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HIBISPEPTIN A, A NOVEL CYCLIC PEPTIDE FROM *HIBISCUS SYRIACUS*

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Abstract : A new cyclic peptide, hibispeptin A, has been isolated from the root bark of *Hibiscus syriacus* and the structure was assigned as *cyclo*[-Ahabpa(-pyro-Glu)-Pro-Leu-Phe-] on the basis of various spectroscopic analyses. © 1998 Elsevier Science Ltd. All rights reserved.

Many cyclic peptides with unique structures and biological activities have been isolated from microbial and marine origins. But only a few compounds including lyciumins[1], citrusins[2], curcacycline A[3], cleromyrine I[4], yunnanins[5], astins[6], segetalins[7] and dichotomins[8] were isolated from higher plants. Some of them have abnormal amino acids in their structures. We herein deal with a peptide with unusual amino acid unit in cyclic.

In the course of screening for biologically active novel constituents from higher plants using as the traditional Chinese medicines[9], we have isolated a unique cyclic peptide, named hibispeptin A[10], from the root bark of *Hibiscus syriacus* Linne (Malcaceae), which has been used as antipyretic, anthelmintic and antifungal agents in the Orient[11]. Previously some flavonoids, polyphenols and fatty acids have been isolated from *H. syriacus*[12]. In this paper, we describe the isolation and structural elucidation of hibispeptin A.

A methanolic extract of the dried root bark (1.6 kg) of *H. syriacus* was washed with hexane and then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction was chromatographed on silica gel and

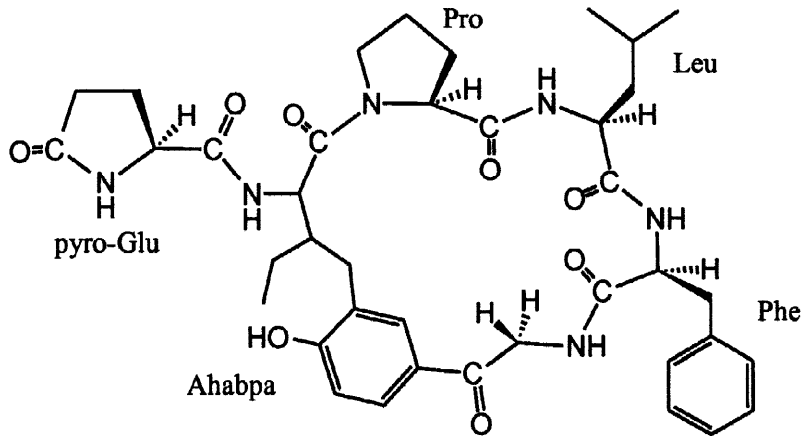

Fig. 1. The structure of hibispeptin A.

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Sephadex LH-20 columns followed by preparative RP-TLC developed with 65% aq. MeOH to give hibispeptin A (18 mg).

The molecular formula of hibispeptin A obtained as white powder was established as $C_{39}H_{50}N_6O_8$ by HR-FAB mass spectroscopy (glycerol/PEG, *m/z* 731.3748 $(M+H)^+$ -2.1mmu). The IR absorptions near 1695(sh), 1665 and 1540 cm^{-1} attributed to the α,β-unsaturated carbonyl, amide carbonyl and amide NH groups, respectively, indicated that this compound had peptidic character. The UV absorption at 276 nm in MeOH suggested the presence of aromatic functions in hibispeptin A. ^1H NMR spectrum in DMSO-d_6 indicated seven α protons between δ 3.58 to δ 4.55 and six exchangeable protons at δ 10.50, 8.79, 8.58, 7.96, 7.66 and 7.08, which were collapsed on shaking with D_2O. A DEPT experiment established the multiplicities of the carbon resonances while the HMQC[13] data assigned all of the proton-bearing carbons. The DQF-COSY[14] data in combination with ^{13}C spectral data suggested the presence of 1 mol each of the glutamine (Gln) or pyro-glutamic acid (pyro-Glu), proline (Pro), leucine (Leu), phenylalanine (Phe) and glycine, together with 1,2,5-trisubstituted benzene and an unusual amino acid. Of these partial units, pyro-glutamic acid, but not glutamine, was assigned by the long range correlations from the amide proton at δ 7.96 and α methine proton at δ 4.36 of pyro-Glu to the carbonyl carbon at δ 177.7 that was long range coupled with β and γ protons of pyro-Glu. Also the methylene protons at δ 2.34 and 3.06 of an unusual amino acid with isoleucine moiety showed the long range correlations to three sp^2 carbons at δ 124.8 (C-1), 159.9 (C-2) and 132.5 (C-6) of 2-hydroxyphenyl, and H-4 and H-6 of hydroxyphenyl were correlated to the carbonyl carbon of glycine moiety at δ 196.2, revealing the presence of 2-amino-3-(2-hydroxy-5-aminoacetylbenzyl)pentanoic acid (Ahabpa). From the above results, we found that hibispeptin A was composed of pyro-Glu, Pro, Leu, Phe and Ahabpa. The chemical connectivities of these partial structures were further established by the HMBC experiment[15], as shown in Fig. 2.

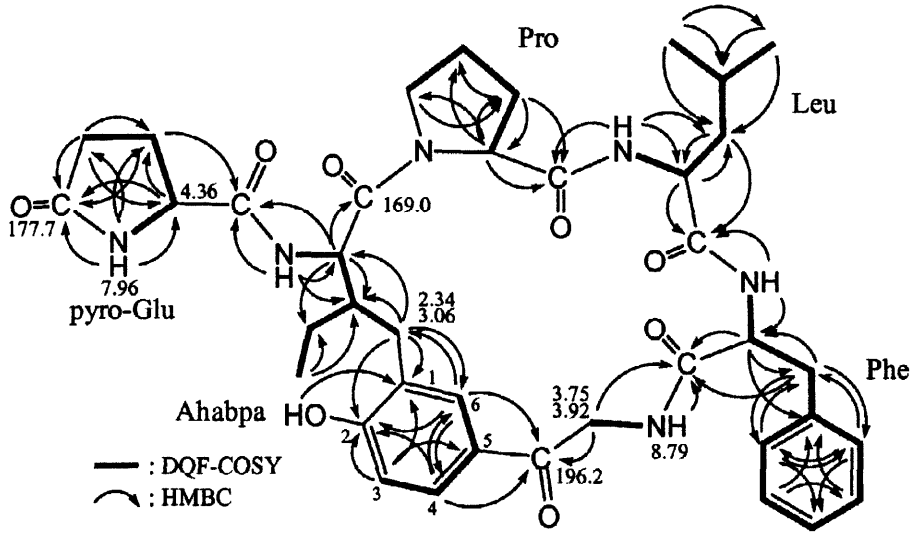

Fig. 2. The structure of hibispeptin A elucidated by the DQF-COSY and HMBC experiments.

It showed long-range correlation(s) from amide and/or α proton(s) of each amino acid to carbonyl carbon of neighboring amino acid. Namely, the long range couplings from the amide and α protons of

Ahabpa to the carbonyl carbon of pyro-Glu, from the amide proton at δ 8.79 and methylene protons at δ 3.75 and 3.92 of Ahabpa to the carbonyl carbon of Phe, from the amide proton of Phe to the carbonyl carbon of Leu and from the amide proton of Leu to the carbonyl carbon of Pro were observed. Thus the sequencing of the amino acids for hibispeptin A was determined to be Pro-Leu-Phe-Ahabpa-pyro-Glu. By the process of elimination, the remaining carbonyl carbon of Ahbpa at δ 169.0 should be connected to nitrogen of Pro, assigning the structure of hibispeptin A as a unique cyclic peptide composed of five amino acids. The ^{1}H and ^{13}C NMR spectral data are summarized in Table 1.

Table 1. ^{1}H and ^{13}C NMR spectral data of hibispeptin A in DMSO-d_6[a].

Position	δ_H	δ_C	Position	δ_H	δ_C
pyro-Glu			Pro		
α	4.36 (1H, dd, 8.0, 4.5)[b]	54.4	α	4.24 (1H, br. d, 7.5)	59.3
β	1.92 (1H, m)	25.4	β	1.88 (1H, m)	30.6
	2.38 (1H, m)			2.28 (1H, m)	
γ	2.08 (1H, m)	29.3	γ	1.40 (2H, m)	21.2
	2.18 (1H, m)		δ	3.31 (2H, m)	45.6
δ		177.7	CO		170.0
NH	7.96 (1H, s)		Leu		
CO		175.0	α	4.04 (1H, m)	53.9
Ahabpa			β	1.19 (1H, m)	41.1
α	3.59 (1H, br. s)	53.2		1.44 (1H, m)	
β	2.27 (1H, m)	37.0	γ	1.29 (1H, m)	24.2
γ	1.38 (1H, m).	22.0	δ	0.79 (3H, d, 7.0)	22.9
	1.62 (1H, m)			0.69 (3H, d, 7.0)	20.8
δ	0.95 (3H, t, 7.0)	12.3	NH	7.66 (1H, d, 9.0)	
CH_2	2.34 (1H, d, 13.0)	33.0	CO		171.8
	3.06 (1H, dd, 13.0, 4.0)		Phe		
1		124.8	α	4.55 (1H, br. ddd, 9.0, 8.0, 6.5)	54.1
2		159.9	β	2.78 (1H, dd, 13.5, 6.5)	38.0
2-OH	10.50 (1H, s)			2.89 (1H, dd, 13.5, 8.0)	
3	6.72 (1H, d, 8.5)	113.8	γ		137.5
4	7.36 (1H, d, 8.5)	129.0	δ	7.18 (2H, br. d, 7.5)	129.1
5		126.8	ε	7.23 (2H, m)	128.3
5-CO		196.2	ζ	7.12 (1H, m)	126.6
5-CH_2	3.75 (1H, br. d, 15.0)	47.7	NH	7.08 (1H, d, 9.0)	
	3.92 (1H, dd, 15.0, 6.5)		CO		170.8
5-NH	8.79 (1H, br. t)				
6	7.38 (1H, br. s)	132.5			
NH	8.58 (1H, d, 4.5)				
CO		169.0			

[a] taken in 600 MHz for ^{1}H and 150 MHz for ^{13}C at 298 K

[b] Proton resonance integration, multiplicity and coupling constant (J=Hz) are in parenthesis

In order to determine the absolute stereochemistry, hibispeptin A was subjected to complete hydrolysis with 6N HCl at 110°C for 24h in a sealed tube. Each amino acid from the hydrolysate was purified and then analysed by comparing the Rf-values with standard amino acids on chiral-TLC. Consequently, all normal component amino acids (Gln, Pro, Leu and Phe) in hibispeptin A were determined to be L-configuration. The stereochemistries of α and β positions in Ahabpa remain to be established. The geometry of the proline amide bond was determined to be *cis* by the ^{13}C chemical shifts at δ 30.6 and 21.2 for

β and γ positions, respectively[16]. The biological activities of hibispeptin A are now under investigation.

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10. Hibispeptin A White powder ; UV λ_{max} nm (ε) in MeOH : 206 (31,200), 224 (sh, 11,300), 276 (8,600) ; IR (KBr) : 3360, 3320, 1695 (sh), 1665, 1640, 1595, 1540, 1440, 1285 cm^{-1} ; $[\alpha]_D$ = -38° (*c*=0.21, $CHCl_3$-MeOH (1:1)) ; HRFAB-MS : *m/z* 731.3748 $(M+H)^+$, $C_{39}H_{50}N_6O_8$ requires 731.3769.
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