



Cyclopeptides from the seeds of *Annona glabra*

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Abstract

From the seeds of *Annona glabra*, two new cyclopeptides, glabrin C [cyclo-(prolyl-glycyl-tyrosyl-valyl-leucyl-alanyl-leucyl-valyl)] and glabrin D [cyclo-(prolyl-prolyl-valyl-tyrosyl-glycyl-prolyl-glutamyl)], have been isolated. Their structures were elucidated by chemical and spectral methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Annona glabra*; Annonaceae; Seeds; Cyclopeptides; Glabrin C, D

1. Introduction

In our previous paper (Li et al., 1998) we have reported two new cyclopeptides glabrin A and B from *Annona glabra* (Annonaceae). As a part of continuing investigations on Annonaceae cyclopeptides (Li et al., 1995, 1997, 1998), two new cyclopeptides named glabrin C and D have been isolated from seed extracts of *Annona glabra*.

2. Results and discussion

The cyclopeptides, glabrin C (**1**) and D (**2**), were isolated from the CHCl₃ fraction of the alcoholic extract of *Annona glabra* seeds by column chromatography as previously (Li et al., 1998) described.

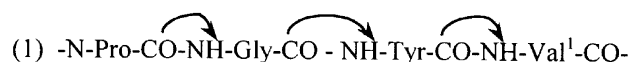
Glabrin C (**1**), amorphous powder, gave a negative ninhydrin reaction and showed a high resolution positive FAB-MS spectral quasimolecular ion peak at m/z 813.4813 ((M + 1)⁺, ∇ 6.1 mDa), corresponding to molecular formula C₄₁H₆₄N₈O₉. IR absorption maxima at 3320 and 1655 cm⁻¹ indicated that the compound might be a peptide (Tan et al., 1993). The 400 MHz ¹H NMR and ¹³C NMR spectra clearly showed seven amide NH at δ 9.82, 9.28, 8.66, 8.66, 8.66, 8.56, 7.85 and eight amide CO at δ 174.4, 174.4, 173.4, 172.8, 172.5, 172.0, 172.0, 170.2. Using ¹H-¹H COSY, ¹³C-¹H COSY and COLOC spectra, the composition of amino acid residues were determined

as Pro (1eq), Gly (1eq), Tyr (1eq), Val (2eq), Leu (2eq) and Ala (1eq), which indicated that glabrin C appeared to be a cyclic octapeptide. The spectral data are shown in Tables 1 and 2.

The amino acid sequence was determined primarily by positive FAB-MS which showed the fragments of 1 to 7 as follows:

1. m/z 155 [Pro-Gly + H]⁺
2. m/z 318 [Pro-Gly-Tyr + H]⁺
3. m/z 417 [Pro-Gly-Tyr-Val + H]⁺
4. m/z 530 [Pro-Gly-Tyr-Val-Leu + H]⁺
5. m/z 601 [Pro-Gly-Tyr-Val-Leu-Ala + H]⁺
6. m/z 714 [Pro-Gly-Tyr-Val-Leu-Ala-Leu + H]⁺
7. m/z 813 [Pro-Gly-Tyr-Val-Leu-Ala-Leu-Val + H]⁺

So the structure of the compound, named glabrin C, a cyclic octapeptide was deduced as cyclo-(Pro-Gly-Tyr-Val-Leu-Ala-Leu-Val). This was also confirmed by the partial sequence of the following peptides (Equations (1–2)) from the correlations between amide CO and NH in the COLOC experiment ($J = 10$ Hz) (Tan et al., 1993):



Glabrin D (**2**), amorphous powder, gave a negative ninhydrin reaction and showed a high resolution positive FAB-MS spectral quasimolecular ion peak at m/z 739.3624 ((M)⁺, ∇ -8.3 mDa), corresponding to molec-

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Table 1

¹H and ¹³C NMR spectral data of glabrin C (1) and D (2) (in pyridine-d, 400 MHz for δ_{H} , 100 MHz for δ_{C} , TMS)

Glabrin C (1)			Glabrin D (2)		
	H	C		H	C
1			1		
2	4.42 (m)	62.2 ^b	2	4.36 (m)	62.8
3	2.05 (m)	29.6	3	2.02 (m), 2.10 (m)	29.1
4	1.65 (m), 1.91 (m)	25.8	4	1.56 (m), 2.02 (m)	25.1
5	3.61 (m), 3.72 (m)	48.3	5	3.53 (m), 3.66 (m)	47.9
6		173.4	6		172.4 ^d
7	9.82 (br.)		7		
8	3.72 (m), 4.57 (m)	44.1	8	4.66 (t, 6.5)	61.4
9		170.2	9	1.82 (m), 2.37 (m)	31.8
10	8.56 (d, 8.4)		10	0.96 (m), 1.37 (m)	21.9
11	5.20 (m)	56.0	11	3.41 (m)	47.9
12	3.21 (m), 3.36 (m)	37.3	12		171.8 ^d
13		128.9	13	7.75 (d, 7.6)	
14	7.40 (d, 8.3)	131.0	14	5.02 (d, 6.1)	55.9
15	7.16 (d, 8.3)	116.4	15	2.37 (m)	32.9
16		157.7	16	1.12 (d, 5.9)	17.9
17		174.4	17	1.78 (d, 6.4)	21.2
18	9.28 (d, 3.1)		18		171.6
19	4.57 (m)	63.3 ^b	19	8.50 (d, 7.4)	
20	2.51 (m)	30.4	20	5.12 (m)	59.5
21	1.25 (d, 6.6) ^a	19.6 ^c	21	3.41 (m)	38.8
22	1.33 (d, 6.6) ^a	20.0 ^c	22		128.4
23		174.4	23	7.36 (d, 7.2)	130.7
24	8.66 (m)		24	7.10 (d, 6.9)	116.6
25	4.42 (m)	54.3	25		157.9
26	2.18 (m)	38.6	26		173.2
27	1.91 (m)	25.4	27	9.80 (br.)	
28	0.90 (d, 6.5)	21.6	28	4.70 (m), 4.05 (dd)	43.7
29	1.02 (d, 6.4)	23.6	29		170.0
30		172.0	30		
31	8.66 (m)		31	4.80 (t, 4.2)	59.9
32	4.93 (m)	49.9	32	1.82 (m), 2.10 (m)	28.8
33	1.85 (d, 7.2)	18.0	33	1.56 (m), 1.82 (m)	26.0
34		172.5	34	3.41 (m)	47.1
35	8.66 (m)		35		171.1 ^d
36	4.57 (m)	54.8	36	8.61 (d, 7.8)	
37	1.76 (m), 1.91 (m)	39.9	37	5.20 (m)	51.8
38	1.76 (m)	25.4	38	2.50 (m), 2.70 (m)	28.2
39	0.75 (d, 5.9)	21.5	39	2.70 (m), 2.84 (m)	33.1
40	0.80 (d, 6.1)	22.9	40	8.19 (s)	175.1
41		172.8	41		170.1 ^d
42	7.82 (br.)				
43	5.20 (m)	56.0			
44	2.4 (m)	32.3			
45	1.12 (d, 6.7) ^a	18.3 ^c			
46	1.18 (d, 6.8) ^a	19.3 ^c			
47		172.0			

Data of Leu¹ and Leu² in glabrin C (1), Pro¹, Pro² and Pro³ in glabrin D (2) and the pairs with superscripts a, b, c, d are interchangeable.

ular formula C₃₆H₄₉N₇O₁₀. Its IR absorptions maxima were at 3321, 1664 and 1622 cm⁻¹.

The 400 MHz ¹H NMR and ¹³C NMR spectra clearly showed four amide NH at δ 9.80, 8.61, 8.50, 7.75 and one OH of COOH at δ 8.19 and seven amide CO at δ

173.2, 172.4, 171.8, 171.6, 171.1, 170.1, 170.0 and one CO of COOH at δ 175.3. Using ¹H-¹H COSY, ¹³C-¹H COSY and COLOC spectra, the composition of amino acid residues were determined as Pro (3eq), Val (1eq), Tyr (1eq), Gly (1eq), Glu (1eq), which indicated that

Table 2
 ^1H – ^1H COSY and ^{13}C – ^1H COSY spectra of glabrin C (1)

Amino acid residues			^1H	Coupling in ^1H – ^1H COSY spectra	Coupling in ^{13}C – ^1H COSY spectra
Pro	N	1			
	α	2	4.42 (m)	β -H ₂ (2.05)	62.2 (CH)
	β	3	2.05 (m)	α -H (4.42)	29.6 (CH ₂)
	γ	4	1.65 (m), 1.91 (m)	δ -H ₂ (3.61, 3.72)	25.8 (CH ₂)
	δ	5	3.61 (m), 3.72 (m)	γ -H ₂ (1.65, 1.91)	48.3 (CH ₂)
		6			173.4 (CO)
Gly	NH	7	9.82 (br.)	α -H ₂ (3.72, 4.57)	
	α	8	3.72 (m), 4.57 (m)	NH (9.82)	44.1 (CH ₂)
		9			170.2 (CO)
Tyr	NH	10	8.56 (d)	α -H (5.20)	
	α	11	5.20 (m)	NH (8.56), β -H ₂ (3.21, 3.36)	56.0 (CH)
	β	12	3.21 (m), 3.36 (m)	α -H (5.20)	37.3 (CH ₂)
	Ar	13			128.9 (Ar-C)
		14	7.40 (d)	15-H (7.16)	131.9 (Ar-CH)
		15	7.16 (d)	14-H (7.40)	116.4 (Ar-CH)
		16			157.7 (Ar-C)
		17			174.4 (CO)
Val ¹	NH	18	9.28 (d)	α -H (4.57)	
	α	19	4.57 (m)	NH (9.28), β -H (2.51)	63.3 (CH)
	β	20	2.51 (m)	α -H (4.57), γ -H ₃ (1.25), γ -H ₃ (1.33)	30.4 (CH)
	γ	21	1.25 (d)	β -H (2.51)	19.6 (CH ₃)
	γ	22	1.33 (d)	β -H (2.51)	20.0 (CH ₃)
		23			174.4 (CO)
Leu ¹	NH	24	8.66 (m)	α -H (4.42)	
	α	25	4.42 (m)	NH (8.66), β -H ₂ (2.18)	54.3 (CH)
	β	26	2.18 (m)	α -H (4.42)	38.6 (CH ₂)
	γ	27	1.91 (m)	δ -H ₃ (0.90), δ -H ₃ (1.02)	25.4 (CH)
	δ	28	0.90 (d)	γ -H (1.91)	21.6 (CH ₃)
	δ	29	1.02 (d)	γ -H (1.91)	23.6 (CH ₃)
		30			172.0 (CO)
Ala	NH	31	8.66 (m)	α -H (4.93)	
	α	32	4.93 (m)	NH (8.66), β -H ₃ (1.85)	49.9 (CH)
	β	33	1.85 (d)	α -H (4.93)	18.0 (CH ₃)
		34			172.5 (CO)
Leu ²	NH	35	8.66 (m)	α -H (4.57)	
	α	36	4.57 (m)	NH (8.66), β -H ₂ (1.76, 1.91)	54.8 (CH)
	β	37	1.76 (m), 1.91 (m)	α -H (4.57)	39.9 (CH ₂)
	γ	38	1.76 (m)	δ -H ₃ (0.75), δ -H ₃ (0.80)	25.4 (CH)
	δ	39	0.75 (d)	γ -H (1.76)	21.5 (CH ₃)
	δ	40	0.80 (d)	γ -H (1.76)	22.9 (CH ₃)
		41			172.8 (CO)
Val ²	NH	42	7.85 (br.)	α -H (5.20)	
	α	43	5.20 (m)	NH (7.85), β -H (2.41)	56.0 (CH)
	β	44	2.41 (m)	α -H (5.20), γ -H ₃ (1.12), γ -H ₃ (1.18)	32.3 (CH)
	γ	45	1.12 (d)	β -H (2.41)	18.3 (CH ₃)
	γ	46	1.18 (d)	β -H (2.41)	19.3 (CH ₃)
		47			172.0 (CO)

glabrin D appeared to be a cyclic heptapeptide. The spectral data are shown in Tables 1 and 3.

The amino acid sequence was determined by positive FAB-MS which showed the fragments of 1 to 6 as the following:

1. m/z 154 [Gly-Pro]⁺

2. m/z 283 [Gly-Pro-Glu]⁺

3. m/z 457 [Pro-Pro-Val-Tyr+H]⁺

4. m/z 494 [Gly-Pro-Glu-Pro-Pro-CONH₂+H]⁺

5. m/z 656 [Tyr-Gly-Pro-Glu-Pro-Pro-CONH₂]⁺

6. m/z 739 [Pro-Pro-Val-Tyr-Gly-Pro-Glu]⁺

With the partial sequence of amino acid residues as the

Table 3
 ^1H – ^1H COSY and ^{13}C – ^1H COSY spectra of glabrin D (2)

Amino acids residue			^1H	Coupling in ^1H – ^1H COSY spectra	Coupling in ^{13}C – ^1H COSY spectra
Pro ¹	N	1			
	α	2	4.36 (m)	β -H ₂ (2.02, 2.10)	62.8 (CH)
	β	3	2.02 (m), 2.10 (m)	α -H (4.36), γ -H ₂ (1.56, 2.02)	29.1 (CH ₂)
	γ	4	1.56 (m), 2.02 (m)	δ -H ₂ (3.53, 3.66), β -H ₂ (2.02, 2.10)	25.1 (CH ₂)
	δ	5	3.53 (m), 3.66 (m)	γ -H ₂ (1.56, 2.02)	47.9 (CH ₂)
		6			172.4 (CO)
Pro ²	N	7			
	α	8	4.66 (t)	β -H ₂ (1.82, 2.37)	61.4 (CH)
	β	9	1.82 (m), 2.37 (m)	α -H (4.66), γ -H ₂ (0.96, 1.37)	31.8 (CH ₂)
	γ	10	0.96 (m), 1.37 (m)	δ -H ₂ (3.41), β -H ₂ (1.82, 2.37)	21.9 (CH ₂)
	δ	11	3.41 (m)	γ -H ₂ (0.96, 1.37)	47.9 (CH ₂)
		12			171.8 (CO)
Val	NH	13	7.75 (d)	α -H (5.02)	
	α	14	5.02 (d)	NH (7.75), β -H (2.37)	55.9 (CH)
	β	15	2.37 (m)	α -H (5.02), γ -H ₃ (1.12), γ -H ₃ (1.78)	32.9 (CH)
	γ	16	1.12 (d)	β -H (2.37)	17.9 (CH ₃)
	γ	17	1.78 (d)	β -H (2.37)	21.2 (CH ₃)
		18			171.6 (CO)
Tyr	NH	19	8.50 (d)	α -H (5.12)	
	α	20	5.12 (m)	NH (8.50), β -H ₂ (3.41)	59.5 (CH)
	β	21	3.41 (m)	α -H (5.12)	38.8 (CH ₂)
	Ar	22			128.4 (Ar-C)
		23	7.36 (d)	24-H (7.10)	130.7 (Ar-CH)
		24	7.10 (d)	23-H (7.36)	116.6 (Ar-CH)
Gly		25			157.9 (Ar-C)
		26			173.2 (CO)
	NH	27	9.80 (br.)	α -H ₂ (4.05, 4.70)	
	α	28	4.70 (m), 4.05 (m)	NH (9.80)	43.7 (CH ₂)
		29			170.0 (CO)
		30			
Pro ³	N	31			
	α	32	4.80 (t)	β -H ₂ (1.82, 2.10)	59.9 (CH)
	β	33	1.82 (m), 2.10 (m)	α -H (4.80), γ -H ₂ (1.56, 1.82)	28.8 (CH ₂)
	γ	34	1.56 (m), 1.82 (m)	δ -H ₂ (3.41), β -H ₂ (1.82, 2.10)	26.0 (CH ₂)
	δ	35	3.41 (m)	γ -H ₂ (1.56, 1.82)	47.1 (CH ₂)
		36			171.1 (CO)
Glu	N	37	8.61 (d)	α -H (5.20)	
	α	38	5.20 (m)	β -H ₂ (2.50, 2.70), NH (8.61)	51.8 (CH)
	β	39	2.50 (m), 2.70 (m)	α -H (5.20), γ -H ₂ (2.70, 2.84)	28.2 (CH ₂)
	γ	40	2.70 (m), 2.84 (m)	β -H ₂ (2.50, 2.70)	33.1 (CH ₂)
	δ	41	8.19 (m)		175.3 (COOH)
					170.1 (CO)

following peptide eq. (3) from the correlations between amide CO and NH in the COLOC experiment ($J=10$ Hz), the structure



of the compound named glabrin D, a cyclic heptapeptide, was elucidated as cyclo-(prolyl–prolyl–valyl–tyrosyl–glycyl–prolyl–glutamyl).

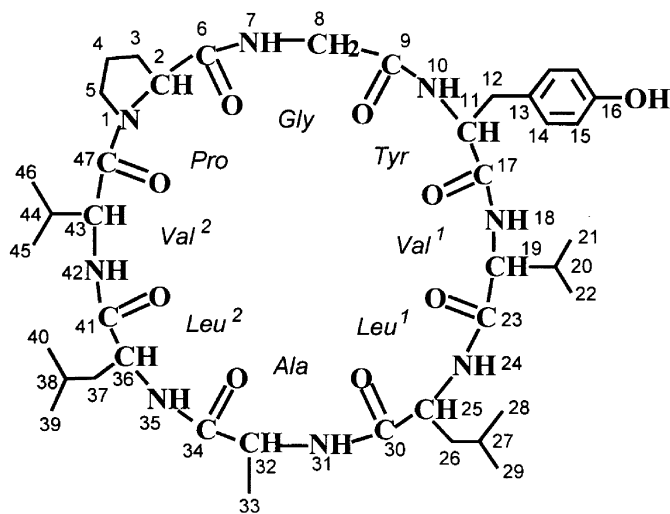
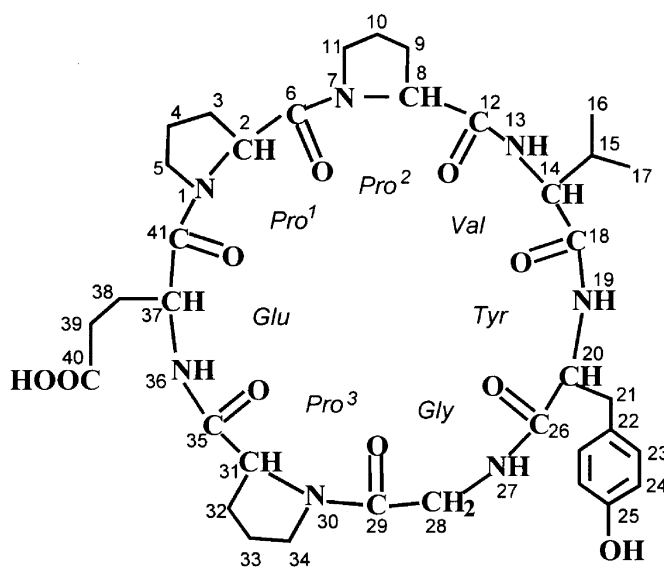
3. Experimental

Glabrin C (**1**, 565 mg) and glabrin D (**2**, 174 mg) were obtained from the CHCl_3 fraction of the alcohol extract

of *Annona glabra* seeds by column chromatography as described in the experimental section in our previous paper (Li et al., 1998).

3.1. Glabrin C (**1**)

Yield $1.0 \times 10^{-2}\%$, amorphous powder, m.p. 153°C , $[\alpha]_{\text{D}}^{29.0} -35.11^\circ$ (MeOH; C 0.235). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 202.5 (4.49), 221.5 (3.99), 279.5 (2.86). IR ν_{max} cm^{-1} : 3320, 1655. For the ^1H and ^{13}C NMR see Table 1. Pos. FAB-MS m/z : 813 $[\text{M}+1]^+$, 714 $[-\text{N-Pro-Gly-Tyr-Val-Leu-Ala-Leu-CO}+\text{H}]^+$, 601 $[-\text{N-Pro-Gly-Tyr-Val-Leu-Ala-CO}+\text{H}]^+$, 530 $[-\text{N-Pro-Gly-Tyr-Val-Leu-CO}+\text{H}]^+$, 417 $[-\text{N-Pro-Gly-Tyr-Val-CO}+\text{H}]^+$,

**glabrin C (1)****glabrin D (2)**

318 [$-N$ -Pro-Gly-Tyr-CO+H] $^+$ and 155 [$-N$ -Pro-Gly-CO+H] $^+$.

3.2. Glabrin D (2)

Yield $5.8 \times 10^{-3}\%$, amorphous powder, m.p. 219°C , $[\alpha]_{29.1}^{20} -53.54^\circ$ (MeOH; C 0.551). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 203.5 (4.54), 220.5 (4.14), 279.5 (3.17). IR ν_{max} cm^{-1} : 3321, 1664, 1622. For the ^1H and ^{13}C NMR see

Table 1. Pos. FAB-MS m/z : 739[M] $^+$, 656 [$-NH$ -Tyr-Gly-Pro-Glu-Pro-Pro-CONH $_2$] $^+$, 494 [$-NH$ -Gly-Pro-Glu-Pro-Pro-CONH $_2$ +H] $^+$, 457 [$-N$ -Pro-Pro-Val-Tyr-CO+H] $^+$, 283[$-NH$ -Gly-Pro-Glu-CO-] $^+$ and 154 [$-NH$ -Gly-Pro-CO-] $^+$.

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