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Structures and Conformation of Antitumour Cyclic Pentapeptides, Astins A, B and C, from *Aster tataricus* [1)]

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Abstract : Astins A, B and C, three antitumour chlorine-containing cyclic pentapeptides were isolated from *Aster tataricus* and their structures were elucidated by spectroscopic and chemical evidences. Astins A and B, each containing an *allo* threonine at residues 5 and 2, respectively, and both of which were isomers, showed more potent antitumour activity than astin C. Conformational analysis of astin B in solid state was conducted by X-ray crystallographic analysis, showing a cis configuration in one of the amide bonds.

Introduction

During the survey of novel antitumour compounds from medicinal plants,[2)] the alcoholic extract of the roots of *Aster tataricus* showed potent antitumour activity. As active components, three antitumour chlorine-containing cyclic pentapeptides, named astins A, B and C, being analogous to cyclochlorotine from *Penicillium islandicum* Sopp,[3)] have been isolated and their structures were solved by extensive NMR analysis and chemical conversion. Furthermore, the X-ray structure of astin B shows that it possesses a cis proline amide bond and is different to that in cyclochlorotine.[4)] In the present work, we describe a full account of structure elucidation of astins A (**1**), B (**2**) and C (**3**), and solid state conformation of astin B (**2**) with a cis proline amide bond.

Results and Discussion

Isolation

The *n*-butanol soluble portion of a methanolic extract of *Aster tataricus* (10.0 kg), showing a significant antitumour effect on Sarcoma 180 ascites in mice,[5] was subjected to Diaion HP-20 column chromatography. The fraction eluted with 80% methanol was further separated by silica gel column chromatography. The active fraction was employed reversed-phase high performance liquid chromatography (HPLC) to give three cyclic pentapeptides, named astin A (**1**: 0.01%), astin B (**2**: 0.03%) and astin C (**3**: 0.05%).

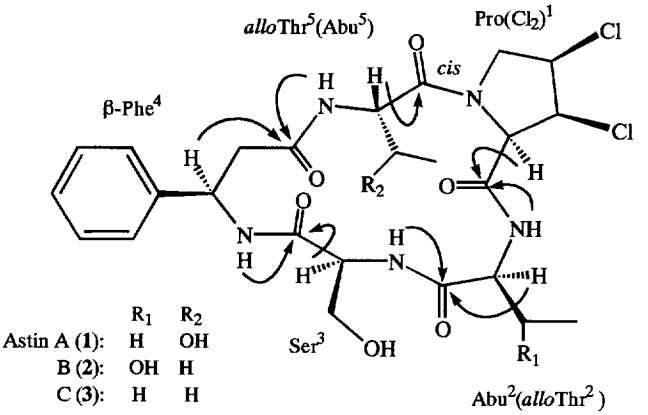


Fig. 1. Structures of astins A-C, and some important HMBC correlations; Pro in **1** was provisionally numbered as the first amino acid. The structure of cyclochlorotine is cyclo(Pro(Cl_2)-Abu-Ser-β-Phe-Ser).

Structure determination

Astin A (**1**), colourless needles, mp 192 - 194°C, $[\alpha]_D$ -77.0° (c 0.37, MeOH), exhibited a high-resolution FAB-mass spectral protonated molecular ion peak at *m/z* 586.1814, corresponding to molecular formula, $C_{25}H_{33}N_5O_7Cl_2$. Hydrolysis of **1** with 6N HCl, followed by derivatization with Marfey's reagent and HPLC analysis,[6] suggested the presence of Ser, *allo* Thr, β-Phe and Abu, and showed that all of the amino acids had the L configuration. The IR absorptions at 3350 and 1650 cm^{-1} were attributed to amino and amide carbonyl groups, respectively. The peptide nature of **1** was evident from its ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of **1** (Table 1) showed four amide NH and ^{13}C NMR spectrum (Table 2) five amide carbonyl groups, indicating **1** was a pentapeptide. Further, the relatively high intensity of the molecular ion and the lack of terminal amino group protons in the ^1H NMR suggested **1** to be a cyclic pentapeptide. The last amino acid containing two chlorine atoms was disclosed to be 2,3-dichloroproline by the coupling sequence from

Table 1. ^{1}H NMR chemical shifts (ppm) for **1** - **3**

Proton	1	2	3
Pro(Cl_2)[1]			
Hα	5.32 (d, 5.9)	4.88 (d, 5.3)	4.83 (d, 5.8)
Hβ	5.17 (dd, 4.4, 5.9)	5.13 (dd, 4.5, 5.3)	5.13 (dd, 4.6, 5.8)
Hγ	4.54 (ddd, 4.4, 6.7, 8.8)	4.77 (ddd, 4.5, 6.7, 9.6)	4.80 (ddd, 4.6, 6.6, 8.7)
Hδ1	3.50 (dd, 8.8, 11.7)	3.40 (dd, 9.6, 11.3)	3.51 (dd, 8.7, 11.6)
Hδ2	4.40 (dd, 6.7, 11.7)	4.35 (dd, 6.7, 11.3)	4.29 (dd, 6.6, 11.6)
Abu[2](*allo* Thr)[2]			
Hα	4.42 (dt, 5.0, 8.9)	4.24 (t, 9.4)	4.39 (m)
Hβ1	1.75 (m)	4.20 (ddd, 5.8, 5.9, 9.4)	1.76 (m)
Hβ2	1.94 (m)	5.79 (d, 5.9; OH)	1.99 (m)
Hγ	0.92 (t, 7.4)	1.22 (d, 5.8)	0.93 (t, 7.3)
NH	7.98 (d, 8.9)	8.39 (d, 9.4)	7.98 (d, 9.0)
Ser[3]			
Hα	3.73 (m)	3.80 (m)	3.75 (m)
Hβ	3.73 (m)	3.66 (br s)	3.71 (m)
	4.92 (br t, 5.5; OH)		
NH	8.11 (br d, 4.6)	8.84 (d, 4.2)	7.98 (d, 4.2)
β-Phe[4]			
Hα1	2.29 (dd, 10.9, 13.9)	2.10 (t, 12.7)	2.29 (dd, 11.2, 13.4)
Hα2	2.71 (dd, 4.7, 13.9)	2.82 (dd, 4.7, 12.7)	2.76 (dd, 4.7, 13.4)
Hβ	4.86 (ddd, 4.7, 6.5, 10.9)	4.91 (ddd, 4.7, 6.8, 12.7)	4.90 (ddd, 4.7, 6.6, 11.2)
Hδ			
Hε	7.20 - 7.27 (m)	7.21 - 7.31 (m)	7.20 - 7.33 (m)
Hζ			
NH	7.79 (d, 6.5)	7.38 (d, 6.8)	7.70 (d, 6.6)
allo Thr[5](Abu[5])			
Hα	4.29 (dd, 6.2, 9.4)	4.32 (td, 3.7, 9.3)	4.27 (m)
Hβ1	3.68 (ddd, 4.8, 6.1, 9.4)	1.50 (m)	1.48 (m)
Hβ2	5.43 (d, 4.8; OH)	1.75 (m)	1.74 (m)
Hγ	1.12 (d 6.1)	0.97 (t, 7.4)	0.91 (t, 7.4)
NH	8.28 (d, 6.2)	8.58 (d, 3.7)	8.34 (d, 5.1)

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

α-proton as shown in Table 1 and chemical conversion to proline residue as described below. Dechlorination of **1** by tributyltinhydride afforded dechlorinated product **4**. Amino acid analysis of the acid hydrolysate of **4**, followed by derivatization with Marfey's reagent revealed the presence of a L-proline in addition to the above four amino acids. Extensive analyses of ^{1}H-^{1}H COSY and HOHAHA[7] spectra led to assignment of an individual amino acid unit (Table 1). The five peptide bonds in the cyclic backbone region were elucidated by long-range CH correlation found in the carbonyl region of the HMBC[8] spectrum (Figure 1) in combination with HMQC[9] spectrum. As can be seen from Figure 2, the configurations of two chlorines attached to the β and γ carbons in Pro(Cl_2) were established to be cis relation each other by the NOEs observed between Hα and Hβ, between Hα and Hγ, and between Hβ and Hγ of Pro(Cl_2) in NOESYPH spectrum.[10]

Astin B (**2**), colourless needles, mp 183-185°C, $[\alpha]_D$ -84.9° (c 0.31, MeOH), exhibited the same protonated molecular ion peak as that of astin A, corresponding to molecular formula,

$C_{25}H_{33}N_5O_7Cl_2$. The amino acid analysis and the NMR properties of **2** indicated the same amino acid composition as that of **1**. From the HMBC correlation as shown in Fig. 1, the positions of Abu and *allo* Thr were disclosed to be reversed, compared from those of **1**. The characteristic feature of astin A having both a *allo*Thr residue and a cis peptide bond formed by the proline residue exists also in compound **2**. Therefore, the structure of **2** was elucidated to be cyclo (Pro(Cl_2)-*allo* Thr-Ser-β-Phe-Abu).

Astin C (**3**), colourless needles, mp 158-160°C, $[\alpha]_D$ -52.0° (c 0.20, MeOH), showed a protonated molecular ion at *m/z* 570, and the molecular formula has been shown as $C_{25}H_{33}N_5O_6Cl_2$ by HR FAB-mass analysis. Amino acid analysis of the acid hydrolysate, followed by derivatization with Marfey's reagent revealed the presence of one L-Ser, one L-β-Phe and two L-Abu. HMBC correlation indicated **3** to be a cyclic pentapeptide, composed of Abu at both of 2 and 5 residues. The NMR properties including NOE relation revealed the structure of **3** to be a cyclo (Pro(Cl_2)-Abu-Ser-β-Phe-Abu).

In astins A-C, base catalyzed cleavage reaction of proline amide bond with ammonia produced the corresponding linear peptides **5** - **7**, respectively, as shown in Figure 3. These peptides (**5** - **7**) showed strong UV absorption bands at about 266 nm (ε ca. 10000) characteristic of pyrrole ring. Furthermore, in ^1H and ^{13}C NMR spectra, the signals based on α-substituted pyrrole ring were also observed as shown in Tables 3 and 4. From these results,

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

Carbon	**1**	**2**	**3**
Pro(Cl_2)[1]			
Cα	64.42	64.45	64.42
Cβ	63.61	65.23	63.80
Cγ	55.77	54.79	55.37
Cδ	51.21	51.02	51.28
$C_{C=O}$	166.23	166.33	166.10
Abu[2](*allo* Thr[2])			
Cα	53.75	56.84	53.98
Cβ	24.09	65.91	23.78
Cγ	10.28	21.96	10.49
$C_{C=O}$	170.87	169.78	170.75
Ser[3]			
Cα	59.37	58.27	59.18
Cβ	59.63	60.21	59.78
$C_{C=O}$	169.07	169.14	169.05
β-Phe[4]			
Cα	41.71	42.84	41.90
Cβ	50.72	51.33	50.89
Cγ	142.61	142.80	142.59
Cδ	125.85	125.58	125.78
Cε	128.11	128.16	128.12
Cζ	126.55	126.60	126.55
$C_{C=O}$	169.90	171.07	170.34
allo Thr[5] (Abu[5])			
Cα	57.23	53.32	52.59
Cβ	68.03	22.65	23.30
Cγ	21.17	10.43	10.20
$C_{C=O}$	172.87	172.12	171.92

Measurements were performed in DMSO-d_6 at 125 MHz.

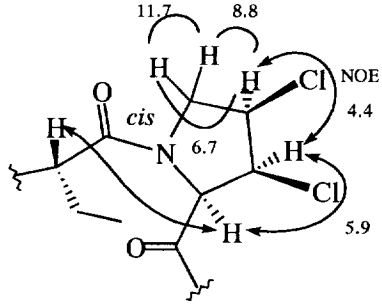


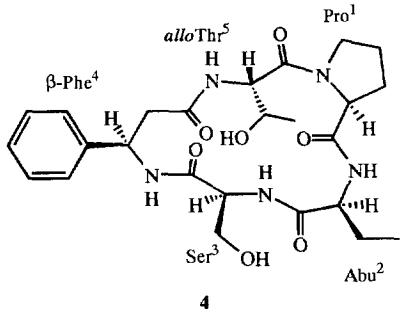
Fig. 2. Fractional NOEs in **1** - **3** and coupling correlations in **1**; The arrows show the NOE relationship and the numbers indicate coupling constant in Hz.

compounds **5** - **7** were considered to be produced by dechlorination and aromatization from Pro(Cl_2)[1] to pyrrole under basic condition, following the cleavage of amide bond in Pro. Thus, astins A-C indicated the similar characterisity to cyclochlorotine, cyclo(Pro(Cl_2)-Abu-Ser-β-Phe-Ser), which has been isolated from *Penicillium islandicum* Sopp.[3)]

Table 3. ^{1}H NMR chemical shifts (ppm) for **5** - **7**

Proton	5	6	7
Pyrrole			
Hβ1	6.88 (m)	6.88 (m)	6.88 (m)
Hβ2	6.09 (t, 2.8)	6.10 (t, 3.0)	6.09 (t, 2.6)
Hα2	6.88 (m)	6.88 (m)	6.88 (m)
NH	11.42 (br s)	11.45 (br s)	11.43 (br s)
Abu^2(*allo* Thr)^2			
Hα	4.35 (dt, 5.1, 8.4)	4.42 (t, 8.3)	4.35 (dt, 5.1, 8.3)
Hβ1	1.63 (m)	3.93 (m)	1.62 (m)
Hβ2	1.75 (m)	5.25 (d, 5.3; OH)	1.75 (m)
Hγ	0.86 (t, 7.4)	1.15 (d, 6.2)	0.86 (t, 7.4)
NH	7.91 (d, *)	7.88 (d, 8.3)	7.92 (d, *)
Ser^3			
Hα	4.30 (br q, 5.8)	4.27 (m)	4.30 (br q, 5.8)
Hβ	3.57 (br s))	3.60 (m)	3.57 (br s)
	4.79 (t, 5.5; OH)	4.82 (t, 5.5; OH)	4.82 (br s)
NH	7.91 (d, *)	8.05 (d, 7.8)	7.91 (d, *)
β-Phe^4			
Hα1	2.61 (dd, 7.6, 14.4)	2.50 (dd, *)	2.57 (dd, 7.2, 14.2)
Hα2	2.69 (dd, 7.1, 14.4)	2.71 (dd, 8.2, 14.2)	2.70 (dd, 7.5, 14.2)
Hβ	5.20 (ddd, 7.1, 7.6, 8.3)	5.21 (br t, 7.9)	5.19 (ddd, 7.2,7.5,8.3)
Hδ			
Hε	7.16 - 7.30 (m)	7.17 - 7.31 (m)	7.18 - 7.30 (m)
Hζ			
NH	8.32 (d, 8.3)	8.25 (d, 8.4)	8.31 (d, 8.3)
allo Thr^5(Abu^5)			
Hα	4.08 (dd, 6.7, 8.6)	4.04 (dt, 5.1, 8.0)	4.04 (dt, 5.2, 8.2)
Hβ1	3.73 (m)	1.39 (m)	1.40 (m)
Hβ2	4.73 (d, 5.2; OH)	1.54 (m)	1.56 (m)
Hγ	0.88 (d 6.3)	0.62 (t, 7.4)	0.66 (t, 7.4)
NH	7.91 (d, *)	7.81 (d, 8.0)	7.91 (d, *)
$CONH_2$	7.00 (br s)	6.95 (br s)	6.94 (br s)
	7.20 (br s)	7.26 (br s)	7.29 (br s)

Measurements were performed in DMSO-d_6 at 400 MHz. Multiplicity and coupling constants (*J*/Hz) were in parenthesis. * not determined in the present study because of the signal overlapping



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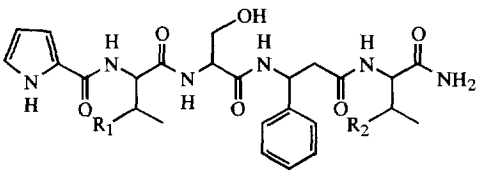

	R1	R2
5:	H	OH
6:	OH	H
7:	H	H

Fig. 3. Structures of **4** - **7**

Solid State Conformation of Astin B

The complete structure, stereochemistry and solid state conformation of **2** were established unequivocally by single-crystal X-ray analysis. Compound **2** was crystallized from CH_2Cl_2 - MeOH solution in orthorhombic crystals of space group $P2_12_12_1$. Figure 4 shows the backbone of compound **2** in a stereoscopic view. It is a cyclic pentapeptide consisting entirely of L-amino acid residues. In Figure 5, the ϕ and ψ-angles along the main chain of astin B and cyclochlorotine are summarized in the Ramachandran plot, which shows that *allo*Thr^2 of astin B have the (ϕ, ψ)-value in the energetically not favorable region.[11]

The turns are formed by the residues 5 → 1 and 3 → 4. The 5 → 1 turn is formed by the cis-amide linkage, which was identical with NOE data described above. While the residue 3 → 4 serve the turn by taking a bend structure similar to type I.[12] All constituted amino acids were not present in the energetically favorable region without forming an appropriate intramolecular hydrogen bond and characteristic β-turn was not found. Especially, the residue 2 lies almost outside the permissible region for the usual L-amino acid.

Table 4. ^{13}C-NMR chemical shifts (ppm) for **5** - **7**

Carbon	**5**	**6**	**7**
Pyrrole^1			
Cα1	125.73	125.70	125.76
Cβ1	110.87	110.97	110.89
Cα2	121.47	121.54	121.47
Cβ2	108.52	108.52	108.52
C$_{C=O}$	160.60	160.33	160.61
Abu^2(*allo* Thr^2)			
Cα	54.11	58.39	54.15
Cβ	25.03	67.31	24.99
Cγ	10.44	20.34	10.44
C$_{C=O}$	171.82	170.73	171.82
Ser^3			
Cα	55.08	55.39	55.08
Cβ	61.53	61.35	61.63
C$_{C=O}$	168.83	168.77	168.79
β-Phe^4			
Cα	41.99	42.22	42.03
Cβ	49.85	49.97	49.94
Cγ	142.26	142.13	142.23
Cδ	126.42	126.47	126.46
Cε	127.93	127.90	127.91
Cζ	126.63	126.64	126.63
C$_{C=O}$	169.39	169.03	169.27
allo Thr^5 (Abu^5)			
Cα	58.05	53.45	53.47
Cβ	66.75	24.94	24.99
Cγ	19.46	9.75	9.88
C$_{C=O}$	172.21	173.31	173.38

Measurements were performed in DMSO-d_6 at 100 MHz.

Cyclic peptides are constrained as they contain turns in the backbones and these turns are often stabilized by intramolecular hydrogen bonds. Such structures may provide good models for the studies of various possible types of turns containing intramolecular hydrogen bonds and for establishing the molecular dimensions and conformational angles of such turns. In the crystal structure of **2**, however, only an intramolecular H bond between Ser^3-HN3 and an hydroxyl oxygen (O21) in the side chain of *allo*Thr^2 is present [N3---O21 of 2.688(17) Å and HN3---O21 of 1.82(16) Å]. Interatomic short distances found in the crystal are summarized in Table 5. Obvious intramolecular H bonds involving an amide carbonyl oxygen and amide hydrogen were not observed, however, weak intramolecular H bonds between HN2 in *allo*Thr^2 and O4 in β-Phe^4 [N2---O4 of 2.967 (16) Å and HN2---O4 of 2.42 (15) Å], and between HN4 in β-Phe^4 and O4 in the same

Table 5. Interatomic short distances found in the crystal of Astin B

Donor atom (D)	H	Acceptor atom (A)		D ... A (Distance/Å)	H(D) ... A (Distance/Å)	<H-D ... A (Angle/°)	
N2	HN2	N1	[1]	2.735 (17)	2.29 (16)	54 (9)	**
N2	HN2	O4	[1]	2.967 (16)	2.42 (15)	49 (9)	**
N3	HN3	O21	[1]	2.688 (17)	1.82 (16)	29 (9)	*
O21		O5	[2]	2.711 (16)			***
N4	HN4	O2	[1]	3.197 (18)	3.30 (18)	86 (9)	
O2		O4	[1]	3.332 (17)			
N3	HN3	O31	[1]	2.903 (17)	2.78 (16)	73 (9)	
N5	HN5	O31	[3]	3.017 (18)	2.02 (21)	15 (10)	***
O31		O3	[4]	2.603 (19)			***
O1W		O2W	[1]	2.948 (21)			***
N4	HN4	O1W	[1]	3.681 (17)	2.74 (19)	24 (10)	
N4	HN4	O4	[1]	2.963 (16)	2.46 (18)	52 (9)	

[1] x, y, z; [2] x, y, -1+z; [3] x, y, 1+z; [4] 11/2-x, -y, -1/2+z
* Intra-molecular H bond suggested.
** Intra-molecular bifurcated H bond suggested.
*** Inter-molecular H bond suggested.

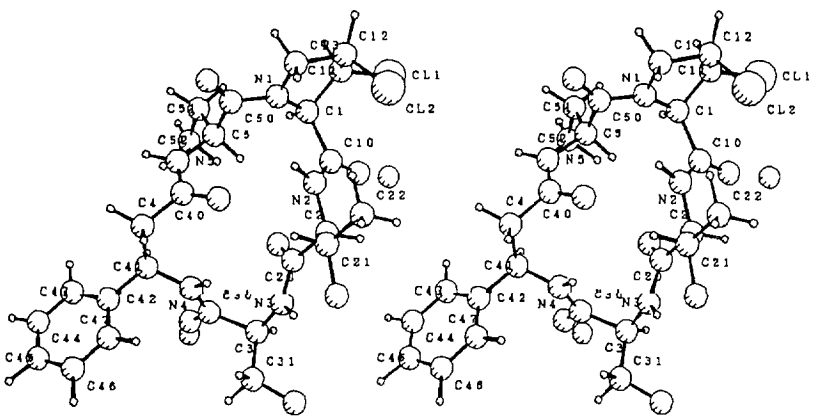

Fig. 4. A stereoscopic view of the crystal structure of astin B (**2**) by PLUTO drawing. Each number refers to the carbon, oxygen, nitrogen and chlorine atoms of **2**.

Table 6. X-ray calculated backbone dihedrals in astin B (**2**) and cyclochlorotine

Residue	Dihedral angle	Astin B(2)	Cyclochlorotine
Pro(Cl2)[1]	ϕ	-96.2	-60.4
	ψ	7.4	-22.8
	ω	169.2	176.8
alloThr[2]	ϕ	-83.3	-105.4
(Abu)	ψ	-149.5	5.4
	ω	-171.4	-178.8
Ser[3]	ϕ	-69.0	-144.6
	ψ	-26.5	-110.3
	ω	176.9	177.6
β-Phe[4]	ϕ^*	-159.1	-141.6
	ψ_1^{**}	56.6	63.5
	ψ_2^{**}	87.9	-93.5
	ω	-168.2	170.8
Abu[5]	ϕ	-71.4	-69.2
(Ser)	ψ	140.7	175.7
	ω	15.4	-163.3

The amino acids in parenthesis indicate those in cyclochlorotine.

*ϕ in β-Phe[4]=C4-C41-N4-C30

**ψ_1 in β-Phe[4]=C40-C4-C41-N4, ψ_2 in β-Phe[4]=N5-C40-C4-C41

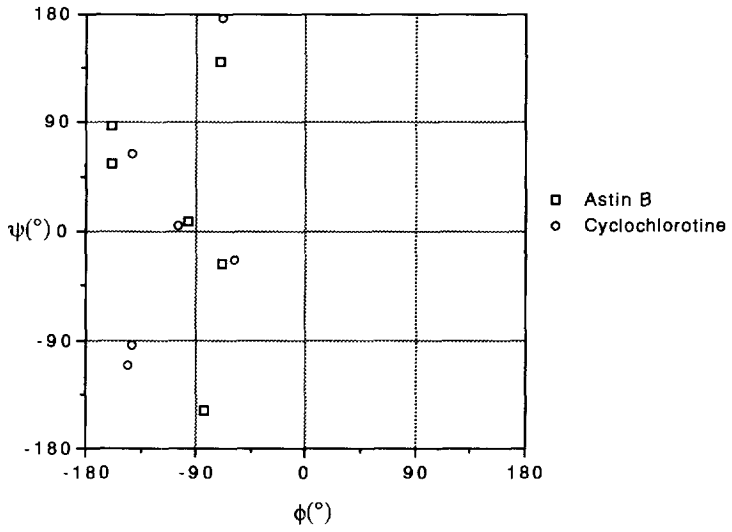

Fig. 5. Ramachandran plot of astin B (**2**) and cyclochlorotine, ϕ and ψ angles calculated by X-ray .

residue, β-Phe[4] were observed [N4---O4 of 2.963(16) Å and HN4---O4 of 2.46(18) Å]. On the other hand, the analogous cyclic pentapeptide, cyclochlorotine[4] adopted a stable type I β-turn structure between Pro(Cl_2) and Abu with a trans proline amide bond and a transannular hydrogen bond. In this manner, astin B (**2**) and cyclochlorotine were found to take quite different conformations in solid states. Table 6 and Fig. 5 show the backbone dihedral angles in compound **2**, compared with those of cyclochlorotine.[4]

Antitumour activity

Allo-threonines, though more rarely encountered in nature, are found as constituents of biologically active peptides.[13] Two chlorine- and *allo*Thr-containing astins are novel cyclic pentapeptides, which are characterized by one cis peptide bond. Antitumor activity was examined by the total packed cell volume method using Sarcoma 180 ascites in mice.[5] The effectiveness was evaluated in terms of the tumor growth ratio (GR(%)=(test group packed cell volume/control group packed cell volume)×100). The GR values of astins A, B and C were 40% (++) at 0.5 mg/kg/day dose, 26% (++) at 0.5 mg/kg/day dose and 45% (+) at 5 mg/kg/day, respectively, for 5 consecutive days. The effective doses of astins A and B were ten-fold stronger than astin C. Efforts are currently underway to determine the precise backbone conformation and biological activity relationship.

Experimental

M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 spectrometer and the $[\alpha]_D$ values were given in 10^{-1}deg cm^2 g^{-1}. FAB and high resolution mass spectra were taken with a VG Autospec spectrometer. IR spectrum was recorded on a Perkin Elmer 1710 spectrophotometer. High-pressure liquid chromatography (HPLC) was performed with an Inertsil PREP-ODS column (20mm i.d.× 250mm and 30mm i.d.× 250mm, GL Science Inc.) packed with 10μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by spraying Dragendorff reagent. ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers (AM400 and AM500) at 303K and processed on a Bruker data station with an Aspect 3000 computer. NOESYPH experiments were made with a mixing time of 0.6s. The NMR coupling constants (*J*) are given in Hz.

Materials

Roots of *Aster tataricus*, used in this experiment, were purchased from Uchida Wakanyaku Co. in Japan.

Extraction and isolation of **1** - **3**

Dry roots (10.0 kg) of *A. tataricus* were extracted with methanol to give a methanol extract (4 kg) which was treated with methylene chloride and then *n*-butanol. The *n*-butanol soluble fraction (500 g), showing antitumor activity, 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** - **3** as colourless needles.

Astin A (1). - Colourless needles, mp 192-194°C, $[\alpha]_D$ -77.0° (c 0.37, MeOH); *m/z* 586 $(M+H)^+$, 516, 485 and 338 (Found: $(M+H)^+$, 586.1814. $C_{25}H_{34}N_5O_7Cl_2$ requires, 586.1835); ν_{max} (KBr)/cm^{-1} 3350, 1650, 1540, 1460, 1420 and 1310.

Astin B (2). - Colourless needles, mp 183-185°C, $[\alpha]_D$ -84.9° (c 0.31, MeOH); *m/z* 586 $(M+H)^+$, 516, 501, 481, 437, 393 and 349 (Found: $(M+H)^+$, 586.1837. $C_{25}H_{34}N_5O_7Cl_2$ requires, 586.1835); ν_{max} (KBr)/cm^{-1} 3350, 1650, 1540, 1440 and 1320.

Astin C (3). - Colourless needles, mp 158-160°C, $[\alpha]_D$ -52.0° (c 0.20, MeOH); *m/z* 570$(M+H)^+$, 501, 485 and 338 (Found: $(M+H)^+$, 570.1863. $C_{25}H_{34}N_5O_6Cl_2$ requires, 570.1886); ν_{max} (KBr)/cm^{-1} 3325, 1655, 1540, 1325 and 1270.

Dechlorination of **1**

A solution of **1** (20.0 mg) and *n*-Bu_3SnH (10.0 mg) in 4 ml tetrahydrofuran with 4 mg azoisobutyronitrile was heated in a sealed tube at 110°C for 12 h. Reaction mixture was concentrated and subjected to ODS-HPLC with 25% MeOH to give compound **4** (10 mg).

Compound **4**. - Colourless needles, mp 263-265°C, $[\alpha]_D$ -121.7° (c 0.29, MeOH), *m/z* 518$(M+H)^+$, 332, 304, 287, 270, 246, 207, 185, 154 and 137; ν_{max} (KBr)/cm^{-1} 3400, 3300, 1630, 1535, 1505 and 1430; δ_H (pyridine-d_5) Pro^1: 5.62 (1H, d, J 8.2, Hα), 2.21 (1H, m, Hβ1), 2.55 (1H, dd, J 6.0, 12.3, Hβ2), 1.74 (1H, m, Hγ1), 1.87 (1H, m, Hγ2), 3.81 (2H, m, Hδ); Abu^2: 5.14 (1H, m, Hα), 2.12 (2H, m, Hβ), 1.07 (3H, t, J 7.3, Hγ), 8.57 (1H, d, J 8.1, NH); Ser^3: 4.63 (1H, m, Hα), 4.50 and 4.59 (each 1H, m, Hβ), 9.35 (1H, d, J 6.0, NH); β-Phe^4: 2.82 (1H, dd, J 9.1, 13.6, Hα1), 3.10 (1H, dd, J 4.9, 13.6, Hα2), 5.53 (1H, ddd, J 4.9, 6.1, 9.1, Hβ), 7.47 (2H, d, J 7.5, Hδ), 7.25 (2H, t, J 7.5, Hε), 7.15 (1H, t, J 7.5, Hζ), 9.14 (1H, d, J 6.1, NH); *allo* Thr^5: 5.16 (1H, m, Hα), 4.41 (1H, m, Hβ), 1.52 (1H, d, J 6.1, Hγ), 9.73 (1H, d, J 6.4, NH); δ_C (pyridine-d_5) 10.61 (q), 21.60 (q), 22.94 (t), 25.98 (t), 31.81 (t), 42.16 (t), 47.25 (t), 52.30 (d), 55.51 (d), 59.49 (d), 60.07 (d), 60.95 (t), 62.14 (d), 68.40 (d), 126.78 (d × 2), 127.12 (d), 128.78 (d × 2), 143.20 (s), 170.27 (s), 171.67 (s), 172.21 (s), 172.55 (s), 172.73 (s).

Absolute Configuration of Amino Acids[6]

Solutions of **1** - **4** (each containing 1 mg of peptides) in 6N HCl were heated at 110° for 12h. 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 1h. After being cooled, 2M HCl was added and then concentrated to dryness. This residue was subjected to HPLC (Lichrospher 100, RP-18 (10μm), Merck), flow rate 2 ml/min, detection 340nm, solvent : 10 - 50% CH_3CN / 50mM triethylamine phosphate (TEAP) buffer. The t_R values were L-

Ser 13.33, L-*allo*Thr 15.13, L-Abu 22.04, L-β-Phe 32.67 and L-Pro 21.20 min, respectively (Standard amino acids: 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, L-Pro 21.18 and D-Pro 24.48 min, respectively). Though Marfey's method for β-amino acids has not been reported, the method was applicable to β-Phe residue in this experiment, whose absolute configuration was confirmed by X-ray analysis.

Alkaline catalyzed cleavage of **1** - **3**

Each of Astins A - C (20 mg) was treated with 29% NH_3 in tetrahydrofuran for 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 by methanol was concentrated to give each of compounds **5** - **7**, quantitatively.

Compound 5. - Colourless needles, mp 248-250℃, $[\alpha]_D$ -16.0° (c 0.16, MeOH), *m/z* 531 $(M+H)^+$, 514, 397, 338 and 179 (Found: $(M+H)^+$, 531.2526. $C_{25}H_{35}N_6O_7$ requires, 531.2567); ν_{max} (KBr)/cm^{-1} 3300, 1650, 1548, 1540, 1520, 1440, 1410, 745 and 710; λ_{max}(MeOH)/nm 266 (ε 13100).

Compound 6. - Colourless needles, mp 214-216℃, $[\alpha]_D$ -39.1° (c 0.13, MeOH); *m/z* 531 $(M+H)^+$, 514, 443, 320, 235 and 185 (Found: $(M+H)^+$, 531.2595. $C_{25}H_{35}N_6O_7$ requires, 531.2567); ν_{max} (KBr)/cm^{-1} 3300, 1638, 1540, 1520, 750 and 710; λ_{max}(MeOH)/nm 267 (ε 9290).

Compound 7. - Colourless needles, mp 229-231℃, $[\alpha]_D$ -17.4° (c 0.26, MeOH), *m/z* 515 $(M+H)^+$, 498, 413, 337, 266, 235, 216, 179 and 151 (Found: $(M+H)^+$, 515.2619. $C_{25}H_{35}N_6O_6$ requires, 515.2618); ν_{max} (KBr)/cm^{-1} 3300, 1640 and 1550; λ_{max}(MeOH)/nm 266 (ε 12300). The ^1H and ^{13}C NMR data of **5** - **7** were shown in Tables 3 and 4.

Crystallographic analysis of compound **2**

Crystal data: $C_{25}H_{33}N_5O_7Cl_2 \cdot 2H_2O$, orthorhombic, space group $P2_12_12_1$, $Z=4$, $a=17.4404(19)$, $b=20.1230(22)$, $c=9.3888(7)$ Å, $V=3295Å^3$, $Dx=1.255$ gcm^{-3}, $F(000)=1312$. A colourless prismatic crystal of approximately 0.58 × 0.33 × 0.20 mm in length was sealed in a thin walled glass capillary and was mounted on a Nonius CAD4 diffractometer with graphite-monochromated CuKα radiation ($\mu=22.4$ cm^{-1}) at 23℃. A total of 3104 reflections were observed above the $2\sigma(I)$ level, with the 2θ range from 6° through 150°. The structure was determined by the direct method using the SHELXS-86 program[14] and the refinement was carried out by the block-diagonal-matrix least-squared method. The final R value was 0.168 ($Rw=\Sigma w(|Fo|-|Fc|)^2/\Sigma w|Fo|^2=0.0347$, where, $\sqrt{w}=0.1$ when $|Fo|\leq 0.5$, $\sqrt{w}=1$ when $0.5<|Fo|\leq 50$, $\sqrt{w}=50/|Fo|$ when $|Fo|>50$). The number of atoms refined were 41 C, N, O and Cl atoms with anisotropic thermal parameters and 31 hydrogen atoms with isotropic parameters which were found on the difference electron-density map and located at the calculated positions. Final shift/esd values were ranged in 0.044 - 0.061 for non-hydrogen atoms. The maximum residual electron densities were 0.53 e/$Å^3$ with the average values, 0.34 e/$Å^3$. The molecular structure determined by this method is illustrated in Fig. 4. The refined fractional atomic coordinates, the bond lengths, the bond angles, the hydrogen-atom coordinates and the thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

Assay of activity against Sarcoma 180 ascites

ICR male mice, 5 weeks old, supplied by Clea Japan Co., Ltd., were used in groups of 6 animals. Sarcoma 180 ascites, provided by the National Cancer Center Research Institute and maintained in successive generations by us, was implanted i.p. at 1×10^6 cells/body. Administration of a test drug was started at 1 day after the implantation and continued for 5 day by the i.p. route. The effectiveness was evaluated by means of the total packed cell volume method[5]: growth ration(GR%)=(packed cell volume (PCV) of test groups/PCV of control groups) × 100; GR=0 - 10% (+++), 11 - 40% (++), 41 - 65% (+) and over 66% (-).

Drug Treatment. - A 0.5% solution of carboxymethylcellulose (CMC) in isotonic sodium chloride was used as a vehicle for the infection of test drugs. Control group mice received equal volumes of normal saline containing 0.5% CMC. The results were evaluated according to the standard methods described above.

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