ANTITUMOR SUBSTANCES FROM HIGHER PLANTS

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Abstract - In this review, we summarize the isolation, structural determination and bio-activity of antitumor and cytotoxic substances from higher plants which were collected in Japan, China, Korea, Southeast Asia and South America by us.

1. Introduction

To date many kinds of compounds have been obtained from plant 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 agents of blood sugar. When the extract of this plant was given non-orally, leucopenie 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 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, camptothecine isolated from Camptotheca acuminata is also an antineoplastic alkaloid, but is very toxic. Chemical modification has been tried to decrease its toxicity. This compound will be permitted to use as a 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 substancial anticancer activity in patients. Supply of this drug has severely limited for full exploration of its antineoplastic potencial. Some efforts are continued at 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 clinically 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 America[25-27] 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 antitumor and cytotoxic substances of the higher plants selected from above screening tests.

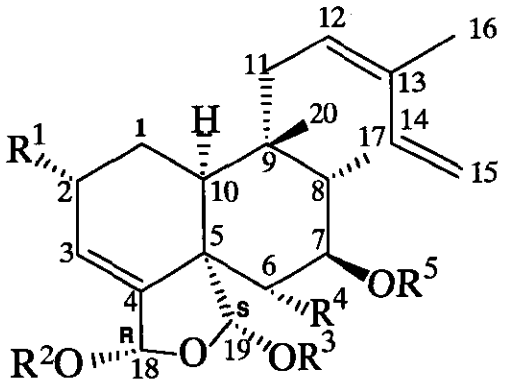
In 1982, it was given a definition for expression of activity, that is, the word cytotoxicity must be used only for *in vitro* activity, the words antineoplastic and antitumor must be used only for *in vivo* test using animal. We should call anticancer, when it shows activity in clinical trials of human.[12]

2. Antitumor Clerodane Diterpenes from Casearia sylvestris[28-30]

Casearia sylvestris Sw. (Flacourtiaceae), Paraguayan name "Burro-Kaa" and Brazilian name "Guassatonga", is one of the South American medicinal plants and is used as a tonic and an antispasmodic in native peoples. Its chromatographic purification with the guidance of bioassay against Sarcoma 180A in mice led us to isolate new antitumor clerodane diterpenes, named casearins A - F (**1** - **6**). The further investigation of the active fractions led to the isolation of minor clerodane diterpenes, named casearins G - R (**7** - **18**).

The basic skeleton of casearin A (**1**) was elucidated by ^{1}H-^{1}H and ^{1}H-^{13}C correlated spectroscopy (COSY) and 2D-incredible natural abundance double quantum transfer experiment (INADEQUATE). The positions of ester functions were determined by the ^{1}H-^{13}C long range COSY (COLOC) spectrum. The relative structure of casearin A (**1**) was established on the basis of the coupling constants of each proton and the nuclear Overhauser effect (NOE) correlated spectrum. The structures of casearins B - R (**2 - 18**) were determined by comparing

Figure 1 Structures of Casearins and and Cytotoxic Activities against V-79 Cells



Casearins	R^1	R^2	R^3	R^4	R^5	IC_{50}(μmol/l)*
A(1)	OCH_3	$COCH_3$	$COCH_3$	OH	$CO(CH_2)_2CH_3$	1.0
B(2)	OCH_3	$COCH_3$	$COCH_3$	$OCOCH_3$	$CO(CH_2)_2CH_3$	8.5
C(3)	OH	$COCH_3$	$COCH_3$	$OCOCH_3$	$CO(CH_2)_8CH_3$	0.77
D(4)	OH	$CO(CH_2)_2CH_3$	$COCH_3$	OH	$CO(CH_2)_2CH_3$	1.8
E(5)	OH	CH_2CH_3	$COCH_3$	OH	$CO(CH_2)_8CH_3$	4.7
F(6)	OH	CH_2CH_3	$COCH_3$	OH	$CO(CH_2)_2CH_3$	29
G(7)	OCH_3	$COCH_3$	$COCH_3$	H	$CO(CH_2)_2CH_3$	0.17
H(8)	OH	$COCH_3$	$COCH_3$	H	$CO(CH_2)_2CH_3$	0.37
I(9)	OH	$COCH_3$	$CO(CH_2)_2CH_3$	H	$CO(CH_2)_2CH_3$	0.51
J(10)	OCH_3	$CO(CH_2)_2CH_3$	$COCH_3$	OH	$CO(CH_2)_2CH_3$	1.1
K(11)	$OCOCH_3$	$COCH_3$	$COCH_3$	OH	$CO(CH_2)_2CH_3$	0.52
L(12)	OCH_3	$CO(CH_2)_2CH_3$	$COCH_3$	$OCOCH_3$	H	1.6
M(13)	OH	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	$OCOCH_3$	H	1.8
N(14)	OCH_3	$COCH_3$	$CO(CH_2)_2CH_3$	$OCOCH_3$	$CO(CH_2)_2CH_3$	5.9
O(15)	OCH_3	$CO(CH_2)_2CH_3$	$COCH_3$	$OCOCH_3$	$CO(CH_2)_2CH_3$	6.0
P(16)	OCH_3	$COCH_3$	$COCH_3$	$OCOCH_3$	$COCH_3$	7.8
Q(17)	OH	$COCH_3$	$COCH_3$	$OCOCH_3$	$CO(CH_2)_2CH_3$	4.3
R(18)	=O	$COCH_3$	$COCH_3$	OH	$CO(CH_2)_2CH_3$	5.4
Aa(21)	OCH_3	$COCH_3$	$COCH_3$	=O	$CO(CH_2)_2CH_3$	0.55
Ab(22)	OCH_3	$COCH_3$	$COCH_3$	$OCOCH_2CH_3$	$CO(CH_2)_2CH_3$	17
Ac(23)	OCH_3	$COCH_3$	$COCH_3$	$OCO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	38
Da(24)	=O	$CO(CH_2)_2CH_3$	$COCH_3$	=O	$CO(CH_2)_2CH_3$	19

* Cytotoxic activities against V-79 cells

their spectral data with those of casearin A (**1**). Also, the absolute structures of casearins shown in Figure 1 were confirmed by the X-ray analysis of casearin B (**2**) and the exciton chirality method of the allylic benzoate derivative of casearin C (**3**).

Cytotoxic activities (IC_{50} value) of casearins A - R (**1** - **18**) against V-79 cells are listed in Figure 1, and classified in several groups, groups I - IV, by remarking the point of substituent groups at C-6. Group I which showed the strongest activity includes casearins G (**7**), H (**8**) and I (**9**), whose structures were characterized by the lack of oxygen-bearing function at C-6. Casearins J (**10**) and K (**11**), whose structures were characteristic of a hydroxyl moiety at C-6, belong to the next group II and possess similar or slightly weaker activity than group I. The structures of casearins L (**12**) and M (**13**) are characterized by possession of a hydroxyl moiety at not C-6 but C-7, and this group III had a little weaker activity than group II. The other casearins N (**14**), O (**15**), P (**16**), Q (**17**) and R (**18**) belong to the last group IV which showed only weak activity. Further, this activity was found to be little influenced by whether the substituents of R^1 (C-2) were methoxyl, hydroxyl or oxygen. By this classification, casearins A (**1**) and D (**4**) belong to group II and casearin B (**2**) belong to group IV. The only exception among these groups is casearin C (**3**), whose structural features include possession of decanoate substituent at C-7 and which belongs to group IV. Casearin C (**3**) exhibited about ten-fold more activity than the other casearins in group IV and its hydrophobicity is considered to be remarkably larger than those of the other casearins. For this reason, affinity for the membrane of V-79 cells may increase and exhibit strong activity.

Then, to investigate the influence of substituent groups at C-6, the derivatives A^a (**19**), A^b (**20**), A^c (**21**) and D^a (**22**) at R^4 were prepared from casearins A (**1**) and D (**4**). As shown in Figure 1, the introduction of acyl substituent at C-6 was found to cause marked weakness in the activity. These results support that bulkiness of the substient at C-6 has the greatest influence on the activity. Also, the antitumor activities of casearins A (**1**), B (**2**), C (**3**) and F (**6**), which are major constituents in diterpenes of C. sylvestirs, against Sarcoma 180 ascites in mice are summerized in Table I. Casearin C (**3**) showed the best effect in this bio-assay.

Table I Antitumor Activity of Casearins A, B, C and F against Sarcoma 180A in Mice

Casearin	Dose	BWC	PCV/TV	GR(%)	Assessment
A	15	-3.0	0.10	4.0	+++
B	15	+3.6	0.36	76.9	-
C	15	-1.9	0.07	1.9	+++
F	15	+2.4	0.33	81.3	-

The effectiveness was evaluated by means of the total packed cell volume method. Dose, mg/kg/d for 5 consecutive day; BWC, body weight change = (day 7 weight - TV) / day 0 weight; PCV, packed cell volume; TV, total volume; GR, growth ratio = PCV (test group) / PCV (control group) x 100.

3. An Antitumor Morphinane Alkaloid, Sinococuline, from Cocculus trilobus and the Related Compounds[31-33]

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 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,[34] however, it was confirmed by measuring the CD spectrum (positive maximum at 238 nm).[35] Further, in order to determine the absolute configuration using the exciton chirality

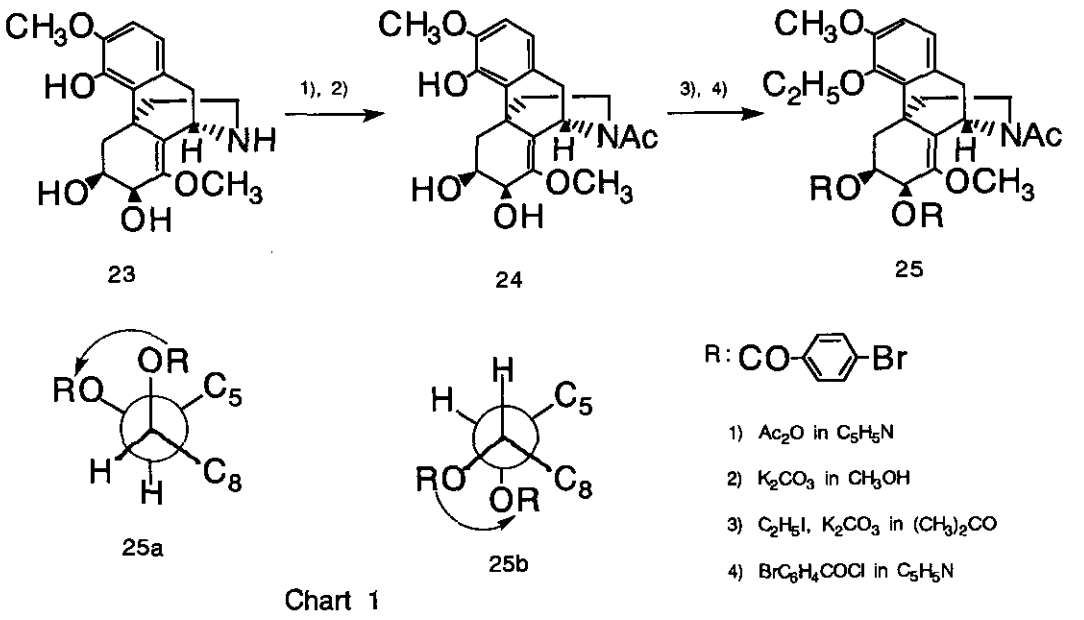


Chart 1

rule,[36] 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. These facts suggested both structures (**25a**) (6S, 7S) and (**25b**) (6R, 7R) in Chart 1. However, the NOE between H_6 and H_{15} 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 (**23**: 40 mg/kg/d dose for 5 consecutive days, GR (growth ratio = T/C): 56% (+)) and P388 leukemia in mice shown in Table II. Also, these various derivatives were prepared and

applied to P388 in vivo test, however, any more effective substance than sinococulin (**23**) could not be obtained.[34]

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

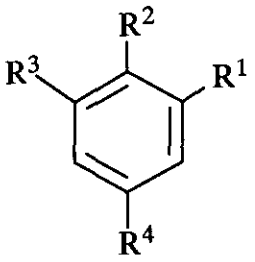
Compounds	Dose (mg/kg)	Survival Time (d, mean + S.E.)	T/C (%)	BWC (g)
	10	12.5 + 0.48	154.6	+0.9
23	25	13.5 + 0.34	167.0	+0.6
	50	14.3 + 0.49	177.0	-0.6
	100	16.2 + 1.92	200.0	-4.7
26	10	11.3 + 0.21	140.2	+0.9
	25	12.7 + 0.42	156.7	-1.1
	6.25	13.8 + 0.31	131.7	+0.8
27	12.5	14.5 + 0.50	138.1	+0.1
	25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

4. Antitumor Long-Chain Phenols from Ginkgo biloba [38,39]

Ginkgo biloba L. (Ginkgoaceae) is a tree from 30 to 40 m in hight 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 Figure 2. Also, the antitumor activity of them was summerized in Table III. This results a speculation that the antitumor activity of long-chain phenols against Sarcoma 180A in mice appears not to require the carboxyl group.

Further, a bioassay based on the cytotoxic activity against Chinese humster 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 III.



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

Figure 2 The Structures of Compounds **28a-30a'**

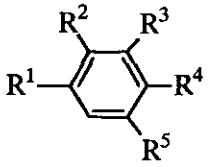
Thirty long-chain phenol derivatives, which were divided into six groups consisting of five compounds having the same aromatic ring contribution and a different alkyl side chain moiety, were synthesized by Grignard reaction of alkyl bromide and hydroxybenzaldehyde in the usual way. Each compound was tested for cytotoxic activity against V-79 cells and each IC_{50} 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 method[40] as the hydrophobic parameter. As the electronic parameter, the energy of the lowest unoccupied molecular orbital (E_{LUMO}) was calculated by using the modified neglect of diatomic differential overlap (MNDO) method,[41,42] because Hammett's substituent constants[43,44] were not suitable for both ortho- and disubstituted aromatic rings.

Table III 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 IV. 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.

Table IV Structures and Parameters for Multiple Regression Analysis



Compound HOMO	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_7H_{15}	OH	H	H	H	63.5	-	192	1.16	4.45	0.142	-8.86
A-9 (3 2)	C_9H_{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_5H_{11}	H	OH	H	H	50.0	-	164	-	3.13	0.135	-8.88
B-9 (3 7)	C_9H_{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_7H_{15}	H	H	OH	H	82.4	-	192	1.43	4.15	0.179	-8.82
C-9 (4 2)	C_9H_{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_7H_{15}	OH	OH	H	H	48.8	-	208	1.76	3.59	0.020	-8.60
D-9 (4 7)	C_9H_{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_7H_{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_9H_{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_5H_{11}	H	OH	OH	H	37.5	-	180	1.57	3.13	0.098	-9.06
F-9 (5 7)	C_9H_{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				

ED_{50} in mM, E_{LUMO} and E_{HOMO} in eV.

Then, as electronic parameters, 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

$$-\log IC_{50} = -4.47E_{LUMO} + 1.97 \qquad (1)$$

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

and many workers have discussed the charge transfer interaction between a drug and its receptor using the E_{LUMO} values.[45] 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 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.

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

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} value.[46]

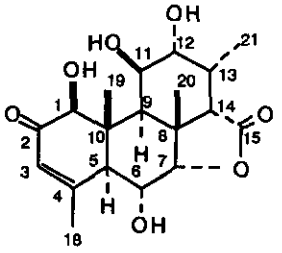
Among the synthesized long-chain phenols (**31** - **60**), the activity of **59** was stronger by about 10 times than others agaist V-79 cells, and **59** also showed antitumor activity against Sarcoma 180A in mice at a low dose, 10 mg/kg/d. Natural compounds (**61** - **63**) from G.biloba did not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

5. Cytotoxic Quassinoids, Linear Triterpenes and Canthin Alkaloids from Eurycoma longifolia[47,48]

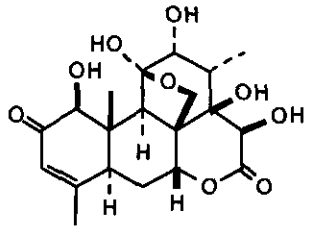
Eurycoma longifolia Jack (Simaroubaceae) is one of famous folk medicines named "Pasak Bumi" in the Southeast Asia and has been used for antimalaria and tonic etc. The roots of E. longifolia collected in Indonesia were extracted with 50% aqueous methanol. The extract was partitioned between water and 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 Figure 3 by various spectral data or comparison with various data in literatures. These compouds exhibited cytotoxic activities as can be seen also from Figure 3. From the structure-activity relationships discussed among quassinoids, it has been reported that the partial structures of the C_1-OH, C_{12}-OH, 2-keto-3-ene and oxide-bridge are important in essential features for antileukemic activity.

In the 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**),[49,51] 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 of Moshor's method.[52]

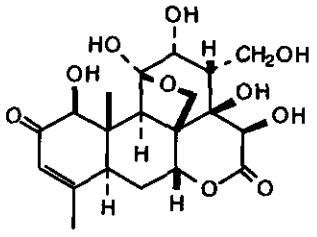
The structures and cytotoxic activities of **73** and **74** are shown in Figure 4. The activities of **73** against V-79, P388 and KB cells were stronger than those of **74**. The perspective views of both compounds from their X-ray



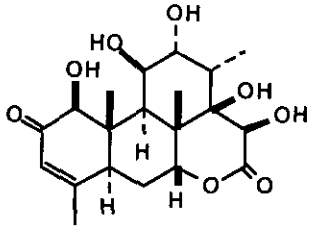
67 KB: 3.4, P388: 1.3
V-79: 4.2



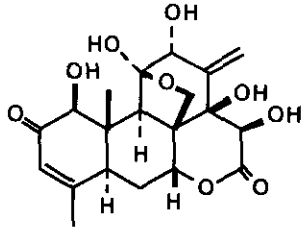
68 KB: 0.33, P388: 1.2
V-79: 1.6



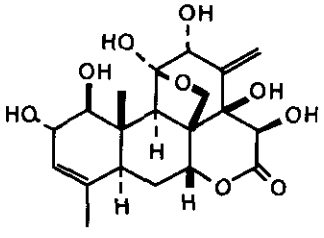
69 KB: 20, P388: 10
V-79: 46



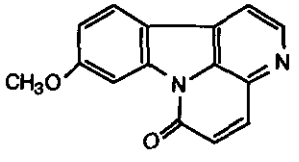
70 KB: 0.38, P388: 0.29
V-79: 1.3



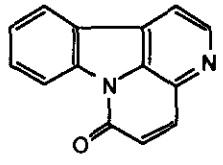
71 KB: 0.4, P388: 10
V-79: 14



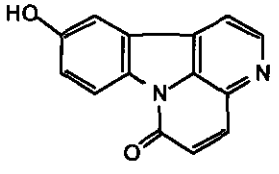
72 KB: 3.6, P388: 58
V-79: 40



64 V-79: 6.8

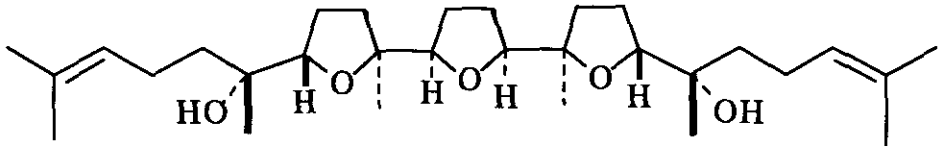


65 V-79: 2.0

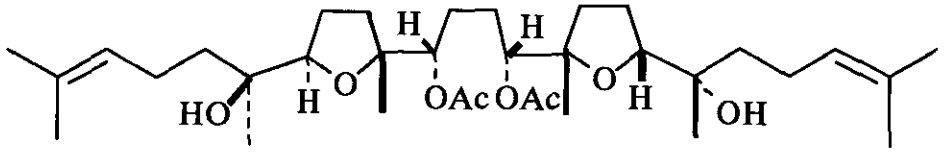


66 V-79: 4.2

Figure 3 IC_{50} Values (μg/ml) of Canthins and Quassinoids against KB, P388 and V-79 Cells



teurilene (**73**), KB: 27.5, P388 2.3, V-79: 4.8



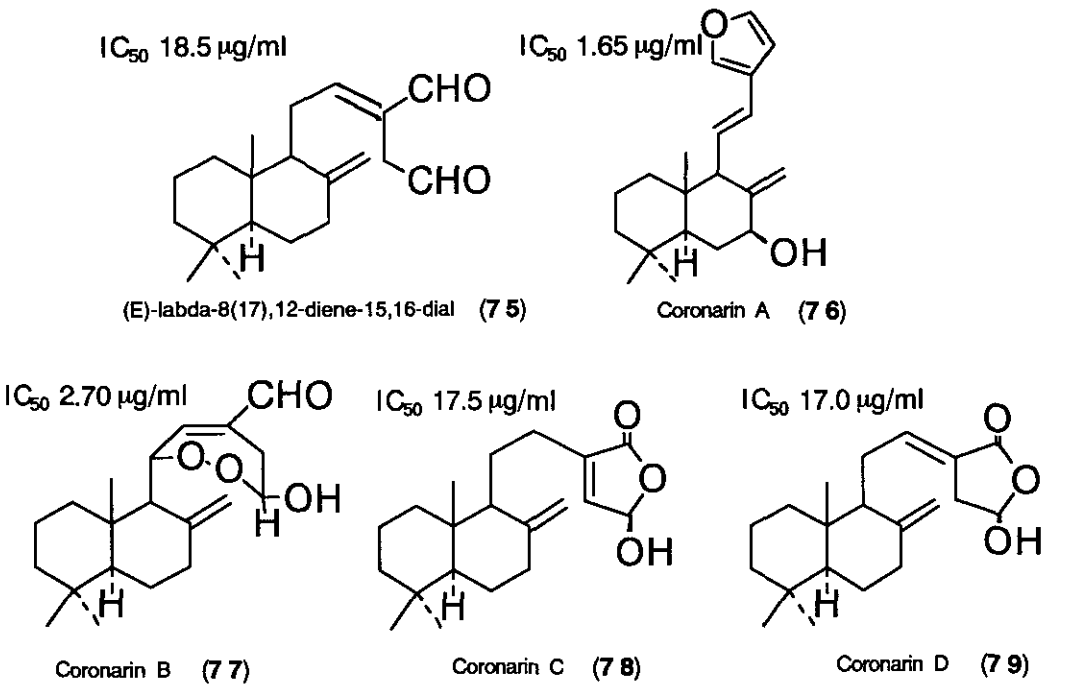
eurylene (**74**), KB: >30, P388: 55, V-79: >100

Figure 4 IC_{50} Values (μg / ml) of Teurilene and Eurylene

analyses gave a curvature form in **73**[49] and a linear one in **74**.[48] These molecular forms are presumed to be correlated with the cytotoxic activities from the related compounds.

6. Cytotoxic Diterpenes from Hedychium coronarium[53, 54]

The chloroform extract prepared from the rhizomes of Hedychium coronarium Koeng (Zingiberaceae, Brazilian name "Lirio-do-brejo"), which is used for rheumatism in Brazil,[55] showed a significant effect against V-79 cells and Sarcoma 180A in mice. Fractionation of the chloroform extract was made with the guidance of bioassay against V-79 cells. The extract was subjected to silica gel column chromatogaraphy and separated to seven



(E)-labda-8(17),12-diene-15,16-dial (**75**) Coronarin A (**76**)

Coronarin B (**77**) Coronarin C (**78**) Coronarin D (**79**)

Figure 5 Diterpenes from *Hedychium coronarium* and Their Cytotoxity against V-79 Cells

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**), 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 Figure 5. Coronarins A (**76**) and B (**77**) of them exthibited a particularly significant cytotoxic activity.

7. Cytotoxic Triterpenes from Maytenus ilicifolia[56, 57]

Maytenus ilicifolia Mart. ex Reiss. (Celastraceae) is used for analgesic, antipyretic, antiseptic and anticarcer etc. in South America, especially named "Cangorosa" in Paraguay is employed for birth control[58] and the presence of maitenin and pristimerin as toxic principles so far have been revealed.[56-58]

The methanolic extract of M. ilicifolia was fractionated by partitioning between chloroform and water, and then n-butanol and water. Purification of each extract by the guidance of cytotoxic activity led us to isolate friedelane- and pristimerin-type triterpenes, D:A-friedoolean-24-al-3-en-3-ol-2-on-29-oic acid (**82**), D:A-friedoolean-1-

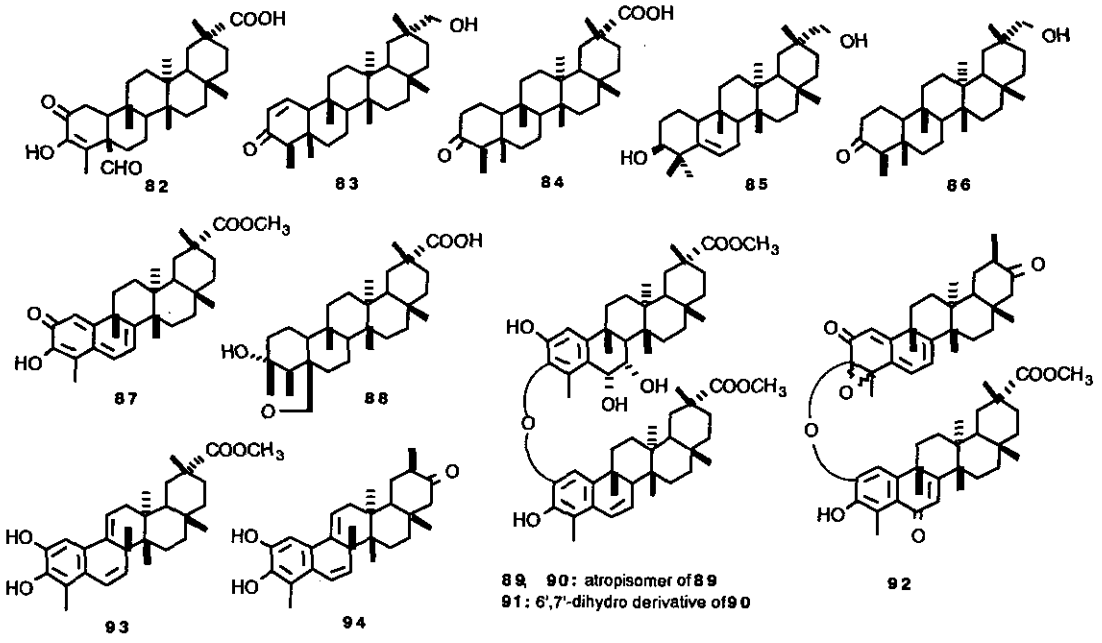


Chart 2 Triterpenes from *Maytenus ilicifolia*

en-29-ol-3-one (**83**), maytenoic acid (**84**), D:B-friedoolean-5-en-3β,29-diol (**85**), D:A-friedoolean-29-ol-3-one (**86**), pristimerin (**87**) and salaspermic acid (**88**), and triterpene dimers named as cangorosinA (**89**), atropcangorosin A (**90**), dihydroatropcangorosin A (**91**) and cangorosin B (**92**) from the chloroform soluble extract, and isopristimerin III (**93**) and isotingenone III (**94**) from the n-butanol soluble extract. The isolation of **82**, from M. ilicifolia, whose 24-positional carbon is oxidized to formyl moiety, is worthwhile on the viewpoint of biogenesis, because it would be suggesting a biogenetic route produced by the oxidative elimination of the

Table V Cytotoxic Activity of Triterpenes from Maytenus ilicifolia

compounds	IC_{50} values (μg/ml)		
	V-79 cells	KB cells	P388 cells
D:A-friedoolean-1-en-29-ol-3-one	> 100	> 100	> 100
D:A-friedooleanan-3-on-29-oic acid	38	12	23
D:B-friedoolean-5-en-3β,29-diol	> 100	1.1×10^{2}	95
D:A-friedooleanan-29-ol-3-one	1.1×10^{2}	1.0×10^{2}	98
pristimerin	1.3×10^{-1}	2.3×10^{-1}	5.2×10^{-2}
isopristimerin III	9.4	1.7	2.0
isotingenone III	1.4	1.1	1.8×10^{-1}

24-Me from friedelin to pristimerin-type triterpenes, and furthermore to triterpene dimers. On the other hand, cytotoxic activities of isolated compounds against V-79, KB and P388 cells were examined. The result is summarized in Table V. Pristimerin (**87**) among the isolated compounds exhibited the strongest activity against each one.

8. Antitumor Phenylpropanoids from Alpinia galanga [62]

Alpinia galanga Willd. (=Languas galanga Stuntz, Zingiberaceae) is growing in south-east Asia, and is widely cultivated in this region. The rhizomes are used for flavouring foods in the preparation of meat dishes and curries[63,64] and showed anti-ulcer,[65] antifungal[66] and xanthine oxidase inhibitor activitites.[67] The alcoholic

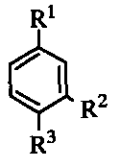
Table VI Antitumor Activity against Sarcoma 180 Ascites in Mice

	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	-
copound **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 volum; 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.

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 bioassay as shown in Table VI. 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'-acetoxy-eugenol acetate (**96**), *trans*-3,4-dimethoxycinnamyl alcohol (**99**), *trans*-4-methoxycinnamyl alcohol (**100**) and *trans*-4-hydroxy-cinnamaldehyde (**101**). Also, compounds (**95**), (**96**), 1'-hydroxychavicol acetate (**97**) and 1'-hydroxychavicol (**98**) were synthesized according to the previously outlined procedure.[65]

Antitumor activity of compounds (**95 - 101**) against Sarcoma 180A in mice is summarized in Table VI. As can be seen from Table VI, it was evaluated that **96** was a more useful agent than **95** from the viewpoint of



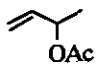
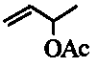
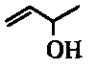
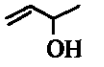
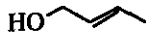
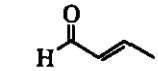
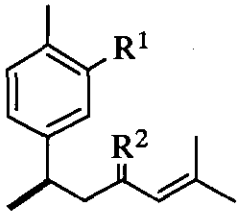
	R^1	R^2	R^3
95		H	OAc
96		OCH_3	OAc
97		H	OAc
98		H	OH
99		OCH_3	OCH_3
100		H	OCH_3
101		H	OH

Figure 6 Structures of Phenylpropanoids from *Alpinia galanga*

antitumor activity and toxicity. The results for **95 - 98** suggested that a 1'-acetoxyl group in the chavicol and eugenol analogs was required for the antitumor 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'-acetoxyl group. The elimination seems to be regulated by variation of the functional groups attached to the benzene ring.

9. Antitumor Bisabolane Sesquiterpenes from Curcuma xanthorrhiza[68]



102: R^1=H, R^2=H_2
103: R^1=H, R^2=O
104: R^1=OH, R^2=H_2

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	++

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

Curcuma xanthorrhiza (Zingiberaceae, named Temu Lawak in Indonesia) is utilized as a tonic in south-east Asia and as a choleretic 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 (**102**), ar-turmerone (**103**) and xanthorrhizol (**104**) and the minor related compounds.[69,70] The structures and antitumor activity against Sarcoma 180A in mice are shown in Figure 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.

10. A Cytotoxic Phenolic Compound from Croton palanostigma[71]

Sangre de Grado is red viscous sap produced by several Croton species (Euphorbiaceae) growing in the upper Amazon basin in Peru. This sap has been largely used by Peruvian natives for several medicinal purposes, including wound healing and cancer treatment. Sangre de Grade obtained from C. palanostigma was found to be cytotoxic to V-79 cells (IC_{50} value = 3.7 μg/ml) *in vitro*. Bio-assay directed purification guided by cytotoxicity against V-79 cells led to the isolation of taspine (**105**), which showed strong cytotoxicity against V-79 (IC_{50} =0.17 μg/ml) and KB cells (IC_{50} ≈ 0.39 μg/ml).

11. A Cytotoxic Alkaloid from Evodia rutaecarpa[72]

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 (IC_{50} = 5.2 μg/ml). The cytotoxic activity on V-79 cells was concetrated in the chloroform sub-extract (IC_{50} = 5.6 μg/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.

12. An Antitumor Ingerol-type Diterpene from Euphorbia lathyris[73]

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 lathyrane diterpenes.[74] Though **108** is well known as a tumor-promoting agent, it showed antitumor activity agaist Sarcoma 180A in mice. This result indicates a paradoxical action, which is cocarcinogenic and antitumor, of the diterpene esters of the Euphorbiaceae.

13. Cardenolides and Pregnanes from Antitumor Fraction of Periploca sepium[75-80]

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 chlorofom was subjected to column chromatography on silica gel using the solvent system of benzene, benzene-$CHCl_3$, $CHCl_3$, $CHCl_3$-CH_3OH (10:1) and (1:1) successively, the antitumor activity against Sarcoma 180A in mice was concentrated in the fraction eluted with $CHCl_3$-CH_3OH (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 $CHCl_3$-CH_3OH (10:1) fraction.

14. Oligopeptides

14-1. Antineoplastic Components from Rubiae Radix [81-87]

Rubiae Radix is originated to Rubiaceous plants Rubia akane in Japan, R. cordifolia in China and R. tincutorum 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, hemostasis 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 ssp. Ruberitorin, which is a kind of alizarin glycoside, purpurin glycoside, rubiadin glycoside are contained in R. tinctorum. Purpurin, morgin, alizarin, rucidin, primeroside, ruberitorin and anthraquinone etc. are found in oriental Rubia spp. However, these pigments were assumed to be non antineoplastic constituents.[81]

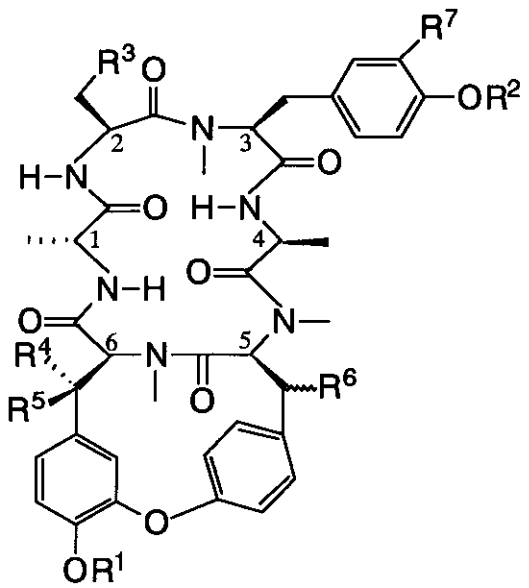
Because the extract of Rubiae Radix showed antineoplastic activity against Sarcoma 180A, the compounds were pursued as the active principles. After repeating fractionation and purification of extract, some oligopeptides were obtained as active principles against P388 leukemia. The extract was partitioned 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 (**110** - **116**) after R. akane.

Recently, the structure of RA-VI (**115**) was decided. Moreover, RA-VIII (**117**), RA-IX (**118**), and RA-X (**119**) were also obtained, and the structures of them were elucidated.

14-1-1. 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 ^{13}C-nmr of RA-VII (**116**) showing that there were three of C-CH_3, three of -CH_2-, three of N-CH_3, two of O-CH_3, 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*-methyltyrosine dimer having ether linkage. Thus, the derivatives having tyrosine moiety were identified by deriving to acetate and methylate. Then it was assumed to 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*-methyltyrosine. From these results, the structure of RA-VII was assumed to be a two-cyclic hexapeptide



Rubia cordifolia

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
RA-I (**110**)	H	CH_3	OH	H	H	H	H
RA-II (**111**)	CH_3	H	H	H	H	H	H
RA-III (**112**)	CH_3	CH_3	OH	H	H	H	H
RA-IV (**113**)	CH_3	CH_3	H	OH	H	H	H
RA-V (**114**)	H	CH_3	H	H	H	H	H
RA-VI (**115**)	CH_3	CH_3	OH	H	H	H	H
RA-VII (**116**)	CH_3	CH_3	H	H	H	H	H
RA-VIII (**117**)	CH_3	CH_3	CH_3,OH	H	H	H	H

Bouvardia ternifolia

bouvardin	H	CH_3	H	H	H	β-OH	H
deoxybouvardin (RA-V)	H	CH_3	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

Figure 8

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 Figure 8.

Recently, the structures of RA-VI (**115**) and RA-VIII (**117**) were elucidated. RA-VI was elucidated as the configurational isomer of RA-III (**112**) 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 (**116**) and RA-V (**114**) were noticed to be main components in these oligopeptides.

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]

14-1-2. Cytotoxicity and Antineoplastic Activity[85,86]

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 1.85×10^{-3} μg/ml and 7.50×10^{-3} μg/ml, respectively, in KB cells, 1.15×10^{-2} μg/ml and 6.40×10^{-3} μg/ml in P388 cells, and 4.40×10^{-2} μg/ml and 9.60×10^{-3} 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 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 (Cβ) of Tyr-6 by comparing the ^{13}C chemical shift values of RA-IV with those of RA-VII; Cβ 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.

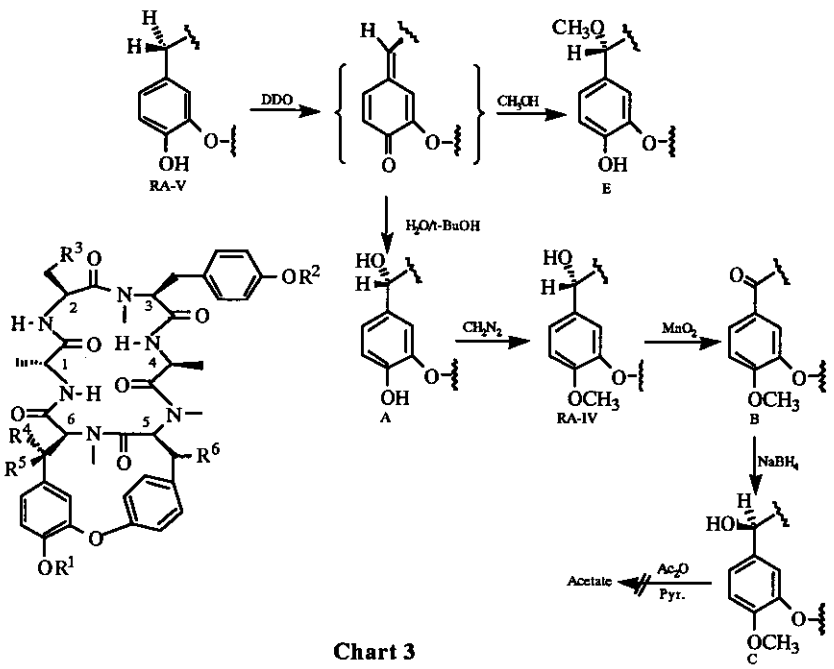
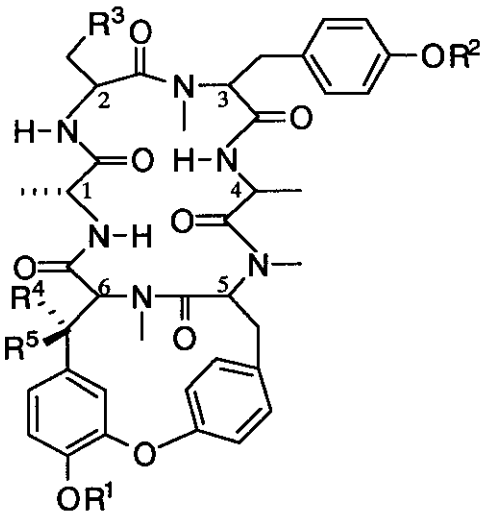


Chart 3

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) agaist 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 Figure 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 Cβ-position of Tyr-6 was replaced with bulky substituent groups. In spite of a similar replacement at Cβ, 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.



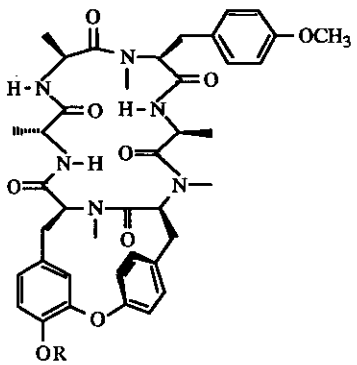
	R^1	R^2	R^3	R^4	R^5	Antitumor activity[a] T/C (%)
RA-I	H	CH_3	OH	H	H	169.3
RA-I-diAc	Ac	CH_3	OAc	H	H	182.8
RA-II	CH_3	H	H	H	H	142.2
RA-III	CH_3	CH_3	OH	H	H	179.4[b]
RA-VI	CH_3	CH_3	H	OH	H	149.0
RA-V	H	CH_3	H	H	H	187.4
RA-VII	CH_3	CH_3	H	H	H	173.6[c]
A	H	CH_3	H	OH	H	126.3
A-diAc	Ac	CH_3	H	OAc	H	98.2
B	CH_3	CH_3	H	=O	=O	171.9
C	CH_3	CH_3	H	H	OH	160.0
E	H	CH_3	H	OCH_3	H	118.5
E-Me	CH_3	CH_3	H	OCH_3	H	132.0
E-Ac	Ac	CH_3	H	OCH_3	H	116.9

a) P388 : 10^6 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.

Figure 9 Structures and Antitumor Activities of Native Cyclic Hexapeptides and Related Compounds

14-1-3. Relationships between Structure and Activity[84,86,87]

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 $1/IC_{50}$) 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 log P values of ILS 150 and 160% differed from that of MLD, it was considered that the most suitable ether



RA-V : R=H
RA-VII: R=CH_3

Table VII 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	
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
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
CH_2CH_3		137.3[c]	165.4[c]	162.2	Toxic	5 10 1/3 3/3
$(CH_2)_2CH_3$		138.4[c]	146.0[a]	93.7	Toxic	5 10 1/3 3/3
$CH(CH_3)_2$		142.2[b]	175.1[c-e]	105.4	Toxic	5 10 3/3 3/3
$(CH_2)_3CH_3$		133.0[c]	144.9[a]	Toxic	Toxic	5 10 0/3 3/3
$(CH_2)_4CH_3$		122.2[c]	142.7[c]	165.4[c]	Toxic	5 10 20 0/3 0/3 3/3
$(CH_2)_5CH_3$		110.3[b]	137.3[c]	153.5[c]	173.0	10 20 30 0/3 1/3 3/3
$(CH_2)_6CH_3$		115.8[c]	144.7[c]	150.1[c]	164.0[b]	
$(CH_2)_7CH_3$		136.1	146.8[c]	162.9[c]	152.2[b]	
$(CH_2)_8CH_3$		112.5[b]	141.5[b]	150.1[c]	155.4[b]	
$(CH_2)_9CH_3$		101.0	120.2[b]	132.7[c]	137.5[a]	
$(CH_2)_{10}CH_3$		115.4	108.7	121.2[b]	123.1[c]	
$(CH_2)_{11}CH_3$		93.0	101.0	105.8	112.5[a]	
$(CH_2)_{12}CH_3$		93.0	99.0	115.4	125.0[c]	
$(CH_2)_{17}CH_3$		101.0	98.1	101.0	108.7	
		126.5	162.2	164.3[b]	Toxic	
		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.

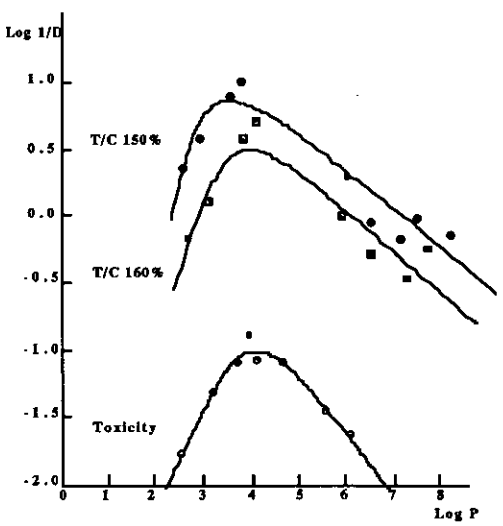


Figure 10 Structure-Antitumor Activity and Toxicity Relationships of Alkyl Ethers of RA-V on P-388 Leukemia in Mice

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 mitomycin C (MMC). Mechanism of action of RA-VII was also investigated and was assumed to be 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 that of MMC, and

Table VIII Therapeutic Effects of RA-700 on P388 Leukemia

Group	Dose (mg/kg)	Route	Survival effects: Survival time (d, mean±S. E.)	T/C (%)	B. W. (g)	
Control	10 ml	i. p.	10.1 ± 0.18	100.0	+5.0	
RA-700	0.005	i. p.	11.0 ± 0.26	109.2	+3.8	
	0.01	i. p.	13.3 ± 1.15	132.3	+2.6	
	0.05	i. p.	15.5 ± 1.12	153.8	+2.1	
	0.5	i. p.	16.7 ± 0.33	165.4	+1.3	T. R. = 400
	2.0	i. p.	18.6 ± 1.21	184.3	+0.4	
	4.0	i. p.	23.6 ± 2.62[a)]	234.2	-0.6	
	6.0	i. p.	6.00 ± 2.61	62.7		
MMC	0.005	i. p.	10.8 ± 0.31	107.5	+4.8	
	0.01	i. p.	10.5 ± 0.22	104.2	+5.2	
	0.1	i. p.	13.7 ± 0.67	135.6	+2.8	T. R. = 10
	0.5	i. p.	15.8 ± 0.31	157.1	+0.8	
	1.0	i. p.	18.0 ± 0.68	178.1	-0.3	
	2.0	i. p.	12.7 ± 0.33	125.7	-1.8	
Control	10 ml	i. v.	9.50 ± 0.15	100.0	+4.0	
RA-700	0.25	i. v.	10.0 ± 0.27	105.3	+3.2	
	1.0	i. v.	11.0 ± 0.19	115.8	+1.9	
	2.5	i. v.	13.4 ± 0.18	140.8	-0.3	
	4.0	i. v.	14.5 ± 1.25	152.6	-2.0	
	6.0	i. v.	15.9 ± 0.23	167.1	-4.7	
MMC	0.1	i. v.	10.5 ± 0.19	110.5	+4.4	
	0.5	i. v.	12.5 ± 0.19	131.6	+2.3	
	1.0	i. v.	13.6 ± 0.18	143.4	+0.9	
	2.0	i. v.	12.1 ± 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.

RA-V was concluded to be a "time-dependent drug" like vincristine. Furthur, 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.

14-1-4. Recent Reports on RA-Series Compounds

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

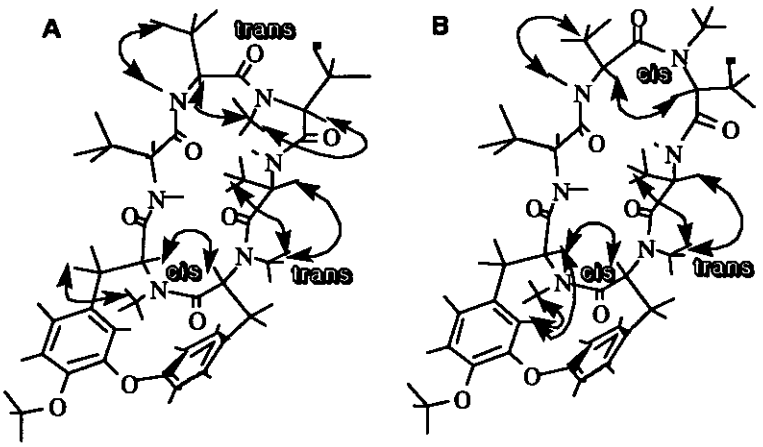
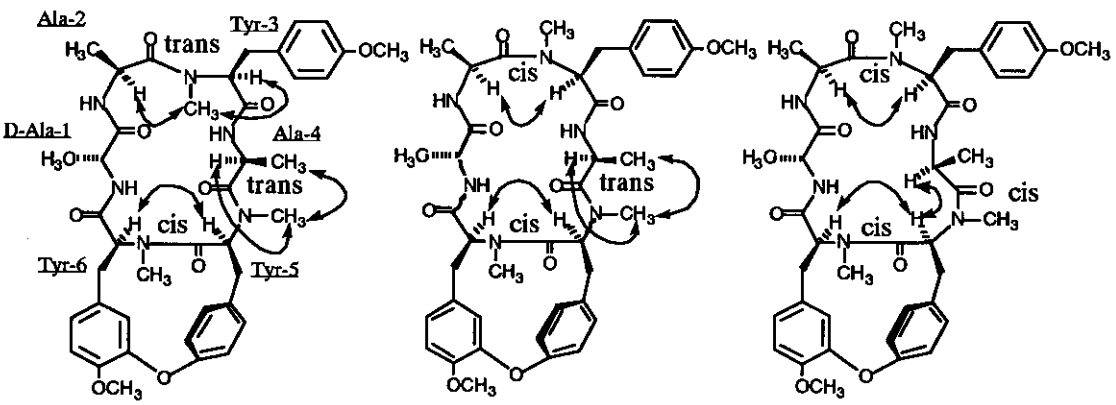


Figure 11 NOE Enhancements in Conformers A and B of RA-VII
The arrows show the NOE relationships confirmed by 1D-NOE and NOESY experiments in $CDCl_3$ at 303K.

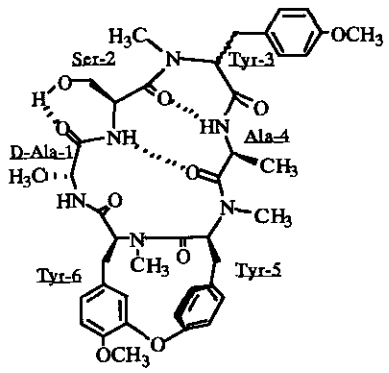
conformers A and B of RA-VII in $CDCl_3$. The Structures of the three conformers (A, B and C) in DMSO-d_6 were also determined by 2D-nmr techniques, temperature effects on NH protons and NOE experiments. Distances deduced from the nmr measurements were used for the refinements by the restrained molecular 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 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.[88]



Conformer A Conformer B Conformer C

Figure 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 NOESYPH experiments.

Using ^1H- and ^{13}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

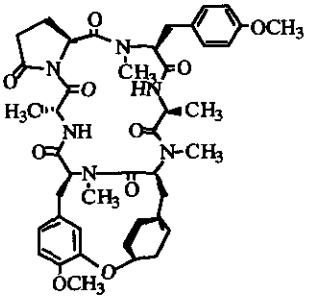


RAI-III (**112'**) : L-Tyr-3
RAI-VI (**115'**) : D-Tyr-3

Figure 13. Structures of RAI-III and VI.

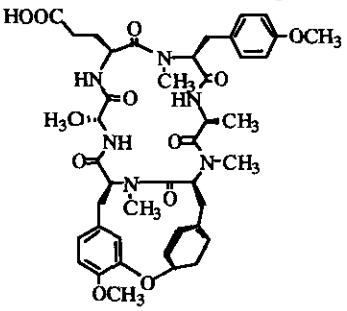
Tyr-5 and Tyr-6. The second conformational isomer, accounting 32%, has adopted *cis* conformations between both Tyr-5 and Tyr-6, and between Ala-2 and Ala-3. The third isomer, accounting to 4%, was determined to have *cis* conformations for all of the three *N*-methyl amide bond.[89]

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.[90] On the other hand, by the conformational analysis of RA-III and VI using spectroscopic and computational chemical methods, Minor constituents RAI-III (**112'**) and RAI-VI (**115'**) 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.[91]

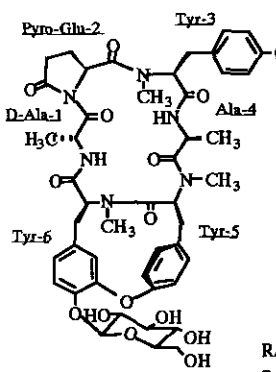


RA-IX (**118**)

Figure 14

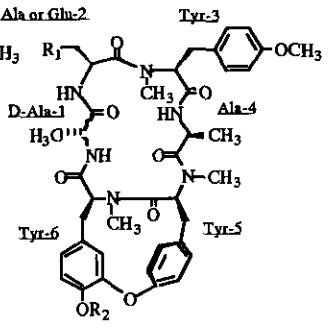


RA-X (**119**)



RA-XIV (**123**)

Figure 15



RA-XI (**120**): R_1=CH_2COOH; R_2=H
RA-XII (**121**): R_1=H; R_2=β-D-glucose
RA-XIII (**122**): R_1=CH_2COOH; R_2=β-D-glucose

Recently, many other RA-series components were isolated from R. cordifolia. The structures of new anti-tumor hexapeptide RA-VI (**115**) and RA-VIII (**117**) 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 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 $CDCl_3$, 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 ^{13}C 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.[92]

Moreover, RA-IX (**118**) and -X (**119**) 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.[93] Further, four new bicyclic hexapeptides, RA-XI (**120**), -XII (**121**), -XIII (**122**) and -XIV (**123**) were isolated from same plant and showed potent antitumor activity against P388. The structures were elucidated from spectroscopic and chemical evidences.[94]

14-2. The Oligopeptides Related to RA Compounds

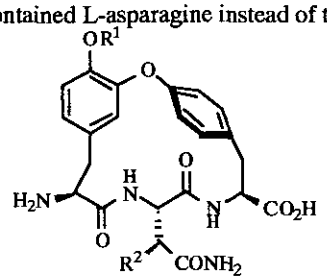
14-2-1. Bouvardin

Bouvardin was the first compound as this type of cyclic hexapetides, which was isolated from Bouvardia ternifolia belonging to Rubiaceae by Cole *et al.*[95] Microbial transformations of bouvardin to *O*-desmethylbouvardin catechol were performed by Petrosky *et al.*[96] Zalacain *et al.* reported that bouvardin was an antitumor drug which inhibited protein synthesis in intact eukaryotic cells and cell-free systems. Bouvardin acted at the level of the 80 S ribosome in a site somehow involved with the interaction of elongation factors EF1 and EF2. Bouvardin inhibited EF1-dependent binding of aminoacyl-tRNA and EF2-dependent translocation of peptidyl-tRNA but did not affect the non-enzymic translocation since this reaction did not require EF2. The site of the 80 S ribosome involved in the interaction with bouvardin appeared to be independent from the cycloheximide and the cryptopleurine binding sites, since yeast mutants resistant to cycloheximide or cryptopleurine are sensitive to bouvardin.[97]

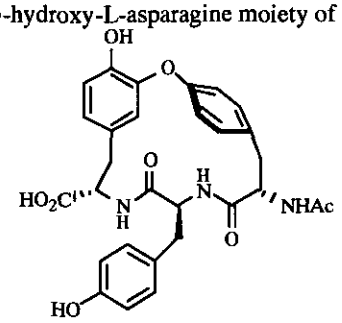
14-2-2. OF4949[98-100]

OF4949-I, II, III and IV were isolated as new aminopeptidase B inhibitor from the culture broth of a fungus, Penicillium rugulosum OF4949. The structures of them were identified by analysis of the products of their

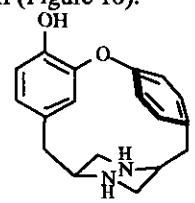
chemical degradation and by ^1H nmr, and mass spectrometry. These compounds were new cyclic tripeptides containing diphenylether as a chromophore. OF4949-I had two amino acids, β-hydroxy-L-asparagine and 4-methylisodityrosine. The structural differences between I and II and between III and IV lay solely in the diphenyl ether moiety; the phenolic hydroxyl group in II and IV was methylated in I and III. OF-4949-III and IV contained L-asparagine instead of the β-hydroxy-L-asparagine moiety of I and II (Figure 16).



$R^1 = CH_3$ $R^2 = OH$ *OF4949-I*
$R^1 = H$ $R^2 = OH$ OF4949-II
$R^1 = CH_3$ $R^2 = H$ OF4949-III
$R^1 = H$ $R^2 = H$ OF4949-IV



K-13



piperazinomycin

Figure 16 The Structures of OF4949 Serious, K-13 and Piperazinomycin

14-2-3. K-13[101-103]

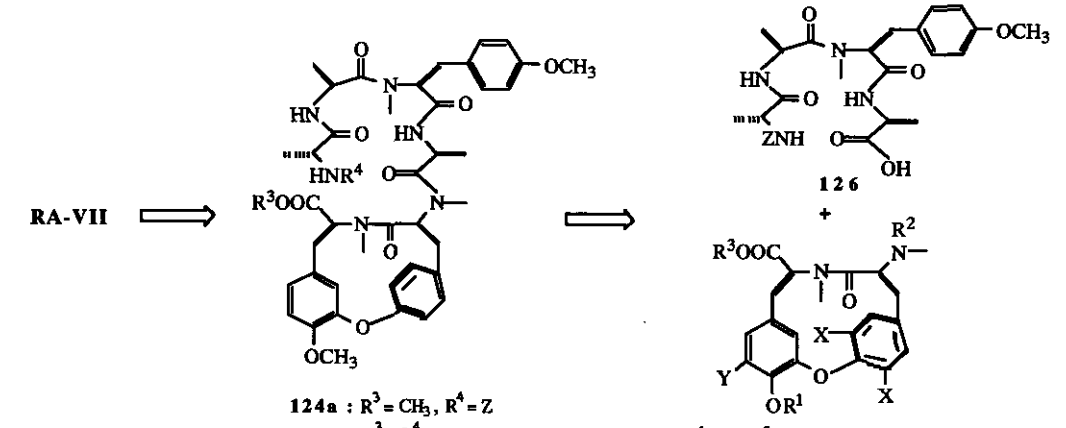
K-13 was isolated as a novel inhibitor of angiotensin I converting enzyme (ACE) from the culture broth of Micromonospora harospora subsp. exilisia K-13. K-13 inhibited ACE non-competitively when hippuryl-L-histidyl-L-leucine was used as a substrate. When K-13 was administered intravenously to rats, it inhibited the pressor response to angiotensin I. The structure of K-13 was determined to be a cyclic tripeptide composed of tyrosine and unusual diamino dicarboxylic acid, isodityrosine, by spectral and chemical studies of K-13 and its derivatives. K-13 has been synthesized from *N*-acetyl-3,5-dichloro-L-tyrosyl-*O*-benzyl-L-tyrosyl-3,5-diiodo-L-tyrosine methyl ester, whose oxidation with thallium trinitrate (TTN) as a key step followed by zinc reduction affords the corresponding diphenyl ether with the same heterocyclic skeleton as that of K-13, indicating that K-13 is biosynthesized from three molecules of L-tyrosine (Figure 16).

14-2-4. Piperazinomycin[104,105]

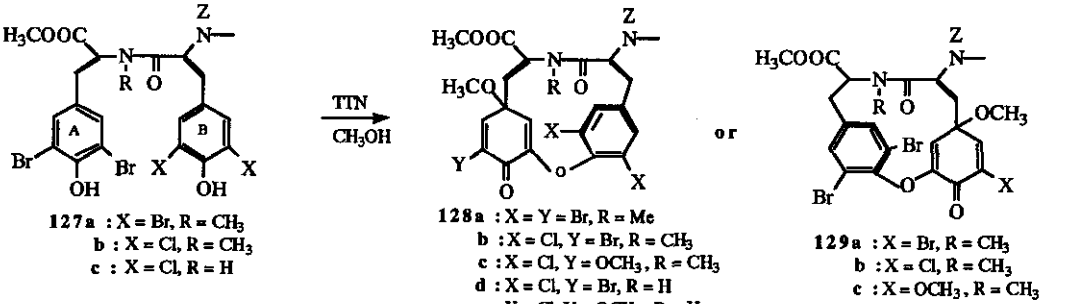
A new antifungal antibiotic, named piperazinomycin, was isolated from the cultured broth of Streptoverticillium olivoreticuli subsp. neoenacticus. Piperazinonomycin showed inhibitory activity against fungi and yeasts, especially against Tricophyton. The molecular formular of piperazinomycin was determined to be $C_{18}H_{20}N_2O_2$ by high resolution mass spectrum and the spectroscopic and chemical properties. The structure and absolute configuration of piperazinomycin have been established by X-ray crystallographic analysis of its monohydrobromide (Figure 16).

14-3. Total Synthesis of RA-Series and Related Compounds

First of all, Itokawa *et al.* completed the total synthesis of RA-series skeleton. Intramolecular oxidative coupling of two phenolic parts of protected 2,6-dibromotyrosyl-2,6-dichlorotyrosine with thallium trinitrate (TTN) afforded a highly strained 14-membered ring system, from which deoxybouvardin and RA-VII (**116**) were synthesized.[106,107] The synthetic schemes are shown in Figure 17. At first, **127** was treated with TTN to give diphenyl ether linkage of natural type (**128**). Then, **126** and **125** were condensed to synthesize RA-VII totally according to the arrows reversely. Yamamura *et al.* developed the new method for consisting diphenyl ether by oxidative coupling with TTN. OF4949-III has been synthesized from *N*-benzyloxycarbonyl-3,5-dibromo-L-tyrosyl-3,4-dichloro-L-tyrosine methyl ester, whose oxidation with TTN as a key step followed by zinc reduction affords the corresponding diphenyl ether with the same heterocyclic skeleton as that of OF4949-III.[108]



Scheme I



Scheme II

Figure 17 Schemes for Total Synthesis of RA-VII (116)

K-13 was synthesized from *N*-acetyl-3,5-dichloro-L-tyrosyl-*O*-benzyl-L-tyrosyl-3,5-diiodo-L-tyrosine methyl ester, whose oxidation with TTN as a key step followed by zinc reduction affords the corresponding diphenyl ether with the same heterocyclic skeleton as that of K-13, indicating that K-13 is biosynthesized from three molecules of L-tyrosine.[109] K-13 was also synthesized by using an Ullmann reaction by Boger *et al.*[110]

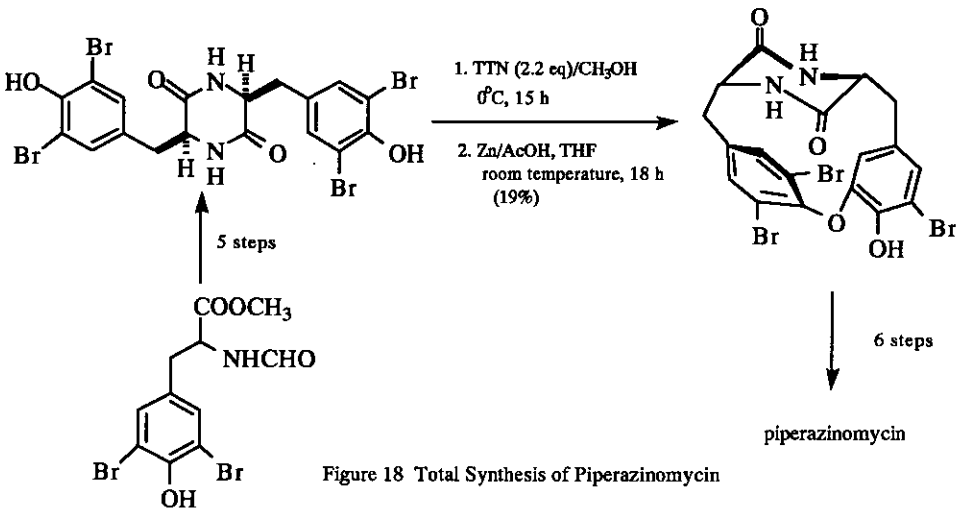


Figure 18 Total Synthesis of Piperazinomycin

Scheme III

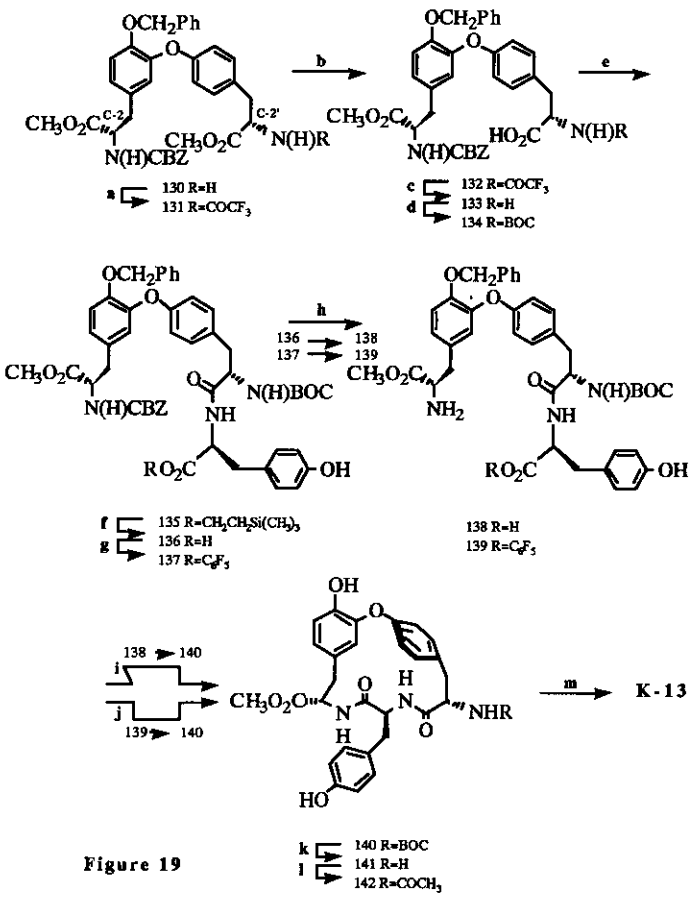


Figure 19

Yamamura *et al.* synthesized also piperazinomycin from tetrabromotyrosyltyrosine, whose oxidation with TTN leads to the formation of a strained 14-membered ring similar to that of piperazinomycin as a key step.[111] (Figure 18)

Evans and Ellman examined the asymmetric synthesis of the two cyclic tripeptides OF4949-III and K-13. The absolute stereochemical assignment of the former compound has been confirmed, while the absolute configuration of the latter has been established for the first time. The expedient bidirectional synthesis of a fully differenciated isodityrosine, a common intermediate to both

Scheme IV

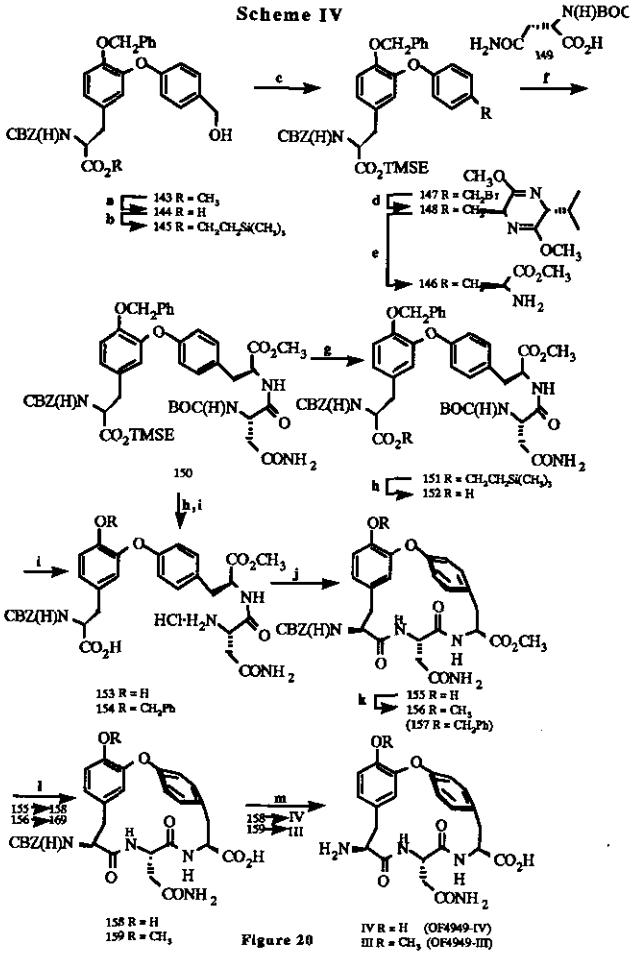


Figure 20

molecules, was achieved by employing the recently developed direct electrophilic azidation of chiral imide enolates. In completing these synthesis, the utility of the azide as an amino-protecting group in peptide-coupling reactions and in peptide cyclizations was also evaluated. These studies have established that α-azido carboxilic acids are practical *N*-protected α-amino acid synthons and may be used as such as in "racemization-free" peptide synthesis.[112]

Recently, Bogar and Yohannes reported a series of papers about the total synthesis of OF4949, K-13 and RA-compounds. A study of reaction conditions for implementation of an activated Ullmann diaryl ether condensation that may be conducted without amino acid racemization and that

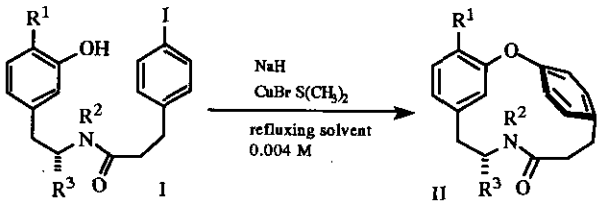


Table IX

I	R^1	R^2	R^3	NaH, equiv	$CuBr\cdot S(CH_3)_2$, equiv	solvent[a]	time, h	II	yield, % (RSM,[b] %)	S:R[c]
Ia	H	H	H	2.0	10	pyridine	18	IIa	58 (20)	
Ia	H	H	H	2.0	10	dioxane	18	IIa	51 (24)	
Ib	H	CH_3	H	1.2	10	pyridine	18	IIbd	49 (36)	
Ic	OCH_3	H	H	2.0	10	pyridine	18	IIc	46 (29)	
Id	OCH_3	CH_3	H	1.2	10	pyridine	18	IId	45 (22)	
Ie	OH	H	CO_2CH_3	2.0	10	pyridine	9	IIe	51 (20)	nd
If	OCH_3	H	CO_2CH_3	2.0	10	pyridine	9	IIf	51 (8)	55:45
If	OCH_3	H	CO_2CH_3	2.0	10	dioxane	9	IIf	31 (17)	96:4
If	OCH_3	H	CO_2CH_3	2.0	10	collidine	9	IIf	50 (12)	93:7

a Reaction temperatures: pyridine, 130∞C (bath); collidine, 185∞C (bath); dioxane, 115∞C (bath). b RSM = recovered starting material. c Ratio of *S*:*R* enantiomers; nd = not determined. Starting If, *S*:*R* = 99:1. d Structure established by X-ray.

Scheme V

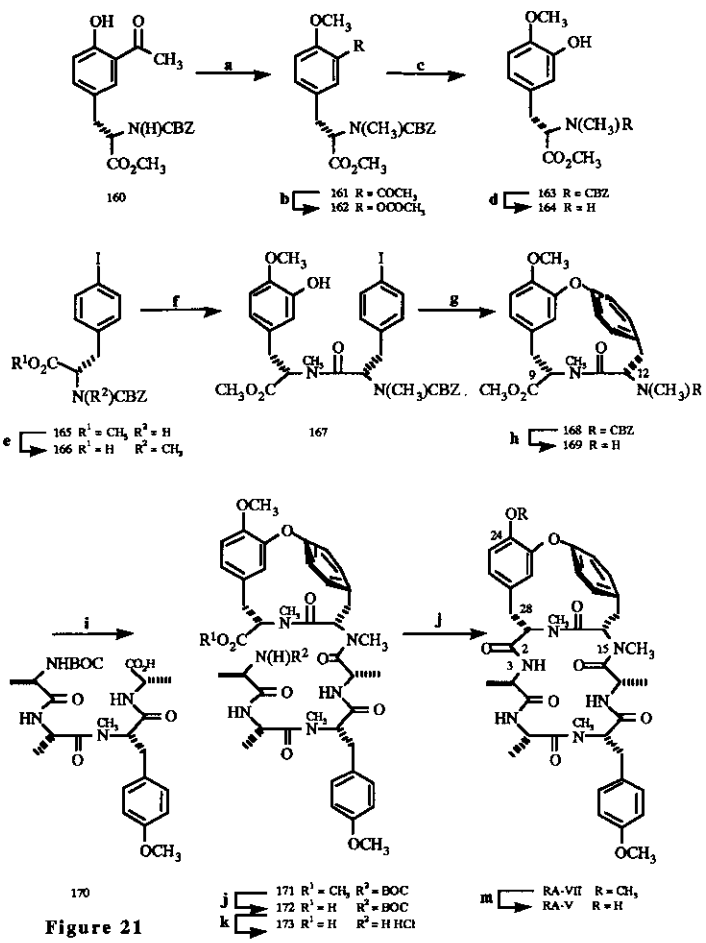


Figure 21

proved suitable for incorporation of a selectively protected catechol was described and its application to the synthesis of L,L-isodityrosine was also communicated.[113]

The total synthesis of OF4949-III and OF4949-IV was also completed by Boger *et al.* A study of the unusual effects remote substituents might have on the rate of the key macrocyclization leading to 17-membered cyclic tripeptides incorporating a diaryl ether linked meta- and paracyclophane structural subunit was described.[114] The development of reaction conditions for implementation of an activated Ullmann condensation reaction that may be conducted without amino acid racemization and that have proven suitable for incorporation of the selectively protected catechol of functionalized L-Dopa derivatives are reported. The application of this procedure in the total synthesis of L,L-isodityrosine, K-13, and OF4949-III /OF4949-IV is detailed. Full details of a study of the macrocyclization reaction required for formation of the 17-membered tripeptides incorporating a diaryl ether linked meta- and paracyclophane structural submit are provided and illustrated that the cyclization in route to K-13 / OF4949-I - IV is optimally conducted on substrates bearing a carbamate derivative of the C-15 / C-9 amine and a C-4 free phenol with C^{11}-N^{10} / C^{10}-N^{11} amide bond closure.[115] The total synthesis of K-13 was performed as shown in Scheme III (Figure 19). Moreover, OF4949 series compounds were also synthesized according to Scheme IV (Figure 20). The demonstration and definition of the scope of the intramolecular Ullmann condensation reaction suitable for use in macrocyclization reactions leading to 14-membered para- and metacyclophanes possessing a diaryl ether are detailed. One of the results of intramolecular Ullmann reaction, which was reported by Boger *et al.* is illustrated in Table IX.[116]

With the established viability of the key Ullmann macrocyclization reaction and the modifications that effectively address potential substrate racemization in hand, its application to the total synthesis of RA-VII and RA-V were persuaded. Single step *O*- and *N*-methylation of *N*-carbobenzyloxy-3-acetyl-L-tyrosine methyl ester (**160**) followed by Bayer-Villiger oxidation and subsequent acid-catalyzed methanolysis of the resulting acetate provided the selectively protected *N*-methyl-L-DOPA derivatives (**163**, Figure 21) Catalytic hydrogenolysis of **163** served to remove the CBZ protecting group, and coupling of the resultant amine (**164**) with *N*-carbobenzyloxy-*N*-methyl-4-iodo-L-phenylalanine (**166**) provided the key dipeptide (**167**). Subjection of **167** to the prescribed conditions for effecting the strategic intramolecular Ullmann condensation reaction provided **168** (30%) without evidence of racemization. In contrast to the natural products but consistent with expectations based on conformational analysis, **168** exists in a rigid solution conformation ($CDCl_3$) possessing a *trans* C^{11}-N^{10} amide bond. Amide deprotecting, CBZ hydrolysis, N-3 BOC deprotection, and diphenyl phosphorazidate promoted macrocyclization with C^2-N^3 amide bond formation strategically conducted employing a D-amino acid amine terminus under the recently induced and improved reaction conditions provided RA-VII. Selective C-24 methyl ether removal provided deoxybouvardin (RA-V).[117] In our laboratory, a lot of derivatives started from RA series oligopeptides are now under investigation to develop better anticancerous agents.[118]

15. Conclusiom

Recently, we have isolated many other compounds from Rubia cordifolia, that is; anthraquinones, naphthoquinones and naphthohydroquinone dimers,[119-121] and triterpenoids.[122,123] 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 materials 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 agents 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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