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CYCLOPEPTIDE FROM THE SEEDS OF *ANNONA MURICATA*

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Key Word Index—*Annona muricata*; Annonaceae; seeds; cyclopeptide; annomuricatin B.

Abstract—From the seeds of *Annona muricata* one new cyclopeptide, annomuricatin B [cyclo-(prolyl-asparaginyl-alanyl-tryptophyl-leucyl-glycyl-thryl)], has been isolated. The structure was elucidated by chemical and spectral methods. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our previous paper [1] we have reported one new cyclopeptide annomuricatin A from *Annona muricata* (Annonaceae). In this paper we report another new cyclopeptide named annomuricatin B obtained from the same plant seeds. The fruit of *Annona muricata* Linn. is edible in Yunnan province (China). As a part of continuing studies on Annonaceae cyclopeptides [1–3], in this paper we describe the isolation and structure determination of one new cyclopeptide annomuricatin B from the plant based on chemical and spectral methods.

RESULTS AND DISCUSSION

The cyclopeptide, annomuricatin B (**1**), was isolated from the $CHCl_3$ fraction of the alcohol extract of *Annona muricata* seeds by column chromatography as described in the Experimental. Annomuricatin B (**1**), needles, gave a negative ninhydrin reaction, and showed a high resolution positive FAB-MS spectral quasimolecular iron peak at *m/z* 740.3795 [$(M+1)^+$, $\nabla -6.3$ mDa], corresponding to molecular formula $C_{35}H_{49}N_9O_9$. IR_{max} absorptions at 3300, 1650 (*br*) cm^{-1} indicated that the compound might be a peptide [4]. The 400 MHz ^1H NMR and ^{13}C NMR spectra clearly showed eight amide NH at δ 10.42, 9.44, 9.29, 9.17, 8.75, 8.75, 8.57, 8.36, and one NH at δ 11.79, and eight amide CO at δ 174.8, 173.0, 172.8, 172.7, 172.6, 172.1, 170.2, 169.7. Using ^1H-^1H COSY, ^{13}C-^1H COSY, and COLOC spectra, the composition of amino acid residues was determined as Pro (1eq), Asn (1eq), Ala (1eq), Trp (1eq), Leu (1eq), Gly (1eq) and Thr (1eq). The spectral data are shown in Table 1. The sequence of individual amino acids was assembled by COLOC experiments ($J = 6$ Hz and 10 Hz) as summarized in Fig. 1 [5]. The sequence was —N—Pro—Asn—Ala—Trp—Leu—Gly—Thr—CO—.

To further corroborate the peptide to be a cyclopeptide, pos. FAB-MS was pursued. The compound gave $(M+1)^+$ at *m/z* 740 which proved the M_r was in agreement with that of the sequence above after cyclization. Several useful fragment ions at *m/z* 683 [—N—Thr—Pro—Asn—Ala—Trp—Leu—CO—]$^+$, 610 [—CON—Pro—Asn—Ala—Trp—Leu—CO—]$^+$, and 470 [—Asn—Ala—Trp—Leu—CO—]$^+$ were obtained. Therefore, the structure of the cyclopeptide named annomuricatin B (**1**), a heptacyclopeptide, was determined as cyclo-(prolyl-asparaginyl-alanyl-tryptophyl-leucyl-glycyl-thryl).

EXPERIMENTAL

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

Extraction and isolation of cyclopeptide

Crushed dry seeds of *A. muricata* (6 kg, collected in Xishuangbanna, Yunnan province in China) were macerated at room temp. with 95% EtOH and the extracts concd *in vacuo*. The EtOH extract was partitioned with $CHCl_3$ to yield the $CHCl_3$ fr. which was then partitioned between petrol and 90% aq. MeOH to yield the 90% aq. MeOH soluble fr. (382 g). The

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Table 1. ^1H and ^{13}C NMR spectra data of annomuricatin B (**1**) (in pyridine-*d*, 400 MHz for δ_H, 100 MHz for δ_C, TMS)

	Annomuricatin B (**1**)	
	H	C
1		
2	4.42 (*t*, 8.0)	62.0
3	2.04 (*m*), 1.94 (*m*)	29.7
4	1.80 (*m*), 1.35 (*m*)	25.0
5	3.89 (*m*), 3.68 (*m*)	48.8
6		172.6
7	9.17 (*d*, 5.2)	
8	5.04 (*m*)	51.3
9	3.89 (*m*), 3.68 (*m*)	36.2
10		174.8
11	9.44 (*s*), 8.57 (*s*)	
12		172.1
13	9.29 (*d*, 3.2)	
14	4.50 (*m*)	53.1
15	1.12 (*d*, 7.2)	16.8
16		172.7
17	8.75 (*d*, 8.4)	
18	5.75 (*m*)	55.7
19	3.89 (*m*)	29.5
20		123.0*
21	7.25 (*t*, 7.4)	121.6
22	11.79 (*s*)	
23		137.5
24	7.18 (*t*, 7.4)§	119.2
25	7.54 (*dd*, 2.6, 17.0)	112.2†
26	7.54 (*dd*, 2.6, 17.0)	112.0†
27	7.83 (*d*, 7.6)§	119.2
28		129.2*
29		173.0
30	8.36 (*d*, 9.6)	
31	5.44 (*m*)	57.9
32	1.94 (*m*), 1.80 (*m*)	44.7
33	1.94 (*m*)	25.1
34	0.90 (*m*)	22.6
35		172.8‡
36	10.42 (*dd*, 4.4, 8.0)	
37	4.82 (*dd*, 8.2, 17.0), 3.89 (*m*)	44.0
38		169.7
39	8.75 (*d*, 8.4)	
40	5.44 (*m*)	54.5
41	4.50 (*m*)	69.1
42	1.65 (*d*, 5.2)	20.3
43		170.2‡

*†‡§indicate that data with same symbol are interchangeable.

90% aq. MeOH fr. was repeatedly chromatographed on a silica gel column and eluted with EtOAc–MeOH or $CHCl_3$–MeOH, affording annomuricatin B (544 mg).

Annomuricatin B (**1**). Yield: 9.0×10^{-3}%, needles ($CHCl_3$–MeOH), mp 213°, $[\alpha]_D^{19}$ −37.25° (MeOH; *c* 0.51). UV λ_{max}^{MeOH} nm: 204 (3.49), 221.5 (3.53), 282 (2.65), 290 (2.61). IR ν_{max} cm^{-1}: 3300, 1650. ^1H and ^{13}C NMR see Table 1. Pos. FAV-MS *m/z*: 740[M + 1]$^+$, 683 [—N—Thr—Pro—Asn—Ala—Trp —Leu—CO—]$^+$, 610 [—CON—Pro—Asn—Ala —Trp—Leu—CO—]$^+$, and 470 [—Asn—Ala —Trp—Leu—CO—]$^+$.

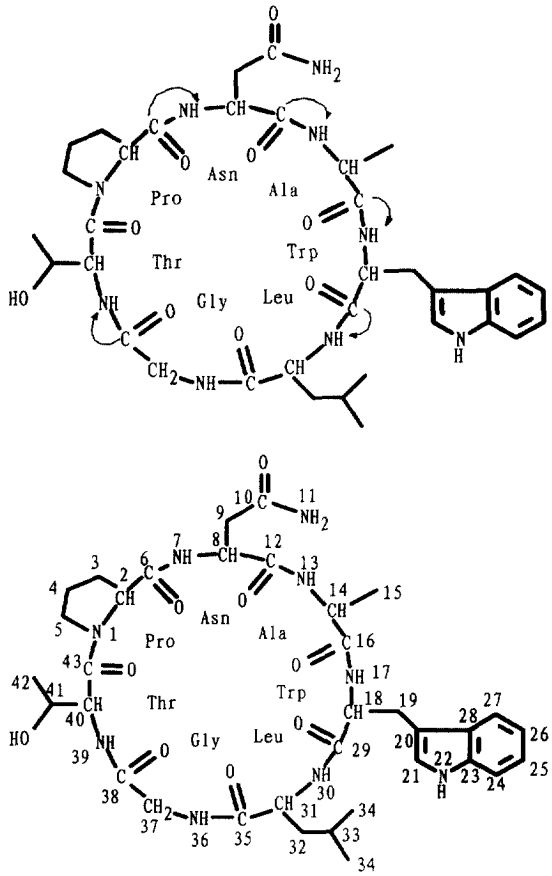

Fig. 1. The sequence is shown by arrows for annomuricatin B by COLOC spectra.

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