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Squamotacin: An Annonaceous Acetogenin with Cytotoxic Selectivity for the Human Prostate Tumor Cell Line (PC-3)

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Abstract: The bark extracts of *Annona squamosa* yielded a new bioactive acetogenin, squamotacin (**1**), and the known compound, molvizarin (**2**), which is new to this species. Compound **1** is identical to the potent acetogenin, bullatacin (**3**), except that the adjacent bis-tetrahydrofuran (THF) rings and their flanking hydroxyls are shifted two carbons toward the γ-lactone ring. Compound **1** showed cytotoxic selectively for the human prostate tumor cell line (PC-3), with a potency of over 100 million times that of Adriamycin.

The Annonaceous acetogenins are a new group of powerful bioactive agents, and nearly 200 of these compounds have been found to date.[1−4] *Annona squamosa*, the custard apple, is a fruit tree, native to the new world and naturalized throughout the tropics, and belongs to this plant family.[5] Research on the seeds of this plant has yielded 27 different acetogenins.[1−3] By contrast, only four have been reported from the bark, and these are bullatacin (**3**),[6] 2,4-*cis*- and 2,4-*trans*-bullatacinones,[6] and squamone.[7] Through further work with the bark,[8] each of these four known compounds have now been reisolated. Additionally found were asimicin,[9] which is new to the bark of this species, molvizarin (**2**),[10] which is new to the species, and squamotacin (**1**), which is a new acetogenin (Chart 1). These compounds were isolated using column chromatography followed by HPLC and by directing the fractionation with the brine shrimp lethality test (BST).[11] Structure elucidation was carried out using NMR and MS analyses;[3] the relative stereochemistry of **1** was suggested based on the close agreement with the NMR signals of **3** whose absolute stereochemistry is now known through Mosher ester analyses.[12]

Table 1. ^{1}H (500 MHz, $CDCl_3$, J in Hz) and ^{13}C NMR (125 MHz, $CDCl_3$) Data of Molvizarin (**2**)[10]

no.	δ_H	δ_C	no.	δ_H	δ_C
1		174.53	18	3.84 m	82.71
2		131.00	19−20	1.96, 1.62 m	28.30, 24.40
3a	2.50 m	33.12	21	3.92 m	82.13
3b	2.35 m		22	3.84 m	71.26
4	3.84 m	69.77	23	1.36 m	32.29
5	1.46 m	37.25	24	1.36 m	25.47
6	1.36 m	25.94	25−29	1.25 m	29.50−29.21
7−10	1.25 m	29.50−29.21	30	1.25 m	31.76
11	1.36 m	25.47	31	1.25 m	22.55
12	1.36 m	33.00	32	0.86 t (7.0)	14.00
13	3.37 tt	74.02	33	7.16 d (1.5)	151.75
14	3.84 m	83.18	34	5.04 dq (1.5, 7.0)	77.87
15−16	1.96−1.62 m	28.87, 28.30	35	1.42 d (1.5)	18.97
17	3.92 m	82.44			

Molvizarin (**2**) was isolated from a partitioned fraction (F005)[13] of the ethanol extract using open columns and then purified with repeated reversed-phase and normal-phase HPLC. On the basis of NMR data, this compound was at first mistaken for bullatacin (**3**).[6,12] The respective ^{1}H and ^{13}C NMR signals at δ 3.37, 74.02, 3.84, 83.18, and 3.92, 82.44 (Table 1) indicated that two adjacent THF rings with flanking hydroxyls were present in the structure of **2** with a *threo/trans/threo/trans/erythro* relative configuration.[1,2] Compound **2** was distinguished from **3** by the CIMS which showed a molecular ion with an m/z of 594, indicating that **2** contained only 35 carbons, while **3** has 37 carbons and a molecular weight of 622.

Squamotacin (**1**)[14] was also separated from the other constituents of the extract by multiple open columns followed by normal-phase and reversed-phase HPLC.[15] The respective ^{1}H and ^{13}C NMR signals at δ 3.39, 74.08, 3.85, 83.20, 3.93, 82.52, 3.85, 82.81, 3.93, 82.28, and 3.85, 71.36 suggested that **1** contained adjacent THF

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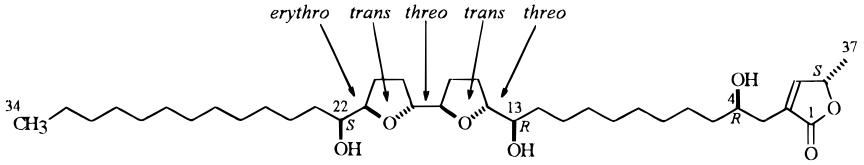
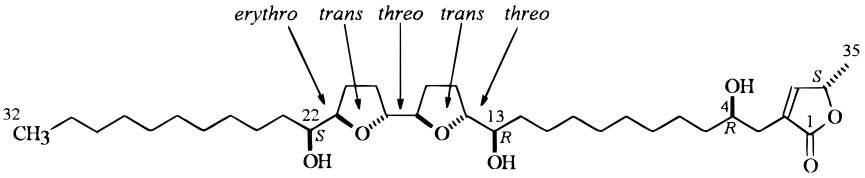
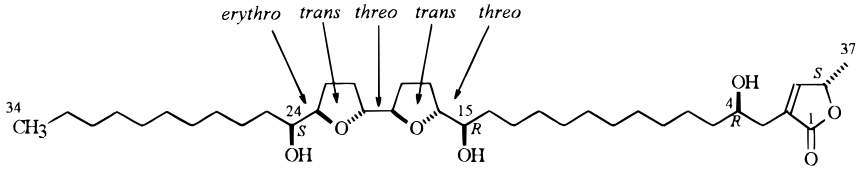
Chart 1



1



2



3

Table 2. ^{13}C NMR (125 MHz, $CDCl_3$) and ^{1}H NMR (500 MHz, $CDCl_3$, *J* in Hz) Data of **1** and **3**

	squamotacin (**1**)		bullatacin (**3**)			squamotacin (**1**)		bullatacin (**3**)	
no.	δ_C	δ_H	δ_C	δ_H	no.	δ_C	δ_H	δ_C	δ_H
1	174.61		174.58		19	28.90	1.63 m	82.49	3.92 m
2	131.19		131.15		20	24.51	1.98 m	82.24	3.83 m
3a	33.35	2.40 m	33.28	2.41 m	21	82.28	3.93 m	28.92	1.3−2.0 m
3b	33.35	2.53 m	33.28	2.53 m					
4	70.1	3.85 m	69.94	3.80 m	22	71.36	3.85 m	28.35	1.3−2.0 m
5	37.39	1.45 m	37.38	1.3−2.0 m	23	32.45	1.45 m	82.77	3.92 m
6	26.05	1.36 m	26.02	1.25 m	24	25.55	1.45 m	71.28	3.83 m
7−10	29.64−29.35	1.25 m	29.66−29.30	1.25 m	25	29.64−29.35	1.25 m	32.39	1.3−2.0 m
11	25.55	1.36 m	29.66−29.30	1.25 m	26	29.64−29.35	1.25 m	25.54	1.25 m
12	33.35	1.36 m	29.66−29.30	1.25 m	27−31	29.64−29.35	1.25 m	29.66−29.30	1.25 m
13	74.08	3.39 m	25.54	1.25 m	32	31.92	1.25 m	31.87	1.25 m
14	83.20	3.85 m	33.28	1.35 m	33	22.68	1.25 m	22.65	1.25 m
15	28.35	1.63 m	74.08	3.38 m	34	14.11	0.88 t (7.0)	14.09	0.88 t (7.0)
16	28.90	1.98 m	83.25	3.83 m	35	151.78	7.16 d (1.5)	151.76	7.17 d (1.5)
17	82.52	3.93 m	28.92	1.3−2.0 m	36	77.98	5.06 dq (1.5, 6.5)	77.94	5.06 dq (1.5, 7.0)
18	82.81	3.85 m	28.35	1.3−2.0 m	37	19.11	1.43 d (1.5)	19.08	1.44 d (1.5)

rings with flanking hydroxyls. The presence of a hydroxyl group at the four position was indicated by the characteristic signals for the Ha and Hb protons at C-3 (Table 2). The relative stereochemistry of *threo/trans/threo/trans/erythro* was established on the basis of the very close similarities between the ^{1}H and ^{13}C NMR spectra of **1** and bullatacin (**3**), which has this ring arrangement (Table 2).[6,12] These two compounds showed virtually identical ^{1}H and ^{13}C NMR spectra both with respect to the positions and splitting patterns of the resonances. Because of this close relationship, and the unlikely possibility that the *ent*-isomer is naturally occurring, the absolute stereochemistry of **1** is suggested to be the same as that for **3**, whose absolute stereochemistry is known.[12]

The molecular weight of 622 for **1** was assigned on the basis of the MH^+ peak at *m/z* 623 in the CIMS. The EIMS showed fragment peaks at *m/z* 283 (100) and *m/z* 335 (80) representing cleavage between C-13 and C-14. On the basis of this information, it was hypothesized that the bis-THF ring system, versus that of **3**, was shifted two carbon units toward the lactone ring along the aliphatic chain. The movement of the THF ring units and their flanking hydroxyls from C-15 and C-24 to C-13 and C-22 was confirmed by the EIMS fragmentation pattern of the TMSi derivative (Figure 1). The fragment at *m/z* 427 was the most intense and indicated major cleavage between C-13 and C-14. Further support was given by the fragment at *m/z* 271, indicating a cleavage between C-22 and C-23.

Due to their structural similarities, **1** was expected to show highly potent cytotoxicities to human tumor cells that would be similar to those of **3**. Compounds **1**−**3** were then submitted for evaluation of cytotoxicities, in the same run, against a panel of six human solid tumor cell lines (Table 3). Surprisingly, **1** showed less activity than **3** in five of the six cell lines tested, HT-29 (colon) being the exception. However, in the prostate cell line (PC-3), **1**, unexpectedly, showed a high selectively (Table 3). In this cell line, **1** was over 100 million times as active as the positive control, Adriamycin. Molvizarin (**2**)[10] is nearly identical to **1** in structure with the only difference being that it is two methylene units shorter on the hydrocarbon end. It still, however, has

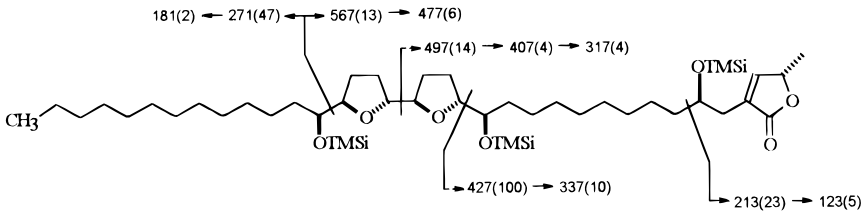


Figure 1. EIMS fragmentation of the TMSi derivative of squamotacin (**1**) (intensities indicated in parentheses).

Table 3. Bioactivities of **1**−**3**

compd	BST[a] (LC_{50}, μg/mL)	cytotoxicity (ED_{50}, μg/mL) A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PACA-2[g]
1	6.80×10^{-3}	2.77×10^{-2}	>1	1.00×10^{-3}	>1	1.72×10^{-9}	1.33×10^{-4}
2	5.26×10^{-2}	6.30×10^{-2}	>1	7.32×10^{-3}	7.09×10^{-1}	4.47×10^{-8}	7.66×10^{-3}
3[h]	1.59×10^{-3}	2.44×10^{-6}	6.96×10^{-1}	>1	4.85×10^{-5}	$<10^{-9}$	$<10^{-9}$
adriamycin[h]	2.57×10^{-1}	2.84×10^{-2}	3.47×10^{-1}	4.16×10^{-2}	2.49×10^{-2}	3.42×10^{-1}	3.17×10^{-3}

[a] Brine shrimp lethality test.[11] [b] Human lung carcinoma.[19] [c] Human breast carcinoma.[20] [d] Human colon adenocarcinoma.[21] [e] Human renal carcinoma.[19] [f] Human prostate adenocarcinoma.[22] [g] Human pancreatic carcinoma.[23] [h] Brine shrimp LC_{50} taken from ref 24.

the same spatial relationships between the terminal unsaturated γ lactone and the adjacent bis-THF rings. The spectra of cytotoxic selectivities of **1** and **2** were quite similar (Table 3). The fact that molvizarin (**2**) also showed selectivity for PC-3 strongly suggests that the distance between the γ lactone and the bis-THF ring system may be a critical factor influencing the activity of the acetogenins in certain cell types. The acetogenins exact their bioactive effects, at least in part, by inhibition of mitochondrial NADH ubiquinone oxidoreductase (complex I)[16] and by inhibition of the ubiquinone-linked NADH oxidase that is peculiar to plasma membranes of cancerous cells.[17] These effects deplete ATP levels and likely induce programmed cell death (apoptosis).[18]

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(8) Dried stem bark of *Annona squamosa* was purchased from United Chemical and Allied Products in Calcutta, India.
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(14) *Squamotacin* (**1**): white powder (3.5 mg); $[\alpha]_D$ 2.59° ($c = 0.0027$); IR ν max (KBr) 3450 (br, OH), 2924, 2853, 2358, 1750, 1456, 1073 cm^{-1}; UV λ max (MeOH) 207 nm ($\epsilon = 7.9 \times 10^3$); HRFABMS (thioglycerol) *m/z* [MH^+] 623.4867 for $C_{37}H_{66}O_7$ (calcd 623.4887); CIMS *m/z* 623 (31), 605 (100), 587 (69), 569 (28); EIMS *m/z* 387 (33) 335 (80), 317 (33) 283 (100); EIMS of TMSi derivative, see Figure 1; ^{1}H-NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2.
(15) Approximately 7.4 kg of the dried and pulverized stem bark was extracted with ethanol and then further partitioned[13] to give 545.5 g of F005 (BST $LC_{50} = 1.5155$). From this, 500.5 g was added to an open column containing Si gel (1.5 kg) and was developed using hexane with increasing amounts of chloroform followed by chloroform with increasing amounts of methanol. Bioactive fractions 24−29 appeared on TLC to contain acetogenins. These were combined (34.96 g) and treated with hexane and dichloromethane (2:1). The soluble portion (12.9 g, BST $LC_{50} = 0.0013$) was added to an open column loaded with Si gel (220 g) and developed with hexane and increasing amounts of acetone followed by acetone and increasing amounts of methanol. Compounds **1**−**3** were purified by reversed-phase HPLC using MeOH and H_2O (90:10, flow rate 10 mL/min) and normal phase HPLC using a solvent system of hexane−MeOH−THF (93:6:1, flow rate 10 mL/min) to yield each as a white waxy powder.
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