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Amoxantin A: A New Bisnorlabdane Diterpenoid from *Amomum xanthioides*

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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, $[\alpha]_D^{25}$ -5.5^o (*c* 0.12, $CHCl_3$). Its molecular formula was established as $C_{18}H_{28}O$ (5 degrees of unsaturation) from the $[M + Na]^+$ peak at *m/z* 283.2041 (calcd. for $C_{18}H_{28}ONa$, 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 ^{13}C 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 ^{13}C NMR data showed resonances 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.

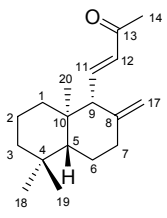

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Figure 1. Structure of amoxanthin A.

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 H_2-2/C-4, C-10; H_3-18/C-3, C-4; H_3-19/C-3, C-4; H_3-20/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 ^{13}C NMR data and HMBC correlations for **1**

C/H	$^1H^a$/δ	$^{13}C^b$/δ	HMBC (H→C)
1α	1.40 m	41.1 (t)[d]	C-3, 20
β	1.04 ddd (12.5, 12.5, 5.0)[c]		
2α	1.55 m	19.2 (t)	C-4, 10
β	1.55 m		
3α	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
6α	1.74 m	23.4 (t)	C-4, 8, 10
β	1.46 m		
7α	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 [a]500 and [b]125 MHz in $CDCl_3$, respectively. [c]*J* values (in Hz) are in parentheses. [d]Multiplicity was deduced by DEPT and is indicated by the usual symbols.

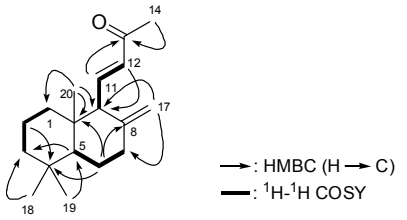
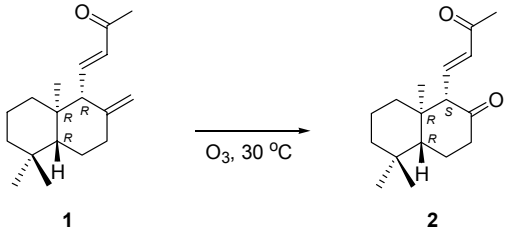


Figure 2. The ^{1}H-^{1}H COSY and key HMBC correlations of **1**.



Scheme 1. Ozonolysis of **1**

C-20; H_2-6/C-4, C-8, C-10; H_2-7/C-5, C-9; H_3-18/C-4, C-5; H_3-19/C-4, C-5; H_3-20/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 H_2-17/C-7, C-9. The remaining acetyl group was positioned at C-12 by HMBC correlations between H-11/C-13 and H_3-14/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/H_3-20, 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 quarternary methyl at C-10 (H_3-20), and a *cis*-relation between the methyl at C-10 (H_3-20) and the side chain residue at C-9 (H-11). The optical rotation of **1** ($[\alpha]_D^{25}$ −5.5°) was almost of the same value but of opposite sign to that of (*E*)-15,16,-bisnorlabda-8(17),11-diene-13-one ($[\alpha]_D^{25}$ +6.6°/ +4.5°),[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 → π* band near 290 nm.[8] The CD spectrum of **2** showed a positive Cotton effect at 290 nm ($\Delta\varepsilon$ = +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 (IC_{50}: 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 Schimadzu 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 ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) 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 5μ column (250 × 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% H_2SO_4 in C_2H_5OH (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), $CHCl_3$ (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 × 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 CH_2Cl_2-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; $[\alpha]_D^{25}$ −5.5° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (4.8) nm; IR (KBr) ν_{max} 3070, 1678, 1645, 1461, 1259, 1085, 885 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1. FABMS *m*/*z* 260 $[M]^+$; HRFABMS (positive-ion mode) *m*/*z* 283.2041 $[M + Na]^+$ (calcd. for $C_{18}H_{28}ONa$, 283.2038).

Ozonolysis of compound 1. Compound **1** (3.5 mg) in MeOH (5 mL) was bubbled with O_3 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) (Δε): 290 (+3.32) nm; ^{1}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 $[M + H]^+$.

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., ≥ 98%) was used as a positive control.

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

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