CYCLIC PEPTIDES FROM HIGHER PLANTS. PART 8.[1] THREE NOVEL CYCLIC PENTAPEPTIDES, ASTINS F, G AND H FROM *ASTER TATARICUS*

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Abstract - Three novel cyclic pentapeptides, named astins F (**1**), G (**2**) and H (**3**), which two of them contain one chlorine atom, have been isolated from *Aster tataricus* (Compositae) and their structures were elucidated by spectroscopic evidence, chemical degradation and chemical transformation from astin C to **2**.

During the course of our investigations in search of new biologically active cyclic peptides from higher plants,[2, 3] we have already isolated five mono- or dichlorinated antitumor cyclic pentapeptides, named astins A - E, from *Aster tataricus* (Compositae) and characterized their structures and antitumor activities.[2] Continued investigation of the roots of *A. tataricus* has now resulted in isolation of three new related cyclic pentapeptides, named astins F (**1**), G (**2**) and H(**3**), which two of them contain one chlorine atom. Here we report the isolation and structural characterization of these congeneric peptides (**1** - **3**).

Repeated fractionation of *n*-BuOH soluble phase of the MeOH extract by Diaion HP-20, silica gel and ODS chromatography led us to the isolation of three new cyclic pentapeptides, astins F (**1**), G (**2**) and H(**3**).

Astin F (**1**) was obtained as colorless needles, mp 237 - 239°C: $[\alpha]_D$ -68.6° (c 0.54, MeOH); ir (KBr): 3325 (NH), 3075, 2980, 2950 and 1650 (amide C=O) cm^{-1}. The FAB ms of **1** showed protonated molecule at *m/z* 536, and the molecular formula has been shown as $C_{25}H_{34}N_5O_6Cl$ by HR-FAB ms analysis. The peptide nature of **1** was evident from its ^1H and ^{13}C nmr spectra. Extensive 2D nmr analysis, including ^1H-^1H COSY, HOHAHA,[4] HMQC[5] and HMBC,[6] was used to determine the identity of the five amino acids and to assign the nmr signals. As shown in Tables 1 and 2, all proton and carbon signals in the nmr spectrum closely resembled those of astin C,[2] which possess β, γ-dichloroproline at residue 1, except for the signals ascribable to the proline residue.

The Hα in Pro[1] at δ 4.71 was coupled with a Hβ methine proton at δ 4.89 attached to a chlorine atom-bearing carbon at δ 59.37, which was coupled with two Hγ protons at δ 2.11 and 2.33 attached to a methylene carbon at δ 32.96. Further, the presence of nOe and HMBC correlations as shown in Figure 2, suggested that monochlorine atom must be attached at β position in Pro[1]. Therefore, astin F was shown to contain Pro(Cl)[1] residue. The configuration of chlorine atom was suggested to be β by the presence of nOe between Hα and Hβ protons. Furthermore, a strong cross peak between Hα in Pro(Cl)[1] and Hα in Abu[5] was observed in NOESYPH spectrum,[7] indicating a cis peptide bond like that of astin B,[2] which was confirmed by the similar nOe and X-ray crystallographic analysis.[2] Additional evidence concerning the amino acid composition and the sequence was obtained from the HMBC experimental results (Figure 1). Absolute configuration of each amino acid was confirmed to be all L-configuration by Marfey's derivatization of acid hydrolysate, followed by hplc analysis.[8]

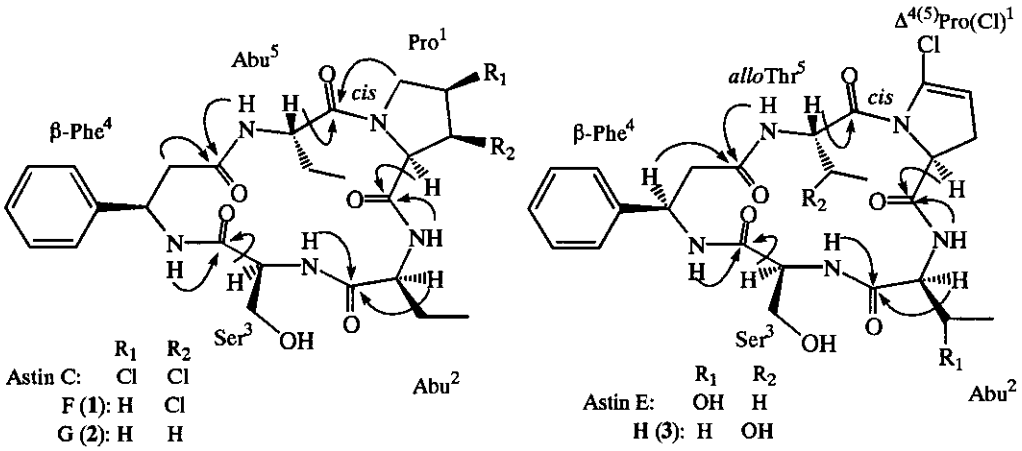

Figure 1. Structures of astins C, E, F, G and H, and some important HMBC correlations; Pro was provisionally numbered as a first amino acid. Arrow show HMBC correlations.

Astin G (**2**), colorless needles, mp 289-291°C: $[\alpha]_D$ -107.9° (c 1.14, MeOH), exhibited a high-resolution FAB-ms spectral protonated molecular ion peak at *m/z* 502.2711, corresponding to molecular formula, $C_{25}H_{35}N_5O_6$. Amino acid analysis of acid hydrolysate, followed by Marfey's method[8] showed the presence of two L-Abu and each one L-Ser, L-Pro, L-β-Phe. Dechlorination of astin C,[2] possessing Pro(Cl_2)[1] residue, with tributyltin hydride afforded dechlorinated product, which was completely identical with astin G (**2**) by direct comparison. Furthermore, the sequencing was also confirmed by the HMBC correlation as shown in Figure 1.

Table 1. ^{1}H-Nmr chemical shifts (ppm) for **1**, **2** and **3**.

Proton	**1**	**2**	**3**
Pro[1]			
Hα	4.71 (d, 6.6)	4.46 (d, 7.9)	5.33 (dd, 2.2, 4.5)
Hβ	4.89 (m)	2.06 (m)	4.34 (m)
		2.26 (dd, 6.2, 12.3)	4.37 (m)
Hγ	2.11 (m)		6.25 (br d, 1.9)
	2.33 (m)		
Hδ1	3.62 (m)	3.43 (dd, 4.7, 9.3)	
Hδ2	3.79 (m)		
Abu[2]			
Hα	4.31 (m)	4.35 (m)	4.42 (m)
Hβ1	1.71 (m)		1.59 (m)
Hβ2	1.89 (m)		1.72 (m)
Hγ	0.90 (t, 7.3)	0.87 (t, 7.3)	0.81 (t, 7.4)
NH	7.80 (d, 8.5)	8.09 (d, 9.3)	7.63 (d, 8.7)
Ser[3]			
Hα	3.79 (m)	3.87 (dd, 6.3, 11.7)	3.74 (m)
Hβ	3.70 (m)	3.69 (m)	3.74 (m)
	4.89 (br s; OH)	4.97 (br t, 5.5; OH)	4.82 (m; OH)
NH	8.17 (br d, 6.4)	7.95 (d, 6.3)	8.47 (d, 6.1)
β-Phe[4]			
Hα1	2.45 (dd, 9.6, 13.9)	2.37 (dd, 11.5, 13.5)	2.55 (m)
Hα2	2.67 (dd, 4.9, 13.9)	2.77 (dd, 4.4, 13.5)	2.55 (m)
Hβ	4.86 (ddd, 4.9, 6.5, 9.6)	4.85 (ddd, 4.4, 6.3, 11.5)	4.81 (m)
Hδ			
Hε	7.19 - 7.33 (m)	7.21 - 7.30 (m)	7.18 - 7.27 (m)
Hζ			
NH	8.25 (d, 6.5)	7.89 (d, 6.3)	8.74 (d, 6.2)
Abu[5](*allo* Thr[5])			
Hα	4.26 (m)	4.16 (m)	4.24 (dd, 7.8, 9.4)
Hβ1	1.47 (m)		3.66 (ddq, 5.2, 7.8, 6.1)
Hβ2	1.69 (m)		5.22 (d, 5.2: OH)
Hγ	0.85 (d 7.4)	0.90 (t, 7.4)	1.07 (d 6.1)
NH	8.17 (d, 6.4)	8.32 (d, 4.7)	8.13 (d, 7.8)

Measurements were performed in DMSO-d_6 at 500 MHz. Multiplicity and coupling constants (J/Hz) were in parenthesis.

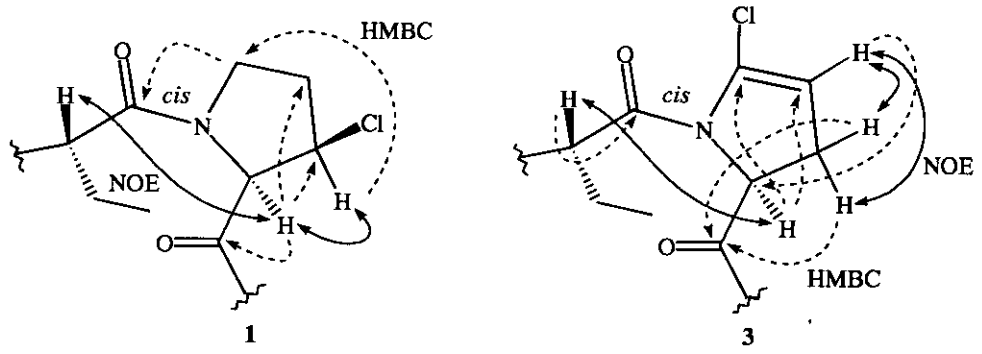

Figure 2. Fractional nOe and HMBC correlations in **1** and **3**. Arrows show nOe and dashed arrows show HMBC correlations.

Table 2. ^{13}C-Nmr chemical shifts (ppm) for **1**, **2** and **3**.

Carbon	1	2	3
Pro[1]			
Cα	65.19	60.67	69.13
Cβ	59.37	30.94	52.33
Cγ	32.96	21.69	124.48
Cδ	44.96	46.19	125.37
C=O	167.07	170.69	167.12
Abu[2]			
Cα	54.66	54.37	54.15
Cβ	24.68	24.13	26.28
Cγ	10.47	10.35	10.09
C=O	171.11	171.03	171.76
Ser[3]			
Cα	58.52	58.55	58.51
Cβ	59.61	59.97	59.26
C=O	168.89	169.04	168.73
β-Phe[4]			
Cα	40.89	41.46	39.79
Cβ	50.77	51.08	50.46
Cγ	142.61	142.57	142.62
Cδ	126.00	125.76	126.25
Cε	128.10	128.12	128.06
Cζ	126.48	126.55	126.43
C=O	169.98	170.22	169.41
Abu[5] (*allo* Thr[5])			
Cα	52.28	52.86	56.51
Cβ	23.72	23.82	68.26
Cγ	10.02	9.82	21.00
C=O	171.24	172.22	170.95

Measurements were peformed in DMSO-d$_6$ at 125 MHz.

Astin H (**3**), colorless needles, mp 265-266°C, $[\alpha]_D$ -107.3° (c 0.11, MeOH), exhibited the same molecular ion peak as that of astin E,[2] corresponding to molecular formula, $C_{25}H_{32}N_5O_7Cl$. The amino acid analysis and the nmr properties of **3** indicated the same amino acid composition as that of astin E. From the HMBC correlation as shown in Figure 1, the positions of Abu and *allo*Thr were disclosed to be reversed, compared from those of astin E. The characteristic feature of astin H having both an *allo*Thr residue and a *cis* peptide bond formed by the proline residue exists also in astin E. Furthermore, the substituted pattern of the chlorine atom and a double bond in Pro[1] was verified with the fractional nOe and HMBC correlations as shown in Figure 2. Therefore, the structure of **3** was elucidated to be cyclo ($\Delta^{4(5)}$Pro(Cl)-Abu-Ser-β-Phe-*allo*Thr).

Precise antitumor activities of these astins and derived astins are now under investigation.

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EXPERIMENTAL

General Details. - Mp's were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Mass, uv, and ir spectra were taken with a VG-Autospec spectrometer, a Hitachi 557 spectrophotomer and a JASCO A-302 spectrophotometer, respectively. Hplc was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. Tlc was conducted on

precoated Kieselgel 60 F_{254} (Art. 5715; Merck). ^1H and ^{13}C nmr spectra were recorded on Bruker spectromers (AM 400 and AM 500) at 303K and processed on a Bruker data station with an Aspect 3000 computer. NOESYPH experiments were made with a mixing time of 0.6 s. The value of the delay to optimize one-bond correlations in the HMQC spectrum and suppress them in the HMBC spectrum was 3.2 msec and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 msec. The nmr coupling constants (J) are given in Hz.

Materials. - The roots of *Aster tataricus* were purchased from Uchida Wakanyaku Co. Ltd. and a voucher specimen has been deposited in the herbarium of Tokyo College of Pharmacy.

Extraction and isolation of 1 - 3. - The roots (5 kg) of *A. tataricus* were extracted with a MeOH (50 l) at 50°C for 24 h at three times to give a MeOH extract (1100 g) which was partitioned between CH_2Cl_2 and H_2O, and *n*-BuOH and H_2O. The *n*-BuOH soluble fraction (118 g) was subjected to Diaion HP-20 cc using an H_2O - MeOH gradient system (1;0 - 0;1) to give six fractions. 80 and 100 % MeOH eluted fractions were further subjected to silica gel cc using an CH_2Cl_2 - MeOH gradient system (1:0 - 0:1) and finally purified by an ODS hplc with a MeOH - H_2O and MeCN - H_2O solvent system to give **1** (100 mg), **2** (100 mg) and **3** (10 mg), as colorless needles.

Astin F (1). - Colorless needles, mp 237-239°C, $[\alpha]_D$ -68.6° (c 0.54, MeOH), *m/z* : 536 (Found : $[M+H]^+$, 536.2231 $C_{25}H_{34}N_5O_6Cl$; requires : 536.2276), $\nu_{max}^{(KBr)}/cm^{-1}$: 3325, 3075, 2980, 2950, 1650, 1540 and 1440, ^1H nmr(DMSO-d_6) : listed in Table 1, ^{13}C nmr(DMSO-d_6) : listed in Table 2.

Astin G (2). - Colorless needles, mp 289-291°C, $[\alpha]_D$ -107.9° (c 1.14, MeOH), *m/z* : 502 (Found : $[M+H]^+$, 502.2711 $C_{25}H_{35}N_5O_6$; requires : 502.2666), $\nu_{max}^{(KBr)}/cm^{-1}$: 3325, 3080, 2990, 2950, 1645, 1520 and 1435, ^1H nmr(DMSO-d_6) : listed in Table 1, ^{13}C nmr(DMSO-d_6) : listed in Table 2.

Astin H (3). - Colorless needles, mp 265-266°C, $[\alpha]_D$ -107.3° (c 0.11, MeOH), *m/z* : 550 (Found : $[M+H]^+$, 550.2089 $C_{25}H_{33}N_5O_7Cl$; requires : 550.2068), $\nu_{max}^{(KBr)}/cm^{-1}$: 3270, 1640, 1535, 1520, 1435, 1328, 1320, 1285 and 1210, ^1H nmr(DMSO-d_6) : listed in Table 1, ^{13}C nmr(DMSO-d_6) : listed in Table 2.

Dechlorination of astin C. - A solution of astin C (20 mg), *n*-Bu_3SnH (65 mg) and azoisobutyronitrile (4 mg) in 4 ml tetrahydrofuran was heated in a sealed tube at 100°C for 12 h. Reaction mixture was concentrated and subjected to ODS-hplc with 23% MeCN to give astin G (**2**; 1.9 mg).

Acid Hydrolysis of 1 - 3. - Solutions of **1** - **3** (each containing 1 mg of peptide) in 6N HCl (1 ml) were heated at 110°C for 24 h. After cooling, each solution was concentrated to dryness. The hydrolysates were soluble in 0.02N HCl and applied to the analysis by an amino acid analyzer.

Absolute Configuration of Amino Acids. - Solutions of **1- 3** (each containing 1 mg of peptides) in 6N HCl (1 ml) were heated at 110° for 12 h. After being cooled, each solution was concentrated to dryness. The residue was soluble in water and treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) and 1M $NaHCO_3$ at 35° for 1 h. After being cooled, 2M HCl was added and then concentrated to dryness. This residue was subjected to hplc (Lichrospher 100, RP-18 (10mm), Merck), flow rate 2 ml/min, detection 340nm, solvent : 10 - 50% MeCN / 50mM triethylamine phosphate (TEAP) buffer. The t_R values were L-Ser 13.58, D-Ser 15.46, L-*allo*Thr 15.13, D-*allo*Thr 17.93, L-Abu 22.29, D-Abu 28.71, L-β-Phe 32.33 and D-β-Phe 39.42 min, respectively.

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