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Additional Bioactive Annonaceous Acetogenins from *Asimina triloba* (Annonaceae)

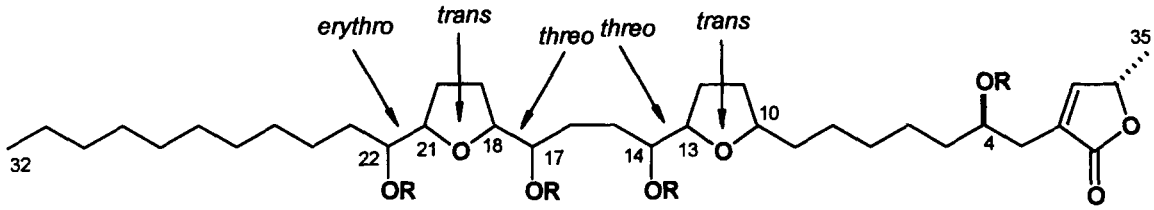
Kan He, Geng-Xian Zhao, Guoen Shi, Lu Zeng, Jin-Feng Chao and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

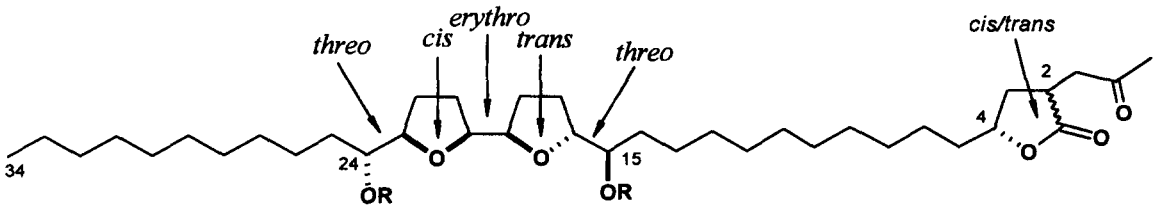
Abstract—Trilobalicin (**1**), a new nonadjacent bis-THF ring annonaceous acetogenin, 2,4-*cis*- (**2**) and 2,4-*trans*-trilobacinone (**3**), the ketolactones of trilobacin, an adjacent bis-THF ring acetogenin, were isolated from the stem bark of *Asimina triloba* (L.) Dunal (Annonaceae). Their structures were established based on chemical and spectral evidence. The relative stereochemistry of **1** was determined as *trans/threo/threo/trans/erythro* from C-10 to C-22 by comparisons of NMR data with those of model compounds. Compound **1** is the first example of a nonadjacent bis-THF acetogenin being isolated from the title species and represents a new type of these compounds. Bioactivities of these new structures against brine shrimp larvae and six human solid tumor cell lines were determined, and cytotoxic selectivities were shown for the lung (A-549) and breast (MCF-7) cell lines with up to a million times the potency of adriamycin.

Introduction

To date, more than 40 annonaceous acetogenins have been isolated from *Asimina triloba* (L.) Dunal (Annonaceae), the North American native paw paw tree.[1–9] The adjacent bis-THF ring acetogenins with three hydroxyl groups (the asimicin-type, bullatacin-type, and trilobacin-type compounds) are found to be the most potent cytotoxic and pesticidal agents of this species;[11] their four hydroxylated isomers show less bioactivities within the series of each type.[10] In our continuing effort to find new antitumor agents, we have isolated three additional novel acetogenins from the stem bark. The structures were identified as trilobalicin (**1**), 2,4-*cis*-trilobacinone (**2**), and 2,4-*trans*-trilobacinone (**3**), based on spectroscopic analysis and chemical derivatives. Compounds **2** and **3** possess the trilobacin-type relative stereoconfiguration of *threo/trans/erythro/cis/threo* between the two carbinol centers in the adjacent bis-THF ring system; this arrangement is only found, so far, in acetogenins from this species (trilobacin, trilobin, and asitribin). Compound **1** was elucidated as a new type of nonadjacent bis-THF ring acetogenin and is the first of this type to be isolated from this species. In this paper, we report the structural determination and bioactivities in the brine shrimp



1: R = H, **1a**: R = TMSi



2: R = H, 2,4-*cis*, **2a**: R = TMSi, **2r**: R = (*R*)-MTPA, **2s**: R = (*S*)-MTPA
3: R = H, 2,4-*trans*, **3a**: R = TMSi, **3r**: R = (*R*)-MTPA, **3s**: R = (*S*)-MTPA

Table 1. NMR data of trilobalicin (**1**) ($CDCl_3$, δ in ppm, *J* in Hz)

No.	^{1}H NMR	^{13}C NMR
1		174.5
2		131.2
3	2.54 ddt (15.1, 3.3, 1.5)	
	2.41 ddt (15.1, 8.3, 1.4)	33.4
4	3.86 m	70.0
5	1.24–1.72 m	37.3
6–8	1.24–1.72 m	22.7–31.9
9	1.24–1.72 m	35.6
10	3.87 m	79.3
11	2.00 m, 1.65 m	32.5
12	2.00 m, 1.65 m	28.4
13	3.80 m	82.0
14	3.41 m	74.4
15–16	1.24–1.73 m	22.7–31.9
17	3.41 m	74.6
18	3.86 m	83.3
19	2.00 m, 1.65 m	28.7
20	2.00 m, 1.65 m	25.3
21	3.87 m	82.2
22	3.80 m	71.6
23	1.24–1.73	32.5
24	1.24–1.73	26.0
25–31	1.24–0.73	22.7–31.9
32	0.88 t (7.0)	14.1
33	7.18 ddd (1.5, 1.5, 1.5)	151.9
34	5.06 qddd (7.0, 1.5, 1.5, 1.5)	78.0
35	1.44 d (7.0)	19.1

lethality test (BST) and cytotoxicity data in six human solid tumor cell lines of these new compounds.

Results and Discussion

Compound **1** was isolated as a white wax. The molecular ion was determined at *m/z* 610 by LRFABMS. The HRFABMS gave the $[MH]^+$ at *m/z* 611.4529 corresponding to the molecular formula of $C_{35}H_{62}O_8$ (calcd 611.4523). Four consecutive losses of H_2O $[MH^+ - 18]$ in the FABMS indicated the existence of four hydroxyl groups in **1**.

The ^{1}H signals at δ 2.54 (H-3a), 2.41 (H-3b), 3.86 (H-4), 7.18 (H-33), 5.06 (H-34), and 1.44 (H-35), as well as the ^{13}C NMR signals at δ 33.4 (C-3), 70.0 (C-4), 151.9 (C-33), 78.0 (C-34), and 19.1 (C-35), indicated the existence of a methylated α,β-unsaturated γ-lactone with a 4-OH group in **1** (Table 1); this moiety was confirmed by a peak at *m/z* 213, a characteristic fragment for all acetogenins of this type, in the EIMS spectrum of the TMSi derivative (**1a**) (Fig. 1).[10] In addition to the signal for H-4 at δ 3.86, there were five proton signals around δ 3.80–3.88 and two proton signals at δ 3.41 in the ^{1}H NMR for **1**, suggesting the existence of two nonadjacent THF ring systems with three flanking hydroxyl groups.[12] The carbon signal at δ 79.3 was especially useful, as an indication for the presence of a THF ring without one flanking hydroxyl on one side, since it appeared relatively upfield compared with the flanking THF ring carbon signals at δ 82.0, 82.2, and 83.3 in the ^{13}C NMR spectrum of **1**.[11] Consequently, **1** was concluded to be an acetogenin with nonadjacent bis-THF rings with three flanking hydroxyl groups.

The carbon skeleton and positions of the THF rings, as well as the hydroxyl groups along the hydrocarbon chain, were deduced from the EIMS of the TMSi derivative (**1a**). As shown in Figure 1, the fragmentation patterns clearly indicated that the two THF rings were located at C-10 and C-18 by the displacement of three prominent peaks at *m/z* 313, 353, and 585, which were confirmed further by high resolution EIMS of the TMS derivative (**1a**) at *m/z* 313.2567 (calcd 313.2563), 353.2140 (calcd 353.2148), and 585.3457 (calcd 585.3463), respectively. The positions of the three flanking hydroxyl groups were also suggested by the fragment ions and were located at C-14, C-17, and C-22. The planar structure of **1** was, therefore, established as similar to that of the bullatalicin-type acetogenins[12] but with a two-carbon unit shift of the THF ring system toward the lactone ring; the THF ring system starts at C-10 in **1** compared with bullatalicin in which it starts at C-12.

The relative stereochemistries of C-10, C-13, C-14, C-17, C-18, C-21, and C-22 in **1** were determined by comparing the ^{1}H and ^{13}C NMR signals with those of published data and model compounds.[13,14] The observed two proton signals at ca. δ 3.41 suggested two *threo* relative configurations either at C-13/14, C-17/18, or C-21/22 between the flanking hydroxyls and their THF rings. The third hydroxyl and its adjacent THF ring was assigned an *erythro* relative configuration due to the corresponding proton signal at ca. δ 3.80.[11] The ^{13}C NMR signals at δ 74.4 and 74.6 were also quite indicative of two *threo* flanking hydroxyls while an *erythro* signal appeared relatively upfield at δ 71.6.

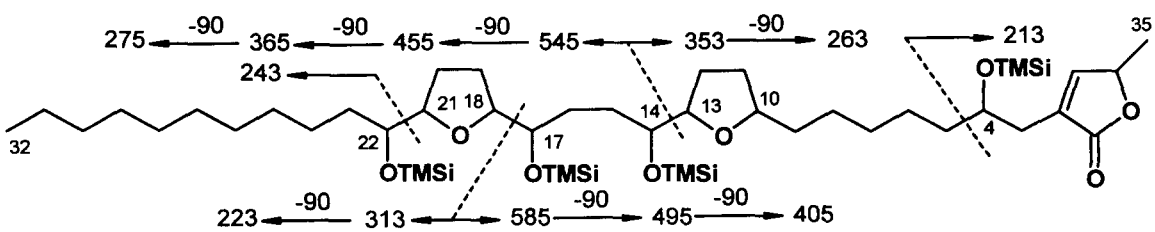


Figure 1. Diagnostic EIMS fragment ions of **1a**.

Considering just these proton and carbon signals, however, it was difficult to determine the exact position of the *erythro* arrangement among the above three possibilities.

Both THF rings of **1** possess a *trans* configuration based on the observation that the methylene proton signals resonated at ca. δ 2.00 (H-11a, H-12a, H-19a, and H-20a) and δ 1.65 (H-11b, H-12b, H-19b, and H-20b).[11] A comparison of partial structures of **1** with those of synthetic model compounds revealed that the ^{1}H and ^{13}C NMR signals of **1** matched well with those of *threo/trans/erythro* (dihydroxyl flanked) mono-THF ring and *trans/threo* (single hydroxyl) flanked mono-THF ring compounds.[13–15] Compound **1** was, thus, suggested to be similar to the bullatalicin-type structure with the *trans/threo/threo/trans/erythro* relative configuration but with the molecule two carbons shorter (C-35 versus C-37). On direct comparison with the literature data, **1** was found to exhibit the exact same ^{13}C NMR data from C-10 to C-23 as those of bullatalicin from C-12 to C-25.[12] Due to the insufficient quantities of **1** (only 1.5 mg was isolated), the preparation of per-Mosher ester derivatives for **1** was not successful and left the absolute stereochemistry unresolved. Absolute stereochemistries at C-4 and C-34 were assumed as *R* and *S* as in all other known acetogenins.[16] The structure of **1** was, thus, assigned as a new compound of the rare C-35 nonadjacent bis-THF ring acetogenins with the bullatalicin-type relative configuration, and it was named trilobalicin in honor of the specific epithet. Among the 28 currently known nonadjacent bis-THF acetogenins, only one C-35 compound has previously been reported.[17]

Compounds **2** and **3** were isolated as white waxes, and the molecular weights were established from their HRFABMS at *m/z* 623.4895 and 623.4892 $[MH]^+$ corresponding to the molecular formula $C_{37}H_{66}O_7$ (calcd 623.4887). The spectra of **2** and **3**, for the aliphatic side chain and THF rings, proved to be identical, indicating that these two compounds share the same structural features in these regions. The ^{1}H NMR at δ 3.37 (H-15), 3.83 (H-16), 3.97 (H-19), 4.04 (H-20), 3.83 (H-23), and 3.39 (H-24), as well as ^{13}C NMR at δ 74.5 (C-15), 83.2 (C-16), 81.7 (C-19), 80.8 (C-20), 82.6 (C-23), and 74.0 (C-24), showed closely similar NMR signals to those reported for trilobacin, at C-15 to C-24,[6] and suggested an adjacent bis-THF ring moiety with a trilobacin-type *threo/trans/erythro/cis/threo* configuration from C-15 to C-24 for **2** and **3** (Table 2). The placement of the bis-THF rings at C-16 to C-23 was diagnosed by the exhibition of characteristic fragments at *m/z* 383, 523, and 243 in their bis-TMSi derivatives (**2a** and **3a**) in the EIMS spectra (Fig. 2).

The absolute configurations at the C-15 and C-24 carbinol centers for **2** and **3** were determined by ^{1}H NMR analysis of the 2,4-*cis/trans* trilobacinone per-Mosher esters derivative values (**2s**, **3s** and **2r**, **3r**).[18] The $\Delta\delta_{S-R}$ showed positive values on the aliphatic chain side (H-14 and H-25) and negative values on the THF rings side (H-16 to H-23), indicating *R* and *R* configurations for C-15 and C-24, based on the Mosher model (Table 3). Identification of the *cis*- and *trans*-ketolactone rings was straightforward due to their distinguishable NMR signals from H-2 to H-5 and C-2 to C-5 in a comparison of their ^{1}H and ^{13}C NMR shifts with published data.[19,20] The *cis*-isomer (**2**) exhibited

Table 2. ^{1}H and ^{13}C NMR data of 2,4-*cis*-trilobacinone (**2**) and 2,4-*trans*-trilobacinone (**3**) ($CDCl_3$, δ in ppm, *J* in Hz)

	^{1}H NMR (500 MHz)		^{13}C NMR (125 MHz)	
No.	**2**	**3**	**2**	**3**
1			178.3	178.8
2	3.05 m	3.03 m	34.4	36.7
3	1.49 m, 2.60 m	1.98 m, 2.23 m	43.7	44.2
4	4.39 dddd (11.5, 7.4, 5.5, 5.0)	4.55 dddd (8.5, 9.0, 5.5, 2.5)	79.3	79.0
5	1.48 m, 1.74 m	1.54 m, 1.69 m	36.6	35.5
6–13	1.22–1.80 m	1.22–1.80 m	25.1–35.3	25.1–35.3
14	1.41 m	1.41 m	33.5	33.5
15	3.37 ddd (8.0, 5.0, 5.0)	3.37 m	74.5	74.5
16	3.83 m	3.83 m	83.2	83.2
17	1.74 m, 1.96 m	1.74 m, 1.96 m	25.1–35.3	25.1–35.3
18	1.87 m, 1.93 m	1.87 m, 1.93 m	25.7	25.7
19	3.97 ddd (8.5, 6.5, 5.0)	3.97 ddd (8.5, 6.5, 5.0)	81.7	81.5
20	4.04 ddd (8.5, 6.0, 5.0)	4.04 ddd (8.5, 6.0, 5.0)	80.8	80.8
21	1.70 m, 2.05 m	1.70 m, 2.05 m	25.7	25.7
22	1.74 m, 1.96 m	1.74 m, 1.96 m	27.0	27.0
23	3.83 m	3.83 m	82.6	82.6
24	3.39 ddd (6.5, 7.0, 6.0)	3.39 m	74.0	73.8
25	1.41 m	1.41 m	34.1	34.1
26–33	1.22–1.60 m	1.22–1.60 m	25.1–35.3	25.1–35.3
34	0.88 t (7.0)	0.88 t (7.0)	14.0	14.0
35a	3.11 dd (18.5, 3.5)	3.02 dd (18.5, 3.5)	43.8	44.2
35b	2.61 dd (18.5, 8.5)	2.64 dd (15.3, 8.5)		
36			205.6	205.6
37	2.20 s	2.20 m	29.9	29.9

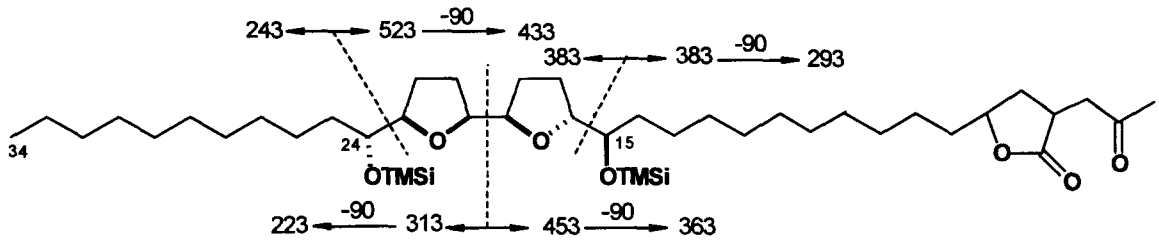


Figure 2. Diagnostic EIMS fragment ions of **2a** and **3a**.

Table 3. ^{1}H NMR data of the (*S*)- and (*R*)-Mosher esters of **2s**, **3s** and **2r**, **3r**

Protons	H-14	H-16	H-17	H-18	H-19	H-20	H-21	H-22	H-23	H-25
S-MTPA (**2s**)	1.61	3.93	1.78 1.47	1.80 1.65	3.63	3.71	1.76 1.48	1.91 1.50	3.96	1.61
R-MTPA (**2r**)	1.46	3.94	1.91 1.58	1.97 1.80	3.79	3.75	1.99 1.66	1.95 1.59	3.98	1.46
$\Delta\delta_{S-R}$	+0.15	−0.01	−0.13 −0.11	−0.17 −0.15	−0.16	−0.04	−0.23 −0.18	−0.04 −0.09	−0.02	+0.05
S-MTPA (**3s**)	1.61	3.93	1.78 1.48	1.80 1.65	3.63	3.71	1.76 1.48	1.91 1.49	3.96	1.61
R-MTPA (**3r**)	1.46	3.95	1.92 1.59	1.96 1.80	3.77	3.75	1.99 1.66	1.95 1.59	3.97	1.46
$\Delta\delta_{S-R}$	+0.15	−0.02	−0.14 −0.11	−0.16 −0.15	−0.14	−0.04	−0.23 −0.18	−0.04 −0.10	−0.01	+0.05
config		15*R*							24*S*	

Table 4. Bioactivities of compounds **1** and **2**[a]

	BST[b]	A-549[c]	MCF-7[d]	HT-29[e]	A-498[f]	PC-3[g]	PaCa-2[h]
1	5.79	5.78×10^{-8}	1.59×10^{-7}	2.28	5.97×10^{-2}	9.80×10^{-1}	2.75×10^{-1}
2	2.0×10^{-2}	4.64×10^{-6}	1.25×10^{-8}	1.57	4.91×10^{-1}	2.75	6.86×10^{-2}
Adriamycin[i]	8.0×10^{-2}	2.54×10^{-3}	9.98×10^{-2}	1.81×10^{-2}	2.62×10^{-3}	4.20×10^{-2}	3.38×10^{-3}

[a]The BST LC_{50} value of **3** was 5.3×10^{-2} ppm; sufficient **3** was not available for cytotoxicity testing.
[b]Brine shrimp lethality test.
[c]Human lung carcinoma.
[d]Human breast carcinoma.
[e]Human colon adenocarcinoma.
[f]Human kidney carcinoma.
[g]Human prostate adenocarcinoma.
[h]Human pancreatic carcinoma.
[i]Standard positive control.

the characteristic proton signals at δ 3.05 (H-2), 2.60, 1.49 (2 × H-3), 4.39 (H-4), 1.48, 1.74 (2 × H-5) and carbon signals at δ 34.4 (C-2), 43.7 (C-3), 79.3 (C-4), and 36.6 (C-5), which were quite consistent with other *cis*-ketolactones. Similarly, the proton signals at δ 3.03 (H-2), 2.23, 1.98 (2 × H-3), 4.55 (H-4), 1.54, 1.69 (2 × H-5) and carbon signals at δ 36.7 (C-2), 44.2 (C-3), 79.0 (C-4), and 35.5 (C-5) suggested the *trans*-ketolactone structure for **3**. Compounds **2** and **3** were, therefore, identified as 2,4-*cis*- and 2,4-*trans*-trilobacinones, respectively. The complete assignments of carbon signals were achieved by using HMQC results (Table 2).

Biological activities of **1** and **2** are summarized in Table 4. They show potent activities against brine shrimp larvae and human solid tumor cell lines. Selective cytotoxicities, with 10^3–10^6 times the potency of adriamycin against the lung (A-549) and breast (MCF-7) carcinoma cell lines were exhibited. Annonaceous acetogenins act as powerful inhibitors of ATP production through inhibition of the NADH-ubiquinone oxidoreductase (complex I) of mitochondrial electron transport systems and as inhibitors of the ubiquinone-linked NADH oxidase in the plasma membranes of cancerous cells.[21,22] They are active against drug-resistant cell lines and are relatively nontoxic to normal cells.[23]

Experimental

Instrumentation

Optical rotations were determined on a Perkin–Elmer 241 polarimeter. The IR was recorded on a Perkin–Elmer 1600 FTIR spectrophotometer. The low and high resolution EIMS were taken on Finnigan 4000 and on Kratos 50 spectrometers, respectively. The NMR spectra were recorded on a Varian VXR-500 (^{1}H at 500 MHz and ^{13}C at 125 MHz) or a Bruker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometer with $CDCl_3$ as solvent and TMS as internal reference. A Rainin HPLC system with Dynamax software and a Dynamax UV-1 variable wavelength detector were used for preparative separations.

Plant material

The stem bark of *Asimina triloba* (L.) Dunal (Annonaceae) was collected from stands growing wild at the Purdue University Horticultural Research Farm, West Lafayette, Indiana, U.S.A. The species was identified by Dr George R. Parker, Department of Forestry and Natural Resources, Purdue University. The voucher specimen is deposited in the pharmacognosy herbarium.

Bioassays

The brine shrimp (*Artemia salina* Leach) test (BST) was routinely employed for evaluating the extracts, fractions, and isolated compounds from the title plant.[24,25] Seven-day MTT in vitro cytotoxicities against A-549 (human lung carcinoma),[26] MCF-7 (breast carcinoma),[27] HT-29 (colon adenocarcinoma),[28] A-498 (kidney carcinoma),[26] PC-3 (prostate adenocarcinoma),[29] MIA PaCa-2 (pancreatic carcinoma)[30] human tumor cell lines were performed at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive control.

Extraction and isolation

The bark extraction procedures were the same as those described in our previous reports.[4–9] The partitioned fraction (F005) obtained was repeatedly chromatographed over open silica-gel columns using gradients of CH_2Cl_2–MeOH and then separated by repeated normal phase HPLC (Dynamax-60 A 8 μm silica gel, 250 × 21.4 mm i.d. or 250 × 4.6 mm i.d.), eluted with hexane–90% MeOH/THF (8:2), and by a C-18 column (250 × 21.4 mm i.d.), eluted with an CH_3CN–H_2O (8:2) isocratic solvent system, to yield **1** (1.5 mg), **2** (2.8 mg), **3** (2.4 mg), and the mixture of **2** and **3** (15.2 mg).

Trilobalicin (1). White wax, $[\alpha]^{22}_D$+13.6 (*c* 0.125, $CHCl_3$); IR ν^{film}_{max} 3333, 2919, 2866, 1738, 1594, 1456, 1398, 1116 cm^{-1}; FABMS (GLY/S-GLY) *m/z* 611 $[M+H]^+$; HRFABMS (GLY/S-GLY) *m/z* 611.4529 for $C_{35}H_{62}O_8$ $[M+H]^+$, calcd 611.4523; EIMS (Fig. 1); ^{1}H and ^{13}C NMR (Table 1).

Formation of TMSi Derivatives (1a–3a). Approximately 10–50 μg of pure compound was placed in a 100 μL conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h. The sample was treated with 2 μL pyridine and 20 μL *N,O*-bis-(trimethylsily) acetamide and heated at 70 °C for 30 min.

Formation of per-(*R*)- and per-(*S*)-Mosher ester derivatives. One mg of **1** and **2** was dissolved in 0.5 mL dry CH_2Cl_2, respectively, and 0.2 mL pyridine and 0.2 mg 4-dimethylaminopyridine, as well as 25 mg of (*R*)-(−)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride was sequentially introduced to this solution and the reaction mixture was stored in refrigerator overnight. The product was purified over a small pipette eluted with hexane, hexane–CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2–EtOAc to give *S*-Mosher esters. *R*-Mosher esters were prepared by same way using (*S*)-(+)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride reagent; ^{1}H NMR (Table 3).

2,4-*cis*-Trilobacinone (2). White wax, $[\alpha]^{22}_D$+17.3 (*c* 0.22, $CHCl_3$); IR ν^{film}_{max} 3354, 2923, 2845, 1742, 1722, 1591, 1456, 1403, 1361, 1116, 1170, 1121, 1079 cm^{-1}; FABMS (GLY/S-GLY) *m/z* 623 $[MH]^+$; HRFABMS (GLY/S-GLY) *m/z* 623.4887 for $C_{37}H_{66}O_7$ $[MH]^+$, calcd 623.4895; EIMS (Fig. 2); ^{1}H and ^{13}C NMR (Table 2).

2,4-*trans*-Trilobacinone (3). White wax, $[\alpha]^{22}_D$+9.2 (*c* 0.22, $CHCl_3$); IR ν^{film}_{max} 3354, 2922, 2845, 1743, 1724, 1590, 1456, 1411, 1359, 1120, 1171, 1121, 1074 cm^{-1}; FABMS (GLY/S-GLY) *m/z* 610 $[MH]^+$; HRFABMS (GLY/S-GLY) *m/z* 623.4882 for $C_{37}H_{66}O_7$ $[MH]^+$, calcd 623.4895; EIMS (Fig. 2); ^{1}H and ^{13}C NMR (Table 2).

Acknowledgments

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References

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

2. Woo, M.-H.; Zeng, L.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1731.

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

4. Zhao, G.-X.; Rieser, M. J.; Hui, Y. H.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Phytochemistry* **1993**, *33*, 1065.

5. Zhao, G.-X.; Hui, Y. H.; Rupprecht, J. K.; McLaughlin, J. L. *J. Nat. Prod.* **1992**, *55*, 347.

6. Zhao, G.-X.; Gu, Z.-M.; Zeng, L.; Chao, J.-F.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L. *Tetrahedron* **1995**, *51*, 7149.

7. Zhao, G.-X.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *J. Med. Chem.* **1995**, *37*, 1971.

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

9. Zhao, G.-X.; Ng, J. H.; Kozlowzki, J. F.; Smith, D. L.; McLaughlin, J. L. *Heterocycles* **1994**, *38*, 1897.

10. Gu, Z.-M.; Zeng, L.; Schwedler, J. T.; Wood, K. V.; McLaughlin, J. L. *Phytochemistry* **1995**, *40*, 467.

11. 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, p 249.

12. Fang, X.-P.; Gu, Z.-M.; Rieser, M. J.; Hui, Y.-H.; McLaughlin, J. L.; Nonfon, M.; Lieb, F.; Moeschler, H.-F.; Wendisch, D. *J. Nat. Prod.* **1993**, *56*, 1095.

13. Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175.

14. Born, L.; Lieb, F.; Lorentzen, J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312.

15. Hoye, T. R.; Zhuang, Z. *J. Org. Chem.* **1988**, *53*, 5578.

16. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8529.

17. Ratnayake, S.; Gu, Z.-M.; Miesbauer, L. R.; Smith, D. L.; Wood, K. V.; Evert, D. R.; McLaughlin, J. L. *Can. J. Chem.* **1994**, *72*, 287.

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

19. Duret, P.; Gromek, D.; Hocquemiller, R.; Cave, A.; Cortes, D. *J. Nat. Prod.* **1994**, *57*, 911.

20. Gu, Z.-M.; Fang, X.-P.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *J. Nat. Prod.* **1993**, *56*, 870.

21. Ahammadsahib, K. I.; Hollingworth, R. M.; McGovern, J. P.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113.

22. Morre, D. J.; Cabo, R. D.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

23. Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; McLaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55.

24. Meyer, B. M.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31.

25. McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic: London, 1991; Vol. 6, p 1.

26. Giard, D. J.; Aronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer. Inst.* **1973**, *51*, 1417.

27. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer. Inst.* **1973**, *51*, 1409.

28. Fogh, J.; Trempe, G. In *Human Tumor Cells in Vitro*; Fogh, J., Ed.; Plenum: New York, 1975; p 115.

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

30. Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128.

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