J. Microbiol. Biotechnol. (2010), **20**(8), 1189–1191
doi: 10.4014/jmb.1004.04028
First published online 11 June 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

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Hydroxyhibiscone A, a Novel Human Neutrophil Elastase Inhibitor from *Hibiscus syriacus*

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Received: April 19, 2010 / Revised: May 26, 2010 / Accepted: May 27, 2010

In an ongoing investigation of compounds from natural products that exhibit anti-aging properties, hydroxyhibiscone A (1), a new furanosesquiterpenoid, together with hibiscone D (2), was isolated from the root bark of *Hibiscus syriacus*. Utilizing UV, IR, NMR, and MS spectroscopic analyses, these chemical structures were revealed. Compounds 1 and 2 were found to posses significant anti-aging properties on the human neutrophil elastase (HNE) assay, exhibiting HNE inhibitory activities with IC_{50} values of 5.2 and 4.6 μM, respectively.

Keywords: *Hibiscus syriacus*, hydroxyhibiscone A, human neutrophil elastase

Aging is a process of progressive decreases in the maximal functioning and reserve capacity of all organs in the body, including the skin. Skin aging is degeneration of the extracellular matrix (ECM), composed of collagen and elastin. The detrimental effects of ECM degeneration are winkles and diminished structural integrity. Histologically, in wrinkled skin, there is an accumulation of altered elastic fibers and a degradation or degeneration of collagen bundles in the dermis. Elastin is an important component of elastic fibers in the skin. Recently, many studies have reported that elastin is also involved in the inhibition or repair of wrinkle formation, although collagen is a major factor in the skin wrinkle formation [1, 10]. The term elastase refers to endopeptidases that can degrade insoluble elastin and release soluble elastin fragments [2]. Human neutrophil elastase (HNE) is a serine protease stored mainly in the azurophil granules of neutrophil granulocytes, which is formed during the promyelocyte phase. It plays a role in the degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens, and elastin [11]. Biologically, elastase activity significantly increases with age, which results in reduced skin elasticity [6, 14]. It has been suggested that an inhibition in elastase activity could be a useful method for protecting against skin aging [13]. These finding not only illustrate an essential role for elastase in the alteration of elastic fibers, but also highlight the importance of elastase inhibitors as novel anti-wrinkle agents.

Hibisus syriacus L. (Malvaceae) is widely distributed in eastern and southern Asia. The dried flower of *H. syriacus* is used as a folk medicine in the Orient for the cure of hematochezia, dysentery, obstruction due to wind phlegm, and vomiting of food [4, 5]. Previous phytochemical investigations performed on this species resulted in the isolation of some fatty acids and the flavonoids, saponarin, phenols, triterpenoids, and lignans [7, 9, 12]. In the search for effective new anti-aging compounds from the methanol extract of *H. syriacus* that showed moderate activity in HNE assay, hydroxyhibiscone A (**1**), a new furanosesquiterpenoid, was isolated from the methanol extract of this plant, together with hibiscone D (**2**). This paper describes the isolation, structure elucidation, and biological activities of compounds **1** and **2**.

The dried root bark of *Hibiscus syriacus* (1.6 kg) was ground into a powder and extracted with methanol at room temperature for 2 days. The methanol extract was concentrated under reduced pressure to get an aqueous solution, which was washed with *n*-hexane and then partitioned with chloroform. Compounds **1** and **2** were obtained from the chloroform layer by monitoring with HNE inhibitory activity. The concentrated chloroform extract was applied to a column of silica gel and eluted with a *n*-hexane/ethyl acetate

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Table 1. Physicochemical properties of compounds **1** and **2**.

Compound	Hydroxyhibiscone A	Hibiscone D
Appearance	Colorless plate	Colorless needles
Molecular formula	$C_{15}H_{20}O_3$	$C_{15}H_{16}O_3$
Molecular weight	248	244
EI–MS (*m/z*)	248.1	244.1
HR–EI-MS (*m/z*)		
found	248.1410 $(M)^+$	
calcd.	248.1413	
UV λ_{max}^{MeOH} nm (ε)	281 (4.09)	243 (3.93), 330 (3.12)
IR ν_{max}^{KBr} cm^{-1}	3100, 1650	3210, 1680

gradient mixture. The active fractions were concentrated and then chromatographed over a silica gel column with chloroform/methanol (100:1–5:1). The crude active constituents were purified by repetitive Sephadex LH-20 column chromatography eluting with methanol:water [7:3 (v/v)], followed by preparative HPLC using a YMC-pack ODS-a column (10 mm id×150 mm, 5 μm) eluted with 70% aqueous methanol with a retention time of 11.0 min to give compound **1**. The physicochemical properties of compound **1** are summarized in Table 1. Compound **1** was obtained as a colorless plate and its molecular formula, $C_{15}H_{20}O_3$, was determined by high-resolution EI–MS $[(M)^+$, *m/z* 249.1410 (–0.3 mmu error)]. The UV spectrum of **1** showed an absorption maximum at 281 nm. The IR spectrum revealed characteristic absorption bands for a hydroxyl group at 3,100 cm^{-1} and a carbonyl group at 1,650 cm^{-1}. The ^1H NMR spectrum of **1** showed a singlet aromatic proton at δ_H 7.53 (1H, s, H-2), a CH_3-CH system [a methyl signal at δ_H 1.29 (3H, d, *J*=7.7 Hz, H-9) and methine at δ_H 2.60 (1H, ddq, *J*=7.7, 4.5, 2.2 Hz, H-7)], an isopropyl side chain [two methyl signals at δ_H 1.26 (3H, s, H-11), 1.31 (3H, s, H-12)], three methylenes [δ_H 2.5 (1H, ddd, *J*=15.6, 11.4, 5.7 Hz, H-3) and 2.8 (1H, dd, *J*=15.6, 5.1 Hz, H-3); 1.51 (1H, m, H-4) and 2.1 (1H, dd, *J*=11.4, 5.7 Hz, H-4); 2.0 (1H, ddd, *J*=13.8, 10.8, 4.5 Hz, H-6) and 2.6 (1H, dd, *J*= 13.8, 4.5 Hz, H-6)], and two methines [δ_H 1.5 (1H, m, H-5) and 3.1 (1H, td, *J*=10.8, 4.5 Hz, H-5a)].

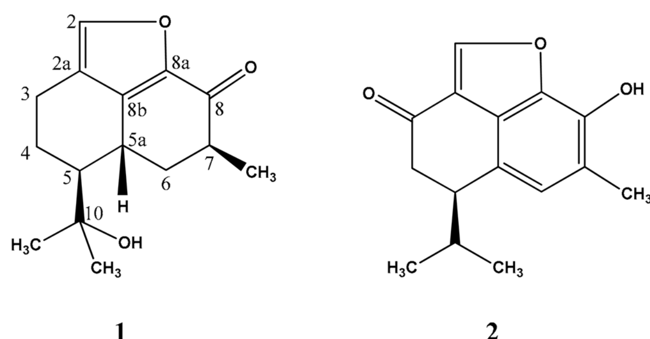

Fig. 1. The chemical structures of compounds **1** and **2**.

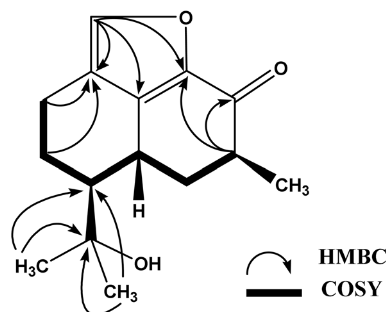

Fig. 2. Key correlations in the HMBC and COSY of compound **1**.

The ^{13}C NMR spectrum of **1** exhibited 15 carbon resonances consisting of an α,β-unsaturated carbonyl group (δ_C 191.5), three *sp*[2] quaternary carbons (δ_C 146.9, 143.0, 123.8), one *sp*[2] methine (δ_C 145.6), one oxygenated quaternary carbon (δ_C 74.2), three methines (δ_C 51.7, 44.2, 32.8), three methylenes (δ_C 40.9, 27.8, 20.7), and three methyls (δ_C 30.2, 25.1, 16.3). The presence of a partial structure of CH_3-CH-CH_2-CH-CH-CH_2-CH_2- in **1** was supported by the ^1H-^1H COSY experiment (Fig. 2). The above data suggest that **1** is a furanosesquiterpenoid, similar to that of hibiscone A already isolated from *Hibiscus elatus* [3]. Furthermore, the ^1H NMR spectrum of **1** was closely similar to that of hibiscone A, except for the absence of a methine signal (H-10) of hibiscone A. The chemical shift of C-10 (δ_C 74.2) was much more downfield shifted than that of hibiscone A, suggesting that a hydroxyl group was located at C-10 in 1. The location of a hydroxy

Table 2. ^1H and ^{13}C NMR data of compound **1** in $CDCl_3$.

Position	δ_C	δ_H (*J* in Hz)
2	145.6	7.53 (s)
2a	123.8	
3	20.7	2.50 (ddd, *J*=15.6, 11.4, 5.7 Hz)
		2.80 (dd, *J*=15.6, 5.1 Hz)
4	27.8	1.51 (m)
		2.10 (dd, *J*=11.4, 5.7 Hz)
5	51.7	1.50 (m)
5a	32.8	3.10 (td, *J*=10.8, 4.5 Hz)
6	40.9	2.00 (ddd *J*=13.8, 10.8, 4.5 Hz)
		2.60 (dd, *J*=13.8, 4.5 Hz)
7	44.2	2.60 (ddq, *J*=7.7, 4.5, 2.2 Hz)
8	191.5	
8a	146.9	
8b	143.0	
9	16.3	1.29 (3H d, *J*=7.7 Hz)
10	74.2	
11	25.1	1.26 (3H, s)
12	30.2	1.31 (3H, s)

^1H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively; TMS was used as internal standard; assignments were based on ^1H-^1H COSY, DEPT, HMQC, and HMBC spectra.

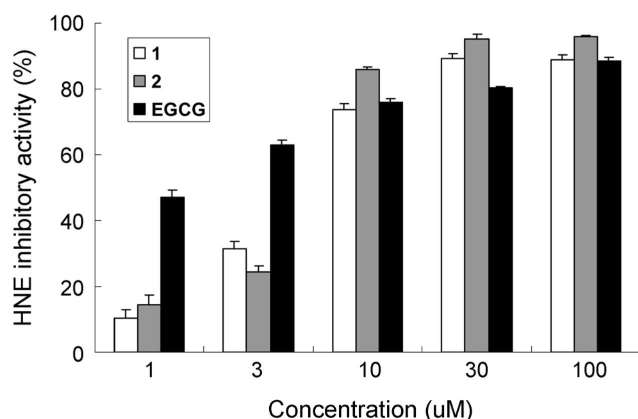

Fig. 3. HNE inhibitory activity of compounds **1** and **2**.

group at C-10 was also confirmed from the HMBC correlations of Me-11 (δ_H 1.26) with C-10 (δ_C 74.2) and C-5 (δ_C 51.7), and Me-12 (δ_H 1.31) with C-10 (δ_C 74.2) and C-5 (δ_C 51.7) (Fig. 2). The relative stereochemisty of **1** was deduced from the NOE correlations of H-5a with H-4_{ax} and Me-9, and H-5 with H-3_{ax}. Accordingly, the structure of compound **1** was determined to be hydroxyhibiscone A (Fig.1). Compound **2** was obtained as colorless needles and identified as hibiscone D, by comparing its physicochemical and spectral data with those in the literature (Table 1.) [3].

The HNE inhibitory activities of compounds **1** and **2** were evaluated using a previously described procedure [8]. Briefly, to each well of a 96-well plate containing 80 μl of substrate solution [1.4 mM *N*-methoxysuccinyl-ala-ala-pro-val-*p*-nitroanilide, in 10 mM Tris-HCl buffer (pH 7.5)] and 1 μl of test solution (stock solutions of the test compounds were dissolved in dimethyl sulfoxide and diluted with Tris-HCl buffer to give the final sample concentrations), 20 μl of an enzyme solution (0.18 Unit HNE) was added and the mixture was incubated for 1 h at 37°C in the dark. After the reaction was quenched by adding 100 μl of soybean trypsin inhibitor at a concentration of 0.2 mg/ml, the absorbance was immediately measured at 405 nm using an ELISA reader. As a result, compounds **1** and **2** dose-dependently inhibited HNE activity with IC_{50} values of 5.2 and 4.6 μM, respectively (Fig. 3). To the best of our knowledge, this is the first report showing the HNE inhibitory activity of furanosesquiterpenoid derivatives.

Acknowledgment

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A-050432).

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