L-6

Antitumor constituents from some Korean medicinal plants

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For the screening of bioactive natural products, the benzene or methanol extracts from 93 medicinal plants of Korea were prepared, and tested for the cytotoxicity against L1210 cells and for the antitumor action (Bae *et al.*, 1992 and 1996). Of 93 extracts tested, 6 samples showed a cytotoxicity in both benzene and methanol extract, 39 samples in benzene and 13 samples in methanol extract. The benzene extract of the root of *Scutellaria indica* L., *Sophora flavescens* Solander ex Aiton, *Carpesium abrotanoides* L., *Gymnaster koraiensis* (Nakai) Kitamura, *Pyrola japonica* Klenze, and Forsythiae Fructus showed a potent cytotoxic activity. This observation led to isolate active cytotoxic components, some of which demonstrated some antitumor action. In addition, the structure-activity relationship was discussed.

INTRODUCTION

In cancer therapy, natural product is one of useful tools not only in their isolated form, but also as templates for the formation of analogues with improved activity and as proves for stduying, biochemical processess at the molecular level (O'Dwyer *et al.*, 1985). Numerous cytotoxic compounds have been isolated from natural resources using a cytotoxicity-based screening system. However, most of these compounds lack of selectivity in attacking tumor cells and display minimal therapeutic indices (Jayatilake *et al.*, 1993). Some active compounds such as vincristine, vinblastine, taxol have been isolated from plants, and used for clinical treatments (Kingston *et al.*, 1990).

In a continuing research to find novel antitumor agents from Korean medicinal plants, the cytotoxic and antitumor activities were examined. The cytotoxicity was tested against L1210, A549, K562 and HeLa cells *in vitro*, and antitumor activity tested in hybrid female mouse (BDF1-KIST) implanted with P388 cells or in mouse bearing Sarcoma 180 cells *in vivo*.

RESULTS AND DISCUSSION

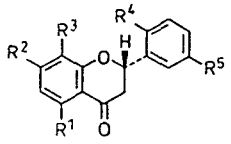
1. The cytotoxic and antitumor flvavonoids from *Scutellaria indica*

The whole herb of *Scutellaria indica,* known as "Han-xin-cao", is used for hemoptysis, hematemesis, anticancer, and other diseases in traditional medicine (Chiang, 1977), and is distributed widely in Korea, Japan, China and Taiwan. The methanolic extract of the *S. indica* was found to have potent cytotoxicity against L1210 and HL60 cells (Bae *et al.*, 1992). In our continuing investigations to clarify the bioactive constituents, 5 cytotoxic flavonoids were isolated and identified. The cytotoxic activity and antitumor activity were examined *in vitro* and *in vivo* against various tumor cell lines. Moreover, the analogues of cytotoxic component were synthesized, and their cytotoxicity were evaluated.

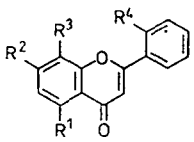
The dried root (505 g) of *S. indica* was extracted with hot MeOH and concentrated. The MeOH extract (120 g) was suspended in distilled water. The water suspension was successively fractionated with *n*-hexane, diethyl ether, ethyl acetate, BuOH, and water, respectively. The ether extract (7.6 g, ED_{50} values of 4.2 μg/ml) was chromatogaphed on a silica gel column gradiently with n-hexane:ethyl acetate as the eluent to obtain 5 cytotoxic constituents. The structures (Fig. 1) of **1-5** were identified by physical and instrumental analyses in comparsion with those of authentic specimens (Tomimori *et al.*, 1985 and 1984; Myaichi *et al.*, 1987). The constituents **1**, **2**, **3**, **4**, and **5** were identified as 2(*S*)-5,7-dihydroxy-8,2'-dimethoxyflavanone, wogonin, 5,7-dihydroxy-8,2'-dimethoxy-flavone,

2(*S*)-5,7,2'-trihydroxy-8-methoxyflavanone, and 2(*S*)-5,2',5'-trihydroxy-7,8-dimethoxy-flavanone, respectively.

Among the cytotoxic constituents, **5** showed the most potent activity against L1210 cells with an ED_{50} value of 0.9 μg/ml (Table 1). Also, it expressed a potent and wide spectrum of activity against the other cell lines tested, the ED_{50} values for HL-60, K562, and SNU-1 cells were 0.6, 1.3, and 2.7 μg/ml, respectively. Meanwhile, the activity of the other constituents was insignificant cytotoxicity. The compoud **5**, which contains the hydroxy group at C-5' in the B-ring, was much more potent than the other constituents (**1**, **2**, **3**, and **4**) devoid of the hydroxy group at the same position. In addition, **5** have only one hydroxy group in the A-ring, in constrast to less cytotoxic constituents (**1-4**) containing two hydroxy groups. Based on these results, it is proposed that the presence of one more hydroxy group in the A-ring decreased the cytotoxicity greatly, whereas the degree of substitution may not be an important factor for the enhancement of the cytotoxicity.



	R^1	R^2	R^3	R^4	R^5
1	OH	OH	OCH_3	OCH_3	H
4	OH	OH	OCH_3	OH	H
5	OH	OCH_3	OCH_3	OH	OH



	R^1	R^2	R^3	R^4
2	OH	OH	OCH_3	H
3	OH	OH	OCH_3	OCH_3

Fig. 1 Cytotoxic flavonoids isolated from the root of *Scutellaria indica*.

Table 1 Cytotoxic activity of 1–5 against various cultured tumor cell lines.

Constituents	ED_{50} Values (μg/ml)[a] (n = 5) L1210	HL-60	K562	SNU-1
1	19.2 ± 2.6	19.5 ± 3.2	17.5 ± 3.7	16.6 ± 3.3
2	10.2 ± 1.3	8.6 ± 1.6	13.1 ± 2.4	5.6 ± 1.2
3	18.1 ± 2.5	14.6 ± 1.9	> 20.0	7.8 ± 1.7
4	19.0 ± 2.8	15.9 ± 2.2	17.7 ± 3.4	12.3 ± 2.2
5	0.9 ± 0.2	0.7 ± 0.1	1.3 ± 0.2	2.7 ± 0.2
skullcapflavone II[b]	1.5	0.9		

[a] A value of ED_{50} below 4.0 μg/ml is considered to be of significant cytotoxicity (9, 11).
[b] Cited from reference 1.

In vivo antitumor activity

Each of the female ICR mice weighing 20-25 g was implanted with 10^6 of Sarcoma 180 cells into the peritonium and the test compounds were administered *i.p.* once daily for consecutive 10 days. The mortality of mice was recorded for 60 days and survival rate was calculated (Geran *et al.*, 1972). The life span effect of constituent **5** was 142 % at 5 mg/Kg concentration compared with the control.

Table 1. Effect of contituent 5 against Sarcoma 180 implanted i.p. in ICR mouse

Compound	Dose (mg/kg)	Survival day	T/C %	60 day (Survival)
Saline	-	21.8		0/9
Constituent 5	0.5	24.5	112.4	0/8
	5.0	30.9	141.7	0/8
Cisplatin[a]	2.0	>37.7	>186	2/10

[a]Positive control

2. The cytotoxic constituents from *Sophora flavescens*

The ether fraction of the root has been found to show a moderate cytotoxic activity against L1210 cells. By means of activity-directed chromatogrphic fractionation, constituent 1 was isolated, a pale yellow powder, mp 173-175℃, showed a positive result in $FeCl_3$ test. The compound was identified as sophoraflavanone G, which had been already been isolated from the root of *S. moocrofting* (Shirataki *et al.*, 1988).

The cytotoxicity of **1** was tested against four cancer cells, which had originated from human and murine. As indicated Table II, **1** showed significant cytotoxicity against A549, K562 and HeLa cells with ED_{50} values of 0.78, 2.14 and 1.57 μg/ml, respectively. The cytotoxicity against L1210 cells (ED_{50} value, 8.59 μg/ml) was lower than that against three human cell

lines. The cytotoxicity of 1 against A549 cells was two to three times higher than that against K562 and HeLa cells. Thus, 1 showed the strongest cytotoxicity against A549 (human lung carcinoma) among cancer cell lines tested. The constituent 1 was more cytotoxic than 5,2,5'-trihydroxy-7,8-dimethoxyflavanone, which was isolated from *S. indica*. Also, the cytotoxicity of 1 against A549 cells was about twice greater in comparison with that of 5-fluorouracil which are currently widely used in cancer therapy. 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.

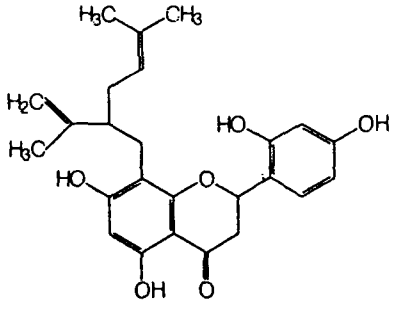


Fig. 1. Structure of sophoraflavanone G (constituent I).

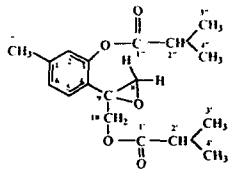
Table 1. Cytotoxicities of sophoraflavanone G against four cancer cells

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

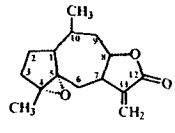
[a]5-FU: 5-fluorouracil, Positive control
[b]The values were the mean of at least three experiments
[c]Not tested

3. The cytotoxic and antitumor constituents from *Carpesium abrotanoides*

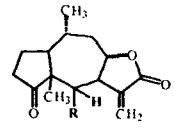
The *Carpesium* genus including *Carpesium abrotanoides* exhibited a significant cytotoxicity against L1210 and HL60 cancer cells with ED_{50} values (<5 μg/ml), respectively (Bae *et al.*, 1996). Therefore, we isolated cytotoxic constituents from *C. abrotanoides*. The aerial part of *C. abrotanoides* was extracted with MeOH. The MeOH extract was suspended in distilled water. The water suspension was sussessively extracted with pet. ether and chloroform. Of two extracts, the chloroform extract showed exhibited a significant cytotoxicity against L1210 cells. Cytotoxic constituents, **1-9**, were isolated from chloroform extract using activity-guided and repeated column chromatography. The structure of cytotoxic constituents 1-9 was identified by physical and spectral analyses in comparsion with those of authentic specimens (Dong *et al.*, 1988, Maruyama *et al.*, 1977 and Bohlmann *et al.* 1978). The constituents were identified as 10-isobutyryloxy-8,9-thymol-isobutyrate (**1**), 4α,5α-epoxy-10α,14H-inuviscolide (**2**), 2,3-dihydroaromaticin (**3**), carabrone (**4**), telekin (**5**), carpesiolin (**6**), carabrol (**7**), 11(13)-dehydroivaxillin (**8**), and ivalin (**9**), respectively.



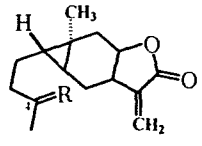
Comp I : 10-isobutyryloxy-8,9-epoxy thymol-isobutyrate



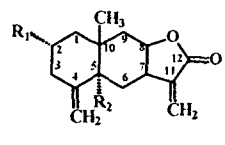
II : 4,5-epoxy-10,14H inuviscolide



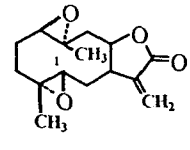
III : R = H : 2,3-dihydro-aromaticin
VI ; R = OH : carpesiolin



IV ; R = O ; carabrone
VII : R = H, OH ; carabro



V ; R_1 = H, R_2 = OH ; telekin
IX ; R_1 = OH, R_2 = H ; ivalin



VIII ; 11(13)-dehydro-ivaxillin

Fig. Cytotoxic constituents isolated from *C. abrotanoides*

In the cytotoxicity test, isolated constituents showed a significant activity against L1210 (mouse leukemia) and cultured human tumor cell lines such as A549 (lung carcinoma), SK-OV-3 (adenocarcinoma, ovary malignant ascites), SK-MEL-2 (malignant melamnoma), XF498 (central nerve system tumor), and HCT15 (colon adenocarcinoma). They, except constituent I, exihibited a potent cytotoxicity with ED_{50} values of below 4.0 μg/ml against all the cell lines used. In enzyme inhibitory activity assay using the enzymes such as Topoisomerase I (Ross, 1985) or FPTase (Gibbs *et al.*, 1991), and angiogenesis (Auerbach *et al.*, 1994), isolated constituents did not an show inhibition. But, **5** exhibited a moderate inhibitory activity on FPTase with IC_{50} values (300 μM) and a potent inhibitory activity in angiogenesis with IC_{50} values (0.5 μg/egg).

4. The cytotoxic and antitumor constituent from *Pyrola japonica*

The hexane and ethyl acetate fractions of the whole plants have been found to show a moderate cytotoxic activity against L1210 and K562 cells. By means of activity-directed chromatogrphic fractionation, constituent **1** was isolated, a yellowish needle crystal, mp 113.5-114.5℃. The compound was identified as chimaphylline (Yazaki *et al.*, 1989), which had already been isolated from the whole plant of *P. incarnate.*

The cytotoxicity of **1** was tested against two cancer cells, which had originated from murine and human. As indicated in Table I, **1** showed significant cytotoxicity against L1210, and K562 cells with ED_{50} values of 1.17 and 0.88 μg/ml, respectively. The antitumor activity of **1** was tested in hybrid female mouse (BDF1-KIST) implanted with P388 cells *in vivo*. The life span was prolonged with 110%.

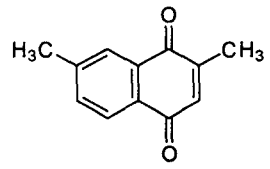


Fig. 1 Structure of chimaphilline

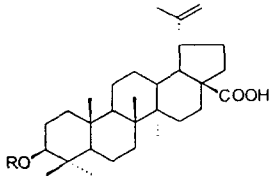
Table 1 ED_{50} values of the cytotoxic constituents

compound	ED_{50} (μg/ml)	
	L1210	HL60
chimaphilline	1.2±0.2	0.9±0.1

5. The cytotoxic constituents from Forsythiae Fructus

The hexane fraction has been found to show a cytotoxic activity against L1210 and HL60 cells. By means of activity-directed chromatogrphic fractionation, constituent **1** (mp 297-298℃, a colorless neddle crystal) and **2** (mp 262-264℃, a colorless neddle crystal) were isolated. The compounds were identified as acetylbetulinic acid and betulinic acid, respectively, in comparison with the data reported by Budzikiewicz *et al.*, 1963, and Kang *et al.*, 1986.

The cytotoxicity of **1** and **2** was tested against two cancer cells (L1210 and HL 60 cells), which had originated from murine and human. As indicated Table I, **1** and **2** showed a moderate cytotoxicity against L1210, but showed strong activity in HL60 cells.



R= H, betulinic acid
R= Ac, acetylbetulinic acid

Fig. 1 Structures of acetylbetulinc acid and betulinc acid

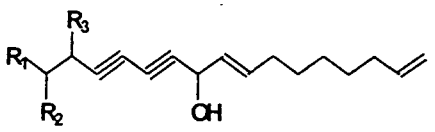
Table 1 ED_{50} values of the cytotoxic constituents

	ED_{50} (μg/ml)	
	L1210	HL60
betulinic acid	16.4±1.0	2.4±0.8
acetylbetulinic acid	9.1±1.9	2.7±0.7

6. The cytotoxic and antitumor polyacetylenes from *Gymnaster koraiensis*

This plant is an endemic in Korea and its methanol extract showed cytotoxic activity against L1210. The methanol extract was suspended with water and fractionated with dichloromethane, and n-buthanol, successively. By means of activity-directed chromatogrphy of the dichloromethane fraction, four cytotoxic constituents were isolated. They were identified as 2,3-epoxy-9,16-heptadecadiene-4,6-diyne-8-ol (**1**), 8-hydroxy-falcarinol acetate (**2**), 1,9,16-heptadecatriene-4,6-diyne-3,8-diol (**3**), and 9,16-heptadeca-diene-4,6-diyne-2.3.8-triol (**4**) in comparison with the data reported (Hu *et al.*).

The compounds **1-4** showed a strong cytotoxic activity (ED_{50} 0.04~9.7 $\mu g/m\ell$) against tumor cell lines, with **3** being the most potent (ED_{50}, 0.04~0.73 $\mu g/m\ell$). They showed a more potent specific against melanoma (SK-MEL-2), colon (HCT15) and ovarian (SK-OV-3) cell lines than L1210, A549, XF498 and HCT15. A comparison of the cytotoxicity of **1-4** indicated that the unsaturation between C-1 and C-2 contributed to enhance the cytotoxicity as evidenced from the cytotoxicity of **2** and **3**. Noteworthy, the acetylation of hydroxyl group at C-3 remarkably the cytotoxicity of **3**.



	R_1	R_2	R_3
1	**CH3**	**-O-**	
2	**CH2**	**H**	**OAc**
3	**CH2**	**H**	**OH**
4	**CH3**	**OH**	**OH**

Figure 1, Cytotoxic compounds isolated from *G. koraiensis.*

Table 1, Cytotoxicity of polyacetylene compounds against various tumor cells.

compound	ED_{50} values ($\mu g/m\ell$)					
	L1210	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	3.28	3.92	1.64	0.40	3.11	1.47
2	2.08	3.65	0.83	0.31	1.90	1.31
3	0.12	0.73	0.38	0.04	0.46	0.37
4	9.64	13.4	6.58	3.66	6.91	5.04

L1210(mouse leukemia), A549(human lung), SK-OV-3(human ovarian), SK-MEL-2(human melanoma), XF498(human CNS), HCT15(human colon)

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