Cyclic peptides from higher plants. Part 21.[1] Thionation of the antitumour cyclic pentapeptides, astins A, B and C, from *Aster tataricus*

Hiroshi Morita, Shinji Nagashima, Koichi Takeya and Hideji Itokawa*
Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

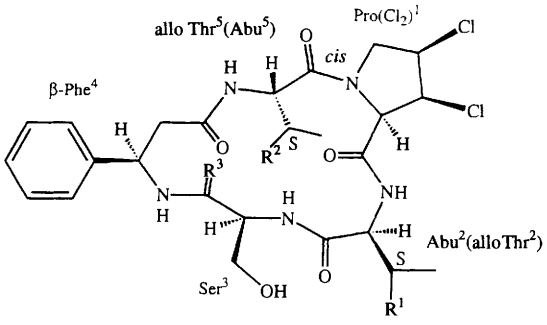
Thionation of the potent antitumour cyclic pentapeptides, the astins A, B and C, with Lawesson's reagent gave [Ser-3-ψ(CS-NH)-β-Phe-4]astin A (thioastin A), [Ser-3-ψ(CS-NH)-β-Phe-4]astin B (thioastin B) and [Ser-3-ψ(CS-NH)-β-Phe-4]astin C (thioastin C), respectively. Conformational analysis of the thioastins was conducted by comparing the 2D NMR data, temperature effects on NH protons, vicinal NH-CαH coupling constants, and NOE results, with those of the corresponding astins. Thioastins showed more promising antitumour activity than the corresponding astins.

Introduction

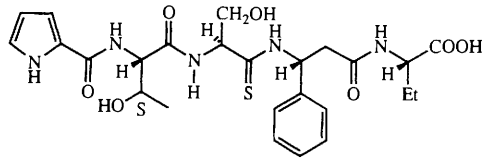
Astins A **1**, B **2** and C **3**, cyclic pentapeptides with a dichlorinated proline residue, were isolated in 1993 from the roots of *Aster tataricus* (Compositae),[2] and their structures were characterized by NMR spectroscopic or X-ray diffraction studies.[2,3] As shown in Fig. 1, astins A, B and C consist of one 16 membered-ring system containing a unique β,γ-dichlorinated proline [Pro(Cl_2)], an allothreonine [alloThr], a serine [Ser], a β-phenylalanine [β-Phe] and an α-aminobutyric acid [Abu]. In an earlier paper,[4] the conformation of astin B in the solid state was shown to be different from that of cyclochlorotine,[5] a toxic principle, isolated from *Penicillium islandicum* Sopp., and to possess a *trans* proline amide bond and a typical type I β-turn between Pro[1] and Abu[2], by X-ray diffraction studies. In solution, the conformation of astin B and those of astins A and C were shown to be different from each other by NOE correlation studies around the residues 2 and 3 in Fig. 1.[1]

Recently, several procedures have been reported for modification of the structures and conformations of peptide backbones. The replacement of amide bonds in physiologically active peptides with thioamide bonds is one of several backbone modifications used frequently in the hope of obtaining more potent and/or selective compounds than the parent ones.[6] Since thionation can strongly affect the secondary structure of cyclic peptides, the reaction may provide us with valuable information about the conformational structure–activity relationships of bioactive peptides.[7] Conformational analysis of cyclic oligopeptides is of interest since it shows that many cyclic peptide backbones are not rigid in solution and have limited conformational flexibility.

The present paper describes the thionation of the antitumour astins A **1**, B **2** and C **3**, with Lawesson's reagent[8] to produce the mono thionated astins A **4**, B **5** and C **6** having more promising antitumour activity than the parent compounds; the structures of compounds **4–6** together with their conformational analysis on the basis of NMR spectroscopic results are also described.



	R^1	R^2	R^3
Astin A **1**:	H	OH	O
Astin B **2**:	OH	H	O
Astin C **3**:	H	H	O
Thioastin A **4**:	H	OH	S
Thioastin B **5**:	OH	H	S
Thioastin C **6**:	H	H	S



Acyclic thioastin B **7**

Fig. 1 Structures of astins A–C (**1–3**), thioastins A–C (**4–6**) and acyclic thioastin B (**7**); Pro was provisionally numbered as the first amino acid

Results and discussion

Thionation of astins A, B and C

Although thionation of cyclic peptides with Lawesson's reagent often gives complex mixtures of products,[7] with 2 mol equiv. of Lawesson's reagent in dry dioxane at 50 °C for 12 h the astins A, B and C gave the thioastins A, B and C, respectively, as the sole products, in 30, 40 and 43% yields, respectively. The following spectroscopic properties of thiopeptides were recorded by Lawesson *et al.*[9] In their ^1H NMR spectra, the thioamide proton and α proton resonate at lower field than those in the parent peptides (NH: Δδ 1.60–2.03; Hα: Δδ 0.24–0.26), and in their ^{13}C NMR spectra, the thiocarbonyl carbon and α carbon of thiopeptides resonate to lower field than those of the parent compounds (C=O: Δδ 29.9–32.3; C^{α} Δδ 7.7–11.3). Further, thiopeptides exhibit UV absorption at 265 nm (log ε 3.8–4.1) characteristic of a π-π* transition associated with the C=S group.[10] The ^1H and ^{13}C signals of the thionation products of compounds **1–3**, which are shifted downfield, are listed in Tables 1 and 2. As can be seen from these Tables, it is apparent that in each compound, only the amide carbonyl

Table 1 ^{1}H NMR chemical shifts (ppm) of thioastins (**4**, **5** and **6**)[a]

Proton	4	5	6
Pro(Cl_2)[1]			
Hα	5.31 (d, 5.9)	4.87 (d, 5.3)	4.82 (d, 5.8)
Hβ	5.16 (dd, 4.4, 5.9)	5.12 (dd, 4.7, 5.3)	5.13 (dd, 4.7, 5.8)
Hγ	4.54 (ddd, 4.4, 6.5, 8.9)	4.77 (ddd, 4.7, 7.2, 9.5)	4.80 (ddd, 4.7, 6.5, 8.6)
Hδ1	3.51 (dd, 8.9, 11.5)	3.40 (dd, 9.5, 11.4)	3.52 (dd, 8.6, 11.7)
Hδ2	4.39 (dd, 6.5, 11.5)	4.34 (dd, 7.2, 11.4)	4.28 (dd, 6.5, 11.7)
Abu[2](alloThr[2])			
Hα	4.45 (dt, 5.1, 8.9)	4.25 (m)	4.41 (m)
Hβ1	1.79 (m)	4.25 (m)	1.80 (m)
Hβ2	1.93 (m)		1.95 (m)
Hγ	0.94 (t, 7.4)	1.22 (d, 5.6)	0.95 (t, 7.3)
NH	7.98 (d, 8.9)	3.83 (d, 9.4)	7.95 (d, 9.0)
Ser[3]			
Hα	4.22 (m)	4.36 (m)	4.25 (m)
Hβ1	3.84 (m)	3.73 (m, 2 H)	3.85 (m, 2 H)
Hβ2	3.88 (m)		
	5.04 (brs, OH)		5.03 (t, 5.9, OH)
NH	8.25 (d, 5.9)	8.88 (d, 4.1)	8.09 (d, 5.7)
β-Phe[4]			
Hα1	2.45 (dd, 10.6, 14.4)	2.36 (t, 12.8)	2.44 (dd, 10.5, 14.0)
Hα2	2.83 (dd, 4.7, 14.4)	2.89 (dd, 4.7, 12.8)	2.86 (dd, 4.8, 14.0)
Hβ	5.44 (ddd, 4.7, 6.7, 10.6)	5.51 (ddd, 4.7, 7.3, 12.8)	5.49 (ddd, 4.8, 6.9, 10.5)
Hδ			
Hε	7.21–7.32	7.22–7.31	7.21–7.32
Hζ			
NH	9.97 (d, 6.7)	9.38 (d, 7.3)	9.86 (d, 6.9)
alloThr[5](Abu[5])			
Hα	4.25 (m)	4.27 (m)	4.21 (m)
Hβ1	3.68 (m)	1.47 (m)	1.44 (m)
Hβ2		1.73 (m)	1.71 (m)
	5.41 (d, 4.8, OH)		
Hγ	1.12 (d, 6.0)	0.95 (t, 7.4)	0.90 (t, 7.4)
NH	8.36 (d, 6.1)	8.62 (d, 3.9)	8.47(d,5.3)

[a] Measurements were performed in [$^2H_6$]-DMSO at 400 MHz. Multiplicity and coupling constants (J/Hz) are in parentheses.

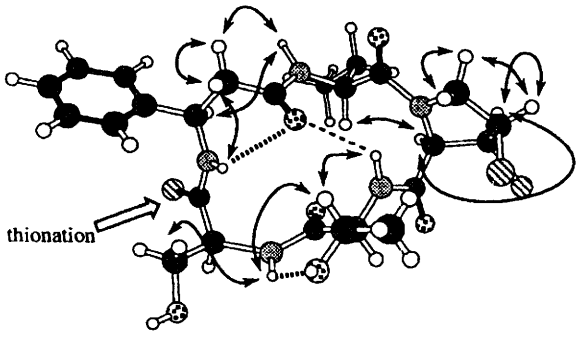
Table 2 ^{13}C NMR chemical shifts (ppm) of thioastins (**4**, **5** and **6**)

Carbon	4	5	6
Pro(Cl_2)[1]			
Cα	64.49	64.51	64.49
Cβ	63.63	65.18	63.75
Cγ	55.76	54.83	55.39
Cδ	51.19	51.06	51.28
CC=O	166.25	166.35	166.07
Abu[2](alloThr[2])			
Cα	53.78	57.40	53.96
Cβ	24.09	65.76	23.79
Cγ	10.33	21.83	10.47
CC=O	171.34	169.79	171.09
Ser[3]			
Cα	67.39	65.89	67.10
Cβ	61.01	62.16	61.22
CC=S	199.12	199.51	198.99
β-Phe[4]			
Cα	41.62	42.42	41.56
Cβ	56.10	56.90	56.17
Cγ	140.78	140.91	140.76
Cδ	126.16	126.03	126.11
Cε	128.22	128.18	128.22
Cζ	126.74	126.73	126.73
CC=O	169.58	170.71	170.08
alloThr[5](Abu[5])			
Cα	57.35	53.34	52.71
Cβ	67.96	22.64	23.30
Cγ	21.24	10.50	10.38
CC=O	172.90	172.26	172.03

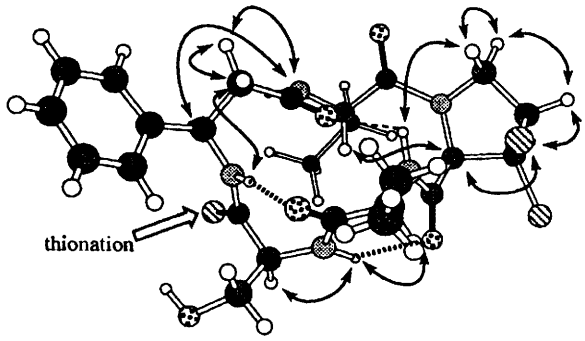
Measurements were performed in [$^2H_6$]-DMSO at 100 MHz.

group of Ser[3] was thionated. The UV absorption band at 265 nm (log ε: **4** 4.1, **5** 4.1, **6** 4.0) observed in each of the thioastins A, B and C further supported production of thiopeptides. From the foregoing evidence, the structures of thioastins A, B and C were established as [Ser-3-ψ(CS-NH)-β-Phe-4]astin A **4**, [Ser-3-ψ(CS-NH)-β-Phe-4]astin B **5** and [Ser-3-ψ(CS-NH)-β-Phe-4]astin C **6**, respectively.

Upon thionation of compound **2**, a minor thiopeptide **7** was also obtained in 4% yield. This was an amorphous powder and had the molecular formula, $C_{25}H_{33}N_5O_7S$, (FAB MS). The spectroscopic data for compound **7** suggested that it was an acyclic thiopeptide, like astin J.[11] In the ^{1}H NMR spectrum, five discrete spin-coupled systems were observed in the ^{1}H-^{1}H COSY spectrum, four of which could be attributed to one Ser, one alloThr, one Abu, and one β-Phe. The remaining system was assigned to a pyrrole residue on the basis of three coupled olefinic protons and a strong UV absorption at 266 (ε 9500) characteristic of the pyrrole ring. The position of the mono thionated carbonyl group was determined by the downfield shift of the ^{1}H and ^{13}C resonance chemical shifts. The β-Phe[4]-NH signal (δ 10.15) was to considerably lower field than that (δ 8.25) in the corresponding linear peptide[3] produced by astin B under basic condition. In addition, the signal for Ser[3]-Hα was also slightly shifted to lower field (δ 4.64, Δδ 0.37). The ^{13}C NMR spectrum also showed downfield shifts for the thiocarbonyl carbon (δ 199.39, Δδ 30.62) in Ser[3] and Ser[3]-Cα (δ 61.81, Δδ 6.42). Therefore, the thioamide position of **7** was the same as that in thoastin B. One reason for the low yields of cyclic thioastins may be that dechlorination and aromatization from Pro(Cl_2) to pyrrole, following the cleavage of amide bond in Pro, take place.



thioastin B **5**



thioastin C **6**

Fig. 2 Perspective view of the structures of thioastins B and C; these pictures are based on the conformations found for astins B and C.[1,4] The bold dashed lines indicate strong intramolecular hydrogen bonds and dotted lines weak ones. The arrows show the NOE relationships in [$^2H_6$]-DMSO.

Conformation of thioastins A, B and C

Conformational analysis of astins A, B and C in the solid and in solution reported previously have indicated that the conformation around the residues 2 and 3 of astin B was different from those of astins A and C.[1] In addition, it has been suggested that the conformation influences the antitumour activities of astins.[12] Thionation which can strongly affect the secondary structure of cyclic peptides,[7] may give valuable information about the conformational structure–activity relationships of astins. Solution forms of thioastins were determined on the basis of NMR spectral data including NOE experiment results, temperature effects on NH protons and vicinal NH-Cα coupling constants.

NMR spectral studies of the thioastins, **4–6** have shown that they exist in a single, stable conformational state in [$^2H_6$]-DMSO. The complete assignments of ^1H and ^{13}C NMR signals of **4–6** were made by a combination of ^1H-^1H COSY, HMQC[13] and HMBC[14] spectral analysis (Tables 1 and 2). The observation of NOEs around Pro(Cl$_2$)^1 and β-Phe^4 as shown in Fig. 2 allowed stereospecific assignments to the methylene protons of Pro(Cl$_2$)^1 and β-Phe^4.

The first step in determining the secondary structure of peptides in solution by NMR spectroscopy is to distinguish between the NH protons exposed to the solvent or shielded from the solvent either sterically or through hydrogen bonding. The common procedure for that purpose is to determine the temperature effects on the NH protons,[15] the NH protons exposed to solvents showing higher temperature dependence. The temperature coefficients ($d\delta/dT$) of NH protons of compounds **4–6** in 10 intervals over the range 300–330 K are compared with those of compounds **1–3** in Table 3, which clearly show that Ser^3-NH and β-Phe^4-NH are shielded from the solvent, whereas Abu^5-NH is exposed to the solvent in both astins and thioastins. There is a similar propensity between the peptides **1–3** and the corresponding thiopeptides, **4–6**.

In thioastin B, Ser^3-NH and β-Phe^4-NH were obviously involved in intramolecular hydrogen bonds, because the temperature coefficients of the NHs of Ser^3 and β-Phe^4 were lower than those in the active conformer of astin B, whereas that of the NH in alloThr^2 was higher than that in astin B. Therefore, stable hydrogen bonds between alloThr^2-O and Ser^3-NH, and between β-Phe^4-NH and β-Phe^4-CO were implied. In thioastins A and C, on the other hand, the coefficients of Ser^3 and β-Phe^4 were lower than those in astins A and C, whereas, that of Abu^2 was higher than those in astins A and C. This indicated stable hydrogen bonds between β-Phe^4-NH and Abu^2-CO, and between Ser^3-NH and Pro(Cl$_2$)^1-CO. As a result of these stable hydrogen bonds, the hydrogen bond between Abu^2-NH and β-Phe^4-CO had disappeared.

Three-bond couplings gave very useful information for the determination of the backbone conformation because they can directly be converted into dihedral angles *via* the Karplus-type equations proposed by Bystrov *et al.*[16] The dihedral angles, φ in compounds **1–6**, calculated from vicinal NH-CαH (NH-CβH for β-Phe^4) coupling constants are shown in Table 4, which shows that the dihedral angles of thioastin B and astin B are about the same except for that in β-Phe^4. The calculated φ angle in β-Phe^4 indicated that the orientation of the thioamide proton

Table 3 Temperature coefficients, $-d\delta/dT$ (10^3 ppm/K), of NH protons of astins A–C (**1–3**) and thioastins A–C (**4–6**) in 10 intervals over the range 300–330 K in [$^2H_6$]-DMSO

Compounds	Abu^2(alloThr^2)	Ser^3	β-Phe^4	alloThr^5(Abu^5)
Astin A **1**	3.5	1.7	0.5	5.3
Thioastin A **4**	5.0	1.3	0.7	5.0
Astin B **2**	3.0	2.2	2.8	4.5
Thioastin B **5**	3.7	1.7	1.7	4.0
Astin C **3**	3.5	1.3	0.0	5.7
Thioastin C **6**	4.7	0.7	0.0	5.0

Table 4 Backbone dihedrals (φ) in astins A–C (**1–3**) and thioastins A–C (**4–6**), calculated from vicinal NH-CαH coupling constants (Hz)

	Astin A **1**		Thioastin A **4**	
Residues	Hz	φ angle*	Hz	φ angle*
Abu^2	8.9	−**93**, −147	8.9	−**93**, −147
Ser^3	4.6	−68, −172, 17, **103**	5.9	−75, −165, 26, **94**
β-Phe^4	6.5	−79, −**161**, 30, 90	6.7	−80, −**160**, 32, 88
alloThr^5	6.2	−**77**, −163, 28, 92	6.1	−**76**, −164, 27, 93
	Astin B **2**		Thioastin B **5**	
Residues	Hz	φ angle*	Hz	φ angle*
alloThr^2	9.4	−**97**, −143	9.4	−**97**, −143
Ser^3	4.2	−**66**, −175, 14, 106	4.1	−**65**, −175, 14, 106
β-Phe^4	6.8	−80, −**160**, 32, 88	7.3	−83, −**157**, 37, 83
Abu^5	3.7	−**63**, −177, 11, 109	3.9	−**64**, −176, 12, 108
	Astin C **3**		Thioastin C **6**	
Residues	Hz	φ angle*	Hz	φ angle*
Abu^2	9.0	−**94**, −146	9.0	−**94**, −146
Ser^3	4.2	−65, −175, 14, **106**	5.7	−74, −166, 24, **96**
β-Phe^4	6.6	−79, −**161**, 31, 89	6.9	−80, −**159**, 33, 87
Abu^5	5.1	−**71**, −169, 20, 100	5.3	−**72**, −168, 22, 98

* Calculated by using the Karplus–Bystrov equation: $^3J_{HN\alpha} = 9.4\cos^2|60 - \varphi| - 1.1\cos|60 - \varphi| + 0.4$. The calculated dihedral angles in thioastins A, B and C shown by bold letters are corresponding to those in astins A, B and C, respectively, whose solution conformation have already been reported.[1,4]

inside the backbone and the presence of an intramolecular hydrogen bond between β-Phe[4]-CO and β-Phe[4]-NH is stronger than that in astin B. In the case of thioastins A and C, only the calculated φ angle in Ser[3] was slightly different from those in astins A and C, respectively. This indicates that the orientation of the amide protons of Ser[3] in **4** and **6** inside the backbone and the presence of an intramolecular hydrogen bond between Ser[3]-NH and Pro[1]-CO stronger than that in **1** and **3**. In this manner, the φ angles in thioamide-neighbouring residues showed a slight difference from those in the parent astins.

The conformation of thioastins A, B and C, thus proposed, is illustrated in Fig. 2, which also indicates the relationship of NOE enhancements observed by phase-sensitive NOESY spectrum.[17] These conformations are almost the same as those in the corresponding astins. In thioastins A and C, the characteristic NOE observed between Abu[2]-NH and Pro[1]-Hδ1 provides evidence in favour of weak hydrogen bond between Abu[2]-NH and β-Phe[4]-CO. It seems likely that the Abu[2]-NH is directed outside the backbone ring more than in the case of astins A and C.

Antitumour activity of thioastins

Antitumour activity was examined by the total packed-cell volume method using Sarcoma 180 ascites in mice.[18] The effectiveness was evaluated in terms of the tumour growth ratio [GR(%) = (test group packed-cell volume/control group packed-cell volume) × 100]. When administered for 5 days at a dose of 0.5 mg kg^{-1} day^{-1} **1**, 0.5 mg kg^{-1} day^{-1} **2** and 5.0 mg kg^{-1} day^{-1} **3**, astins A, B and C gave the GR values of 40, 26 and 45%, respectively. Whereas thiastins A and B gave the GR values of 26 and 14%, respectively, at 0.5 mg kg^{-1} day^{-1}. Thioastin C gave the GR value of 56% at a dose of 1.0 mg kg^{-1} day^{-1}. Accordingly, thioastins showed more promising antitumour activity on S-180A than the corresponding astins.

Experimental

General details

Mps 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 are 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 (20 mm i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. TLC was conducted on pre-coated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by spraying Dragendorff reagent[19] and iodine. ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometers (AM400 and AM500) at 303 K and processed on a Bruker data station with an Aspect 3000 computer. NOESYPH experiments were made with a mixing time of 0.6 s. The NMR coupling constants (J) are given in Hz.

Materials

Astins A, B and C were prepared according to the previous procedure.[3]

Thionation of compounds 1–3

Solutions of compounds **1**–**3** (each 10 mg, 0.01 mmol) in 1,4-dioxane (2 cm^3) and Lawesson's reagent (10 mg, 0.03 mmol) were stirred at 50 °C. After 12 h, each mixture was diluted with water (1 cm^3), left for 12 h and then concentrated to dryness. The resulting residues were chromatographed on alumina with CH_2Cl_2–MeOH (15:1). Finally, reversed phase HPLC using 30% MeCN in water as eluent gave the respective thioastins A, B and C (**4**: 3.1 mg, **5**: 4.0 mg, **6**: 4.4 mg). In the thionation of **2**, an acyclic thioastin B **7** was also obtained in 4% yield.

Thioastin A 4. Colourless needles, mp 177–179 °C, $[\alpha]_D$ −73.6 (c 0.24, MeOH); m/z 602 $(M + H)^+$ [Found: $(M + H)^+$, 602.1600. $C_{25}H_{34}Cl_2N_5O_6S$ requires, 602.1607]; ν_{max}(KBr)/cm^{-1} 3325, 1650, 1530, 1420 and 1310; λ_{max}(MeOH)/nm 265 (log ε 4.1).

Thioastin B 5. Colourless needles, mp 178–180 °C, $[\alpha]_D$ −115.8 (c 0.19, MeOH); m/z 602 $(M + H)^+$ [Found: $(M + H)^+$, 602.1609. $C_{25}H_{34}Cl_2N_5O_6S$ requires, 602.1607]; ν_{max}(KBr)/cm^{-1} 3325, 1645, 1525, 1415 and 1315; λ_{max}(MeOH)/nm 265 (log ε 4.1).

Thioastin C 6. Colourless needles, mp 178–180 °C, $[\alpha]_D$ −100.5 (c 0.22, MeOH); m/z 586 $(M + H)^+$ [Found: $(M + H)^+$, 586.1660. $C_{25}H_{34}Cl_2N_5O_5S$ requires, 586.1658]; ν_{max}(KBr)/cm^{-1} 3300, 1645, 1525 and 1410; λ_{max}(MeOH)/nm 265 (log ε 4.0).

Acyclic thioastin B 7. Amorphous powder; m/z 548 $(M + H)^+$; λ_{max}(MeOH)/nm 266 (ε 9500); δ_H(400 MHz, [$^2H_6$]-DMSO, J/Hz) 6.88 (2 H, m, pyrrole-Hβ1 and Hα2), 6.09 (1 H, m, pyrrole-Hβ2), 11.47 (1 H, br s, pyrrole-NH), 4.43 (1 H, t, 8.6, alloThr-Hα), 3.97 (1 H, m, alloThr-Hβ), 1.19 (3 H, d, 6.2, alloThr-Hγ), 5.20 (1 H, d, 5.3, alloThr-OH), 7.92 (1 H, d, 8.6, alloThr-NH), 4.64 (1 H, m, Ser-Hα), 3.67 (2 H, m, Ser-Hβ), 4.93 (1 H, t, 5.6, Ser-OH), 8.11 (1 H, d, 7.3, Ser-NH), 2.63 (1 H, dd, 5.8, 14.3, β-Phe-Hα), 2.88 (1 H, dd, 9.0, 14.3, β-Phe-Hα), 5.92 (1 H, ddd, 5.8, 8.2, 9.0, β-Phe-Hβ), 7.22–7.32 (5 H, m, β-Phe-Hδ, Hε, Hζ), 10.15 (1 H, d, 8.2, β-Phe-NH), 4.08 (1 H, m, Abu-Hα), 1.49 and 1.59 (each 1 H, m, Abu-Hβ), 0.68 (3 H, t, 7.4, Abu-Hγ) and 8.30 (1 H, d, 7.6, Abu-NH); δ_C(100 MHz, [$^2H_6$]-DMSO, J/Hz) 111.03 (pyrrole-Cβ1), 108.57 (pyrrole-Cα2), 121.59 (pyrrole-Cβ2), 160.45 (pyrrole-CO), 58.36 (alloThr-Cα), 67.02 (alloThr-Cβ), 20.30 (alloThr-Cγ), 170.38 (alloThr-CO), 61.81 (Ser-Cα), 63.89 (Ser-Cβ), 199.39 (Ser-CS), 40.53 (β-Phe-Cα), 55.46 (β-Phe-Cβ), 140.05 (β-Phe-Cγ), 127.08 (β-Phe-Cδ), 128.01 (β-Phe-Cε), 127.08 (β-Phe-Cζ), 169.05 (β-Phe-CO), 53.09 (Abu-Cα), 24.13 (Abu-Cβ), 9.97 (Abu-Cγ) and 172.31 (Abu-CO).

Assay of antitumour activity on sarcoma 180 ascites

5-Week old ICR male mice (weighing 18–20 g, 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, were implanted i.p. at 1×10^6 cells/body. A test drug was administered i.p. for 5 days starting on the following day of the implantation. The effectiveness was evaluated by the total packed-cell volume method:[18] growth ratio (GR%) = (packed-cell volume (PCV) of test groups/PCV of control groups) × 100.

Drug treatment

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

Acknowledgements

The authors thank the Ministry of Education, Science and Culture, Japan, for financial support through Grant-in-Aid for Encouragement of Young Scientists.

References

1 Part 20, H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, 1995, **43**, 1395.
2 H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, 1993, **41**, 992.

3 H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Tetrahedron*, 1995, **51**, 1121.
4 H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Tetrahedron*, 1994, **50**, 11613.
5 H. Yoshioka, K. Nakatsu, M. Sato and T. Tatsuno, *Chem. Lett.*, 1972, 1319.
6 A. F. Spatola, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein, ed., Dekker, 1983, vol. 7, pp. 267–357.
7 H. Kessler, A. Geyer, H. Matter and M. Kock, *Int. J. Peptide Protein Res.*, 1992, **40**, 25.
8 B. S. Pederson, S. Scheibye, N. H. Nilsson and S.-O. Lawesson, *Bull. Soc. Chim. Belg.*, 1978, **87**, 223; S. Scheibye, B. S. Pederson and S.-O. Lawesson, *Bull. Soc. Chim. Belg.*, 1978, **87**, 229.
9 O. E. Jensen, S.-O. Lawesson, R. Bardi, A. M. Piazzesi and C. Toniolo, *Tetrahedron*, 1985, **41**, 5595.
10 K. Clausen, M. Thorsen, S.-O. Lawesson and A. F. Spatola, *J. Chem. Soc., Perkin Trans. 1*, 1984, 785.
11 H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, 1995, **43**, 271.
12 H. Morita, S. Nagashima, K. Takeya, H. Itokawa and Y. Iitaka, 36th Symposium on the Chemistry of Natural Products, Hiroshima, 1994, p. 445.
13 A. Bax and S. Subramanian, *J. Magn. Reson.*, 1986, **67**, 565.
14 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2094.
15 H. Kessler, *Angew. Chem.*, 1982, **94**, 509; *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 512.
16 V. F. Bystrov, V. T. Ivanov, S. L. Portnova, T. A. Balashova and Yu. A. Ovchinnikov, *Tetrahedron*, 1973, **29**, 873.
17 G. Bodenhauser, H. Koger and R. R. Ernst, *J. Magn. Reson.*, 1984, **58**, 370.
18 A. Hoshi and K. Kuretani, *Farmacis*, 1973, **9**, 464.
19 R. Munier, *Bull. Soc. Chim. Biol.*, 1953, **35**, 1225.

Paper 5/02023I
Received 30*th March* 1995
Accepted 17*th May* 1995