Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 569–575

BIOORGANIC &
MEDICINAL
CHEMISTRY

Three New Bioactive Bis-adjacent THF-ring Acetogenins from the Bark of *Annona squamosa*

D. Craig Hopp, Feras Q. Alali, Zhe-ming Gu and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907-1333, USA

Received 25 September 1997; accepted 10 November 1997

Abstract—Continuing work on the bioactivity-directed fractionation of the bark of *Annona squamosa* has resulted in the discovery of three new Annonaceous acetogenins, (2,4-*cis* and *trans*)-squamolinone (**1**), (2,4-*cis* and *trans*)-9-oxo-asimicinone (**2**), and bullacin B (**3**). Compounds **1**–**3** are all adjacent bis-THF ring acetogenins with **2** representing the first bis-ring acetogenin to contain a carbonyl along its aliphatic chain. Compound **3** was selectively cytotoxic in a panel of six human tumor cell lines with a potency of nearly a million times that of adriamycin against the MCF-7 (human breast adenocarcinoma) cell line. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Annona squamosa Rich. (Annonaceae), commonly known as the custard apple, is a fruit tree, native to Central America which is now cultivated throughout the tropics. Folkloric records report its use as an insecticide and an antitumor agent.[1] Fractionation of the seeds of this plant species by Fujimoto et al. resulted in the isolation of 26 different acetogenins.[2,3] Our previous work on the bark, using the brine shrimp lethality assay to guide fractionation,[4] led to six new and eight known acetogenins.[5–7] Herein, through further fractionation work, we report the discovery of three new adjacent bis-THF ring acetogenins, (2,4-*cis* and *trans*)-squamolinone (**1**), (2,4-*cis* and *trans*)-9-oxo-asimicinone (**2**), and bullacin B (**3**). Compound **1** is similar to isomolvizarin-1[8] but the bis-THF ring system is shifted from C-13/C-22 to C-15/C-24. Compound **2** is identical to asimicinone[9] except that it possesses a ketone functionality at the C-9 position; it is the first reported bis-ring acetogenin with a carbonyl along its aliphatic chain. Compound **3** is very similar to asimicin[10] but with the 4-hydroxyl shifted to the C-6 position; it differs from bullacin,[11] the first 6-hydroxylated acetogenin, by being two methylene units longer (C-37 compared to C-35). Compounds **1**–**3** all showed significant activity against a panel of six human tumor cell lines.

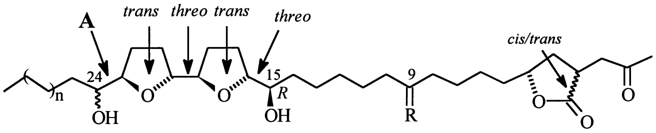
Results and Discussion

(2,4-*cis* and *trans*)-Squamolinone (**1**) is an analogue of isomolvizarin-1[8] in which the THF ring system has been shifted two methylene units further from the γ-lactone ring, i.e., from C-13/C-22 to C-15/C-24 (Figure 1). It was isolated as a white amorphous powder. The mass of **1** was established by the molecular ion peak in the CIMS at *m*/*z* 595. A formula of $C_{35}H_{62}O_7$ was confirmed by high resolution CIMS of the MH^+ ion at *m*/*z* 595.4555 (calcd. 595.4573). The lactone ring in **1** has undergone a translactonization rearrangement to form a mixture of *cis* and *trans* ketolactone isomers.[12,13] As is usual for these mixtures, the isomers were not separated in the purification process. Diagnostic peaks in the ^{1}H NMR at δ 4.40 and 4.54 (H-4), 2.20 (H-35) and between 1.45 and 3.11 (H-2, 3, 5, and 33), and ^{13}C NMR signals at δ 180.50 (C-1), 43.88 and 44.29 (C-2), 78.85 and 79.28 (C-4), and 205.8 (C-34), for positions around the lactone ring, indicated the presence of the *cis*/*trans* mixture. A UV λ_{max} of 203 nm supported this conclusion. Integration of the ^{1}H NMR signals at δ 4.40 and 4.54 indicated that the *trans* isomer was present in a slight excess (55:45). The existence of hydroxyl groups in the molecule was evidenced by an absorption band in the IR at 3426 cm^{-1} and was validated by the successive losses of two molecules of water from the MH^+ ion in the CIMS. The presence of two THF rings was suggested by signals in the ^{1}H NMR at δ 3.40 (H-15) and between δ 3.86 and 3.93 (H-16, 19, 20, 23, and 24), which integrated for a total of 6 protons, and signals in the ^{13}C NMR for 6

Key words: *Annona squamosa*; Annonaceae; annonaceous acetogenins.

*Corresponding author. Tel: (765) 494-1455; Fax: (765) 494-1414.

PII: S0968-0896(98)00018-2



Compd	R	A	n
1	H,H	*erythro*	3
2	O	*threo*	4

Figure 1. Structures of **1** and **2**.

oxygenated carbons at δ 74.09 (C-15), 83.18 (C-16), 82.39 (C-19), 82.18 (C-20), 82.83 (C-23), and 71.56 (C-24). The relative stereochemistry across the rings was determined to be *threo*/*trans*/*threo*/*trans*/*erythro* based on comparison with bullatacin,[12] which has known stereochemistry,[14] and with synthetic models published by Hoye.[15,16] The location of the rings was set between C-15 and C-24 based on fragment ions in the EIMS at *m*/*z* 311 and 363 (loss of 18 a.m.u. from *m*/*z* 381).

The absolute stereochemistry was established through the use of advanced Mosher ester methodology.[14,17,18] By preparing the *S* and *R* Mosher ester derivatives and comparing the change in chemical shift on either side of the carbinol center, the absolute stereochemistry of each stereocenter can be determined. The di-*S* and di-*R* Mosher esters of **1** were prepared (**1a** and **1b**, respectively). Analysis of the ^{1}H–^{1}H COSY for **1a** and **1b** enabled assignment of the configurations as C-15*R* and C-24*S* (Table 1). The absolute stereochemistry at C-4 was assumed to be *R* based on comparison with bullatacinone and the fact that all known acetogenins are 4*R*.[12] The structure of **1** is, thus, suggested as illustrated and named (2,4-*cis* and *trans*)-squamolinone.

(2,4-*cis* and *trans*)-9-Oxo-asimicinone (**2**), another white amorphous powder, shares the same structure as (2,4-*cis* and *trans*)-asimicinone[9] with the addition of a carbonyl group at C-9. There are no previous reports of a bis-ring acetogenin containing a ketone in its structure, although several mono-ring Annonaceous acetogenins, bearing a carbonyl group at C-9, have been reported from *A. squamosa*.[6] The molecular weight of **2** was determined based on the $[MH]^+$ peak at *m*/*z* 637 in the CIMS. HRCIMS of the molecular ion gave an exact mass at *m*/*z* 637.4665 (calcd. 637.4679) and confirmed the elemental composition as $C_{37}H_{64}O_8$. As with **1**, **2** is a mixture of 2,4-*cis* and *trans* ketolactone isomers. Similar resonances in the ^{1}H NMR and ^{13}C NMR for C-1 to C-4 and C-35 to C-37 indicated the presence of the ketolactone mixture. Once again the *trans* isomer was present in a slight excess (56:44) as indicated by ^{1}H NMR. Loss of two molecules of water from the molecular ion in the CIMS, as well as the existence of a broad IR absorption at 3400 cm^{-1}, gave evidence for the presence of two hydroxyl groups in **2**. The signals in the ^{1}H NMR spectrum at δ 3.39 (H-15, 24), 3.83 (H-16, 23), and 3.86 (H-19, 20), integrating for a total of six protons, suggested the existence of a bis-THF ring system with two flanking hydroxyls in a *threo*/*trans*/*threo*/*trans*/*threo* relative stereochemistry, identical to that of asimicin.[10] This assignment was corroborated by three pairs of ^{13}C NMR signals at δ 74.05 and 73.93 (C-15, 24), 83.11 and 83.16 (C-16, 23) and 81.80 and 81.77 (C-19, 20). A pair of triplets at δ 2.41 and 2.39 (H-8, 10) in the ^{1}H NMR and a pair of signals at δ 211.05 and 211.02 (C-9) in the ^{13}C NMR were reminiscent of the carbonyl signals seen at C-9 in other acetogenins isolated from this species.[6] The position of the ketone was suggested to be at C-9 based on a peak in the EIMS of **2** at *m*/*z* 225. HREIMS of this fragment gave an exact mass of *m*/*z* 225.1117 (calcd. 225.1127), representing a molecular formula of $C_{12}H_{17}O_4$, and supported this assignment. Other signals in the EIMS at *m*/*z* 325 and *m*/*z* 377 helped place the ring system between C-15 and C-24. The absolute stereochemistry of **2** was established by preparing the di-*S* and di-*R* Mosher esters (**2a** and **2b**, respectively). Analyses of the spectral data allowed designation of the carbinols as C-15*R* and C-24*R* (Table 2). As in **1**, the absolute stereochemistry at C-4 is assumed to be *R*. Therefore, the structure of **2** is suggested as illustrated and named (2,4-*cis* and *trans*)-9-oxo-asimicinone.

Table 1. ^{1}H NMR (500 MHz, CDCl$_3$) data (δ) for MTPA derivatives of **1**

MTPA ester	14	16	17	18	19	20	21	22	23	25
1a	1.63	4.03	1.94, 1.53	1.81, 1.69	3.80	3.80	1.81, 1.69	1.84, 1.71	3.99	1.54
1b	1.49	4.00	2.01, 1.56	1.89, 1.74	3.83	3.64	1.76, 1.66	1.79, 1.64	3.93	1.60
Δ(δ*S*-δ*R*)	+0.14	+0.03	−0.07, −0.03	−0.08,−0.05	−0.03	+0.16	+0.05, +0.03	+0.05, +0.07	+0.06	−0.06
Configuration			C-15 *R*					C-24 *S*		

Table 2. ^{1}H NMR (500 MHz, $CDCl_3$) data (δ) for MTPA derivatives of **2**

MTPA ester	**14**, **25**	**16**, **23**	**17**, **22**	**18**, **21**	**19**, **20**
2a	1.59	3.95	1.84, 1.45	1.74, 1.65	3.78
2b	1.48	3.99	1.94, 1.55	1.94, 1.84	3.93
Δ(δ*S*-δ*R*)	+0.11	−0.04	−0.10, −0.10	−0.20,−0.19	−0.15
Configuration			C-15, C-24 *R*		

Bullacin B (**3**) has a 6-hydroxyl and is identical to bullacin[11] except that it is two methylene units longer, with the hydroxyl-flanked THF ring system beginning at C-15 for **3** rather than at C-13. Also, the stereochemistry at C-6 is *S* for bullacin but *R* for **3**. The mass of **3** was established by the $[MH]^+$ peak at *m/z* 623 in the CIMS. An exact mass of *m/z* 623.4874 (calcd. 623.4887) in the HRCIMS of **3** confirmed the molecular formula to be $C_{37}H_{66}O_7$. The presence of three hydroxyl groups in **3** was obvious from the loss of three molecules of water from the molecular ion in the CIMS and the broad IR absorbance at 3418 cm^{-1}. An α,β-unsaturated γ-lactone was indicated by the UV λ_{max} at 211 nm and an IR absorbance at 1756 cm^{-1}. The existence of the lactone moiety was further substantiated by ^{1}H NMR resonances at δ 7.03 (H-35), 5.01 (H-36), and 1.41 (H-37) and cross-peaks in the HMQC and HMBC spectra at δ 173.90 (C-1), 134.14 (C-2), 149.09 (C-35), 77.34 (C-36), and 18.93 (C-37). Two THF rings in the *threo/trans/threo/trans/threo* relative configuration were indicated by ^{1}H NMR signals at δ 3.39 and between 3.84 and 3.88, and by HMQC correlations at δ 73.80 (C-15, 24), 82.86 (C-16, 23), and 81.65 (C-19, 20). The bis-THF ring system was placed between C-15 and C-24 by the EIMS fragment ions at *m/z* 311 and 381. The presence of a third hydroxyl was evidenced by a ^{1}H NMR signal at δ 3.62 and an HMQC signal at δ 71.37. Its position was predicted to be at C-6 based on the shift of the lactone signals and resonances for protons H-3, 4a, 4b, 5a, and 5b, established from ^{1}H–^{1}H COSEY, at δ 2.30, 1.70, 1.63, 1.50, and 1.46, respectively. These signals were in agreement with other six hydroxylated acetogenins.[19] Further corroboration came from the peak in the EIMS at *m/z* 169 corresponding to cleavage next to the 6-OH group. HREIMS of this fragment gave an exact mass at *m/z* 169.0859 (calcd. 169.0865) corresonding to an elemental composition of $C_9H_{13}O_3$. The absolute stereochemistry of **3** was determined by preparing the tri-*S* and tri-*R* Mosher ester derivatives (**3a** and **3b**, respectively). Examination of the ^{1}H NMR and COSEY spectra led to the assignment of the absolute stereochemistries as C-6*R*, C-15*R*, and C-24*R* (Table 3). Since there was no hydroxyl at the C-4 position, a CD spectrum was obtained in order to elucidate the absolute stereochemistry at C-36. A negative Cotton effect was observed at 238 nm, matching data for another 4-deoxy acetogenin, squamocin,[2,20] and allowing the assignment of the stereochemistry at C-36 as *S*. The structure of **3** is, therefore, suggested to be as illustrated and named bullacin B (Figure 2).

In a seven day MTT assay, **1**–**3** all showed significant cytotoxicitities against a panel of six human tumor cell lines (Table 4). The MTT assay uses a tetrazolium salt, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] for the colorimetric quantitation of cell survival and proliferation. The tetrazolium ring of MTT is cleaved by dehydrogenase enzymes in the active

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$) data (δ) for MTPA derivatives of **3**

MTPA ester	**35**	**34**	**33**	**3**	**4**	**14**	**16**, **23**	**17**, **22**	**19**, **20**	**18**, **21**	**25**
3a	1.39	4.97	6.86	2.20	1.45	1.60	3.95	1.83, 1.45	3.78	1.77,1.64	1.60
3b	1.40	4.99	6.96	2.28	1.60	1.56	4.00	1.93, 1.56	3.93	1.93,1.84	1.56
Δ(δ*S*-δ*R*)	−0.01	−0.02	−0.10	−0.08	−0.15	+0.04	−0.05	−0.11	−0.15	−0.16,−0.20	+0.04
Configuration			C-6 *R*						C-15, C-24 *R*		

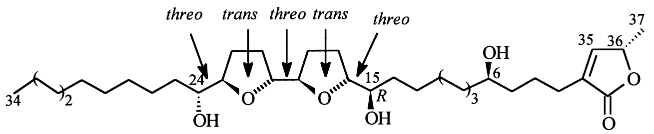


Figure 2. Structure of **3**.

Table 4. Biological data for compounds **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	7.42×10^{-2}	1.85	1.76	1.70	1.80	2.62	1.10
2	6.69×10^{-1}	5.71×10^{-3}	2.59×10^{-1}	4.03×10^{-3}	4.55×10^{-1}	1.12	1.22
3	1.86×10^{-2}	8.98×10^{-7}	2.47×10^{-7}	4.51×10^{-3}	>1	>1	4.11×10^{-1}
Adriamycin[h]	2.57×10^{-1}	1.33×10^{-2}	1.95×10^{-1}	4.58×10^{-2}	1.01×10^{-2}	1.86×10^{-2}	2.16×10^{-2}

[a] Brine shrimp lethality test.[4]
[b] Human lung carcinoma.[27]
[c] Human breast adenocarcinoma.[28]
[d] Human colon adenocarcinoma.[29]
[e] Human kidney carcinoma.[28]
[f] Human prostrate adenocarcinoma.[30]
[g] Human pancreatic carcinoma.[31]
[h] Positive control, BST LC$_{50}$ value taken from Ref. 32.

Table 5. ^{1}H NMR and ^{13}C NMR data (δ) for **1**

Position	^{1}H NMR (500 MHz, CDCl$_3$, *J* in Hz)		^{13}C NMR (125 MHz, CDCl$_3$)	
	1 *trans*	**1** *cis*	**1** *trans*	**1** *cis*
1	—	—	180.5	180.5
2	3.02 m (1H)	3.03 m (1H)	43.88	44.29
3a	1.99 m (1H)	1.50 m (1H)	34.53	34.53
3b	2.22 ddd (1H) (12.9,9.6,3.4)	(2.60 ddd (1H) (12.3,9.4,5.6)	34.53	34.53
4	4.55 dddd (1H) (8.3,8.2,5.7,3.2)	4.40 dddd (1H) (10.7,7.4,5.4,5.4)	78.85	79.28
5a	1.55 m (1H)	1.45 m (1H)	36.77	
5b	1.70 m (1H)	1.75 m (1H)	36.77	
6	1.26 br s (2H)		26.01[a]	
7–12	1.26 br s (12H)		29.29–29.68	
13	1.26 br s (2H)		25.27[a]	
14	1.41 m (2H)		33.48	
15	3.40 m (1H)		74.09	
16	3.86 m (1H)		83.18[b]	
17	1.99 m, 1.63 m (2H)		28.87[c]	
18	1.99 m, 1.63 m (2H)		28.36[c]	
19	3.93 m (1H)		82.39[b]	
20	3.86 m (1H)		82.18[b]	
21	1.99 m, 1.63 m (2H)		28.87[c]	
22	1.89 m, 1.81 m (2H)		28.36[c]	
23	3.93 m (1H)		82.83[b]	
24	3.86 (1H)		71.56	
25	1.36 m (2H)		32.56	
26	1.26 br s (2H)		25.64[a]	
27–29	1.26 br s (6H)		29.29–29.68	
30	1.26 br s (2H)		31.89	
31	1.28 m (2H)		22.64	
32	0.88 t (7.0) (3H)		14.03	
33a	2.67 dd (19.5, 9.0) (1H)	2.61 dd (18.5, 9.0) (1H)		
33b	3.04 dd (19.5, 3.0) (1H)	3.11 dd (18.5, 3.0) (1H)	35.62	35.50
34	—	—	205.8	205.8
35	2.20 s (3H)	2.20 s (3H)	24.66	24.66

[a–c] Values may be interchangeable.

mitochondria to produce a purple formazan product.[21] The product is only formed in viable cells. The resulting water insoluble formazan is dissolved by adding DMSO, and the color is read by a scanning multiwell spectrophotometer (ELISA reader). Optical densities read at 570 nm are directly proportional to the number of living cells. Compound **2** shows 100-fold selectivity for the lung (A-549) and colon (HT-29) tumor cell lines. Compound **3** is especially active against the lung (A-549) and breast (MCF-7) cell lines. Against these two tumor types, **3** was 100,000 to 1,000,000 times as active as the positive control, adriamycin. The acetogenins exert their cytotoxic and their in vivo antitumor effects, in part, by inhibiting complex I of the electron transport system in the mitochondria and by blocking the NADH oxidase enzyme peculiar to the plasma membranes of cancerous cells.[22,23] Both modes of action result in the depletion of ATP within the cell. As a result, the acetogenins are particularly effective against pesticide-resistant strains of insects and against tumors which possess the ATP-dependent multiple-drug resistance (MDR) efflux pump.[24–26]

Experimental

Instruments

UV spectra were measured on a Beckman DU 640 series spectrophotometer. IR data were collected using a Perkin–Elmer 1600 series FTIR instrument. Optical

Table 6. ^{1}H NMR and ^{13}C NMR data (δ) for **2**

Position	^{1}H NMR (500 MHz, $CDCl_3$, *J* in Hz)		^{13}C NMR (125 MHz, $CDCl_3$)	
	2 *trans*	**2** *cis*	**2** *trans*	**2** *cis*
1	—	—	178.26	178.78
2	3.02 m (1H)	3.03 m (1H)	43.67	44.15
3a	1.99 m (1H)	1.45 m (1H)	33.18	34.36
3b	ddd (1H) (12.9,9.6,3.4)	ddd (1H) 12.3,9.4,5.6)	33.18	34.36
4	dddd (1H) (8.3,8.2,5.7,3.2)	dddd (1H) (10.7,7.4,5.4,5.4)	78.59	79.03
5a	1.55 m (1H)	1.50 m (1H)	35.37	36.61
5b	1.70 m (1H)	1.75 m (1H)	35.37	36.61
6–7	1.26 br s (4H)		24.88–25.60	
8	1.41 m (2H)		42.34	42.36
9	—		211.02	211.05
10	1.41 m (2H)		42.73	
11	1.26 m (2H)		24.88–25.60	
12	1.26 m (2H)		29.56–29.95	
13	1.26 m (2H)		24.88–25.60	
14	1.39 m (2H)		33.35[a]	
15	3.39 m (1H)		74.05[b]	
16	3.83 m (1H)		83.11[c]	
17	1.98 m, 1.65 m (2H)		28.32–29.29	
18	1.98 m, 1.65 m (2H)		28.32–29.29	
19	3.86 m (1H)		81.80[d]	
20	3.86 m (1H)		81.77[d]	
21	1.98 m, 1.65 m (2H)		28.3229.29	
22	1.98 m, 1.65 m (2H)		28.32–29.29	
23	3.83 m (1H)		83.16[c]	
24	3.39 m (1H)		73.93[b]	
25	1.39 m (2H)		33.09[a]	
26	1.26 br s (2H)		24.88–25.60	
27–31	1.26 br s (10H)		29.56–29.95	
32	1.26 br s (2H)		31.86	
33	1.29 m (2H)		22.64	
34	0.88 t (7.0) (3H)		14.07	
35a	2.68 dd (19.5, 9.0) (1H)	2.61 dd (18.5, 9.0) (1H)	35.16	35.30
35b	3.05dd (19.5, 3.0) (1H)	3.09 dd (18.5, 3.0) (1H)	35.16	35.30
36	—	—	205.56	205.62
37	2.20 s (3H)	2.20 s (3H)	23.23	23.30

[a–d]Values may be interchangeable.

rotations were obtained on a Perkin–Elmer model 241 polarimeter. Circular dichroism (CD) spectra were collected on a JASCO model J600 CD Spectropolarimeter. ^1H NMR and ^{13}C NMR spectra were obtained on a Varian VXR-500S spectrometer. Low-resolution EIMS and CIMS data were collected on a Finnigan 4000 spectrometer. High-resolution EIMS and CIMS were obtained on a 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.

Plant material

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

Bioassays

The toxicity of column fractions and pure compounds was monitored using the brine shrimp lethality test (BST).[4] Cell culture assays were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in seven day MTT assays with adriamycin as the positive control.[21]

Extraction and isolation

The dried and pulverized bark (7.4 kg) was extracted with EtOH (1.83 kg F001, BST $LC_{50} = 1.55$ ppm). The residue was partitioned between CH_2Cl_2 and H_2O to yield a CH_2Cl_2 soluble residue (842 g F003, BST $LC_{50} = 1.68$ ppm) and a H_2O soluble residue (128.6 g F002, BST $LC_{50} = 950.14$ ppm). F003 was further partitioned between 90% aq. MeOH and hexane resulting in a MeOH soluble residue (545.5 g F005, BST $LC_{50} = 1.52$ ppm) and a hexane soluble residue (162.9 g F006, BST $LC_{50} = 122.97$ ppm). 500.5 g of F005 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 further resolved on another Si gel column eluted with hexane and Me_2CO. The pools from this column, that were bioactive in the BST,[4] were subjected to a third Si gel column eluted with $CHCl_3$ and MeOH. Compounds **1**–**3** were purified by repeated normal-phase and reversed-phase HPLC using solvent systems of hexane:MeOH:THF (90:9:1) and acetonitrile:H_2O (70:30), respectively.

Derivatizations

To **1**–**3** (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. After 4 h at room temp. the mixture was passed through a small pipet column (0.6×6 cm) packed with Si gel and eluted with 5 mL CH_2Cl_2. This residue was then 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 **3**. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride yielded the *R*-Mosher ester of **3**.

(2,4-*cis* and *trans*)-Squamolinone (1). White amorphous powder (4.5 mg); $[\alpha]_D^{23} = +21.4°$ (*c* 0.073) (CH_2Cl_2); UV (MeOH) $\lambda_{max} = 203$ nm (log $\varepsilon = 2.85$); IR ν_{max} film cm^{-1}: 3426, 2920, 2850, 1767; CIMS *m/z* $[MH]^+$ 595 (78), $[MH\text{-}H_2O]^+$ 578 (47), $[MH\text{-}2H_2O]^+$ 561 (23); EIMS *m/z* 363 (17), 345 (12), 311 (35), 283 (7); HRCIMS *m/z* 595.4555 for $C_{35}H_{62}O_7$ (calcd. 595.4573); HREIMS *m/z* 311.2228 for $C_{18}H_{31}O_4$ (calcd. 311.2222); ^1H NMR ($CDCl_3$, 500MHz) and ^{13}C NMR ($CDCl_3$, 125MHz), see Table 5.

(2,4-*cis* and *trans*)-9-*oxo*-Asimicinone (2). White amorphous powder (6.1 mg); $[\alpha]_D^{23} = +19.7°$ (*c* 0.10) (CH_2Cl_2); UV(MeOH) $\lambda_{max} = 203$ nm (log $\varepsilon = 3.55$); IR

Table 7. ^1H NMR and ^{13}C NMR data (δ) for **3**

Position	^1H NMR (500 MHz, $CDCl_3$, *J* in Hz)	^{13}C NMR (125 MHz, $CDCl_3$)
1	—	173.90
2	—	134.14
3	2.30 m (2H)	24.85
4	1.70 m, 1.63 m (2H)	23.24
5	1.50 m, 1.46 m (2H)	36.50
6	3.62 m (1H)	71.37
7	1.42 m (2H)	37.13
8–13	1.26 br s (12H)	28.51–29.97
14	1.41 m (2H)	33.15
15	3.39 m (1H)	73.80
16	3.84 m (1H)	82.86
17	1.98 m, 1.67 m (2H)	28.31
18	1.98 m, 1.67 m (2H)	28.31
19	3.88 m (1H)	81.65
20	3.88 m (1H)	81.65
21	1.98 m, 1.67 m (2H)	28.31
22	1.98 m, 1.67 m (2H)	28.31
23	3.84 m (1H)	82.86
24	3.39 m (1H)	73.80
25	1.41 m (2H)	33.15
26–31	1.26 br s (12H)	28.51–29.97
32	1.26 br s (2H)	31.65
33	1.30 m (2H)	22.44
34	0.88 t (7.0) (3H)	13.78
35	7.03 m (1H)	149.09
36	5.01dq (1.5, 6.5) (1H)	77.34
37	1.41 d (6.5) (3H)	18.93

λ_{max} (film) cm^{-1}: 3390, 2921, 2851, 1770, 1705; CIMS *m/z* $[MH]^+$ 637 (7), $[MH\text{-}H_2O]^+$ 619 (55), $[MH\text{-}2H_2O]^+$ 601 (22); EIMS *m/z* 377 (1), 359 (4), 341 (1), 325(6), 307 (6), 289 (3), 225 (3), 207 (9); HRCIMS *m/z* 637.4665 (calcd. 637.4679); HREIMS *m/z* 225.1117 (calcd. 225.1127); ^{1}H NMR ($CDCl_3$, 500 MHz), and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 6.

Bullacin B (3). White amorphous powder (1.8 mg); $[\alpha]_D^{23} = +43.8°$ (*c* 0.030) (CH_2Cl_2); UV (MeOH) $\lambda_{max} = 211$ nm (log $\varepsilon = 3.79$); IR λ_{max} film cm^{-1}: 3418, 2925, 2853, 1756; CIMS *m/z* $[MH]^+$ 623 (100), $[MH\text{-}H_2O]^+$ 605 (70), $[MH\text{-}2H_2O]^+$ 587 (34), $[MH\text{-}3H_2O]^+$ 569 (39); EIMS *m/z* 433 (2), 415 (5), 397 (15), 363 (3), 345 (65), 327 (6), 311 (25), 293 (100), 275 (7), 169 (14), 151 (8); HRCIMS *m/z* 623.4874 (calcd. 623.4887); HREIMS *m/z* 169.0859 (calcd. 169.0865); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 7.

Acknowledgements

This work was supported, in part, by RO1 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.

References

1. Morton, J. *Fruits of Warm Climates*; Media: Miami, 1973; pp 75–80.
2. Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakenuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175.
3. Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.
4. Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31.
5. Hopp, D. C.; Zeng, L.; Gu, Z.-M.; McLaughlin, J. L. *J. Nat. Prod.* **1996**, *59*, 97.
6. Hopp, D. C.; Zeng, L.; Gu, Z.-M.; Kozlowski, J. F.; McLaughlin, J. L. *J. Nat. Prod.* **1997**, *60*, 581.
7. Hopp, D. C.; Alali, F. Q.; Gu, Z.-M.; McLaughlin, J. L. *Phytochemistry* **1998**, accepted for publication.
8. Duret, P.; Gromek, D.; Hocquemiller, R.; Cavé, A. *J. Nat. Prod.* **1994**, *57*, 911.
9. Zhao, G.-X.; Chao, J.-F.; Zeng, L.; McLaughlin, J. L. *Nat. Toxins* **1996**, *4*, 128.
10. Rupprecht, J. K.; Chang, C.-J.; Cassady, J. M.; McLaughlin, J. L. *Heterocycles* **1986**, *24*, 1197.
11. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1993**, *36*, 2221.
12. Hui, Y.-H.; Rupprecht, J. K.; Liu, Y. M.; Anderson, J. E.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463.
13. Duret, P.; Laurens, A.; Hocquemiller, R.; Cortes, D.; Cavé, A. *Heterocycles* **1994**, *39*, 741.
14. Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203.
15. Hoye, T. R.; Suhadolnik, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 4402.
16. Hoye, T. R.; Zhuang, Z.-P. *J. Org. Chem.* **1988**, *53*, 5578.
17. Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *95*, 512.
18. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
19. Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L.; In *Recent Advances in Phytochemistry*, Romeo, J. T.; et al. eds., Plenum Press: New York, 1995, 29, pp. 249–310.
20. Zhao, G.-X.; Chao, J.-F.; Zeng, L.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 25.
21. Monks, A.; Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Vistica, D.; Hose, C.; Langley, J.; Cronice, P.; Vaigio-Wolff, A.; Gray-Goodrich, M.; Cambell, H.; Mayo, H.; Boyd, M. R. *J. Nat. Cancer Inst.* **1991**, *83*, 757.
22. Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427.
23. Morre, J. D.; Decabo, R.; Farley, C.; Oberlies, H. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.
24. Oberlies, N. H.; Croy, V. L.; Harrison, M. L.; McLaughlin, J. L. *Cancer Lett.* **1997**, *115*, 73.
25. Oberlies, N. H.; Chang, C.-J.; McLaughlin, J. L. *J. Med. Chem.* **1997**, *40*, 2102.
26. Alali, F. Q.; Kaakeh, W.; Bennett, G. W.; McLaughlin, J. L. *J. Econ. Entomol.* **1998**, accepted for publication.
27. Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.
28. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.
29. Fogh, J.; Trempe, G. *Human Tumor Cells*; Fogh, J.; Ed.; Plenum Press: New York, **1973**, pp 115–119.
30. Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16.
31. Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128.
32. Ratnayake, S.; Gu, Z.-M.; Miesbauer, L. R.; Smith, D. L.; Wood, K. V.; Evert, D. R.; McLaughlin, J. L. *Can. J. Chem.* **1994**, *72*, 287.