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# A new 3, 4-epoxyfurocoumarin from *Heracleum moellendorffii* Roots

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**Abstract** – Activity-guided isolation of *Heracleum moellendorffii* roots led to four coumarin derivatives as acetylcholinesterase inhibitors. The structures of these isolates were characterized by spectroscopic method to be angelicin (**1**), isobergapten (**2**), pimpinellin (**3**), and (3*S*, 4*R*)-3, 4-epoxypimpinellin (**4**). All the isolated compounds **1**, **2**, **3**, and **4** showed moderate inhibition activities against acetylcholinesterase with the $IC_{50}$ values of 10.2, 18.1, 21.5 and 22.9 μM, respectively. (3*S*, 4*R*)-3, 4-Epoxypimpinellin (**4**) was newly isolated from the plant source.
**Keywords** – *Heracleum moellendorffii*, Angular furanocoumarins, (3*S*, 4*R*)-3, 4-epoxypimpinellin, Acetylcholinesterase inhibition

## Introduction

*Heracleum moellendorffii* is distributed in Korea and China.[1] The roots of this plant has been used as a common cold, headache and analgesics in China.[2] Young and Tender aerial parts of this plant has been used as an edible vegetable in Korea. Coumarins,[3,4] flavonoids,[5] polyacetylenes[6] and essential oils[7] have been isolated from this plant, possessing anti-inflammatory activity, peroxynitrite-scavenging effect, and antiproliferative activity. To the best of our knowledge the acetylcholinesterase inhibitory activity of *Heracleum moellendorffii*, though it is a good source of coumarins, has not been reported, meanwhile some *Herecleum* species has been reported on their anticholinesterase activity.[8-10] Therefore, we focused on the isolation of acetylcholinesterase inhibitors from the roots of *H. moellendorffii*. This study deals with isolation, structure elucidation and determination of acetylcholinesterase inhibitory activity of compounds from the roots of *H. moelledorffii*.

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## Experimental

**General experimental procedures** – Melting point was determined using a Fisher-Johns melting point apparatus (uncorrected) (Fisher-Johns, USA), UV/Vis spectra were recorded using a V-530 spectrophotometer (JASCO, Tokyo, Japan). MS spectra were measured using an API 3200 LC/MS/MS system (AB Sciex, Concord, Canada) and JMS-700 (JEOL, Tokyo, Japan). NMR spectra were recorded using a Bruker AVANCE 600 (Bruker, Rheinstetten, Germany). The chemical shifts are represented as parts per million (ppm) using the residual solvent signal as an internal standard. Optical rotation was recorded using a DIP-1000 digital polarimeter (JASCO, Tokyo, Japan). CD spectrum was recorded using a Chirascan ECD spectrometer (Applied Photophysics, Leatherhead, UK).Column chromatography was carried out using a Kieselgel 60, 63 - 200 μm and 40 - 63 μm (Merck, Darmstadt, Germany) and YMC gel ODS-A, 150 μm (YMC, Kyoto, Japan). Flash column chromatography was carried out using CombiFlash®, Retrieve™ (Teledyne Isco Inc., NE, USA). Medium pressure liquid chromatography was carried out using a Buchi 682 chromatography pump system (Buchi, Flawil, Switzerland). TLC was performed on a glass backed Kieselgel 60 $F_{254}$ and RP $F_{254s}$ plates. All other chemicals and reagents used were of analytical grade. Electric eel acetylcholinesterase, acetylthiocholine

iodide, and 5-5-thiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (St. Louis, Mo, USA.).

**Plant materials** – The roots of *Heracleum moelldendorffii* were collected at the Yanggu agricultural technology center, Yanggu, Korea in September, 2015 and identified by professor Yongsoo Kwon (College of Pharmacy, Kangwon National University). A voucher specimen (KNUH-R-1509-1) was deposited in the Herbarium of College of Pharmacy, Kangwon National University, Korea.

**Extraction and isolation** – The air dried roots of *Heracleum moelldendorffii* was meshed into the rough powder and extracted with hot MeOH (1.4 kg, 3 L × 3) for 4hrs. The extracts were combined and concentrated *in vacuo* at 40 ºC. The MeOH extract (179 g) was suspended in water and successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, leaving a residual water soluble fraction. Each soluble fraction was evaporated *in vacuo* to yield the residues of *n*-hexane fraction (fr.) (19.9 g), $CHCl_3$ fr. (16.9 g), and *n*-BuOH fr. (15.4 g). Among the extract and solvent soluble fractions, the $CHCl_3$ fraction showed an acetylcholinesterase inhibitory activity with $IC_{50}$ value of 63.1 μg/mL. Among the three fractions, *n*-hexane and $CHCl_3$ fraction showed very similar patterns by TLC analysis. These two fractions were combined and purified by various chromatography. Two fractions (35 g) were applied to silica gel column (63 - 200 μm, 10 × 50 cm, 1.0 kg) using stepwise gradient elution with *n*-hexane : EtOAc (4:1 → 3:1 → 2:1; 2 L each), to divide it into five fractions (Fr. 1 – Fr. 5). Fr. 3 (12.8 g) was applied to ODS medium pressure liquid chromatography (Buchi 680 pump; 45 × 5 cm; 400 g) using an isocratic elution with MeOH : $H_2O$ (65 : 35) to yield ten sub-fractions (Fr. 3-1 – Fr. 3-10). Fr. 3-1 was further purified by *n*-hexane-EtOA to give compound **1** (300 mg). Fr. 3-3 (1.2 g) was applied to normal phase flash column chromatography (RediSep®, 80 g) using an isocratic elution with $CHCl_3$ : MeOH (99 : 1) to yield four sub-fractions (Fr. 3-3-1 – Fr. 3-3-4). Fr. 3-3-1 (0.7 g) was applied to silica gel column chromatography (40 - 63 μm, 100 g, 3 × 20 cm) using isocratic elution with $CHCl_3$ to give compounds **2** (170 mg) and **3** (580 mg). Fr. 3-9 (4.3 g) was applied to silica gel column chromatography (40 - 63 μm, 80 g, 3 × 40 cm) using isocratic elution with $CHCl_3$ : MeOH (19:1) to yield four sub-fractions (Fr. 3-9-1 – Fr. 3-9-4). Fr. 3-9-2 (2.4 g) was re-chromatographed on a silica gel (40 - 63 μm, 100 g, 3 × 20 cm) using elution with *n*-hexane : EtOAc (4:1) to yield four sub-fractions (Fr. 3-9-2-1 – Fr. 3-9-2-4). Fr. 3-9-2-**3** (0.5 g) was purified by Sephadex LH20 using isocratic elution with MeOH : $H_2O$ (70:30) to give compound **4** (197 mg).

Compound **1** – $^1$H-NMR (600 MHz, $CDCl_3$) δ : 7.82 (1H, d, $J$ = 9.5 Hz, H-4), 7.70 (1H, d, $J$ = 2.1 Hz, H-2'), 7.45 (1H, d, $J$ = 8.6 Hz, H-5), 7.39 (1H, d, $J$ = 8.6 Hz, H-6), 7.14 (1H, d, $J$ = 2.1 Hz, H-3'), 6.40 (1H, $J$ = 9.5 Hz, H-3); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ : see Table 1; ESI-MS (positive mode) *m/z* : 209 $[M+H]^+$.

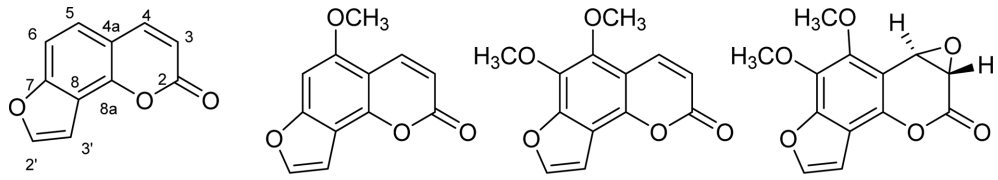
Compound **2** – $^1$H-NMR (600 MHz, $CDCl_3$) δ : 8.16 (1H, d, $J$ = 9.7 Hz, H-4), 7.57 (1H, d, $J$ = 2.2 Hz, H-2'), 7.02 (1H, d, $J$ = 2.2 Hz, H-3'), 6.89 (1H, s, H-6), 6.31 (1H, $J$ = 9.7 Hz, H-3), 3.97 (3H, s, $OC\underline{H}_3$); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ : see Table 1; ESI-MS (positive mode) *m/z* : 239 $[M+H]^+$.

Compound **3** – $^1$H-NMR (600 MHz, $CDCl_3$) δ : 8.07 (1H, d, $J$ = 9.7 Hz, H-4), 7.65 (1H, d, $J$ = 2.0 Hz, H-2'),

**Table 1.** $^{13}$C-NMR data of **1 - 4** (150 MHz, $CDCl_3$)

| No | **1** | **2** | **3** | **4** | 5,8-dimethoxyetane 3,4-epoxy-furanocoumarin[12] |
|---|---|---|---|---|---|
| 2 | 160.89 | 161.02 | 160.84 | 164.69 | 164.7 |
| 3 | 114.12 | 112.21 | 113.72 | 39.80 | 37.5 |
| 4 | 144.55 | 139.89 | 139.92 | 38.2 | 39.1 |
| 4a | 113.52 | 105.81 | 109.44 | 106.45 | 107.5 |
| 5 | 123.83 | 154,27 | 144.44 | 147.55 | 133.8 |
| 6 | 108.83 | 90.53 | 135.12 | 134.56 | 112.9 |
| 7 | 157.37 | 157,98 | 149.80 | 148.19 | 147.2 |
| 8 | 116.94 | 110.06 | 114.09 | 113.81 | 147.4 |
| 8a | 148.50 | 148.79 | 143.17 | 139.53 | 139.1 |
| 2' | 145.89 | 144.32 | 145.39 | 144.58 | 145.8 |
| 3' | 104.11 | 103.73 | 104.29 | 103.97 | 103.4 |
| $OCH_3$ | | 56.31 | 62.38 | 60.77 | 60.4 |
| $OCH_3$ | | | 61.23 | 60.77 | 60.4 |

Chemical shifts are represented parts per million (δ)



**1** **2** **3** **4**

**Fig. 1.** Structures of **1** - **4**.

7.07 (1H, d, $J$ = 2.0 Hz, H-3'), 6.36 (1H, $J$ = 9.7 Hz, H-3), 4.13 (3H, s, OC$\underline{H_3}$), 4.03 (3H, s, OC$\underline{H_3}$); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ : see Table 1; ESI-MS (positive mode) *m/z* : 269 $[M+H]^+$.

Compound **4** – White needles; mp : 248~250°C; $[\alpha]_D^{22°C}$ : −1.8° (c 1.0, $CHCl_3$); CD (c 0.1, $CHCl_3$) $\lambda_{max}$ (Δε) : 238 (0.25), 210 (-0.36); UV (MeOH, $\lambda_{max}$, log ε) nm : 225 (3.34), 253 (s, 2.55), 289 (0.55); IR (ATR, $\nu_{max}$) $cm^{-1}$; 1756 (C=O), 1480, 1406, 1360 (C=C), 1215, 120, 1046 (C-O); $^1$H-NMR (600 MHz, $CDCl_3$) δ : 7.47 (1H, d, $J$ = 2.1 Hz, H-2'), 6.75 (1H, d, $J$ = 2.1 Hz, H-3'), 4.40 (1H, $J$ = 9.6 Hz, H-4), 4.04 (1H, d, $J$ = 9.6 Hz, H-3), 3.85 (3H, s, OC$\underline{H_3}$), 3.69 (3H, s, OC$\underline{H_3}$); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ : see Table 1; HR-EI-MS *m/z* : 262.0474 (calcd for $C_{13}H_{10}O_6$, 262.0477); EI-MS (rel. int. %) *m/z* : 262 ($M^+$, 0.22), 246 (100), 231 (99.79), 216 (8.05), 203 (12.99), 188 (15.13), 175 (23.76), 160 (25.50), 147 (30.78), 132 (7.11), 119 (7.26), 109 (3.00), 104 (9.66), 91 (7.59), 76 (5.28).

**Determination of acetylcholinesterase inhibitory activity** – The acetylcholinesterase inhibition assay was measured according to the method of Ellman *et. al*.[11] with slight modification.

## Result and Discussion

Compounds **1**, **2**, and **3** were identified as angelicin,[3] isobergapten,[3] and pimpinellin,[9] respectively, by comparing their physico-chemical and spectral data with those of literature values. The $^1$H-NMR spectrum of **4** exhibited two pairs of doublets at δ 7.47 and 6.75 (each 1H, $J$ = 2.1 Hz), and δ 4.40 and 4.04 (each 1H, $J$ = 9.6 Hz). Two methoxyl signals showed at δ 3.85 and 3.69. These signals are very similar to those of 5, 8-dimethoxyetane 3, 4-epoxy-furanocoumarin.[12] The $^{13}$C-NMR and HMBC spectra of **4** were slightly different to that of 5, 8-dimethoxyetane 3, 4-epoxyfuranocoumarin. In the HMBC spectrum of **4**, C-3' proton at $\delta_H$ 6.75 correlated to C-2' carbon at $\delta_C$ 144.58 and C-7 carbon at $\delta_C$ 148.19, C-8 carbon at $\delta_C$ 113.81, and C-8a carbon at $\delta_C$ 139.53. The C-4 proton at $\delta_H$ 4.40 correlated to C-2 carbon at $\delta_C$ 164.69, C-3 carbon at $\delta_C$ 39.80, C-4a carbon at $\delta_C$ 106.45, and C-8a at $\delta_C$ 139.53. Furthermore, the two methoxyl groups are attached at C-5 ($\delta_C$ 144.58) and C-6 ($\delta_C$ 134.56), which were assigned by HSQC and HMBC spectra. These result strongly suggested that **4** is an angular furanocoumarin derivative.[13,14] The HR-EIMS spectrum showed a molecular ion peak at *m/z* 262.0474, consistent with $C_{13}H_{10}O_6$ (calcd for 262.0477). The large coupling constants ($J$ = 9.6 Hz) between H-3 and H-4 showed a 3, 4 – *trans* configuration of **4**. The CD spectrum of **4** showed a positive cotton effect at 238 nm and a negative cotton effect at 210 nm, respectively. These data indicated 4β-oriented epoxy moiety.[15,16] Based on the above data, the absolute configuration of C-3 and C-4 could be determined as 3*S*, 4*R*. Thus, the structure of **4** identified as (3*S*, 4*R*)-3,4-epoxypimpinellin, which is newly isolated from the plant sources. All the isolated compounds **1**, **2**, **3**, and **4** were tested for acetylcholinesterase inhibition activity, showing inhibition activities against acetylcholinesterase with the $IC_{50}$ values of 10.2, 18.1, 21.5 and 22.9

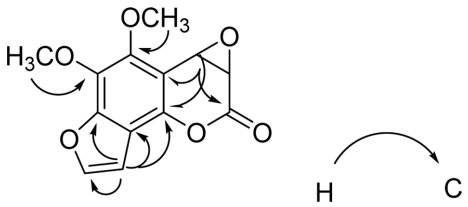


**Fig. 2.** Important HMBC correlations of **4**.

**Table 2.** Acetylcholinesterase inhibitory activity of compounds **1** - **4**.

| Tested compounds | $IC_{50}$[a] (μg/ml) | $IC_{50}$ (μM) |
|---|---|---|
| **1** | 1.9 | 10.2 |
| **2** | 3.9 | 18.1 |
| **3** | 5.3 | 21.5 |
| **4** | 6.0 | 22.9 |
| Eserine[b] | 0.007 | 0.03 |

[a] The inhibitory activity dose that reduced 50% of acetylcholinesterase activity and expressed as mean of two different experiments.
[b] A positive control

μM, respectively. Though the number of tested compounds is too small, this result suggested that the methoxyl group and 3, 4-epxoy moiety did not affect the acetylcholinesterase inhibitory activity, indicating that α-pyrone may has a key role against the acetylcholinesterase inhibitory activity in the angular furanocoumarins.

In conclusion, angelicin (**1**), isobergapten (**2**), pimpinellin (**3**), and (3*S*, 4*R*) - 3, 4-epoxypimpinellin (**4**) were isolated from the *n*-hexane and chloroform soluble fractions of *H. moellendorfii* roots as acetylcholinesterase inhibitors. (3*S*, 4*R*) - 3, 4-Epoxypimpinellin (**4**) was newly isolated from the plant source. All the isolated compounds showed mild acetylcholinesterase inhibitory activity. These results suggested that the extract of root of *H. moellendorffii* might to be a good resource for angular furanocoumarins as acetylcholinesterase inhibitors.

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