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Antimicrobial Constituents of *Foeniculum vulgare*

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A phenyl propanoid derivative, dillapional(**1**) was found to be a antimicrobial principle of the stems of *Foeniculum vulgare* (Umbelliferae) with MIC values of 125, 250 and 125/ against *Bacillus subtilis*, *Aspergillus niger* and *Cladosporium cladosporicides*, respectively. A coumarin derivative, scopoletin(**2**) was also isolated as marginally antimicrobial agent along with inactive compounds, dillapiol(**3**), bergapten(**4**), imperatorin(**5**) and psolaren(**6**) from this plant. The isolates **1-6** were not active against the *Escherichia coli*.

Key words: *Foeniculum vulgare*, Dillapional, Scopoletin, Dillapicl, Bergapten, Imperatorin, Psolaren, Antimicrobial activity

INTRODUCTION

Foeniculum vulgare (Umbelliferae) is widely cultivated worldwide, and its fruit is used as flavoring, spice and gastrointestinal mobility stimulator (Kim *et al*, 1997). Studies on the chemical constituents of this plant have been carried out by some investigators, and various constituents have been found (Ceska *et al*, 1986; Ono *et al*, 1995; Ono *et al*, 1996). In the course of a screening program to evaluate antimicrobial constituents from medicinal plants, we found that $CHCl_3$ soluble fraction from the stems of *F. vulgare* exhibited a potent antimicrobial activity against bacteria and fungi. Repeated column chromatography of the $CHCl_3$ soluble fraction led to the isolation of six compounds. This paper deals with the structure elucidation of these compounds and their antimicrobial activities.

MATERIALS AND METHOD

Instruments and reagents

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. Nuclear magnetic resonances (^{1}H-NMR and ^{13}C-NMR spectra aken at 200 and 50MHz, respectively) were recorded on a Varian Gemini 200 spectrometer using deuterated solvents as the internal standard. The EI/MS (70 eV) spectra were determined using a Autospec Micromass, UV spectra were obtained using a Hitachi U-2000, and IR spectra in a KBr disk using a Bio-Rad FTS-7. TLC work was carried out using plates coated with silica gel 60 F254 (Merck Co.). All solvents were routinely distilled prior to use. Silica gel column chromatography was performed on Merck silica gel 60 (70-230 mesh and 230-400 mesh). Other reagents were commercial grade without purification. Microorganisms were purchased from Korea Research Institute of Bioscience and Biotechnology (*Escherichia coli* KTCC 1045, *Bacillus subtilis* KTCC 1332, *Aspergillus niger* KTCC 6960 and *Cladosporium cladosporioides* KTCC 6167)

Plant materials

The stem of *F. vulgare* was collected at Herbal garden, Kangwon National University, Korea in September 1999 and identified taxonomically with respect to morphology. A voucher specimen of the plant was deposited at the College of Pharmacy, Kangwon National University. Deposit number : KNUP-F-062-1.

Extraction and isolation

The air-dried stems (0.6 kg) were ground and extracted three times with hot MeOH over a total 4hr period. The resultant extracts were combined and concentrated under reduced pressure to afford 120 g of the residue. This

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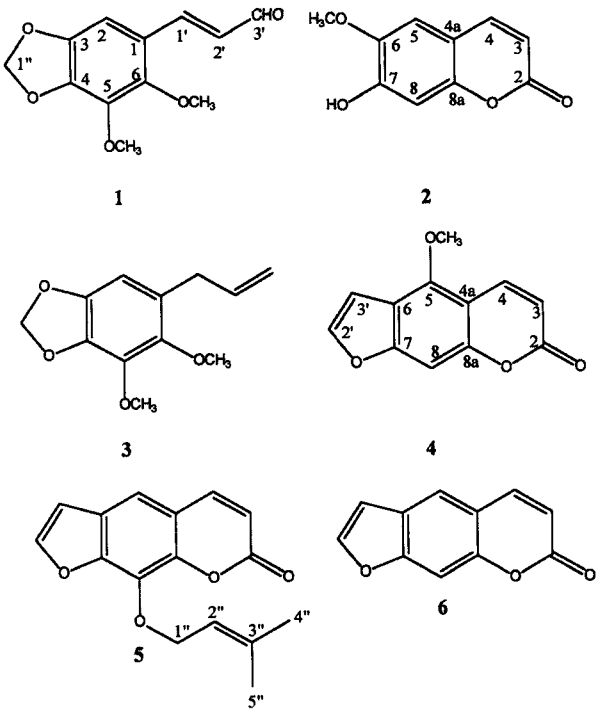

Fig. 1. The structures of Compounds **1-6**

MeOH extract was suspended in 10 volumes of water and then partitioned successively with equal volumes of $CHCl_3$, and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vaccuo to yield the residues of $CHCl_3$ fr., (15 g), and *n*-BuOH fr., (22 g).

The $CHCl_3$ soluble fraction (15 g) was column chromatographed on a silica gel column (250 g, 70-230 mesh, 15×50 cm) using stepwise gradient elution with the solvents *n*-hexane-EtOAc (4:1, 2:1, 1:1, v/v) to divide the fraction into four sub-fractions (Fr.1-Fr.4).

Sub-fraction 1 was re-chromatographed on a silica gel column (70g, 70-230 mesh, 2×50 cm) with benzene-EtOAc(9:1) to give compound **1** (25 mg). Sub-fraction 2 was re-chromatographed on ODS (70 g, YMC gel, ODS-A, S-150 μm) column by elution with 50% MeOH to give compound **2** (10 mg), **3** (30 mg), **4** (70 mg), **5** (10 mg) and **6** (35 mg).

Compound 1(dillapional); mp : 101-102°; IR λ_{max} (KBr)cm^{-1}: 2850 (CH_2), 1656(C=O), 1486, 1449, 1402(C=C); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 9.69(1H, d, *J*=7.8Hz, H-3'), 7.77(1H, d, *J*=16Hz, H-1'), 6.76(1H, s, H-2), 6.57(1H, dd, *J*=7.8, 16Hz, H-2'), 6.01(2H, s, H-1"), 4.06(3H, s, -OC$\underline{H}_3$), 3.87(3H, s, -OC$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ_C (ppm):194.17 (C-3'), 152.03(C-1'), 147.26(C-2), 146.92(C-5), 138.25(C-4), 136.50(C-3), 127.78(C-6), 121.10(C-2'), 102.10(C-1), 99.19(C-1"), 62.26(-O$\underline{C}H_3$), 60.22(-O$\underline{C}H_3$); MS : m/z (rel. int) 236(M^+, 16), 205(100.0), 190(8.4), 163(13.7), 135 (12.4), 120(6.4), 92(7.3), 71(9.6)

Compound 2 (scopoletin); mp : 213-215°; UV λ_{max}: 341, 296, 252, 222 nm; IR ν_{max} (KBr)cm^{-1}: 1677(C=O), 1469, 1440, 1408(C=C), 1132, 1084(C-O); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.62(1H, d, *J*=9.6Hz, H-4), 6.94(1H, s, H-5), 6.87(1H, s, H-8), 6.28(1H, d, *J*=9.6Hz, H-3), 3.97(3H, s, -OC$\underline{H}_3$)

Compound 3(dillapiol); mp : 29.5; IR ν_{max} (KBr)cm^{-1}: 2898, 3000 (=CH_2), 1460, 1430, 1390(C=C); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 6.33(1H, s, H-2), 5.89(1H, m, H-2'), 5.85(2H, s, H-1"), 5.05(2H, dd, *J*=1.8 and 6.4Hz, H-1'), 3.99(3H, s, -OC$\underline{H}_3$), 3.74(3H, s, -OC$\underline{H}_3$), 3.29(2H, d, *J*=6.4Hz, H-3'); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 145.17(C-2), 144.86(C-5), 138.18(C-4), 137.97(C-2'), 136.50(C-3), 126.54(C-6), 116.02(C-3'), 103.25(C-1), 102.62(C-1"), 61.69(-O$\underline{C}H_3$), 60.37(-O$\underline{C}H_3$), 34.40(C-1')

Compound 4 (bergapten); mp : 188; UV λ_{max}: 310, 266, 248, 222nm; IR $_{max}$ (KBr)cm^{-1}: 1677(C=O), 1460, 1430, 1390(C=C), 1131, 1085(C-O); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 8.17(1H, d, *J*=9.6Hz, H-4), 7.62(1H, d, *J*=2.4Hz, H-2'), 7.15(1H, s, H-8), 7.05(1H, d, *J*=2.4Hz, H-3'), 6.30(1H, d, *J*=9.6Hz, H-3), 4.29(3H, s, -OC$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 160.47(C-2), 158.52(C-7), 152.02(C-8a), 149.69(C-5), 144.92(C-2'), 139.43(C-4), 112.68(C-3 and C-6), 105.17(C-4a and C-3'), 94.23(C-8), 59.82(-O$\underline{C}H_3$)

Compound 5(imperatorin); mp : 101-102; UV_{max}: 299, 263, 248, 222nm; IR $_{max}$ (KBr)cm^{-1}: 1674(C=O), 1465, 1437, 1400(C=C), 1131, 1081(C-O); ^{1}H-NMR ($CDCl_3$) $_H$ (ppm): 7.80(1H, d, *J*=9.8Hz, H-4), 7.72(1H, d, *J*=2.2Hz, H-2'), 7.38(1H, s, H-5), 6.84(1H, d, *J*=2.2Hz, H-3'), 6.30(1H, d, *J*=9.8Hz, H-3), 5.63(1H, t, *J*=7.2Hz, H-2"), 5.03(2H, d, *J*=7.2Hz, H-1"), 1.76, 1.74(each 3H, s, gem-($C\underline{H}_3$)$_2$); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 160.71(C-2), 148.69(C-7), 146.75(C-2'), 144.54(C-8a), 143.90(C-4), 139.89(C-3"), 131.73(C-8), 125.99(C-6), 119.87(C-2"), 116.57(C-4a), 114.74(C-3), 113.32(C-5), 106.84(C-2'), 70.23(C-4"), 25.89(C-4"), 18.18(C-5")

Compound 6 (psolaren); mp : 163-164; UV λ_{max}: 245, 289, 327.5, 341nm; IR ν_{max} (KBr)cm^{-1}: 1677(C=O), 1469, 1440, 1408(C=C), 1132, 1084(C-O); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.82(1H, d, *J*=10.0Hz, H-4), 7.72(1H, d, *J*=2.0Hz, H-2'), 7.71(1H, s, H-5), 7.50(1H, d, J=0.8Hz, H-8), 6.86(1H, dd, *J*=0.8 and 2.0Hz, H-3'), 6.37(1H, d, *J*=10.0Hz, H-3)

Antimicrobial test against *Escherichia coli* KTCC 1045, *Bacillus subtilis* KTCC 1332, *Aspergillus niger* KTCC 6960 and *Cladosporium cladosporioides* KTCC 6167

Antimicrobial tests against bacteria and fungi were

performed by the 2-fold dilution method. Bacteria were precultured in 10 ml of a nutrient- broth medium for 12h at 27 on a shaker, and then diluted 100-fold with the same medium. Fungi were inoculated into 10 ml of a potato-molt extract-sucrose agar medium, and incubated at 27°C for 7days to form a well-expanded fungal mat with spores. These spores were collected by filtration and suspended in 50 of a Henneberg medium (Sucrose 100g, KNO_3 2g, KH_2PO_4. $7H_2O$ 0.5g, $CaCl_2$ 0.1g, H_2O 1). The liquid cultures of bacteria or the spore suspension of fungi containing the various concentrations of test materials were placed in the wells of a 96-well microplate and incubated at 27°C for 24h. The growth of bacteria was evaluated by the degree of turbidity of the culture with naked eye, and the spore germination was examined under a microscope. The MIC values were determined by the comparison with the control.

RESULTS AND DISCUSSION

The $CHCl_3$ extracts of the stems of *F. vulgare* showed significant antimicrobial activity against the bacteria and fungi. Thus, detailed laboratory investigation was performed on the active $CHCl_3$ extract. Bioassay-guided fractionation led to the isolation of compound **1** as a active principle along with marginally active compound **2**, and inactive constituents, **3-6**. The IR (1656cm^{-1}) and ^{1}H-NMR (δ 9.69) spectra showed that compound **1** has an aldehyde group. Besides showing two methoxyls (δ 3.87 and 4.06) and a methylenedioxy group (δ 6.01) in its ^{1}H-NMR spectrum, the compound showed the presence of an , -unsaturated aldehyde group (a doublet at 9.69 for -CHO, J=7.8Hz; a double doublet at 6.57 for H-2", J= 7.8 and 16.0Hz and a doublet at 7.97 for H-3", J=16.0Hz). The large coupling constant (J=16.0Hz) for H-2" and H-3" is consistent with its *trans*-stereochemistry. Based on these results and on values previously reported in the literature (Tomar and Mukerjee, 1981), compound **1** was identified as dillapional. Compound **2** was identified as a simple coumarin, scopoletin on the basis of spectral analysis as well as comparison of physical constants with those reported in the literature (Steck and Mazurk, 1972). The ^{1}H-NMR spectrum of compound **3** similar to those of compound **1** except for presence of propenyl group (a double doublet at δ 5.05 for H-1', J=1.8 and 6.4Hz; a multiplet at 5.89 for H-2' and a doublet at 3.29 for H-3', J=6.4Hz). Based on these results on values previously reported in the literature (Orjala *et al.*, 1993), compound **3** was identified as dillapiol. Compound **4** exhibited the presence of a 5-oxygenated furocoumarin skeleton (Lee and Soine, 1969). This result further supported by the fact that the methoxy and H-4 signals at 4.29 and 8.17, respectively, in its ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum of compound **4** exhibited signals due to protons of the C-3 and C-4 position of the coumarin ring at δ 6.30 (1H, d, J=9.6Hz) and 8.17 (1H, d, J=9.6Hz), due to protons of the C-2' and C-3' positions at 7.62(1H, d, J=2.4Hz) and 7.05 (1H, d, J=2.4Hz), due to proton of the C-8 position at 7.15 (1H, s), and due to the methoxyl group at 4.29 (3H, s). Based on these results on values previously reported in the literature (Steck and Mazurk, 1972; Elgamal *et al.*, 1979), compound **4** was identified as bergapten. Compound **5** exhibited the presence of a 8-oxygenated furocoumarin skeleton (Lee and Soine, 1969). The ^{1}H-NMR spectrum of compound **5** similar to those of compound **4** except for presence of isoprenyl group (a triplet at 5.63 for H-2", J=7.2Hz; a doublet at 5.03 for H-1", J=7.2Hz, and two singlets at 1.76 and 1.74 (each 3H, s, gem-$(CH_3)_2$). Based on these results on values previously reported in the literature (Steck and Mazurk, 1972; Elgamal *et al.*, 1979; Harkar *et al.*, 1984), compoun **5** was identified as imperatorin. The UV spectrum of compound **6** showed furocoumarin skeleton (Lee and Soine, 1969). The ^{1}H-NMR spectrum of compound **6** exhibited signals due to protons of the C-3 and C-4 position of the coumarin ring at δ 6.37 (1H, d, J=10.0Hz) and 7.82 (1H, d, J=10.0Hz), due to protons of the C-5 and C-8 positions at 7.71 (1H, s) and 7.50 (1H, dd, J=0.8 and 2.0Hz), and due to protons of the C-2' and C-3' positions at δ 7.72 (1H, d, J=2.0Hz) and 6.83 (1H, dd, J=0.8 and 2.0Hz). Based on these results on values previously reported in the literature (Steck and Mazurk, 1972), compound **6** was identified as psolaren. Dillapional (**1**) exhibited potent antimicrobial activity against *Bacillus subtilis*, *Aspergillus niger* and *Cladosporium cladosporioides* with the MIC values of 125, 250 and 125 μg/, respectively. Scopoletin (**2**) showed weak antimicrobial activity against *Bacillus subtilis Aspergillus niger* and *Cladosporium cladosporioides* with the MIC values of 125, 500 and 250/ respectively. Dillapiol (**3**), bergapten (**4**), imperatorin (**5**) and psolaren (**6**) were considered to be inactive in the antimicrobial assay system used in the

Table. 1. Antimicrobial activity of compounds **1-6**

Compounds	MIC(μg/ml)			
	Escherichia coli	Bacillus subtilis	Aspergillus niger	Cladosporium cladosporioides
1	1000	125	250	125
2	>500	125	500	250
3	>500	>500	>500	>250
4	>500	1000	1000	500
5	1000	500	1000	1000
6	>500	>1000	500	>500
Tetracycline*	1.6	0.8	-	-
Cycloheximide**	-	-	6.3	12.5

*, **: positive controls against bacteria and fungi, respectively.

present study as shown in Table Ⅰ. Among isolated compounds, dillapional (**2**) and dillapiol (**3**) were isolated for the first time from this plant.

REFERENCES

Ceska, O., Chaudgary, S. K., Warrington, P. J., Ashwood-Smith, M. J., Photoactive furocoumarins in fruits of some umbellifers. *Phytochemistry*, 26, 165-169 (1986).

Elgamal, M. H. A., Elewa, N. H., Elkhrisy, E. A. M. and Duddeck, H., ^{13}C NMR chemical shift and carbon-proton coupling constants of some furocoumrins and furochromones. *Phytochemistry*, 18, 139-143 (1979).

Harkar, S., Razden, T. K., Waight, E. S., Steroids, chromone and coumrins from *Angelica officinalis*. *Phytochemistry*, 23, 419-426 (1984).

Kim, C. M., Sin, M. K., Lee, K. S. and Ahn, D. K., The dictionary of Chinese medicines, Jungdam press, Seoul, pp. 6597-6604, 1997.

Lee, K. H. and Soine, T. O., Coumain : Spectral studies on some linear furocoumarins, *J. Pharm. Sci.*, 58, 681-683 (1969).

Ono, M., Ito, Y., Ishikawa, T., Kitajima, J., Tanaka, Y., Niiho, Y. and Nohara, T., Five new monoterpene glycosides and other compounds from Foeniculi Fructus (Fruit of *Foeniculum vulgare* Miller). *Chem. Pharm. Bull.*, 44, 337-342 (1996).

Ono, M., Ito, Y., Kinjo, J., Yahara, S., Nohara, T. and Niiho, Y., Four new glycosides of stilbene trimer from Foeniculi Fructus (Fruit of *Foeniculum vulgare* Miller). *Chem. Pharm. Bull.*, 43, 868-871 (1995).

Orjala, J., Erdelmeier, C. A. J., Wright, A. D., Rali, T. and Sticher, O., Five new prenylated *p*-hydroxybenzoic acid derivatives with antimicrobial and molluscicidal activity from *Piper aduncum* leaves. *Planta Med.*, 59, 546-551 (1993).

Steck W. and Mazurek, M., Identification of natural coumarins by nmr spectroscopy. *Lloydia*, 35, 418-439 (1972).

Tomar, S. S. and Mukerjee, Dillapional, a new constituent of *Anethum sowa* Roxb. *Indian J. Chem.*, 20B, 723-724 (1981).