Cytotoxic Sesquiterpenoid from the Seeds of *Amomum xanthioides*

Ki Hyun Kim¹, Jung Wook Choi¹, Sang Un Choi², and Kang Ro Lee¹,*

¹Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
²Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

Abstract – As parts of our continuing search for biologically active compounds from medicinal plants, we investigated the constituents of the seeds of *Amomum xanthioides* and isolated a sesquiterpenoid, a nerolidol derivative from its MeOH extract. The chemical structure was determined by spectroscopic methods, including 1D and 2D NMR to be (2'S,2'R,5'S')-2-(5'-ethenyltetrahydro-5'-methylfuran-2'-yl)-6-methylhept-5-en-2-ol (1). Compound 1 was isolated for the first time from nature source. Compound 1 exhibited a good cytotoxicity against SK-OV-3 and SK-MEL-2 cells (IC₅₀: 16.7 and 8.6 µM, respectively) using a SRB bioassay. In this study, we also determined the absolute configuration of 2 reported in previous paper.

Keywords – *Amomum xanthioides*, Zingiberaceae, Sesquiterpenoid, Cytotoxicity

Introduction

*Amomum xanthioides* (Zingiberaceae) is a perennial herb and its seeds, listed in the Japanese Pharmacopoeia as Amomum seed, have been used in traditional medicinal purposes for the treatment of stomach and digestive disorders (Kitajima et al., 2003). Previous chemical investigations on this herb have demonstrated that the essential oil (1 - 1.5%) of this plant was rich in monoterpenoids (borneol, linalool, camphene and nerolidol) (Kitajima et al., 2003; Zhang et al., 1989). The extract of this plant was reported to exhibit antidiabetic activity (Park et al., 2001). As parts of our continuing search for biologically active compounds from medicinal plants, we investigated the constituents of the seeds of *A. xanthioides* and have reported cytotoxic constituents including terpenoids, phenolics and flavonoids (Choi et al., 2009; Kim et al., 2010a; Kim et al., 2010b). We conducted a further chemical investigation of the seeds of *A. xanthioides*, which led to isolation of a nerolidol derivative, (2'S,2'R,5'S')-2-(5'-ethenyltetrahydro-5'-methylfuran-2'-yl)-6-methylhept-5-en-2-ol (1). The structure of 1 was elucidated by spectroscopic methods, including 1D and 2D NMR. Compound 1 was isolated for the first time from nature source. Compound 1 was tested for cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15 cells) *in vitro* using a SRB bioassay. In this study, we also determined the absolute configuration of 2 reported in previous paper (Choi et al., 2009). This paper describes the isolation, structural elucidation, and cytotoxic activity of 1.

Experimental

General – Optical rotations were measured on a Jasco P-1020 polarimeter in CHCl₃. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. 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 (¹³C) 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 × 22 mm i.d.). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) was used for column chromatography. TLC was performed using Merck precoated silica gel F₂₅₄ plates. 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**—Seeds of *A. xanthioides* (2.5 kg) were extracted with 80% MeOH at room temperature and filtered. The filtrate was evaporated under reduced pressure to give a MeOH extract (210 g), which was suspended in water (800 mL) and then successively partitioned with *n*-hexane, CHCl₃, and *n*-BuOH, yielding 18, 11, and 23 g of residue, respectively. The *n*-hexane-soluble fraction (18 g) was chromatographed on a silica gel (230-400 mesh, 500 g, 6 × 90 cm) column eluted with *n*-hexane-EtOAc (10:1 → 1:1, gradient system) to yield seven fractions (H1-H7). Fraction H3 (1.3 g) was subjected to column chromatography (CC) over a silica gel (230-400 mesh, 100 g) eluted with a solvent system of *n*-hexane-EtOAc (1:1) to give six sub-fractions (H31-H36). Sub-fraction H34 (350 mg) 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 (1:1) over 30 min at a flow rate of 2.0 mL/min (Agilent silica 5 μ column; Shodex refractive index detector) to yield 1 (7 mg). Compound 2 was isolated from the *n*-hexane-soluble fraction by CC over a silica gel, Sephadex LH-20 and semi-preparative HPLC as described in previous paper (Choi et al., 2009).

**Results and Discussion**

Compound 1 was obtained as a colorless oil. The molecular formula was determined to be C₁₅H₂₇O₂ from the molecular ion peak [M + H]⁺ at m/z 239.2018 (caled for C₁₅H₂₇O₂, 239.2011). The 1H-NMR (CDCl₃, 500 MHz): δ 5.94 (1H, dd, J = 17.5, 10.5 Hz, H-5a), 5.18 (1H, dd, J = 17.5, 1.5 Hz, H-5b), 5.11 (1H, m, H-5), 5.00 (1H, dd, J = 10.5, 1.5 Hz, H-5b), 3.88 (1H, t, J = 7.0 Hz, H-2'), 2.09 (2H, m, H-4'), 1.95-1.74 (4H, m, H-3', 4'), 1.68 (3H, s, H-7), 1.62 (3H, s, CH₃-7), 1.51 (2H, m, H-3), 1.31 (3H, s, CH₃-5), 1.22 (3H, s, H-1); 13C-NMR (CDCl₃, 125 MHz): δ 144.4 (C-5a), 132.3 (C-6), 125.6 (C-5), 111.8 (C-5b), 85.3 (C-2'), 83.5 (C-5'), 72.9 (C-2), 40.7 (C-4'), 38.1 (C-3), 26.5 (CH₃-5'), 26.2 (C-4), 26.1 (CH₃-7), 22.1 (C-3'), 21.7 (C-1), 17.9 (C-7).

**3(S,E)-Nerolidol (2)**—Colorless oil, [α]₂⁰°⁰ : +11.2⁰ (c 1.75, CHCl₃); IR νmax cm⁻¹: 3413, 2970, 1661, 1534, 1454, 1376, and 1108; FAB-MS m/z: 223 [M + H]⁺; 1H-NMR (CDCl₃, 500 MHz): δ 5.92 (1H, dd, J = 18.0, 11.0 Hz, H-2), 5.22 (1H, d, J = 18.0 Hz, H-1b), 5.13 (1H, m, H-10), 5.08 (1H, m, H-6), 5.04 (1H, d, J = 11.0 Hz, H-1a), 2.08-1.97 (4H, m, H-4, 8), 1.67 (3H, s, H-15), 1.60 (3H, pro, H-12), 1.59 (3H, pro, H-14), 1.27 (3H, pro, H-14); 13C-NMR (CDCl₃, 125 MHz): δ 145.2 (C-2), 135.7 (C-7), 131.6 (C-11), 124.4 (C-10), 124.4 (C-6), 111.8 (C-1), 73.6 (C-3), 42.2 (C-4), 39.8 (C-8), 28.0 (C-13), 26.8 (C-9), 25.8 (C-12), 22.9 (C-5), 17.8 (C-15), 16.2 (C-14).

**Cytotoxicity assay**—A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines (Skelan et al., 1990). The assays were performed at the Korea Research Institute of Chemical Technology. 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. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were IC₅₀ 0.01, 0.06, 0.12, and 0.16 μM, respectively.
DEPT and HMQC experiments as one trisubstituted double bond (\(\delta 132.3\) and 125.6), one monosubstituted double bond (\(\delta 144.4\) and 111.8), four methyls, four methylenes, an oxygenated methine at \(\delta 85.3\), and two oxygenated quaternary carbons at \(\delta 83.5\) and 72.9. The \(^1\)H- and \(^{13}\)C-NMR data of 1 were similar to those of 2, except that the proton and carbon resonances of the \(\Delta^2\)-double bond were absent, and resonances of one oxygenated methine [\(\delta 3.88\) (t, \(J = 7.0\) Hz); \(\delta 85.3\)] and one oxygenated quaternary carbon [\(\delta 72.9\)] were present in 1. The structure of 1 was verified by HMBC correlations from H-1 to C-2, C-3, and C-2', from CH\(_3\)-5' to C-4', C-5', and C-5'a, and from H-2' to C-5', confirming the presence of an OH at C-2 and an epoxy function at C-2'/C-5', forming a tetrahydrofuran ring in the structure (Fig. 2). The presence of a tetrahydrofuran ring was also supported by the signal for C-5' (\(\delta 83.5\)) shifted to lower field in comparison with that of \(\delta 73.6\) (Holmes et al., 1990). The relative configuration of 1 was deduced from analysis of NOESY spectra (Fig. 2), and the configuration of methyl (H-1) at C-2 and proton at C-2' was determined to be same orientated on the basis of the process of bioconversion (Holmes et al., 1990). This was confirmed by the NOESY spectra showing correlations from H-1 to H-2' and from H-2' to CH\(_3\)-5' (Fig. 2). A plausible biosynthetic pathway for 1 from the (3S,E)-nerolidol (2) isolated from this plant was proposed as shown in Scheme 1. Epoxidation of 2 in the double bond of C-6/C-7, the following cyclization forming a tetrahydrofuran ring, and finally ring-opening of the epoxide could lead to the formation of 1. Thus, based on all the above evidence, the structure of 1 was assigned as (2S*,2'R*,5S*)-2-(5'-ethenyltetrahydro-5'-methyl furan-2'-yl)-6-methylhept-5-en-2-ol. According to the survey of literature, compound 1 and relative derivatives were synthesized (Kaiser et al., 1979; Holmes et al., 1990), but here, compound 1 was isolated for the first time from nature source.

Compound 2 was reported as nerolidol, the absolute configuration of which has not been confirmed in our previous paper (Choi et al., 2009). In this paper, compound 2 was identified as (3S,E)-nerolidol by comparison of physicochemical and spectroscopic data with previously reported values (Blanc et al., 2005; Morikawa et al., 2002). In the \(^{13}\)C-NMR spectrum of 2, the chemical shift value (\(\delta 16.2\)) of C-14 suggested that compound 2 is the (E)-form of nerolidol because the corresponding value was observed at \(\delta 23.4\) in (Z)-nerolidol (Blanc et al., 2005). The positive optical rotation value ([\(\alpha\])_D\(^{25}\) +11.2°) of 2 supported that the absolute configuration of C-3 was S (Morikawa et al., 2002). Thus, the structure of 2 was assigned as (3S,E)-nerolidol.

The cytotoxicities of compound 1 against the A549 (a non small cell lung carcinoma), SK-OV-3 (ovarian malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma) human cancer cell lines were evaluated using the SRB assay (Skehan et al., 1990). Compound 1 exhibited a good cytotoxicity against the SK-OV-3 and SK-MEL-2 cells (IC\(_{50}\); 16.7 and 8.6 \(\mu\)M, respectively). But, compound 1 was essentially non-cytotoxic against the other tested cell lines (IC\(_{50}\) > 30 \(\mu\)M). Compound 2

\[\text{Scheme 1. Proposed biosynthetic pathway for 1.}\]
also exhibited significant cytotoxic activity against the SK-OV-3 and SK-MEL-2 in previous paper (Choi et al., 2009). Above biological data suggest that nerolidol derivatives have relatively good cytotoxicity against the SK-OV-3 and SK-MEL-2 cells though more nerolidol derivatives need to be tested to confirm this hypothesis.

Acknowledgements

The authors would like to thank Mr. Do Kyun Kim, Dr. Eun Jung Bang, and Dr. Jung Ju Seo at the Korea Basic Science Institute for the NMR and MS spectra measurements.

References


