Two New Epimeric Pairs of Acetogenins Bearing a Carbonyl Group from *Annona cherimolia* Seeds

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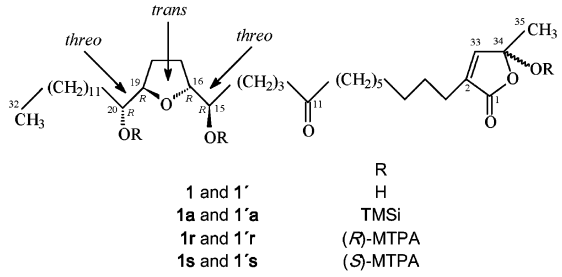
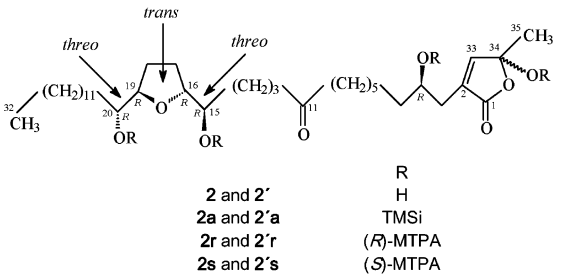
Further studies on the seeds of *Annona cherimolia* have led to the isolation of two pairs of Annonaceous acetogenins, a mixture of epimers of annomolon A (**1**) and 34-*epi*-annomolon A (**1′**), and a mixture of epimers of annomolon B (**2**) and 34-*epi*-annomolon B (**2′**), containing a rare γ-hydroxymethyl-γ-lactone moiety and a carbonyl group. Their structures were elucidated on the basis of spectroscopic and chemical methods, and their absolute stereochemistry was solved by preparing their respective per-Mosher ester derivatives. These acetogenins showed substantial cytotoxicity, comparable to that of adriamycin, against a human pancreatic tumor cell line (MIA PaCa-2).

The Annonaceous acetogenins are an expanding class of potent long-chain fatty acid derivatives that are found only in certain species of the Annonaceae. Interest in these compounds has become widespread, as knowledge of their potent antitumor and pesticidal activities has become better understood.[1] *Annona cherimolia* Mill. is the only species in its genus cultivated in Europe and is grown principally in southern Spain, between Almuòecar and La Herradura (Granada coast).[2] In previous investigations by Cortes et al., eight new acetogenins, cherimolin, dihydrocherimolin,[3] molvizarin, motrilin,[4] itrabin, jetein,[5] cherimolin-2, and almunequin,[6] were isolated from *A. cherimolia* seeds. We have reported the isolation of 10 acetogenins from the seeds of this plant.[7−10] In this paper, we report the identification of two pairs of Annonaceous acetogenins, a mixture of epimers of annomolon A (**1**) and 34-*epi*-annomolon A (**1′**), and a mixture of epimers of annomolon B (**2**) and 34-*epi*-annomolon B (**2′**). Their structures, characterized by the unusual presence of a γ-hydroxymethyl-γ-lactone, were elucidated by spectroscopic analysis and chemical derivatization.



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



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

Results and Discussion

The seeds of *A. cherimolia* were extracted with 95% EtOH, and the extracted residue, F001, was partitioned by a standard extraction scheme (see Experimental Section). The aqueous MeOH residue (F005), which was the most bioactive fraction in the brine shrimp lethality test (BST),[11,12] was subjected to column chromatography and reversed-phase HPLC.

A mixture of epimers of **1** and **1′**, $[\alpha]^{23}_{D}$ −5.0° (*c* 0.02, CH_2Cl_2), was obtained as a white powder. The HRFABMS gave a $[M + H]^+$ ion at *m*/*z* 595.4574 (calcd 595.4580) corresponding to the formula $C_{35}H_{63}O_7$. The ^{13}C NMR spectrum revealed a duplication of several signals that appeared at δ 105.2/105.3, 132.0/132.3, 149.6/150.5, and 172.0/172.6, suggesting the presence of two epimeric compounds.[13,14] In the ^1H NMR spectrum, no H-34 signal was apparent. The H-35 protons exhibited a singlet at δ 1.64, while in the ^{13}C NMR spectrum the C-34 signal appeared at δ 105.2/105.3. These data indicated that for the mixture of epimers of **1** and **1′**, H-34 was substituted by a hydroxyl group.

The ^{13}C NMR spectrum of the mixture of epimers of **1** and **1′** displayed a signal at δ 212.2 (C-11) for a carbonyl group and two signals at δ 42.5 (C-10) and 42.4 (C-12) corresponding to methylene carbons adjacent to the carbonyl.[15] The position of the carbonyl group in the alkyl chain of the mixture of epimers of **1** and **1′** was deduced by mass spectral analysis. The position of the carbonyl group was determined from the EIMS fragment ions at *m*/*z* 253, 323, and 305 (Figure 1), which can be explained by a C-11/C-12 cleavage in the mixture of epimers of **1** and **1′**.[7]

The EIMS data of the tri-TMSi derivative of the mixture of epimers of **1a** and **1a′** clearly resolved the carbon skeleton and the placement of the tetrahydrofuran (THF) ring (Figure 1). The EIMS fragmentation patterns indicated that the mono-THF ring was located at C-16 and C-19 along the hydrocarbon chain and also supported the placement of the three hydroxyl groups at C-15, C-20, and C-34, as originally indicated by the NMR data. The presence of a mono-THF ring in the mixture of epimers of **1** and **1′**, with two OH groups at the adjacent carbons of the ring, was deduced by the ^1H NMR resonances at δ 3.80 (H-16/H-19) and 3.40 (H-15/H-20) and the ^{13}C NMR peaks at δ 74.1 (C-15), 82.3(C-16), 82.7 (C-19), and 74.3 (C-20), which are characteristic for mono-THF acetogenins having two OH groups adjacent to a mono-THF ring.[16,17] The

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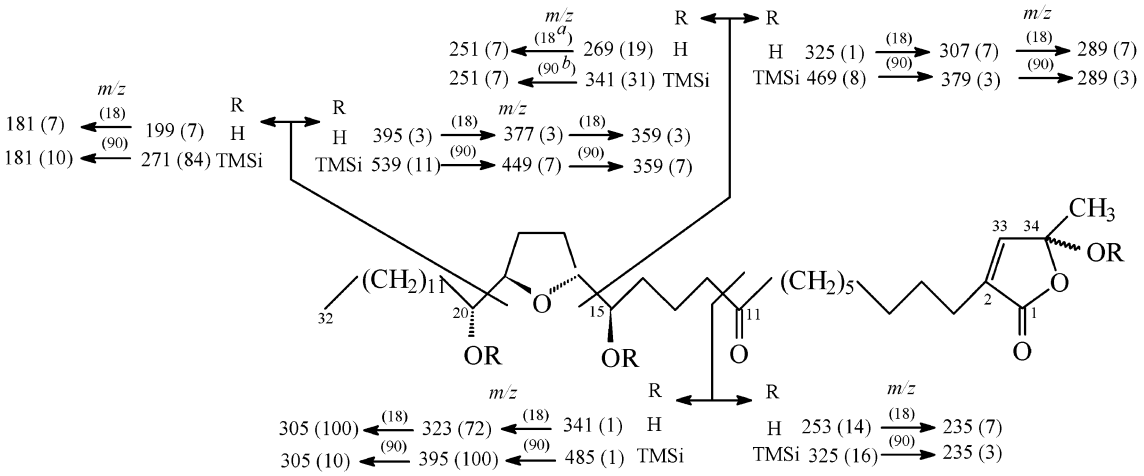

Figure 1. Diagnostic EIMS peaks (*m*/*z* ratios) of the mixture of **1** and **1′** and the tri-TMSi (**1a** and **1′a**) derivative (intensities are indicated in parentheses). [a]H_2O. [b]TMSiOH.

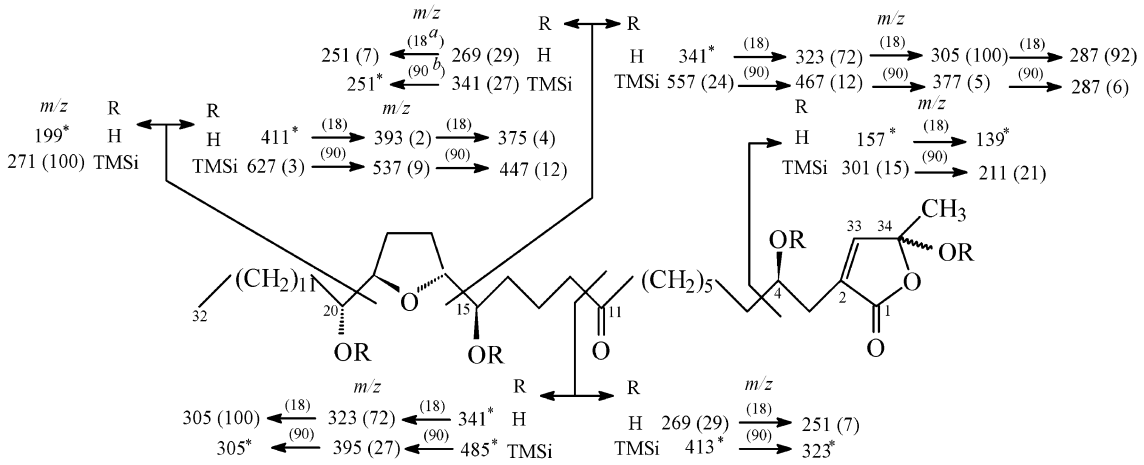

Figure 2. Diagnostic EIMS peaks (*m*/*z* ratios) of the mixture of **2** and **2′** and the tetra-TMSi (**2a** and **2′a**) derivative (intensities are indicated in parentheses). [a]H_2O. [b]TMSiOH. *Ions not observed.

relative stereochemistries of C-15/16 and C-19/20 were both defined as *threo* by comparing the ^{1}H NMR signals of the mixture of epimers of **1** and **1′** for H-15, H-16, H-19, and H-20 with those of model compounds of known relative stereochemistry.[18] The mixture of epimers of **1** and **1′** showed ^{1}H NMR signals at δ 1.97 (H-17a and 18a) and 1.67 (H-17b and 18b), which are typical methylene proton signals for a *trans*-THF ring. The mixture of epimers of **1** and **1′** are C-35 mono-THF acetogenins with novel γ-hydroxymethyl-γ-lactone moieties and have been named annomolon A (**1**) and 34-*epi*-annomolon A (**1′**).

The mixture of epimers of **2** and **2′**, $[\alpha]^{23}_D$ +6.0° (*c* 0.02, CH_2Cl_2), was obtained as a white powder. The HRFABMS gave a $[M + Na]^+$ ion at *m*/*z* 633.4359 (calcd 633.4342), corresponding to the formula $C_{35}H_{62}O_8Na$. The ^{13}C NMR spectrum revealed a duplication of several signals that appeared at δ 171.6/172.7, 146.9/147.2, 134.4/134.6, and 110.0, suggesting the presence of two epimeric compounds. The H-3a and H-3b ^{1}H NMR signals of the mixture of epimers of **2** and **2′** were observed at δ 2.26 and 2.55, respectively, correlating with the ^{1}H NMR signal at δ 3.90 (H-4). Several spectral characteristics in the terminal lactone moiety of **2** and **2′** were identical to those of the above-mentioned lactol acetogenins, annomolon A (**1**) and 34-*epi*-annomolon A (**1′**).

The placement of the THF ring unit and their flanking hydroxyls from C-15 to C-20 was confirmed by the EIMS fragmentation pattern of the TMSi derivative of the mixture of **2** and **2′**. The fragment at *m*/*z* 271 was the most intense and indicated major cleavage between C-19 and C-20 (Figure 2). Further support was given by the fragment at *m*/*z* 557, indicating a cleavage between C-15 and C-16. The relative stereocheimisty of the mono-THF ring with two flanking hydroxyl groups in the mixture of epimers of **2** and **2′** was assigned as *threo*/*trans*/*threo*, as in the mixture of epimers of **1** and **1′**. Thus, the new epimers **2** and **2′** were named annomolon B (**2**) and 34-*epi*-annomolon B (**2′**).

To determine the absolute stereochemistry of the carbinol centers at C-15 and C-20 in the mixture of epimers of **1** and **1′**, and at C-4, C-15, and C-20 in the mixture of epimers of **2** and **2′**, tri-(*R*)- and tri-(*S*)-methoxytrifluoromethyl phenylacetic acid (MTPA) esters (Mosher esters) in the mixture of **1** and **1′** and tetra-(*R*)- and tetra-(*S*)-MTPA esters in the mixture of **2** and **2′** were prepared.[19−21] The ^{1}H NMR chemical shift data of **2s**/**2′s** and **2r**/**2′r** showed that the absolute configuration at C-4 of the mixture of epimers of **2** and **2′** is *R*. This result was identical to all acetogenins examined so far that possess an OH group at C-4. Similarly, the Mosher ester data allowed the absolute stereochemical assignments of the carbinol centers adjacent to the mono-THF ring as C-15 *R* and C-20 *R* in the mixture of epimers of **1** and **1′** and the mixture of epimers of **2** and **2′**.

Bioactivity data obtained with the mixture of epimers of **1** and **1′** and the mixture of epimers of **2** and **2′** are

Table 1. Characteristic ^{1}H NMR Data of the Mosher Esters of **1s** and **1′s**, **1r** and **1′r**, **2s** and **2′s**, and **2r** and **2′r**

position	**1s** and **1′s** δ *S*	**1r** and **1′r** δ *R*	$\Delta\delta$ *S*−*R*	position	**2s** and **2′s** δ *S*	**2r** and **2′r** δ *R*	$\Delta\delta$ *S*−*R*
14	1.65	1.56	+0.09	5	1.74	1.72	+0.02
15	4.96	5.02	*R*	4	4.55	4.56	*R*
16	3.92	4.00	−0.08	3	2.61	2.62	−0.01
17	1.40	1.60	−0.20	14	3.05	3.06	−0.01
18	1.64	1.94	−0.30	15	1.57	1.56	+0.01
19	1.40	1.60	−0.20	16	4.96	5.02	*R*
20	1.64	1.94	−0.30	19	3.92	4.01	−0.09
21	3.92	4.00	−0.08	20	3.92	4.01	−0.09
	4.96	5.02	*R*	21	4.96	5.02	*R*
	1.65	1.56	+0.09		1.57	1.55	+0.02

Table 2. Brine Shrimp Lethality and Cytotoxicity in Human Solid Tumor Cell Lines for **1** and **1′** and **2** 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 and **1′**	3.75×10^{-1}	1.26	3.03×10^{-1}	1.93×10^{-1}	9.30×10^{-1}	1.98×10^{-1}	3.12×10^{-3}
2 and **2′**	7.00×10^{-2}	1.37	4.70×10^{-2}	7.19×10^{-2}	3.77×10^{-1}	5.53×10^{-2}	7.48×10^{-3}
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.

summarized in Table 2. The mixture of **1** and **1′** and the mixture of **2** and **2′** showed cytotoxicity comparable to adriamycin against the human pancreatic tumor cell line (MIA PaCa-2). The introduction of a hydroxyl group at C-4 in the mixture of **2** and **2′** appears to account for an order of magnitude more potency than the mixture of **1** and **1′** against breast (MCF-7), colon (HT-29), and prostate (PC-3) cell lines. The mechanism of action of the acetogenins has been determined, and they are powerful inhibitors of complex I in mitochondrial electron transport systems.[22−24]

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. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. IR spectra were measured on a JASCO FT-IR 300E spectrophotometer. ^{1}H, ^{13}C, and COSY NMR spectra were recorded on Varian VXR 300S 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, 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). A μBondapak C_{18} column (19 × 300 mm and 7.8 × 300 mm) was used for preparative purposes.

Plant Material. The seeds of *Annona cherimolia* were obtained in September 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 (CUDP 96003) 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).[11,12] 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),[25] MCF-7 (human breast carcinoma),[26] HT-29 (human colon adenocarcinoma),[27] A-498 (human kidney carcinoma),[25] 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 and H_2O (1:1) to yield the H_2O-soluble fraction (F002, 300 g) and the CH_2Cl_2-soluble fraction (F003, 400 g). F003 was then partitioned between 90% aqueous MeOH and 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). An aliquot of 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 profiles. Further purification of the most bioactive BST fraction (F_2-6, BST $LC_{50} = 2.00 \times 10^{-4}$ μg/mL) was carried out by HPLC to afford a mixture of epimers of **1** and **1′** and a mixture of epimers of **2** 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 20.0 min (a mixture of **1** and **1′**) and 24.5 min (a mixture of **2** and **2′**)].

Mixture of Epimers of Annomolon A (1) and 34-*epi*-Annomolon A (1′): white powder (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} 3444, 1731 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (1H, s, H-33), 3.80 (2H, m, H-16, H-19), 3.40 (2H, m, H-15, H-20), 1.97 (2H, m, H-17a, H-18a), 1.67 (3H, m, H-17b, H-18b, H-35), 0.68 (1H, t, J = 6.5 Hz, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 212.2 (s, C-11), 172.0/172.6 (s, C-1), 149.6/150.5 (d, C-33), 132.0/132.3 (s, C-2), 105.2/105.3 (s, C-34), 82.7 (d, C-19), 82.3 (d, C-16), 74.3 (d, C-20), 74.1 (d, C-15), 42.5 (t, C-10), 42.4 (t, C-12), 29.0 (t, C-17, C-18), 24.9 (q, C-35), 14.1 (q, C-32); FABMS *m/z* 595 $[M + H]^+$, 577 $[M + H - H_2O]^+$, 559 $[M + H - 2H_2O]^+$, 541 $[M + H - 3H_2O]^+$; EIMS *m/z* 253 $[C_{14}H_{21}O_4]^+$, 341 $[C_{21}H_{41}O_3]^+$, 323 $[C_{21}H_{41}O_3 - H_2O]^+$, 305 $[C_{21}H_{41}O_3 - 2H_2O]^+$; HRFABMS *m/z* $[MH]^+$ 595.4574 for $C_{35}H_{63}O_7$ (calcd 595.4580).

Mixture of Epimers of Annomolon A (1) and 34-*epi*-Annomolon A (1′) and Tri-TMSi Derivative (1a and 1′a). Approximately 10 μg of the mixture of epimers of **1** and **1′** was treated with 0.2 μL of pyridine and 2 μL of *N,O*-bis(trimeth-

ylsilyl)acetamide for 5 h to give the mixture of epimers of **1a** and **1a′**: EIMS *m/z*, see Figure 1.

Mixture of Epimers of Annomolon B (2) and 34-*epi*-Annomolon B (2′): white powder (10 mg); mp 86.3−87.2 °C; $[\alpha]^{23}_{D}$ +6.0° (*c* 0.02, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 224 (3.6) nm; IR (film) ν_{max} 3427, 1743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.96 (1H, s, H-33), 3.90 (1H, m, H-4), 3.78 (2H, m, H-16, H-19), 3.42 (2H, m, H-15, H-20), 2.55 (1H, m, H-3b), 2.26 (1H, m, H-3a), 1.99 (2H, m, H-17a, H-18a), 1.67 (2H, m, H-17b, H-18b), 1.65 (1H, s, H-35), 0.88 (1H, t, J = 7.0 Hz, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 212.3 (s, C-11), 171.6/172.7 (s, C-1), 146.9/147.2 (d, C-33), 134.4/134.6 (s, C-2), 110.0 (s, C-34), 82.7 (d, C-16), 82.4 (d, C-19), 74.4 (d, C-15), 74.0 (d, C-20), 69.0 (d, C-4), 42.5 (t, C-10), 42.3 (t, C-12), 33.0 (t, C-3), 28.9 (t, C-17, C-18), 24.7 (q, C-35), 14.1 (q, C-32); FABMS *m/z* 633 $[M + Na]^+$, 615 $[M + Na - H_2O]^+$, 597 $[M + Na - 2H_2O]^+$, 579 $[M + Na - 3H_2O]^+$, 561 $[M + Na - 4H_2O]^+$; EIMS *m/z* 269 $[C_{14}H_{22}O_5]^+$, 341 $[C_{21}H_{41}O_3]^+$, 323 $[C_{21}H_{41}O_3 - H_2O]^+$, 305 $[C_{21}H_{41}O_3 - 2H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 633.4359 for $C_{35}H_{62}O_8Na$ (calcd 633.4342).

Mixture of Epimers of Annomolon B (2) and 34-*epi*-Annomolon B (2′) and Tetra-TMSi Derivative (2a and 2′a). Approximately 10 μg of the mixture of epimers of **2** and **2′** was treated with 0.2 μL of pyridine and 2 μL of *N*,*O*-bis-(trimethylsilyl)acetamide for 5 h to give a mixture of epimers of **2a** and **2a′**: EIMS, see Figure 2.

Preparation of Mosher Esters. A previously described method was used.[19−21] To each of 1 mg of the mixture of epimers of **1** and **1′** and the mixture of epimers of **2** and **2′**, both 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). The eluate was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed with 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 the mixture of epimers of **1** and **1′** and the mixture of epimers of **2** and **2′**, respectively. The use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride afforded the *R* Mosher ester analogues. 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: Bogotá, 1974; p 340.
(2) Fies, R. E. In *Die Natürlichen Pflanzenfamilien*, 2nd ed.; Engler, A., Prantl, K., Eds.; Dunker and Humbolt: Berlin, 1959; Vol. 17.
(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.; Cave, 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.; Ma, E. S.; Suk, K. D.; Son, J. K.; Lee, J. S.; Woo, M. H. *J. Nat. Prod*. **2001**, *64*, 502−506.
(9) Kim, D. H.; Woo, M. H. *Yakhak Hoeji* **1999**, *43*, 584−590.
(10) Woo, M. H.; Chung, S. O.; Kim, D. H. *Arch. Pharm. Res.* **1999**, *22*, 524−528.
(11) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1−35.
(12) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med*. **1982**, *45*, 31−34.
(13) Chen, Y.; Chen, R. R.; Jiang, Z.; Yu, D. Q. *Planta Med.* **1998**, *64*, 242−245.
(14) Jiang Z.; Yu. D. Q. *J. Nat. Prod*. **1997**, *60*, 122−125.
(15) Gallardo, T.; Saez, J.; Granados, H.; Tormo, J. R.; Velez, I. D.; Brun, N.; Torres, B.; Cortes, D. *J. Nat. Prod*. **1998**, *61*, 1001−1005.
(16) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod*. **1990**. *53*, 237−278.
(17) Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal*. **1993**, *4*, 27−67.
(18) Araya, H.; Fujimoto, Y.; Hirayama, K. *J. Synth. Org. Chem*. **1994**, *52*, 765−777.
(19) Dale, J. A.; Mosher, H. S. *J. Org. Chem*. **1973**, *95*, 512−519.
(20) 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.
(21) Rieser, M. J.; Fang, X.-P.; Anderson, E.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1994**, *76*, 2433−2444.
(22) Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H. *Pestic. Sci*. **1991**, *33*, 427−438.
(23) Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K. *Pestic. Biochem. Physiol*. **1993**, *45*, 15−23.
(24) Ahammadsahib, K. I.; Hollingworth, R. M.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci*. **1993**, *53*, 1113−1120.
(25) Giard, D. L.; Aaronson, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Nat. Prod*. **1973**, *51*, 1417−1423.
(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. *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.

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