ent-Pimarane-Type Diterpenoids from *Siegesbeckia orientalis* L.

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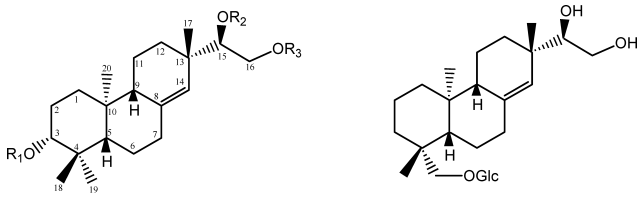
A new *ent*-pimarane glucoside, named hythiemoside B (4), was isolated from the aerial part of *Siegesbecikia orientalis* L. (Asteraceae) together with four known *ent*-pimarane-type diterpenoids: darutigenol (1), darutoside (2), hythiemoside A (3), and *ent*-(15*R*),16,19-trihydroxypimar-8(14)-ene 19-*O*-β-D-glucopyranoside (5). The structure of the new compound was elucidated by spectroscopic analyses and chemical transformation. The NMR data of compounds 1 (^{1}H-) and 5 (^{1}H- and ^{13}C-) were also compiled in this study on the basis of 2D experiments.

Key words *Siegesbeckia orientalis*; Asteraceae; *ent*-pimarane; diterpenoid; hythiemoside

The herbaceous plant *Siegesbeckia orientalis* L. (syn. *S. glutinosa* WALL.)[1] of the family Asteraceae, is the only species of the genus *Siegesbeckia* L. identified in Vietnam.[2] Known as *Hy thiem* in Vietnam, the plant is widely distributed in North Vietnam and its aerial parts have been used in traditional medicine to treat rheumatism, acute arthritis, furunculosis, impetigo and menstrual disorders.[2] Hundreds of tons of *S. orientalis* plant (Herba Siegesbeckiae) have been exploited annually in Vietnam for medicinal purposes.[2] Melampolides,[3—5] germacranolides,[4] *ent*-pimarenes,[4,5] and geranylnerol derivatives[5] were reported from *S. orientalis*. β-Sitosterol, stigmasterol and their 3-*O*-β-D-glucosides, rutin, caffeic acid and two *ent*-pimarane glucosides were isolated in our preliminary study on the *S. orientalis* species growing in Vietnam.[6,7] In the present reinvestigation, we report the isolation of a new *ent*-pimarane glucoside **4** (Fig. 1), named hythiemoside B, together with four known *ent*-pimarane-type diterpenoids: darutigenol (**1**),[4] darutoside (**2**),[6] *ent*-16-acetoxypimar-8(14)-ene-3β,(15*R*)-diol 3-*O*-β-D-glucopyranoside (**3**),[7] which is newly named hythiemoside A, and *ent*-(15*R*),16,19-trihydroxypimar-8(14)-ene 19-*O*-β-D-glucopyranoside (**5**)[8] from the MeOH extract of the aerial parts of *S. orientalis*.

Compound **4** was isolated as a white amorphous powder, $[\alpha]_D^{25}$ −110° (*c*=0.10, MeOH). Its molecular formula was determined as $C_{28}H_{46}O_9$ from the quasi molecular ion peak at *m*/*z* 525.3071 $[M-H]^-$ (Calcd 525.3064) in the negative-ion high-resolution (HR)-FAB-MS. The IR spectrum indicated the presence of a hydroxy (3396 cm^{-1}), an ester (1722 cm^{-1}) and a double bond (1641 cm^{-1}). The ^{1}H- (Table 1) and ^{13}C-NMR (Experimental) spectra of **4** showed the presence of four methyl singlets (δ_H 0.84, 0.86, 0.89, 1.02) and a 1,2-dioxygenated ethyl side chain [δ_H 5.02 (dd, *J*=9.2, 2.5 Hz), δ_C 79.5; δ_H 3.58 (dd, *J*=12.2, 9.2 Hz), 3.75 (dd, *J*=12.2, 2.5 Hz), δ_C 62.1 (correlations from the heteronuclear single quantum correlation (HSQC) spectrum of **4**)], suggesting a pimarane skeleton of **4**. An anomeric carbon (δ_C 101.7), attached to a proton doublet at δ_H 4.30 in the HSQC experiment, was detected. Complete set of the ^{1}H- (Table 1) and ^{13}C-NMR signals (δ_C 101.7, 74.9, 78.0, 71.7, 77.4, 62.8) assigned a glucopyranosyl unit. The absolute configuration of the D-glucose, obtained from **4** by enzymatic hydrolysis, was determined by a direct co-TLC method of its thiazolidine derivative [methyl 2-(D-gluco-pentahydroxypentyl)-thiazolidine-(4*R*)-carboxylate],[9] and a β-configuration of the anomeric position was deduced from the value of the coupling constant of H-1′ (d, *J*=8.0 Hz). Additionally, a trisubstituted double bond [δ_H 5.15 (s), δ_C 140.9 and 127.8] and an oxymethine [δ_H 3.35 (dd, *J*=11.7, 3.9 Hz) and δ_C 85.8] were also present in the structure of **4**. A comparison of the ^{13}C-NMR spectra of **4** and darutoside (**2**),[10] revealed the location of the double bond at C-8(C-14) and the oxymethine at C-3, meanwhile the β-glucopyranoside was located at C-3 on the basis of the detection of long-range heteronuclear multiple bond correlation (HMBC) cross-peaks between H-3 (δ_H 3.35) and C-1′ (δ_C 101.7) and beween H-1′ (δ_H 4.30) and C-3 (δ_C 85.8) (Fig. 2). HMBC cross-peaks between H-3 and C-18 (δ_C 28.9), between H-18 (δ_H 1.02) and C-3, as well as between H-14 (δ_H 5.15) and C-9 (δ_C 51.7), C-12 (δ_C 33.3) and C-17 (δ_C 23.2) (Fig. 2) confirmed the placement of H-3 and C-8/C-14 double bond. The additional acetyl signal [δ_H 2.06 (s), δ_C 172.8 and 20.9] in **4** when compared with darutoside (**2**) deshielded C-15 position (δ_H 5.02, δ_C 79.5) and



	R_1	R_2	R_3
1	H	H	H
2	Glc	H	H
3	Glc	H	Ac
4	Glc	Ac	H

Fig. 1. Chemical Structures of *ent*-Pimarane-Type Diterpenoids

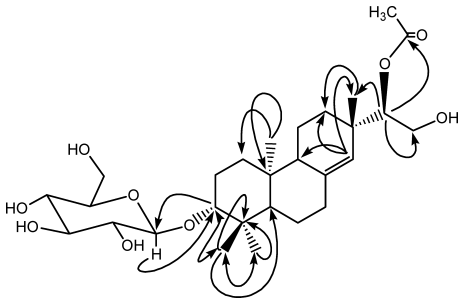


Fig. 2. HMBC Correlations of Compound **4**

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Table 1. ^{1}H-NMR Spectroscopic Data of Compounds **1**, **4** and **5** (δ in ppm, J in Parentheses in Hz, 500 MHz, CD_3OD)

H	**1**	**4**	**5**
1	1.10 td (12.9, 4.8)	1.13 m	0.99 ddd (13.3, 13.1, 3.5)
	1.61[a)]	1.73[a)]	1.63 m
2	1.50[a)]	1.52[a)]	1.32 dquint. (14.2, 3.5)
	1.50[a)]	1.75[a)]	1.44[a)]
3	3.10 dd (11.0, 4.8)	3.35 dd (11.7, 3.9)	0.85[a)]
			1.88[a)]
5	0.97 dd (10.3, 1.8)	1.08 dd (12.4, 2.5)	1.11 dd (13.1, 2.9)
6	1.28 qd (12.9, 4.4)	1.38 m	1.25 qd (13.1, 4.4)
	1.53[a)]	1.61 m	1.64[a)]
7	1.95 m	1.95[a)]	1.91 ddd (13.4, 13.1, 5.0)
	2.18 dt (13.4, 2.1)	2.18 br d (14.2)	2.17 br d (13.4)
9	1.61[a)]	1.70[a)]	1.65[a)]
11	1.44[a)]	1.50[a)]	1.46[a)]
	1.44[a)]	1.50[a)]	1.46[a)]
12	0.82 m	0.84 m	0.80[a)]
	1.88 br d (13.1)	1.97[a)]	1.88 br d (13.3)
14	5.07 s	5.15 s	5.04 s
15	3.46 br d (8.9)	5.02 dd (9.2, 2.5)	3.47 dd (11.3, 2.3)
16	3.36 dd (10.8, 8.9)	3.58 dd (12.2, 9.2)	3.35 dd (11.3, 9.2)
	3.58 br d (10.8)	3.75 dd (12.2, 2.5)	3.58 dd (9.2, 2.3)
17	0.74 s	0.89 s	0.74 s
18	0.89 s	1.02 s	0.94 s
19	0.72 s	0.84 s	3.14 d (9.7)
			4.07 d (9.7)
20	0.71 s	0.86 s	0.73 s
15-OAc		2.06 s	
Glc 1′		4.30 d (8.0)	4.08 d (8.1)
2′		3.13 dd (8.0, 8.9)	3.07 dd (8.1, 8.7)
3′		3.31[a)]	3.23[a)]
4′		3.29[a)]	3.21[a)]
5′		3.20[a)]	3.14[a)]
6′a		3.63 dd (11.7, 5.5)	3.57 dd (11.9, 5.7)
b		3.82 dd (11.7, 2.3)	3.76 dd (11.9, 2.3)

a) Overlapped signals.

thus was assigned at C-15. It was supported by HMBC cross-peaks between H-15 and C-17, C-16 (δ_C 62.1), and the carbonyl carbon of the acetyl group (δ_C 172.8). On alkaline hydrolysis, compound **4** was converted quantitatively to **2**, identified by comparison of the optical rotation,[4)] ^{13}C-NMR data with literature values,[10)] and ^{1}H-NMR data with those of authentic sample from the same plant, thus **4** was ascertained to possess *enantio* absolute stereochemistry and the configuration at C-15 was assigned as *R*. Therefore, the structure of hythiemoside B (**4**) was determined to be *ent*-(15*R*)-acetoxypimar-8(14)-ene-3β,16-diol 3-*O*-β-D-glucopyranoside as depicted in Fig. 1.

Darutigenol (**1**) was isolated with physical (mp, $[\alpha]_D$)[4)] and ^{13}C-NMR spectroscopic data[10)] comparable with literature values. Additionally, in this study, the assignment of the ^{1}H-NMR signals of **1** was compiled in Table 1 on the basis of the correlations observed in the HSQC spectrum. After structural elucidation of the *ent*-pimarane glucoside **5** by spectral means which included 2D NMR experiments HSQC and HMBC, it was determined that a compound under the name *ent*-15,16,19-trihydroxypimar-8(14)-en-19-*O*-β-D-glucopyranoside had appeared in the literature.[8)] Since the spectroscopic data of the title compound are not readily available for the comparison with our data, we included the ^{1}H- and ^{13}C-NMR data of **5** in Table 1 and Experimental, respectively.

Table 2. ^{13}C-NMR Spectroscopic Data of Compounds **4** and **5** (δ in ppm, 400 MHz, CD_3OD)

C	**4**	**5**
1	36.9	40.3
2	24.2	19.5
3	85.8	37.2
4	39.2	39.2
5	55.1	57.3
6	23.6	23.6
7	37.8	37.5
8	140.9	139.9
9	51.7	52.5
10	38.9	39.0
11	19.2	19.7
12	33.3	33.3
13	37.8	38.4
14	127.8	129.4
15	79.5	77.5
16	62.1	64.3
17	23.2	23.0
18	28.9	28.2
19	17.1	73.6
20	15.1	16.4
15-OAc	20.9	172.8
Glc-1	101.7	105.0
Glc-2	74.9	75.2
Glc-3	78.0	78.2
Glc-4	71.7	71.6
Glc-5	77.4	77.7
Glc-6	62.8	62.7

Experimental

General Procedure Optical rotations were measured on a Union Giken PM-101 digital polarimeter at 25 °C. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 500 spectrometer and a JEOL JNM α-400 NMR spectrometer, respectively. Negative-ion HR-FAB-MS were measured on a JEOL SX-102 mass spectrometer with PEG-400 as the calibration matrix. HPLC was carried out with a JASCO PU-1580 pump and UV-2075 Plus detector (set at 210 nm) on YMC ODS columns (150×4.6 mm i.d. in analytical and 150×20 mm i.d. in preparative scales) at the corresponding flow rates of 0.5 and 5 ml/min. Sephadex LH-20 (25—100 μm, Fluka) and reversed-phase octadecyl silica (ODS) gel (YMC) were used for open column chromatography. TLC was carried out on Merck precoated TLC sheets (silica gel 60 F_{254}), and detected by spraying with 10% H_2SO_4 in 50% EtOH, followed by heating on a hot plate at 200 °C.

Plant Material The aerial parts of *S. orientalis* were collected and identified by Dr. Tran Ngoc Ninh of the Institute of Ecology and Biological Resources, Vietnam National Center of Natural Science and Technology, in Ha Giang province, Northern Vietnam, in October 2002. A voucher specimen (HCTN No. 1002) was deposited in the Herbarium of the same Institute.

Extraction and Isolation The powdered, dried aerial parts of *S. orientalis* (100 g) were extracted three times, each time for two days, with MeOH by percolation at room temperature. The combined extracts were concentrated under reduced pressure and the obtained residue (4.0 g) was subjected to column chromatography on Sephadex LH-20 eluted with MeOH. Three fractions were collected on the basis of the TLC pattern (developing system: $CHCl_3$–MeOH, 10 : 1). Fraction 2 (2.32 g) was chromatographed on ODS (MeOH–H_2O, 7 : 3) to give six fractions. Fraction 1 (0.74 g) was subjected to an ODS column chromatography (CH_3CN–H_2O, 11 : 9) and the three fractions obtained were purified repeatedly by ODS HPLC (MeOH–H_2O, 7 : 3) to give darutoside (**2**) (36.3 mg), hythiemoside A (**3**) (16.0 mg), and hythiemoside B (**4**) (16.8 mg). The same separation procedures were applied for fraction 2 (0.47 g) to afford darutigenol (**1**) (40 mg), **2** (68.7 mg), **3** (26.2 mg), **4** (17.4 mg) and *ent*-(15*R*),16,19-trihydroxypimar-8(14)-ene 19-*O*-β-D-glucopyranoside (**5**) (6.0 mg).

Hythiemoside B (**4**): White amorphous powder. $[\alpha]_D^{25}$ −110° (c=0.10, MeOH). IR ν_{max} (film) cm^{-1}: 3396, 2939, 2875, 1722, 1641, 1369, 1257, 1077, 1033. ^{1}H-NMR: see Table 1. ^{13}C-NMR (CD_3OD) δ: 15.1 (C-20), 17.1 (C-19), 19.2 (C-11), 20.9 ($\underline{C}H_3COO$–), 23.2 (C-17), 23.6 (C-6), 24.2 (C-2), 28.9 (C-18), 33.3 (C-12), 36.9 (C-7), 37.8 (C-1), 38.4 (C-13), 38.9 (C-10), 39.2 (C-4), 51.7 (C-9), 55.9 (C-5), 62.1 (C-16), 62.8 (C-6′), 71.7 (C-4′), 74.9 (C-2′), 77.4 (C-5′), 78.0 (C-3′), 79.5 (C-15), 85.8 (C-3), 101.7 (C-1′), 127.8 (C-14), 140.9 (C-8), 172.8 ($CH_3\underline{C}OO$–). Negative-ion HR-FAB-MS: m/z 525.3071 $[M-H]^-$ (Calcd for $C_{28}H_{45}O_9$: 525.3064).

ent-(15*R*),16,19-Trihydroxypimar-8(14)-ene 19-*O*-β-D-Glucopyranoside (**5**): White amorphous powder. $[\alpha]_D^{25}$ −35.5° (c=0.60, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2936, 2874, 1642, 1078, 1032. ^{1}H-NMR: see Table. ^{13}C-NMR (CD_3OD) δ: 16.4 (C-20), 19.5 (C-2), 19.7 (C-11), 23.0 (C-17), 23.6 (C-6), 28.2 (C-18), 33.3 (C-12), 37.2 (C-3), 37.5 (C-7), 38.4 (C-13), 39.0 (C-10), 39.2 (C-4), 40.3 (C-1), 52.5 (C-9), 57.3 (C-5), 62.7 (C-6′), 64.3 (C-16), 71.6 (C-4′), 73.6 (C-19), 75.2 (C-2′), 77.5 (C-15), 77.7 (C-5′), 78.2 (C-3′), 105.0 (C-1′), 129.4 (C-14), 139.9 (C-8). Negative-ion HR-FAB-MS: m/z 483.2990 $[M-H]^-$ (Calcd for $C_{26}H_{43}O_8$: 483.2958).

Enzymatic Hydrolysis of 4 Compound **4** (2.6 mg), β-D-glucosidase from almond (10 mg), and H_2O (1 ml) were mixed and incubated at 37 °C for 24 h. The mixture was washed with EtOAc and the aqueous layer was centrifuged to remove the precipitate. The supernatant which showed a spot of glucose (Rf 0.2, $CHCl_3$–MeOH–H_2O 15 : 6 : 1) on silica gel TLC was concentrated *in vacuo* and the residue was treated with L-cysteine methyl ester hydrochloride (3.0 mg) in pyridine (100 μl) at 60 °C for 1 h. After removal of the solvent, the residue was dissolved in water (200 μl) and extracted with *n*-BuOH (200 μl). Two spots observed on the TLC (Rf 0.49 and 0.38, $CHCl_3$–MeOH–H_2O 15 : 6 : 1) of the organic layer containing the thiazolidine derivative established the absolute configuration of the sugar as D-glucose. Standard thiazolidine derivatives obtained from D- and L-glucose gave spots at Rf 0.49 and 0.38, and 0.45, respectively.

Alkaline Hydrolysis of 4 Compound **4** (10 mg) was dissolved in MeOH (5 ml) and KOH (10 mg) was added. The reaction was stirred at 25 °C for one week and the solvent was removed to give a product in quantitative yield which was identified as **2** by comparison of the optical rotation ($[\alpha]_D^{25}$ −36° (c=0.10, MeOH), lit.[4] $[\alpha]_D$ −35°), ^{13}C-NMR data with literature values,[10] and ^{1}H-NMR data with those of an authentic sample from the same plant.

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