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NEW GLYCOSIDES OF ERIODICTYOL FROM *Dracocephalum palmatum*

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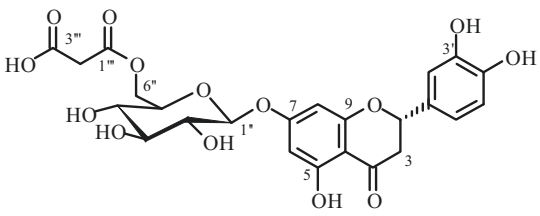
Two new glycosides of eriodictyol were isolated from the aerial part of Dracocephalum palmatum *and identified using UV, NMR, and CD spectroscopy and mass spectrometry as (*S*)-eriodictyol-7-*O*-(6″-*O*-malonyl)-β-D-glucopyranoside (pyracanthoside-6″-*O*-malonate, **1**) and (*S*)-eryodictyol-7-*O*-(4″-*O*-malonyl)-β-D-glucopyranoside (pyracanthoside-4″-*O*-malonate, **2**). The stabilities of **1** and **2** were studied under simulated stomach and intestinal conditions.*

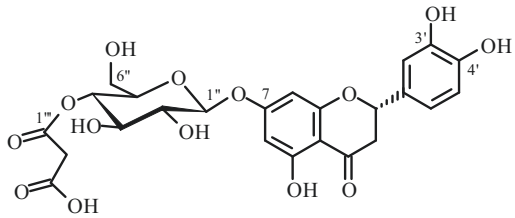
Keywords: *Dracocephalum palmatum*, Lamiaceae, (*S*)-eriodictyol-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside, (*S*)-eriodictyol-7-*O*-(4″-*O*-malonyl)-*β*-D-glucopyranoside, pyracanthoside-6″-*O*-malonate, pyracanthoside-4″-*O*-malonate.

Dracocephalum palmatum Steph. ex Willd. (Lamiaceae) is indigenous to northern Yakutia and is used by nomads as a medicinal and food plant [1]. Previous chemical investigations of *D. palmatum* identified phenylpropanoids, coumarins, flavonoids, triterpenoids [1, 2], lipids, essential oil, simple phenols, and carbohydrates [3]. Herein, two new flavonoids isolated from *D. palmatum* are reported.

Chromatographic separation of fraction F3-2 (prep. HPLC, CC) isolated eight compounds (**1**–**8**) including the known flavonoids acacetin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (agastachoside, **3**) [4], apigenin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**4**) [5], luteolin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**5**) [6], acacetin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**6**) [7], apigenin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**7**) [7], luteolin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**8**) [7], and two new compounds **1** and **2**.



1



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Compound **1** had molecular formula $C_{24}H_{24}O_{14}$ based on ^{13}C NMR spectroscopic and mass spectrometric data (*m*/*z* 535, $[M - H]^-$). Acid hydrolysis of **1** gave eriodictyol and D-glucose. The ESI-MS contained fragment ions with *m*/*z* 449 and 287 that were consistent with loss of species with *m*/*z* 86 (malonyl) and 162 (glucosyl) [8]. PMR and ^{13}C NMR spectra were similar to that of eriodictyol-7-*O*-*β*-D-glucopyranoside (pyracanthoside, miscanthoside; **1a**) [9] with the exception of additional resonances [δ_H 3.26 (2H, s); δ_C 41.2, 167.1, 167.8] due to the malonyl group (Table 1) [10].

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TABLE 1. PMR (500 MHz) and ^{13}C NMR Spectra (125 MHz) of **1** and **2** (MeOH-d_4, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
		Eriodictyol		
2	5.46 (1H, dd, J = 12.0, 3.0)	78.5	5.33 (1H, dd, J = 12.1, 3.0)	78.6
3	3.15 (1H, dd, J = 17.1, 12.0)	42.0	3.12 (1H, dd, J = 17.0, 12.1)	42.2
	2.70 (1H, dd, J = 17.1, 3.0)		2.71 (1H, dd, J = 17.0, 3.0)	
4	–	197.0	–	196.8
5	–	163.0	–	163.2
6	6.03 (1H, m)	96.4	6.00 (1H, m)	96.2
7	–	165.5	–	165.0
8	6.08 (1H, m)	95.2	6.12 (1H, m)	95.5
9	–	162.6	–	162.3
10	–	102.5	–	102.4
1′	–	129.4	–	129.8
2′	6.95 (1H, m)	115.6	7.01 (1H, m)	115.3
3′	–	145.7	–	145.4
4′	–	145.1	–	145.0
5′	6.75 (2H, m)	114.8	6.72 (2H, m)	114.3
6′		117.6		117.5
		7-*O*-*β*-D-Glucopyranosyl		
1″	5.06 (1H, d, J = 7.1)	99.7	5.02 (1H, d, J = 7.0)	99.8
2″	3.36–3.39 (2H, m)	73.1	3.31–3.40 (2H, m)	72.7
3″		76.3		75.2
4″	3.30 (1H, m)	69.9	4.40 (1H, m)	71.2
5″	3.72 (1H, m)	74.1	3.75 (1H, m)	73.4
6″	4.46 (1H, dd, J = 1.7, 12.1)	64.1	3.61 (1H, d J = 12.0)	60.0
	4.12 (1H, dd, J = 7.0, 12.1)		3.51 (1H, dd, J = 5.4, 12.0)	
	6″-*O*-Malonyl		4″-*O*-Malonyl	
1‴	–	167.1	–	166.9
2‴	3.26 (2H, s)	41.2	3.24 (2H, s)	41.0
3‴	–	167.8	–	167.9

The acyl group was positioned on C-6″ according to weak-field shifts of glucose resonances for C-6″ (δ 64.1) and H-6″ [δ 4.46 (1H, dd, J = 1.7, 12.1 Hz), 4.12 (1H, dd, J = 7.0, 12.1 Hz)] as compared to resonances of **1a** (δ_C 60.4; δ_H 3.66, 3.43) [9] and correlations in HMBC spectra between glucose H-6″ (δ_H 4.12, 4.46) and malonyl C-1‴ (δ_C 167.1) [11]. The absolute configuration of the eriodictyol C-2 phenyl was determined using circular dichroism. A positive Cotton effect at 327 nm and a negative effect at 298 nm indicated that C-2 had the *S*-configuration [12]. Thus, the structure of **1** was determined as (*S*)-eriodictyol-7-*O*-(6″-*O*-malonyl)- *β*-D-glucopyranoside or pyracanthoside-6″-*O*-malonate.

Compound **2** had molecular formula $C_{24}H_{24}O_{14}$ and mass and UV spectra that were similar to those of **1**. This indicated that **2** was also an eriodictyol-7-*O*-*β*-D-glucopyranoside derivative with a malonic-acid substituent. A comparison of PMR and ^{13}C NMR spectra of **2** and those of **1** and **1a** showed that they were similar. However, weak-field shifts were observed for resonances of glucopyranose C-4″ (δ 71.2) and H-4″ [δ 4.40 (1H, m)] relative to those of **1a** (δ_C 69.1; δ_H 3.18) (Table 1). HMBC spectra showed a correlation between glucopyranose H-4″ (δ 4.40) and δ_C 166.9, indicating that the malonyl moiety was bonded to glucopyranose C-4″ [10].

The absolute configuration of C-2 was determined as *S* from the positive Cotton effect at 325 nm and a negative effect at 300 nm. The results established the structure of **2** as (*S*)-eriodictyol-7-*O*-(4″-*O*-malonyl)-*β*-D-glucopyranoside or pyracanthoside-4″-*O*-malonate.

Esters of malonic acid and eriodictyol glycosides have not previously been isolated from plants. Until now, the only known flavanone containing a malonic-acid fragment was naringin-6″-malonate from leaves and fruit of *Citrus paradisi* Macfad. [13] and fruit of *C.* × *aurantium* L. (Rutaceae) [14].

TABLE 2. Products from Reactions of **1** and **2** with Simulated Physiological Media[1,2]

Simulated medium	**1**	**2**
Control (H_2O)	**1** (100)	**2** (100)
Stomach juice	**1** (80), **2** (15), **1a** (5)	**1** (62), **2** (31), **1a** (7)
Intestinal juice	**1** (21), **2** (25), **1a** (53), **1b** (< 1)	**1** (39), **2** (2), **1a** (58), **1b** (< 1)
Intestinal microflora	**1a** (2), **1b** (98)	**1a** (1), **1b** (99)

[1]Component peak area (% of total peak areas) is shown in parentheses; [2]**1a**, eriodictyol-7-*O*-*β*-D-glucopyranosyl; **1b**, eriodictyol.

The stabilities of **1** and **2** under simulated gastrointestinal-tract (GIT) conditions showed that both compounds in stomach juice underwent chemical reactions involving acyl migration and deacylation (Table 2). Compound **1** with malonyl on glucopyranose C-6″ was more stable than **2** to acyl migration. Incubation in stomach juice converted ~15% of **1** into **2** whereas **2** was >60% converted into **1**. Only 5–7% of the total compound mass was deacylated under these same conditions. However, the content of deacylated **1a** increased to 53–58% in juice of later GIT stages. Intestinal microflora caused more extensive changes of **1** and **2** that led to total hydrolysis of the compounds to eriodictyol (**1b**). Previously, the same transformation pathway in physiological fluids was demonstrated for naringenin and hesperetin glycosides [15] and is probably common for flavanone glycosides.

EXPERIMENTAL

General comments were published [1–3]. Spectrophotometric studies used an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia). Circular dichroism spectra were recorded on a J-1500 spectrometer (JASCO, Easton, MD, USA). Mass spectrometric studies used an LCMS-8050 TQ-mass-spectrometer (Shimadzu, Columbia, MD, USA). The conditions were electrospray ionization (ESI, negative-ion mode); ESI interface temperature 300°C; desolvation line temperature 250°C; heating block 400°C, sprayer-gas (N_2) flow rate 3 L/min; heating-gas (air) flow rate 10 L/min; collision-induced dissociation (CID) gas (Ar) pressure 270 kPa; Ar flow rate 0.3 mL/min; capillary potential 3 kV; and mass scan range (*m*/*z*) 100–1000. NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian, Palo Alto, CA, USA). Preparative HPLC used a Summit liquid chromatograph (Dionex, Sunnyvale, CA, USA); LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm; Supelco, Bellefonte, PA, USA); mobile phase H_2O (A) and MeCN (B); flow rate 1 mL/min; column temperature 30°C; and UV detector at 280 and 330 nm.

Isolation of 1–8. The extraction conditions for obtaining fraction F3-2 were described before [1]. Fraction F3-2 was chromatographed over a polyamide column (CC, 4 × 120 cm) with elution by H_2O–MeOH mixtures (100:0→0:100) to produce subfractions F3-2-1–F3-2-10. Subfractions F3-2-2 and F3-2-3 were combined and separated over Sephadex LH-20 (CC, 3 × 110 cm, MeOH–H_2O eluent, 100:0→0:100) and by prep. HPLC [gradient mode (%B): 0–10 min, 10–70%; 10–60 min, 70–100%] to isolate acacetin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (agastachoside, **3**, 17 mg) [4]; apigenin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**4**, 10 mg) [5], and luteolin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**5**, 22 mg) [6]. Subfractions F3-2-7–F3-2-9 were combined and chromatographed over RP-SiO_2 (CC, 3 × 100 cm, H_2O–MeCN eluent, 100:0→0:100) and by prep. HPLC [gradient mode (%B): 0–35 min, 5–45%; 35–50 min, 45–60%; 50–70 min, 60–90%; 70–90 min, 90–100%]. This produced **1** (18 mg), **2** (10 mg), acacetin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**6**, 15 mg) [7], apigenin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**7**, 14 mg) [7], and luteolin-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (**8**, 27 mg) [7].

(*S*)-Eriodictyol-7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside (6″-*O*-malonylpyracanthoside, 1). $C_{24}H_{24}O_{14}$. (–)ESI-MS (*m*/*z*): 535 $[M - H]^-$, 449 $[(M - H) - 86]^-$, 287 $[(M - H) - 86–162]^-$. UV spectrum (MeOH, λ_{max}, nm): 284. CD spectrum (MeOH, *c* $4.01 \cdot 10^{-4}$ M; λ_{max}, Δε): 298 (–25.4), 327 (+15.2). Table 1 lists PMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data.

(*S*)-Eriodictyol-7-*O*-(4″-*O*-malonyl)-*β*-D-glucopyranoside (4″-*O*-malonylpyracanthoside, 2). $C_{24}H_{24}O_{14}$. (–)ESI-MS (*m*/*z*): 535 $[M - H]^-$, 449 $[(M - H) - 86]^-$, 287 $[(M - H) - 86 - 162]^-$. UV spectrum (MeOH, λ_{max}, nm): 283. CD spectrum (MeOH, *c* $3.92 \cdot 10^{-4}$ M; λ_{max}, Δε): 300 (–21.7), 325 (+16.1). Table 1 lists PMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data.

Acid Hydrolysis of 1 and 2. The compound (2 mg) was dissolved in TFA (5%, 3 mL) and heated at 110°C for 2 h. The hydrolysate was concentrated *in vacuo*, dissolved in MeOH, and chromatographed over polyamide (CC, 10 g) with elution by H_2O (50 mL, eluate I) and EtOH (90%, 50 mL, eluate II). The eluates were concentrated *in vacuo* and analyzed by HPLC (conditions 1, monosaccharides as 3-methyl-1-phenyl-2-pyrazolin-5-one derivatives [3]; conditions 2, phenolic compounds). Eluate 1 was also analyzed to determine D- and L-monosaccharides after derivatization with L-tryptophan [16]. Eluate I from hydrolysis of **1** and **2** contained D-glucose (t_R 12.52 min); eluate II, eriodictyol (t_R 5.52 min).

HPLC. Conditions 1: ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm; Metrohm AG); mobile phase: NH_4OAc (100 mM, pH 4.5) (A) and MeCN (B); gradient mode (%B): 0–20 min, 20–26%; flow rate 150 μL/min; column temperature 35°C; and UV detector at 250 nm. The retention times of reference standards (t_R, min) were mannose 6.83; glucose 12.52; and galactose 13.54. Conditions 2: ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm; Metrohm AG); mobile phase $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B); gradient mode (%B): 0–18 min, 25–100%; 18–20 min, 100%; flow rate 150 μL/min; column temperature 35°C; and UV detector at 270 nm. Retention times of reference standards (t_R, min) were eriodictyol 5.53; naringenin 6.72; sakuranetin 8.82; and isosakuranetin 9.45.

The stabilities of the compounds were studied using simulated GIT media that were described by us before [17]. The composition of the reaction products was determined using analytical HPLC [3].

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