Sesquiterpenes from the Leaves of *Tithonia diversifolia*

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Two new compounds, a germacrane sesquiterpene, 1-acetyltagitinin A (**1**), and a guaianane sesquiterpene, 8β-isobutyryloxycumambranolide (**2**), were isolated from leaves of *Tithonia diversifolia*, together with two known compounds, methyl 3α-acetoxy-4α-hydroxy-11(13)-eudesmen-12-oate and tagitinin A. The structures of compounds **1** and **2** were elucidated on the basis of spectral and chemical evidence.

Germacrane, cadinane, eudesmane, chromene, and flavone derivatives have been reported in previous investigations on *Tithonia diversifolia* (Hemsl.) A. Gray (Compositae).[1−4] The aerial parts of this plant have been used in traditional Chinese medicine for the treatment of hepatitis.[5] Accordingly, a methanolic extract was prepared and then partitioned with *n*-BuOH and water. The *n*-BuOH layer gave a novel rearranged eudesmene sesquiterpene, diversifolol, together with the known methyl 4α-hydroxy-11(13)-eudesmen-12-oate as described in a previous communication.[6] From further purification of the *n*-BuOH layer, we isolated two new sesquiterpenes, 1-acetyltagitinin A (**1**), and 8β-isobutyryloxycumambranolide (**2**), together with two known compounds, methyl 3α-acetoxy-4α-hydroxy-11(13)-eudesmen-12-oate[7] and tagitinin A.[1]

Compound **1** was obtained as colorless needles, mp 181−182 °C. HRMS data of **1** determined the formula as $C_{21}H_{30}O_8$. Analysis of the IR spectrum of **2** suggested that it contained a hydroxyl group (3482 cm^{-1}), an ester (1729 cm^{-1}), and an α-methylene γ-lactone [1650 and 1755 cm^{-1}]. The UV absorption at λ_{max} 212 nm was consistent with the presence of the latter functionality. The ^{1}H NMR spectrum of **1** exhibited signals for one tertiary and one secondary methyl group [δ 1.46 (3H, s) and 1.09 (d, J = 7.1 Hz)], one acetoxy group [δ 2.06 (3H, s)], one isobutyryloxy group [δ 1.04 and 1.07 (3H each, d, J = 6.9 Hz), 2.43 (1H, sep, J = 6.9 Hz)], an α-methylene γ-lactone [δ 5.53 and 6.25 (each 1H, d, J = 2.9 Hz)], and three methine protons attached to ester groups [δ 4.54 (1H, m, H-6), 5.06 (1H, dd, J = 9.4, 6.3 Hz, H-1), and 5.58 (1H, m, H-8)]. Comparison of the ^{1}H and ^{13}C NMR (Table 1) spectral data of compound **1** and tagitinin A,[1] suggested that compound **1** has an acetoxy group instead of a hydroxy group at C-1 as in tagitinin A [the signal of H-1 at δ 4.21 (m)]. The relative configuration of **1** was determined by the NOESY technique (see Figure 1). The chemical correlation between 1-acetyltagitinin A **(1)** and tagitinin A was performed by acetylation.

Table 1. ^{1}H and ^{13}C NMR (δ values) Data for **1** and **2** (300 and 75 MHz in $CDCl_3$)

	1	**2**	
position	δ_C	δ_C	δ_H
1	79.1	54.9	2.68 br t (10.4)
2	44.5	34.1	2.21 m, 2.25 m
3	106.4	125.2	5.48 br s
4	43.7	143.4	
5	37.7	55.1	2.50 br dd (10.4, 10.5)
6	81.0	80.2	4.43 dd (8.9, 10.5)
7	47.8	46.7	3.84 m
8	69.1	66.6	5.62 m
9	35.4	38.4	1.82 dd (14.8, 7.9) 2.28 dd (14.8, 7.9)
10	80.9	73.4	
11	136.8	135.0	
12	169.2	169.8	
13	121.7	121.4	5.39 d (2.9), 6.22 d (2.9)
14	25.4	32.9	1.26 s
15	19.2	17.6	1.89 br d (1.3)
CH_3COO	20.9		
CH_3COO	170.1		
$(CH_3)_2CHCOO$	18.4	18.9	1.04 s
	18.7	19.2	1.06 s
$(CH_3)_2CHCOO$	34.0	34.1	2.42 sep (7.0)
$(CH_3)_2CHCOO$	176.2	176.4	

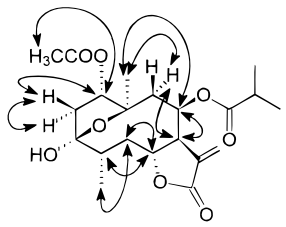
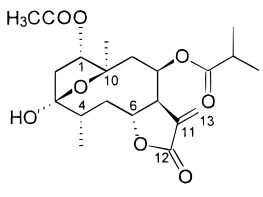
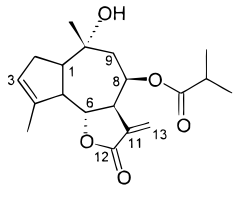


Figure 1. NOESY data for compound **1**.



1



2

Compound **2** was isolated as a light yellow oil and has the formula $C_{19}H_{26}O_5$ on the basis of exact mass measurement (HRMS) at m/z 334.1777. IR analysis

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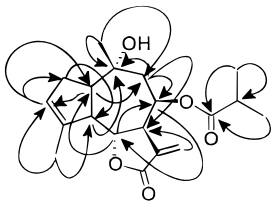

Figure 2. HMBC data for compound **2**.

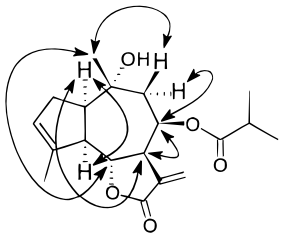

Figure 3. NOESY data for compound **2**.

showed hydroxyl (3482 cm^{-1}), ester (1720 cm^{-1}), and α-methylene γ-lactone (1655 and 1755 cm^{-1}) absorption bands. The ^1H NMR spectrum revealed that **2** has a methyl group attached to a quaternary carbon-bearing hydroxyl group [δ 1.26 (3H, s)], a methyl group attached to an olefin [δ 1.89 (3H, br d, J = 1.3 Hz)], an isobutyryloxy group [δ 1.04 and 1.06 (3H each, d, J = 7.0 Hz), 2.42 (1H, sep, J = 7.0 Hz)], and an α-methylene γ-lactone [δ 5.39 and 6.22 (1H each, d, J = 2.9 Hz)]. Other protons and coupling patterns are shown in Table 1. From data obtained using the HMQC technique, several correlations were established (Table 1). Analysis of HMBC spectral data (see Figure 2) and decoupling studies established the structure of **2** as shown. Compound **2** exhibited very similar ^1H NMR data to 8α-isobutyryloxycumambranolide.[8] In addition, the MS fragmentation pattern of **2** was similar to that of 8α-isobutyryloxycumambranolide. The only major difference in the ^1H NMR data of **2** and 8α-isobutyryloxycumambranolide was for the H-8 proton, which occurred at δ 5.62 and 5.27, respectively. The isobutyryloxy group located at C-8 in 8α-isobutyryloxycumambranolide occurs in the α-orientation;[8] therefore, the isobutyryloxy group in **2** was established to be in the β-orientation. The relative configuration of **2** was further proved by the NOESY technique (see Figure 3). Based on the above evidence, the structure of **2** was elucidated as 8β-isobutyryloxycumambranolide.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on Perkin−Elmer 983G spectrophotometer. ^1H and ^{13}C NMR spectra were run on a Bruker AM-300 spectrometer. EIMS, UV, and specific rotations were taken on a JEOL JMS−HX 300 mass spectrometer, a Hitachi S-3200 spectrometer, and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed over Si gel (Merck 70−230 mesh, 230−400 mesh, ASTM).

Plant Material. The leaves of *Tithonia diversifolia* (Hemsl.) A. Gray were collected in Nan-Tou, Taiwan, in 1994. The plant material was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University, and a voucher specimen (voucher no. 216086) has been deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

Extraction and Isolation. The dried leaves of *T. diversifolia* (3.8 kg) were extracted with MeOH (40 L) at room temperature (10 days × 3). The extract was evaporated in vacuo to yield a residue, which was suspended in H_2O (1 L), and this phase was then partitioned twice with 1 L of *n*-BuOH. The combined *n*-BuOH layer afforded a black syrup (275 g). Part of this syrup (140 g) was chromatographed over Si gel repeatedly with hexane−EtOAc gradient solvent systems. Methyl 4α-hydroxy-11(13)-eudesmen-12-oate (21 mg),[6] diversifolol (8 mg),[6] methyl 3α-acetoxy-4α-hydroxy-11(13)-eudesmen-12-oate (12 mg),[7] 8β-isobutyryloxycumambranolide (**2**) (10 mg), 1-acetyltagitinin A (**1**) (30 mg), and tagitinin A (25 mg)[1] were eluted with 10%, 20%, 30%, 30%, 40%, and 80% EtOAc in hexane, respectively. The four known compounds were identified by comparison of their physical data with those in the literature.

1-Acetyltagitinin A (1): colorless needles; mp 181−182 °C; $[\alpha]^{24}_D$ −85.9° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (3.97) nm; IR (dry film) ν_{max} 3482, 1755, 1729, 1306, 1233, 1203, 1151, 1022 cm^{-1}; ^{13}C NMR, see Table 1; EIMS *m/z* 410 $[M]^+$ (3), 392 $[M^+ - H_2O]$ (2), 322 (8), 71 [100, $(CH_3)_2CHC{=}O^+$]; HREIMS *m/z* 410.1956 (calcd for $C_{21}H_{30}O_8$, 410.1941).

8β-Isobutyryloxycumambranolide (2): light yellow oil; $[\alpha]^{24}_D$ −19.7° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (3.94) nm; IR (dry film) ν_{max} 3482, 1755, 1720, 1655, 1240, 1149, 916 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 334 $[M]^+$ (3), 316 $[M^+ - H_2O]$ (47), 247 (8), 228 (18), 213 (8), 71 (100); HREIMS *m/z* 334.1777 (calcd for $C_{19}H_{26}O_5$, 334.1780).

Acetylation of Tagitinin A with Ac_2O Anhydride and Pyridine. A solution of tagitinin A (5 mg) in pyridine (0.5 mL) and Ac_2O (0.5 mL) was left at room temperature overnight. The reaction mixture was poured into crushed ice H_2O and was extracted with ether (20 mL × 3). The combined organic layers were washed with 3N HCl, saturated aqueous $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure produced **1** (5 mg).

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