **5** was elucidated to be methyl 4-(α-L-rhamnopyranosyloxy)-benzylcarbamate, previously isolated from the fruits of *M. oleifera* (Francis et al., 2004) without the physical and spectroscopic data. Therefore, these data were given in this present study.

3. Concluding remarks

The presence of nitrogen-containing phenolic glycosides (**1**–**5**) from plant source of Thai origin was related to those isolated compounds from other plant sources. Niazirin (**1**) and methyl 4-(α-L-rhamnopyranosyloxy)benzylcarbamate (**5**) have been previously reported from *M. oleifera* (Faizi et al., 1994a,b; Francis et al., 2004). Pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside (**2**) was the first pyrrole alkaloid isolated from this plant. Marumosides A (**3**) and B (**4**) were isolated as glycosides of 4′-hydroxyphenylethanamide. This aglycone was also isolated from roasted seeds of this plant (Villasenor et al., 1989). However, the thiocarbamate glycoside could not be found. In this study, the toxicological and pharmacological properties of the isolated compounds have not been investigated. But there are some report for mutagenic activity for niazirin (**1**) and its aglycone (4-hydroxyphenylacetonitrile) as well as the aglycone of marumosides A (**3**) and B (**4**) (4′-hydroxyphenylethanamide) (Villasenor et al., 1989). Marumosides A (**3**) and B (**4**), which are present in large amounts in the plant material from Thailand, can be readily changed to 4′-hydroxyphenylethanamide by acid hydrolysis. It seems, therefore that the use of this plant as daily food supplement for internal treatments should be evaluated. Further biological and toxicological studies will thus be needed to establish the safety and efficacy of these compounds.

4. Experimental

4.1. General experimental procedures

^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) spectrometer. The MS data was obtained on a Bruker Micro TOF-LC mass spectrometer. FTIR spectra were recorded on a universal attenuated total reflectance attached (UATR) to a Perkin–Elmer Spectrum One spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography (cc), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 × 250 mm i.d., Vertisep™ AQS) with a Jasco UV-970 detector at 220 nm. The flow rate was 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

4.2. Plant material

Leaves of *M. oleifera* Lam. were collected in February 2010 from Khon Kaen province, Thailand, with plant identification done by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0064) is in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

4.3. Extraction and isolation

Dried leaves of *M. oleifera* (2.2 kg) were extracted with MeOH (each 20 L for 24 h, 3 times) at room temperature. After removal of the solvent, the residue (632.1 g) was partitioned with Et_2O and H_2O (each 1.0 L, 3 times). The aqueous soluble fraction

(409.5 g) was subjected to a Diaion HP-20 column, and successfully eluted with H_2O, MeOH and acetone, successively. The fraction eluted with MeOH (90.7 g) was subjected to silica gel cc using solvent systems EtOAc–MeOH (9:1, 4.0 L), EtOAc–MeOH–H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 6.0 L) and EtOAc–MeOH–H_2O (6:4:1, 12.0 L), respectively, to provide seven fractions. Fraction 1 (1.9 g) was applied to a RP-18 column using a gradient solvent system H_2O-MeOH (90:10 → 20:80, v/v) to give five fractions. Fraction 1–3 was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (85:15, v/v) to afford compounds **1** (11.2 mg) and **5** (8.8 mg). Fraction 2 (14.3 g) was subjected to a RP-18 column using a gradient solvent system, H_2O–MeOH (90 :10→ 20:80, v/v) to provide fifteen fractions. Fraction 2–6 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (90:10, v/v) to provide compound **6** (357.7 mg). Fraction 2–5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compound **2** (154.8 mg). Fraction 3 (22.2 g) was applied to a RP-18 column using a gradient solvent system H_2O–MeOH (90:10 → 20:80, v/v) to afford twelve fractions. Fraction 3–1 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to give compound **3** (171.9 mg). Fraction 3–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to yield compound **7** (313.0 mg). Fraction 4 (20.6 g) was separated on a RP-18 column using a solvent system H_2O–MeOH (90:10 → 20:80, v/v) to give seven fractions. Fraction 4–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **4** (786.6 mg) and **10** (104.3 mg). Fraction 4–5 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (80:20) to obtain compounds **8** (756.4 mg) and **9** (190.6 mg). Finally, Fraction 6 (4.9 g) was similarly subjected to RP-18 cc using a solvent system H_2O–MeOH (90:10 → 20:80, v/v) to afford five fractions. Faction 6–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to give compound **11** (244.5 mg).

4.4. Pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside (**2**)

Amorphous powder; $[\alpha]_D^{27}$63.2 (MeOH, *c* 0.49); IR (UATR)ν_{max} 3361, 2934, 1646, 1610, 1509, 1371, 1231, 1060, 1021, 983 cm^{-1}; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 1; negative HR-APCI-TOFMS, *m/z*: 412.1163 $[M+Cl]^-$ (calcd for $C_{19}H_{23}ClNO_7$, 412.1169).

4.5. Acid hydrolysis of pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside (**2**)

A solution of pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside (*ca* 20 mg) in 1,4-dioxane (0.5 ml) and 2 N HCl (4.5 ml) was heated at 80 °C for 4 h. After cooling, H_2O (5 ml) was added and neutralized with 2 N KOH. The mixture was extracted with EtOAc (30 ml × 2) and the combined organic parts were concentrated *in vacuo* to provide pyrrolemarumine (**2a**, 7.2 mg). Its structure was assigned by NMR spectroscopic analyses.

4.6. Pyrrolemarumine (**2a**)

Amorphous powder; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 1; positive HR-APCI-TOFMS, *m/z*: 232.0971 $[M+H]^+$ (calcd for $C_{13}H_{14}NO_3$, 232.0968).

4.7. Marumoside A (**3**)

Amorphous powder, $[\alpha]_D^{28}$ 94.8 (MeOH, *c* 1.00); IR (UATR)ν_{max} 3351, 2933, 1667, 1610, 1510, 1383, 1230, 1063, 1021, 983 cm^{-1}; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 2; negative HR-ESI-TOFMS, *m/z*: 332.0916 $[M+Cl]^-$ (calcd for $C_{14}H_{19}ClNO_6$, 332.0906).

Table 2
NMR Spectroscopic data of compounds **3** and **4** (DMSO-d_6).

Position	**3**		**4**	
	Carbon	Proton	Carbon	Proton
Aglycone				
1	172.8		173.2	
2	41.5	3.30 (2H, s)	41.7	3.30 (2H, s)
1′	129.9		130.4	
2′,6′	130.2	7.17 (2H, d, *J* = 8.2 Hz)	130.5	7.18 (2H, d, *J* = 8.6 Hz)
3′,5′	116.3	6.94 (2H, d, *J* = 8.2 Hz)	116.9	6.97 (2H, d, *J* = 8.6 Hz)
4′	154.8		154.9	
NH_2		7.46 (1H, br s)		7.46 (1H, br s)
		6.86 (1H, br s)		6.85 (1H, br s)
Sugar moiety				
1″	98.6	5.32 (1H, br s)	98.9	5.35 (1H, d, *J* = 1.3 Hz)
2″	70.5	3.82 (1H, br s)	69.3	4.09 (1H, br s)
3″	70.3	3.63 (1H, dd, *J* = 9.0, 3.2 Hz)	81.4	3.76 (1H, dd, *J* = 9.0, 3.1 Hz)
4″	71.9	3.28 (1H, dd, *J* = 9.4, 9.0 Hz)	71.1	3.49 (1H, dd, *J* = 9.0, 9.0 Hz)
5″	69.5	3.45 (1H, m)	69.8	3.57 (1H, m)
6″	18.3	1.09 (3H, d, *J* = 6.0 Hz)	18.2	1.11 (3H, d, *J* = 5.9 Hz)
1‴			104.9	4.48 (1H, d, *J* = 7.7 Hz)
2‴			74.4	3.10 (1H, dd, *J* = 8.7, 7.7 Hz)
3‴			77.0	3.18 (1H)[a]
4‴			70.2	3.13 (1H)[a]
5‴			76.6	3.21 (1H, m)
6‴			61.3	3.67 (1H, br d, *J* = 11.3 Hz)
				3.48 (1H)[a]

[a] Chemical shifts obtained approximately by HMQC.

4.8. Marumoside B (**4**)

Amorphous powder, $[\alpha]_D^{28}$ 65.2 (MeOH, *c* 0.42); IR (UATR)ν_{max} 3355, 2920, 1668, 1610, 1509, 1376, 1229, 1070, 1022, 983 cm^{-1}; for ^1H and ^{13}C NMR (DMSO-d_6): spectroscopic data, see Table 2; negative HR-ESI-TOFMS, *m/z*: 494.1448 $[M+Cl]^-$ (calcd for $C_{20}H_{29}ClNO_{11}$, 494.1434).

4.9. Methyl 4-(α-L-rhamnopyranosyloxy)benzylcarbamate (**5**)

Amorphous powder, $[\alpha]_D^{27}$123.0 (MeOH, *c* 0.46); IR (UATR)ν_{max} 3337, 2933, 1701, 1533, 1509, 1263, 1229, 1063, 1020, 983 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.62 (1H, *t*, *J* = 6.1 Hz, N–H), 7.17 (2H, *d*, *J* = 8.6 Hz, H-2, 6), 6.97 (2H, *d*, *J* = 8.6 Hz, H-3, 5), 5.32 (1H, *d*, *J* = 1.6 Hz, H-1″), 4.10 (2H, *d*, *J* = 6.1 Hz, H-7), 3.80 (1H, *br s*, H-2″), 3.62 (1H, *dd*, *J* = 9.7, 1.8 Hz, H-3″), 3.53 (3H, *s*, OCH_3), 3.45 (1H, *m*, H-5″), 3.26 (1H, *dd*, *J* = 9.7, 9.6 Hz, H-4″), 1.08 (3H, *d*, *J* = 6.2 Hz, H-6″); ^{13}C NMR (DMSO-d_6): δ 157.3 (C-1′), 155.4 (C-4), 133.6 (C-1), 128.7 (C-2, 6), 116.7 (C-3, 5), 98.9 (C-1″), 72.2 (C-4″), 70.9 (C-2″), 70.6 (C-3″), 69.8 (C-5″), 51.8 (OCH_3), 43.7 (C-7), 18.3 (C-6″); negative HR-ESI-TOFMS, *m/z*: 362.1018 $[M+Cl]^-$ (calcd for $C_{15}H_{21}ClNO_7$, 362.1012).

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NOTE

Phenylethanoid and flavone glycosides from *Ruellia tuberosa* L.

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Abstract A new phenylethanoid glycoside, isocassifolioside (**8**), and two new flavone glycosides, hispidulin 7-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-β-D-glucuronopyranoside (**11**) and pectolinaringenin 7-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-β-D-glucuronopyranoside (**12**) were isolated from the aerial portions of *Ruellia tuberosa* L., together with verbascoside (**1**), isoverbascoside (**2**), nuomioside (**3**), isonuomioside (**4**), forsythoside B (**5**), paucifloside (**6**), cassifolioside (**7**), hispidulin 7-*O*-β-D-glucuronopyranoside (**9**) and comanthoside B (**10**). The structure elucidations were based on analyses of chemical and spectroscopic data including 1D- and 2D-NMR. The isolated compounds **1**–**12** exhibited radical scavenging activity using ORAC assay.

Keywords *Ruellia tuberosa* · Acanthaceae · Flavone glycoside · Phenylethanoid glycoside · Antioxidant activity

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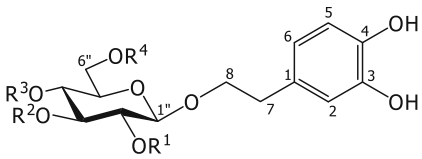
Introduction

Ruellia tuberosa L. (Acanthaceae, Thai name: Toi-ting) is a perennial herb widely distributed in tropical areas of Asian countries. It has been externally used in Thai tradition medicine as an anti-inflammatory, an antiseptic as well as an antidote for detoxification of poisons. Previous phytochemical studies of this plant revealed the presence of steroids, terpenoids, long-chain aliphatic compounds and flavonoids [1–6]. In pharmacological studies, the aerial part extracts showed antioxidant, antinociceptive, and anti-inflammatory activities [7–9]. This present study deals with the isolation and structure determination of polar constituents including a new phenylethanoid (**8**) and two new flavone glycosides (**11**, **12**) together with nine known compounds. Also, their antioxidant properties using DPPH and ORAC assays were evaluated.

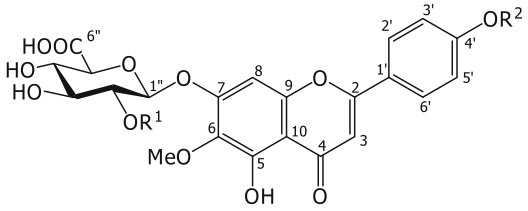
Results and discussion

The methanolic extract of the aerial part of *R. tuberosa* was suspended in H_2O and extracted with Et_2O. The aqueous layer was applied to a column of Diaion HP-20, with H_2O, MeOH and Me_2CO as eluants, successively. The portion eluted with MeOH was separated by a combination of chromatographic procedures to provide 12 compounds (Fig. 1). Nine were identified as known compounds, including verbascoside (**1**), isoverbascoside (**2**), nuomioside (**3**), isonuomioside (**4**), forsythoside B (**5**), paucifloside (**6**), cassifolioside (**7**), hispidulin 7-*O*-β-D-glucuronopyranoside (6-*O*-methyl-scutellarein, **9**) and comanthoside B (pectolinaringenin 7-*O*-β-D-glucuronopyranoside, **10**) on the basis of spectroscopic evidence and comparison of the physical data with reported values [10–16].

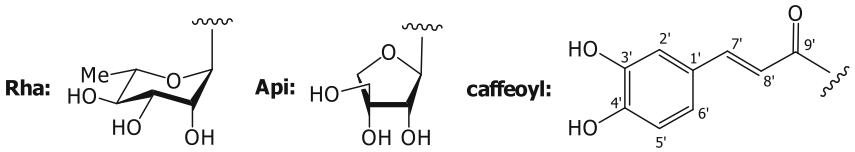
Fig. 1 The structures of compounds **1**–**12**



	R^1	R^2	R^3	R^4
1:	H	Rha	caffeoyl	H
2:	H	Rha	H	caffeoyl
3:	H	Api	caffeoyl	H
4:	H	Api	H	caffeoyl
5:	H	Rha	caffeoyl	Api
6:	H	Api	caffeoyl	Api
7:	Rha	Rha	caffeoyl	H
8:	Rha	Rha	H	caffeoyl



	R^1	R^2
9:	H	H
10:	H	Me
11:	Rha	H
12:	Rha	Me



Compound **8** was isolated as a yellow amorphous powder, and its molecular formula was determined as $C_{35}H_{46}O_{19}$ by high-resolution atmospheric pressure chemical ionization time-of-flight (HR-APCI-TOF) mass spectrometric analysis. The ^{1}H- and ^{13}C-NMR spectroscopic data (Table 1) indicated that compound **8** is an analogue of compounds **1**–**7** with three sugar moieties. The chemical shifts were related to those of isoverbascoside (**2**) except for a set of additional signals arising from an α-L-rhamnopyranosyl moiety, deduced from the chemical shifts of the carbon signals at δ_C 102.9, 72.3, 72.3, 73.6, 70.1 and 18.0. This sugar moiety was suggested to be attached at C-2″ of the glucopyranosyl moiety due to the downfield shift of this carbon atom at δ_C 80.1 as compared to isoverbascoside (**2**). The assignment was confirmed by means of COSY, HMQC and HMBC. In the HMBC spectrum, the significant correlations were observed from H-1″ (δ_H 4.43) to C-8 (δ_C 72.3), H-1‴ (δ_H 4.98) to C-2″ (δ_C 80.1), H-1‴ (δ_H 4.93) to C-3″ (δ_C 87.2), and H-6″ (δ_H 4.50 and 4.34) to C-9′ (δ_C 169.1) as illustrated in Fig. 2. In addition, acid hydrolysis provided D-glucose and L-rhamnose, identified by HPLC analysis using the optical rotation detector. Consequently, the structure was determined as shown. Since compound **8** was an isomer of crassifolioside (**7**), different on the location of the caffeoyl moiety at C-6″ instead of C-4″, therefore the name isocrassifolioside was proposed for this compound.

Compound **11** had the molecular formula $C_{28}H_{30}O_{16}$ based on HR-APCI-TOF mass spectrometric analysis. The ^{1}H- and ^{13}C-NMR spectroscopic data (Table 2) were similar to those of hispidulin 7-*O*-*β*-D-glucuronopyranoside (**9**) except for the presence of an α-L-rhamnopyranosyl unit. This sugar moiety was assigned to be attached at C-2″ of the *β*-D-glucuronopyranosyl because the chemical shifts of C-1″ and C-2″ were significantly changed to 97.6 and 76.3 ppm, respectively, relative to compound **9**. Moreover, HMBC correlation was observed between H-1‴ (δ_H 5.23) and C-2″ (δ_C 76.3), as shown in Fig. 2. The absolute configuration of two sugar units were identified as D-glucuronic acid and L-rhamnose by HPLC analysis. Consequently, the structure of this compound was elucidated to be hispidulin 7-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-*β*-D-glucuronopyranoside.

The molecular formula of compound **12** was determined as $C_{29}H_{32}O_{16}$by HR-APCI-TOF mass spectrometric analysis. Inspection of the ^{1}H- and ^{13}C-NMR spectroscopic data (Table 2) revealed that this compound had the same aglycone as pectolinaringenin 7-*O*-*β*-D-glucuronopyranoside (**10**), while the sugar moieties were identical to those of compound **11**. Accordingly, this compound was determined as pectolinaringenin 7-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-*β*-D-glucuronopyranoside.

In this study, the isolated compounds **1**–**12** were evaluated for their radical scavenging activities using both

Table 1 ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of compound **8** (MeOH-d_4)

Position	δ_C	δ_H
Aglycone		
1	131.2	
2	117.1	6.67 (1H, d, 1.9)
3	146.1	
4	144.7	
5	116.5	6.63 (1H, d, 8.1)
6	121.3	6.53 (1H, dd, 8.1, 1.9)
7	36.7	2.79 (2H, m)
8	72.3	3.95 (1H, m)
		3.70 (1H, m)
Caffeoyl moiety		
1′	127.7	
2′	115.2	7.03 (1H, d, 1.9)
3′	146.7	
4′	149.5	
5′	116.6	6.77 (1H, d, 8.2)
6′	123.2	6.87 (1H, dd, 8.2, 1.9)
7′	147.2	7.55 (1H, d, 15.9)
8′	114.9	6.28 (1H, d, 15.9)
9′	169.1	
Glc		
1″	102.8	4.43 (1H, d, 7.7)
2″	80.1	3.46 (1H, dd, 8.9, 7.7)
3″	87.2	3.61 (1H, dd, 8.9, 8.9)
4″	70.7	3.44 (1H)[a]
5″	75.2	3.53 (1H)[a]
6″	64.5	4.50 (1H, dd, 11.7, 1.7)
		4.34 (1H, dd, 11.7, 5.9)
Rha-I		
1‴	103.7	4.93 (1H, brs)
2‴	72.4	3.89 (1H)[a]
3‴	72.3	3.71 (1H)[a]
4‴	73.9	3.42 (1H)[a]
5‴	70.7	3.98 (1H)[a]
6‴	17.8	1.19 (3H, d, 6.0)
Rha-II		
1⁗	102.9	4.98 (1H, brs)
2⁗	72.3	3.89 (1H)[a]
3‴	72.3	3.71 (1H)[a]
4⁗	73.6	3.42 (1H)[a]
5⁗	70.1	3.98 (1H)[a]
6⁗	18.0	1.21 (3H, d, 6.0)

[a] Chemical shifts were assigned by HMQC

DPPH and ORAC assays (Table 3) [17]. In DPPH assay, phenylethanoid glycosides (**1**–**8**) displayed relative scavenging activity in same range of the positive control, ascorbic acid, while flavonoid glycosides (**9**–**12**) were inactive. In the ORAC assay, the unit values of all isolated compounds were in the range of 2–6 folds more potent than the positive control, Trolox. The predominantly antioxidant activity of phenylethanoid glycosides (**1**–**8**) and was due to the presence of a caffeoyl and free two hydroxyl groups on phenyl ring. Position of a caffeoyl group locating on C-4″ or C-6″ of sugar moiety seemed to be no effect on the activity. In addition, flavonoid glycosides having free hydroxyl group on phenyl ring (**9**, **11**) were active about 2 folds more potent than methoxy analogues (**10**, **12**).

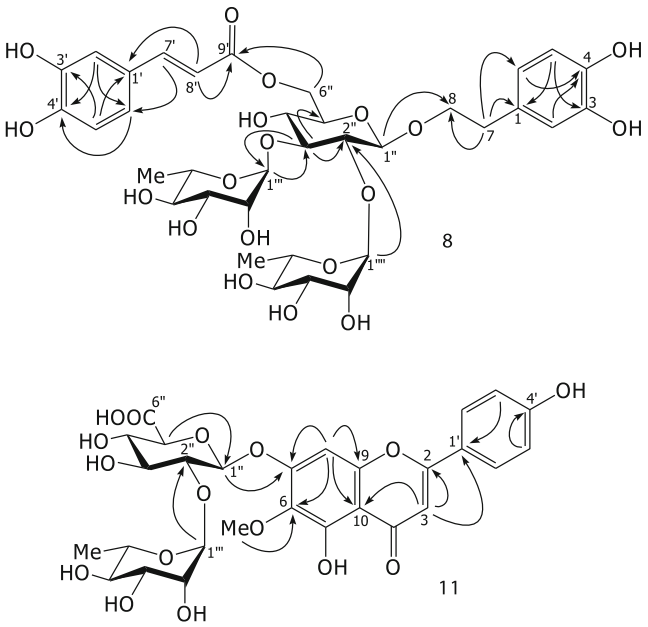


Fig. 2 HMBC correlations of compounds **8** and **11**

Experimental section

General procedures

^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker AV-400 and a Brucker AVANCE III ultrashield 300 MHz spectrometers. Mass spectra were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on ODS column (21.2 × 250 mm i.d., Vertisep^{TM} AQS, 8 mL/min) with a Jasco MD-2010 detector at 220 nm. The spraying reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

Table 2 ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of compounds **11** and **12** (DMSO-d_6)

No.	**11**		**12**	
	δ_C	δ_H	δ_C	δ_H
2	164.5		163.9	
3	102.3	6.65 (1H, s)	103.3	6.88 (1H, s)
4	182.2		182.3	
5	152.0		152.1	
6	132.9		133.0	
7	155.9		156.0	
8	94.1	6.86 (1H, s)	94.5	7.02 (1H, s)
9	152.5		152.5	
10	105.8		105.9	
1′	120.3		122.7	
2′,6′	128.3	7.76 (2H, d, 8.5)	128.4	8.01 (2H, d, 8.9)
3′,5′	116.1	6.78 (2H, d, 8.5)	114.7	7.09 (2H, d, 8.9)
4′	162.2		162.5	
6-OMe	60.4	3.78 (3H, s)	60.3	3.78 (3H, s)
4′-OMe			55.6	3.83 (3H, s)
5-OH		13.0 (1H, brs)		12.9 (1H, brs)
GlcA				
1″	97.6	5.33 (1H, d, 7.3)	98.0	5.33 (1H, d, 7.6)
2″	76.3	3.60 (1H)[a]	76.1	3.61 (1H, dd, 8.8, 7.6)
3″	77.7	3.55 (1H)[a]	77.7	3.54 (1H, dd, 9.3, 8.8)
4″	72.1	3.27 (1H, dd, 9.5, 9.0)	72.0	3.26 (1H, dd, 9.4, 9.3)
5″	73.9	3.66 (1H, d, 10.2)	73.9	3.67 (1H, d, 10.0)
6″	172.7		172.2	
Rha				
1‴	100.2	5.23 (1H, brs)	100.1	5.27 (1H, brs)
2‴	70.6	3.70 (1H)[a]	70.5	3.72 (1H)
3‴	70.5	3.33 (1H, dd, 9.6, 2.9)	70.5	3.38 (1H, dd, 9.4, 3.2)
4‴	72.0	3.16 (1H, dd, 9.6, 9.2)	72.0	3.18 (1H, dd, 9.4, 9.3)
5‴	68.7	3.70 (1H, m)	68.6	3.72 (1H, m)
6‴	18.1	1.08 (3H, d, 6.0)	18.1	1.08 (3H, d, 6.1)

[a] Chemical shifts were assigned by HMQC

Plant material

The aerial portions of *Ruellia tuberosa* L. were collected in March 2010, Bangkok, Thailand. The identification of the plant was done by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0069) is on file in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation

The air-dried aerial portions of *R. tuberosa* (3.2 kg) were macerated with MeOH three times (12 L for each extraction) at room temperature. The MeOH extract was concentrated *in vacuo* to dryness. This residue (318.1 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 3 times). The aqueous soluble fraction (255.0 g) was subjected to a Diaion HP-20 column, and eluted with H_2O, MeOH and $(CH_3)_2CO$, successively. The fraction eluted with MeOH (41.5 g) was subjected to a silica gel column using solvent systems EtOAc–MeOH (9:1, 5.0 L), EtOAc–MeOH–H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 5.0 L) and EtOAc–MeOH–H_2O (6:4:1, 10.0 L), respectively, to afford seven fractions. Fraction 3 (6.6 g) was applied to a RP-18 column using a gradient solvent system H_2O–MeOH (90:10 → 20:80, v/v) to provide eight fractions. Fraction 3–4 (2.4 g) was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (80:20, v/v) to give compounds **1** (792.3 mg), **2** (171.8 mg), **3** (19.4 mg) and **4** (17.7 mg). Fraction 5 (9.7 g) was

Table 3 Radical scavenging activities of isolated compounds **1–12**

Compounds	DPPH assay (SC_{50}, μM)[a]	ORAC (ROO·, unit)[b]
1	13.1 ± 0.8	5.0 ± 0.3
2	13.6 ± 0.6	4.0 ± 0.4
3	19.7 ± 0.5	3.9 ± 0.4
4	18.7 ± 0.4	2.9 ± 0.2
5	16.9 ± 0.1	5.1 ± 0.4
6	18.9 ± 1.7	4.5 ± 0.3
7	16.9 ± 0.4	5.2 ± 0.3
8	15.7 ± 1.7	4.4 ± 0.1
9	I (38%)[c]	5.7 ± 0.4
10	I (18%)[c]	2.2 ± 0.3
11	225 ± 9	4.8 ± 0.6
12	I (34%)[c]	2.3 ± 0.3
L-ascorbic acid	21.2 ± 1.4	–[d]
Trolox	–[d]	1

[a] SC_{50} is half-maximal scavenging concentration

[b] 1 ORAC unit equals the net protection of fluorescein produced by 1 μM Trolox

[c] Numbers in parentheses indicate the percentage of scavenging

[d] Not determined

subjected to a RP-18 column using solvent system H_2O–MeOH (90:10 → 20:80, v/v) to provide eleven fractions. Fraction 5–6 (3.4 g) was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (80:20, v/v) to provide compounds **5** (332.5 mg), **6** (32.6 mg), **7** (27.4 mg) and **8** (66.2 mg). Fraction 6 (5.8 g) was applied to a RP-18 column using solvent system H_2O–MeOH (90:10 → 20:80, v/v) to afford six fractions. Fraction 6–2 (2.1 g) was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to obtain compounds **9** (87.8 mg), **10** (59.9 mg), **11** (89.0 mg) and **12** (90.1 mg).

*Isocassifolioside (**8**)*

Yellow amorphous powder; $[\alpha]_D^{27}$ -54.5 (MeOH, *c* 1.05); IR spectrum: $\nu_{max} = 3386, 2936, 1693, 1604, 1278, 1040\ cm^{-1}$; negative HRMS (APCI-TOF): $[M-H]^-$, found 769.2545. $C_{35}H_{45}O_{19}$ requires 769.2561. ^{1}H- and ^{13}C-NMR data: see Table 1.

*Hispidulin 7-O-α-L-rhamnopyranosyl-(1‴ → 2″)-O-β-D-glucuronopyranoside (**11**)*

Yellow amorphous powder; $[\alpha]_D^{26}$-44.2 (DMSO, *c* 1.00); IR spectrum: $\nu_{max} = 3266, 2926, 1604, 1458, 1351, 1286, 1023\ cm^{-1}$; negative HRMS (APCI-TOF): $[M-H]^-$, found 621.1444. $C_{28}H_{29}O_{16}$ requires 621.1461. ^{1}H- and ^{13}C-NMR data: see Table 2.

*Pectolinaringenin 7-O-α-L-rhamnopyranosyl-(1‴ → 2″)-O-β-D-glucuronopyranoside (**12**)*

Yellow amorphous powder; $[\alpha]_D^{26}$-69.4 (DMSO, *c* 1.03); IR spectrum: $\nu_{max} = 3320, 2926, 1603, 1459, 1354, 1250, 1022\ cm^{-1}$; negative HRMS (APCI-TOF): $[M-H]^-$, found 635.1604. $C_{29}H_{31}O_{16}$ requires 635.1617. ^{1}H- and ^{13}C-NMR data: see Table 2.

Determination of the absolute configurations of sugars

Monosaccharide subunits of new compounds (**8**, **11** and **12**) were obtained by acid hydrolysis. Each sample (ca. 5 mg) in 2 N HCl-dioxane (6:1, 3.5 ml) was heated at 80 °C for 6 h. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous layer was concentrated to dryness providing the sugar fraction. Each of these was dissolved in H_2O (1 mL) and analysed by HPLC (Jasco OR-2090 plus chiral detector; column A: Vertisep^{TM} sugar LMP or column B: Vertisep^{TM} OA, 7.8 × 300 mm i.d.; mobile phase A: water or B: 0.003 M H_2SO_4 aq.; flow rate 0.4 ml/min; temperature 40 or 80 °C) and comparison of their retention times and optical rotations with authentic samples.

Hydrolysis of compound **8** gave peaks corresponding to D-glucose at 19.0 min and L-rhamnose at 23.0 min (both positive optical rotation) (column A, mobile phase A, 80 °C).

Hydrolysis of compound **11** gave peaks of D-glucuronic acid at 12.6 min and L-rhamnose at 15.2 min (both positive optical rotations) (column B, mobile phase B, 40 °C).

Hydrolysis of compound **12** gave peaks of D-glucuronic acid at 12.6 min and L-rhamnose at 15.2 min (both positive optical rotations) (column B, mobile phase B, 40 °C).

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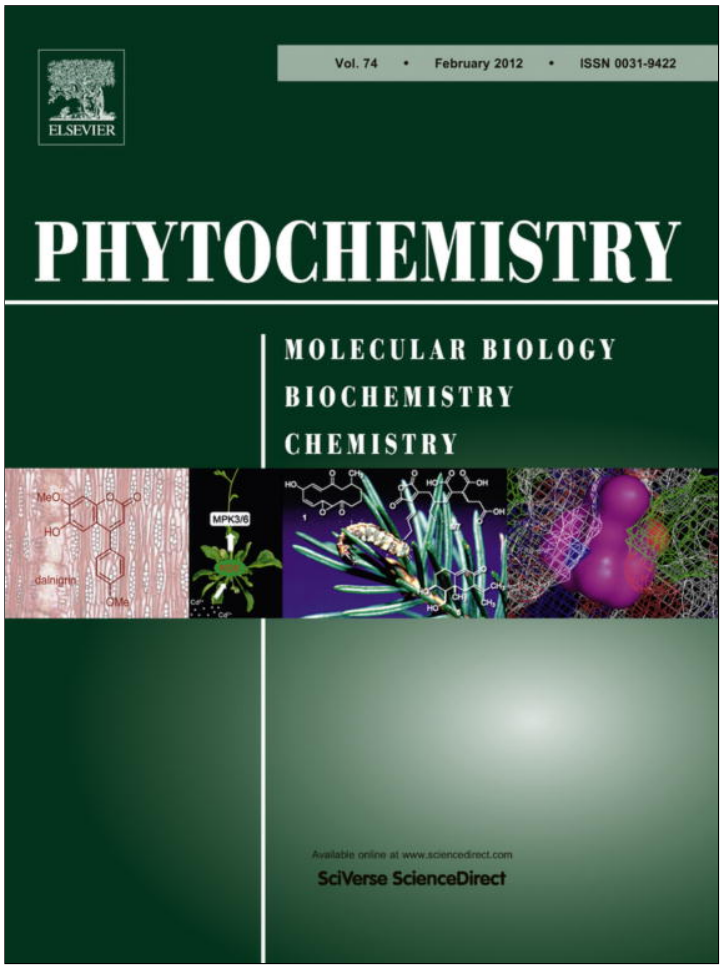

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Unprecedented furan-2-carbonyl *C*-glycosides and phenolic diglycosides from *Scleropyrum pentandrum*

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ABSTRACT

Five unprecedented furan-2-carbonyl *C*-glycosides, scleropentasides A–E, and two phenolic diglycosides, 4-hydroxy-3-methoxybenzyl 4-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside and 2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside, were isolated from leaves and twigs of *Scleropyrum pentandrum* together with potalioside B, luteolin 6-*C*-β-D-glucopyranoside (isoorientin), apigenin 8-*C*-β-D-glucopyranoside (vitexin), apigenin 6,8-di-*C*-β-D-glucopyranoside (vicenin-2), apigenin 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranoside (isoschaftoside), apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-xylopyranoside, adenosine and L-tryptophan. Structure elucidations of these compounds were based on analyses of chemical and spectroscopic data, including 1D and 2D NMR. In addition, the isolated compounds were evaluated for their radical scavenging activities using both DPPH and ORAC assays.

1. Introduction

Scleropyrum pentandrum (Dennst.) Mabb. [Santalaceae; Thai name: Khi-Non; syn. *S. wallichianum* (Wight & Arn.) Arn.] is a small tree, commonly found in the evergreen dipterocarp forests in North-eastern Thailand (Macklin and Parnell, 2005). In previous phytochemical investigation, nonpolar compounds such as acetylenic acids, fatty acids and sterol derivatives have been reported (Wang et al., 1992; Suksamrarn et al., 2005). The present paper describes the isolation and structure elucidation of 15 polar compounds, including five unprecedented furan-2-carbonyl *C*-glycosides (**1–5**, Fig. 1), two new phenolic glycosides (**7–8**, Fig. 1) in addition to five flavone *C*-glycosides (**9–13**), a phenolic glycoside (**6**), a nucleoside (**14**) and an amino acid (**15**) from the aqueous soluble fraction of leaves and twigs of this plant. Also, the isolated compounds **1–13** were evaluated for their antioxidant activity using DPPH and ORAC assays.

2. Results and discussion

2.1. Structure elucidation of new compounds

The methanolic extract of the leaves and twigs of *S. pentandrum* was partitioned with Et_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to provide 15 compounds. Eight compounds were identified as potalioside B (**6**) (Li et al., 2005), luteolin 6-*C*-β-D-glucopyranoside (isoorientin, **9**) (Kumarasamy et al., 2004), apigenin 8-*C*-β-D-glucopyranoside (vitexin, **10**) (Eldahshan et al., 2008), apigenin 6,8-di-*C*-β-D-glucopyranoside (vicenin-2, **11**), apigenin 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranoside (isoschaftoside, **12**) (Xie et al., 2003), apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-xylopyranoside (**13**), (Fiorentino et al., 2008), adenosine (**14**) (Kanchanapoom et al., 2001) and L-tryptophan (**15**) (Kanchanapoom et al., 2007) by comparison of physical data with literature values and from spectroscopic evidence.

Compound **1** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{11}H_{14}O_7$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^1H NMR spectrum (Table 1) indicated the presence of a 2-furanyl group from the chemical shifts at δ_H 8.03 (d, J = 1.6 Hz), 7.60 (d, J = 3.6 Hz), and 6.73 (dd, J = 3.6, 1.6 Hz), and the resonances from δ_H 3.0 to 4.4 were suggested to be the signals of

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Table 1
NMR spectroscopic data of scleropentasides A (**1**) and its reduced forms (**1a** and **1b**) (DMSO-d_6).

Position	1		1a		1b	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		151.5		154.6		156.7
3	7.60 (1H, d, *J* = 3.6 Hz)	121.4	6.34 (1H, d, *J* = 3.1 Hz)	107.6	6.36 (1H, s)	106.5
4	6.73 (1H, dd, *J* = 3.6, 1.6 Hz)	112.8	6.38 (1H, dd, *J* = 3.1, 1.8 Hz)	110.3	6.36 (1H, s)	110.3
5	8.03 (1H, d, *J* = 1.6 Hz)	148.8	7.54 (1H, d, *J* = 1.8 Hz)	141.4	7.52 (1H, s)	141.2
6		184.6	4.77 (1H, d, *J* = 2.8 Hz)	66.5	4.75 (1H, br s)	64.2
1′	4.30 (1H, d, *J* = 9.5 Hz)	79.7	3.37 (1H, dd, *J* = 9.7, 2.8 Hz)	81.5	3.29 (1H, br d, *J* = 9.5 Hz)	79.7
2′	3.47 (1H, dd, *J* = 9.5, 9.1 Hz)	71.7	2.91 (1H, dd, *J* = 9.7, 8.7 Hz)	71.2	3.34 (1H, dd, *J* = 9.5, 8.3 Hz)	69.3
3′	3.31 (1H, dd, *J* = 9.1, 8.4 Hz)	78.1	3.15 (1H, dd, *J* = 8.7, 8.7 Hz)	78.3	3.19 (1H, dd, *J* = 8.5, 8.3 Hz)	78.2
4′	3.15 (1H, dd, *J* = 9.1, 8.4 Hz)	70.1	2.93 (1H, dd, *J* = 9.5, 8.7 Hz)	70.5	3.13 (1H, dd, *J* = 9.2, 8.5 Hz)	69.5
5′	3.27 (1H, m)	81.7	3.07 (1H, m)	80.9	3.09 (1H, m)	80.1
6′	3.68 (1H, dd, *J* = 11.8, 1.2 Hz)	61.3	3.65 (1H, br d, *J* = 11.2 Hz)	62.0	3.61 (1H, br d, *J* = 11.2 Hz)	60.8
	3.45 (1H, dd, *J* = 11.8, 6.3 Hz)		3.40 (1H, dd, *J* = 11.2, 3.5 Hz)		3.45 (1H)[a]	

[a] Chemical shifts were assigned from COSY and HMQC spectra.

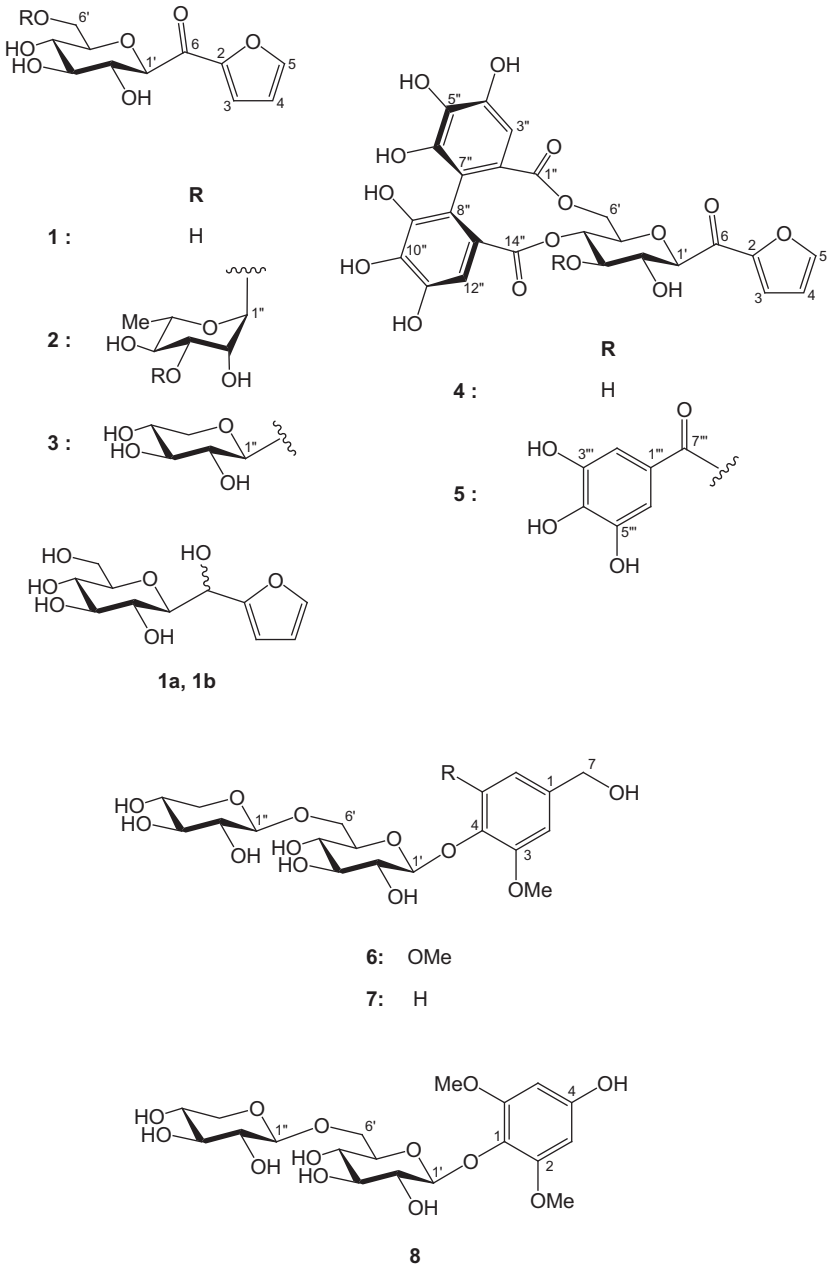

Fig. 1. Structures of isolated compounds **1–8**.

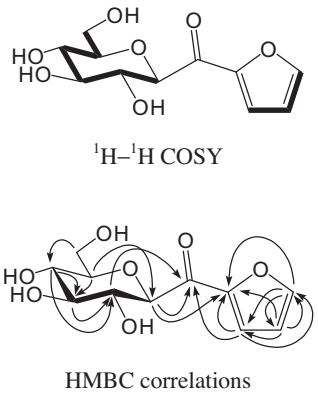

Fig. 2. ^{1}H–^{1}H COSY and HMBC correlations of scleropentaside A (**1**).

one sugar moiety. Analysis of the ^{13}C NMR spectroscopic data showed that this compound contained a furan-2-carbonyl part from a set of the chemical shifts at δ_C 151.5, 121.4, 112.8 and 148.8, and one carbonyl group at δ_C 184.6 (Pouchert and Behnke, 1993). The remaining six carbons at δ_C 79.7, 71.7, 78.1, 70.1, 81.7 and 61.3 belonging to the sugar part could be identified as a *C*-β-glucopyranosyl unit by comparing the chemical shifts with those of compounds **9–13**, and this was confirmed by the detailed analysis of ^{1}H–^{1}H COSY, HMQC (Fig. 2) along with the results from the splitting pattern of each axial protons in the sugar moiety (Table 1). A HMBC experiment provided the structure assignment (Fig. 2) from the correlations between (i) H-1′ and C-2 and C-6, (ii) H-2′ and C-6, and (iii) H-3 and C-6. Consequently, the structure of this compound was elucidated as furan-2-carbonyl *C*-β-glucopyranoside, namely scleropentaside A. However the presence of a *C*-glycoside connecting with the carbonyl group was unusual from a natural source. In order to confirm the structure, the carbonyl group of compound **1** was reduced to a hydroxyl group (see Section 4), affording two hydroxyl forms (**1a** and **1b**). The chemical shifts from analysis of ^{1}H and ^{13}C NMR spectra around the secondary alcohol in **1a** and **1b** significantly changed as compared to those of **1** (Table 1). Also, the partial connectivities of the structures **1a** and **1b** were observed from C-6 to C-6′ by ^{1}H–^{1}H COSY and HMQC spectral analysis. HMBC experiments of both forms displayed significant correlations from H-6 to C-2, C-3, C-1′ and C-2′, providing strong evidence of the structure of scleropentaside A (**1**).

Compound **2** was obtained as an amorphous powder and its molecular formula was determined to be $C_{17}H_{24}O_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectra were related to those of compound **1**, except for a set of additional signals of α-L-rhamnopyranosyl unit deduced from the chemical shifts of its anomeric proton at δ_H 4.53 (1H, d, J = 1.5 Hz) and a secondary methyl protons at δ_H 1.09 (3H, d, J = 5.2 Hz). In the ^{13}C NMR spectrum, a set of the carbon signals for this sugar unit at δ_C 100.7, 70.5, 70.7, 72.0, 68.4 and 18.0 was observed. This additional moiety was assigned to be located at C-6′ of the glucopyranosyl moiety due to the downfield shift of this carbon atom to δ_H 67.0 as compared to compound **1**. A HMBC spectrum provided strong confirmation of the structure from the three bond correlation from H-1″ (δ_H 4.53, d, J = 1.5 Hz) to C-6′. In addition, acid hydrolysis yielded scleropentaside A (**1**) and L-rhamnose, which were identified by HPLC analysis using the optical rotation detector (see Section 4). Accordingly, this compound was characterized as furan-2-carbonyl *C*-(6″-*O*-α-L-rhamnopyranosyl)-β-glucopyranoside, namely scleropentaside B.

Compound **3** was isolated as an amorphous powder. The molecular formula was identified as $C_{16}H_{22}O_{11}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The NMR spectra were very similar to those of compounds **1** and **2**, indicating that this compound was a functional group analogue of both compounds. From the ^{13}C NMR spectrum, a set of the chemical shifts of β-D-xylopyranosyl unit at δ_C 104.0, 73.4, 76.7, 69.7 and 68.8 were found instead of those of α-L-rhamnopyranosyl unit in compound **2**. This sugar moiety was also assigned to be attached to C-6′ of a β-D-glucopyranosyl moiety, since the appearance of this carbon atom at δ_C 65.8. The structure was also confirmed by HMBC spectrum, in which correlation were found between H-1″ (δ_H 4.12, d, J = 7.5 Hz) and C-6′. Acid hydrolysis liberated scleropentaside A (**1**) and D-xylose. Therefore, the structure of compound **3** was identified as furan-2-carbonyl *C*-(6″-*O*-β-D-xylopyranosyl)-β-glucopyranoside, namely scleropentaside C.

Compound **4** was isolated as an amorphous powder. Its molecular formula was identified as $C_{25}H_{20}O_{15}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data (Table 3)

Table 2
NMR spectroscopic data of scleropentasides B (**2**) and C (**3**) (DMSO-d_6).

Position	2		3	
	δ_H	δ_C	δ_H	δ_C
2		151.1		151.4
3	7.59 (1H, d, J = 3.6 Hz)	121.8	7.61 (1H, d, J = 3.5 Hz)	121.7
4	6.73 (1H, dd, J = 3.6, 1.7 Hz)	112.8	6.72 (1H, dd, J = 3.5, 1.6 Hz)	112.9
5	8.03 (1H, d, J = 1.7 Hz)	148.6	8.02 (1H, d, J = 1.6 Hz)	148.8
6		184.4		184.5
1′	4.26 (1H, d, J = 9.6 Hz)	79.8	4.29 (1H, d, J = 9.5 Hz)	79.7
2′	3.42 (1H)[a]	71.7	3.43 (1H, dd, J = 9.5, 9.2 Hz)	71.6
3′	3.28 (1H, dd, J = 8.8, 8.3 Hz)	77.8	3.30 (1H, dd, J = 9.2, 9.0 Hz)	77.9
4′	3.12 (1H, dd, J = 9.2, 8.3 Hz)	70.0	3.16 (1H, dd, J = 9.1, 9.0 Hz)	69.9
5′	3.42 (1H, m)	80.1	3.45 (1H, m)	80.1
6′	3.82 (1H, br d, J = 9.9 Hz) 3.45 (1H)[a]	67.0	3.66 (1H, dd, J = 11.2, 5.3 Hz) 2.98 (1H, br d, J = 11.2 Hz)	65.8
1″	4.53 (1H, d, J = 1.5 Hz)	100.7	4.12 (1H, d, J = 7.5 Hz)	104.0
2″	3.58 (1H, dd, J = 3.2, 1.5 Hz)	70.5	2.95 (1H)[a]	73.4
3″	3.42 (1H)[a]	70.7	3.06 (1H, dd, J = 8.7, 8.5 Hz)	76.7
4″	3.16 (1H, dd, J = 9.2, 9.2 Hz)	72.0	3.26 (1H, m)	69.7
5″	3.37 (1H, m)	68.4	3.96 (1H, br d, J = 10.6 Hz) 3.56 (1H, dd, J = 10.6, 5.2 Hz)	68.8
6″	1.09 (3H, d, J = 6.2 Hz)	18.0		

[a] Chemical shifts were assigned from COSY and HMQC spectra.

Table 3
NMR spectroscopic data of scleropentasides D (**4**) and E (**5**) (DMSO-d_6).

Position	4		5	
	δ_H	δ_C	δ_H	δ_C
2		151.5		151.4
3	7.62 (1H, d, *J* = 3.6 Hz)	121.5	7.71 (1H, d, *J* = 3.6 Hz)	122.1
4	6.78 (1H, dd, *J* = 3.6, 1.6 Hz)	112.9	6.81 (1H, dd, *J* = 3.6, 1.5 Hz)	113.0
5	8.08 (1H, d, *J* = 1.6 Hz)	149.1	8.13 (1H, d, *J* = 1.5 Hz)	149.5
6		183.4		182.5
1′	4.53 (1H, d, *J* = 9.2 Hz)	79.7	4.79 (1H, d, *J* = 9.5 Hz)	79.0
2′	3.63 (1H, dd, *J* = 9.2, 8.8 Hz)	71.8	3.94 (1H, dd, *J* = 9.5, 9.3 Hz)	69.3
3′	3.57 (1H, dd, *J* = 9.2, 8.8 Hz)	75.5	5.35 (1H, dd, *J* = 9.5, 9.4 Hz)	75.9
4′	4.61 (1H, dd, *J* = 9.6, 9.2 Hz)	71.7	4.78 (1H, dd, *J* = 9.9, 9.9 Hz)	69.9
5′	4.01 (1H, m)	75.8	4.31 (1H, m)	75.6
6′	4.96 (1H, dd, *J* = 12.9, 5.7 Hz)	63.0	5.06 (1H, dd, *J* = 13.4, 6.2 Hz)	62.6
	3.70 (1H, br d, *J* = 12.9 Hz)		3.78 (1H, br d, *J* = 13.4 Hz)	
1″		168.0		167.6
2″		124.7		123.9
3″	6.34 (1H, s)	105.5	6.39 (1H, s)	105.7
4″		144.1		144.3
5″		135.1		135.3
6″		144.9		144.8
7″		115.4		115.4
8″		115.8		115.3
9″		145.0		144.8
10″		135.4		135.4
11″		144.1		144.3
12″	6.34 (1H, s)	106.2	6.27 (1H, s)	105.5
13″		124.5		124.4
14″		167.2		167.0
1‴				119.2
2‴,6‴			6.89 (2H, s)	109.0
3‴,5‴				145.3
4‴				138.5
7‴				165.7

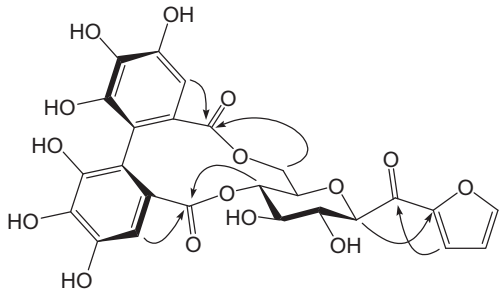

Fig. 3. Significant HMBC correlations of scleropentaside D (**4**).

suggested the presence of furan-2-carbonyl *C*-β-glucopyranoside as a core structure. In addition, the signals of the hexahydroxydiphenoyl (HHDP) group were observed in the NMR spectra (Okuda et al., 1983). The structure assignment of compound **4** was based on the results from $^1H-^1H$ COSY, HMQC and HMBC spectroscopic methods. $^1H-^1H$ COSY and HMQC were used to determine protons for observing the splitting pattern of each sugar protons (Table 3), confirming that the sugar part of this compound was a β-glucopyranosyl moiety. Analysis of the HMBC spectrum provided assignment of the HHDP group to C-4′and C-6′ of the glucopyranosyl part from the three bond correlations between H-4′ (δ_H 4.61, dd, *J* = 9.6, 9.2 Hz) and C-14" (δ_C 167.2) as well as H_2-6′ (δ_H 4.96, dd, *J* = 12.9, 5.7 Hz and δ_H 3.70, br d, *J* = 12.9 Hz) and C-1″ (δ_C 168.2) as illustrated in Fig. 3. The absolute configuration of the hexahydroxydiphenoyl group was established as *S* configuration from the CD spectrum, which showed extreme values for Δε (nm) +9.1 (238), −2.7 (274), and −1.1 (312), relating to the data of (*S*)-configurated compounds (Okuda et al., 1983). Therefore, the structure of compound **4** was elucidated to be furan-2-carbonyl *C*-[4,6-di-*O*-(*S*)-hexahydroxydiphenoyl]-β-glucopyranoside, namely scleropentaside D.

Compound **5** was obtained as an amorphous powder and the molecular formula was identified to be $C_{32}H_{24}O_{19}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. The NMR spectroscopic data were very similar to those of compound **4**, except for a set of signals arising from a galloyl group at δ_H 6.89 (2H, s) in the ^1H NMR spectrum and δ_C 119.2, 109.0(2C), 145.3(2C), 138.5, 165.7 in the ^{13}C NMR spectrum in compound **5**. This galloyl unit was suggested to be located at C-3′ of the glucopyranosyl moiety with the noted downfield shift of this carbon atom to δ_C 75.9 together with the upfield shifts of C-2′ and C-4′ to δ_C 69.3 and 69.9, respectively, as compared to **4**. The complete assignment was confirmed by $^1H-^1H$ COSY, HMQC and HMBC spectroscopic methods, in which long range correlations were observed from the HMBC spectrum between (i) H-3′ (δ_H 5.35, dd, *J* = 9.5, 9.4 Hz) and C-7‴ (δ_C 165.7), (ii) H-4′ (δ_H 4.78, dd, *J* = 9.9, 9.9 Hz) and C-14″ (δ_C 167.0), and iii) H_2-6′ (δ_H 5.06, dd, *J* = 13.4, 6.2 Hz and δ_H 3.78, br d, *J* = 13.4 Hz) and C-1″ (δ_C 168.2) as shown in Fig. 4. The biphenyl configuration was assigned to be *S* by its CD spectrum, which established extreme values for Δε (nm) +8.0 (237), −1.9 (269), +0.7 (292), and −0.6 (315) as compared to compound **4**. Consequently, compound **5** was identified to be 3′-*O*-galloyl-derivative of compound **4**, namely scleropentaside E.

Compound **7** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{28}O_{12}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis of the ^1H NMR spectrum suggested the presence of 1,2,4-trisubstituted aromatic ring system from the chemical shifts at δ_H 7.07 (1H, d, *J* = 8.3 Hz), 6.93 (1H, br s) and 6.80 (1H, br d, *J* = 8.3 Hz), one singlet methylene proton signal at δ_H 4.42 (2H, s)

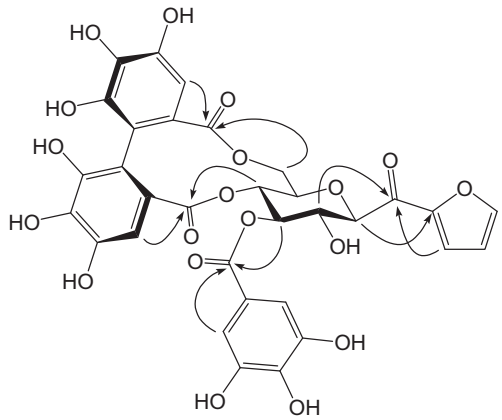

Fig. 4. Significant HMBC correlations of scleropentaside E (**5**).

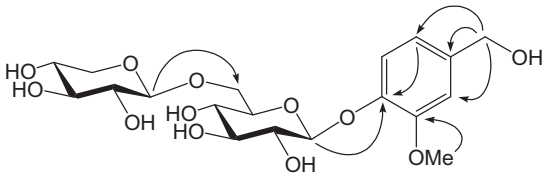

Fig. 5. Significant HMBC correlations of 4-hydroxy-3-methoxybenzyl 4-*O*-β-xylopyranosyl-(1 → 6)-β-glucopyranoside (**7**).

and one methoxyl group at δ_H 3.75 (3H, s) in addition to two anomeric resonances at δ_H 4.83 (1H, d, J = 7.0 Hz) and 4.14 (1H, d, J = 7.5 Hz). The ^{13}C NMR spectrum showed 19 carbon signals, of which eight were assignable to two oxy-aryl carbons at δ_C145.3, 148.8; three aryl-methines at δ_C 111.2, 115.6, 119.0; one aryl quarternary carbon at δ_C 136.5; one hydroxymethyl at δ_C 62.9; and one methoxyl group at δ_C 55.7 for the aglycone moiety. The remaining 11 carbons belonging to the sugar part could be identified as β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl unit by comparing the chemical shifts with those of potalioside B (**6**). The monosaccharide subunits were confirmed to be D-xylose and D-glucose by acid hydrolysis and identified by HPLC analysis using optical rotation detector. All protonated carbons were assigned by the result from HMQC spectrum. The structure of this compound was related to compound **6**, differing by the absence of one methoxyl group from the aromatic ring, and confirmed by the HMBC experiment. Significant correlations were observed between (i) H-7 (δ_H 4.42), and C-2 (δ_C 111.2), C-6 (δ_C 119.0); (ii) H-6 (δ_H 6.80) and C-4 (δ_C 145.3); (iii) MeO-3 (δ_H 3.75) and C-3 (δ_C 148.8); H-1′ (δ_H 4.83) and C-4 (δ_C 145.3); and H-1″ (δ_H 4.14); and C-6′ (δ_C 68.2) as illustrated in Fig. 5. On the basis of these evidence, the structure of compound **7** was identified to be 4-hydroxy-3-methoxybenzyl alcohol 4-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **8** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{28}O_{13}$ by high resolution electrospray time-of-flight (HR-ESI-TOF) mass spectrometric analysis. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data indicated the presence of a tetrasubstituted symmetrical aromatic ring, two equivalent methoxyl groups for the aglycone moiety together with a β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl unit for the sugar part, as compared to compounds **6** and **7**. Acid hydrolysis provided D-xylose and D-glucose. The chemical shifts of the core structure were in agreement with those of 2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-D-glucopyranoside (Otsuka et al., 1989). However, the additional signals of the xylopyranosyl unit were observed. The positions of two methoxyl groups were confirmed to be located at C-2 and C-6 by an NOE difference experiment, in which irradiation of the anomeric resonance of the glucopyranosyl unit at δ_H 4.62 gave an NOE enhancement of the methoxy signal at δ_H 3.75. Therefore, the structure of this compound was determined to be 2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl unit.

Table 4
NMR spectroscopic data of compound **7** (DMSO-d_6).

Position	δ_H	δ_C
1		136.5
2	6.93 (1H, br s)	111.2
3		148.8
4		145.3
5	7.07 (1H, d, J = 8.3 Hz)	115.6
6	6.80 (1H, br d, J = 8.3 Hz)	119.0
7	4.42 (2H, s)	62.9
MeO-3	3.75 (3H, s)	55.7
1′	4.83 (1H, d, J = 7.0 Hz)	100.3
2′	3.25 (1H)[a]	73.3
3′	3.25 (1H)[a]	76.8
4′	3.16 (1H, dd, J = 9.2, 8.3 Hz)	69.7
5′	3.47 (1H, m)	76.0
6′	3.90 (1H, br d, J = 11.0 Hz)	68.2
	3.57 (1H, dd, J = 11.0, 6.1 Hz)	
1″	4.14 (1H, d, J = 7.5 Hz)	103.8
2″	2.94 (1H, dd, J = 8.8, 7.5 Hz)	73.6
3″	3.05 (1H, dd, J = 8.8, 8.7 Hz)	76.5
4″	3.25 (1H)[a]	69.7
5″	3.64 (1H, dd, J = 11.2, 5.0 Hz)	65.7
	2.89 (1H, br d, J = 11.2 Hz)	

[a] Chemical shifts assigned from COSY and HMQC spectra.

Table 5
NMR spectroscopic data of compound **8** (DMSO-d_6).

Position	δ_H	δ_C
1		127.4
2, 6		153.3
3, 5	6.05 (2H, s)	93.8
4		154.0
MeO-2,6	3.75 (6H, s)	56.2
1′	4.62 (1H, d, J = 7.0 Hz)	103.4
2′	3.21 (1H)[a]	74.1
3′	3.24 (1H)[a]	76.4
4′	3.16 (1H)[a]	69.8
5′	3.40 (1H, m)	76.0
6′	3.84 (1H, br d, J = 11.2 Hz)	68.2
	3.52 (1H, dd, J = 11.2, 4.5 Hz)	
1″	4.08 (1H, d, J = 7.5 Hz)	103.6
2″	2.90 (1H, dd, J = 8.7, 7.5 Hz)	73.4
3″	3.04 (1H, dd, J = 8.7, 8.6 Hz)	76.3
4″	3.25 (1H)[a]	69.6
5″	3.64 (1H, dd, J = 11.2, 5.2 Hz)	65.5
	2.95 (1H, br d, J = 11.2 Hz)	

[a] Chemical shifts assigned from COSY and HMQC spectra.

2.2. Antioxidant activities

In this study, the isolated compounds **1–13** were evaluated for their radical scavenging activities using both DPPH and ORAC assays (Table 6) (Rangkadilok et al., 2007; Disadee et al., 2011). Previous studies reported that furan derivatives showed radical scavenging activity and cytoprotective effects (Nishio et al., 2008). The furan-2-carbonyl derivatives, scleropentasides A–C (**1–3**), bearing with mono- or disaccharides moiety displayed relative scavenging activity in the same range of Trolox, using ORAC assay, while in DPPH assay they were inactive. However when furan-2-carbonyl part connects with sugar moieties esterified by

Table 6
Radical scavenging activities of isolated compounds **1–13.**

Compounds	DPPH assay (SC_{50}, μM)[a,c]	ORAC ($ROO^{\cdot}$, unit)[b]
1	318 ± 2	0.3
2	I (43%)	0.6
3	I (6%)	0.2
4	17.5 ± 5.4	1.5
5	5.9 ± 0.4	1.5
6	I (4%)	0.2
7	I (21%)	0.5
8	I (48%)	3.0
9	21.9 ± 5.6	2.2
10	160 ± 2	4.9
11	134 ± 6	7.7
12	I (33%)	6.5
13	137 ± 4	6.6
Ascorbic acid	21.2 ± 1.4	ND
Trolox	ND	1

[a] SC_{50} is half-maximal scavenging concentration.
[b] 1 ORAC unit equals the net protection of fluorescein produced by 1 μM Trolox.
[c] I is inactive and numbers in parentheses indicate the percentage of scavenging.

HHDP group (scleropentaside D, **4**) and/or galloyl group (scleropentaside E, **5**), they were more potent than positive controls in both assays. These results were consistent with the previous report that polyphenols related compounds have been known to have antioxidant properties (Okuda and Ito, 2011). HHDP and galloyl groups were an important contributor towards the radical scavenging activities of scleropentasides D–E (**4**–**5**). It has been known that the antioxidant activities of phenolic natural products are predominantly due to their redox properties (Kumarasamy et al., 2004). Therefore, phenolic glycosides having no free hydroxyl group on phenyl ring (**6–7**) were inactive, whereas the other one having free hydroxyl group (**8**) was about 3 folds more potent than Trolox. In addition flavone *C*-glycosides (**9–13**) were also exhibited the most active in the ORAC assay with 2–8 folds more potent than Trolox.

3. Concluding remarks

Among the compound isolated, the present study reports 13 secondary metabolites including five furan-2-carbonyl *C*-glycosides (**1–5**), three phenolic diglycosides (**6–8**), and five flavones *C*-glycosides (**9–13**) from the leaves and twigs of *S. pentandrum*. Phenolic glycosides and flavone *C*-glycosides are commonly isolated from plant kingdom. However, this is the first report on the occurrence of unusual structure furan-2-carbonyl *C*-glycosides, scleropentasides A–E (**1**–**5**), from plant kingdom. These specific constituents might be useful for further chemotaxonomic studies of this family.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded in DMSO-d_6 using a Bruker AV-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers, whereas MS data were obtained on a Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured with a Jasco P-1020 digital polarimeter. CD spectra were recorded on a Jasco J-815 spectropolarimeter. For column chromatography (CC), Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.), silica gel 60 (70–230 mesh, Merck), and RP-18 (50 μm, YMC) were used. HPLC (Jasco PU-980 pump) was carried out on an ODS column (21.2 × 250 mm i.d., Vertisep™ AQS) with a Jasco MD-2010 detector at 220 nm. The flow rate was 8 ml/min. The spraying reagent used for TLC was 10% H_2SO_4 in H_2O–EtOH (1:1, v/v).

4.2. Plant material

Dried leaves and twigs of *S. pentandrum* (Dennst.) Mabb. were collected in April 2010 from Khon Kaen Province, Thailand. The plant was identified by Mr. Nopporn Nontapa of Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher specimen (TK-PSKKU-0067) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

4.3. Extraction and isolation

Dried powder of leaves and twigs of *S. pentandrum* (2.4 kg) was extracted with MeOH at room temperature (each 20 L for 24 h, 3 times). The combined MeOH extracts were concentrated *in vacuo* to dryness. The resulting residue (374.4 g) was suspended in H_2O, and partitioned with Et_2O (each 1.0 L, 4 times). The aqueous soluble fraction (235.9 g) was applied to a Diaion HP-20 column, and eluted with H_2O, H_2O–MeOH (1:1, v/v), MeOH and acetone, successively. The fraction eluted with H_2O–MeOH (1:1, v/v) (38.3 g) was subjected to silica gel CC using solvent systems EtOAc–MeOH (9:1, 4.0 L), EtOAc–MeOH-H_2O (40:10:1, 4.0 L), EtOAc–MeOH–H_2O (70:30:3, 8.0 L) and EtOAc–MeOH–H_2O (6:4:1, 6.0 L), respectively, to obtain six fractions. Fraction 1 (1.3 g) was applied to a RP-18 column using a gradient solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to provide seven fractions. Fraction 1–6 was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (80:20, v/v) to afford compound **10** (113.1 mg). Fraction 2 (4.0 g) was subjected to a RP-18 column using solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to provide seven fractions. Fraction 2–1 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to provide compounds **1** (149.7 mg) and **2** (32.0 mg). Fraction 4 (12.4 g) was applied to a RP-18 column using solvent system, H_2O–MeOH (90:10 → 20:80, v/v) to afford thirteen fractions. Fraction 4–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to give compounds **3** (81.8 mg), **7** (74.2 mg) and **8** (59.6 mg). Fraction 4–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **6** (264.0 mg) and **14** (15.1 mg). Compound **11** (24.3 mg) was crystallized from fraction 4–7. Fraction 4–8 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to provide compound **12** (106.5 mg). Fraction 4–9 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to yield compound **13** (191.2 mg). Finally, fraction 5 (5.0 g) was similarly subjected to a RP-18 colum using, H_2O–MeOH (90:10 → 20:80, v/v) to afford eleven fractions. Faction 5–2 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (90:10, v/v) to provide compound **15** (15.0 mg). Fraction 5–3 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (95:5, v/v) to afford compounds **4** (21.7 mg) and **5** (22.7 mg). Fraction 5–4 was purified by preparative HPLC-ODS with solvent system H_2O–MeCN (85:15, v/v) to give compounds **9** (23.0 mg) and **12** (47.4 mg).

4.4. Scleropentaside A (*1*)

White amorphous powder; $[\alpha]_D^{30}$ −28.8 (MeOH, *c* 1.00); for ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, *m/z*: 293.0432 [M+Cl] (calcd for $C_{11}H_{14}ClO_7$, 293.0434).

4.5. Reduction of scleropentaside A (*1*)

To a solution of ketone **1** (45 mg) in EtOH (5 mL) was added solid $NaBH_4$ (40 mg) in small portion at 0 °C, and the reaction was further stirred at room temperature for an hour. The mixture was

quenched with 5% NH_4Cl aq. (5 mL) and evaporated to dryness *in vacuo* to afford a mixture of compounds **1a** and **1b** which was applied to a Diaion HP-20 column, and eluted with H_2O and MeOH, successively. The obtained solid from MeOH part was purified by preparative HPLC-ODS using solvent system H_2O–MeCN (95:5, v/v) to afford compounds **1a** (14.0 mg) and **1b** (17.6 mg).

4.6. Reduced form of scleropentaside (*1a*)

White amorphous powder; $[\alpha]_D^{28}$ +18.4 (MeOH, *c* 1.32); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, *m/z*: 295.0584 [M+Cl] (calcd for $C_{11}H_{16}ClO_7$, 295.0590).

4.7. Reduced form of scleropentaside (*1b*)

White amorphous powder; $[\alpha]_D^{28}$ −32.9 (MeOH, *c* 1.47); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 1; negative HR-ESI-TOFMS, *m/z*: 295.0580 [M+Cl] (calcd for $C_{11}H_{16}ClO_7$, 295.0590).

4.8. Scleropentaside B (*2*)

White amorphous powder; $[\alpha]_D^{29}$ −36.9 (MeOH, *c* 1.20); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 2; negative HR-ESI-TOFMS, *m/z*: 439.1008 $[M+Cl]^-$ (calcd for $C_{17}H_{24}ClO_{11}$, 439.1013).

4.9. Scleropentaside C (*3*)

White amorphous powder; $[\alpha]_D^{29}$ −39.8 (MeOH, *c* 1.00); for ^1H NMR (DMSO-d_6) spectroscopic data, see: Table 2; negative HR-ESI-TOFMS, *m/z*: 425.0849 $[M+Cl]^-$ (calcd for $C_{16}H_{22}ClO_{11}$, 425.0856).

4.10. Scleropentaside D (*4*)

White amorphous powder; $[\alpha]_D^{28}$ −42.0 (MeOH, *c* 0.19); CD (MeOH) Δε (nm) +9.1 (238), −2.7 (274), and −1.1 (312); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 3; negative HR-ESI-TOFMS, *m/z*: 559.0720 $[M-H]^-$ (calcd for $C_{25}H_{19}O_{15}$, 559.0729).

4.11. Scleropentaside E (*5*)

White amorphous powder; $[\alpha]_D^{28}$ +5.0 (MeOH, *c* 1.07); CD (MeOH) Δε (nm) +8.0 (237), −1.9 (269), +0.7 (292), and −0.6 (315); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 3; negative HR-ESI-TOFMS, *m/z*: 711.0843 $[M-H]^-$ (calcd for $C_{32}H_{23}O_{19}$, 711.0839).

4.12. 4-Hydroxy-3-methoxybenzyl alcohol 4-O-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (*7*)

Yellow amorphous powder; $[\alpha]_D^{29}$ −56.9 (MeOH, *c* 1.02); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 4; negative HR-ESI-TOFMS, *m/z*: 483.1287 $[M+Cl]^-$ (calcd for $C_{19}H_{28}ClO_{12}$, 483.1275).

4.13. 2,6-Dimethoxy-p-hydroquinone 1-O-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (*8*)

White amorphous powder; $[\alpha]_D^{28}$ −57.0 (MeOH, *c* 1.05); for ^1H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see: Table 5; negative HR-ESI-TOFMS, *m/z*: 499.1223 $[M+Cl]^-$ (calcd for $C_{19}H_{28}ClO_{13}$, 494.1224).

4.14. Determination of the absolute configurations of sugars

Monosaccharide subunits of scleropentasides B,C (**2**,**3**) and compounds **7**,**8** were obtained by acid hydrolysis. Each sample (*ca* 10 mg) in 2 N HCl-dioxane (1:1, 4 ml) was heated at 80 °C for 5 h, except for compounds **7**, **8** whose reaction conditions included was heating for 15 h. After cooling, each reaction was diluted with H_2O and extracted with EtOAc. Each aqueous layer was neutralized with 2 N KOH, concentrated to dryness providing the sugar fraction. Each of these was dissolved in H_2O (2 mL) and partitioned with *n*-BuOH (2 ml). Each organic part was concentrated *in vacuo* to a provide residue, which was analysed by HPLC (Jasco OR-2090 plus chiral detector; Vertisep™ sugar LMP column, 7.8 × 300 mm i.d.; mobile phase water; flow rate 0.4 ml/min; temperature 80 °C) and comparison of their retention times and optical rotations with authentic samples.

Hydrolysis of scleropentasides B (**2**) gave peaks corresponding to L-rhamnose at 22.5 min (positive optical rotation) and scleropentaside A (**1**) at 32.5 min (negative optical rotation).

Hydrolysis of scleropentasides C (**3**) gave peaks of D-xylose (positive optical rotation) at 20.5 min and scleropentaside A (**1**) at 32.5 min (negative optical rotation).

Hydrolysis of compound **7** gave peaks of D-glucose and D-xylose at 19.0 min and 20.8 min, respectively (both positive optical rotations).

The hydrolysis of compound **8** gave peaks of D-glucose and D-xylose at 19.0 min and 20.5 min, respectively (both positive optical rotations).

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