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Squamin-A, Novel Cyclopeptide from *Annona squamosa*

Jian Xia SHI[1], Hou Ming WU[2]*, Fa Hu HE[2], Kenichiro INOUE[3], Zhi Da MIN[1]

[1] Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009
[2] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese academy of Sciences, Shanghai 200032
[3] Department of Pharmacognosy, Gifu Pharmaceutical University, Gifu 502, Japan

Abstract: A novel cyclopeptide, squamin-A has been isolated from the seeds of *Annona squamosa* Linn.. The structure was elucidated by extensive 2D-NMR, FABMS and amino acid analysis in combination with Marfey's reagents as cyclo-[*l*-prolyl-*l*-(S-oxo)-methionyl-*l*-tyrosyl-*l*-glycyl-*l*-threonyl-*l*-valyl-*l*-alanyl-*l*-isoleucyl].

Keywords: Squamin-A, cyclopeptide, *Annona squamosa*.

Recently a number of cyclopeptides with unique structures were reported from higher plants[1,2], especially from *Annona squamosa* Linn.[3,4]. During our study on the chemical constituents of the seeds of *Annona squamosa* Linn., a novel cyclopeptide named squamin-A was isolated. In this letter we report the structure of squamin-A.

The well powdered dried seeds (2.5kg) of *Annona squamosa* were degreased by petroleum ether and percolated by 95% EtOH. The EtOH extracts were partitioned between $CHCl_3$ and water and the chloroform soluble portion was separated by repeated silica gel column chromatography to obtain squamin-A.

Squamin-A, white crystal, m.p. >300℃, $[\alpha]_D^{18}$ -48.4 (MeOH), is negative to ninhydrin reaction. However, upon treating with 12mol L^{-1} HCl, it shows positive reaction to ninhydrin indicating a cyclopeptide. Amino acid analysis of its hydrolysates revealed that it contains Pro, Tyr, Gly, Thr, Val, Ala, Ile, and a modified Met (1 mol each) residue corresponding to $C_{39}H_{60}O_{10}N_8S$. Its molecular formula was assigned as $C_{39}H_{60}O_{11}N_8S$ by high resolution FABMS [$(M+1)^+$ at 849.4164, calc. 849.4180] suggesting the presence of an extra oxygen atom.

The complete assignments of proton spin system for the particular residue was achieved using TOCSY and DQF-COSY spectra. The carbon resonance assignments were determined by HMQC and HMBC experiments and were summarized in **Table 1**. The chemical shifts (δ 23.68, 48.86 and 36.97) of C_β, C_γ and C_ε of the modified Met residue are quite similar to those (δ 24.4, 49.0 and 37.0) of authentic S-oxo-methionine other than those (δ 30.5, 29.5 and 15.2) of authentic methionine sample. Thus, it was assigned as S-oxo-methionine. This assignment is coincident with the FAB-MS data.

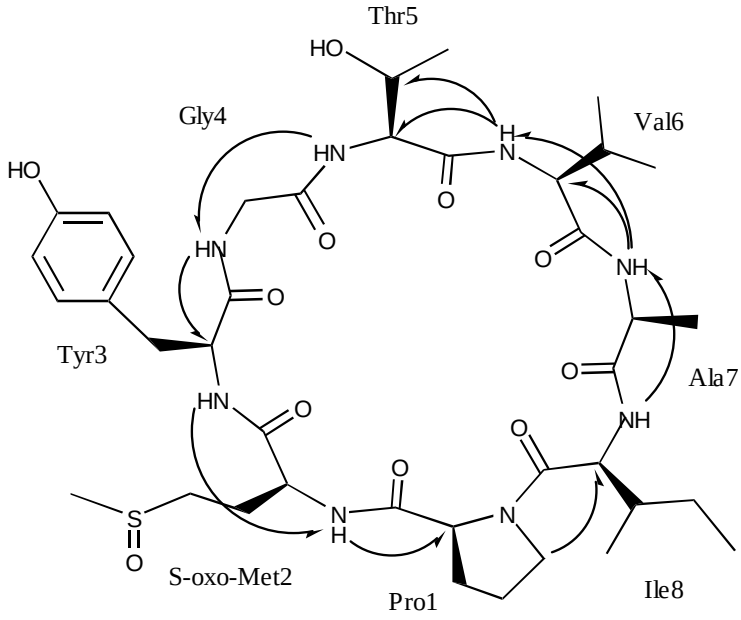
Table 1, The chemical shifts of ^{1}H and ^{13}C NMR of Squamin-A in pyridine-d_5[a]

Position	δ_C	δ_H	$^{3}J_{NH-\alpha H}$	HMBC
Pro-1, CO	176.7			S-oxo-Met-2, NH
-α	63.84	5.23		
-β	29.96	2.31, 1.91		
-γ	25.23	2.11, 1.81		
-δ	47.91	3.98, 3.97		
S-oxo-Met-2, CO	172.1			Try-3, NH
-NH		9.55	2.7	
-α	55.58	4.59	2.7	
-β	23.68	2.51, 2.43		
-γ	48.86	2.90, 2.77		
ε	36.97	2.36		
Tyr-3, CO	172.9			Gly-4, NH
-NH		8.63	9.3	
-α	52.89	5.58	9.3, 2.7	
-β	36.65	4.16, 3.22		
-γ	129.00			
-δ	129.63	7.32		
-χ	115.94	7.12		
-ε	157.11			
Gly-4, CO	170.5			Thr-5, NH
-NH		8.65	5.8, 6.7	
-α	44.35	4.61, 3.90		
Thr-5, CO	172.1			Val-6, NH
-NH		7.95	9.8	
-α	56.61	5.59	9.8, 2.2	
-β	70.31	5.01		
-γ	19.51	1.38		
Val-6, CO	172.4			Ala-7, NH
-NH		9.15	3.5	
-α	62.99	4.08	3.5, 6.7	
-β	29.47	2.36		
-γ	19.51	1.11, 1.07		
Ala-7, CO	174.0			Ile-8, NH
-NH		7.81	6.7	
-α	51.81	4.74	6.7, 7.6	
-β	17.96	1.58		
Ile-8, CO	172.1			
-NH		7.67	9.4	
-α	55.85	4.81	9.4	
-β	36.44	2.26		
-γ	24.27	1.57, 1.20		
-δ	17.96	0.96		
-ε	11.13	0.64		

[a] The chemical shifts of ^{1}H and ^{13}C are referenced to C_5D_5N at 7.19 and 123.5, respectively.

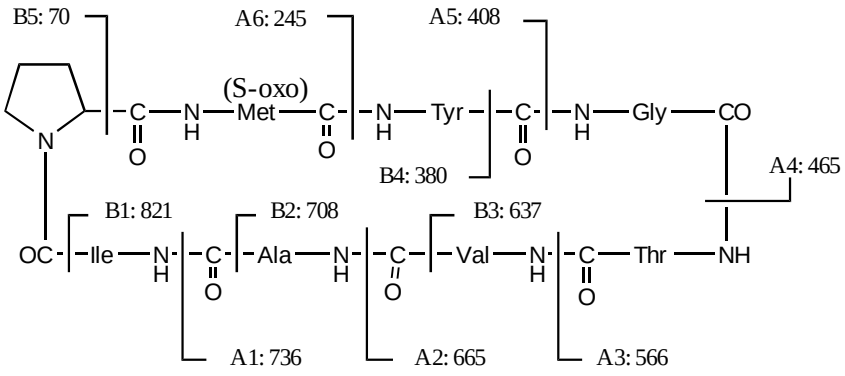
The sequence from Pro-1 to Ile-8 was established by the $^{2}J_{CO-NH}$ HMBC correlations between neighboring residues (**Table 1**) and was confirmed by the NOE connectivities $d_{NH-\alpha H}$ and d_{NH-NH} observed in the ROESY experiment (shown in **Figure 1**). The connectivity $d_{\alpha H-\delta H}$ instead of $d_{NH-\alpha H}$ afforded the evidence for the linkage between Ile-8 and Pro-1.

Figure 1. The sequence of squamin-A. The arrows denoted inter-residue NOEs

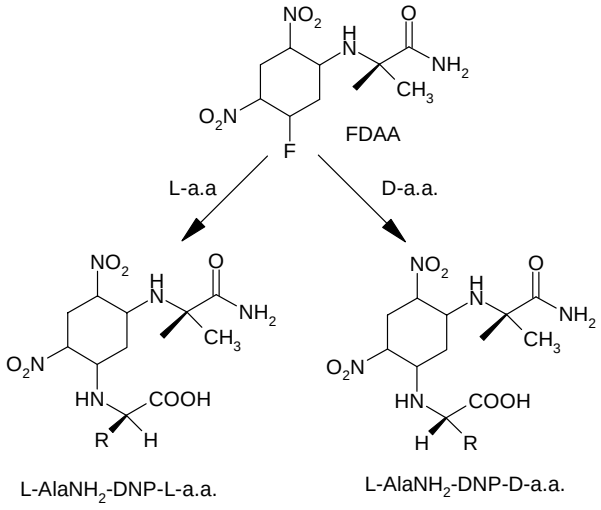


To further corroborate the sequence of the compound, a detailed analysis on FAB-MS was performed. A complete set of fragment ion peaks (**Figure 2**) were assigned for the sequence, which is agreeable with the sequence determined by HMBC and ROESY data.

Figure 2. The MS fragmentation of squamin-A



The absolute stereo-chemistry of the molecule was determined by Marfey's method. The hydrolysate of squamin A was treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent) as following:



The elution positions of the FDAA derivatives of the compositional residues on HPLC chromatography were compared with those of the FDAA derivatives of relevant *l*- and *d*- authentic amino acid residues. The results demonstrated that all compositional residues have *S* configuration. Therefore, the structure of squamin-A was elucidated as cyclo-[*l*-prolyl-*l*-(s-oxo)-methionyl-*l*-tyrosyl-*l*-glycyl-*l*-threonyl-*l*-valyl-*l*-alanyl-*l*-isoleucyl].

The structure of squamin-A has been confirmed by X-ray analysis of the single crystal, which will be reported elsewhere. It was noted that the amino acid sequence of the molecule is different from that of annosquamosin, a cyclopeptide, which was isolated from the same plant and possessed the same amino acid composition[4].

Acknowledgments

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