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A Novel Cyclic Pentapeptide with a β-Hydroxy-γ-chloroproline from *Aster tataricus* [1)]

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A novel cyclic pentapeptide with a β-hydroxy-γ-chloroproline residue, named astin I was isolated from the roots of *Aster tataricus* and the structure was elucidated by the spectroscopic methods.

A series of cyclic pentapeptides, named astins, exhibiting potent antitumour activity, have been isolated from the roots of *Aster tataricus*.[2)] During a survey of bioactive peptidic compounds from the higher plant,[1-3)] a novel cyclic pentapeptide, named as astin I (**1**), with a β-hydroxy-γ-chloroproline residue as an unusual amino acid was isolated. We now report about the structure of **1** containing the β-hydroxy-γ-chloroproline residue.

The 1-butanol soluble portion of a methanolic extract of *Aster tataricus* showing a significant antitumour effect on Sarcoma 180A[4)] was subjected to HP-20 and silica gel column chromatography. The fractions obtained were further separated by reversed-phase MPLC and/or HPLC to give a new cyclic pentapeptide, named astin I (**1**: 0.00015%).

Astin I (**1**),[5)] mp 174.1 - 176.5 °C, $[\alpha]_D$ -78.8° (c 0.13, MeOH), gave a quasi-parent ion $(M+H)^+$ in the FAB-MS at *m/z* 552.2246 appropriate for a molecular formula of $C_{25}H_{34}N_5O_7Cl$ (DM +2.1 mmu). The ^1H and ^{13}C NMR spectra of **1** (see ref. 5) contained resonances that were characteristic of cyclic pentapeptides. 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 (β-Phe), two α-amino-*n*-butyric acids (Abu), and a serine (Ser) residues. Hydrolysis of **1** with 6N HCl, followed by derivatization with Marfey's reagent and HPLC analysis,[6)] suggested the presence of one Ser, one β-Phe and two Abu, and showed that all of the amino acids had the L configuration. The last unusual amino acid containing both a chlorine atom and a hydroxyl group was disclosed to be β-hydroxy-γ-chloroproline residue by the coupling sequence from α-proton and NOE correlations as shown below. In the ^1H-NMR spectrum, the signal resonated at δ 6.20, which disappeared on addition of D_2O, was assignable to the hydroxyl proton and after its disappearance, a broad multiplet peak at δ 4.56 attached to the hydroxy bearing carbon at δ 72.76 was changed to a double doublet peak. The coupling network around Pro residue makes clear as shown in Fig. 2. HMQC 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 as indicated in Fig. 1. The

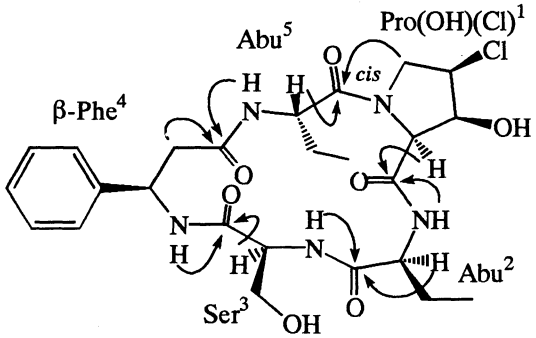

Fig. 1. Structure of astin I (**1**); Pro in **1** was provisionally numbered as a first amino acid. The arrows show HMBC correlations.

stereochemistry of the hydroxyl group and chlorine atom attached to the β and γ carbons in Pro(OH)(Cl) was established to be cis each other by the NOEs observed between Hα and Hβ, between Hα and Hγ, and between Hβ and Hγ of Pro(OH)(Cl) residue. Then the presence of a cis amide bond between Pro(OH)(Cl)[1] and Abu[5] was secured by an NOE correlation between two α protons in Pro(OH)(Cl)[1] and Abu[5], as in the other astins.

Cyclic peptides with various pharmacological activities are known in natural origin, however, not so many examples are present in higher plants.[7] The isolation of the new cyclic pentapeptide, astin I, especially, containing the β-hydroxy-γ-chloroproline residue, from a higher plant is very interesting. Precise antitumor activity of astin I and derived astins are now under investigation.

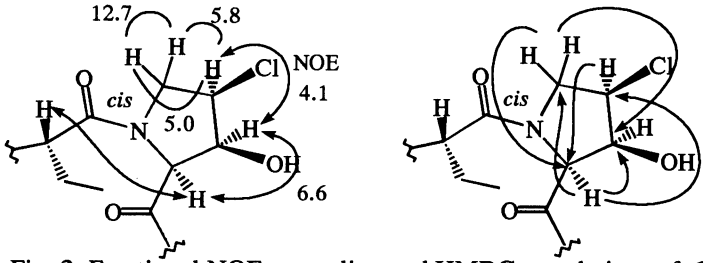

Fig. 2. Fractional NOEs, coupling and HMBC correlations of **1** in DMSO-d_6; Left: The arrows show the NOE relationship and the numbers indicate coupling constants in Hz which were measured on addition of D_2O. Right: The arrows show HMBC correlations.

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5) Astin I (**1**): IR ν max/KBr cm^{-1} 3325 and 1650. ^{1}H-NMR (DMSO-d_6, 500 MHz), Pro(OH)(Cl)[1]: 4.47 (d, 6.6, Hα), 4.56 (br m, Hβ), 6.20 (d, 6.3, OH); 4.50 (ddd, 5.4, 6.0, 9.9, Hγ), 4.08 (dd, 6.0, 12.6, Hδ), 5.48 (dd, 5.4, 12.6, Hδ); Abu[2]: 4.40 (dt, 4.6, 9.5, Hα), 1.76 and 1.96 (each m, Hβ), 0.92 (t, 7.3, Hγ), 8.20 (d, 9.3, HN); Ser[3]: 3.82 (td, 6.1, 11.6, Hα), 3.68 and 3.71 (Hβ), 7.97 (t, 5.8, HN); β-Phe[4]: 2.31 (dd, 11.7, 13.3, Hα), 2.77 (dd, 4.6, 13.3, Hα), 4.87 (ddd, 4.6, 6.6, 11.7, Hβ), 7.21 - 7.30 (m), 7.69 (d, 6.6, HN); Abu[5]: 4.18 (td, 4.7, 9.3, Hα), 1.52 and 1.73 (each m, Hβ), 0.90 (t, 7.4, Hγ), 8.36 (d, 4.6, HN). ^{13}C-NMR (DMSO-d_6, 125MHz), Pro(OH)(Cl)[1]: 60.49 (Cα), 72.76 (Cβ), 58.24 (Cγ), 51.57 (Cδ), 169.34 (C=O); Abu[2]: 53.88 (Cα), 23.94 (Cβ), 10.47 (Cγ), 170.88 (C=O); Ser[3]: 58.97 (Cα), 59.90 (Cβ), 169.14 (C=O); β-Phe[4]: 41.95 (Cα), 51.04 (Cβ), 142.59 (Cγ), 125.74 (Cδ), 128.17 (Cε), 126.61 (Cζ), 170.41 (C=O); Abu[5]: 52.74 (Cα), 23.45 (Cβ), 10.06 (Cγ), 171.41 (C=O).

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