

Amoxanthin A: A New Bisnorlabdane Diterpenoid from *Amomum xanthioides*Ki Hyun Kim, Jung Wook Choi, Sang Un Choi,[†] Eun-Kyoung Seo,[‡] and Kang Ro Lee*

Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

*E-mail: krlee@skku.ac.kr

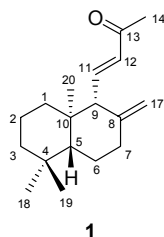
[†]Korea Research Institute of Chemical Technology, Teajon 305-600, Korea[‡]The Center for Cell Signaling & Drug Discovery Research, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

Received December 8, 2009, Accepted January 15, 2010

Key Words: *Amomum xanthioides*, Zingiberaceae, Bisnorlabdane, Amoxanthin A, Cytotoxicity

Amomum xanthioides (Zingiberaceae) is a perennial herb and its seeds, listed in the Japanese Pharmacopoeia as *Amomum* seed, have been used in folk medicines for the treatment of stomach and digestive disorders.¹ Previous chemical investigations have demonstrated that the essential oil (1 ~ 1.5%) of this plant was rich in monoterpenoids.^{2,3} As a part of our search for new bioactive substances from medicinal plants,^{4,5} we conducted a further chemical investigation of the seeds of *A. xanthioides*, which led to isolation of an unusual 15,16-bisnorlabdane diterpenoid, named amoxanthin A (**1**). The structure of **1**, including the absolute stereochemistry, was elucidated by spectroscopic methods and CD data analysis.

Amoxanthin A (**1**) was obtained as a colorless gum, [α]_D²⁵ -5.5° (c 0.12, CHCl₃). Its molecular formula was established as C₁₈H₂₈O (5 degrees of unsaturation) from the [M + Na]⁺ peak at *m/z* 283.2041 (calcd. for C₁₈H₂₈ONa, 283.2038) in the positive HRFABMS. Its IR spectrum exhibited the presence of carbonyl (1678 cm⁻¹), exomethylene (3070 and 885 cm⁻¹), and double bond (1645 cm⁻¹) units. The UV spectrum of **1** showed an absorption maxima at 228 nm, corresponding to an α,β -unsaturated ketone. The ¹H NMR spectrum (Table 1) of **1** displayed signals for three quarternary methyl protons at δ 0.86 (3H, s) and 0.91 (6H, s) and for two exomethylene protons at δ 4.42 (1H, d, *J* = 1.5 Hz), 4.81 (1H, d, *J* = 1.5 Hz), suggesting that **1** possesses the bicyclic carbon skeleton of labdane.⁶ The ¹H NMR spectra also showed signals for an olefinic bond at δ 6.08 (1H, d, *J* = 16.0 Hz), 6.88 (1H, dd, *J* = 16.0, 10.0 Hz), and a quarternary methyl proton at δ 2.28 (3H, s), indicating the presence of a side chain, α,β -unsaturated ketone. The ¹³C NMR and DEPT spectra (Table 1) of **1** showed 18 carbon signals, composed of four methyl, six methylene (one terminal olefinic), four methine (two olefinic), and four quaternary carbons (one ketone, one olefinic). The ¹³C NMR data showed resonances

**Figure 1.** Structure of amoxanthin A.

for four olefinic carbons at δ 108.8 (C-17), 133.8 (C-12), 146.9 (C-11), and 148.8 (C-8), and a carbonyl carbon at δ 198.3 (C-13), suggesting that **1** is a bicyclic bisnorditerpenoid, due to the remaining degrees of unsaturation and a total of 18 carbon signals.

The bicyclic bisnorditerpene skeleton of **1** was further confirmed by 2D NMR studies. Its ¹H-¹H COSY spectrum showed the presence of three different structural units (Figure 2), which were assembled with the assistance of HMBC experiment (Table 1 and Figure 2). Key HMBC correlations between H₂-2/C-4, C-10; H₃-18/C-3, C-4; H₃-19/C-3, C-4; H₃-20/C-1, C-10 established connectivities between C-1 and C-10 and between C-3 and C-4. HMBC correlations between protons and remaining quarternary carbons of **1**, such as H-5/C-7, C-9, C-18, C-19,

Table 1. ¹H and ¹³C NMR data and HMBC correlations for **1**

C/H	¹ H ^a / δ	¹³ C ^b / δ	HMBC (H→C)
1 α	1.40 m	41.1 (t) ^d	C-3, 20
β	1.04 ddd (12.5, 12.5, 5.0) ^c		
2 α	1.55 m	19.2 (t)	C-4, 10
β	1.55 m		
3 α	1.39 m	42.3 (t)	C-1, 5, 18
β	1.21 ddd (12.5, 12.5, 5.0)		
4		33.8 (s)	
5	1.11 dd (12.5, 2.5)	54.6 (d)	C-3, 7, 9, 18, 19, 20
6 α	1.74 m	23.4 (t)	C-4, 8, 10
β	1.46 m		
7 α	2.45 m	36.8 (t)	C-5, 9, 17
β	2.11 ddd (12.5, 12.5, 5.0)		
8		148.8 (s)	
9	2.48 d (10.0)	61.0 (d)	C-5, 7, 12, 15, 17
10		39.5 (s)	
11	6.88 dd (16.0, 10.0)	146.9 (d)	C-8, 10, 13
12	6.08 d (16.0)	133.8 (d)	C-9, 14
13		198.3 (s)	
14	2.28 s	27.4 (q)	C-12, 13
17	4.42 d (1.5)	108.8 (t)	C-7, 9
	4.81 d (1.5)		
18	0.91 s	33.7 (q)	C-3, 4, 5, 19
19	0.86 s	22.1 (q)	C-3, 4, 5, 18
20	0.91 s	15.3 (q)	C-1, 5, 9, 10

Spectra were recorded at ^a500 and ^b125 MHz in CDCl₃, respectively. ^c*J* values (in Hz) are in parentheses. ^dMultiplicity was deduced by DEPT and is indicated by the usual symbols.

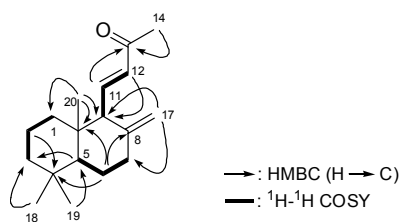
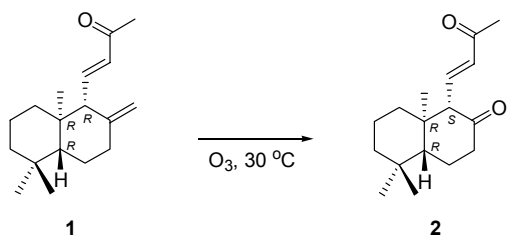


Figure 2. The ^1H - ^1H COSY and key HMBC correlations of **1**.



Scheme 1. Ozonolysis of **1**

C-20; H₂-6/C-4, C-8, C-10; H₂-7/C-5, C-9; H₃-18/C-4, C-5; H₃-19/C-4, C-5; H₃-20/C-9, C-10, confirmed the presence of a bicyclic bisnorditerpene skeleton. The presence of an exocyclic double bond attached at C-8 established by the HMBC correlations between H₂-17/C-7, C-9. The remaining acetyl group was positioned at C-12 by HMBC correlations between H-11/C-13 and H₃-14/C-13.

The relative configurations of the three chiral centers at C-5, C-9, and C-10 of **1** were elucidated by analyzing NOESY data. The NOESY spectrum of **1** displayed correlations between H-5/H-9 and H-11/H₃-20, but no correlations between H-5/H-11 or H-20 and between H-9/H-11 or H-20. These led to the assignment of a *trans*-relation between the proton at the ring junction C-5 (H-5) and the quaternary methyl at C-10 (H₃-20), and a *cis*-relation between the methyl at C-10 (H₃-20) and the side chain residue at C-9 (H-11). The optical rotation of **1** ($[\alpha]_{\text{D}}^{25} -5.5^\circ$) was almost of the same value but of opposite sign to that of (*E*)-15,16-bisnorlabda-8(17),11-diene-13-one ($[\alpha]_{\text{D}}^{25} +6.6^\circ/+4.5^\circ$),^{7,8} isolated from *Alpinia* genus. To establish the absolute structure of **1**, compound **2** was prepared *via* the ozonolysis of **1** (Scheme 1).⁸ The absolute stereochemistry of **1** could be determined by application of the octant rule using the Cotton effect of the $n \rightarrow \pi^*$ band near 290 nm.⁸ The CD spectrum of **2** showed a positive Cotton effect at 290 nm ($\Delta\epsilon = +3.32$), which indicated 5*R*, 9*S*, and 10*R* of **2** based on reported data.⁸ On the basis of above findings, the structure of **1** was established and the absolute configuration of the chiral centers of **1** were assigned as 5*R*, 9*R*, and 10*R*, and named as amoxanthin A.

It is worth noting that bisnorlabdane diterpenoid was rarely found in natural sources.^{9,10} In this paper, we suggest that the C-1 (δ 36.6) and C-7 (δ 40.9) assignments of the bisnorlabdane diterpene, (*E*)-15,16-bisnorlabda-8(17),11-diene-13-one,^{7,8} determined by the Itokawa group should be corrected since those of its stereoisomer, amoxanthin A (**1**) were unequivocally assigned as δ 41.1 (C-1) and δ 36.8 (C-7) by detailed analysis of 2D NMR data in the present study. The cytotoxicity of **1** was evaluated against the A549 (a non small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma),

and HCT15 (colon adenocarcinoma) human tumor cell lines *in vitro* using the SRB assay.¹¹ Compound **1** was found to have moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (IC₅₀: 13.9, 15.2, 11.8 and 12.6 μM , respectively).

Experimental Section

General procedures. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using a Shimadzu UV-1601 UV-visible spectrophotometer. CD spectra were measured on a JASCO J-715 spectropolarimeter. FAB and HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^1H - ^1H COSY, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Apollo Silica 5 μ column (250 \times 10 mm). Silica gel 60 (Merck, 70 ~ 230 mesh and 230 ~ 400 mesh) was used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Spots were detected on TLC under UV light or by heating after spraying with 10% H₂SO₄ in C₂H₅OH (v/v).

Plant materials. The seeds of *A. xanthioides* (2.5 kg), which were imported from China, were bought at Kyungdong Market (Seoul) in December 2007 and identified by one of the authors (K.R.L.). A voucher specimen (SKKU-2007-12B) of the plant was deposited at the School of Pharmacy at Sungkyunkwan University, Suwon, Korea.

Extraction and isolation. The seeds of *A. xanthioides* (2.5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (210 g), which was dissolved in water (800 mL) and partitioned with solvent to give *n*-hexane (18 g), CHCl₃ (11 g), and *n*-BuOH (23 g) soluble portions. The *n*-hexane soluble fraction (18 g) was subjected to column chromatography (CC) over a silica gel (230 ~ 400 mesh, 500 g, 6 \times 90 cm), eluting with a gradient solvent system of *n*-hexane-EtOAc (10 : 1 and 1 : 1, 2 L of each solvent) to yield seven crude fractions (F1 – F7). F3 (1.3 g) was applied to CC over Sephadex LH-20 (Pharmacia Co.), eluting with a solvent system of CH₂Cl₂-MeOH (1 : 1) and purified further by semi-preparative HPLC, using *n*-hexane-EtOAc (10 : 1) over 30 min at a flow rate of 2.0 mL/min (Apollo Silica 5 μ column; Shodex refractive index detector) to yield **1** (5 mg).

Amoxanthin A (1). Colorless gum; $[\alpha]_{\text{D}}^{25} -5.5^\circ$ (*c* 0.12, CHCl₃); UV (EtOH) λ_{max} (log ϵ) 228 (4.8) nm; IR (KBr) ν_{max} 3070, 1678, 1645, 1461, 1259, 1085, 885 cm⁻¹; ^1H and ^{13}C NMR: see Table 1. FABMS m/z 260 [M]⁺; HRFABMS (positive-ion mode) m/z 283.2041 [M + Na]⁺ (calcd. for C₁₈H₂₈ONa, 283.2038).

Ozonolysis of compound 1. Compound **1** (3.5 mg) in MeOH (5 mL) was bubbled with O₃ for 15 minutes at 0°C. The reaction mixture was stirred for 1 hr at 30°C with acetic acid (0.25 mL) and zinc powder (10 mg). Then the solvent was evaporated and the product was subjected to HPLC (*n*-hexane-EtOAc, 5 : 2) to give compound **2** (1.3 mg).

Compound 2. Colorless gum; CD (MeOH) ($\Delta\epsilon$): 290 (+3.32) nm; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 0.89 (3H, s), 0.91 (3H, s), 0.99 (3H, s), 2.30 (3H, s), 2.86 (1H, d, $J=10.0$ Hz), 5.97 (1H, d, $J=16.0$ Hz), 6.90 (1H, dd, $J=10.0, 16.0$ Hz); FABMS m/z 263 $[\text{M} + \text{H}]^+$.

Cytotoxicity assay. A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines.¹¹ The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (Sigma Chemical Co., $\geq 98\%$) was used as a positive control.

Acknowledgments. We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their assistance with the NMR spectroscopic and mass spectrometric measurements.

Supporting Information. 1D and 2D NMR data of **1** are available on request from the correspondence author.

References

1. *Japanese Pharmacopoeia*, 14th ed.; Hirokawa Publishing Co.: Tokyo, 2001; pp 2627-2628.
2. Kitajima, J.; Ishikawa, T. *Chem. Pharm. Bull.* **2003**, *51*, 890.
3. Zhang, S.; Lan, Y.; Qin, X. *Yaowu Fenxi Zazhi* **1989**, *9*, 219.
4. Choi, J. W.; Kim, K. H.; Lee, I. K.; Choi, S. U.; Lee, K. R. *Nat. Prod. Sci.* **2009**, *15*, 44.
5. Kim, K. H.; Choi, J. W.; Choi, S. U.; Lee, K. R. *Planta Med.* **2010**, *76*, 461.
6. Itokawa, H.; Morita, H.; Takeya, K.; Motidome, M. *Chem. Pharm. Bull.* **1988**, *36*, 2682.
7. Itokawa, H.; Morita, M.; Mihashi, S. *Chem. Pharm. Bull.* **1980**, *28*, 3452.
8. Itokawa, H.; Yoshimoto, S.; Morita, H. *Phytochemistry* **1988**, *27*, 435.
9. Muhammad, I.; Mossa, J. S.; El-Feraly, F. S. *Phytother. Res.* **1996**, *10*, 604.
10. Marco, J. A.; Sanz-Cervera, J. F.; Mangano, E. *Phytochemistry* **1993**, *33*, 875.
11. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; MaMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.