

CYCLOPEPTIDES FROM THE SEEDS OF *ANNONA GLABRA*

LI CHAO-MING*, TAN NING-HUA, MU QING, ZHENG HUI-LAN†, HAO XIAO-JIANG, LIANG HUI-LING,
 ZHOU JUN

Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming, 650204, People's Republic of China; † Xishuangbanna Tropical Botanic Garden, Kunming Institute of Botany, Academia Sinica, Mengla, 666303, People's Republic of China

(Received 16 June 1997)

Key Word Index—*Annona glabra*; Annonaceae; seeds; cyclopeptides; glabrin A, B.

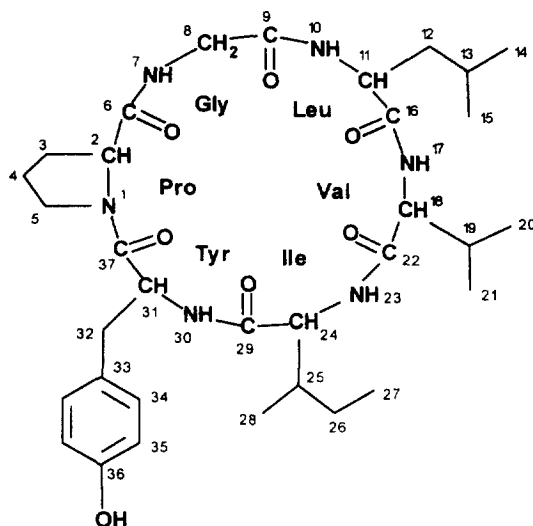
Abstract—From the seeds of *Annona glabra* two new cyclopeptides, glabrin A [cyclo-(prolyl-glycyl-lencyl-valyl-isolencyl-tyrosyl)] and B [cyclo-(prolyl-S-oxomethyl-valyl-alanyl-valyl-tyrosyl-glycyl-thryl)], have been isolated. Their structures were elucidated by chemical and spectral methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our previous paper [1] we have reported a new cyclopeptide annosquamosin A from *Annona squamosa*. As a part of a series of investigations on Annonaceae cyclopeptides, in this paper we report two new cyclopeptides named glabrin A and B obtained from the seeds of *Annona glabra*. The fruits of *A. glabra* Linn. are eaten in Yunnan province. Its whole herb can be used as an anti-cancer drug and its leaves can be used to treat chronic bronchitis [2]. In a preliminary communication [3] we reported a new cyclopeptide glabrin A from *A. glabra* which was at this time the first cyclopeptide found in the family of Annonaceae. In this paper we describe the isolation and structure determination of glabrin A and another new cyclopeptide glabrin B, based on chemical and spectral methods.

RESULTS AND DISCUSSION

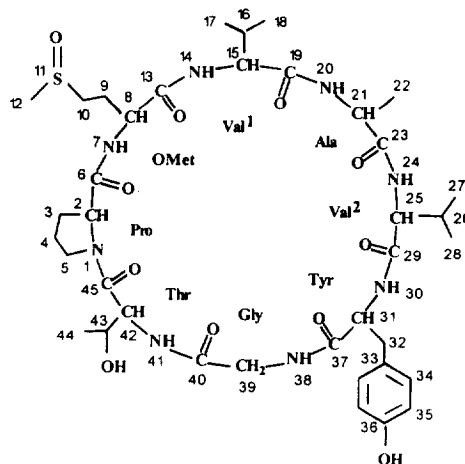
The cyclopeptides, glabrin A (1) and B (2), were isolated from the CHCl_3 fraction of the alcohol extract of *Annona glabra* seeds by column chromatography as described in the Experimental. Glabrin A (1), needles, gave a negative ninhydrin reaction, and showed a high resolution positive FAB-MS spectral quasimolecular ion peak at m/z 643.3791 $[\text{M} + 1]^+$, ∇ 2.9 mDa, corresponding to molecular formula $\text{C}_{33}\text{H}_{51}\text{N}_6\text{O}_7$. IR maxima absorptions at 3280, 1635 (*br*) cm^{-1} indicated that the compound might be a peptide [4]. Amino acid analysis of the peptide after hydrolysis with 6M HCl



glabrin A (1)

at 110° gave the composition: Gly (1eq), Val (1eq), Ile (1eq), Leu (1eq), Tyr (1eq), Pro (1eq). The 400 MHz ^1H NMR and ^{13}C NMR spectra clearly showed five amide NH at δ 10.23, 9.91, 9.17, 9.04, 8.40, and six amide CO at δ 174.6, 173.2, 172.6, 172.2, 171.3, 170.3. Using ^1H - ^1H COSY, ^{13}C - ^1H COSY, and COLOC spectra, six protein amino acids were found to be identical with those of amino acid analysis. The spectral data are shown in Table 1. The sequence of individual amino acids was assembled by COLOC experiments ($J = 6$ Hz and 12.1 Hz) as summarized in Fig.

* Author to whom correspondence should be addressed.



glabrin B (2)

1 [5], i.e. the sequence was -N-Pro-Gly-Leu-Val-Ile-Tyr-CO-.

To further corroborate the peptide to be a cyclopeptide, pos. FAB-MS was pursued. The compound gave $[M+1]^+$ at m/z 643 which proved the M_r was in agreement with that of the sequence above after cyclizing and several useful fragment ions at m/z 480 $[-N\text{-Pro-Gly-Leu-Val-Ile-CO}]^+$, 367 $[-N\text{-Pro-Gly-Leu-Val-CO}]^+$, 268 $[-N\text{-Pro-Gly-Leu-CO}]^+$, 431 $[-NH\text{-Ile-Tyr-Pro-Gly-CO}]^+$ or $[-NH\text{-Tyr-Pro-Gly-Leu-CO}]^+$, 403 $[431\text{-CO}]^+$, 339 $[367\text{-CO}]^+$, 303 $[-CO\text{-NH-Ile-Tyr-CO}]^+$, 227 $[303\text{-CO}]^+$, 240 $[-CO\text{-NH-Val-Ile-CO}]^+$ or $[-CO\text{-NH-Ile-Val-CO}]^+$, and 213 $[240\text{-CO}]^+$ which showed that the sequence was identical with

the sequence above. Therefore, the structure of the cyclopeptide named glabrin A (1), a hexacyclopeptide, was determined as cyclo-(prolyl-glycyl-leucyl-valyl-isoleucyl-tyrosyl).

Glabrin B (2), needles, gave a negative ninhydrin reaction, and showed a high resolution positive FAB-MS spectral quasimolecular ion peak at m/z 835.4054 $[M+1]^+$, $\nabla -3.0$ mDa, corresponding to molecular formula $C_{38}H_{59}N_8O_{11}S_1$. Its IR maxima absorptions were similar to 1. Amino acid analysis of 2 revealed it to consist of Thr (1eq), Gly (1eq), Ala (1eq), Val (2eq), Tyr (1eq), Pro (1eq), and a non-protein amino acid. The 400 MHz 1H NMR and ^{13}C NMR spectra clearly showed seven amide NH at δ 9.59, 9.14, 8.69, 8.63, 8.03, 7.74, 7.65 and eight amide CO at δ 176.6, 173.7, 173.0, 172.6, 172.0, 172.0, 172.0, 170.6. Using 1H - 1H COSY, ^{13}C - 1H COSY, and COLOC spectra, seven protein amino acids were identical with those of amino acid analysis, and the remaining NMR signals consisted of one independent spin system of the type -NH-CH(CO)-CH₂-CH₂-SO-CH₃, which is a non-protein amino acid, named S-oxomethionine (OMet). The spectral data are shown in Table 1. In a similar manner to 1, the sequence of amino acids was summarized by COLOC experiments ($J = 6$ Hz and 10 Hz) in Fig. 1 [5], and was shown to be -N-Pro-OMet-Val¹-Ala-Val²-Tyr-Gly-Thr-CO-.

The M_r was in accordance with that of the sequence above after ring formation. Finally the structure of the cyclopeptide named glabrin B (2), an octacyclopeptide, was determined as cyclo-(prolyl-S-oxomethyl-valyl-alanyl-valyl-tyrosyl-glycyl-thryl).

EXPERIMENTAL

General. Mp: uncorr. Optical rotation was recorded at room temp. using a 1 dm cell. FAB-MS was measured at 6 kV for an Ar beam source. NMR was taken

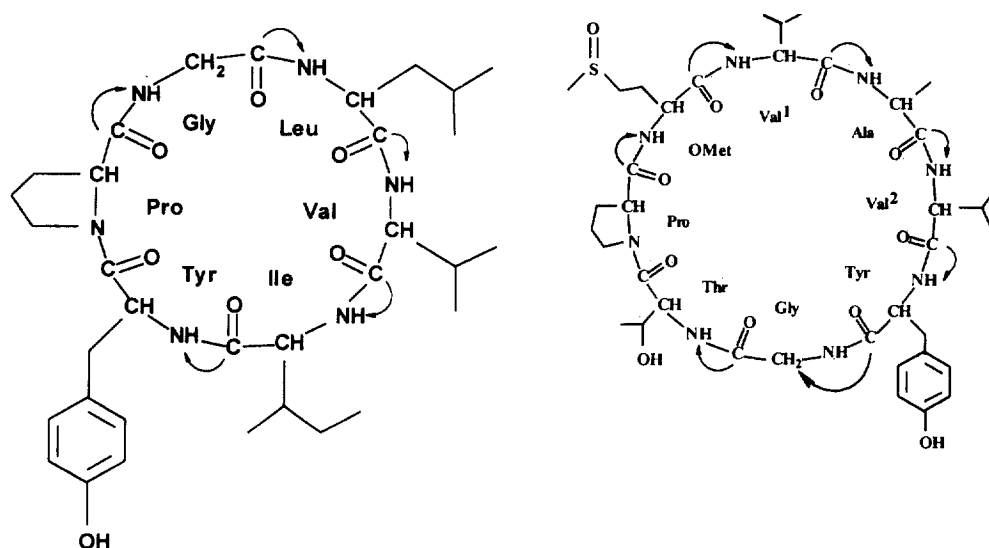


Fig. 1.

Table 1. ^1H and ^{13}C NMR spectral data of glabrin A **1** and B **2** (in pyridine- d_5 , 400 MHz for δ_{H} , 100 MHz for δ_{C} , TMS)

glabrin A 1			glabrin B 2		
H		C	H		C
1			1		
2	3.95 (<i>m</i>)	61.6	2	5.26 (<i>t</i>)	64.0
3	2.44 (<i>m</i>), 1.40 (<i>m</i>)	30.9	3	1.82 (<i>m</i>), 2.42 (<i>m</i>)	30.0
4	1.47 (<i>m</i>)	22.4	4	1.70 (<i>m</i>), 2.12 (<i>m</i>)	25.4
5	3.64 (<i>m</i>)	47.1	5	3.95 (<i>m</i>)	48.1
6		172.6	6		176.6
7	9.91 (<i>dd</i> , 2.4, 9.6)		7	9.59 (<i>d</i> , 4.2)	
8	3.98 (<i>m</i>), 5.08 (<i>dd</i> , 9.6, 16.2)	42.9	8	4.59 (<i>m</i>)	55.7
9		170.3	9	2.42 (<i>m</i>)	24.3
10	9.17 (<i>d</i> , 8.8)		10	2.75 (<i>m</i>), 2.95 (<i>m</i>)	49.1
11	4.71 (<i>m</i>)	54.6	11		
12	1.81 (<i>m</i>)	41.3	12	2.33 (<i>s</i>)	36.8
13	1.81 (<i>m</i>)	25.2	13		172.0
14	0.71 (<i>m</i>)	21.8	14	9.14 (<i>d</i> , 3.0)	
15	0.71 (<i>m</i>)	22.8	15	4.14 (<i>m</i>)	63.1
16		174.6	16	2.42 (<i>m</i>)	30.7
17	9.04 (<i>d</i> , 5.6)		17	1.09 (<i>d</i> , 6.6)	19.5
18	4.47 (<i>t</i> , 9.9)	64.9	18	1.12 (<i>d</i> , 7.0)	19.7
19	2.65 (<i>m</i>)	29.9	19		172.0
20	1.12 (<i>d</i> , 5.6)	19.9	20	7.74 (<i>d</i> , 6.9)	
21	1.12 (<i>d</i> , 5.6)	20.0	21	4.80 (<i>m</i>)	56.6
22		172.2	22	1.40 (<i>d</i> , 6.1)	18.3
23	8.40 (<i>d</i> , 9.6)		23		173.7
24	4.98 (<i>t</i> , 9.9)	56.8	24	7.65 (<i>d</i> , 9.4)	
25	2.11 (<i>m</i>)	37.7	25	4.80 (<i>m</i>)	51.9
26	1.40 (<i>m</i>), 1.59 (<i>m</i>)	25.3	26	2.42 (<i>m</i>)	29.8
27	0.71 (<i>m</i>)	10.5	27	0.91 (<i>d</i> , 6.8)	18.8
28	1.26 (<i>d</i> , 6.8)	15.7	28	1.04 (<i>d</i> , 6.4)	22.4
29		173.2	29		172.0
30	10.23 (<i>d</i> , 2.4)		30	8.69 (<i>d</i> , 10.2)	
31	5.19 (<i>m</i>)	55.6	31	5.62 (<i>m</i>)	53.5
32	3.06 (<i>dd</i> , 4.6, 12.6), 3.27 (<i>t</i> , 12.1)	37.7	32	3.23 (<i>dd</i>), 4.23 (<i>d</i> , 13.1)	37.1
33		126.7	33		129.5
34	7.24 (<i>d</i> , 8.4)	131.3	34	7.38 (<i>d</i> , 8.4)	130.1
35	7.07 (<i>d</i> , 8.4)	116.7	35	7.16 (<i>d</i> , 8.4)	116.2
36		158.3	36		157.4
37		171.3	37		173.0
			38	8.63 (<i>t</i> , 6.2)	
			39	3.95 (<i>m</i>), 4.69 (<i>dd</i> , 6.6, 17.0)	44.6
			40		170.6
			41	8.03 (<i>d</i> , 9.8)	
			42	5.62 (<i>m</i>)	56.9
			43	4.98 (<i>m</i>)	70.7
			44	1.56 (<i>d</i> , 7.3)	19.7
			45		172.6

at 400 MHz in pyridine- d_5 soln using TMS as int. standard.

Extraction and isolation of cyclopeptide. Crushed dry seeds of *A. glabra* (3 kg, collected in Xishu-angbanna in Yunnan province in China) were macerated at room temp. with 95% EtOH, after being degreased with petrol, and the extracts concd *in vacuo*. The EtOH extract was partitioned with CHCl_3 . Removal of solvent furnished CHCl_3 fr. (200 g). The CHCl_3 fr. was repeatedly chromatographed on a silica gel

column and eluted with petrol–EtOAc–MeOH, affording glabrin A (690 mg) and glabrin B (179 mg).

Glabrin A (1). Yield $2.3 \times 10^{-2}\%$, needles (CHCl_3 –MeOH), mp 300 – 303° , $[\alpha]_{\text{D}}^{28} -195.86^\circ$ (MeOH; c 0.845). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 204 (4.27), 225 (4.06), 279 (3.19). IR ν_{max} cm^{-1} : 3280, 1635. ^1H and ^{13}C NMR see Table 1. Pos. FAB-MS m/z : 643 $[\text{M}+1]^+$, 480 $[-\text{N-Pro-Gly-Len-Val-Ile-CO-}]^+$, 367 $[-\text{N-Pro-Gly-Len-Val-CO-}]^+$, 268 $[-\text{N-Pro-Gly-Leu-CO-}]^+$, 431 $[-\text{NH-Ile-Tyr-Pro-Gly-CO-}$ or $-\text{NH-Tyr-Pro-Gly-Leu-}$

CO-]⁺, 403[431-CO]⁺, 339[367-CO]⁺, 303[-CO-NH-Ile-Tyr-CO-]⁺, 277[303-CO]⁺, 240[-CO-NH-Val-Ile-CO- or -CO-NH-Leu-Val-CO-]⁺, and 213[240-CO]⁺. Amino acid analysis (standard method): Gly (1eq), Val (1eq), Leu (1eq), Ile (1eq), Tyr (1eq), Pro (1eq).

Glabin B (2). Yield $6.0 \times 10^{-3}\%$, needles (MeOH), mp 205, $[\alpha]_D^{29} -76.67^\circ$ (MeOH; c 0.375). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 202 (2.94), 225 (3.01), 279 (2.11). IR $\nu_{\max}$ cm⁻¹: 3300, 1660. ¹H and ¹³C NMR see Table 1. Pos. FAB-MS m/z : 835[M+1]⁺. Amino acid analysis (standard method): Thr (1eq), Gly (1eq), Ala (1eq), Val (2eq), Tyr (1eq), Pro (1eq), and a non-protein amino acid.

Acknowledgements—We thank Mr He Yi-neng (Kunming Institute of Botany, China) for NMR. This work was supported by Grants No. 95C083m from The Fund of Applied and Basic Science Research of Yunnan Province.

REFERENCES

1. Li Chao-ming, Tan Ning-hua, Mu Qing, Zheng Hui-lan, Hao Xiao-jiang, Wu Yu and Zhou Jun (1997) *Phytochemistry* in press.
2. Yunnan company of medicinal materials, *Yunnan Zhongyao Ziyuan Minglu*. Academic Press, Beijing, 1993, p. 153.
3. Li Chao-ming, Tan Ning-hua, Zheng Hui-lan, Hao Xiao-jiang and Zhou Jun, *Chinese Chemical Letters*, 1995, **6**, 39.
4. Tan Ning-hua, Zhou Jun, Chen Chang-xiang and Zhao Shou-xun, *Phytochemistry*, 1993, **32**, 1327.
5. Tan Ning-hua, Wang De-zu, Zhang Hong-Jie, Chen Chang-xiang, Zhou Jun and Zhao Shou-xun, *Chinese Journal of Magnetic Resonance*, 1993, **10**, 69.