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CYCLOPEPTIDE FROM THE SEEDS OF *ANNONA SQUAMOSA*

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Key Word Index—*Annona squamosa*; Annonaceae; seeds; cyclopeptide; annosquamosin A.

Abstract—From the seeds of *Annona squamosa* a new cyclopeptide, annosquamosin A (cyclo-(prolyl-*S*-oxomethyl-thryl-alaryl-isoleucyl-valyl-glycyl-tyryl), has been isolated. The structure was elucidated by chemical and spectral methods. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Annonaceous acetogenins from the Annonaceae are now of great interest for their anti-tumour properties. The activities of some of these compounds are stronger than taxol by as much as 40–300 times [1, 2]. In order to further investigate Annonaceae plants in the Yunnan province (China) we collected more than 20 species in the Xishuangbanna tropical region and undertook a series of chemical and pharmacological studies. We isolated 22 acetogenins of which eight were new [3, 4], we also found cyclopeptides in some species [5, 6]. This paper reports a new cyclopeptide named annosquamosin A obtained from the seeds of *Annona squamosa*. The fruit of this plant is popular in the Xishuangbanna region and its immatured fruits and seeds can kill parasites. It is said that its roots are used to treat acute dysentery, depression, spinal marrow diseases, and its leaves for prolapse of the anus, sores and swelling [7]. We now describe the isolation and structure determination of one novel cyclopeptide annosquamosin A, based on chemical and spectral methods.

RESULTS AND DISCUSSION

Annosquamosin A (**1**) was isolated from the $CHCl_3$ fraction of the alcohol extract of *Annona squamosa* seeds by column chromatography as described in the Experimental. Annosquamosin A (**1**), gave a negative ninhydrin reaction, and showed a high resolution positive FAB–mass spectrometry spectral quasimolecular ion peak at *m/z* 849.4196 [$(M+1)^+$, ∇ −1.5 mDa), corresponding to a molecular formula of $C_{39}H_{61}O_{11}N_8S_1$. IR maxima absorptions at 3300, 1650 (br) cm^{-1} indicated that the compound might be a peptide [8]. Amino acid analysis of the peptide after hydrolysis with 6 M HCl at 110° gave the composition: Thr (1 eq), Gly (1 eq), Ala (1 eq), Val (1 eq), Ile (1 eq), Tyr (1 eq), Pro (1 eq), and a non-protein amino acid. The 400 MHz ^1H and ^{13}C NMR spectra clearly showed a seven amide NH at δ 9.72, 9.14, 8.76, 8.72, 8.07, 7.77, 7.73 and an eight amide CO at δ 176.9, 174.1, 173.2, 172.5, 172.5, 172.3, 172.3, 170.9. 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)-CH_2-CH_2-SO-CH_3, which is a non-protein amino acid, named *S*-oxomethionine (OMet). The spectral data are shown in Table 1. The sequence of individual amino acids was assembled by COLOC experiments as summarized in Fig. 1 [9]. In two COLOC experments we chose J = 6 and 10 Hz, respectively, and the results indicated that the compound contains the following peptide residues: -N-Pro-OMet-Thr-Ala-Ile-Val-CO-; and -NH-Gly-Tyr-CO-. The M_r of the cyclopeptide, associated with the peptide residues was in agreement with that of the FAB-mass spectrometry. Therefore, the structure of the cyclopeptide named annosquamosin A, an octacyclopeptide, was determined as cyclo-(prolyl-*S*-oxomethyl-thryl-alanyl-isoleucyl-valyl-glycyl-tyryl).

EXPERIMENTAL

Mp: uncorr. Optical rotation was recorded at 24.3° 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

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

	H	C
1		
2	5.27 (*t*, 8.4)	64.1
3	2.35 (*m*), 1.95 (*m*)	30.3
4	2.20 (*m*), 1.88 (*m*)	25.5
5	4.01 (*m*)	48.3
6		176.9
7	9.72 (*d*, 3.5)	
8	4.60 (*m*)	56.0
9	2.56 (*m*), 2.35 (*m*)	24.8
10	2.95 (*m*), 2.82 (*m*)	49.4
11		
12	2.56 (*s*)	37.7
13		172.3
14	8.72 (*d*, 9.9)	
15	5.65 (*m*)	53.5
16	5.03 (*m*)	70.8
17	1.43 (*d*, 6.0)	19.9
18		172.5
19	7.73 (*d*, 9.3)	
20	4.86 (*t*, 9.8)	56.1
21	1.61 (*d*, 7.4)	18.3
22		174.1
23	7.77 (*d*, 11.0)	
24	4.80 (*t*, 7.2)	52.0
25	2.35 (*m*)	36.8
26	1.58 (*m*), 1.28 (*m*)	24.7
27	0.64 (*t*, 15.2)	11.4
28	0.99 (*d*, 10.2)	17.5
29		172.3
30	9.14 (*d*, 3.4)	
31	4.14 (*dd*, 3.5, 6.4)	63.2
32	2.35 (*m*)	29.8
33	1.15 (*d*, 6.7)	19.7
34	1.12 (*d*, 6.8)	19.6
35		172.5
36	8.76 (*t*, 6.2)	
37	4.67 (*dd*, 6.5, 16.9), 4.01 (*m*)	44.7
38		170.9
39	8.07 (*d*, 8.1)	
40	5.65 (*m*)	57.0
41	4.25 (*d*, 14.8), 3.30 (*dd*, 12.6, 15.4)	37.2
42		129.4
43	7.18 (*d*, 8.4)	116.3
44	7.38 (*d*, 8.4)	129.9
45		157.4
46		173.2

seeds of *A. squamosa* (2.6 kg, collected in Xishuangbanna in Yunnan province in China) were macerated at room temp with 95% EtOH after being defatted with petrol, and the extracts concd *in vacuo*. The EtOH extract was partitioned with $CHCl_3$. Removal of solvent furnished a $CHCl_3$ fr. (82.5 g). The $CHCl_3$ fr. was repeatedly chromatographed on a silica gel column and eluted with petrol–EtOAc–MeOH, affording annosquamosin A (195 mg).

Annosquamosin A (**1**), Yield 7.5×10^{-3}%, needles (MeOH), mp 215–216°, $[\alpha]_D^{24.3}$ −65.27° (MeOH; *c* 0.429). IR ν_{max} cm^{-1}: 3300, 1650. ^1H and ^{13}C NMR see Table 1. Pos. FAB-MS *m/z*: 849$[M+1]^+$, 785$[M-SOCH_3]^+$, 736$[M+1-Ile]^+$, 637$[M+1-Ile-Val]^+$, 465$[M+1-Ile-Val-Ala-Thr]^+$, 221$[M+1-Ile-Val-Ala-Thr-OMet-Pro]^+$, 136$[M+1-Ile-Val-Ala-Thr-OMet-Pro-Gly-CO]^+$. Amino acid analysis (standard method): Thr (1 eq), Gly (1 eq), Ala (1 eq), Val (1 eq), Ile (1 eq), Tyr (1 eq), Pro (1 eq), and a non-protein amino acid.

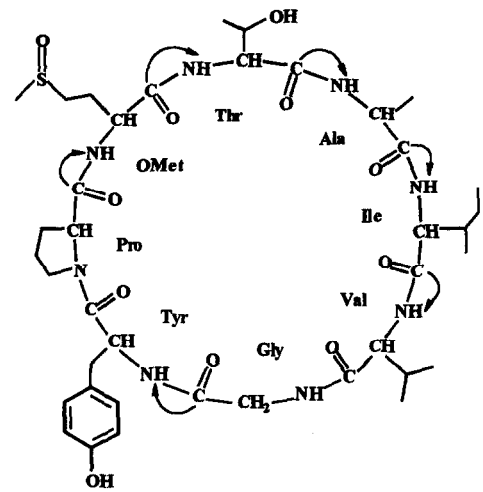
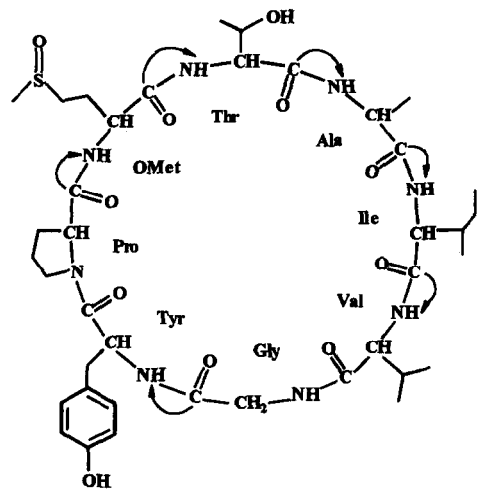


Fig. 1. The sequence is shown by arrows for annosquamosin A by COLOC spectra.



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