ASTINS A AND B, ANTITUMOR CYCLIC PENTAPEPTIDES FROM *ASTER TATARICUS*

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Two novel antitumor chlorine- and *allo*Thr-containing cyclic pentapeptides, named astins A and B, both of which were isomers and took a *cis* configuration in one of the amide bonds, were isolated from *Aster tataricus* together with astin C. Their structures were solved by spectroscopic analysis and chemical degradation.

KEYWORDS Astin A; Astin B; Astin C; cyclic pentapeptide; *Aster tataricus*; antitumor activity

During the survey of novel antitumor compounds from medicinal plants, *n*-butanol extract from *Aster tataricus* showed potent antitumor activity. *A. tataricus* (Compositae) is known as a Chinese medicine containing several terpenoids[1] and saponins[2] and is also popular as a garden flower. Efforts at chromatographic purification of the *n*-butanol extract guided by antitumor activity led to the isolation of three cyclic pentapeptides, named astins A (**1**), B (**2**) and C (**3**).

Astin A (**1**)[3] gave a quasi-parent ion $(M+1)^+$ in the FAB-MS at *m/z* 586.1814 appropriate for a molecular formula of $C_{25}H_{33}N_5O_7Cl_2$ (DM +2.1 mmu). The ^1H and ^{13}C NMR spectra of **1** (see Ref.) contained resonances that were characteristic of peptides. A detailed analysis of the COSY and HOHAHA spectra recorded in DMSO-d_6 showed that it contained a β-amino-β-phenyl propionic acid, an α-amino-*n*-butyric acid, a threonine and a serine. Hydrolysis of **1** with 6N HCl, followed by derivatization with Marfey's reagent and HPLC analysis,[4] suggested the presence of Ser, *allo*Thr, β-Phe and Abu, and showed that all of the amino acids had the L configuration. The last amino acid containing two chlorine atoms was disclosed to be 2,3-dichloroproline by the coupling sequence from α-proton. HMQC[5] data were used to assign the carbon resonances to the individual amino acids in **1**, and the amino acid sequence was determined by an analysis of HMBC spectrum.[6] 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).

Astin B (**2**)[7] gave the same molecular formula and was composed of the same amino acids as **1**. HMBC correlations indicated that the positions of Abu and *allo*Thr were changing each other, compared from those of **1**; *i.e.*, the only different point between **1** and **2** was in the amino acid sequence. This difference was also corroborated by the fragmentation pattern of FAB-MS spectra (Fig. 1).

Furthermore, NOE relationships suggested that each compound contains one cis amide bond as follows. The NOEs between Pro-Hα and Thr-Hα in **1** and between Pro-Hα and Abu-Hα in **2** were observed, suggesting that each amide bond was *cis* (Fig. 1).

Astin C (**3**) was identical with asterin,[8] which was recently isolated from the same plant.[9]

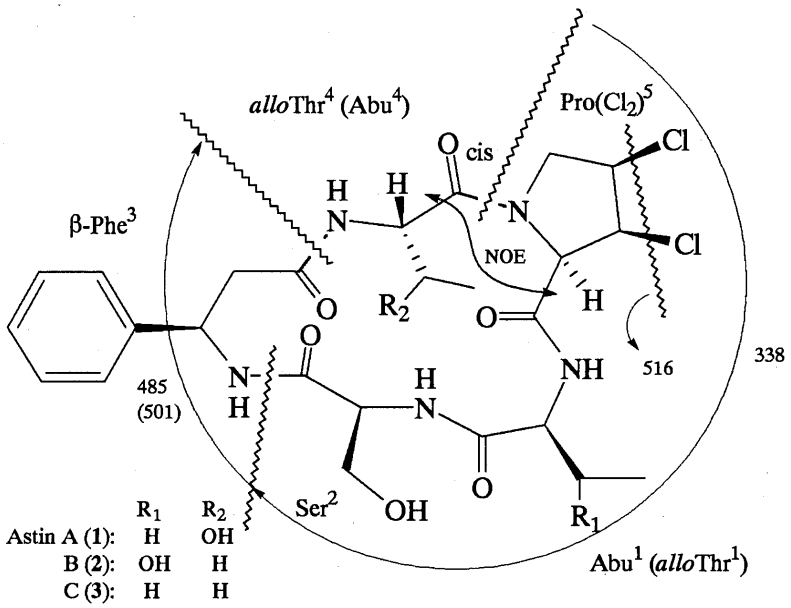

Fig. 1 Structures of Astins A, B and C; Arrows show NOE relationship and fragmentation ion in FAB-MS spectra of **1** and **2**. Abu in **1** was provisionally numbered as a first amino acid. The data in parentheses are for **2**.

Allo-threonines, though more rarely encountered in nature, are found as constituents of biologically active peptides.[10] 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.[11] 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.

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3) Astin A: colorless needles, mp 192-194°C, $[\alpha]_D$ -77.0° (c 0.37, MeOH). ^{1}H-NMR (DMSO-d_6, 500MHz), Abu^1: 4.42 (Hα), 1.75 and 1.94 (Hβ), 0.92 (t, 7.4, Hγ), 7.98 (d, 8.9, HN); Ser^2: 3.73 (Hα), 3.73 (Hβ), 4.92 (OH), 8.11 (d, 4.6, HN); β-Phe^3: 2.29 (dd, 10.9, 13.9, Hα), 2.71 (dd, 4.7, 13.9, Hα), 4.86 (ddd, 4.7, 6.5, 10.9, Hβ), 7.27 (Hδ), 7.27 (Hε), 7.20 (Hζ), 7.79 (d, 6.5, HN); Thr^4: 4.29 (dd, 6.2, 9.4, Hα), 3.68 (ddd, 4.8, 6.1, 9.4, Hβ), 1.12 (d, 6.1, Hγ), 5.43 (d, 4.8, OH), 8.28 (d, 6.2, HN); Pro(Cl$_2$)^5: 5.32 (d, 5.9, Hα), 5.17 (dd, 4.4, 5.9, Hβ), 4.54 (ddd, 4.4, 6.7, 8.8, Hγ), 3.50 (dd, 8.8, 11.7, Hδ), 4.40 (dd, 6.7, 11.7, Hδ). ^{13}C-NMR (DMSO-d_6, 125MHz), Abu^1: 53.75 (Cα), 24.09 (Cβ), 10.28 (Cγ), 170.87 (C=O); Ser^2: 59.37(Cα), 59.63 (Cβ), 169.07 (C=O); β-Phe^3: 41.71 (Cα), 50.72 (Cβ), 142.61 (Cγ), 125.85 (Cδ), 128.11 (Cε), 126.55 (Cζ), 169.90 (C=O); Thr^4: 57.23 (Cα), 68.03 (Cβ), 21.17 (Cγ), 172.87 (C=O); Pro(Cl$_2$)^5: 64.42 (Cα), 63.61 (Cβ), 55.77 (Cγ), 51.21 (Cδ), 166.23 (C=O).

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7) Astin B: colorless needles, mp 183-185°C, $[\alpha]_D$ -84.9° (c 0.31, MeOH). FAB-MS $(M+1)^+$: Calcd. for $C_{25}H_{34}N_5O_7Cl_2$, 586.1835; Found 586.1837. ^{1}H-NMR (DMSO-d_6, 500MHz), Thr^1: 4.24 (t, 9.4, Hα), 4.20 (ddd, 5.8, 5.9, 9.4, Hβ), 1.22 (d, 5.8, Hγ), 5.79 (d, 5.9, OH), 8.39 (d, 9.4, HN); Ser^2: 3.80 (Hα), 3.66 (Hβ), 8.84 (d, 4.2, HN); β-Phe^3: 2.10 (t, 12.7, Hα), 2.82 (dd, 4.7, 12.7, Hα), 4.91 (ddd, 4.7, 6.8, 12.7, Hβ), 7.31 (Hδ), 7.31 (Hε), 7.21 (Hζ), 7.38 (d, 6.8, HN); Abu^4: 4.32 (td, 3.7, 9.3, Hα), 1.50 and 1.75 (Hβ), 0.97 (t, 7.4, Hγ), 8.58 (d, 3.7, HN); Pro(Cl$_2$)^5: 4.88 (d, 5.3, Hα), 5.13 (dd, 4.5, 5.3, Hβ), 4.77 (ddd, 4.5, 6.7, 9.6, Hγ), 3.40 (dd, 9.6, 11.3, Hδ), 4.35 (dd, 6.7, 11.3, Hδ). ^{13}C-NMR (DMSO-d_6, 125MHz), Thr^1: 56.84 (Cα), 65.91 (Cβ), 21.96 (Cγ), 169.78 (C=O); Ser^2: 58.27 (Cα), 60.21 (Cβ), 169.14 (C=O); β-Phe^3: 42.84 (Cα), 51.33 (Cβ), 142.80 (Cγ), 125.58 (Cδ), 128.16 (Cε), 126.60 (Cζ), 171.07 (C=O); Abu^4: 53.32 (Cα), 22.65 (Cβ), 10.43 (Cγ), 172.12 (C=O); Pro(Cl$_2$)^5: 64.45 (Cα), 65.23 (Cβ), 54.79 (Cγ), 51.02 (Cδ), 166.33 (C=O).

8) The name of asterin is not suitable for compound **3**, because it has already been used for the antitumor agent showing immunomodulating action.[12] We renamed as astin C for compound **3**.

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(Received March 6, 1993)