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Aromatic glycosides from *Eulophia andamanensis*

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ABSTRACT

Two new phenolic glycosides, eulophiosides A and B (**3**, **4**), were isolated from *Eulophia andamanensis* in addition to six known compounds. The known compounds were identified as gastrodin (**1**), vitexnegheteroin A (**2**), grammatophylloside A (**5**), grammatophylloside B (**6**), pleionoside E (**7**), and pleionoside F (**8**). Their structures were determined based on the physical data and the spectroscopic evidence including 1D and 2D NMR experiments.

1. Introduction

Eulophia andamanensis Rchb.f. (Thai name: Chang-Pa-Som-Khong) is a species of the family Orchidaceae, distributed in India and Southeast Asia. In Thai traditional medicine, the dried pseudobulbs are externally used to treat wounds for antiseptic purposes. The phytochemical investigation of this species has not been carried out. However, some species of *Eulophia* were reported to contain phenanthrenes (Tuchinda et al., 1998, 1989; Blitzke et al., 2000; Temkitthawon et al., 2017). In this article, we described the isolation and structural identification of four simple aromatic glycosides (**1–4**, Fig. 1), of which compounds **3** and **4** were new, in addition to four known glucosyloxybenzyl succinate derivatives (**5–8**) from the n-BuOH soluble fraction of this plant.

2. Results and discussion

The methanolic extract of the leaves of *E. andamanensis* was suspended in water and partitioned with Et_2O and n-BuOH. The n-BuOH fraction fraction was separated by combination of chromatographic methods to obtain two new aromatic glycosides (**3**, **4**), and six known compounds. The known compounds were identified as gastrodin (**1**) (Sahakitpicham et al., 2013), vitexnegheteroin A (**2**) (Hu et al., 2015), grammatophylloside A (**5**), grammatophylloside B (**6**) (Sahakitpicham et al., 2013), pleionoside E (**7**), and pleionoside F (**8**) (Han et al., 2019) by comparison of physical data with literature values and from spectroscopic evidence.

Compound **3** was isolated as amorphous powder, and the molecular formula, $C_{20}H_{22}O_9$, was determined by using HR-ESI-TOF-MS (*m/z*: 405.1184 $[M-H]^-$). The ^1H NMR spectroscopic data (Table 1) showed the presence of two sets of 1,4-disubstituted aromatic rings from the chemical shifts at δ_H 7.16 and 7.04 (each 2H, d, J =8.5 Hz), and δ_H 7.90 and 6.85 (each 2H, d, J =8.7 Hz); a hydroxymethyl group at $\delta\delta_H$ 4.50 (2H, s) in addition to an anomeric proton signals at δ_H 4.91 (1H, d, J =7.3 Hz) for a β-D-glucopananosyl moiety. Acid hydrolysis of **3** afforded D-glucose. In the ^{13}C NMR spectrum, the chemical shifts were similar to those of gastrodin (**1**), except for the presence of the additional signals at δ_C 116.2 (2C), 122.0 (1C), 132.9 (2C), 163.5 (1C) and 167.8 (1C). These carbons belonged to the 4-hydroxybenzoyl moiety, related to the structure part of vitexnegheteroin A (**2**) (Table 2). Comparison of the chemical shifts of **3** with those of **1** indicated that the 4-hydroxybenzoyl moiety was connected to C-6′ of the glucopyranosyl unit since this carbon atom appeared downfield at δ_C 65.0. Also, the chemical shifts of H_2-6′ of the glucopyranosyl moiety were observed downfield at δ_H 4.65 (1H, dd, J = 11.8, 1.8 Hz) and 4.35 (1H, dd, J =7.6 Hz). The assignment was confirmed by the HMBC experiment, in which the correlation was observed from H-6′ (δ_H 4.68 and 4.35) to C"-7 (δ_C 167.8) as shown in Fig. 2. Therefore, this compound was identified as 6′-*O*-4-hydroxybenzoyl-gastrodin, and named eulophioside A.

Compound **4** was isolated as amorphous powder. The molecular formula, $C_{26}H_{32}O_{14}$, was determined by using HR-ESI-TOF-MS (*m/z*: 567.1705 $[M-H]^-$). Inspection of the ^1H NMR and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated that this compound was a derivative compound of **1-3**. In addition, the ^{13}C NMR signals arising from a β-D-glucopyranosyl moiety (δ_C 103.0, 75.1, 78.1, 71.7, 78.0 and 62.8)

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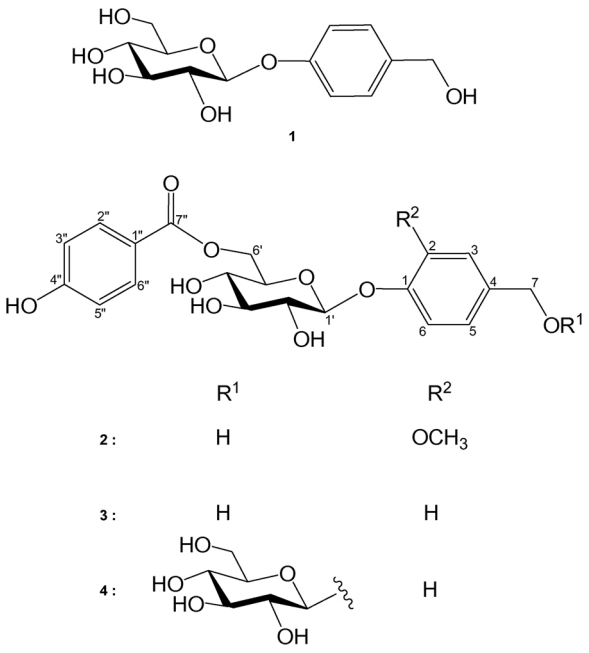

Fig. 1. Structures of compounds **1-4.**

Table 1
^{1}H NMR spectroscopic data of compounds **3** and **4** (400 MHz for ^{1}H NMR, in CD_3OD).

Position	3	4
2, 6	7.16 (2H, d, *J* =8.5 Hz)	7.24 (2H, d, *J* =8.6 Hz)
3, 5	7.04 (2H, d, *J* =8.5 Hz)	7.04 (2H, d, *J* =8.6 Hz)
7	4.50 (2H, s)	4.85 (1H, d, *J* =11.5 Hz) 4.58 (1H, d, *J* =11.5 Hz)
Glc		
1'	4.91 (1H, d, *J* = 7.3 Hz)	4.92 (1H, d, *J* = 7.3 Hz)
2'	3.51 (1H, dd, *J* = 9.2, 7.3 Hz)	3.50 (1 H)[a]
3'	3.53 (1H, dd, *J* = 9.2, 8.9 Hz)	3.51 (1 H)[a]
4'	3.44 (1H, dd, *J* = 9.4, 8.9 Hz)	3.42 (1H, dd, *J* = 9.5, 8.9 Hz)
5'	3.78 (1H, ddd, *J* = 9.4, 7.6, 1.8 Hz)	3.78 (1H, ddd, *J* = 9.5, 7.6, 2.1 Hz)
6'	4.68 (1H, dd, *J* = 11.8, 1.8 Hz) 4.35 (1H, dd, *J* = 11.8, 7.6)	4.68 (1H, dd, *J* = 11.8, 2.1 Hz) 4.35 (1H, dd, *J* = 11.8, 7.6)
Ester moiety		
2", 6"	7.90 (2H, d, *J* =8.7 Hz)	7.90 (2H, d, *J* =8.8 Hz)
3", 5"	6.85 (2H, d, *J* =8.7 Hz)	6.87 (2H, d, *J* =8.8 Hz)
Glc		
1"'		4.33 (1H, d, *J* =7.8 Hz)
2"'		3.23 (1H, dd, *J* = 9.0, 7.8 Hz)
3"'		3.28 (1 H)[a]
4"'		3.29 (1 H)[a]
5"'		3.32 (1H, m)
6"'		3.91 (1H, dd, *J* = 12.0, 2.1 Hz) 3.70 (1H, dd, *J* = 12.0, 5.6)

[a] Signal was assigned by HSQC.

were observed in **4**, as compared to **3**. The additional glucopyranosyl unit was assigned to be attached at C-7 (δ_C 71.3) of the aglycone moiety because of the glycosylation shift effect to this carbon atom (Kasai et al., 1977). Moreover, the HMBC spectrum provided further confirmation with a significant correlation from H-7 (δ_H 4.85 and 4.58, each d, *J* =11.5 Hz) of the aglycone moiety to C-1"' (δ_C 103.0) of the additional sugar unit. Besides, acid hydrolysis of **4** gave D-glucose. Consequently, the structure of compound **4** was assigned as shown, named eulophioside B.

The phytochemical investigation of *E. andamanensis* isolated eight compounds (**1-8**), which contained the glucosyloxybenzyl moiety as part of the structure. These results were closely related to those of the

Table 2
^{13}C NMR spectroscopic data of compounds **1-4** (100 MHz for ^{13}C NMR, in CD_3OD).

Position	1	2	3	4
Aglycone				
1	136.5	137.7	136.5	132.9
2	129.4	112.5	129.3	130.6
3	117.5	150.8	117.6	117.5
4	158.3	147.0	158.1	158.5
5	117.5	117.8	117.6	117.5
6	129.4	120.5	129.3	130.6
7	64.7	64.9	64.8	71.3
Glc				
1'	102.2	102.7	102.1	102.1
2'	74.8	74.9	74.8	75.0
3'	77.9	77.8	77.8	77.9
4'	71.2	72.0	71.9	72.0
5'	77.8	75.6	75.5	75.6
6'	62.3	64.9	65.0	65.0
Ester moiety				
1"		122.1	122.0	122.1
2", 6"		133.0	132.9	133.0
3", 5"		116.2	116.2	116.3
4"		163.7	163.5	163.7
7"		167.9	167.8	167.9
Glc				
1"'				103.0
2"'				75.1
3"'				78.1
4"'				71.7
5"'				78.0
6"'				62.8

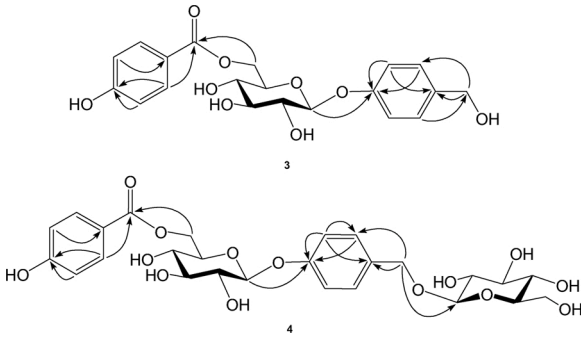

Fig. 2. HMBC correlations of compounds **3** and **4.**

secondary metabolites obtaining from members of the subfamily Epidendroideae of the orchid family, such as compounds **1**, **5**, and **6** from *Grammatophyllum speciosum*; and compounds **5–8** from *Pleione bulbocodioides* (Mochizuki et al., 1992; Simmler et al., 2011; Yoshikawa et al., 2013, 2014; Sahakitpicham et al., 2013; Han et al., 2019; Auberona et al., 2019; Liu et al., 2019). From the chemotaxonomical point of view, the occurrence of these specific constituents provided further confirmation of the typical profile of the secondary metabolites found in this subfamily.

3. Experimental

3.1. General procedures

NMR spectra were recorded in CD_3OD using Bruker AV-400 spectrometer. The MS data was obtained from the Bruker Micro TOF-LC mass spectrometer. Optical rotations were measured using a Jasco P-1020 digital polarimeter. SiliaFlash® P60 (230–400 mesh, SiliCycle Inc.), and RP-18 (50 μm, YMC) were used to create a column chromatography. HPLC (Jasco PU-980 pump) was carried out for ODS columns (column 20 mm i.d. × 250 mm length, YMC ODS-AQ) with a Jasco UV-970

detector at 210 nm and flow rates were 6 mL/min. The TLC spraying reagent was 10 % H_2SO_4 in H_2O-EtOH (1:1, v/v).

3.2. Plant material

The whole plants of *E. andamanensis* Rchb.f. were collected from Phitsanulok Province, Thailand, in July 2020. Plant specimen was identified by one researcher (TK). Voucher specimens (TK-PSKKU-0094) are deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3. Extraction and isolation

The air-dried whole plants of *E. andamanensis* (5.8 kg) were extracted three times with MeOH, and the extracted solution was then concentrated to dryness. The residue (196.1 g) was suspended in H_2O and partitioned with Et_2O and *n*-BuOH. The *n*-BuOH part (13.1 g) was applied to a silica gel column using solvent systems of EtOAc (2.0 L); EtOAc-MeOH (9:1, 6.0 L); EtOAc-MeOH-H_2O (40:10:1, 4.0 L); EtOAc-MeOH-H_2O (70:30:3, 2.0 L); and EtOAc-MeOH-H_2O (6:4:1, 2.0 L), respectively to produce seven fractions (A to G). Fraction C (170.8 mg) was applied to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to yield compound **3** (74.6 mg). Fraction D (1.25 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. Compound **1** (192.7 mg) was precipitated from sub-fraction D-1. Sub-fraction D-4 was purified by preparative HPLC-ODS with solvent system H_2O-MeCN (80:20, v/v) to afford compound **2** (7.9 mg). Fraction E (2.8 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 sub-fractions. Sub-fraction E-3 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (85:15, v/v) to provide compounds **4** (0.8 mg) and **8** (20.4 mg). Sub-fraction E-6 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (80:20, v/v) to produce compound **6** (40.2 mg). Fraction F (1.78 g) was applies to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide eight sub-fractions. Sub-fraction F-3 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (85:15, v/v) to afford compound **5** (1.2 mg). Finally, sub-fraction F-5 was purified by preparative HPLC-ODS using solvent system H_2O-MeCN (80:20, v/v) to obtain compound **7** (14.4 mg).

3.4. Eulophioside A (3)

Amorphous powder, $[\alpha]_D^{26}$ −35.2 (CH_3OH, *c* 1.17); ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2; Negative HRESITOFMS, *m/z*: 405.1184 $[M-H]^-$ (calcd for $C_{20}H_{21}O_9$, 405.1191).

3.5. Eulophioside B (4)

Amorphous powder, $[\alpha]_D^{26}$ −80.1 (CH_3OH, *c* 0.08); ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2; Negative HRESITOFMS, *m/z*: 567.1705 $[M-H]^-$ (calcd for $C_{26}H_{31}O_{14}$, 567.1719).

3.6. Determination of the absolute configuration of sugar

Each compound was dissolved in 2 N HCl–dioxane (6:1, 2.0 mL) and heated at 80 °C 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; VertisepTM sugar LMP, 7.8 mm x 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 an authentic sample. Hydrolysis of compounds **3** (2.1 mg) and **4** (0.8 mg) gave peaks corresponding to D-glucose at 19.3 min with positive optical rotation.

Declaration of Competing Interest

The authors report no declarations of interest.

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