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Cytotoxic Constituents of *Psoralea corylifolia*

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A coumestan derivative, psoralidin (**1**) was found to be a cytotoxic principle of the seeds of *Psoralea corylifolia* L. (Leguminosae) with the IC_{50} values of 0.3 and 0.4 μg/ml against the HT-29 (colon) and MCF-7 (breast) human cancer cell lines, respectively. A coumarin, angelicin (**2**) was also isolated as a marginally cytotoxic agent along with an inactive compound, psoralen (**3**) from the plant. The isolates **1-3** were not active against the A541 (lung) and HepG2 (liver hepatoma) cancer cell lines.

Key words: *Psoralea corylifolia,* Psoralidin, Angelicin, Psoralen, Cytotoxicity

INTRODUCTION

Psoralea corylifolia Linn. (Leguminosae) is an annual herbaceous plant that has been distributed widely throughout India and the Southeast Asia. The plant has been inserted to the formulations of traditional medicine in the Southeast Asia as a prophylactic against osteoporosis caused by senescence (Miura *et al.*, 1996), and for the treatment of impotence, premature ejaculation (Tsai *et al.*, 1996), cold, painful lower back, enuresis, alopecia, psoriasis, and vitiligo (Bensky and Gamble, 1993). Several flavonoids with antiplatelet activity (Tsai *et al.*, 1996) and coumarins have been reported previously from *P. corylifolia* (Kondo *et al.*, 1990; Mehta *et al.*, 1973; Miura *et al.*, 1996). (+)-Bakuchiol was reported as a cytotoxic agent from this plant before (Ryu *et al.*, 1992).

As a part of our research program to discover potential anticancer agents of plant origin, an ethyl acetate extract of *P. corylifolia* was investigated. We report herein the cytotoxic activity of three isolates, psoralidin (**1**), angelicin (**2**), and psoralen (**3**) from the plant against HT-29 (colon), MCF-7 (breast), A541 (lung), and HepG2 (liver hepatoma) human cancer cell lines for the first time.

MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The IR spectra were gained on a JASCO FT/IR-5300 spectrometer. The NMR spectra were measured on a Gemini 2000 (300 MHz) instrument, and chemical shifts were referenced to TMS.

Plant materials

The seeds of *Psoralea corylifolia* Linn. (Leguminosae) were purchased from herb markets in Seoul and voucher specimens have been deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Seoul, Korea.

Extraction and isolation

The dried seeds of *P. corylifolia* (1 kg) were ground and extracted 3 times with MeOH (3 × 5 l) overnight at room temperature. The MeOH extract was concentrated *in vacuo*, and then diluted with H_2O to give an aqueous MeOH solution. The aqueous solution was subsequently partitioned into *n*-hexane, ethyl acetate and aqueous fractions. The ethyl acetate extract (99 g) displayed significant cytotoxic activity, thus, further fractionations were performed using silica gel column chromatography. Repetition of silica gel column chromatography using gradient solvent systems of $CHCl_3$-MeOH and hexane-EtOAc afforded psoralidin (**1**, 114 mg), angelicin (**2**, 132 mg), and psoralen (**3**, 9.0 mg).

Chemical structures of 1-3

Compound 1 (psoralidin): pale yellowish white crystal (MeOH, EtOAc); mp. 286-291°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.70 (3H, s, 5'-Me), 1.73 (3H, s, 4'-Me), 3.35

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(2H, d, 1'-H_2, overlapped with H_2O), 5.34 (1H, br t, J = 7.2 Hz, H-2), 6.91 (1H, s, H-4), 6.93 (1H, br d, J = 9.9 Hz, H-8), 7.16 (1H, br s, H-10), 7.60 (1H, s, H-1), 7.67 (1H, d, J = 8.7 Hz, H-7); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 17.9, 25.8 (C-4' & C-5'), 27.7 (C-1'), 98.9 (C-10), 102.1 (C-4), 114.1 (C-8), 120.7 (C-7), 121.1 (C-1), 121.9 (C-2'), 132.7 (C-3'), 157.9 (C=O), 102.6, 104.0, 114.9, 126.6, 153.0, 156.1, 157.1, 159.1, 159.7.

Compound 2 (angelicin): white crystal (MeOH, hexane); mp. 133-134°C; IR ν_{max} (KBr): 1712 (C=O), 1616 (C=C), 833, 743 cm^{-1}; UV λ_{max} log ε (EtOH): 247.5 (4.42), 299 (4.08); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.39 (1H, d, J = 9.6 Hz , H-3), 7.81 (1H, d, J = 9.6 Hz , H-4), 7.38 (1H, brd, J = 8.4 Hz , H-5), 7.44 (1H, dd, J = 0.6, 8.4 Hz , H-6), 7.70 (1H, d, J = 2.1 Hz , H-2'), 7.14 (1H, dd, J = 0.6, 2.1 Hz, H-3'); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 161.0 (C-2), 114.2 (C-3), 144.6 (C-4), 123.9 (C-5), 108.8 (C-6), 157.5 (C-7), 117.0 (C-8), 148.6 (C-9), 113.6 (C-10), 146.0 (C-2'), 104.1 (C-3'); MS *m/z* (rel. int.): 186 $[M]^+$(91), 158 (100), 130 (18.0), 102 (31.5).

Compound 3 (psoralen): yellowish white crystal (MeOH); mp. 154-156°C; IR ν_{max} (KBr) 3156, 3123 (furan), 1718 (C=O), 1633, 1577 (C=C) cm^{-1}; UV λ_{max} log ε (EtOH): 245.5 (4.38), 291 (3.97), 329.5 (3.74); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.39 (1H, d, J = 9.6Hz, H-3), 7.81 (1H, d, J = 9.6Hz, H-4), 7.69 (1H, s, H-5), 7.49 (1H, d, J=0.9 Hz, H-8), 7.70 (1H, d, J = 2.4 Hz, H-2), 6.83 (1H, dd, J = 0.9, 2.4 Hz, H-3'); MS *m/z* (rel. int.): 186$[M]^+$(100), 158 (64.5), 130 (11.0), 102 (19.5).

Cell culture

HT-29 (human colorectal adenocarcinoma, ATCC HTB -38) cell lines were cultured in DMEM with 4 mM L-glutamine, 4.6 g/l glucose, 100 U/ml penicillin and 10 μg/ml streptomycin, supplemented with 10% heat-inactivated fetal bovine serum. MCF-7 (human breast adenocarcinoma, ATCC HTB-22) and A549 (human lung carcinoma, ATCC CCL-185) cell lines were cultured in RPMI 1640. HepG2 (human hepatocellular carcinoma, ATCC HB-8065). All cells were maintained at 37°C, 5% CO_2 in a humidified atmosphere incubator. The confluent cells were used for the MTT assay.

Cytotoxicity

Cytotoxicity was assessed by using the MTT (3-[4,5-dimethyl (thiazol-2-yl)]-2,5-diphenyl tetrazolium bromide) assay. It was performed according to Mosmann (1983). In short, 5,000~10,000 cells were seeded in each well of 96-well plate in a final volume of 200 μl of culture medium supplemented with 10% FBS (fetal bovine serum). Cells were exposed to different concentrations of samples for 72 h at 37°C in an atmosphere of 5% CO_2 in air. At the end of the treatments, MTT (0.5 mg/ml final concentration) in PBS was added to the wells and after incubation for 4 h at 37°C, media were removed. In viable cells, MTT is converted to the purple formazan dye, which is measured spectrophotometrically following solubilization in dimethyl sulfoxide (DMSO). The formazan crystals in viable cells were solubilized with DMSO, to each well followed by agitation of the plate on a microplate shaker for 5 min. The absorbancies of each well were then read at 540 nm in a microplate reader.

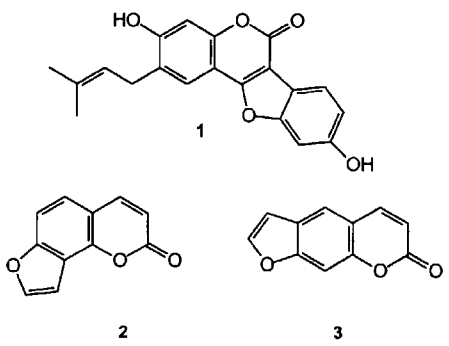

Fig. 1. Structures of compounds **1-3** from *P. corylifolia*

RESULTS AND DISCUSSION

The ethyl acetate extracts of the seeds of *P. corylifolia* showed significant cytotoxic activity against the HT-29 and MCF-7 cancer cell line. Thus, detailed laboratory investigation was performed on the active ethyl acetate extract. Bioassay-guided fractionation led to the isolation of compound **1** as an active principle along with a marginally active compound **2** and an inactive constituent, **3**. Structures of **1-3** were identified as psoralidin (Yang *et al.*, 1996; Ryu *et al.*, 1992), angelicin (Inocenti et al., 1991), and psoralen (Baskin et al., 1967; Inocenti *et al.*, 1991), respectively, mainly, by analysis of their NMR data as well as by comparison of their physical and spectral data with those of literature values. Psoralidin (**1**) exhibited potent cytotoxic activity against the HT-29 (colon) and MCF-7 (breast) cancer cell lines with the IC_{50} values of 0.3 and 0.4 μg/ml, respectively. Angelicin (**2**) showed weak cytotoxicity against HT-29 (colon) and MCF-7 (breast) cancer cell lines with the IC_{50} values of 17.7 and 11.9 μg/ml, respectively. Psoralen (**3**) was considered to be inactive in

Table 1. Cytotoxic activity of compounds **1-3**

Compounds	IC_{50} (μg/ml)			
	HT-29	MCF-7	A549	HepG2
1	0.3	0.4	15.3	21.0
2	17.7	11.9	31.3	35.8
3	43.9	32.9	45.2	44.6
Ellipticin*	0.1	0.1	0.8	0.8

*Used as a positive control

the cytotoxicity assay system used in the present study as shown in Table I.

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REFERENCES

Baskin, J. M., Ludlow, C. J., Harris, T. M., and Wolf, F. T. Psoralen, an inhibitor in the seeds of *Psoralea subacaulis*. *Phytochemistry*, 6, 1209-1213 (1967).

Bensky, D. and Gamble, A. Chinese herbal medicine: Materia Medica. Revised edition. Eastland Press, Seattle, Washington, pp. 344-345 (1993).

Inocenti, G., Cappelletti, E. M., and Caporale, G. Furocoumarin contents in the vegetative organs of cultivated Psoralea species. *Int. J. Pharmacog.*, 29, 311-316 (1991).

Kondo, Y., Kato, A., Kubota, Y., and Nozoe, S. Bakuchicin, a new simple furanocoumarin from *Psoralea corylifolia*. *Heterocyles*, 31, 187-190 (1990).

Mehta, G., Naysak, U. R., and Dev, S. Monoterpenoids. I. *Psoralea corylifolia* Linn. 1. bakuchiol, a novel monoterpene phenol. *Tetrahedron* 29, 1119-1125 (1973).

Miura, H., Nishida, H., and Iinuma, M. Effect of crude fractions of *Psoralea corylifolia* seed extract on bone calcification. *Planta Med.* 62, 150-153 (1996).

Ryu, S. Y., Choi, S.U., Lee, C. C., and Zee, O. P. Antitumor activity of *Psoralea corylifolia*. *Arch. Pharm. Res.*, 15, 356-359 (1992).

Tsai, W. J., Hsin, W. C., and Chen, C. C. Antiplatelet flavonoids from seeds of *Psoralea corylifolia*. *J. Nat. Prod.*, 59, 671-672 (1996).

Yang, Y. -M., Hyun, J. -W., Sung, M. -S., Chung, H. -S., Kim, B. -K., Paik, W. -H., Kang, S. -S., and Park, J.-G. The cytotoxicity of Psoralidin from *Psoralea corylifolia*. *Planta Med.*, 62, 353-354 (1996).