Asimitrin and 4-Hydroxytrilobin, New Bioactive Annonaceous Acetogenins from the Seeds of *Asimina triloba* Possessing a Bis-tetrahydrofuran Ring

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Bioactivity-directed fractionation of the seeds of *Asimina triloba* resulted in the isolation of asimitrin (**1**) and 4-hydroxytrilobin (**2**). Compound **1** represents an adjacent ring-hydroxylated bis-tetrahydrofuran (THF) acetogenin. Compound **2** has an adjacent bis-THF ring with two flanking hydroxyl groups and a α,β-unsaturated γ-lactone with a 4-hydroxyl group. Compounds **1** and **2** showed cytotoxic selectivity, with 100−10 000 times the potency of adriamycin against prostate adenocarcinoma (PC-3) and colon adenocarcinoma (HT-29) cell lines.

The paw paw tree (*Asimina triloba* Dunal) is a temperate representative of the tropical plant family Annonaceae, distributed abundantly in the eastern region of North America.[1] Previous phytochemical studies on the species have resulted in the isolation of 18 new acetogenins.[2−7] Our continuing search for new members of this same compound class in the seeds of the same species has led to the isolation of two acetogenins, asimitrin (**1**) and 4-hydroxytrilobin (**2**). Compounds **1** and **2** showed significant cytotoxicity against several human tumor cell lines, with selectivities for prostate adenocarcinoma (PC-3) and colon adenocarcinoma (HT-29) cell lines.

Results and Discussion

Directing the fractionation with the brine shrimp lethality test (BST),[8,9] both **1** and **2** were isolated as bioactive compounds after repeated chromatographic treatment of the aqueous MeOH partition (F005).[2] Compound **1**, $[\alpha]^{23}_{D}$ +20.0° (*c* 0.01, CH_2Cl_2), was isolated as a white wax. Its molecular weight was suggested by the mass peak at *m/z* 639 $[M + H]^+$ in the FABMS. The HRFABMS gave *m/z* 639.4821 for the $[M + H]^+$ ion (calcd 639.4836), corresponding to the molecular formula $C_{37}H_{67}O_8$. Compound **1** showed an IR carbonyl absorption at 1763 cm^{-1}, a UV λ_{max} (MeOH) at 228 nm (log ϵ 3.06), ^{1}H NMR resonances at δ 7.19, 5.06, 3.84, 2.53, and 2.40, and ^{13}C NMR resonances at δ 174.6, 151.7, 131.2, 78.0, 70.0, and 19.1, all of which provided characteristic spectroscopic features for an α,β-unsaturated γ-lactone fragment with an OH-4 group.[10−12] The presence of four hydroxyl functionalities in **1** was evident from the IR absorption at 3367 cm^{-1} and four successive losses (*m/z* 926, 836, 746, and 656) of TMSiOH (*m/z* 90) from the $[M]^+$ in the EIMS of **1b** (Figure 1). Furthermore, the ^{13}C NMR spectrum of **1** showed four resonances due to oxygen-bearing carbons at δ 70.0, 72.0, 73.4, and 73.4, confirming these four hydroxyl groups.

The positions of the unusual adjacent ring-hydroxylated bis-THF and hydroxyl groups on the hydrocarbon chain were determined by careful analysis of the ^{1}H NMR, COSY, ^{13}C NMR, HMQC, and HMBC spectra of **1**. The OH-17 position was proposed on a rigid ring system rather than an open-ended hydrocarbon chain because of the large δ value difference of its neighboring methylene protons (δ 1.94 for H-18a and 2.36 for H-18b).[10−12] In turn, H-19, a methine proton at δ 4.14, was identified by tracing its COSY cross-peaks to both H-18a and H-18b. At this point, a hydroxylated THF ring across C-16/19 was established. This inference was supported by the three new peaks at C-19/H-17, C-17/H-15, and C-16/H-18ab in the HMBC spectrum. In addition, the ^{13}C NMR chemical shift of **1** for C-16 shifted downfield to δ 91.4 and C-19 was shifted upfield to δ 79.9 and was also supported by the α,β-effect and a γ-gauche effect[13] due to the hydroxyl group at C-17. Typically, the carbons at C-16 and C-19 having no hydroxyl group at C-17 appear at δ 81−82 and 83−84, respectively. The assignment of the second THF ring at C-20/23 was made possible by the H-20/23 cross-peak (δ 4.00/3.91) in the double-relayed COSY spectrum. In the ^{1}H−^{1}H COSY spectrum of **1**, the correlations observed at H-23/24 (δ 3.91/3.43) confirmed the placement of the hydroxyl flanking the adjacent bis-THF rings. Finally, the two hydroxyls flanking the adjacent ring-hydroxylated bis-THF rings were clearly present. The above structural proposal was supported by the EIMS of the tetraacetate (**1a**) and tetra-TMSi (**1b**) derivatives of **1** (Figure 1).

The relative stereochemistry of the adjacent bis-THF flanking hydroxyls at C-15/16 and C-23/24 was assigned as *threo* to the ring system by comparing the ^{1}H NMR signals of **1** for H-15, H-16, H-23, and H-24 with those of model compounds of known relative stereochemistry.[14] To determine the relative stereochemistry across the THF rings at C-16/19, C-20/23, and C-16/17, a 2D NOESY experiment was conducted. Both THF rings were suggested to be *trans* because no NOESY correlation across the rings was detected. The lack of any correlation between H-16 and H-17 indicated a *trans* configuration.[15]

The absolute stereochemistry of **1** was established by using Mosher ester methodology.[16−18] The $\Delta\delta_H$ ($\delta_{1S} - \delta_{1R}$) values of diagnostic protons in the adjacent bis-THF moiety

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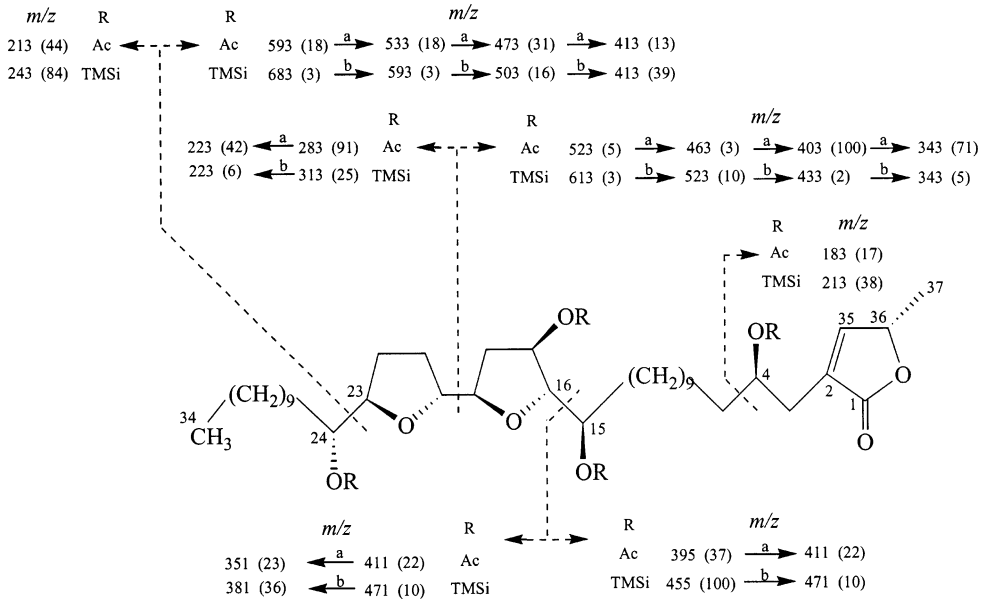
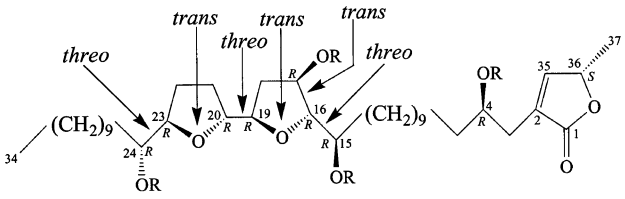


Figure 1. Diagnostic EIMS fragmentation (*m/z*) of the tetraacetate (**1a**) and tetra-TMSi (**1b**) derivatives: (a) loss of HOAc (60 amu); (b) loss of TMSiOH (90 amu) (intensities are indicated in parentheses).

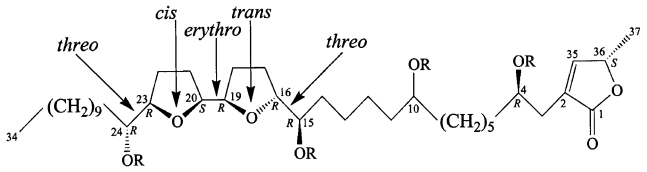
Table 1. Characteristic ^{1}H NMR Data of Mosher Esters **1s** and **1r**

position	**1s** δS	**1r** δR	$\delta S-R$
3a	2.57	2.59	−0.02
3b	2.59	2.67	−0.08
4	5.31	5.38	*R*
5a	1.64	1.62	+0.02
5b	1.70	1.69	+0.01
14	1.60	1.58	+0.02
15	5.01	5.11	*R*
16	3.96	4.06	−0.10
17	5.08	5.16	*R*
18a	1.67	1.74	−0.07
18b	2.00	2.30	−0.30
19	3.80	3.92	−0.12
20	3.80	3.87	−0.07
21a	1.49	1.59	−0.10
21b	1.65	1.74	−0.09
22a	1.49	1.54	−0.05
22b	1.82	1.91	−0.09
23	3.96	3.97	−0.01
24	4.96	4.98	*R*
25	1.67	1.43	+0.24

from C-14 to C-25 of the Mosher ester derivatives, **1s** and **1r**, showed a positive value on the aliphatic chain side (H-14 and H-25) and a negative value on the THF ring side (H-16 to H-23) (Table 1). According to the Mosher configurational correlation model, the *R* configuration was assigned for both C-15 and C-24.[16−18] Thus, the absolute stereochemistries for C-15, C-16, C-19, C-20, C-23, and C-24 were all deduced as *R*. At this point, the *threo* relationship at C-19/20 became self-evident. Using the Hoye model,[19,20] the absolute stereochemistry at C-4 and C-36 was determined from the Mosher esters as *R* and *S*, respectively. The *R* configuration for the carbinol center at C-17 was assigned from the *trans* C-16/17 bond. Therefore, the absolute configuration of **1** was concluded to be C-4*R*, C-15*R*, C-16*R*, C-17*R*, C-19*R*, C-20*R*, C-23*R*, C-24*R*, and C-36*S*. Compound **1** is the second adjacent ring-hydroxylated bis-THF to be reported and further indicates the existence of a novel acetogenin type that is different from mucoxin,[21] the first adjacent ring-hydroxylated bis-THF, at the location of ring-hydroxylation. Thus, the structure of **1** was elucidated as illustrated, and it was named asimitrin.



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



	R
2	H
2a	TMSi

Compound **2**, $[\alpha]^{20}_D$ +20.0° (*c* 0.07, $CHCl_3$), was obtained as a white powder. The HRFABMS gave a $[M + Na]^+$ ion at *m/z* 661.4648 (calcd 661.4655), corresponding to the formula $C_{37}H_{66}O_8Na$. The ^{1}H NMR spectral peaks at δ 7.18 (H-35) and 5.06 (H-36), as well as the ^{13}C NMR resonances at δ 174.6 (C-1), 131.2 (C-2), 151.7 (C-35), 78.0 (C-36), and 19.1 (C-37), again suggested the presence of an α,β-unsaturated γ-lactone ring with an OH-4.[10−12] The signals in the ^{1}H and ^{13}C NMR spectra of **2** at δ 3.59 and 71.8 are characteristic of a hydroxyl group in an alkyl chain. The carbon skeleton and the placement of the hydroxyl group were determined on the basis of the EIMS fragmentation of the TMSi derivative (**2a**) of **2** (Figure 2). The position of the OH group was determined by the fragment at *m/z* 385, which indicated that this hydroxyl was at C-10.[22]

An adjacent bis-THF ring with two flanking hydroxyls was indicated to be preserved in **2** from the signals at δ

Table 2. Brine Shrimp Lethality and Cytotoxicity against Human Solid Tumor Cell Lines of **1** and **2**

compound	BST[a] LC_{50} (μg/mL)	human cancer cell line ED_{50} (μg/mL)					
		A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	MIA PaCa-2[g]
1	2.07×10^{-2}	1.19	2.12	1.19×10^{-4}	7.50×10^{-1}	1.72×10^{-6}	2.11×10^{-4}
2	7.00×10^{-2}	1.54	3.79	1.54×10^{-6}	3.62×10^{-2}	2.01×10^{-4}	2.01×10^{-4}
adriamycin[h]	NT[i]	6.22×10^{-3}	9.53×10^{-1}	2.87×10^{-2}	2.86×10^{-3}	5.77×10^{-2}	6.10×10^{-3}

[a] Brine shrimp test. [b] Human lung carcinoma. [c] Human breast carcinoma. [d] Human colon adenocarcinoma. [e] Human kidney carcinoma. [f] Human prostate adenocarcinoma. [g] Human pancreatic carcinoma. [h] Positive control standard. [i] NT: Not tested.

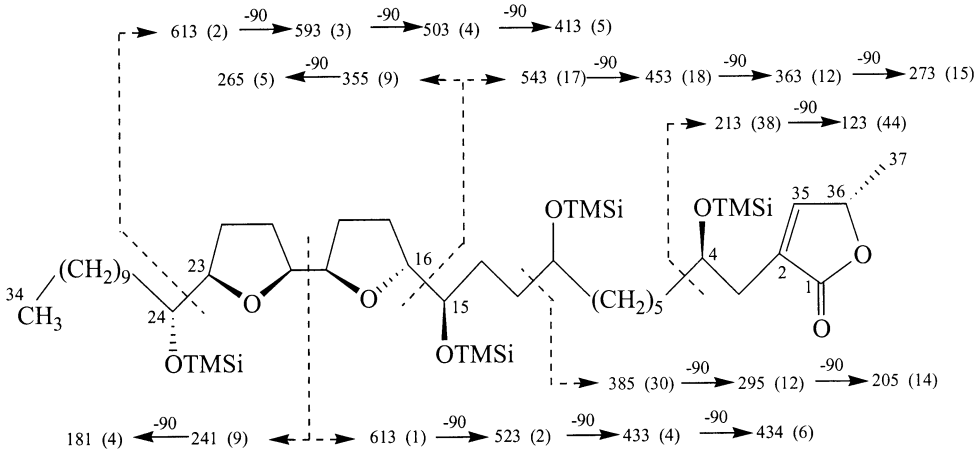


Figure 2. Diagnostic EIMS peaks for the tetra-TMSi derivative (**2a**) (intensities are indicated in parentheses). *Ions not observed.

3.38 (H-15, 24), 3.85 (H-16), 3.97 (H-19), 4.05 (H-20), and 3.84 (H-23) in the ^{1}H NMR spectrum and from the signals at δ 74.6 (C-15), 83.2 (C-16), 81.6 (C-19), 80.9 (C-20), 82.6 (C-23), and 73.8 (C-24) in the ^{13}C NMR spectrum. These proton and carbon signals were very similar to those observed with trilobacin[23] and trilobin.[24] The bis-THF ring was located from C-16 to C-23 in the hydrocarbon chain on the basis of typical fragments observed in the EIMS of **2a** (Figure 2). All OH-4 acetogenins found, so far, have the *R* stereochemistry at C-4 in **2**.[25] Thus, compound **2** was named 4-hydroxytrilobin and is a new natural annonaceous acetogenin.

Compounds **1** and **2** were significantly active in the brine shrimp lethality test (BST)[8,9] and were also cytotoxic for six human solid tumor cell lines in a 7-day MTT [26−30] assay using adriamycin as the positive control. Compound **1** was selectively cytotoxic against prostate adenocarcinoma (PC-3) at about 10 000 times and against colon adenocarcinoma (HT-29) at about 100 times the potency of adriamycin, respectively. In turn, compound **2** was especially active against the prostate adenocarcinoma (PC-3) at about 100 times and against colon adenocarcinoma (HT-29) cell line at about 10 000 times the potency of adriamycin. The acetogenins exert their cytotoxic and their in vivo antitumor effects, in part, by inhibiting complex I of the electron transport system in the mitochondria and by blocking the NADH oxidase enzyme peculiar to the plasma membranes of cancerous cells.[31−34]

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micro melting point apparatus and are 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. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR spectra were recorded on a Varian VXR 300S or a Varian 500S spectrometer in $CDCl_3$ using TMS as an internal standard. Low- and high-resolution FABMS data were collected on a JEOL JMS-HX110 spectrometer. EIMS were recorded on a Quattro II 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 performed on a Waters 600 apparatus equipped with a Waters 486 UV detector at 225 nm using the Autochrowin software system (Waters Korea Co., Seoul, Korea). μBondapak C_{18} columns (Waters, 19 × 300 mm and 7.8 × 300 mm) were used for preparative purpose.

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, Washington, DC. The identification was confirmed by R. Neal Peterson. A voucher specimen (CUDP 93001) of the seeds is preserved at the Department of Pharmacy, Catholic University of Daegu, Korea.

Bioassays. The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST).[8,9] Seven-day in vitro MTT cytotoxicity tests against human tumor cell lines were carried out at the Cell Culture Laboratory, Purdue Cancer Center, West Lafayette, IN, using standard protocols for A-549 (human lung carcinoma),[26] MCF-7 (human breast carcinoma),[27] HT-29 (human colon adenocarcinoma),[28] A-498 (human kidney carcinoma),[26] PC-3 (human prostate adenocarcinoma),[29] and MIA PaCa-2 (human pancreatic carcinoma),[30] with adriamycin as a positive control.

Extraction and Isolation. Steps for extraction and chromatographic fractionation were identical to those reported previously.[2] The BST-active fractions F (BST, $LC_{50} = 2.28 \times 10^{-1}$ μg/mL) and H (BST, $LC_{50} = 1.38 \times 10^{-1}$ μg/mL) were separately subjected to further repeated separation by Si gel (60−200 mesh) column chromatography eluted with hexane/Me_2CO gradients. Further purification of the most bioactive fractions were carried out by HPLC to yield two new acetogenins, **1** and **2** [preparative HPLC: μBondapak C_{18} column (10 μm, 19 × 300 mm i.d.), elution with acetonitrile/H_2O (80:20) at flow rate 10 mL/min, t_R 15.3 min (**1**) and 14.3 min (**2**)].

Asimitrin (1): white wax (20 mg); mp 82.1−82.7°C; $[\alpha]^{23}_D$ −5.0° (*c* 0.02, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 230 (3.1) nm; IR (film) ν_{max} 3367, 1763 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (1H, q, J = 1.5 Hz, H-35), 5.06 (1H, dq, J = 7.0, 1.5 Hz, H-36), 4.14 (1H, dt, J = 4.3, 1.0 Hz, H-19), 4.09 (1H, br d, J = 7.0, H-17), 4.00 (1H, dt, J = 7.5,1.5 Hz, H-20), 3.91 (1H, m, H-23), 3.84 (1H, m, H-4), 3.75 (1H, dd, J = 5.0, 2.5 Hz, H-16),

3.43 (1H, m, H-24), 3.33 (1H, m, H-15), 2.53 (1H, dt, $J = 15.0$, 1.5 Hz, H-3b), 2.40 (1H, dd, $J = 15.0$, 8.2 Hz, H-3a), 2.36 (1H, m, H-18b), 2.08 (1H, m, H-21b), 2.02 (1H, m, H-22b), 1.98 (1H, m, H-21a), 1.94 (1H, m, H-18a), 1.78 (1H, m, H-22a), 1.43 (1H, d, $J = 7.0$ Hz, H-37), 0.88 (1H, d, $J = 7.0$ Hz, H-34); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6 (s, C-1), 151.7 (d, C-35), 131.2 (s, C-2), 91.4 (d, C-16), 83.6 (d, C-23), 82.2 (d, C-20), 79.9 (d, C-19), 78.0 (d, C-36), 73.4 (d, C-24), 73.4 (d, C-17), 72.0 (d, C-15), 70.0 (d, C-4), 38.0 (s, C-18), 33.3 (t, C-3), 28.7 (t, C-22), 28.1 (t, C-21), 19.1 (q, C-37), 14.1 (q, C-34); FABMS *m/z* 639 $[MH]^+$, 577 $[MH - H_2O]^+$, 621 $[MH - 2\ H_2O]^+$, 603 $[MH - 3\ H_2O]^+$, 585 $[MH - 4\ H_2O]^+$; HRFABMS *m/z* $[MH]^+$ 639.4821 for $C_{37}H_{67}O_8$ (calcd 639.4836).

Asimitrin Tetraacetate (1a). Treatment of **1** (2 mg) with anhydrous pyridine and acetic anhydride (at room temperature overnight) and subsequent workup gave **1a**: EIMS, see Figure 1

Asimitrin Tetra-TMSi Derivative (1b). Approximately 10 μg of **1** was treated with 0.2 μL of pyridine and 2 μL of *N*,*O*-bis(trimethylsilyl)acetamide for 5 h to give compound **1b**: EIMS, see Figure 1.

4-Hydroxytrilobin (2): white powder (10 mg); mp 95.0−96.0 °C; $[\alpha]^{23}_D$ +20.0° (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205.2 (3.8) nm; IR (film) ν_{max} 3414, 1752 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (1H, q, $J = 1.5$ Hz, H-35), 5.06 (1H, qq, $J = 6.8$, 1.5 Hz, H-36), 4.05 (1H, m, H-20), 3.97 (1H, m, H-19), 3.85 (1H, m, H-16), 3.84 (2H, m, H-4, H-23), 3.59 (1H, m, H-10), 3.38 (2H, m, H-15, H-24), 2.53 (1H, dt, $J = 15.0$, 1.5 Hz, H-3b), 2.40 (1H, ddt, $J = 15.3$, 8.5, 1.5 Hz, H-3a), 2.05 (1H, m, H-21b), 1.95 (2H, m, H-17b, H-22b), 1.69 (21H, m, H-17a, H-22a), 1.67 (1H, m, H-21a), 1.43 (1H, d, $J = 6.5$ Hz, H-37), 0.88 (1H, t, $J = 7.0$ Hz, H-34); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6 (s, C-1), 151.8 (d, C-35), 131.2 (s, C-2), 83.2 (d, C-16), 82.6 (d, C-23), 81.6 (d, C-19), 80.9 (d, C-20), 78.0 (d, C-36), 74.6 (d, C-15), 73.8 (d, C-24), 71.8 (d, C-10), 69.9 (d, C-4), 33.5 (t, C-3), 28.9 (t, C-17), 28. 3 (t, C-22), 28.2 (t, C-18), 26.9 (t, C-21), 19.1 (q, C-37), 14.1 (q, C-34); FABMS *m/z* 639 $[M + H]^+$, 621 $[MH - H_2O]^+$, 603 $[MH - 2\ H_2O]^+$, 585 $[MH - 3\ H_2O]^+$, 567 $[MH - 4\ H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 661.4648 for $C_{37}H_{66}O_8Na$ (calcd 661.4655).

4-Hydroxytrilobin Tetra-TMSi Derivative (2a). Approximately 10 μg of **2** was treated with 0.2 μL of pyridine and 2 μL of *N*,*O*-bis(trimethylsilyl)acetamide for 5 h to give **2a**: EIMS, see Figure 2.

Preparation of Mosher Esters. A previously described method was used.[16−18] To 1 mg of **1**, in 0.5 mL of CH_2Cl_2, were added sequentially 0.2 mL of pyridine, 0.5 mg of 4-(dimethylamino)pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride. 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). Then 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 ester of **1**. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride afforded the *R* Mosher ester. Their pertinent ^{1}H NMR chemical shifts are given in Table 1.

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References and Notes

(1) Callaway, M. B. In *The PawPaw* (*Asimina triloba*); Kentucky State University Press: Frankfort, KY, 1990; p 1.
(2) Woo, M. H.; Cho, K. Y.; Zhang, Y.; Zeng, L.; Gu, Z.-M.; McLaughlin, J. L. *J. Nat. Prod*. **1995**, *58*, 1533−1542.
(3) Woo, M. H.; Zeng, L.; Ye, Q.; Gu, Z.-M.; Zhao, G.-Z.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1135−1140.
(4) Woo, M. H.; Zeng, L.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1731−1742.
(5) Woo, M. H.; Kim, D. H.; McLaughlin, J. L. *Phytochemistry* **1999**, *50*, 1033−1040.
(6) Woo, M. H.; Chung, S. O.; Kim, D. H. *Bioorg. Med. Chem.* **2000**, *8*, 285−290.
(7) Kim, E. J.; Tian, F.; Woo, M. H. *J. Nat. Prod*. **2000**, *63*, 1503−1506.
(8) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1−35.
(9) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.
(10) Cavé, A.; Figadére, B.; Laurens, A.; Cortes, D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, 1997; Vol. 70, pp 81−287.
(11) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep*. **1996**, *13*, 275−306.
(12) 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 Press: New York, 1995; Vol. 29, pp 249−310.
(13) Eggert, H.; VanAntwerp, C. L.; Bhacca, N. S.; Djerassi, C. *J. Org. Chem.* **1976**, *41*, 71−78.
(14) Born, L.; Lieb, F. J.; Lorentzen, P.; Moeschler, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med*. **1990**, *56*, 312−316.
(15) Alali, F. Q.; Rogers, L.; Zhang, Y.; McLaughlin, J. L. *J. Nat. Prod*. **1999**, *62*, 31−34.
(16) Dale, J. A.; Mosher, H. S. *J. Org. Chem*. **1973**, *95*, 512−519.
(17) Rieser, M. J.; 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*, 10203−10213.
(18) Rieser, M. J.; Fang, X.-P.; Anderson, E.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1994**, *76*, 2433−2444.
(19) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett*. **1994**, *35*, 8525−8528.
(20) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett*. **1994**, *35*, 8529−8532.
(21) Shi, G.; Kozlowski, J. F.; Schwedler, J. T.; Wood, K. V.; MacDougal, J. M.; McLaughlin, J. L. *J. Org. Chem*. **1996**, *61*, 7988−7989.
(22) Cortes, D.; Myint, S. H.; Dupont, B.; Davoust, D. *Phytochemistry* **1993**, *32*, 1475−1482.
(23) Zhao, G. X.; Hui, Y. H.; McLaughlin, J. L. *J. Nat. Prod*. **1992**, *55*, 347−356.
(24) Zhao, G. X.; Gu, Z. M.; Zeng, L.; Chao, J. F.; Kozlowski, J. F.; Woo, K. V.; McLaughlin, J. L. *Tetrahedron* **1995**, *51*, 7149−7160.
(25) Alali, F. Q.; Zhang, Y.; Rogers, L.; McLaughlin, J. L. *Phytochemistry* **1998**, *49*, 761−768.
(26) Giard, D. L.; Aaronson, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Nat. Prod*. **1973**, *51*, 1417−1423.
(27) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennam, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409−1416.
(28) Fogh, J.; Trempe, G. In *Human Tumor Cells*; Fogh, J., Ed.; Plenum Press: New York, 1975; pp 115−119.
(29) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol*. **1979**, *17*, 16−23.
(30) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128−135.
(31) Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H. *Pestic. Sci*. **1991**, *33*, 427−438.
(32) Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K. *Pestic. Biochem. Physiol*. **1993**, *45*, 15−23.
(33) Ahammadsahib, K. I.; Hollingworth, R. M.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci*. **1993**, *53*, 1113−1120.
(34) Alali, F. Q.; Liu X. X.; McLaughlin, J. L. *J. Nat. Prod*. **1999**, *62*, 504−540.

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