NEW ANTITUMOR BICYCLIC HEXAPEPTIDES, RA-XI, -XII, -XIII AND -XIV FROM *RUBIA CORDIFOLIA*

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Four new bicyclic hexapeptides, named as RA-XI, -XII, XIII and -XIV were isolated from *Rubia cordifolia* and showed potent antitumor activities against P-388. The structures were elucidated from spectroscopic and chemical evidences. RA-XII, XIII and -XIV were proved to be unique bicyclic hexapeptidic glucosides.

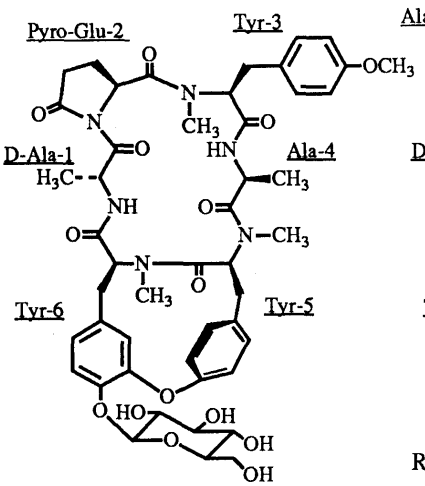
KEYWORDS RA-XI; RA-XII; RA-XIII; RA-XIV; *Rubia cordifolia*; hexapeptide; antitumor activity; glucoside

Bicyclic hexapeptide, RA series, isolated from *Rubia cordifolia* and *R. akane* (Rubiaceae) are potent antitumor agents. Their structures,[1] antitumor activities[2] and total synthesis[3] have already been reported. Recently, followed by the conformational analysis of the RA series, we are in the progress of studying conformation-antitumor activity relationships.[4] The major active principle, RA-VII, is now being tested for its clinical usefulness. However, there are some problems with administering it intravascularly because of its hydrophobicity. Chemical examination of aqueous antitumor principles from *R. cordifolia* led us to isolate four novel cyclic hexapeptides, named RA-XI, -XII, -XIII and -XIV, of which three are glucosides of RA-V, -IX and -X.[1] In this communication, we describe the structure determination of RA-XI, -XII, -XIII and -XIV and their antitumor activities.

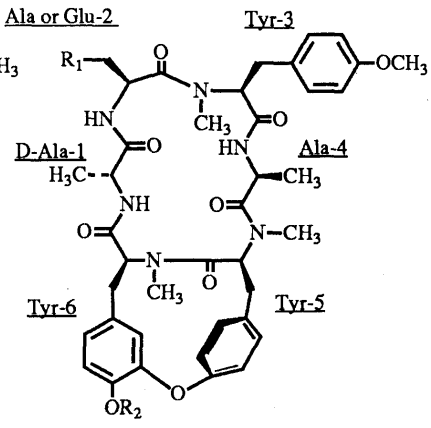
The aqueous solution of methylene chloride - methanol (1:1) extract from *R. cordifolia* was successively partitioned with methylene chloride, n-butanol and water. The methylene chloride fraction was rich in the RAs reported in our previous paper.[1] At this time, chromatographic purification of more polar n-butanol fraction led us to isolate four bicyclic hexapeptides (RA-XI, -XII, -XIII and -XIV) showing potent antitumor activities against P-388.

RA-XI,[5] colorless needles, mp 255.5°C (dec.), $[\alpha]_D$ -235.8° (c 0.24, MeOH) showed the molecular formula, $C_{42}H_{50}N_6O_{11}$ (MH^+, 815.3618). The ^1H and ^{13}C NMR spectra suggested the lack of methoxyl moiety in those of RA-X. Methylation of RA-XI by diazomethane gave RA-X. Therefore, RA-XI was confirmed to be [O-demethyl-Tyr-6]RA-X.

RA-XII and -XIII were elucidated to be glucosides of RA-V and XI, as follows. RA-XII,[6] an amorphous powder, mp 252-255°C, $[\alpha]_D$ -270.0° (c 0.2, MeOH), showed the molecular formula $C_{46}H_{58}N_6O_{14}$ by the high resolution FAB-MS spectrum (MH^+, 919.4091). In the NMR spectra of RA-XII, the signals ascribable to an anomeric position of a sugar were shown at δ 4.99 (^1H) and 102.94 (^{13}C). Thus, the ^{13}C signal pattern of the sugar (Table 1) and the coupling constant (d, J=7.6Hz) of the anomeric proton suggested that it contained a β-glucose. Acidic



RA-XIV



RA-XI: R_1=CH_2COOH; R_2=H
RA-XII: R_1=H; R_2=β-D-glucose
RA-XIII: R_1=CH_2COOH; R_2=β-D-glucose

hydrolysis of RA-XII using 10% H_2SO_4 - MeOH gave RA-V, which had been isolated from the same plant, and a β-D-glucose. Then enzymatic hydrolysis using β-D-glucosidase furnished RA-V. Based on these data, the structure of RA-XII was elucidated as β-D-glucoside of RA-V. Another newly isolated peptide, RA-XIII[7] as an amorphous powder has the following physical properties; mp. 273-276°C, $[\alpha]_D$ -109.3° (c 0.08, MeOH), $C_{48}H_{60}N_6O_{16}$ (MNa^+, Calcd. 999.3962, found 999.3969). Methylation of RA-XIII by diazomethane gave mono methyl ester drivative (RA-XIII-OMe: $C_{49}H_{62}N_6O_{16}$, MH^+, Calcd. 991.4299, found 991.4294).[8] Acidic hydrolysis of RA-XIII using 10% H_2SO_4 - MeOH gave RA-X and a β-D-glucose. Therefore, RA-XIII was proved to be β-D-glucoside of RA-X.

RA-XIV,[9] colorless powder, mp 264-267°C, $[\alpha]_D$ -257.8° (c 0.26, MeOH) with the molecular formula, $C_{48}H_{58}N_6O_{15}$ (MH^+, 959.4038), was determined to be β-D-glucoside of RA-IX by acidic hydrolysis of RA-XIV.

Using a combination of various 2D-NMR techniques, such as ^1H-^1H COSY, ^{13}C-^1H COSY and COLOC spectra enabled us to perform complete assignments of ^{13}C signals of RA-XI - RA-XIV, and these data were also corroborated by the above structures (Table I).

In particular, RA-XII was soluble in water and showed potent antitumor activity against P-388 (Table II). We newly nominate the RA-XII as an antitumor principle with water solubility.

Table II. Cytotoxic Activities of RA-XI~XIV on P 388

Compounds	IC_{50} (μg/ml)
RA-XI	5.2
RA-XII	0.046
RA-XIII	6.3
RA-XIV	>10.0

Table I. ^{13}C-NMR Chemical Shifts of RA-XI~XIV[1]

Amino acid	Carbon	RA-XI	XII	XIII	XIV
D-Ala-1	Cα	47.81	49.00	49.00	50.11
	Cβ	20.64	21.03	21.11	20.04
	CC=O	171.68	173.37	173.74	173.41
Glu-2	Cα	48.43	45.53	50.38	56.03
(Ala-2 or	Cβ	25.66	16.44	28.51	22.36
Pyro Glu	Cγ	30.47		34.77	33.24
-2)[2]	Cδ	172.77		180.98	177.01
	CC=O	172.12	174.47	174.18	174.60
Tyr-3	Cα	68.52	68.70	69.06	68.47
	Cβ	32.91	33.69	34.00	33.92
	Cγ	130.43	132.30	132.44	132.37
	Cδ	130.13	131.44	131.44	131.50
	Cε	114.25	115.03	115.23	115.16
	Cζ	158.47	159.94	160.03	160.08
	CC=O	168.22	170.88	170.98	170.90
	CN	39.90	40.34	40.34	40.16
	CO	55.24	55.72	55.71	55.72
Ala-4	Cα	46.43	47.77	47.75	48.03
	Cβ	18.20	18.85	18.85	18.56
	CC=O	168.22	172.95	173.06	172.84
Tyr-5	Cα	54.36	55.78	55.71	56.18
	Cβ	36.76	37.39	36.59	37.20
	Cγ	135.35	136.88	136.69	137.17
	Cδ1	132.95	134.04	134.07	134.20
	Cδ2	130.79	131.93	131.94	131.73
	Cε1	124.19	125.09	125.09	125.10
	Cε2	125.91	127.25	127.28	127.18
	Cζ	158.15	159.65	159.76	159.76
	CC=O	169.30	171.22	171.40	171.05
	CN	30.47	31.10	31.08	31.23
Tyr-6	Cα	57.50	58.65	58.77	58.72
	Cβ	35.48	36.58	37.41	36.78
	Cγ	127.57	131.65	131.78	131.58
	Cδ1	121.58	122.62	122.67	122.76
	Cδ2	113.40	115.78	115.93	115.83
	Cε1	116.04	118.80	118.91	118.84
	Cε2	151.27	154.66	154.72	154.72
	Cζ	143.14	145.35	145.39	145.50
	CC=O	170.63	172.09	172.14	172.03
	CN	29.35	30.12	30.08	30.23
Glucose	C1'		102.94	103.04	103.00
	C2'		74.92	74.98	74.95
	C3'		77.89	77.89	77.93
	C4'		71.34	71.44	71.42
	C5'		78.16	78.24	78.25
	C6'		62.57	62.55	62.58

1)The measurements were made in CD_3OD at 303K (125MHz)
2)The residue 2 of RA-XII is alanine and that of RA-XIV is pyro glutamic acid.

ACKNOWLEDGMENT The authors thank the Ministry of Education, Science and Culture, Japan, for financial support through the grants of the Cancer Research Program.

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5) RA-XI: IR (KBr) cm^{-1}: 3397 (NH), 1657 (C=O); UV (EtOH) nm: 280 (ε 3636); ^{1}H-NMR (CD_3OD) δ: D-Ala-1: 4.47 (Hα), 1.25 (Hβ); Glu-2: 4.80 (Hα), 1.97 (Hβ), 2.32 (Hγ); Tyr-3: 3.90 (Hα), 3.30 (Hβ), 7.13 (Hδ), 6.91 (Hε), 2.97 (NCH_3), 3.78 (OCH_3); Ala-4: 4.75 (Hα), 1.12 (Hβ), 6.97 (NH); Tyr-5: 5.46 (Hα), 3.58 (Hβ1), 2.67 (Hβ2), 7.21 (Hδ1), 7.52 (Hδ2), 6.81 (Hε1), 7.29 (Hε2), 3.08 (N-CH_3); Tyr-6: 4.69 (Hα), 3.09 (Hβ1), 2.97 (Hβ2), 6.55 (Hδ1), 4.60 (Hδ2), 6.73 (Hε1), 2.63 (NCH_3).

6) RA-XII: IR (KBr) cm^{-1}: 3397 (NH), 1662 (amide C=O); UV (EtOH) nm: 278 (ε 3060); ^{1}H-NMR (CD_3OD) δ: D-Ala-1: 4.47 (Hα), 1.25 (Hβ); Ala-2: 4.75 (Hα), 1.29 (Hβ); Tyr-3: 3.82 (Hα), 3.25 (Hβ), 7.07 (Hδ), 6.87 (Hε), 2.90 (NCH_3), 3.76 (OCH_3); Ala-4: 4.75 (Hα), 1.10 (Hβ), 6.98 (NH); Tyr-5: 5.44 (Hα), 3.60 (Hβ1), 2.66 (Hβ2), 7.21 (Hδ1), 7.47 (Hδ2), 6.81 (Hε1), 7.29 (Hε2), 3.06 (NCH_3); Tyr-6: 4.69 (Hα), 3.09 (Hβ), 6.66 (Hδ1), 4.61 (Hδ2), 7.10 (Hε1), 2.61 (NCH_3); Glucose: 4.99 (J=7.6, H1'), 3.58 (H2'), 3.50 (H3'), 3.45 (H4'), 3.44 (H5'), 3.71 and 3.88 (H6').

7) RA-XIII: IR (KBr) cm^{-1}: 3425 (NH), 1641 (C=O); UV (EtOH) nm: 276 (ε 3960); ^{1}H-NMR (CD_3OD) δ: D-Ala-1: 4.49 (Hα), 1.27 (Hβ); Glu-2: 4.70 (Hα), 1.98 (Hβ), 2.22 (Hγ); Tyr-3: 3.83 (Hα), 3.25 (Hβ), 7.13 (Hδ), 6.90 (Hε), 2.98 (NCH_3), 3.78 (OCH_3); Ala-4: 4.70 (Hα), 1.12 (Hβ); Tyr-5: 5.46 (Hα), 3.58 (Hβ1), 3.69 (Hβ2), 7.22 (Hδ1), 7.49 (Hδ2), 6.82 (Hε1), 7.29 (Hε2), 3.07 (NCH_3); Tyr-6: 4.61 (Hα), 3.12 (Hβ1), 3.04 (Hβ2), 6.67 (Hδ1), 4.65 (Hδ2), 7.10 (Hε1), 2.61 (NCH_3); Glucose: 5.00 (J=7.5, H1'), 3.60 (H2'), 3.50 (H3'), 3.45 (H4'), 3.44 (H5'), 3.73 and 3.89 (H6').

8) RA-XIII-OMe: mp 264-267°C, $[\alpha]_D$ -257.8° (c 0.26, MeOH), IR (KBr) cm^{-1}: 3397 (NH), 1735 (C=O), 1662 (amide C=O).

9) RA-XIV: IR (KBr) cm^{-1}: 3425 (NH), 1748 (C=O), 1636 (amide C=O); UV (EtOH) nm: 276 (ε 4300); ^{1}H-NMR (CD_3OD) δ: D-Ala-1: 5.56 (Hα), 1.28 (Hβ); Pyro-Glu: 5.08 (Hα), 2.22 (Hβ1), 2.05 (Hβ2), 3.10 (Hγ1), 2.58 (Hγ2); Tyr-3: 3.91 (Hα), 3.25 (Hβ), 7.13 (Hδ), 6.89 (Hε), 2.92 (NCH_3), 3.78 (OCH_3); Ala-4: 4.80 (Hα), 0.95 (Hβ), 6.85 (HN); Tyr-5: 5.46 (Hα), 3.61 (Hβ1), 2.67 (Hβ2), 7.22 (Hδ1), 7.49 (Hδ2), 6.81 (Hε1), 7.29 (Hε2), 3.01 (NCH_3); Tyr-6: 4.68 (Hα), 3.16 (Hβ1), 3.04 (Hβ2), 6.66 (Hδ1), 4.65 (Hδ2), 7.10 (Hε1), 2.63 (NCH_3); Glucose: 4.99 (J=7.6, H1'), 3.60 (H2'), 3.50 (H3'), 3.45 (H4'), 3.44 (H5'), 3.71 and 3.89 (H6').

(Received March 18, 1992)