Tetrahedron Letters, Vol. 34, No. 8, pp. 1291-1294, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Isolation and Structure of Asterin, A New Halogenated Cyclic Penta-Peptide from *Aster tataricus*

Seiji KOSEMURA*
Department of Chemistry, Faculty of Science and Technology, Keio University,
Hiyoshi, Yokohama 223, Japan

Toshiko OGAWA and Kazuo TOTSUKA
Ohme Research Laboratories Tobishi Phamaceutical Co., Ltd. 1-7-1 Suehiro-cho,
Ohme, Tokyo 198, Japan

Key Words: Structure elucidation; Chlorinated cyclic penta-peptide; Asterin; *Aster tataricus*

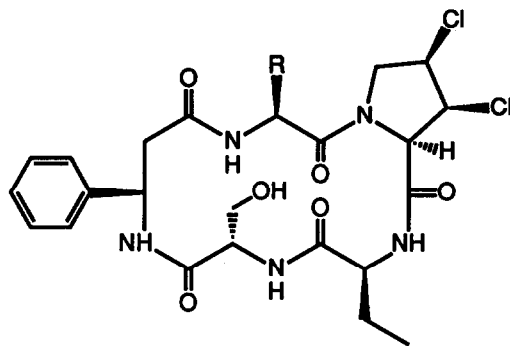
Summary: A new dichlorinated cyclic penta-peptide, asterin, has been isolated from the plant *Aster tataricus* L.f. (*Compositae*), and its structure has also been elucidated on the basis of its spectral data coupled with some chemical evidences.

From a view point of biological activity, many structural studies have been reported on a number of cyclic peptides including bouvardin,[1] ulithiacycliclamide,[2] cyclochlorotine (1),[3] and others. In this communication, we wish to report the isolation and the structure elucidation of a new halogenated cyclic penta-peptide, asterin (2), from the plant *Aster tataricus* L. f. (*Compositae*) (*Shion* in Japanese).

Commercially available dried roots of *Aster tataricus* (5.0 Kg) were immersed in MeOH at room temperature, and then MeOH extract was concentrated and partitioned between *n*-BuOH and water. The *n*-BuOH extract was adsorbed on activated charcoal (ca. 250 g), and the eluate with $CHCl_3$-MeOH (9 : 1) was further purified with normal phase (SiO_2, $CHCl_3$-MeOH (9 : 1)) followed by reverse phase column chromatography (RP-8, 60% MeOH) to give a halogenated cyclic peptide, named asterin (2) in 0.05% yield,[4] and this compound was also obtained in 0.038% yield from the dried fresh roots of *Aster tataricus* (300 g).

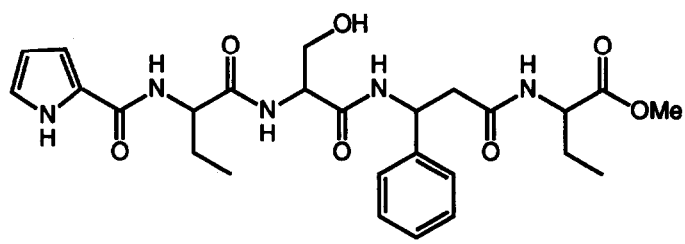
The IR spectrum of **2** showed the presence of hydroxyl groups (3400 cm^{-1}) and amide groups(1650 cm^{-1}) and the ^{13}C NMR spectrum indicated the presence of five amide type carbonyls (δ (C_5D_5N) 167.38, 170.50, 171.96, 172.25, and 173.37), accordingly asterin (**2**) has five amide bonds. Moreover asterin gave a positive at Beilstein test and the isotopic abundances in EI mass spectral fragments indicated that two chlorine atoms were present. The moleculer formula could be deduced by counting carbon (in the ^{13}C NMR spectrum) and hydrogens (in the ^1H and ^{13}C NMR data) and comparing those totals with molecular weight determined by FD mass spectrometory m/z 569 (M+). Six oxygens were mandated by the amide and hydroxyl functionarities; addition of five nitrogens gave the formula $C_{25}H_{33}N_5O_6Cl_2$.

In support of these assigment, dechlorination of **2**[5] (Bu_3SnH, AIBN, THF, 100 °C, overnight) followed by hydrolysis with 6N-HCl (105 °C, 20 h) yielded L-serine, L-β-phenyl alanine, L-proline, and L-α-amino-n-butyric acid in an approximately 1 : 1 : 1 : 2 ratio. The absolute configurations for all amino acids were established by HPLC retention correlation of them on a column coated with an optically active solid phase (CHIRAL PAK WH type, DAICEL; 0.25 mmol $CaSO_4aq$, 1.5 ml/min, 50 °C).



1 : R=CH_2OH

2 : R=CH_2CH_3



3

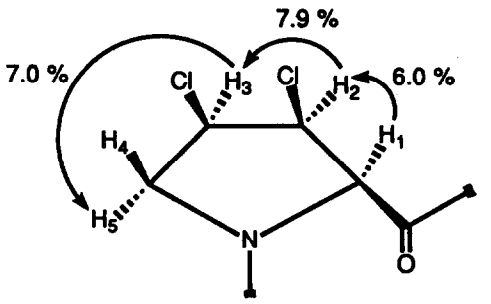


Fig. 1. Results of differential NOEs on dichlorinated proline for asterin (2) in pyridine-d_5.

	1 (ppm)	2 (ppm)		1 (Hz)	2 (Hz)
H_1	6.20	5.51	J_{1-2}	5.7	5.7
H_2	5.51	5.63	J_{2-3}	4.2	4.2
H_3	4.78	5.00	J_{3-4}	6.5	6.5
H_4	4.90	4.84	J_{3-5}	8.4	8.4
H_5	3.95	4.00	J_{4-5}	11.1	11.7

Table 1. Chemical shifts and ^1H-^1H coupling constants of cyclochlorotine (1) and asterin (2) on proline moiety in pyridine-d_5.

Asterin was subjected to regioselective methanolysis using two equivalents of K_2CO_3 in MeOH (room temp., 30 min.) to afford the dehydrochlorinated acyclic penta-peptide methyl ester (3) in almost quantitative yield,[6] then, amino acid sequence was resolved by partial hydrolysis of 3 (1.0 N-HCl, 70 °C, 3h) coupled with DNS-Edman's method. In the light of the above-mentioned results together with co-occurance of cyclochlorotine (1) and its ^1H and ^{13}C-NMR spectra, a tentative structure (2) is given to asterin except for the stereochemistry of two chlorine atoms on proline.

Finally, the stereochemistry of the dichlorinated proline of 2 was elucidated by the NOE difference experiments as shown in Fig. 1. Irradiation at H-1 signal resulted in 6.0 % NOE for H-2, irradiation at H-2 signal resulted in 7.9 % NOE for H-3, and irradiation at H-3 signal resulted in 7.0 % NOE for H-5, thereby, indicating that two chlorine atoms are in a β-*cis* configuration. Moreover, ^1H NMR spectral data of cyclochlorotine (1) and asterin (2), of which the structure of cyclochorotine (1) has been elucidated by means of an X-ray crystallographic analysis, are quite similar to each other as shown in Table 1. Consequently the structure 2 was established for asterin.

Asterin is a hepatotoxic substance as well as cyclochlorotine, however we examined which asterin has an antitumor activity against salcoma-180. Further studies on this point are in progress.

The authors are grateful to Prof. Shosuke Yamamura (KEIO University) for helpful suggestions.

References and Notes

1. S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo, and G. R. Kriek, *J. Am. Chem. Soc.*, **1977**, *99*, 8040.
2. C. Ireland and P. J. Scheuer, *J. Am. Chem. Soc.*, **1980**, *102*, 5688.
3. a) T. Tatsuno, M. Tsukioka, Y. Sakai, Y. Suzuki, and Y. Asami, *Chem. Pham. Bull.*, **1955**, *3*, 476. b) H. Yoshioka, K, Nakatsu, M. Sato, and T. Tatsuno, *Chem. Lett.*, **1973**, 1319. Cyclochlorotine is one of the toxic metabolites of *Penicillium islandium* Sopp, the mold of islandia yellow rice.
4. 2 as a colorless needles (from MeOH); mp 183-187 °C; $[\alpha]_D^{25}$ -65.4° (c 0.11, EtOH); m/z 569 (M+); UV_{max}^{EtOH} nm(ε) 250 (4550), 257 (5000), and 265 (4670); IR (KBr) 3600 and 1650 cm^{-1}; $\delta_H(C_5D_5N)$ 1.02 (t, J= 7.0 Hz, 3H),1.16 (t, J= 7.0 Hz, 3H), 1.80 (m, 2H), 2.20 (m, 2H), 2.80 (dd, J= 10.0, 13.2 Hz, 1H), 3.16.(dd, J=.5.0, 13.2 Hz, 1H), 4.02 (dd, J= 8.8, 11.6 Hz, 1H), 4.45 (dd, J= 4.5, 10.8 Hz, 1H), 4.50-4.65 (complex, 2H), 4.84 (dd, J=6.3, 11.6 Hz, 1H), 5.01 (m, 1H), 5.19 (complex), 5.52 (5.52d, J= 5.8 Hz, 1H), 5.64 (complex, 2H), 7.1-7.5 (complex, 5H), 8.76 (d, J= 8.9 Hz, 1H), 8.78 (d, J= 8.8Hz, 1H), 8.97 (d, J= 5.3 Hz, 1H), 9.77 (d, J= 5.5 Hz, 1H); $\delta_C(C_5D_5N)$ 10.73 (q), 11.12 (q), 24.76 (t), 25.03 (t), 42.79 (t), 52.32 (d), 52.45 (t), 54.00 (d), 55.51 (d), 56.41 (d), 60.30 (d), 60.91 (t), 64.83 (d), 66.16 (d), 126.66 (d x 2), 127.20 (d), 128.78 (d x 2), 143.15 (s), 167.38 (s), 170.50 (s), 171.96 (s), 172.25 (s), 173.37 (s).
5. Dechlorinated product of 2: mp 215-220 °C (decomp); found; m/z 501.2583 (M+), calcd for $C_{25}H_{35}N_5O_6$; M, 501.2585; $[\alpha]_D^{25}$ -136.1° (c 0.08, EtOH).
6. Compound 3; found: m/z 511.2444 (M+ - H_2O), calcd for $C_{26}H_{33}N_5O_6$; M+ - H_2O 511.2429, $[\alpha]_D^{25}$ -30.4° (c 0.13, EtOH), mp 238 - 243°C (decomp); δ_H(DMSO-d_6) 3.60 (s, 3H), 6.10 (like q, 1H), 6.88 (like t, 2H), 11.44 (s, 1H, exchangenable in presence of D_2O).

(Received in Japan 22 October 1992)