PERGAMON

Phytochemistry 50 (1999) 1033–1040

PHYTOCHEMISTRY

Asitrilobins A and B: cytotoxic mono-THF Annonaceous acetogenins from the seeds of *Asimina triloba*

Mi-Hee Woo[a,*], Dal-Hwan Kim[a], Jerry L. McLaughlin[b]

[a]*Department of Pharmacy, College of Pharmacy, Catholic University of Taegu-Hyosung, Kyongsan 712-702, South Korea*
[b]*Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, USA*

Received 20 April 1998; accepted 26 August 1998

Abstract

The seeds of *Asimina triloba* have yielded two novel cytotoxic mono-tetrahydrofuran (THF) Annonaceous acetogenins, asitrilobins A (**1**) and B (**2**). In addition, annonacin, asimin and asiminacin, which are known, and annomontacin and xylomaticin, which are known but are new in this species, were obtained. Compounds **1** and **2** have a relative stereochemical relationship of *erythro*/*cis*/*threo* across the mono-THF ring with its two flanking hydroxyls and they, thus, represent a new type of acetogenin. Their structures were established on the basis of chemical and spectral evidence. **1** and **2** showed potent bioactivities in the brine shrimp lethality test (BST) and among six human solid tumor cell lines with notable selectivity for the pancreatic cell line (MIA PaCa-2) at ten to one-hundred times the potency of adriamycin.

Keywords: Index-*Asimina triloba*; Annonaceae; Seed; Mono-tetrahydrofuran Acetogenins; Asitrilobins A and B; Cytotoxicities

1. Introduction

Asimina triloba (L.) Dunal (Annonaceae), commonly known as the pawpaw tree, is native to the eastern United States (Callaway, 1990). Extracts of the seeds showed toxicity in the brine shrimp lethality test (BST) and demonstrated cytotoxicity against six human solid tumor cell lines. Bioactivity-directed isolation using the BST (Meyer et al., 1982; McLaughlin, 1991) has led to the discovery of approximately 46 bioactive acetogenins from the seeds and stem bark of the pawpaw (Zhao, Hui, & McLaughlin, 1992; Zhao et al., 1993; Zhao, Ng, Kozlowski, Smith, & McLaughlin, 1994; Zhao, Miesbauer, Smith, & McLaughlin, 1994; Zhao et al., 1995; Woo et al., 1995aWoo et al., 1995b; Woo, Zeng, & McLaughlin, 1995; Zhao, Chao, Zeng, Rieser, & McLaughlin, 1996; Zhao, Chao, Zeng, & McLaughlin, 1996; He et al., 1996He et al., 1997). As part of our continuing efforts to find new antitumor agents, we have isolated two novel acetogenins from the seeds; these are named asitrilobins A (**1**) and B (**2**), and they have a relative stereochemical relationship of *erythro*/*cis*/*threo* across the mono-THF ring moiety with its two flanking hydroxyl groups. Compounds **1** and **2** showed significant cytotoxicities among human tumor cell lines with selectivities for the lung (A-549), breast (MCF-7) and pancreatic (MIA PaCa-2) cell lines. In addition, annonacin, asimin and asiminacin, which are known, and annomontacin and xylomaticin, which are known but are new in this species, were obtained.

2. Results and discussion

As reported previously, the seeds of *Asimina triloba* were extracted with 95% EtOH, and the residue of the extract (F001) was partitioned through a standard extraction scheme under the guidance of the lethality test to brine shrimp larvae (BST) (Meyer et al., 1982; McLaughlin, 1991). The most bioactive extract, as tested by the BST, was F005, the aqueous methanol partitioned fraction. F005 was submitted to successive fractionations by silica gel open column chromatography and HPLC, directed by the BST at each step, to yield two new compounds (**1** and **2**).

The molecular weight of asitrilobin A (**1**) was determined by the $[M+H]^+$ ions at m/z 625 and $[M+Na]^+$ ions at m/z 646 in its FABMS spectrum, indicating a molecular mass of 624; HRFABMS $[M+H]^+$ ions at m/z

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00632-3

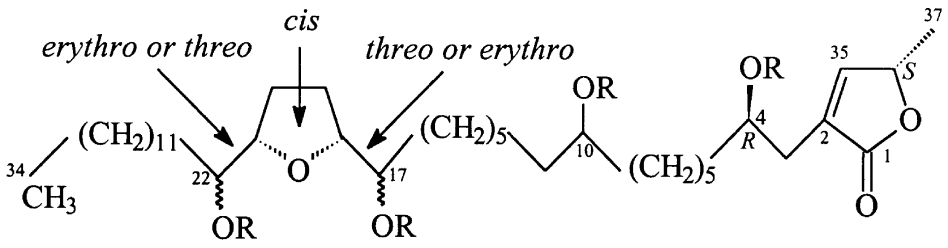
625.5068 (calcd 625.5043) corresponded to the formula $C_{37}H_{68}O_7$. The existence of an α,β-unsaturated γ-lactone in **1** was suggested by an IR carbonyl absorption at 1736 cm^{-1}, a UV λ_{max} at 225.60 nm (log ε 4.3), and six resonances at δ 7.18 (H-35), 5.06 (H-36), 2.53 (H-3a), 2.40 (H-3b), 3.83 (H-4), and 1.43 (H-37) in the ^{1}H NMR; these are characteristic spectral features for the α,β-unsaturated γ-lactone fragment with a 4-OH group in the Annonaceous acetogenins (Fang, Rieser, Gu, Zhao, & McLaughlin, 1993).

The existence of four OH groups in **1** was evidenced by an IR absorption at 3448 cm^{-1} and resonances due to oxygen-bearing carbons at δ 69.88, 71.52, 71.84 and 74.30, correlated with proton signals at δ 3.83 (2H), 3.39 (1H) and 3.58 (1H). These were further confirmed by preparation of a tetraacetyl derivative (**1a**). The ^{1}H NMR spectrum of **1a** showed four proton singlets at δ 2.03, 2.04, 2.05 and 2.08 and multiplet proton resonances at δ 4.88 (3H) and 5.10 (1H) corresponding to downfield shifts on four secondary OH-bearing carbons as compared with **1**. The mono-THF ring, with the usual flanking OH groups on each side, was indicated in **1** by ^{1}H NMR chemical shifts (Table 1) at δ 3.39 (H-17 or H-22) and 3.83 (H-22 or H-17) and the ^{13}C NMR signals (Table 1) at δ 74.30 (C-17 or C-22) and 71.84 (C-22 or C-17). These structural units were further confirmed by ^{1}H–^{1}H COSY data in which the proton coupling correlations from H-3↔H-4 and (H-17 and H-22)↔(H-16, H-18 and H-21, H-23) could be clearly seen.

The positions of the OH groups in **1** were assigned at C-4, C-10, C-17 and C-22 by careful analysis of the fragments in the EIMS spectrum at *m/z* 551, 481, 325 and 183 (Figs. 1 and 3), in its tetraacetate (**1a**) and at *m/z* 641, 571, 385 and 213 (Fig. 3), in its tetra-TMSi derivative (**1b**). The relative stereochemistry at C-17/C-18 and C-21/C-22 was defined by comparing the ^{13}C NMR signals of the hydroxylated carbons at δ 74.30 (C-17 or C-22) and 71.84 (C-22 or C-17) and the ^{1}H NMR signals at δ 3.39 (H-17 or H-22) and 3.83 (H-22 or H-17) in **1**. These data suggested that the relative configurations at C-17/C-

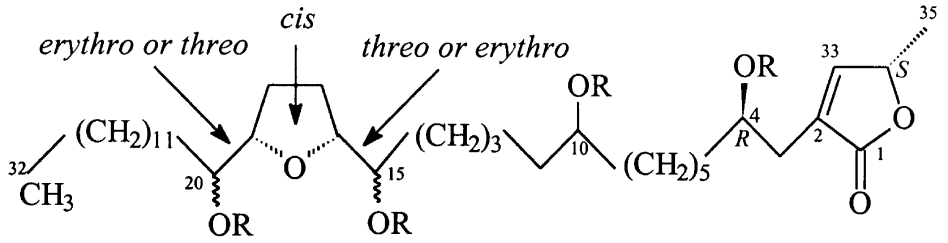
Table 1
^{1}H and ^{13}C NMR spectral data of **1**, **1a**, **2**, and **2a** ($CDCl_3$, δ)

Position	^{1}H NMR (300 MHz)				^{13}C NMR (75.5 MHz)	
	1	1a	2	2a	1	2
1	–	–	–	–	174.62	174.61
2	–	–	–	–	131.13	13.66
3a	2.53 m	2.54 m	2.54 m	2.56	33.19	33.64m
3b	2.40 m	2.52 m	2.44 m	2.53 m		
4	3.83 m	5.10 m	3.83 m	5.10 m	69.88	70.40
5–9	1.1–1.6 m	1.1–1.6 m	1.1–1.6 m	1.1–1.6 m	22.66–37.40	23.14–37.85
10	3.58 m	4.88 m	3.60 m	4.85 m	71.52	72.09
11–14	1–1.6 m	1.1–1.6 m	1.1–1.6 m	1.1–1.6 m	22.66–37.40	23.14–37.85
15	1.1–1.6 m	1.1–1.6 m	3.41[b] m	4.85 m	22.66–37.40	74.72[d]
16	1.1–1.6 m	1.1–1.6 m	3.83 m	3.97 m	22.66–37.40	82.63
17a	3.39[a] m	4.88 m	1.84m	1.72 m	74.30[c]	28.40
17b			1.99 m	1.98 m		
18a	3.83 m	3.96 m	1.84 m	1.72 m	82.15	25.77
18b			1.99 m	1.98 m		
19a	1.86 m	1.71 m	3.83 m	3.97 m	28.58	83.63
20a	1.86 m	1.71 m	3.83[b] m	4.85 m	25.21	72.26[d]
20b	1.98 m	1.93 m				
21	3.83 m	3.96 m	1.1–1.6 m	1.1–1.6 m	83.18	23.14–37.85
22	3.83[a] m	4.88 m	1.1–1.6 m	1.1–1.6 m	71.84[c]	23.14–37.85
23–31	1.1–1.6 m	1.1–1.6 m	1.1–1.6 m	1.1–1.6 m	22.66–37.40	23.14–37.85
32	1.1–1.6 m	1.1–1.6 m	0.88 t	0.88 t	22.66–37.40	14.56
33	1.1–1.6 m	1.1–1.6 m	7.19 s	7.09 s	22.66–37.40	152.26
34	0.87 t	0.88 t	5.06 q	5.02 m	14.10	78.42
35	7.18 s	7.08 s	1.44 d	1.44 d	151.84	19.58
36	5.06 q	5.10 m			77.84	
37	1.43 d	1.40 d			19.08	
4-OAc		2.03 s		2.03 s		
10-OAc		2.04 s		2.04 s		
15-OAc				2.08 s		
17-OAc		2.08 s				
20-OAc				2.06 s		
22-OAc		2.05 s				



1 R=H
1a R=Ac
1b R=TMSi
1r R=(*R*)-MTPA
1s R=(*S*)-MTPA

Fig. 1. Structures of **1** and its derivatives.

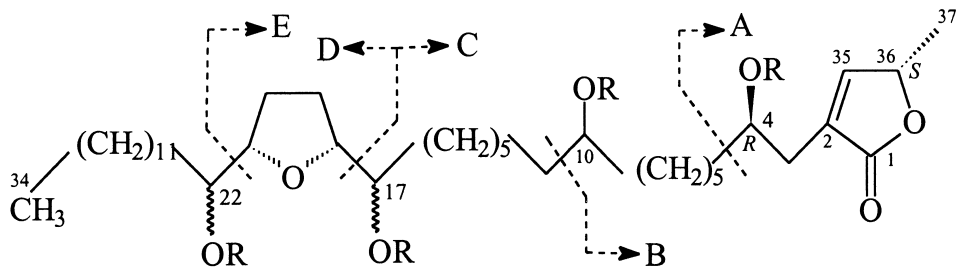


2 R=H
2a R=Ac
2b R=TMSi
2r R=(*R*)-MTPA
2s R=(*S*)-MTPA

Fig. 2. Structures of **2** and its derivatives.

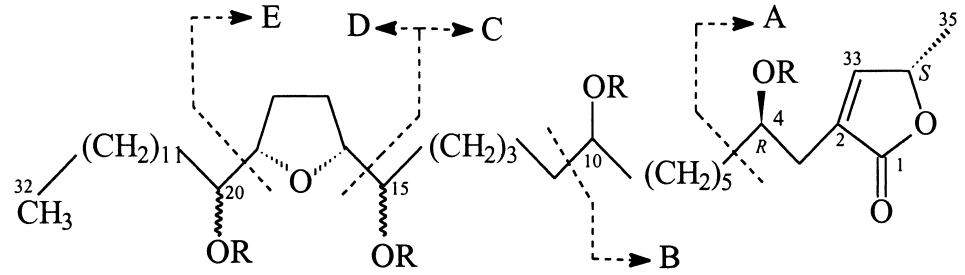
18 and C-21/C-22 were either *erythro* and *threo* or *threo* and *erythro* (Gu et al., 1994). The ^{1}H NMR signals at δ 1.98 and 1.86, corresponding to H-19a/H-20a and H-19b/H-20b, are typical methylene proton signals of a *cis*-THF ring configuration, whereas these are at δ 1.98 and 1.66 for the *trans*-THF ring configuration. Thus, the relative configuration for these four chiral centers in **1** was assigned as *threo*/*cis*/*erythro* or *erythro*/*cis*/*threo* and, in either case, represents a new type of mono-THF acetogenin [Gu, Zhao, Oberlies, Zeng, & McLaughlin, 1995; Zeng et al., 1996; Cave, Figadere, Laurens, & Zechmeister, 1997).

The FABMS spectrum (Figs. 2 and 4) of asitrilobin B (**2**) showed a $[M+H]^+$ ion at *m*/*z* 597, indicating a molecular weight of 596; HRFABMS $[M+H]^+$ ions at *m*/*z* 597.4752 (calcd 597.4730) corresponded to the formula $C_{35}H_{64}O_7$. The prominent absorption peak at 3448 cm^{-1} in the IR spectrum and four successive losses of H_2O (*m*/*z* 18) from the molecular ion in the FABMS (*m*/*z* 578, 560, 542, 524) again indicated the presence of four OH groups. The EIMS showed successive losses of four HOAc units (*m*/*z* 60) from the tetraacetate (**2a**) (*m*/*z* 704, 644, 584, 524) as well as four TMSiOH units (*m*/*z* 90) from the tetra-TMSi derivative (**2b**) (*m*/*z* 794, 704, 614, 524). The IR, UV, ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The ^{1}H NMR spectrum of **2** showed resonances at δ 7.19 (H-33), 5.06 (H-34), 1.44 (H-35) and 3.83 (H-4) attributed to the α,β-unsaturated γ-lactone fragment with a 4-OH group in acetogenins. On the basis of the spectral data described above, the



R	$H^+/M(Ac)_4^+$ /$M(TMSi)_4^+$	A	B	C	D	E
H (FABMS)	624, 606(a), 588(a), 570(a), 552(a)	141, 123(a)	241, 223(a), 205(a)	355, 337(a), 319(a)	269, 251(a)	425, 407(a), 388(a)
Ac (EIMS)	792, 732(b), 672(b), 612(b), 552(b)	183, 123(b)	325, 265(b) 205(b)	481, 421(b), 361(b)	311, 251(b),	551, 491(b), 431(b)
TMSi (EIMS)	912, 822(c),732(c), 642(c), 552(c)	213, 123(c)	385, 295(c), 205(c)	571, 481(c), 391(c)	341, 251(c)	641, 551(c), 461(c)

Fig. 3. Diagnostic FABMS and EIMS fragmentations of **1** and its tetraacetate (**1a**) and tetra-TMSi (**1b**) derivatives. (a) Loss of H_2O (m/z 18); (b) loss of HOAc (m/z 60); (c) loss of TMSiOH (m/z 90).



R	$H^+/M(Ac)_4^+$ /$M(TMSi)_4^+$	A	B	C	D	E
H (FABMS)	596, 578(a), 560(a), 542(a), 524(a)	141, 123(a)	241, 223(a), 205(a)	327, 309(a), 291(a)	269, 251(a)	397, 379(a), 361(a)
Ac (EIMS)	764, 704(b), 644(b), 584(b), 524(b)	183, 123(b)	325, 265(b) 205(b)	453, 393(b), 333(b)	311, 251(b),	523, 463(b), 403(b)
TMSi (EIMS)	884, 794(c), 704(c), 614(c), 524(c)	213, 123(c)	385, 295(c), 205(c)	543, 453(c), 363(c)	341, 251(c)	613, 523(c), 433(c)

Fig. 4. Diagnostic FABMS and EIMS fragmentations of **2** and its tetraacetate (**2a**) and tetra-TMSi (**2b**) derivatives. (a) Loss of H_2O (m/z 18); (b) loss of HOAc (m/z 60); (c) loss of TMSiOH (m/z 90).

Table 2
^{1}H NMR chemical shifts for the determination of the absolute configurations at C-4, C-17, and C-22 of the tetra-(*S*)- and -(*R*)-MTPA esters (**1r** and **1s**) of **1** (500 MHz, δ, $CDCl_3$)

MTPA ester	5-Hab	4-H	3-Hab	35-H	36-H	37-H	16-Hab	17-H	18-H	21-H	22-H	23-Hab
1r	1.57	5.3	2.65	6.95	4.92	1.32	1.33[a]	4.98[e]	3.74[g]	3.99[g]	5.26[e]	1.60[a]
	1.65	5	2.60				1.40[b]					1.57[b]
1s	1.60	5.2	2.58	6.72	4.86	1.29	1.59[c]	5.02[f]	3.92[h]	3.92[h]	5.21[f]	1.52[c]
	1.69	9	2.56				1.61[d]					1.47[d]
$\Delta\delta$**1s**–**1r**	+0.03	*R*[a]	−0.07	−0.23	−0.06	−0.03	+0.26	+0.04	+0.18	−0.07	−0.05	−0.08
	+0.04		−0.04				+0.21					−0.10
Absolute configuration	C-4: *R*, C-17: *R* or *S*, C-22: *S* or *R*											

[a–h]May be reversed.

Table 3
^{1}H NMR chemical shifts for the determination of the absolute configurations at C-4, C-15, and C-20 of the tetra-(*S*)- and -(*R*)-MTPA esters (**2r** and **2s**) of **2** (500 MHz, δ, $CDCl_3$)

MTPA ester	5-Hab	4-H	3-Hab	33-H	34-H	35-H	14-Hab	15-H	16-H	19-H	20-H	21-Hab
2r	1.56	5.35	2.65	6.95	4.92	1.32	1.33[a]	4.97[e]	3.73[g]	3.99[g]	5.25[e]	1.61[a]
	1.65		2.60				1.40[b]					1.56[b]
2s	1.60	5.29	2.58	6.72	4.86	1.29	1.58[c]	4.96[f]	3.91[h]	3.91[h]	5.21[f]	1.52[c]
	1.69		2.56				1.60[d]					1.46[d]
$\Delta\delta$**2s**–**2r**	+0.04	*R*[a]	−0.07	−0.23	−0.06	−0.03	+0.25	−0.01	+0.18	−0.08	−0.04	−0.09
	+0.04		−0.04				+0.20					−0.10
Absolute configuration	C-4: *R*, C-15: *R* or *S*, C-20: *S* or *R*											

[a-h]May be reversed.

THF ring was placed between C-15 and C-20, and the four OH groups were assigned at the C-4, C-10, C-15, and C-20 positions in **2**. The relative configuration of the mono-THF ring with two flanking hydroxyl groups in **2** is either *threo*/*cis*/*erythro* or *erythro*/*cis*/*threo* such as in **1**. Thus, **2** was named asitrilobin B and is also a new natural compound.

To determine the absolute stereochemistry of the carbinol centers at C-4, C-10, C-17 and C-22 in **1**, and at C-4, C-10, C-15 and C-20 in **2**, their tetra-(*R*)- and tetra-(*S*)-methoxytrifluoromethyl phenylacetic acid (MTPA) esters (Mosher esters) [**1r**, **1s**, **2r** and **2s**] were prepared (Hui et al., 1992; Rieser et al., 1993, 1994). ^{1}H–^{1}H COSY analysis of these Mosher ester derivatives was then performed. The ^{1}H NMR chemical shift data of **1r** and **1s** showed that the absolute configuration at C-4 is *R*, and those at C-17 and C-22 are *R* and *S* or *S* and *R* in **1** (Table 2). Also, the absolute configuration at C-4 is *R*, and those at C-15 and C-20 are *R* and *S* or *S* and *R* in **2** (Table 3). Hoye, Hanson, Hasenwinkel, Raminez, and Zhuang (1994) synthesized (+)-*SS* (like) and (±)-*RS* (unlike) model butenolides and permitted the assignments of the relative configurations between C-4 and C-34 in acetogenins by using the magnitudes of the $\Delta\delta$ values for the ^{1}H and ^{19}F nuclei in their Mosher esters (Hoye, Hanson, Hasenwinkel, Raminez, & Zhuang, 1994). The $\Delta\delta_H$ values for H-35 and H-36 in **1r** and **1s**, and for H-33 and H-34 in **2r** and **2s**, at 0.23 and 0.03, suggested that **1** has the 4*R*, 36*S*, and **2** has the 4*R*, 34*S* configurations, as is usual. Therefore, the absolute configuration of **1** is C-22*S*, C-21*R*, C-18*R*, C-17*R*, C-4*R* and C-36*S* or C-22*R*, C-21*R*, C-18*R*, C-17*S*, C-4*R* and C-36*S*, and that of **2** is C-20*S*, C-19*R*, C-16*R*, C-15*R*, C-4*R* and C-34*S* or C-20*R*, C-19*R*, C-16*R*, C-15*S*, C-4*R* and C-34*S*.

Bioactivity data obtained with **1** and **2** are summarized in Table 4. All of these acetogenins were toxic to the brine shrimp larvae and showed potent cytotoxicities against six human solid tumor cell lines in culture. Cytotoxic selectivities in **1** and **2**, e.g. with activities against the pancreatic cell line (MIA PaCa-2) of 10 to 100× that of adriamycin, were exhibited. The acetogenins exert their effects through inhibition of mitochondrial electron transport (complex I) and the inhibition of the plasma membrane NADH oxidase of cancer cells (Ahammadahib, Hollingworth, McGovren, Hui, & McLaughlin, 1993; Moore, de Caro, Farley, Oberlies, & McLaughlin, 1995).

Table 4
Brine shrimp lethality and cytotoxicity against human solid tumor cell lines for **1** and **2**

		Human cancer cell line					
Compound	BST[a] LC_{50} (μg/ml)	A-549[b] ED_{50} (μg/ml)	MCF-7[c] ED_{50} (μg/ml)	HT-29[d] ED_{50} (μg/ml)	A-498[e] ED_{50} (μg/ml)	PC-3[f] ED_{50} (μg/ml)	MIA PaCa-2[g] ED_{50} (μg/ml)
1	1.31×10^{-1}	4.39×10^{-3}	2.11×10^{-3}	2.09	2.78	2.28	3.99×10^{-5}
2	4.29×10^{-3}	1.65×10^{-3}	1.69×10^{-3}	4.40×10^{-1}	2.19	1.06	2.88×10^{-4}
Adriamycin[h]	not tested	1.74×10^{-2}	4.40×10^{-1}	1.16×10^{-2}	1.16×10^{-2}	4.61×10^{-2}	7.81×10^{-3}

[a] Brine shrimp test (Meyer et al., 1982; McLaughlin, 1991).
[b] Lung carcinoma (Giard et al., 1973).
[c] Breast carcinoma (Soule et al., 1973).
[d] Colon adenocarcinoma (Fogh and Trempe, 1975).
[e] Kidney carcinoma (Giard et al., 1973)
[f] Prostate adenocarcinoma (Kaighn et al., 1979).
[g] Pancreatic carcinoma (Yunis et al., 1977).
[h] Positive control standard.

3. Experimental

3.1. General experimental procedures

Mps were determined on a Yanaco micro melting point apparatus and were uncorrected. Optical rotations were taken on a Jasco DIP-370 digital polarimeter. IR spectra were measured on a Jasco FT/IR 300E spectrophotometer. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. ^1H, ^{13}C and COSY NMR spectra were taken on a Bruker AM-300 or AM-500 spectrophotometer in $CDCl_3$ using TMS as an internal standard. Low- and high-resolution FABMS data were collected on a JEOL JMS-HX110 spectrometer. EIMS spectra were recorded on a Quattro spectrometer. For TLC, silica gel 60 F-254 (EM 5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in MeOH and heating. HPLC was carried out with a Waters 600E HPLC instrument using the Autochrowin software system (Young Lin Scientific Co., South Korea) and a C_{18} column equipped with a Waters 486 detector set at 230 nm.

3.2. Plant material

The seeds of *Asimina triloba* were collected in the fall of 1993, from a plantation of authenticated pawpaw trees grown at the University of Maryland, Western Agricultural Research Station, Keadysville, MD, and were provided through the cooperation of R. Neal Peterson and the PawPaw Foundation, Washington, DC. The identification was confirmed by R. Neal Peterson.

3.3. Extraction and isolation

Steps for extraction and chromatographic fractionation were identical to those reported previously (Woo et al., 1995a). The BST active fractions F (BST, $LC_{50}=1.31\times10^{-1}$) and H (BST, $LC_{50}=4.20\times10^{-3}$) were further subjected to repeated open column chromatography and HPLC to yield pure compounds **1** and **2**.

3.4. Asitrilobin A (*1*)

White amorphous powder; mp 79.3–82.5°C; $[\alpha]^{22}_D$: −2.6° (CH_2Cl_2, $c=0.03$); IR ν_{max} cm^{-1}: 3448 (OH), 1736 (C═O, α,β-unsaturated γ-lactone); UV λ_{max} MeOH ($\log\varepsilon$): 225.6 (4.3); FABMS *m/z* see Fig. 3; HRFABMS *m/z* $[M+H]^+$ 625.5068 for $C_{37}H_{68}O_7$ (calcd 625.5043); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75.5 MHz, $CDCl_3$) see Table 1.

3.5. Asitrilobin A tetraacetate (*1a*)

Treatment of **1** (2 mg) with anhydrous pyridine and acetic anhydride (at room temperature overnight) and subsequent workup gave **1a**: EIMS *m/z* see Fig. 3; ^1H NMR (300 MHz, $CDCl_3$) see Table 1.

3.6. Asitrilobin A tetra-TMSi derivative (*1b*)

Approximately 10 μg of compound **1** was treated with 0.2 μl pyridine and 2 μl of N, *O*-bis-(trimethylsilyl)acetamide for 5 h to give a **1b**: EIMS *m/z* see Fig. 3.

3.7. Asitrilobin B (*2*)

White amorphous powder; mp 65.3–68.4°C; $[\alpha]^{22}_D$: +23.3° (CH_2Cl_2, $c=0.03$); IR ν_{max} cm^{-1}: 3448 (OH), 1736 (C═O, α,β-unsaturated γ-lactone); UV λ_{max} MeOH ($\log\varepsilon$): 225.9 (4.1); FABMS *m/z* see Fig. 4; HRFABMS

m/z $[M+H]^+$ 597.4752 for $C_{35}H_{64}O_7$ (calcd 597.4730); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75.5 MHz, $CDCl_3$) see Table 1.

3.8. Asitrilobin B tetraacetate (**2a**)

Treatment of **2** (2 mg) with anhydrous pyridine and acetic anhydride (at room temperature overnight) and subsequent workup gave **2a**: EIMS m/z see Fig. 4; ^1H NMR (300 MHz, $CDCl_3$) see Table 1.

3.9. Asitrilobin B tetra-TMSi derivative (**2b**)

Approximately 10 μg of **2** was treated with 0.2 μl pyridine and 2 μl of *N*,*O*-bis-(trimethylsilyl)acetamide for 5 h to give **2b**: EIMS m/z see Fig. 4.

3.10. S- and R-MTPA esters of asitrilobins A and B

A previously described method was used (Hui et al., 1992; Rieser et al., 1993, 1994). To 1 mg of **1** or **2** in 0.5 ml of CH_2Cl_2 were added sequentially 0.2 ml pyridine, 0.5 mg 4-(dimethylamino)-pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride, separately. The mixture was left at room temperature overnight and purified over a microcolumn (0.6×6 cm) of silica gel (230–400 mesh) eluted with 3–4 ml of hexane–CH_2Cl_2 (1:2); the eluate was dried, CH_2Cl_2 (5 ml) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 ml × 3) and H_2O (5 ml × 2); the washed eluate was dried in vacuo to give the *S* Mosher esters of **1** and **2**, respectively. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride afforded the *R* Mosher esters. Their pertinent ^1H NMR chemical shifts are given in Tabs. 2–3.

3.11. Bioassay

The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) (Meyer et al., 1982; McLaughlin, 1991). Seven-day in vitro MTT cytotoxicity tests against human tumor cell lines were carried out at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols 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 and Trempe, 1975), A-498 (human kidney carcinoma) (Giard et al., 1973), PC-3 (human prostate adenocarcinoma) (Kaighn et al., 1979) and MIA PaCa-2 (human pancreatic carcinoma) (Yunis et al., 1977) with adriamycin as a positive control.

Acknowledgements

This work was supported by a research grant from the Korea Science and Engineering Foundation (KOSEF: 961-0720-115-2) and was presented as a poster at the 1997 spring and autumn meetings of the Pharmaceutical Society of Korea. Thanks are due to the Cell Culture laboratory, Purdue Cancer Center, for cytotoxicity testing, and to R. Neal Peterson and the PawPaw Foundation for the collection of seeds.

References

Ahammadahib, K. I., Hollingworth, R. M., McGovren, J. P., Hui, Y. -H., & McLaughlin, J. L. (1993). *Life Sci.*, *53*, 1113.

Callaway, M. B. (1990). In *The PawPaw (Asimina triloba)* (pp. 1). Kentucky State University Press, Frankfort.

Cave, A., Figadere, B., Laurens, A. and Zechmeister, D. C. (1997). In W. Herz, G. W., Kirby, R. E. Moore, W. Steglich, Ch. Tamm (Eds.), *Progress in the Chemistry of Organic Natural Products* (pp. 81). Spring Verlag, New York.

Fang, X. P., Rieser, R. J., Gu, Z. M., Zhao, G. X., & McLaughlin, J. L. (1993). *Phytochem. Anal.*, *4*, 49.

Fogh, J., & Trempe, G. (1975). In J. Fogh (Ed.), *Human tumor cells in vitro* (pp. 115). Plenum Press, New York.

Giard, D. L., Aaronson, G. J., Arnstein, P., Kersey, J. H., Dosik, H., & Parks, W. P. (1973). *J. Nat. Prod.*, *51*, 1417.

Gu, Z. -M., Fang, Z. -P., Zeng, L., Song, R., Ng, J. H., Wood, K. V., Smith, D. L., & McLaughlin, J. L. (1994). *J. Org. Chem.*, *59*, 3472.

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

He, K., Shi, G., Zhao, G. -X., Zeng, L., Ye, Q., Schwedler, J. L., Wood, K. V., & McLaughlin, J. L. (1996). *J. Nat. Prod.*, *59*, 1029.

He, K., Zhao, G. -X., Shi, G., Zeng, L., Chao, J. -F., & McLaughlin, J. L. (1997). *Bioorg. Med. Chem.*, *5*, 501.

Hoye, T. R., Hanson, P. R., Hasenwinkel, L. E., Raminez, E. A., & Zhuang, Z. (1994). *Tetrahedron Lett.*, *35*, 8525.

Hoye, T. R., Hanson, P. R., Hasenwinkel, L. E., Raminez, E. A., & Zhuang, Z. (1994). *Tetrahedron Lett.*, *35*, 8529.

Hui, Y. -H., Rupprecht, J. K., Kozlowski, J. F., Wood, K. V., McLaughlin, J. L., Hanson, P. R., Zhuang, Z. -P., & Hoye, T. R. (1992). *J. Am. Chem. Soc.*, *114*, 10203.

Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., & Jones, L. W. (1979). *Invest. Urol.*, *17*, 16.

McLaughlin, J. L. (1991). In K. Hostettmann (Ed.), *Methods in Plant Biochemistry* (Vol. 6, pp. 1). Academic Press, London.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). *Planta Med.*, *45*, 31.

Moore, D. J., de Caro, R., Farley, C., Oberlies, N. H., & McLaughlin, J. L. (1995). *Life Sci.*, *56*, 343.

Rieser, M. J., Fang, X. -P., Anderson, J. E., Miesbauer, L. R., Smith, D. L., & McLaughlin, J. L. (1993). *Helv. Chim. Acta*, *76*, 2433.

Rieser, M. J., Fang, X. -P., Anderson, J. E., Miesbauer, L. R., Smith, D. L., & McLaughlin, J. L. (1994). *Helv. Chim. Acta*, *77*, 882.

Soule, H. D., Vazquez, J., Long, A., Albert, S., & Brennam, M. J. (1973). *Natl. Cancer Inst.*, *51*, 1409.

Woo, M. H., Cho, K. Y., Zhang, Y., Zeng, L., Gu, Z. -M., & McLaughlin, J. L. (1995). *J. Nat. Prod.*, *58*, 1533.

Woo, M. H., Zeng, L., & McLaughlin, J. L. (1995). *Heterocycles*, *41*, 1731.

Woo, M. H., Zeng, L., Ye, Q., Gu, Z. -M., Zhao, G. -X., & McLaughlin, J. L. (1995). *Bioorg. Med. Chem. Lett.*, *5*, 1135.

Yunis, A. A., Arimura, G. K., & Russin, D. (1977). *Int. J. Cancer*, *19*, 128.

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

Zhao, G. -X., Chao, J. -F., Zeng, L., & McLaughlin, J. L. (1996). *Natural Toxins*, *4*, 128.

Zhao, G. -X., Chao, J. -F., Zeng, L., Rieser, M. J., & McLaughlin, J. L. (1996). *Bioorg. Med. Chem.*, *4*, 25.

Zhao, G. -X., Gu, Z. -M., Zeng, L., Chao, J. -F., Kozlowski, J. F., Wood, K. V., & McLaughlin, J. L. (1995). *Tetrahedron*, *26*, 7149.

Zhao, G. -X., Hui, Y. -H., & McLaughlin, J. L. (1992). *J. Nat. Prod.*, *55*, 347.

Zhao, G. -X., Miesbauer, L. R., Smith, D. L., & McLaughlin, J. L. (1994). *J. Med. Chem.*, *37*, 1971.

Zhao, G. -X., Ng, J. H., Kozlowski, J. F., Smith, D. L., & McLaughlin, J. L. (1994). *Heterocycles*, *38*, 1897.

Zhao, G. -X., Rieser, M. J., Hui, Y. -H., Miesbauer, L. R., Smith, D. L., & McLaughlin, J. L. (1993). *Phytochemitry*, *33*, 1065.