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ASIMILOBIN AND *CIS*- AND *TRANS*-MURISOLINONES, NOVEL BIOACTIVE ANNONACEOUS ACETOGENINS FROM THE SEEDS OF *ASIMINA TRILOBA*

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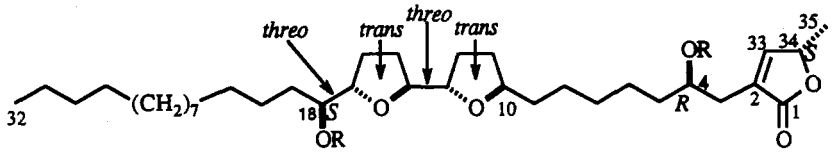
ABSTRACT.—Three new bioactive Annonaceous acetogenins, asimilobin [**1**], *cis*-murisolinone [**2**], and *trans*-murisolinone [**3**], have been isolated from an ethanolic extract of the seeds of *Asimina triloba* by directing the fractionation with brine shrimp lethality. The structures were elucidated based on spectroscopic and chemical methods. In addition, *cis*- and *trans*-bullatacinone, which are known compounds, were obtained. Asimilobin [**1**] has adjacent bis-THF rings, located at C-10 to C-17 and having only one flanking hydroxyl group at C-18. Compounds **1–3** showed cytotoxicity values comparable with adriamycin against six human solid tumor cell lines.

Asimina triloba (L.) Dunal (Annonaceae), commonly known as the Paw Paw tree, is native to the eastern United States and, since before the first European settlers arrived, has been prized for its delicious, custard-like fruit. The distribution of *A. triloba* covers the eastern portion of the United States, and it occurs as far west as eastern Nebraska and Oklahoma (1). Previous phytochemical studies have led to the isolation of oil, lipids, fatty acids, and proteins from its fruits and seeds (2–5); also tannins (6), sitosterol (3), caffeic acid (7), some phenols such as procyanidin, quercetin (7), and quercetin glycosides (8), and a number of isoquinoline alkaloids have been isolated (9–11). The discovery, from the seeds and stem bark, of asimicin, a novel acetogenin which is highly cytotoxic and has promising antitumor and pesticidal activities, was reported by Rupprecht *et al.* (12). Further studies of the stem bark by Zhao *et al.* led to the isolation and structural elucidation of an additional eleven novel bioactive acetogenins [trilobacin (13), *cis*- and *trans*-annonacin-A-one, *cis*- and *trans*-gigantetrocin-A-one, *cis*-isoannonacin (14), asimin, asiminacin, asiminecin (15), bullatin and bullanin (16)], in addition to eleven known compounds [asimicin, bullatacin, *cis*- and *trans*-bullatacinone, *N*-*p*-coumarolytyramine, *N*-*trans*-feruloyltyramine, (+)-syringaresinol (13), *trans*-isoannonacin, squamolone (14), squamocin and motrilin (16)]. From the seeds, we have recently identified two new mono-THF acetogenins, murisolin A and 16,19-*cis*-murisolin, as well as murisolin (17,18).

To continue our search for new potential anticancer and pesticidal constituents from this plant, an EtOH extract of the seeds was investigated, using brine shrimp lethality (BST) to direct the fractionation (19,20). This work has resulted in the isolation of three new bioactive Annonaceous acetogenins, namely, asimilobin [**1**] (Figure 1), *cis*-murisolinone [**2**], and *trans*-murisolinone [**3**] (Figure 2), as well as *cis*- and *trans*-bullatacinone, which are known.

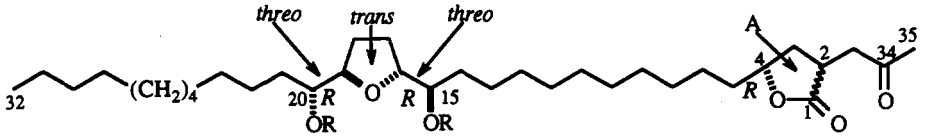
RESULTS AND DISCUSSION

The most active fraction, F005 (BST $LC_{50} = 1.425 \times 10^{-1}$ μg/ml) was obtained from the EtOH extract (F001) of the dried seeds, by partitioning between CH_2Cl_2 (F003) and H_2O (F001) and subsequent partitioning of F003 between hexane and 90% aqueous MeOH (F005) under the guidance of a test for lethality to brine shrimp larvae (19,20).



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

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



Compound	A	R	Compound	A	R
2	cis	H	**3**	trans	H
2a	cis	Ac	**3a**	trans	Ac
2b	cis	TMSi	**3b**	trans	TMSi
2r	cis	(*R*)-MTPA	**3r**	trans	(*R*)-MTPA
2s	cis	(*S*)-MTPA	**3s**	trans	(*S*)-MTPA

FIGURE 2. Structures of **2** and **3** and their derivatives.

The MeOH extract (F005) was submitted successively to Si gel open column and hplc to yield the three new acetogenins, **1–3**. The known acetogenins, *cis*- and *trans*-bullatacinone, were also isolated.

Compound **1** (40 mg), mp 56–57°, $[\alpha]_D$ +6.0° (c=0.05, $CHCl_3$), was obtained in a needle-like form. Its mol wt of 578 was determined by hrcims $[MH]^+$ at 579.4608 (calcd 579.4625), corresponding to a molecular formula of $C_{35}H_{62}O_6$. The presence of two OH groups in **1** was suggested by the formation of a diacetate [**1a**] which gave the expected molecular ion, M^+ at *m/z* 662 in the eims, and exhibited two singlet proton peaks at δ 2.03 and 2.08 in the ^{1}H-nmr spectrum of **1a**. This was supported in the ir spectrum of **1** by a prominent OH absorption at 3440 cm^{-1}, and the carbonyl absorption at 1755 cm^{-1} suggested an α,β-unsaturated γ-lactone, confirmed by a positive response to Kedde's reagent (12,20,21). Compound **1** showed six resonances at δ 7.19 (H-33), 5.06 (H-34), 3.85 (H-4), 2.53 (H-3a), 2.40 (H-3b), and 1.43 (H-35) in the ^{1}H-nmr spectrum, and six peaks at δ 174.62 (C-1), 151.84 (C-33), 131.11 (C-2), 77.97 (C-34), 69.89 (C-4), and 19.08 (C-35) in the ^{13}C-nmr spectrum (Table 1). These are all characteristic spectral features for the methylated α,β-unsaturated γ-lactone fragment, bearing an OH-4 group, as is prevalent in many of the Annonaceous acetogenins (23–25).

Adjacent bis-THF rings were suggested in **1** by the signals for protons at δ 3.85–3.94 (H-10, H-13, H-14, H-17) in the ^{1}H-nmr spectrum (Table 1) and by the signals for carbons at δ 83.09 (C-17), 82.03 (C-14), 81.25 (C-13), and 79.88 (C-10) in the ^{13}C-nmr spectrum (Table 1) (22–24). There were two methine protons attached by hydroxylated carbons at δ 3.85 (H-4) and 3.38, although only the signal at δ 3.38 was observed to have a correlation cross-peak with one of the THF methine protons at δ 3.85 in the ^{1}H-^{1}H COSY spectrum. This indicated that there was only one OH group adjacent to one of the THF rings. The signal for the carbon at δ 79.88 in the ^{13}C-nmr spectrum

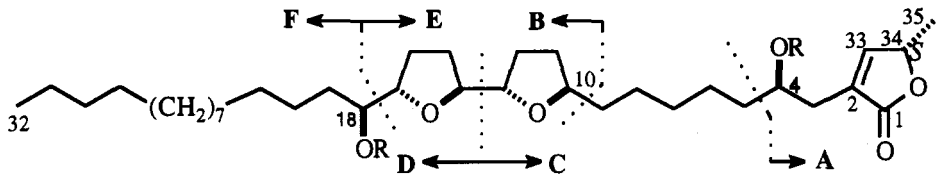
TABLE 1. ^{1}H-Nmr Spectral Data of **1** and **1a**, and ^{13}C-Nmr Spectral Data of **1** ($CDCl_3$, δ).

Position	^{1}H nmr (500 MHz) (Hz)		^{13}C nmr (125 MHz)
	1	**1a**	**1**
1	—	—	174.62
2	—	—	131.11
3a	2.40 dd (15.0, 8.2)	2.51 dd (15.2, 8.0)	33.28
3b	2.53 dt (15.0, 1.5)	2.56 dt (15.5, 1.6)	—
4	3.85 m	5.09 m	69.89
5	1.48 m	1.56 m	37.26
6–8	1.26 br s	1.24 br s	25.45–31.89
9	1.63 m	1.70 m	35.64
10	3.85 m[a]	3.89 m	79.88
11a	1.47 m	1.47 m	29.33
11b	2.02 m	2.02 m	—
12a	1.47 m	1.40 m	28.70
12b	1.63 m	1.59 m	—
13	3.94 m[a]	3.89 m	81.25
14	3.85 m[a]	3.89 m	82.03
15a	1.63 m	1.59 m	28.89
15b	1.96 m	1.96 m	—
16a	1.63 m	1.59 m	28.36
16b	1.96 m	1.96 m	—
17	3.85 m[a]	4.02 m	83.09
18	3.38 m	4.87 m	74.13
19	1.40 m	1.59 m	33.31
20–30	1.26 br s	1.24 br s	25.64–32.03
31	1.29 m	1.29 m	22.66
32	0.88 t (7.0)	0.88 t (7.0)	14.10
33	7.19 q (1.0)	7.08 q (1.5)	151.84
34	5.06 qq (7.0, 1.5)	5.01 qq (7.0, 1.5)	77.97
35	1.43 d (7.0)	1.39 d (6.5)	19.08
4-OAc		2.08 s	
18-OAc		2.03 s	

[a]Assignments may be interchangeable.

supported this suggestion, because a ^{13}C-nmr chemical shift at ca. δ 79 has been found to be characteristic for the oxygenated carbons of THF rings that lack adjacent OH groups; examples of such acetogenins are gigantecin, bullatalicin and gigantetrocin A (23–25). ^{1}H- and ^{13}C-nmr chemical shifts around the bis-THF rings were in good agreement with those of *cis*- and *trans*-bulladecinones, isolated from *Annona bullata* by Gu *et al.* (26). This unusual structural moiety was confirmed by the eims fragmentation analysis of the TMSi derivative [**1b**], which also placed the adjacent bis-THF ring system at C-10 to C-17 (Figure 3).

The relative stereochemistry around the bis-THF rings was determined by comparing the ^{1}H- and ^{13}C-nmr signals of **1** and its diacetate [**1a**] with those of model compounds of known relative stereochemistry (27–29). The comparison suggested that the relative stereochemistry at C-17/C-18 was threo, from the signal for H-18 at δ 3.38 in **1** and from the signal for the protons of the acetyl methyl of C-18 at δ 2.08 and H-18 at δ 4.87 in **1a** (27,28). The relative stereochemistry between C-13 and C-14 was determined as threo by the ^{1}H-nmr signals for H-13 and H-14 at δ 3.85 in **1** and at δ 3.89 in **1a**. Previous to this report, three acetogenins, namely, trilobacin, trilobin, and asitribin have been found to have an erythro relationship between the two THF rings; the signals for the adjacent protons of the two rings were at δ 4.01 and 3.93, respectively



R	$MH^+/M(Ac)_2^+/M(TMSi)_2^+$	A	B	C	D	E	F
H (cims)	579,561 (a), 543 (a)	141,123 (a)	367*,349 (a)*	281,263 (a)	297*,279 (a)*	351,333 (a)	227*,209 (a)*
Ac (eims)	662,602(b), 542 (b)	183*,123 (b)*	409,349 (b)	323,263 (b)	339,279 (b)*	393,333 (b)	268*,208 (b)*
TMSi (eims)	722,632 (c), 542 (c)	213,123 (c)	439,349 (c)	353,263 (c)	369,279 (c)	423,333 (c)	299,209 (c)

FIGURE 3. Diagnostic cims and eims fragmentation ions (*m/z*) of **1** and its diacetate and di-TMSi derivatives. (a) loss of H_2O (*m/z* 18); (b) loss of HOAc (*m/z* 60); (c) loss of TMSi-OH (*m/z* 90). Ions indicated with an asterisk (*) were not observed.

(13,30,31). The relative stereochemistry of both THF rings was suggested as trans by the ^1H- and ^{13}C-nmr signals for the protons attached to the oxygenated carbons of the THF rings in **1** and **1a** as well as the ^1H-nmr resonances for the methylene protons of the THF rings (26–28). The methylene protons of H-11a, H-12a, H-15a, and H-16a were at δ 1.47–1.63 (shifted upfield), and those of H-11b, H-12b, H-15b, and H-16b were at δ 1.63–2.02 (shifted downfield), compared with those of the model compounds having cis configurations across the THF ring (at ca. δ 1.94–1.95 and 1.75–1.80, respectively) (32).

TABLE 2. ^1H-Nmr Spectral Data (δ) of **2**, **2a**, **3**, and **3a**, and ^{13}C-Nmr Spectral Data of **2** and **3** ($CDCl_3$, δ).

Position	^1H nmr (500 MHz) (Hz)				^{13}C nmr (125 MHz)	
	2	**2a**	**3**	**3a**	**2**	**3**
1	—	—	—	—	178.32	178.92
2	3.03 m	3.03 m	3.02 m	3.02 m	35.38	33.49
3a	1.48 m	1.46 m	1.99 m	1.99 m	25.60–31.91	25.60–29.94
3b	2.61 ddd (12.3,9.4,5.6)	2.60 ddd (12.3,9.4,5.6)	2.22 ddd (12.9,9.6,3.4)	2.24 ddd (12.9,9.6,3.4)	—	—
4	4.39 dddd (10.7,7.4,5.4,5.4)	4.40 dddd (10.7,7.4,5.4,5.4)	4.55 dddd (8.3,8.2,5.7,3.2)	4.54 dddd (8.3,8.2,5.7,3.2)	79.40	78.95
5a	1.60 m	1.57 m	1.48 m	1.68 m	36.71	35.46
5b	1.76 m	1.76 m	1.56 m	1.72 m	—	—
6–13	1.26 br s	1.25 br s	1.26 br s	1.25 br s	25.60–31.91	25.60–29.94
14	1.40 m	1.53 m	1.40 m	1.55 m	33.50	33.28
15	3.40 m	4.86 m	3.40 m	4.86 m	74.04[a]	74.03
16	3.80 m	3.97 m	3.80 m	3.97 m	82.61	82.61
17a	1.69 m	1.57 m	1.69 m	1.55 m	28.72	28.72
17b	1.99 m	1.95 m	1.99 m	1.95 m	—	—
18a	1.69 m	1.57 m	1.69 m	1.55 m	28.72	28.72
18b	1.99 m	1.95 m	1.99 m	1.95 m	—	—
19	3.80 m	3.97 m	3.80 m	3.97 m	82.61	82.61
20	3.40 m	4.86 m	3.40 m	4.86 m	74.03[a]	74.03
21	1.40 m	1.53 m	1.40 m	1.55 m	33.50	33.28
22–30 ...	1.26 br s	1.25 br s	1.26 br s	1.25 br s	25.60–31.91	25.60–29.94
31	1.29 m	1.29 m	1.29 m	1.29 m	22.68	22.68
32	0.88 t (7.0)	0.88 t (6.5)	0.88 t (7.0)	0.88 t (6.5)	14.11	14.11
33a	2.61 dd (18.5,9.0)	2.60 dd (19.0,9.0)	2.67 dd (18.5,9.0)	2.67 dd (19.0,9.0)	43.83	44.27
33b	3.11 dd (18.5,3.0)	3.11 dd (18.0,3.0)	3.04 dd (18.5,3.0)	3.04 dd (18.0,3.0)	—	—
34	—	—	—	—	205.61	205.57
35	2.20 s	2.20 s	2.20 s	2.20 s	25.19	25.59
15-OAc ..		1.07 s		207.s		
20-OAc ..		2.07 s		2.07 s		

[a]Assignments may be interchangeable.

Thus, the relative stereochemistry around the THF rings from C-10 to C-18 was concluded to be trans/threo/trans/threo.

To determine the absolute stereochemistry of the carbinol centers at C-4 and C-18 in **1**, the di-(*R*)- and -(*S*)-methoxytrifluoromethyl phenylacetic acid (MTPA) esters (Mosher esters) [**1r** and **1s**] were prepared (33,34). ^{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 that at C-18 is *S* (Table 3). Hoye *et al.* synthesized (+)-*SS* (like) and (±)-*RS* (unlike) model butenolides (34) 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 (36). The $\Delta\delta_H$ values for H-33 and H-34 in **1r** and **1s**, at 0.25 and 0.06, suggested that **1** has the 4*R*,34*S* configuration, as is usual. Therefore, the structure of **1** was concluded to be as illustrated and was named asimilobin; this name should not be confused with that of the alkaloid, asimilobine (10). The absolute configuration of **1** is C-18*S*, C-17*S*, C-14*S*, C-13*S*, C-10*R*, C-4*R*, and C-34*S*. Compound **1** was simultaneously obtained and identified in our laboratory from the bark of *Goniothalamus giganteus* (37).

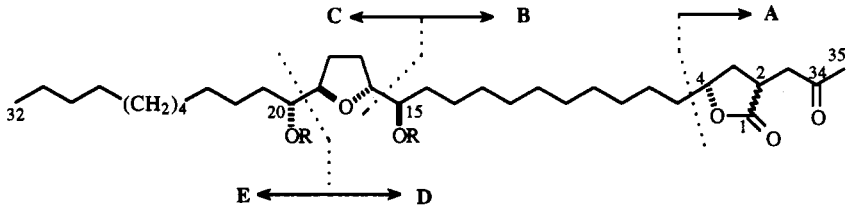
TABLE 3. ^{1}H-Nmr Chemical Shifts for the Determination of Absolute Configurations at C-4 and C-18 of the Di-(*S*)- and -(*R*)-MTPA Esters of **1**.

MTPA Configuration	Proton Chemical Shift (δ_H)											
	H-5a/ H-5b	H-4	H-3a	H-3b	H-33	H-34	H_3-35	H-16a	H-16b	H-17	H-18	H-19a/ H-19b
R	1.62/ 1.69	4.90	2.59	2.67	6.97	5.37	1.31	1.52	1.94	4.05	5.06	1.64/ 1.68
S	1.64/ 1.70	4.85	2.57	2.59	6.72	5.31	1.28	1.60	2.04	4.05	5.06	1.49/ 1.52
$\Delta\delta_{S-R}$	+0.02/ +0.01	−0.05	−0.02	−0.08	−0.25	−0.06	−0.03	+0.08	+0.10	0	0	−0.15/ −0.16
Carbinol Configuration	C-4: *R*							C-18: *S*				

Compound **2** (12 mg), mp 92–93°, $[\alpha]_D$ +13.3° (*c*=0.1, CH_2Cl_2), was obtained as an amorphous powder. A molecular ion peak at *m/z* 581 in the cims (isobutane) spectrum of **2** (Figure 4) indicated a mol wt of 580 daltons. The hrcims (isobutane) spectrum showed an exact mass peak at *m/z* 581.4769, which matched the molecular formula $C_{35}H_{64}O_6$ (calcd 581.4781). The ir spectrum showed strong absorption at 1764 cm^{-1} for a γ-lactone and 1723 cm^{-1} for a ketone. Compound **2** was not positive to Kedde's reagent suggesting that the lactone ring is not α,β-unsaturated. The ^{1}H- and ^{13}C-nmr spectra of **2** clearly indicated the presence of a ketolactone moiety (22–24). In the ^{1}H-nmr spectrum, the resonance at δ 4.39 was assigned to H-4 at the ketolactone ring moiety, as is typical with these (2,4-*cis*)-ketolactones (38). In the ^{13}C-nmr spectrum (Table 2), signals at δ 178.32, 43.83, 35.38, 79.40, and 35.59 were assigned to C-1, C-2, C-3, C-4, and C-34, respectively. The absolute configuration of C-4 was deduced as *R* since all acetogenins whose absolute configurations have so far been determined have 4*R* configurations (34). The further assignments of H-2, H-3a, H-3b, H-33a, and H-33b were based on analysis of the ^{1}H-^{1}H COSY spectrum of **2**.

The remaining portion of **2** exhibited identical ^{1}H- and ^{13}C-nmr signals for a long aliphatic chain, a mono-THF ring, and two OH groups. In the cims of **2**, peaks at *m/z* 581, 563, and 545, arising from the successive losses of H_2O molecules, were observed, confirming the presence of two OH groups. The OH groups were also identified by the broad ir absorption band at 3494 cm^{-1} and by the acetate methyl signals at δ 2.08 (6H)

in the ^{1}H-nmr spectrum of its diacetate, **2a** (Table 2). The placement of the mono-THF ring system at C-16 to C-19 and the two OH groups in **2** was further confirmed based on the ms fragmentation pattern of the di-TMSi derivative [**2b**] (Figure 4).



R	Compound	MH$^+$/M(Ac)$_2^+$/ M(TMSi)$_2^+$	A	B	C	D	E
H (cims)	**2**	581,563 (a), 545 (a)	141	311,293 (a)	269,251 (a)	381,363 (a)	199,187 (a)
H (cims)	**3**	581,563 (a), 545 (a)	141	311,293 (a)	269,251 (a)	381,363 (a)	199,187 (a)
Ac (eims)	**2a**	664,604 (b), 544 (b)	141	352,292 (b)	310,250 (b)	422,362 (b)	242,182 (b)
Ac (eims)	**3a**	664,604 (b), 544 (b)	141	352,292 (b)	310,250 (b)	422,362 (b)	242,182 (b)
TMSi (eims)	**2b**	724*,634 (c), 544 (c)	141	383,293 (c)	341,251 (c)	453,363 (c)	271,181 (c)
TMSi (eims)	**3b**	724*,634 (c), 544 (c)	141	383,293 (c)	341,251 (c)	453,363 (c)	271,181 (c)

FIGURE 4. Diagnostic cims and eims fragmentation ions (*m/z*) of **2** and **3** and their diacetates and di-TMSi derivatives. (a) loss of H_2O (*m/z* 18); (b) loss of HOAc (*m/z* 60); (c) loss of TMSi-OH (*m/z* 90). Ions indicated with an asterisk (*) were not observed.

The relative stereochemistry of the mono-THF ring of **2** was determined as threo/trans/threo using methods of Hoye *et al.* (27,28) and Born *et al.* (29), as well as by comparison with murisolin (39). ^{1}H- and ^{13}C-nmr spectral data around the mono-THF ring had good agreement with murisolin. To determine the absolute configuration of the carbinol centers in **2**, the di-(*R*)- and -(*S*)-MTPA esters, **2r** and **2s**, were prepared, and ^{1}H-^{1}H COSY analyses were made (Table 4). ^{1}H-Nmr chemical shift data of **2r** and **2s** indicated that the absolute configurations of C-15 and C-20 are *R*. Therefore, **2** was identified and named as (2,4-*cis*)-murisolinone, which is a new natural compound; however, the 2,4-*cis* and *trans* mixture has been prepared by translactonization from murisolin (18).[1] Hui *et al.* (38) and Duret *et al.* (39) have demonstrated that these ketolactones are formed by translactonization to the OH-4 from α,β-unsaturated γ-lactones. That this translactonization occurred during our extraction is possible since we have already demonstrated the presence of murisolin in these extracts (17).[2]

Compound **3** (3 mg), mp 101–102°, [α]D +20.0° (*c*=0.1, CH_2Cl_2), was also obtained in an amorphous state. Cims gave a [MH]$^+$ at *m/z* 581 indicating a mol wt of 580 daltons. The molecular formula was established as $C_{35}H_{64}O_6$ by the hrcims (MH$^+$ *m/z* 581.4787; calcd 581.4781). The ir, uv, and ms data of **3** and its diacetate and tri-

[1]Because science usually honors precedent (38), we suggest that further workers use the "-one" suffix, rather than the "iso-" prefix (39,40) to name new acetogenin ketolactones. Similarly, to honor precedent, this class of compounds should properly be called the "acetogenins," as they were originally named by Jolad *et al.* (21), and not "polyketides" as suggested by some authors (40, *inter alia*). In this manner, it is hoped to avoid nomenclatural inconsistencies that may add confusion to the literature.

[2]However, the lesser quantity isolated of the trans-isomer (compound **3**) suggests that translactonization in the plant may be stereospecific and, thus, a natural process.

TMSi derivatives (Figure 4) were quite similar to those of **2**, suggesting that **3** is a diastereomer of **2**. In the ^{1}H-nmr spectrum of **3**, the resonance at δ 4.55 was assigned to H-4 of the (2,4-*trans*)-ketolactone ring. Signals for Ha-3, Hb-3, Ha-5, Hb-5, Ha-33, and Hb-33 appeared at δ 1.99, 2.22, 1.48, 1.56, 2.67, and 3.04, respectively, and also suggested the trans configuration of the ketolactone moiety (38). The relative and absolute stereochemistries around the mono-THF ring in **3** were the same as those of **2** (Table 4). Thus, **3** was determined to be (2,4-*trans*)-murisolinone, which is also a new natural compound but was in the mixture prepared from murisolin as described above (18). The separation of the (2,4-*cis* and *trans*)-ketolactones is difficult but, for **2** and **3**, was achieved by hplc (41).

TABLE 4. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configurations at C-15 and C-20 of the Di-(*S*)- and -(*R*)-MTPA Esters of **2** and **3**.

MTPA Configuration	Proton Chemical Shift (δ_H)									
	H-14a/ H-14b	H-15	H-16	H-17a	H-17b	H-18a	H-18b	H-19	H-20	H-21a/ H-21b
2 (*R*)	1.54	5.03	4.01	1.55	1.92	1.55	1.92	4.01	5.03	1.54
2 (*S*)	1.59	4.96	3.92	1.37	1.65	1.37	1.65	3.92	4.96	1.59
$\Delta\delta_{2(S)-2(R)}$	+0.05	−0.07	−0.09	−0.18	−0.27	−0.18	−0.27	−0.09	−0.07	+0.05
3 (*R*)	1.55	5.03	4.02	1.55	1.92	1.55	1.92	4.02	5.03	1.54
3 (*S*)	1.59	4.96	3.93	1.37	1.65	1.37	1.65	3.93	4.96	1.59
$\Delta\delta_{3(S)-3(R)}$	+0.04	−0.07	−0.09	−0.18	−0.27	−0.18	−0.27	−0.09	−0.07	+0.05
Carbinol Configuration	C-15: *R*					C-20: *R*				

Bioactivity data obtained with **1–3** are summarized in Table 5. All of these acetogenins were toxic to brine shrimp larvae and showed cytotoxic activity against six human tumor cell lines in culture. Compound **1** exhibited similar activities to adriamycin across the human tumor cell lines. The diastereomeric differences of the ketolactone moiety in **2** and **3** did not exert any major effects on the bioactivities except with the pancreatic carcinoma (MIA PaCa-2) cell line, in which **3** was about 50 times more potent. The acetogenins exert their biological effects through inhibition of mitochondrial electron transport (complex I) and the inhibition of the plasma membrane NADH oxidase of cancer cells (42,43).

TABLE 5. Brine Shrimp Lethality and Cytotoxicity against Human Solid Tumor Cell Lines for **1–3**.

Compound	BST[a] LC_{50} (μg/ml)	Human Cancer Cell Line					
		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	1.06	3.01×10^{-2}	2.14	6.30×10^{-2}	2.26×10^{-2}	1.47	1.04×10^{-1}
2	1.23×10	1.48×10^{-1}	7.93×10^{-2}	7.54×10^{-1}	3.44	1.48	1.07×10^{-1}
3	1.82×10	2.76×10^{-2}	2.96×10^{-2}	1.16	1.23	2.14×10^{-1}	5.90×10^{-3}
Adriamycin[h]	Not tested	1.01×10^{-3}	1.03×10^{-2}	2.62×10^{-2}	3.66×10^{-3}	1.96×10^{-2}	1.32×10^{-3}

[a]Brine shrimp test (18,19).
[b]Lung carcinoma (41).
[c]Breast carcinoma (42).
[d]Colon adenocarcinoma (43).
[e]Kidney carcinoma (41).
[f]Prostate adenocarcinoma (44).
[g]Pancreatic carcinoma (45).
[h]Positive control standard.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mel-Temp apparatus and are reported uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. Ir spectra were obtained on a Perkin-Elmer 1600 Ftir spectrophotometer. Uv spectra were measured on a Beckman DU-7 uv spectrophotometer. ^{1}H, ^{13}C-, and COSY nmr spectra were recorded on a Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer in $CDCl_3$ with TMS as internal reference. Low-resolution cims and eims data were collected on a Finnigan 4000 spectrometer. Eims for TMSi derivatives and exact masses on ms measurements were obtained on a Kratos MS-50 spectrometer through peak matching. For tlc, Si gel 60 F_{254} (EM 5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Hplc was carried out with a Rainin hplc instrument using the Dynamax software system and a Si gel column (250×21 mm) equipped with a Rainin UV-1 detector set at 230 nm. Compounds **2** and **3** were transparent and were detected by their retention times (hexane-MeOH-THF, 90:9:1; **2** in 79 min, **3** in 82 min).

PLANT MATERIAL.—The seeds of *Asimina triloba* were collected in the fall of 1993, from plantations of Paw Paw trees grown at the University of Maryland and were purchased from the Paw Paw Foundation. The identification was confirmed by R. Neal Peterson. A voucher specimen of the seeds is preserved at the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

EXTRACTION AND ISOLATION.—Air-dried seeds of *Asimina triloba* (6 kg) were ground into a powder and then extracted with 95% EtOH (8 liters×4) at room temperature and evaporated, under rotary vacuum, to yield 2 kg of the EtOH extract (F001, BST LC_{50} 2.48 μg/ml) which was partitioned between H_2O (3 liters) and CH_2Cl_2 (3 liters×5), yielding 298 g of the H_2O-soluble fraction (F002, BST LC_{50} 1.66×10^{2} μg/ml), 1.7 kg of the CH_2Cl_2 soluble fraction (F003, BST LC_{50} 8.19×10^{-1} μg/ml), and 2 g of an insoluble interface (F004). F003 was further partitioned between hexane (3 liters) and 90% MeOH aqueous solution (3 liters×6) and yielded 600 g of the MeOH fraction (F005, BST LC_{50} 1.43×10^{-1} μg/ml) and 1.1 kg of the hexane-soluble fraction (F006, BST LC_{50} 1.05×10^{2} μg/ml). Directed by the BST bioassay, the most bioactive fraction, F005 (440 g) was further fractioned by open cc on Si gel (3.7 kg, 60–200 mesh), eluting with hexane/$CHCl_3$ and $CHCl_3$/MeOH gradients; 14 pools (from A to N) were made from the collected fractions, according to their tlc patterns, and evaluated by the BST bioassay. The most active pools, D (BST LC_{50} 6.85×10^{-2} μg/ml) and E (BST LC_{50} 1.49×10^{-1} μg/ml), were separately subjected to further repeated separation by Si gel (60–200 mesh) cc eluted with hexane/Me_2CO gradients. Further purification of the most bioactive fractions were carried out by hplc eluted with 10% THF in MeOH/hexane gradients (5–10%) to yield the three new acetogenins, **1–3**, in addition to a mixture of the two known compounds, (2,4-*cis*- and *trans*)-bullatacinone.

Asimilobin [**1**].—White needles (40 mg), mp 56–57°; [α]D +6.0° (*c*=0.05, $CHCl_3$); uv λ max MeOH (nm) 228 (log ε 2.98); ir ν max (film) 3440, 2924, 2853, 1755, 1597, 1448, 1319, 1065, 845 cm^{-1}; hrcims (isobutane) $[MH]^+$ *m/z* 579.4608 for $C_{35}H_{62}O_6$ (calcd 579.4625); eims of di-TMSi and diacetate derivatives, see Figure 3; ^{1}H- and ^{13}C-nmr data, see Table 1; COSY in $CDCl_3$ (500 MHz).

Asimilobin diacetate [**1a**].—Treatment of **1** (3 mg) with Ac_2O/pyridine (at room temperature, overnight) and subsequent workup gave **1a** as a wax: [α]D +14.3° (*c*=0.3, $CHCl_3$); ir ν max (film) 2925, 2854, 1756, 1739, 1600, 1446, 1372, 1240, 1026 cm^{-1}; eims see Figure 3; ^{1}H-nmr data, see Table 1; COSY in $CDCl_3$ (500 MHz).

cis-*Murisolinone* [**2**].—White powder (12 mg), mp 92–93°, [α]D +13.3° (*c*=0.1, CH_2Cl_2); uv λ max (CH_2Cl_2) 220 (log ε 3.80) nm; ir ν max (film) 3494, 2916, 2848, 1764, 1723, 1587, 1531, 1467, 1188, 1069 cm^{-1}; hrcims (isobutane) $[MH]^+$ *m/z* 581.4769 for $C_{35}H_{64}O_6$ (calcd 581.4781); eims of di-TMSi and diacetate derivatives see Figure 4; ^{1}H- and ^{13}C-nmr data, see Table 2; COSY in $CDCl_3$ (500 MHz).

cis-*Murisolinone diacetate* [**2a**].—Treatment of **2** (1 mg) with Ac_2O/pyridine (at room temperature, overnight) and subsequent work up gave **2a** as a wax; eims see Figure 4; ^{1}H-nmr data, see Table 1; COSY in $CDCl_3$ (500 MHz).

trans-*Murisolinone* [**3**].—White powder (3 mg), mp 101–102°, [α]D +20.0° (*c*=0.1, CH_2Cl_2); uv λ max (EtOH) (nm) 218 (log ε 3.43); ir ν max (film) 3490, 2916, 2848, 1744, 1713, 1589, 1183, 1070 cm^{-1}; hrcims (isobutane) $[MH]^+$ *m/z* 581.4787 for $C_{35}H_{64}O_6$ (calcd 581.4781); eims of di-TMSi and diacetate derivatives see Figure 4; ^{1}H- and ^{13}C-nmr data, see Table 2; COSY in $CDCl_3$ (500 MHz).

trans-*Murisolinone diacetate* [**3a**].—Treatment of **3** (1 mg) with Ac_2O/pyridine (at room temperature, overnight) and subsequent work up gave **3a** as a wax; eims *m/z*, see Table 4; ^{1}H-nmr data, see Table 1; COSY in $CDCl_3$ (500 MHz).

TMSi derivatives of **1–3** [**1b–3b**].—10–50 μg of **1**, **2**, or **3** were placed separately in a 100 μl conical

reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h, respectively. The sample was treated with 2 μl pyridine (silylation grade) and 20 μl of *N,O-bis*-(trimethylsilyl)-acetamide (BSA) and heated at 70° for 30 min. Eims, see Figures 3 and 4.

S- *and* R *MTPA-Esters of* **1–3**.—To 1 mg of **1**, **2**, or **3** 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 micro-column (0.6×6 cm) of Si 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 *S* Mosher esters of **1**, **2**, and **3**, respectively. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride afforded the *R* Mosher esters. Their pertinent ^{1}H-nmr chemical shifts are given in Tables 3 and 4.

BIOLOGICAL TESTING.—The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) (19,20). Seven-day in vitro MTT cytotoxicity tests against human tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma) (44), MCF-7 (human breast carcinoma) (45), HT-29 (human colon adenocarcinoma) (46), A-498 (human kidney carcinoma) (44), PC-3 (human prostate adenocarcinoma) (47), and MIA PaCa-2 (human pancreatic carcinoma) (48) with adriamycin as a positive control. The reported ED_{50} values in μg/ml (Table 5) were tabulated from the same run in order to facilitate comparisons for the SAR conclusions.

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