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Antibiotic Components from the Rhizomes of *Curcuma zedoaria*

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Abstract – Two terpenoids, including one uniquely aromatized one (**1**), were isolated from CH_2Cl_2-soluble fraction of MeOH extracts of *Curcuma zedoaria*. They were identified to be a sesquiterpene ketolactone (**1**) and orobanone (**2**), respectively on the basis of their NMR data. The structure of compound **1** was confirmed by X-ray chrystallography and the reported NMR assignments for **1** were revised in this study. Antibiotic activities for compounds **1** and **2** were evaluated using disk diffusion assay. Compound **1** showed potent antibacterial activities against *Listeria monocytogenes* and *Staphylococcus pseudointermedius* while compound **2** was active against *Bacillus cereus*.

Keywords – *Curcuma zedoaria*, Zingiberaceae, Sesquiterpene Ketolactone, Antibacterial activity

Introduction

Curcuma zedoaria Rosc (Zingiberaceae) is perennial herb and indigenous to Bangladesh, Sri Lanka, and India, and is also cultivated in China, Japan, Brazil, Nepal, and Thailand.[1] Rhizomes of this plants, known as white turmeric, zedoaria, or gajutsu, has been used as an aromatic stomachic, emmenagogus, or for the treatment of 'Oketsu' syndrome caused by blood stagnation in Korea, Japan, and China.[2] Many researches on zedoaria has also been carried out so far, and it exhibited a variety of biological activities including antimicrobial, analgesic, antiallergic, hepatoprotective, anti-inflammatory, and antioxidant activities.[3-5] Our previous phytochemical investigations on *C. zedoaria* led to the isolation of a dozen of compounds including known sesquiterpenoids, a known flavonoid, a known diarylhepatoid, a unique diterpene, and three new sesquiterpenoids.[6-7] Our ongoing research on zedoaria led to isolation of two antibiotic terpenoids. The present study describes isolation and structural determination of two known sesquiterpenoid (**1 - 2**), revision of NMR assignments, and their antibiotic activities.

Experimental

General experimental procedures – NMR spectra were recorded with Varian's standard pulse program of Varian VNS spectrometer at 250 MHz, 300 MHz, and 600 MHz. EI-MS spectra were recorded with Micromass spectrum (AUTOSPEC, UK). TLC was done using Kieselgel 60F254 (Merck) and RP-18 (Whatman). Column chromatography was done using silica gel (70 - 230 mesh, Merck). Agilent 1200 series HPLC system equipped with a quaternary pump, a degasser, an injector, a column thermostat, and diode array detector (DAD) was used for purification of compounds. All HPLC separations were carried out using an Eclipse XDB-C_{18} semi-preparative column (9.4 × 250 mm, 5 μm, Agilent Technologies, USA) at a flow rate of 2 mL/min.

Plant materials – The dried rhizomes of *Curcuma zedoaria* were purchased from Daegu pharmacopoeia market, South Korea. A voucher specimen was deposited in the College of Pharmacy, Duksung Women's University.

Extraction and isolation – The dried rhizomes of *C. zedoaria* (2 kg) were extracted three times with methanol (4 L) under reflux for 3 hrs to give a MeOH extract (241.2 g). The extract was successively partitioned with *n*-hexane, CH_2Cl_2, and EtOAc to yield 50.9, 73.2, and 12.1 g respectively. The CH_2Cl_2-soluble fraction (73.2 g) was subjected to vacuum liquid chromatography (70 - 230 mesh, Merck) with elution of a gradient of *n*-hexane-ethyl acetate, followed by EtOAc-MeOH gradient, to afford eleven fractions (Fr. 1~Fr. 11). Fr. 10 (1.2 g) was further subjected to silica gel column chromatography eluted with CH_2Cl_2-MeOH (100:0 → 100:1) to give 20 fractions (Fr. 10-1~10-20). Fr. 10-12 (0.56 g) was further subjected to Sephadex LH-20 column chromatography with an isocratic solvent system of $CHCl_3$-MeOH (1:1) to give 13

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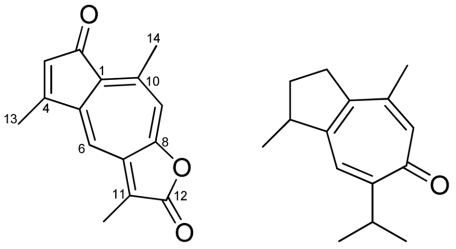

1 **2**

Fig. 1. Chemical structures of compounds **1** - **2**.

fractions (Fr. 10-12-1~10-12-13). Fr. 10-12-5 (50.2 mg) was subjected to semi-preparative reverse-phase HPLC (Luna 5u Phenyl-Hexyl column; 10.00 × 250 mm; flow rate, 2 mL/min; 50% MeOH in H_2O for 10 min, followed by 50 - 75% MeOH in H_2O for 10 min, followed by 75 - 80% MeOH in H_2O for 10 min; UV detection at 210 nm) to afford compounds **1** (5.6 mg, t_R = 25.2 min) and **2** (3.5 mg, t_R = 20.2 min) (Fig. 1).

A sesquiterpene ketolactone (1) – Yellow crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.83 (1H, s, H-6), 6.52 (1H, s, H-9), 6.21 (1H, s, H-3), 2.68 (3H, s, H-14), 2.30 (3H, s, H-13), 2.09 (3H, s, H-15); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 195.1 (C-2), 169.2 (C-12), 160.7 (C-5), 156.0 (C-8), 145.9 (C-4), 144.7 (C-10), 143.4 (C-7), 132.6 (C-3), 127.0 (C-1), 116.9 (C-11), 116.9 (C-9), 114.6 (C-6), 22.3 (C-14), 14.3 (C-13), 8.5 (C-15); HMBC correlations (H-# → C-#) H-3 → C-1, C-2, C-4, C-5, and C-13; H-6 → C-1, C-5, C-7, and C-11; H-9 →C-1, C-7, C-8, and C-14; H-14 →C-1 and C-9; H-15 →C-11 and C-12; EIMS *m/z* (rel. int.), 240 $[M]^+$ (100).

Orobanone (2) – Colorless crystals. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.33 (1H, s, H-6), 7.01 (1H, s, H-9), 3.39 (1H, sept, *J* = 6.6 Hz, H-7), 3.26 (1H, m, H-4), 2.99 (1H, m, H-2a), 2.87 (1H, m, H-2b), 2.30 (3H, s, H-14), 2.25 (1H, m, H-3a), 1.65 (3H, d, *J* = 6.7 Hz, H-15), 1.62 (1H, m, H-3b), 1.26 (3H, d, *J* = 6.6 Hz, H-13), 1.18 (3H, d, *J* = 6.7 Hz, H-12); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 187.0 (C-8), 159.2 (C-7), 152.6 (C-1), 148.6 (C-5), 148.5 (C-10), 139.3 (C-9), 132.7 (C-6), 46.2 (C-4), 35.4 (C-2), 31.7 (C-3), 31.2 (C-11), 25.4 (C-14), 22.8 (C-12), 22.7 (C-15), 20.8 (C-13); EIMS *m/z* (rel. int.), 216 $[M]^+$ (25).

X-ray Crystallography – The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX CCD diffractometer equipped with Cu K_α radiation (λ = 1.5478). A 0.21 × 0.05 × 0.03 mm orange needle was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. Crystal-to-detector distance was 60 mm using variable exposure time (2s-10s) depending on θ with a scan width of 1.0°. Data collection was 99.1% complete to 68.00° in *θ*. A total of 12363 reflections were collected covering the indices, −13 <= h <= 13, −18 <= k <= 17, −16 <= l <= 16. 2044 reflections were found to be symmetry independent, with a R_{int} of 0.0338. Indexing and unit cell refinement indicated a *C*-centered, monoclinic lattice. The space group was found to be *C*2/c. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SHELXS) produced a complete phasing model consistent with the proposed structure.

All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97. Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Center (CCDC no. 979489).

Result and Discussion

Compound **1** was obtained as yellow crystals. The molecular formula of **1** was determined to be $C_{15}H_{12}O_3$ (ten saturations) by a combination of EI-MS and ^{13}C NMR spectroscopy.

Compound **1** showed ^{1}H and ^{13}C-NMR spectra containing signals for three isolated sp^2 methine groups, three methyl groups attached to an sp^2 quaternary carbon, a ketone, and a lactone group. Accordingly, a tricyclic structure was required for **1** to account for the unsaturation number. Whole assignment of the structure was based on HMBC correlations. HMBC correlation of a methyl at δ_H 2.09 with the lactonyl carbon at δ_C 169.2 indicated that the methyl was placed in the lactone ring. HMBC correlations of a methine proton at δ_H 6.21 with the ketone carbon, a methyl carbon at δ_C 14.3, and two quaternary sp^2 carbons at δ_C 160.6 and 127.0 indicated that **1** has a cyclopentenone moiety and a methyl group-bearing carbon was adjacent to the methine carbon, which is positioned next to the ketone carbon (Fig. 2). In addition, the rest methyl proton at δ_H 2.68 showed HMBC correlations with the quaternary sp^2 carbon at δ_C 127.0 and a methine at δ_C 116.9, suggesting the existence of C-C(CH_3)-CH-C spin system. Finally, HMBC correlations of the rest methine proton at δ_H 6.83 with the quaternary carbon at δ_C 160.7 of the cyclopentenone ring and the quaternary carbon at δ_C 143.4 of the lactone ring indicated that **1** has a 5-7-5 ring system. In this way, whole NMR assignment of **1** was elucidated (Fig. 1).

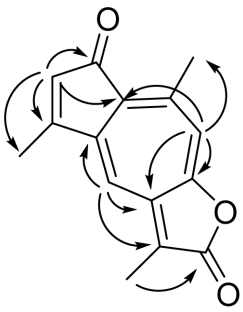

Fig. 2. Key HMBC correlations for compound **1**.

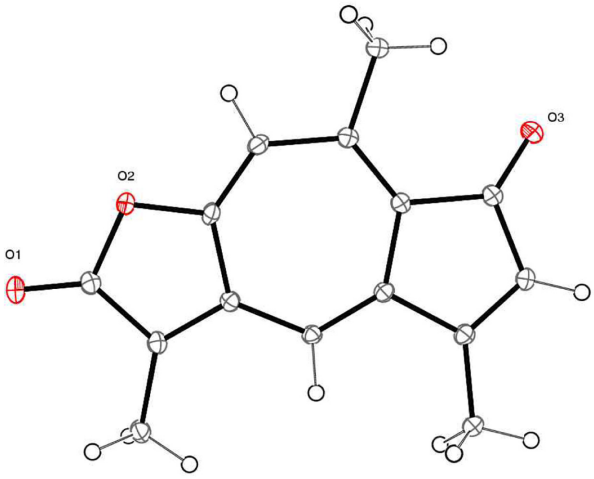

Fig. 3. X-ray crystallographic structure for **1**.

Crystals of **1** were obtained from methanol, and X-ray crystallographic analysis led to confirmation of the structure (Fig. 3). Compound **1** was first found as a photo-oxidation artefact of lindazulene isolated from *Paramuricea chamaeleon*.[8] And then, it was isolated from *Taraxacum wallichii* and *Artemisia gilvescens*.[9-10] To the best of our knowledge, this is the third isolation of **1** from natural resources and first report of the isolation from Zingiberaceae. In addition, NMR assignments for C-4, C-7, C-8, and C-10 were herein revised on the basis of HMBC correlations.

Compound **2** was isolated pale yellow crystals. Its ^{1}H and ^{13}C-NMR spectra indicated that it is a sesquiterpenoid and identified to be orobanone by comparison of the NMR data with those in the literature (Fig.1).[11-12] Orobanone was isolated from *Orobanche rapum* and *Croton argyroglossum*.[11-12] To the best of our knowledge, this is first isolated from this genus.

The isolated compounds **1** and **2** were evaluated for antibacterial activities against *Bacillus cereus* (ATCC 13061), *Listeria monocytogenes* (ATCC 19114), *Escherichia coli* (ATCC 35150), *Salmonella typhimurium* (ATCC 43174), and *Staphylococcus pseudointermedius* (ATCC 49444). The antibacterial activities were determined by disc diffusion assay.[13] Compound **1** showed potent antibacterial activity at 100 μg/disk against *L. monocytogenes* and *S. pseudointermedius*, causing inhibitory zones of 24 and 16 mm, respectively. Compound **2** was active against *B. cereus* at the same level, affording zones of 18 mm. An ampicillin gave 10- to 15-mm clear zones in these antibacterial assays at 25 μg/disk.

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