Novel Mono-Tetrahydrofuran Ring Acetogenins, from the Bark of *Annona squamosa*, Showing Cytotoxic Selectivities for the Human Pancreatic Carcinoma Cell Line, PACA-2

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Received February 14, 1997[⊗]

The bark of *Annona squamosa* yielded three new mono-tetrahydrofuran (THF) ring acetogenins, each bearing two flanking hydroxyls and a carbonyl group at the C-9 position. These compounds were isolated using the brine shrimp lethality assay as a guide for the bioactivity-directed fractionation. (2,4-*cis* and *trans*)-Mosinone A (**1**) is a mixture of ketolactone compounds bearing a *threo*/*trans*/*threo* ring relationship and a double bond two methylene units away from the flanking hydroxyl. The other two new acetogenins differ in their stereochemistries around the THF ring; mosin B (**2**) has a *threo*/*trans*/*erythro* configuration across the ring, and mosin C (**3**) possesses a *threo*/*cis*/*threo* relative stereochemistry. Also found was annoreticuin-9-one (**4**), a known acetogenin that bears a *threo*/*trans*/*threo* ring configuration and a C-9 carbonyl and is new to this species. The structures were elucidated based on spectroscopic and chemical methods. Compounds **1**−**4** all showed selective cytotoxic activity against the human pancreactic tumor cell line, PACA-2, with potency 10−100 times that of Adriamycin.

The annonaceous acetogenins are a well-established class of compounds with significant bioactivities. The recent discoveries of new structural types and acetogenins with selectivities for particular cell lines has kept interest high and encourages continued work on plants in the Annonaceae family. Research on a number of members in this family has suggested that different species and genera tend to synthesize particular types or structurally similar series of acetogenins. *Annona squamosa* Rich. (Annonaceae) produces a large number of compounds containing carbonyls in their structures, although this functionality is fairly rare among the acetogenins as a whole.[1]

A. squamosa, also known as the custard apple, is a new world fruit tree that has been naturalized throughout the tropics.[2] Our recent work on the bark of this species has resulted in the isolation of one new and six known annonaceous acetogenins;[3] Fujimoto's group has isolated about 30 acetogenins from the seeds.[1] Herein, we report the discovery from the bark of three novel (**1**−**3**) and one known (**4**) acetogenin, each bearing one tetrahydrofuran (THF) ring and a carbonyl at the C-9 position (Figures 1 and 2).

Results and Discussion

Approximately 7.4 kg of dried bark was pulverized and extracted with EtOH then further partitioned[4] to yield 545.5 g of F005 [brine shrimp lethality test (BST) $LC_{50} = 1.5\ \mu g/mL$]. From this, 500.5 g was loaded onto a Si gel column and eluted with hexane and increasing percentages of $CHCl_3$, then $CHCl_3$ and increasing percentages of MeOH. In all, 60 fractions were collected, and fractions 30−36 were combined (21.67 g). This material was separated on successive open columns packed with Si gel. Repeated HPLC of fractions active in the BST[5] yielded compounds **1**−**4**.

(2,4-*cis* and *trans*)-Mosinone A (**1**) was isolated in a mixture as a white, waxy solid. The molecular weight

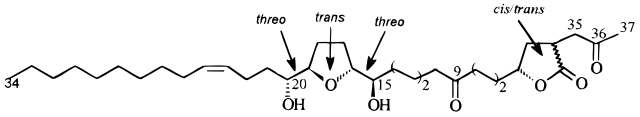


Figure 1. Structure of mosinone A (**1**).

of **1** was shown to be 620 based on the MH^+ peak at 621 in the CIMS. The compound was established as $C_{37}H_{64}O_7$ by the high resolution CIMS peak for the MH^+ at *m*/*z* 621.4723 (621.4730 calcd). Signals in the ^1H NMR of **1** at δ 4.39 and 4.55 (Table 1), with a combined integration for one proton, were assigned to H-4 and indicated the presence of a (2,4-*cis* and *trans*)-mixture at the ketolactone moiety, which is common for acetogenins of this type.[6,7] Resonances in the ^1H NMR of **1** at δ 2.65 and 3.07 (H-35) and at 2.20 (H-37) further substantiated this assignment. In the ^{13}C NMR of **1**, signals at δ 205.5 (C-36), 178.7 and 178.1 (C-1), 44.2 and 43.7 (C-2), 79.0 and 78.5 (C-4), and 23.7 (C-37) (Table 2) also confirmed that **1** is a *cis*/*trans* mixture of ketolactone isomers. The stereochemistry at C-4 was assumed as *R*, based on spectral comparisons with (2,4-*cis* and *trans*)-bullatacinone, which has known chirality,[7] and the fact that all 4-oxygenated acetogenins known to date are 4-*R*.

Resonances at δ 3.40 (H-15, H-20) and 3.80 (H-16, H-19) in the ^1H-NMR spectrum and δ 73.5 (C-15), 82.6 (C-16, 19), and 73.9 (C-20) in the ^{13}C-NMR spectrum indicated the presence of a single THF ring and two flanking hydroxyls with a *threo*/*trans*/*threo* relative stereochemistry.[8,9] The presence of the two hydroxyls was supported by two successive losses of H_2O from the CIMS MH^+ ion at *m*/*z* 621. The ring was placed between C-15 and C-20, based on EIMS peaks at *m*/*z* 325 and 395. The presence of hydroxyl groups was corroborated by a broad absorbance in the IR (3439 cm^{-1}), as was the existence of a carbonyl (1713 cm^{-1}) somewhere along the aliphatic chain. A carbonyl in the structure was also suggested by a pair of triplets in the ^1H NMR with resonances at δ 2.40 (H-8) and 2.42 (H-

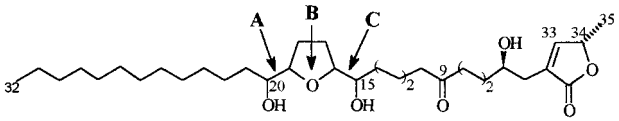
[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.

S0163-3864(97)00128-6 CCC: $14.00

Table 1. ^{1}H NMR Spectral Data (δ) for **1**−**4**

	^{1}H NMR (500 MHz, $CDCl_3$, *J* in Hz)				
position	**1** *trans*	**1** *cis*	**2**	**3**	**4**
1					
2	3.02 m	3.03 m			
3a	1.99 m	1.48 m	2.40 dddd (15.0, 8.2, 1.5, 1.5)	2.40 dddd (15.0, 8.2, 1.5, 1.5)	2.40 dddd (15.0, 8.2, 1.5, 1.5)
3b	2.22 ddd (12.9, 9.6, 3.4)	2.61 ddd (12.3, 9.4, 5.6)	2.53 dddd (15.0, 3.5, 1.5, 1.5)	2.52 dddd (15.0, 3.5, 1.5, 1.5)	2.52 dddd (15.0, 3.5, 1.5, 1.5)
4	4.55 dddd (8.3, 8.2, 5.7, 3.2)	4.39 dddd (10.7, 7.4, 5.4, 5.4)	3.87 m	3.86 m	3.86
5a	1.48 m	1.60 m	1.48	1.47 m	1.47 m
5b	1.56 m	1.76 m	1.48 m	1.47 m	1.47 m
6−7	1.26 br s	1.26 br s	1.26 br s	1.26 br s	1.26 br s
8	2.40 t (7.5)[a]	2.40 t (7.5)[a]	2.40 t (7.5)[a]	2.40 t (7.5)[a]	2.40 t (7.0)[a]
9					
10	2.42 t (7.5)[a]	2.42 (7.5)[a]	2.42 t (7.5)[a]	2.42 t (7.5)[a]	2.42.t (7.5)[a]
11−13	1.26 br s	1.26 br s	1.26 br s	1.26 br s	1.26 br s
14	1.41 m	1.41 m	1.41 m	1.41 m	1.41 m
15	3.40 m	3.40 m	3.38 m[b]	3.42 m	3.40 m
16	3.80 m	3.80 m	3.81 m[c]	3.81 m	3.79 m
17	1.69 m, 1.99 m	1.69 m, 1.99 m	1.97 m, 1.56 m[d]	1.94 m, 1.75 m	1.99 m, 1.69 m
18	1.69 m, 1.99 m	1.69 m, 1.99 m	1.87 m, 1.83 m[d]	1.94 m, 1.75 m	1.99 m, 1.69 m
19	3.80 m	3.80 m	3.82 m[c]	3.81 m	3.79 m
20	3.41 m	3.41 m	3.87 m[b]	3.42 m	3.40 m
21	2.00 m	2.00 m	1.41	1.41 m	1.41 m
22	2.20 m	2.20 m	1.26 br s	1.26 br s	1.26 br s
23	5.36 m	5.36 m	1.26 br s	1.26 br s	1.26 br s
24	5.39 m	5.39 m	1.26 br s	1.26 br s	1.26 br s
25	2.04 m	2.04 m	1.26 br s	1.26 br s	1.26 br s
26−30	1.26 br s	1.26 br s	1.26 br s	1.26 br s	1.26 br s
31	1.29 m	1.29 m	1.30 m	1.29 m	1.29 m
32	1.26 br s	1.26 br s	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)
33	1.26 br s	1.26 br s	7.19 d (1.5)	7.19 d (1.5)	7.19 d (1.5)
34	0.88 t (7.0)	0.88 t (7.0)	5.06 dq (7.0, 1.5)	5.06 dq (6.5, 1.5)	5.06 dq (6.5, 1.5)
35a	2.65 dd (18.5, 9.0)	2.61 dd (18.5, 9.0)	1.44 d (6.5)	1.44 d (7.0)	1.44 d (7.0)
35b	3.07 dd (18.5, 3.0)	3.11 (18.5, 3.0)			
36					
37	2.20 s	2.20 s			

[a−d] Values may be interchangeable in each column.



Compound	A	B	C	C-15/C-20
2, mosin B	*threo/erythro*	*trans*	*threo/erythro*	*R/S* or *S/R*
3, mosin C	*threo*	*cis*	*threo*	*R/S*
4, annoreticuin-9-one	*threo*	*trans*	*threo*	*R/R*

Figure 2. Structures of **2**−**4**.

10) and by a carbonyl signal at δ 210.8 (C-9) in the ^{13}C NMR. The carbonyl position was suggested to be at C-9 based on a peak at *m*/*z* 225 in the EIMS of **1**. This assignment was predicated on the assumption that cleavage was between C-9 and C-10, assuming that the oxygen was included in the fragment ion. An EIMS peak at *m*/*z* 225 would also be seen for a carbonyl at C-11 if cleavage were between C-10 and C-11. The carbonyl was placed conclusively at C-9 by the HREIMS of the fragment peak at *m*/*z* 225. The *m*/*z* of 225.1131 (225.1127 calcd) dictated that the composition of the fragment was $C_{12}H_{17}O_4$, as predicted by the above cleavage. The structure of **1** also contained a double bond, as evidenced by multiplet ^{1}H-NMR resonances at δ 5.36 and 5.39 and ^{13}C-NMR resonances at δ 128.9 and 130.8.[10−13] The configuration of the double bond was assigned as *cis* by comparing the NMR spectra with other double-bond-containing acetogenins of known configuration.[4] This double bond was placed two methylene units away from the flanking hydroxyl, based on a cross-peak in the double-relayed COSY spectrum between the methine protons at δ 5.36 (H-23) and 3.41 (H-20).

The absolute stereochemistries of the chiral centers in **1** were determined by preparing the di-(*R*)- and (*S*)-methoxy(trifluoromethyl)phenylacetate (MTPA) ester derivatives (Mosher esters).[14−16] According to advanced Mosher ester methodology,[15] the absolute stereochemistry of a secondary alcohol is found by analyzing the difference in ^{1}H-NMR chemical shifts between the *S*- and *R*-MTPA ester derivatives on both sides of the carbinol center. Analysis of the ^{1}H−^{1}H COSY for mosinone A−*S*-MTPA (**1a**) and mosinone A−*R*-MTPA (**1b**) suggested that, based on Mosher's arguments, the absolute stereochemistry for mosinone A was C-15*R* and C-20*R* (Table 3). Thus, the structure of **1** was elucidated as illustrated and named mosinone A.

Compound **2** was isolated as a white, waxy solid. The CIMS showed an MH^+ peak at *m*/*z* 595, indicating that this compound was only 35 carbons long, unlike **1**, which was two methylene units longer. The molecular composition of $C_{35}H_{62}O_7$ was confirmed by HRFABMS. Signals in the ^{1}H-NMR of **2** at δ 7.19 (H-35), 5.06 (H-34), and 1.44 (H-33) (Table 1) implied that the structure of **2** contained an α,β-unsaturated γ-lactone. ^{13}C NMR resonances at δ 174.6 (C-1), 131.04 (C-2), 151.9 (C-33), and 78.0 (C-34) (Table 2) substantiated this hypothesis. Further evidence for the presence of an α,β-unsaturated γ-lactone was provided by the IR carbonyl peak at 1755 cm^{-1}. The existence of hydroxyl groups in the structure

Table 2. ^{13}C NMR Spectral Data (δ) for **1**−**4**

	^{13}C NMR (125 MHz in $CDCl_3$ for **1**, **3**; 75 MHz in $CDCl_3$ for **2**, **4**)				
position	**1** *cis*	**1** *trans*	**2**	**3**	**4**
1	178.66	178.12	174.63	174.70	174.61
2	44.17	43.68	131.04	131.09	131.03
3	34.41	34.41	33.34	33.35	33.30
4	78.98	78.54	69.60	69.62	69.56
5	36.65	35.39	37.01	37.00	36.98
6	24.97[a]	24.97[a]	25.95[a]	25.67[a]	25.55[a]
7	24.90−35.32	24.90−35.32	25.12−29.64	23.44−29.67	23.44−33.30
8	42.71[b]	42.71[b]	42.67[b]	42.64[b]	42.64[b]
9	210.80	210.80	211.38	211.50	211.40
10	42.36[b]	42.36[b]	42.51[b]	42.51[b]	42.51[b]
11−12	23.68−33.46	24.90−35.32	25.12−29.64	23.44−29.67	23.44−33.30
13	25.32[a]	25.32[a]	25.28[a]	25.32[a]	25.26[a]
14	33.19[c]	33.19[c]	32.94[c]	34.07[c]	33.39[c]
15	73.49[d]	73.49[d]	74.21[d]	74.38[d]	74.05[d]
16	82.63	82.63	83.18[e]	82.66	82.66[e]
17	28.69	28.69	28.57[f]	28.10	28.73
18	28.69	28.69	25.23[f]	28.10	28.73
19	82.63	82.63	82.14[e]	82.66	82.58[e]
20	73.91[d]	73.91[d]	71.51[d]	74.28[d]	73.89[d]
21	33.46[c]	33.46[c]	32.51[c]	33.73[c]	33.39[c]
22	23.27[a]	23.27[a]	25.12[a]	25.14[a]	25.13[a]
23	128.92	128.92	25.12−29.64	23.44−29.67	23.44−33.30
24	130.78	130.78	25.12−29.64	23.44−29.67	23.44−33.30
25	27.21[a]	27.21[a]	25.12−29.64	23.44−29.67	23.44−33.30
26−29/31	23.68−33.46	24.90−35.32	25.12−29.64	23.44−29.67	23.44−33.30
30/32	31.87	31.87	31.89	31.90	31.86
31/33	22.63	22.63	22.64	22.66	22.66
32/34	14.05	14.05	14.09	14.09	14.07
33/35	35.39	35.39	151.93	151.98	151.92
34/36	205.50	205.50	78.01	78.03	77.99
35/37	23.68	23.68	19.07	19.07	19.05

[a−f] Values may be interchangeable in each column.

Table 3. ^1H NMR (500 MHz, $CDCl_3$) Data (δ) for MTPA Derivatives of Compound **1**

MTPA ester	14	16	17	18	19	21
1a	1.64, 1.60	3.93	1.64, 1.38	1.64, 1.38	3.93	1.64, 1.60
1b	1.60, 1.56	4.01	1.91, 1.57	1.91, 1.57	4.01	1.60, 1.56
$\Delta(\delta S-\delta R)$	pos	neg	neg	neg	neg	pos
confign		15*R*			20*R*	

Table 4. ^1H NMR (500 MHz, $CDCl_3$) Data (δ) for MTPA Derivatives of Compound **2**

MTPA ester	H-5	H-3a	H-3b	H-33	H-34	H-35
2a	1.72, 1.62	2.61	2.55	6.73	4.86	1.28
2b	1.61, 1.56	2.67	2.59	6.97	4.91	1.30
$\Delta(\delta S-\delta R)$	pos	neg	neg	neg	neg	neg
config			4*R*			

was suggested by a broad absorbance in the IR (3441 cm^{-1}) as well as peaks in the eims at *m*/*z* 577, 559, and 541, indicating three successive losses of H_2O from the molecular ion at *m*/*z* 595. Examination of the regions around δ 3.80 (H-4, H-15/20, H-16/19) and δ 3.40 (H-15/20) in the ^1H-NMR spectrum of **2** and ^{13}C-NMR signals for **2** at δ 74.2 (C-15/20), 83.2 (C-16/19), 82.1 (C-16/19), and 71.5 (C-15/20) indicated a mono-THF ring acetogenin with two flanking hydroxyls. Other ^1H signals for the ring methines (H-17/18) at δ 1.97, 1.87, 1.83, and 1.56, and ^{13}C-NMR signals at δ 32.9 and 32.5 (C-14/21) and at δ 28.6 and 25.2 (C-17/18) suggested a relative stereochemistry of *threo*/*trans*/*erythro*. These resonances matched those of a synthetic model compound and supported this assignment.[9] The fragment peak in the EIMS at *m*/*z* 325 placed the THF ring between C-15 and C-20. As in **1**, a carbonyl moiety along the aliphatic chain in **2** was evidenced by a second carbonyl peak in the IR (1702 cm^{-1}), ^1H-NMR signals at δ 2.40 (H-8) and 2.42 (H-10), and a ^{13}C-NMR carbonyl signal at δ 211.4 (C-9). Similarly, the position of the carbonyl was placed at C-9 by an EIMS fragment peak at *m*/*z* 225 and verified by HREIMS spectral analysis of that fragment as described above for **1**.

Although the relative stereochemistry of *threo*/*trans*/*erythro* could be assigned in **2** based on NMR comparisons with model compounds,[9,17−21] it was unknown whether the *erythro* hydroxyl was at C-15 or C-20. Analysis of the $^1H-^1H$ COSY spectra for the tri-(*S*)- and tri-(*R*)-MTPA esters of **2** (**2a** and **2b**, respectively) provided no information that could stereochemically differentiate the H-16, H-17ab, H-18ab, and H-19 protons (Table 4), although the absolute stereochemistry at C-4 was conclusively determined to be *R*. The absolute stereochemistry at C-34 was established as *S* by the method of Hoye *et al.*[22] Due to the coplanarity of the *threo*/*trans*/*erythro* relationship in **2**, these protons experienced shielding effects of the phenyl groups of both flanking MTPA esters. Therefore, am-

Table 5. ^{1}H-NMR (500 MHz, $CDCl_3$) Data (δ) for MTPA Derivatives of Compounds **3** and **4**

MTPA ester	5	3	33	34	35	14	16	17	18	19	21
3a	1.64, 1.72	2.56, 2.61	6.73	4.87	1.28	1.32, 1.36	3.86	1.44, 1.42	1.37, 1.82	4.10	1.63, 1.69
3b	1.61, 1.67	2.59, 2.66	6.97	4.92	1.31	1.31, 1.35	3.88	1.46, 1.43	1.36, 1.81	4.11	1.65, 1.70
$\Delta(\delta S-\delta R)$	pos	neg	neg	neg	neg	pos	neg	neg	pos	neg	neg
confign		4*R*					15*R*			20*S*	
4a	1.71, 1.63	2.55, 2.60	6.73	4.85	1.28	1.60, 1.54	3.92	1.65, 1.36	1.65, 1.36	3.92	1.60, 1.54
4b	1.70, 1.61	2.59, 2.68	6.97	4.91	1.31	1.55, 1.48	4.00	1.91, 1.53	1.91, 1.53	4.00	1.55, 1.48
$\Delta(\delta S-\delta R)$	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	pos
confign		4*R*					15*R*			20*R*	

Table 6. Bioactivities of Compounds **1**−**4**

		cytotoxicity (ED_{50}, μg/mL)					
compd	BST[a] (LC_{50}, μg/mL)	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PACA-2[g]
1	4.4×10^{-1}	>1	>1	>1	>1	3.2×10^{-2}	2.2×10^{-3}
2	2.9×10^{-1}	9.4×10^{-1}	>1	>1	>1	3.5×10^{-1}	2.5×10^{-4}
3	1.5×10^{-1}	6.0×10^{-1}	>1	>1	>1	>1	1.2×10^{-4}
4	4.1×10^{-1}	2.7×10^{-1}	>1	>1	>1	9.6×10^{-3}	2.4×10^{-4}
adriamycin[h]	2.6×10^{-1}	5.3×10^{-3}	2.0×10^{-1}	2.0×10^{-2}	1.0×10^{-2}	3.2×10^{-2}	1.8×10^{-2}

[a] Brine shrimp lethality test.[5] [b] Human lung carcinoma.[29] [c] Human breast adenocarcinoma.[30] [d] Human colon adenocarcinoma.[31] [e] Human kidney carcinoma.[30] [f] Human prostate adenocarcinoma.[32] [g] Human pancreatic carcinoma.[33] [h] Positive control, BST LC_{50} value taken from Ratnayake *et al.* 34.

biguity remains concerning the relative and absolute stereochemistries of **2**.

The structure of **3** differs from **2** only in the relative stereochemistry around the THF ring. The CIMS and EIMS for **3** were identical to those of **2** inasmuch as since they have the same planar structure. As in **2**, analysis of the IR, ^{1}H-NMR and ^{13}C-NMR spectra of **3** indicated the presence of an α,β-unsaturated γ-lactone and a single THF ring with two flanking hydroxyls. A carbonyl in the structure was again suggested by IR, ^{1}H-NMR (Table 1), and ^{13}C-NMR (Table 2) spectra. The assignment of the carbonyl to the C-9 position was made as before by the EIMS peak at *m*/*z* 225 and was corroborated by HREIMS of that fragment ion.

For **3**, the *cis* assignment across the THF ring was made based on ^{1}H-NMR signals for the ring methines at δ 1.94 and 1.75 compared to δ 1.99 and 1.69 for a *trans* ring configuration.[10,13,17,23] ^{13}C-NMR resonances at δ 74.4, 82.7, 34.1, 33.7, and 28.1 also suggested a *cis* assignment for the THF ring.[10,13,17,23] These signals showed a close resemblance to those of a synthetic model compound with a *threo*/*cis*/*threo* ring configuration and supported this assignment.[9] To determine the absolute stereochemistry of **3**, the tri-(*S*)- and (*R*)-MTPA ester derivatives (**3a** and **3b**, respectively) were prepared. Analysis of the ^{1}H-NMR and ^{1}H−^{1}H COSY spectral data indicated that the absolute configurations at C-4 and C-15 are *R*, and at C-20 the configuration is *S* (Table 5). As in **2**, the stereochemistry at C-34 was determined to be *S*.[22] The irregular $\Delta\delta$ value for C-19 has been observed in other acetogenins and has been explained by Zhao *et al.*[24] The structure of **3** is, thus, suggested as illustrated and named mosin C.

Annoreticuin-9-one (**4)** is known previously from *Annona reticulata* but is new to this species.[25] It, too, was obtained as a white, waxy solid. Compound **4** is closely related to squamone, which we previously isolated from *A. squamosa*.[3] The only difference is that **4** possesses an α,β-unsaturated γ-lactone with a hydroxyl at C-4 instead of the ketolactone moiety seen in squamone. Examination of the diagnostic peaks in the ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) of **4** indicated the presence of the aforementioned lactone and hydroxyl at C-4 and a single THF ring bearing a *threo*/*trans*/*threo* relationship.

Although the planar structure of **4** is known, its absolute stereochemistry has not been reported previously. The tri-(*R*)- and (*S*)-Mosher esters of **4** were prepared (**4a** and **4b**, respectively) and analyzed using ^{1}H-NMR and ^{1}H−^{1}H COSY spectral data (Table 5). The absolute stereochemistry of **4** was determined to be 4*R*,-15*R*,20*R*,34*S*, and it can now be assumed that squamone[3] has the same stereochemistry.

Compounds **1**−**4** all showed moderate activity against brine shrimp (Table 6). In cell culture, these acetogenins all exhibited up to 10 000-fold selectivities for the pancreatic cell line, PACA-2, and were 10−100 times more active than the positive control, Adriamycin (Table 6). It is interesting to compare this group of compounds to murisolin, murisolin A, and 16,19-*cis*-murisolin, a related series of acetogenins which differ from **2**, **3**, and **4** only in that they do not contain a carbonyl at the C-9 position.[17] These compounds did not show any selectivity for PACA-2 but did exhibit strong potency against other cell lines. Furthermore, unlike the compounds described in this paper, which all displayed a similar profile of activity, the murisolin series showed different activities compared to each other. For example, 16,19-*cis*-murisolin was several orders of magnitude less active than the other two compounds against the A-549, HT-29, and A-498 cell lines, but was 100 times more potent than them against the MCF-7 cell line. From this data, it can be suggested that the carbonyl at C-9 decreases activity in five of the six cell lines tested but magnifies the potency toward the pancreatic cell line, PACA-2. Knowing that acetogenins act by inhibiting ubiquinone-linked NADH oxidases that are membrane bound,[26−28] a possible explanation for the observed selectivity is that the target enzymes in PACA-2 cells have a peculiar geometry making them more susceptible to acetogenins with a carbonyl at C-9.

Experimental Section

General Experimental Procedures. UV spectra were measured on a Beckman DU 640 series spectrophotometer. IR data were collected using a Perkin-Elmer 1600 series FTIR. ^{1}H NMR and ^{13}C NMR were obtained on a Varian VXR-500S spectrometer. LREIMS and LRCIMS data were collected on a Finnigan 4000 spectrometer. HREIMS, HRCIMS, and HRFABMS were obtained on the Kratos MS50 through peak matching. HPLC was carried out using a Dynamax UV-1 detector coupled with a Rainin model HPXL solvent delivery system for normal phase and Dynamax Model SDS-200 solvent delivery system for reversed phase.

Biological Testing. The cytotoxicity of column fractions and pure compounds was monitored using the BST.[5] Cell culture assays were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in 7-day MTT assays, with Adriamycin as the positive control.

Plant Material. The dried stem bark of *A. squamosa* Rich. was purchased from United Chemical and Allied Products in Calcutta, India.

Extraction and Isolation. The dried and pulverized bark (7.4 kg) was extracted with EtOH (1.83 kg F001, BST LC_{50} = 1.6 μg/mL). The residue was partitioned between CH_2Cl_2 and H_2O to yield a CH_2Cl_2-soluble residue (842 g F003, BST LC_{50} = 1.7 μg/mL) and an H_2O-soluble residue (128.6 g F002, BST LC_{50} = 950.1 μg/mL). F003 was further partitioned between 90% aqueous MeOH and hexane, resulting in a MeOH-soluble residue (545.5 g F005, BST LC_{50} = 1.5 μg/mL) and a hexane-soluble residue (162.9 g F006, BST LC_{50} = 123.0 μg/mL). F005 (500.5 g) was separated by column chromatography over Si gel using hexane and $CHCl_3$, then $CHCl_3$ and MeOH as solvent systems. Fractions 30−36 were combined on the basis of TLC and were further resolved on another Si gel column eluted with hexane and Me_2CO. The pools from this column that were bioactive in the BST were subjected to a third Si gel column eluted with $CHCl_3$ and MeOH. Compounds **1**−**4** were purified by repeated normal-phase and reversed-phase HPLC using solvent systems of MeCN-*N*H_2O (70:30) and hexane*N*MeOH*N*THF (90:9:1), respectively.

Derivatizations. To **1**−**4** (0.5 mg in 0.5 mL CH_2Cl_2) were sequentially added 0.2 mL pyridine, 0.5 mg 4-(dimethylamino)pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The mixture was stirred for 4 h at room temperature, then passed through a small pipet column (0.6 × 6 cm) packed with Si gel, and eluted with 5 mL CH_2Cl_2. The residue was redissolved in 5 mL CH_2Cl_2 and washed with 5 mL 1% $NaHCO_3$ and 2 × 5 mL H_2O. The organic layer was evaporated to give the *S*-Mosher esters of **1**−**4**. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride yielded the *R*-Mosher esters of **1**−**4**.

Mosinone A (1). White, waxy solid (12 mg); $[\alpha]^{23}_D$ + 4.8°; (c = 0.016, CH_2Cl_2) UV (MeOH) λ_{max} 202 nm (log ϵ 2.96); CIMS (isobutane) m/z $[MH]^+$ 621 (64), $[MH - H_2O]^+$ 603 (100), $[MH - 2H_2O]^+$ 585 (6); EIMS m/z 395 (5), 377 (20), 359 (19), 325 (100), 307 (40), 289 (13), 225(16), 207 (20); HRCIMS (isobutane) m/z 621.4723 for $C_{37}H_{60}O_7$ (calcd 621.4730); HREIMS m/z 225.1131 for $C_{12}H_{17}O_4$ (calcd 225.1127); ^{1}H-NMR data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Mosin B (2). White, waxy solid (7 mg); $[\alpha]^{23}_D$ +11.5° (c = 0.005, CH_2Cl_2); UV (MeOH) λ_{max} 222 nm (log ϵ 3.57); CIMS (isobutane) m/z $[MH]^+$ 595 (30), $[MH - H_2O]^+$ 577 (71), $[MH - 2H_2O]^+$ 559 (37); EIMS m/z 325 (16), 307 (91), 289 (62), 225 (7), 207 (69); HRFABMS m/z 595.4578 for $C_{35}H_{62}O_7$ (calcd 595.4574); HREIMS 225.1133 for $C_{12}H_{17}O_4$ (calcd 225.1127); ^{1}H-NMR data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-NMR data ($CDCl_3$, 75 MHz), see Table 2.

Mosin C (3). White, waxy solid (6 mg); $[\alpha]^{23}_D$ −2.7° (c = 0.007, CH_2Cl_2); UV (MeOH) λ_{max} 216 nm (log ϵ 3.56); CIMS (isobutane) m/z $[MH]^+$ 595 (74), $[MH - H_2O]^+$ 577(100), $[MH - 2H_2O]^+$ 559 (53); EIMS m/z 325, (16), 307 (14), 289 (9), 225 (4), 207 (10); HRFABMS m/z 595.4578 for $C_{35}H_{62}O_7$ (calcd 595.4574); HREIMS 225.1135 for $C_{12}H_{17}O_4$ (calcd 225.1137); ^{1}H-NMR data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-NMR data ($CDCl_3$, 125 MHz), see Table 2.

Acknowledgment. This work was supported in part by R01 Grant no. CA30909 from the National Cancer Institute, National Institutes of Health, and Predoctoral Training Grant GM08298-07 from Public Health Services, National Institute of General Medical Sciences, National Institutes of Health. Cytotoxicity data were provided by the Cell Culture Laboratory, Purdue University Cancer Center.

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NP9701283