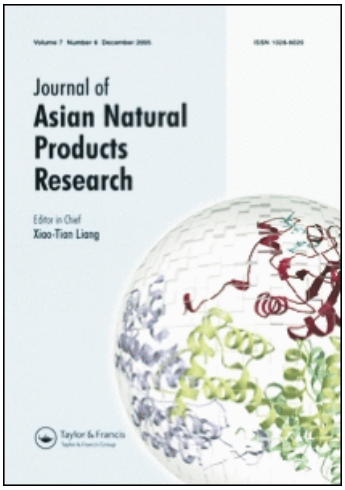

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Cuneatoside, a new megastigmane diglycoside from *Erythroxylum cuneatum* Blume

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Note

Cuneatoside, a new megastigmane diglycoside from *Erythroxylum cuneatum* Blume

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A new megastigmane diglycoside, inamoside 6′-*O*-L-α-arabinofuranoside (cuneatoside), was isolated from the leaves and branches of *Erythroxylum cuneatum* together with seven known compounds, (+)-catechin, quercetin 3-*O*-α-L-rhamnoside, apocynol **B**, (6*S*,9*R*)-roseoside, vomifoliol 9-*O*-α-L-arabinofuranosyl (1 → 6)-β-D-glucopyranoside, inamoside, and citroside A The structural elucidations were based on analyses of physical and spectroscopic data.

Keywords: *Erythroxylum cuneatum*; Erythroxylaceae; Megastigmane diglycoside; Apocynol B; Vomifoliol diglycoside; Cuneatoside

1. Introduction

Erythroxylum cuneatum Blume (Erythroxylaceae, Thai name: Krai-Tong) is a tree up to 5 m high, widely distributed in South-East Asia. It is used in Thai traditional medicine for anti-fever purposes, as well as an anti-inflammatory agent. In the course of our continuing studies on Thai medicinal plants, the constituents of this plant were investigated. In a previous study, tropane alkaloids were reported [1]. The present paper deals with the isolation and structural determination of eight compounds from the leaves and branches of this plant, including one flavan (**1**), one flavonol glycoside (**2**) and six megastigmanes (**3**–**8**), of which compound 7 is new (see figure 1).

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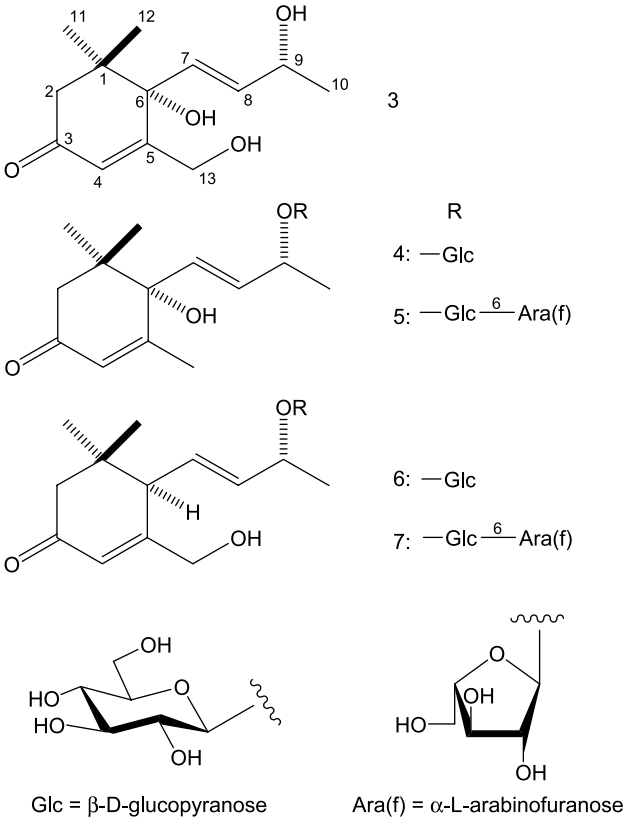

Figure 1. The structures of compounds **3**–**7**.

2. Results and discussion

The methanolic extract was suspended in H_2O and partitioned with $CHCl_3$ and n-BuOH, successively. The n-BuOH portion was subjected to a Diaion HP-20 column, using H_2O, 50% aqueous MeOH, MeOH and Me_2CO successively. The MeOH eluent was repeatedly chromatographed on columns of silica gel, RP-18, and preparative HPLC-ODS to afford eight compounds. Compounds **1** and **2** were assigned as (+)-catechin [2] and quercetin 3-*O*-α-L-rhamnoside [4], respectively. The spectroscopic and physical data of compound **3** were consistent with those of apocynol **B**, previously characterized as a product from enzymatic hydrolysis of apocynoside II [4]. It should be noted that compound **3** was isolated from a plant source for the first time. Compounds **4**, **6** and **8** were elucidated as (6*S*,9*R*)-roseoside [5], inamoside [6] and citroside A [7], respectively, by comparison of physical data with values reported in the literature and from spectroscopic evidence. Compound **5** was identical to vomifoliol 9-*O*-α-L-arabinofuranosyl (1 → 6)-β-D-glucopyranoside based on our spectroscopic and physical data. This compound was first published as a constituent of wine by Mainos et al. [8], based on the combination of the results from mass spectra and the reported occurrence of α-L-arabinofuranosyl (1 → 6)-β-D-glucopyranosyl unit in the constituents of *Vitis vinifera* [9,10], without full spectroscopic data. The NMR spectral data of compound 5 were also given here.

The molecular formula of compound **7** was determined to be $C_{24}H_{38}O_{12}$ by negative HRFAB-MS (*m/z* 517.2306 $[M - H]^-$). The ^1H NMR and ^{13}C NMR spectra indicated the presence of two sugar units and signals for the aglycone moiety. The chemical shifts of the aglycone moiety were superimposable with those of inamoside (**6**) [6], and the chemical shifts of two sugar units were the same as those of **5**, also in accordance with the reported data [11]. Moreover, the CD spectrum showed extreme values for $\Delta\epsilon$ (nm): + 15.8 (242.5) and − 0.9 (318), indicating the *R*-configuration of C-6, which was in agreement with that of **6**. The appearance of the chemical shifts of C-9 (δ_C 77.2) and C-10 (δ_C 21.1) led us to conclude the absolute configuration at C-9 to be *R* [12]. Consequently, the structure of compound **7** was identified as inamoside 6′-*O*-L-α-arabinofuranoside, namely, cuneatoside.

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded in MeOH-d_4 using a JEOL JNM α-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a union PM-1 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 Toyo Soda RI-8000 refractive index detector. The flow rate was 6 ml/min. The solvent systems were: (I) EtOAc/MeOH (9:1); (II) EtOAc/MeOH/H_2O (40:10:1); (III) EtOAc/MeOH/H_2O (70:30:3); (IV) 10–50% aqueous MeOH; and (V) 15% aqueous MeCN. The spraying reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2 Plant material

The leaves and branches of *Erythroxylum cuneatum* Blume were collected in August 2003 from the Botanical Garden, Faculty of Pharmaceutical Sciences, Prince of Songkhla University, Songkhla, Thailand. The plant was identified by Dr. Thaweesak Thitimetharoch, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (PSKKU 0048) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

3.3 Extraction and isolation

Dried leaves and branches of *E. cuneatum* (1.2 kg) were extracted three times with hot MeOH (5 l for each extraction, under reflux). The solvent was concentrated *in vacuo* to give a greenish powder (193.4 g). This residue was suspended in H_2O and partitioned with $CHCl_3$ and n-BuOH, successively. The n-BuOH portion (32.8 g) was applied to a column of Diaion HP-20 and eluted with H_2O, MeOH, and Me_2CO successively. The fraction eluted with MeOH (25.1 g) was concentrated to dryness and subjected to a silica gel column using solvent systems I (4.0 l), II (7.0 l) and III (7.0 l). Seven fractions were collected. Fraction 2 (2.3 g) was further applied to a column of RP-18 using solvent system IV to give 11 fractions

(fr. 2-1 to 2-11). Fractions 2-3 and 2-4 were combined and purified by preparative HPLC-ODS using solvent system V to afford compound **1** (376.4 mg). Fraction 3 (6.2 g) was separated by a column of RP-18 with solvent system IV, affording 10 fractions (fr. 3-1 to 3-10). Fraction 3-4 was purified by preparative HPLC-ODS with solvent system V to provide compounds **3** (6.0 mg) and **4** (28.7 mg). Compound **2** was crystallized from fraction 3-6 (325.8 mg). Similarly, fraction 4 (4.6 g) was separated on a column of RP-18 using solvent system IV to give 10 fractions (fr. 4-1 to 4-10). Fraction 4-4 was purified by preparative HPLC-ODS with solvent system V to obtain compound **5** (34.2 mg). Finally, fraction 4-8 was purified by preparative HPLC-ODS using solvent system V to give compounds **6** (13.7 mg), **7** (8.1 mg) and **8** (21.0 mg).

3.3.1 Vomifoliol 9-*O*-α-L-arabinofuranosyl (1 → 6)-β-D-glucopyranoside (5). Amorphous powder, $[\alpha]_D^{24}$?52.9° (MeOH, *c* 1.02); ^{1}H NMR and ^{13}C NMR (MeOH-d_4): see table 1; CD (MeOH, *c* 0.0019) $\Delta\epsilon$ (nm): + 12.6 (242.5) (positive max), − 0.7 (318) (negative max); Negative HRFAB-MS, *m/z*: 517.2306 $[M - H]^-$ (calcd for $C_{24}H_{37}O_{12}$, 517.2285).

3.3.2 Cuneatoside (7). Amorphous powder, $[\alpha]_D^{24}$?101.2° (MeOH, *c* 0.53); ^{1}H NMR and ^{13}C NMR (MeOH-d_4): see table 1; CD (MeOH, *c* 0.0019) $\Delta\epsilon$ (nm): + 15.8 (242.5) (positive

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **5** and **7**

No.	*5*		*7*	
	δ_H	δ_C	δ_H	δ_C
1		42.5		37.3
2	2.42 (1H, d, J = 17.1 Hz)	50.7	2.50 (1H, d, J = 16.8 Hz)	49.1
	2.06 (1H, d, J = 17.1 Hz)		2.10 (1H, d, J = 16.8 Hz)	
3		201.3		202.1
4	5.80 (1H, br s)	127.2	6.13 (1H, br s)	122.4
5		167.3		168.2
6		80.1	2.70 (1H, d, J = 7.8 Hz)	52.2
7	5.75 (1H, d, J = 3.4 Hz)	131.6	5.70 (1H, d, J = 6.3 Hz)	129.0
8	5.76 (1H, d, J = 1.4 Hz)	134.9	5.72 (1H, d, J = 8.1 Hz)	138.0
9	4.33 (1H, m)	76.8	4.35 (1H, m)	77.2
10	1.20 (3H, d, J = 6.3 Hz)	21.1	1.27 (3H, d, J = 6.3 Hz)	21.1
11	0.94 (3H, s)	24.7	1.03 (3H, s)	27.9
12	0.95 (3H, s)	23.4	1.01 (3H, s)	27.6
13	1.83 (3H, d, J = 1.2 Hz)	19.7	4.18 (1H, dd, J = 17.8, 1.5 Hz)	64.1
			4.09 (1H, dd, J = 17.8, 1.2 Hz)	
1′	4.27 (1H, d, J = 7.8 Hz)	102.6	4.33 (1H, d, J = 7.8 Hz)	102.7
2′	3.08 (1H, dd, J = 8.5, 7.8 Hz)	75.2	3.15 (1H, dd, J = 8.7, 7.8 Hz)	75.2
3′	3.22 (1H)[a]	77.9	3.31 (1H)[a]	78.0
4′	3.19 (1H, dd, J = 9.5, 9.0 Hz)	71.9	3.28 (1H, dd, J = 9.5, 9.0 Hz)	71.9
5′	3.30 (1H, m)	76.7	3.37 (1H, m)	76.8
6′	3.89 (1H)[a]	68.0	3.97 (1H)[a]	68.0
	3.49 (1H, dd, J = 11.0, 5.6 Hz)		3.58 (1H, dd, J = 11.5, 5.9 Hz)	
1″	4.86 (1H, br s)	109.9	4.95 (1H, br s)	109.9
2″	3.90 (1H)[a]	83.0	3.97 (1H)[a]	83.3
3″	3.73 (1H, dd, J = 5.6, 3.2 Hz)	78.9	3.81 (1H, dd, J = 6.1, 3.4 Hz)	79.0
4″	3.87 (1H)[a]	85.9	3.94 (1H)[a]	85.9
5″	3.66 (1H, dd, J = 12.0, 3.2 Hz)	63.1	3.74 (1H, dd, J = 11.7, 3.4 Hz)	63.1
	3.55 (1H, dd, J = 12.0, 5.4 Hz)		3.63 (1H, dd, J = 11.7, 5.4 Hz)	

[a] Chemical shifts obtained approximately by HSQC.

max), −0.9 (318) (negative max); Negative HRFAB-MS, *m/z*: 517.2306 $[M - H]^-$ (calcd for $C_{24}H_{37}O_{12}$, 517.2285).

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