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THIONATION OF AN ANTITUMOUR CYCLIC PENTAPEPTIDE, ASTIN B, FROM ASTER TATARICUS[1)]

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Abstract: Astin B, an antitumour cyclic pentapeptide, isolated from the roots of *Aster tataricus*, was thionated with Lawesson's reagent to give [Ser-3-ψ(CS-NH)-β-Phe-4]astin B (thioastin B). NMR spectroscopy showed that S atom was introduced at Ser^3. The conformation of thioastin B was studied by NOE experiments, temperature effects on NH protons and vicinal NH-Cα coupling constants. Thioastin B was shown to retain the restricted bioactive conformation of astin B and had a more promising antitumour activity than astin B.

Antitumour cyclic pentapeptides, astins, containing two chlorine atoms, were originally isolated from the roots of *Aster tataricus* (Compositae), and their structures[2)] and conformation[3)] were investigated by high field NMR, X-ray and MD simulation studies. Astins, containing one chlorinated proline, has a conformation different from that of an analogous cyclic peptide, cyclochlorotine[4)] isolated from *Penicillium islandicum* Sopp, and the unique structure stimulated us to derive the analogue with higher potency.

Recently, various procedures have been reported on modification of structures and conformations of peptide backbones. Such changes may also modify the biological properties of active peptides. Of them, substitution of a sulfur atom for the oxygen atom seems to be a highly isosteric replacement. Since thionation can strongly influence the secondary structure of cyclic peptides, it may provide us with valuable information about conformational structure-activity relationships of bioactive peptides.[5)]

In the present communication, astin B (**1**) having a potent antitumour activity, was thionated with Lawesson's reagent,[6)] to produce thioastin B (**2**) having a more promising antitumour activity, and the structure and conformation of thioastin B (**2**) were studied by NMR spectroscopy.

Thionation of cyclic peptides with Lawesson's reagent often give complicated mixture of products.[5)] However, in the case of astin B, use 2 mol equiv. of Lawesson's reagent in dry dioxane at 50°C for 12 h, gave a single major product, thioastin B.[7)]

Thioastin B (**2**), mp. 178 - 180 °C, $[\alpha]_D$ -115.8° (c 0.19, MeOH), showed a quassimolecular ion $(M+H)^+$ by FAB-MS spectrum, corresponding to the molecular formula, $C_{25}H_{33}N_5O_6SCl_2$.[8)] The assignments of ^1H and ^{13}C-NMR signals of **2** were made by the combination of ^1H-^1H COSY, HMQC[9)] and HMBC[10)] spectra

(Table 1). The position of the mono thionated carbonyl group was determined by NMR spectroscopy. In the ^{1}H NMR spectrum (DMSO-d_6) of thioastin B, the β-Phe-NH signal (δ 9.38) was in a considerably lower field than that (δ 7.38) in astin B, and the chemical shift of carbonyl carbon of Ser^3 was observed at δ 199.51. Therefore, the structure of thioastin B was determined to be [Ser-3-ψ(CS-NH)-β-Phe-4]astin B.

Thionation is a useful method for modification of peptides for conformation-activity investigations.[5] We have already reported about the solution conformation of astin B[3] and that the activities of astins are influenced by the conformations.[11] Therefore, we analyzed the conformational changes introduced by thionation into astin B and compared the conformation of thioastin B, showing more promising activity than astin B, with that of astin B.

The first step in the determination of the secondary structure of cyclic peptides in solution by NMR is to distinguish the NH protons exposed to the solvent or shielded from the solvent either sterically or through hydrogen bonding. The most common procedure for that is to determine the temperature effects on the NMR signals of NH protons.[12] The temperature coefficients (dδ/dT) of thioastin B (Table 2) clearly suggest that Ser^3-NH and β-Phe^4-NH are involved

Table 1. ^{1}H and ^{13}C-NMR chemical shifts of thioastin B (2)

	proton	carbon
Pro(Cl$_2$)^1		
α	4.87 (d, 5.3)	64.51
β	5.12 (t, 5.3)	65.18
γ	4.77 (m)	54.83
δ	3.35 (m)	51.06
	4.34 (m)	
C$_{C=O}$		166.35
allo Thr^2		
α	4.25 (t, 9.4)	57.04
β	4.20 (m)	65.76
	5.79 (d, 5.9; OH)	
γ	1.22 (d, 5.6)	21.83
NH	8.38 (d, 9.4)	
C$_{C=O}$		169.79
Ser^3		
α	4.36 (m)	65.39
β	3.73 (br s)	62.16
NH	8.88 (d, 4.1)	
C$_{C=O}$		199.51
β-Phe^4		
α	2.39 (t, 12.5)	42.43
	2.89 (dd, 4.7, 12.5)	
β	5.52 (m)	56.90
γ		140.91
δ		126.03
ε	7.22 - 7.31 (m)	128.18
ζ		126.73
NH	9.38 (d, 7.3)	
C$_{C=O}$		170.71
Abu^5		
α	4.27 (td, 3.9, 9.3)	53.34
β	1.47 (m)	22.64
	1.73 (m)	
γ	0.95 (t, 7.4)	10.50
NH	8.62 (d, 3.9)	
C$_{C=O}$		172.26

Measurements were peformed in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C).

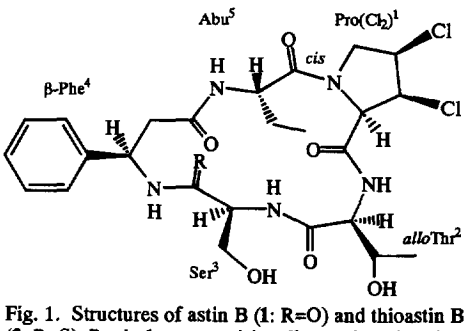

Fig. 1. Structures of astin B (1: R=O) and thioastin B (2: R=S); Pro in 1 was provisionally numbered as the first amino acid.

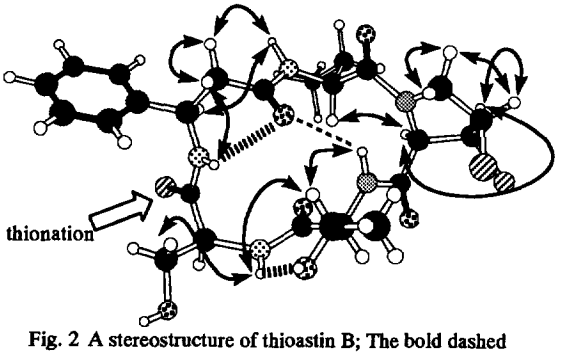

Fig. 2 A stereostructure of thioastin B; The bold dashed lines indicate the strong intramolecular hydrogen bonds and normal dashed line the weak one. The arrows show the NOE relationships in DMSO-d_6.

Table 2. Temperature coefficients, $-d\delta/dT$ (10^3 ppm/K), of NH chemical shifts of astin B (**1**) and thioastin B (**2**) in DMSO-d_6.

compounds	*allo*Thr^2	Ser^3	β-Phe^4	Abu^5
astin B	3.0	2.2	2.8	4.5
thioastin B	3.7	1.7	1.7	4.0

Table 3. Backbone dihedrals (ϕ) in astin B (**1**) and thioastin B (**2**), calculated from vicinal NH-CαH coupling constants (Hz).

	astin B (1)		thioastin B (2)	
Residues	Hz	ϕ angle*	Hz	ϕ angle*
allo Thr^2	9.4	-97	9.4	-97
Ser^3	4.2	-66	4.1	-65
β-Phe^4	6.8	-160	7.3	-157
Abu^5	3.7	-63	3.9	-64

* Calculated by using the Karplus-Bystrov equation: $^3J_{HN\alpha}=9.4\cos^2|60-\phi|-1.1\cos|60-\phi|+0.4$

in intramolecular hydrogen bonds, because the coefficients of Ser^3 and β-Phe^4 were lower than those in the active conformer of astin B. The temperature coefficient of the NH in *all*Thr^2 was higher than that in astin B. Therefore, formation of stable hydrogen bonds between *allo*Thr^2-O and Ser^3-NH, and between β-Phe^4-NH and β-Phe^4-CO was implied.

The allowed dihedral angles were calculated from the vicinal NH-CαH coupling constants obtained by ^{1}H NMR spectrum, by Karplus type equation proposed by Bystrov et al..[13] The J values and the corresponding calculated dihedral angles, ϕ, are shown in Table 3. The corresponding dihedral angles of the two compounds have about the same values except for that in β-Phe^4. The calculated ϕ angle in β-Phe^4 indicates that the thioamide proton orients inside the backbone and the presence of a stronger intramolecular hydrogen bond between β-Phe^4-CO and β-Phe^4-NH than in astin B.

Sulfur is a weaker hydrogen bond acceptor than oxygen: the distance of a hydrogen bond between NH and S is about 50 pm longer than that between NH and O in amides, due to its larger covalent and van der Waals radius.[14] The C=O of Ser^3 and the corresponding C=S are regarded not to involve in intramolecular hydrogen bonds. In contrast, the amide nitrogen next to the thiocarbonyl group is a stronger hydrogen donor,[15] which is also corresponded to the above results in thioastin B.

The conformation of thioastin B, thus proposed, is illustrated in Fig. 2. This conformation was also supported by ROE relationship in ROESYPH spectrum,[16] which is almost the same as that in astin B.[3]

The conformational analyses thioastin B resulted in the strong intramolecular hydrogen bond between β-Phe^4-NH and β-Phe^4-CO in thioastin B more than that in astin B. The main conformer with 5 → 1 and 3 → 4 turns may play an important role in its antitumor activity. The biologically active conformation of thioastin B may be retained mainly by the intramolecular hydrogen bond between Ser^3-NH and Ser^3-OH, which is verified by the fact that the acetylated analog with different conformation from that in astin B, whose intramolecular hydrogen bond was severed by acetylation of Ser-OH, showed less activity.[11]

From the results of our present studies, substitution of sulfur for amide oxygen at Ser^3 in astin B was shown to modify the antitumor activity and backbone conformation of the original compound. Thioastin B possessing the biologically active conformer with rigid backbone showed an antitumor activity on S-180A more promising than astin B.[17, 18]

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7. A solution of **1** (10 mg, 0.01 mmol) in 1,4-dioxane (2 ml) and Lawesson's reagent (10 mg, 0.03 mmol) were stirred at 50 ℃. After 12 h, water (1 ml) was added to the mixture which was left stands for 12 h. The reaction mixture was concentrated to dryness and the residue was chromatographed on alumina with CH_2Cl_2 - MeOH (15 : 1). Finally, reversed phase HPLC using 30 % CH_3CN in H_2O as eluent gave thioastin B (**2**) as a single product (4 mg).
8. HR FAB MS of **1** m/z: 602 ($(M+H)^+$, Calcd for $C_{25}H_{34}N_5O_6SCl_2$ 602.1607, Found 602.1609).
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18. Preliminary assay of *in vivo* antitumor activity was evaluated using Sarcoma 180 ascites in mice. When astin B and thioastin B were administered at 0.5 mg/kg/day for 5 consecutive days, tumor growths were 26 % and 10 %, respectively, of the control.

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