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A New Cytotoxic Acetogenin from the Seeds of *Annona squamosa*

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Abstract: A new Annonaceous acetogenin, squamostolide (**1**), was isolated from the seeds of *Annona squamosa*. Its structure was elucidated based on spectroscopic methods and comparison with known compounds. It is the first example of Annonaceous acetogenin with each of the two ends of the aliphatic chain bearing a γ-lactone. The new compound exhibited cytotoxic activity in vitro against bel-7402 and CNE_2 human tumor cell lines.

Keywords: *Annona squamosa*, *Annona*, acetogenins, squamostolide, cytotoxicity.

Annona squamosa L. (Annonaceae) is known for its edible tropical fruits called Fan-Li-Zhi (foreign litchi) in China. As a part of our program for antitumor Annonaceous acetogenins, we investigated the seeds of this plant cultivated in Guangdong Province. A new Annonaceous acetogenin which we have named squamostolide (**1**) was isolated. Its structure was established based on spectroscopic methods and comparison with known compounds. In this paper, we report the structure elucidation and cytotoxic activity of this new compound.

Squamostolide (**1**) was isolated as colorless needles (MeOH), mp 94-96°C (uncorrected), $[\alpha]_D^{24}$ −3.3 (*c* 0.122, acetone). Its formula, $C_{22}H_{36}O_5$, was determined by HRFABMS measurements of the molecular ion at *m/z* 381.2647 $[MH]^+$ (calcd. 381.2641) and from EIMS, ^1H and ^{13}C NMR data.

Three signals at δ 6.96 (d, 1H, *J* = 1.2 Hz, H-20), 4.96 (qd, 1H, *J* = 6.8, 1.2 Hz, H-21) and 1.37 (d, 3H, *J* = 6.8 Hz, H-22) in the ^1H NMR spectrum (see **Table 1**) and six peaks at 173.9 (C-1), 134.3 (C-2), 148.9 (C-20), 77.4 (C-21) and 19.2 (C-22) in the ^{13}C NMR spectrum (see **Table 1**) are characteristic of the methylated α, β-unsaturated γ–lactone fragment with the absence of an OH group at the C-4 position, as commonly found among many of the Annonaceous acetogenins[1]. The proton signals at δ 3.54 (m, 1H, H-15), 4.39 (m, 1H, H-16), 2.09 (m, 1H, H-17a), 2.22 (m, 1H, H-17b), 2.52 (m, 1H, H-18a) and 2.57 (m, 1H, H-18b) and the carbon signals at δ 73.6 (C-15), 82.9 (C-16), 24.1 (C-17), 28.7 (C-18) and 177.2 (C-19) indicated the presence of a saturated γ-lactone ring with an OH group nearby the ring, as that in muricatacin (**2a**, **2b**)[2]. EIMS showed ion peaks at *m/z* 381 ($[MH]^+$, 9), 362 ($[M - H_2O]^+$, 70), 344 (13), 334 (8), 295 (98), 277 (12), 251 (18), 223 (6), 112 (65), 111 (51), 95 (78), 85 (100), 68 (44), 67 (65), and the

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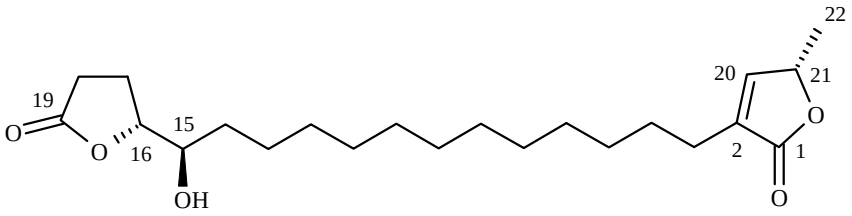
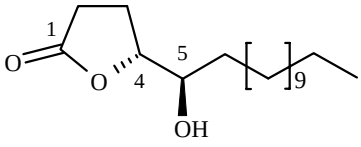
significant fragments were showed in **Figure 1**. On the basis above, compound **1** was determined as shown, disregarding stereochemistry.

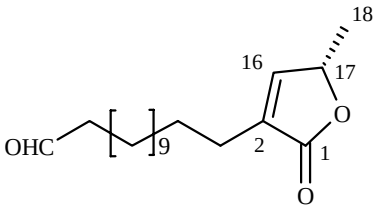


1



2a: 4*R*, 5*R*

2b: 4*S*, 5*S*



3: 17*S*

The stereochemistry at C-21 was assigned to be *S* from biogenetic consideration, as all of natural Annonaceous acetogenins possess the *S* absolute configuration at this chiral center[3]. The identity of ^{1}H and ^{13}C NMR data for the moiety of the saturated γ–lactone ring and its vicinity in **1** with those for that in muricatacin[2] indicated that the relative configuration at C-15/C-16 in **1** was *threo* and the absolute configuration was either *R, R* or *S, S*. To determine the absolute configuration at C-15 and C-16 in **1**, we compared the $[\alpha]_D$ of **1** with those of related known compounds, **2a**, **2b** and **3**. Compound **2a**, with 4*R*/5*R* configuration, displayed an $[\alpha]_D$ at -22.3°, while **2b**, the enantiomer of **2a**, displayed that at +22.6[4], and the $[\alpha]_D$ of **3**, a natural product previously isolated from the seeds of the same plant[5], was reported at +21.1[6]. Based on these facts, the minus $[\alpha]_D$ value of **1** proposed *R* and *R* configuration at C-15 and C-16 in **1**. Therefore, the 15*R*, 16*R*, 21*S* stereochemistries could be suggested for **1**.

The cytotoxicities of **1** against human liver carcinoma (bel-7402) and human nasopharyngeal carcinoma (CNE_2) cell lines were assayed using standard MTT method. The assays showed the IC_{50} of **1** to these two cell lines were 4.79 and 1.24 μg/mL, respectively. The potency was approximately equivalent to that of cisplatin.

Figure 1 EIMS Fragmentation of **1**

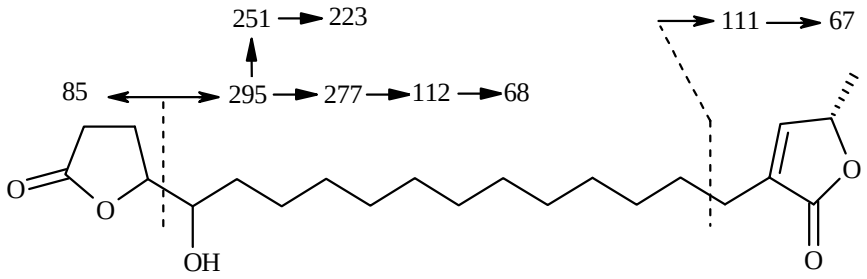


Table 1 ^{1}H and ^{13}C NMR Chemical Shifts and Assignments for **1**

Position	^{1}H (*J* in Hz)	^{13}C	Position	^{1}H (*J* in Hz)	^{13}C
1		173.9	17a	2.09 m	24.1
2		134.3	17b	2.22 m	
3	2.22 t (7.6)	25.1	18a	2.52 m	28.7
4	1.49-1.52 m	27.4	18b	2.57 m	
5-12	1.23-1.27 m	29.1-29.4	19		177.2
13	1.45 m	25.4	20	6.96 d (1.2)	148.9
14	1.49-1.52 m	32.9	21	4.96 dq (6.8, 1.2)	77.4
15	3.54 m	73.6	22	1.37 d (6.8)	19.2
16	4.39 m	82.9			

Note: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded in $CDCl_3$ and referenced to the residual $CHCl_3$ at δ 7.24 and δ 77.0, respectively. The assignments were accomplished by the aid of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra.

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