

Antitumor Substances from Higher Plants in Asian Region

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Abstract - In this review, we summarize the isolation, structural determination, antitumor activity of substances from higher plants which were collected in Asian region.

1. Introduction

To date many kinds of compounds have been obtained from plants kingdom as antineoplastic and anticancerous agents. However, there is no special type of compounds for cancer therapy. Various types of substances are effective for various types of cancers and tumors: for instance, alkaloids, lignans, terpenes and steroids etc.¹⁾ First of all, most important components obtained from higher plants are Vinca alkaloids and Podophyllum lignans. Vinca rosea (= Catharanthus roseus) has been used as inhibiting agent for milk secretion, hypotensor, astringent and emetics as folk medicines in Madagascar. Moreover, native people in West Indian Island have been using Vinca spp. as depression agent of blood sugar. When the extract of this plant was given non-orally, leucopenic and indirect inhibiting action of nuclear division of cells were observed. Above 60 kinds of alkaloids have been isolated from Vinca spp. Vinblastine and vincristine are most active substances among of them. The former is effective to Hodgkin disease and the latter to leukemia. Podophyllotoxin is a representative lignan isolated from the rhizomes of Podophyllum peltatum. Podophyllum rhizome had been used as an emetic and an anthelmintic by American Indians traditionally. Because podophyllotoxin was also found to have inhibiting action for cell-division, antineoplastic activity was noticed.

The others, curcumol obtained from Curcuma aromatica was tested and noticed to be effective against cancer of the uterine cervix clinically. Oridonin isolated from Rabdosia ssp. is now investigated for clinical trials in China. Moreover, camptothecin isolated from Camptotheca acuminata is also antineoplastic alkaloid, but is very toxic. Chemical modification has been tried to decrease its toxicity. This compound will be permitted to use as clinical agent later.²⁾ Colchicine derivatives are also said to have inhibiting action of cell-division. Demecolcine and colchicine have activity against mammary cancer. Harringtonin was investigated as an anticancerous drug in China. Taxol, a compound with a taxane ring isolated from the bark of Taxus brevifolia,

has been demonstrated to have substantial anticancer activity in patients with solid tumors refractory standard chemotherapy. Supply of this drug has severely limited full exploration of its antineoplastic potential. Some efforts are continued in National Cancer Institute (NCI) Washington for surveying various Taxus species for optimal taxol content, improvement in semi-synthesis from baccatin III, improvement in method of extraction, and development of alternative renewable resources.³⁾ Further, there are many compounds which have been reported as antineoplastic agents.

Development of novel clinical useful anticancer agents would be dependent on the screening system and the sample sources for the bioassay. The search for potential anticancer agents from natural sources mainly has been carried out with the guidance of bioassays confirmed by the NCI,⁴⁻¹⁰⁾ because the large number of natural products screened at the NCI program have also been discussed from an overview of the relationship of assessment between experimental animals and clinical patients for drug development, and the screening protocols for each tumor system have been well-established. It is considered that these are "compound-oriented" *in vivo* screenings. These screenings could not lead to develop some new drug for solid cancers.¹¹⁻¹⁴⁾

Recently, NCI has established a "disease-oriented" approach to antitumor activity screening^{5,15,16)} and the biological response modifiers (BRM)^{17,18)} program from a viewpoint of the diversity and specificity of tumor, and the requirements of novel structure types and novel action-mechanistic types of anticancer agents. These screening system led to isolate many antineoplastic compounds from plants,¹⁹⁻²²⁾ microorganism^{23,24)} and marine metabolites^{4,12)} etc. On the other hand, we have screened on higher plants collected in Japan, China, Korea, Southeast Asia and South America²⁵⁻²⁷⁾ for antineoplastic activity, which has been done using Sarcoma 180 ascites in mice, P388 lymphocytic leukemia in mice, Chinese hamster lung V-79 cells, P388 cells and nasopharynx carcinoma (KB) cells in our laboratory, as primary screening. In this review we will describe on antitumor and cytotoxic substances of the higher plants selected from above screening tests.

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 clinical trials of human.¹²⁾

2. An Antitumor Morphinane Alkaloid, Sinococuline, from Cocculus trilobus and the Related Compounds²⁸⁻³⁰⁾

Cocculus trilobus DC. (Menispermaceae) growing in the mountainous areas of East Asia has been used in folk medicine as a diuretic, analgesic and anti-inflammatory crude drug. When an aqueous solution of the methanolic extract prepared from the stems and rhizomes of C. trilobus was partitioned successively with n-hexane and ethyl acetate, the antitumor activity against Sarcoma 180 ascites in mice was concentrated in the

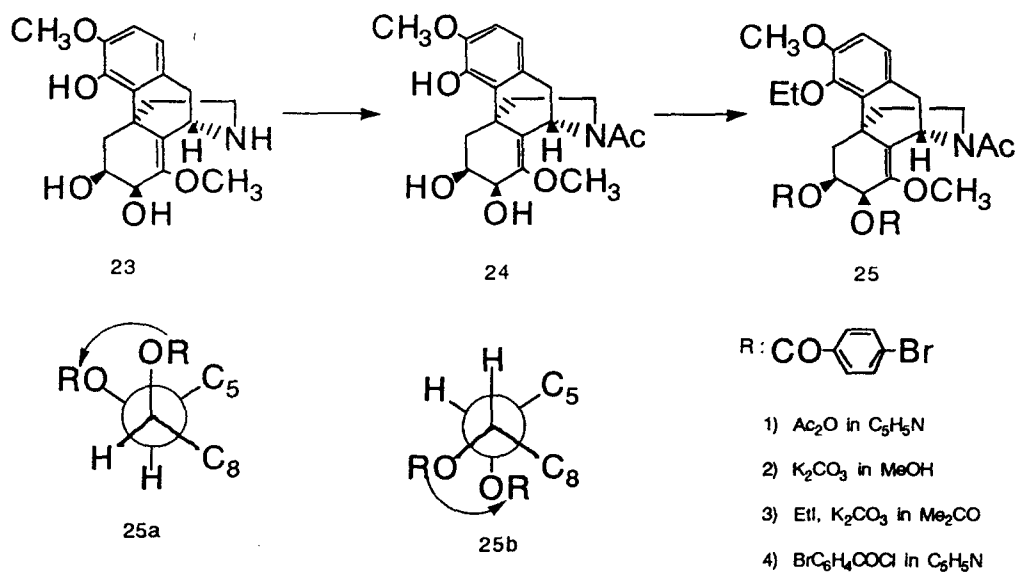


Chart 1

aqueous-layer residue. Repeated purification of the residue gave an antitumor alkaloid, named sinococuline (**23**). The relative structure was established by various spectroscopic method and the C_9 configuration was assumed to be S from the viewpoint of chemotaxonomy,³¹⁾ however, it was confirmed by measuring the CD spectrum (positive maximum at 238 nm).³²⁾ Further, in order to determine the absolute one using the exciton chirality rule,³³⁾ sinococuline (**23**) was converted to the 6,7-dibenzoate derivative as shown in Chart 1. Its CD spectrum showed a negative Cotton effect at 252 nm and the coupling constant between H_6 and H_7 was 3.5 Hz.

Table I Antitumor Activity of Sinococuline (**23**) and Its Related Compounds **26** and **27** against P388 Leukemia in Mice

Compounds	Dose (mg/kg)	Survival Time (d, mean + S.E.)	T/C (%)	BWC (g)
23	10	12.5 + 0.48	154.6	+0.9
	25	13.5 + 0.34	167.0	+0.6
	50	14.3 + 0.49	177.0	-0.6
	100	16.2 + 1.92	200.0	-4.7
26	10	11.3 + 0.21	140.2	+0.9
	25	12.7 + 0.42	156.7	-1.1
27	6.25	13.8 + 0.31	131.7	+0.8
	12.5	14.5 + 0.50	138.1	+0.1
	25	16.0 + 0.52	152.4	-1.5

P388: 10^6 cells/0.1 ml, *i.p.*, CDF1 mice (n=6); Drug: *i.p.*, d 1 - 5.

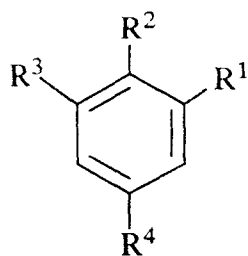
Their fact was suggested both structures **25a** (6S, 7S) and **25b** (6R, 7R) in Chart 1. However, the NOE between H₆ and H₁₅ supported the structure **25a** only. The continuous research of antitumor substances from *Cocculus* plants led us to isolate **23** and the related compounds **26** and **27** from *C. sarmentosus*. These compounds had antitumor activity against Sarcoma 180A (40 mg/kg/d dose for 5 consecutive days, GR (growth ratio = T/C): 56% (+) in **23**) and P388 leukemia in mice shown in Table I. Also, these various derivatives were prepared and applied to P388 in vivo test, however, a more effective substance than sinococulin (**23**) could not get anymore.³⁴⁾

3. Antitumor Long-Chain Phenols from *Ginkgo biloba* ^{35,36)}

Ginkgo biloba L. (Ginkgoaceae) is a tree from 30 to 40 m in height and is a native of China. The seeds are used for allaying coughing and tonic. The methanolic extract from the sarcotesta of *G. biloba* L. showed remarkable antitumor activity against Sarcoma 180A in mice. The extract was subjected to silica gel and/or alumina column chromatography to give some fractions containing anacardic acid, bilobol and cardanol. Their further purification with ODS column furnished anacardic acid (**28a, b, c**), bilobol (**29a, b**) and cardanol (**30a, b**) as shown in Fig.1. Also, the antitumor activity of them was summarized in Table II. This result is speculated that the antitumor activity of long-chain phenols against Sarcoma 180A in mice appears not to require the carboxyl group.

Further, a bio-assay based on the cytotoxic activity against Chinese hamster lung V-79 cells instead of the antitumor activity against Sarcoma 180A in mice was employed in a search for antitumor principles by means of quantitative structure-activity relationship (QSAR) analysis, because there was a good correlation between the results of the biological tests of long-chain phenols using V-79 cells and Sarcoma 180A in mice. We considered that the activities of antitumor long-chain phenols were controlled by both hydrophobic and electronic parameters based on the alkyl side chain moiety and the aromatic ring contribution of hydroxyl function, respectively, because acetates and methyl esters of the long-chain phenols did not show antitumor activity against Sarcoma 180A in mice as can be seen from Table II.

Thirty long-chain phenol derivatives, which were divided into six groups consisting of five compounds having the same aromatic ring contribution and a different alkyl side chain moiety, were synthesized by Grignard reaction of alkyl bromide and hydroxybenzaldehyde in the usual way. Each compound was tested for cytotoxic activity against V-79 cells and each IC₅₀ value was determined. Also, for all synthesized compounds (**30 - 60**), the log P values (P stands for the n-octanol - water partition coefficient) were measured by the HPLC method³⁷⁾ as the hydrophobic parameter. As the electronic parameter, the energy of the lowest unoccupied molecular orbital (ELUMO) was calculated by using the modified neglect of diatomic differential overlap (MNDO)



28 a:	$R^1=(CH_2)_{12}CH_3$	$R^2=COOH$	$R^3=OH$	$R^4=H$
28 b:	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=COOH$	$R^3=OH$	$R^4=H$
28 c:	$R^1=(CH_2)_9CH=CH(CH_2)_5CH_3$	$R^2=COOH$	$R^3=OH$	$R^4=H$
28 a':	$R^1=(CH_2)_{12}CH_3$	$R^2=COOCH_3$	$R^3=OH$	$R^4=H$
28 b':	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=COOCH_3$	$R^3=OH$	$R^4=H$
28 c':	$R^1=(CH_2)_9CH=CH(CH_2)_5CH_3$	$R^2=COOCH_3$	$R^3=OH$	$R^4=H$
29 a:	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=H$	$R^3=R^4=OH$	
29 b:	$R^1=(CH_2)_9CH=CH(CH_2)_5CH_3$	$R^2=H$	$R^3=R^4=OH$	
29 a':	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=H$	$R^3=R^4=OCOCH_3$	
30 a:	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=R^4=H$	$R^3=OH$	
30 b:	$R^1=(CH_2)_9CH=CH(CH_2)_5CH_3$	$R^2=R^4=H$	$R^3=OH$	
30 a':	$R^1=(CH_2)_7CH=CH(CH_2)_5CH_3$	$R^2=R^4=H$	$R^3=OCOCH_3$	

Fig. 1 The Structures of Compounds 28a-30a'

method,^{38,39}) because Hammett's substituent constants^{40,41}) were not suitable for both ortho- and di-substituted aromatic rings.

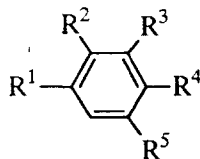
Table II Antitumor Activity on Sarcoma 180A in Mice

Compound	Dose (mg/kg)	GR (%)	Assessment
28b	40	17.4	++
29a	40	0.4	+++
30a	40	0.0	+++
28b'	60	110.4	-
29a'	40	81.9	-
30a'	40	105.7	-

The synthesized long-chain phenols and their parameters used in this work are listed in Table III. In the comparison of the cytotoxic activity in each group, the compounds having 11, 13 or 15 carbons in the alkyl side chain moiety usually showed strong activity in comparison with others in each group. In a further comparison among groups, groups D (46 - 50) and F (56 - 60) exhibited ten-fold stronger activity than the other groups. The optimum log P existed in groups A, D and F from the multiple regression analysis of each group, but the activity was modified by electronic effects based on the aromatic ring contributions.

Then, as electronic parameter, we used the E_{LUMO} and E_{HOMO} values which are related to the drug-receptor interaction processes. The E_{LUMO} value is noted as a measure of relative electron-acceptor property of a molecule and many workers have discussed the charge transfer interaction between a drug and its receptor using the E_{LUMO} values⁴²). When the correlation between the cytotoxic activity (-log IC_{50} value) and the E_{LUMO} value was examined by single regression analysis, a good correlative equation (Eq.1) was derived from compounds having 13 carbons at the alkyl side chain moiety, whose activity was usually stronger than others in

Table III Structures and Parameters for Multiple Regression Analysis



Compound	R ¹	R ²	R ³	R ⁴	R ⁵	Yield	mp (°C)	MS (M ⁺)	-log ED ₅₀	log P	E _{LUMO}	E
A-7 (3 1)	C ₇ H ₁₅	OH	H	H	H	63.5	-	192	1.16	4.45	0.142	-8.86
A-9 (3 2)	C ₉ H ₁₉	OH	H	H	H	59.0	-	220	1.38	5.76	0.142	-8.86
A-11 (3 3)	C ₁₁ H ₂₃	OH	H	H	H	75.4	32.0-33.0	248	1.39	7.17	0.142	-8.86
A-13 (3 4)	C ₁₃ H ₂₇	OH	H	H	H	70.4	42.5-43.5	276	1.42	8.61	0.142	-8.86
A-15 (3 5)	C ₁₅ H ₃₁	OH	H	H	H	29.3	53.0-54.0	304	1.43	10.07	0.142	-8.86
B-5 (3 6)	C ₅ H ₁₁	H	OH	H	H	50.0	-	164	-	3.13	0.135	-8.88
B-9 (3 7)	C ₉ H ₁₉	H	OH	H	H	56.4	-	220	1.16	5.61	0.135	-8.88
B-11 (3 8)	C ₁₁ H ₂₃	H	OH	H	H	57.6	-	248	1.14	6.98	0.135	-8.88
B-13 (3 9)	C ₁₃ H ₂₇	H	OH	H	H	31.7	41.0-42.0	276	1.34	8.84	0.135	-8.88
B-15 (4 0)	C ₁₅ H ₃₁	H	OH	H	H	11.2	50.0-51.0	304	1.33	10.11	0.135	-8.88
C-7 (4 1)	C ₇ H ₁₅	H	H	OH	H	82.4	-	192	1.43	4.15	0.179	-8.82
C-9 (4 2)	C ₉ H ₁₉	H	H	OH	H	35.7	41.0-42.5	220	1.29	5.76	0.179	-8.82
C-11 (4 3)	C ₁₁ H ₂₃	H	H	OH	H	86.6	56.5-57.0	248	1.14	7.18	0.179	-8.82
C-12 (4 4)	C ₁₂ H ₂₅	H	H	OH	H	79.8	67.5-68.0	262	1.14	7.91	0.179	-8.82
C-13 (4 5)	C ₁₃ H ₂₇	H	H	OH	H	84.5	68.0-69.0	276	1.39	8.20	0.179	-8.82
D-7 (4 6)	C ₇ H ₁₅	OH	OH	H	H	48.8	-	208	1.76	3.59	0.020	-8.60
D-9 (4 7)	C ₉ H ₁₉	OH	OH	H	H	46.6	-	236	2.04	4.86	0.020	-8.60
D-11 (4 8)	C ₁₁ H ₂₃	OH	OH	H	H	63.5	51.8-52.5	264	2.34	6.30	0.020	-8.60
D-13 (4 9)	C ₁₃ H ₂₇	OH	OH	H	H	43.0	56.0-56.5	292	2.08	7.75	0.020	-8.60
D-15 (5 0)	C ₁₅ H ₃₁	OH	OH	H	H	41.0	60.5-61.0	320	2.01	9.27	0.020	-8.60
E-7 (5 1)	C ₇ H ₁₅	OH	H	OH	H	3.9	70.8-71.3	208	1.10	2.90	0.098	-8.75
E-9 (5 2)	C ₉ H ₁₉	OH	H	OH	H	7.6	72.0-72.7	236	1.11	4.10	0.098	-8.75
E-11 (5 3)	C ₁₁ H ₂₃	OH	H	OH	H	2.9	72.5-73.0	264	1.43	5.43	0.098	-8.75
E-13 (5 4)	C ₁₃ H ₂₇	OH	H	OH	H	6.2	72.3-73.0	292	1.36	6.84	0.098	-8.75
E-15 (5 5)	C ₁₅ H ₃₁	OH	H	OH	H	2.2	84.5-85.1	320	1.68	8.29	0.098	-8.75
F-5 (5 6)	C ₅ H ₁₁	H	OH	OH	H	37.5	-	180	1.57	3.13	0.098	-9.06
F-9 (5 7)	C ₉ H ₁₉	H	OH	OH	H	35.6	75.5-77.0	236	1.82	5.54	0.098	-9.06
F-11 (5 8)	C ₁₁ H ₂₃	H	OH	OH	H	73.6	84.0-85.0	264	2.28	6.94	0.098	-9.06
F-13 (5 9)	C ₁₃ H ₂₇	H	OH	OH	H	80.0	90.0-91.5	292	2.33	8.42	0.098	-9.06
F-15 (6 0)	C ₁₅ H ₃₁	H	OH	OH	H	40.6	88.5-91.0	320	2.11	9.90	0.098	-9.06
6 1	C ₁₅ H ₂₉	COOH	OH	H	H	-	40.0-41.0	346	-	-	-	-
6 2	C ₁₅ H ₂₉	H	OH	H	OH	-	30.0-31.0	318	-	-	-	-
6 3	C ₁₅ H ₂₉	H	OH	H	H	-	-	302	-	-	-	-

ED₅₀ in mM, E_{LUMO} and E_{HOMO} in eV.

each group. Since the sign of the E_{LUMO} coefficient in Eq.1 was negative, we found that a drug with lower-lying E_{LUMO} value interacted strongly with the receptor.

$$-\log \text{IC}_{50} = -4.47E_{\text{LUMO}} + 1.97 \quad (1)$$

$$n=6, r=0.96, s=0.14, F=50.03$$

The results of the multiple regression analysis based on Eq.2 gave that the cytotoxic activity mainly depended on the log P and a low-lying E_{LUMO}. It has been suggested that receptor protein tryptophan residues

containing an aromatic ring moiety should be the best electron donor for the charge transfer interactions with phenols because of the high E_{HOMO} value⁴³).

$$-\log IC_{50} = a(\log P)^2 + b \log P + c E_{LUMO} + d E_{HOMO} + e \quad (2)$$

Among the synthesized long-chain phenols (31 - 60), the activity of 59 was stronger by about 10 times than others against V-79 cells, and 59 also showed antitumor activity against Sarcoma 180A in mice at a low dose, 10 mg/kg/d. Natural compounds (61 - 63) from *G. biloba* did not show activity at the same dose. Furthermore, 59 exhibited significant activity against P388 lymphocytic leukemia in mice at 100 mg/kg.

4. Cytotoxic Quassinoids, Linear Triterpenes and Canthin Alkaloids from *Eurycoma longifolia*^{44,45}

Eurycoma longifolia Jack (Simaroubaceae) is one of famous folk medicines named "Pasak Bumi" in the Southeast Asia and has been used for antimalaria and tonic etc. The roots of *E. longifolia* collected in Indonesia were extracted with 50% aqueous methanol. The extract was partitioned between water and ether, then n-butanol successively. The chromatographic purification of ether and n-butanol soluble fractions furnished canthin alkaloids (64 - 66) and quassinoids (67 - 72), respectively. Their structures were confirmed as shown in Fig.3

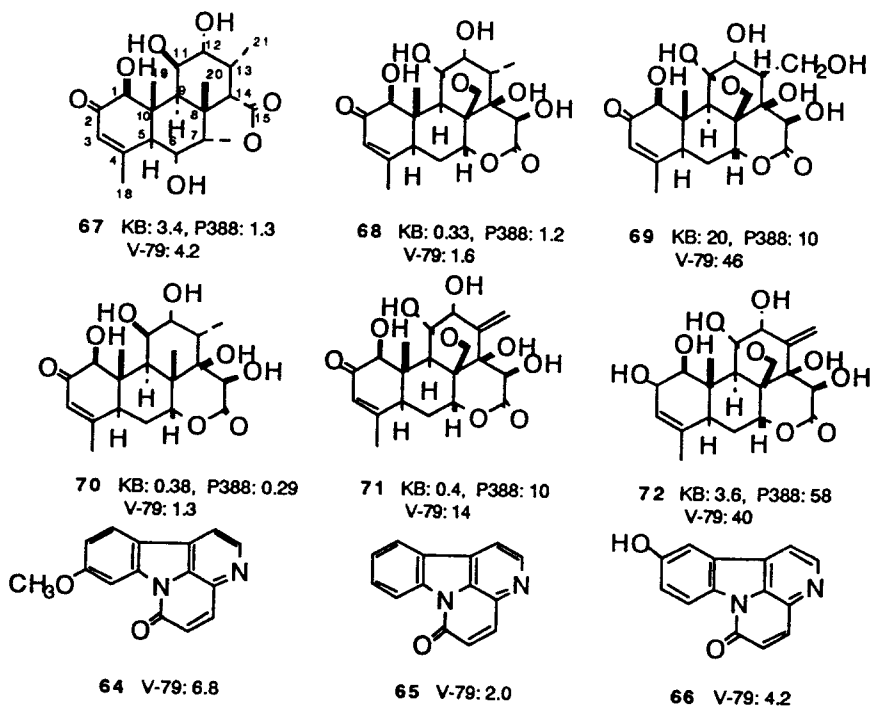


Fig. 3 Cytotoxic Activities of Canthins and Quassinoids against KB, P388 and V-79 Cells

by various spectral data or comparison with various data in literatures. These compounds exhibited cytotoxic activities as can be seen also from Fig.3. From the structure-activity relationships discussed among quassinoids, it has been reported that the partial structures of the C₁-OH, C₁₂-OH, 2-keto-3-ene and oxide-bridge are important in essential features for antileukemic activity.

In this continuing studies on cytotoxic compounds of *E.longifolia*, two unique squalene-type triterpenes, characterized by eight asymmetric carbons and two or three tetrahydrofuran rings, were isolated from the woods of *E.longifolia*. While one of them was identified as the marine meso-triterpene ether, teurilene (73),^{46,48} the other was found to be a new compound, named eurylene (74), whose relative structure was established by spectroscopic data and X-ray analysis. The absolute stereostructure was determined by an advance Mosher's method.⁴⁹

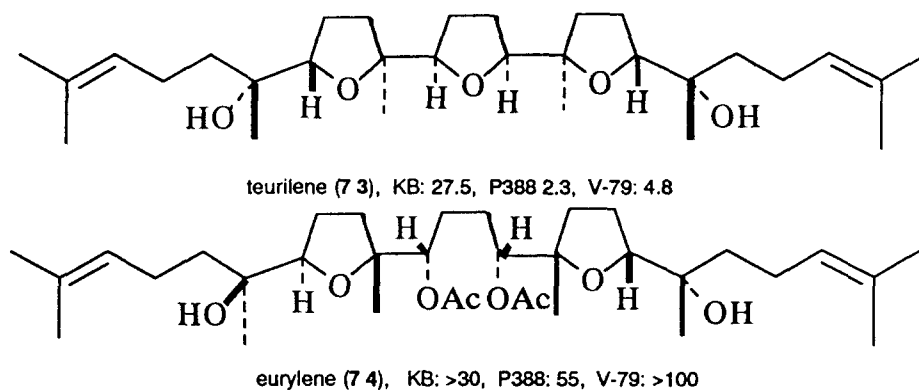


Fig. 4 IC₅₀ Values ($\mu\text{g} / \text{ml}$) of Teurilene and Eurylene

The structures and cytotoxic activities of 73 and 74 are shown in Fig.4. The activities of 73 against V-79, P388 and KB cells were stronger than those of 74. The perspective views of both compounds from their X-ray analyses gave a curvature form in 73⁴⁶) and a linear one in 74.⁴⁵) These molecular forms are presumed to be correlated with the cytotoxic activities from the related compounds.

5. Cytotoxic Diterpenes from *Hedychium coronarium*^{50, 51})

The chloroform extract prepared from the rhizomes of *Hedychium coronarium* Koeng (Zingiberaceae), which is used for rheumatism⁵²), showed a significant effect against V-79 cells and Sarcoma 180A in mice. Fractionation of the chloroform extract was made with the guidance of bio-assay against V-79 cells. The extract was subjected to silica gel column chromatography and separated to seven fractions A - G. A significant cytotoxic activity of the fractions D, E, F and G against V-79 cells led us to isolate the known (E)-labda-8(17),12-diene-15,16-dial (75), six new labdane-type diterpenes, named coronarins A (76), B (77),

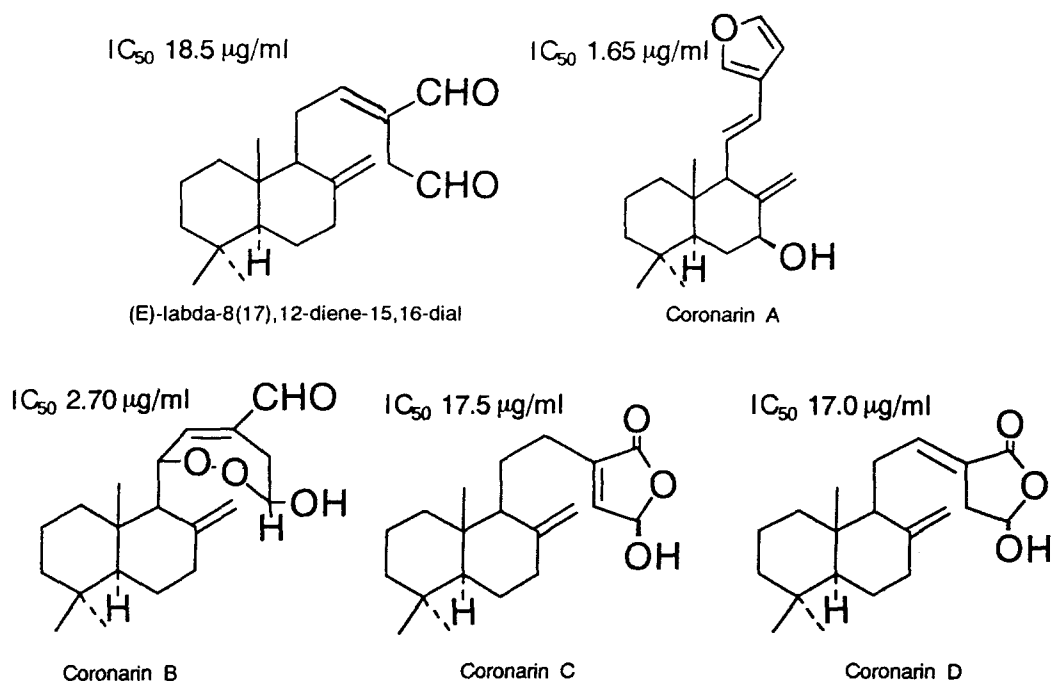


Fig. 5 Diterpenes from *Hedychium coronarium* and Their Cytotoxicity against V-79 Cells

C (78), D (79), E (80) and F (81) by means of repeated chromatography of each fraction. The structures and IC_{50} values of their labdane-type diterpenes against V-79 cells are presented in Fig.5. Coronarins A (76) and B (77) of them exhibited a particularly significant cytotoxic activity.

6. Antitumor Phenylpropanoids from *Alpinia galanga*⁵³⁾

Alpinia galanga Willd. (= *Languas galanga* Stuntz, Zingiberaceae) is growing in south-east Asia, and is widely cultivated in this area. The rhizomes are used for flavouring foods in the preparation of meat dishes and curries^{54,55)} and showed anti-ulcer,⁵⁶⁾ antifungal⁵⁷⁾ and xanthine oxidase inhibitor's activities.⁵⁸⁾ The alcoholic extract prepared from the rhizomes of *A. galanga* (Indonesian name "Lengkuwas") showed a significant effect against Sarcoma 180A in mice. Fractionation of the extract was made with the guidance of above bio-assay as shown in Table IV. Repeated column chromatography of the active n-hexane extract gave 1'-acetoxychavicol acetate (95) as a major antitumor substance. So, with the aim of obtaining the analogs of 95, the MeOH extract from the fruits of *A. galanga* was fractionated in a similar manner as described above to furnish 1'-acetoxyeugenol acetate (96), *trans*-3,4-dimethoxycinnamyl alcohol (99), *trans*-4-methoxycinnamyl alcohol (100) and *trans*-4-hydroxycinnamaldehyde (101). Also, compounds 95, 96, 1'-hydroxychavicol acetate (97) and 1'-hydroxychavicol (98) were synthesized according to the previously outlined procedure.⁵⁶⁾

Table IV Antitumor Activity against Sarcoma 180 Ascites in Mice

	dose (mg/kg/day)	deaths due to toxicity	administration schedule	BWC (g)	PCV /TV	GR (%)	assessment
MeOH extract	100	0	1 - 5	+0.4	0.35	1.8	+++
n-hexane extract (Y: 17)	5	0	1 - 5	+2.4	0.44	122.9	-
	10	3	1 - 3	-0.3	0.30	26.3	++
CHCl ₃ extract (Y: 5)	20	0	1 - 5	+1.2	0.32	103.7	-
residue extract (Y: 78)	80	0	1 - 5	+1.7	0.36	102.2	-
compound 95	5	0	1 - 5	+3.4	0.31	79.3	-
	7	3	1 - 3	+0.1	0.31	54.6	+
	7	0	1 - 2	+0.7	0.52	36.4	++
	10	3	1	+2.4	0.22	26.7	++
	10	3	1 - 2	-1.8	0.40	1.0	+++
compound 96	10	1	1 - 5	+0.2	0.32	10.0	+++
compound 97	10	0	1 - 5	+3.4	0.36	92.5	-
compound 98	10	0	1 - 5	+2.4	0.37	76.3	-
compound 99	10	0	1 - 5	+4.1	0.37	94.7	-
compound 100	10	0	1 - 5	+3.6	0.34	95.7	-
compound 101	10	0	1 - 5	+2.7	0.37	92.6	-

The effectiveness was evaluated by means of the total packed cell volume method. PCV, packed cell volume; TV, total volume; GR, growth ratio = PCV (test groups) / PCV (control groups) x 100; BWC, body weight change = (day 7 weight - TV) / day 0 weight. Y means yield (%) from the MeOH extract.

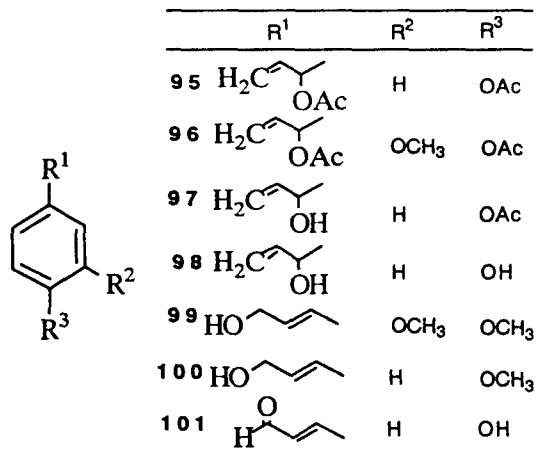


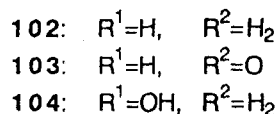
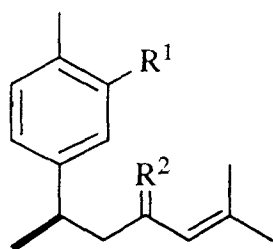
Fig. 6 Structures of Phenylpropanoids from *Alpinia galanga*

Antitumor activity of compounds **95** - **101** against Sarcoma 180A in mice is summarized in Table IV. As can be seen from Table VI, it was evaluated that **96** was a more useful agent than **95** from the viewpoint of antitumor activity and toxicity. The results for **95** - **98** suggested that a 1'-acetoxy group in the chavicol and eugenol analogs was required for appearance of the anti-tumor activity. Therefore, the action mechanism would be estimated as nucleophilic reaction, which

would be caused by transfer of the double bond resulting from elimination of the 1'-acetoxy group. The elimination seems to be regulated by variation of the functional groups attached to the benzene ring.

7. Antitumor Bisabolane Sesquiterpenes from *Curcuma xanthorrhiza*⁵⁹⁾

Curcuma xanthorrhiza (Zingiberaceae, named Temu Lawak in Indonesia) is utilized as a tonic in south-east Asia and as a choleric drug in Europe. The active n-hexane extract against Sarcoma 180A in mice was fractionated by repeated column chromatography to give antitumor bisabolane sesquiterpenes, α -curcumene



Compound	Dose (mg/kg)	BWC	PCV / TV	GR (%)	Assessment
102	10	+4.2	0.31	97	-
	20	+2.1	0.16	31	++
	50	+2.4	0.00	0	+++
103	25	+3.1	0.39	77	-
	50	+2.7	0.12	22	++
104	50	+3.6	0.13	18	++

Fig. 7 Antitumor Activity of **102** - **104** against Sarcoma 180A in Mice

(**102**), ar-turmerone (**103**) and xanthorrhizol (**104**) and the minor related compounds.^{60,61}) The structures and antitumor activity against Sarcoma 180A in mice are shown in Fig.7. α -Curcumene exhibited a dose-dependent effect: (-) at 10 mg/kg, (++) at 20 mg/kg and (+++) at 50 mg/kg. ar-Turmerone and xanthorrhizol showed lower activity (++) at 50 mg/kg than curcumene. On the other hand, curcumene showed no significant activity against P388 lymphocytic leukemia in mice, in the dose range of 50 to 200 mg/kg.

8. A Cytotoxic Alkaloid from *Evodia rutaecarpa*⁶²)

The fruits of *Evodia rutaecarpa* (Rutaceae) is one of the crude drugs in Chinese medicine. The alcoholic extract exhibited a significant effect against V-79 cells (IC₅₀ = 5.2 μ g/ml). The cytotoxic activity on V-79 cells was concentrated in the chloroform sub-extract (IC₅₀ = 5.6 μ g/ml) by partitioning between aqueous solution of the alcoholic extract and each organic solvent. The sub-extract was fractionated with the guidance of bio-assay to give (+)-evodiamine (**106**) and rutaecarpine (**107**) from a cytotoxic fraction. In the cytotoxic test using V-79, KB and P388 cells, it is of interest to note that **106** showed an effective activity, while **107** did not in spite of the similarity of the two structures.

9. An Antitumor Ingerol-type Diterpene from *Euphorbia lathyris*⁶³)

The extract of seeds of *Euphorbia lathyris* (Euphorbiaceae) showed antitumor activity against Sarcoma 180A in mice. Systematic fractionation of the extract led to the characterization of ingenol-3-hexadecanoate (**108**) as an active principle, together with inactive diterpenes ingenol-20-hexadecanoate (**109**) and lathyranes diterpenes.⁶⁴) Though **108** is well known as a tumor-promoting agent, it showed antitumor activity against

Sarcoma 180A in mice. This result indicates a paradoxical action, which is cocarcinogenic and antitumor, of the diterpene esters of the Euphorbiaceae.

10. Cardenolides and Pregnanes from Antitumor Fraction of *Periploca sepium*⁽⁶⁵⁻⁷⁰⁾

The crude drug "beiwujiapi", the root bark of *Periploca sepium* (Asclepiadaceae) is one of the famous "wujiapi" in Chinese literature and has been widely used as a tonic. When the chloroform extract obtained by partitioning the methanolic extract of *P. sepium* between water and chloroform was subjected to column chromatography on silica gel using the solvent system of benzene, benzene-CHCl₃, CHCl₃, CHCl₃-MeOH (10:1) and (1:1) successively, the antitumor activity against Sarcoma 180A in mice was concentrated in the fraction eluted with CHCl₃-MeOH (10:1). Only this fraction exhibited powerful antineoplastic activity (growth ratio: 4.6%, +++) at the dose of 10 mg/kg/day for 5 consecutive days and was a mixture consisted of pregnanes, cardenolides and their glycosides. The antitumor activity of each compound was weaker than that of the CHCl₃-MeOH (10:1) fraction.

11-1. Antineoplastic Components from *Rubiae Radix*⁽⁷¹⁻⁷⁷⁾

Rubiae Radix is originated to Rubiaceae plants *Rubia akane* in Japan, *R. cordifolia* in China and *R. tinctorum* in Europe. Two of the former showed antineoplastic activity, but the latter one did not show activity. From the ancient times, *Rubiae Radix* has been mainly used as antipyretic, homeostasis and tonic. Further in China, it is useful clinically as a component of prescriptions for cancer of uterine cervix. Many pigments were isolated from *Rubia* spp. Ruberitorin, which is a kind of alizarin glycoside, purpurin glycoside, rubiadin glycoside are contained in *R. tinctorum*. Purpurin, morigin, alizarin, rucidin, primeroside, ruberitorin and anthraquinone etc. are found in oriental *Rubia* spp. However, these pigments were assumed to be non antineoplastic constituents.⁽⁷¹⁾

Because the extract of *Rubiae Radix* showed antineoplastic activity against Sarcoma 180A, the compounds were pursued as the active principles. After repeating fractionation and purification of extract, some oligopeptides were obtained as active principles against P388 leukemia. The extract was partitioned with water and benzene, and water and ethyl acetate. From the both of benzene and ethyl acetate fractions, seven components were isolated as crystal, and named as RA-I - VII after *R. akane*.

Recently, the structure of RA-VI was decided. Moreover, RA-VIII, RA-IX, and RA-X were also obtained, and the structures of them were elucidated.

11-2. Structural Elucidation of RA-Series of Compounds

These compounds were assumed to be small peptides from the IR data showing 3390, 1640 cm⁻¹ due to amide bonding. It was found the data of ¹³C-NMR of RA-VII showing that there were three of C-Me, three

of -CH₂-, three of N-Me, two of O-Me, six of CH, eighteen of aromatic carbons, eleven of tertiary carbon, seven of quaternary carbons (three of C-C and four of C-O bonds), six of carbonyl carbons.

By hydrolysis of RA-VII, there were obtained some amino acids, D-alanine, two molecules of L-alanine, N-methyl-4-methoxy-L-phenylalanine, and N-methyl-tyrosine dimer having ether linkage. Thus, the derivatives having tyrosine moiety were identified by deriving to acetate and methylate. Then it was assumed to

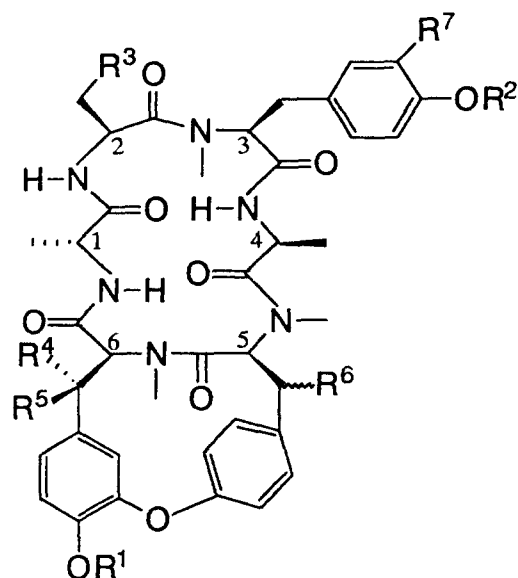


Fig. 8

be cyclic hexapeptide consisted of three alanine and three molecules of tyrosine derivatives. 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 a two-cyclic hexapeptide having ether linkage. However, it was difficult to decide the sequence of amino acids 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.8.

Recently, the structures of RA-VI and RA-VIII were elucidated. RA-VI was elucidated as the configurational isomer of RA-III at the moiety of O-methyl-D-tyrosine (Tyr-3). RA-VIII has L-threonine instead of L-serine in RA-III molecule. However, RA-VII and RA-V were noticed to be main components in these oligopeptides.

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-VI	Me	Me	OH	H	H	H	H
RA-VII	Me	Me	H	H	H	H	H
RA-VIII	Me	Me	Me,OH	H	H	H	H

Bouvardia ternifolia

bouvardin	H	Me	H	H	H	β-OH	H
deoxybouvardin (RA-V)	H	Me	H	H	H	H	H

microbial transformation products

O-desmethyl -bouvardin	H	H	H	H	H	β-OH	H
bouvardin -catechol	H	H	H	H	H	β-OH	OH

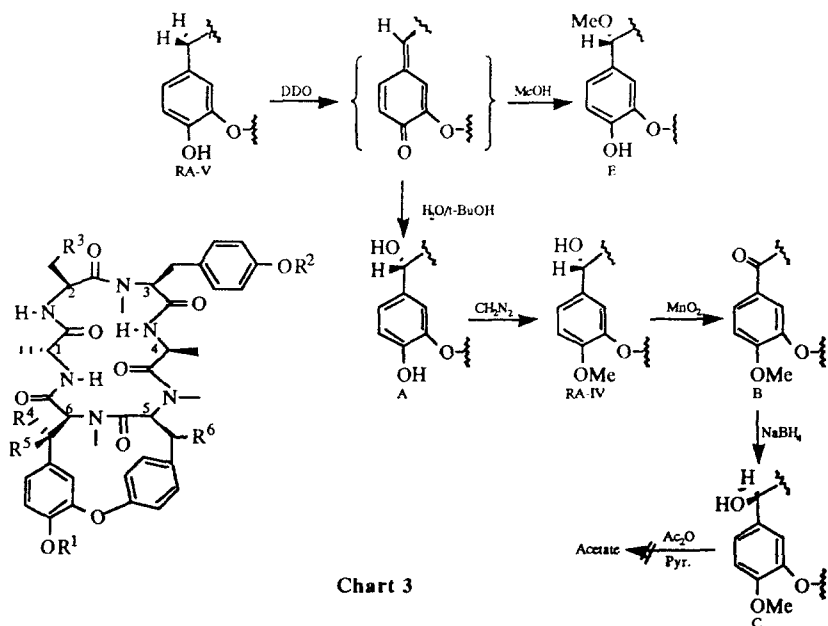
On the other hand, Cole *et al* had isolated bouvardin type oligopeptides from Bouvardia ternifolia (Rubiaceae). RA-V is same compound with deoxybouvardin isolated from same plant.¹⁵⁾

10-3. Cytotoxicity and Antineoplastic Activity^{75,76)}

The cell growth inhibitory effects were examined against KB cells, P388 lymphocytic leukemia cells and MM2 mammary carcinoma cells by using the lead compound RA-VII and n-hexylether derivative, which had shown the strongest antitumor activity *in vivo* assay. RA-V and the n-hexylether showed clear growth inhibitory effects at concentrations higher than $1.85 \times 10^{-3} \mu\text{g/ml}$ and $7.50 \times 10^{-3} \mu\text{g/ml}$, respectively, in KB cells, $1.15 \times 10^{-2} \mu\text{g/ml}$ and $6.40 \times 10^{-3} \mu\text{g/ml}$ in P388 cells, and $4.40 \times 10^{-2} \mu\text{g/ml}$ and $9.60 \times 10^{-3} \mu\text{g/ml}$ 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 dose-dependency. Under microscope, mitomycin C-treated KB cells showed enlargement of the nuclei, deformation of the cells and abnormality of nuclei, whereas KB cells treated with RA-V and its n-hexylether derivative showed globularization as compared with control cells.

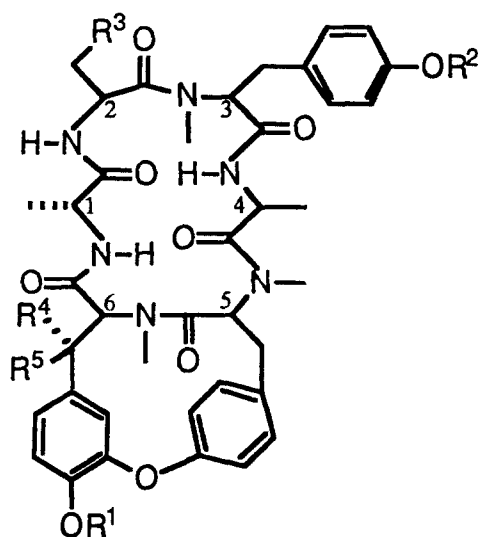
RA-IV was considered to have an additional alcoholic hydroxyl group as compared with RA-VII. It was concluded that the hydroxyl group in RA-IV is linked to the β -carbon ($C\beta$) of Tyr-6 by comparing the ^{13}C chemical shift values of RA-IV with those of RA-VII; $C\beta$ signal at δ 35.56 (t) due to Tyr-6 of RA-VII was shifted down field 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 Chart 3. 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 anhydrous acetic acid pyridine at room temperature. The above results can be reasonably explained by the following stereochemical consideration: the reagent in this series of reactions can approach only from the α -side, because the β -side at the benzyl location of Tyr-6 is strongly blocked by the N-methyl group of this tyrosine moiety as from the X-ray conformation. Consequently, the hydroxyl group of RA-IV was determined to have S configuration.

We also examined the antineoplastic activity of six native cyclic hexapeptides (RA-I, II, III, IV, V and VII) and seven related compounds (A - E) against P388 lymphocytic leukemia in mice. The mice received 10 mg/kg/day (except for RA-VII and RA-III: 4.0 and 2.0 mg/kg/day) *i.p.* for 5 consecutive days. The antineoplastic activities are shown in Fig.9. 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, compound A, A-diAc, E, E-Me and E-Ac, whose α -proton at the C β -position of Tyr-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 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.



	R ¹	R ²	R ³	R ⁴	R ⁵	Antitumor activity ^{a)} T/C (%)
RA-I	H	Me	OH	H	H	169.3
RA-I-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-VI	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	=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 : 10⁶ cells/0.1 ml, i. p., CDF1 mice (n = 6).

Dose : 10.0 mg/kg., i. p., day 1-5.

b) Dose : 2.0 mg/kg.



c) Dose : 4.0 mg/kg.

Fig.9 Structures and Antitumor Activities of Native Cyclic Hexapeptides and Related Compounds

11-4. Relationships between Structure and Activity^{74,76,77)}

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-

Table V Antitumor Activities on P-388 Lymphocytic Leukemia and Toxicities of Ether Derivatives of RA-V

Comp.	R	T/C (%)				Toxicity ^{f)} Dose (mg/kg)							
		0.05 mg/kg	0.5 mg/kg	2.0 mg/kg	4.0 mg/kg								
No.													
1	H (RA-V)	131.1 ^{c)}	152.5 ^{c)}	164.2 ^{c)}	165.3 ^{c)}					20	30	40	50
										0/7	2/7	5/7	5/5
2	CH ₃ (RA-VII)	138.6 ^{c)}	156.7 ^{c)}	164.2 ^{c)}	173.6 ^{b)}	10	15	20	30	0/3	3/3	3/3	3/3
3	CH ₂ CH ₃	137.3 ^{c)}	165.4 ^{c)}	162.2	Toxic	5	10			1/3	3/3		
4	(CH ₂) ₂ CH ₃	138.4 ^{c)}	146.0 ^{a)}	93.7	Toxic	5	10			1/3	3/3		
5	CH(CH ₃) ₂	142.2 ^{b)}	175.1 ^{c-e)}	105.4	Toxic	5	10			3/3	3/3		
6	(CH ₂) ₃ CH ₃	133.0 ^{c)}	144.9 ^{a)}	Toxic	Toxic	5	10			0/3	3/3		
7	(CH ₂) ₄ CH ₃	122.2 ^{c)}	142.7 ^{c)}	165.4 ^{c)}	Toxic	5	10	20		0/3	0/3	3/3	
8	(CH ₂) ₅ CH ₃	110.3 ^{b)}	137.3 ^{c)}	153.5 ^{c)}	173.0	10	20	30		0/3	1/3	3/3	
9	(CH ₂) ₆ CH ₃	115.8 ^{c)}	144.7 ^{c)}	150.1 ^{c)}	164.0 ^{b)}								
10	(CH ₂) ₇ CH ₃	136.1	146.8 ^{c)}	162.9 ^{c)}	152.2 ^{b)}								
11	(CH ₂) ₈ CH ₃	112.5 ^{b)}	141.5 ^{b)}	150.1 ^{c)}	155.4 ^{b)}								
12	(CH ₂) ₉ CH ₃	101.0	120.2 ^{b)}	132.7 ^{c)}	137.5 ^{a)}								
13	(CH ₂) ₁₀ CH ₃	115.4	108.7	121.2 ^{b)}	123.1 ^{c)}								
14	(CH ₂) ₁₁ CH ₃	93.0	101.0	105.8	112.5 ^{a)}								
15	(CH ₂) ₁₂ CH ₃	93.0	99.0	115.4	125.0 ^{c)}								
16	(CH ₂) ₁₇ CH ₃	101.0	98.1	101.0	108.7								
17		126.5	162.2	164.3 ^{b)}	Toxic								
18		127.6	140.5 ^{a)}	149.2 ^{c)}	143.8								

Significantly different from control at a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.
from RA-V at d) $p < 0.05$, from RA-VII at e) $p < 0.05$.

f) Toxicity: number dead/number tested.

activity relationship (QSAR) were investigated from the viewpoint of molecular hydrophobicities. The activity values ($\log 1/IC_{50}$) 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 relationship among the ILS (150 and 160%), the minimum lethal dose (MLD) and hydrophobic coefficient of the ether series of RA-V were analyzed according to both the Hansch-Fujita model, and the bilinear 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

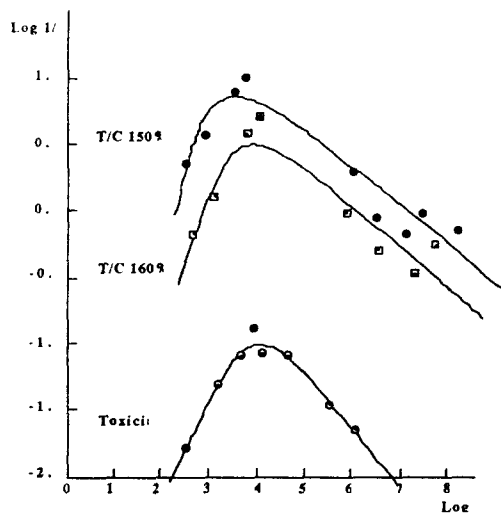


Fig. 10 Structure-Antitumor Activity and Toxicity Relations of Alkyl Ethers of RA-V on P-388 Leukemia

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. Thus, RA-VII and the n-hexylether of RA-V should be useful compounds on this basis.

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 be inhibition of protein biosynthesis, since ^3H -leucine was not taken in

Table VI Therapeutic Effects of RA-700 on P388 Leukemia

Group	Dose (mg/kg)	Route	Survival effects		B. W. (g)	
			Survival time (d, mean \pm S. E.)	T/C (%)		
Control	10 ml	i. p.	10.1 \pm 0.18		100.0	+5.0
RA-700	0.005	i. p.	11.0 \pm 0.26		109.2	+3.8
	0.01	i. p.	13.3 \pm 1.15		132.3	+2.6
	0.05	i. p.	15.5 \pm 1.12		153.8	+2.1
	0.5	i. p.	16.7 \pm 0.33		165.4	+1.3
	2.0	i. p.	18.6 \pm 1.21		184.3	+0.4
	4.0	i. p.	23.6 \pm 2.62 ^{a)}		234.2	-0.6
	6.0	i. p.	6.00 \pm 2.61		62.7	
MMC	0.005	i. p.	10.8 \pm 0.31		107.5	+4.8
	0.01	i. p.	10.5 \pm 0.22		104.2	+5.2
	0.1	i. p.	13.7 \pm 0.67		135.6	+2.8
	0.5	i. p.	15.8 \pm 0.31		157.1	+0.8
	1.0	i. p.	18.0 \pm 0.68		178.1	-0.3
	2.0	i. p.	12.7 \pm 0.33		125.7	-1.8
Control	10 ml	i. v.	9.50 \pm 0.15		100.0	+4.0
RA-700	0.25	i. v.	10.0 \pm 0.27		105.3	+3.2
	1.0	i. v.	11.0 \pm 0.19		115.8	+1.9
	2.5	i. v.	13.4 \pm 0.18		140.8	-0.3
	4.0	i. v.	14.5 \pm 1.25		152.6	-2.0
	6.0	i. v.	15.9 \pm 0.23		167.1	-4.7
MMC	0.1	i. v.	10.5 \pm 0.19		110.5	+4.4
	0.5	i. v.	12.5 \pm 0.19		131.6	+2.3
	1.0	i. v.	13.6 \pm 0.18		143.4	+0.9
	2.0	i. v.	12.1 \pm 0.13		127.6	-3.6

a) 1/6 animal survived 60 d. P388 was implanted i. p. (1×10^6 cells/0.1 ml) in CDF1 mice at day 0. Drugs were given daily at indicated doses for consecutive 9 d from day 1 to 9.

The lethal effect of RA-V on KB cells was clearly different from that of MMC, and RA-V was concluded to be a "time-dependent drug" like vincristine. Further, RA-VII was effective to Colon 38 (*s.c. - i.p.*, *s.c. - i.v.*), P388 (*i.p. - i.v.*), L1210 (*i.p. - i.v.*), Meth A (*s.c. - i.v.*), M5076 (*i.p. - i.p.*). The inhibition was found from the effectiveness to B16-BL-6 (*s.c. - i.p.*, *s.c. - i.v.*).

RA-V is the compound with deoxybouvardin. Bouvardin has been investigated to develop as an antitumor drug in NCI of U.S.A. Adryamycin has $-CH_2OH$ in its molecule instead of $-CH_3$ in daunomycin. Even only such chemical differences, adryamycin revealed more strong activity and less toxicity than daunomycin. So, it is also expected that RA-VII will show the different activity from that of bouvardin. RA-VII (RA-700) is now under investigation for Phase I clinical trials in NCI in Japan.

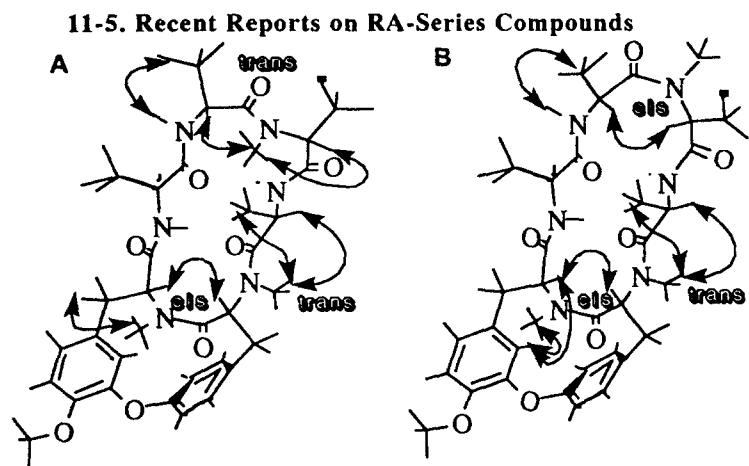


Fig. 11 NOE enhancements in conformers A and B of RA-VII
The arrows show the NOE relationships confirmed by 1D-NOE and NOESY experiments in $CDCl_3$ at 303K.

Conformational analysis of an antitumor cyclic hexapeptides, RA and its analogues was conducted by the spectroscopic and computational chemical methods. A combination of different homo- and heteronuclear 2D NMR techniques at 500MHz have enabled us to perform complete assignment of the 1H and ^{13}C signals of

the two conformers A and B of RA-VII in $CDCl_3$. The Structures of the three conformers (A, B and C) in $DMSO-d_6$ were also determined by 2D-NMR techniques, temperature effects on NH protons and NOE experiments. Distances deduced from the NMR measurements were used for the refinements by the restrained molecular dynamics calculations using AMBER program. These conformational analysis showed that these conformers were caused by geometrical isomerization and that the predominant conformer A exhibits a typical type II β -turn structure, which is similar to the crystal structure analyzed by the X-ray diffractions. The reduced biological activity of the N-methyl derivative of RA-VII in comparison with RA-VII may be responsible for the more weakly populated conformer A in solution. Further, the presence of a highly strained 14-membered ring

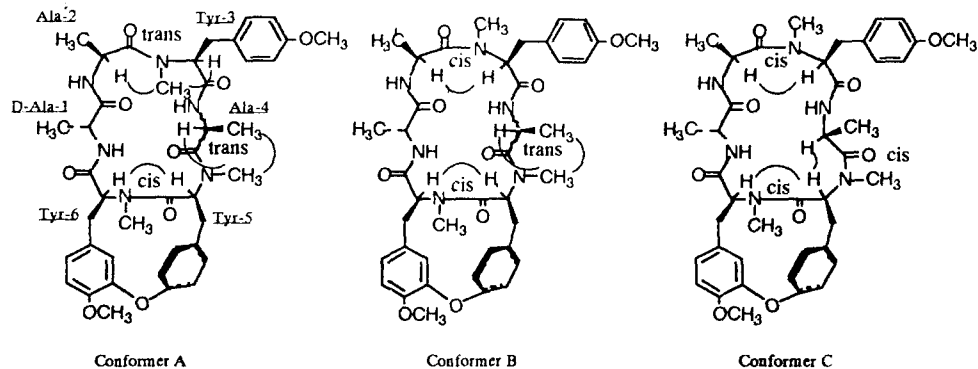
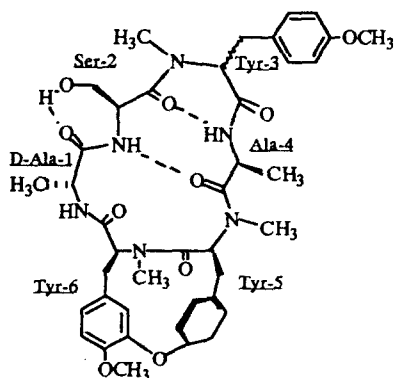


Fig.12 Molecular structures of three different conformers A, B and C of RA-VII in DMSO- d_6 . The arrows show the NOE relationships confirmed by NOESY/CPHARPH experiments.

was necessary to maintain the typical type II β -turn structure of conformer A, and the ring system and turn structure were considered to play an important role in its antitumor activity.⁷⁸⁾

Using $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ experiments, discernible conformational isomers observed in DMSO- d_6 for RA-VII. The largest isomer amounting to 64%, has been assigned as conformer A with only a *cis* conformation between Tyr-5 and Tyr-6. The second conformational isomer, accounting 32%, has adopted *cis* conformations between both Tyr-5 and Tyr-6, and between Ala-2 and Ala-3. The third isomer, accounting to 4%, was determined to have *cis* conformations for all of the three N-methyl amide bond.⁷⁹⁾



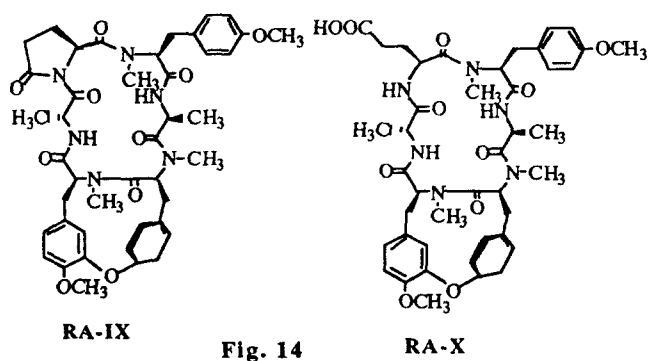
RAI-III : L-Tyr-3
RAI-VI : D-Tyr-3
Fig.13. Structures of RAI-III and VI.

A molecular design was carried out in a way that locked the type II β -turn confirmation of RA-VII, by removing the N-methyl group of the Tyr-3 residue. Conformational analysis of [N-demethyl-Tyr(OCH $_3$)-3]RA-VII, by hepatic microsomal biotransformation, was conducted by 2D-NMR techniques, temperature effect on NH protons, and NOE experiments. It showed a restricted conformational state with a typical type II β -turn structure between Ala-2 and Tyr-3 in solution.⁸⁰⁾ On the other hand, by the conformational analysis of RA-III and VI using spectroscopic and computational chemical methods, they were shown to have γ -turn structures at residues 2, 3 and 4, which were

stabilized by a hydrogen bond between Ser-2-OH and D-Ala-1-CO.⁸¹⁾

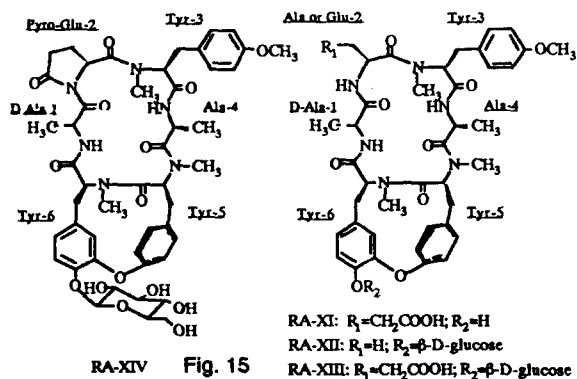
Recently, many other RA-series components were isolated from *R.cordifolia*. The structures of new antitumor hexapeptide RA-VI and RA-VIII were elucidated. A combination of 2D-NMR techniques and NOE

relationships showed that amino acids constituting the β -turn of RA-VI are Ser-2 and D-Tyr-3 and those of RA-VIII, Thr-2 and those of RA-VIII, Thr-2 and Tyr-3. By the conformational analysis of RA-VI in its crystalline state using the X-ray diffractometric technique, RA-VI was shown to have, in its solid state, a type V β -turn structure at the residues Ser-2 and D-Tyr-3, while other RAs have type II β -turns. Further, by 2D-NMR techniques, temperature effects on NH protons and NOE experiments. In solution of CDCl₃, RA-VI was shown to exist only as conformer A and RA-VIII as conformers A, B and C. The difference between solid and solution state conformations of RA-VI was also shown the refinement the restrained molecular dynamics calculations using AMBER program. RA-VIII, having a small population of conformer A with type II β -turn than other RAs, showed a reduced biological activity, and the N-methyl derivative of RA-VIII, whose conformer A content is further reduced, gave further reduced activity, suggesting that conformer A contributes to the activity. However, RA-VI existing in solution 100% as conformer A, showed a very low activity and N-methylation increased the activity. This shows that the stereochemistry and molecular mobility of the aromatic



side chain of Tyr-3 over this turn, as elucidated by the ¹³C spin lattice relaxation time, plays a more important role in the antitumor activity of the compounds of this series in addition to the type II β -turn structure.⁸²⁾

Moreover, RA-IX and -X were also added to these RA-series obtained from same *R. cordifolia*. The structures of them were determined by the spectroscopic and chemical methods. RA-IX contained pyroglutamic acid portion instead of Ala-2 in the molecule of RA-VII, and RA-X had glutamic acid instead of Ala-2 in the same compound.⁸³⁾ Further, four new bicyclic hexapeptides, RA-XI, -XII, -XIII and -XIV were isolated from same plant and showed potent antitumor activity against P388.



The structures were elucidated from spectroscopic and chemical evidences.⁸⁴⁾

12. Conclusion

Recently, we have isolated many other compounds from Rubia cordifolia that is; anthraquinones, naphthoquinones and naphthohydroquinones,⁸⁵⁻⁸⁷) and triterpenoids.^{88,89}) However, these compounds are assumed to be non active components against various tumors for the present.

At the present it is better method to obtain RA compounds from plant material than synthesis, for supplying the samples for clinical trials. The yield obtained by synthesis is not yet so good and the steps should be improved more briefly. Concerning to the antineoplastic agent isolated from higher plants, there are many kinds of compounds to follow as the candidates for the clinical trials. It might be expected some strong anticancerous agents will be come out later.

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