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Novel cytotoxic monotetrahydrofuranic Annonaceous acetogenins from *Annona montana*

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Abstract—Four new monotetrahydrofuranic Annonaceous acetogenins, montalicins G (**1**) and H (**2**) and monlicins A (**3**) and B (**4**), and two new linear acetogenins, (+)-monhexocin (**5**) and (−)-monhexocin (**6**), as well as three known compounds, murisolin (**7**), 4-deoxyannomontacin (**8**), and muricatacin (**9**), were isolated from the seeds of *Annona montana* by high performance liquid chromatographic (HPLC) method. The absolute stereochemical structures of new isolates were elucidated and characterized by spectral and chemical methods. Interestingly, these compounds show special cytotoxicity against human hepatoma cells, Hep G2.

1. Introduction

The Annonaceous acetogenins are a unique class of secondary metabolites of Annonaceous plants. A literature survey indicated that more than 400 Annonaceous acetogenins were isolated from the plants of this family.[1–3] This natural product class displays anticancer, cytotoxic, antiparasitic, insecticidal, and immunosuppressive effects and is regarded as a likely source for the development of potential drugs. In continuation of our studies on acetogenins from the Formosan Annonaceous plant, *Annona montana*, six new Annonaceous acetogenins, montalicins G (**1**) and H (**2**), monlicins A (**3**) and B (**4**), and (+)-monhexocin (**5**) and (−)-monhexocin (**6**), together with three known compounds, murisolin (**7**),[4] 4-deoxyannomontacin (**8**),[5] and muricatacin (**9**),[6] were isolated. All compounds were isolated and purified by reversed-phase HPLC, and their structures were elucidated and characterized by ^{1}H, ^{13}C, and 2D NMR spectral and MS data. The absolute stereochemistries of new compounds **1**, **3**, and **4** were also determined by the preparation and analysis of their Mosher ester derivatives. Among them, compounds **1** and **2** contain a mono-tetrahydrofuranic (THF) ring flanked by two hydroxyl groups together with a 1,3 diol moiety, and compounds **3** and **4** have a mono-THF ring moiety flanked by one hydroxyl together with a 1,2 diol moiety, the latter type of which has been isolated from *A. montana* for the first time.[7] (+)-Monhexocin (**5**) and (−)-monhexocin (**6**), which are linear acetogenins, were also isolated.

The present report also describes the in vitro cytotoxic activities of these new compounds against several human cancer cell lines, including Hep G2 (hepatocellular carcinoma), Hep 3B (hepatocellular carcinoma with hepatitis B surface antigen [HBsAg] [+]), 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). In these assays, these new compounds showed significant activity against the Hep G2 cancer cell line.

2. Results and discussion

Compounds **1** and **2** were successively isolated as colorless waxy solids with $[\alpha]_D^{25}$ +17.2 (*c* 1.6, $CHCl_3$) and +13.0 (*c* 0.81, $CHCl_3$), respectively, from the same

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fraction by reversed-phase HPLC. The $[M+Na]^+$ peaks of both compounds **1** and **2** in the FABMS at *m/z* 635 suggested the molecular weight to be 612, and their molecular formulas of $C_{35}H_{64}O_8Na$ were also suggested by HR-FABMS at *m/z* 635.4483 and 635.4493 (calcd 635.4499), respectively. The successive EIMS peaks at *m/z* 612, 595, 577, 559, 541, and 523 implied the presence of five hydroxyl groups. The UV absorption at 208 nm and the IR absorption at 1747 cm^{-1} indicated the presence of an α,β-unsaturated γ-lactone group, positive to Kedde's reagent.

The ^1H and ^{13}C NMR spectral data of compound **1** showed the characteristic subunits of mono-THF Annonaceous acetogenin, a mono-THF ring with two flanking hydroxyls and an α,β-unsaturated γ-lactone ring (Table 1). Proton signals at δ 7.22 (1H, H-33), 5.06 (1H, H-34), 3.85 (1H, H-4), 2.50 (1H, H-3a), 2.39 (1H, H-3b), and 1.43 (3H, H-35) confirmed the presence of an α,β-unsaturated γ-lactone ring with a hydroxyl group at the C-4 position. Proton signals at δ 3.78 (2H, H-16 and 19), and 3.40 (2H, H-15 and 20), as well as the ^{13}C NMR peaks at δ 83.0 (C-16), 82.8 (C-19), 74.4 (C-15), and 74.3 (C-20), indicated the presence of a mono-THF ring with two flanking hydroxyls in a *threo*/*threo* conformation.[11,12] The proton resonances for the two methylene groups of the mono-THF ring were observed at δ 1.98 (2H, H-17a and 18a), and 1.61 (2H, H-17b and 18b), corresponding to the *trans* configuration (see Fig. 1).

On the basis of the above spectral data, three of the five OH groups were assigned at C-4, C-15, and C-20 according to a close examination of the EIMS fragmentation of **1**. The significant peaks at *m/z* 377 (cleavage between C-19/C-20–$2H_2O$), 325 (C-15/C-16–H_2O), 307 (C-15/C-16–$2H_2O$), and 289 (C-15/C-16–$3H_2O$) allowed placement of the THF system at C-16 and C-19. The fragment ions at *m/z* 225 (cleavage between C-9/C-10–H_2O), 213 (C-8/C-9), and 195 (C-8/C-9–H_2O), as well as the ^{13}C NMR signals at δ 72.8 and 72.6, suggested that the remaining two methines with OH groups at δ 3.85 (2H) should be assigned at C-7 and C-9 (Fig. 2).

To determine the absolute stereochemistry of the carbinol chiral centers in these compounds, the (*S*)- and (*R*)-form Mosher ester derivatives of compound **1** were prepared by the modified methodology of Kinghorn and co-workers.[15] The correlations between H-34 and -35, H-3 and -4, H-15 and -16, and H-19 and -20 were established on the basis of the ^1H–^1H COSY spectrum of these MTPA derivatives. The absolute configuration at C-34 of **1** was determined by the CD method.[13] Thus, it was concluded that compound **1** has the aforementioned relative stereochemistry as well as the 4*R*, 15*R*, 16*R*, 19*R*, 20*R*, and 34*S* absolute configuration (Table 2). On the basis of these

Table 1. ^1H and ^{13}C NMR chemical shifts of montalicins G (**1**) and H (**2**)

	Montalicin G (**1**)		Montalicin H (**2**)	
	δ(^1H)[a]	δ(^{13}C)[b]	δ(^1H)[a]	δ(^{13}C)[b]
1		174.6		174.8
2		131.0		131.0
3	2.50 (m)	33.3[c]	2.50 (m)	33.8[c]
	2.39 (m)		2.40 (m)	
4	3.85 (m)	69.4	3.85 (m)	69.3
5	1.25–1.60	37.9[d]	1.25–1.61	37.4[d]
6	1.25–1.60	36.9[d]	1.25–1.61	36.8[d]
7	3.85 (m)	72.6[f]	3.85 (m)	72.5[f]
8	1.25–1.60	43.0[c]	1.25–1.61	42.9[c]
9	3.85 (m)	72.8[f]	3.85 (m)	72.6[f]
10	1.25–1.60	37.7[d]	1.25–1.61	38.0[d]
11–13	1.25–1.60	21.3–29.7	1.25–1.61	21.3–29.6
14	1.25–1.60	33.4[c]	1.25–1.61	33.3[c]
15	3.40 (m)	74.2[e]	3.39 (m)	74.4[e]
16	3.78 (m)	82.7[g]	3.80 (m)	82.7
17	1.98, 1.61 (m)	28.8	1.93, 1.71 (m)	28.1
18	1.98, 1.61 (m)	28.8	1.93, 1.71 (m)	28.1
19	3.78 (m)	82.6[g]	3.80 (m)	82.7
20	3.40 (m)	74.1[e]	3.39 (m)	74.0[e]
21	1.25–1.60	33.1[c]	1.25–1.61	34.1[c]
22–29	1.25–1.60	25.5–29.7	1.25–1.61	25.5–29.6
30	1.25–1.60	31.9	1.25–1.61	31.9
31	1.25–1.60	22.6	1.25–1.61	22.7
32	0.88 (t, *J* = 6.9 Hz)	14.0	0.88 (t, *J* = 6.9 Hz)	14.1
33	7.22 (d, *J* = 1.5 Hz)	151.9	7.22 (d, *J* = 1.5 Hz)	152.0
34	5.06 (qd, *J* = 6.9, 1.5 Hz)	78.0	5.06 (qd, *J* = 6.9, 1.5 Hz)	78.0
35	1.43 (d, *J* = 6.9 Hz)	19.0	1.43 (d, *J* = 6.9 Hz)	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–g] Assignments may be interchanged.

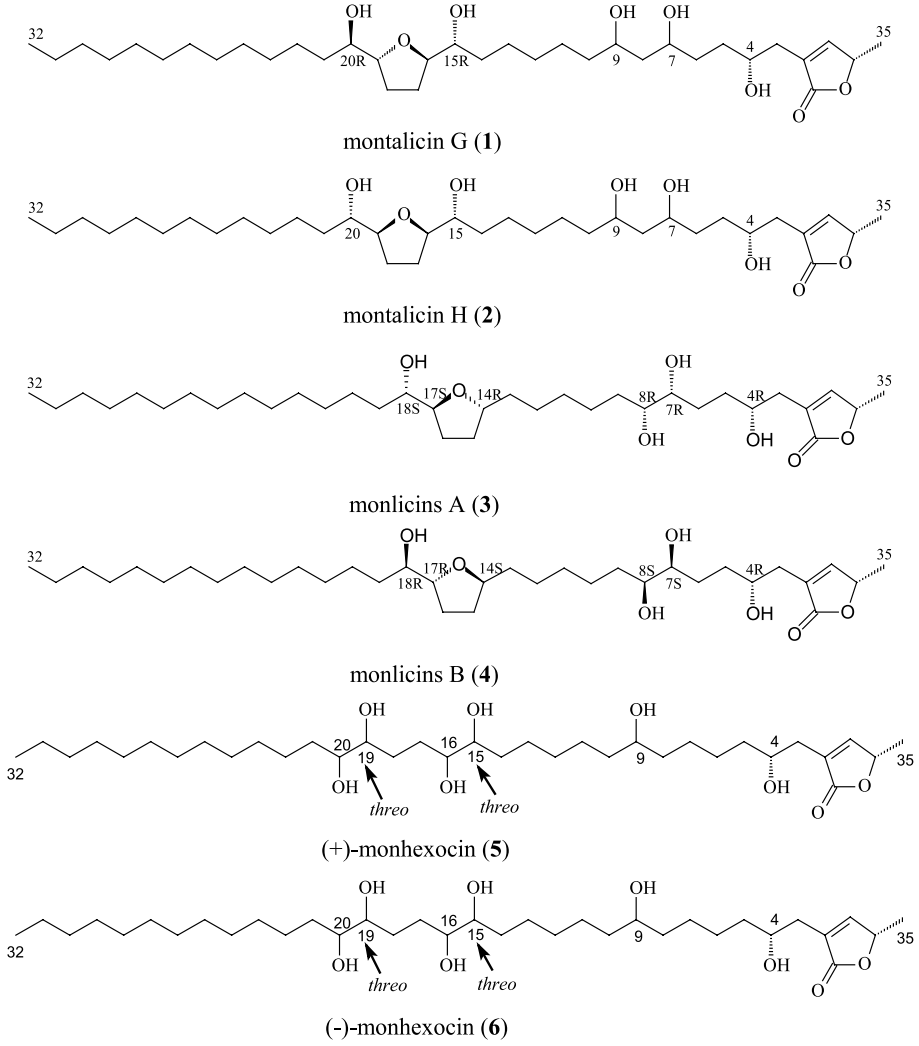

Figure 1. Annonaceous acetogenins isolated from *A. montana*.

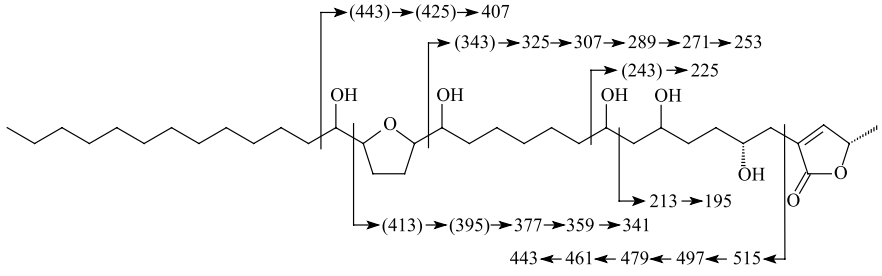

Figure 2. EIMS fragmentations (*m*/*z*) of montalicin G (**1**) and montalicin H (**2**). Data in parentheses refer to peaks that were not observed.

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

	Proton	33	34	3	4	7	9	15, 20	16, 19
1	*S*-MTPA	7.082	4.981	2.710	5.690	5.331	5.430	5.183	4.076
	R-MTPA	7.281	5.023	2.729	5.640	5.313	5.313	5.313	4.188
	$\Delta\delta_{S-R}$	−0.199	−0.042	−0.019	0.05	0.018	0.127	−0.130	−0.112
	Configuration		*S*		*R*	—[a]	—[a]	*R*, *R*	

[a] Indistinguishable.

data, the structure of **1** was fully established and the compound was named montalicin G (**1**).

The molecular formula of compound **2** was confirmed as $C_{35}H_{64}O_8$ by FABMS and HR-FABMS. The EIMS of **2** was almost superimposable with that of **1**. The ^{1}H NMR spectra of compounds **1** and **2** differed only in proton signals at δ 1.93 (2H) and 1.71 (2H) for compound **2**, rather than at δ 1.98 and 1.61 for **1**, which indicated that the mono-THF ring system in **2** likely has a *threo*/*cis*/*threo* conformation, confirmed by the slight up-shift of the ^{13}C NMR signals of the methylene groups from δ 28.7 to 28.1.[12] On the basis of these data, the structure of **2** was fully established and the compound was named as montalicin H (**2**).

Compounds **3** and **4** were isolated as colorless waxy solids with $[\alpha]_D^{25}$ +7.2 (*c* 0.25, $CHCl_3$) and 0 (*c* 0.11, $CHCl_3$), respectively, by reversed-phase HPLC. Their spectral data were almost identical. The ^{1}H and ^{13}C NMR spectra of compounds **3** and **4** showed a common feature of Annonaceous acetogenins, an α,β-unsaturated γ-lactone ring with a hydroxyl group at the C-4 position. In the FABMS, both compounds **3** and **4** showed $[M+Na]^+$ peaks at *m*/*z* 619, which suggested the molecular weight to be 596, and their molecular formulas of $C_{35}H_{64}O_7Na$ were confirmed by HR-FABMS at *m*/*z* 619.4561 and 619.4554 (calcd 619.4550), respectively. The successive FABMS peaks at *m*/*z* 597, 579, 561, 543, and 525 implied the presence of four hydroxyl groups. The UV absorption maximum at 208 nm and the IR absorption band at 1749 cm^{-1} verified the presence of an α,β-unsaturated γ-lactone group, which responds positively to Kedde's reagent.

The signals at δ 3.81 (2H, H-14 and 17) and 3.44 (1H, H-18), as well as the ^{13}C NMR peaks at δ 81.7 (C-17), 79.3 (C-14), and 74.6 (C-18), indicated the presence of a mono-THF ring with one flanking hydroxyl in a *threo* conformation (Table 3). A closer examination of the ^{1}H NMR spectrum showed that the proton resonances for two methylene groups of the mono-THF ring were observed at δ 2.00 (2H, H-15a and 16a), 1.72 (1H, 16b), and 1.62 (1H, H-15b), corresponding to a *trans* conformation.[14] Two of the hydroxyl groups were suspected to be a vicinal diol in a *threo* conformation due to the proton signal at δ 3.44 (2H) and the ^{13}C NMR peaks at δ 74.4 and 74.2.

The placements of the THF ring and the diol were established by close examination of the EIMS fragmentation of both compounds. The THF ring was placed at C-14 and C-17 based on the EIMS fragments at *m*/*z* 333 (cleavage between C-17/C-18–2H_2O) and 281 (C-13/C-14–H_2O), and the vicinal diol was located at C-7/C-8 based on the EIMS fragment at *m*/*z* 199 (cleavage between C-7/C-8) (Fig. 3).

To determine the absolute configuration of compound **3**, the (*R*)- and (*S*)-Mosher ester derivatives were prepared by the modified methodology of Kinghorn and co-workers.[15] At the same time, we made the MTPA

Table 3. ^{1}H and ^{13}C NMR chemical shifts of monlicins A (**3**) and B (**4**)

Position	Monlicin A (**3**)		Monlicin B (**4**)	
	δ(^{1}H)[a]	δ(^{13}C)[b]	δ(^{1}H)[c]	δ(^{13}C)[d]
1		174.7		174.5
2		131.1		131.2
3	2.52 (dd, *J* = 2.0, 14.8 Hz)	33.4[e]	2.53 (dd, *J* = 1.8, 15.3 Hz)	33.7[e]
	2.40 (dd, *J* = 8.0, 14.8 Hz)		2.40 (dd, *J* = 8.1, 15.3 Hz)	
4	3.89 (m)	69.9	3.88 (m)	69.9
5	1.25–1.65	37.2	1.25–1.65	37.3
6	1.25–1.65	31.9[e]	1.25–1.65	31.9[e]
7	3.44 (m)	74.4[d]	3.42 (m)	74.5[d]
8	3.44 (m)	74.2[d]	3.42 (m)	74.4[d]
9	1.25–1.65	31.9	1.25–1.65	33.4
10–12	1.25–1.65	25.5–29.9	1.25–1.65	25.5–29.7
13	1.25–1.65	35.4	1.25–1.65	35.4
14	3.81 (m)	79.3	3.81 (m)	79.3
15	2.00, 1.65 (m)	32.4	2.00, 1.62 (m)	32.4
16	2.00, 1.72 (m)	28.4	2.00, 1.72 (m)	28.4
17	3.81 (m)	81.7	3.83 (m)	81.8
18	3.44 (m)	74.6	3.42 (m)	74.8
19	1.25–1.65	33.4[e]	1.25–1.65	33.4[e]
20–29	1.25–1.65	25.5–29.7	1.25–1.65	25.5–29.7
30	1.25–1.65	31.9	1.25–1.65	31.9
31	1.25–1.65	22.7	1.25–1.65	22.7
32	0.88 (t, *J* = 6.9 Hz)	14.1	0.88 (t, *J* = 6.9 Hz)	14.1
33	7.19 (d, *J* = 1.5 Hz)	151.9	7.20 (d, *J* = 1.2 Hz)	151.8
34	5.06 (dq, *J* = 6.9, 1.5 Hz)	78.0	5.06 (qd, *J* = 6.9, 1.2 Hz)	77.9
35	1.45 (d, *J* = 6.8 Hz)	19.1	1.44 (d, *J* = 6.9)	19.1

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

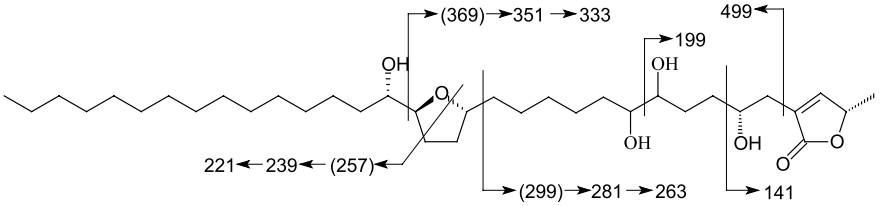

Figure 3. EIMS fragmentations (*m/z*) of monlicin A (**3**) and monlicin B (**4**). Data in parentheses refer to peaks that were not observed.

Table 4. ^{1}H NMR (300 MHz) data of the (*S*)- and (*R*)-Mosher esters of **3** and **4**

	Proton	33	34	3	4	7	8	14[a]	17	18
3	*S*-MTPA	7.054	4.977	2.759	5.692	5.505	5.577	—	4.076	5.331
	R-MTPA	7.393	5.031	2.826	5.737	5.677	5.697	—	4.074	5.342
	$\Delta\delta_{S-R}$	−0.236	−0.054	−0.067	−0.045	−0.172	−0.080	—	0.002	−0.011
	Configuration		*S*		*R*	*R*	*R*			*S*
4	*S*-MTPA	7.050	4.965	2.739	5.711	5.624	5.711	—	4.085	5.371
	R-MTPA	7.297	5.020	2.818	5.703	5.590	5.703	—	4.108	5.369
	$\Delta\delta_{S-R}$	−0.263	−0.055	−0.079	0.004	0.034	0.008	—	−0.023	−0.022
	Configuration		*S*		*R*	*S*	*S*			*R*

[a] Values are indistinguishable.

derivatives by the conventional method.[16] Both of the methods indicated the same results and confirmed that the absolute configurations at C-4, C-7, C-8, C-14, C-17, and C-18 of compound **3** are *R*, *R*, *R*, *R*, *S*, and *S*, respectively (Table 4). Finally, the absolute configuration at C-34 of **3** was determined to be *S* by the CD method.[13]

Compound **4** was separated following compound **3** on reversed-phase HPLC. The NMR spectral and MS data of compound **4** were very similar to those of compound **3**. Like compound **3**, the ^{1}H and ^{13}C NMR signals indicated the presence of an α,β-unsaturated γ-lactone with a hydroxyl group at the C-4 position, a mono-THF ring with one flanking hydroxyl group in a relative conformation of *trans*/*threo*, and a diol (Table 3). The normal-form tail of **4** was corroborated by the IR absorptions at 1747 cm^{-1} and the UV absorption maximum at 209 nm. The conformation of the vicinal diol was determined as *threo* based on the comparison of the ^{1}H and ^{13}C NMR data with those of compound **3**. The EIMS data of compounds **3** and **4** were similar and determined the placement of the THF ring and the diol at C-14/C-17 and C-7/C-8, respectively. The absolute configuration at C-34 of **4** was determined to be *S* by the CD method.[13]

All of the NMR and MS data indicated that compounds **3** and **4** are stereoisomers. To determine the absolute stereochemistries, their Mosher ester derivatives were prepared and compared. Compounds **3** and **4** differ stereochemically in the configurations of the THF ring and the diol. The clear difference between the (*S*)- and (*R*)-MTPA ester derivatives of compound **4** permitted us to conclude that the configurations of C-7, C-8, C-14, C-17, and C-18 of **4** are *S*, *S*, *S*, *R*, and *R*, while the configurations of C-7, C-8, C-14, C-17, and C-18 of **3** are *R*, *R*, *R*, *S*, and *S* (Table 4). Therefore, the structures of compounds **3** and **4** were determined as shown and named as monlicins A (**3**) and B (**4**), respectively.

Compounds **5** and **6** were isolated and purified by reversed-phase HPLC. The proton signals and ^{13}C NMR peaks indicated the presence of an α,β-unsaturated γ-lactone with a hydroxyl group at C-4 position (Table 5), which was also corroborated by the IR absorption at 1745 cm^{-1}, the UV maximum at 212 nm, and a positive reaction to Kedde's reagent. In contrast to the aforementioned mono-THF acetogenins, both compounds **5** and **6** contained a free hydroxyl group due to the ^{1}H NMR signal at δ 3.50 (1H, br s) and the ^{13}C NMR peak at δ 71.3, and two vicinal diol groups, instead of the mono-THF ring moiety, on the basis of the presence of ^{1}H NMR signals at δ 3.34 (4H, br s) in compound **5** and 3.37 (4H, br s) in compound **6** and ^{13}C NMR peaks at δ 73.7–74.4, as well as the absence of ^{1}H NMR signals at δ 3.80 and ^{13}C NMR peaks at δ 82–83. The configuration of these vicinal diols was assigned as *threo* based on a comparison of the NMR chemical shifts with the literature data.[16]

From a close examination of EI-MS, the diols were placed at C-15/C-16 and C-19/C-20 due to successive loss of water from two groups of the EIMS fragments: *m/z* 379 (cleavage between C-19/C-20–2H_2O) to 361 (C-19/C-20–3H_2O) to 343 (C-19/C-20–4H_2O), and *m/z* 309 (C-15/C-16–H_2O) to 291 (C-15/C-16–2H_2O) to 273 (C-15/C-16–3H_2O). The free hydroxyl group was located at C-9 based on the EIMS fragments at *m/z* 227 (cleavage between C-9/C-10), 191 (C-9/C-10–2H_2O), and 197 (C-8/C-9) (Fig. 4).

Literature surveys indicated that five compounds, including donhexocin ($[\alpha]_D^{30}$ +8.0 MeOH, $[\alpha]_D^{16}$ +11.7 $CHCl_3$),[17,18] gardnerilin A ($[\alpha]_D^{11}$ +21.9 MeOH),[19] muricatins A ($[\alpha]_D^{16}$ +7.2 $CHCl_3$) and B ($[\alpha]_D^{16}$ +11.68 $CHCl_3$),[20] and murihexol ($[\alpha]_D^{16}$ +7.2 $CHCl_3$),[18] belong to the same linear Annonaceous acetogenins. According to the spectral analysis, muricatin A corresponds with donhexocin having six hydroxyls at C-4, -10, -15, -16,

Table 5. ^{1}H and ^{13}C NMR chemical shifts of (+)-monhexocin (**5**) and (−)-monhexocin (**6**)

Position	(+)-Monhexocin (**5**)		(−)-Monhexocin (**6**)	
	$\delta(^{1}H)$[a]	$\delta(^{13}C)$[b]	$\delta(^{1}H)$[a]	$\delta(^{13}C)$[b]
1		175.0		174.9
2		130.8		130.9
3	2.42 (dd) (dd, J = 2.0, 14.8 Hz) 2.32 (dd) (dd, J = 8.0, 14.8 Hz)	33.2[c]	2.44 (m) (dd, J = 1.8, 15.3 Hz) 2.37 (dd) (dd, J = 8.1, 15.3 Hz)	33.4[c]
4	3.76 (br s)	69.4	3.78 (m)	69.6
5	1.20–1.64	36.9[d]	1.22–1.63	36.8[d]
6–7	1.20–1.64	25.2–29.6	1.22–1.63	25.1–29.8
8	1.20–1.64	36.9[d]	1.22–1.63	36.9[d]
9	3.50 (br s)	71.3	3.54 (m)	71.4
10	1.20–1.64	36.9[d]	1.22–1.63	37.0[d]
11–13	1.20–1.64	25.2–29.6	1.22–1.63	25.1–29.8
14	1.20–1.64	33.0[c]	1.22–1.63	33.1[c]
15	3.34 (br s)	73.7[e]	3.37 (m)	74.0[e]
16	3.34 (br s)	74.2[e]	3.37 (m)	74.2[e]
17	1.20–1.64	33.0[c]	1.22–1.63	33.1[c]
18	1.20–1.64	33.0[c]	1.22–1.63	33.1[c]
19	3.34 (br s)	74.2[e]	3.37 (m)	74.4[e]
20	3.34 (br s)	74.0[e]	3.37 (m)	74.1[e]
21	1.20–1.64	33.0[c]	1.22–1.63	33.1[c]
22–29	1.20–1.64	25.2–29.6	1.22–1.63	25.1–29.8
30	1.20–1.64	31.8	1.22–1.63	31.8
31	1.20–1.64	22.6	1.22–1.63	22.6
32	0.82 (t, J = 6.9 Hz)	13.9	0.84 (t, J = 6.9 Hz)	14.0
33	7.17 (d, J = 1.5 Hz)	152.2	7.18 (d, J = 1.5 Hz)	152.2
34	5.01 (qd, J = 6.9, 1.5 Hz)	78.2	5.03 (qd, J = 6.9, 1.5 Hz)	78.2
35	1.38 (d, J = 6.8 Hz)	18.8	1.40 (d, J = 6.8. Hz)	18.7

[a] Measured at 400 MHz, in $CDCl_3$. Chemical shifts are in δ values.
[b] Measured at 100 MHz, in $CDCl_3$.
[c,d,e]Assignments may be interchanged.

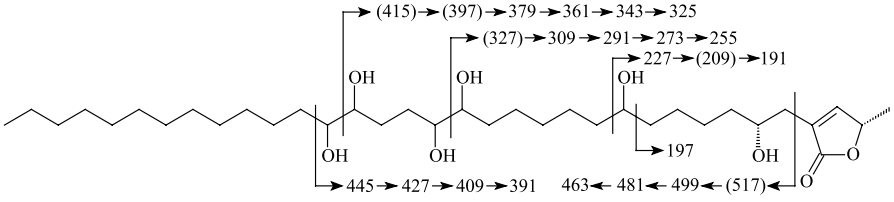

Figure 4. EIMS fragmentations (*m/z*) of (+)-monhexocin (**5**) and (−)-monhexocin (**6**). Data in parentheses refer to peaks that were not observed.

-19, and -20 with a *threo*/*threo* configuration of the diols, while muricatin B corresponds with murihexol having six hydroxyls at C-4, -10, -15, -16, -19, and -20 but with a *threo*/*erythro* configuration. The absolute configuration at C-34 of **5** and **6** was determined to be *S* by the CD method.[13] Interestingly, the isolation of the optical isomers of these linear acetogenins has not been reported. Unfortunately, the absolute stereochemistry of the carbinol centers at C-15/C-16 and C-19/C-20 (*threo*/*threo*) was undefined due to the indistinguishable chemical shifts of H-14, -17, -18, and -21 in the ^{1}H NMR spectra. Herein, (+)-monhexocin (**5**) and (−)-monhexocin (**6**), which have six hydroxyls at C-4, 9, 15/16 (*threo*), and 19/20 (*threo*), would be suspected as the precursor of annoreticuin, which is also found in the same species.[21]

The isolated compounds **7**, **8**, and **9** were verified as the known compounds, murisolin (**7**), 4-deoxyannomontacin (**8**), and muricatacin (**9**), by comparing UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with the published values.[4–6] Among them, 4-deoxyannomontacin (**8**) and muricatacin (**9**) were isolated from this plant for the first time. Interestingly, muricatacin (**9**) is a special linear acetogenin without terminal γ-lactone moiety that was only reported from *A. muricata.*

Compounds **1**–**9** were subjected to a 3-day MTT cytotoxicity assay toward three human cancer cell lines, A549 (lung), Hep G2, and 3B (hepatoma). Interestingly, the bioassay data showed that all compounds **1**–**9** were significantly cytotoxic toward Hep G2 cells, but displayed only moderate cytotoxic activities toward Hep 3B and A549 (Table 6). From a structure–activity relationship (SAR) viewpoint, the terminal α,β unsaturated γ-lactone ring moiety with a hydroxyl group at C-4 appears to be very important for the cytotoxicity of Annonaceous acetogenins against Hep G2 cell line, because compounds **8** and **9** were much less active than the

Table 6. In vitro cytotoxicity data of compounds **1–9** by the MTT assay

Compound	Cell lines[a]/ED_{50} (μg/mL)		
	Hep G2	Hep 3B	A549
Montalicin G (**1**)	0.078	6.09	6.55
Montalicin H (**2**)	0.075	2.02	6.38
Monlicin A (**3**)	0.020	1.59	4.37
Monlicin B (**4**)	0.023	3.77	5.61
(+)-Monhexocin (**5**)	0.081	NA	ND
(−)-Monhexocin (**6**)	0.098	NA	2.64
Murisolin (**7**)	0.020	3.44	13.92
4-Deoxyannomontacin (**8**)	0.53	8.97	14.57
Muricatacin (**9**)	0.31	7.41	16.52
Doxoribicin	0.18	0.31	0.75
Paclitaxol	0.04	0.1	ND[b]

[a] Hep G_2, human hepatoma cell (hepatitis B surface antigen [HBsAg] [−]); Hep 3B, human hepatoma cell (hepatitis B surface antigen [HBsAg] [+]); and A549, lung cancer cell lines.

[b] NA, not active (inhibition 5%) at the highest concentration. ND, not determined.

remaining compounds. In addition, compounds **1–6** were subjected to a SRB cytotoxicity assay against eight human cancer cell lines, MCF-7 (breast), HCT-8 (ileocecal), SK-MEL-2 (melanoma), KB (epidermoid nasopharyngeal carcinoma), KB-VIN (vincristine-resistant KB), U-87-MG (glioblastoma), PC-3 (prostate), 1A9 (ovarian), and PTX10 (ovarian cancer cell line with β-tubulin mutation).[9,10] The bioassay data showed that only compound **4** had significant bioactivity against 1A9 cells in a 3-day assay and compound **3** had improved bioactivity against 1A9 cells in the 6-day assay. Compounds **1**, **2**, and **4–6** had only moderate bioactivities against these eight human cancer cell lines (Table 7).

3. Conclusion

Annonaceous acetogenins are a specific type of active natural products from Annonaceae. In our study, two types of Annonaceous acetogenins, mono-THF (**1-4**) and non-THF (**5** and **6**), were isolated from *A. montana*. All of these compounds showed significant selective cytotoxic activity toward hepatoma cells (Hep G2) and two compounds, **3** and **4**, also were active against ovarian cancer cell (1A9). Detailed mechanism of action studies is under investigation.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. The IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^1H and ^{13}C NMR spectra (all in $CDCl_3$) were recorded with Varian Gemini 300 MHz (UNC-CH) or Varian Unity 400 NMR (KMU) spectrometers, using TMS as internal standard. LRFABMS 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 (UNC-CH). HR-FABMS were measured on a JEOL JMS-700 MStation (Okayama University of Science, Japan). Si gel 60 (Macherey-Nagel, 230–400 mesh) was used for column chromatography; precoated Si gel plates (Macherey-Nagel, SIL G-25 UV254, 0.25 mm) were used for analytical TLC. The spots were detected by spraying with Dragendorff's reagent, Kedde's reagent, or 50% H_2SO_4 aqueous solution and then heating on a hot plate. HPLC was performed on a Shimadzu LC-10AT apparatus equipped with a Shimadzu SPD-10A UV–vis detector. Hypersil ODS-5 (250 × 4.6 mm i.d.), preparative ODS-5 (250 × 20 mm i.d.) columns, Purosphere® STAR RP-18 (250 × 4 mm i.d.) and preparative RP-18 (250 × 25 mm i.d.), and Altima C18 5 μm (250 × 4.6 mm i.d.) and preparative C18 5 μm (250 × 25 mm i.d.) columns were used for analytical and preparative purposes, respectively.

4.2. Plant material

The seeds of *A. montana* 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.

4.3. Extraction and isolation

The seeds of *A. montana* (2 kg) were extracted with MeOH (2L × 3). The MeOH extracts of the seeds were partitioned with $CHCl_3$ and water to yield $CHCl_3$ and aqueous extracts. After removing the solvent in vacuo,

Table 7. In vitro cytotoxicity data of new compounds **1–6** by the SRB assay

Compound	Cell lines[a]/ED_{50} (μg/mL)									
	MCF-7	HCT-8	KB	KB-VIN	U-87-MG	PC-3	1A9 (3 day)	PTX10 (3 day)	1A9 (6 day)	PTX10 (6 day)
Montalicin G (**1**)	4.8	11.5	5.8	12.7	12.8	9.2	2	3.5	1.3	4.6
Montalicin H (**2**)	>20 (30)	>20 (24)	>20 (41)	>20 (7)	>20 (23)	>20 (27)	>20 (45)	20	>20 (43)	20
Molicin A (**3**)	6.9	14.6	9.5	14	15.6	15.6	5.2	7.6	0.085	1.1
Molicin B (**4**)	4.9	ND[b]	5.1	3.2	ND[b]	ND[b]	0.03	ND[b]	ND[b]	ND[b]
(+)-Monhexocin (**5**)	9.6	>20 (16)	9.6	>20 (17)	>20 (31)	>20 (31)	>10 (44)	9.6	4.2	3.2
(−)-Monhexocin (**6**)	10	>20 (10)	>20 (46)	NA	NA	NA	>10 (43)	>10 (38)	3.1	3.6

[a] MCF-7, breast cancer cells; HCT-8, ileocecal cancer cells; KB, epidermoid nasopharyngeal carcinoma cells; KB-VIN, vincristine-resistant KB cells; U-87-MG, glioblastoma cancer cells; PC-3, prostate cancer cells; 1A9, ovarian cancer cells; and PTX10, ovarian cancer cells with β-tubulin mutation.

[b] ND, not determined.

the $CHCl_3$ residue was partitioned using MeOH 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, and 1:5, pure $CHCl_3$) and $CHCl_3$–MeOH (100:1, 50:1, 20:1, 10:1, and 5:1).

Fraction 4, eluted with *n*-hexane–$CHCl_3$ (1:5) to pure $CHCl_3$, was isolated and purified by column and HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, and Purosphere RP-18 column, 250 × 4.6 mm, acetonitrile–water, 95:5, flow rate 1 mL/min; UV detector set at 225 nm) to give murisolin (**7**) (2.1 mg) and 4-deoxyannomontacin (**8**) (7.8 mg). 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 monlicin A (**3**) (11.0 mg), monlicin B (**4**) (7.9 mg), and muricatacin (**9**) (7.7 mg). Fraction 8, eluted with $CHCl_3$–MeOH (20:1), was isolated and further purified by column chromatography and HPLC (Alltima C18 5 μm column, 250 × 4.6 mm, acetonitrile–water, 70:30, flow rate 1 mL/min; UV detector set at 225 nm) to give montalicin G (**1**) (22.2 mg) and montalicin H (**2**) (10.9 mg). Fraction 9, eluted with $CHCl_3$–MeOH (20:1), was isolated and further purified by column chromatography and HPLC (Purosphere® STAR RP-18, 250 × 4.6 mm, MeOH–water, 80:20, flow rate 1 mL/min; UV detector set at 225 nm) to give (+)-monhexocin (**5**) (20.2 mg) and (−)-monhexocin (**6**) (13.1 mg).

4.3.1. Montalicin G (1). It was obtained as a colorless waxy solid. $[\alpha]_D^{25}$ +17.2 (*c* 1.6, $CHCl_3$). UV (MeOH) λ_{max} ($\log\varepsilon$) 208 (3.81) nm. IR (Neat) ν_{max} 3424, 2919, 2850, 1735 (OC=O), 1070 cm^{-1}. EI-MS (70 eV), see Figure 2. ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1. HR-FABMS $[M+Na]^+$ at *m*/*z* 635.4483 (calcd for $C_{35}H_{64}O_8Na^+$, 653.4499). CD (MeCN) $\Delta\varepsilon > 0$ (*c* 3.1×10^{-4}) [θ] (242 nm) n–π* −4.98E+0.2, (209 nm) π–π* 2.49E+03.

4.3.2. (*R*)- and (*S*)-MTPA derivatives by Kinghorn's modified method. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of **1** was carried out 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 ^1H NMR. The reaction was found to be complete after 2 h. ^1H NMR (300 MHz, pyridine-d_5) data of the (*R*)-MTPA ester derivative (**1r**) of **1** were obtained directly and were assigned on the basis of the correlations of the ^1H–^1H COSY spectrum. The (*S*)-MTPA ester derivative (**1s**) was prepared in the same way using (*R*)-(−)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride reagent. (*R*)- and (*S*)-MTPA ester derivatives of **3** and **4** were also prepared by the same method.

4.3.3. Montalicin H (2). It was obtained as a colorless waxy solid. $[\alpha]_D^{25}$ +13.0 (*c* 0.81, $CHCl_3$). UV (MeOH) λ_{max} ($\log\varepsilon$) 208 (3.77) nm. IR (Neat) ν_{max} 3424, 2919, 2850, 1746 (OC=O), 1070 cm^{-1}. EI-MS (70 eV), see Figure 2. ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1. HR-FABMS $[M+Na]^+$ at *m*/*z* 635.4493 (calcd for $C_{35}H_{64}O_8Na^+$, 653.4499). CD (MeCN) $\Delta\varepsilon > 0$ (*c* 1.8×10^{-4}) [θ] (242 nm) n–π* −3.04E+0.2, (209 nm) π–π* 7.40E+03.

4.3.4. Monlicin A (3). It was obtained as a colorless waxy solid. $[\alpha]_D^{25}$ +7.2 (*c* 0.25, $CHCl_3$). UV (MeOH) λ_{max} ($\log\varepsilon$) 224 (3.91) nm. IR (Neat) ν_{max} 3392, 2921, 2852, 1747 (OC=O), 1080 cm^{-1}. EI-MS (70 eV), see Figure 3. ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 3. HR-FABMS $[M+Na]^+$ at *m*/*z* 619.4561 (calcd for $C_{35}H_{64}O_7Na^+$, 619.4550). CD (MeOH) $\Delta\varepsilon > 0$ (*c* 1.51×10^{-4}) [θ] (238 nm) n–π* −2.59E+03, (212 nm) π–π* 2.29E+04.

4.3.5. (*R*)- and (*S*)-MTPA derivatives of 3 and 4 by the conventional method. Compounds **3** (4 mg) were dissolved in 1 mL of dry CH_2Cl_2, 0.5 mL of pyridine, and 1 mg of 4-dimethylaminopyridine, and 100 mg of (*R*)-(−)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride was introduced to the solution. After the reaction mixture was allowed to remain for more than 6 h at room temperature (the reaction progress was monitored by TLC), saturated $NaHCO_3$ (~3 mL) and Et_2O (~3 mL) were added. The organic phase was removed, and the aqueous phase was extracted with Et_2O (~5 mL, 2×). The organic phases were combined, washed three times with $NaHSO_4$ (5% aqueous solution, to remove pyridine) and brine, dried ($MgSO_4$), and concentrated under reduced pressure to leave a crude yellow oil, which was purified by preparative TLC to give the (*S*)-MTPA esters (**3s**). The (*R*)-MTPA esters (**3r**) were prepared in the same way using (*S*)-(+)-methoxyl-α-(tri-fluoromethyl)-phenylacetyl chloride reagent. (*R*)- and (*S*)-MTPA ester derivatives of **4** were also prepared by the same method.

4.3.6. Monlicin B (4). It was obtained as a colorless waxy solid. $[\alpha]_D^{25}$ 0 (*c* 0.11, $CHCl_3$). UV (MeOH) λ_{max} ($\log\varepsilon$) 224 (3.91) nm. IR (Neat) ν_{max} 3391, 2921, 2851, 1745 (OC=O), 1080 cm^{-1}. EI-MS (70 eV), see Figure 3. ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 3. HR-FABMS $[M+Na]^+$ at *m*/*z* 619.4554 (calcd for $C_{35}H_{64}O_7Na^+$, 619.4550). CD (MeOH) $\Delta\varepsilon > 0$ (*c* 1.68×10^{-4}) [θ] (238 nm) n–π* −1.70E+03, (213 nm) π–π* 170E+04.

4.3.7. (+)-Monhexocin (5). It was obtained as a white waxy solid. $[\alpha]_D^{25}$ +8.7 (*c* 0.21, $CHCl_3$). UV (MeOH) λ_{max} ($\log\varepsilon$) 224 (3.91) nm. IR (Neat) ν_{max} 3364, 2915, 2845, 1743 (OC=O), 1070 cm^{-1}. EI-MS (70 eV), see Figure 4. FABMS $[M+Na]^+$ *m*/*z* 637, molecular formula ($C_{35}H_{66}O_6Na^+$). ^1H NMR ($CDCl_3$ with few drops of methanol-d_4, 400 MHz) and ^{13}C NMR ($CDCl_3$ with few drops of methanol-d_4, 100 MHz) data, see Table 5. CD (MeCN) $\Delta\varepsilon > 0$ (*c* 1.30×10^{-4}) [θ] (242 nm) n–π* −7.20E+02, (209 nm) π–π* 2.12E+03.

4.3.8. (−)-Monhexocin (6). It was obtained as a white waxy solid. $[\alpha]_D^{25}$ −5.7 (*c* 0.43, $CHCl_3$). UV (MeOH) λ_{max} (log ε) 224 (3.91) nm. IR (Neat) ν_{max} 3364, 2915, 2845, 1741 (OC=O), 1070 cm^{-1}. EI-MS (70 eV), see Figure 4. FABMS $[M+Na]^+$ *m/z* 637, molecular formula $C_{35}H_{66}O_8Na^+$. ^1H NMR ($CDCl_3$ with few drops of methanol-d_4, 400 MHz) and ^{13}C NMR ($CDCl_3$ with few drops of methanol-d_4, 100 MHz) data, see Table 5. CD (MeCN) $\Delta\varepsilon > 0$ (*c* 1.80×10^{-4}) [θ] (242 nm) n–π* −2.06E+03, (209 nm) π–π* 1.38E+04.

4.3.9. Murisolin (7). It was obtained as colorless oil. $[\alpha]_D^{25}$ 8.2 (*c* 0.11, $CHCl_3$). UV (MeOH) λ_{max} 211 nm. IR (Neat) ν_{max} 3450, 2918, 2850, 1748 (OC=O), 1468 cm^{-1}. EI-MS (70 eV) *m/z* 393, 381, 363, 345, 311, 293, 263, 141. FAB-MS *m/z* 603 $[M+Na]^+$, 581 $[M+H]^+$, molecular formula $C_{35}H_{64}O_6$. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.18 (1H, d, *J* = 1.2 Hz, H-33), 5.05 (1H, qd, *J* = 6.4, 1.2 Hz, H-34), 3.80 (3H, m, H-4, 16, 19), 3.38 (2H, m, H-15, 20), 2.57 (1H, m, H-3a), 2.41 (1H, m, H-3b), 2.0–1.2 (aliphatic CH_2), 1.40 (3H, d, *J* = 6.4 Hz, H-35), 0.87 (3H, t, *J* = 6.8 Hz, H-32). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.6 (C-1), 151.8 (C-33), 131.2 (C-2), 82.6 (C-16, 19), 78.0 (C-34), 74.1 (C-15, 20), 70.0 (C-4), 31.9 (C-3), 37–22 (aliphatic CH_2), 19.1 (C-35), 14.1 (C-32) (identical with the literature.)[4]

4.3.10. 4-Deoxyannomontacin (8). It was obtained as a white waxy solid. $[\alpha]_D^{25}$ +14.4 (*c* 0.5, $CHCl_3$). UV (MeOH) λ_{max} (log ε) 213 (3.91) nm. IR (Neat) ν_{max} 3443, 2919, 2850, 1742 (OC=O), 1455 cm^{-1}. EI-MS (70 eV) *m/z* 403, 373, 355, 321, 303, 285, 225, 207. FABMS $[M+Na]^+$ *m/z* 647, molecular formula $C_{37}H_{68}O_6$. ^1H NMR ($CDCl_3$, 300 MHz) 6.98 (1H, d, *J* = 1.5 Hz, H-35), 5.00 (1H, qd, *J* = 6.9, 1.5 Hz, H-36), 3.80 (2H, m, H-18, 21), 3.59 (1H, m, H-10), 3.40 (2H, m, H-17, 22), 2.0–1.2 (aliphatic CH_2), 1.41 (3H, d, *J* = 6.5 Hz, H-37), 0.88 (3H, t, *J* = 6.9 Hz, H-34). ^{13}C NMR ($CDCl_3$,75 MHz) 174.0 (C-1), 148.9 (C-35), 134.3 (C-2), 82.7 and 82.6 (C-18, 21), 77.4 (C-36), 74.0 (C-17, 22), 71.9 (C-10), 37–22 (aliphatic CH_2), 19.2 (C-37), 14.1 (C-34) (identical with the literature.)[5]

4.3.11. Muricatacin (9). It was obtained as colorless oil. $[\alpha]_D^{20}$ −13.3 (*c* 0.02, $CHCl_3$). IR (Neat) ν_{max} 3424, 2919, 2850, 1755 (OC=O), 1070 cm^{-1}. EI-MS (70 eV) *m/z* 267, 249, 199. FAB-MS *m/z* 307 $[M+Na]^+$, 285 $[M+H]^+$, molecular formula $C_{17}H_{32}O_3$. ^1H NMR ($CDCl_3$, 400 MHz) δ 4.42 (1H, td, *J* = 7.6, 4.8 Hz, H-4), 3.57 (1H, br td, *J* = 7.6, 4.4 Hz, H-35), 2.62 (1H, ddd, *J* = 17.6, 9.6, 4.8 Hz, H-2), 2.53 (1H, td, *J* = 17.6, 9.2 Hz, H-2), 2.25 (1H, m, H-3), 2,11 (1H, m, H-3), 2.0–1.2 (aliphatic CH_2), 0.87 (3H, t, *J* = 6.8 Hz, H-17). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.1 (C-1), 82.9 (C-4), 73.7 (C-5), 32.9 (C-2), 31–22 (aliphatic CH_2), 14.1 (C-17) (identical with the literature.)[6]

4.4. Cytotoxic assay

The anti-tumor activity of compounds isolated from *A. montana* was evaluated. The MTT and SRB assays were used in Taiwan and US, respectively. The MTT assay toward human cancer cells, Hep G2, Hep 3B, and A549, uses a tetrazolium salt, [3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT)], for the colorimetric quantization of cell survival and proliferation.[8] Optical densities read at 570 nm are directly proportional to the number of living cells. Doxoribicin and paclitaxel are tested as the positive control.

In addition, compounds were assayed for cytotoxic activity on human tumor cell lines, 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), as well as 1A9 (ovarian cancer), and PTX10 (ovarian cancer cell line with β-tubulin mutation), using a reported procedure.[9,10] 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 absorbancy 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. ED_{50} values are calculated and activity less than 4 μg/mL for pure compounds and less than 20 μg/mL for extracts is considered significant.

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