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Research on Antineoplastic Compounds Obtained from Natural sources Especially from Higher Plants

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Abstract

Vincristine and vinblastine isolated from *Vinca* spp., and podophyllotoxine derivatives isolated from *Podophyllum* spp. are useful as anticancerous components obtaned from higher plants. More than ten antineoplastic compounds are now following them as anticancerous agents from higher plants. In my laboratory, Sarcoma 180A has been used as the first screening test. By this method, I have found out some kinds of antineoplastic constituents from active plants extracts. For instance, bisaborane type compounds were isolated from *Curcuma xanthorrhiza*, one of Indonesian plants; a morphinane type compound from *Cocculus trilobus*; cyclic hexapeptides from Rubia akane and *R. cordifolia*. Seven components having antineoplastic activity were isolated from *Rubia* spp. except. R. tinctoria. Their structures were elucidated except RA-VI by chemical reaction and various instrumental analysis as shown in Fig. Among of them, RA-VII showed strong activity against P388 lymphocytic leukemia, L20, B16 melanoma, Lewis lung carcinoma, colon 38 and Ehrlich carcinoma. RA-V revealed excellent activity against MM2 mammary carcinoma. The value of acute LD50 of RA-VII were 10.0mg/kg(ip) and 16.5mg/kg(po) respectively. Theraprutic ratio was 400, compared with 10 of mitomycin C. QSAR was also applied to these compounds by elongation of ether and ester side chains at R'. Mechanism of action of RA-VII was also investigated and was assumed to be inhibition of protein biosynthesis.

Introduction

Chemotherapeutic approaches against cancer have been increased yer to year. Generally, tumor is divided into nonmalignant tumor(10%) and malignant type of tumor(90%). There are so many types of cancer. It is said that there is no efficacious agent which is useful against all types of cancer.

Recently, anticancer agents used crincially are divided into about eight classes according to the actions and the differences of origin as follows:

1. alkylating agents
   a) nitrogen mustards
   b) erhyleneimine derivatives
   c) alkyl sulfonates
   d) nitrosoureas

2.	antimetabolites	a) Falic acid analogs
		b) purine analogs
		c) pyrimidine analogs
		d) glutamine analogs
3.	antibiotics	blemoycin, mitomycin C, adriamycin
4.	hormones	adrenocorticosteroids, androgens, estrogens
5.	enzymes	L-asparaginase
6.	compounds (from higher plants)	a) vinca alkaloids b) podophyllum lignans
7.	polysaccharides (from fungi, microorganisms)	Lentinan, PSK
8.	miscellaneous	

Among of them, compounds belonging to classes Nos. 3,5,6 and 7 are originating so called natural sources. Especially, compounds of No. 6 are isolated from higher plants. In this reivew, the author wishes to mention mainly about constitutents from higher plants.

In 1982, it was given a definition for expression of activity, that is, the word cytotoxicity must be used only for in vitro activity, the words antineoplastic and antitumor must be used only for in vivo test using animal. We should call anticancer, when it shows activity in crinical trials of human.[1)]

Status quo of anticancer agents obtained from higher plants[2.3.4)]

Many kinds of components have been obtained from plants kingdom as antineoplastic and anticancerous agents till now. However, there is no special type of compunds structurally. Various types of substances are effective for various types of cancers and tumors: for instance, alkaloids, lignans, terpenes and steroids etc.

First of all, most important components isolated from higher plants are Vinca alkaloids and Podophyllum lignans. *Vinca rosea (=Catharanthus roseus)* has been used as inhibiting agent of milk secretion, hypotensor, astringent and emetics as folks medicines traditionally. Moreover, native people in West Indian Islands have been using *Vinca* spp. as depression agent of blood sugar. When the extract of this plant was given non-orally, leukopenie and indirect inhibiting action of nuclear division were observed. Above 60 kinds of alkaloids have been isolated from *Vinca* spp. Vinblastine(I) and vincristine(II) are most active substances among of them. The former is effective to Hodgkin disease and the latter to leulemia.

Podophyllotoxin(III) is a representative lignan isolated from the rhizome of *Podophyllum peltatum*. *Podophyllum rhizome* had been used as an emtic and an anthelmintic by American Indians traditionally. Because podophylltoxin was also found to have inhibiting action for cell-division, antineoplastic activity was noticed. At present, VP-16 and VM-26, a kind of podophyllotoxin derivatives are now in progress to be developped as an anticancer agents.

The others, curcumol obtained from *Curcuma aromatica* was tested and noticed to be effective against cancer of the uterine cervix crinically. Oridonin(V) isolated from *Rabdosia* spp. is now tested

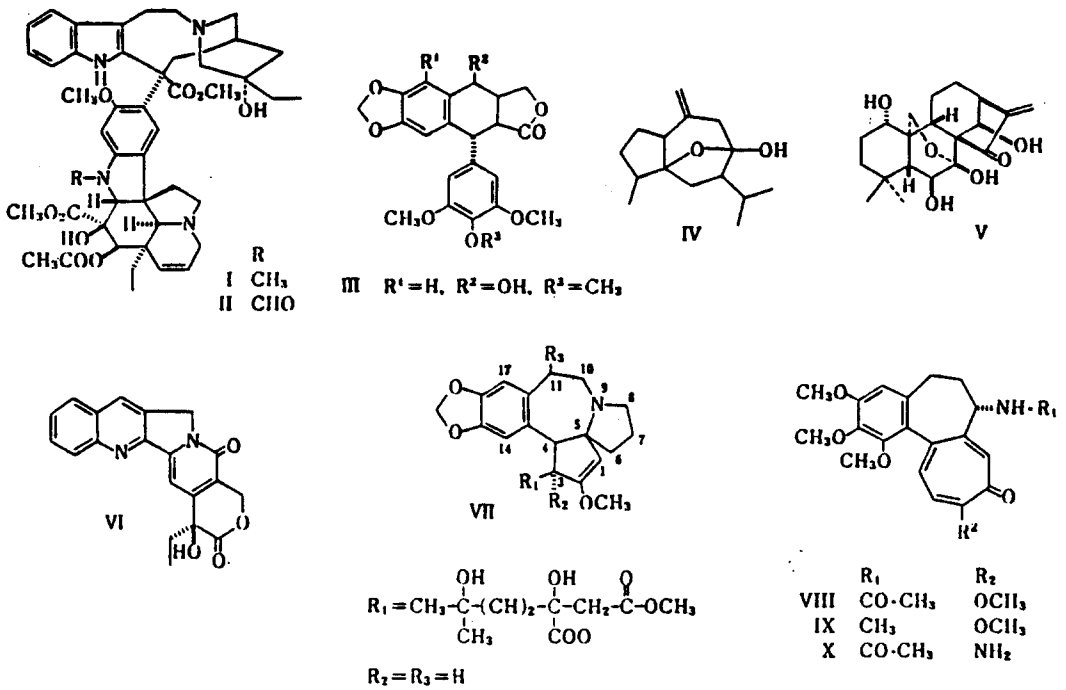


Fig. 1 The structures of anticancerous compounds obtained from higher plants

crinically in China. Moreover, camptothecine(VI) isolated from *Camptotheca acuminata* is also antineoplastic alkaloid, but is very toxic. Chemical modification has been tried to decrease its toxicity. Harringtonin were investigated as an anticancerous drugs in China. Colchicine(VIII) derivatives are also said to have inhibiting action of cell-division. Demecolcine(IX) and colchicine amide (X) have activity against mammary cancer. Further, there are many compounds which have been reported as antineoplastic agents.
In near future, it will be developped more effective anticancer drugs from these natural products, since screening metods have been improved by the efforts of many people.

Screening method

The efforts to develop anticancer substances from natural sources have been continued for a long time in Cancer Chemotherapy National Service Center(CCNSC) of America. At present, it was renamed as National Cancer Institute(NCI), and Drug Research & Development(Dr & D), branch of NCI, are doing the screening test about 10,000 samples collected from the world every year.

It is always a problem that a compound having antineoplstic activity against some kinds of tumors, but doesn't show activity against the other tumors. For instance, Vinca alkaloids were not

activity by above 130(T/C%) in this method.

Table 1 Antineoplastic Activity of Crude Drugs with Sarcoma 180 Ascites Mice

Crude Drug	Original Plant	Part Used	Solvent	Deaths due to Toxicity	BWC (g)	PCW/TV	GR(%)
1 梅寄生	*Fomes* spp.	fruit body	W	0	-2.1	0.15	22.1 ++
2 香附子	*Cyperus rotundus*	rhizome	W	0	-4.6	0.15	27.7 ++
3 檳榔子	*Areca catechu*	seed	E	0	-1.8	0.07	9.1 ++
			W	0	-0.9	0.04	6.1 +++
4 羊夏	*Pinellia ternata*	tuber	W	0	-0.9	0.13	31.0 ++
5 縮砂	*Amomum xanthioides*	seed	W	0	-3.3	0.23	14.0 ++
6 白豆蔻	*Amomum cardamon*	fruit	W	0	-3.5	0.06	11.2 ++
7 宇金	*Curcuma longa*	rhizome	W	0	-2.2	0.14	38.3 ++
8 莪述	*Curcuma zedoaria*	rhizome	W	0	-2.5	0.15	20.0 ++
9 生姜	*Zingiber officinale*	rhizome	W	0	-2.0	0.09	17.8 ++
10 山奈	*Kaempferia galanga*	rhizome	W	0	-4.3	0.08	9.2 ++
11 益智	*Alpinia oxyphylla*	fruit	W	0	-1.1	0.15	25.4 ++
12 胡椒	*Piper nigrum*	fruit	W	0	-3.0	0.12	19.4 ++
13 虎杖根	*Reynoutria japonica* var. *typica*	root	W	0	-0.5	0.13	32.0 ++
14 地膚子	*Kochia scoparia*	seed	W	0	-0.5	0.13	30.0 ++
15 藤榴	*Wisteria floribunda*	gall	W	0	-0.9	0.22	15.8 ++
16 蒺莉子	*Tribulus terrestris*	fruit	W	0	-2.0	0.18	36.3 ++
17 續隨子	*Euphorbia Lathyris*	seed	E	0	-1.9	0.14	32.8 ++
			W	0	-1.7	0.05	8.0 ++
18 枳椇子	*Hovenia dulcis*	fruit	W	0	-2.8	0.14	28.9 ++
19 オトギリソウ	*Hypericum erectum*	herb	W	0	-4.2	0.10	32.3 ++
20 タラ根皮	*Aralia elata*	bark	W	0	-2.6	0.28	39.1 ++
21 益母草	*Nepeta japonica*	herb	W	0	-1.1	0.11	21.4 ++
22 延命草	*Isodon japonicus*	herb	W	0	-0.3	0.46	9.3 +++
23 菱の實	*Trapa quadrispinosa*	fruit	W	0	-0.1	0.24	40.0 ++
24 蛇床子	*Torilis japonica*	fruit	W	0	-0.3	0.09	12.3 ++
25 蘭草	*Eupatorium fortunei*	herp	E	0	+1.3	0.25	18.0 ++
26 北五加皮	*Periploca sepium*	root bark	E	0	-5.9	0.13	30.5 ++
27 太瓜子	*Benincasa cerifera*	seed	M	0	-1.1	0.07	11.3 ++
28 繁縷	*Stellaria media*	herb	W	0	+0.1	0.31	39.1 ++
29 茜草根	*Rubia cordifolia*	root	M	0	-3.4	0.27	20.0 ++
30 芭蕉根	*Musa basjoo*	rhizome	W	0	-1.4	0.06	11.3 ++
31 卷柏	*Selaginella tamariscina*	herb	W	0	-0.5	0.14	38.8 ++

W : water, E:ethanol, M: methanol, dose: 100 mg/kg/day

Various screening systems are shown in Table 2.

Table 2. Experiment System

Tumor	Host animal	Inoculation size	Inoculation site	Administration route	Administration period	Criteria
Leukemias and ascites tumors						
P388	CDFI	10^6	i.p.	i.p. i.p. i.p. i.v.	1 1,5,9 1 - 9 1 - 9	MST[a]
L1210	CDFI	10^5	i.p.	i.p. i.v.	1 - 9 1 - 9	MST
Meth-A	BALB/C	2×10^5	i.p.	i.p.	1 - 9	MST
M5076	BDFI	Homogenate	i.p.	i.p.	1 - 9	MST
MOPC-104E	CDFI	10^6	i.p.	i.p.	1 - 9	MST
Yoshida sarcoma	Donryu rat	10^6	i.p.	i.p. i.v.	1 - 9 1 - 9	MST
AH-13	Donryu rat	10^6	i.p.	i.p.	1 - 9	MST
Solid tumors						
Meth-A	BALB/C	10^6	s.c.	i.p.	1 - 11	TWD 14[b]
LLC	BDFI	5×10^5	s.c.	i.p. i.p. i.v. i.v.	1 - 11 7 - 17 1 - 11 7 - 17	TWD 12,22[c] and MST
Colon-38	BOFI	Homogenate	s.c.	i.p. i.v.	1 - 11 1 - 11	TWO 17[c] TWO 22[c]
B-16-BL6	C57BL/6	2.5×10^5	s.c.	i.v.	1 - 11	d)

a) ; Mean survival time.

b) ; Tumor weight determined by extirpation on the day.

c) ; Tumor weight determined with callipers($LXW^2/2$)on the day.

d) ; Tumor size on the day 18 and No. of pulmonary nodules and weight of lymph node on the day 35.

found from cancer screening research, but from another biological evaluation. So, it is considered to be important to select any tumor for first screening.

Generally, Sarcoma-180A, Ehrlich carcinoma and KB cell etc are used for first screening. Then, leukemia system of P388 and L1210 will be following as an next step. As to be agreable method, we have started screening by using Sarcoma 180A.[9]

1, It is necessary to be able to predict crinical effectiveness.
2, To be able to have relinance(or reappearance) of results.
3, Convenience and efficiency for experimental techniques.
4, To be able to supply the test material fully for screening.

By this method, we have tested about 700 samples. A part of the results in shown in Table 1.[7,8] Judgment was evaluated by growth ratio(GR) of ascites sarcoma; GR=0-, 10% +++, 11-40% ++, 41-61% +, 66%-.

After this screening, P388 leukemia is recommended as next screening system. Elongation of survival represented by T/C% is usefull for evaluation. In the case of natural product extracts, it was noticed to have antineoplastic activity by above 130(T/C%) in this method.

Bisabolane type sesquiterpenoids[5] and sinococulin[6]

We have isolated two types of antineoplastic components, one is sesquiterpense and the other is Cocculus alkaloids. Eight bisabolane type sesquiterpenoids were obtained from *Curcuma xanthorrhiza*, an Indonesian plants, which has antineoplastic activity against Sarcoma 180A.

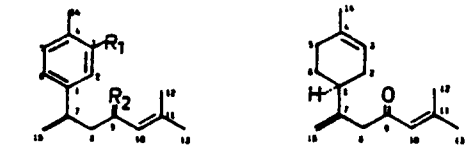
Cocculus alkaloid named sinococulone was isolated from Cocculus trilobus to be active substance against Sarcoma 180A, P388 and L1210. This compound is now waiting for crinacal trials for anticancer agent.

Antineoplastic components from Rubiae Radix

Among of the crude drugs showing activity against Sarcoma 180A in Table 1, the extracts of *Euphoriba lathyris* and *Rubia cordifolia* revealed elongation effect of survival to P388 leukemia mice, above 130(T/C%).

Euphorbia spp. have been famous in the world to contain antineoplstic and carcinogenic diterpenoids. TPA(12-0-tetradeca-noylphorbol-13-acetate) which is useful as a promotor to produce carcinoma was also isolated from *Euphoriba* spp.

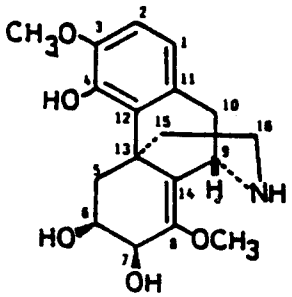
Rubiae Radix is originated to *Rubia akane* in Japan, *R. cordifolia* in China and *R. tinctorum* in Europa. Two of the former showed antineoplastic activity, but the latter one didn't show activity. From the ancient times, *Rubiae Radix* has been mainly used as a pigments. As the medical treatment, it was used as antipyretic, hemostasis and tonic. Further in China, it is usefull crinically as a component of prescriptions for cancer of uterine cervix. Many pigments were isolated from *Rubia* spp. Ruberitorin, which is a kind of alizarin



I: R_1=H, R_2=H_2
II: R_1=H, R_2=O
IV: R_1=OH, R_2=H_2

III

Bisabolanes from *C. xanthorrhiza*



sinococulin

Table 3

RA-VII: colorless needles, mp $>300^0$ (from methanol)
MS m/z: 770 (M^+, $C_{41}H_{50}O_9N_6$)
$[\alpha]_D$-229° (chloroform)

RA-V : colorless powder, mp $>300^0$ (from methanol)
MS m/z: 756 (M^+, $C_{40}H_{46}O_9N_6$)
$[\alpha]_D$-225° (chloroform)

RA-IV : colorless powder, mp 247-255° (from methanol)
MS m/z: 786 (M^+, $C_{41}H_{50}O_{10}N_6$), 768(M^+,H_2O)
$[\alpha]_D$-126° (chloroform)

RA-III : colorless needles, mp $>300^0$ (from methanol)
MS m/z: 786 (M^+, $C_{41}H_{50}O_{10}N_6$), 768 (M^+, H_2O)
$[\alpha]_D$-199° (chloroform)

RA-II : colorless needles, mp 261° (dec., from methanol)
MS m/z: 756(M^+, $C_{40}H_{48}O_9N_6$)
$[\alpha]_D$-201° (chloroform)

RA-I : colorless powder, mp 284° (dec., from methanol)
MS m/z: 772 (M^+, $C_{40}H_{48}O_{10}H_6$)
$[\alpha]_D$-216° (chloroform-methanol(9:1))

glycoside, purpurin glycoside, rubiadin glycoside are contained in *R tinctorum*. Purpurin, morgin, arizarin, rucidin, primeroside, ruberitorin and anthraquinon etc. are found in oriental *Rubia* spp.[10] However, these pigments were assumed to be non antineoplastic constituents. So, we have started our investigation to find out antineoplastic principles from *Rubia* spp. After repeating fractionation and purification of extract, oligopeptides were obtained as active

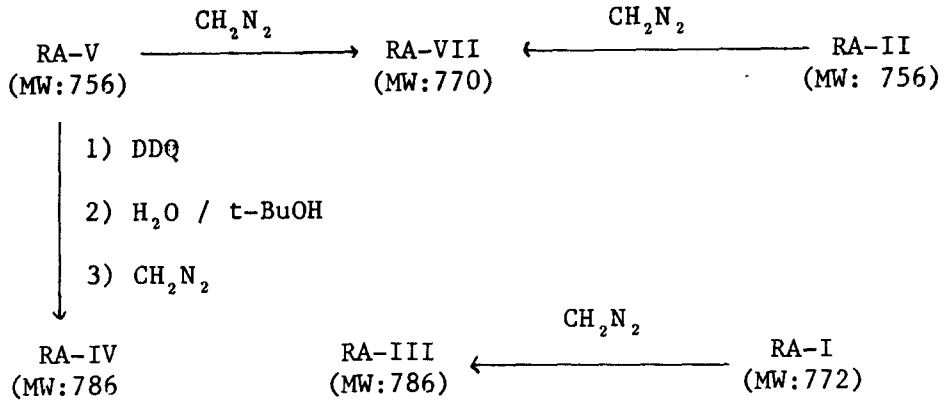
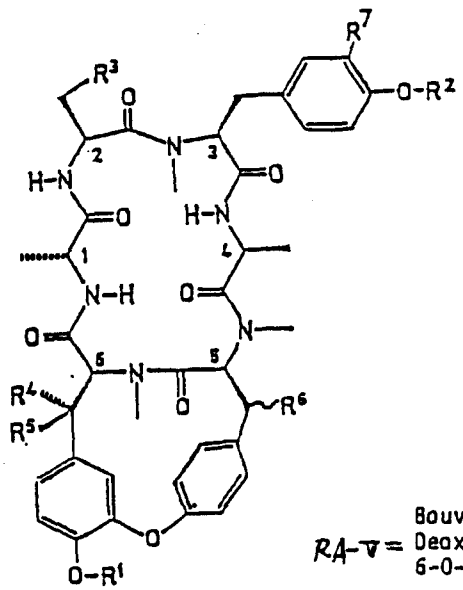


Fig. 2 Structural Relationships of RA Compds



Rubia cordifolia

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
RA-I	H	Me	OH	H	H	H	H
RA-II	Me	H	H	H	H	H	H
RA-III	Me	Me	OH	H	H	H	H
RA-IV	Me	Me	H	OH	H	H	H
RA-V	H	Me	H	H	H	H	H
RA-VII	Me	Me	H	H	H	H	H.
Products derived from RA- V by DDQ							
A	H	Me	H	OH	H	H	H
A-diAc	Ac	Me	H	OAc	H	H	H
B	Me	Me	H	=O		H	H
C	Me	Me	H	H	OH	H	H
E	H	Me	H	OMe	H	H	H
E-Me	Me	Me	H	OMe	H	H	H
E-Ac	Ac	Me	H	OMe	H	H	H

Bouvardia ternifolia (Rubiaceae)[68,69]

RA-V =

Bouvardin	H	Me	H	H	H	β-OH	H
Deoxybouvardin	H	Me	H	H	H	H	H
6-O-Me-bouvardin	Me	H	H	H	H	β-OH	H
Microbial transformed products[102]							
O-desMe-bouvardin	H	H	H	H	H	β-OH	H
Bouvardin catechol	H	H	H	H	H	β-OH	OH

Fig.3 Structures of RA-VII,V,IV,III,II,I and RA-IV related compounds

principles against P388 leukemia. Commercial extract was partitioned with water and benzen, and water and ethyl acetate. Both of benzen and ethylacetate fractions, seven components were isolated as crystal, and named as RA I-VII, in relation to *R. akane*.

Structural elucidation of RA compounds

Physical data of RA series are shown in Table 3. These compounds

were assumed to be small peptides from the IR data showing 3390, 1640 cm due to amide bonding.

It was found the data of [13] C-NMR of RA-VII that there were three of C-Me, three of $-CH_2-$, three of N-Me, two of O-Me, six of CH, eighteen of aromatic carbon, eleven of tertiary carbon, seven of quatery carbon(three of C-C bond and four of C-O bond), and six of carbonyl. Then, it was assumed to be cyclic hexapeptide consisted of three alanin and three molecules of tylosine derivative. Further, complete hydrolysis afforded to produce one of D-alanine, two of L-alanine, N-methyl-4-methoxy-L-phenylalanine and a dimer of N-methyl-tyrosine. From these results, the structure of RA-VII was assumed to be two cyclic hexapeptides having ether linkage. However, it was difficult to decide the sequence of amino acid and the configuration stereochemically. Lastly, X-ray analysis was applied to p-bromobenzoate of RA-V. From various reactions and instrumental analysis, structural relationships and the structures were determined as illustrated in Fig. 2 and 3.

Cytotoxicity and antineoplastic activity[15]

We first examined the cell growth inhibitory effects against KB cells, P388 lymphocytic leukemia cells and MM2 mammary carcinoma cells of the lead compound RA-V and n-hexylether derivative, which had shown the strongest antitumor activity in an in vivo assay. These results are shown in Fig. 4. RA-V and the n-hexylether showed clear growth-inhibitory effects at concentrations higher than 5×10 μg/ml and 5×10^{-2} μg/ml, respectively, in KB cells, 1×10^{-1} μg/ml and 1×10 μg/ml in mm2 cells. Thus the growth inhibitory effect of the n-hexylether derivative was stronger than that of RA-V in each cell line, and the effect showed dose-dependency.

Under microscope, mitomycin C-treated KB cells showed enlargement of the nuclei, deformation of the cells and abnormality of the nuclei, whereas KB cells treated with RA-V and its n-hexylether derivative showed globularization as compared with control cells.

RA-IV was considered to have an additional alcoholic hydroxyl group as compared with RA-VII, It was concluded that the hydroxyl group in RA-IV is linked to the β-carbon (C_β) of Tyr-6 by comparing the [13] C chemical shift values of RA-IV with those of RA-VII; C_β signal at δ 35.56(t) due to Tyr-6 of RA-VII was shifted downfield to 73.49 (d) in RA-IV, while other carbon signals in both peptides were similar. Next, in order to introduce an oxygen functional group into the benzyl position of Tyr-6 in RA-V, it was oxidized with 2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ) as shown in Fig. 5. This reaction gave selectively compound E in methanol and compound A in 90% aqueous tert BuOH solution. Compound A was methylated with diazomethane to provide RA-IV. Further, to confirm the configuration of the hydroxyl group in RA-IV, its epimer(c) was synthesized by reducing the oxidation product(B) with $NaBH_4$. This epimer could not be acetylated with abhydrous acetic acidpyridine at room temperature. The above results can be reasonably explained by the following stereochemical considerations: the reagent in this series of reactions can approach only from the α-side, because the β-side at the bonzyl

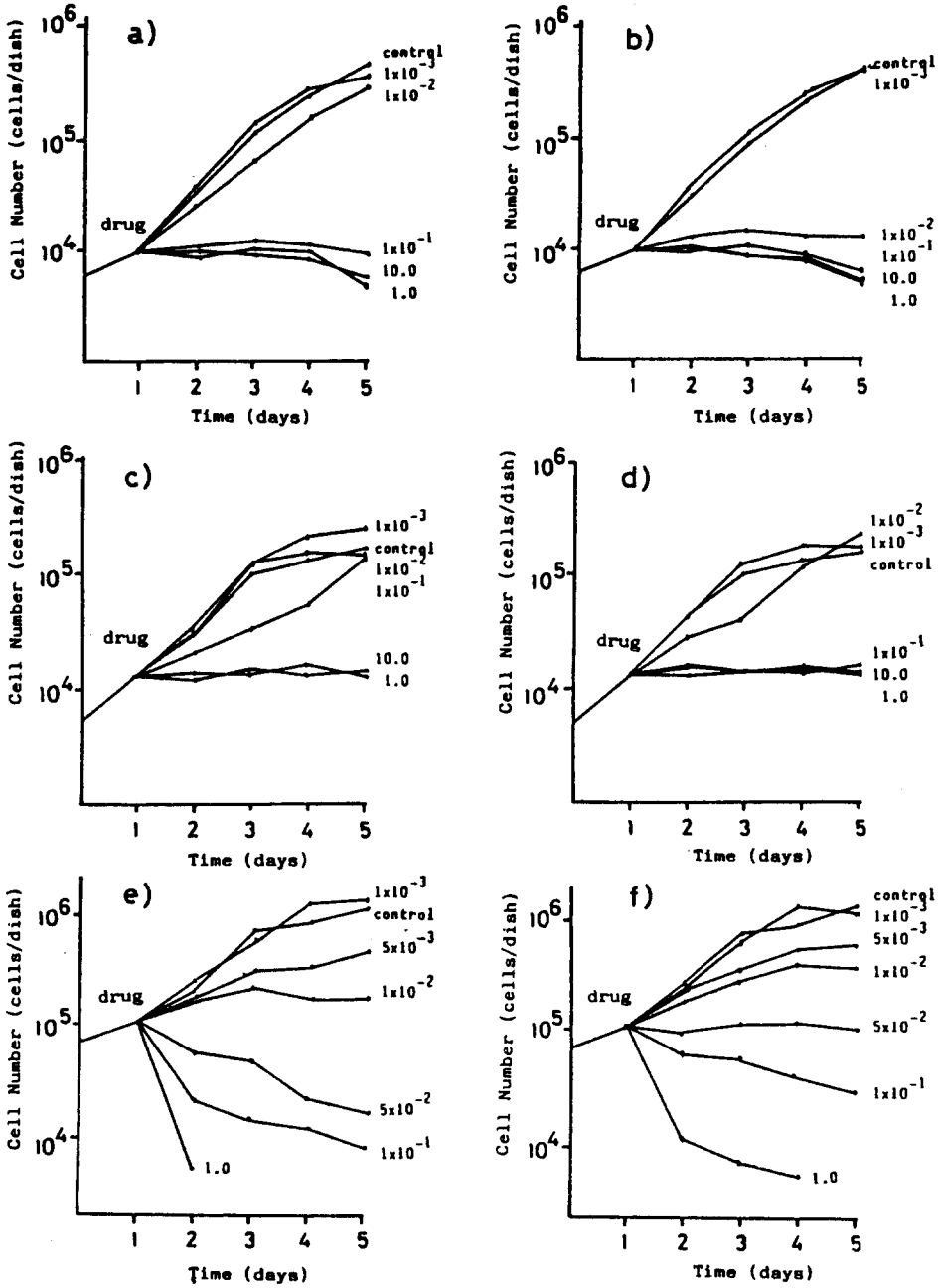


Fig. 4 Effects of RA-V and its *n*-hexylether on the growth of P388, MM2 and KB cells. P388 (9.70×10^3, a and b), MM2 (1.24×10^4, c and d) and KB cells (1.04×10^5, e and f) were treated with various concentrations of drugs and cell growth was followed daily with a Coulter counter. Drugs: a, c and e; RA-V. b, d and f; *n*-hexylether of RA-V.

location of Tyr-6 is strongly blocked by the N-methyl group of this tyrosine moiety as from the X-ray conformation. Consequently, the

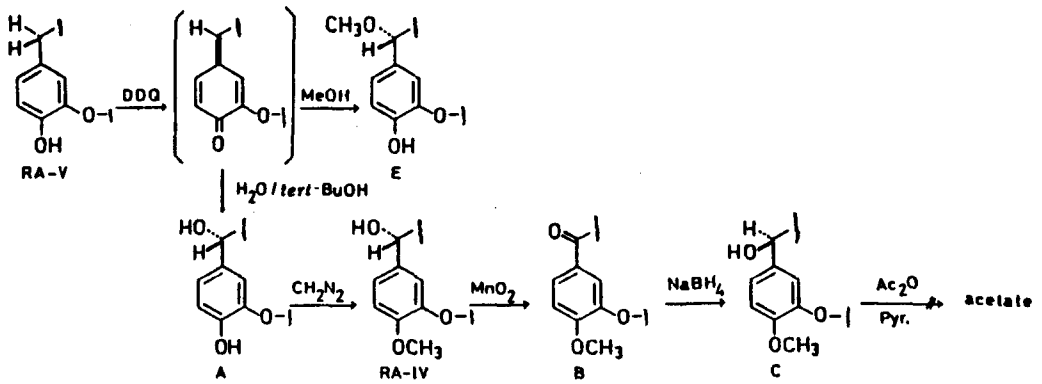
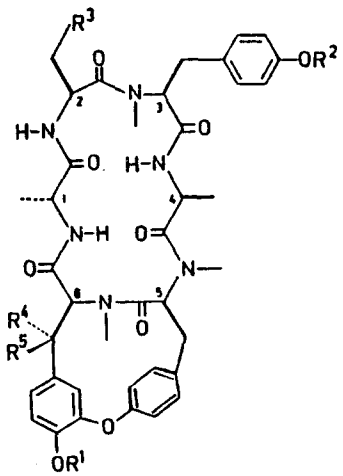


Fig. 5 Preparation of RA-IV and Related Compounds



	R^1	R^2	R^3	R^4	R^5	Antitumor activity[a] T/C(%)
RA-1	H	Me	OH	H	H	169.3
RA-1-diAc	Ac	Me	OAc	H	H	182.8
RA-II	Me	H	H	H	H	142.2
RA-III	Me	Me	OH	H	H	179.4[b]
RA-IV	Me	Me	H	OH	H	149.0
RA-V	H	Me	H	H	H	187.4
RA-VII	Me	Me	H	H	H	173.6[c]
A	H	Me	H	OH	H	126.3
A-diAc	Ac	Me	H	OAc	H	98.2
B	Me	Me	H	=O		171.9
C	Me	Me	H	H	OH	160.0
E	H	Me	H	OMe	H	118.5
E-Me	Me	Me	H	OMe	H	132.0
E-Ac	Ac	Me	H	OMe	H	116.9

a)P388:10^6cells/0.1ml,i.p., CDFI mice(n=6). Dose: 10.0mg/kg. i.p., day 1-5. b)Dose:2.0mg/kg. c)Dose:4.0mg/kg.

Fig. 6 Structures and Antitumor Activities of Native Cyclic Hexapeptides and Related Compounds

hydroxyl group of RA-IV was determined to have S configuration. We also examined the antineoplastic activity of six native cyclic

hexapeptides and seven related compounds(A-E) against P388 lymphocytic leukemia in mice. The mice received 10mg/kg/day(except for RA-VII and RA-III: 4.0 and 2.0mg/kg day) i.p. for 5 consecutive days. The antineoplastic activities are shown in Fig. 1. The small differences of antitumor activity among these compounds could be explained to some extent by the molecular hydrophobicities as previously mentioned, but a remarkable decrease of antitumor activity was observed in RA-IV, compounds A, A-diAc, E, E-Me and E-Ac, whose α-proton at the C_β position of Try-6 was replaced with bulky substituent groups. In spite of a similar replacement at C_β, the activity of compounds B and C did not decrease. From the above findings, it may be concluded that introduction of large substituent groups at the α-side of the RA series brings about a decrease of antitumor activity. This area seems to play an important role in the mechanism of antitumor activity. The antitumor activity decrease of RA-II can rather be explained from the viewpoint of the molecular hydrophobicity than the α-block hypothesis.

Relationships between structure and activity

In order to obtain RA-analogs with higher pharmacological and lower toxicological activities, several derivatives were synthesized by substituting the phenol moiety of RA-V, and their quantitative structure-activity relationship (QSAR) were investigated from the viewpoint of molecular hydrophobicities. The activity values(log I/IC50) of ether derivatives of RA-V gave an upward parabolic or bilinear relationship when plotted against log P(P: partition coefficient determined with the 1-octanol/water system) as the carbon number of the side chain at the phenol moiety of RA-V was increased, the optimum log P values being in the range from 3.5 to 4.9. The ester derivatives showed a similar relationship, the optimum log P values being 6.3-6.7, which is higher than that of the ether derivatives.

The relationships among the ILS (150 and 160%), the minimum lethal dose(MLD) and the hydrophobic coefficient of the ether series of RA-V were analyzed according to both the Hansch-Fujita model, and the bilinear model of Kubinyi. When the parabolic model obtained from the Hansch-Fujita equation was applied to the ILS and MLD, significant results could not be obtained.

However, since the optimum log P values of ILS 150 and 160% differed from that of MLD, it was considered that the most suitable ether derivatives of RA-V for antitumor activity might be selected from the region away from the optimum log P of MLD and approximating the log 1/D value in the optimum log P of ILS (Fig. 7). Thus, RA-VII and the n-hexylether of RA-V should be useful compounds on this basis.[13]

Therapeutic ratio of RA-VII was 400, compared with 10 of MMC. Mechanism of action of RA-VII was also investigated and was assumed to inhibition of protein biosynthesis, since ^{3}H-leucine was not taken in. The lethal effect of RA-V on KB cells was clearly different from

Table 4 IC_{50} of the RA-V Ethers on Some Tumor Cell Lines

Comp. No.	R	mp(°C) (decomp.)	$[\alpha]_D^{20-25}$ ($CHCl_3$)	log P	IC_{50} ($\mu g/m\ell$) KB	P388	MM2
1	H(RA-V)	>300	-220	2.72	1.85×10^{-3}	1.15×10^{-3}	4.40×10^{-2}
2	CH_3(RA-VII)	>280	-224	3.17	2.10×10^{-3}	2.30×10^{-3}	1.10×10^{-2}
3	CH_2CH_3	219-225	-194	3.70	7.80×10^{-4}	1.05×10^{-3}	3.50×10^{-3}
4	$(CH_2)_2CH_3$	212-217	-193	4.20	8.50×10^{-4}	1.05×10^{-3}	2.30×10^{-3}
5	$CH(CH_3)_2$	213-230	-194	4.00	8.30×10^{-4}	8.00×10^{-4}	2.10×10^{-3}
6	$(CH_2)_3CH_3$	210-216	-192	4.70	9.10×10^{-4}	9.40×10^{-4}	2.00×10^{-3}
7	$(CH_2)_4CH_3$	214-221	-191	5.30	2.05×10^{-3}	1.90×10^{-3}	1.75×10^{-3}
8	$(CH_2)_5CH_3$	261-268	-178	5.80	7.50×10^{-3}	6.40×10^{-3}	9.60×10^{-3}
9	$(CH_2)_6CH_3$	245-250	-189	6.30	1.90×10^{-2}	8.30×10^{-2}	1.85×10^{-3}
10	$(CH_2)_7CH_3$	243-249	-178	6.80	1.00×10^{-1}	1.50×10^{-2}	2.00×10^{-2}
11	$(CH_2)_8CH_3$	245-248	-169	7.30	5.40×10^{-1}	1.50×10^{-2}	3.80×10^{-2}
12	$(CH_2)_9CH_3$	242-247	-198	7.90	2.45×10^{-1}	6.70×10^{-2}	2.30×10^{-1}
13	$(CH_2)_{10}CH_3$	228-235	-179	8.40	6.60×10^{-1}	3.40×10^{-1}	8.40×10^{-1}
14	$(CH_2)_{11}CH_3$	240-242	-189	8.90	$>1.00^{-1}$	2.45	2.10
15	$(CH_2)_{12}CH_3$	238-242	-186	9.40	>1.00	1.60	2.05
16	$(CH_2)_{17}CH_3$	228-235	-153	12.00	>1.00	>10,00	>10,00
17	cyclopentyl	229-233	-155	4.80	4.20×10^{-4}	6.20×10^{-4}	1.80×10^{-3}
	mitomycin				70×10^{-3}	4.90×10^{-2}	8.50×10^{-2}

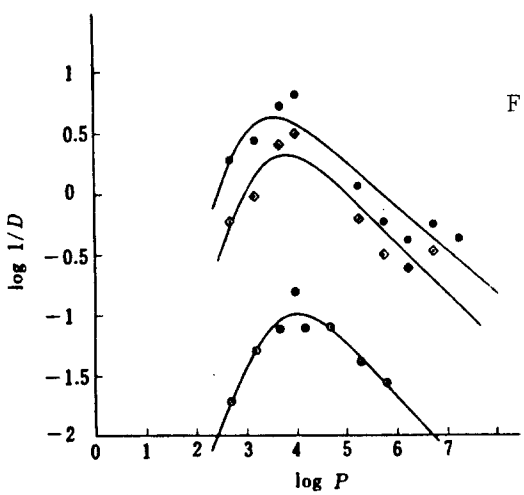


Fig. 7. Structure-Antitumor Activity and Toxicity Relationships of Alkyl Ethers of RAV on P-388 Leukemia in Mice

These results were analyzed on the basis of the bilinear model (see the text).

●, T/C 150%; ◇, T/C 160%; ⊙, toxicity.

that of MMC, and RA-V was concluded to be a "time-dependent drug" like vinblastine as shown in Fig. 8.[16] Further, RA-VII was effective to Colon 38 (s.c.-i.p., s.c.-i.v.), p. 388 (i.p.-i.v.), L1210 (i.p.-i.v), Meth A (s.c.-i.p.), M5076 (i.p.-i.p.). The inhibition was found

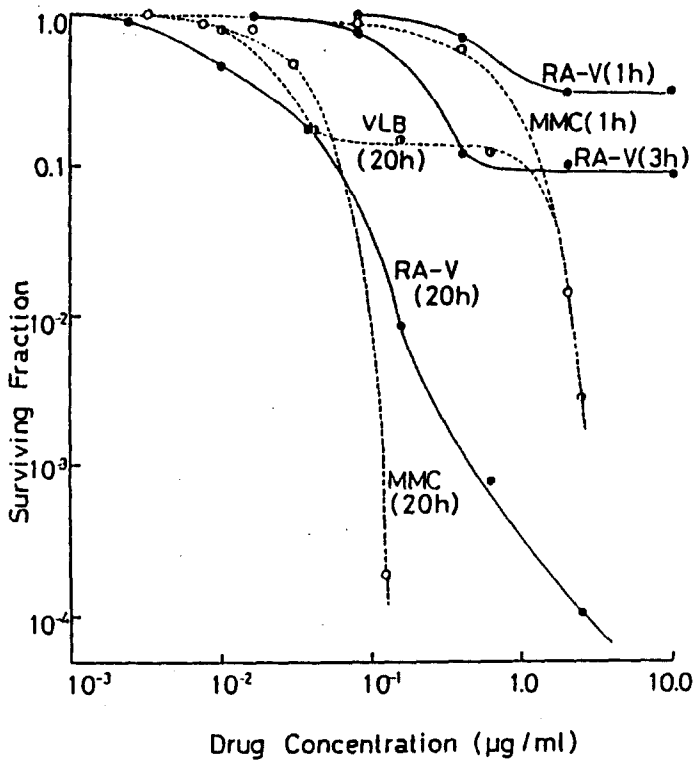


Fig. 8. Lethal Effect of RA-V, Mitomycin C and Vinblastine on KB Cells
Survivals were assayed by exposure of KB cells to RA-V, VLB and MMC, and then by their incubation for colonal growth.
MMC : moitomycin C (··· ○ ···), exposure 1 and 20 hours
VLB : vinblastine (... ◑ ...), exposure 20 hours
RA-V: (——●——), exposure 1,3 and 20 hours

from the effectiveness to B16-BL-6 (s.c.-i.p., s.c.-i.v.).

To conclusion

In seasonal variation, content of RA-V has been increased from July to August in root and aerial part. We have found out RA series compounds only from *R. akane* and *R. cordiforia*, although many other relative plants were investigated.

Ra-V is the same compound with deoxybouvardin. Bouvardin has been investigated to develop as an antitumor drug in NCI of U.S.A. connecting with Arizona University. Adriamycin has CH_2OH in its molecule instead of CH_3 in daunomycin. Even only such chemical difference, adriamycin revealed more strong activity and less toxicity than daunomycin. So, it is also expected that RA-VII with show the different activity from that of bouvardin.

Recently, preferable result was observed, when RA-VII (RA-700) was taken with cyclophosphamide at the same time.[18]

On the other hand, plant tissure culture has been also investigated to supply RA compounds. Moreover, total synthesis of RA-VII

was performed. Although synthetic substances are usefull, it was noticed historically lead compounds isolated from natural sources to be necessary. Sooner or later, some one will find out usefull compounds from natural kingdom.

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