PERGAMON

Phytochemistry 50 (1999) 815–821

PHYTOCHEMISTRY

Two bioactive mono-tetrahydrofuran acetogenins, annoglacins A and B, from *Annona glabra*

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Received 3 March 1998

Abstract

Two new bioactive mono-THF Annonaceous acetogenins, annoglacins A and B, have been isolated from the fractionated ethanolic extracts of the leaves of *Annona glabra*, directing the fractionation with the brine shrimp lethality test (BST). Their structures were elucidated based on spectroscopic and chemical methods and the absolute stereochemistries were determined by the advanced Mosher ester method. Annoglacins A and B were selectively 1000 and 10,000 times, respectively, more potent than adriamycin against the human breast carcinoma (MCF-7) and pancreatic carcinoma (PACA-2) cell lines in our panel of six human solid tumor cell lines.

Keywords: *Annona glabra*; Annonaceae; Acetogenins; Annoglacin A; Annoglacin B; Cytotoxicities; Brine shrimp

1. Introduction

The Annonaceous acetogenins are reported to possess numerous biological properties including antitumor, pesticidal, antimalarial, antimicrobial and anthelmintic activities (Zeng et al., 1996). *Annona glabra* L. (Annonaceae), commonly known as pond-apple, is a tropical tree distributed mainly in the Americas and in southeast Asia. It is used in traditional medicines as an insecticide and a parasiticide (Ohsawa, Atsuzawa, Mitsui, & Yamamoto, 1991 (Padmaja, Thankamany, Hara, Fujimoto, & Hisham, 1995). Several bioactive Annonaceous acetogenins were previously isolated from the bark and leaves (Ohsawa et al., 1991; Liu, Alali, Craig, Rogers, Pilarinou, & McLaughlin, 1998; Liu, Alali, Pilarinou, & McLaughlin, 1998). As part of our continuing efforts to find new antitumor and pesticidal agents, two additional bioactive acetogenins, annoglacins A (**1**) and B (**2**), were isolated from the ethanolic extracts of the leaves, obtained from trees native to Florida, using bioactivity-directed fractionation with the brine shrimp lethality test (BST) (McLaughlin, 1991). The structures of **1** and **2** were identified as mono-tetrahydrofuran (mono-THF) ring acetogenins by NMR and mass spectroscopic techniques. The absolute configurations of **1** and **2** were determined through analyses of their respective per-Mosher esters (Rieser et al, 1992; Rieser et al, 1994. The new compounds were selectively cytotoxic among six human solid tumor cell lines with, respectively, 1000 and 10,000 times the potency of adriamycin against human breast carcinoma (MCF-7) and pancreatic carcinoma (PACA-2) cell lines (Fig. 1).

2. Results and discussion

Compound **1** was isolated as a whitish waxy solid. Its molecular weight was suggested by the mass peak at m/z 625 $[MH]^+$ in the CIMS. The suggested molecular formula of $C_{37}H_{69}O_7$ was confirmed by HRCIMS of the molecular ion which gave an exact mass of m/z 625.5042 (calcd. 625.5043). Compound **1** showed an IR carbonyl absorption at 1742 cm^{-1}, a UV (MeOH) λ_{max} at 219 nm ($\log \varepsilon$, 3.62), six resonances at δ 7.19 (q, H-35), 5.06 (qd, H-36), 1.43 (d, H-37), 2.41 and 2.53 (dddd, H-3) and 3.86 (H-4) in the

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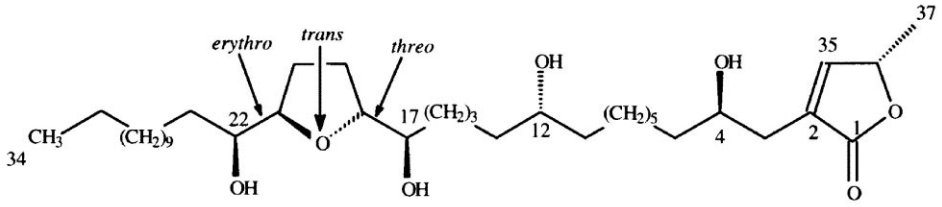
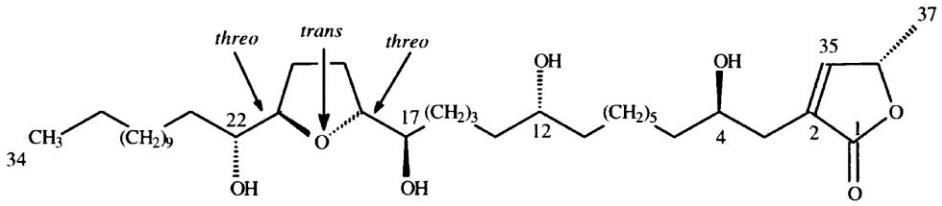
0031-9422/99/$ - see front matter
PII: S0031-9422(98)00466-X



1: annoglacin A



2: annoglacin B

Fig. 1.

^{1}H NMR spectrum and six peaks at δ 174.65 (C-1), 151.84 (C-35), 131.18 (C-2), 77.99 (C-36), 19.09 (C-37) and 69.95 (C-4) in the ^{13}C NMR spectrum (Table 1). These are characteristic spectral features for the methylated α,β-unsaturated γ-lactone fragment, with the presence of an OH group at the C-4 position, as commonly found among many of the Annonaceous acetogenins (Zeng et al., 1996; Cavé, Figadére, Laurens, & Cortes, D., 1997).

The presence of four OH moieties in **1** was suggested by a prominent IR OH absorption at 3413 cm^{-1} and four successive losses of H_2O (m/z −18) from the [MH^+] in the CIMS. The ^{13}C NMR of **1**, which showed four resonances due to oxygen-bearing carbons at δ 69.95 (C-4), 71.84 (C-12), 74.28 (C-17) and 71.54 (C-22), also indicated the existence of four secondary hydroxyls. The presence of a mono-THF ring, with two OH groups flanking the ring, was apparent by the presence of ^{13}C NMR resonances at δ 71.54 (C-22) and 74.28 (C-17) due to hydroxylated carbons and δ 82.15 (C-21) and 83.18 (C-18) due to oxygenated carbons of the THF-ring. These signals and their corresponding ^{1}H NMR resonances at δ 3.40 (H-17) and δ 3.80 (H-18, H-21, and H-22) are also indicative of di-hydroxyl THF moieties.

The placements of the hydroxyl groups and the mono-THF ring system along the aliphatic chain were determined based on the analysis of the EIMS spectral data (Fig. 2), where the diagnostic fragments at m/z 425 and 355 were characteristic of the hydroxylated mono-THF ring system located from C-17 to C-22. The HREIMS gave an ion at m/z 269.1752 (calcd. 269.1753) for a fragment with the formula $C_{15}H_{25}O_4$ which confirmed the position of the fourth OH at C-12.

Signals at δ 1.96 and 1.64 for the protons at C-19/20 indicated the *trans* configuration according to Fujimoto's models (Fujimoto et al., 1994). However, in **1** the OH at C-17 is five bonds away from the C-12 position and conversion of this 1,6-diol into a cyclic intramolecular formaldehyde acetal has not been successful (Gu et al, 1994). Thus, it was not possible to use such information to identify which side of the THF ring bears the *erythro* configuration. However, based on the similar chemical shifts in the ^{1}H- and ^{13}C-NMR spectra, as compared with annosenegelin (Sahpaz, Gonzalez, Hocquemiller, Zafra-Polo, & Cortes, 1996), jetein (Cortes, Myint, Lebouef, Cave, 1991) and synthetic models (Fujimoto et al., 1994), the stereochemistries were reasoned to be *threo* at C-17/C-18 and *erythro* at C-21/C-22 in **1**. Thus, the relative configuration of **1** was proposed as *threo*/*trans*/*erythro* from C-17 to C-22. This conclusion is supported by the fact that no mono-THF acetogenins with *erythro*/*trans*/*threo* configurations, proceeding down the hydrocarbon chain, have ever been reported (Zeng et al., 1996).

The (*S*)- and (*R*)-methoxy (fluoromethyl) phenylacetic acid (MPTA) esters (Mosher esters) of **1** were prepared and careful examination of the ^{1}H NMR and

Table 1
^{13}C NMR and ^{1}H NMR (δ, J in Hz) of **1** and its *S*- and *R*-Mosher esters

	^{13}C NMR **1** (125 MHz)	^{1}H (500 MHz)			$\Delta\delta_H$, $\delta_S-\delta_R$
		1 (J in Hz)	**1**-*S*-MPTA	**1**-*R*-MPTA	
1	174.65	–			
2	131.18	–			
3a	33.34	2.53 (dddd, 15.0, 3.3, 1.0, 1.0)	2.59	2.67	negative
3b	–	2.41 (dddd, 15.0, 8.6, 1.0, 1.0)	2.56	2.60	negative
4	69.95	3.86 (m)	5.30	5.37	*R*
5	37.41	1.26–1.68 (m)	1.65	1.53	positive
6–9	29.33–29.66	1.26–1.68 (m)			
10	25.49–25.96	1.26–1.68 (m)			
11	37.35	1.26–1.68 (m)			
12	71.84	3.60 (m)	5.02	4.92	*R*
13	37.32	1.26–1.68 (m)			
14–15	25.49–25.96	1.26–1.68 (m)			
16	32.54	1.26–1.68 (m)	1.47	1.31	positive
17	74.28	3.40 (m)	4.95	4.90	*R*
18	83.18	3.80 (m)	3.81	3.68	positive
19a, 20a	28.57	1.68 (m)	1.75, 1.34	1.76, 1.39	negative
19b, 20b	25.22	1.99 (m)	1.75, 1.65	1.79, 1.68	negative
21	82.15	3.80 (m)	3.91	3.96	negative
22	71.54	3.80 (m)	5.21	5.26	*S*
23	33.11	1.26–1.68 (m)	1.51	1.55	negative
24	25.49–25.96	1.26–1.68 (m)			
25–31	29.33–29.66	1.26–1.68 (m)			
32	31.89	1.26–1.68 (m)			
33	22.60	1.26–1.68 (m)			
34	14.09	0.88 (t, 7.0)			
35	151.84	7.19 (q, 1.4)	6.72	6.95	negative
36	77.99	5.06 (qd, 6.5, 1.4)	4.86	4.90	*S*
37	19.09	1.43 (d, 6.5)	1.30	1.32	negative

^{1}H–^{1}H COSY analysis of these two derivatives was used to establish the absolute configuration. In the per-Mosher ester of **1**, the two phenyl rings on the Mosher esters flanking the THF ring take the same orientation, causing H-18, 19, 20 and 21 to receive both shielding and deshielding effects at the same time. Thus, it was difficult to establish the absolute stereochemistries of the carbinol centers at C-17 and C-22. However, it is anticipated that the chemical shift of H-23 would be significantly influenced by the C-22-OMPTA group, but much less by the MPTA group at C-17. With this assumption, the data was interpretable and the absolute stereochemistry was assigned as *S* at C-22 (Table 1). Since the relative stereochemistry of the mono-THF ring system was known, the absolute stereochemistry at C-17 was, thus, automatically defined as *R*. Configurations at C-4 and C-36 were *R* and *S*, respectively, as determined by Hoye's method (Hoye, Hanson, Hasenwinkel, Ramirez, & Zhuang, 1994); all 4-OH acetogenins that have been found so far have the *R* configuration at C-4 and the *S* at C-36 (Zeng et al., 1996). The ^{13}C NMR chemical shift of **1** was assigned by comparison of the NMR data of Fujimoto's model compounds and the well-established annosenegalin (Fujimoto et al., 1994; Sahpaz et al, 1996).

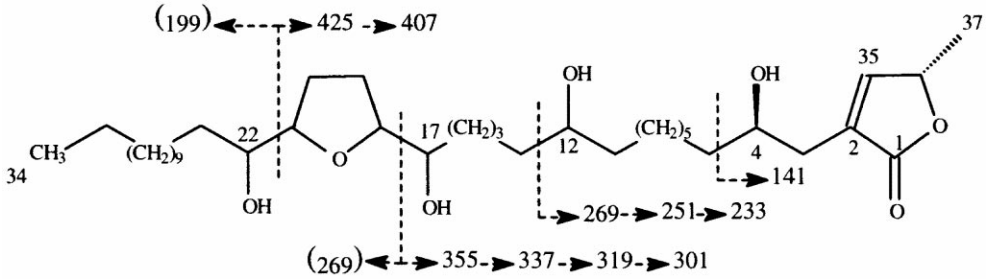


Fig. 2.

Annoglacin B (**2**) was also isolated in the form of a whitish waxy solid. The molecular weight was determined to be 624 amu based on a peak in the CIMS at m/z 625 corresponding to the $[MH]^+$ ion. The suggested molecular formula of $C_{37}H_{69}O_7$ was proven by the exact mass analysis of the $[MH]^+$ peak in the HRCIMS at m/z 625.5043 (calcd. 625.5043). This molecular formula was identical to that of **1**. The IR spectrum showed strong absorptions at 1735 cm^{-1} for the carbonyl and at 3453 cm^{-1} for the OH moieties. In the CIMS, a series of peaks at m/z 607, 589, 571 and 553, arising from the successive losses of four molecules of H_2O (m/z-18), were observed, again suggesting the presence of four OH groups. Four resonances, due to oxygen-bearing carbons at δ 69.95 (C-4), 71.83 (C-12), 74.00 (C-17) and 74.08 (C-22) in the ^{13}C NMR of **2**, also suggested the existence of four secondary hydroxyls. As with **1**, resonances at δ 7.19 (q, H-35), 5.06 (qd, H-36), 1.43 (d, H-37), 3.85 (H-4) and 2.41 and 2.53 (dddd, H-3) in the ^1H NMR spectrum and further evidence in the ^{13}C NMR spectrum, which showed six peaks at δ 174.68 (C-1), 151.87 (C-35), 131.17 (C-2), 78.00 (C-36), 69.95 (C-4) and 19.08 (C-37) (Table 2), suggested the existence of the methylated α,β-unsaturated γ-lactone fragment, with the presence of an OH group at C-4. The presence of a mono-THF ring, with two OH groups flanking the ring, was apparent by the presence of ^{13}C NMR resonances at δ 74.00 (C-17) and 74.08 (C-22) due to hydroxylated carbons and δ 82.57 (C-18) and 82.64 (C-22) due to oxygenated carbons of the THF-ring. These signals and their corresponding ^1H NMR resonances at δ 3.41 (H-17 and H-22) and δ 3.80 (H-18 and H-21) are also indicative of di-hydroxyl THF moieties.

The carbon skeleton and placement of the ring and these four OH groups along the hydrocarbon chain were determined based on the EIMS spectral analysis of **2** (Fig. 2). The EIMS displayed peaks at m/z 425 and m/z 355 which, as in **1**, suggested the placement of the mono-THF ring system and its two flanking hydroxyls from C-17 to C-22. The fourth OH was, again as in **1**, placed at C-12 by analysis of the fragments in the EIMS spectra of **2**. The location of the fourth hydroxyl at C-12, rather than at C-10, was also indicated because the ^1H NMR signals of H-17 and H-22 were a pseudo-quintet; if, the fourth OH was placed at C-10,

Table 2
^{13}C NMR and ^1H NMR (δ, J in Hz) of **2** and its *S*- and *R*-Mosher esters

	^{13}C NMR **2** (125 MHz)	^1H (500 MHz)			$\Delta\delta_H$, $\delta_S-\delta_R$
		2 (J in Hz)	**2**-*S*-MPTA	**2**-*R*-MPTA	
1	174.68	–			
2	131.17	–			
3a	33.32	2.53 (dddd, 15.0, 3.3, 1.0, 1.0)	2.56	2.60	negative
3b	–	2.41 (dddd, 15.0, 8.6, 1.0, 1.0)	2.60	2.70	negative
4	69.95	3.85 (m)	5.09	5.08	*R*
5	37.37	1.26–1.68 (m)	1.56	1.52	positive
6–9	29.33–29.66	1.26–1.68 (m)			
10	25.48–25.64	1.26–1.68 (m)			
11	37.33[c]	1.26–1.68 (m)			
12	71.83	3.60 (m)	5.05	5.02	*R*
13	37.28[c]	1.26–1.68 (m)			
14–15	25.48–25.64	1.26–1.68 (m)			
16	33.43[d]	1.26–1.68 (m)	1.58	1.52	positive
17	74.00[a]	3.41 (m)	4.93	5.01	*R*
18	82.57[b]	3.80 (m)	3.90	4.01	negative
19a, 20a	28.73	1.68 (m)	1.64, 1.35	1.90, 1.56	negative
19b, 20b	28.73	1.99 (m)	1.64, 1.35	1.90, 1.56	negative
21	82.64[b]	3.80 (m)	3.90	4.01	negative
22	74.08[a]	3.41 (m)	4.90	5.01	*R*
23	33.32[d]	1.26–1.68 (m)	1.51	1.50	positive
24	25.48–25.64	1.26–1.68 (m)			
25–31	29.33–29.66	1.26–1.68 (m)			
32	31.89	1.26–1.68 (m)			
33	22.66	1.26–1.68 (m)			
34	14.09	0.88 (t, 7.0)			
35	151.87	7.19 (q, 1.4)	6.72	6.96	negative
36	78.00	5.06 (qd, 6.5, 1.4)	4.85	4.90	*S*
37	19.08	1.43 (d, 6.5)	1.28	1.30	negative

[a–d]Signals may be interchangeable.

wherein the C-10 and C-17 protons would have been separated by six methylenes, the ^{1}H NMR signals of H-17 and H-22 would have appeared as a pseudo-quartet, as in annomontacin (Gu, Zhao, Oberlies, Zeng, & McLaughlin, 1995). The HREIMS of the fragmentation peak at *m/z* 269.1747 (calcd. 269.1753), representing $C_{15}H_{25}O_4$, also validated this assignment.

The relative configuration of **2** was deduced as *threo*/*trans*/*threo* from C-17 to C-22 based on the similar chemical shifts in the ^{1}H- and ^{13}C-NMR spectra as compared with those of the model compounds, annonacin and xylomaticin (Alkofahi, Rupprecht, Smith, Chang, & McLaughlin, 1988; Colman-Saizarbitoria et al., 1994). Again, the absolute stereochemistry of **2** was established by using advanced Mosher ester methodology. Analysis of the differences between the (*S*)- and (*R*)-Mosher derivatives, combined with the prior knowledge of relative stereochemistry, allowed the absolute stereochemical assignments of the carbinol centers as C-4*R*, C-12*R*, C-17*R*, C-22*R* and C-36*S* (Table 2). As in **1**, the ^{13}C NMR chemical shift of **2** was assigned by comparison of the NMR data of Fujimoto's model compounds (Fujimoto et al., 1994).

The biological activities of **1** and **2** are summarized in Table 3. These compounds are approximately equipotent and more selective than adriamycin across the six human tumor cell lines in our seven-day MTT human solid tumor cytotoxicity tests. Compounds **1** and **2** showed activity with potency comparable with that of adriamycin against the lung carcinoma (A-549) (Giard et al., 1973), prostate carcinoma (PC-3) (Kaighn, Narayan, Ohnuki, Lechner, & Jones, 1979) and colon adenocarcinoma (HT-29) cell lines (Fogh & Trempe, 1975). Selectivities were exhibited for the breast carcinoma (MCF-7) (Soule, Vazquez, Long, Albert, & Brennan, 1973) and pancreatic carcinoma (PACA-2) cell lines (Yunis, Arimura, & Russin, 1977), with up to 10,000 times the potency of the positive control, adriamycin. Most mono-THF ring acetogenins are not so potent, and **1** and **2** suggest enhancement of potency with a 12-OH and placement of the ring and its flanking hydroxyls further down the chain at C-17 (Alfonso et al., 1996; He et al, 1997; Shimada, Grutzner, Kozlowski, & McLaughlin, 1998).

All of the acetogenins tested, so far, decrease oxygen uptake in mitochondrial tests (Landolt et al., 1995). The acetogenins exert their bioactive effects, at least in part, by inhibition of NADH ubiquinone oxidoreductase (complex I) (Londershausen et al., 1991) and also by inhibition of the NADH oxidase peculiar to the plasma membranes of cancerous cells (Morré, Decabo, Farley, Oberlies, & McLaughlin, 1995). These effects deplete ATP levels and cause programmed cell death (apoptosis) (Rieser et al, 1994; Londershausen et al., 1991). Recently, the acetogenins were found to inhibit cells that are multiple drug resistant due to ATP dependent efflux purges (Oberlies, Chang, & McLaughlin, 1997). The acetogenins, by nature of their ATP-depleting modes of action, are more toxic to these cells than they are against the wild-type cells which do not possess the MDR pump and show more of a cytostatic response to the acetogenins (Oberlies et al., 1997). They also inhibit pesticide resistant German cockroaches (Alali, Kaakeh, Bennett, & McLaughlin, 1998). Thus, the acetogenins offer an excellent potential for development as new antitumor and pesticidal agents which thwart such resistance mechanisms.

Table 3
Bioactivities of **1** and **2** [ED_{50} (μg/ml)]

Human tumor cell lines	Compounds [BST LC_{50} (μg/ml)][a]		
	1 (4.9×10^{-1})	**2** (1.7×10^{-1})	adriamycin[g]
A-549[b]	5.3×10^{-3}	2.8×10^{-3}	7.2×10^{-3}
MCF-7[c]	9.6×10^{-4}	6.2×10^{-4}	1.2×10^{-1}
HT-29[d]	5.3×10^{-3}	5.3×10^{-3}	3.3×10^{-3}
A-498[e]	9.0×10^{-1}	4.0×10^{-1}	5.2×10^{-3}
PC-3[f]	2.0×10^{-3}	8.4×10^{-4}	1.3×10^{-2}
PACA-2[h]	5.1×10^{-7}Fig.	1.2×10^{-6}	2.7×10^{-3}

[a]Brine shrimp lethality test McLaughlin, 1991.
[b]Human lung carcinoma Giard et al., 1973.
[c]Human breast carcinoma Soule, Vazquez, Long, Albert, & Brennan, 1973.
[d]Human colon adenocarcinoma Fogh & Trempe, 1975.
[e]Human kidney carcinoma Giard et al., 1973.
[f]Human prostate adenocarcinoma Kaighn, Narayan, Ohnuki, Lechner, & Jones, 1979.
[g] Human pancreatic carcinoma Yunis, Arimura, & Russin, 1977.
[h]Positive control standard.

3. Experimental

3.1. Instrumentation

Optical rotations were determined on a Perkin 241 polarimeter. IR spectra (film) were measured on a Perkin-Elmer 1600 FTIR spectrometer. UV spectra were taken in MeOH on a Beckman DU-7 UV spectrophotometer. Low resolution EIMS and CIMS data were collected on a Finnigan 4000 spectrometer. High resolution EIMS and CIMS data were collected on a Kratos MS50 spectrometer through peak matching. ^{1}H NMR, ^{1}H–^{1}H COSY and ^{13}C NMR spectra were obtained on a Varian VXR-500S (^{1}H at 500 MHz and ^{13}C at 125 MHz) or a Brucker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometers with $CDCl_3$ as solvent and TMS as the internal reference. HPLC separations were performed with a Rainin Dynamax UV-1 detector coupled with a Rainin model HPXL solvent delivery system for normal phase and a Dynamax model DSD-200 solvent delivery system for reversed phase.

3.2. Bioassays

The brine shrimp (*Artemia salina* Leach) test (BST) was routinely employed for evaluating the extracts, fractions and isolated compounds from the title plant. In vitro cytotoxicities against human tumor cell lines were carried out at the Purdue Cancer Center, Cell Culture Laboratory, using standard 7-day MTT assays for A-549 (human lung carcinoma) (Giard et al., 1973), MCF-7 (human breast carcinoma) (Soule et al. 1973), HT-29 (human colon adenocarcinoma) (Fogh, J. & Trempe, G., 1975), A-498 (human kidney carcinoma) (Soule et al., 1973), PC-3 (human prostate adenocarcinoma) (Kaighn et al., 1979) and PACA-2 (human pancreatic carcinoma) (Colman-Saizarbitoria et al., 1994). Adriamycin is always used as a positive cytotoxic control in the same runs.

3.3. Plant material

The leaves of *Annona glabra* L. were collected in Florida in May, 1996. The material was collected and the species identified by EP (Flora, 1993). A voucher specimen is deposited in the Pharmacognosy Herbarium.

3.4. Extraction and isolation

The air dried leaves (1360 g) were ground and percolated with 95% ethanol. The extract residue (122 g) (F001) was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer (F002) and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer (54 g) (F003) was partitioned between 90% MeOH and hexane, giving a MeOH layer (31 g) (F005) and a hexane layer (17 g) (F006). The MeOH layer (F005) was the most active fraction in the BST (LC_{50} 0.15 μg/ml). Thus, F005 was repeatedly chromatographed over open silica gel columns, using gradients of CH_2Cl_2–MeOH (directed by the BST test) and then purified by repeated normal phase HPLC (Dynamax-60 A 8 μm silica gel, 250 × 21.4 mm i.d. or 250 × 4.6 mm i.d.), eluted with hexane (90%)/[MeOH/THF (9:1), 10%] and by a C-18 column (250 × 21.4 mm i.d.), eluted by an CH_3CN–H_2O (7:3) isocratic solvent system.

3.5. Preparation of Mosher ester derivatives

To an acetogenin (1 mg, in 0.5 ml of CH_2Cl_2), were sequentially added pyridine (0.2 ml), 4-(dimethylamino)pyridine (0.5 mg) and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride. The reaction mixture was stirred from 4 h to overnight, checked with TLC to make sure that the reaction was complete and passed through a disposable pipet (0.6 × 6 cm) containing silica gel (60–200 mesh) and eluted with CH_2Cl_2 (3 ml). The CH_2Cl_2 residue, after washing with 1% $NaHCO_3$ (5 ml) and H_2O (2 × 5 ml), was dried in vacuo to give the *S* Mosher esters. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride yielded the *R*-Mosher ester of the acetogenin.

3.6. Annoglacin A (1)

A whitish waxy solid (4.5 mg); $[\alpha]_D^{25}$ +15.0° ($CHCl_3$); UV(MeOH) λ_{max} 219 nm (log ε, 3.62); IR cm^{-1} (film on NaCl plate) 3413, 2920, 2850, 2280, 1742, 1657, 1630, 1551, 1528, 1467, 1321, 1075, 850, 791; CIMS (isobutane) *m/z* (%) $[MH]^+$ 625 (100), $[MH–H_2O]^+$ 607 (70), $[MH–2H_2O]^+$ 589 (75), $[MH–3H_2O]^+$ 571 (13) $[MH–4H_2O]^+$ 553 (2); HRCIMS (isobutane) *m/z* 625.5042 for $C_{37}H_{69}O_7$ $[MH]^+$ (calcd 625.5043); EIMS (Fig. 2) *m/z* (%): 425 (2), 407 (3), 355 (5), 337 (50), 319 (100), 301 (10), 269 (18), 251 (6), 233 (4), 141 (3); HREIMS *m/z* 269.1752 for $C_{15}H_{25}O_4$ (calcd. 269.1753); ^{1}H and ^{13}C NMR: see Table 1.

3.7. Annoglacin B (2)

A whitish waxy solid (11.6 mg); $[\alpha]_D^{25}$ +15.7° ($CHCl_3$); UV(MeOH) λ_{max} 218 nm (log ε, 3.48); IR cm^{-1} (film on NaCl plate) 3453, 2920, 2851, 1735, 1548, 1530, 1512, 1462, 1409, 1318, 1275, 1106, 1075, 1022, 965, 855, 673 cm^{-1}; CIMS (isobutane) *m/z* (%) $[MH]^+$ 625 (100), $[MH–H_2O]^+$ 607 (21), $[MH–2H_2O]^+$ 589 (15), $[MH–3H_2O]^+$ 571 (7); HRCIMS (isobutane) *m/z* 625.5043 for $C_{37}H_{69}O_7$ $[MH]^+$ (calcd 625.5043); EIMS (Fig. 2) *m/z* (%): 425 (3), 407 (10), 355 (4), 337 (55), 319 (100), 301 (10), 269 (19), 251 (6),

233 (4), 141 (5); HREIMS m/z 269.1747 for $C_{15}H_{25}O_4$ (calcd. 269.1753); ^1H and ^{13}C NMR: see Table 2.

Acknowledgements

This investigation was supported by RO1 grant No. CA 30909 from the National Cancer Institute, NIH. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing.

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