

Antineoplastic Natural Products and the Analogues(IX). A Review of the Series

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Abstract—Bioassay-directed isolation has yielded some cytotoxic substances against L1210 cell from the Korean traditional medicine. These include 5,2'-dihydroxy-6,7,8,6'-teramethoxyflavone (IV, scutellaria root, $ED_{50}=1.7 \mu\text{g/ml}$), 7-geranyloxy coumarin (XXXII, poncirus fruit, $10.2 \mu\text{g/ml}$) and panaxydol (I, white ginseng, $0.03 \mu\text{g/ml}$). IV, XXXII and their derivatives were synthesized in the purpose of in vivo tests and for observation of structure-activity relations. Among the flavone derivatives, 5,2',6'-trihydroxy-6,7,8-trimethoxy flavone (XVIII), 5-hydroxy-6,7,8-trimethoxy-6'-benzyloxyflavone (XVII) and 5,8-dihydroxy-6,7-dimethoxyflavone (X) showed the cytotoxicity which has no correlation to the flavone structures. Of the coumarins synthesized, 7,8-dihydroxycoumarin (XXVI), 6-7-dihydroxycoumarin (XXIX) and 6-hydroxy-5,7-dimethoxycoumarin (XXXI) showed considerable activities. Acetylated XXXI has moderate activity ($ED_{50}=17.2 \mu\text{g/ml}$). Monohydroxycoumarins or their methyl and allyl ether were inactive. IV inhibits the growth of the solid form of S-180 by 70% at 40 mg/kg and shows T/C of 166% on the ascitic S-180 at 40 mg/kg. It strongly inhibits the activity of the membrane bounded ATPase from L1210 cell. The most cytotoxic fraction of the antitumor materials studied is the one from the trichosanthes root showing $ED_{50}=0.0003 \mu\text{g/ml}$ against L1210 cell. This fraction, obtained from ethyl acetate extract, showed T/C of 130 and 135% on ICR mice bearing S-180 and BDF₁ mice bearing L1210 at 10 mg/kg and 7.5 mg/kg, respectively.

Keywords—Antitumor · panaxydol · skullkapflavone II · auraptin · structure-activity relation

A search for antitumor agents from the Korean traditional medicine is a necessary undertaking in the respect of its systematically and abundantly accumulated experiences in handling the natural materials and in treating the diseases with them.

An early screening regarding the crude drugs for antileukemic activity reported that six of 38 materials screened were cytotoxic against L1210

cell¹⁾. In addition to it, we have screened further 32 drugs, which were found in the antitumor formulas or believed to have been applied for treatment of tumors and tumor like diseases in the folklore.

The solvent fractions representing 22 materials of these exhibited ED_{50} values less than $20 \mu\text{g/ml}$ against L1210 cell. The materials with an interesting cytotoxic activity are as follows;

Korean ginseng (white, red, leaves, stems), *Scutellaria baicalensis* (root), *Coptis japonica* (root), *Trichosanthes kirilowii* (root, fruit, seed), *Lithospermum erythrorhizon* (root), *Curcuma domestica* (root), *Poncirus trifoliata* (unripe fruit, ripe fruit), *Picrasma ailanthoides* (seed), *Cinnamomum cassia* (bark), *Phellodendron amurense* (bark) and *Agrimonia pilosa* (herb).

Bioassay-directed isolation has yielded some cytotoxic substances with potent or moderate activity from Ginseng, *S. baicalensis* and *P. trifoliata*.

Though no definitive substance was obtained so far from the root of *T. kirilowii*, its fractions showed the strongest cytotoxic activity among the materials screened, thus led to the *in vivo* studies.

The active substances of *S. baicalensis* and *P. trifoliata* were synthesized for observation of structure-activity relationship in the cytotoxicity and for animal tests, if the amount available.

1. The Ginseng Root

It was previously reported that the petroleum ether fraction of the Korean ginseng root shows cytotoxic activity against L1210 cell, Hela cell, L5178 Y cell and S-180 cell *in vitro*.²⁾

In present study³⁾ the cytotoxic substance against L1210 cell was isolated over a silica gel column and a subsequent preparative HPLC, followed by the cytotoxic assay. Bioassay was carried out due to instruction of Thayer.⁴⁾ This substance was found to show a very strong cytotoxic activity against L1210 cell ($ED_{50} = 0.03 \mu\text{g/ml}$).

IR absorptions at 3,450, 2,250, 1,630 and 1,110 cm^{-1} suggested that it is an alcoholic acetylenic compound with double bond. The multiplets at 6.15~5.15 ppm and 3.25~2.75 ppm in its NMR (Fig. 1) correspond to the typical peak shapes of an allylic system, $\text{CH}_2=\text{CH}-\underset{\text{O}}{\underset{|}{\text{C}}}$, and a butylene oxide moiety, $-\text{CH}_2-\underset{\text{O}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-$, respectively. The PMR of its trimethyl-

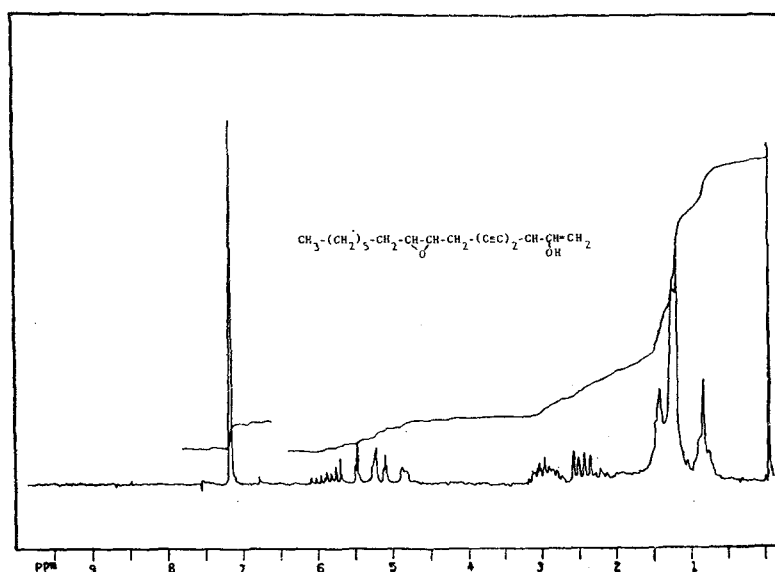
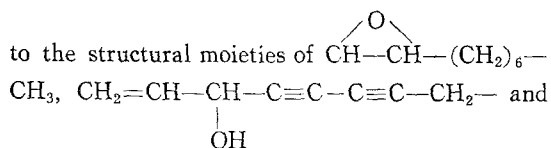


Fig. 1. NMR of panaxydol

silylated product shows only one trimethylsilyl group at 0.20 ppm, so that the active substance is a monohydroxy compound. These interpretation was supported by its mass spectra. The isobutane chemiionization spectrum shows an $M^+ + 1$ peak at $261m/z$ and $M^+ + 1 - H_2O$ peak at $243m/z$ as major peaks. Thus the molecular weight of the active substance is 260. In the EI mass spectrum, there appeared the main peaks at 161, 141, 119 and $99m/z$ (Fig. 2).

The peaks of 141, 119 and $99m/z$ correspond



n-heptyl, respectively.

In order to chemically verify the presence of the epoxy group, the active substance was hydrolysed with dil- $H_2SO_4/EtOH$ at room temperature for 24 hrs. The hydrolysed product was

oxidized by periodic acid to result in isolation of n-octanal (Fig. 3).

A literature search revealed that six acetylenic compounds were isolated from the Korean ginseng root.⁵⁻¹⁰ By comparing the physical and chemical properties, the active substance turned out to be identical with panaxydol (I)⁷.

The hydrolysed products mentioned above (Fig. 3) consisted of two main substances which were separated on a silica gel column. According to NMR and oxidative behavior, the substance with lower Rf value proved to be heptadeca-1-en-4, 6-diyne-3, 9, 10-triol (III), where the vicinal hydroxyls at C-9 and C-10 have come from cleavage of the epoxy group, and another substance with higher Rf-value was an ethoxydiol (II) with ethoxy group at C-9 or C-10 (Fig. 4 and 5).

The sharpness of the ethyl peaks in the PMR indicates that one kind of two epoxy carbons

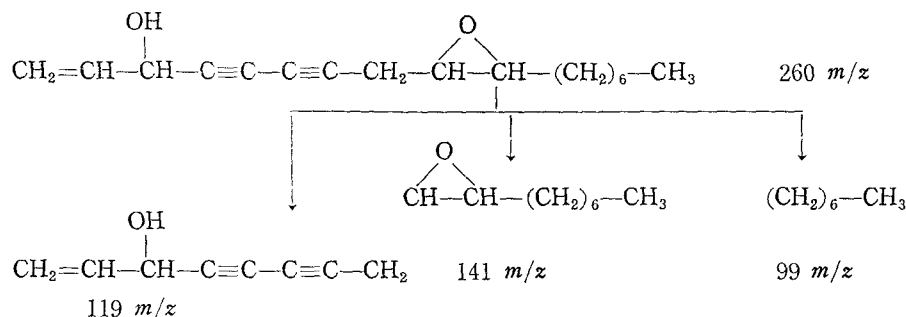


Fig. 2. Mass spectrum of the active substance

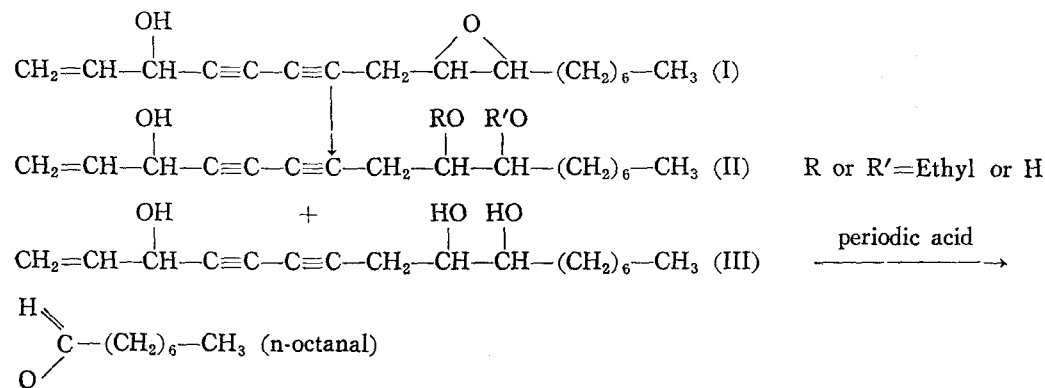


Fig. 3. Hydrolysis of the active substance.

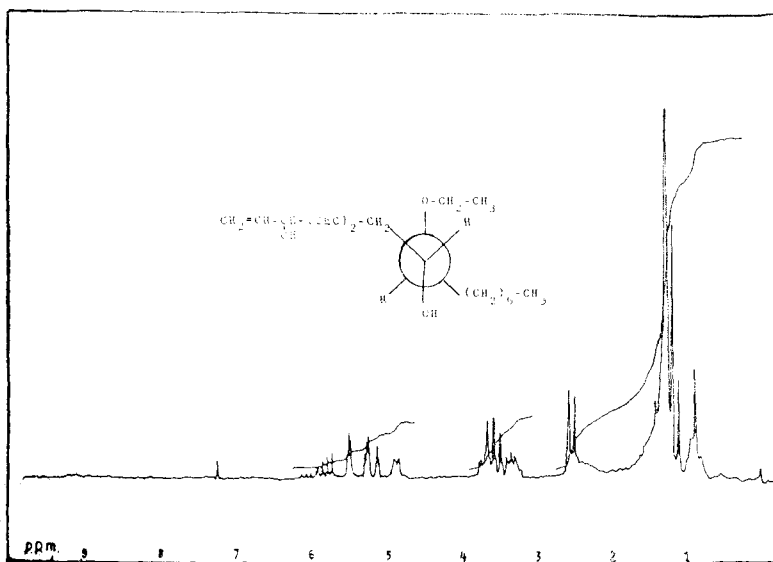


Fig. 4. NMR spectrum of the hydrolysed product (II)

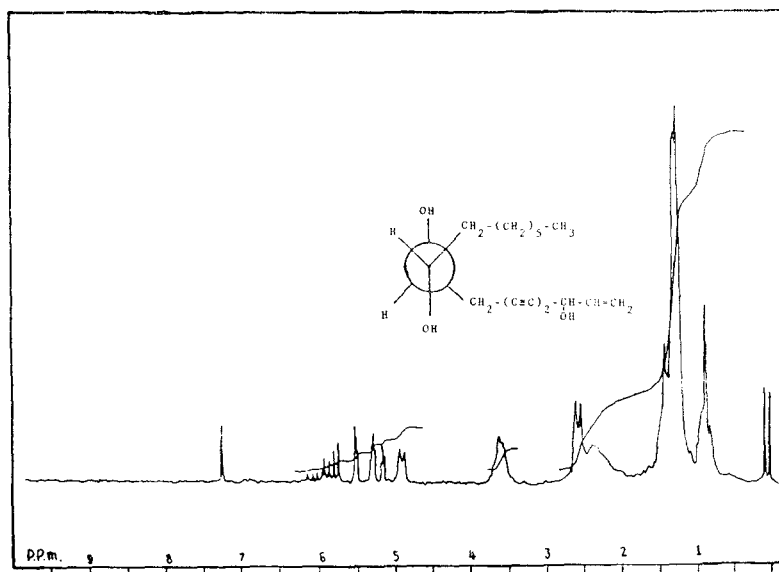


Fig. 5. NMR of the hydrolysed product (III)

was ethoxylated.

At the moment it is not clear which one is ethoxylated.

Both substances show the ED_{50} values less than $1.0 \mu\text{g/ml}$.

According to the chromatographic behaviors and the NMR interpretations, the red ginseng,

which showed strong cytotoxic activity against L1210, contains the same triol (III). Recently this triol was isolated from the red ginseng without a bioactivity study.¹⁰ From the activity considered above, it is natural to suppose that this triol is partly responsible for the cytotoxicity of the red ginseng.

Furthermore, the cytotoxic activity of the ethoxydiol (II) has provided a clue for a detailed study on a relation between structure (R or R'; variables) and cytotoxicity in this kind of the substances.

2. The Root of *Scutellaria baicalensis*, "Whanggum"

The ether extract of "Whanggum", the Korean name of the scutellaria root, shows ED₅₀ value of 10.4 µg/ml against L1210 cell. The active principle, isolated from the ether extract over a silica gel column, turned out to be 5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone known as skullcapflavone II (IV) and has ED₅₀=1.5 µg/ml¹³⁾.

Attention was given to the difference in the ED₅₀ values of IV and the ether extract. HPLC analysis revealed that the ether extract contains 2% of IV. Accordingly, 10.4 µg of the ether extract contains 0.21 µg of IV. Considered to be ED₅₀=1.5 µg/ml of IV, the ether extract exhibits seven times stronger activity than IV.

Thus, it was assumed that certain kind of synergistic principle could exist in the extract.

The other flavones of the root such as baicalin, baicalein, wogonin, oroxyline A and 5,8,2'-trihydroxy-6,7-dimethoxyflavone showed no significant activity.

Intraperitoneal injection of IV (40 mg/kg) resulted in a significant (T/C=166%) and a moderate (T/C=122%) prolongation of the life spans of ICR mice bearing ascitic S-180 and BDF₁ mice bearing L1210, respectively (Table

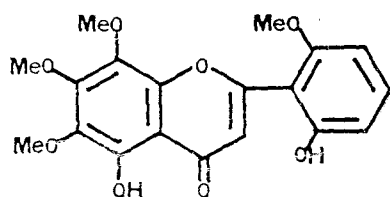


Fig. 6. the active flavone (IV)

I and II).¹²⁾

Peritumoral injection of IV around the transplantation point of solid S-180 in the left groin of ICR mice inhibited the tumor growth by 71% (Table III).

As the results shown, IV exhibited the signi-

Table I. Effect of the intraperitoneal administration of skullcapflavone II on the life span of BDF₁ mice bearing L1210 cells.

Dose (mg/kg)	Mean survival days	30day survivors	T/C (%)
0	12.0(10~15) ^a	0/16	100
20	14.1(11~20)	0/16	116
40	14.6(10~24)	0/16	122
90	10.4(9~13)	0/16	87

^a Range

Table II. Effect of the intraperitoneal administration of skullcapflavone II on the ascitic form of sarcoma 180.

Dose (mg/kg)	Mean survival days	60 day survivors	T/C (%)
0	21.3(14~38) ^a	0/36(0) ^b	100
1	23.2(18~51)	0/13(0)	109
10	24.6(15~49)	2/26(8)	115
20	28.3(20~27)	3/24(13)	133
40	35.4(17~34)	7/24(29)	166
60	30.9(20~40)	3/24(13)	145
90	29.6(22~35)	3/24(13)	139

^a Range for the dead mice

^b %

Table III. Effect of the peritumoral administration of skullcapflavone II on the solid forms of sarcoma 180.

Dose (mg/kg)	Tumor weight (g, mean±S.D.)	Complete regression	Inhibition rate(%)
0	6.96±2.39	0/17(0) ^a	0
1	5.68±1.74	0/16(0)	18
10	3.16±1.94	3/17(18)	55
20	2.95±2.10	4/16(25)	58
40	1.99±2.15	6/16(38)	71
60	2.06±2.04	4/16(25)	70

^a %

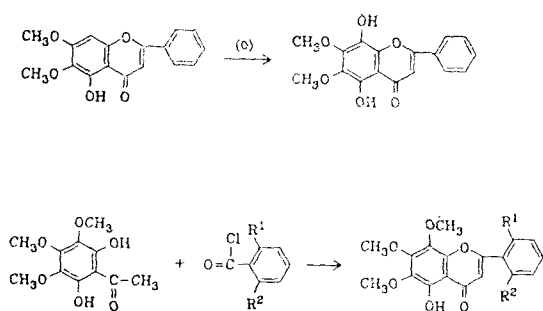
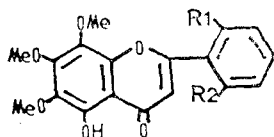


Fig. 8. Synthesis of flavones with the fully oxygenated A-ring.

Table V. ED_{50} values of the synthesized flavones on L1210 cell



Flavone	Substitution		ED_{50} ($\mu\text{g/ml}$)	$\text{Log}\epsilon_1/\text{Log}\epsilon_1^a$
	R1	R2		
XIV	H	H	20.1	1.067
XV	H	OH	20.2	1.072
XVI	H	OMe	>40.0	1.065
XVII	H	OBz ^b	15.4	1.073
XVIII	OH	OH	4.5	1.091
IV	OH	OMe	2.3	1.109
XIX	OH	OBz	21.8	1.115
XX	OMe	OMe	>40.0	1.212
XXI	OMe	OBz	26.9	1.214
XXII	OBz	OBz	>40.0	1.322

^a ϵ_1 ; molar extinction coefficient for "Band I"

ϵ_2 ; molar extinction coefficient for "Band II"

^bBz = $-\text{CH}_2\text{C}_6\text{H}_5$

The structural difference of IV from other inactive flavones such as baicalein, wogonin, oroxylin A and 5,8,2'-trihydroxy-6,7-dimethoxy flavone (Tab. IV) lies in the A-ring of the flavone structure.

This observation led to the synthesis of several flavones with the fully oxygenated A-rings (Fig. 8). Among the flavones synthesized, 5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone (IV iden-

tical with the natural skullcapflavone II), 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone (XVIII) and 5,8-dihydroxy-6,7-dimethoxyflavone (X) have the cytotoxic effect (Table V).

As indicated in the literature,¹⁵⁾ we could not find a correlation between flavone structure and cytotoxicity against L1210 cell.

3. The Unripe Dried Fruit of *Poncirus trifoliata*, "Jisil"

It was made a methanol extract of "Jisil", which was further extracted with petroleum ether, ether and ethyl acetate in sequence. Bioassaying the solvent fractions demonstrated that the activity was concentrated in the petroleum ether fraction ($ED_{50}=12.5 \mu\text{g/ml}$ against L1210 cell).

As shown in Fig. 9, administration of the petroleum ether fraction prolonged the life span of ICR mice bearing S-180 by 126%, a significant T/C value.

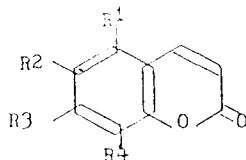
Repeated chromatography of the petroleum ether fraction on a silica gel column yielded three active substances. One of these was recrystallized in n-hexane. m.p. 71° and $ED_{50}=10.2 \mu\text{g/ml}$.

Other two substances were of too small amount for a further manipulation.

From comparison of its m.p., NMR and IR with those of the coumarins isolated from *P. trifoliata*, the crystalline substance was proven to be identical with auraptin, 7-geranyloxycoumarin.¹⁶⁾

In order to find the cytotoxic structural moiety, the active substance was hydrolysed, and found to give umbelliferone and geraniol. Only geraniol possesses the cytotoxic property ($ED_{50}=6.7 \mu\text{g/ml}$).

The coumarins with the oxygenated A-ring and some of their ethers were synthesized. The products and their activities were shown in

Table VI. Coumarins synthesized and their ED₅₀ values against L1210

Compound	Substitution				ED ₅₀ (ug./ml)
	R1	R2	R3	R4	
XXIII Umbelliferone	H	H	OH	H	>20.0
XXIV Herniarin	H	H	OCH ₃	H	>20.0
XXV 7-allyloxy coumarin	H	H	Oallyl	H	>20.0
XXVI Daphnetin	H	H	OH	OH	8.8
XXVIII Dimethyl daphnetin	H	H	OCH ₃	OCH ₃	>20.0
XXVIII 6-Hydroxy 7,8-dimethoxycoumarin	H	OH	OCH ₃	OCH ₃	>20.0
XXIX Esculetin	H	OH	OH	H	4.3
XXX Acetylfraxinol	OCH ₃	OAc	OCH ₃	H	17.2
XXXI Fraxinol	OCH ₃	OH	OCH ₃	H	5.5
XXXII Auraptin	H	H	Ogeranyl	H	10.2
XXXIII Geraniol					6.5

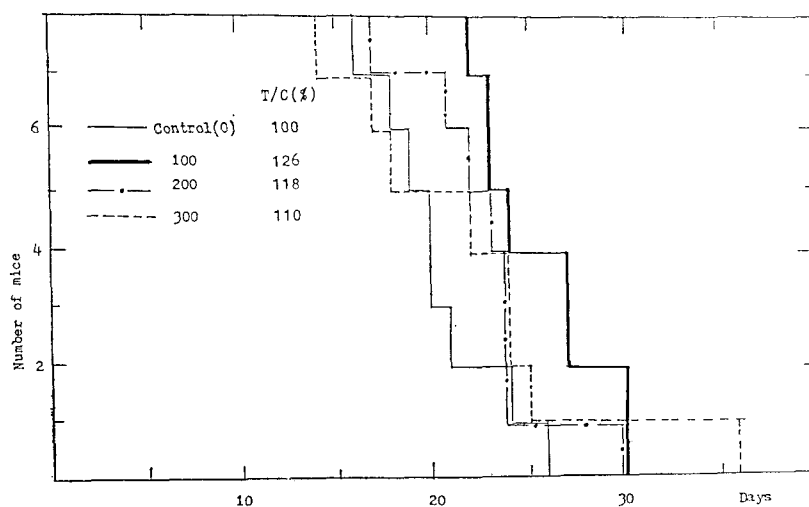
**Fig. 9.** Effect of the i.p. administration of petroleum ether fraction of *P. trifoliata* on the life span of ICR mice bearing sarcoma 180 cells. Numerals indicate the dose of petroleum ether fraction of *P. trifoliata* (mg/kg).

Table VI. Umbelliferone and its methyl and allyl ethers were inactive. 6,7-dihydroxy and 7,8-dihydroxycoumarins showed moderate activity, while permethylation deactivated them. Among the coumarins with three oxygen functions in benzene ring, 5,6,7-trioxygenated ones

were active, but not the 6,7,8-trioxygenated one.

With these findings, it is to argue that coumarins with more oxygen functions in the benzene ring have more chance for the cytotoxic action.

Other coumarins such as daphnoretin,¹⁷⁻¹⁹⁾ cleomiscosin²⁰⁾ and ellagic acid derivatives²¹⁾ showed cytotoxic activities. Daphnoretin and 6,7-dihydroxycoumarin synthesized above are structurally related to each other in the respect of the 6,7-oxygenated benzene ring.

4. The Root of *Trichosanthes kirilowii*

Methanol extract of the trichosanthes root has $ED_{50}=10.0 \mu\text{g/ml}$. As shown in Table VII, the ethyl acetate solubles from the methanol extract

Table VII. Solvent fractions of *Trichosanthes kirilowii* and their cytotoxic activity

Fractions	$ED_{50}(\mu\text{g/ml})$
Methanol	10.0
Petr. ether	20.0
Ethyl acetate	2.0
Water	20.0

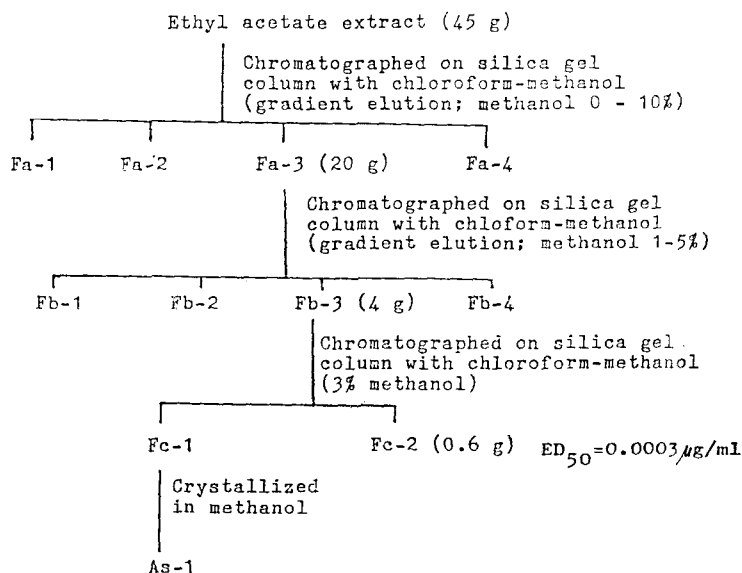
exhibited strong activity, while other solvent fractions had no significant activities.

The ethyl acetate fraction was chromatographed over a silica gel column, yielding a fraction C-2 with the strongest cytotoxic activity ($ED_{50}=0.0003 \mu\text{g/ml}$) among other fractions (Scheme 1).

The fraction C-2 has T/C=130% at 10 mg/kg on ICR mice bearing S-180 and T/C=135% at 7.5 mg/kg on BDF₁ mice bearing L1210 respectively.

The solubility in ethyl acetate and the chromatographic behavior under the described conditions exclude the possibility that the active fraction might contain a peptide such as trichosanthin, which is claimed to possess antitumor action,^{22,23)}

For study of mode of action, it is noteworthy that the L1210 cells, which were survived after treatment of the fraction C-2, were enlarged three times in average in their diameter.



Scheme 1. Fraction of ethyl acetate extract.

5. Concluding Remarks

This presentation has summarized the results

of the studies which enclosed isolation, structure identification, structural modification and synthesis of the cytotoxic substances, originating from the Korean traditional medicine.

Isolation of antitumor agents from each component drug of an antitumor formula and determination of their properties would contribute to form a more rationalized regimen than the original formula. Besides finding the lead compounds for development of new antitumor agents, such a research should engage also in the studies on synergism of drug components of the formulas, indirect antitumor effects of drugs such as hematopoietic and immunostimulating ones and the others enhancing the general health conditions.

Attention would be given to the fact that skullcapflavone II lowered the activity of the membrane bounded ATPase of L1210 cell.

It demonstrates a rare cytotoxic mechanism compared to the intracellular mechanism of the antitumor agents studied so far.

At a dose of 120 mg/kg, skullcapflavone II demonstrated no toxic effects on ICR mice.

A combined administration of skullcapflavone II with other cytotoxic substances is necessary to observe if it ensued synergistic or other additional effects.

In vivo studies using panaxydol and coumarins are planned. Isolation of the active principles from *Trichosanthes kirilowii* and synthesis of panaxydol and its derivatives are on the way.

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