Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 959–965

BIOORGANIC &
MEDICINAL
CHEMISTRY

Glabracins A and B, Two New Acetogenins from *Annona glabra*

Xiao-Xi Liu,[a] Feras Q. Alali,[a] D. Craig Hopp,[a] Lingling L. Rogers,[a]
Elsa Pilarinou[b] and Jerry L. McLaughlin[a,*]

[a]*Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, USA*
[b]*Walker Cancer Research Institute, 1634A Metropolitan Blvd, Tallahassee, FL 32308, USA*

Received 14 November 1997; accepted 26 January 1998

Abstract—Two new bioactive bis-THF Annonaceous acetogenins, glabracins A (**1**) and B (**2**), and two previously known acetogenins, javoricin (**3**) and bullatanocin (**4**), have been isolated from the leaves of *Annona glabra* by activity-directed fractionation using the brine shrimp lethality test (BST). The structures of **1** and **2** were elucidated based on spectroscopic and chemical methods, and the absolute stereochemistries were partially determined by the advanced Mosher ester method. **1** and **2** showed selective cytotoxicities to certain human tumor cell lines, and **1** was significantly more potent although **1** and **2** differ only in the stereochemistry of their vicinal diols at C-23/24. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

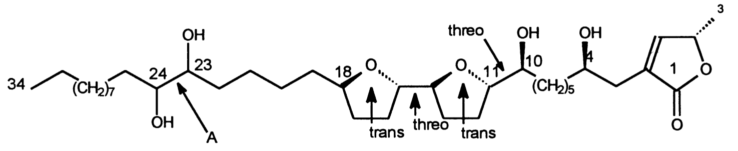
The Annonaceous acetogenins are a relatively new class of natural compounds. Chemically, they are C_{35}–C_{37} fatty acid derivatives with long chain hydrocarbon portions connecting a variable number of tetrahydrofuran (THF) or tetrahydropyran (THP) rings and terminated at one end with a 2,4-disubstituted-γ-lactone moiety.[1] All of the acetogenins tested, so far, decrease oxygen uptake in mitochondrial tests.[2] These results indicate that they act, at least in part, as potent inhibitors of ATP production via blocking at complex I in mitochondria.[3] In addition, they act as potent inhibitors of the ubiquinone-linked plasma membrane NADH oxidase of cancerous cells; this action decreases cytosolic ATP production and results in apoptosis.[4,5] Recently, the acetogenins were found to inhibit cells that are multiple drug resistant, and they offer an excellent potential for development as new antitumor agents.[6] So far, they are found only in the Annonaceae and over 250 of them have been found.[1,7–10] *Annona glabra* L. (Annonaceae), a tropical tree distributed mainly in South America and in southeast Asia, is used in traditional medicines as an insecticide and a parasiticide.[11,12] Several bioactive Annonaceous acetogenins have been previously isolated from the bark.[11] As part of our continuing efforts to find new antitumor agents, two new bioactive acetogenins, glabracins A (**1**) and B (**2**), and two already known acetogenins, javoricin (**3**) and bullatanocin (**4**), have been isolated from the leaves, obtained from trees native in Florida, using bioactivity-directed fractionation with the brine shrimp lethality test (BST).[21] The structures of **1** and **2** were identified as adjacent bis-tetrahydrofuran (bis-THF) ring acetogenins by NMR and MS spectroscopic techniques and chemical derivation. The absolute configurations of **1** and **2** were partially determined through analyses of their respective per-Mosher esters.[5,13] The selective cytotoxicities of the new compounds among six human solid tumor cell lines are also reported and demonstrate the importance of stereochemical differences in biological SARs. The two previously known acetogenins, javoricin (**3**) and bullatanocin (**4**), are reported here for the first time in this plant species (Figure 1).

Key words: *Annona glabra*; acetogenins; glabracin A; glabracin B; cytotoxicities; brine shrimp.

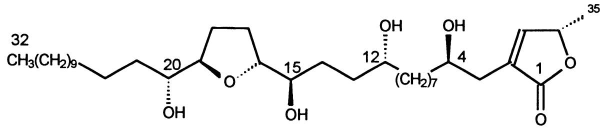
*Corresponding author. Tel.: 317 494 1455; Fax: 317 494 6790.

PII: S0968-0896(98)00059-5

1 glabracin A (**A** = *erythro*) **2** glabracin B (**A** = *threo*)



3 javoricin



4 Bullatanocin

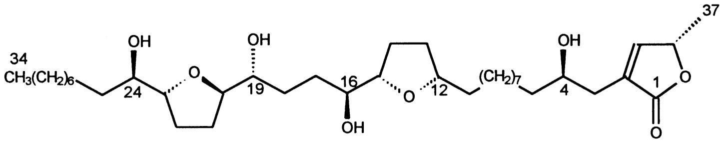


Figure 1. Structures of **1**–**4**.

Results and Discussion

Compound **1** was isolated as a colorless wax. Its molecular weight was suggested by the mass peak at *m/z* 639 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 639.4844 for the $[MH]^+$ ion (calcd 639.4836) corresponding to the molecular formula $C_{37}H_{66}O_8$. The structural determination of **1** was primarily based on the analyses of NMR spectra and MS fragmentation data. **1** showed an IR carbonyl absorption at 1748 cm^{-1}, a UV (MeOH) λ_{max} at 223 mn (log ε 3.58), five resonances at δ 7.20 (q, H-35), 5.06 (qq, H-36), 1.43 (d, H-37), and 2.41 and 2.53 (dddd, H-3) in the ^1H NMR spectrum and five peaks at δ 174.68 (C-1), 151.90 (C-35), 131.08 (C-2), 77.99 (C-36), and 19.05 (C-37) 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.[1,7–10]

The presence of OH moieties in **1** was suggested by a prominent OH absorption at 3382 cm^{-1} in the IR spectrum. The ^{13}C NMR of **1**, which showed four resonances due to oxygen-bearing carbons at δ 69.90 (C-4), 74.13 (C-10), 74.48 (C-23) and 74.71 (C-24), indicated the existence of four secondary hydroxyls. The presence of a bis-THF ring, with one OH group flanking the ring, was suggested by ^1H NMR resonances at δ 3.42 (H-10), δ 3.80 (H-11) and δ 3.77–3.89 (H-14, 15, 18) and the ^{13}C NMR peaks at δ 74.13 (C-10), 82.65 (C-11), 82.07 (C-14), 81.30 (C-15) and 79.89 (C-18); these were directly analogous to similar peaks of other bis-THF acetogenins with one flanking hydroxyl group, such as asimilobin and goniodenin.[14,15]

The placements of the hydroxyl groups and the adjacent bis-THF ring system along the aliphatic chain were determined by the EIMS of **1** (Figure 2), where the diagnostic fragments at *m/z* 241, 311, 327, and 397 were characteristic of the hydroxylated bis-THF ring system located from C-10 to C-19. The EIMS fragment at *m/z* 141 was characteristic of the methylated α,β-unsaturated γ-lactone with a 4-OH group present. The flanking OH group of the bis-THF system was placed at C-10 instead of C-19 because HREIMS gave the exact mass *m/z* 241.1450 (calcd 241.1440) for cleavage between C-10 and C-11. The ^1H NMR of the acetonide derivative of **1** showed the downfield shifts of two protons from δ 3.61 ppm to δ 4.02 ppm for two of the four methine protons on OH-bearing carbons which was consistent with their assignment as the vicinal diol OH groups. The 1,2-diol group was placed at C-23/24 because EIMS of **1** gave a prominent fragment at *m/z* (171/467→449→431→413) (Figure 2). This position was also supported by HREIMS of the fragment at *m/z* 449.2884 (calcd 449.2903).

The stereochemistries at C-10/C-11 in **1** were concluded to be *threo*, the stereochemistry of each of the THF rings was determined as *trans*, and the relative configuration of **1** was deduced as *threo*/*trans*/*threo*/*trans* from C-10 to C-19 based on the similar chemical shifts in the ^1H and ^{13}C NMR spectra as compared with asimilobin and goniodenin.[14,15] The (*S*)- and (*R*)-methoxy(fluoromethyl) phenylacetic acid (MPTA) esters (Mosher

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

Compd	^{13}C NMR 1 (125 MHz)	^{1}H (500 MHz)			$\Delta\delta_H$ δ_S-δ_R
		1(*J* in Hz)	*S*-MPTA	*R*-MPTA	
1	174.68	—			
2	131.08	—			
3a	33.21	2.53 (dddd, 15.1, 3.5, 1.4, 1.4)			
3b	—	2.41 (dddd, 15.1, 8.3, 1.4, 1.4)			
4	69.99	3.80 (m)			
5	37.33	1.40–1.55 (m)			
6–8	22.65–31.98	1.20–1.40 (m)			
9	22.65–31.98	1.40–1.55 (m)	1.47	1.48	−0.01
10	74.13	3.42 (m)			*S*
11	82.65	3.80(m)	4.02	3.91	+0.11
12–13	22.65–31.98	1.58–2.04 (m)			
14	82.07	3.77–3.89 (m)			
15	82.30	3.77–3.89 (m)			
16–17	22.65–31.98	1.58–2.04 (m)			
18	79.89	3.77–3.89 (m)			
19	35.61	1.40–1.50 (m)			
20–22	22.65–31.98	1.40–1.70 (m)			
23	74.48[a]	3.61 (m)			
24	74.71[a]	3.61 (m)			
25	22.65–31.98	1.40–1.70 (m)			
26–34	22.65–31.98	1.20–1.40 (m)			
34	14.08	0.88 (t, 7.0)			
35	151.90	7.20 (q, 1.0)			
36	77.99	5.06 (qq, 7.0, 1.5)			
37	19.05	1.43 (d, 7.0)			

[a]Signals may be interchangeable.

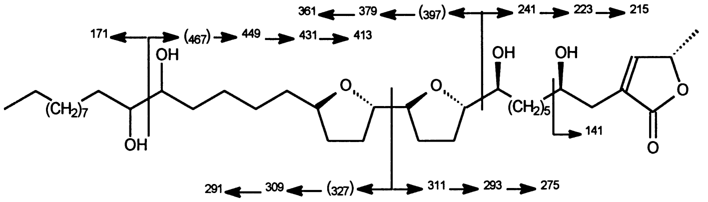


Figure 2. Diagnostic mass fragmentation ions of **1** and **2**. Peaks in parentheses were not observed. Losses of H_2O indicated by −18 *m*/*z*.

esters) of **1** were prepared, and ^{1}H COSY analysis of these derivatives allowed the assignment of the absolute configuration at C-10 as *S* (Table 1).[13] Since the relative stereochemistry of the bis-THF ring system was known, the absolute stereochemistries at C-11, 14, 15, and 18 were automatically defined. Configurations for C-4 and C-36 were *R* and *S*, respectively, since all 4-OH acetogenins found have the *R* stereochemistry at C-4 and *S* at C-36.

The 1,2-diol at C-23/24 was deduced to possess an *erythro* configuration from a direct comparison of its NMR chemical shifts with literature data.[4] Usually, the ^{1}H NMR spectrum of a *threo* diol shows an oxygenated signal at δ 3.40, while its *erythro* isomer will appear at δ 3.60.[4] The chemical shift at δ 3.61 indicated an *erythro* diol for **1**, and this was supported by the close comparison of similar data of reported *erythro* 1,2-diols.[16] Though determination of the absolute stereochemistry of the carbinol centers at C-23/24 was attempted by advanced Mosher methodology, the absolute stereochemistry at these positions remains undefined because the chemical shifts of H-23 and H-24 are indistinguishable by ^{1}H NMR.

Glabracin B (**2**) was also isolated in the form of an amorphous waxy powder. The molecular weight of **2** was indicated by a peak at *m*/*z* 639 for the $[MH]^+$ in the

CIMS. The HRCIMS gave *m/z* 639.4822 (calcd 639.4836) for the $[MH]^+$ corresponding to the molecular formula $C_{37}H_{66}O_8$, identical to that of **1**. The IR spectrum showed a strong absorption at $1747\,cm^{-1}$ for a γ-lactone carbonyl, $1717\,cm^{-1}$ for a ketone carbonyl, and $3433\,cm^{-1}$ for the OH moieties. Four resonances due to oxygen-bearing carbons at δ 74.35, 69.99, 74.18 and 74.05 in the ^{13}C NMR of **2** showed the existence of four secondary hydroxyls. Resonances at δ 7.20 (q, H-35), 5.06 (qq, H-36), 1.43 (d, H-37), and 2.41 and 2.53 (dddd, H-3) in the ^1H NMR spectrum and five peaks at δ 174.68 (C-1), 151.90 (C-35), 131.08 (C-2), 77.99 (C-36), and 19.05 (C-37) in the ^{13}C NMR spectrum (Table 1) suggested the existence of the methylated α,β-unsaturated γ-lactone fragment, with the presence of an OH group at the C-4 position in **2**. As with **1**, the presence of a bis-THF ring with one OH group flanking the ring was suggested by proton resonances at δ 3.42 (H-10), δ 3.80 (H-11) and δ 3.77–3.92 (H-14, 15, 18), and the carbon peaks at δ 82.78 (C-11), 82.60 (C-14), 81.81 (C-15), 79.32 (C-18) and 74.18 (C-10).

The carbon skeleton and placement of the ring and three OH groups along the hydrocarbon chain were determined based on the EIMS spectral analysis of **2** (Figure 2). The EIMS displayed intense ion peaks at *m/z* 379 and *m/z* 309 which suggested the placement of the hydroxyl bis-THF ring system at C-10 to C-19. This was confirmed by HREIMS for the fragment at *m/z* 309.2800 (calcd 309.2794). The flanking OH was placed at C-10 because of the distinctive HREIMS fragment at *m/z* 241.1434 (calcd 241.1440). The acetonide derivative of compound **2** suggested the existence of one *threo* 1,2-diol because the ^1H NMR of the acetonide derivative of **2** showed the shifts of two protons downfield from δ 3.43 ppm to δ 3.60 ppm for two of the four methine protons on OH-bearing carbons, and in the EIMS (Figure 2) the positions of the diol OH groups was determined by the fragment at *m/z* 467 which indicated that these hydroxyls were, as in **1**, located at C-23/24.

Similar to compound **1**, the relative configuration of **2** was deduced as *threo/trans/threo/trans* from C-10 to C-19 based on the similar chemical shifts in the ^1H and ^{13}C NMR spectra as compared with those of the model compounds asimilobin and goniodenin.[14,15] Configurations for C-4 and C-36 were assigned as *R* and *S*, respectively, since all 4-OH acetogenins found have the *R* stereochemistry at C-4 and *S* at C-36.

Again, the partial absolute stereochemistry of **2** was established by using advanced Mosher ester methology (Table 2). Analysis of the difference between the (*S*)- and (*R*)-Mosher derivatives around C-10, which exhibited negative results for H-9 and positive results for H-11, suggested an *S* configuration for C-10 (Table 2). As in compound **1**, because the chemical shifts of H-23 and H-24 were virtually indistinguishable by ^1H NMR, the absolute carbinol centers at C-23/24 were not solved by spectral analysis of the per-Mosher ester derivatives. Thus, compounds **1** and **2** have the same structures and absolute stereochemistries except at the C-23/24 position where **1** is *erythro* and **2** is *threo*, but the absolute stereochemistries at these two positions remain undefined.

The biological activities of **1** and **2** are summarized in Table 3. These compounds were active in the BST; they also showed significant cytotoxicities against the human tumor cell lines in our seven-day MTT human solid tumor cytotoxicity tests. **1** and **2** appeared to be more selective than adriamycin across the six human tumor cell lines. Selectivity in **1** was exhibited for the lung carcinoma (A-549),[17a] breast carcinoma (MCF-7),[17b] and pancreatic carcinoma (HT-29).[17e] The cytotoxic potency of **1** against MCF-7 was over 100,000 times that of adriamycin and against A-549 and HT-29 over 10,000 times that of adriamycin. Compound **2** was significantly less potent than **1** but showed comparable cytotoxicity to adriamycin against the lung carcinoma (A-549), breast carcinoma (MCF-7), colon adenocarcinoma (HT-29) and pancreatic carcinoma (PACA-2) cells.[17c] Stereochemistry seems to play an extremely important role in determining the cytotoxicity of these acetogenins as **1**, the *erythro* diol, is selectively over 1000 times more toxic than **2**. This type of stereochemistry-sensitive selectivity has also been found among other acetogenins.[18]

So far, among the large number of reported adjacent bis-THF ring acetogenins, bis-THF ring acetogenins with four OH groups are relatively rare. Those acetogenins with four OH groups generally show less potent activities against human solid tumor cell lines. The decrease in bioactive potency might be due to the extra OH which would increase the polarity of the molecules and, thus, affect the transport of the compounds into cells leading to reduction of activity. Similar decreases of activity were also observed for those mono-THF ring acetogenins with five and six hydroxyls when compared with the four hydroxylated mono-THF ring molecules; optimum potency seems to remain with the adjacent bis-THF compounds that bear three hydroxyls.[19,20]

Experimental

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

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

Compd	^{13}C NMR 2 (125 MHz)	^{1}H (500 MHz)			$\Delta\delta_H$ $\delta_{S\text{-}\delta_R}$
		1(*J* in Hz)	*S*-MPTA	*R*-MPTA	
1	174.68	—			
2	131.08	—			
3a	33.21	2.53 (dddd, 15.1, 4.0, 1.6, 1.6)			
3b	—	2.40 (dddd, 15.1, 8.0, 1.6, 1.6)			
4	69.94	3.80 (m)			
5	37.34	1.41–1.53 (m)			
6–8	22.66–34.28	1.20–1.40 (m)			
9	22.66–34.28	1.41–1.53 (m)	1.46	1.48	−0.02
10	74.18	3.42 (m)			*S*
11	82.78	3.80 (m)	4.02	3.91	+0.11
12–13	22.66–34.28	1.65–2.04 (m)			
14	82.60	3.77–3.92 (m)			
15	81.81	3.77–3.92 (m)			
16–17	22.66–34.28	1.65–2.04 (m)			
18	79.31	3.77–3.92 (m)			
19	35.48	1.41–1.53 (m)			
20–22	22.66–34.28	1.41–1.64 (m)			
23	74.05[a]	3.43 (m)			
24	74.35[a]	3.43 (m)			
25	22.66–34.28	1.40–1.70 (m)			
26–34	22.66-34.28	1.20–1.40 (m)			
34	14.09	0.88 (t, 7.0)			
35	151.86	7.20 (q, 1.0)			
36	77.99	5.06 (qq, 7.0, 1.5)			
37	19.08	1.43 (d, 7.0)			

[a] Signals may be interchangeable.

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 Brucker 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. Analytical TLC was carried out on silica gel plates (0.25 mm), developed with $CHCl_3$-CH_3COCH_3 (5:5) and visualized with 5% phosphomolybdic acid in EtOH followed by heating.

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

Compounds		**1**	**2**	Adriamycin
BST[a] LC_{50} (μg/mL)		0.13	0.31	
Human	A-549[b]	2.8×10^{-6}	1.6×10^{-6}	1.5×10^{-2}
Tumor	MCF-7[c]	4.2×10^{-6}	1.8×10^{-3}	2.5×10^{-1}
Cell	HT-29[d]	5.7×10^{-6}	1.9×10^{-2}	4.0×10^{-2}
Lines	A-498[e]	1.0	1.4	4.2×10^{-2}
ED_{50}	PC-3[f]	1.1	1.2	2.8×10^{-2}
(μg/mL)	PACA-2[g]	1.8	2.8×10^{-1}	1.3×10^{-2}

[a] Brine shrimp lethality test.[21]
[b] Human lung carcinoma.[17a]
[c] Human breast carcinoma.[17b]
[d] Human colon adenocarcinoma.[17c]
[e] Human kidney carcinoma.[17a]
[f] Human prostate adenocarcinoma.[17d]
[g] Human pancreatic carcinoma.[17e]
Positive control standard.

Bioassays

The brine shrimp (*Artemia salina* Leach) test (BST) was routinely employed for evaluating the extracts, fractions, and isolated compounds from the title plants. 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),[17a] MCF-7 (human breast carcinoma),[17b] HT-29 (human colon adenocarcinoma),[17c] A-498 (human kidney carcinoma),[17a] PC-3 (human prostate adenocarcinoma)[17d] and PACA-2 (human pancreatic carcinoma).[17e] Adriamycin is always used as a positive antitumor control in the same runs.

Plant material

The leaves of *Annona glabra* L. were collected in Florida, USA in May, 1996. The species was collected and identified by one of us (Dr Elsa Pilarinou).[22] A voucher specimen is deposited in the Pharmacognosy Herbarium.

Extraction and isolation

The 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 id or 250×4.6 mm id), eluted with hexane (90%)/(MeOH: THF, 9:1, 10%), and by a C-18 column (250×21.4 mm id), eluted by an CH_3CN:H_2O(7:3) isocratic solvent system.

Preparation of Mosher esters

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 from 4 h to overnight, checked with TLC to make sure that the reaction was complete, and passed through a disposable pipet (0.6×4 cm) containing silica gel (60–200 mesh) and eluted with 3 mL CH_2Cl_2. The CH_2Cl_2, after washing with 1% $NaHCO_3$ (5 mL) and H_2O (2×5 mL), was dried in vacuo to give the (*S*)-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%.

Preparation of acetonide derivatives

Compound **1** or **2** (1–2 mg) and 0.1 mg of toluenesulfonic acid monohydrate were dissolved in 0.5 mL acetone. The mixture was allowed to stand at room temperature for 5 h using TLC to monitor the conversion. The product was then purified over Si gel in a small pipette eluted with hexane:acetone (20:1) and was dried in vacuo to give the respective acetonide derivative.

Glabracin A (1). A whitish wax (4 mg); $[\alpha]_D^{25}$ −11.6° (*c* 0.013, $CHCl_3$); UV(MeOH) λ_{max} 220 nm (log ε 3.89); IR (film on NaCl plate) 3330, 2919, 2850, 1747, 1717, 1469, 1320,1203, 1064, 956, 758; CIMS (isobutane) *m/z* (%) $[MH]^+$ 639 (79), $[MH\text{-}H_2O]^+$ 621 (14), $[MH\text{-}2H_2O]^+$ 603 (100), $[MH\text{-}3H_2O]^+$ 585 (7); HRCIMS (isobutane) *m/z* 639.4844 for $C_{37}H_{68}O_7$ $[MH]^+$ (calcd 639.4836); EIMS (Figure 2) *m/z* (%): 449 (9.0), 431(8.8), 413 (6.8), 379 (7.5), 361(21.3), 311 (8.8), 309 (100), 293 (6.6), 291 (10.7), 275 (2.0), 241 (2.8), 223 (2.0), 215 (1.7), 171 (2.3), 141 (4.5); ^1H and ^{13}C NMR: see Table 1.

Glabracin B (2). A whitish wax (5 mg); $[\alpha]_D^{25}$ +16.9° (*c* 0.050, $CHCl_3$); UV (MeOH) λ_{max} 223 nm (log ε 3.58); IR (film on NaCl plate) 3382, 2926, 2854, 1748, 1540, 1456, 1318, 1270, 1199, 1072 cm^{-1}; CIMS (isobutane) *m/z* (%) $[MH]^+$ 639 (100), $[MH\text{-}H_2O]^+$ 621 (6), $[MH\text{-}2H_2O]^+$ 603 (10); HRCIMS (isobutane) *m/z* 639.4822 for $C_{37}H_{68}O_6$ $[MH]^+$ (calcd 639.4836); EIMS (Figure 2) *m/z* (%): 449 (10.7), 431(7.5), 413 (7.4), 379 (11.3), 361(32.8), 311 (12.4), 309 (100), 293 (6.0), 291 (12.0), 275 (2.3), 241 (4.7), 223 (2.7), 215 (1.9), 171 (3.0), 141 (6.7); ^1H and ^{13}C NMR: see Table 2.

Acknowledgements

This investigation was supported by R01 grant No. CA 30909 from the National Cancer Institute, National Institute of Health. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing.

References

1. Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237.

2. (a) Landolt, J. L.; Ahammadsahib, K. L; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G.P.; McLaughlin, J. L. *Chemico-Biol. Interact*. **1995**, *98*, 1. (b) Alfonso, D.; Johnson, H. A.; Colman-Saizarbitoria, T.; Preslay, C. P.; McCabe, G. P.; McLaughlin, J. L. *Natural Toxins* **1996**, *4*, 181.

3. (a) Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427. (b) Ahammadsahib, K. L; Hollingworth, R. M.; McGrovren, J. P.; Hui, Y.-K; McLaughlin, J. L. *Life Sciences* **1993**, *53*, 1113. (c) Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K.; McLaughlin, J. L. *Pesticide Biochem. Physiol.* **1993**, *45*, 15.

4. Morré, J. D.; Decabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

5. Wolvetang, E. J.; Johnson, K. L.; Krauer, K.; Ralph, S. L; Linnane, A. W. *FEBS Lett*. **1994**, *339*, 40.

6. (a) Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; McLaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55. (b) Oberlies, N. H.; Croy, V. L.; Chang, C.-J.; McLaughlin, J. L. *Cancer Lett.* **1997**, *115*, 73. (c) Oberlies, N. K; Chang, C.-J.; McLaughlin, J. L. *J. Med. Chem.* **1997**, *40*, 2102.

7. Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27.

8. Gu, Z.-M.; Zhao, G. X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T.; Mata. R.; Romeo, J. T., Eds; Plenum: New York, 1995; Vol. 29, pp 249–310.

9. Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275.

10. (a) Cavé, A. In *Phytochemistry of Plants Used in Traditional Medicine*; Hostettmann, K.; Marston, A.; Maillard, M.; Hamburger, M., Eds; Clarendon: Oxford, 1995; pp 247–248. (b) Cavé, A; Figadére, B.; Laurens, A.; Cortes, D. In *Progress in The Chemistry of Organic Natural Products*; Herz, W.; Kirby, G. W.; Morre, R. E.; Steglich, W.; Tamm, Ch., Eds.; Springer-Verlag: New York, 1997; pp 81–287.

11. Ohsawa, K.; Atsuzawa, S.; Mitsui, T.; Yamamoto, I. *J. Pesticide Sci.* **1991**, *16*, 93.

12. Padmaja, V.; Thankamany, K.; Hara, N.; Fujimoto, Y.; Hisham, A. *J. Ethnopharmacol.* **1995**, *48*, 21.

13. Rieser, M. L; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, A.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *144*, 10203.

14. Woo, M.-K.; Cho, K.-Y.; Zhang, Y.; Zeng, L.; Gu, Z.-M.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, *58*, 1533.

15. Zhang, Y.; Zeng, L.; Woo, M.-K; Gu, Z.-M.; Ye, Q.; Wu, F.-E.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1743.

16. Shi, G.; MacDougal, J. M.; McLaughlin, J. L. *Phytochemistry* **1997**, *45*, 719.

17. (a) Giard, D. L; Aaronson, S. A.; Todaro, G. L; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417. (b) Soule, H. D.; Vazquez, L; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409. (c) Fogh, L.; Trempe, G. In *Human Tumor Cells*; Fogh, J., Ed.; Plenum: New York, 1975, p 115. (d) Kaighn, M. R; Narayan, K. S.; Ohnuki, Y.; Lechner, J. R; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16. (e) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer*, **1977**, *19*, 128.

18. Shi, G.; Gu, Z.-M.; He, K.; Wood, K. V.; Zeng, L.; Ye, Q; MacDougal, J. M.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1281.

19. Zeng, L.; Wu, F.-E.; Gu, Z.-M.; McLaughlin, J. L. *Tetrahedron Lett.* **1995**, *36*, 5291.

20. Hui, Y. H.; Rupprecht, J. K.; Anderson, J. E.; Liu, Y. M.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463.

21. McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettman, K., Ed.; Academic: London, 1991; Vol. 6, pp 1–33.

22. Flora of North America Editorial Committee, *Flora of North America*, Oxford University: New York, 1997; Vol. 3, pp 11–20.