Annomolin and Annocherimolin, New Cytotoxic Annonaceous Acetogenins from *Annona cherimolia* Seeds

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Two new cytotoxic annonaceous acetogenins, annomolin (**1**) and annocherimolin (**2**), were isolated from an ethanolic extract of the seeds of *Annona cherimolia*. Annomolin has a mono-THF ring with one flanking hydroxyl and possesses a 1,2-diol at C-7/8 of the aliphatic chain. Annocherimolin has a mono-THF ring with two flanking hydroxyls and possesses a double bond at C-21/22. Their structures were elucidated by spectral data and chemical derivatization. Compound **1** showed cytotoxic selectivity for the human prostate tumor cell line (PC-3), with a potency of over 10 000 times that of adriamycin. Compound **2** showed cytotoxic potencies about 10 000 times those of adriamycin in the breast (MCF-7) and colon (HT-29) cancer cell lines.

Annona cherimolia Mill. is a tropical tree native to Peru and is used in traditional medicine as an insecticide and a parasiticide.[1] As cytotoxic activity had been observed with a seed extract of this plant, a systematic investigation of the chemical content of the plant has been undertaken.[2] In previous studies by Cortes *et al.*, eight novel acetogenins, cherimolin, dihydrocherimolin,[3] molvizarin, motrilin,[4] itrabin, jetein,[5] cherimolin-2, and almunequin,[6] were isolated from *Annona cherimolia* seeds. We have reported the isolation of eight acetogenins from the seeds of this plant. Three of them are new, annocherin and (2,4)-*cis*- and *trans*-annocherinones,[7] and the five others, *cis*-annonacin, corrosolin,[8] (2,4)-*cis*- and *trans*-isoannonacins, and compound 2,[9] are known acetogenins. Our continuing brine shrimp lethality test directed isolation has now yielded two new acetogenins, annomolin (**1**) and annocherimolin (**2**) (Chart 1).

Results and Discussion

The seeds of *A. cherimolia* were extracted with 95% EtOH, and the residue of the extract, F001, was partitioned through a standard extraction scheme (see Experimental Section). The extract F005 was subjected to repeated open column chromatography and HPLC to yield two novel acetogenins.

Compound **1**, $[\alpha]^{23}_{D}$ +4.0° (*c* 0.02, CH_2Cl_2), was obtained as a white powder. The HRFABMS gave a $[M + Na]^+$ ion at *m/z* 619.4548 (calcd 619.4550) corresponding to the formula $C_{35}H_{64}O_7Na$. The IR spectrum contained absorptions for hydroxyl (3446 cm^{-1}) and α,*β*-unsaturated *γ*-lactone (1747 cm^{-1}) functionalities. Sequential losses of four molecules of H_2O from the $[M + Na]^+$ in the FABMS as well as the formation of the tetra-TMSi derivative (**1a**) confirmed the existence of four hydroxyl groups in compound **1** (Figure 1).

The presence of an α,*β*-unsaturated *γ*-lactone with a hydroxyl group at C-4 in **1** was suggested by the ^{1}H NMR resonances at *δ* 7.18 (H-33), 5.06 (H-34), 3.89 (H-4), 2.41 (H-3a), 2.53 (H-3b), and 1.43 (H-35) corresponding in the ^{13}C NMR spectrum to the resonances at *δ* 174.7 (C-1), 151.9 (C-33), 131.2 (C-2), 78.0 (C-34), 69.9 (C-4), 33.4 (C-3), and 19.1 (C-35). These are all characteristic spectral features for the methylated α,*β*-unsaturated *γ*-lactone fragment, with the presence of an OH group at the C-4 position, as commonly found among many of the annonaceous acetogenins.[10,11]

The positions of the OH groups in **1** were assigned at C-4, C-7, C-8, and C-18 by careful analysis of the fragments in the EIMS of the tetra-TMSi derivative (**1a**) at *m/z* 585, 541, 445, 439, 343, 299, and 213 (Figure 1). The mono-THF ring with one flanking hydroxyl was indicated by the proton signals in **1** at *δ* 3.83 (H-14), 1.48 (H-15a), 2.02 (H-15b), 1.68 (H-16a), 1.99 (H-16b), 3.80 (H-17), and 3.43 (H-18) and carbon signals at *δ* 79.3 (C-14), 32.4 (C-15), 28.4 (C-16), 81.8 (C-17), and 74.6 (C-18). These data also indicated that the relative stereochemistry of the carbon centers C-17/C-18 was *threo* and the configuration across the THF ring was *trans*, by comparison with a series of model compounds of known relative stereochemistries.[12,13] The ^{1}H NMR *δ* values of the two carbinyl methines of a *threo* 1,2-diol should be relatively low (*ca.* *δ* 3.40) when compared with that of an *erythro* 1,2-diol (*ca.* *δ* 3.60).[14,15] Thus, the vicinal diols in **1** were concluded to be *threo* from the signal of H-7 and H-8 at *δ* 3.43.

Tetra-Mosher ester derivatives (**1r** and **1s**) of **1** were prepared, and their ^{1}H NMR data were assigned according to ^{1}H−^{1}H COSY spectra (Table 1). For **1**, the positive value of δ_H (*S*-*R*) at H-14 (+0.12), H-15 (+0.06, +0.15), H-16 (+0.03, +0.07), and H-17 (+0.02) suggested an *S* configuration at C-18.[14] The 1,2,5-triol group is a fairly common structural feature among the annonaceous acetogenins. The chemical shifts of the esterified methine proton signals appear at ca. *δ* 4.91−4.94 and 5.01−5.03 (*δ* 4.91−4.94 and 5.05 in cases where another double bond is located two carbons away) for the *S*-Mosher esters in the *R*,*R*-1,2-diols, and at ca. *δ* 5.10−5.15 and 5.16−5.19 (*δ* 5.16 and 5.18−5.20 in cases where another double bond is located two carbons away) for the *R*-Mosher esters in *R*,*R*-1,2-diols. In contrast, these signals are located at ca. *δ* 5.03−5.06 and 5.10−5.16 for the *S*-Mosher esters in the *S*,*S*-1,2-diols and at ca. *δ* 5.03−5.04 and 5.17 for the *R*-Mosher esters in the *S*,*S*-1,2-diols. By using this comparative chemical shift

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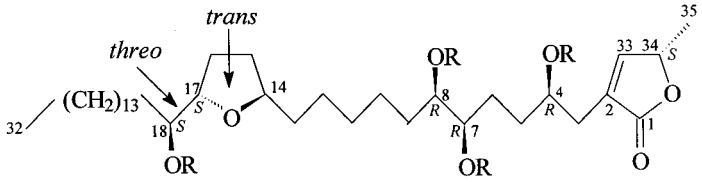
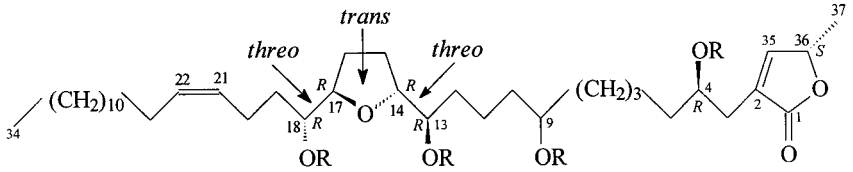
Chart 1



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



Compound	R
2	H
2a	TMSi
2r	(*R*)-MTPA
2s	(*S*)-MTPA

pattern data, we have analyzed and assigned the chemical shifts of compound **1**. The chemical shifts of the 1,2-diol at C-7,8 appeared at δ 5.03 and 4.94 in the *S*-Mosher ester and at δ 5.18 and 5.14 in the *R*-Mosher ester. These values indicated that **1** has *R*,*R*-1,2 diols at C-7,8.[10,16] On the other hand, the chemical shifts of murihexocins A and B[17] having *S*,*S*-1,2-diols at C-7,8 appeared at δ 5.03 and 5.10 (in both murihexocins A and B) in the *S*-Mosher ester and at δ 5.03 and 5.17 (murihexocin A) and δ 5.03 and 5.20 (murihexocin B) in the *R*-Mosher ester. Hoye *et al.* 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.[18,19] The $\Delta\delta_H$ values for H-33 and H-34 in **1r** and **1s** at 0.24 and 0.04 suggested that **1** has the 4*R*,34*S* arrangement. All of the C_{35}, OH-4 annonaceous acetogenins, so far, are 4*R* and 34*S*. Therefore, the structure of **1** was determined as illustrated and named annomolin.

Compound **2**, $[\alpha]^{23}_D$ −21° (*c* 0.02, CH_2Cl_2), was isolated as a white powder. Its molecular weight was suggested by the peak at *m*/*z* 645 $[M + Na]^+$ in the FABMS. The HRFABMS gave *m*/*z* 645.4707 for the $[M + Na]^+$ ion (calcd 645.4706) corresponding to the molecular formula $C_{37}H_{66}O_7$-Na. Compound **2** showed an IR carbonyl absorption at 1743 cm^{-1}, a UV (MeOH) λ_{max} at 230 nm (log ϵ 3.1), six

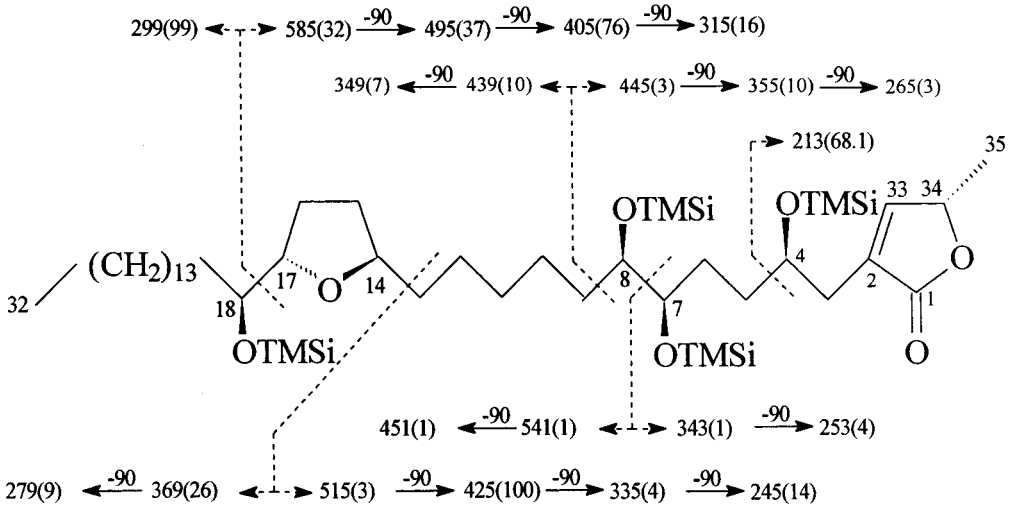


Figure 1. Diagnostic EIMS peaks (*m*/*z*) for the tetra-TMSi derivative **1a** (intensities are indicated in parentheses).

Table 1. Characteristic ^{1}H NMR Data of Mosher Esters of **1s**, **1r**, **2s**, and **2r** for Determinations of Stereochemistry

position	**1s** δ *S*	**1r** δ *R*	δ *S*-*R*	position	**2s** δ *S*	**2r** δ *R*	δ *S*-*R*
5	1.59	1.58	+0.01	5	1.59	1.57	+0.02
4	5.31	5.34	*R*	4	5.30	5.36	*R*
3	2.56	2.58	−0.02	3	2.56	2.60	−0.04
	2.59	2.67	−0.08		2.59	2.63	−0.04
33	6.72	6.96	−0.24	35	6.72	6.95	−0.23
34	4.85	4.89	−0.04	36	4.86	4.91	−0.05
35	1.27	1.30	−0.03	37	1.25	1.30	−0.05
7	5.03	5.18	−0.15	12	1.59	1.57	+0.02
8	4.94	5.14	−0.20	13	4.86	5.03	*R*
14	3.83	3.71	+0.12	14	3.87	4.01	−0.14
15	1.40	1.34	+0.06	15	1.44	1.60	−0.16
	1.98	1.83	+0.15		1.93	1.96	−0.03
16	1.33	1.30	+0.03	16	1.44	1.60	−0.16
	1.85	1.78	+0.07		1.93	1.96	−0.03
17	3.88	3.86	+0.02	17	3.87	4.01	−0.14
18	4.85	4.89	*S*	18	4.86	5.03	*R*
19	1.58	1.59	−0.01	19	1.59	1.57	+0.02

resonances at δ 7.18 (H-35), 5.06 (H-36), 1.43 (H-37), 2.40 (H-3a), 2.53 (H-3b), and 3.89 (H-4) in the ^{1}H NMR spectrum, and six peaks at δ 174.6 (C-1), 151.9 (C-35), 131.2 (C-2), 78.0 (C-36), 19.1 (C-37), and 70.0 (C-4) in the ^{13}C NMR spectrum.

The presence of the mono-THF ring with a flanking OH group on each side was indicated by the proton signals at δ 3.82 (H-14 and 17), 3.44 (H-13 and 18), 1.73 (H-15a and 16a), and 1.99 (H-15b and 16b) and the carbon resonances at δ 82.7 (C-14 and 17), 74.4 (C-13), and 74.3 (C-18) in **2**. These NMR data also indicated that the relative stereochemistries of the carbon centers C-13/14 and C-17/18 were *threo* and the configuration across the THF ring was *trans*, by comparison with a series of model compounds of known relative stereochemistries.[13,20] The carbon skeleton and the placement of the THF ring were determined on the basis of the EIMS fragmentation of the TMSi derivative (**2a**) of **2**. The signals at δ 3.59 and 71.9 in the ^{1}H and ^{13}C NMR spectra of **2** are characteristic of a hydroxyl group in an alkyl chain. The position of the OH group was determined by the fragment at *m*/*z* 371, which indicated that this hydroxyl was at C-9.[21] The presence of an isolated double bond in **2** was determined by the proton signals at δ 5.35 and 5.40 and the carbon signals at δ 129.0 and 130.9. The configuration of the double bond was assigned as *cis* by comparing the NMR spectra with other double-bond-containing acetogenins of known configuration.[22] The position of the double bond was determined at C-21/C-22 from the single-relayed COSY spectrum, which showed a correlation cross-peak between H-18 (δ 3.44) and H-20 (δ 2.20).

The absolute stereochemistry of the carbinol stereocenters in **2** has been determined using Mosher ester methodology based on the differences between the ^{1}H NMR chemical shifts of (*S*)- and (*R*)-MPTA ester derivatives (Table 1). According to the Mosher arguments, C-13 and C-18 were assigned with the *R* absolute configuration, since the signs of $\Delta\delta_H$ (*S*-*R*) were negative for H-14 and H-17, showing relatively less shielding for this side in the (*S*)-MTPA ester.[23] Using the Hoye models, the absolute stereochemistries at C-4 and C-36 were determined from the Mosher esters as *R* and *S*, respectively. Thus, the structure of **2** was elucidated as illustrated, and it was named annocherimolin.

These new isolates (**1** and **2**) were isolated by activity-directed fractionation and are very active in the brine shrimp lethality test (BST),[24,25] and, as expected, they were all significantly cytotoxic to human solid tumor cells in culture (Table 2). Compound **1** exhibited potent and selective cytotoxicities against the breast (MCF-7),[26] colon (HT-29),[27] and prostate (PC-3)[28] cell lines with 100 to 10 000 times the potency of adriamycin. Compound **2** was especially active against the breast (MCF-7), colon (HT-29), and pancreatic (MIA PaCa-2)[29] cell lines. Against these three tumor types, **2** was 100 to 10 000 times as active as the positive control, adriamycin.

Annonaceous acetogenins inhibit cancerous cells by the blockage of mitochondrial complex I (NADH-ubiquinone oxidoreductase)[30] and also through the inhibition of the NADH oxidase prevalent in the plasma membranes of tumor cells.[31]

Experimental Section

General Experimental Procedures. Melting points 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. ^{1}H, ^{13}C, and COSY NMR spectra were recorded on Varian VXR300S or 500S spectrometers 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, Si 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 (Young Su Scientific Co., Seoul, Korea). A μBondapak C_{18} column (19 × 300 mm and 7.8 × 300 mm) was used for preparative purposes.

Plant Material. The seeds of *A. cherimolia* were obtained in the fall of 1996 from fruits grown commercially in plantations in southern California and purchased from Hurov Botanicals and Seeds, located in Chula Vista, CA. A voucher specimen 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).[24,25] 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),[32] MCF-7 (human breast carcinoma),[26] HT-29 (human colon adenocarcinoma),[27] A-498 (human kidney carcinoma),[32] PC-3 (human prostate adenocarcinoma),[28] and MIA PaCa-2 (human pancreatic carcinoma)[29] with adriamycin as a positive control.

Extraction and Isolation. The dried seeds of *A. cherimolia* (8 kg) were repeatedly percolated with 95% EtOH to yield 700 g of an extract (F001), on removal of solvent. F001 was partitioned between CH_2Cl_2−H_2O (1:1) to yield the H_2O-soluble

Table 2. Brine Shrimp Lethality and Cytotoxicities in Human Solid Tumor Cell Lines for **1** and **2**

		human cancer cell line ED_{50} (μg/mL)					
compound	BST[a] LC_{50} (μg/mL)	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	MIA PaCa-2[g]
1	9.40×10^{-3}	2.37	1.15×10^{-4}	8.92×10^{-5}	6.88×10^{-4}	5.39×10^{-6}	2.18
2	5.80×10^{-3}	1.56	4.06×10^{-6}	2.49×10^{-6}	1.53×10^{-1}	1.02	1.20×10^{-5}
adriamycin[h]	NT[i]	1.13×10^{-3}	1.82×10^{-2}	1.28×10^{-2}	2.26×10^{-3}	5.02×10^{-2}	2.62×10^{-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.

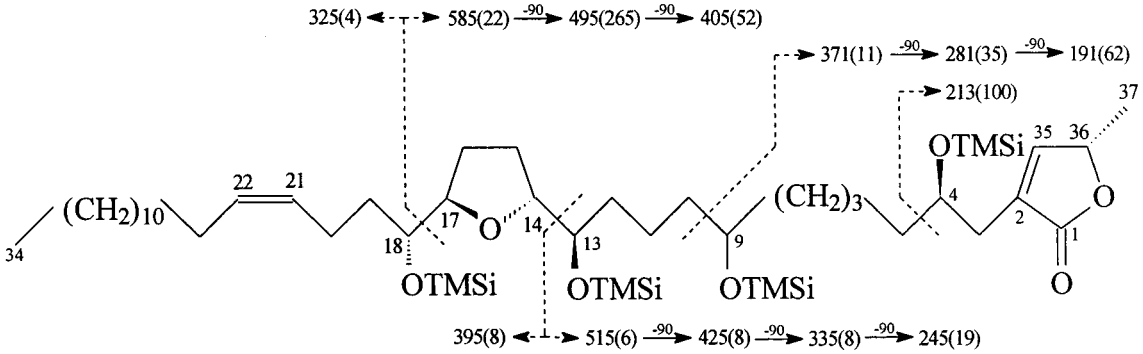


Figure 2. Diagnostic EIMS peaks (*m/z*) for the tetra-TMSi derivative **2a** (intensities are indicated in parentheses).

fraction (F002, 300 g) and the CH_2Cl_2-soluble fraction (F003, 400 g). F003 was then partitioned between 90% aqueous MeOH−hexane (1:1) to yield a hexane-soluble fraction (F006, 150 g) and an aqueous MeOH-soluble fraction (F005, 250 g). All fractions were subjected to the BST, with the most active fraction being F005 (BST $LC_{50} = 1.13 \times 10^{-2}$ μg/mL). F005 (250 g) was subjected to open column chromatography over Si gel (2.8 kg) eluted with hexane−$CHCl_3$ and $CHCl_3$−MeOH gradients. Fractions (F_1-1 to F_1-18) were collected and pooled according to their similar TLC patterns. The BST active pool F_1-12 was further resolved on another Si gel (1.5 kg) open column, eluted with hexane−$CHCl_3$ and $CHCl_3$−MeOH gradients. Fractions (F_2-1 to F_2-13) were collected into 13 pools on the basis of similar TLC patterns. Further purifications of the most bioactive BST fractions (F_2-6, BST $LC_{50} = 2.00 \times 10^{-4}$ μg/mL) were carried out by HPLC to afford compounds **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 29.0 min (**1**) and 34.8 min (**2**).

Annomolin (1): white powder (20 mg); mp 60.5−61.2 °C; $[\alpha]^{23}_D$ +4.0° (*c* 0.02, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 230 (3.2) nm; IR (film) ν_{max} 3446, 2915, 2848, 1747, 1646, 1465, 1321, 1062 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.18 (1H, q, J = 1.5 Hz, H-33), 5.06 (1H, qq, J = 7.0, 1.5 Hz, H-34), 3.89 (1H, m, H-4), 3.83 (1H, m, H-14), 3.80 (1H, m, H-17), 3.43 (3H, m, H-7, H-8, H-18), 2.53 (1H, dt, J = 15.0, 1.5 Hz, H-3b), 2.41 (1H, dd, J = 15.0, 8.5 Hz, H-3a), 2.02 (1H, m, H-15b), 1.99 (1H, m, H-16b), 1.68 (1H, m, H-16a), 1.48 (1H, m, H-15a), 1.43 (1H, d, J = 6.5 Hz, H-35), 0.88 (1H, t, J = 7.0, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7 (s, C-1), 151.9 (d, C-33), 131.2 (s, C-2), 81.8 (d, C-17), 79.3 (d, C-14), 78.0 (d, C-34), 74.6 (d, C-18), 74.4 (d, C-8), 74.3 (d, C-7), 69.9 (d, C-4), 33.4 (t, C-3), 32.4 (t, C-15), 28.4 (t, C-16), 19.1 (q, C-35), 14.1 (q, C-32); FABMS *m/z* 619 $[M + Na]^+$, 601 $[M + Na - H_2O]^+$, 583 $[M + Na - 2H_2O]^+$, 565 $[M + Na - 3H_2O]^+$, 547 $[M + Na - 4H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 619.4548 for $C_{35}H_{64}O_7Na$ (calcd 619.4550).

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

Annocherimolin (2): white powder (10 mg); mp 57.9−58.7 °C; $[\alpha]^{23}_D$ −21° (*c* 0.02, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 230 (3.1) nm; IR (film) ν_{max} 3421, 2925, 2854, 1743, 1646, 1457, 1319, 1079 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.18 (1H, q, J = 1.5 Hz, H-35), 5.40 (1H, m, H-22), 5.35 (1H, m, H-21), 5.06 (1H, qq, J = 7.0, 1.5 Hz, H-36), 3.89 (1H, m, H-4), 3.82 (2H, m, H-14, H-17), 3.59 (1H, m, H-9), 3.44 (2H, m, H-13, H-18), 2.53 (1H, dt, J = 15.0, 1.5 Hz, H-3b), 2.40 (1H, dd, J = 15.0, 8.5 Hz, H-3a), 2.20 (1H, m, H-20), 2.03 (1H, m, H-23), 1.99 (2H, m, H-15b, H-16b), 1.73 (2H, m, H-15a, H-16a), 1.48 (1H, m, H-19), 1.43 (1H, d, J = 6.5 Hz, H-37), 0.88 (1H, t, J = 7.0, H-34); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6 (s, C-1), 151.9 (d, C-35), 131.2 (s, C-2), 130.9 (d, C-22), 129.0 (d, C-21), 82.7 (d, C-14, C-17), 78.0 (d, C-36), 74.4 (d, C-13), 74.3 (d, C-18), 71.9 (d, C-9), 70.0 (d, C-4), 28.8 (t, C-15, C-16), 27.3 (t, C-23), 23.5 (t, C-20), 19.1 (q, C-37), 14.1 (q, C-34); FABMS *m/z* 645 $[M + Na]^+$, 627 $[M + Na - H_2O]^+$, 609 $[M + Na - 2H_2O]^+$, 591 $[M + Na - 3H_2O]^+$, 573 $[M + Na - 4H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 645.4707 for $C_{35}H_{64}O_7$ (calcd 645.4706).

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

Preparation of Mosher Esters. A previously described method was used.[33,34] To each of 1 mg of **1** and **2** 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, separately. The mixture was left at room temperature overnight and purified over a microcolumn (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 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 Table 1.

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References and Notes

(1) Barriga, H. G. *Flora Medicinal de Colombia*; Botánica Médica: Bogatá, 1974; p 340.
(2) Alali, F. Q.; Liu, X.-X.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 504−540.
(3) Cortes, D.; Rios, A.; Villar, A.; Valverde, S. *Tetrahedron Lett.* **1984**, *25*, 3199−3202.
(4) Cortes, D.; Myint, S. H.; Hocquemiller, R. *Tetrahedron* **1991**, *47*, 8195−8202.
(5) Cortes, D.; Myint, S. H.; Leboeuf, M.; Cavé, A. *Tetrahedron Lett.* **1991**, *32*, 6133−6134.
(6) Cortes, D.; Myint, S. H.; Dupont, B.; Davonst, D. *Phytochemistry* **1993**, *32*, 1475−1482.
(7) Woo, M. H.; Kim, D. H.; Fotopoulos, S. S.; McLaughlin. J. L. *J. Nat. Prod.* **1999**, *62*, 1250−1255.
(8) Kim, D. H.; Woo, M. H. *Yakhak Hoeji* **1999**, *43*, 584−590.
(9) Woo, M. H.; Chung, S. O.; Kim, D. H. *Arch. Pharmacal Res.* **1999**, *22*, 524−528.
(10) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306.
(11) 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; pp 81−287.
(12) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175−1184.
(13) Harmange, J. C.; Figadere, B.; Cavé, A. *Tetrahedron Lett.* **1992**, *33*, 5749−5752.
(14) 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.
(15) Shi, G.; Gu, Z.-M.; He, K.; Wood, K. V.; Zeng, L.; Ye, Q.; MacDougal, J. M.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1281−1286.
(16) Zeng, L.; Zhang, Y., Ye, Q.; Shi, G. E.; He, K.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1271−1279.
(17) Zeng, L.; Wu, F.-E.; Gu, Z.-M.; McLaughlin, J. L. *Tetrahedron Lett.* **1995**, *36*, 5291−5294.
(18) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8525−8528.
(19) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8529−8532.

(20) Born, L.; Lieb, F.; Lorentzen J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med*. **1990**, *56*, 312−316.
(21) Ye. Q.; He, K.; Oberlies, N. H.; Zeng, L.; Shi, G. E.; Evert, D.; McLaughlin, J. L. *J. Med. Chem*. **1996**, *39*, 1790−1796.
(22) Woo, M. H.; Zeng, L.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1731−1742.
(23) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(24) McLaughlin, J. L. *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1−35.
(25) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med*. **1982**, *45*, 31−34.
(26) Soule, H. D.; Vazquez, J.; Long., A.; Albert, S.; Brennam, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409−1416.
(27) Fogh, J.; Trempe, G. In *Human Tumor Cells*; Fogh, J., Ed.; Plenum Press: New York, 1975; pp 115−119.
(28) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol*. **1979**, *17*, 16−23.
(29) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128−135.
(30) Oberlies, N. H.; Chang, C.-J.; McLaughlin, J. L. *J. Med. Chem*. **1997**, *40*, 2102−2106.
(31) Ahammadsahib, K. I.; Hollingworth, R. M.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci*. **1993**, *53*, 1113−1120.
(32) Giard, D. L.; Aaronson, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Nat. Prod*. **1973**, *51*, 1417.
(33) Dale, J. A.; Mosher, H. S. *J. Org. Chem*. **1973**, *95*, 512−519.
(34) 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.

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