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Two novel iridoids with an unusual δ-lactone-containing skeleton from *Triosteum himalayanum*

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ABSTRACT

Two novel iridoids triohimas A (**1**) and C (**3**) with an unusual δ-lactone-containing skeleton were isolated from *Triosteum himalayanum* Wall. Their structures were determined by NMR spectroscopic analyses and X-ray crystallography. The absolute configuration was established by computational methods. They were also tested for the *in vitro* cytotoxicity against L1210 cell line.

Common iridoids are monoterpenes based on the cyclopenta[*c*]pyranoid skeleton represented by iridane (*cis*-2-oxabicyclo[4.3.0]nonane).[1] Usually, they are found in a large number of plant families as natural glucosides,[2] including the iridoid glycoside dimers,[2,3] and even tetramers.[4] Non-glycosidic iridoids have been rarely reported. This class of compounds displays various biological activities,[1] such as cardiovascular, antitumor, antiviral, and immunomodulator activity. The Caprifoliaceae family is well known for the constituents of iridoids and flavonoids.[5] In our search for bioactive iridoids from natural resources, *Triosteum himalayanum* Wall (Caprifoliaceae), which has been traditionally applied for many medical purposes, including inducing diuresis and promoting blood circulation,[6] was studied phytochemically for the first time. Two novel iridoids (**1** and **3**) with an unusual δ-lactone-containing skeleton and a known compound **2**[7] (triohima B), which has been reported as the synthetic product, were isolated from this plant. In this Letter, we describe the structural elucidation and bioactivity evaluation of these three metabolites.

The air dried and ground *T. himalayanum* (6.8 kg) was extracted successively with acetone. The extract (279 g) was subjected to fractionation using silica gel column chromatography, and eluted fractions containing products were further purified by Sephadex LH-20 and compounds **1** (20 mg), **2** (500 mg), and **3** (7 mg) were obtained (Fig. 1).

Triohima A (**1**) was colorless needles, mp 143–145 °C, $[\alpha]_D^{20} + 37$ (*c* 1.0, $CHCl_3$). Its molecular formula was determined as $C_{11}H_{12}O_5$ by HRESIMS (*m/z* 247.0584 $[M+Na]^+$; calcd 247.0577), indicating six degrees of unsaturation. Its UV spectrum (λ_{max} 236.5 nm) and IR spectrum (ν_{max} 1711 and 1620 cm^{-1}) suggested the presence of α,β-unsaturated ester groups. The ^1H NMR spectrum (Table 1) displayed the signals (δ 7.42, H-3; δ 3.69, COOMe) corresponding to an enol ether system conjugated with a carbomethoxy group as found in various iridoid glycosides.[8,2c] In addition to the signals attributable to the above-mentioned fragment, there were signals for the protons of an oxymethine (δ 5.95), two other methines (δ 3.53 and 6.52), a methylene group (δ 2.26 and 2.06), and a methyl group (δ 2.13). The ^{13}C NMR and DEPT spectral data (Table 1) indicated the presence of 11 carbons including two quaternary (δ 161.8 and 165.8) carbons, four CH (150.3, 145.3, 93.6 and 30.7), one CH_2 (26.2), and two CH_3 (15.9 and 51.4). Among them, the signals at δ 165.8, 150.3, 110.6, and 51.4 corresponded to an enol ether system with a carbomethoxy group, and the signals at δ 161.8, 145.3, and 125.8 corresponded to another unsaturated ester group.[9] the deshielded nature of the methine (δ_H 5.95 and δ_C 93.6) was indicative of a methine linked directly to two oxygen atoms.[8d] Four of the six double bond equivalents were occupied by the aforesaid functionalities, and the remaining two required compound **1** possessing two other rings. Comprehensive analysis of the 2D NMR spectra, especially ^1H–^1H COSY and HMBC (Fig. 2), allowed the full establishment of the planar structure of **1**. The ^1H–^1H COSY spectrum indicated by bold lines in Figure 2. The key HMBC correlations of H-1/C-3, C-7; H-3/C-5; and H-11/C-5 further constructed the rings system of an unusual monoterpenoid.

The *Z*-configuration of the 6–11 double bond of **1** was assigned based on the NOE experiment. Upon irradiation of H-11, NOE enhancement for H-5 was observed, but this enhancement was not observed for the known **2**. The full structure of **1** was finally

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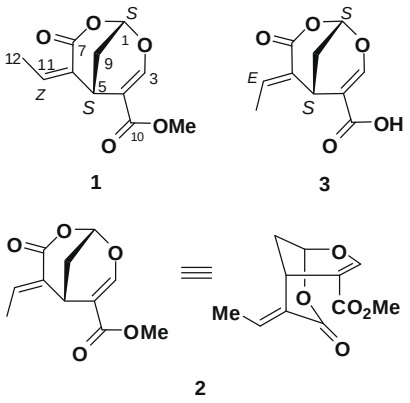

Figure 1. Structures of compounds **1–3**.

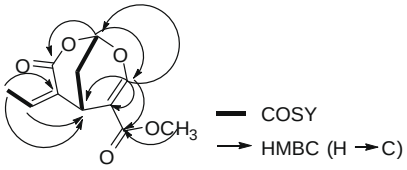

Figure 2. Selected COSY and HMBC correlations for **1**.

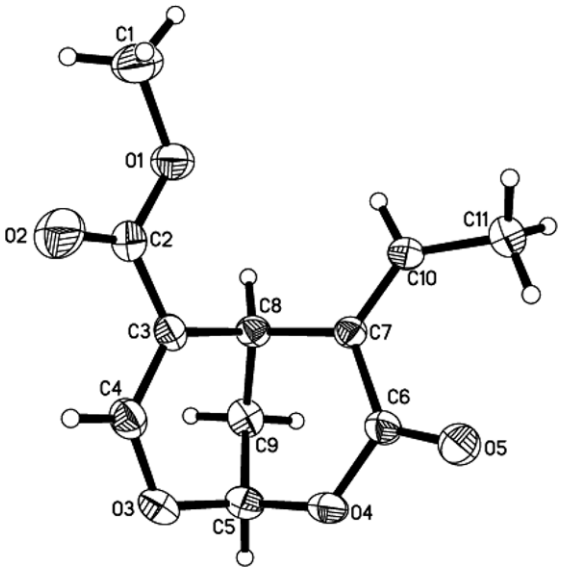

Figure 3. ORTEP drawing of **1**.

determined by X-ray diffraction analysis (Fig. 3),[10] which was consistent with that deduced from the spectroscopic analyses.

Recently, Gaussian package has become a popular method to predict the optical rotation in chiral rigid compounds.[11] For the assignment of absolute configuration, the optical rotation values for compounds **1**, **2** (Fig. 1) and their enantiomers (1*R*,5*R*)-**1**, (1*R*,5*R*)-**2** were calculated, respectively. The geometry of the molecules was optimized with GAUSSIAN 98 package,[12] at B3LYP/6-31G(d) computational level. The minimum nature of the structure was confirmed by frequency calculations at the same computational level. These geometries were used to evaluate the optical rotation at B3LYP/6-311++G(d,p) computational level. The calculated optical rotation for **1** is +28.08° and that for its enantiomer is −67.31°. The former is very close to the experimental value of +37°. The calculated optical rotation for **2** is +55.11° and that for its enantiomer is −103.99°, while the experimental value of **2** is +80° (*c* 1.12 $CHCl_3$). The results suggested that the absolute configuration of **1** was also 1*S*,5*S*, which was same as that reported for **2**.[7]

To the best of our knowledge, **2** was only reported previously as a synthetic product. In our investigation on *T. himalayanum,* 500 mg of **2** and 20 mg of **1** were isolated firstly as the natural products. When comparing **1** and **2**, we found that **1** is nearly 4.58 kJ/mol higher in energy than **2**, according to B3LYP/6-31G(d) and 4.43 kJ/mol higher according to B3LYP/6-311++G(d,p). The energies were corrected by the zero-point energies calculated at B3LYP/6-31G(d). These energy differences clearly demonstrate the stability[13] of **2** over **1**.

Triohima C (**3**) was obtained as colorless needles, mp 165–167 °C, $[\alpha]_D^{20}$ + 24 (*c* 0.68 $CHCl_3$), with the molecular formula $C_{10}H_{10}O_5$ as determined by HRESIMS (*m/z* 228.0872 $[M+NH_4]^+$; calcd 228.0866), indicating six degrees of double-bond equivalence and 14 mass units lighter than that of **2**. Similar NMR studies were also conducted for **3**. The ^1H NMR spectroscopic pattern of **3** showed the same characteristics as that of **2**. Further comparison of the ^{13}C NMR data obtained for **3** (Table 1) with the data for **2** showed that the structures of **2** and **3** were closely related. The major difference in the ^1H and ^{13}C NMR of **3** compared to those of **2** was the absence of a methoxyl group in **3**. The band between 3000 and 3500 cm^{-1} in the IR spectrum of **3** also suggested the presence of a hydroxyl group in the structure. The stereochemistry of **3** was deduced to be the same as that of **2** from the consideration of its positive optical rotation and the biogenetic relationship between **2** and **3**.

Compounds **1–3** were tested for the in vitro cytotoxicity against L1210 (mouse lymphocytic leukemia) cell line. It showed 13%, 11%, 18% inhibition in proliferation assay at 50 μM concentration,

Table 1
NMR data for **1–3** in $CDCl_3$ (300 MHz for ^1H, 75 MHz for ^{13}C)

No.	1		2		3	
	δ_H (mult) *J* (Hz)	δ_C	δ_H (mult) *J* (Hz)	δ_C	δ_H (mult) *J* (Hz)	δ_C
1	5.95 (t) 2.1	93.6	5.94 (t) 1.8	93.5	6.02 (dd) 3.9, 2.7	93.6
3	7.42 (s)	150.3	7.44 (s)	151.1	7.63 (s)	153.4
4		110.6		109.9		109.5
5	3.53 (dd) 2.1, 3.6	30.7	3.90 (dd) 1.8, 2.4	23.2	3.95 (m)	23.1
6		125.8		127.3		127.1
7		161.8		163.3		163.2
9a	2.26 (dt) 14.1, 2.1	26.2	2.20 (dt) 14.1, 1.8	26.1	2.26 (dt) 13.8, 2.7	26.3
9b	2.06 (ddd) 14.1, 3.6, 2.1		2.08 (ddd) 14.1, 1.8, 2.4		2.12 (ddd) 13.8, 1.8, 3.9	
10		165.8		165.8		171.1
11	6.52 (q) 7.2	145.3	6.95 (q) 7.2	141.6	7.05 (q) 7.2	142.3
12	2.13 (d) 7.2	15.9	2.00 (d) 7.2	14.2	2.03 (d) 7.2	14.5
OMe	3.69 (s)	51.4	3.66 (s)	51.4		

respectively. In addition, the total extract had significant inhibitory activity toward plant pathogenic fungus *Peronophythora litchi* (85% inhibition at 5 mg/mL). Compound **2** exhibited inhibitory activity against *P. litchi* with EC_{50} value of 34.0 μg/mL. The antifungal activity was not evaluated for **1** and **3** due to the limited quantities.

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Supplementary data

^{1}H and ^{13}C NMR spectra of **1**–**3**, NOEs of **1** and **2**, and DEPT, COSY, HMBC spectra of **1** were available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.111.

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