Glacins A and B: Two Novel Bioactive Mono-tetrahydrofuran Acetogenins from *Annona glabra*

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Two new bioactive mono-THF Annonaceous acetogenins, glacins A (**1**) and B (**2**), have been isolated, from the fractionated ethanolic extracts of the leaves of *Annona glabra*, directing the fractionation with the brine shrimp lethality test (BST). The structures of **1** and **2** were elucidated on the basis of spectroscopic and chemical methods, and the absolute stereochemistries were solved by preparing their respective per-Mosher ester derivatives. **1** and **2** showed potent and selective in vitro cytotoxicities among several human solid tumor cell lines.

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.[1,2] Several bioactive Annonaceous acetogenins have been previously isolated from the bark.[1] As part of our continuing efforts to find new bioactive leads, two new bioactive acetogenins, glacins A (**1**) and B (**2**) (Figure 1), have been 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).[3] The structures of **1** and **2** were identified as new mono-tetrahydrofuran (mono-THF) ring acetogenins by NMR and MS spectroscopic techniques and by preparing chemical derivatives. The absolute configurations of **1** and **2** were determined through analyses of their respective per-Mosher esters.[4,5] The new compounds were selectively cytotoxic among six human solid tumor cell lines with approximately 10 times the potency of adriamycin.

Results and Discussion

Compound **1** was isolated as a whitish waxy solid. Its molecular weight was suggested by the mass peak at *m/z* 597 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 597.4725 for the $[MH]^+$ ion (calcd 597.4730 for $C_{35}H_{65}O_7$) corresponding to the molecular formula $C_{35}H_{64}O_7$. **1** showed an IR carbonyl absorption at 1734 cm^{-1}, a UV (MeOH) λ_{max} at 218 nm (log ϵ 3.83), six resonances at δ 7.19 (q, H-33), 5.06 (qq, H-34), 1.43 (d, H-35), 2.41 and 2.53 (dddd, H-3), and 3.84 (H-4) in the ^{1}H NMR spectrum and six peaks at δ 174.68 (C-1), 151.90 (C-33), 131.10 (C-2), 78.01 (C-34), 19.10 (C-35), and 69.96 (C-4) in the ^{13}C NMR spectrum (Table 1). These are all 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.[6, 7]

The presence of four OH moieties in **1** was suggested by a prominent IR OH absorption at 3461 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 methine carbons at δ 69.96 (C-4), 71.82 (C-12), 74.07 (C-17), and 73.98 (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 suggested by ^{1}H NMR resonances at δ 3.41 (H-17 and H-22) and δ 3.80 (H-18 and H-21) and the ^{13}C NMR peaks at δ 74.07 (C-17), 82.65 (C-18), 82.59 (C-21), and 73.98 (C-22).

The placements of the hydroxyl groups and the mono-THF ring system along the aliphatic chain were determined on the basis of the analysis of the EIMS spectral data (Figure 2), where the diagnostic fragments at *m/z* 171/425 and 241/355 were characteristic of the hydroxylated mono-THF ring system located from C-17 to C-22. The placement of the OH groups at C-4, C-12, C-17, and C-22 was based on the analysis of the fragments in the EIMS spectrum.

On the basis of the similar chemical shifts in the ^{1}H and ^{13}C NMR spectra, as compared with annonacin, the stereochemistries at C-17/C-18 and C-21/C-22 in **1** were concluded to be threo, and the stereochemistry of the THF ring was determined as trans; signals at δ 1.96 and 1.64 for the protons at C-19/20 indicated the trans configuration according to Fujimoto's models.[8] Thus, the relative configuration of **1** was deduced as threo/trans/threo from C-17 to C-22. The (*S*)- and (*R*)-methoxy(trisfluoromethyl)phenylacetic acid (MPTA) esters (Mosher esters) of **1** were prepared, and the COSY ^{1}H NMR analysis of these derivatives allowed the assignment of the absolute configurations as C-12*R*, C-17*R*, and C-22*R* (Table 1).[4] The absolute configuration at C-12 was also confirmed by comparing the (*S*)- and (*R*)-Mosher ester chemical shifts of H-12 of **1** to those of the per-Mosher esters of 4-acetylannonacin and 4-acetylxylomaticin, which have the same absolute configurations as **1**.[9] The absolute configurations at C-4 and C-36 were assigned *R* and *S*, respectively, as determined by Hoye's method;[10] all of the 4-OH aceto-

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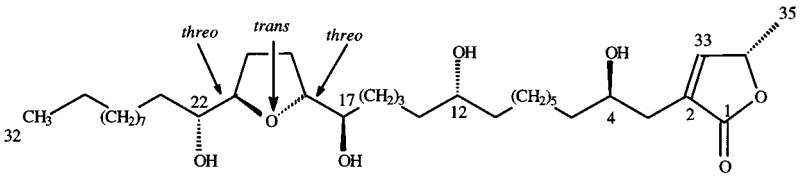

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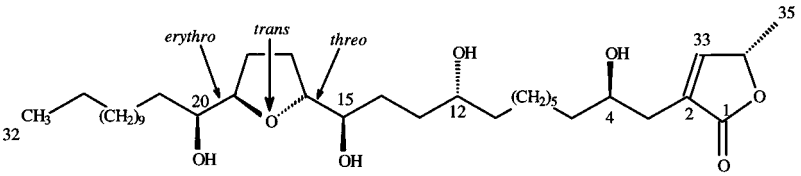

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Figure 1. Structures of glacins A (**1**) and B (**2**).

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

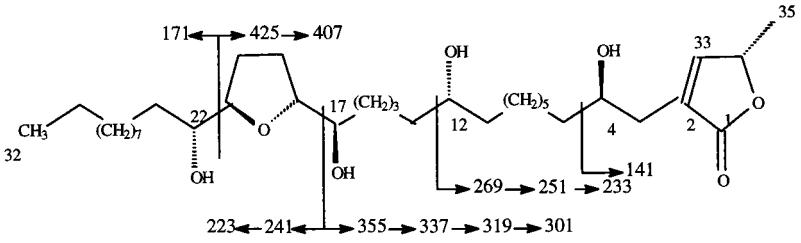
	^{13}C NMR (125 MHz)	^{1}H (500 MHz)			$\Delta\delta_H$	abs
	1	**1** (J in Hz)	**1**−(*S*)- MPTA	**1**−(*R*)-MPTA	$\delta_S-\delta_R$	config
1	174.68					
2	131.10					
3a	33.44	2.53 (dddd, 15.0, 3.3, 1.0, 1.0)	2.57	2.65	neg	
3b	33.44	2.41 (dddd, 15.0, 8.6, 1.0, 1.0)	2.60	2.70	neg	
4	69.96	3.84 (m)	5.31	5.37	neg	4*R*
5	37.30	1.20−1.70 (m)	1.64	1.62	pos	
6−10	25.49−33.32	1.20−1.70 (m)				
11	37.34	1.38 (m)				
12	71.82	3.60 (m)	5.05	5.04	pos	12*R*
13	37.34	1.42 (m)				
14−15	25.49−33.32	1.20−1.70 (m)				
16	25.49−33.32	1.39 (m)	1.56	1.50	pos	
17	74.07[a]	3.41 (m)	4.89	4.98	neg	17*R*
18	82.65	3.80 (m)	3.88	3.99	neg	
19a, 20a	25.49−33.32	1.64 (m)	1.35	1.56	neg	
19b, 20b	25.49−33.32	1.96 (m)	1.65	1.90	neg	
21	82.59	3.80 (m)	3.91	3.99	neg	
22	73.98[a]	3.41 (m)	4.95	4.98	neg	22*R*
23	33.32−33.43	1.35 (m)	1.54	1.51	pos	
24−29	25.49−31.90	1.20−1.70 (m)				
30	22.60	1.20−1.70 (m)				
31	31.90	1.20−1.70 (m)				
32	14.11	0.88 (t, 7.0)				
33	151.90	7.19 (q, 1.4)	6.72	6.96	neg	
34	78.01	5.06 (qq, 6.5, 1.4)	4.85	4.91	neg	34*S*
35	19.10	1.43 (d, 6.5)	1.28	1.30	neg	

[a] Signals may be interchangeable.

genins that have been found so far have the *R* configuration at C-4 and the *S* at C-34 or C-36. [6]

Glacin B (**2**) was also isolated in the form of a whitish waxy powder. The molecular weight of **2** was indicated by the peak at *m/z* 597 for the $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 597.4721 (calcd 597.4730 for $C_{35}H_{65}O_7$) for the $[MH]^+$ corresponding to the molecular formula $C_{35}H_{64}O_7$ and identical to that of **1**. The IR spectrum showed a strong absorption at 1736 cm $^{-1}$ for the carbonyl and 3423 cm^{-1} for the presence of OH moieties. In the CIMS, a series of peaks at *m/z* 579, 561, 543, and 525, arising from the successive losses of four molecules of H_2O, were observed, confirming the presence of four OH groups. Four resonances, due to oxygen-bearing carbons at δ 69.90 (C-4), 71.66 (C-12), 74.57(C-15), and 71.56 (C-20) in the ^{13}C NMR of **2**, also suggested the existence of four secondary hydroxyls. As with **1**, resonances at δ 7.20 (q, H-33), 5.06 (qq, H-34), 1.43 (d, H-35), 3.84 (H-4), and 2.41 and 2.53 (dddd, H-3) in the ^{1}H NMR spectrum and six peaks at δ 174.72 (C-1), 151.91 (C-33), 131.13 (C-2), 78.02 (C-34), 69.90 (C-4), and 19.05 (C-35) in the ^{13}C NMR spectrum (Table 2) suggested the existence of the methylated α,β-unsaturated γ-lactone fragment, with the presence of an OH group at the C-4 position. The presence of a mono-THF ring with OH groups on each side of the ring was indicated by proton resonances at δ 3.40 (H-15), δ 3.80−3.89 (H-16, 19, 20) and the carbon peaks at δ 74.57 (C-15), 83.06 (C-16), 82.19 (C-19), 71.56 (C-20)

As for **1**, the carbon skeleton and placement of the ring and these four OH groups along the hydrocarbon chain were determined on the basis of the EIMS spectral analysis of **2** (Figure 2). The EIMS displayed intense ion peaks at *m/z* 379/199 and 327/269, which suggested the placement of the mono-THF ring system and its two flanking hydroxyls at C-15 to C-20. The fourth OH was placed at C-12 by analysis of the fragments in the EIMS spectra of **2**.



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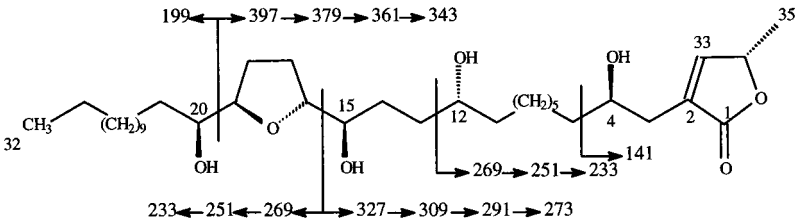

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Figure 2. Diagnostic mass fragmentation ions (*m/z*) of **1** and **2**.

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

	^{13}C NMR (125 MHz)	^{1}H (500 MHz)			$\Delta\delta_H$	abs
	2	**2** (*J* in Hz)	**2**-(*S*)-MPTA	**2**-(*R*)-MPTA	δ_S-δ_R	config
1	174.72					
2	131.13					
3a	33.35	2.53 (dddd, 15.0, 3.5, 1.4, 1.4)	2.56	2.65	neg	
3b	33.35	2.41 (dddd, 15.0, 8.0, 1.4, 1.4)	2.59	2.70	neg	
4	69.90	3.84 (m)	5.30	5.39	neg	4*R*
5	37.29−37.37	1.22−1.72 (m)	1.65	1.63	neg	
6−10	25.18−33.35	1.22−1.72 (m)				
11	37.29−37.37	1.22−1.72 (m)				
12	71.66[a]	3.63 (m)	5.02	4.92	pos	12*R*
13	25.18−33.35	1.22−1.72 (m)				
14	25.18−33.35	1.22−1.72 (m)	1.45	1.31	pos	
15	74.57	3.44 (m)	4.93	4.91	pos	15*R*
16	83.06	3.80−3.89 (m)	3.81	3.68	pos	
17	25.18−33.35	1.64−1.94 (m)	1.75, 1.34	1.76, 1.39	neg	
18	25.18−33.35	1.64−1.94 (m)	1.75, 1.64	1.78, 1.68	neg	
19	82.19	3.80−3.89 (m)	3.89	3.96	neg	
20	71.56[a]	3.80−3.89 (m)	5.20	5.28	neg	20*S*
21	25.18−33.35	1.22−1.72 (m)	1.50	1.55	neg	
22−31	25.18−33.35	1.22−1.72 (m)				
32	14.08	0.88 (t, 6.9)				
33	151.91	7.20 (q, 1.5)	6.32	6.96	neg	
34	78.02	5.06 (qq, 6.9, 1.4)	4.84	4.90	neg	34*S*
35	19.05	1.43 (d, 6.9)	1.28	1.30	neg	

[a] Signals may be interchangeable.

The relative configuration of **2** was deduced as threo/trans/erythro from C-15 to C-20 on the basis of the similar chemical shifts in the ^{1}H and ^{13}C NMR spectra as compared with those of the model compounds, longicin and rollinecins A and B.[11,12] The location of the *threo* assignment should be at the C-15/16 position rather than at the C-19/20 junction because the δ value of the threo carbinol methine was δ 3.44; if, on the contrary, the reverse were to be true, this value would have been δ 3.40.[6] Again, the absolute stereochemistry of **2** was established by using advanced Mosher ester methology. Analysis of the differences between the (*S*)- and (*R*)-Mosher derivatives allowed the absolute stereochemical assignments of the carbinol centers as C-4*R*, C-15*R*, C-20*S*, and C-36*S* (Table 2). We were unable to assign the absolute stereochemistry at C-12 directly from COSY analysis due to overlapping signals. However, C-12 was assigned as *R* by comparing the *(S)*- and *(R)*-Mosher ester chemical shifts of H-12 in **2** to those of the per-Mosher ester of longicin and rollinecin A, which have the same stereochemistry as **2**.[11,12] The absolute stereochemistry of longicin has been previously confirmed by preparing the *(S)*- and *(R)*-Mosher esters of the intramolecular formaldehyde acetal derivative across C-12/15.[11]

The biological activities of **1** and **2** are summarized in Table 3. These compounds appeared to be more selective than adriamycin across the six human tumor cell lines in our 7-day MTT human solid tumor cytotoxicity tests. Selectivity in **1** was exhibited for the breast carcinoma (MCF-7),[14] prostate carcinoma (PC-3),[16] and pancreatic carcinoma (PACA-2) cells.[17] Com-

Table 3. Bioactivities of **1** and **2**

human tumor cell lines ED_{50} (μg/mL)	**1** (BST[a] LC_{50} (μg/mL) = 1.1×10^{-1})	**2** (BST[a] LC_{50} (μg/mL) = 2.7×10^{-1})	adriamycin[h]
A-549[b]	1.53	1.29	1.91×10^{-2}
MCF-7[c]	4.42×10^{-3}	5.20×10^{-2}	1.79×10^{-1}
HT-29[d]	2.30	8.68×10^{-2}	2.78×10^{-2}
A-498[e]	1.25	2.61	6.20×10^{-3}
PC-3[f]	9.81×10^{-3}	5.59×10^{-2}	4.60×10^{-2}
PACA-2[g]	8.57×10^{-3}	2.74×10^{-2}	1.53×10^{-2}

[a] Brine shrimp lethality test.[3] [b] Human lung carcinoma.[13] [c] Human breast carcinoma.[14] [d] Human colon adenocarcinoma.[15] [e] Human kidney carcinoma.[13] [f] Human prostate adenocarcinoma.[16] [g] Human pancreatic carcinoma.[17] [h] Positive control standard.

pound **2** showed activity selectively comparable with that of adriamycin against the breast carcinoma (MCF-7),[14] colon adenocarcinoma (HT-29),[15] prostate carcinoma (PC-3),[16] and pancreatic carcinoma (PACA-2) cells.[17] Surprisingly, compound **2** has significantly reduced bioactivities against the six human cancer cells, compared to longicin, although the only difference between **2** and longicin is that the mono-THF ring and its flanking OH groups shift two methylene units along the aliphatic chain toward the C-32 terminal methyl group.[11]

All of the acetogenins tested, so far, decrease oxygen uptake in mitochondrial tests.[18,19] These results indicate that they act, at least in part, as potent inhibitors of ATP production via blocking at complex I in mitochondria.[20−22] In addition, they act as potent inhibitors of the ubiquinone-linked plasma membrane NADH oxidase of cancerous cells;[23] this action decreases cytosolic ATP production, and such a depletion of ATP results in apoptosis.[28] Recently, we have found that the acetogenins inhibit cells that are multiple drug resistant due to ATP-dependent efflux pumps; thus, the acetogenins offer an excellent potential for development as new antitumor and pesticidal agents that thwart such resistance mechanisms.[24−27]

Experimental Section

General Experimental Procedures. 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- and high-resolution MS data were collected on Finnigan 4000 and Kratos MS50 spectrometers, respectively. ^{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 Bruker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometer with $CDCl_3$ as solvent and TMS as internal reference. HPLC separations were performed with a Rainin Dynamax solvent delivery system (model SD-200) using a Dynamax software system and a Dynamax absorbance detector (model UV-1) set at 225 nm.

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),[13] MCF-7 (human breast carcinoma),[14] HT-29 (human colon adenocarcinoma),[15] A-498 (human kidney carcinoma),[13] PC-3 (human prostate adenocarcinoma),[16] and PACA-2 (human pancreatic carcinoma).[17] Adriamycin is always used as a positive antitumor control in the same runs.

Plant Material. The leaves of *A. glabra* L. were collected in Florida in May, 1996. The species was collected and identified by one of us (Dr. Elsa Pilarinou).[29] A voucher specimen is deposited in the Pharmacognosy Herbarium.

Extraction and Isolation. The air-dried leaves (1360 g) were ground into a powder 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 a CH_3CN-H_2O (7:3) isocratic solvent system.

Preparation of Mosher Ester Derivatives. To an acetogenin (1 mg, in 0.5 mL of CH_2Cl_2) were sequentially added pyridine (0.1 mL), 4-(dimethylamino)-pyridine (0.1 mg), and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The mixture was stirred at reaction from 4 h to overnight, checked with TLC to make sure that the reaction was complete, passed through a disposable pipet (0.6 × 4 cm) containing silica gel (60−200 mesh), and eluted with 3 mL of CH_2Cl_2. The CH_2Cl_2 residue, dried in vacuo, was redissolved in 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL); the CH_2Cl_2 layer was dried in vacuo to give the (*S*)-Mosher esters.

Glacin A (1): whitish waxy solid (4 mg); $[\alpha]^{25}_D + 6.0°$ ($CHCl_3$); UV (MeOH) λ_{max} 218 nm (log ϵ 3.83); IR (film on NaCl plate) 3461, 2919, 2851, 1734, 1717, 1464, 1318, 1203, 1075; CIMS (isobutane) *m/z* $[MH]^+$ 597 (100), $[MH - H_2O]^+$ 579 (57), $[MH - 2H_2O]^+$ 561 (87), $[MH - 3H_2O]^+$ 543 (29); HRCIMS (isobutane) *m/z* 597.4725 for $C_{35}H_{65}O_7$ $[MH]^+$ (calcd 597.4730); EIMS see Figure 2; ^{1}H and ^{13}C NMR see Table 1.

Glacin B (2): whitish waxy solid (7 mg); $[\alpha][\alpha]^{25}_D$ +4.8° ($CHCl_3$); UV (MeOH) λ_{max} 221 nm (log ϵ 3.65); IR (film on NaCl plate) 3423, 2921, 2850, 1736, 1543, 1452, 1318, 1275, 1120, 1066 cm^{-1}; CIMS (isobutane) *m/z* $[MH]^+$ 597 (77), $[MH - H_2O]^+$ 579 (60), $[MH - 2H_2O]^+$ 561 (100), $[MH - 3H_2O]^+$ 543 (10); HRCIMS (isobutane) *m/z* 597.4721 for $C_{35}H_{65}O_7$ $[MH]^+$ (calcd 597.4730); EIMS see Figure 2; ^{1}H and ^{13}C NMR see Table 2.

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