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NOTE

Isodeoxyhelicobasidin, a novel human neutrophil elastase inhibitor from the culture broth of *Volvariella bombycina*

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Elastin, an important structural protein of extracellular matrix (ECM), is the main component of elastic fiber, which provides resilience and elasticity to many tissues, such as the skin, lungs, ligaments and arterial walls.[1,2] Human neutrophil elastase (HNE), a serine protease primarily located in the azurophil granules of polymorphonuclear leukocytes, is the only enzyme capable of degrading ECM proteins, such as elastin, collagen, fibronectin, laminin and proteoglycan.[3] Biologically, elastase activity significantly increases with age and results in a reduced skin elastic property.[4]

In the course of our screening program for HNE inhibitors, we isolated a novel compound, isodeoxyhelicobasidin (**1**), from the culture broth of *Volvariella bombycina* (Figure 1). We report herein the fermentation, isolation, structure elucidation and biological activities of **1**.

The strain of *V. bombycina* (MKACC 53745) was provided by the Korea Agricultural Culture Collection of the National Institute of Agricultural Biotechnology, Suwon, Republic of Korea. The producing strain of *V. bombycina* pre-grown on a potato dextrose agar (PDA; Difco, Sparks, MD, USA) slant was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of yeast peptone sucrose (YPS) medium consisting of 2% glucose, 0.5% polypeptone, 0.2% yeast extract, 0.1% KH_2PO_4 and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 6.6), and cultured on a rotary shaker (153 r.p.m.) for 7 days at 27 °C. For fermentation, the seed culture was aseptically transferred into a 5-l jar fermenter containing 3.5 l of the above medium, and cultivation was carried out at 28 °C for 7 days with aeration of 2 l min^{-1} and agitation of 250 r.p.m.[5,6] The collected mycelial cake from the whole fermented broth (10 liters) was extracted with acetone and the extract was concentrated *in vacuo* to an aqueous solution, which was then extracted thrice with equal volume of EtOAc. The EtOAc layer (5 g) was loaded on a silica gel column and eluted with CH_2Cl_2–MeOH in a gradient mode (20:1 → 1:1), the active fraction was subjected to Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) column chromatography and eluted with CH_2Cl_2–MeOH (1:1), and then purified by YMC C_{18} preparative HPLC (20×250 mm, flow rate=4 ml min^{-1}, MeOH–H_2O=85:15) to afford **1** (6 mg, t_R=33 min).

Compound **1** was obtained as a yellowish powder; $[\alpha]_D^{20}$–25.0 (*c* 0.2, MeOH); UV ($CHCl_3$) λ_{max} nm (log ε): 266 (4.02); IR (KBr) ν_{max} (cm^{-1}): 3434, 2964, 1650, 1633, 1368, 1304, 1210, 896; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.55 (1H, s, 5-OH), 6.44 (1H, q, *J*=1.6 Hz, H-2), 2.93 (1H, m, H-8a), 2.04 (3H, d, *J*=1.60 Hz, H-15), 1.76-1.74 (1H, m, H-9a), 1.69-1.67 (1H, m, H-8b), 1.66-1.64 (1H, m, H-10a), 1.63-1.59 (1H, m, H-9b), 1.51-1.44 (1H, m, H-10b), 1.33 (3H, s, H-14), 1.12 (3H, s, H-12), 0.84 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz): *ä* 188.9 (C-1), 184.8 (C-4), 152.1 (C-5), 139.0 (C-3), 138.4 (C-2), 126.3 (C-6), 51.5 (C-7), 46.3 (C-11), 41.6 (C-10), 39.1 (C-8), 27.8 (C-13), 25.9 (C-12), 24.2 (C-14), 21.3 (C-9), 14.5 (C-15); HR-ESI-MS (*m/z*): 247.1342 $[M-H]^-$ (calcd for $C_{15}H_{19}O_3$, 247.1340). The molecular formula of **1**, $C_{15}H_{20}O_3$, was determined by high-resolution mass spectrometry. The UV spectrum of **1** showed an absorption maximum at 266 nm, indicating the presence of 1,4-benzoquinone chromophore.[7,8] The IR spectrum revealed characteristic absorption bands for hydroxyl group at 3434 cm^{-1} and conjugated carbonyl group at 1650 cm^{-1}.[9] The ^1H NMR spectrum of **1** displayed an enolic hydroxyl proton at δ_H 7.55 (1H, s, 5-OH), a quinonoid proton at δ_H 6.44 (1H, q, *J*=1.6 Hz, H-2) and a quinonoid methyl at δ_H 2.04 (3H, d, *J*=1.6 Hz, H-15). In addition, it also displayed signals for three tertiary methyl and three methylene groups, which were attributed to cyclopentane ring bearing three tertiary methyl groups. The ^{13}C NMR spectrum of **1** exhibited 15 carbon resonances consisting of three tertiary methyls, one quinonoid methyl, three methylenes, two quaternary aliphatic carbons, two carbonyl groups, one quinonoid methine

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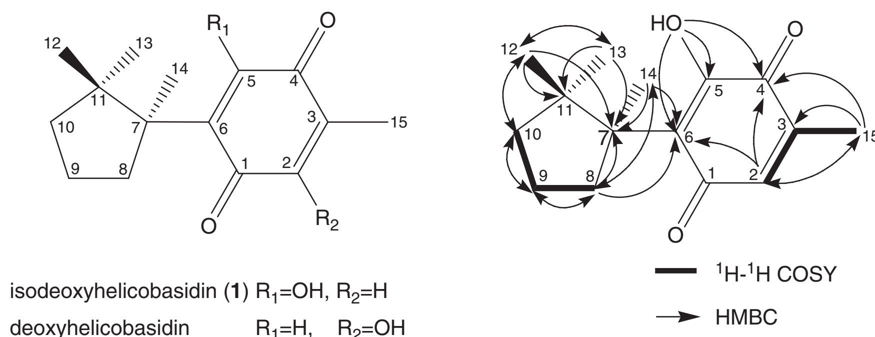

Figure 1 Structure, 1H-1H COSY and HMBC correlations of isodeoxyhelicobasidin (**1**).

Table 1 HNE inhibitory activity of isodeoxyhelicobasidin (1)[a]

	Inhibition ratio for HNE (%)					
Compound	*100 μM*	*30 μM*	*10 μM*	*3 μM*	*1 μM*	*IC_{50}[b] (μM)*
1	70.6±0.7	64.4±2.0	57.9±0.8	38.3±1.4	27.2±0.3	9.0±0.9
EGCG	65.9±1.3	62.4±0.8	47.3±1.3	25.8±1.4	17.3±0.8	12.9±0.3

Abbreviations: EGCG, epigallocatechin gallate; HNE, human neutrophil elastase.
[a]Results are expressed as means±s.d. (*n*=3).
[b]IC_{50} indicates the concentration (μM) at which the inhibition percentage of HNE activity was 50%, and the values were determined by regression analysis.

and three quaternary aromatic carbons. All protonated carbons and their protons were assigned by ^{1}H-^{1}H COSY and heteronuclear multiple quantum correlation (HMQC) experiments. The above mentioned spectroscopic data suggested that compound **1** was a cuparene-type sesquiterpenoid,[10] and the gross structure was further confirmed by COSY and heteronuclear multiple-bond correlation (HMBC) experiments (Figure 1). The COSY correlation of the quinonoid methyl protons at δ_H 2.04 (H-15) with the quinonoid proton at δ_H 6.44 (1H, q, *J*=1.6 Hz, H-2) and HMBC correlations of H-15 with C-2 at δ_C 138.4, C-3 at δ_C 139.0 and C-4 at δ_C 184.8 suggested that the quinonoid methyl group was at C-5 and the quinonoid methine was at C-2. The hydroxyl proton at δ_H 7.55 (OH-5) was long-range coupled to C-4, C-5 at δ_C 152.1 and C-6 at δ_C 126.3 in HMBC spectrum. In addition, HMBC correlations of the tertiary methyl protons at δ_H 1.33 (H-14) with C-6, C-7 at δ_C 51.5 and C-8 at δ_C 39.1 were observed. These spectral data indicated that **1** was a derivative hydroxylated at C-5 and dehydroxylated at C-2 of deoxyhelicobasidin, which has been isolated from *Helicobasidium mompa* Tanaka.[11] The stereochemistry at C-7 of **1** was assigned as *S* configuration by comparison with deoxyhelicobasidin, which also showed a negative optical rotation. Thus, the structure of **1** was established to be (*S*)-5-hydroxy-3-methyl-6-(1,2,2-trimethylcyclopentyl)-1,4-benzoquinone and named as isodeoxyhelicobasidin.

The inhibitory activity of **1** on HNE was evaluated with earlier described procedure.[12] Briefly, each well of a 96-well plate containing 100 μl of the following reagents: 10 mM Tris-HCl buffer (pH 7.5), 1.4 mM MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide, 0.18 U HNE and the sample at various concentrations were incubated for 1 h at 37 °C in the dark. After the reaction was stopped by addition of 100 μl soybean trypsin inhibitor of 0.2 mg ml^{-1}, absorbance was immediately measured at 405 nm. Epigallocatechin gallate (EGCG) was used as a positive control. As a result, compound **1** dose-dependently inhibited HNE activity with an IC_{50} value of 9.0 μm, which was comparable to the positive control, EGCG (IC_{50}, 12.9 μm) (Table 1). Compound **1** also showed antibacterial activity against several gram-positive bacteria including *S. aureus* 503, methicillin-resistant *S. aureus* CCARM 3167 (MRSA), quinolone-resistant *S. aureus* CCARM 3505 (QRSA), *Bacillus subtilis* 1021, *Staphylococcus epidermidis* 3958 and *Streptococcus mutans* 3065 with MIC values of 3.1–12.4 μg ml^{-1}.[13] In conclusion, compound **1** was a new analog of helicobasidin and lagopodin B, which were earlier isolated from *H. mompa* Tanaka and *Coprinus cinereus*, respectively,[14,15] and the potent HNE inhibitory activity of **1** suggested that it could be useful for the development of anti-aging cosmetics.

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