PERGAMON

PHYTOCHEMISTRY

Phytochemistry 50 (1999) 1047–1052

Cyclopeptides from the seeds of *Annona glabra*

Chao-Ming Li[a,*], Ning-Hua Tan[a], Hui-Lan Zheng[b], Qing Mu[a], Xiao-Jiang Hao[a], Yi-Neng He[a], Jun Zhou[a]

[a]*Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Heilongtan, Kunming 650204, People's Republic of China*
[b]*Xishuangbanna Tropical Botanic Garden, Kunming Institute of Botany, Academia Sinica, Mengla 666303, People's Republic of China*

Received 12 March 1998; accepted 22 September 1998

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.

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 $CHCl_3$ 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 $C_{41}H_{64}N_8O_9$. 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 ^{13}C 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, ^{13}C–^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):

(1) -N-Pro-CO-NH-Gly-CO - NH-Tyr-CO-NH-Val[1]-CO-

(2) -N-Ala-CO - NH-Leu[2]-CO-

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-

* Corresponding author. Tel.: +86-871-515-0660; fax: +86-871-5150-227.

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00631-1

Table 1
^{1}H and ^{13}C 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 Leu[1] and Leu[2] in glabrin C (**1**), Pro[1], Pro[2] and Pro[3] in glabrin D (2) and the pairs with superscripts a, b, c, d are interchangeable.

ular formula $C_{36}H_{49}N_7O_{10}$. Its IR absorptions maxima were at 3321, 1664 and 1622 cm^{-1}.

The 400 MHz ^{1}H NMR and ^{13}C 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 ^{1}H–^{1}H COSY, ^{13}C–^{1}H 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
^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectra of glabrin C (**1**)

Amino acid residues			^{1}H	Coupling in ^{1}H–^{1}H COSY spectra	Coupling in ^{13}C–^{1}H 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 $[Gly–Pro–Glu–Pro–Pro–CONH_2+H]^+$
5. m/z 656 $[Tyr–Gly–Pro–Glu–Pro–Pro–CONH_2]^+$
6. m/z 739 $[Pro–Pro–Val–Tyr–Gly–Pro–Glu]^+$

With the partial sequence of amino acid residues as the

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

Amino acids residue			^{1}H	Coupling in ^{1}H–^{1}H COSY spectra	Coupling in ^{13}C–^{1}H 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)
		25			157.9 (Ar–C)
		26			173.2 (CO)
Gly	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)
Pro[3]	N	30			
	α	31	4.80 (t)	β-H_2 (1.82, 2.10)	59.9 (CH)
	β	32	1.82 (m), 2.10 (m)	α-H (4.80), γ-H_2 (1.56, 1.82)	28.8 (CH_2)
	γ	33	1.56 (m), 1.82 (m)	δ-H_2 (3.41), β-H_2 (1.82, 2.10)	26.0 (CH_2)
	δ	34	3.41 (m)	γ-H_2 (1.56, 1.82)	47.1 (CH_2)
		35			171.1 (CO)
Glu	N	36	8.61 (d)	α-H (5.20)	
	α	37	5.20 (m)	β-H_2 (2.50, 2.70), NH (8.61)	51.8 (CH)
	β	38	2.50 (m), 2.70 (m)	α-H (5.20), γ-H_2 (2.70, 2.84)	28.2 (CH_2)
	γ	39	2.70 (m), 2.84 (m)	β-H_2 (2.50, 2.70)	33.1 (CH_2)
	δ	40	8.19 (m)		175.3 (COOH)
		41			170.1 (CO)

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

(3) -N-Pro-CO -NH-Val-CO-NH-Tyr-CO-NH-Gly-CO-

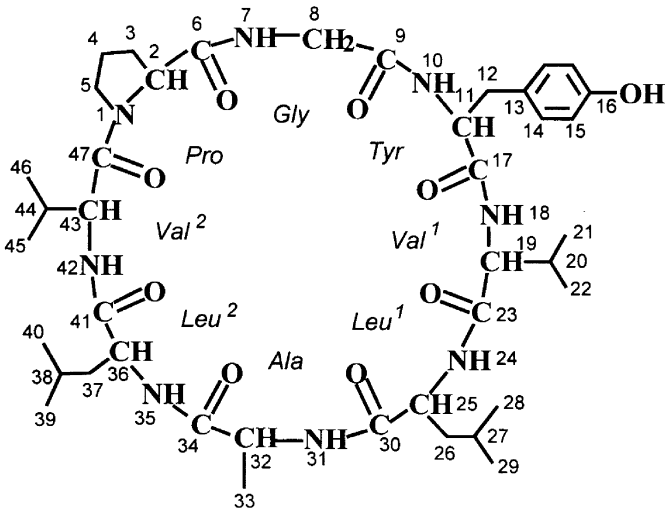
of the compound named glabrin D, a cyclic heptapeptide, was elucidated as cyclo-(prolyl–prolyl–valyl–tyrosyl–glycyl–prolyl–glutamyl).

3. Experimental

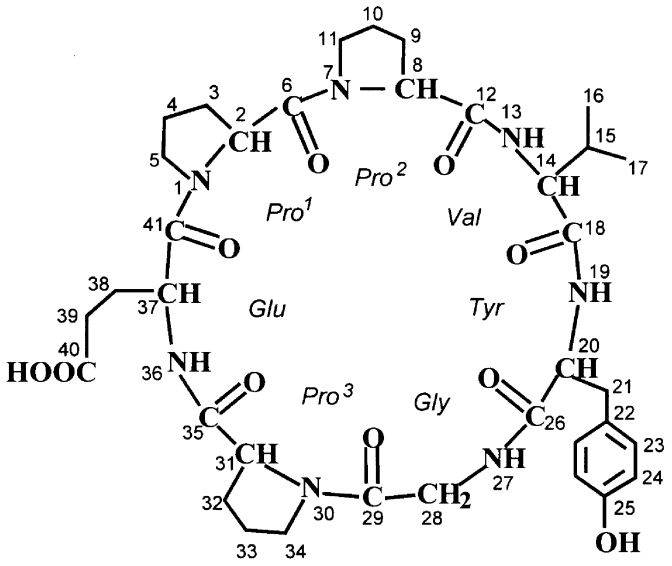
Glabrin C (**1**, 565 mg) and glabrin D (**2**, 174 mg) were obtained from the $CHCl_3$ fraction of the alcohol extract of *Annona glabra* seeds by column chromatography as described in the experimental section in our previous paper (Li et al., 1998).

3.1. Glabrin C (*1*)

Yield 1.0×10^{-2}%, amorphous powder, m.p. 153°C, $[\alpha]^{29.0}_{D}$ −35.11° (MeOH; C 0.235). UV λ^{MeOH}_{max} nm (log ε): 202.5 (4.49), 221.5 (3.99), 279.5 (2.86). IR ν_{max} cm^{-1}: 3320, 1655. For the ^{1}H and ^{13}C NMR see Table 1. Pos. FAB-MS *m/z*: 813 $[M+1]^+$, 714 [–*N*-Pro–Gly–Tyr–Val–Leu–Ala–Leu–CO + H]$^+$, 601 [–*N*-Pro–Gly–Tyr–Val–Leu–Ala–CO + H]$^+$, 530 [–*N*-Pro–Gly–Tyr–Val–Leu–CO + H]$^+$, 417 [–*N*-Pro–Gly–Tyr–Val–CO + 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 5.8×10^{-3}%, amorphous powder, m.p. 219°C, $[\alpha]^{29.1}_D$ −53.54° (MeOH; C 0.551). UV λ^{MeOH}_{max} 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 ^{1}H 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–]$^+$.

Acknowledgements

We thank Ms. Liang Hui-ling (Kunming Institute of Botany, China) for MS. This work was supported by

Grant No. 95C083m from The Fund of Applicated and Basic Science Research of Yunnan Province.

References

Li, C. -M., Tan, N. -H., Lu, Y. -P., Liang, H. -L., Mu, Q., Zheng, H. -L., Hao, X. -J., & Zhou, J. (1995). *Acta Botanica Yunnanica*, *17*, 459.

Li, C. -M., Tan, N. -H., Mu, Q., Zheng, H. -L., Hao, X. -J., Wu, Y., & Zhou, J. (1997). *Phytochemistry*, *45*, 521.

Li, C. -M., Tan, N. -H., Mu, Q., Zheng, H. -L., Hao, X. -J., Liang, H. -L., & Zhou, J. (1998). *Phytochemistry*, *47*, 1293.

Li, C.-M., Tan, N.-H., Zheng, H.-L., Mu, Q., Hao, X.-J., He, Y.-N., & Zhou, J. (1998). *Phytochemistry*, *48*, 555.

Tan, N. -H., Zhou, J., Chen, C. -X., & Zhao, S. -X. (1993). *Phytochemistry*, *32*, 1327.

Tan, N. -H., Wang, D. -Z., Zhang, H. -J., Chen, C. -X., Zhou, J., & Zhao, S. -X. (1993). *Chin. J. Mag. Reson.*, *10*, 69.