New Cytotoxic Monotetrahydrofuran Annonaceous Acetogenins from *Annona muricata*

Chih-Chuang Liaw, Fang-Rong Chang, Chih-Yuan Lin, Chi-Jung Chou, Hui-Fen Chiu, Ming-Jung Wu, and Yang-Chang Wu*

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China

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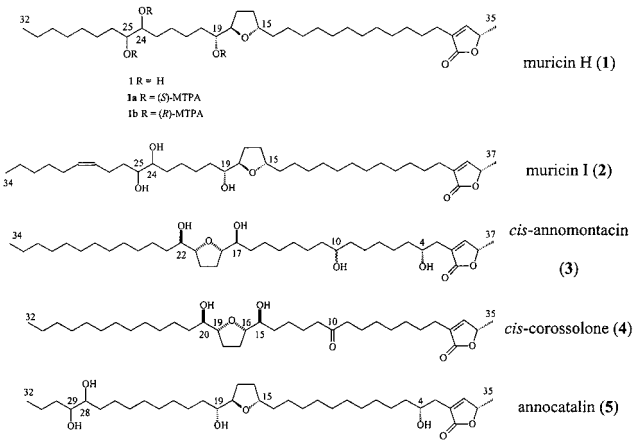
Three new monotetrahydrofuran annonaceous acetogenins, muricin H (**1**), muricin I (**2**), and *cis*-annomontacin (**3**), along with five known acetogenins, annonacin, annonacinone, annomontacin, murisolin, and xylomaticin, were isolated from the seeds of *Annona muricata*. Additionally, two new monotetrahydrofuran annonaceous acetogenins, *cis*-corossolone (**4**) and annocatalin (**5**), together with four known ones, annonacin, annonacinone, solamin, and corossolone, were isolated from the leaves of this species. The structures of all new isolates were elucidated and characterized by spectral and chemical methods. These new acetogenins exhibited significant activity in in vitro cytotoxic assays against two human hepatoma cell lines, Hep G_2 and 2,2,15. Compound **5** showed a high selectivity toward the Hep 2,2,15 cell line.

In continuation of our studies on acetogenins from Formosan annonaceous plants, including *Annona reticulata*,[1−3] *A. montana*,[4] *A. cherimola*,[5] *A. atemoya*,[6] and *Rollinia mucosa*,[7,8] *A. muricata* L. was chosen for investigation. This plant is a well-known tropical fruit tree, named "sour sop" or "guanabana", that is distributed in the Americas, Africa, and Southeast Asia. To date, there have been more than 50 annonaceous acetogenins isolated from the stems, leaves, and seeds of this species.[9,10] In our previous investigation of this species, a series of unusual monotetrahydrofuran (mono-THF) annonaceous acetogenins were found from the stem bark and the seeds.[11,12]

The present report describes the isolation and structural elucidation of compounds from the leaves and the seeds of *A. muricata*. Three new mono-THF annonaceous acetogenins, muricin H (**1**), muricin I (**2**), and *cis*-annomontacin (**3**), along with five known compounds, annonacin,[13] annonacinone,[14] annomontacin,[15] murisolin,[16] and xylomaticin,[17] were isolated from the seeds. In turn, two new mono-THF annonaceous acetogenins, *cis*-corossolone (**4**) and annocatalin (**5**), as well as four known compounds, annonacin,[13] annonacinone,[14] solamin,[18] and corossolone,[19] were isolated from the leaves. All of isolates from both the seeds and leaves of this species were isolated and purified by reversed-phase HPLC, and their structures were elucidated and characterized by spectral and chemical methods. Annonacin and annonacinone were obtained as two major annonaceous acetogenins from both the leaves and seeds. In in vitro cytotoxic assays, the new compounds showed potent activity against two human hepatoma cell lines, Hep G_2 and 2,2,15. Interestingly, compound **5** demonstrated significant selective cytotoxicity toward the human hepatoma Hep 2,2,15 cell line.

Results and Discussion

Compound **1** was isolated as a colorless waxy solid, $[\alpha]^{25}_D$ +9.5° (*c* 0.76, $CHCl_3$). The $[M + Na]^+$ peak in the FABMS at *m*/*z* 603 suggested the molecular weight to be 580, and the molecular formula of $C_{35}H_{64}O_6$ was confirmed by HRFABMS. The UV spectral absorption at 208 nm and the IR spectral absorption at 1743 cm^{-1} indicated the presence of an α,β-unsaturated γ-lactone group. The successive FABMS peaks at *m*/*z* 563, 545, and 527 implied the presence of three hydroxyl groups. In the ^1H NMR spectrum, the signals at δ 6.98 (1H, H-33), 5.04 (1H, H-34), 2.24 (2H, H-3), and 1.39 (3H, H-35) verified the presence of an α,β-unsaturated γ-lactone (see Table 1). The assignment was also confirmed by the ^{13}C NMR resonances at δ 173.9 (C-1), 148.9 (C-33), 134.2 (C-2), 77.6 (C-34), and 19.1 (C-35). The signals at δ 3.82 (2H, H-15, 18) and 3.41 (1H, H-19), as well as the ^{13}C NMR peaks at δ 81.8 (C-18), 79.3 (C-15), and 74.4 (C-19), indicated the presence of a mono-THF ring with one flanking hydroxyl in a *threo* conformation.[12] A close examination of the ^1H NMR spectrum showed that the proton resonances for the two methylene groups of the mono-THF ring, which were observed at δ 1.98 (H-16a, 17a) and 1.62 (H-16b, 17b), corresponded to the *trans* configuration.[20] By making the (*R*)- and (*S*)-Mosher ester derivatives and carrying out Hoye's methodology,[8,12] the absolute configuration at C-19 of compound **1** could be confirmed as *R* (see Table 2). Therefore, the absolute configurations at C-15 and C-18 were assigned as *S* and *R*, respectively. Two of the hydroxyl groups were suspected to be a vicinal diol due to the proton signal at δ 3.41 (2H) and the ^{13}C NMR peaks at δ 74.9 and 74.2. The configuration of this vicinal diol was assigned as *threo* on



* To whom correspondence should be addressed. Tel: +886-7-3121101, ext. 2197. Fax: +886-7-3114773. E-mail: yachwu@cc.kmu.edu.tw.

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **1**−**3**

	muricin H (**1**)		muricin I (**2**)		*cis*-annomontacin (**3**)	
position	δ (^{1}H)[a]	δ (^{13}C)[b]	δ (^{1}H)[a]	δ (^{13}C)[b]	δ (^{1}H)[a]	δ (^{13}C)[b]
1		173.9		174.0		174.6
2		134.2		134.2		131.2
3	2.24 (m)	25.1	2.26 (m)	25.1	H-a 2.56 (m) H-b 2.48 (m)	33.4
4	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	3.80 (m)	69.9
5	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	1.2−1.5	37.3[c]
6−8	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	1.2−1.5	25.5−29.7
9	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	1.2−1.5	37.2[c]
10	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	3.54	70.8
11	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	1.2−1.5	37.4[c]
12−13	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	1.2−1.5	25.5−29.7
14	1.2−1.6	35.5	1.2−1.5	35.4	1.2−1.5	25.5−29.7
15	3.82 (m)	79.3	3.82 (m)	79.3	1.2−1.5	25.5−29.7
16	1.98, 1.62 (m)	25.7−29.9	1.99, 1.65 (m)	26.2−29.0	1.2−1.5	34.0[d]
17	1.98, 1.62 (m)	25.7−29.9	1.99, 1.65 (m)	26.2−29.0	3.40 (m)	74.3
18	3.82 (m)	81.8	3.82 (m)	81.8	3.80 (m)	82.7
19	3.41 (m)	74.4[c]	3.45 (m)	74.2[c]	1.93, 1.76 (m)	25.5−29.7
20	1.2−1.6	35.5	1.2−1.5	35.4	1.93, 1.76 (m)	25.5−29.7
21	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	3.80 (m)	82.7
22	1.2−1.6	25.7−29.9	1.2−1.5	26.2−29.0	3.40 (m)	74.3
23	1.2−1.6	33.4[d]	1.2−1.5	32.4[d]	1.2−1.5	34.1[d]
24	3.41 (m)	74.5 [c]	3.45 (m)	74.5[c]	1.2−1.5	25.5−29.7
25	3.41 (m)	74.2 [c]	3.45 (m)	74.5[c]	1.2−1.5	25.5−29.7
26	1.2−1.6	32.3[d]	1.2−1.5	33.4[d]	1.2−1.5	25.5−29.7
27	1.2−1.6	25.7−29.9	1.2−1.5	23.5	1.2−1.5	25.5−29.7
28	1.2−1.6	25.7−29.9	5.37	130.8	1.2−1.5	25.5−29.7
29	1.2−1.6	25.7−29.9	5.37	129.0	1.2−1.5	25.5−29.7
30	1.2−1.6	31.9	1.2−1.5	26.2−29.0	1.2−1.5	25.5−29.7
31	1.2−1.6	22.6	1.2−1.5	26.2−29.0	1.2−1.5	25.5−29.7
32	0.86 (t, J = 6.7)	14.1	1.2−1.5	31.9	1.2−1.5	31.9
33	6.98 (d, J = 1.2)	148.9	1.2−1.5	22.7	1.2−1.5	22.7
34	5.04 (qd, J = 6.8, 1.2)	77.6	0.87 (t, J = 6.8)	14.1	0.87 (t, J = 6.8)	14.1
35	1.39 (d, J = 6.8)	19.2	6.98 (d, J = 1.2)	148.9	7.17 (d, J = 1.4)	152.2
36			5.05 (qd, J = 6.8, 1.2)	77.4	5.02 (qd, J = 6.8, 1.4)	78.0
37			1.39 (d, J = 6.8)	19.2	1.42 (d, J = 6.8)	19.1

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

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

proton	*S*-MTPA	*R*-MTPA	$\Delta\delta_{S-R}$	configuration
H-15	3.82	3.92	−0.10	19 *R*
H-18	3.77	3.79	−0.02	19 *R*

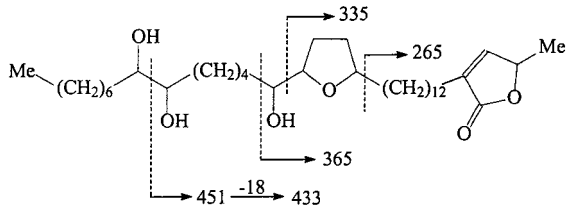


Figure 1. EIMS fragmentation (*m*/*z* values) of muricin H (**1**).

the basis of a comparison of its NMR chemical shifts with literature data.[12]

The placements of the THF ring and the diol were established by close examination of the EIMS fragmentation of **1** (Figure 1). The THF ring was placed between C-15 and C-18 based on the fragments at *m*/*z* 335 and 265, while the diol was determined at C-24/C-25, from the fragments at *m*/*z* 451 and 433 (cleavage between C-24/C-25 and loss of a H_2O moiety).

Finally, the absolute configuration at C-34 of **1** was determined by a CD method.[21] A positive π−π^* Cotton effect ($\Delta\epsilon$ >0) clearly indicated the stereochemistry at C-34 on the γ-lactone fragment to be in the *S* configuration. The absolute stereochemistry of the carbinol centers at C-24/C-25 (*threo*) was undefined due to the indistinguishable chemical shifts of H-23 and H-26 in the ^{1}H NMR spectrum. Thus, the structure of **1** was elucidated and this compound named muricin H.

Compound **2** was isolated as a white waxy solid, $[\alpha]^{25}_D$ +88.0° (*c* 0.25, $CHCl_3$). The $[M + Na]^+$ peak in the FABMS at *m*/*z* 629 suggested a molecular weight of 606, and the molecular formula of $C_{37}H_{66}O_6$ was confirmed by HR-FABMS. Successive FABMS fragments at *m*/*z* 589, 571, and 553 were indicative of the presence of three hydroxyls. The IR, UV, and NMR spectral data revealed that **2** has the same moieties as **1**, including an α,β-unsaturated γ-lactone, a mono-THF ring with one flanking hydroxyl group in a *threo*/*trans* configuration, and a vicinal diol with a *threo* configuration.[8,12,22] Additionally, two proton signals at δ 5.37, together with the ^{13}C NMR signals at δ 130.8 and 129.0, showed the presence of a double bond (Table 1). The ^{13}C NMR chemical shifts of the allylic methylene carbons (C-27, 30) at δ 25.5−29.9 suggested the geometry of the double bond as being in the *Z*-conformation.[23]

The structure of **2** was established by close examination of the EIMS fragmentation (Figure 2). The EIMS peaks at *m*/*z* 335 and 265 demonstrated that the THF ring is located between C-15/C-18. Furthermore, the diol was located between C-24/C-25, as suggested by the EIMS peaks at *m*/*z* 451 and 433. According to the anticipated biogenetic pathway and the EIMS peak at *m*/*z* 549, the double bond was located between C-28/C-29. On the basis of the above

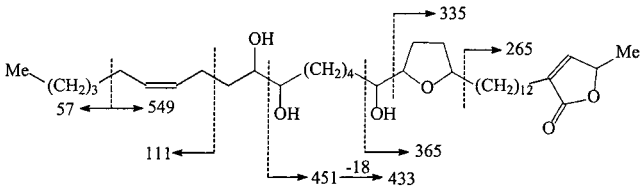


Figure 2. EIMS fragmentation (*m*/*z* values) of muricin I (**2**).

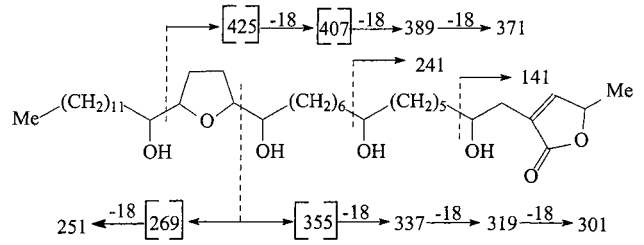


Figure 3. EIMS fragmentation (*m*/*z* values) of *cis*-annomontacin (**3**).

data, the structure of **2** was characterized and the compound named muricin I.

Compound **3** was isolated as a white waxy solid, $[\alpha]^{25}_D$ +36.5° (*c* 0.03, $CHCl_3$). The $[M + Na]^+$ peak in the FABMS at *m*/*z* 647 suggested a molecular weight of 624, and the molecular formula of $C_{37}H_{68}O_7$ was confirmed by HR-FABMS. The UV spectral absorption at 210 nm and the IR spectral absorption at 1741 cm^{-1} again indicated the presence of an α,*β*-unsaturated *γ*-lactone group. The successive FABMS peaks at *m*/*z* 607, 589, 571, and 553 implied the presence of four hydroxyl groups. In the ^1H NMR spectrum, the signals at *δ* 7.17 (1H, H-35), 5.02 (1H, H-36), 3.80 (1H, H-4), 2.56 (1H, H-3a), 2.48 (1H, H-3b), and 1.42 (3H, H-37) confirmed the presence of an α,*β*-unsaturated *γ*-lactone with a hydroxyl group at the C-4 position (Table 1). The ^1H NMR signals at *δ* 3.80 (2H, H-18, 21) and 3.40 (2H, H-17, 22), as well as the ^{13}C NMR peaks at *δ* 82.7 (C-18, 21) and 74.3 (C-17, 22), indicated the presence of a mono-THF ring with two flanking hydroxyls both in a *threo* configuration.[8] The ^1H NMR spectrum showed proton resonances for the two methylene groups of the mono-THF ring, which appeared at *δ* 1.93 (2H, H-19a, 20a) and 1.76 (2H, H-19b, 20b) and corresponded to the *cis* configuration.[22]

The placement of the THF ring and the diol were established by the EIMS fragmentation of **3** (Figure 3). The THF ring was placed between C-18 and C-21 on the basis of the EIMS fragments at *m*/*z* 389 (C-21/C-22 cleavage − 2 H_2O), 337 (C-17/C-18 cleavage − H_2O), and 319 (C-17/C-18 cleavage − 2 H_2O), and the free hydroxyl was located at C-10 on the basis of the EIMS fragments at *m*/*z* 241 (C-11/C-10 cleavage) and 223 (C-11/C-10 cleavage − H_2O).

The absolute configuration at C-36 of **3** was again determined by a CD method.[21] A positive π–π^* Cotton effect ($\Delta\epsilon > 0$) indicated that the stereochemistry at C-36 on the *γ*-lactone fragment was in the *S* configuration. Comparison of the spectral data of compound **3** with those of the known compound, annomontacin,[15] suggested that the only difference between the two structures was the conformation of the THF ring. Thus, the structure of **3** was determined and the compound named *cis*-annomontacin.

Compound **4** was isolated as a white waxy solid, $[\alpha]^{25}_D$ +13.6° (*c* 0.1, MeOH). The $[M + H]^+$ and $[M + Na]^+$ peaks in the FABMS at *m*/*z* 579 and 601 established the molecular weight as 578. The molecular formula, $C_{35}H_{62}O_6$, was confirmed by HRFABMS. Compound **4** had a very close retention time to corossolone on reversed-phase HPLC, with retention times of 19.27 and 18.19 min, respectively,

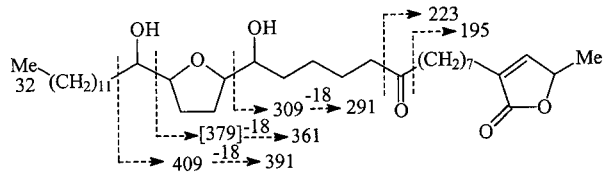


Figure 4. EIMS fragmentation (*m*/*z* values) of *cis*-corossolone (**4**).

Table 3. ^1H and ^{13}C NMR Chemical Shifts of Compounds **4** and **5**

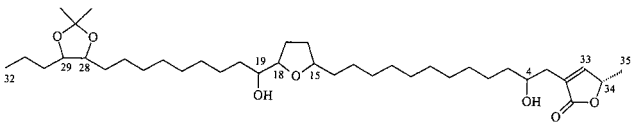
	cis-corossolone (**4**)		annocatalin (**5**)	
position	*δ* (^1H)[a]	*δ* (^{13}C)[b]	*δ* (^1H)[a]	*δ* (^{13}C)[b]
1		173.9		175.0
2		134.2		131.2
3	2.25	25.6	2.51 (m)	31.8
			2.39 (m)	
4	1.43−1.60	27.3	3.88	69.9
5	1.24−1.33	29.7−28.7	1.2−1.6	37.1
6−7	1.24−1.33	29.7−28.7	1.2−1.6	25.5−29.8
8	1.43−1.60	23.7	1.2−1.6	25.5−29.8
9	2.38 (m)	42.8[c]	1.2−1.6	25.5−29.8
10		211.4	1.2−1.6	25.5−29.8
11	2.38 (m)	42.7[c]	1.2−1.6	25.5−29.8
12	1.43−1.60	23.7	1.2−1.6	25.5−29.8
13	1.24−1.33	25.1	1.2−1.6	25.5−29.8
14	1.36−1.42	33.4	1.2−1.6	35.3
15	3.41 (m)	74.3	3.83	79.4
16	3.80 (m)	82.7	1.99, 1.62 (m)	25.5−29.8
17	1.74, 1.92 (m)	28.1	1.99, 1.62 (m)	25.5−29.8
18	1.74, 1.92 (m)	28.1	3.83 (m)	81.7
19	3.80 (m)	82.6	3.42 (m)	74.2[c]
20	3.41 (m)	74.4	1.2−1.6	33.4[d]
21	1.36−1.42	33.2	1.2−1.6	25.5−29.8
22	1.24−1.33	25.2	1.2−1.6	25.5−29.8
23−26	1.24−1.33	29.7−28.7	1.2−1.6	25.5−29.8
27	1.24−1.33	29.7−28.7	1.2−1.6	33.2[d]
28	1.24−1.33	29.7−28.7	3.44 (m)	74.5[c]
29	1.24−1.33	29.7−28.7	3.44 (m)	74.4[c]
30	1.24−1.33	31.8	1.2−1.6	31.8
31	1.24−1.33	22.7	1.2−1.6	22.5
32	0.87 (t, *J* = 6.8)	14.1	0.87 (t, *J* = 6.8)	13.9
33	6.98 (d, *J* = 1.6)	148.9	7.19 (d, *J* = 1.4)	152.2
34	4.99	77.4	5.05	77.4
	(qd, *J* = 6.8, 1.6)		(qd, *J* = 6.8, 1.4)	
35	1.40 (t, *J* = 6.8)	19.2	1.42 (d, *J* = 6.8)	18.9

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

with the solvent system MeOH−H_2O (88:12) (Hypersil C_{18} column, 250 × 4.6 mm i.d., flow rate of 1 mL/min). Analysis of the UV, IR, ^1H and ^{13}C NMR, and mass (see Figure 4) spectra of **4** in comparison to those of corosslone[19] suggested that **4** also possesses a carbonyl group and an α,*β*-unsaturated *γ*-lactone ring and is a mono-THF acetogenin with two flanking hydroxyls in a *threo*, *threo* configuration. The ^1H NMR peaks at *δ* 6.98 (1H, H-33), 4.99 (1H, H-34), and 1.40 (3H, H-35), as well as the ^{13}C NMR resonances at *δ* 173.9 (C-1), 148.9 (C-33), 134.2 (C-2), 77.4 (C-34), and 19.2 (C-35), matched well with the published data of annonaceous acetogenins with an α,*β*-unsaturated *γ*-lactone ring without a hydroxyl group at the C-4 position (see Table 3).[8]

The only differences between **4** and corossolone were signals from two methylene groups observed at *δ* 1.74 and 1.92 (H-17a, H-18a and H-17b, H-18b) in the ^1H NMR spectrum, which corresponded to the *cis*-THF ring configuration.[8,12,19] These stereochemical relationships were further substantiated as *threo*/*cis*/*threo* by the ^{13}C NMR signals of the oxygenated carbons of the THF subunit at *δ* 74.06, 74.34 (C-15, C-20) and 82.7, 82.6 (C-16, C-19), respectively. Moreover, in the same fraction of the leaves

Table 4. ^{1}H NMR Signals for the Protons of the *Threo* and *Erythro* Diols, Acetonide, Compounds **5**, and the Acetonide Derivative **5b**



5b

	methine protons		acetonyl methyls	
	threo	*erythro*	*threo*	*erythro*
diol	3.45 (2H)	3.62, 3.58		
acetonide	3.58 (2H)	4.02, 4.00	1.37 (6H)	1.43, 1.33
5	3.41 (2H)			
5b	3.59 (2H)		1.34 (6H)	

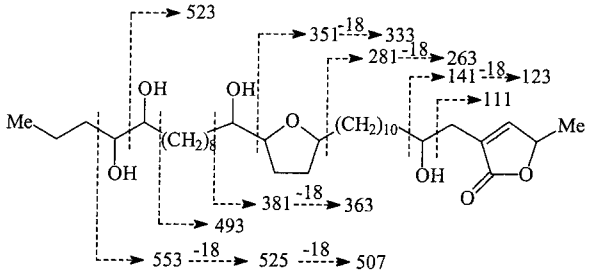


Figure 5. EIMS fragmentation (*m*/*z* values) of annocatalin (**5**).

of this species, we also isolated the known stereoisomeric compound, corossolone.[19] Thus, the structure of **4** was established and the compound has been named *cis*-corossolone.

Compound **5** was isolated as a white waxy solid, $[\alpha]^{25}_D$ +23.2° (*c* 0.05, MeOH). The $[M + H]^+$ and $[M + Na]^+$ peaks in the FABMS at *m*/*z* 597 and 619 established the molecular weight as 596. The molecular formula, $C_{35}H_{64}O_7$, was confirmed by HRFABMS. Successive FABMS fragments at *m*/*z* 579, 561, 543, and 525 suggested the presence of four hydroxyl groups, and the IR and UV spectra indicated the presence of an α,β-unsaturated γ-lactone group. Comparison with the NMR spectral data of muricin A suggested that **5** may possess similar functional moieties, including an α,β-unsaturated γ-lactone with a hydroxyl group at C-4, a mono-THF ring with one flanking hydroxyl group, and a vicinal diol (see Table 3).[12]

Two of the hydroxyl groups were suspected to constitute a vicinal diol unit due to the proton signal at δ 3.44 (2H) and the ^{13}C NMR peaks at δ 74.5 and 74.4. To confirm the presence of a vicinal diol, the acetonide derivative was made. When the original compound and its acetonide derivative were compared, the downfield shifts of two protons of carbinol carbons at δ 3.44 to 3.59 and the chemical shift of two equivalent methyls at δ 1.34 in the ^{1}H NMR spectrum verified the presence of a *threo* vicinal diol group (see Table 4).[12,24]

The placement of the THF ring and hydroxyl groups was established by examination of the EIMS fragmentation of **5** (Figure 5). The THF ring was located between C-15/C-18 on the basis of the EIMS peaks at *m*/*z* 351 and 281, and the vicinal diol was placed on C-28/C-29 according to the EIMS peaks at *m*/*z* 553 (C-29/C-30 cleavage), 523 (C-28/C-29 cleavage), and 493 (C-27/C-28 cleavage). The absolute configuration at C-34 of **5** was determined from the CD spectrum, in which a positive π–π* Cotton effect ($\Delta\epsilon$ >0) indicated the *S* configuration of C-34 on the γ-lactone fragment.[21] Thus, the structure of **5** was determined and the compound named annocatalin.

Table 5. In Vitro Cytotoxicity Data of Compounds **1**–**5**

	cell lines[a]/IC_{50} (μg/mL)	
compound	hep G_2	hep 2,2,15
1	9.51×10^{-2}	1.18×10^{-2}
2	5.09×10^{-2}	2.22×10^{-1}
3	2.98×10^{-1}	1.62×10^{-2}
4	1.65×10^{-1}	4.76×10^{-2}
5	5.70	3.48×10^{-3}
adriamycin	2.41×10^{-1}	4.50×10^{-1}

[a] Hep G_2 is a human hepatoma cell line, and Hep 2,2,15 is a Hep G_2 cell line transfected with HBV.

The identifications of the known annonaceous acetogenins obtained in the investigation were verified by comparing UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with published values of annonacin,[13] annonacinone,[14] annomontacin,[15] murisolin,[16] xylomaticin,[17] solamin,[18] and corossolone.[19]

In 3-day cytotoxicity bioassays, compounds **1**–**5** exhibited significant inhibitory activity against two cancer cell lines, Hep G_2 (human hepatoma cells) and Hep 2,2,15 (human hepatoma cells transfected with hepatitis B virus).[25,26] Adriamycin was taken as the positive control. The IC_{50} values are shown in Table 5. Among them, compound **5** demonstrated selective cytotoxicity toward Hep 2,2,15. In an SAR comparison, compound **5** was further compared with muricins A–C (see Supporting Information),[12] all of which possess a mono-THF ring with one flanking hydroxyl group that originates from the unusual C-15 or C-17 position, an α,β-unsaturated γ-lactone with a hydroxyl group at C-4, and a vicinal diol. All of the examples of this type of annonaceous acetogenin exhibited selectivity against Hep 2,2,15. The acetogenins are shown to act by suppression of ATP production.[9]

Experimental Section

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. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (all in $CDCl_3$) were recorded with Varian NMR 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. 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, and precoated Si gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm) were used for preparative 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-10AT apparatus equipped with a Shimadzu SPD-10A UV–vis detector. Hypersil ODS-5 (250 × 4.6 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant Material. The seeds and leaves of *A. muricata* L. were collected from Chia-Yi City, Taiwan, in March 1997. Voucher specimens (Annona-04) were deposited in the Graduate Institute of Natural Products, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Steps for extraction and chromatographic fractionation of the seeds were identical to those reported previously.[8] The MeOH extracts of the seeds (ca. 1 kg) were partitioned to yield $CHCl_3$ and aqueous extracts. The $CHCl_3$ layer afforded a waxy extract residue (ca. 200.6 g), which was further separated into 10 fractions by column

chromatography on Si gel with gradients of *n*-hexane−$CHCl_3$ and $CHCl_3$−MeOH. Fraction 7, eluted with $CHCl_3$−MeOH (95:5), was isolated and further purified by column chromatography and HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, MeOH−water, 95:5, flow rate 1 mL/min; UV detector set at 225 nm) to give muricin H (**1**) (100 mg, t_R 6.90 min), murisolin (8 mg, t_R 7.42 min), and muricin I (**2**) (10 mg, t_R 8.98 min) and by HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, MeOH−water, 93:7, flow rate 1 mL/min; UV detector set at 225 nm) to give annonacinone (200 mg, t_R 6.03 min). Fraction 9 was isolated and further purified by column chromatography 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 (45 mg, t_R 15.29 min) and *cis*-annomontacin (**3**) (26 mg, t_R 18.15 min) and by HPLC (Hypersil ODS-5 column, 250 × 4.6 mm, MeOH−water, 86:14, flow rate 1 mL/min; UV detector set at 225 nm) to give annonacin (1050 mg, t_R 16.92 min) and xylomaticin (15 mg, t_R 31.61 min).

The dried leaves of *A. muricata* (4.0 kg) were also extracted with MeOH at room temperature repeatedly. The combined MeOH extracts (ca. 400 g) were evaporated and partitioned to yield $CHCl_3$ (ca. 250 g) and aqueous extracts. The $CHCl_3$ layer was further separated into 12 fractions by column chromatography on Si gel with gradients of *n*-hexane−EtOAc (*n*-hexane−EtOAc, 4:1, to pure EtOAc) and EtOAc−MeOH (pure EtOAc to EtOAc−MeOH, 10:1). Fraction 8, eluted with EtOAc−MeOH (98:2), was isolated and further purified by column chromatography and preparative TLC development three times with $CHCl_3$−MeOH (100:1) to give solamin (10 mg, *n*-hexane−EtOAc, 4:1, R_f 0.5). Fraction 9, eluted with EtOAc−MeOH (95:5), was chromatographed on a Si gel column (70−230 mesh, 0−7% MeOH in $CHCl_3$) to give 10 subfractions. Subfraction 5, eluted with $CHCl_3$−MeOH (97:3), was separated and purified by reversed-phase HPLC (Hypersil ODS-5 column, 250−4.6 mm, MeOH−water, 90:10, flow rate 1 mL/min; UV detector set at 225 nm) to give murisolin (4 mg, t_R 7.76 min). The remainder of this subfraction was combined and repurified by reversed-phase 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 corossolone (6 mg, t_R 18.19 min) and *cis*-corossolone (**4**) (5 mg, t_R 19.27 min). Subfraction 6, eluted with $CHCl_3$−MeOH (96:4), was separated and purified by reversed-phase 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 annocatalin (**5**) (5 mg, t_R 41.77 min).

Muricin H (1): colorless waxy solid; $[\alpha]^{25}_D$ +9.5°(*c* 0.76, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (3.64) nm; IR (KBr) ν_{max} 3409 (OH), 2918, 2848, 1743 (OC═O), 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 581 $[M + H]^+$; EIMS (30 eV) data, see Figure 1; HRFABMS *m*/*z* 581.4785 (calcd for $C_{35}H_{65}O_7$, 581.4781).

(*R*)- and (*S*)-MTPA Derivatives of 1. Compound **1** (5 mg) was dissolved in 1 mL of dry CH_2Cl_2, 0.5 mL of pyridine, and 1 mg of 4-(dimethylamino)pyridine, and 100 mg of (*R*)-(−)-methoxyl-α-(trifluoromethyl)phenylacetyl chloride was introduced to the solution. After the reaction mixture was allowed to sit 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,[12] which was purified by preparative TLC to give the (*S*)-MTPA esters. The (*R*)-MTPA esters were prepared in the same way using (*S*)-(+)-methoxyl-α-(trifluoromethyl)phenylacetyl chloride reagent.[12]

Muricin I (2): white waxy solid; $[\alpha]^{25}_D$ +88.0° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (3.54) nm; IR (KBr) ν_{max} 3421 (OH), 2925, 2854, 1749 (OC═O), 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 607 $[M + H]^+$; EIMS (30 eV) data, see Figure 2; HRFABMS *m*/*z* 607.4934 (calcd for $C_{35}H_{65}O_7$, 607.4938).

***cis*-Annomontacin (3):** white waxy solid; $[\alpha]^{25}_D$ +36.5° (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.72) nm; IR (KBr) ν_{max} 3409 (OH), 2921, 2852, 1741 (OC═O), 1079 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 625 $[M + H]^+$; EIMS (30 eV) data, see Figure 3; HRFABMS *m*/*z* 625.5040 (calcd for $C_{35}H_{65}O_7$, 625.5043).

***cis*-Corossolone (4):** white waxy solid; $[\alpha]^{25}_D$ +13.6° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.61) nm; IR (KBr) ν_{max} 3500 (OH), 3018, 2928, 2855, 1749 (OC═O), 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 579 $[M + H]^+$; EIMS (30 eV) data, see Figure 4; HRFABMS *m*/*z* 601.4437 $[M + Na]^+$ (calcd for $C_{35}H_{64}O_7Na$, 601.4444).

Annocatalin (5): white waxy solid; $[\alpha]^{25}_D$ +23.2° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.64) nm; IR (KBr) ν_{max} 3406 (OH), 2924, 2849, 1756 (OC═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 597 $[M + H]^+$; EIMS (30 eV) data, see Figure 5; HRFABMS *m*/*z* 597.4739 (calcd for $C_{35}H_{64}O_7$, 597.4730).

Acetonide Derivative of Compound 5. Compound **5** (2 mg) and 0.1 mg of toluenesulfonic acid monohydrate were dissolved in 0.5 mL of acetone. The mixture was allowed to stand at room temperature for 5 h, and the conversion was monitored by TLC. The product was purified over small column of Si gel (in a small pipet) eluted with 20:1 hexane/acetone and was dried in vacuo to give the acetonide derivative **5b**.[12]

Bioassays. The 3-day bioassays against Hep G_2 and 2,2,15 were carried out according to procedures described in the literature.[25,26] A previous paper described the assay procedure in detail.[8]

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Supporting Information Available: Data for seven known compounds and biological list data for muricins A−C. This information is available free of charge via the Internet at http://pubs.acs.org.

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