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Coumarins and a Pyrimidine from *Angelica gigas* Roots

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Abstract – Five coumarins and a pyrimidine were isolated from the roots of *Angelica gigas*. Their structures were elucidated as bergapten (**1**), decursinol angelate (**2**), decursin (**3**), nodakenetin (**4**), uracil (**5**) and nodakenin (**6**) by spectral analysis. Among them, bergapten (**1**) and uracil (**5**) were isolated for the first time from this plant part.
Keywords – *Angelica gigas*, Umbelliferae, coumarin, pyrimidine.

Introduction

Angelica gigas is genus of the family Umbelliferae. *A. gigas* grows on moist soils of Korea. The roots of this plant have been used as traditional medicine not only for treatment anemia but also as a sedative, an anodyne or a tonic agent (Yook, 1990).

Investigations on the compounds from *A. gigas* have revealed the presence of aegelinol, agasyllin, 6"-acetylnodakenine, columbianetin-β-D-glucoside, decursin, decursidin, decursinol, decursinol angelate, 7-demethylsuberosine, *iso*-apiosylskimmine, *iso*-imperatorin, gigasol, marmesin, marmesinine, nodakenetin, nodakenin, prenyletin, umbelliferone, xanthyletin, xanthotoxin, peucedanone, 7-methoxy-5-prenyloxycoumarin and 7-hydroxy-6-(2-(*R*)-hydroxy-3-methylbut-3-enyl)coumarin (Chi, 1969; Jung *et al.*, 1991; Kang *et al.*, 2001; Konoshima *et al.*, 1968; Pachaly *et al.*, 1996; Ryu *et al.*, 1990; Yook *et al.*, 1973), octadeca-1,9-dien-4,6-diyn-3,8,18-triol and 18-acetoxy-octadeca-1,9-dien-4,6-diyn-3,8-diol (Choi *et al.*, 2000), and essential oils (Chi and Kim, 1988).

A. gigas has been studied extensively and shown to exhibit a variety of activities. Decursin exhibited significant prolongation of hexobarbital-induced hypnosis as well as significant inhibition of hepatic microsomal drug metabolizing enzyme activities (Shin *et al.*, 1996). Decursin and decursinol angelate displayed cytotoxic activity against various human cancer cell lines (Ahn *et al.*, 1996; Ahn *et al.*, 1997). Decursin and decursinol antagonized against the voluntary activity in mice (Kim *et al.*, 1980). Decursinol represented the highest inhibitory activity toward acetyl cholinesterase (Kang *et al.*, 2001). Polyacetylenes inhibited the production of NO in LPS-activated RAW 264.7 cells by suppressing the *i*-NOS enzyme expression (Choi *et al.*, 2000).

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The chromatographic separation of the fractions from this plant led to the isolation of coumarins and a pyrimidine. This paper describes the isolation and structural determination of these compounds.

Experimental

Instruments and reagents – IR spectra were recorded with Jasco FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE 400 NMR spectrometer in $CDCl_3$ or DMSO using TMS as internal standard. MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. Other reagents were commercial grade without purification.

Plant materials – The roots of *Angelica gigas* Nakai were purchased from Kyung Dong Market, Seoul, Korea in March 2001 and verified by Prof. Emeritus H. J. Chi, Seoul National University, Korea. A voucher specimen of this plant was deposited at the Herbarium of Natural Products Research Institute (NPRI), Seoul National University, Korea.

Extraction and isolation – The air-dried powdered roots of *A. gigas* (5 kg) were extracted three times with MeOH under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 1125 g of the residue. The MeOH extract was suspended in water, and then fractionated successively with equal volumes of Et_2O and *n*-BuOH, leaving residual H_2O soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of Et_2O soluble fraction (518 g) and *n*-BuOH soluble fraction (445 g).

The portion of Et_2O fraction (34 g) was chromatographed on a silica gel column (7×60 cm) eluting with a gradient of *n*-hexane-EtOAc to afford compounds **1** (5.8 mg, 38 : 2), **2** (789 mg, 37 : 3), **3** (5 g, 37 : 3) and **4** (4.2 mg, 30 : 10). The portion of *n*-BuOH fraction (34 g) was chromatographed

on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford compounds **5** (3.7 mg, 38:2) and **6** (2.9 g, 37:3).

Compound **1**; EI-MS *m/z* (rel. int. %): 216 $[M]^+$ (100), 201 (32.0), 188 (20.0), 173 (84.9), 145 (38.0), 129 (4.8), 89 (19.2), 75 (7.8); IR ν_{max} (KBr) cm^{-1}: 1732 (α-pyrone ring), 1634, 1560, 1479 (aromatic C=C), 1218, 1121 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.18 (1H, d, J = 9.8 Hz, H-4), 7.62 (1H, d, J = 2.4 Hz, H-2'), 7.17 (1H, s, H-8), 7.05 (1H, d, J = 2.4 Hz, H-3'), 6.30 (1H, d, J = 9.8 Hz, H-3), 4.30 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_c (ppm): 161.3 (C-2), 158.4 (C-7), 152.7 (C-9), 149.5 (C-5), 144.8 (C-2'), 139.2 (C-4), 112.7 (C-6), 112.6 (C-3), 106.4 (C-10), 105.0 (C-3'), 93.9 (C-8), 60.1 (5-O*C*H$_3$).

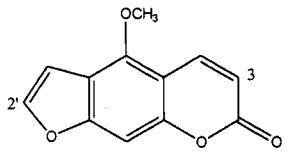
Compound **2**; EI-MS *m/z* (rel. int. %): 328 (5.1) $[M]^+$, 228 (32.7), 213 (100), 147 (1.8). 83 (21.8), 55 (21.5); IR ν_{max} (KBr) cm^{-1}: 1732 (α-pyrone ring), 1626, 1561, 1494 (aromatic C=C), 1229, 1134 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.59 (1H, d, J = 9.5 Hz, H-4), 7.17 (1H, s, H-5), 6.79 (1H, s, H-8), 6.23 (1H, d, J = 9.5 Hz, H-3), 6.11 (1H, q, J = 7.2 Hz, H-3"), 5.14 (1H, t, J = 4.9 Hz, H-3'), 3.24 (1H, dd, J = 17.0, 4.9 Hz, H-4'$_a$), 2.90 (1H, dd, J = 17.0, 4.9 Hz, H-4'$_b$), 1.89 (3H, d, J = 7.2 Hz, H-4"), 1.85 (3H, s, 2"-CH_3), 1.41 (3H, s, gem-CH_3), 1.39 (3H, s, gem-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_c (ppm): 167.0 (C-1"), 161.2 (C-2), 156.4 (C-7), 154.2 (C-9), 143.1 (C-4), 139.4 (C-3"), 128.6 (C-5), 127.3 (C-2"), 115.8 (C-6), 113.2 (C-3), 112.8 (C-10), 104.6 (C-8), 76.6 (C-2'), 70.0 (C-3'), 27.8 (C-4'), 25.0 (gem-*C*H$_3$), 23.2 (gem-*C*H$_3$), 20.5 (2"-*C*H$_3$), 15.7 (C-4").

Compound **3**; EI-MS *m/z* (rel. int. %): 328 (4.6) $[M]^+$, 228 (33.8), 213 (100), 147 (1.8). 83 (38.3), 55 (11.5); IR ν_{max} (KBr) cm^{-1}: 1726 (α-pyrone ring), 1626, 1563, 1494 (aromatic C=C), 1226, 1135 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.58 (1H, d, J = 9.5 Hz, H-4), 7.15 (1H, s, H-5), 6.77 (1H, s, H-8), 6.20 (1H, d, J = 9.5 Hz, H-3), 5.65 (1H, s, H-2"), 5.07 (1H, t, J = 4.8 Hz, H-3'), 3.18 (1H, dd, J = 17.1, 4.7 Hz, H-4'$_a$), 2.90 (1H, dd, J = 17.1, 4.7 Hz, H-4'$_b$), 2.13 (3H, s, 3"- CH_3), 1.86 (3H, s, H-4"), 1.37 (3H, s, gem-CH_3), 1.35 (3H, s, gem-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_c (ppm): 165.7 (C-1"), 161.2 (C-2), 158.4 (C-3"), 156.4 (C-7), 154.1 (C-9), 143.1 (C-4), 128.6 (C-5), 115.9 (C-6), 115.5 (C-2"), 113.1 (C-3), 112.7 (C-10), 104.6 (C-8), 76.7 (C-2'), 69.0 (C-3'), 27.8 (C-4'), 27.4 (C-4"), 24.9 (gem-*C*H$_3$), 23.1 (gem-*C*H$_3$), 20.3 (3"-*C*H$_3$).

Compound **4**; EI-MS *m/z* (rel. int. %): 246 (70.2) $[M]^+$, 228 (4.4), 213 (23.3), 187 (100), 175 (14.2), 160 (22.4), 147 (3.3), 131 (11.1), 115 (2.0), 102 (3.1), 81 (3.7), 69 (5.9), 59 (20.6); IR ν_{max} (KBr) cm^{-1}: 3479 (OH), 1699 (α-pyrone ring), 1630, 1569, 1486 (aromatic C=C), 1268, 1132 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.61 (1H, d, J = 9.5 Hz, H-4), 7.24 (1H, s, H-5), 6.77 (1H, s, H-8), 6.24 (1H, d, J = 9.5 Hz, H-3), 4.76 (1H, t, J = 8.7 Hz, H-2'), 3.24 (2H, m, H-3'), 1.40 (3H, s, CH_3), 1.26 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_c (ppm): 163.1 (C-2), 161.0 (C-7), 155.7 (C-10), 143.6 (C-4), 125.0 (C-6), 123.3 (C-5), 112.8 (C-9), 112.3 (C-3), 97.9 (C-8), 91.0 (C-2'), 71.6 (C-4'), 29.4 (C-3'), 26.1 (C-6'), 24.2 (C-5').

Compound **5**; EI-MS *m/z* (rel. int. %): 112 $[M]^+$ (100), 97 (0.4), 83 (0.6), 69 (49.0), 68 (17.3), 57 (1.8); IR ν_{max} (KBr) cm^{-1}: 3434 (-OH), 1419, 1236; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 11.01 (1H, s, -OH), 10.81 (1H, s, -OH), 7.39 (1H, d, J = 7.6 Hz, H-6), 5.45 (1H, d, J = 7.6 Hz, H-5); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 164.7 (C-4), 151.9 (C-2), 142.6 (C-6), 100.6 (C-5).

Compound **6**; EI-MS *m/z* (rel. int. %): 408 (23.1) $[M]^+$, 229 (66.5), 213 (37.4), 187 (100); IR ν_{max} (KBr) cm^{-1}: 3352



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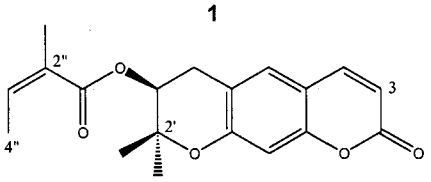

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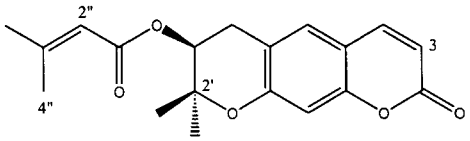

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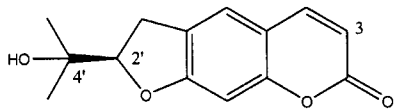

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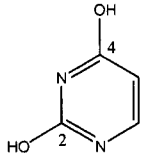

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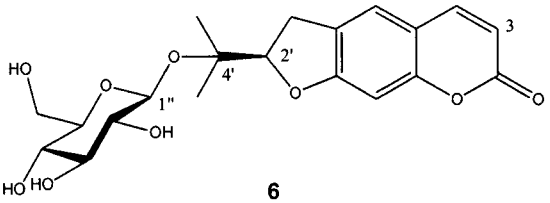

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(OH), 1717 (α-pyrone ring), 1627, 1568, 1487 (aromatic C=C), 1265, 1170 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 7.91 (1H, d, J = 9.5 Hz, H-4), 7.46 (1H, s, H-5), 6.78 (1H, s, H-8), 6.20 (1H, d, J = 9.5 Hz, H-3), 4.85 (1H, m, H-2'), 4.40 (1H, d, J = 7.7 Hz, glycosyl H-1"), 3.19 (2H, m, H-3'), 1.25 (3H, s, CH_3), 1.22 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 163.5 (C-2), 161.0 (C-7), 155.4 (C-10), 145.2 (C-4), 126.1 (C-6), 124.4 (C-5), 112.6 (C-9), 111.6 (C-3), 97.6 (C-1"), 97.2 (C-8), 90.5 (C-2'), 77.5 (C-5"), 77.4 (C-4'), 76.9 (C-3"), 73.9 (C-2"), 70.4 (C-4"), 61.2 (C-6"), 29.2 (C-3'), 23.4 (C-6'), 21.1 (C-5').

Results and Discussion

The chromatographic separation of the roots of *A. gigas* led to the isolation of bergapten (**1**), decursinol angelate (**2**), decursin (**3**) and nodakenetin (**4**) from the Et_2O fraction, and uracil (**5**) and nodakenin (**6**) from the *n*-BuOH fraction, respectively. Among them, the isolation of decursinol angelate (**2**) (Ryu *et al.*, 1990), decursin (**3**) (Konoshima *et al.*, 1968), nodakenetin (**4**) (Chi, 1969) and nodakenin (**6**) (Pachaly *et al.*, 1996) from this plant was already reported.

Compound **1** was obtained as pale yellowish powder. In the ^{1}H-NMR spectrum of **1**, the typical signals of linear furanocoumarin were observed. The H-3 and H-4 signals of coumarin were observed at δ 6.30 (d, J = 9.8 Hz) and δ 8.18 (d, J = 9.8 Hz), respectively. The singlet signals at δ 7.17 and δ 4.30 were shown aromatic H-8 and 5-OCH_3 signals by HMBC assignments, respectively. The doublets at δ 7.62 (J = 2.4 Hz) and δ 7.05 (J = 2.4 Hz) assigned the furan signals of H-2' and H-3', respectively. Its ^{13}C-NMR spectrum of **1** showed C=O signal at δ 161.3 and OCH_3 at δ 60.1. The IR spectrum of **1** showed adsorption bands for α,β-unsaturated C=O at 1732 cm^{-1} and aromatic ring at 1634, 1560 and 1479 cm^{-1}. The EIMS of **1** showed an $[M]^+$ ion at *m/z* 216 as a base peak. The fragment ions of M-15 $[M-CH_3]^+$, M-28 $[M-CO]^+$, M-43 $[M-(CH_3+CO)]^+$ and M-71 $[M-(CH_3+CO+CO)]^+$ were observed. Accordingly, the structure of **1** was elucidated as bergapten. Chung (1970) reported the isolation of bergapten from *Evodia daniellii*. It showed the inhibitory activity of monoamine oxidase (Huong *et al.*, 1999), the inhibition of lipid peroxidation in brain and kidney homogenates (Ng *et al.*, 2000) and the most potent human CYP_3A_4 inhibitor (Ho *et al.*, 2001).

Compound **5** was obtained as white powder from MeOH. In the ^{1}H-NMR spectrum of **5**, the doublets at δ 7.39 (J = 7.6 Hz) and δ 5.45 (J = 7.6 Hz) assigned H-6 and H-5 of pyrimidine, respectively. The each singlet at δ 11.01 and δ 10.81 showed hydroxyl signals. Its ^{13}C-NMR spectrum of **5** showed two C-O signals at δ 164.7 and δ 151.9. The IR spectrum of **5** showed adsorption bands for hydroxy at 3434 cm^{-1} and C-O at 1419, 1236 cm^{-1}. The EIMS of **5** showed an $[M]^+$ ion at *m/z* 112 as a base peak. Accordingly, the structure of **5** was elucidated as uracil, which was lactim type of uracil. Ko *et al.* (1992) reported the isolation of uracil from the roots of *Anthriscus sylvestris*.

Among the isolated compounds, bergapten (**1**) and uracil (**5**) were isolated for the first time from the roots of *A. gigas*.

Acknowledgement

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