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A new eudesmane sesquiterpenoid lactone from *Chloranthus japonicus*

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[ABSTRACT] AIM: To study the chemical constituents of the whole plant of *Chloranthus japonicus*. **METHODS:** Compounds were isolated and purified by column chromatography. Their structures were elucidated on the basis of spectroscopic methods. **RESULTS:** Six sesquiterpenoids were obtained and their structures were identified as chlojaponilactone A (**1**), atractylenolide III (**2**), neolitacumone B (**3**), 10*α*-hydroxy-1-oxoeremophila-7(11), 8(9)-diene-8, 12-olide (**4**), shizukanolide C (**5**), and shizukanolide H (**6**). **CONCLUSION:** Compound **1** is a new eudesmane-type sesquiterpenoid lactone, and compounds **3** and **4** have been isolated from this species for the first time.

[KEY WORDS] *Chloranthus japonicus*; Chemical constituents; Sesquiterpenoid; Eudesmane

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1 Introduction

The genus *Chloranthus* (Chloranthaceae), which includes *ca.* 15 species, is mainly distributed in the east of Asia[1]. Sesquiterpenoid is the major constituent of the *Chloranthus* genus and a series of sesquiterpenoid and sesquiterpenoid oligomers were isolated from this genus previously[2-11], some of which were reported to exhibit antifungal activity[6], potent and selective inhibition on the delayed rectifier (I_K) K^+ current[7-8], and inhibition on the expression of cell adhesion molecules[12]. The perennial herbaceous plant *Chloranthus japonicus* with a Chinese name *yinxiancao* is used as a folk medicine for the treatment of traumatic injuries, rheumatic arthralgia, fractures, pulmonary tuberculosis, and neurasthenia since ancient time[13]. In order to explore biologically active substances from this plant, we studied the chemical constituents from the whole plants of *C. japonicus*. As a result, six sesquiterpenoids were isolated and their structures and relative configurations were elucidated on the basis of spectroscopic analysis and comparison with the related compounds reported in literature, including a new eudesmane-type sesquiterpenoid chlojaponilactone A (**1**) and five known sesquiterpenoids, atractylenolide III (**2**)[14], neolitacumone B (**3**)[15], 10*α*-hydroxy-1-oxoeremophila-7(11), 8(9)-diene-8, 12-olide (**4**)[16], shizukanolide C (**5**)[2-3], and shizukanolide H (**6**)[10] (Fig. 1).

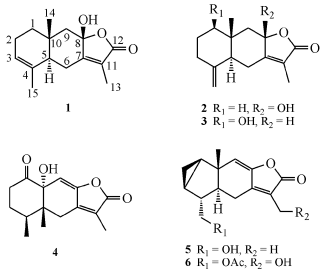

Fig. 1 Structures of compounds 1-6

2 Results and Discussion

Chlojaponilactone A (**1**) was obtained as a white amorphous powder, displaying a molecular formula of $C_{15}H_{20}O_3$ as determined by HREI-MS at *m*/*z* 249.148 4 $[M + H]^+$

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(Calcd. 249.149 0) and indicating 6 degrees of unsaturation. The absorption bands in the IR spectrum at 1 739 and 1 698 cm^{-1}, and the UV maximum at 239 nm indicated the presence of an *α*, *β*-unsaturated *γ*-lactone moiety in **1** [17]. Its ^1H NMR spectrum showed three methyl singlets at δ_H 1.01, 1.68, and 1.73, one olefinic proton at δ_H 5.34 (br s) (Table 1). The ^{13}C NMR spectrum displayed 15 carbon signals comprising three methyl, four methylene, one methine, one quaternary carbon, one ester carbonyl at δ_C 171.9, one hemiacetal at δ_C 104.1, and four olefinic carbons (Table 1). The above spectroscopic analysis indicated that **1** is an eudesm-7(11)-enolide sesquiterpene. Comprehensive analysis of NMR and mass spectra suggested that **1** was a double bond isomer of atractylenolide III (**2**)[14]. The position of the 3,4-double bond was supported by the HMBC correlations from Me-15 (δ_H 1.68, s) to C-3 (δ_C 121.6, d), C-4 (δ_C 133.1, s), and C-5 (δ_C 48.3, d). Moreover, the HMBC correlations from Me-13 (δ_H 1.73, s) to C-7 (δ_C 161.5, s), C-11 (δ_C 120.9, s), and C-12 (δ_C 171.9, s), from H_2-9 to C-7 (δ_C 161.5, s), C-8 (δ_C 104.1, s), and C-10 (δ_C 33.1, s), and from OH-8 (δ_H 7.22, s) to C-7 (δ_C 161.5, s), C-8 (δ_C 104.1, s), and C-9 (δ_C 50.3, t) confirmed the hemiacetal located at C-8 (δ_C 104.1, s) and the presence of a *γ*-lactone moiety between C-8 and C-12. The relative configuration of **1** was established by a ROESY experiment, in which the correlations H-1α/H-5, H-1α/H-9α, and H-5/H-6α indicated that they were cofacial and were arbitrarily assigned having an α-orientation. Consequently, the ROESY correlations of Me-14 with H-6*β*, and OH-8 suggested that they were *β*-oriented. Therefore, the structure of **1** was elucidated as 8*β*-droxyeudesm-3, 7(11)-dien-12, 8*α*-olide.

Table 1 NMR data for Compound 1 in DMSO-d_6 (400 MHz)

Position	δ_H (*J* in Hz)	δ_C	HMBC
1*α*	1.38 (m)	36.9 (t)	C-2, C-3, C-5, C-10
1*β*	1.27 (m)		C-2, C-5, C-10, C-14
2*α*	2.05 (m)	22.4 (t)	C-1, C-3, C-4, C-10
2*β*	1.94 (m)		C-1, C-3, C-4, C-10
3	5.34 (br s)	121.6 (d)	C-1, C-2, C-4, C-5, C-15
4		133.1 (s)	
5	1.92 (m)	48.3 (d)	C-1, C-3, C-7, C-9, C-10, C-14, C-15
6*α*	2.80 (dd, 12.9, 3.0)	23.9 (t)	C-4, C-5, C-7, C-8, C-10, C-11
6*β*	2.10 (d, 12.9)		C-4, C-5, C-7, C-8, C-10, C-11
7		161.5 (s)	
8		104.1 (s)	
9*α*	2.09 (d, 13.3)	50.3 (t)	C-5, C-7, C-8, C-10, C-14
9*β*	1.33 (d, 13.3)		C-1, C-8, C-10, C-14
10		33.1 (s)	
11		120.9 (s)	
12		171.9 (s)	
13	1.73 (s)	8.0 (q)	C-7, C-8, C-11, C-12
14	1.01 (s)	15.8 (q)	C-1, C-5, C-9, C-10
15	1.68 (s)	21.1 (q)	C-3, C-4, C-5
-OH	7.22 (s)		C-5, C-7, C-8, C-9

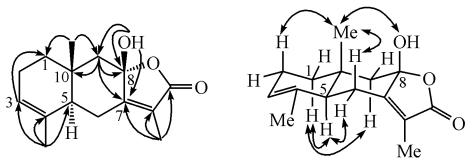

Fig. 2 Selected HMBC (→) and ROESY (↔) correlations of chlojaponilactone A (1).

3 Experimental

3.1 *General experimental procedures*

Optical rotations were taken on a Horiba SEAP-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were run on Bruker AM-400 and DRX-500 instruments. FABMS was performed on a VG Auto Spec-3000 spectrometer. ESI-MS and HRESI-MS were performed on an APIQSTAR time-of-flight spectrometer. Column chromatography was performed on silica gel (48−75 μm, Qindao Marine Chemical Inc., Qingdao, China), or Rp-18 gel (LiChroprep, 40-63 μm, Merck, Darmstadt, Germany), or Sephadex LH-20 (Amersham Biosciences, Sweden). Fractions were monitored by thin layer chromatography (TLC), and spots were detected with a UV 254 nm lamp and by spraying with 10% H_2SO_4 in EtOH followed by heating at 105 ºC for 5 min.

3.2 *Plant material.*

The whole plants of *C. japonicus* were collected in August 2008 from Panshi, Jilin Province, China and identified by Dr. LIU En-De of Kunming Institute of Botany. A voucher sample (No. HY0003) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

3.3 *Extraction and isolation.*

The dried and powdered plant material (10.5 kg) was extracted three times with MeOH under reflux. The filtrate was evaporated under reduced pressure to give a residue (800 g), which was subsequently subjected to silica gel chromatography eluted with EtOAc to yield 426 g eluate, which was passed through a column containing MCI gel, eluted with MeOH-H_2O mixtures in a gradient (3 : 7→5 : 5→7 : 3→1 : 0). The 70% MeOH fraction (109.4 g) was chromatographed over a silica gel column eluted with $CHCl_3$-MeOH (100 : 1→80 : 1→60 : 1→40 : 1) to yield six fractions A-F. Fraction B was then subjected to column chromatography over silica gel eluted with $CHCl_3$-MeOH (150 : 1→120 : 1→ 80 : 1→60 : 1) to afford **1** (40 mg) and **2** (55 mg). Fraction C (21.1 g) was subjected to column chromatography over a Rp-18 column eluted with a MeOH-H_2O gradient system (35%, 40%, 45%, 50%, and 55%) to obtain eight fractions, C_1-C_8. Compound **4** (20 mg) powder was obtained from C_1. Fraction E was subjected to column chromatography over silica gel eluted with $CHCl_3$-MeOH (130 : 1→120 : 1→100 : 1→ 60 : 1) to afford compound **3** (19 mg). The 50% MeOH