Tetrahedron Letters, Vol. 34, No. 3, pp. 501-504, 1993
Printed in Great Britain

0040-4039/93 $5.00 + .00
Pergamon Press Ltd

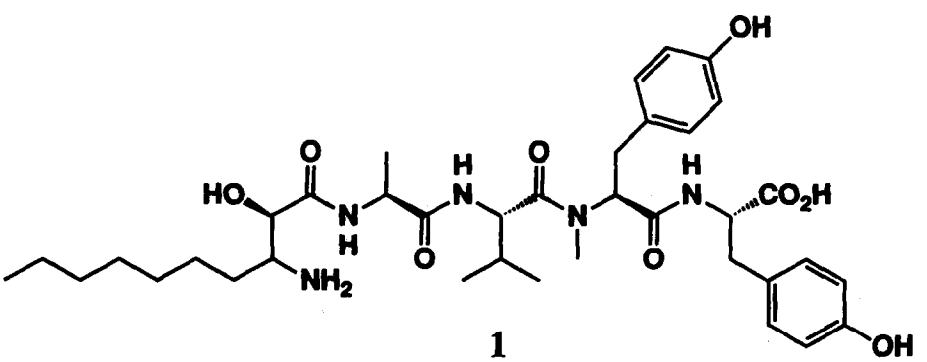
MICROGININ, AN ANGIOTENSIN-CONVERTING ENZYME INHIBITOR FROM THE BLUE-GREEN ALGA *Microcystis aeruginosa*

Tatsufumi Okino, Hisashi Matsuda, Masahiro Murakami* and Katsumi Yamaguchi
Laboratory of Marine Biochemistry, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Abstract: Microginin, an angiotensin-converting enzyme inhibitory pentapeptide, was isolated from the freshwater blue-green alga *Microcystis aeruginosa*. Its structure was elucidated to be **1** on the basis of 2D NMR data and chemical degradation.

Microalgae have recently received much attention as sources of novel bioactive compounds.[1-5] Especially, blue-green algae have been shown to be excellent producers for cytotoxins and fungicides by Moore *et al.*[6] In the course of our screening program of enzyme inhibitors from microalgae,[7] we isolated an angiotensin-converting enzyme inhibitory peptide from the cultured freshwater blue-green alga *Microcystis aeruginosa* (NIES-100), which is well known to produce hepatotoxic peptides microcystins.[8] Angiotensin-converting enzyme inhibitors have been developed as antihypertensive agents.[9] In this paper, we describe the isolation and structure elucidation of a linear pentapeptide microginin (**1**).

M. aeruginosa was obtained from the NIES-collection (Microbial Culture Collection, the National Institute for Environmental Studies, Japan) and cultured in 10 L glass bottles containing MA medium [$Ca(NO_3)_2 \cdot 4H_2O$ 5 mg, KNO_3 10 mg, $NaNO_3$ 5 mg, Na_2SO_4 4 mg, $MgCl_2 \cdot 6H_2O$ 5 mg, β-Na_2glycerophosphate 10 mg, $Na_2EDTA \cdot 2H_2O$ 0.5 mg, $FeCl_3 \cdot 6H_2O$ 0.05 mg, $MnCl_2 \cdot 4H_2O$ 0.5 mg, $ZnCl_2$ 0.05 mg, $CoCl_2 \cdot 6H_2O$ 0.5 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.08 mg, H_3BO_3 2 mg, BICINE 50 mg, distilled water 100 mL, pH 8.6][10]



1

Table 1. ^{13}C and ^{1}H NMR Spectra of Microginin in DMSO-d_6

Carbon	position	C (mult)	H (mult, J Hz)	HMBC correlations from C to
Ahda	1	170.2 (s)		Ahda H-2,H-3, Ala H-2,NH
	2	69.4 (d)	4.05 (brs)	Ahda H-3
	2-OH		6.48 (br)	
	3	53.0 (d)	3.22 (m)	Ahda H-2
	3-NH_2		7.76 (br)	
	4	28.7 (t)	1.42 (m)	Ahda H-2,H-3
			1.58 (m)	
	5	24.6 (t)	1.30 (m)	Ahda H-3
	6	28.6 (t)*	1.23 (m)	
	7	28.4 (t)*	1.23 (m)	
	8	31.1 (t)	1.22 (m)	Ahda H-10
	9	22.0 (t)	1.23 (m)	Ahda H-10
	10	13.9 (q)	0.85 (t, 6.6)	
Ala	1	171.6 (s)		Ala H-2,H-3, Val H-2,NH
	2	47.7 (d)	4.33 (dd, 7.4, 6.9)	Ala H-3
	3	18.5 (q)	1.10 (d, 6.9)	Ala H-2
	NH		7.99 (d, 7.4)	
Val	1	171.5 (s)		Val H-2, MeTyr H-2,*N*-Me
	2	53.7 (d)	4.41 (dd, 9.0, 7.8)	Val H-3,H-4
	3	30.2 (d)	1.87 (dq, 7.8, 6.0)	Val H-2,H-4
	4	19.2 (q)	0.77 (d, 6.0)	Val H-2,H-4'
	4'	18.0 (q)	0.78 (d, 6.0)	Val H-2,H-4
	NH		8.05 (d, 9.0)	
MeTyr	1	169.8 (s)		MeTyr H-2,H-3, Tyr H-2,NH
	2	56.7 (d)	5.21 (dd, 8.0, 6.1)	MeTyr H-3,*N*-Me
	3	33.1 (t)	2.63 (dd, 14.5, 6.1)	MeTyr H-2,H-5
			3.02 (dd, 14.5, 6.1)	
	4	127.6 (s)		MeTyr H-2,H-3,H-6
	5	129.4 (d)	6.92 (d, 8.2)	MeTyr H-3
	6	114.9 (d)	6.58 (d, 8.2)	MeTyr H-6,OH
	7	155.6 (s)		MeTyr H-6
	OH		9.15 (brs)	
	N-Me	30.9 (q)	2.77 (s)	MeTyr H-2
Tyr	1	172.8 (s)		Tyr H-2,H-3
	2	53.7 (d)	4.32 (m)	Tyr H-3
	3	35.6 (t)	2.79 (m)	Tyr H-2,H-5
			2.92 (dd, 14.3, 4.7)	
	4	127.6 (s)		Tyr H-2,H-3,H-6
	5	129.9 (d)	6.93 (d, 8.2)	Tyr H-3
	6	114.9 (s)	6.62 (d, 8.2)	Tyr H-6,OH
	7	155.9 (s)		Tyr H-6
	OH		9.22 (brs)	
	NH		7.96 (d, 7.7)	

*:Interchangeable

under illumination of 80 μE/m^2·s. The 80 % methanol extract of freeze-dried algal cells (27 g from 380 L of culture) was partitioned between water and diethyl ether, and the aqueous layer was further extracted with *n*-butanol. The *n*-butanol layer was subjected to ion-exchange column chromatography (DEAE TOYOPEARL 650-S 5-150 mM $HCOONH_4$) followed by reversed phase HPLC (YMC-Pack ODS AM, 35 % MeCN 0.1 %

TFA) to yield 36 mg of microginin (**1**). Microginin is colorless amorphous powder and positive to ninhydrin: $[\alpha]_D$ -80° (c=0.02, MeOH); UV (MeOH) λmax 224 nm (ε 15400), 276 (2200). The IR spectrum showed the presence of amide carbonyl groups (1640-1660 cm^{-1}) and hydroxyl groups (3400 cm^{-1}). The molecular formula of microginin was determined to be $C_{37}H_{55}N_5O_9$ by HRFABMS [*m/z* 714.4078 $(M+H)^+$ Δ 3.6 mmu] and NMR data. The spectra of ^1H and ^{13}C NMR (Table 1) were characteristic of peptide. Amino acid analysis of the acid hydrolysate of **1** (6 N HCl, 16 hr) gave Ala, Val, Tyr, *N*-MeTyr and an unknown amino acid. The detailed analyses of ^1H-^1H COSY, HOHAHA,[11] HMQC[12] and HMBC[13] spectra supported the presence of the usual amino acids. And the HMBC correlation (*N*-Me δH 2.77, δC 30.9/CH δH 5.21, δC 56.7) indicated the presence of *N*-MeTyr. The unknown amino acid, *N*-MeTyr and Tyr were isolated by column chromatography (Hitachi Custom Ion Exchange Resin 2614, Et_3N/AcOH) followed by HPLC (YMC-Pack ODS AM, H_2O and 60 % MeOH). The structure of the unknown amino acid was decided to be a new β amino acid, 3-amino-2-hydroxy-decanoic acid (Ahda) by the interpretation of ^1H NMR (δ 0.85, 3H, t; δ 1.2-1.3, 12H, m; δ 3.08, 1H, br; δ 3.54, 1H, m), ^{13}C NMR (δ 13.95, 22.07, 25.20, 28.48, 28.90 (2C), 31.17, 52.30, 69.60), ^1H-^1H COSY, ^1H-^{13}C COSY and positive FABMS (*m/z* 204, $[M+H]^+$). The CD spectrum of Ahda exhibited a negative Cotton effect at 215 nm, suggesting the stereochemistry of C-2 of α-hydroxy-β-amino acid to be R.[14] Long chain β-amino-α-hydroxy-amino acids were found in puwainaphycins isolated from a blue-green alga *Anabaena* sp.[2] The configuration of *N*-MeTyr was determined to be L by the CD spectrum (a positive Cotton effect at 224 nm) of isolated one. The stereochemistries of the usual amino acids were assigned to be L by the chiral GC analyses (Chirasil-Val, Alltech) of *N*-trifluoroacetyl methyl ester derivatives of the acid hydrolysate. The sequence of microginin was deduced by HMBC correlations (Ahda CO/Ala NH and α-H, Ala CO/Val NH and α-H, Val CO/*N*-MeTyr *N*-CH_3 and α-H, *N*-MeTyr CO/Tyr NH and α-H)(Table 1) and ROESY correlations[15] (Ahda α-H/Ala NH, Ala α-H/Val NH, Val α-H/N-MeTyr *N*-Me and Tyr NH/ *N*-MeTyr *N*-Me and α-H) (Fig. 1). Furthermore fragmentation of FABMS (*m/z* 533) supported that Tyr was the terminal amino acid.

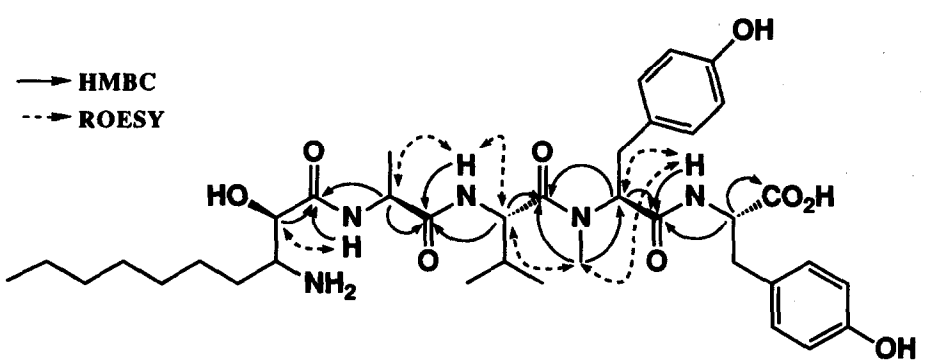


Fig.1 HMBC and ROESY correlations of microginin

Microginin inhibits angiotensin-converting enzyme with an IC_{50} of 7.0 μg/mL, but doesn't inhibit papain, trypsin, chymotrypsin and elastase at 100 μg/mL. Study on stereochemistry of C-3 of Ahda is under progress.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

1) J. M. Gregson, J. L. Chen, G. M. L. Patterson and R. E. Moore, *Tetrahedron*, **48**, 3727 (1992).
2) F. E. Koehn, R. E. Longley and J. K. Reed, *J. Nat. Prod.* **55**, 613 (1992).
3) M. Murakami, H. Matsuda, K. Makabe and K. Yamaguchi, *Tetrahedron Lett.*, **32**, 2391 (1991).
4) M. Satake, M. Murata, T. Yasumoto, T. Fujita and H. Naoki, *J. Am. Chem. Soc.*, **113**, 9859 (1991).
5) M. Murakami, K. Makabe, K. Yamaguchi, S. Konosu and M. R. Wälchli, *Tetrahedron Lett.*, **29**, 1149 (1988).
6) R. E. Moore, S. Banarjee, V. Bornemann, F. R. Caplan, J. L. Chen, D. G. Corley, L. K. Larsen, B. S. Moore, G. M. L. Patterson, V. J. Paul, J. B. Stewart and D. E. Williams, *Pure & Appl. Chem.*, **61**, 521 (1989).
7) K. Yamaguchi, M. Murakami and T. Okino, *J. Appl. Phycol.*, **1**, 271 (1989).
8) W. W. Carmichael: *Handbook of Natural Toxins*; Tu A. T. Ed., Marcel Dekker, New York, pp 121 (1988).
9) M. J. Wyvratt and A. A. Patchett, *Med. Res. Rev.*, **5**, 483 (1985).
10) M. M. Watanabe and K. N. Satake, *NIES-Collection List of Strains Third Edition Microalgae and Protozoa* Microbial Culture Collection, Natl. Inst. Environ. Stud., Tsukuba, Japan, pp 32 (1991).
11) D. G. Davis and A. Bax, *J. Am. Chem. Soc.*, **107**, 2820 (1985).
12) A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
13) A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
14) B.Sjöberg, *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*; G. Snatzke Ed., Holden-Day, San Francisco, pp 304 (1965).
15) A. Bax and D. G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).

(Received in Japan 24 September 1992)