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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-5en-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 (IC_{50}: 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-5en-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**.

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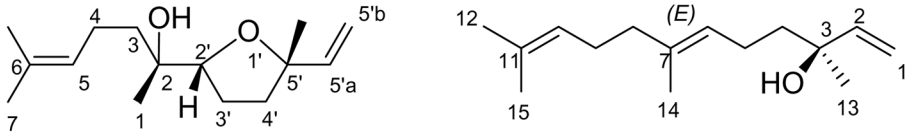
Experimental

General – Optical rotations were measured on a Jasco P-1020 polarimeter in $CHCl_3$. 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 (^{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 × 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 F_{254} 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% 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



1 2

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, $CHCl_3$, 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 CH_2Cl_2-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-5en-2-ol (1)** – Colorless oil, $[\alpha]_D^{25}$: +20.6° (*c* 0.35, $CHCl_3$); 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 $C_{15}H_{27}O_2$, 239.2011); ^{1}H-NMR ($CDCl_3$, 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, CH_3-7), 1.51 (2H, m, H-3), 1.31 (3H, s, CH_3-5'), 1.22 (3H, s, H-1); ^{13}C-NMR ($CDCl_3$, 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 (CH_3-5'), 26.2 (C-4), 26.1 (CH_3-7), 22.1 (C-3'), 21.7 (C-1), 17.9 (C-7).

(3*S*,*E*)-Nerolidol (2) – Colorless oil, $[\alpha]_D^{25}$: +11.2° (*c* 1.75, $CHCl_3$); IR ν_{max} cm^{-1}: 3415, 2973, 1674, 1534, 1454, 1376, and 1108; FAB-MS *m/z*: 223 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 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 IC_{50} 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 $C_{15}H_{26}O_2$ from the molecular ion peak $[M + H]^+$ at *m/z* 239.2018 (calcd for $C_{15}H_{27}O_2$, 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 ^{1}H-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 ^{13}C-NMR spectrum indicated 15 carbon resonances, which were classified by

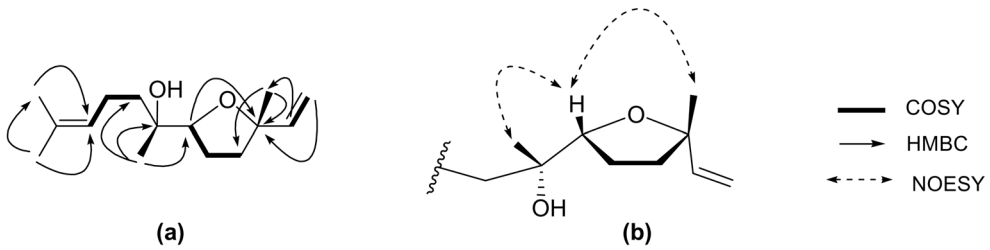
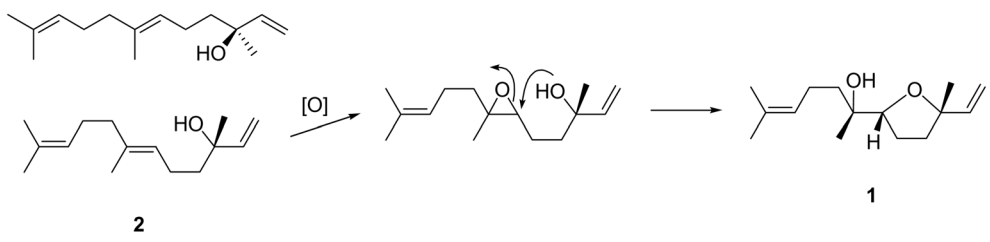


Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations **(a)** and key NOESY correlations **(b)** of **1**.



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 ^{1}H- and ^{13}C-NMR data of **1** were similar to those of **2**, except that the proton and carbon resonances of the $\Delta^{2,2'}$-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-5en-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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