Arch. Pharm. Res. Vol.20, No.4, pp. 342-345, 1997

A Cytotoxic Constituent from *Sophora flavescens*

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(Received May 12, 1997)

A cytotoxic constituent was isolated by bioassay-guided procedure from the roots of *Sophora flavescens* Aiton (Leguminosae). The constituent was identified as sophoraflavanone G (**I**) by means of chemical methods and in comparsion with spectral data of standard compound. The ED_{50} values of constituent I were 0.78, 1.57, 2.14 and 8.59 μg/ml against A549, HeLa, K562 and L1210 cell lines, respectively. Constituent **I** exhibited highly cytotoxic activities against A 549, K562 and HeLa cells, but showed a mild activity (ED_{50} value, 5 μg/ml) against L1210 cells. Among the tested cell lines, A549 cells were the most sensitive to constituent **I**.

Key words : *Sophora flavescens, Sophora moorcroftiang,* Leguminosae, sophoraflavanone G, cytotoxicity, A549

INTRODUCTION

As a part of our continuing research to find novel antitumor agents from Korea medicinal plants, we have screened compounds to express the cytotoxicities against L1210 cells (Bae *et al.*, 1992). Among the medicinal plants tested, the benzene soluble fraction of the roots of *Sophora flavescens* has exhibited a cytotoxic activity against L1210 cells. In folk medicine, the roots of *Sophora flavescens* (Leguminosae), which is called Kosam (Sophorae Radix), has been used as antipyretic, diuretic and anthelmintic.

In earliest investigations, the chemical constituents of this plant investigated so far were sophoraflavoside I, soyasapogenol (Yashikawa *et al.*, 1985), sophoraflavoside II-IV (Ding *et al.*, 1992), matrine (Okuda *et al.*, 1965), (—)-$\triangle^7$-dehydrosophoramine (Ueno *et al.*, 1978), (—)-5α-hydroxysophocarpine (Saito, *et al.*, 1990), kushequinone A (Wu *et al.*, 1986), kushenol J, K, L, M (Wu *et al.*, 1985a), kurarinol (Kyogoku *et al.*, 1973), kushenol A, B, E, F (Wu *et al.*, 1985b, 1985c) and sophoraflavanone A, B, G (Shirataki *et al.*, 1983; Shirataki *et al.*, 1988). Matrine had been reported to possess a preventive activity of stress ulcer (Yamazaki *et al.*, 1984), and also reported to have antibiotic (Yamaki *et al.*, 1990), antifungal activities (Yagi *et al.*, 1989) and antitumoral activities. However, no paper has been so far published on the antitumoral activities of flavanone isolated from *S. flavescens.*

In this paper, we report on the isolation, the identification and the cytotoxicity of constituent from the roots of *S. flavescens.*

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MATERIALS AND METHODS

Apparatus and methods

All commercial reagents were used of analytical grade and all solvents were purified prior to use by the procedures of Perrin *et al.* Melting points were determined on an Electrothermal melting point apparatus. IR spectra in KBr disk were run on a JASCO Report-100 Infrared spectrophotometer. UV/VIS spectrum were measured on Milton Roy 3000 array and Pye Unicam PU 8800. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a JEOL LNM-EX90 (90, 22.5MHz). Column chromatography was carried out on silica gel (Merck, 70-230 mesh).

Plant material

Sophorae Radix was purchased from crude drug market in Taejon, Korea. The crude drug was identified by Prof. KiHwan Bae (College of Pharmacy, Chungnam National University).

Isolation of cytotoxic constituent I

The Sophorae Radix (3 Kg) was extracted with hot MeOH and concentrated. The MeOH extract (312 g) was suspended in distilled water (2 L). The water suspension was sucessively partitioned with *n*-hexane, ether and ethyl acetate. The respective fraction was evaporated *in vacuo* to dryness. The distribution and purification of the cytotoxic constituents against L1210 cells were monitored by the procedures of Geran *et.*

Table I. Cytotoxicities of solvent fractions on the roots of *Sophora flavescens* against L1210 cells[a]

Fraction	ED_{50} value (μg/ml)
n-hexane	>100[b]
Ether	19.5
EtOAc	> 40
Water	>100
5-FUc	<0.02

[a]L1210 cells were cultured in Fisher's medium with 10% horse serum.
[b]The values were the mean of at least three experiments.
[c]5-FU: 5-Fluorouracil, Positive control.

al., 1972 and Thayer *et. al.*, 1971. The ether fraction showed an ED_{50} value of 19.5 μg/ml, but the other fractions showed no significant activity (Table 1). The ether fraction (96 g) was chromatographed on silica gel column gradiently with *n*-hexane:acetone (10:1→0:1) as eluent to obtain five fractions. Among five fractions, the fraction 3 exhibited a remarkable cytotoxic activity. The fraction was further purified by silica gel column chromatography using *n*-hexane:acetone (5:1) as eluent. Further purification of sub-fraction 3-2 over silica gel column with *n*-hexane:acetone (7:1) as solvent gave the active constituent **I** (230 mg), which was recrystallized from the solvent mixture (*n*-hexane:acetone=20:1).

Constituent **I** (sophoraflavanone G, Fig 1): pale yellow powder, mp 173-5°C, $FeCl_3$ test: black-blue color, UV λ_{max} (log ε): (MeOH) nm 294 (4.25), 342 (3.60), (MeOH-$AlCl_3$) nm 316 (4.37), 398 (3.63), (MeOH-$AlCl_3$-HCl) nm 314 (4.33), 398 (3.60), (MeOH-NaOMe) nm 333 (4.42), (MeOH-NaOAc) nm 333 (4.34), IR (KBr, cm^{-1}): 3,300 (OH), 1,625 (C=O), 1,600, 1,520 (C=C of benzene), ^{1}H-NMR (Pyridine-d_5, ppm): 1.58 (6H, s, -CH=C(CH_3)$_2$), 1.90 (3H, s, CH=C-CH_3), 2.42, 3.10 (2H, m, -CH_2-), 4.93 (2H, s, =CH_2), 5.29 (1H, s, H of B ring), 11.57, 11.92 (1H, s, -OH), 12.90 (1H, s, chelated -OH), ^{13}C-NMR (Pyridine-d_5, ppm): 197.2 (C-3), 158.6 (C-4'), 155.5 (C-2'), 147.8 (C-8''), 130.6 (C-5''), 127.6 (C-6'), 123.4 (C-4''), 115.9 (C-1'), 110.8 (C-9''), 106.4 (C-8), 106.3 (C-5'), 101.6 (C-3'), 95.1 (C-6), 73.9 (C-2), 17.2-46.6 (C^3 of lavandulyl group).

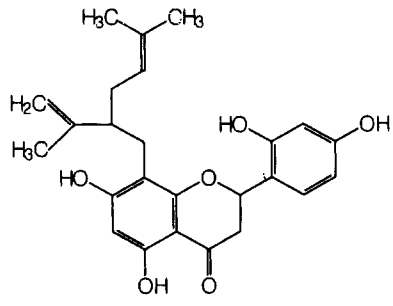

Fig. 1. Structure of sophoraflavanone G (constituent I).

Cytotoxic assay

Cytotoxicity of constituent I was measured against murine leukemia L1210, human lymphoma K562, human lung carcinoma A549 and human cervix carcinoma HeLa cell lines using sulfurhodamine B (SRB) method (Skehan *et al.*, 1990). ED_{50} value was determind graphically by plotting the viability versus the concentration of tested sample.

RESULTS AND DISCUSSION

The ether fraction of the roots of *S. flavescens* has been found to show moderate cytotoxic activity against L1210 cells (Table I). By means of activity-directed chromatographic fractionation, constituent I was isolated from the ether fraction of the roots of *S. flavescens*. Constituent **I**, a pale yellow powder, mp 173-175°C, showed a positive result in $FeCl_3$ test. The IR spectrum of constituent **I** showed a strong absorbance of hydroxyl group (3300 cm^{-1}) and carbonyl group (1625 cm^{-1}), suggesting the existence of a strong intramolecular hydrogen bond between both groups. Its UV spectrum in MeOH solution displayed UV maxima at 294 (band II) and 342 nm (band I) charcteristic of B-ring and A-ring of flavonoid skeleton. When $AlCl_3$ reagent was added in MeOH solution, the band II exhibited 20 nm bathochromic shift. Meanwhile, $AlCl_3$/HCl spectrum showed no differece from $AlCl_3$ spectrum. The UV spectrum after the addition of NaOMe and NaOAc reagents showed a maximum absorbance at 333 nm. Thus, the UV spectra of constituent **I** was characteristic of the 5,7-dihydroxyflavanone (Mabry *et al.*, 1970). The ^{1}H-NMR spectrum of constituent **I** exhibited the signals of two hydroxyls on B-ring (11.57 and 11.92 ppm), one chelated hydroxyl (12.90 ppm), an ABX type grouping due to the C-2 (6.22 ppm) and C-3 (3.18, 3.34 ppm), and alkane and alkene of lavandulyl group (1.58, 1.90, 2.42, 3.10, 4.93, and 5.29 ppm). In the aromatic region of the spectrum, the signals of the remaining four protons occurred as a singlet (6.49 ppm) due to the A-ring proton, and a doublet (7.82 ppm) and a multiplet (6.97 ppm) due to the B-ring. In the ^{13}C-NMR spectrum, the signals of three hydroxyl bonded carbons (155.46, 158.35, 160.77 ppm), one hydrogen bonded carbonyl carbon (197.17 ppm), and lavandulyl bonded carbon (106.4 ppm) were shown. On the basis of all spectral data, constituent **I** was found to be a flavanone with lavandulyl substituent at C-8. The direct comparison of the chemical and spectral data of constituent **I** with those of sophoraflavanone G revealed both constituents to be identical. Thus, constituent **I** was identified as sophoraflavanone G, which had already been isolated from the roots of *Sophora moorcroftiang* (Shirataki *et al.*, 1988).

The cytotoxicities of constituent **I** were tested against

Table II. Cytotoxicities of sophoraflavanone G against four cancer cells

Cancer cells	ED_{50} value (μg/ml)	
	Sophoraflavanone G	5-FU[a]
A549	0.78[b]	1.52
K562	2.14	0.21
HeLa	1.57	—[c]
L1210	8.59	<0.02

[a]5-FU: 5-fluorouracil, Positive control
[b]The values were the mean of at least three experiments
[c]Not tested

four cancer cell lines, which had originated from human and murine. As indicated in Table II, constituent **I** showed significant cytotoxicities against A549, K562 and HeLa cells with ED_{50} values of 0.78, 2.14 and 1.57 μg/ml, respectively. The cytotoxicity (ED_{50} value, 8.59 μg/ml) against L1210 cells was lower than that against three human cell lines. The cytotoxicity of constituent **I** against A549 cells was two to three times higher than that against K562 and HeLa cells. Thus, constituent I showed the strongest cytotoxicity against A549 (human lung carcinoma) among cancer cell lines tested. Constituents **I** was more cytotoxic than 5,2',5'-trihydroxy-7,8-dimethoxyflavanone, which was isloated from *Scutellaria indica*, or synthetic analogous of 5,2',5'-trihydroxy-7,8-dimethoxyflavanone (Bae *et al.*, 1994, Min *et al.*, 1996). Also, the cytotoxicity of constituent **I** against A549 cells was about twice greater in comparsion with that of 5-fluorouracil which are currently widely used in cancer therapy. Among synthetic analogous of 5,2',5'-trihydroxy-7,8-dimethoxyflavanone, the remarkable cytotoxicities against A549 cells were exhibited only the compounds benzyloxylated on flavanone B-ring. In our present study, the existence of lipophilic lavandulyl or benzyloxyl group on A or B-ring of flavanone was considered to be one of the important factors to express a cytotoxic activity against A549 cells.

REFERENCES CITED

Bae, K. H., Min, B. S., Do, D. S., Kim, N. S., Yang, K. J. and Ahn, B. Z., Screening on Cytotoxicity of Medicinal Plants against L1210 Cell. *Yakhak Hoeji*, 36, 491-495 (1992).

Bae, K. H., Min, B. S., Park, K. L. and Ahn, B. Z., Cytotoxic Flavonoids from *Scutellaria indica*. *Planta Medica*, 60, 280-281 (1994).

Ding, Y., Tian, R., Kinio, J., Nohara, T. and Kitagawa, J., Three new oleanene glycosides from Sophora flavescens. *Chem. Pharm. Bull.*, 40, 2990-2994 (1992).

Kyogoku, K., Hatayama, K. and Komatsu, M., Constituents of Chinese crude drug "Kushen" (the root of *Sophora flavescens* Aiton.). Isolation of five new flavonoids and formononetic. *Chem. Pharm. Bull.*, 21, 2733-2738 (1973).

Mabry, T. J., Markham, K. R. and Thomas, M. B., *The systematic identification of flavonoids*, Spring-Verlag, New York, Chapter V. p.41-226 (1970).

Min, B. S., Ahn, B. Z. and Bae, K. H., Snthesis and structure-activity relationship of cytotoxic 5,2', 5'-trihydroxy-7,8-dimethoxyflavanone analogues. *Arch. Pharm. Res.*, 19, 543-550 (1996).

Okuda, S., Murakoshi, I., Kamata, H., Kashida, Y., Haginiwa, J. and Tsuda, K., Studies on lupine alkaloids I. The minor alkaloids of Japanese *Sophora flavescens*. *Chem. Pharm. Bull.*, 13, 482-487 (1965).

Perrin, D. D. and Armarego, W. L. F., *Purification of Laboratory Chemicals*, 3rd ed., Pergamon, Oxford, 1988.

Saito, K., Arai, M., Sekine, T., Ohmiya, S., Kubo, H., Otomasu, H. and Murakoshi, I., (—)-5α-hydroxysophocarpine, a new lupine alkaloid from the seeds of *Sophora flavescens* var. *angustifolia*. *Planta Medica.*, 56, 487-488 (1990).

Shirataki, Y., Endo, M., Yokoe, I. and Komatsu, M., Studies on the constituents of *Sophora species* XVIII. Constituents of the root of *Sophora tomentosa* L. (3)., *Chem. Pharm. Bull.*, 31, 2859-2863 (1983).

Shirataki, Y., Yokoe, I., Noguchi, M., Tomimori, T. and Komatsu, M., Studies on the constituents of Sophora species XXII. Constituents of the root of Sophora moorcroftiag Benth. ex Baker (1). *Chem. Pharm. Bull.*, 36, 2220-2225 (1988).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1113 (1990).

Thayer, P. S., Himmelfarb, P. and Watts, G. L., Cytotoxicity assay with L1210 Cells *in vitro*. Comparision with L1210 Cells *in vitro* and KB Cells *in vitro*. *Cancer Chemother.* Rep.(part 2) 2, 1 (1971).

Ueno, A., Morinaga, K., Fukushima, S. and Okuda, S., Studies on Lupine alkaloids VII. (—)-$\triangle^7$-dehydrosophoramine. *Chem. Pharm. Bull.*, 26, 1832-1836 (1978).

Wu, L. T., Miyase, T., Ueno, A., Kuroyanagi, M., Noro, T., Fukushima, S. and Sasaki, S., Studies on the constituents of *Sophora flavescens* Aiton V., *Yakugaku Zasshi*, 106, 22-26 (1986).

Wu, L. T., Miyase, T., Ueno, A. and Kuroyanaga, M., Studies on the constituents of *Sophora flavescens* Aiton IV. *Yakugaku Zasshi*, 105, 1036-1039 (1985a).

Wu, L. T., Miyase, T., Ueno, A. and Kuroyanaga, M., Studies on the constituents of *Sophora flavescens* Aiton II. *Chem. Pharm. Bull.*, 33, 3231-3236 (1985b).

Wu, L. T., Miyase, T., Ueno, A. and Kuroyanaga, M., Studies on the constituents of *Sophora flavescens* Aiton III. *Yakugaku Zasshi,* 105, 736-741 (1985c).

Yagi, A., Fukunaga, M., Okuzako, N., Mifuchi, I. and Kawamoto, F., Antifungal substances from *Sophora flavescens. Shoyakugaku Zasshi,* 43, 343-347 (1989).

Yamazaki, M., Arai, A., Suzuki, S. and Takechi, T., Protective effects of matrine and oxymatrine on stress ulcer in relation to there effects on the central nervous system. *Yakugaku Zasshi,* 104, 293-301 (1984).

Yamaki, M., Kashihara, M. and Takagi, S., Activity of Kushen compounds against *Staphylococcus aureus* and *Streptococcus mutants. Phytother. Res.,* 4, 235-236 (1990).

Yashikawa, M., Wang, H. K., Kayakiri, H., Taniyama, T. and Kitagawa, I., Saponin and sapogenol XI. Structure of sophoraflavoside I, a bisdesmoside of soyasapogenol B, from *Sophorae Radix, the root of Sophora flavescens* Aiton. *Chem. Pharm. Bull.,* 33, 4267-4274 (1985).