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BIOACTIVE CYCLIC PEPTIDES FROM HIGHER PLANTS

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Abstract – Cyclic peptides comprise a class of naturally occurring molecules, which exhibit a range of biological activities, and have attracted great interest from a biogenetic point of view as well as providing challenging targets for total synthesis. This review covered the structure elucidation and biological activity of cyclic peptides recently isolated from higher plants such as Amaranthaceae, Annonaceae, Caryophyllaceae, Compositae, Linaceae, Rutaceae, and Rubiaceae.

INTRODUCTION

Cyclic peptides comprise a class of naturally occurring molecules, which exhibit a range of biological activities.¹ These cyclic peptides often have higher lipophilicity and membrane permeability, because of their reduced zwitterionic character. Furthermore, restricted bond rotation make these molecules retain rigid backbone conformation resulting in higher affinity and selectivity to certain specific target molecules to give relevant biological activities.

During research on bioactive peptides from medicinal plants, from *Celocia argentea* (Amaranthaceae), we isolated celogentins, a series of unique bicyclic peptides, remarkably inhibiting the tubulin polymerization, from *Rubia* species (Rubiaceae), a series of RAs exhibiting a potent antitumor activity *in vitro* and *in vivo*, and from *Aster tataricus* (Compositae), a series of astins showing antitumor activity *in vivo*. We also isolated, from some higher plants belonging to the families Annonaceae, Caryophyllaceae, Linaceae, Rutaceae, and others, monocyclic oligopeptides consisting of five to twelve amino acid residues and having estrogenic, vasorelaxant, and immunosuppressive activities. Structures and biological activities of some kinds of cyclic peptides isolated from higher plants were discussed in this review.

[†]Dedicated to Professor Emeritus Akira Suzuki, Hokkaido University, on the occasion of his 80th birthday.

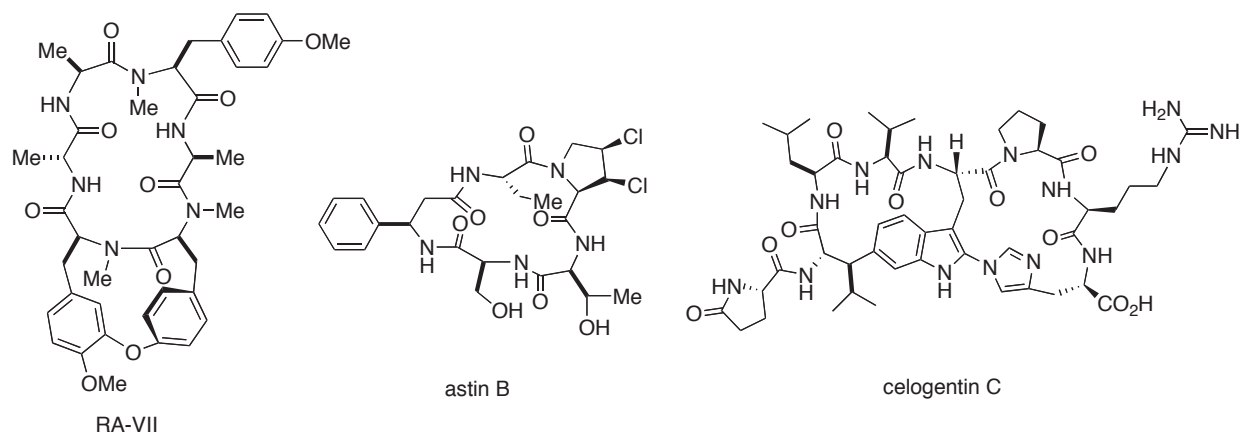


Figure 1. Interesting representative bioactive cyclic peptides from higher plants.

In 1983, Itokawa and co-workers isolated a new cyclic peptide, RA-VII from *Rubia akane* (Rubiaceae) as one of the active principles against Sarcoma 180A.² In vitro cell growth, RA-VII had a potent cytotoxicity against KB cells, P388 lymphocytic leukemia cells, and MM2 mammary carcinoma cells.³ RA-VII was shown to have a potent antitumor activity on various tumors as a promising candidate for clinical study.^{4,5} Astin B and celogentin C were isolated from medicinal plants, *Aster tataricus*⁶ and *Celosia argentea*,⁷ respectively. Astins containing a 16-membered ring system containing an unique β,γ -dichlorinated proline showed an antitumor activity against Sarcoma 180A⁸ and also induced apoptosis against a human papillary thyroid carcinoma cell line⁹ associated with activation of caspases, whereas celogentin C showed antimetabolic activity on inhibition of polymerization of tubulin protein.⁷ These unusual bicyclic ring system and the inherent antitumor activity have attracted great interest as challenging targets for total synthesis and SAR studies.¹⁰⁻¹³ Certainly, research on bioactive cyclic peptides in higher plants is a very promising strategy to get lead for developing new drugs. Tan *et al.* established TLC protosite reaction with ninhydrin reagent, which is a sensitive method for detection of cyclic peptides in these sources, and this approach benefits the extensive effective isolation and evaluation of cyclic peptides derived from higher plants.¹⁴ The biosynthesis of cyclic peptides is still not fully elucidated except in some limited area.¹⁵⁻¹⁹

The number of cyclic peptides reported now reaches about 500. There are some reviews of the chemistry and biology of cyclic peptides or cyclopeptides from plants.²⁰⁻²³ This present review summarizes recent studies on the structure elucidation and biological activity of these cyclic peptides isolated and reported mainly by us from higher plants such as the families Amaranthaceae, Annonaceae, Caryophyllaceae, Compositae, Linaceae, Rutaceae, and Rubiaceae.

A. CELOGENTINS FROM *CELOCIA ARGENTEA* (AMARANTHACEAE)

The seeds of *Celosia argentea* (Amaranthaceae) are a Chinese herbal medicine used as a therapeutic drug for eye and hepatic diseases in China and Japan. From the seeds of *C. argentea*, we isolated a unique

bicyclic peptide, moroidin originally isolated from *Laportea moroides* (Labiatae),^{24,25} which effectively inhibits the tubulin polymerization.²⁶ Then, from a MeOH extract of the plant, we isolated of nine new moroidin-type bicyclic peptides, celogentins A – H and J (1–9)^{7,27} and a new cyclic peptide, celogentin K (10) having a 3-hydroxyoxindole ring.²⁸ Their structures including absolute stereochemistry were determined by extensive NMR studies, MS/MS, and CD spectral analysis, chemical means, and X-ray crystallography. Celogentins A – H and J (1–9) are new type bicyclic peptides related to moroidin, different only in the right-hand side backbone ring size.

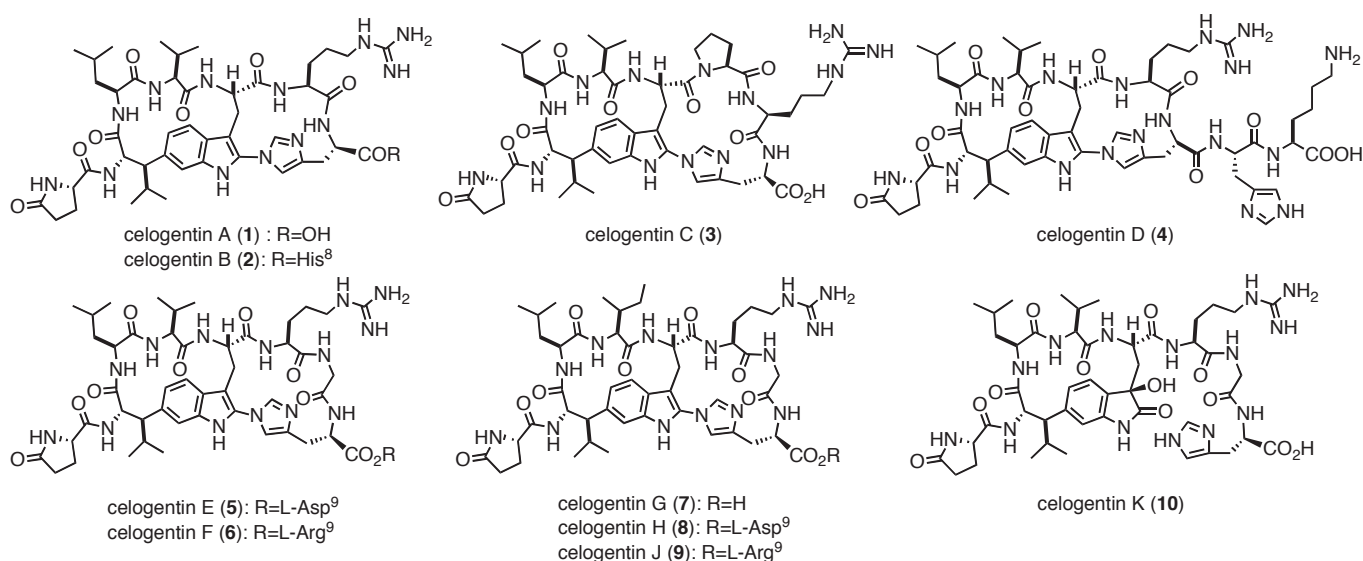


Figure 2. Structures of celogentins A–K (1–10) from *Celosia argentea*.

Microtubules are known to play a pivotal role in mitotic spindle assembly and cell division. These cytoskeletal elements are formed by the self-association of the $\alpha\beta$ tubulin heterodimers. A number of natural compounds are reported to inhibit the microtubule formation and the mitotic arrest of eucaryotic cells. It is known that antimitotic peptides such as ustiloxin A, arenastatin A, phomopsin A, and dolastatin 10 bind to vinca alkaloid binding site. The antimitotic agents have potential applications in drug development.

The present celogentins A – H and J, and moroidin have been found to inhibit the polymerization of tubulin in a concentration-dependent manner.^{7,27} Celogentins A and B, lacking the Gly residue of moroidin, were showed less effective (IC_{50} , celogentin A, 20 μ M; celogentin B, 30 μ M) than moroidin (3.0 μ M) in inhibition of the tubulin polymerization. Celogentin C was more potent (IC_{50} 0.8 μ M) than moroidin. Stephanotic acid from *Stephanotis floribunda*²⁹ corresponding to the left-hand side portion of the two backbone rings of celogentins and moroidin, did not show such inhibition. These results suggest that the bicyclic ring system of celogentins and moroidin including unusual non-peptide connections between β^5 -Leu, Trp, and His residues, and their ring size and conformations are suitable for interaction with tubulin, and that they are important factor for their biological activity.²⁷

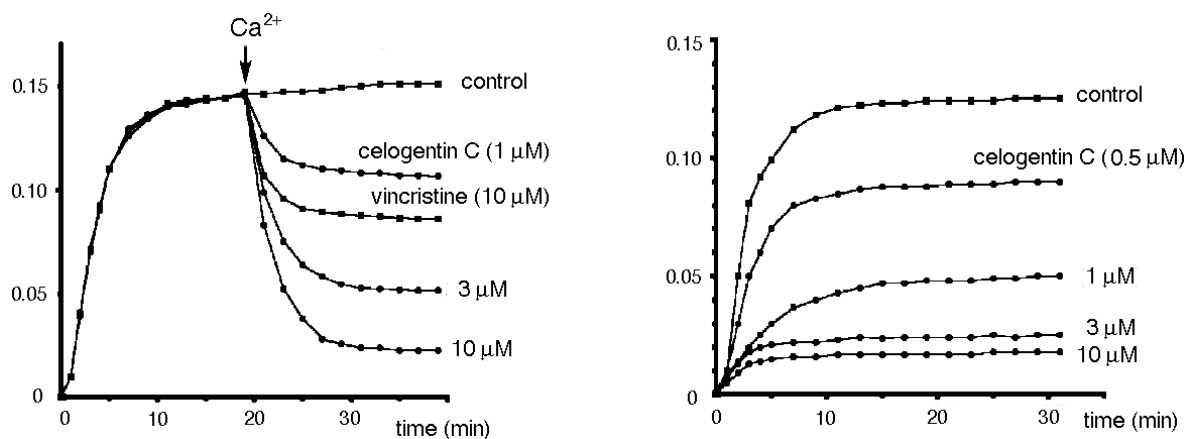


Figure 3. Antimitotic activity of celogentin C (**3**) from *Celosia argentea*.

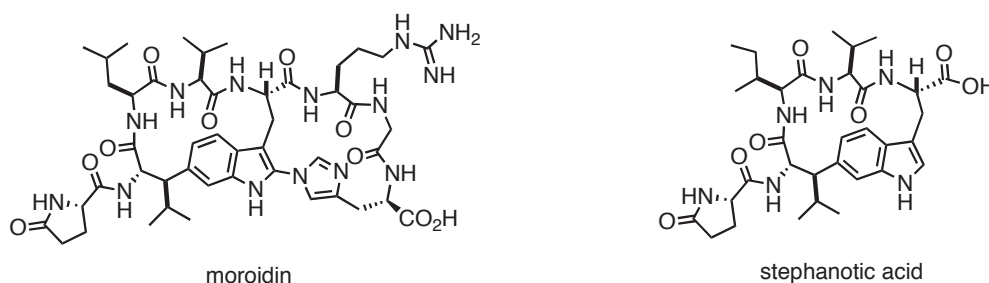


Figure 4. Structures of moroidin from *L. moroides* and stephanotic acid from *S. floribunda*.

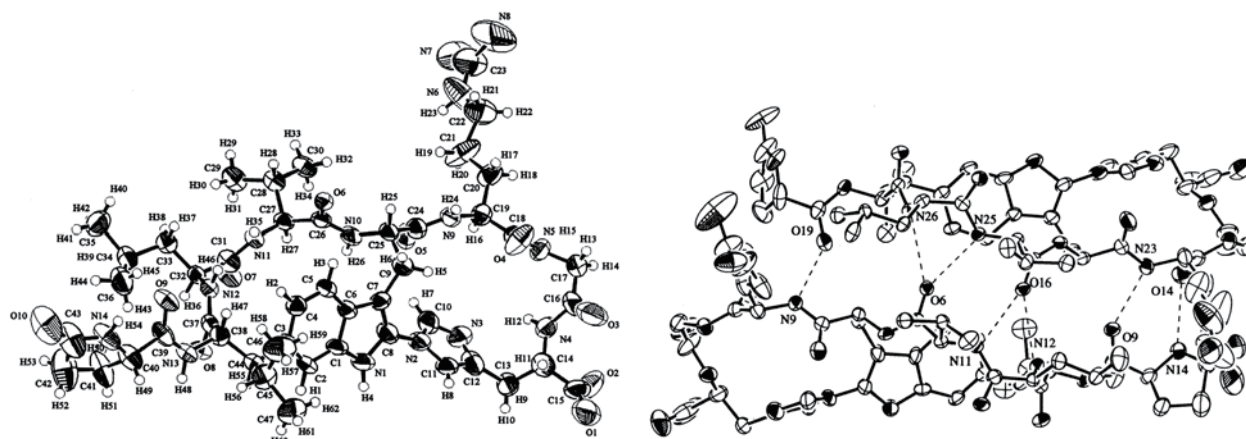


Figure 5. ORTEP drawing for moroidin and two conformationally different molecules in the asymmetric unit. Dashed lines show H-bonded contacts between two conformers.²⁸

Celogenamide A (**11**) was also isolated from the same plant source together with lyciumin A and C³⁰ methylates, and its structure was elucidated by the spectroscopic method to be as shown in Figure 6.³¹ A series of lyciumins isolated from the roots of *Lycium chinense* (Solanaceae) show an inhibitory activity on angiotensin-converting enzyme.³⁰ Celogenamide A (**11**) is a new 17-membered cyclic peptide containing a unique connection between the C- α carbon of Gly⁴ and a nitrogen of the indole ring of Trp.⁸ Celogenamide A (**11**) and lyciumin A and C³⁰ methylates, however, did not inhibit the polymerization of tubulin.

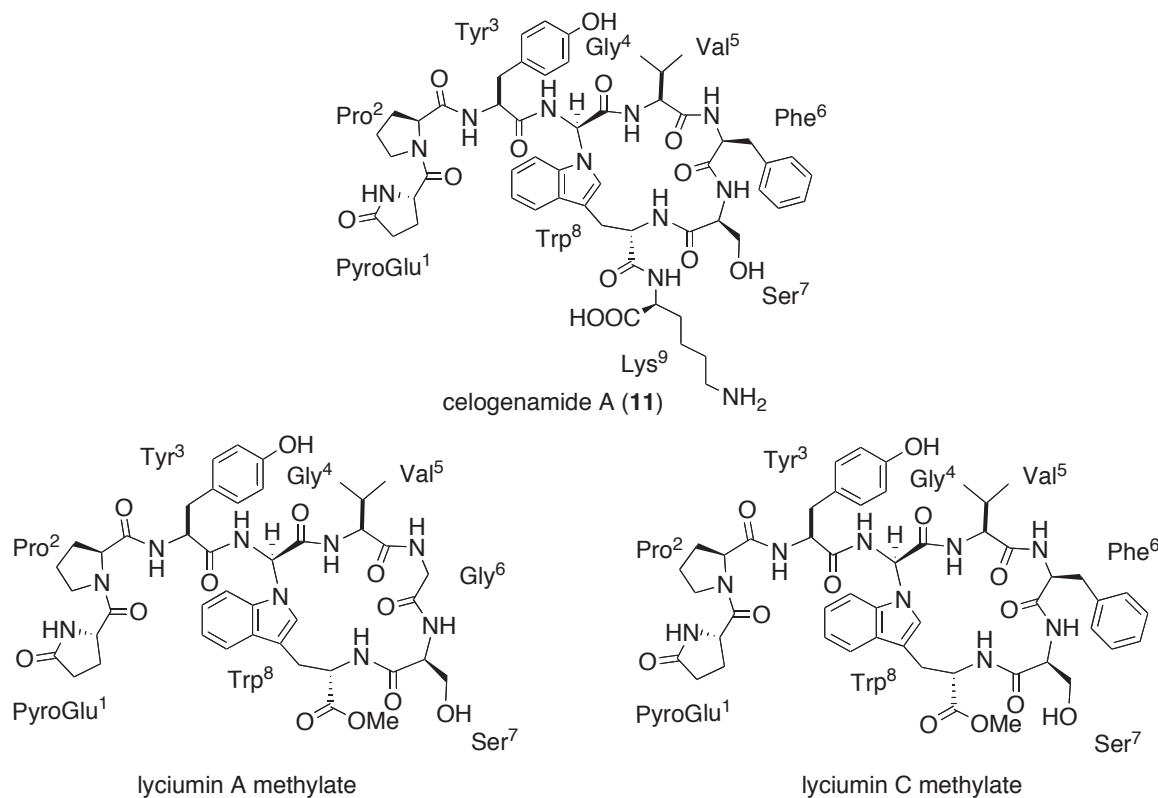


Figure 6. Celogenamide A (11) and lyciumins A and C methylates from *Celosia argentea*.

B. ANTITUMORE CYCLIC HEXAPEPTIDES FROM *RUBIA CORDIFOLIA* (RUBIACEAE)

Rubiae Radix means radix of *Rubia akane* in Japan, that of *R. cordifolia* in China and that of *R. tinctorum* in Europe. The former two show antineoplastic activity, whereas the third does not. From an extract of Rubiae Radix showing an antineoplastic activity on Sarcoma 180A, after repeated fractionation and purification of the extract, eight crystalline antitumor cyclic hexapeptides, RA-I - VIII (**12** - **19**) were isolated. These compounds were assumed to be small peptides from the IR values showing 3390 and 1640 cm^{-1} due to amide bonding. It was determined from the ^{13}C -NMR data of RA-VII (**18**) that there were three C-Me, three CH_2 -, Three N-Me, two O-Me, six CH, eighteen aromatic carbons, eleven tertiary carbons, seven quaternary carbons (three C-C bonds and four C-O bonds), and six carbonyl carbon groups. By analysis of complete and partial hydrolysate of RA-VII (**18**), one D-alanine, two molecules of L-alanine, N-methyl-4-methoxy-L-phenylalanine and N-methyltyrosine dimer having ether linkage were obtained. RA-VII was assumed to be a cyclic hexapeptide consisting of three alanines and three molecules of tyrosine derivatives and having an ether linkage. The exact sequence of amino acids and the stereochemistry were determined by the X-ray analysis of p-bromobenzoate of RA-V (**16**).^{2,3} A series of reactions and instrumental analysis showed the structural relationships among the structures of RA-I (**12**) - RA-VIII (**19**) to be as illustrated in Figure 7. Of this series of oligopeptides in *Rubia cordifolia*, RA-VII and RA-V were the main components, and RA-IX (**20**) - RA-XVI (**27**) were minor components in this RA-series.³²⁻³⁴ The structures of these minor components were determined mainly by

spectroscopic and chemical methods to be as listed in Figure 7. RA-IX (**20**) and -XIV (**25**) contained a pyroglutamic acid instead of the Ala-2 found in RA-VII, and RA-X (**21**), -XI (**22**) and -XIII (**24**) had glutamic acid instead of Ala-2. RA-XII (**23**) - XVI (**27**) were isolated also as glucosides from the same plant.

Recently, from *R. cordifolia*, RA-dimer A (**29**) was isolated and the structure was determined by the analysis of its spectroscopic data and the chemical synthesis of a methyl ether of **29**.³⁵ RA-V (deoxybouvardin, **16**) was selectively brominated to produce monobromide **30**, which was successively *O*-methylated to afford a bromide of RA-VII, **31**. Compound **31** was then coupled with RA-V (**16**) under Ullmann conditions to afford a dimeric compound **32**, which was proved to be identical to RA-dimer A methyl ether obtained by the *O*-methylation of **29** by the HPLC profiles, 500 MHz ¹H NMR and IR spectra, and optical rotations (Scheme 1). RA-dimer A (**29**) gave an IC₅₀ value of 0.26 µg/mL when tested on P-388 leukemia cells.

Another novel antitumor bicyclic hexapeptide RA-XVII (**28**) was isolated from the root of *R. cordifolia*. By spectral studies and synthetic approach, its structure was determined to be [D-2-aminobutyric acid-1]RA-V as shown in Figure 8. Studies on the effect of the side chain at residue 1 showed that it had little effect on the conformation of the molecule, but that the longer the side chain was, the more decreased the activity was.³⁶ On the other hand, the two congeners, bouvardin and deoxybouvardin (RA-V), have been isolated from *Bouvardia ternifolia* (Rubiaceae).³⁷ The antitumor activities of these cyclic hexapeptides which are structurally closely related to RA-VII (**18**), are considered to be due to their inhibition of protein synthesis through interaction with eukaryotic ribosome.^{38,39} Recently, RA-VII was shown to cause conformational changes of F-actin and stabilization of actin filaments to induce G2 arrest.⁴⁰ RA-VII has already been evaluated clinically,^{4,5} but it has serious drawbacks of low water solubility and strong toxicity in humans. In order to synthesize rationally designed analogues for the solution of the above problems, further extensive studies of the detailed structure-activity relationships (SAR) are required.

The SAR studies of cyclic peptides have been carried out by us and by the Prof. Boger group. We studied the SAR focusing on the ζ positional substituent effect in the Tyr-3 and Tyr-6 residues, the effect of the side-chain structures in the amino acid-2 residue, the effect of the modification of amino acid skeleton, and the absolute configuration of constituent amino acids, etc.^{10,41-44} Also, efficient synthetic method of the important isodityrosine was studied for the synthesis of various synthetic derivatives.¹¹ In order to obtain further information on SAR and on the effect of the side-chain conformation in Tyr-3 residue whose substituent at the ζ position is known to be closely related to the activity, we attempted to obtain more new cyclic hexapeptides from *Rubia cordifolia*.

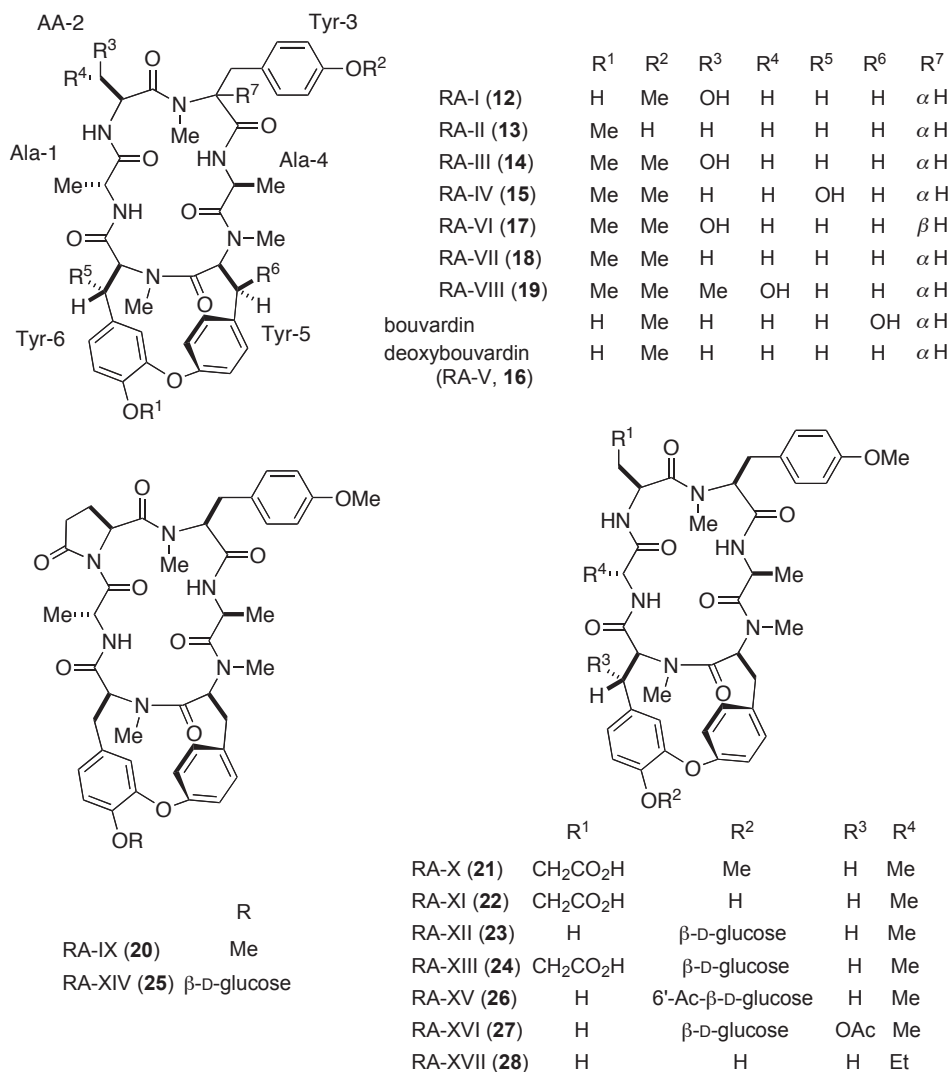
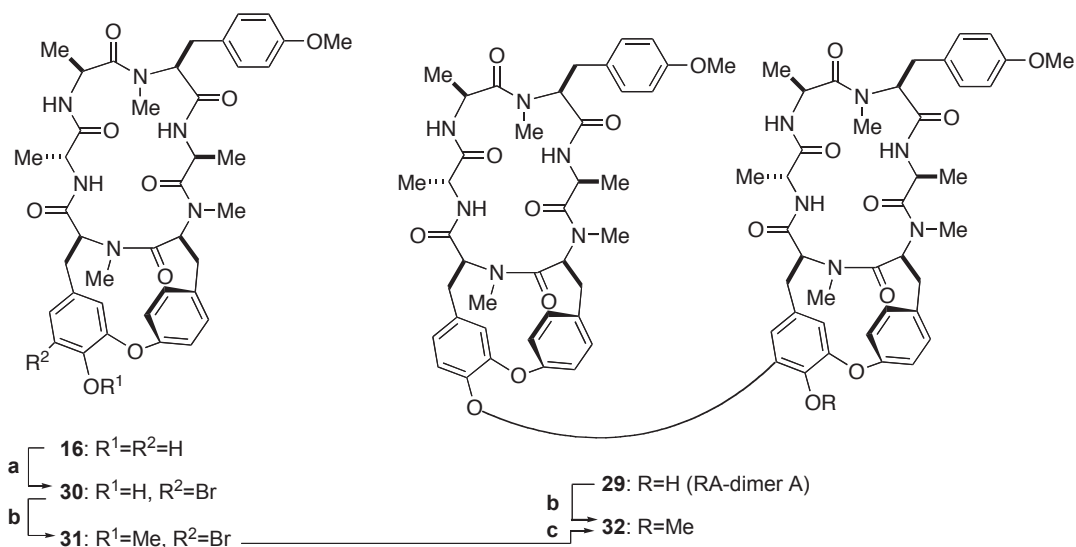


Figure 7. Structures of antitumor cyclic hexapeptides (12-28) from *Rubia cordifolia*.



Scheme 1. Reagents and conditions: (a) C₅H₅N-HBr₃, AcONa, AcOH-CHCl₃, room temp., 20 min, 93%; (b) MeI, K₂CO₃, acetone-MeOH, room temp., 24 h, 95% for 31, for 32; (c) 16 (1.5 equiv.), CuBr-SMe₂, K₂CO₃, C₅H₅N-MeCN, 90°C, 24 h, 8.7%

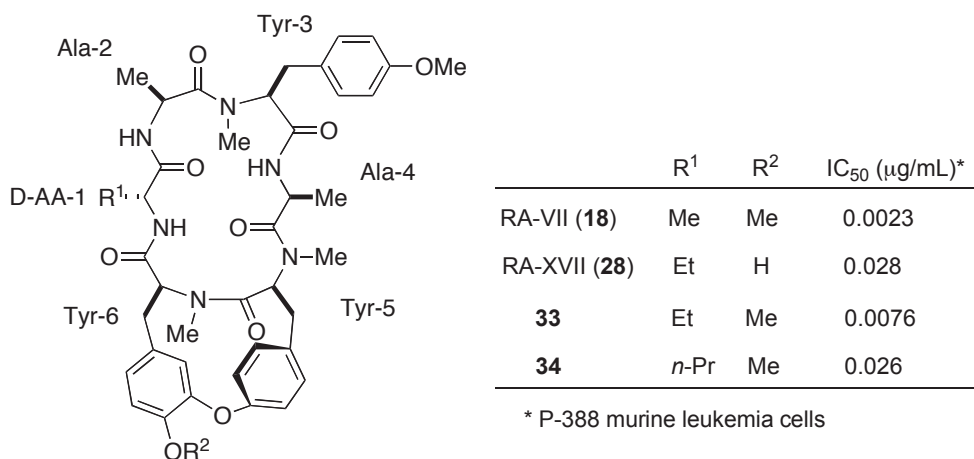


Figure 8. RA-XVII (**28**) from *Rubia cordifolia* and its related compounds.

1. Structure and Activity on New Cyclic Peptide RA-XVIII⁴⁵

From the chloroform-methanol (10:1) soluble portion of a methanol extract of *R. cordifolia*, a new cyclic hexapeptide RA-XVIII (**35**) was isolated (yield: 8.7×10^{-6} %). Its ¹H and ¹³C NMR spectra showed signals typical of RA-series peptides, and also demonstrated that it was of a mixture of two conformers in a ratio of 89:11. The structure of **35** was assumed to be as shown in Figure 9 by using the resonances caused by the major conformer, which was confirmed by the semi-synthesis of **35** from RA-V (**16**). An isomer **39** whose hydroxyl group of Tyr-6 was at a different position from that in **35** was synthesized from RA-VII (**18**) by the above synthetic method. The new peptide **35** and synthesized analogues **36** – **40**, and as references, **18** and **16**, were evaluated for their cytotoxicity on P388 leukemia cells. The IC₅₀ value of **35** was 0.012 μg/mL, indicating that its cytotoxicity was one-fourth of that of **18** (0.0030 μg/mL), though slightly higher than that of **16** (0.014 μg/mL). Analogues **36** – **40** were all less cytotoxic than peptide **18**, thus suggesting that introduction of a hydroxyl, a nitro, or an amino group at the δ_a or the ε_a position of Tyr-6 causes a decrease in the cytotoxic activity.

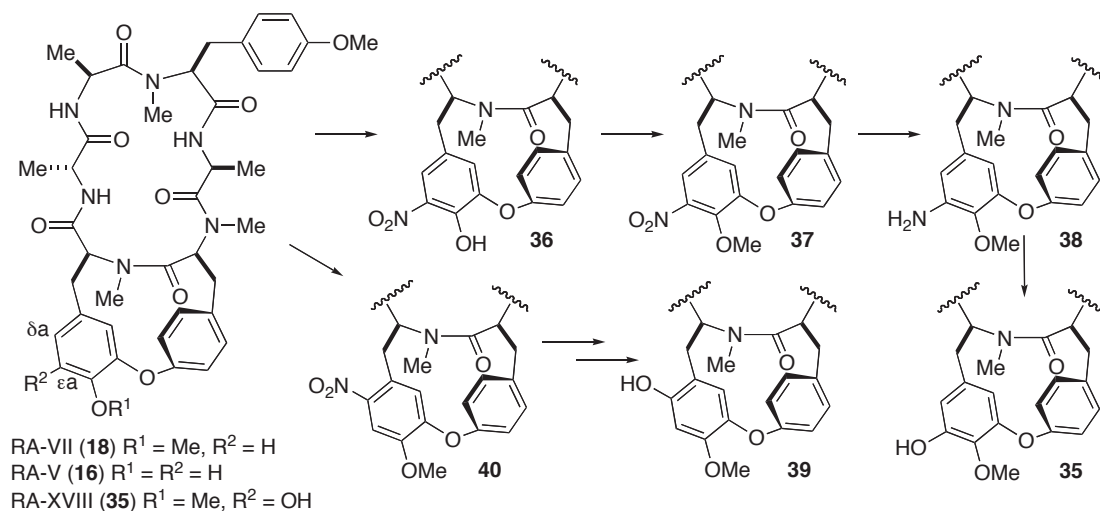


Figure 9. Synthesis of RA-XVIII (**35**) from RA-V and their related compounds.

2. Structures and Activity on New Cyclic Peptides RA-XIX, -XX, -XXI, -XXII, -XXIII, and -XXIV^{46,47}

From the roots of *R. cordifolia*, six new RA-series bicyclic hexapeptides, RA-XIX (**41**, yield: 5.2×10^{-6} %), -XX (**42**, 9.2×10^{-6} %), -XXI (**43**, 1.1×10^{-5} %), -XXII (**44**, 6.9×10^{-5} %), -XXIII (**45**, 2.8×10^{-4} %) and -XXIV (**46**, 5.6×10^{-4} %) were further isolated. The structures of RA-XIX (**41**) and RA-XX (**42**) were established by semisynthesis from a cycloisodityrosine which was derived from previously reported RA-VII (**18**), and those of RA-XXI (**43**) and RA-XXII (**44**) by chemical correlation with RA-XX (**42**). The absolute structure of RA-XXIII (**45**) was confirmed by the X-ray crystallography of bromide **45a** derived from **45**. The structure of RA-XXIV (**46**) was presumed to be the one in which the O-methyl group in **45** was replaced by a hydroxyl group. Treatment of **46** with (trimethylsilyl)diazomethane afforded a product which was shown to be identical to natural **45** by their spectroscopic data and optical rotations. The IC_{50} values of these peptides **41** - **46** against P-388 leukemia cells were in the range of 0.013–0.63 $\mu\text{g/mL}$. Peptides **45** and **46** both showed moderate cytotoxicity on P-388 leukemia cells with IC_{50} values of 0.16 and 0.48 $\mu\text{g/mL}$, respectively.

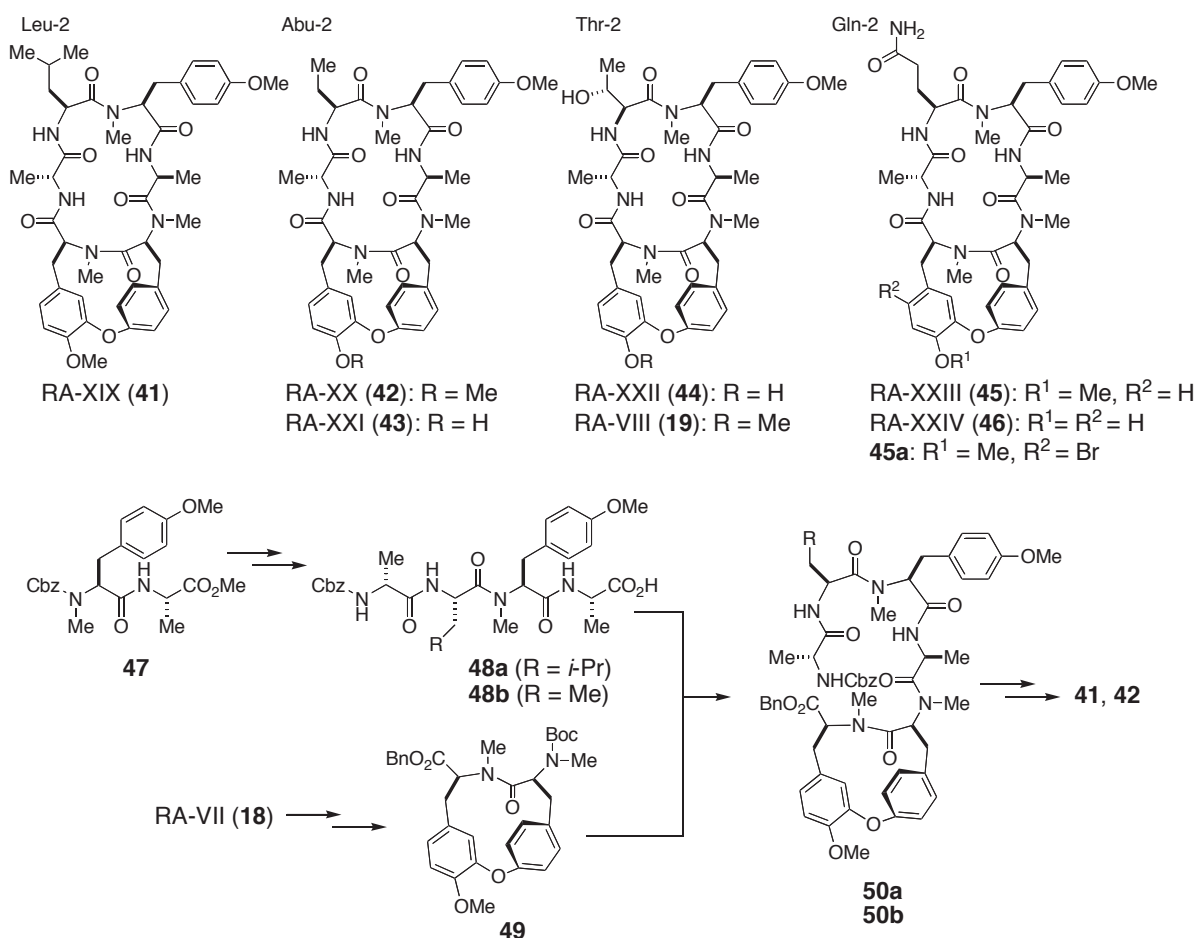


Figure 10. Structures of RA-XIX (**41**) – RA-XXIV (**46**) and their synthesis.

3. Design and Synthesis of a Bis(Cycloisodityrosine) Analogue RA-VII, an Antitumor Bicyclic Hexapeptide¹²

RA-VII (**18**) is known to exist as a mixture of two or three conformers in solution,^{48,49} and the most populated conformer, having *trans, trans, trans, trans, cis, and trans (t-t-t-t-c-t)* configuration at the peptide bonds between D-Ala-1/Ala-2, Ala-2/Tyr-3, Tyr-3/Ala-4, Ala-4/Tyr-5, Tyr-5/Tyr-6, Tyr-6/D-Ala-1, respectively, has been identified as an active conformer. Of the three tyrosines at positions-3, -5, and -6 in RA-VII, Tyr-5 and Tyr-6 form a cycloisodityrosine unit by forming a linkage between the phenolic oxygen of Tyr-5 and the C_ε of Tyr-6. Due to a planar amide bond and 1,3-disubstituted- and 1,4-disubstituted-phenyl rings included in the 14-membered ring of the cycloisodityrosine unit, the rotation of the side chains of those residues is restricted. The remaining side chain at Tyr-3 rotates about the C_α-C_β (χ_1) and C_β-C_γ (χ_2) bonds.

Since the substituent at the zeta position of Tyr-3 is known to be greatly related to the activity, the χ_1 and χ_2 angles of Tyr-3, defining the spatial orientation of the Tyr-3 phenyl ring, appear to play a critical role in the cytotoxicity. Then, to evaluate the effect of the side-chain conformation of Tyr-3 upon the activity, we synthesized an analogue in which Tyr-3 side-chain rotation was restricted by introduction of cycloisodityrosine unit in place of the Ala-2/Tyr-3 moiety of RA-VII. The structure of this analogue having two cycloisodityrosine units in one molecule was as shown in **54** (Figure 11).

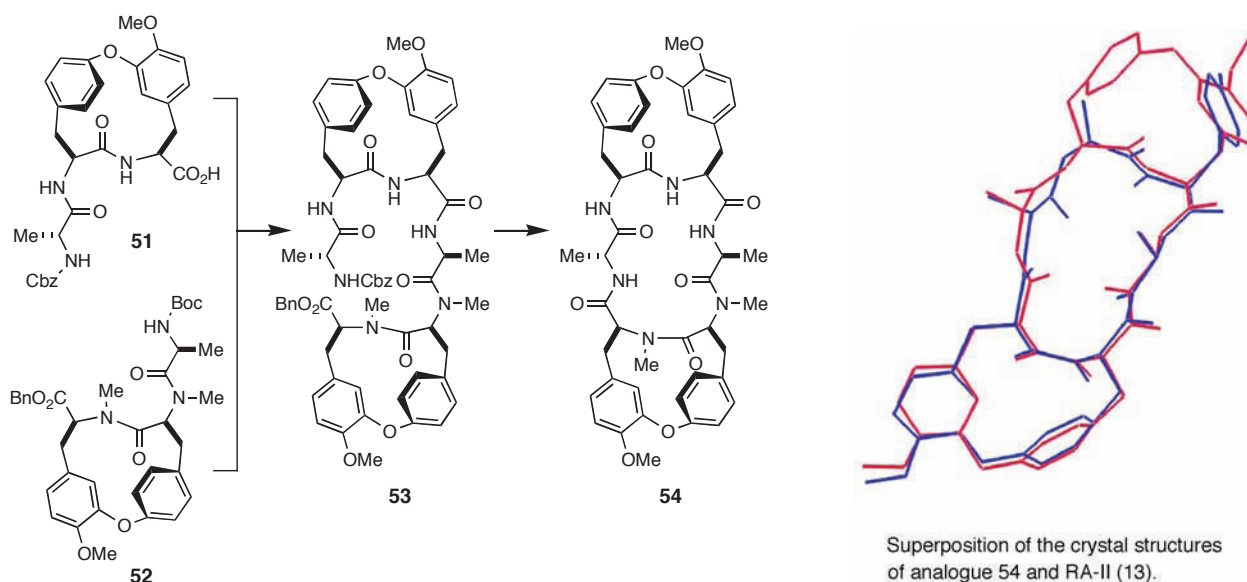


Figure 11. The synthesis of an analogue having a restricted Tyr-3 side-chain rotation and its conformation analysis.

The route of synthesis of **54** is illustrated in Figure 11. The starting material, cycloisodityrosine, corresponding to Tyr-2 and Tyr-3 of **54**, was prepared from 3-iodo-L-tyrosine according to the procedure

reported previously.⁴⁴ It was deprotected and coupled with Cbz-D-Ala-OH to afford tripeptide, which was then converted to acid **51**. The other portion of the above starting material was *N,N'*-dimethylated under phase-transfer catalysis conditions to prepare the other cycloisodityrosine unit, corresponding to Tyr-5 and Tyr-6 of the analogue. Subsequent conversion of the methyl ester functionality of it to a benzyl ester group, which, after removal of the Boc group, was coupled with Boc-Ala-OH to afford tripeptide. After deprotection, tripeptide **52** was coupled with acid **51** to give hexapeptide **53**. Removal of the N- and C-terminal protecting groups of **53** and subsequent formation of the macrocycle with diphenylphosphoryl azide (DPPA) and triethylamine under high-dilution conditions in DMF (0.001 M) furnished **54** in 45% yield from **53**. In this analogue **54**, the rotations about the C_α-C_β and C_β-C_γ bonds of Tyr-3 are restricted simultaneously as in the case of those in Tyr-6. However, Tyr-3 in the newly introduced second cycloisodityrosine unit bears no *N*-methyl group, so that, in contrast to the Tyr-5/Tyr-6 bond of the original cycloisodityrosine unit, the Tyr-2/Tyr-3 bond adopts a *trans* configuration as in the active conformer of RA-VII.

The similarity in the three-dimensional structural features of RA-VII (**18**) and **54** was highlighted by superimposing the crystal structure of **54** over that of RA-II (**13**), whose conformational property is known to be identical to that of **7** (Figure 11).⁵⁰ The spatial positions of the phenyl rings of the three tyrosines and the peptide backbone conformation at residues 2–6 of these two peptides **54** and **13** are almost superimposable, which indicated that analogue **54** may effectively mimic one of the lowest-energy conformations in peptide **18** including the side chain of Tyr-3. Analogue **54** and as reference, **18**, were evaluated for their cytotoxicity on P-388 leukemia cells. Their IC₅₀ values were 7.5 and 0.0015 μg/mL, respectively. The result apparently does not agree with our hypothesis that the side-chain conformation at Tyr-3 of peptide **18**, as shown in the crystal structure of **13**, is a major factor which determines the cytotoxic activity of the compounds of this series. The bulky phenoxy ether connecting the C_β of Ala-2 and the C_ε of Tyr-3 in the present compound **54**, however, may be hampering its necessary close access to the relevant binding site, resulting in giving low cytotoxicity. Synthesis of further analogues and their analyses may give further information to this problem.

C. ANTITUMORE CYCLIC PENTAPEPTIDES FROM *ASTER TATARICUS* (COMPOSITAE)

Aster tataricus (Compositae) is a Chinese medicine known to contain several terpenoids and saponins, and is also a popular garden flower. From a *n*-butanol extract of the root of this plant showing a potent antitumor activity, three new cyclic pentapeptides, astins A–C (**55–57**)^{6,8} containing a dichlorinated proline residue were isolated.^{51,52} Their structures, containing a novel dichlorinated proline residue, were determined on the basis of 2D NMR and FAB MS spectroscopy analysis and by degradation reaction, followed by HPLC analysis using Marfey's method. Astin A consists of *L-allo* threonine,

L-serine (Ser), β -phenyl alanine (β -Phe), L- α -amino-butyric acid (Abu), and L- β,γ -dichlorinated proline [Pro(Cl₂)] residues. *Allo*-threonine is often found as a constituent in biologically active peptides. The amino acid sequence of astin A was determined on the basis of HMBC correlations and MS fragmentation patterns.

Cyclic astins D–I (**58**–**63**) were also isolated from the same source as minor constituents and were characterized on the basis of degradation reactions and NMR studies.^{51,53} These cyclic astins contain several different types of unique chlorinated proline residues in the molecule. The β,γ -dichlorinated proline is in astins A, B, and C, a $\Delta^{4(5)}$, δ -chlorinated proline in astins D (**58**), E (**59**),⁵¹ and H (**62**),⁵³ a β -chlorinated proline in astin F (**60**),⁵³ and a β -hydroxy- γ -chlorinated proline in astin I (**63**).⁵⁴ All of the chlorine atom and hydroxy group configurations in the above proline residues were elucidated to be β -orientated by NOEs, ¹H coupling, and HMBC correlations.

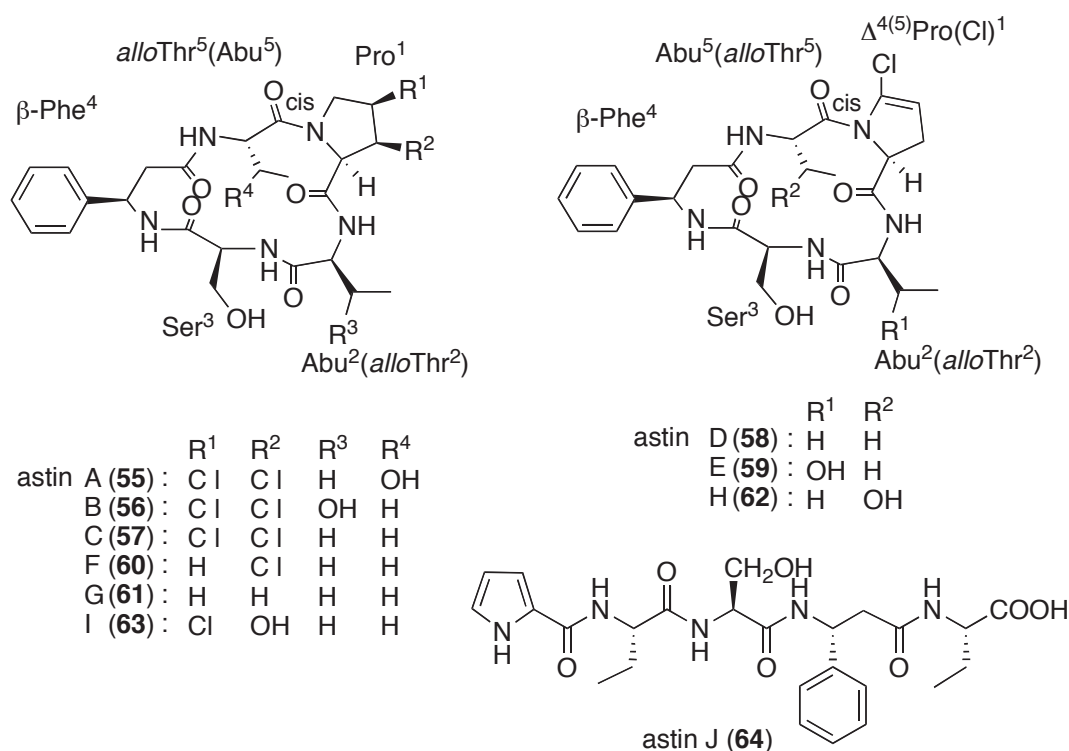


Figure 12. Structures of astins A–J (**55**–**64**) from *Aster tataricus*.

Astins A – C (**55** – **57**), containing chlorines and an *allo* Thr, and characterized by one *cis* peptide bond, showed antitumor activities, as determined by the total packed cell volume method using Sarcoma 180 ascites in mice.^{6,8} This assay estimates this activity in terms of the tumor growth ratio (GR(%))=(test group packed cell volume/control group packed cell volume) \times 100). The GR results for astins A, B and C were 40% (++) (dose 0.5 mg/kg/day), 26% (++) (dose 0.5 mg/kg/day) and 45% (+) (dose 5 mg/kg/day), respectively, for 5 consecutive days. The effective doses for astins A and B (**55** and **56**) were 1/10 of

that for astin C (**57**). The other astins D–J (**58–64**) did not inhibit tumor growth even at 10.0 mg/kg/day. Various congeners with no dichlorinated proline residues, prepared by chemical derivatization from astins A–C (**55–57**) and by a hepatic microsomal biotransformation in rats, did not show antitumor activities either, suggesting that the 1,2-*cis* dichlorinated proline residue might play an important role in the antitumor activity of astins.⁵⁵ It was suggested that the backbone conformations of astins A and C (**55** and **57**), having a weaker activity than astin B (**56**), were different from that of astin B, and that such difference in the backbone conformation might affect antitumor activity.^{56,57} However, the presence of *cis* dichlorinated proline residues was concluded to be a more essential structural motif for the astins to show antitumor activity on S-180A.

The effect of the backbone conformational difference between astin B (**56**) and astin C (**57**) was more clearly seen by the backbone modification using Lawesson's reagents, and comparison of the activities of the resulting these derivatives. The produced thionated derivatives, [Ser-3-ψ(CS-NH)-β-Phe-4]astin A (thioastin A), [Ser-3-ψ(CS-NH)-β-Phe-4]astin B (thioastin B) and [Ser-3-ψ(CS-NH)-β-Phe-4]astin C (thioastin C), showed more promising antitumor activity than the corresponding parent astins.^{58,59}

Table 1. Antitumor Activity of Astins. Antitumor activity was examined by the total packed cell volume method by using Sarcoma 180A in mice. Drugs were given daily at indicated doses for consecutive 5 days from 1 day to 5 day. The effectiveness was evaluated in terms of the tumor growth ratio [GR% = (test group packed cell volume / control group packed cell volume) × 100].

compounds	dose (mg/kg/day)	route	T/C (%)
astin A (55)	0.5	i.p.	40.00 (++)
astin B (56)	0.5	i.p.	26.00 (++)
astin C (57)	5.0	i.p.	45.00 (+)
astin D (58)	10.0	i.p.	96.62 (-)
astin E (59)	10.0	i.p.	107.14 (-)
astin F (60)	10.0	i.p.	122.62 (-)
astin G (61)	10.0	i.p.	90.26 (-)
astin H (62)	NT*	NT*	NT*
astin I (63)	NT*	NT*	NT*
astin J (64)	10.0	i.p.	120.62 (-)
thioastin A	0.5	i.p.	25.56 (++)
thioastin B	0.5	i.p.	14.00 (++)
thioastin C	1.0	i.p.	55.90 (+)

* NT: not tested.

Recently, a new chlorinated cyclic pentapeptide, hydroxycyclochlorotine, was isolated from a fungus *Penicillium islandicum*.⁶⁰ Both hydroxycyclochlorotine and the present astin B (**56**) containing an *allo* threonine as residue 2, have a *cis* proline configuration, but cyclochlorotine, one of hepatotoxic mycotoxins, is known to be in two conformational states in solution, which may be due to *cis-trans* isomerization of the proline amide bond. The presence of an intramolecular hydrogen bond between Ser³-NH and a hydroxyl oxygen atom of *allo*Thr² may serve to maintain the backbone conformation.

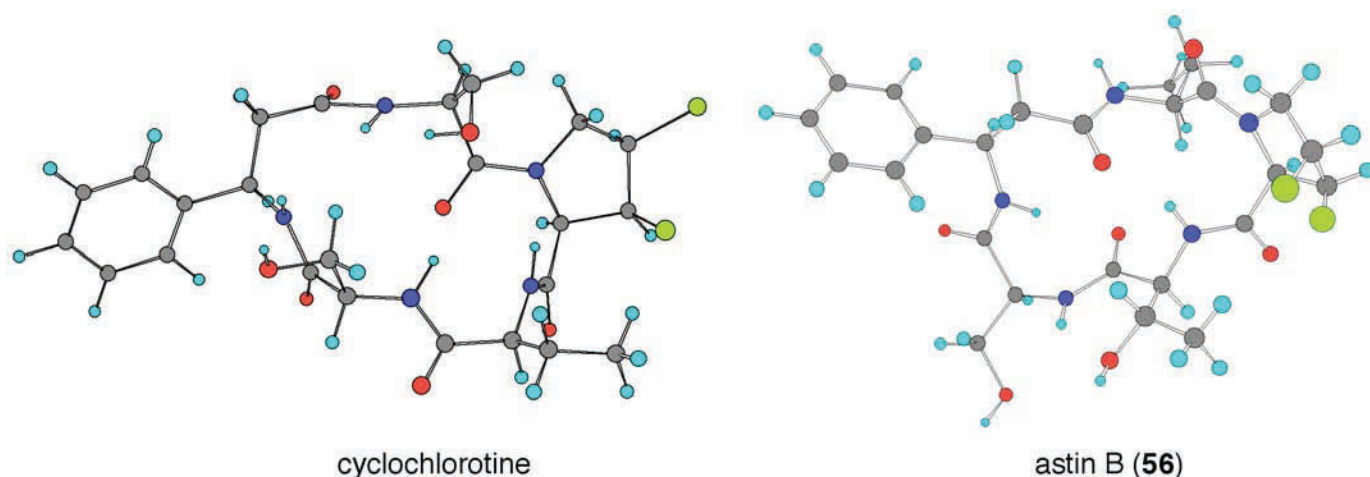


Figure 13. X-Ray crystallographic structures of cyclochlorotine and astin B (**56**)⁶⁰

D. SEGETALINS FROM *VACCARIA SEGETALIS* (CARYOPHYLLACEAE)

Seeds of *Vaccaria segetalis* (Caryophyllaceae) have been used as a Chinese drug for invigorating blood circulation, regulating menstrual disturbance and dispelling edema.⁶¹⁻⁶⁴ From a MeOH extract of the seeds of *V. segetalis*, we isolated new cyclic peptides, segetalins A–H (**65–72**).⁶¹⁻⁶⁶ Their structures including absolute stereochemistry were elucidated by using 2D NMR and Marfey's methods.

AcOEt and MeOH extracts of *V. segetalis* and the cyclic peptides, segetalins A and B (**65** and **66**) were shown to have a moderate estrogen-like activity. When the peptides were administrated to rats for 14 consecutive days, the weight of uterus increased dose-dependently. This estrogenic activity was also confirmed by recording the oxytocin-induced uterine contractions. It is interesting that the non-steroidal cyclic peptides have an estrogenic activity.⁶⁷⁻⁶⁹

Thionation of segetalins A (**65**) and B (**66**) with Lawesson's reagent gave the corresponding thiosegetalins, of which only thiosegetalin A2 showed estrogen-like activity in ovariectomized rats. The backbone conformation was considered to play an important role in the estrogen-like activity by segetalins.

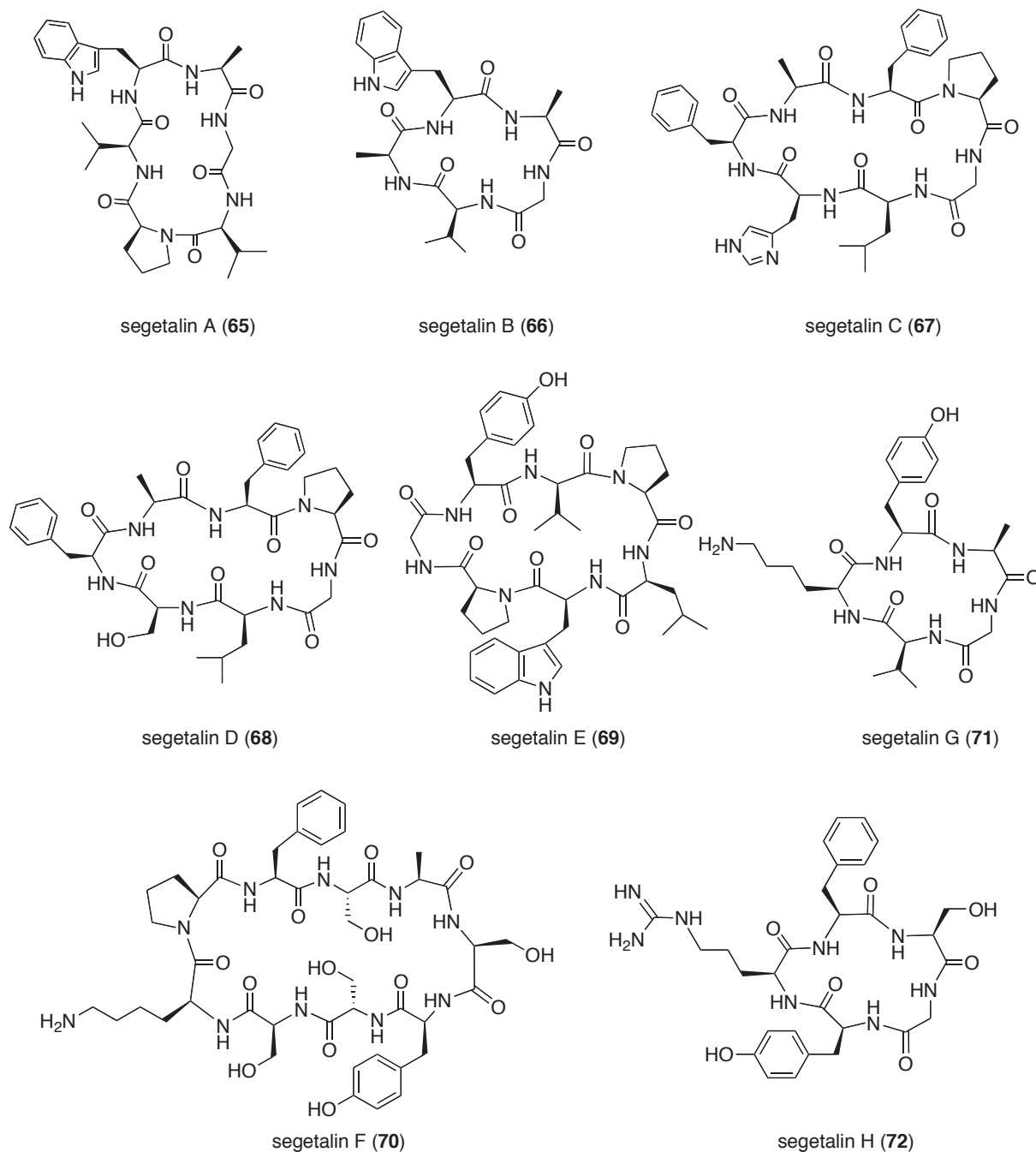


Figure 14. Structures of segetalins A–H (65–72) from *Vaccaria segetalis*.

Vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. When NE 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, segetalins A–H (65–72) were added at 10^{-4} M. Segetalins A, D, F, G, and H (65, 68, 70, 71, and 72) showed slow vasorelaxant actions.⁶⁵ Segetalins F (70), G (71), and H (72) having a basic amino acid such as Lys and Arg exhibited a relatively potent relaxant activity. Interestingly, segetalin B (66) with a Trp residue and without a basic amino acid showed a contractile activity. In addition, a vasodilation effect seems not to be related to the ring size. The same relaxant actions were seen in the sample of aortic rings without endothelium.

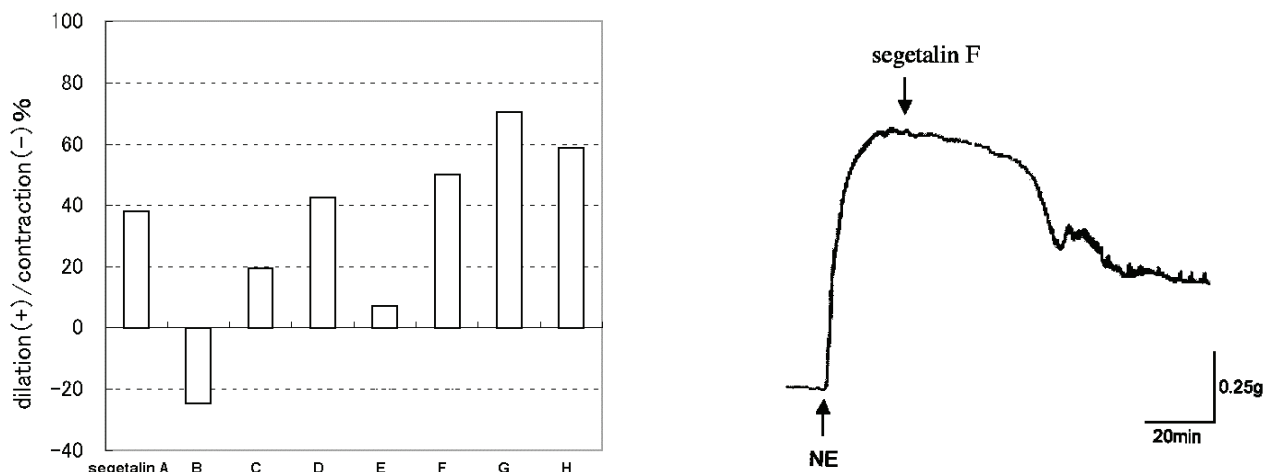


Figure 15. Relaxation effects of 10^{-4} M segetalins A–H (**65–72**) on aortic rings precontracted with 3×10^{-7} M norepinephrine (NE). Positive values show vasodilator effects and negative ones show vasoconstriction effects. Typical recording of the slow relaxation effect of segetalin F (**70**: 10^{-4} M) on NE (3×10^{-7} M) - contracted aorta.

So far, from plants belonging to the Caryophyllaceae, a variety of cyclic peptides, such as segetalins,⁶¹⁻⁶⁶ yunnanins,⁷⁰⁻⁷² dichotomins,⁷³⁻⁷⁵ delavayins,⁷⁶ and pseudostellarins,⁷⁷⁻⁸⁰ have been obtained. Recent investigation by Tan *et al.* is increasing the number of new cyclic peptides.⁸¹⁻⁸⁴ These cyclic peptides are apparently present in various parts of the plants such as seeds, roots, fruit, and whole plants.

E. CYCLOSQUAMOSINS FROM *ANNONA SQUAMOSA* (ANNONACEAE)

Annona squamosa (Annonaceae) is a fruit tree and its seeds is known to contain many acetogenins consisting of long chain fatty acids. From a MeOH extract of the seeds of *A. squamosa* showing a vasorelaxant activity on rat aorta, we isolated seven cyclic peptides, cyclosquamosins A–G (**73–79**), whose structures were established by spectroscopic data.^{85,86}

In an assay, norepinephrine (NE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, and then cyclosquamosins A–G (**73–79**) at 10^{-4} M were added so that slow vasorelaxant actions were observed (Figure 17). Cyclosquamosin B (**74**) showed the most potent vasorelaxant effect, whereas cyclosquamosin C (**75**) with methionine sulfoxide (Mso) had no vasorelaxant effect, indicating that Met residue was necessary for the vasodilation. On the other hand, cyclic octapeptide, cyclosquamosin G (**79**) with Met residue was found to be less effective than cyclosquamosin B (**74**), although both cyclic octapeptides had common structural features as shown in Figure 16. Cyclosquamosin B (**74**) has 2 Pro residues, whereas cyclosquamosin G (**79**) has 1 Pro, which may cause them take different conformation in solution. The geometry between two Pro residues in cyclosquamosin B (**74**) was shown to be *cis* by the strong NOE correlation between the two H α in Pro residues, which was supported also by the ^{13}C chemical shifts (δ 31.6 and 24.9) of β and γ positions in the second Pro residue and the occurrence of a doublet signal of H α in the second Pro residue (δ 4.74, d,

$J=7.3\text{Hz}$). The presence of the second Pro residue might allow limited space for conformational movement. Appropriate amide geometry may be important in deciding the conformation of the whole molecule.⁸⁶

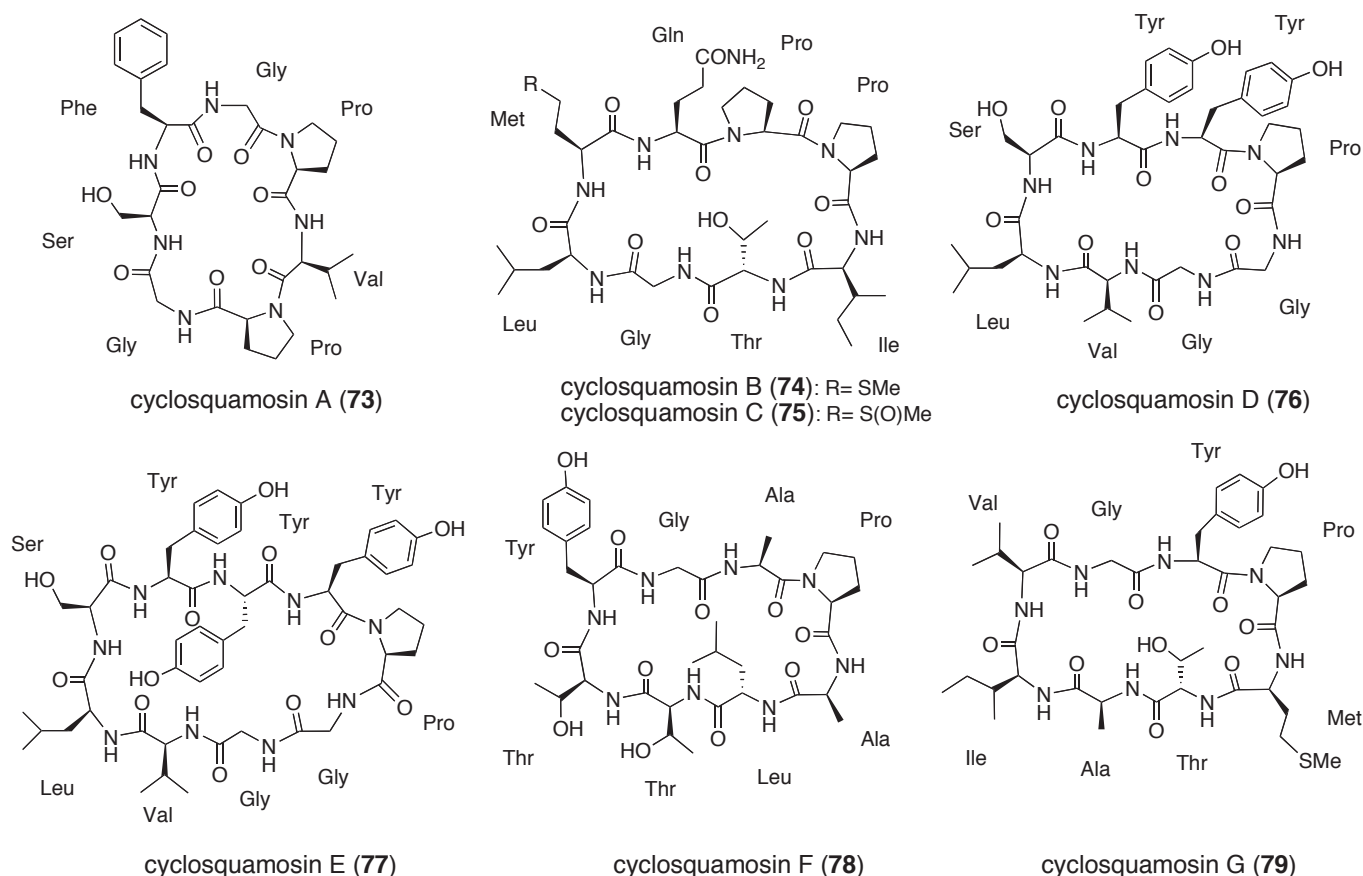


Figure 16. Structures of cyclosquamosins A–G (**73–79**) from *Annona squamosa*.

As mentioned above, cyclosquamosin B (**74**) has vasorelaxant effect. Figure 17 shows that at a concentration of 10^{-4} to 10^{-6} M, it decreased NE-induced vasoconstrictions in a concentration-dependent manner and the same relaxant action was seen in the sample of aortic rings without endothelium. Thus the findings suggest that the inhibitory effect of cyclosquamosin B (**74**) on aortic rings is not dependent on the presence of endothelium.

Ca^{2+} can contract aortic rings concentration dependently in Ca^{2+} -free KHS after depolarization with isotonic high K^+ (60 mM) by the influx *via* voltage-dependent Ca^{2+} channels (VDCs); this contraction was significantly inhibited by cyclosquamosin B (**74**) at the concentration of 10^{-5} M (Figure 17).

In addition, the NE (10^{-6} M)-induced contractions of the aortic rings in the presence of nifedipine (10^{-6} M) in Ca^{2+} -free KHS occurred in a Ca^{2+} (10^{-5} M to 10^{-3} M) concentration-dependent manner, presumably due to $[\text{Ca}^{2+}]_i$ *via* receptor-operated Ca^{2+} channels (ROCs). Cyclosquamosin B (**74**) inhibited these contractions at the concentrations of 10^{-5} M moderately, suggesting that cyclosquamosin B exerts

inhibitory effects on $[Ca^{2+}]_i$. Consequently, the vasorelaxant activity of cyclosquamosin B (**74**) may be attributed to the inhibition of VDC and partially dependent on ROC.

Recently, cyclosquamosin D (**76**) was shown to have an inhibitory effect on the production of pro-inflammatory cytokines within lipopolysaccharide and Pam3Cys-stimulated J774A.1 macrophages.⁸⁷

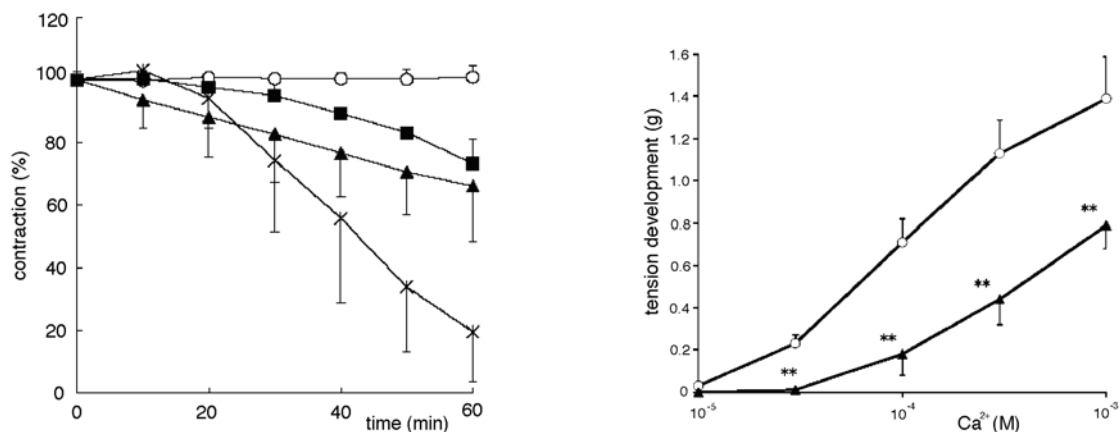


Figure 17. Left: relaxation responses induced by cyclosquamosin B (**74**) in aortic rings precontracted with 3×10^{-7} M norepinephrine (NE); symbols: -○- control, -x-: cyclosquamosin B at 10^{-4} M, -▲-: cyclosquamosin B at 10^{-5} M, -■-: cyclosquamosin B at 10^{-6} M, Right: concentration-response relationships for contractile responses of the aortic rings to Ca^{2+} in a Ca^{2+} -free medium preincubated with high potassium (60mM); symbols: -○- control, -▲-: cyclosquamosin B (**74**) at 10^{-5} M. Values are the mean \pm S.E. (n=4). ** P < 0.01

F. CYCLONATSUDAMINE A FROM *CITRUS NATSUDAIDAI* (RUTACEAE)

From a MeOH extract of the peels of *Citrus natsudaidai* (Rutaceae), showing a vasorelaxant effect on rat aorta, a cyclic heptapeptide, cyclonatsudamine A (**80**) was isolated as a new vasodilator.⁸⁸

The structure of cyclonatsudamine A (**80**) was elucidated to be *cyclo* (-Gly-Tyr-Leu-Leu-Pro-Pro-Ser-) by using a combination of 2D-NMR experiments on a 800 MHz NMR machine, such as ¹H-¹H COSY, HOHAHA, HMQC, and HMBC spectra.

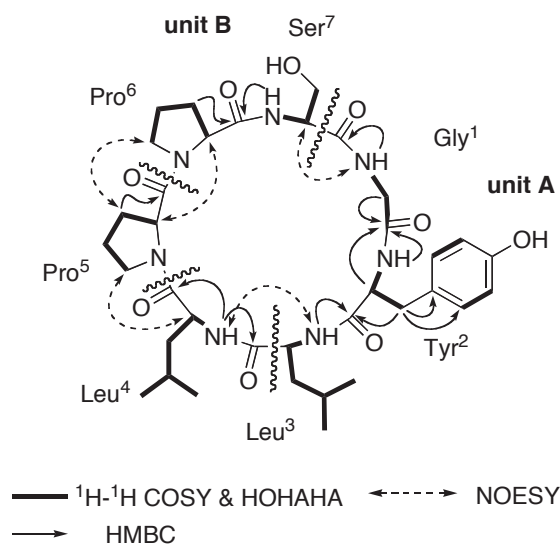


Figure 18. Selected 2D NMR correlations of cyclonatsudamine A (**80**).

After achieving a maximal response to thoracic aortic rings with endothelium by NE (3×10^{-7} M), cyclonatsudamine A (**80**) showed vasorelaxant action at 10^{-4} M (Figure 19). The vasorelaxant activity of cyclonatsudamine A (**80**) was observed in a concentration-dependent manner (10^{-4} M, 80% relaxation; 3×10^{-7} M, 46 % relaxation) and did not cause vascular relaxation in endothelium-denuded aortic tissues. Treatment with N^G -monomethyl-L-arginine (L-NAME, 10^{-4} M), an inhibitor of nitric oxide (NO) synthase, also inhibited cyclonatsudamine A-induced vasorelaxation. The vasodilator effect of cyclonatsudamine A (**80**) may be mediated through the increased release of NO from endothelial cells.⁸⁸

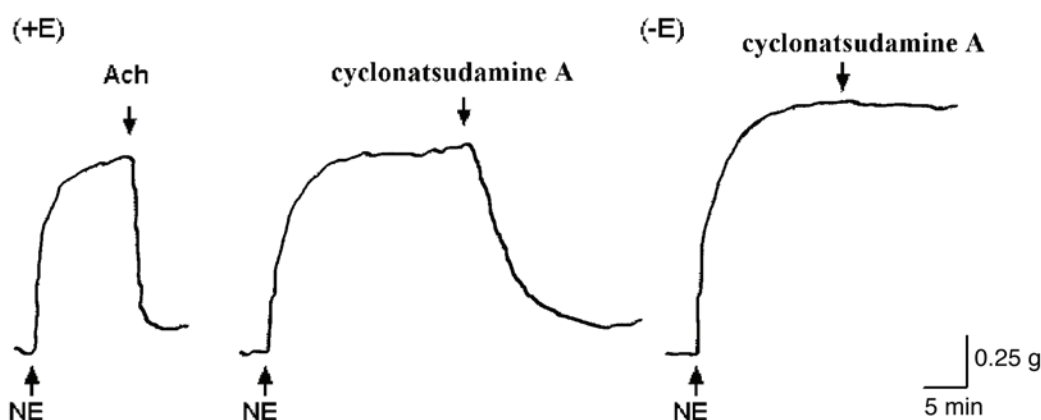


Figure 19. Typical recording of the relaxation effect of cyclonatsudamine A (**80**: 10^{-4} M) on aortic rings precontracted with 3×10^{-7} M norepinephrine (NE) with endothelium (+E) and without endothelium (-E).

So far, a series of citrucins I – X have already been isolated from the fruit peels of various Citrus plants, such as *Citrus unshiu*, *C. sinensis*, *C. natsudaidai*, *C. medica* var. *sarcodactylis*, and *C. aurantium*. Few report is available which describes the biological activity of these cyclic peptides.⁸⁹⁻⁹¹

Table 2. Structures of cyclic peptides (citrucins) from the seeds of *Citrus* species.

compounds	structures	molecular formula
citrusin I	cyclo (-Ala-Thr-Gly-Thr-Phe-Leu-Ile-)	$C_{34}H_{53}N_7O_9$
citrusin II	cyclo (-Ala-Pro-Phe-Trp-Gly-Gly-Pro-)	$C_{37}H_{44}N_8O_7$
citrusin III	cyclo (-Gly-Ser-Pro-Leu-Leu-Pro-Trp-)	$C_{36}H_{53}N_7O_9$
citrusin IV	cyclo (-Ala-Glu-Trp-Gly-Glu-Val-Pro-Glu-)	$C_{41}H_{55}N_9O_{14}$
citrusin V	cyclo (-Gly-Leu-Val-Leu-Pro-Ser-)	$C_{27}H_{46}N_6O_7$
citrusin VI	cyclo (-Gly-Gly-Leu-Leu-Leu-Pro-Pro-Phe-)	$C_{41}H_{62}N_8O_8$
citrusin VII	cyclo (-Asp-Leu-Thr-Val-Tyr-Phe-Gly-)	$C_{39}H_{53}N_7O_{11}$
citrusin VIII	cyclo (-Ala-Ala-Gly-Leu-Pro-Trp-Leu-Ile-)	$C_{42}H_{63}N_9O_8$
citrusin IX	cyclo (-Glu-Ile-Gly-Ile-Phe-Pro-Pro-)	$C_{38}H_{56}N_8O_8$
citrusin X	cyclo (-Gly-Gly-Pro-Pro-Trp-Pro-Phe-)	$C_{39}H_{46}N_8O_7$

G. CYCLOLIANOPEPTIDES FROM *LINUM USITATISSIMUM* (LINACEAE)

Cyclolinopeptide A (**81**), a cyclic nonapeptide, was the first natural cyclic peptide isolated from linseed oil. Cyclolinopeptide A (**81**) was found to exhibit a distinct immunosuppressive activity and is currently under intensive chemical, biological, and pharmacological investigation.^{92,93}

From a MeOH extract of the seeds of *L. usitatissimum*, another new cyclic peptide, cyclolinopeptide B (**82**) was isolated along with cyclolinopeptide A.⁹⁴ Cyclolinopeptide B (**82**) showed an inhibitory effect on mitogen (concanavalin A)–induced response of human peripheral-blood lymphocytes.

The structures were elucidated by extensive 2D NMR studies and chemical degradations to be as shown in Figure 20. Further analysis of extracts from *L. usitatissimum* led to isolation of additional related cyclic peptides, cyclolinopeptides C–K, whose structures are listed in Table 3.^{95,96}

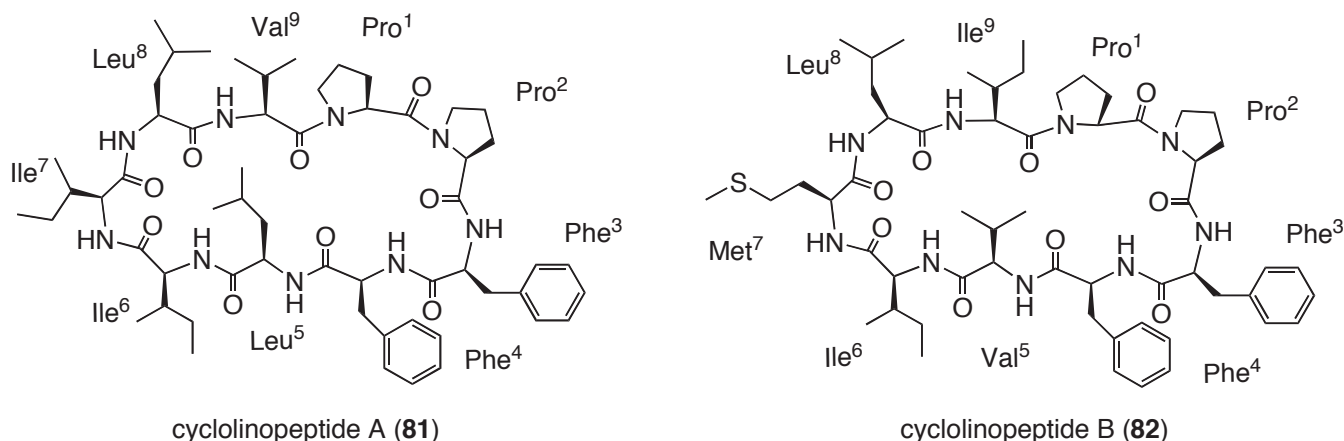


Figure 20. Structures of cyclolinopeptides A and B (**81** and **82**) from *Linum usitatissimum*.

Table 3. Structures and immunosuppressive effect of cyclic peptides from the seeds of *Linum usitatissimum*.

compounds	structures	IC ₅₀ (μg/mL)*
cyclolinopeptide A (81)	cyclo (-Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val-)	2.5
cyclolinopeptide B (82)	cyclo (-Pro-Pro-Phe-Phe-Val-Ile-Met-Leu-Ile-)	39.0
cyclolinopeptide C (83)	cyclo (-Pro-Pro-Phe-Phe-Val-Ile-Mso-Leu-Ile-)	>100
cyclolinopeptide D (84)	cyclo (-Pro-Phe-Phe-Trp-Ile-Mso-Leu-Leu-)	>100
cyclolinopeptide E (85)	cyclo (-Pro-Leu-Phe-Ile-Mso-Leu-Val-Phe-)	43.0
cyclolinopeptide F (86)	cyclo (-Pro-Phe-Phe-Trp-Val-Mso-Leu-Mso-)	>100
cyclolinopeptide G (87)	cyclo (-Pro-Phe-Phe-Trp-Ile-Mso-Leu-Mso-)	>100
cyclolinopeptide H (88)	cyclo (-Pro-Phe-Phe-Trp-Ile-Mso-Leu-Met-)	>100
cyclolinopeptide I (89)	cyclo (-Pro-Phe-Phe-Trp-Val-Met-Leu-Mso-)	>100
cyclolinopeptide J (90)	cyclo (-Pro-Leu-Phe-Ile-Msn-Leu-Val-Phe-)	28.1
cyclolinopeptide K (91)	cyclo (-Pro-Pro-Phe-Phe-Val-Ile-Msn-Leu-Ile-)	25.2

* Inhibitory effect on mitogen–induced response of human peripheral–blood lymphocytes.

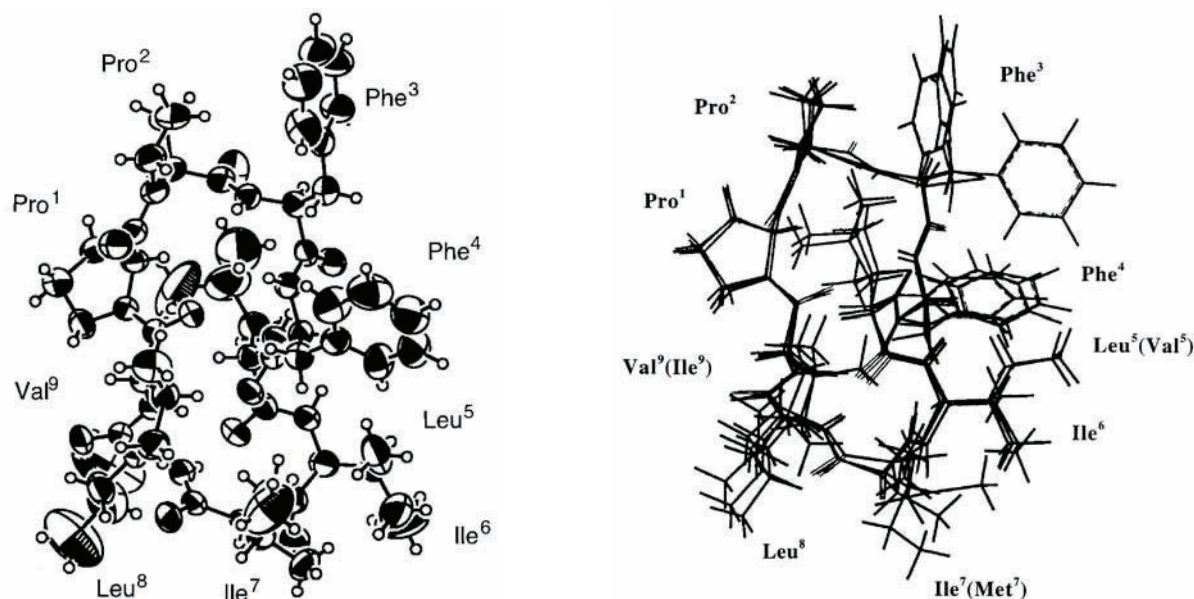


Figure 21. Left: ORTEP drawing of cyclolinopeptide A (**81**). Right: superposition of the backbone of cyclolinopeptides A and B (**81** and **82**), RMSD=0.25.

Cyclolinopeptides A (**81**) and B (**82**) showed an inhibitory effect on mitogen (concanavalin A)–induced response of human peripheral–blood lymphocytes (IC_{50} : 2.5 and 39 $\mu\text{g/ml}$, respectively).⁹⁴ It is known that the biological activity of cyclolinopeptide A is critically dependent on the sequence and conformation of the molecule. Three dimensional structures of cyclolinopeptides A (cPPFFLILV) and B (cPPFFVIMLI) isolated from the seeds of *Linum usitatissimum* were analyzed by X-ray crystallography and the distance geometry (DG) calculations using nuclear Overhauser effect (NOE) constraints. Conformation in the solid state of cyclolinopeptide A was similar to those of cyclolinopeptides A and B in solution. Their common conformational features may shed light on the relations between the biological activity and conformation.⁹⁷

CONCLUSIONS

Our recent studies on the cyclic peptides from medicinal plants are reviewed, on the special attention to their recent studies in their bioactivity and unique cyclic structures of celogentins, RAs, astins, segetalins, cycloquamosins, cyclonatsudamine, and cyclolinopeptides. There are currently more than 500 cyclic peptides from higher plants. Further phytochemical investigations will shed light on the more various cyclic peptides from higher plants.

Total syntheses of some of the cyclic peptide skeletons have been established, whereas some still remain to be accomplished. It is of value to elucidate the structural and pharmacological features of more cyclic peptides. Further efforts for understanding the properties of these complex cyclic peptides will definitely bring forthcoming developments in this field.

ACKNOWLEDGMENTS

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REFERENCES

1. H. Morita, In International Congress Series, Elsevier Science B. V., 1998, Vol. 1157 (Towards Natural Medicine Research in the 21st Century), p. 467.
2. H. Itokawa, K. Takeya, K. Mihara, N. Mori, T. Hamanaka, T. Sonobe, and Y. Iitaka, *Chem. Pharm. Bull.*, 1983, **31**, 1424.
3. H. Itokawa, K. Takeya, N. Mori, T. Hamanaka, T. Sonobe, and K. Mihara, *Chem. Pharm. Bull.*, 1984, **32**, 284.
4. H. Majima, S. Tsukagoshi, H. Furue, M. Suminaga, K. Sakamoto, R. Wakabayashi, S. Kishino, H. Niitani, A. Murata, A. Genma, N. Nukariya, K. Uematsu, T. Furuta, M. Kurihara, F. Yoshida, S. Isomura, T. Takemoto, M. Hirashima, T. Izumi, I. Nakao, Y. Ohashi, K. Ito, and R. Asai, *Jpn. J. Cancer Chemother.*, 1993, **20**, 67.
5. F. Yoshida, R. Asai, H. Majima, S. Tsukagoshi, H. Furue, M. Suminaga, K. Sakamoto, H. Niitani, A. Murata, M. Kurihara, T. Izumi, I. Nakao, Y. Ohashi, and K. Ito, *Jpn. J. Cancer Chemother.*, 1994, **21**, 199.
6. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, 1993, **41**, 992.
7. J. Kobayashi, H. Suzuki, K. Shimbo, K. Takeya, and H. Morita, *J. Org. Chem.*, 2001, **66**, 6626.
8. H. Morita, S. Nagashima, K. Takeya, H. Itokawa, and Y. Iitaka, *Tetrahedron*, 1995, **51**, 1121.
9. R. Cozzolino, P. Palladino, F. Rossi, G. Cali, E. Benedetti, and P. Laccetti, *Carcinogenesis*, 2005, **26**, 733.
10. Y. Hitotsuyanagi and K. Takeya, *J. Synth. Org. Chem., Jpn.*, 2004, **62**, 993.
11. Y. Hitotsuyanagi, H. Ishikawa, S. Naito, and K. Takeya, *Tetrahedron Lett.*, 2003, **44**, 5901.
12. J.-E. Lee, Y. Hitotsuyanagi, Y. Nakagawa, S. Kato, H. Fukaya, and K. Takeya, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 6458.
13. B. Ma, D. N. Litvinov, L. He, B. Banerjee, and S. L. Castle, *Angew. Chem. Int. Ed.*, 2009, **48**, 6104.
14. J. Zhou and N.-H. Tan, *Chin. Sci. Bull.*, 2000, **45**, 1825.
15. G. Gu, L. Smith, N. Wang, H. Wang, and S.-E. Lu, *Biochem. Biophys. Res. Commun.*, 2009, **380**, 328.
16. A. W. Schultz, D.-C. Oh, J. R. Carney, R. T. Williamson, D. W. Udvary, P. R. Jensen, S. J. Gould, W. Fenical, and B. S. Moore, *J. Am. Chem. Soc.*, 2008, **130**, 4507.

17. N. I. Martin, H. Hu, M. M. Moake, J. J. Churey, R. Whittal, R. W. Worobo, and J. C. Vederas, *J. Biol. Chem.*, 2003, **278**, 13124.
18. K. F. Pedley and J. D. Walton, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 14174.
19. B. Walzel, B. Riederer, and U. Keller, *Chem. Biol.*, 1997, **4**, 223.
20. N.-H. Tan and J. Zhou, *Chem. Rev.*, 2006, **106**, 840.
21. A. B. Pomilio, M. E. Battista, and A. A. Vitale, *Curr. Org. Chem.*, 2006, **10**, 2075.
22. M. M. Joullié and D. J. Richard, *Chem. Comm.*, 2004, 2011.
23. H. R. El-Seedi and M. H. Zahra, *Phytochem. Rev.*, 2007, **6**, 143.
24. T.-W. C. Leung, D. H. Williams, J. C. J. Barna, and S. Foti, *Tetrahedron*, 1986, **42**, 3333.
25. T.-W. C. Leung, D. H. Williams, J. C. J. Barna, and S. Foti, *J. Org. Chem.*, 1989, **54**, 1901.
26. H. Morita, K. Shimbo, H. Shigemori, and J. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 469.
27. H. Suzuki, H. Morita, S. Iwasaki, and J. Kobayashi, *Tetrahedron*, 2003, **59**, 5307.
28. H. Suzuki, H. Morita, M. Shiro, and J. Kobayashi, *Tetrahedron*, 2004, **60**, 2489.
29. K. Yoshikawa, S. Tao, and S. Arihara, *J. Nat. Prod.*, 2000, **63**, 540.
30. S. Yahara, C. Shigeyama, K. Wakamatsu, T. Yasuhara, and T. Nohara, *Tetrahedron Lett.*, 1989, **30**, 6041.
31. H. Morita, H. Suzuki, and J. Kobayashi, *J. Nat. Prod.*, 2004, **67**, 1628.
32. H. Itokawa, K. Takeya, Y. Hitotsuyanagi, and H. Morita, *Yakugaku Zasshi*, 1999, **119**, 529.
33. K. Takeya, *The Kanpo and Crude Drugs Textbook for Pharmacist 2nd Edition*, 2005, 152.
34. H. Itokawa, K. Takeya, Y. Hitotsuyanagi, and H. Morita, In *The Alkaloids*, ed. by G. A. Cordell, Academic Press, NY, 1997, **49**, 301.
35. Y. Hitotsuyanagi, T. Aihara, and K. Takeya, *Tetrahedron Lett.*, 2000, **41**, 6127.
36. Y. Hitotsuyanagi, H. Ishikawa, T. Hasuda, and K. Takeya, *Tetrahedron Lett.*, 2004, **45**, 935.
37. S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo, and G. R. Kriek, *J. Am. Chem. Soc.*, 1977, **99**, 8040.
38. M. Zalacaín, E. Zaera, D. Vázquez, and A. Jiménez, *FEBS Lett.*, 1982, **148**, 95.
39. B. V. Sirdeshpande and P. L. Toogood, *Bioorg. Chem.*, 1995, **23**, 460.
40. H. Fujiwara, S. Saito, Y. Hitotsuyanagi, K. Takeya, and Y. Ohizumi, *Canner Lett.*, 2004, **209**, 223.
41. H. Itokawa, K. Takeya, N. Mori, T. Sonobe, N. Serisawa, T. Hamanaka, and S. Mihashi, *Chem. Pharm. Bull.*, 1984, **32**, 3216.
42. H. Itokawa, K. Kondo, Y. Hitotsuyanagi, M. Isomura, and K. Takeya, *Chem. Pharm. Bull.*, 1993, **41**, 1402.
43. H. Itokawa, K. Kondo, Y. Hitotsuyanagi, and K. Takeya, *Heterocycles*, 1993, **36**, 1837.
44. H. Itokawa, K. Kondo, Y. Hitotsuyanagi, A. Nakamura, H. Morita, and K. Takeya, *Chem. Pharm.*

- Bull.*, 1993, **41**, 1266.
45. J.-E. Lee, Y. Hitotsuyanagi, I.-H. Kim, T. Hasuda, and K. Takeya, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 808.
46. J.-E. Lee, Y. Hitotsuyanagi, and K. Takeya, *Tetrahedron*, 2008, **64**, 4117.
47. J.-E. Lee, Y. Hitotsuyanagi, H. Fukaya, K. Kondo, and K. Takeya, *Chem. Pharm. Bull.*, 2008, **56**, 730.
48. R. B. Bates, J. R. Cole, J. J. Hoffmann, G. R. Kriek, G. S. Linz, and S. J. Torrance, *J. Am. Chem. Soc.*, 1983, **105**, 1343.
49. H. Morita, K. Kondo, Y. Hitotsuyanagi, K. Takeya, H. Itokawa, N. Tomioka, A. Itai, and Y. Iitaka, *Tetrahedron*, 1991, **47**, 2757.
50. Y. Hitotsuyanagi, S. Sasaki, Y. Matsumoto, K. Yamaguchi, H. Itokawa, and K. Takeya, *J. Am. Chem. Soc.*, 2003, **125**, 7284.
51. H. Morita, S. Nagashima, O. Shiota, K. Takeya, and H. Itokawa, *Chem. Lett.*, 1993, 1877.
52. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, 1995, **43**, 271.
53. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Heterocycles*, 1994, **38**, 2247.
54. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Chem. Lett.*, 1994, 2009.
55. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, 1996, **44**, 1026.
56. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Tetrahedron*, 1994, **50**, 11613.
57. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, 1995, **43**, 1395.
58. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 677.
59. H. Morita, S. Nagashima, K. Takeya, and H. Itokawa, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2327.
60. K. Mizutani, Y. Hirasawa, Y. Sugita-Konishi, N. Mochizuki, and H. Morita, *J. Nat. Prod.*, 2008, **71**, 1297.
61. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron Lett.*, 1994, **35**, 9593.
62. H. Morita, Y. S. Yun, K. Takeya, H. Itokawa, and K. Yamada, *Tetrahedron*, 1995, **51**, 6003.
63. H. Morita, Y. S. Yun, K. Takeya, H. Itokawa, and O. Shiota, *Phytochemistry*, 1996, **42**, 439.
64. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Bioorg. Med. Chem.*, 1997, **5**, 2063.
65. H. Morita, M. Eda, T. Iizuka, Y. Hirasawa, M. Sekiguchi, Y. S. Yun, H. Itokawa, and K. Takeya, *Bioorg. Med. Chem. Lett.*, 2006, **17**, 4458.
66. Y. S. Yun, H. Morita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1997, **60**, 216.
67. H. Itokawa, Y. S. Yu, H. Morita, K. Takeya, and K. Yamada, *Planta Medica*, 1995, **61**, 561.
68. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron*, 1995, **51**, 5987.
69. H. Morita, Y. S. Yun, K. Takeya, H. Itokawa, and O. Shiota, *Bioorg. Med. Chem.*, 1997, **5**, 631.
70. H. Morita, A. Shishido, T. Kayashita, M. Shimomura, K. Takeya, and H. Itokawa, *Chem. Lett.*, 1994,

2415.

71. H. Morita, T. Kayashita, M. Simomura, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1996, **59**, 280.
72. H. Morita, T. Kayashita, M. Shimomura, K. Takeya, and H. Itokawa, *Heterocycles*, 1996, **43**, 1279.
73. H. Morita, T. Kayashita, A. Shishido, K. Takeya, H. Itokawa, and M. Shiro, *Tetrahedron*, 1996, **52**, 1165.
74. H. Morita, A. Shishido, T. Kayashita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1997, **60**, 404.
75. H. Morita, T. Iizuka, C. Y. Choo, K. L. Chan, H. Itokawa, and K. Takeya, *J. Nat. Prod.*, 2005, **68**, 1686.
76. H. Morita, T. Kayashita, A. Uchida, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1997, **60**, 212.
77. H. Morita, H. Kobata, K. Takeya, and H. Itokawa, *Tetrahedron Lett.*, 1994, **35**, 3563.
78. H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya, and H. Itokawa, *Tetrahedron*, 1994, **50**, 6797.
79. H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya, and H. Itokawa, *Tetrahedron*, 1994, **50**, 9975.
80. H. Morita, T. Kayashita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1995, **58**, 943.
81. A. Q. Jia, N. H. Tan, and J. Zhou, *J. Asian Nat. Prod. Res.*, 2007, **9**, 569.
82. A. Q. Jia, N. H. Tan, and J. Zhou, *Chem. Biodiversity*, 2007, **4**, 241.
83. A. Q. Jia, N. H. Tan, and J. Zhou, *J. Integrative Plant Biol.*, 2006, **48**, 740.
84. A. Q. Jia, N. H. Tan, Y. Yang, S. Wu, L. Wang, and J. Zhou, *Acta Bot. Sin.*, 2004, **46**, 625.
85. H. Morita, Y. Sato, and J. Kobayashi, *Tetrahedron*, 1999, **55**, 7509.
86. H. Morita, T. Iizuka, C. Y. Choo, K. L. Chan, K. Takeya, and J. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4609.
87. Y.-L. Yang, K.-F. Hua, P.-H. Chuang, S.-H. Wu, K.-Y. Wu, F.-R. Chang, and Y.-C. Wu, *J. Agric. Food Chem.*, 2008, **56**, 386.
88. H. Morita, M. Enomoto, Y. Hirasawa, T. Iizuka, K. Ogawa, N. Kawahara, Y. Goda, and K. Takeya, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5410.
89. Y. Matsubara, T. Yusa, A. Sawabe, Y. Iizuka, S. Takekuma, and Y. Yoshida, *Agric. Biol. Chem.*, 1991, **55**, 2923.
90. T. Matsumoto, K. Nishimura, and T. Takeya, *Chem. Pharm. Bull.*, 2002, **50**, 857.
91. T. Matsumoto, N. Tashiro, K. Nishimura, and T. Takeya, *Heterocycles*, 2002, **57**, 477.
92. E. Benedetti and C. Pedone, *J. Peptide Sci.*, 2005, **11**, 268.
93. P. Drygala, J. Olejnik, A. Mazur, K. Kierus, S. Jankowski, M. Zimecki, and J. Zabrocki, *Eur. J. Med. Chem.*, 2009, **44**, 3731.
94. H. Morita, A. Shishido, K. Takeya, H. Itokawa, T. Hirano, K. Oka, and O. Shirota, *Bioorg. Med.*

Chem. Lett., 1997, **7**, 1269.

95. H. Morita, A. Shishido, T. Matsumoto, H. Itokawa, and K. Takeya, *Tetrahedron*, 1999, **55**, 967.
96. T. Matsumoto, A. Shishido, H. Morita, H. Itokawa, and K. Takeya, *Phytochemistry*, 2001, **57**, 251.
97. T. Matsumoto, A. Shishido, H. Morita, H. Itokawa, and K. Takeya, *Tetrahedron*, 2002, **58**, 5135.



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