DOI 10.1007/s10600-018-2311-5
Chemistry of Natural Compounds, Vol. 54, No. 2, March, 2018

SESQUITERPENE LACTONES FROM *Vernonia cinerea*

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*A new hirsutinolide-type sesquiterpene lactone, 8α-(2′Z-tigloyloxy)-1α-methoxyhirsutinolide (**1**), and a new naturally occurring 8α-(2′-hydroxymethylacryloyloxy)-1α-methoxyhirsutinolide-13-*O*-acetate (**2**) were isolated from the $CHCl_3$ extract of the aerial parts of* Vernonia cinerea *(Asteraceae). The structures of the new compounds were determined by 1D and 2D NMR and MS experiments, as well as by comparison of their data with the published values.*

Keywords: *Vernonia cinerea*, Asteraceae, sesquiterpene lactone.

The genus *Vernonia* comprises about one thousand species. The plant *Vernonia cinerea* Less. (Asteraceae) is distributed mainly in Africa and Southeast Asia. It is commonly used for treatment of various diseases such as asthma, bronchitis, cough, malaria fever, skin, and liver diseases [1–3]. There have been phytochemical reports on the diverse compounds from this species, including sesquiterpene lactones [4, 5], flavonoids [6], and triterpenes [7]. Some of the compounds have been shown to have anticancer [8], antimalarial [5], and antifeedant biological activities [9]. In our previous research, we have reported on the sesquiterpene lactones from the aerial parts of *V. cinerea*, along with their structure–activity relationships and their anti-inflammation and anticancer properties [10, 11]. In our ongoing chemical and biochemical studies on this plant, a new hirsutinolide-type sesquiterpene lactone (**1**), together with a new naturally occurring compound (**2**), has been isolated. This paper reports the isolation and structure elucidation of the new compound.

Compound **1** was obtained as a white amorphous powder, and the molecular formula, $C_{21}H_{28}O_7$ was deduced from the positive-ion at *m/z* 415.2012 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_7Na$, 415.2023) in the HR-ESI-MS spectrum. A strong absorption band at λ_{max} 290 nm in the UV spectrum and an IR absorption band at 1762 cm^{-1} were indicative of the presence of a lactone moiety [10]. The ^{13}C NMR spectrum (Table 1) displayed 15 carbon signals, including an ester carbonyl at δ 165.4 (C-12), an oxygenated olefinic quaternary carbon at δ_C 145.9 (C-6), and two olefinic quaternary carbons at δ 147.5 (C-7)/131.4 (C-11), indicating a γ-lactone group [10]. The NMR and HSQC spectra displayed signals for an olefinic group at δ_H 5.87 (s)/δ_C 125.4 (C-5), an oxygenated methylene [δ_H 4.64 (d, J = 13.2 Hz, H-13a) and 4.54 (d, J = 13.2 Hz, H-13b)/δ_C 54.5 (C-13)], three methylenes [δ_H 2.11 m/δ_C 38.0 (C-2), δ_H2.13 m/δ_C 39.5 (C-3), and δ_H 2.44 (dd, J = 15.6, 11.6 Hz, H-9α), 1.88 (m, H-9β)/δ_C 38.0 (C-9)], a methine at δ_H 1.92 (m)/δ_C 41.3 (C-10), a downfield shifted oxymethine at δ_H 6.32 (d, J = 8.0 Hz)/δ_C 70.1 (C-8), a ketal quaternary carbon at δ_C 109.0 (C-1), and an oxygenated quaternary carbon at δ 81.1 (C-4), indicative of a hirsutinolide-type sesquiterpene lactone [4]. In addition, the 1D and 2D NMR spectra revealed an olefinic signal at δ_H 6.17 (q, J = 7.6 Hz)/δ_C 139.9 (C-3′), two methyl groups downfield shifted [δ_H 2.04 (d, J = 7.6 Hz)/δ_C 16.0 (C-4′) and δ_H 1.93 (s)/δ_C 20.5 (C-5′)], an olefinic quaternary carbon at δ_C 127.2 (C-2′), and an ester carbonyl carbon at δ_C 168.3 (C-1′), indicative of a tigloyl group possessing a *Z* configured olefinic (C-2′–C-3′) bond, which was quite similar to that of 8α-(2′*Z*-tigloyloxy)-hirsutinolide [10]. However, the ^1H and ^{13}C NMR spectra of **1** showed an additional signal for a methoxy group at δ_H 3.26 (s)/δ_C 49.5 (1-OCH_3), indicating an attachment of this functional group at C-1 position.

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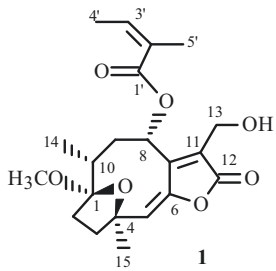
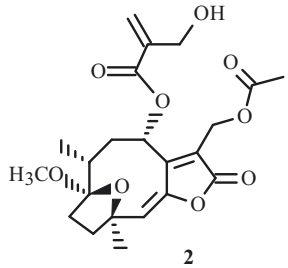
TABLE 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	HMBC
1	109.0	–	
2	38.0	2.11 (m, overl.)	
3	39.5	2.13 (m, overl.)	
4	81.1	–	
5	125.4	5.87 (s)	C-4, C-7
6	145.9	–	
7	147.5	–	
8	70.1	6.32 (d, J = 8.0)	C-7, C-11, C-1′
9α	38.0	2.44 (dd, J = 15.6, 11.6)	
9β		1.88 (m)	
10	41.3	1.92 (m)	
11	131.4	–	
12	165.4	–	
13a	54.5	4.64 (d, J = 13.2)	C-7, C-13
13b		4.54 (d, J = 13.2)	
14	15.7	0.91 (d, J = 6.8)	C-4, C-6
15	27.9	1.49 (s)	
1′	168.3	–	
2′	127.2	–	
3′	139.9	6.17 (q, J = 7.6)	
4′	16.0	2.04 (d, J = 7.6)	
5′	20.5	1.93 (s)	
1-OCH_3	49.5	3.26 (s)	C-1



1



2

This observation was further supported by the HMBC correlation between the methoxy proton (1-OCH_3) and the ketal carbon (C-1). The relative stereochemistry of asymmetric carbons of **1** was deduced to be the same as that of 8α-(2′*Z*-tigloyloxy)hirsutinolide [10], based on its physicochemical data analyses. Thus, compound **1** was elucidated as a new compound, 8α-(2′*Z*-tigloyloxy)-1α-methoxyhirsutinolide.

Compound **2** was isolated for the first time in nature and identified as 8α-(2′-hydroxymethylacryloyloxy)-1α-methoxyhirsutinolide-13-*O*-acetate by comparison of its physical and spectral data with published values [12].

EXPERIMENTAL

General Methods. Specific rotations were measured on a Rudolph Research Autopol IV multiwavelength polarimeter. UV spectra were run on a Shimadzu PharmaSpec-1700 UV/vis spectrophotometer. IR spectra were measured on a Bruker Tensor-27 FT-IR spectrometer. NMR spectroscopic data were recorded at room temperature on Bruker Avance DRX-400 MHz spectrometers, and the data were processed using Top Spin 3.1 software. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained with an Agilent 6530 LC-qTOF High Mass Accuracy mass spectrometer operated in the positive- and negative-ion modes. Analytical TLC was performed on 0.25 mm thick silica gel F_{254} glass-backed plates (Sorbent Technologies). Column chromatography was carried out with silica gel (230–400 mesh; Sorbent Technologies), Sephadex LH-20 gel (GE Healthcare), and RP-18 (YMC·GEL, 12 nm, S-150 μm; Sorbent Technologies). Semipreparative (10 × 150 mm) columns were used for semipreparative HPLC, which was conducted on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech reversed-phase Econosil C_{18} column (10 μm, 10 × 250 mm) with a flow rate of 1.5 mL/min.

Plant Material. The aerial parts of *V. cinerea* Less. (Asteraceae) were provided by Lampang Herb Conservation Club, Lampang Province, Thailand, in May 2011. The plant materials were identified by Dr. Thanapat Songsak (Faculty of Pharmacy, Rangsit University, Thailand). A voucher specimen (No. VCS02) has been deposited at the Natural Product Chemistry Laboratory, Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo.

Extraction and Isolation. The air-dried aerial parts of *V. cinerea* (1 kg) were extracted by maceration in MeOH (3 × 20 L) at room temperature. The solvent was concentrated *in vacuo* to yield 50 g of a crude extract, which was then suspended in distilled water (2 L) and then extracted successively with $CHCl_3$ (3 × 2 L), EtOAc (3 × 2 L), and *n*-butanol (2 × 4 L). The $CHCl_3$-soluble extract (20 g) was separated by column chromatography over Si gel (CC; ∅ 20 cm; 230–400 mesh, 2 kg) using a gradient solvent system of *n*-hexane–EtOAc (100:1 to 0:100) to afford 16 fractions (C1–C10). Combined fractions (30 g) from C3 to C7 were subjected to Si gel column chromatography (CC; ∅ 10 cm; 230–400 mesh, 500 g), with $CHCl_3$–MeOH (100:0 to 1:1) as the solvent system, yielding 10 subfractions (C3S1–C3S10). Subfraction C3S5 (0.5 g) was rechromatographed on a Sephadex LH-20 gel column (CC; ∅ 5 cm; 200 g) to give two subfractions (C3S5L1–C3S5L2). The combined two subfractions were purified by HPLC on a semipreparative RP-18 column using MeOH–H_2O mixtures (50:50 to 0:100) as the solvent system to yield **2** (1.5 mg, t_R 110 min) and **1** (1.0 mg, t_R 115 min).

8α-(2′*Z*-Tigloyloxy)-1α-methoxyhirsutinolide (1). White amorphous powder; $[\alpha]_D^{20}$ +30.0° (*c* 0.2, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 290 (4.0). IR (KBr, ν_{max}, cm^{-1}): 3320, 1762. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1. HR-ESI-MS *m/z* 415.2012 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_7Na$, 415.2023).

8α-(2′-Hydroxymethylacryloyloxy)-1α-methoxyhirsutinolide-13-*O*-acetate (2). White amorphous powder, $[\alpha]_D^{20}$ +25.0° (*c* 0.2, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 285 (3.90). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.42 (1H, d, J = 7.2, H-8), 6.34 (1H, s, H-3a′), 5.95 (1H, s, H-5), 5.77 (1H, s, H-3b′), 5.12, 5.07 (each 1H, d, J = 12.8, H-13a), 4.28 (2H, br.s, H-4′), 3.31 (3H, s, 1-OCH_3), 2.12 (2H, m, H-2), 2.10 (2H, m, H-3), 1.85 (1H, m, H-10), 2.40 (1H, dd, J = 12.5, 12.5, H-9a), 1.91 (1H, ddd, J = 12.5, 7.6, 1.6, H-9b), 1.58 (3H, s, CH_3-15), 0.94 (3H, d, J = 4.8, CH_3-14). ESI-MS *m/z* 459 $[M + Na]^+$.

ACKNOWLEDGMENT

This research was supported by the National Institute of Minority Health and Health Disparities (NIMHD) of the National Institutes of Health under award number U54MD008149 (L.C.C). The content is solely the responsibility of the authors and does not necessarily represent the official view of NIMHD or NIH. This work was also supported by the Korea Polar Research Institute, KOPRI, under the project PE17100 (U.J.Y). We thank H. S. Shin, National Center for Inter-university Research Facilities, Seoul National University, for providing the mass spectrometry facility used in this study.

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