A Novel Bisesquiterpenoid, Biatractylolide, from the Chinese Herbal Plant *Atractylodes macrocephala*

Yongcheng Lin,* Tao Jin, Xiongyu Wu, Zhongqi Huang, and Jingsong Fan

Department of Chemistry, Zhongshan University, Guanzhou 510275, People's Republic of China

Wing Lai Chan

Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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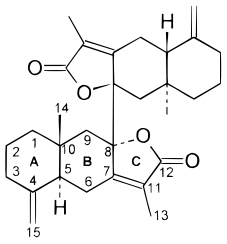
Atractylodes macrocephala Koidz is a traditional medicinal plant in China and is known for the treatment of gastroenteric and splenic disorders. The EtOAc extract of *A. macrocephala* was chromatographed to give a novel bisesquiterpenoid, biatractylolide (**1**). The structure of **1** was determined by spectroscopic methods, mainly 2D-NMR techniques.

Sesquiterpenoid lactones belong to the isoprenoid family, which is one of the most numerous and widespread of natural constituents in plants. They exhibit various biological properties, such as allergenicity[1,2] and cytotoxicity,[3] and plant growth-inhibitory,[4,5] antiinflammatory,[6] and antitumor activities.[7−9] *Atractylodes macrocephala* is used in Chinese folk medicine for the treatment of gastroenteric and splenic disorders. There have been several reports on about *A. macrocephala*,[10,11] and many compounds have been reported isolated. Amongst those, hydroxyatractylolide was found to have an effect on fluid propulsion of ileal and colonic segments from rat and guinea pig.[12] It was our intention to study the chemical constituents of *A. macrocephala*, and this paper reports the isolation and structure elucidation of a novel bisesquiterpenoid, biatractylolide (**1**), based mainly on 2D-NMR techniques.



1

The EtOAc extract of *A. macrocephala* was chromatographed on a silica gel column using a stepwise solvent gradient method (EtOAc/petroleum ether). Compound **1** was obtained from the 12% EtOAc/petroleum ether fraction (0.02% dry wt) and crystallized from EtOAc yielding colorless crystals, mp 210−212 °C, $[\alpha]^{20}_D$ = +256.40 (*c* 0.02, $CHCl_3$). Its molecular formula was determined as $C_{30}H_{38}O_4$ on the basis of the molecular ion peak at *m*/*z* 463 $[M + 1]^+$ in the FABMS spectrum and elemental analysis.

The ^{13}C NMR spectrum of **1** contained 15 signals and confirmed the presence of two CH_3, six CH_2, one CH, and six quaternary carbons, which constituted the structure of half of the molecule. It indicated that **1** was a molecule formed from two identical units. The structure of this "unit" was elucidated by means of spectral data. The IR spectrum of **1** showed an α,β-unsaturated γ-lactone absorption at 1748 cm^{-1}. ^{13}C NMR and the DEPT spectrum at δ 171.7(s), together with the UV absorption at λ_{max} 238 (ε 2000), also indicated the presence of this group. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of **1** showed signals of one terminal double bond at δ 4.65 (1H, brs) and 4.89 (1H, brs). The ^{13}C NMR spectrum also indicated terminal double bond carbons at δ 147.8 (s) and 107.2 (t) and a tetrasubstituted double bond at δ 164.3 (s) and 124.3 (s). All ^1H and ^{13}C NMR signals of **1** were unambiguously assigned by COSY, HMBC, HMQC, and ROESY spectra (Table 1).

The double bond equivalents of the molecule were 12, giving six per "unit". Two of these were assigned to two double bonds, one to the ester carbonyl group, and three to the tricyclic structure.

The COSY experiment revealed the contiguous sequence of coupled signals from H-1 to H-3 and H-5 to H-6. Long-range coupling between CH_3−14 and H-9 was also observed. The HMBC experiment (Table 1) systematically assembled the carbon skeleton and allowed no alternative structure. In particular, the multiple correlations to the C-10 and to C-5 signals can be used to define the ring system. The correlations between C-7 and CH_3−13, C-8 and H-9, C-11 and CH_3−13, and C-12 and CH_3−13 indicated that the α,β-unsaturated γ-lactone was attached at C-7 and C-8, CH_3−13 at C-11. The correlation of CH_3−14 and C-10 suggested that CH_3−14 was connected to C-10.

The stereochemistry of H-5 and H-6 was assigned by the coupling constants and a ROESY experiment, while the coupling constants of H-5 and H-6a (13.2 Hz) and H-5 and H-6b (3.6 Hz) suggested that H-5 and H-6a were axial hydrogens and had an antiperiplanar relationship. H-6b was an equatorial hydrogen. The significant cross peak between H-6b and H-5 implied that they were in the same plane. The absence of a correlation between H-5 and CH_3−14 indicated their trans relationship, and thus, C-6 and C-9 were also trans.

Experimental Section

General Experimental Procedures. ^1H and 2D NMR spectra were recorded on a Bruker MSL-400 spectrometer and ^{13}C NMR spectra on a FX-90Q spec-

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Table 1. NMR Data of Compound **1**

	13C	1H	1H−1H COSY	HMBC	ROESY
1	42.5 (t)	1.23 (ddd, 5.4, 12, 22 Hz, 1Ha)	H-2	H-14, H-9	H-9b, H-2
		1.61 (m, 1Hb)[a]	H-2		H-3b, H-1a
2	22.3 (t)	1.65 (m, 2H)[a]	H-1a,b, H-3a,b	not observed	H-3, H-14
3	35.8 (t)	1.94 (brq, 12 Hz, 1 Ha)	H-2	H-2, H-15	H-2
		2.35 (brd, 12 Hz, 1 Hb)	H-2		H-2, H-15b
4	147.8 (s)			not observed	
5	52.7 (d)	1.72 (dd, 13.2, 3.6 Hz, 1H)	H-6a,b	H-15, H-6, H-14, H-9	H-6b, H-9b
6	27.8 (t)	2.72 (t, 13.2 Hz, 1Ha)	H-5	not observed	H-14, H-15a
		2.65 (dd, 13.2, 3.6 Hz, 1Hb)	H-5		H-5, H-15b
7	164.3 (s)			H-6, H-13, H-9	
8	89.2 (s)			H-9, H-6b	
9	49.6 (t)	2.82 (d, 14.5 Hz, 1Ha)		H-14	H-14, H-1b
		1.42 (d, 14.5 Hz, 1Hb)	H-14 long range		H-5, H-1a
10	36.9 9s)			H-14, H-1, H-9, H-6b	
11	124.3 (s)			H-13	
12	171.7 (s)			H-13	
13	8.3 (q)	1.75 (s, 3H)		not observed	
14	17.1 (q)	1.13 (s)	H-9	H-9b	H-15a, H-9a
					H-2
15	107.2 (t)	4.65 (brs, 1Ha)	H-15b	not observed	H-14, H-6a,b
		4.86 (brs, 1Hb)	H-15a		H-3b

[a] Overlapping.

trometer. The mass spectrum was obtained on VG ZAB mass spectrometer. The IR spectrum was taken with a Nicoler 5DX-FTIR spectrophotometer, and the UV spectrum was recorded on a Shimadzu UV-240 spectrophotometer. The optical rotation was measured with a Perkin-Elmer 241 polarimeter.

Plant Material. The dried branches of the herbal plant, *A. macrocephala*, from Zhejiang Province of China was obtained from a local herbal medicine store and was taxonomically identified by Cangxing Ye (Biology Department, Zhongshan University). A voucher specimen of the plants was deposited at the Research Centre of Organic Natural Products, Zhongshan University, People's Republic of China.

Extraction and Isolation. The dried specimens of *A. macrocephala* (2 kg) were extracted with EtOAc. The extract was chromatographed on a silica gel column, using gradient elution, with EtOAc/petroleum ether. The fraction eluted with 12% EtOAc was collected and evaporated to dryness to give biatractylolide (**1**) (0.02% dry wt). Crystallization from EtOAc gave colorless crystals: mp 210−212 °C; $[\alpha]^{20}_D = +256.40°$ (*c* 0.02, $CHCl_3$); IR (KBr) 3070, 2910, 2860, 1748, 1665, 1640, 1435, 1380, 1310, 1090, 1038, 1000, 978, 880 cm^{-1}; UV ($CHCl_3$) λ_{max} 238 nm (ϵ 2000); FABMS *m/z* (rel int) 463 $[M + 1]^+$, 447, 231 (100), 215, 203, 189, 107, 91, 77, 67, 55. Anal. Calcd for $C_{30}H_{38}O_4$: C, 77.88; H, 8.28. Found: C, 77.66, H, 8.41.

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