

CYCLIC PEPTIDES FROM HIGHER PLANTS. PART 8.<sup>1</sup> THREE NOVEL CYCLIC  
PENTAPEPTIDES, ASTINS F, G AND H FROM *ASTER TATARICUS*

Hiroshi Morita, Shinji Nagashima, Koichi Takeya, and Hideji Itokawa\*

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432-1, Hachioji,  
Tokyo 192-03, Japan

**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,<sup>2, 3</sup> 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.<sup>2</sup> 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;  $[\alpha]_D -68.6^\circ$  (c 0.54, MeOH); ir (KBr): 3325 (NH), 3075, 2980, 2950 and 1650 (amide C=O)  $\text{cm}^{-1}$ . The FAB ms of 1 showed protonated molecule at  $m/z$  536, and the molecular formula has been shown as  $\text{C}_{25}\text{H}_{34}\text{N}_5\text{O}_6\text{Cl}$  by HR-FAB ms analysis. The peptide nature of 1 was evident from its  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra. Extensive 2D nmr analysis, including  $^1\text{H}$ - $^1\text{H}$  COSY, HOHAHA,<sup>4</sup> HMQC<sup>5</sup> and HMBC,<sup>6</sup> 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,<sup>2</sup> which possess  $\beta$ ,  $\gamma$ -dichloroproline at residue 1, except for the signals ascribable to the proline residue.

The  $H_{\alpha}$  in  $Pro^1$  at  $\delta$  4.71 was coupled with a  $H_{\beta}$  methine proton at  $\delta$  4.89 attached to a chlorine atom-bearing carbon at  $\delta$  59.37, which was coupled with two  $H_{\gamma}$  protons at  $\delta$  2.11 and 2.33 attached to a methylene carbon at  $\delta$  32.96. Further, the presence of  $nOe$  and HMBC correlations as shown in Figure 2, suggested that monochlorine atom must be attached at  $\beta$  position in  $Pro^1$ . Therefore, astin F was shown to contain  $Pro(Cl)^1$  residue. The configuration of chlorine atom was suggested to be  $\beta$  by the presence of  $nOe$  between  $H_{\alpha}$  and  $H_{\beta}$  protons. Furthermore, a strong cross peak between  $H_{\alpha}$  in  $Pro(Cl)^1$  and  $H_{\alpha}$  in  $Abu^5$  was observed in NOESYPH spectrum,<sup>7</sup> indicating a *cis* peptide bond like that of astin B,<sup>2</sup> which was confirmed by the similar  $nOe$  and X-ray crystallographic analysis.<sup>2</sup> 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.<sup>8</sup>

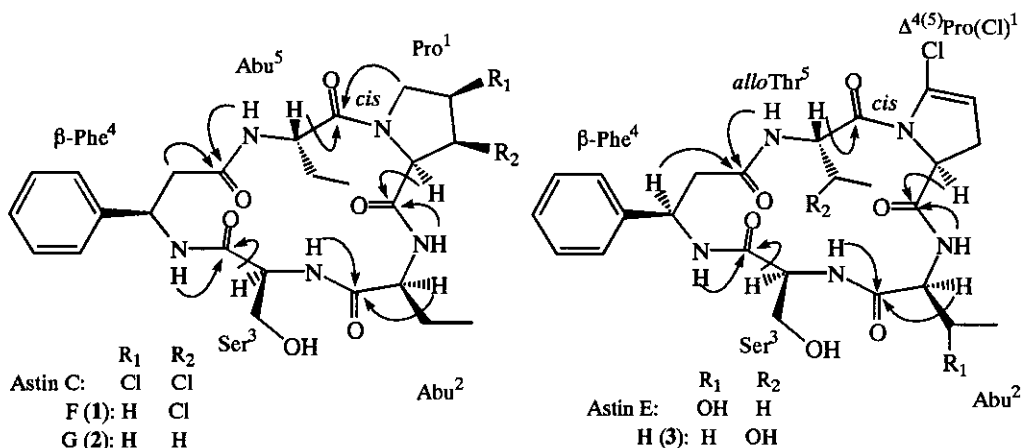


Figure 1. Structures of astins C, E, F, G and H, and some important HMBC correlations; Pro was provisionally numbered as a first amino acid. Arrow show HMBC correlations.

Astin G (2), colorless needles, mp 289-291°C:  $[\alpha]_D -107.9^\circ$  (c 1.14, MeOH), exhibited a high-resolution FAB-*m*s 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<sup>8</sup> showed the presence of two L-Abu and each one L-Ser, L-Pro, L- $\beta$ -Phe. Dechlorination of astin C,<sup>2</sup> 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.  $^1\text{H-Nmr}$  chemical shifts (ppm) for **1**, **2** and **3**.

Proton	<b>1</b>	<b>2</b>	<b>3</b>
<b>Pro<sup>1</sup></b>			
H $\alpha$	4.71 (d, 6.6)	4.46 (d, 7.9)	5.33 (dd, 2.2, 4.5)
H $\beta$	4.89 (m)	2.06 (m)	4.34 (m)
H $\gamma$	2.11 (m)	2.26 (dd, 6.2, 12.3)	4.37 (m)
H $\delta$ <sub>1</sub>	2.33 (m)		6.25 (br d, 1.9)
H $\delta$ <sub>2</sub>	3.62 (m)	3.43 (dd, 4.7, 9.3)	
	3.79 (m)		
<b>Abu<sup>2</sup></b>			
H $\alpha$	4.31 (m)	4.35 (m)	4.42 (m)
H $\beta$ <sub>1</sub>	1.71 (m)		1.59 (m)
H $\beta$ <sub>2</sub>	1.89 (m)		1.72 (m)
H $\gamma$	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)
<b>Ser<sup>3</sup></b>			
H $\alpha$	3.79 (m)	3.87 (dd, 6.3, 11.7)	3.74 (m)
H $\beta$	3.70 (m)	3.69 (m)	3.74 (m)
NH	4.89 (br s; OH)	4.97 (br t, 5.5; OH)	4.82 (m; OH)
	8.17 (br d, 6.4)	7.95 (d, 6.3)	8.47 (d, 6.1)
<b><math>\beta</math>-Phe<sup>4</sup></b>			
H $\alpha$ <sub>1</sub>	2.45 (dd, 9.6, 13.9)	2.37 (dd, 11.5, 13.5)	2.55 (m)
H $\alpha$ <sub>2</sub>	2.67 (dd, 4.9, 13.9)	2.77 (dd, 4.4, 13.5)	2.55 (m)
H $\beta$	4.86 (ddd, 4.9, 6.5, 9.6)	4.85 (ddd, 4.4, 6.3, 11.5)	4.81 (m)
H $\delta$			
H $\epsilon$	7.19 - 7.33 (m)	7.21 - 7.30 (m)	7.18 - 7.27 (m)
H $\zeta$			
NH	8.25 (d, 6.5)	7.89 (d, 6.3)	8.74 (d, 6.2)
<b>Abu<sup>5</sup>(<i>allo</i> Thr<sup>5</sup>)</b>			
H $\alpha$	4.26 (m)	4.16 (m)	4.24 (dd, 7.8, 9.4)
H $\beta$ <sub>1</sub>	1.47 (m)		3.66 (ddq, 5.2, 7.8, 6.1)
H $\beta$ <sub>2</sub>	1.69 (m)		5.22 (d, 5.2; OH)
H $\gamma$	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.

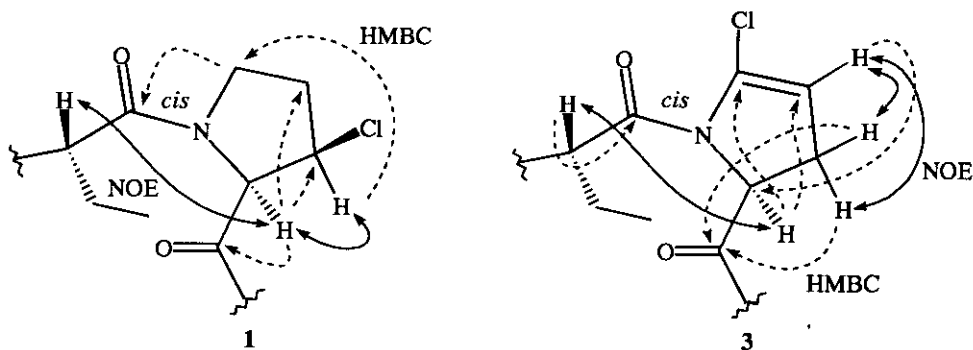


Figure 2. Fractional nOe and HMBC correlations in **1** and **3**. Arrows show nOe and dashed arrows show HMBC correlations.

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

Carbon	1	2	3
<b>Pro<sup>1</sup></b>			
C $\alpha$	65.19	60.67	69.13
C $\beta$	59.37	30.94	52.33
C $\gamma$	32.96	21.69	124.48
C $\delta$	44.96	46.19	125.37
C $\text{C=O}$	167.07	170.69	167.12
<b>Abu<sup>2</sup></b>			
C $\alpha$	54.66	54.37	54.15
C $\beta$	24.68	24.13	26.28
C $\gamma$	10.47	10.35	10.09
C $\text{C=O}$	171.11	171.03	171.76
<b>Ser<sup>3</sup></b>			
C $\alpha$	58.52	58.55	58.51
C $\beta$	59.61	59.97	59.26
C $\text{C=O}$	168.89	169.04	168.73
<b><math>\beta</math>-Phe<sup>4</sup></b>			
C $\alpha$	40.89	41.46	39.79
C $\beta$	50.77	51.08	50.46
C $\gamma$	142.61	142.57	142.62
C $\delta$	126.00	125.76	126.25
C $\epsilon$	128.10	128.12	128.06
C $\zeta$	126.48	126.55	126.43
C $\text{C=O}$	169.98	170.22	169.41
<b>Abu<sup>5</sup> (<i>allo</i> Thr<sup>5</sup>)</b>			
C $\alpha$	52.28	52.86	56.51
C $\beta$	23.72	23.82	68.26
C $\gamma$	10.02	9.82	21.00
C $\text{C=O}$	171.24	172.22	170.95

Measurements were performed in DMSO- $d_6$  at 125 MHz.

Astin H (3), colorless needles, mp 265-266°C,  $[\alpha]_D -107.3^\circ$  (c 0.11, MeOH), exhibited the same molecular ion peak as that of astin E,<sup>2</sup> corresponding to molecular formula, C<sub>25</sub>H<sub>32</sub>N<sub>5</sub>O<sub>7</sub>Cl. 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<sup>1</sup>

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- $\beta$ -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  $\text{cm}^2 \text{g}^{-1}$ . Mass, uv, and ir spectra were taken with a VG-Autospec spectrometer, a Hitachi 557 spectrophotometer and a JASCO A-302 spectrophotometer, respectively. Hplc was performed with an Inertsil PREP-ODS column (20 mm i.d.  $\times$  250 mm, GL Science Inc.) packed with 10  $\mu\text{m}$  ODS. Tlc was conducted on

precoated Kieselgel 60 F254 (Art. 5715; Merck).  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra were recorded on Bruker spectrometers (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  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ , and *n*-BuOH and  $\text{H}_2\text{O}$ . The *n*-BuOH soluble fraction (118 g) was subjected to Diaion HP-20 cc using an  $\text{H}_2\text{O}$  - 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  $\text{CH}_2\text{Cl}_2$  - MeOH gradient system (1:0 - 0:1) and finally purified by an ODS hplc with a MeOH -  $\text{H}_2\text{O}$  and MeCN -  $\text{H}_2\text{O}$  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 :  $[\text{M}+\text{H}]^+$ , 536.2231  $\text{C}_{25}\text{H}_{34}\text{N}_5\text{O}_6\text{Cl}$ ; requires : 536.2276),  $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  : 3325, 3075, 2980, 2950, 1650, 1540 and 1440,  $^1\text{H}$  nmr(DMSO- $d_6$ ) : listed in Table 1,  $^{13}\text{C}$  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 :  $[\text{M}+\text{H}]^+$ , 502.2711  $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_6$ ; requires : 502.2666),  $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  : 3325, 3080, 2990, 2950, 1645, 1520 and 1435,  $^1\text{H}$  nmr(DMSO- $d_6$ ) : listed in Table 1,  $^{13}\text{C}$  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 :  $[\text{M}+\text{H}]^+$ , 550.2089  $\text{C}_{25}\text{H}_{33}\text{N}_5\text{O}_7\text{Cl}$ ; requires : 550.2068),  $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  : 3270, 1640, 1535, 1520, 1435, 1328, 1320, 1285 and 1210,  $^1\text{H}$  nmr(DMSO- $d_6$ ) : listed in Table 1,  $^{13}\text{C}$  nmr(DMSO- $d_6$ ) : listed in Table 2.

**Dechlorination of astin C.** - A solution of astin C (20 mg), *n*-Bu<sub>3</sub>SnH (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<sub>3</sub> 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<sub>R</sub>* 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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