

Cytotoxic Sesquiterpenoid from the Seeds of *Amomum xanthioides*

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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

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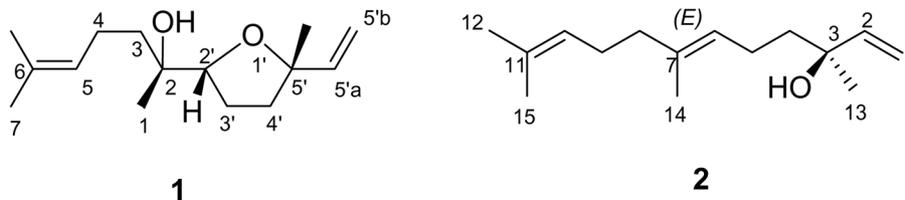


Fig. 1. The structures of **1** and **2** from *A. xanthioides*.

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 (14 : 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 (18 : 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** (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).

(2*S,2'*R**,5'*S**)-2-(5'-Ethenyltetrahydro-5'-methylfuran-2'-yl)-6-methylhept-5-en-2-ol (1)** – Colorless oil, $[\alpha]_D^{25} : +20.6^\circ$ (*c* 0.35, CHCl₃); IR ν_{\max} cm⁻¹: 3413, 2970, 1661, 1534, 1455, 1380, and 1117; FAB-MS *m/z*: 239 [M + H]⁺; HR-FAB-MS *m/z*: 239.2018 [M + H]⁺ (calcd for C₁₅H₂₇O₂, 239.2011); ¹H-NMR (CDCl₃, 500 MHz): δ 5.94 (1H, dd, *J* = 17.5, 10.5 Hz, H-5'a), 5.18 (1H, dd, *J* = 17.5, 1.5 Hz, H-5'b), 5.11 (1H, m, H-5), 5.00 (1H, dd, *J* = 10.5, 1.5 Hz, H-5'b), 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-5'a), 132.3 (C-6), 125.6 (C-5), 111.8 (C-5'b), 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, $[\alpha]_D^{25} : +11.2^\circ$ (*c* 1.75, CHCl₃); IR ν_{\max} cm⁻¹: 3415, 2973, 1674, 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, brs, H-15), 1.60 (3H, brs, H-12), 1.59 (3H, brs, H-14), 1.27 (3H, brs, H-13); 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 (Skehan *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.

Results and Discussion

Compound **1** was obtained as a colorless oil. The molecular formula was determined to be C₁₅H₂₆O₂ from the molecular ion peak [M + H]⁺ at *m/z* 239.2018 (calcd for C₁₅H₂₇O₂, 239.2011) in the positive-ion HR-FAB-MS. The IR spectrum of **1** indicated the presence of hydroxy group (3413 cm⁻¹) and C = C double bond groups (1661 cm⁻¹). The ¹H-NMR spectrum showed the presence of four olefinic proton signals at δ 5.94 (dd, *J* = 17.5, 10.5 Hz), 5.18 (dd, *J* = 17.5, 1.5 Hz), 5.11 (m), and 5.00 (dd, *J* = 10.5, 1.5 Hz), an oxygenated methine at δ 3.88 (t, *J* = 7.0 Hz), and four tertiary methyls at δ 1.68 (s), 1.62 (s), 1.31 (s), and 1.22 (s). The ¹³C-NMR spectrum indicated 15 carbon resonances, which were classified by

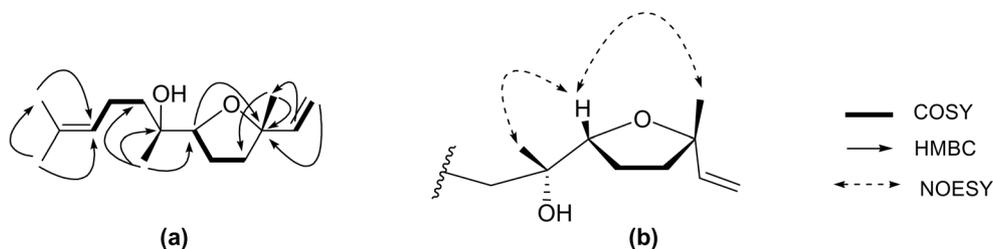
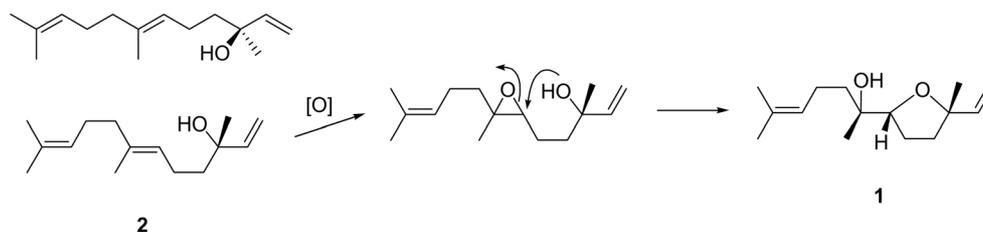


Fig. 2. Key ^1H - ^1H COSY and HMBC correlations (a) and key NOESY correlations (b) of **1**.



Scheme 1. Proposed biosynthetic pathway for **1**.

DEPT and HMQC experiments as one trisubstituted double bond (δ 132.3 and 125.6), one monosubstituted double bond (δ 144.4 and 111.8), four methyls, four methylenes, an oxygenated methine at δ 85.3, and two oxygenated quaternary carbons at δ 83.5 and 72.9. The ^1H - and ^{13}C -NMR data of **1** were similar to those of **2**, except that the proton and carbon resonances of the $\Delta^{2,2'}$ -double bond were absent, and resonances of one oxygenated methine [δ 3.88 (t, J = 7.0 Hz); δ 85.3] and one oxygenated quaternary carbon [δ 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' (δ 83.5) shifted to lower field in comparison with that of **2** (δ 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 (3*S,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 (2*S**,2'*R**,5'*S**)-2-(5'-ethenyltetrahydro-5'-methylfuran-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 (3*S,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 (δ 16.2) of C-14 suggested that compound **2** is the (*E*)-form of nerolidol because the corresponding value was observed at δ 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 (3*S,E*)-nerolidol.

The cytotoxicities of compound **1** 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 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 μM , respectively). But, compound **1** was essentially non-cytotoxic against the other tested cell lines (IC_{50} > 30 μM). Compound **2**

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.

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References

- Blanc, M.C., Bradesi, P., and Casanova, J., Enantiomeric differentiation of acyclic terpenes by ^{13}C NMR spectroscopy using a chiral lanthanide shift reagent. *Magn. Res. Chem.* **43**, 176-179 (2005).
- Choi, J.W., Kim, K.H., Lee, I.K., Choi, S.U., and Lee, K. R., Phytochemical Constituents of *Amomum xanthioides*. *Nat. Prod. Sci.* **15**, 44-49 (2009).
- Holmes, D.S., Ashworth, D.M., and Robinson, J.A., The bioconversion of (3*RS,E*)- and (3*RS,Z*)-nerolidol into oxygenated products by *Streptomyces cinnamonensis*. Possible implications for the biosynthesis of the polyether antibiotic monensin A?. *Helv. Chim. Acta* **73**, 260-271 (1990).
- Kaiser, R. and Lamparsky, D., Constituents of *Osmanthus absolute*. Part 6. The (7*S*,10*S*,5*E*)- and (7*R*,10*S*,5*E*)-2,6,10-trimethyl-7,10-epoxy-2,5,11-dodecatrienes. *Helv. Chim. Acta* **62**, 1887-1892 (1979).
- Kim, K.H., Choi, J.W., Choi, S.U., Seo, E.-K., and Lee, K. R., Amoxantin A: A new bisnorlabdane diterpenoid from *Amomum xanthioides*. *Bull. Korean Chem. Soc.* **31**, 1035-1037 (2010a).
- Kim, K.H., Choi, J.W., Choi, S.U., and Lee, K.R., Terpene glycosides and cytotoxic constituents from the seeds of *Amomum xanthioides*. *Planta Med.* **76**, 461-464 (2010b).
- Kitajima, J. and Ishikawa, T., Water soluble constituents of Amomum seed. *Chem. Pharm. Bull.* **51**, 890-893 (2003).
- Morikawa, T., Matsuda, H., Sakamoto, Y., Ueda, K., and Yoshikawa, M., New farnesane-type sesquiterpenes, hedychiols A and B 8,9-diacetate, and inhibitors of degranulation in RBL-2H3 cells from the rhizome of *Hedychium coronarium*. *Chem. Pharm. Bull.* **50**, 1045-1049 (2002).
- Park, B.H. and Park, J.W., The protective effect of *Amomum xanthioides* extract against alloxan-induced diabetes through the suppression of NFkappaB activation. *Exp. Mol. Med.* **33**, 64-68 (2001).
- Skehan, P., Strohreng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., and Boyd, M.R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **82**, 1107-1112 (1990).
- Zhang, S., Lan, Y., and Qin, X., Gas chromatography analysis of the volatile oil of the imported amomi semen (*Amomum xanthioides* Wall ex Bak.) and adulterants (Hong Ke Sha, Cao Dou Kou, Hong Dou Kou, and Zhu Mu Sha). *Yaowu Fenxi Zazhi* **9**, 219-222 (1989).

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