[Chem. Pharm. Bull. 34(9)3762—3768(1986)]

# Studies on Antitumor Cyclic Hexapeptides RA Obtained from Rubiae Radix, Rubiaceae. VI. Minor Antitumor Constituents[1)]

HIDEJI ITOKAWA,*,[a] KOICHI TAKEYA,[a] NOBORU MORI,[b] TOHRU SONOBE,[b] SUSUMU MIHASHI,[b] and TOSHINORI HAMANAKA[b]

*Tokyo College of Pharmacy,[a] Horinouchi 1432–1, Hachioji, Tokyo 192–03, Japan and Tohbishi Pharmaceutical Co., Ltd., Research Laboratory,[b] Ohmori-Nishi 1–16–18, Ohta-ku, Tokyo 143, Japan*

(Received January 27, 1986)

New antitumor cyclic hexapeptides RA-IV, RA-III, RA-II and RA-I were isolated as minor constituents of *Rubia cordifolia*. Their structures were elucidated by means of various instrumental and chemical analyses. From a comparison of the antitumor activities of 7 products derived by oxidizing RA-V with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone and 6 native cyclic hexapeptides isolated from *R. cordifolia*, it is surmised that the active site in the RA-series is present at the α-side of RA molecules.

**Keywords**——*Rubia cordifolia*; Rubiaceae; cyclic hexapeptide; antitumor activity; antitumor substance; structure–activity correlation

Two antitumor cyclic hexapeptides, named RA-V and RA-VII, have been isolated as active principles from Rubiae Radix (roots of *Rubia cordifolia* and *R. akane*), and their structures were established by means of various instrumental and chemical analyses.[2,3)] RA-V and its derivatives showed antitumor activities against various experimental murine tumors *in vivo* and *in vitro*.[4–6)] In the course of these studies, a large-scale extraction of Rubiae Radix made it possible to isolate significant amounts of the minor antitumor constituents, RA-IV, RA-III, RA-II and RA-I. In this paper, we would like to describe the structural elucidation and antitumor activity of these compounds, and to revise the structure of RA-III proposed in a previous paper.[2)]

An efficient isolation method for the antitumor cyclic hexapeptides (RA) from Rubiae Radix was established by following the activity against murine tumor P388 leukemia, as shown in Chart 1. The final purifications of RA-IV—RA-I were carried out by subjecting the more polar fractions, after the elution of RA-VII and RA-V in the last silica gel column chromatography, to repeated preparative thin layer chromatography (TLC).

RA-III, showing a molecular ion peak at *m/z* 786 in the mass spectrum (MS), has the molecular formula $C_{41}H_{50}N_6O_{10}$ from the elemental analysis, and was considered to have an extra oxygen atom as compared with RA-VII, because the MS showed a dehydration peak at *m/z* 768 ($M^+ - 18$), and acetylation gave a monoacetate (indicating the presence of an alcoholic hydroxyl group). After acid hydrolysis of RA-III, the amino acid composition was determined as D-Ala : L-Ala : L-Ser (1 : 1 : 1) according to the method described by Lam *et al.*[7)] This suggested that either L-Ala-2 or L-Ala-4 of RA-VII (shown in Fig. 1) was replaced by L-Ser in RA-III. When the $^1H$- and $^{13}C$-nuclear magnetic resonance (NMR) spectra of RA-III were compared with those of RA-VII, most of the chemical shift values due to the former were very closely compatible with those of the latter except for a few signals attributable to an alanine residue. Therefore, detailed signal assignments in the $^{13}C$-NMR spectra of the RA series were made according to the method reported by Bates *et al.*[8)] The result is shown in Table II; the assignments due to β-carbon signals of Ala-2 and Tyr-5 in RA-III[2)] were revised.

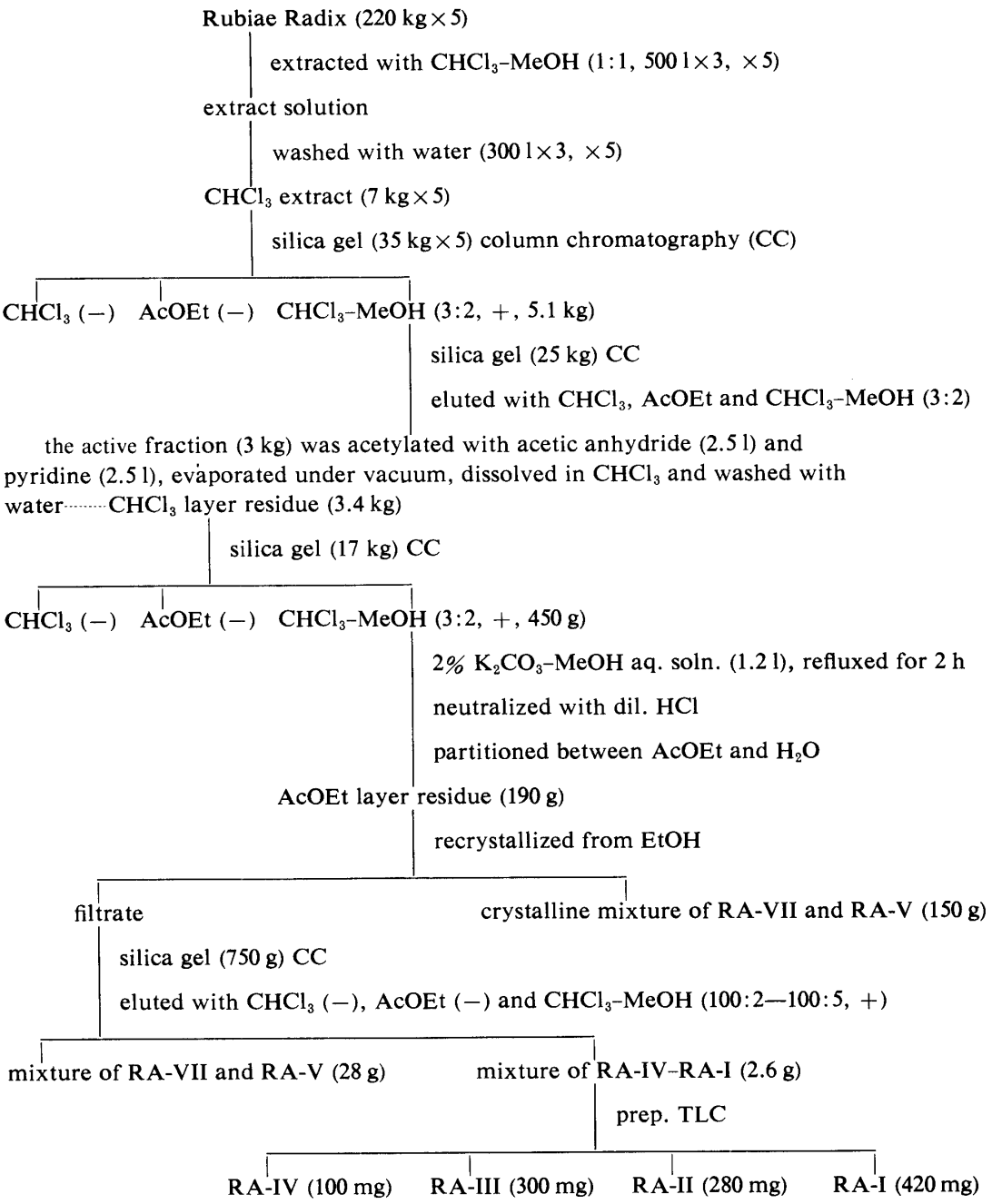


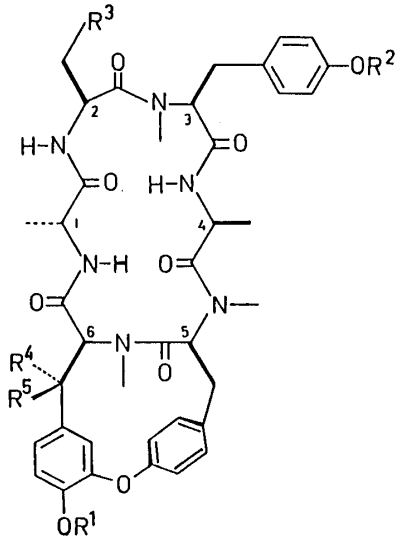

Chart 1. Procedure for Separation of Cyclic Hexapeptides from *Rubia cordifolia*

Methyl and methine carbon signals of Ala-1 and Ala-4 were almost the same in RA-VII and RA-III, but those of Ala-2 appearing at $\delta$ 16.56 (q) and 44.54 (d) in RA-VII were shifted downfield to $\delta$ 61.61 (t) and 49.56 (d) in RA-III. This evidence was also substantiated by the fact that the $^1$H-NMR spectrum of RA-VII showed three methyl signals at $\delta$ 1.11, 1.31 and 1.35, whereas that of RA-III exhibited only two methyl signals at $\delta$ 1.09 and 1.31. The result of a decoupling experiment on RA-III acetate supported the proton sequence, which was extended to the serine amide proton ($\delta$ 7.36) and two other amide protons ($\delta$ 6.44 and 6.75) due to Ala-1 and Ala-4 at 24.0 °C. When the temperature dependence of the signals of these three amide protons at 41.5 °C was determined in chloroform-$d_1$, the serine amide proton signal was significantly shifted upfield to $\delta$ 7.17, whereas the chemical shifts of two the alanine amide protons ($\delta$ 6.43 and 6.75) remained the same. This result suggested that the serine amide

TABLE I. $^1$H-NMR Chemical Shifts for the Major Stereoisomers of RA Series

| | RA-VII | RA-IV | RA-IV-Ac | RA-III | RA-III-Ac | RA-II | RA-I |
|---|---|---|---|---|---|---|---|
| Ala-4$\beta$ | 1.11 d (6.7) | 1.15 d (6.7) | 1.09 d (6.1) | 1.09 d (6.7) | 1.13 d (6.6) | 1.06 d (6.4) | 1.11 d (6.7) |
| Ala-2$\beta$ | 1.31 d (6.9) | 1.27 d (7.0) | 1.24 d (6.8) | — | — | 1.30 d (6.4) | — |
| Ala-1$\beta$ | 1.35 d (6.9) | 1.32 d (6.8) | 1.35 d (6.8) | 1.31 d (6.9) | 1.30 d (6.9) | 1.32 d (5.9) | 1.29 d (7.0) |
| Tyr-6N-Me | 2.70 s | 2.36 s | 2.58 s | 2.69 s | 2.67 s | 2.70 s | 2.68 s |
| Tyr-3N-Me | 2.87 s | 2.84 s | 2.89 s | 2.96 s | 2.95 s | 2.87 s | 2.98 s |
| Tyr-5N-Me | 3.13 s | 3.09 s | 3.10 s | 3.10 s | 3.14 s | 3.08 s | 3.10 s |
| Tyr-3O-Me | 3.79 s | 3.78 s | 3.80 s | 3.78 s | 3.79 s | — | 3.80 s |
| Tyr-6O-Me | 3.94 s | 3.92 s | 3.94 s | 3.93 s | 3.94 s | 3.93 s | — |
| Tyr-6$\delta$b | 4.35 d (1.9) | 4.83 br s | 4.84 d (2.0) | 4.35 d (1.9) | 4.34 d (2.0) | 4.34 br s | 4.42 br s |
| Tyr-6$\delta$a | 6.58 dd (8.4, 1.9) | 6.73 dd (8.5, 2.2) | 6.72 dd (8.5, 2.0) | 6.59 dd (8.5, 1.9) | 6.59 dd (8.4, 2.0) | 6.59 dd (8.5, 1.8) | 6.52 dd (8.2, 2.0) |
| Tyr-6$\varepsilon$a | 6.81 d (8.5) | 6.81 d (8.5) | 6.81 d (8.5) | 6.81 d (8.5) | 6.81 d (8.4) | 6.89 d (8.5) | 6.78 d (8.2) |
| Tyr-3$\varepsilon$ | 6.84 d (8.5) | 6.83 d (8.5) | 6.84 d (8.5) | 6.83 d (8.5) | 6.84 d (8.6) | 6.80 d (8.1) | 6.86 d (8.5) |
| Tyr-5$\varepsilon$b | 6.88 dd (8.4, 2.4) | 6.82 dd (8.4, 2.1) | 6.82 dd (8.4, 2.0) | 6.88 dd (8.5, 2.3) | 6.89 dd (8.4, 2.4) | 6.87 dd (8.5, 1.8) | 6.88 dd (8.5, 2.3) |
| Tyr-3$\delta$ | 7.05 d (8.5) | 7.03 d (8.5) | 7.06 d (8.5) | 7.04 d (8.5) | 7.09 d (8.6) | 6.95 d (8.1) | 7.14 d (8.5) |
| Tyr-5$\varepsilon$a | 7.22 dd (8.4, 2.4) | 7.22 dd (8.4, 2.1) | 7.22 dd (8.4, 2.0) | 7.21 dd (8.5, 2.3) | 7.21 dd (8.5, 2.4) | 7.20 dd (8.4, 1.8) | 7.24 dd (8.5, 2.3) |
| Tyr-5$\delta$b | 7.27 dd (8.4, 2.2) | 7.27 dd (8.4, 2.2) | 7.37 dd (8.4, 2.2) | 7.26 dd (8.5, 2.3) | 7.27 dd (8.4, 2.2) | 7.25 dd (8.5, 1.5) | 7.24 dd (8.5, 2.0) |
| Tyr-5$\delta$a | 7.43 dd (8.4, 2.2) | 7.37 dd (8.4, 2.2) | 7.45 dd (8.4, 2.2) | 7.47 dd (8.5, 2.3) | 7.42 dd (8.5, 2.2) | 7.41 dd (8.4, 1.5) | 7.42 (8.5, 2.0) |
| COMe | | | 1.94 s | | 2.09 s | | |

The measurements were made on a Brucker AM400 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference and are expressed in terms of ppm.




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

*a*) P388: $10^6$ 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.

Fig. 1. Structures and Antitumor Activities of Native Cyclic Hexapeptides and Related Compounds

proton cannot intramolecularly form a hydrogen bond, but the Ala-1 and Ala-4 amide protons can hydrogen bond transannularly to the Ala-4 and Ala-1 carbonyl oxygens, respectively, with only slight changes from the X-ray conformation.[8,9] Consequently, the

TABLE II. $^{13}$C-NMR Chemical Shifts for the Major Stereoisomers of RA Series

| | RA-VII | RA-IV | RA-IV-Ac | RA-III | RA-III-Ac | RA-II | RA-I |
|---|---|---|---|---|---|---|---|
| Ala-2$\beta$ | 16.56 q | 16.89 q | 16.43 q | 61.61 t | 62.57 t | 16.36 q | 61.18 t |
| Ala-4$\beta$ | 18.49 q | 18.24 q | 18.63 q | 18.55 q | 18.55 q | 18.42 q | 18.16 q |
| Ala-1$\beta$ | 20.71 q | 20.69 q | 20.07 q | 20.87 q | 20.72 q | 20.64 q | 20.69 q |
| Tyr-6N-Me | 29.33 q | 30.48 q | 31.94 q | 29.39 q | 29.24 q | 29.44 q | 29.55 q |
| Tyr-5N-Me | 30.55 q | 30.91 q | 30.50 q | 30.56 q | 30.52 q | 30.57 q | 30.60 q |
| Tyr-3$\beta$ | 32.70 t | 32.68 t | 32.72 t | 32.72 t | 32.78 t | 32.70 t | 32.78 t |
| Tyr-6$\beta$ | 35.56 t | 73.49 d | 73.68 d | 35.51 t | 35.36 t | 35.56 t | 35.69 t |
| Tyr-5$\beta$ | 36.99 t | 36.78 t | 36.68 t | 36.92 t | 37.04 t | 36.94 t | 36.96 t |
| Tyr-3N-Me | 39.82 q | 39.78 q | 39.79 q | 40.23 q | 39.95 q | 39.89 q | 40.14 q |
| Ala-2$\alpha$ | 44.54 d | 44.35 d | 44.60 d | 49.56 d | 48.22 d | 44.65 d | 50.29 d |
| Ala-4$\alpha$ | 46.44 d | 46.63 d | 46.42 d | 46.36 d | 46.42 d | 46.50 d | 46.49 d |
| Ala-1$\alpha$ | 47.84 d | 47.88 d | 48.28 d | 47.80 d | 47.96 d | 47.82 d | 47.83 d |
| Tyr-5$\alpha$ | 54.32 d | 54.23 d | 54.13 d | 54.38 d | 54.20 d | 54.41 d | 54.62 d |
| Tyr-3O-Me | 55.28 q | 55.26 q | 55.28 q | 55.27 q | 55.28 q | — | 55.38 q |
| Tyr-6O-Me | 56.22 q | 56.16 q | 56.11 q | 56.19 q | 56.20 q | 56.20 q | — |
| Tyr-6$\alpha$ | 57.42 d | 62.88 d | 60.05 d | 57.38 d | 57.48 d | 57.43 d | 57.67 d |
| Tyr-3$\alpha$ | 68.37 d | 68.20 d | 68.32 d | 68.68 d | 68.72 d | 68.26 d | 68.85 d |
| Tyr-6$\delta$b | 112.43 d | 112.33 d | 112.18 d | 112.41 d | 112.38 d | 112.43 d | 113.70 d |
| Tyr-3$\varepsilon$ | 114.09 d | 114.06 d | 114.11 d | 114.15 d | 114.17 d | 115.71 d | 114.28 d |
| Tyr-6$\varepsilon$a | 113.48 d | 115.08 d | 115.97 d | 113.46 d | 113.47 d | 113.46 d | 116.50 d |
| Tyr-6$\delta$a | 120.97 d | 122.56 d | 124.21 d | 120.99 d | 120.92 d | 121.01 d | 121.65 d |
| Tyr-5$\varepsilon$b | 124.25 d | 123.97 d | 124.03 d | 124.26 d | 124.31 d | 124.25 d | 124.40 d |
| Tyr-5$\varepsilon$a | 125.94 d | 126.01 d | 126.15 d | 125.97 d | 125.95 d | 126.01 d | 126.18 d |
| Tyr-6$\gamma$ | 128.25 s | 131.99 s | 127.94 s | 128.16 s | 128.13 s | 128.13 s | 127.36 s |
| Tyr-3$\delta$ | 130.27 d | 130.27 d | 130.28 d | 130.21 d | 130.17 d | 130.37 d | 130.52 d |
| Tyr-3$\gamma$ | 130.75 s | 130.76 s | 130.70 s | 130.47 s | 130.71 s | 129.77 s | 130.73 s |
| Tyr-5$\delta$a | 131.00 d | 129.94 d | 129.79 d | 130.91 d | 130.98 d | 130.97 d | 130.96 d |
| Tyr-5$\delta$b | 132.81 d | 133.84 d | 134.11 d | 132.78 d | 132.80 d | 132.76 d | 132.95 d |
| Tyr-5$\gamma$ | 135.22 s | 135.18 s | 134.79 s | 135.12 s | 135.05 s | 135.13 s | 135.35 s |
| Tyr-6$\zeta$ | 146.57 s | 148.61 s | 149.11 s | 146.56 s | 146.56 s | 146.58 s | 143.60 s |
| Tyr-6$\varepsilon$b | 153.19 s | 153.42 s | 153.03 s | 153.13 s | 153.16 s | 153.14 s | 151.78 s |
| Tyr-5$\zeta$ | 158.28 s | 158.42 s | 158.48 s | 158.28 s | 158.28 s | 158.28 s | 158.58 s |
| Tyr-3$\zeta$ | 158.47 s | 158.95 s | 159.34 s | 158.45 s | 158.47 s | 155.30 s | 158.58 s |
| Tyr-6CO | 168.14 s | 168.14 s | 168.17 s | 168.09 s | 167.65 s | 168.49 s | 168.81 s |
| Tyr-5CO | 169.38 s | 169.04 s | 168.66 s | 169.35 s | 169.50 s | 169.46 s | 169.76 s |
| Tyr-3CO | 170.75 s | 169.92 s | 168.85 s | 170.90 s | 169.61 s | 170.86 s | 171.06 s |
| Ala-4CO | 171.78 s | 171.32 s | 171.84 s | 171.66 s | 170.58 s | 171.72 s | 171.80 s |
| Ala-1CO | 172.30 s | 172.42 s | 172.24 s | 171.72 s | 171.74 s | 172.72 s | 171.90 s |
| Ala-2CO | 172.64 s | 172.58 s | 172.54 s | 172.63 s | 172.59 s | 172.91 s | 172.77 s |
| MeCO-Me | | | 21.34 q | | 20.72 q | | |
| MeCO-CO | | | 168.55 s | | 170.63 s | | |

The measurements were made on a Brucker AM400 spectrometer in $CDCl_3$ with TMS as an internal reference and are expressed in terms of ppm. The assignments of carbonyl carbons may be reversed.

structure of RA-III was concluded to be as shown in Fig. 1.

RA-I, obtained as colorless powder from MeOH, showed a molecular ion at *m/z* 772 ($C_{40}H_{48}N_6O_{10}$) in the MS and was reacted with diazomethane to give RA-III. The two methoxy-methyl signals of RA-III appeared at $\delta$ 3.78 and 3.93 (55.27 and 56.19) in the $^1$H-($^{13}$C-) NMR spectrum, but only one methyl signal due to Tyr-3 was observed at $\delta$ 3.80 (55.38) in the case of RA-I. Consequently, the methoxyl group of Tyr-6 in RA-III is replaced by a hydroxyl group in RA-I.

RA-II, obtained as colorless needles from MeOH, showed a molecular ion at *m/z* 756 ($C_{40}H_{48}N_6O_9$) in the MS and was methylated with diazomethane to furnish RA-VII. One

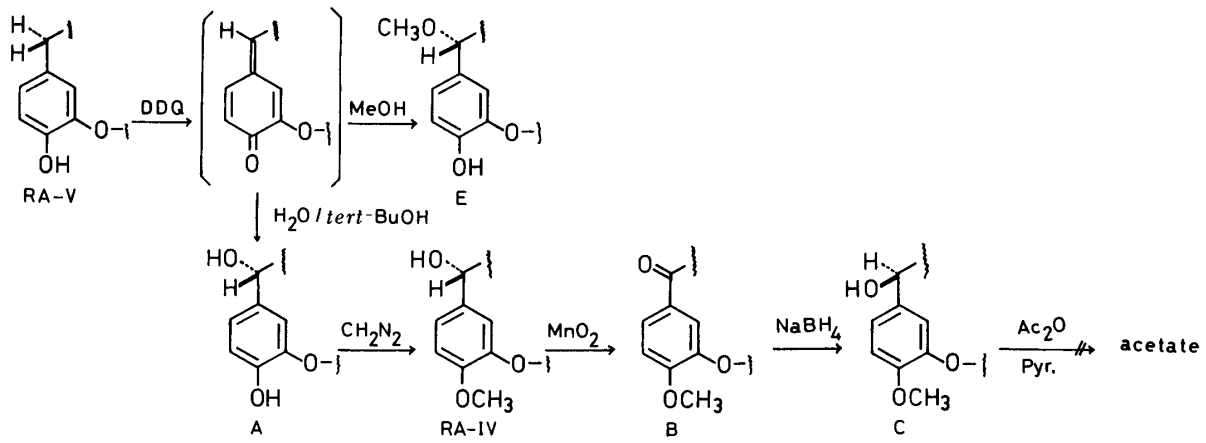



Fig. 2. Preparation of RA-IV and Related Compounds

methoxy-methyl proton (carbon) signal of RA-II at $\delta$ 3.93 (56.20) corresponded to that due to Tyr-6 of the two signals at $\delta$ 3.79 and 3.94 (55.28 and 56.22) in the case of RA-VII. From the above results, it was concluded that the methoxyl group of Tyr-3 in RA-VII is replaced by a hydroxyl group in RA-II.

RA-IV has the molecular formula $C_{41}H_{50}N_6O_{10}$ from the elemental analysis and was considered to have an additional alcoholic hydroxyl group as compared with RA-VII, because the MS exhibited a dehydration peak at $m/z$ 768 ($M^+ - 18$). It was concluded that the hydroxyl group in RA-IV is linked to the $\beta$-carbon ($C_\beta$) of Tyr-6 by comparing the $^{13}C$ chemical shift values of RA-IV with those of RA-VII; the $C_\beta$ signal at $\delta$ 35.56 (t) due to Tyr-6 of RA-VII was shifted downfield to $\delta$ 73.49 (d) in RA-IV, while other carbon signals in both peptides were similar. Next, in order to introduce an oxygen functional group into the benzyl position of Tyr-6 in RA-V, it was oxidized with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) as shown in Fig. 2. 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 considerations: the reagent in this series of reactions can approach only from the $\alpha$-side, because the $\beta$-side at the benzyl location of Tyr-6 is strongly blocked by the N-methyl group of this tyrosine moiety as judged from the X-ray conformation.[9-11] Consequently, the hydroxyl group of RA-IV was determined to have *S* configuration.

In the previous studies,[5,6] 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. We also examined the antineoplastic activity of six native cyclic hexapeptides and seven related compounds (A—E) against P388 lymphocytic leukemia in mice. The mice received 10 mg/kg/d (except for RA-VII and RA-III: 4.0 and 2.0 mg/kg/d) i.p. for 5 consecutive days. The antineoplastic activities are shown in Fig. 1. The small differences of antitumor activity among these compounds could be explained to some extent by the molecular hydrophobicities as previously mentioned, but a remarkable decrease of antitumor activity was observed in RA-IV, compounds A, A-diAc, E, E-Me and E-Ac, whose $\alpha$-proton at the $C_\beta$ position of Tyr-6 was replaced with bulky substituent groups. In spite of a similar replacement at $C_\beta$, the activity of compounds B and C did not decrease. From the above findings, it may be concluded that the introduction of large

substituent groups at the α-side of the RA series brings about a decrease of antitumor activity. This area seems to play an important role in the mechanism of antitumor activity. The antitumor activity decrease of RA-II can rather be explained from the viewpoint of the molecular hydrophobicity than the α-block hypothesis.

## Experimental

All melting points were measured with a Yamato MP-21 apparatus, and are uncorrected. The ultraviolet (UV) spectra were taken with a Hitachi UV-Vis 320 spectrometer, infrared (IR) spectra on a Nihonbunko IRA-I, and $^1$H- and $^{13}$C-NMR spectra on JEOL FX-200 and Brucker AM400 instruments. Chemical shifts are given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard. MS were measured on JEOL-JMS D300 and Hitachi M-80 mass spectrometers.

**Extraction and Isolation of Antitumor Minor Constituents**——As shown in Chart 1, commercial Rubiae Radix (220 kg, roots of *Rubia cordifolia*) purchased in China was extracted three times with $CHCl_3$–MeOH (1 : 1, 500 l). The combined extract solution was washed with water (300 l) and evaporated to dryness *in vacuo*. The obtained syrup (7 kg) was subjected to column chromatography on silica gel (35 kg) and eluted with $CHCl_3$, EtOAc and $CHCl_3$–MeOH (3 : 2) successively. The above process was repeated five times. The combined $CHCl_3$–MeOH (3 : 2) eluate (5.1 kg) was once again subjected to column chromatography over silica gel (25 kg) with $CHCl_3$, EtOAc and $CHCl_3$–MeOH (3 : 2) eluting solutions successively. The obtained $CHCl_3$–MeOH fraction was allowed to stand in anhydrous acetic acid (2.5 l)–pyridine (2.5 l) solution at room temperature overnight. The crude acetylated material (3.4 kg) was purified by silica gel (17 kg) column chromatography in the same manner as above. The $CHCl_3$–MeOH fraction (450 g) was deacetylated with 2% $K_2CO_3$ methanolic aqueous solution (1.2 l) by refluxing for 2 h, and after neutralization with diluted HCl, the reaction mixture was partitioned between AcOEt and $H_2O$. The AcOEt extract (190 g) was recrystallized from MeOH in order to remove a mixture (*ca.* 150 g) of RA-VII and RA-V. The mother liquor was concentrated *in vacuo* and the residue was subjected to column chromatography over silica gel (750 g). Mixtures of $CHCl_3$–MeOH (100 : 2 to 100 : 5) were used stepwise as eluents, and each fraction was developed on 0.25 mm silica gel plates ($60F_{254}$, Merck) with $CHCl_3$–MeOH (100 : 7). The fractions which gave 254 nm positive spots at lower *Rf* values than that of RA-V under UV light were collected and then purified by preparative TLC over silica gel to furnish RA-IV (100 mg), RA-III (300 mg), RA-II (280 mg) and RA-I (420 mg). The physical and spectral data for RA-IV—RA-I were as follows.

RA-IV: Colorless powder, mp 247—255 °C (from MeOH). MS *m/z*: 786 ($M^+$, Calcd for $C_{41}H_{50}N_6O_{10}$ 786.3584, Found 786.3551), 768 ($M^+ - H_2O$). $[\alpha]_D^{21}$ −126° (*c* = 0.07, $CHCl_3$). UV $\lambda_{max}^{EtOH}$ nm (ε): 276 (2600), 284 (2000). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380 (NH), 1640 (amide C=O).

RA-III: Colorless needles, mp >300 °C (from MeOH). MS *m/z*: 786 ($M^+$, Calcd for $C_{41}H_{50}N_6O_{10}$ 786.3584, Found 786.3551), 768 ($M^+ - H_2O$). $[\alpha]_D^{28}$ −199° (*c* = 0.1, $CHCl_3$). UV $\lambda_{max}^{EtOH}$ nm (ε): 276 (2300), 281 (1800). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380 (NH), 1640 (amide C=O).

RA-II: Colorless needles, mp 261 °C (dec., from MeOH). MS *m/z*: 756 ($M^+$), 741 ($M^+ - CH_3$, Calcd for $C_{39}H_{45}N_6O_9$ 741.3245, Found 741.3274). $[\alpha]_D^{28}$ −201° (*c* = 0.1, $CHCl_3$). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380 (NH), 1645 (amide C=O).

RA-I: Colorless powder, mp 284 °C (dec., from MeOH). MS *m/z*: 772 ($M^+$, Calcd for $C_{40}H_{48}N_6O_{10}$ 772.3428, Found 772.3395). $[\alpha]_D^{21}$ −216° (*c* = 0.08, $CHCl_3$–MeOH (9 : 1)). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (NH), 1650 (amide C=O).

**Acid Hydrolysis of RA-III**[7,9]——A solution of RA-III (10 mg) in 6 N HCl was heated at 110 °C for 17 h. After cooling, the solution was concentrated to dryness. The residue was dansylated with 2% $NaHCO_3$ (1 ml) and 5 mM dansyl chloride in acetone (0.5 ml) at 37 °C for 1 h. On the other hand, authentic DL-Ala, D-Ala, DL-Ser and L-Ser were also dansylated in a similar manner. The dansyl amino acids were analyzed by high performance liquid chromatography under the following conditions: column, 4 mm i.d. × 250 mm (Nucleosil 5 μm); solvent, 2% $CH_3CN$ (5 mM L-His, 5 mM $CH_3COONH_4$, 25 mM $CuSO_4 \cdot 5H_2O$); flow rate, 1.0 ml/min; detection, 340 nm; AUFS, 0.16. The $t_R$ values were L-Ala 6.6, D-Ala 7.2, L-Ser 5.0 and D-Ser 4.7 min.

**Preparation of Compound A and RA-IV from RA-V**——DDQ (180 mg) was added to RA-V (200 mg) dissolved in 90% aqueous *tert*-BuOH, and the mixture was stirred for 5 h at room temperature. The reaction mixture was extracted with AcOEt (100 ml). The AcOEt layer was washed with a saturated saline solution (60 ml, 3 times), dried over anhydrous $MgSO_4$ and then evaporated *in vacuo*. The residue (190 mg) was subjected to preparative TLC using Silica gel GF254 with $CHCl_3$–MeOH (10 : 1) solvent. Under ultraviolet irradiation at 254 nm, a main band in the area of lower *Rf* value than the starting material (RA-V) was gathered and eluted sufficiently with $CHCl_3$–MeOH (100 : 5). The eluate was concentrated to give 162 mg of compound A. Compound A was dissolved in a small amount of MeOH, a suitable quantity of diazomethane–ether solution was added, and the mixture was allowed to stand at room temperature overnight. The reaction mixture was concentrated *in vacuo* and purified in the same manner as above to furnish 158 mg of RA-IV. Compound A: colorless powder, mp 225—231 °C (dec., from MeOH). $[\alpha]_D^{21}$ −237° (*c* = 0.32, $CHCl_3$), MS *m/z*: 772 ($M^+$).

**Preparation of Compound B and C from RA-IV**——$MnO_2$ (700 mg) was added to RA-IV (150 mg) dissolved in 20 ml of $CHCl_3$, and the mixture was stirred overnight at room temperature. The reaction mixture was filtered and the residue was washed with a large quantity of $CHCl_3$. The combined filtrate was washed with a saturated saline solution, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give 120 mg of crude material. By preparative TLC, compound B was isolated. Next, compound B (30 mg) dissolved in 5 ml of MeOH was reduced with excess $NaBH_4$ by stirring at room temperature for 30 min. Next 50 ml of AcOEt was added, and the reaction mixture was washed with 30 ml of ice-cold water (once) and with 30 ml of saturated saline solution (twice). The solution was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to furnish 26 mg of residue. This residue was purified by preparative TLC to yield 22 mg of compound C. Compound B: colorless powder, mp 210—215 °C (dec., from MeOH). $[\alpha]_D^{21}$ −123° ($c=0.08$, $CHCl_3$). MS *m/z*: 784 ($M^+$). Compound C: colorless powder, mp 215—220 °C (from MeOH). $[\alpha]_D^{21}$ −172° ($c=0.1$, $CHCl_3$). MS *m/z*: 786 ($M^+$).

**Preparation of Compound E from RA-V**——DDQ (60 mg) was added to RA-V (100 mg) dissolved in 10 ml of MeOH, and the mixture was stirred for 5 h at room temperature. Next 100 ml of AcOEt was added, and the reaction mixture was washed with 60 ml of saturated saline solution, dried over anhydrous $MgSO_4$, and concentrated. By preparative TLC, 80.6 mg of compound E was obtained. Compound E: colorless powder, mp 215—220 °C (from MeOH). $[\alpha]_D^{21}$ −172° ($c=0.25$, $CHCl_3$). MS *m/z*: 786 ($M^+$).

**Preparation of Compounds A-diAc, E-Ac and E-Me**——Acetylation of compounds A and E was carried out with $Ac_2O$–pyridine, and methylation of compound E with diazomethane, in the usual way. Compound A-diAc: colorless powder, mp 224—230 °C (from MeOH). $[\alpha]_D^{21}$ −170° ($c=0.47$, $CHCl_3$). MS *m/z*: 856 ($M^+$). Compound E-Ac: colorless powder, mp 230—239 °C (from MeOH). $[\alpha]_D^{21}$ −256° ($c=0.18$, $CHCl_3$). MS *m/z*: 828 ($M^+$). Compound E-Me: colorless powder, mp 225—227 °C (from MeOH). $[\alpha]_D^{21}$ −267° ($c=0.23$, $CHCl_3$). MS *m/z*: 800 ($M^+$).

**Assay of Activity against P388 Lymphocytic Leukemia**[12]——$CDF_1$ male mice, aged 5 weeks, supplied by Japan Charles River Co., Ltd., were used in groups of 6—7 animals. P388 lymphocytic leukemia, provided by the Cancer Research Institute and maintained in successive generations by us, was implanted i.p. at $1 \times 10^6$ cells/body. Administration of a test drug was started at 1 d after the implantation and continued for 5 d in the case of the i.p. route. The effectiveness was evaluated in terms of the increase of life span (ILS, T/C%).

**Acknowledgements** We thank Mr. K. Kawahara, Mr. K. Yamakawa, Mrs. T. Ogawa and Miss. M. Ohkoshi of the Research Laboratory of Tohbishi Pharmaceutical Co., Ltd., for their assistance. Part of this research was supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

## References and Notes

1) A part of this work was presented at the Annual Meeting of The Pharmaceutical Society of Japan, March 1984, Sendai and The 1984 International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, December 1984.
2) H. Itokawa, K. Takeya, N. Mori, T. Sonobe, T. Hamanaka, K. Mihara and Y. Iitaka, *Chem. Pharm. Bull.*, **31**, 1424 (1983).
3) H. Itokawa, K. Takeya, N. Mori, S. Kidokoro and H. Yamamoto, *Planta Medica*, **51**, 313 (1984).
4) H. Itokawa, K. Takeya, N. Mori, T. Hamanaka, T. Sonobe and K. Mihara, *Chem. Pharm. Bull.*, **32**, 284 (1984).
5) H. Itokawa, K. Takeya, N. Mori, M. Takanashi, H. Yamamoto, T. Sonobe and S. Kidokoro, *Gann*, **75**, 929 (1984).
6) H. Itokawa, K. Takeya, N. Mori, T. Sonobe, N. Serisawa, T. Hamanaka and S. Mihashi, *Chem. Pharm. Bull.*, **32**, 3216 (1984).
7) S. Lam, F. Chow and A. Karmer, *J. Chromatogr.*, **199**, 295 (1980).
8) R. B. Bates, J. R. Cole, J. J. Hoffmann, G. R. Kriek, G. S. Linz and S. J. Torrance, *J. Am. Chem. Soc.*, **105**, 1343 (1983).
9) S. D. Jolad, J. J. Hoffmann, S. G. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo and G. R. Kriek, *J. Am. Chem. Soc.*, **99**, 8040 (1977).
10) H. Itokawa, K. Takeya, K. Mihara, N. Mori, T. Hamanaka, T. Sonobe and Y. Iitaka, 25th Symposium Papers on The Chemistry of Natural Products, Tokyo, October 1982.
11) J. Petroski, R. B. Bates, G. S. Linz and J. P. Rosazza, *J. Pharm. Sci.*, **72**, 1291 (1983).
12) R. J. Geran, N. J. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep.*, **3**, 1 (1972).