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苦蔘의 抗癌活性 및 活性成分에 관한 硏究

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ABSTRACT

In vitro antitumor activity of flavonoids from Sophora flavescens

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The cytotoxicity-guided fractionation of the roots of Sophora flavescens (Leguminosae) extracts led to the isolation of fifteen active principles 1~15, responsible for the cytotoxicity against five kinds of cultured human tumor cell lines, i.e., A549(non small cell lung), SK-OV-3(ovary), SK-MEL-2(skin), XF498(central nerve system) and HCT-15(colon), evaluated by SRB method in vitro. Compounds 2~14 were classified as unusual flavonoid occurred exclusively in this species and the proliferation of each examined tumor cells were significantly inhibited during the continuous exposure to compounds 1~15 for 48 hours, respectively.

Keywords: Sophora flavescens ; cytotoxicity ; flavonoid ; antitumor ; human tumor cells ; SRB method

INTRODUCTION

The species, Sophora flavescens A(Leguminosae) is a shrub spread widely and commonly cultivated in northeast Asian countries. The roots of this species are commercially available as the generic "Kosam" in Korea and it has been applied frequently in folk medicine as an antipyretic, analgesic, anthelmintic and a stomachic(Hur. J. 1994).

We have conducted the cytotoxicity-guided fractionation of the MeOH extract of the roots of S. flavescens, which finally led to the isolation of fifteen active principles. In this paper, we describe the isolation and cytotoxicity of each active components against five cultured human tumor cell lines evaluated by the SRB(sulfrhodamine-B) method in vitro(Skehan et al., 1990).

MATERIALS AND METHODS

General Human tumor cells used in the experiment, i.e., A549(non small cell lung), SK-OV-3(ovary), SK-MEL-2(skin), XF498(central nerve system) and HCT-15(colon) were obtained from the National Cancer Institute(NCI) in the USA, and are currently used in the NCI's in vitro anti-cancer drug screening. Stock cell cultures were grown in T-25(falcon) flasks containing 10 ml of RPMI-1640 medium with glutamine, sodium bicarbonate and 5% fetal calf serum, which were dissociated with 0.25% trypsin and 30mM 1,2-cyclohexane-diaminetetraacetic acid(CDTA) in PBS in case of transfer for experiments. All NMR spectra were obtained on a Bruker AM-300 and Bruker AMX-500 spectrometer. High and low

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resolution MS were taken with a direct inlet and recorded with a JMS-DX303 mass spectrometer(JEOL). The optical rotations were determined on Autopol Ⅲ automatic polarimeter and the CD(circular dichroism) spectra were recorded on JASCO J-720 spectropolarimeter.

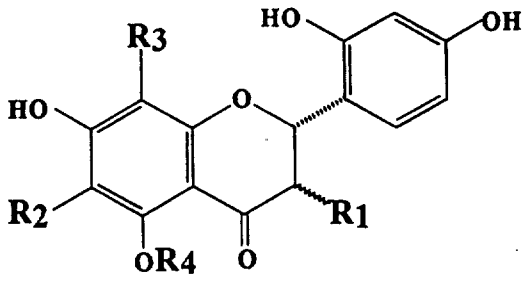
Test for cytotoxicity in vitro Cytotoxicity after treatment of the tumor cells with the test materials was determined using the the SRB method, currently adopted in the NCI's in vitro anti-cancer drug screening(Skehan et al., 1990), i.e., estimating the inhibition rate of cell proliferation after continuous exposure to test materials for 48 hrs. Detailed experimental procedures were described on the previous paper (Ryu et al., 1992). All samples were tested in triplicate and the mean ED50 value(㎍/ml), a concentration that caused 50 % inhibition of cell proliferation, and the S.E.M. was calculated, respectively.

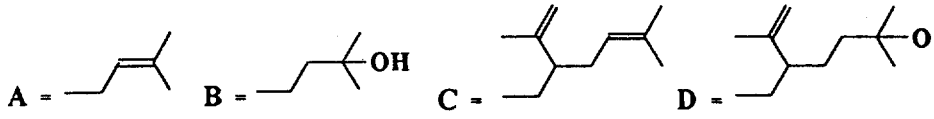
Plant material The roots of S. flavescens were collected from Cheongju, Korea, in October 1993. A sample was identified and a voucher specimen was preserved in the Herbarium of Natural Products Research Institute of Seoul National University, Korea.

Extraction and Isolation The dried and chopped roots(3Kg) were extracted with MeOH at room temperature for one week. Concentration of the solvents afforded an extract of about 240g, which was suspended in H2O and partitioned with CH2Cl2(35g) and EtOAc(50g), successively. The CH2Cl2 soluble fraction(ED50 value against A549 were ca. 50㎍/ml) was divided roughly by six fractions(F1~F6) by silica gel column chromatography. The resultant six fractions were evaluated for cytotoxicity against tumor cells in vitro. Among them, only the F4 and F5 were exhibited a significant cytotoxicity(ED50 value against A549 were ca. 15㎍/ml and 20㎍/ml), which were further divided into several subfractions followed by monitering the cytotoxicity. This procedure, the fractionation followed by cytotoxicity estimation was repeated until the isolation of nine pure active components 1~9 were achieved from F4 and F5, i.e., 1(formononetin, 72mg), 2(kushenol E, 55mg), 3(kushenol B, 250mg), 4(sophoraflavanone G, 480mg), 5(kushenol L, 30mg), 6(kushenol M, 500mg), 7(kuraridin, 110 mg), 8(kurarinone, 120 mg), 9(kushenol N, 20 mg), by the decreasing Rf order. The EtOAc fraction(ED50 value against A549 were ca. 30㎍/ml) was also subjected to the same treatment as for the CH2Cl2 soluble fraction to yield six active principles, 10~15, i.e., 10(kosamol A, 85mg), 11(norkurarinol, 28 mg), 12(kurarinol, 500 mg), 13(kushenol H, 110mg), 14(kushenol K, 100mg) and 15(trifolirhizin, 210mg) by the deceasing Rf order. These compounds 1~15 were identified by physicochemical and spectrospoic analyses such as m.p., NMR, MS and CD and were identical to previously published data(Ryu et al., 1996).

RESULTS AND DISCUSSION

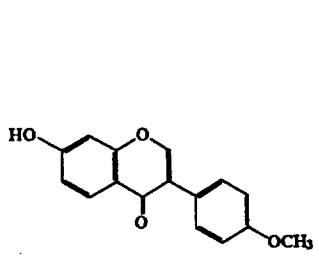
The activity-guided fractionation of the MeOH extract of the roots of S. flavescens was conducted and led to the isolation of active principles responsible for the cytotoxicity against five cultured human tumor cell lines, i e., A549(non small cell lung), SK-OV-3(ovary), SK-MEL-2(skin), XF498(central nerve system) and HCT-15 (colon), in vitro. The preliminary test on the cytotoxicity of S. flavescens extracts suggested that the cytotoxic components in the extracts of the roots of S. flavescens were predominantly concentrated in the CH2Cl2 and EtOAc soluble fractions(ED50 value against A549 were 50㎍/ml and 30㎍/ml, respectively). The repeated chromatographic purification of the CH2Cl2 and EtOAc soluble fraction by the guidance of cytotoxicity monitering afforded fifteen active principles. All isolated active components 1~15 were fully characterised by spectroscopical analyses with UV, MS, NMR and CD experiments



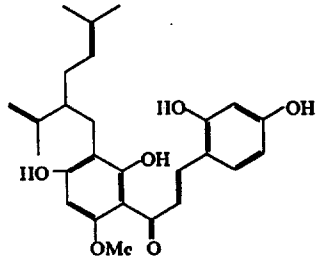


	R_1	R_2	R_3	R_4
2	-H	-A	-A	-H
3	-H	-A	-C	-H
4	-H	-H	-C	-H
5	-OH(β)	-A	-A	-H
6	-OH(β)	-A	-C	-H
8	-H	-H	-C	$-CH_3$

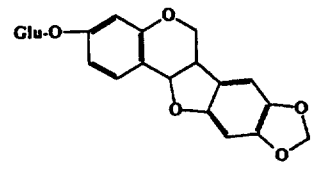
	R_1	R_2	R_3	R_4
9	-OH(α)	-H	-C	$-CH_3$
10	-OH(β)	-B	-C	-H
11	-H	-H	-D	-H
12	-H	-H	-D	$-CH_3$
13	-OH(α)	-H	-D	$-CH_3$
14	-OH(β)	-H	-D	$-CH_3$



1. Formononetin



7. kuraridin



15. Trifolirhizin

Fig. 1. Cytotoxic components from Sophora flavescens

(Ryu et al., 1995 and 1996) and by the direct comparison of spectroscopic data of each components with those of corresponding compounds reported in literatures(Wu et al., 1985a, 1985b and 1986 and Iinuma et al., 1990). The established chemical structure of each components 1~15 are summarized in Fig.1

The cytotoxicity of 1~15 was evaluated against five human tumor cell lines in vitro. As a reference drug the clinically used cytostatic agent cisplatin was used. All components 1~15 exhibited a moderate cytotoxicity against all examined tumor cells(Table I), and cell-specificity on examined tumor cell lines was not observed in any of the components. However, a structure activity-relationship was observed between each active component. The flavanone components(R1=-H) exhibited a slightly higher

cytotoxicity than flavanonol(dihydroflavonol) components(R2= -OH), i.e., the activities of 2, 3, 8 and 12 were twice as potent as those of 5, 6, 9 and 13, respectively. These results correlated with those of previous studies on the cytotoxic property of some flavonoids such as apigenin, luteolin, kaempferol and quercetin, which are commonly occurring in plants(Ryu et al., 1994 and Woerdenbag et al., 1994). The methylation of -OH group at C-5 position(R4) resulted in a slight diminution of activity, i.e., 9 and 13 were observed to give a weaker cytotoxicity than 8 and 12, respectively.

aED50 value of compound against each cancer cell line, which was defined as a concentration that caused 50 % inhibition of cell proliferation in vitro.

bData are mean±S.E.M. of three distinct experiments.

Compounds 2~14 were comprised in unusual flavonoids with isopenteny or lavandulyl side chains which were found exclusively in this species(Fig.1). Even though various biological effects, such as antiulcer activity, inhibition of c-AMP phosphodiesterase activity, antimicrobial and antifungal activity(Yamaki et al., 1990) have been published so far, to our best knowledge, this is the first report on the investigation of cytotoxicity against human tumor cell lines. Research to achieve the mode of cytotoxicity of flavonoids 2~14, of interest from the therapeutical point of view, are currently underway.

Table I. Inhibition of Tumor cell proliferation by some flavonoids from Sophora flavescens

COMPOUND	ED50(μg/mℓ)a				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	⟩50	⟩50	⟩50	⟩50	⟩50
2	6.4±0.2b	6.4±0.3	5.3±0.3	5.0±0.4	4.6±0.3
3	3.8±0.1	4.3±0.2	3.4±0.2	2.7±0.3	3.0±0.2
4	6.4±0.3	7.9±0.3	3.9±0.2	5.8±0.3	5.7±0.3
5	11.4±0.3	11.0±0.3	12.4±0.4	12.0±0.3	8.4±0.3
6	5.5±0.1	5.5±0.2	5.0±0.1	5.7±0.2	5.1±0.3
7	7.1±0.2	5.8±0.1	5.4±0.1	5.8±0.1	5.0±0.1
8	9.0±0.3	9.4±0.2	6.4±0.2	5.9±0.2	8.6±0.3
9	13.1±0.4	13.8±0.5	6.7±0.2	9.6±0.1	14.5±0.1
10	5.9±0.2	6.8±0.2	7.1±0.2	2.7±0.1	6.5±0.2
11	15.4±0.5	14.8±0.5	12.9±0.6	10.7±0.4	16.5±0.3
12	30.3±0.8	25.8±0.6	21.8±0.5	25.9±0.4	28.7±0.4
13	⟩50	⟩50	⟩50	⟩50	⟩50
14	⟩50	⟩50	⟩50	⟩50	⟩50
15	16.3±0.5	24.9±0.7	15.0±0.4	37.8±0.3	21.0±0.4
cisplatin	1.4±0.1	0.9±0.3	0.8±0.2	0.9±0.3	2.2±0.4

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