Structure of a New Peptide, Astin J, from *Aster tataricus*[1)]

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A new peptide called astin J (1) was isolated from the roots of *Aster tataricus*. The structure was elucidated by spectroscopic methods and chemical transformation from a cyclic pentapeptide, astin C, isolated from *A. tataricus* to the analogous acyclic peptide, 2. Antileukemic activities of the series of acyclic peptides are also described.

Key words astin J; astin C; peptide; *Aster tataricus*; antileukemic activity

Cyclic peptides with various pharmacological activities are known to be from natural origins, however, not many examples are present in higher plants.[2)] We studied some of the biologically active cyclic peptides from higher plants,[3)] and the structures, conformations and antitumor activities of a series of cyclic pentapeptides called astins have already been reported.[1,4)]

Further investigation of the peptide fraction prepared from the roots of *Aster tataricus* by chromatographic purification led to the isolation of a new acyclic peptide, astin J (**1**), whose structure was similar to the acyclic peptide obtained by chemical transformation from a cyclic pentapeptide, astin C,[4)] isolated from the title plant. This paper describes the structure of **1** and chemical transformation from the cyclic astin C to an acyclic peptide (**2**). The importance of the cyclic structure of astins to show antitumor activities is also reported.

Results and Discussion

The *n*-BuOH soluble phase prepared from the roots of *A. tataricus* described in a previous paper[4)] was fractionated by Diaion HP-20 and silica gel, and finally purified by octadesil silyl (ODS) silica gel chromatography to isolate a peptidic compound called astin J (**1**).

Astin J (**1**), colorless needles, mp 280 °C (dec.), $[\alpha]_D$ +6.0° (c=0.13, pyridine), showed the molecular formula of $C_{25}H_{33}N_5O_7$ based on the results of high resolution FAB-MS together with NMR data (Tables I and II). The spectroscopic data of **1** suggested that astin J had an acyclic peptidic structure as follows. A combination of two dimensional (2-D) NMR methods including ^1H–^1H correlation spectroscopy (COSY), ^1H-detected heteronuclear multiple quantum coherence (HMQC)[5)] and heteronuclear multiple bond connectivity (HMBC)[6)] spectra revealed that the molecule had five discrete spin-coupled systems. Three of these spin systems could be attributed to well-known amino acids, one serine (Ser) and two α-amino butyric acids (Abu), and another one to a rare amino acid, β-phenylalanine (β-Phe). The remaining spin system was assigned to a pyrrole residue by each of three coupled protons (6.09, 1H and 6.87, 2H). Astin J showed a strong UV absorption band at 266 nm (ε 10000) characteristic of the pyrrole ring. Furthermore, in the ^{13}C-NMR spectrum, the signals coming from α-substituted pyrrole ring were also observed as shown in Table II.

The sequence analysis of **1** was conducted by the ^1H–^{13}C long-range correlations observed in the HMBC spectrum. The correlations between the carbon atoms of the five C=O groups and three bond coupled protons as shown in Fig. 1 unequivocally identified the sequence of astin J as pyrrole–Abu–Ser–β-Phe–Abu.

The structure of **1** was also supported by the chemical transformation from the antitumor cyclic pentapeptide, astin C,[4)] isolated from the same plant to the corresponding acyclic peptide, **2**, under basic conditions, for example, ammonia alkaline solution (Fig. 2). This acyclic derivative was considered to be produced by dechlorination and aromatization from Pro(Cl_2) to pyrrole under basic conditions, following the cleavage of amide bond in Pro, and showed similar spectroscopic propensities to astin J

TABLE I. ^1H-NMR Chemical Shifts (ppm) for **1** and **2**

Proton	**1**	**2**
Pyrrole[1]		
Hβ1	6.87 (m)	6.88 (m)
Hβ2	6.09 (t, 3.0)	6.09 (t, 2.6)
Hα2	6.87 (m)	6.88 (m)
NH	11.44 (br s)	11.43 (br s)
Abu[2]		
Hα	4.36 (ddd, 5.2, 8.0, 8.0)	4.35 (dt, 5.1, 8.3)
Hβ1	1.61 (m)	1.62 (m)
Hβ2	1.75 (m)	1.75 (m)
Hγ	0.86 (t, 7.4)	0.86 (t, 7.4)
NH	7.91 (d, 8.0)	7.92 (d, 8.3)
Ser[3]		
Hα	4.31 (td, 5.7, 7.9)	4.30 (br q, 5.8)
Hβ	3.57 (d, 5.7)	3.57 (br s)
		4.82 (br s, OH)
NH	7.91 (d, 7.9)	7.91 (d, 7.9)
β-Phe[4]		
Hα1	2.54 (dd, 6.7, 14.2)	2.57 (dd, 7.2, 14.2)
Hα2	2.71 (dd, 8.0, 14.2)	2.70 (dd, 7.5, 14.2)
Hβ	5.20 (ddd, 6.7, 8.0, 8.4)	5.19 (ddd, 7.2, 7.5, 8.3)
Hδ		
Hε	7.16—7.30 (m)	7.18—7.30 (m)
Hζ		
NH	8.34 (d, 8.4)	8.31 (d, 8.3)
Abu[5]		
Hα	4.05 (ddd, 5.0, 7.9, 7.9)	4.04 (dt, 5.2, 8.2)
Hβ1	1.47 (m)	1.40 (m)
Hβ2	1.61 (m)	1.56 (m)
Hγ	0.68 (t, 7.4)	0.66 (t, 7.4)
NH	8.08 (d, 7.9)	7.91 (d, 8.2)
$CONH_2$		6.94 (br s)
		7.29 (br s)

Measurements were performed in DMSO-d_6 at 400 MHz. Multiplicity and coupling constants (J/Hz) are in parenthesis.

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(Tables I and II).

The absolute configuration of each of Ser, Abu and β-Phe was determined to be all L by Marfey's method[7] after complete acid hydrolysis.

Recently D. Cheng *et al.* reported the structures of the analogous acyclic peptides, asterinins A—C, from the same plant.[8] Cyclic astins with β,γ-dichloroproline residue were easily transformed to the corresponding acyclic peptides under very mild alkaline conditions. We have also isolated several acyclic peptides with methyl ester at the C-terminal, however, these peptides, like asterinins B and C, were considered to be artifacts derived from the cyclic astins.

The derived astin, **2** showed a moderate cytotoxic activity against cultured tumor cells such as L1210 (IC_{50} 15 μg/ml), P388 (IC_{50} 7 μg/ml) and KB cell lines (IC_{50} 14 μg/ml), however, astin J (**1**) showed none at all ($IC_{50} > 100$ μg/ml). On the other hand, these acyclic astins did not show antitumor activity against S-180 ascites *in vivo*, suggesting that the cyclic nature of astins plays an important role in their antitumor activities. Biological evaluations of a series of astins and derived astins are being made.

TABLE II. ^{13}C-NMR Chemical Shifts (ppm) for **1** and **2**

Carbon	1	2
Pyrrole[1]		
Cα1	125.79	125.76
Cβ1	110.85	110.89
Cα2	121.47	121.47
Cβ2	108.53	108.52
$C_{C=O}$	160.57	160.61
Abu[2]		
Cα	54.07	54.15
Cβ	25.07	24.99
Cγ	10.48	10.44
$C_{C=O}$	171.82	171.82
Ser[3]		
Cα	55.07	55.08
Cβ	61.69	61.63
$C_{C=O}$	168.78	168.79
β-Phe[4]		
Cα	41.82	42.03
Cβ	49.88	49.94
Cγ	142.13	142.23
Cδ	126.54	126.46
Cε	127.92	127.91
Cζ	126.67	126.63
$C_{C=O}$	169.39	169.27
Abu[5]		
Cα	53.04	53.47
Cβ	24.31	24.99
Cγ	9.94	9.88
$C_{C=O}$	173.42	173.38

Measurements were performed in DMSO-d_6 at 100 MHz.

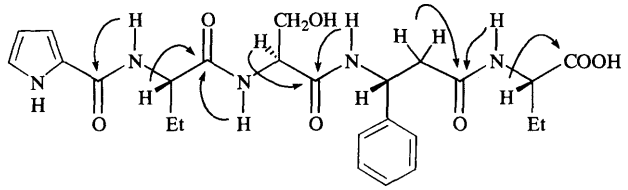
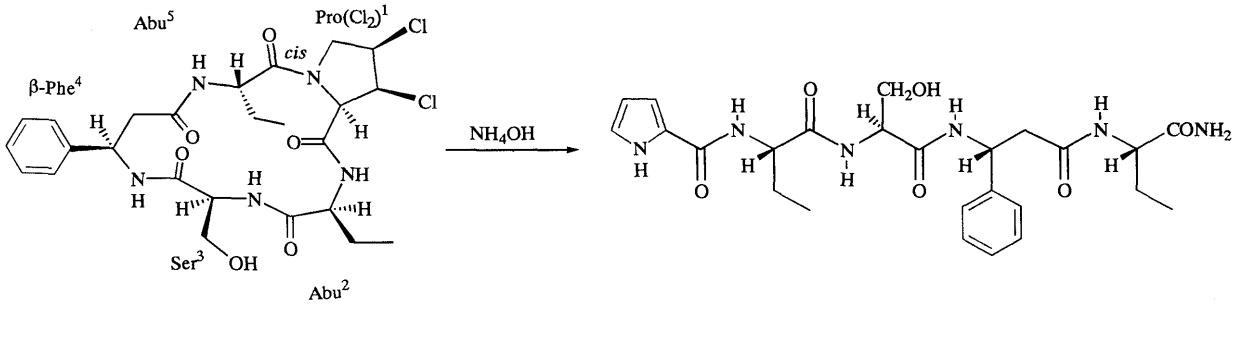


Fig. 1. Structure of Astin J (**1**)
Arrows show some important HMBC correlations.



astin C **2**

Fig. 2. Alkaline Catalyzed Cleavage of Astin C to Compound **2**

Experimental

All melting points were recorded on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 spectrometer. The spectral data were obtained on the following instruments: infrared spectrum (IR) on a JASCO A-302, NMR on a Bruker AM400 and AM500 and mass spectrum (MS) on a VG AutoSpec. High-pressure liquid chromatography (HPLC) was performed with an Inertsil PREP-ODS column (4 i.d. × 250 mm, 20 i.d. × 250 mm and 30 i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715, Merck) and the spots were detected by spraying Dragendorff reagent. The NMR coupling constants (J) are given in Hz.

Materials Roots of *A. tataricus* used in this experiment were purchased from Uchida Wakanyaku Co. in Japan and a voucher specimen has been deposited in the herbarium of Tokyo College of Pharmacy.

Extraction and Isolation of 1 Dry roots (10.0 kg) of *A. tataricus* were extracted with methanol to give an extract (2.2 kg), which was treated with methylene chloride and then *n*-butanol. The *n*-butanol soluble fraction (237 g), showing antitumor activity,[4] was subjected to Diaion HP-20 column chromatography using a water–methanol gradient system (1 : 0—0 : 1). The fraction eluted by 80% methanol was further subjected to silica gel column chromatography using a methylene chloride–methanol gradient system (1 : 0—0 : 1). The fraction eluted by 10% methanol was finally recrystallized and subjected to ODS HPLC with an acetonitrile–water and a methanol–water solvent system to give **1** (300 mg) as colorless needles.

Astin J (**1**): Colorless needles, mp 280 °C (dec.), $[\alpha]_D$ +6.0° (c=0.13, pyridine). High resolution FAB-MS: Calcd for $C_{25}H_{34}N_5O_7$ $[M+1]^+$: 516.2458. Found: 516.2418. IR ν_{max}^{KBr} cm^{-1}: 3309 (NH), 1651 (amide C=O). UV λ_{max}^{MeOH} nm (ε): 266 (10000).

Determination of the Chirality of Amino Acid Residues of 1 A solution of **1** (1 mg) in 6 N HCl was heated at 110 °C for 13 h. After being cooled, the 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 1 M $NaHCO_3$ at 35 °C for 1 h. After being cooled,

2 M HCl was added and the mixture was then concentrated to dryness. This residue was subjected to HPLC (Lichrospher 100, RP-18 (10 μm), Merck), flow rate 2 ml/min, detection 340 nm, solvent: 10—50% CH_3CN/50 mM triethylamine phosphate (TEAP) buffer. The t_R values were L-Ser 13.33, L-Abu 22.04 and L-β-Phe 32.67 min, respectively (standard: L-Ser 13.58, L-Abu 22.29, L-β-Phe 32.33 min).

Cytotoxic Activity against L1210, P388 and KB Cell Lines See previous paper.[9]

Alkaline Catalyzed Cleavage of Astin C Each sample of astin C (20 mg) was treated with 29% NH_3 in tetrahydrofuran overnight. The reaction mixture neutralized with dil. HCl was subjected to Diaion HP-20 column chromatography eluted with H_2O and then methanol. The fraction eluted with methanol was concentrated to give the compound **2**, quantitatively.

Compound **2**: Colorless needles, mp 229—231 °C, $[\alpha]_D$ −17.4° (c=0.26, MeOH). MS m/z: 515 $(M+1)^+$, 498, 413, 337, 266, 235, 216, 179, 151. (Found: 515.2619 $(M^+ + 1)$. $C_{25}H_{35}N_6O_6$ requires 515.2618). IR ν_{max}^{KBr} cm^{-1}: 3300, 1640, 1550. UV λ_{max}^{MeOH} nm: (ε) 266 (12300). The ^{1}H- and ^{13}C-NMR data were shown in Tables I and II.

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