Research on Biologically Active compounds from Natural Sources

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Abstract

RA-VII(RA-700) was selected as a cadidate of anticancer agent from many other components isolated from Rubia akane and many derivatives obtained by chemical modifications of them. RA-VII was a bicyclic hexapeptide and was tested for Phase I clinical trials. Following to this compound, next search for getting more active and less toxic compounds has been performed by chemical modifications of RAs compounds.

By esterification, etherification and thionation, many kinds of derivatives were obtained. They were also tested for antineoplastic activities against various tumor cells and solid tumors. Some of them had stronger antitumor activities than RA-VII.

Astins obtained from Aster tartaricus, had also strong toxicities against some kinds of tumor cells, KB and P388.

Introduction

To date, a lot of anticancer agents have been isolated from natural sources; from microorganisms, from fungi and from higher plants. At present in our laboratory, we are focussing to higher plants for screenig antitumor agents. From higher plants, many kinds of anticancer agents have been isolated and applied for clinical use; vinca alkloids from Vinca rosea, podophyllotoxin from Podophyllum peltatum, camptothecin from Camptotheca acuminata, taxol from Taxus brevifolia and curcumol from Curcuma aromatica. We also collected many materials from all over the world for screening test against Sarcoma 180A and some tumor cell lines.

As the results, we observed that various extracts of them revealed antineoplastic activity. For instance, Zingiberaceous plants, Rubia akane, Menispermaceous plants and so on.

Recently, we have isolated some antitumor cyclic oligopeptides from Rubia akane, and named them as RAs compounds. After the preclinical screenings, one of them, RA-VII was selected as a candidate of anticancerous agent for testing Phase I clinical trials. Moreover, we are now searching for new cyclic oligopeptides having antineoplastic activities from other plants. Consequently, many kinds of monocyclic oligopeptides were isolated from some kinds of plants. Among of them, astins isolated from Aster tartaricus had also cytotoxic activity.

Materials and Methods

By screening test using Sarcoma 180A mice, we have screened many kinds of materials collected from the world; commercially available and collected in the field. Most of them were plant sources and some of them were animal sources.

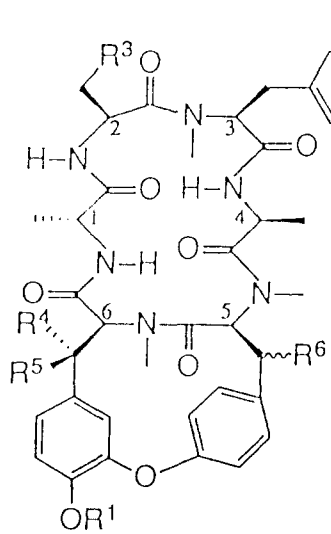
The structural elucidation of isolated components and synthesized compounds was performed by using many kinds of instrmental analysises, UV, IR, NMR, Mass, X-ray, ORD, CD and so on. Antineoplastic test against various tumors were carried out cooperated with National Cancer Institute of Japan and NCI in Washington D.C.

Results and discussion

As the results of the screening test, we have isolated a lot of components from various plants, for instance, Curcuma xanthorrhiza, Alpinia spp., Ginkgoa biloba, Cocculus trilobus and so on.

Finally, RAs[1] components isolated from Rubia akane and R. cordifolia(Rubiaceae) as antineoplastic oligopeptides. These oligopeptides were cyclic hexapeptides showing strong antineoplastic activities against various tumors as illustrated in Fig. 1. Bouvardin and deoxybouvardin were also isolated from Bouvardia ternifolia(Rubiaceae) by the group of Arizona State University. Deoxybouvardine was the same compound with RA-V, which was isolated by us. Cyclic hexapeptides RAs were total 16 as natural components, five of them were glucosides[2].

QSAR was tried to get suitable antineoplastic compound from many derivatives, which were obtained by esterification and by etherification substituted with various size of side chains of RA-V.



Rubia cordifolia

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
RA-I	H	Me	OH	H	H	H	H
RA-II	Me	H	H	H	H	H	H
RA-III	Me	Me	OH	H	H	H	H
RA-IV	Me	Me	H	OH	H	H	H
RA-V	H	Me	H	H	H	H	H
RA-VI	Me	Me	OH	H	H	H	H
RA-VII	Me	Me	H	H	H	H	H
RA-VIII	Me	Me	Me,OH	H	H	H	H

Bouvardia ternifolia

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

Fig. 1 The structures of RAs and bouvardins

As the results, RA-VII was selected as a candidate for antineoplastic compound during the screening tests against various tumor cell lines, leukemia and ascites, and solid tumors. Therapeutic ratio of RA-VII was 400, compared to 10 of mitomycin C (MMC). Mechanism of action of RAs was assumed to be inhibition of protein biosynthesis, since leucine was not taken in. Moreover, RA-V was concluded to be a "time -dependent drug" like vincristine. Further, RA-VII was effective to Colon 38, P388, L1210, Meth A, M5076.

Conformational analysis of an aititumor cyclic hexapeptides, RA and its analogues, were conducted by the spectroscopic and computational chemical method. A combination of different homo- and heteronuclear 2D NMR techniques at 500 MHz have enabled us to perform complete assignment of the 1H and 13C signals of the two conformers A and B of RA-VII in $CDCl_3$. The structures of the three conformers(A, B and C) in DMSO-d_6 were also determined by 2D NMR techniques, temperature effects on NH protons and NOE expeirments.

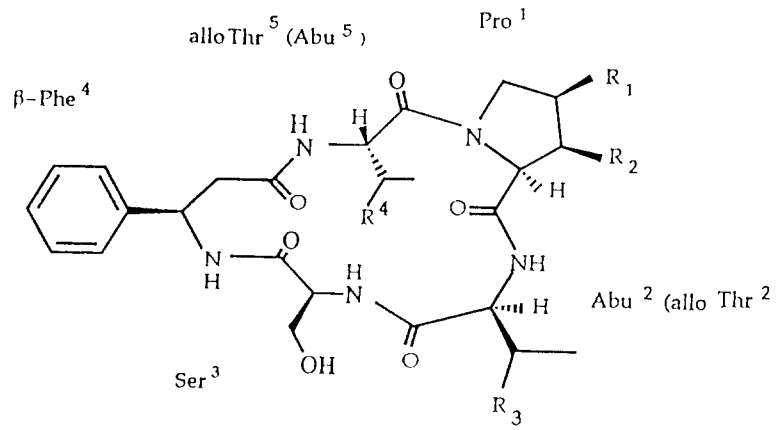
Effects of combinations with other anticancerous agents on P388 Leukemia were

also observed. Mostly, it is assumed that these RAs peptides revealed remarkable effects in the case of combination with cyclophosphamide series compounds.

As the result of in vitro phase II study on the human oncology assay at Jikei University, the chemosensitivity of RA-700 were showed as follows: ovarian cancer(67%), non-small cell lung cancer(22%), breast cancer(17%), and colorectal cancer(10%).

Further searching for getting more active and less toxic compounds has been performed by chemical modifications of RAs compounds[3].

Astins[4] were isolated from Aster tartaricus(Compositae). They were monocyclic pentapeptides and most of them had one or two of chlorine atoms in their molecules as illustrated in Fig. 2. This fact was suggested as distinguished difference compared with other cyclic peptides. Some of them had strong antineoplastic activity against Sarcoma 180A mice.



		R^1	R^2	R^3	R^4
Astin	A	Cl	Cl	H	OH
	B	Cl	Cl	OH	H
	C	Cl	Cl	H	H
	F	H	Cl	H	H

Fig. 2. The structures of Astins

Moreover, the further investigation for searching for cyclic oligopeptides from the other plants are following to this research.

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