

小红参的抗癌环己肽配糖体*

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摘要 从小红参(*Rubia yunnanensis*)中分离得到1个具有抗癌活性的环己肽配糖体新成分 RY-I, 经化学及光谱学方法确定了其结构, 证明此化合物由2个L-丙氨酸、1个D-丙氨酸、3个N-甲基-L-酪氨酸6个氨基酸经肽键缩合与1个葡萄糖构成的环己肽配糖体, 6个氨基酸缩合形成十八员环, 其中两个酪氨酸之间的苯环经氧桥连接又形成1个具有较大张力的十四员环。活性测试表明 RY-I 具有抗癌活性。此外还分离得到 RY-I 的甙元 RA-V, 也是1个有抗癌活性的成分。

关键词 小红参; 茜草科; 抗癌活性; 环肽配糖体; RY-I

ANTITUMOR GLYCOCYCLOHEXAPEPTIDE FROM RUBIA YUNNANENSIS

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Abstract A new antitumor glycocyclohexapeptide, RY-I, was isolated from *Rubia yunnanensis* together with its aglycone, RA-V, which is an antitumor compound. The structure of RY-I was elucidated by means of chemical and spectral methods and its biological activity was studied.

Key words *Rubia yunnanensis*; Rubiaceae; Antitumor; Cyclopeptidic glycoside; RY-I

INTRODUCTION

In the late 1970's some interesting antitumor cyclohexapeptides were isolated from plants of the genera *Bouvardia*⁽¹⁾ and *Rubia*⁽²⁾, thus made the plants possess a new potential application and stimulated further chemical studies on these plants.

Rubia yunnanensis Diels is a species native to Yunnan, China and it can be used as substitute material of *R. cordifolia*⁽³⁾ which is a well-known Chinese traditional medicine. Also the roots of *R. yunnanensis* Diels, known as "Xiao-Hong-Sheng", are used as antitumor drug in Yunnan. As a search for new antitumor drugs, the chemical constituents of roots of *Rubia yunnanensis* were studied. Recently we reported 3 arborane type triterpenoids isolated from the plant^(4,5). In this paper we describe the isolation and structure elucidation of a new antitumor cyclohexapeptide, RY-I, which is the first glycosidic one of the type found in nature together with its aglycone which is also an antitumor compound. Biological activity of RY-I is reported as well.

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RESULTS AND DISCUSSION

An ethanolic extract of *Rubia yunnanensis* was subjected to column chromatography on activated charcoal to yield several fractions. These fractions were further chromatographed repeatedly on silica gel and then silanized silica gel. Compound 2 was identified as deoxybouvardin (RA-V) by comparison of physical and spectral data with those of literature^(6,7).

Compound 1 showed negative reaction when tested with ninhydrin but after hydrolyzed with concentrated HCl the result was opposite, suggesting the presence of a cyclic peptide chain in the molecule. In the ¹H NMR spectrum, three methyl groups adjoining to methine at δ 1.08(3H, d, $J=6.7$ Hz), 1.23(3H, d, $J=7.0$ Hz), and 1.28(3H, d, $J=6.9$ Hz), three N-methyl groups at δ 2.61(3H, s), 2.90(3H, s), and 3.05(3H, s), a O-methyl group at δ 3.76(3H, s), protons of a sugar at δ 3.0—5.5 among which the anomeric signal is at δ 4.98(1H, d, $J=7.6$ Hz), and aromatic protons at δ 6.6—7.8 were observed. The high coupling constant value is consistent only with the β configuration for the anomeric linkage.

Also, the carbon signals due to three C-methyl, three methylene, three N-methyl, an O-methyl, six methine, six carbonyl, eighteen aromatic (eleven tertiary and seven quaternary carbons) and six sugar carbons which were easily identified appeared in the ¹³C NMR DEPT spectrum (Table 1).

Above spectral data are very close to those of cyclohexapeptides isolated from other *Rubia* species^(6,7) indicating that RY-I is also composed of two L-alanines, a D-alanine, and three modified N-methyl-L-tyrosines if the sugar signals were not taken into account temporarily. The ¹³C NMR (DEPT) spectrum showed the sugar signals at δ 102.9(CH, anomeric), 74.9(CH), 78.2(CH), 71.4(CH), 77.9(CH), and 62.6(CH₂), which were coincident with a glycosidic glucose, indicating that the compound was a glucoside with a cyclohexapeptide as aglycone.

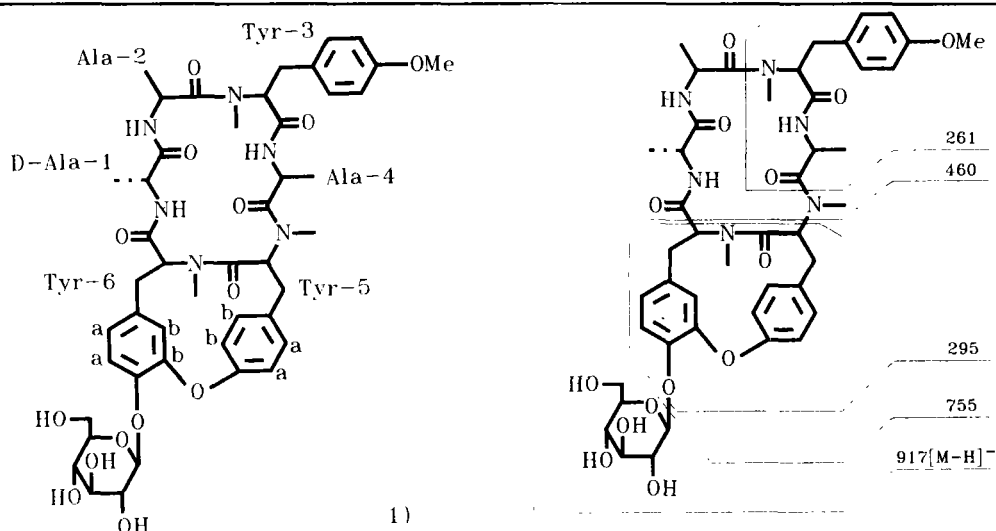
Two positions could be considered possible to link the glucose on the aglycone and one was Tyr-3 ζ while the other Tyr-6 ζ . Usually this kind of problems can be solved by comparing ¹³C NMR spectra of the glycoside with those of its possible aglycones but in this case the results were still ambiguous since the glycoside could not be solved in chloroform in which the NMR spectra of the possible aglycones were measured reportedly^(6,7). Thus the comparison would be affected by solvent effects inevitably. FAB-MS of RY-I (Scheme 1) excluded the former alternative mentioned above and this was confirmed by hydrolysis of RY-I with 1mol/L HCl which afforded glucose and cyclohexapeptide identical with deoxybouvardin or RA-V by comparing its spectral data with literature^(6,7).

The results of antitumor activity of RY-I on P-388 leukemia are shown in table 2.

EXPERIMENTAL

Plant material. Roots of *R. yunnanensis* were collected from Yunnan, China. Mps: uncorr. ¹H and ¹³C NMR with TMS as int. standard. Silica gel G (10–40, Qingdao Marine Chemical factory, China) and silanized silica gel (LiChroprep RP-8, 40–63 μ m, Merck) were used for CC. All solvent systems for chromatography were homogeneous.

Extraction and separation. The ground roots of *Rubia yunnanensis* (10.0kg) were extracted with MeOH (3 \times 15l) and then concentrated to dryness in vacuo. 1.0kg extract was obtained. The extract was subject to activated charcoal chromatography and gradient eluted with CHCl₃, EtOAc and CHCl₃-MeOH (v/v, 3/2). These fractions afforded 21 compounds including RY-I (1, 163mg) and deoxybouvardin (RA-V) (2, 171mg) in total after chromatographed repeatedly with silica gel or silanized silica gel. Both



Scheme 1. Formation of fragments in RY-I upon FAE-MS

Table 1. ^{13}C NMR chemical shifts of RY-I (1) and RA-V(2)

Carbon	1	2	Carbon	1	2
Ala-2 β	16.43	16.31	Tyr-6 γ	131.63	127.45
Ala-4 β	18.84	18.33	Tyr-3 δ	131.46	130.08
Ala-1 β	21.05	20.60	Tyr-3 γ	132.31	130.47
Tyr-6N-Me	30.49	29.34	Tyr-5 δ a	131.90	130.91
Tyr-5N-Me	31.09	30.45	Tyr-5 δ b	134.08	132.87
Tyr-3 β	33.71	32.79	Tyr-5 γ	136.93	135.48
Tyr-6 β	36.63	35.53	Tyr-6 ζ	145.40	142.99
Tyr-5 β	37.40	36.75	Tyr-6 ϵ b	154.87	51.15
Tyr-3N-Me	40.33	39.79	Tyr-5 ζ	159.73	157.92
Ala-2 α	45.56	44.50	Tyr-3 ζ	160.01	158.32
Ala-4 α	47.80	46.33	Tyr-6CO	170.99	168.14
Ala-1 α	48.57	47.81	Tyr-5CO	171.29	169.09
Tyr-5 α	55.69	54.27	Tyr-3CO	172.29	170.61
Tyr-3O-Me	55.69	55.17	Ala-4CO	172.98	171.92
Tyr-6 α	58.69	57.36	Ala-1CO	173.40	172.28
Tyr-3 α	68.74	68.32	Ala-2CO	174.56	172.72
Tyr-6 δ b	115.04	113.10	Glc-1	102.82	
Tyr-3 ϵ	115.81	113.97	2	74.84	
Tyr-6 ϵ a	118.63	115.91	3	78.06	
Tyr-6 δ a	122.64	121.52	4	71.29	
Tyr-5 ϵ b	125.12	124.09	5	77.80	
Tyr-5 ϵ a	127.28	125.84	6	62.59	

Ala-1 = D-alanyl; Ala-2 = Ala-4 = L-alanyl; Tyr-3 = Tyr-5 = Tyr-6 = L-tyrosyl.

Table 2. Antitumor effects of RY-I on P-388 leukemia

Sample concentration ($\mu\text{g} / \text{mL}$)	Inhibition rate(%)
100	100
10	80
1	28

1 and 2 were heated with ninhydrin and no colour change was observed. After hydrolysed in sealed tubes at 120 ° with concentrated HCl for a night 1 and 2 showed red spots when heated with ninhydrin on filter paper.

RY-1 (1). powder, mp 226–228 ° [α]_D²⁵ –267 ° (MeOH, c 0.26). FABMS(negative ion mode) m/z 917[M-H]⁻. ¹³C NMR; (Table 1). ¹H NMR(400 MHz, CD₃OD): 1.08(3H, d, J=6.7Hz, Ala-4 β), 1.23(3H, d, J=7.0Hz, Ala-2 β), 1.28(3H, d, J=6.9Hz, D-ala-1 α), 2.61(3H, s, Tyr-6N-Me), 2.90(3H, s, Tyr-3N-Me), 3.05(3H, s, Tyr-5N-Me), 3.76(3H, s, Tyr-3O-Me), 4.98(1H, d, J=7.6Hz, anomeric H of Glc).

Acid hydrolysis. RY-1 (20mg) in MeOH-H₂O (v/v, 1/1) was refluxed with 1mol/L HCl for 7h. After neutralized with Ag₂CO₃ the mixture was extracted with CHCl₃. Evapn of the extract gave the aglycone 2 (11mg) which was identical with natural deoxybouvardin by mp and TLC comparison with authentic sample. PC (paper Xinhua, ascending mode, solvent: *n*-BuOH-AcOH-H₂O, 4:1:5, upper phase) of the residue indicated the presence of glucose.

Compound 2 was identified as deoxybouvardin (RA-V) by comparing its NMR spectral data with those of literature^[6,7)].

Antitumor effect of RY-1. DMSO was used as a solubilizing agent, and the concentration of sample solution was 100 μ g/mL, 10 μ g/mL, and 1 μ g/mL respectively. The inhibition rates of sample against P-388 cells are shown in table 2. P-388 cells were inoculated; 180 μ L cell solution of (2.5–3) \times 10⁵ cells/mL was added into each hole, these cell solution were laid aside for 30min., and then 20 μ L sample solution was added into each hole and reacting for 24h. Cells were counted by trypan blue colouring method. Contrast groups were cultured as the same as above.

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