Montacin and *cis*-Montacin, Two New Cytotoxic Monotetrahydrofuran Annonaceous Acetogenins from *Annona montana*

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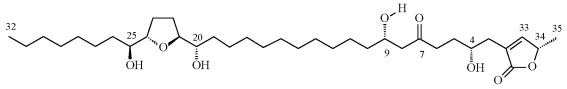
Two new monotetrahydrofuran Annonaceous acetogenins, montacin (**1**), and *cis*-montacin (**2**), along with four known acetogenins, annonacin, *cis*-annonacin, annomontacin, and *cis*-annomontacin, were isolated from the seeds of *Annona montana*. The structures of the new isolates were elucidated by spectral and chemical methods. The new acetogenins exhibited moderate in vitro cytotoxic activity against the 1A9 human ovarian cancer cell line. Interestingly, when Ca^{2+} was present, compounds **1** and **2** became 3- to 10-fold more active against 1A9 cells and the PTX10 subline.

Annonaceous acetogenins are a unique class of secondary metabolites consisting of C_{35} or C_{37} with 0 to 3 tetrahydrofuran (THF) rings, a γ-lactone (either saturated or unsaturated), and a long unbranched aliphatic region. This type of compounds has shown anticancer, cytotoxic, antiparasitic, insecticidal, and immunosuppressive effects and, in recent years, has been regarded as a potential source of new antitumor drugs. Recently, we reported that some acetogenins show selective cytotoxicity against the 1A9 human ovarian cancer cell line.[1]

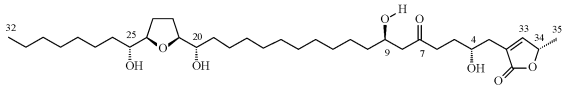
In our continuing studies on acetogenins from Formosan Annonaceous plants,[2] *Annona montana* Macf., also named "mountain soursop", was chosen for investigation. This plant is distributed mainly in the tropical areas of the Americas, Africa, and Southeast Asia. In Taiwan, this plant is scattered in the southern part of the island. A literature survey revealed that 26 Annonaceous acetogenins have been isolated from the leaves and seeds of this species,[3,4] primarily belonging to the mono-THF type. In our previous investigation, a non-THF Annonaceous acetogenin, diepoxymontin, was isolated from the fruit of this species.[5]

The present report describes the isolation and structural elucidation of compounds from the seeds of *A. montana*. Two new mono-THF Annonaceous acetogenins, montacin (**1**) and *cis*-montacin (**2**), along with four known acetogenins, annonacin,[6] *cis*-annonacin,[7] annomontacin,[8] and *cis*-annomontacin,[9] were isolated. Among the known compounds, *cis*-annomontacin was isolated for the first time from this species. Annonacin was the major acetogenin found in the methanol extract of this species. All isolates were purified by reversed-phase HPLC, and their structures were established by ^{1}H, ^{13}C, and 2D NMR spectral data and chemical methods, and further confirmed by ESI-MS/MS.

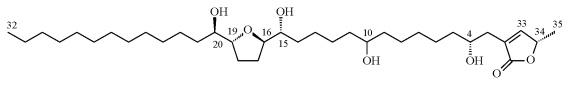
Mass spectral (MS) analysis is a key tool in determining the structures of acetogenins. However, although LC/ESI-MS/MS has been used to identify Annonaceous acetogenins,[10] few examples were reported to form a reliable base for the elucidation of structures of this compound type. Accordingly, we have included a possible mechanism for (+) ESI-MS/MS fragmentation of the new acetogenins (**1** and **2**).



montacin (**1**)



cis-montacin (**2**)



annonacin

The new compounds were evaluated in vitro against 11 human cancer cell lines, including A549 (lung cancer), MCF-7 (breast cancer), HCT-8 (ileocecal cancer), SK-MEL-2 (melanoma cancer), KB (epidermoid nasopharyngeal carcinoma), KB-VIN (vincristine-resistant KB), U-87-MG (glioblastoma cancer), CAKI (renal cancer), PC-3 (prostate cancer), 1A9 (ovarian cancer), and PTX10 (ovarian cancer cell line with β-tubulin mutation).

Results and Discussion

Montacin (**1**) and *cis*-montacin (**2**) were obtained as colorless waxy solids. Their spectral data were almost identical. In a preliminary analysis of the ^{1}H and ^{13}C NMR spectra, montacin (**1**) and *cis*-montacin (**2**) showed two basic features of the Annonaceous acetogenins (see Figure 1): an α,β-unsaturated γ-lactone ring with a hydroxyl group at the C-4 position and a mono-THF ring with two flanking hydroxyls, as discussed below. In the FABMS, compounds **1** and **2** showed $[M + Na]^+$ peaks at *m/z* 633, and molecular formulas of $C_{35}H_{62}O_8Na^+$ were also confirmed by ESIMS, which indicated a molecular weight of 610 ($C_{35}H_{62}O_8$) for both compounds.

In the NMR spectra of compound **1**, signals at δ 7.20 (1H, H-33), 5.06 (1H, H-34), 2.50 (2H, H-3), and 1.43 (3H, H-35) confirmed the presence of an α,β-unsaturated γ-lactone with a hydroxyl group at the C-4 position (see Table 1). The assignment was confirmed by ^{13}C NMR resonances

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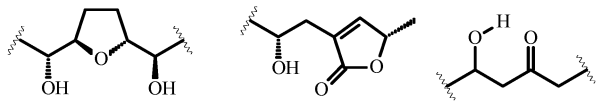

Figure 1. Structural fragments of montacin (**1**) and *cis*-montacin (**2**) based on COSY correlations.

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **1** and **2**

	1		**2**	
position	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]
1		174.6		174.7
2		131.1		131.0
3	2.50 (m)	33.5	2.51 (m)	33.6
4	3.85 (m)	69.6	3.82 (m)	69.5
5	1.2−1.6	25.6−29.7	1.2−1.6	25.0−29.7
6	2.44 (m)	43.5	2.46 (m)	43.4
7		212.2		212.5
8	2.58 (m)	49.1	2.58 (m)	49.0
9	4.04 (m)	67.5	4.03 (m)	67.5
10	1.2−1.6	33.4	1.2−1.6	33.4
11−18	1.2−1.6	25.6−29.7	1.2−1.6	25.0−29.7
19	1.2−1.6	36.1[c]	1.2−1.6	36.0[c]
20	3.40 (m)	73.7[d]	3.41 (m)	74.0[d]
21	3.78 (m)	82.5[e]	3.81 (m)	82.6[e]
22	1.98, 1.64 (m)	28.7	1.94, 1.73 (m)	28.1
23	1.98, 1.64 (m)	28.7	1.94, 1.73 (m)	28.1
24	3.78 (m)	82.7[e]	3.81 (m)	82.7[e]
25	3.40 (m)	74.1[d]	3.41 (m)	74.3[d]
26	1.2−1.6	36.9[c]	1.2−1.6	36.9[c]
27−29	1.2−1.6	25.6−29.7	1.2−1.6	25.0−29.7
30	1.2−1.6	31.9	1.2−1.6	31.9
31	1.2−1.6	22.6	1.2−1.6	22.7
32	0.88 (t, J = 6.8)	14.1	0.88 (t, J = 6.8)	14.1
33	7.20 (d, J = 1.2)	151.9	7.20 (d, J = 1.5)	152.0
34	5.06 (qd, J = 6.9, 1.2)	77.9	5.06 (qd, J = 6.9, 1.5)	78.0
35	1.43 (d, J = 6.9)	19.1	1.43 (d, J = 6.6)	19.1

[a] Measured at 300 MHz, in $CDCl_3$. Chemical shifts are in δ values. [b] Measured at 75 MHz, in $CDCl_3$. Chemical shifts are in δ values. [c−e] Assignments may be interchanged.

at δ 174.6 (C-1), 151.9 (C-33), 131.1 (C-2), 77.9 (C-34), 69.6 (C-4), and 19.1 (C-35) and was consistent with UV and IR absorptions at 203 nm and 1732 cm^{-1}, respectively. Proton signals at δ 3.78 (2H, H-21, 24) and 3.40 (2H, H-20, 25), as well as ^{13}C NMR peaks at δ 82.7 (C-24), 82.5 (C-21), 74.1 (C-25), and 73.7 (C-20), indicated the presence of a mono-THF ring with two flanking hydroxyls in a *threo* conformation according to Born's rule.[11] The proton resonances for the two methylene groups of this ring were observed at δ 1.98 (H-22a, 23a) and 1.64 (H-22b, 23b), which corresponded to a *trans* configuration.[12]

Compound **1** and the known acetogenin annonacin showed the same basic spectral features, except for an unusual proton signal at δ 4.04 (1H, H-9) and indistinguishable peaks between δ 2.58 and 2.44 (4H, H-6, 8) in the former compound, as well as ^{13}C NMR peaks at δ 212.2 (C-7), 67.5 (C-9), 49.1 (C-8), and 43.5 (C-6). Annonacin has a free hydroxyl at the C-10 position; thus, these spectral differences suggested that compound **1** has a different moiety in this molecular region. Additional data were obtained by measuring the ^{1}H NMR spectrum in pyridine-d_5 instead of $CDCl_3$. In pyridine-d_5, the peak at δ 4.04 was shifted to δ 4.46, and the peaks that were originally located between δ 2.58 and 2.44 (4H, H_2-6, H-8) separated into three sets of peaks, δ 2.78 (1H, H-8a), 2.66 (3H, H-3a, 3b, and 8b), and 2.55 (2H, H_2-6). Together with the COSY correlations of compound **1**, these data suggested that a β-hydroxy-carbonyl group was present (see Figure 1).

The placements of the THF ring moiety and the carbonyl group were established by close examination of the EIMS fragmentation of **1** (Figure 2). In our experience, the base peaks of Annonaceous acetogenins are usually due to fragments that are cleaved between the THF ring and its neighboring carbons or subsequent loss of one molecule of water from the flanking hydroxyl. The THF ring was placed between C-20 and C-25 because of the unusual base peak at *m/z* 129 ($C_8H_{17}O$). In addition, a loss of 70 amu from *m/z* 199 to 129, which indicated the loss of a THF ring, confirmed this assignment. The carbonyl group and the β-hydroxyl were located at C-7 and C-9, respectively, because of fragments at *m/z* 381 (cleavage between C-9 and C-8 − H_2O) and 363 (cleavage between C-9 and C-8 − 2 H_2O). The ESI-MS/MS analysis further supported these assignments. The positive-ion ESI-MS/MS of compound **1** exhibited only four peaks at *m/z* 633, 521, 421, and 235. A characteristic loss of 112 amu between *m/z* 633 and 521 indicated that this acetogenin has a hydroxyl at C-4.[13] The fragments at *m/z* 421 $[C_{24}H_{46}O_4Na]^+$ and 235 $[C_{11}H_{16}O_4Na]^+$ (1:2) indicated cleavage between C-9 and C-8. A possible mechanism for formation of these fragment ions is presented in Figure 3.

To determine the stereochemistry of compound **1**, the (*R*)- and (*S*)-Mosher ester derivatives were prepared by the convenient method of Kinghorn et al.[14] To confirm the reliability of this new Mosher ester method with Annonaceous acetogenins, the (*R*)- and (*S*)-Mosher ester derivatives of the known compound, annonacin, were prepared. The experimental results corresponded to the literature data (see Table 2).[15] In this method, the shifts of the proton signals from δ 4.12 (C-4), 3.72 (C-20, −25) to 5.25 (C-4), 5.67 (C-20, −25), respectively, were clearly observed. Moreover, the correlations between H-34 and -35, H-3 and -4, H-8 and -9, H-20 and -21, and H-24 and -25 were established on the basis of the ^{1}H−^{1}H COSY spectrum. Subsequently, the absolute configurations at C-4, C-9, and C-20 of compound **1** could be confirmed as *R*, *S*, and *S* (see Table 2), respectively. In addition to the aforementioned relative stereochemistry, compound **1** has 4*R*, 9*S*, 20*S*, 21*S*, 24*S*, 25*S*, and 34*S* absolute configuration. On the basis of these data, the structure of **1** was fully established and this compound was named montacin.

Compound **2** was purified by reversed-phase HPLC. The molecular formula of $C_{35}H_{64}O_8$ was confirmed by FABMS and ESIMS. The EIMS of **2** was almost superimposable with that of **1**. The UV (204 nm) and IR (1734 cm^{-1}) spectral absorption indicated the presence of an α,β-unsaturated γ-lactone group. The NMR spectra of **1** and **2** were quite similar. As in **1**, the ^{1}H and ^{13}C NMR data of **2** were consistent with the presence of a normal type tail moiety and a mono-THF ring system with two flanking hydroxyls (Table 1). However, on the basis of differences between the chemical shifts of H-22 and H-23 (δ 1.98 and 1.64 for **1**; δ 1.94 and 1.73 for **2**), the mono-THF ring system in **2** likely has a *threo*/*cis*/*threo* conformation.[9] EIMS fragments at *m/z* 129 and 381 and key positive-ion ESI-MS/MS fragments at *m/z* 420 $[C_{24}H_{45}O_4Na]^+$ and 235 $[C_{11}H_{16}O_4Na]^+$ (2:1) again supported the placements of the mono-THF ring system between C-20 and C-25 and the carbonyl group at C-7 and β-hydroxyl at C-9 (Figure 3).

The (*R*)- and (*S*)-Mosher ester derivatives of compound **2** were also prepared to determine the absolute configuration of compounds. The correlations between H-34 and -35, H-3 and -4, H-8 and -9, H-20 and -21, and H-24 and -25 were also established on the basis of the ^{1}H−^{1}H COSY spectrum. It was thus concluded that compound **2** has the

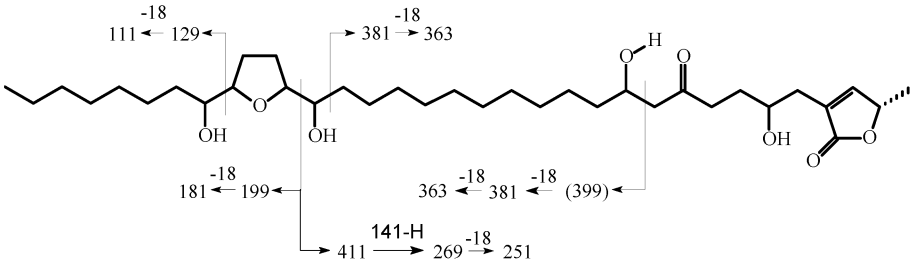

Figure 2. EIMS fragmentation (*m/z* values) of montacin (**1**) and *cis*-montacin (**2**).

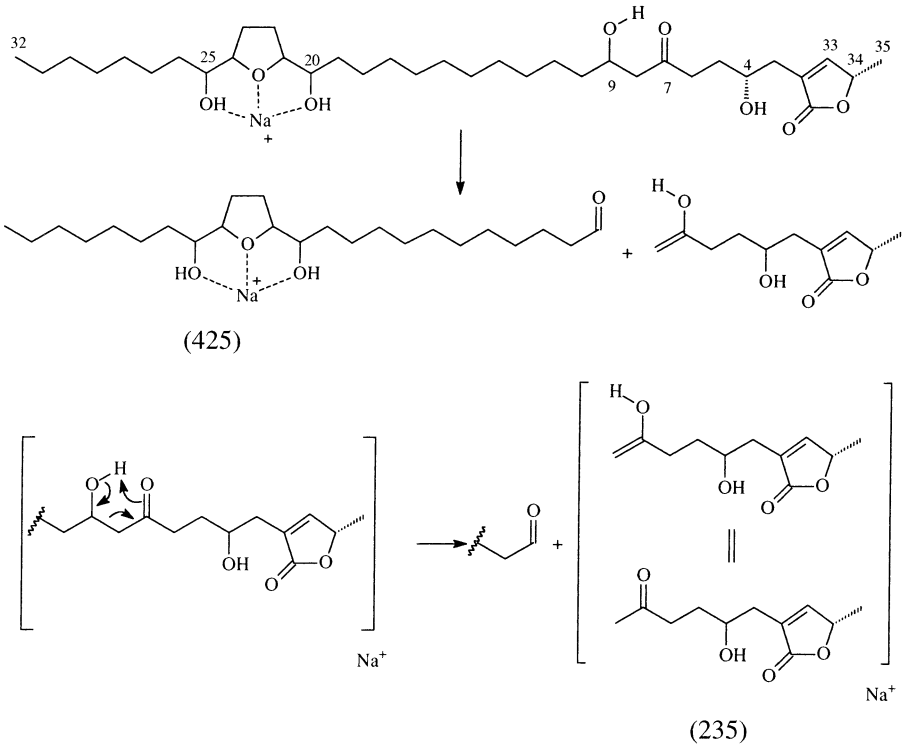

Figure 3. Positive-ion ESI-MS/MS fragmentation (*m/z* values) of montacin (**1**) and *cis*-montacin (**2**).

Table 2. ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1**, **2**, and Annonacin

	proton							
	33	34	3	4	8	9	20, 25	21, 24
1								
S-MTPA	7.019	4.948	2.707	5.668	2.938	5.872	5.250	4.191
R-MTPA	7.296	5.020	2.722	5.648	2.933	5.773	5.298	4.167
$\Delta\delta_{S-R}$	−0.277	−0.072	−0.015	0.02	0.005	0.099	−0.048	0.024
config.		*S*		*R*		*S*	*S*, *S*	

	33	34	3	4	8	9	20	21	24	25
2										
S-MTPA	7.029	4.966		5.691	2.968	5.893	5.355	3.975	4.206	5.149
R-MTPA	7.292	5.031	2.765	5.676	2.990	5.828	5.337	3.967	4.228	5.203
$\Delta\delta_{S-R}$	−0.263	−0.065	−0.039	0.015	−0.022	0.065	0.018	0.008	−0.022	−0.054
config.		*S*		*R*		*R*	*S*[b]			*R*[b]

	33	34	3	4	15, 20	16, 19
annonacin						
S-MTPA	6.926	4.993	2.672	*a*	*a*	4.167
R-MTPA	7.141	5.026	2.785	*a*	*a*	4.196
$\Delta\delta_{S-R}$	−0.215	−0.033	−0.113	*a*	*a*	−0.03
config.		*S*		*R*	*R*	

[a] Indistinguishable. [b] Assignments of the absolute configuration of C-20 and -25 are interchangeable.

aforementioned relative stereochemistry as well as 4*R*, 9*R*, 20*S*, 21*S*, 24*R*, 25*R* (or 20*R*, 21*R*, 24*S*, 25*S*), and 34*S* absolute configuration. On the basis of these data, the structure of **2** was fully established and this compound was named *cis*-montacin.

The known Annonaceous acetogenins obtained in the present investigation were identified by comparing their UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with published values for annonacin,[6] *cis*-annonacin,[7] annomontacin,[8] and *cis*-annomontacin.[9]

Table 3. In Vitro Cytotoxicity of Compounds **1** and **2**

	cell line[a] [ED_{50} (μg/mL)]											
compd	A549	MCF-7	HCT-8	SK- MEL-2	KB	KB-VIN	U-87-MG	CAKI	PC-3	1A9 (3 day)	PTX10 (3 day)	1A9 (6 day)	PTX10 (6 day)
1	12.9	10.0	16.5	11.2	16.6	>20(23)	>20(34)	>20(47)	>20(20)	>10(40)	>10(32)	6.7	7.9
1 + Ca^{2+}	7.4	5.5	12.3	13.3	9.1	13.9	14.2	14.2	14.2	6.7	7.5	4.5	2.8
2	6.0	6.7	12.9	ND[b]	7.7	13.4	15.0	ND[b]	11.9	3.6	5.9	0.54	1.8
2 + Ca^{2+}	5.5	6.4	11.7	ND[b]	8.2	13.8	14.3	ND[b]	12.1	2.0	5.3	0.13	0.11
Ca^{2+} [c]	>20(23)	>20(6)	>20(6)	ND[b]	>20(26)	NA[b]	NA[b]	ND[b]	>20(11)	>20(24)	>20(10)	NA[b]	>20(35)

[a] A549, lung cancer cell; MCF-7, breast cancer cell; HCT-8, ileocecal cancer cell; SK-MEL-2, melanoma cancer cell line; KB, epidermoid nasopharyngeal carcinoma cell; KB-VIN, vincristine-resistant KB; U-87-MG, glioblastoma cancer cell; CAKI, renal cancer cell; PC-3, prostate cancer cell; 1A9, ovarian cancer cell; PTX10, ovarian cancer cell with β-tubulin mutation. [b] NA = not active at 20 μg/mL. ND = not tested. [c] $CaCl_2$ was added as the source of Ca^{2+} in the bioassay.

In three-day cytotoxicity bioassays,[1] compounds **1** and **2** were tested against 11 cancer cell lines and exhibited moderate inhibitory activity against five cancer cell lines, A549 (lung), MCF-7 (breast), HCT-8 (ileocecal), SK-MEL-2 (melanoma), and KB (epidermoid nasopharyngeal carcinoma), with ED_{50} values in a range 10.0−16.6 and 6.0−15.0 μg/mL, respectively (see Table 3). For 1A9 (ovarian cancer) and 1A9 PTX10 (ovarian cancer with β-tubulin mutation) compounds **1** and **2** both showed some activity against the parent 1A9 cell line in three-day or six-day assays, but they showed greater activity in a six-day assay than in a three-day assay (see Table 3). Interestingly, when Ca^{2+} was included in the bioassays, the inhibitory activities of **1** and **2** apparently increased, especially for the 1A9 series cell lines. Both of these new compounds became more active against 1A9 PTX10 when Ca^{2+} was present in a six-day assay. In particular, compound **2** showed the same activity against both 1A9 type cell lines with ED_{50} values of 0.13 and 0.11 μg/mL, respectively. The presence of Ca^{2+} ion apparently improved the bioactivity of compound **2** against 1A9 PTX10. The concentration of calcium ion in cells is an important factor in inducing programmed cell death (apoptosis). Annonaceous acetogenins have been reported to act as calcium ionophores,[16] and our previous study also indicated that Annonaceous acetogenins act like a Ca^{2+} complex to induce a sustained increase in intracellular Ca^{2+} in cultured smooth muscle cells of the human coronary artery.[17] Moreover, cell death can be induced when Ca^{2+} gains entry from extracellular medium via the plasma membrane after treatment with Ca^{2+} ionophores.[18] On the basis of the current bioassay results, we assume that compound **1**, in acting as an ionophore, promotes entry of Ca^{2+} ions into cancer cells and induces cell death. We intend to further study the required concentrations of Ca^{2+} and Annonaceous acetogenins in regard to this effect. The known Annonaceous acetogenins, annonacin, annomontacin, and *cis*-annomontacin, have been reported previously to exhibit potent cytotoxicities in the range of 8.40×10^{-4} to 2.98×10^{-1} μg/mL against human hepatoblastoma cell lines.[19]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-1000 digital polarimeter (cell length 10 mm, unless otherwise indicated). The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Mattson Genesis II spectrophotometer. ^1H and ^{13}C NMR, COSY, and HETCOR spectra were recorded with a Varian Gemini 2000 300 MHz NMR spectrometer, using TMS as internal standard. LR-FABMS and LREIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. ESI-MS/MS was obtained with PE-SCIEX API-3000 mass spectrometer with turbo ion spray source. HRFABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Macherey-Nagel, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm) were used for analytical TLC. The spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and then heating on a hot plate. HPLC was performed on a Shimadzu LC-10AS apparatus equipped with a Shimadzu SPD-M10Avp diode array detector. Hypersil ODS-5 (250 × 4.6 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns and Alltima C_{18} 5 μm (250 × 4.6 mm i.d.) and preparative C_{18} 5 μm (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant Material. The seeds of *A. montana* Macf. were collected from Chia-Yi City, Taiwan, in March 1997. Voucher specimens (Annona-05) were deposited in the Graduate Institute of Natural Products, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The seeds of *A. montana* (2 kg) were extracted with methanol (2 L × 3). The MeOH extracts of the seeds were partitioned with $CHCl_3$ and water to yield $CHCl_3$ and aqueous extracts. After removing solvent in vacuo, the $CHCl_3$ residue was partitioned using methanol and *n*-hexane to yield MeOH and *n*-hexane layers. The MeOH layer afforded a waxy extract residue (ca. 100 g), which was further separated into 10 fractions by column chromatography on silica gel with gradients of *n*-hexane−$CHCl_3$ (2:1, 1:1, 1:2, 1:5, pure $CHCl_3$) and $CHCl_3$−MeOH (100:1, 50:1, 20:1, 10:1, 5:1). Fraction 6, eluted with $CHCl_3$−MeOH (100:1), was isolated and purified by column and HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, MeOH−water, 88:12, flow rate 1 mL/min; UV detector set at 225 nm) to give annomontacin (13 mg, t_R 15.4 min) and *cis*-annomontacin (6 mg, t_R 17.8 min). Fraction 7, eluted with $CHCl_3$−MeOH (50:1), was isolated and purified by column and HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, MeOH−water, 85:15, flow rate 1 mL/min; UV detector set at 225 nm) to give annonacin (1033 mg, t_R 22.1 min) and *cis*-annonacin (t_R 22.4 min). Fraction 8, eluted with $CHCl_3$−MeOH (20:1), was isolated and further purified by column chromatography and HPLC (Alltima C_{18} 5 μm column, 250 × 4.6 mm, MeCN−water, 70:30, flow rate 1 mL/min; UV detector set at 225 nm) to give montacin (**1**) (30.6 mg, t_R 16.1 min) and *cis*-montacin (**2**) (15 mg, t_R 16.9 min).

Montacin (1): colorless waxy solid; $[\alpha]^{25}_D$ +4.7° (*c* 0.38, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 203 (3.64) nm; IR (KBr) ν_{max} 3452 (OH), 2906, 1732 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1; FABMS *m/z* 633 $[M + Na]^+$; HRFABMS *m/z* 611.4534 (calcd for $C_{35}H_{63}O_8$, 611.4523); EIMS (70 eV) data, see Figure 2; ESIMS (4.5 eV) data, see Figure 3.

(*R*)- and (*S*)-MTPA Derivatives of 1. Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1 by a Convenient Mosher Ester Procedure. Compound **1** (2.0 mg) was transferred into a clean NMR tube and was dried completely under vacuum. Deuterated pyridine (0.5 mL) and (*S*)-(+)-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) were added immediately into the NMR tube under a N_2 gas stream, and the NMR tube was shaken carefully to evenly mix the

sample and MTPA chloride. The NMR tube was permitted to stand at room temperature and monitored every 1 h by ^{1}H NMR. The reaction was found to be complete after 2 h. ^{1}H NMR data (300 MHz, pyridine-d_5) of the (*R*)-MTPA ester derivative (**1r**) of **1** were obtained directly and were assigned on the basis of the correlations of the ^{1}H−^{1}H COSY spectrum. The (*S*)-MTPA ester derivative (**1s**) was prepared in the same way using (*R*)-(−)-methoxyl-α-(trifluoromethyl)phenylacetyl chloride reagent.

***cis*-Montacin (2):** white waxy solid; $[\alpha]^{25}_D$ +9.4° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (3.54) nm; IR (KBr) ν_{max} 3454 (OH), 2863, 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1; FABMS *m/z* 633 $[M + Na]^+$; HRFABMS *m/z* 611.4532 (calcd for $C_{35}H_{63}O_8$, 611.4523); EIMS (70 eV) data, see Figure 2; ESIMS (4.5 eV) data, see Figure 3.

Bioassays. Only in vitro cytotoxicity was determined here. Compounds were assayed for cytotoxic activity against human tumor cell lines using a reported procedure.[20] All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with compounds from DMSO-diluted stock. After 3 days in culture, cells attached to the plastic substratum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The ED_{50} is the concentration of agent that reduced cell growth by 50% over a 3-day assay period.

The KB (nasopharyngeal), HCT-8 (ileocecal), A549 (lung), MCF-7 (breast), PC-3 (prostate), HOS (bone), SK-MEL-2 (melanoma), and CAKI (kidney) human tumor cell lines were obtained from ATCC (Rockville, MD). KB-VIN (a vincristine-resistant KB subline) was a generous gift of Dr. Y.-C. Cheng (Yale University). The 1A9 (ovarian) and PTX10 (a 1A9 subline that has a mutated β-tubulin gene) cell lines were generous gifts of Dr. P. Ginnakakou (NCI, Bethesda, MD). All cell lines were cultured in RPMI-1640 medium supplemented with 25 mMM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 mg/mL kanamycin.

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