Two Novel Acetogenins, Annoglaxin and 27-Hydroxybullatacin, from *Annona glabra*

Xiao-Xi Liu,[†] Elsa Pilarinou,[‡] and Jerry L. McLaughlin*[,†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, Walker Cancer Research Institute, 1634A Metropolitan Boulevard, Tallahassee, Florida 32308

Received December 4, 1998

Two new bioactive Annonaceous acetogenins, annoglaxin (**1**) and 27-hydroxybullatacin (**2**), have been isolated from the fractionated ethanolic extracts of the leaves of *Annona glabra*, directing the fractionation with the brine shrimp lethality test (BST). The structures of **1** and **2** were elucidated on the basis of spectroscopic and chemical methods, and the absolute stereochemistries were determined by the advanced Mosher ester method. **1** presents unusual features of an OH at C-8 and a carbonyl at C-12 and, while less potent than **2**, shows interesting selectivity for the human breast carcinoma (MCF-7) cell line. Compound **2** was at least 100 000 times more potent than adriamycin against the human kidney carcinoma (A-498), prostate carcinoma (PC-3), and pancreatic carcinoma (PACA-2) cell lines in our panel of six human solid tumor cell lines.

Annona glabra L. (Annonaceae), commonly known as pond-apple, is a tropical tree distributed mainly in the Americas and in Southeast Asia. It is used in traditional medicines as an insecticide and a parasiticide.[1,2] Our previous work on the leaves, using the brine shrimp lethality assay to guide fractionation,[3] led to six new acetogenins and one new cyclic isoprenylated phenylpropanoid.[4−7] Herein, through further bioactivity-guided fractionation work, we report the discovery of two new acetogenins, annoglaxin (**1**) and 27-hydroxybullatacin (**2**). The structures of **1** and **2** were identified as new mono- and bis-adjacent THF-ring acetogenins, respectively, by NMR and MS spectroscopic techniques and by preparing chemical derivatives. The absolute configurations of **1** and **2** were determined through analyses of their respective per-Mosher esters.[8,9] Compound **2** was at least 100 000 times more potent than adriamycin against the human kidney carcinoma (A-498), prostate carcinoma (PC-3), and pancreatic carcinoma (PACA-2) cell lines in our panel of six human solid tumor cell lines.

Results and Discussion

Compound **1** was isolated as a whitish powder. The CIMS showed an MH^+ peak at *m*/*z* 611 indicating that this molecule was only 35 carbons long. The suggested molecular formula of $C_{35}H_{62}O_8$ was confirmed by HRCIMS of the molecular ion which gave an exact mass of *m*/*z* 611.4516 (calcd 611.4523). Compound **1** showed an IR carbonyl absorption at 1755 cm^{-1}, four resonances at δ 6.99 (d, H-33), 5.00 (dq, H-34), 1.42 (d, H-35), and 2.27 (m, H-3) in the ^1H NMR spectrum and five peaks at δ 172.17 (C-1), 150.59 (C-33), 131.62 (C-2), 78.15 (C-34), and 18.76 (C-35) in the ^{13}C NMR spectrum (Table 1). These are characteristic spectral features for the γ-methyl α,β-unsaturated γ-lactone fragment, without the presence of an OH group at the C-4 position.[10−12]

The existence of four OH moieties in **1** was suggested by a prominent IR OH absorption at 3433 cm^{-1} and four successive losses of H_2O (*m*/*z* −18) from the $[MH^+]$ in the CIMS. The ^{13}C NMR of **1**, which showed four resonances due to oxygen-bearing carbons at δ 71.28 (C-8), 74.15 (C-15), 70.33 (C-20), and 68.85 (C-22), also indicated the existence of four secondary hydroxyls. The presence of a single THF ring, with two OH groups flanking the ring, was apparent from the presence of ^{13}C NMR resonances at δ 74.15 (C-15) and 70.33 (C-20) due to hydroxylated carbons and δ 82.21 (C-16) and 82.57 (C-19) due to oxygenated carbons of the THF-ring. These signals and their corresponding ^1H NMR resonances at δ 3.40 (H-15) and δ 3.80 (H-16, H-19, and H-20) are also indicative of dihydroxyl-flanked THF moieties.

The placements of the hydroxyl groups and the THF ring system along the aliphatic chain were determined on the basis of analysis of the EIMS spectral data (Figure 2), where the diagnostic fragments at *m*/*z* 325 and 395 were

Table 1. ^1H NMR and ^{13}C NMR Data for **1**

position	^1H NMR (500 MHz, *J* in Hz)	^{13}C NMR (125 MHz)
1	−	172.17
2	−	131.62
3	2.27 m	22.54−33.00
4−6	1.27−1.70 m	22.54−33.00
7	1.40 m	22.54−33.00
8	3.60 m	71.28
9	1.40 m	22.54−33.00
10	1.27−1.70 m	22.54−33.00
11	2.40 m	42.47[a]
12	−	209.89
13	2.40 m	42.71[a]
14	1.40 m	22.54−33.00
15	3.40 m	74.15
16	3.80 m	82.21
17−18	1.98m, 1.69 m	22.54−33.00
19	3.80 m	82.57
20	3.80 m	70.33
21	1.58 m	37.09
22	3.80 m	68.85
23−30	1.27−1.70 m	22.54−33.00
31	1.30 m	22.54−33.00
32	0.88 t (7.0)	13.93
33	6.99 d (1.5)	150.59
34	5.00 dq (1.5, 7.0)	78.15
35	1.42 d (1.5)	18.76

[a] Signals may be interchangeable.

* To whom correspondence should be addressed. Tel: (765) 494-1455. Fax: (765) 494-1414. E-mail: jac@pharmacy.purdue.edu.
[†] Purdue University.
[‡] Walker Cancer Research Institute.

10.1021/np980552j CCC: $18.00
Published on Web 04/30/1999

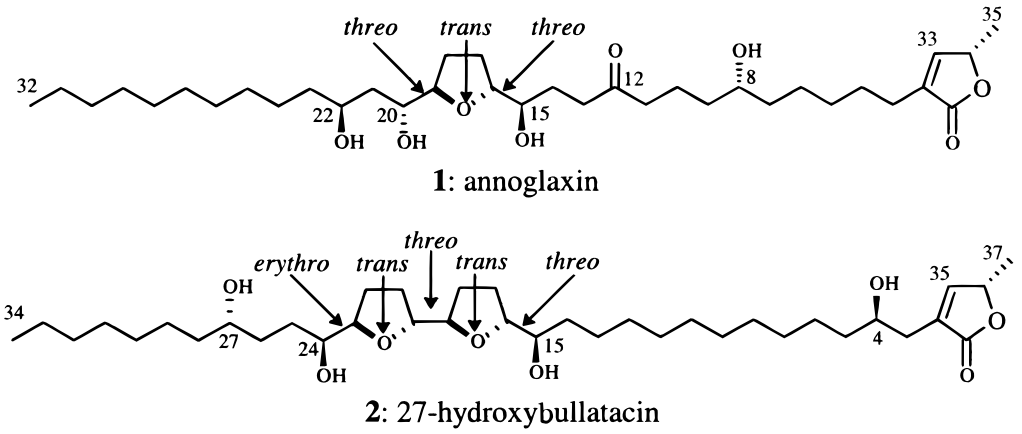

Figure 1. Structures of **1** and **2**.

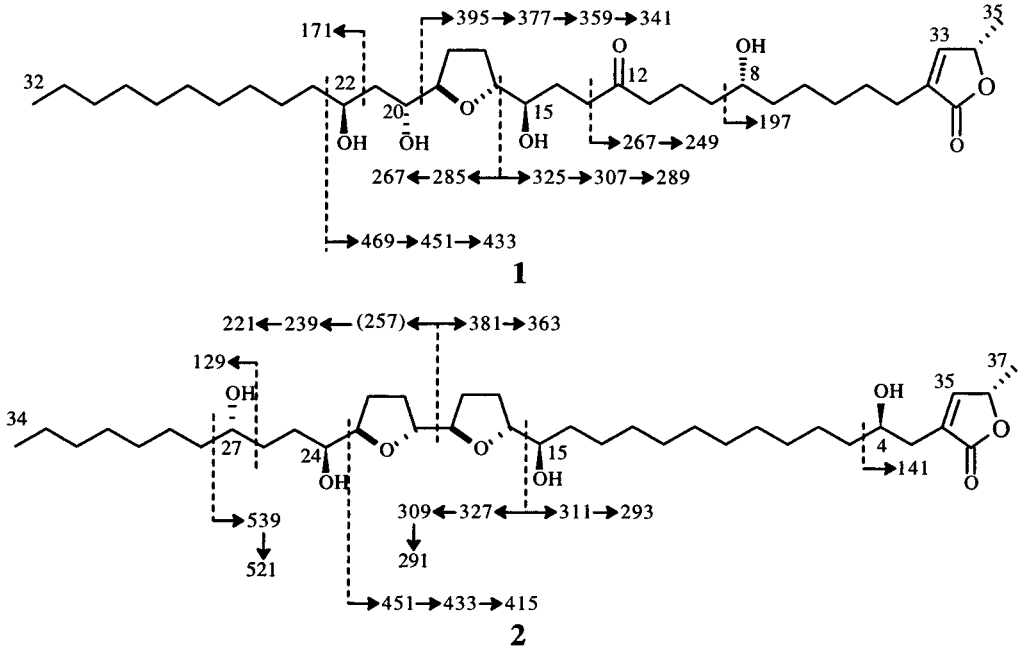

Figure 2. Diagnostic mass fragmentation ions of **1** and **2**. Losses of H_2O indicated by −18 *m/z*. Peak in parentheses was not observed or was very weak.

characteristic of the hydroxylated THF ring system located from C-15 to C-20. The hydroxyl group at C-22 was also suggested by EIMS spectral analysis.

Using the method of Born et al., we observed that the ^{13}C NMR chemical shift of **1** for C-20 at δ 70.33 suggested the erythro relationship between C-19 and C-20.[13] However, when the γ-gauche effect is considered, anticipated due to the presence of the hydroxyl group at C-22, the ^{13}C NMR chemical shift of C-20 should be shifted upfield. Indeed, the ^{13}C NMR shift of **1** for C-22 was shifted upfield to δ 68.85 and supported the presence of the γ-gauche effect. Therefore, the threo relationship between C-19 and C-20 was assigned. This assignment is also supported by the X-ray crystallographic data of a compound of similar structure, uvarigrin. [14] Other ^{1}H signals at δ 3.40 (H-15) and 1.98, 1.69 (H-17/18) suggested a relative threo/trans/threo stereochemistry for the mono-THF ring system.

The IR spectrum of **1** showed an additional strong carbonyl absorption at 1702 cm^{-1}, suggesting the presence of a ketone group. This was confirmed by the ^{1}H NMR signal at δ 2.40 (H-11/13) and a ^{13}C NMR carbonyl signal at δ 209.89 (C-12). Similarly, the position of the carbonyl was placed at C-12 by an EIMS fragment peak at *m/z* 267 and verified by HRCIMS spectral analysis. The HRCIMS gave an ion at *m/z* 268.1675 (calcd 268.1670) for a fragment with the formula $C_{15}H_{23}O_4$ which confirmed the position of the carbonyl at C-12. Both of these functionalities, i.e., a carbonyl at C-12 and an OH at C-8, are unusual.

Although the relative threo/trans/threo configuration was assigned for **1** based on NMR signals, the (*S*)- and (*R*)-α-methoxy α-(trifluoromethyl)phenylacetic acid (MPTA) esters (Mosher esters) of **1** were prepared. Careful examination of the ^{1}H NMR and ^{1}H−^{1}H COSY analysis of these two derivatives was used to establish the absolute configuration. For the carbinol center at C-8, although direct observation of the magnitude of chemical shift changes of H-7 and H-9 was impossible due to the overlapping of their proton signals in the COSY spectra of both MTPA esters, the stereochemistry at C-8 was assigned as *R* on the basis of the small but consistent differences between the chemical shifts of the protons at H-3, H-4, and H-5 of the (*S*)- and (*R*)-Mosher esters. Thus, the absolute stereochemistry was assigned as C-8 *R*, C-15 *R*, C-20 *R*, C-22 *S* (Table 3). The pseudo-threo relationship between the hydroxyl groups at C-20 and C-22 was also evidenced by its ^{13}C NMR peak at δ 68.85. If they were in a pseudo-erythro relationship, the ^{13}C NMR shift of C-22 would be expected to lie between δ 72−73. [10,15] The configuration at C-34 was assigned as *S*

Table 2. ^{1}H NMR and ^{13}C NMR Data for **2**

position	^{1}H NMR (500 MHz, *J* in Hz)	^{13}C NMR (125 MHz)
1	−	174.67
2	−	131.02
3a	2.41 dddd (15.0, 8.0, 1.5, 1.5)	33.38
3b	2.53 dddd (15.0, 3.5, 1.5, 1.5)	33.38
4	3.84 m	69.84
5	1.27 1.70 m	37.33
6−14	1.27−1.70 m	22.62−32.77
15	3.40 m	74.21
16	3.80−3.97 m	82.90
17, 18	1.98 m, 1.65 m	22.62−32.77
19	3.80−3.97 m	82.77
20	3.80−3.97 m	82.55
21, 22	1.98 m, 1.65 m	22.62−32.77
23	3.80−3.97 m	82.23
24	3.80−3.97 m	71.34
25	1.27−1.70 m	22.62−32.77
26	1.40 m	22.62−32.77
27	3.60 m	71.57
28	1.40 m	22.62−32.77
29−32	1.27−1.70 m	22.62−32.77
33	1.33 m	22.62−32.77
34	0.88 t (7.0)	14.07
35	7.18 d (1.5)	151.90
36	5.06 dq (7.0, 1.5)	78.00
37	1.42 d(1.5)	19.02

since all acetogenins that have been found so far have this configuration (or 36*S* in the 37-carbon acetogenins).[10−12]

27-Hydroxybullatacin (**2**) was isolated in the form of a whitish waxy solid. The molecular weight was determined to be 638 by the mass peak at *m*/*z* 639 [MH$^+$] in the low-resolution CIMS. The suggested molecular formula of $C_{37}H_{66}O_8$ was proven by the exact mass analysis of the [MH]$^+$ peak in the HRCIMS at *m*/*z* 639.4829 (calcd 639.4836). As with other common acetogenins, five resonances at δ 7.18 (d, H-35), 5.06 (dq, H-36), 1.42 (d, H-37), 3.84 (m, H-4), and 2.41 and 2.53 (dddd, H-3) in the ^{1}H NMR spectrum and further evidence in the ^{13}C NMR spectrum, which showed six peaks at δ 174.67 (C-1), 151.90 (C-35), 131.02 (C-2), 78.00 (C-36), 69.84 (C-4), and 19.02 (C-37) (Table 2), suggested the existence of the γ-methyl α,β-unsaturated γ-lactone fragment, with the presence of an OH group at C-4.[10−12]

The presence of four hydroxyl groups was concluded from the strong IR hydroxyl absorption band at 3450 cm^{-1} and the series of peaks at *m*/*z* 621, 603, 585, and 567, arising from the successive losses of four molecules of H_2O (*m*/*z* −18) in the CIMS. This was also confirmed by the presence of four hydroxylated carbon peaks at δ 69.84 (C-4), 74.21 (C-15), 71.34 (C-24), and 71.57 (C-27) in the ^{13}C NMR spectra.

The presence of an adjacent bis-THF ring with one flanking hydroxyl group at each side and two isolated hydroxyl groups including the hydroxyl group at C-4 was also suggested by proton resonances at δ 3.40 (H-15), 3.60 (H-27), 3.80 (H-4, 16, 19, 20, 23, and 24), and the carbon peaks at δ 69.84 (C-4), 74.21 (C-15), 71.34 (C-24), 71.57 (C-27), 82.90 (C-16), 82.77 (C-19), 82.55 (C-20), and 82.23 (C-23). Careful comparison of ^{1}H and ^{13}C NMR spectral data of **2** (Table 2) with those of previously reported acetogenins indicated that the structure of **2** was very similar to that of bullatacin. [16] Subsequently, the relative stereochemistry in the adjacent bis-THF moiety of **2** was suggested to be threo/trans/threo/trans/erythro from C-15 to C-24, by comparison of ^{1}H NMR data of relevant protons of **2** with those of model compounds of known relative configurations.[16]

The locations of the hydroxyl groups and the adjacent bis-THF ring unit along the aliphatic chain were determined again by EIMS spectral analysis. The EIMS displayed peaks at *m*/*z* 311 and 381 which suggested the placement of the adjacent bis-THF ring system and its two flanking hydroxyls from C-15 to C-24. That the fourth hydroxyl was situated at C-27 was concluded from the presence of fragment ions at *m*/*z* 539→521 in the EIMS spectrum (Figure 2).

Again, the absolute stereochemistry of **2** was established by analysis and comparison of the ^{1}H NMR spectra of the per-(*S*)-MTPA and per-(*R*)-MTPA derivatives (Table 3). Analysis of the differences between the (*S*)- and (*R*)-Mosher derivatives, combined with the prior knowledge of relative stereochemistry, allowed the absolute stereochemical assignments of the carbinol centers as C-4 *R*, C-15 *R*, C-24 *S*, C-27 *S* (Table 3). As with **1**, C-36 was assigned *S* as in all of the natural acetogenins.

The biological activities of **1** and **2** are summarized in Table 4. As other mono-THF ring acetogenins, compound **1** is equipotent with or less potent than adriamycin. However, compound **2** is much more potent and more selective than adriamycin across the six human tumor cell lines in our seven-day MTT human solid tumor cytotoxicity tests. **2** showed activity with potency comparable with that of adriamycin against the lung carcinoma (A-549),[17] breast carcinoma (MCF-7),[18] and colon adenocarcinoma (HT-29) cell lines.[19] Selectivities were exhibited for the kidney carcinoma (A-498),[17] prostate carcinoma (PC-3),[20] and pancreatic carcinoma (PACA-2) cell lines,[21] with at least 100 000 times the potency of the positive control, adriamycin.

All of the acetogenins tested, so far, exert their cytotoxic and their in vivo antitumor effect, at least in part, by inhibiting NADH ubiquinone oxidoreductase (complex I) and by blocking the NADH oxidase peculiar to the plasma membranes of cancerous cells.[22−28] These effects deplete ATP levels and cause programmed cell death (apoptosis).[29] Recently, the acetogenins were found to inhibit cells that are multiple drug resistant due to ATP dependent efflux purges.[30−32] The acetogenins, by virtue of their ATP-depleting modes of action, are more toxic to these cells than they are to the wild-type cells which do not possess the MDR pump and show more of a cytostatic response to the acetogenins. They also inhibit pesticide-resistant German cockroaches.[33] Thus, the acetogenins offer an excellent potential for development as new antitumor and pesticidal agents which thwart such resistance mechanisms.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra (film) were recorded on a Perkin-Elmer 1600 FTIR spectrometer. UV spectra were taken in MeOH on a Beckman DU-7 UV spectrophotometer. Low-resolution EIMS and CIMS data were collected on a Finnigan 4000 spectrometer. High-resolution EIMS and CIMS data were collected on a Kratos MS50 spectrometer through peak matching. ^{1}H NMR, ^{1}H−^{1}H COSY, and ^{13}C NMR spectra were obtained on a Varian VXR-500S (^{1}H at 500 MHz and ^{13}C at 125 MHz) or Bruker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometers with $CDCl_3$ as solvent and TMS as the internal reference. HPLC separations were performed with a Rainin Dynamax UV-1 detector coupled with a Rainin model HPXL solvent delivery system for normal phase and a Dynamax model DSD-200 solvent delivery system for reversed phase.

Bioassays. The brine shrimp (*Artemia salina* Leach) test (BST)[3] was routinely employed for evaluating the extracts, fractions, and isolated compounds from the title plant. In vitro cytotoxicities against human tumor cell lines were carried out at the Purdue Cancer Center, Cell Culture Laboratory, using

Table 3. ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1** and **2**

proton	*S*-MTPA (**1**)	*R*-MTPA (**1**)	$\Delta\delta_{S-R}$	config	proton	*S*-MTPA (**2**)	*R*-MTPA (**2**)	$\Delta\delta_{S-R}$	config
H-4	1.55	1.63	neg.	8 *R*	H-13	1.31	1.13	pos.	15 *R*
H-5	1.25	1.33	neg.		H-14	1.64	1.48	pos.	
H-14	1.55	1.47	pos.		H-16	4.05	3.96	pos.	
H-16	3.93	3.99	pos.	15 *R*	H-23	3.99	3.87	pos.	24 *S*
H-17/18	1.87	1.64	pos.		H-25	1.54	1.56	neg.	
H-19	3.87	3.94	neg.		H-26	1.58	1.50	pos.	27 *S*
H-21	1.82	1.65	pos.	20 *R*	H-28	1.49	1.55	neg.	
H-23	1.45	1.53	neg.	22 *S*					

Table 4. Bioactivities of **1** and **2**

compounds[a]	**1**	**2**	adriamycin[i]
BST[b] LC_{50} (μg/mL)	2.9×10^{-1}	4.3×10^{-2}	NT
A-549[c]	1.1×10^{0}	2.1×10^{-3}	1.3×10^{-3}
MCF-7[d]	4.3×10^{-4}	7.8×10^{-4}	9.0×10^{-2}
HT-29[e]	1.8×10^{-1}	1.8×10^{-5}	1.0×10^{-2}
A-498[f]	1.8×10^{0}	3.1×10^{-8}	1.9×10^{-3}
PC-3[g]	8.7×10^{-1}	$<10^{-9}$	2.0×10^{-2}
PACA-2[h]	1.6×10^{0}	$<10^{-9}$	4.3×10^{-3}

[a] Human tumor cell lines ED_{50} (μg/mL). [b] Brine shrimp lethality test.[3] [c] Human lung carcinoma.[17] [d] Human breast carcinoma.[18] [e] Human colon adenocarcinoma.[19] [f] Human kidney carcinoma.[17] [g] Human prostate adenocarcinoma.[20] [h] Human pancreatic carcinoma.[21] [i] Positive control standard.

standard 7-day MTT assays for A-549 (human lung carcinoma),[17] MCF-7 (human breast carcinoma),[18] HT-29 (human colon adenocarcinoma),[19] A-498 (human kidney carcinoma),[17] PC-3 (human prostate adenocarcinoma),[20] and PACA-2 (human pancreatic carcinoma). [21] Adriamycin was always used as a positive cytotoxic control in the same runs.

Plant Material. The leaves of *A. glabra* L. were collected near Tallahassee, FL, in May, 1996. The material was collected and the species identified by one of us (Dr. Elsa Pilarinou). [34] A voucher specimen is deposited in the Pharmacognosy Herbarium at Purdue University.

Extraction and Isolation. The air-dried leaves (1360 g) were ground and percolated with 95% ethanol. The extract residue (122 g) (F001) was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer (F002) and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer (54 g) (F003) was partitioned between 90% MeOH and hexane, giving a MeOH layer (31 g) (F005) and a hexane layer (17 g) (F006). The MeOH layer (F005) was the most active fraction in the BST (LC_{50} 0.15 μg/mL). Thus, F005 was repeatedly chromatographed through open silica gel columns, using gradients of CH_2Cl_2−MeOH (directed by the BST test), and the active fractions were purified by repeated normal phase HPLC (Dynamax-60 A 8 μm silica gel, 250 × 21.4 mm i.d. or 250 × 4.6 mm i.d.) eluted with hexane (90%)/(MeOH/THF (9:1), 10%), and by reverse-phase HPLC on a C-18 column (250 × 21.4 mm i.d.) eluted by an CH_3CN-H_2O (7:3) isocratic solvent system.

Preparation of Mosher Ester Derivatives. To an acetogenin solution (1 mg, in 0.5 mL of CH_2Cl_2) were sequentially added pyridine (0.2 mL), 4-(dimethylamino)pyridine (0.5 mg), and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The reaction mixture was stirred from 4 h to overnight, checked by TLC to make sure that the reaction was complete, and passed through a disposable pipet (0.6 × 6 cm) containing silica gel (60−200 mesh) and eluted with CH_2Cl_2 (3 mL). The CH_2Cl_2 residue, after washing with 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL), was dried in vacuo to give the (*S*)-Mosher esters. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride yielded the (*R*)-Mosher ester of the acetogenin.

Annoglaxin (1): whitish waxy powder (3.5 mg); $[\alpha]_D^{25}$ +14.7° ($CHCl_3$); IR cm^{-1} (film on NaCl plate) 3433, 2920, 1755, 1702, 1657, 1075; CIMS (isobutane) *m/z* (%) $[MH]^+$ 611 (21), $[MH - H_2O]^+$ 593 (100), $[MH - 2H_2O]^+$ 575 (72), $[MH - 3H_2O]^+$ 557 (13), $[MH - 4H_2O]^+$ 539 (2); HRCIMS (isobutane) *m/z* 611.4516 for $C_{35}H_{63}O_8$ $[MH]^+$ (calcd 611.4523); EIMS (Figure 2) *m/z* (%) 469 (4), 451 (12), 433 (9), 395 (31), 377 (23), 359 (14), 341 (8), 325 (41), 307 (25), 289 (19), 285 (15), 267 (36), 249 (13), 197 (10); HREIMS *m/z* 268.1675 for $C_{15}H_{24}O_4$ (calcd 268.1670); ^{1}H and ^{13}C NMR, see Table 1.

27-Hydroxybullatacin (2): whitish waxy solid (8.3 mg); $[\alpha]_D^{25}$ +11.0° ($CHCl_3$); IR cm^{-1} (film on NaCl plate) 3450, 2925, 2851, 1745, 1668, 1072; CIMS(isobutane) *m/z* (%) $[MH]^+$ 639 (100), $[MH - H_2O]^+$ 621 (21), $[MH - 2H_2O]^+$ 603 (15), $[MH - 3H_2O]^+$ 585 (7); HRCIMS (isobutane) *m/z* 639.4829 for $C_{37}H_{69}O_7$ $[MH]^+$ (calcd 639.4836); EIMS (Figure 2) *m/z* (%) 539 (3), 521 (7), 451 (5), 433 (11), 415 (13), 381 (38), 363 (32), 327 (9), 311 (31), 309 (23), 293 (50), 291 (15), 239 (13), 221 (5), 141 (5), 129 (12); ^{1}H and ^{13}C NMR, see Table 2.

Acknowledgment. This investigation was supported initially by RO1 Grant No. CA 30909 from the National Cancer Institute, NIH. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing. X.-X.L. acknowledges fellowship support from the Purdue Research Foundation.

References and Notes

(1) Ohsawa, K.; Atsuzawa, S.; Mitsui, T.; Yamamoto, I. *J. Pesticide Sci.* **1991**, *16*, 93−96.
(2) Padmaja, V.; Thankamany, K.; Hara, N.; Fujimoto, Y.; Hisham, A. *J. Ethnopharmacol.* **1995**, *48*, 21−24.
(3) McLaughlin, J. L. *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1−33.
(4) Liu, X.-X.; Alali, F. Q.; Craig, D. C.; Rogers, L.; Pilarinou, E.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1998**, *6*, 959−966.
(5) Liu, X.-X.; Alali, F. Q.; Pilarinou, E.; McLaughlin, J. L. *J. Nat. Prod.* **1998**, *61*, 620−624.
(6) Liu, X.-X.; Alali, F. Q.; Pilarinou, E.; McLaughlin, J. L. *Phytochemistry* **1999**, *50*, 815−821.
(7) Liu, X.-X.; Pilarinou, E.; McLaughlin, J. L. *Tetrahedron Lett.* **1999**, *40*, 399−402.
(8) Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, A.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203−10213.
(9) Rieser, M. J.; Fang, X. P.; Anderson, J. E.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1993**, *76*, 2433−2444.
(10) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Cu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306.
(11) Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. *Progress in The Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Morre, R. E., Steglich, W., Tamn, Ch., Eds.; Springer-Verlag: New York, 1997; pp 81−287.
(12) Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; pp 29, 249−310.
(13) Born, L.; Lieb, F.; Lorentzen, J. P.; Moeschler, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312−316.
(14) Pan, X. P.; Yu, D. Q. *Acta Pharm. Sinica* **1997**, *32*, 286−293.
(15) Wu, F.-E.; Zeng, L.; Gu, Z.-M.; Zhao, G.-X.; Zhang, Y.; Schwedler, J. T.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, *58*, 909−915.
(16) Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163−1174.
(17) Giard, D. L.; Aaronson, S. A.; Todaro, G. L.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417−1423.
(18) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409−1416.
(19) Fogh, J.; Trempe, G. *Human Tumor Cells*; Fogh, J., Ed.; Plenum Press: New York, 1975; pp 115−119.
(20) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16−23.
(21) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128−135.
(22) Landolt, J. L.; Ahammadsahib, K. I.; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G. P.; McLaughlin, J. L. *Chem.-Biol. Interact.* **1995**, *98*, 1−13.
(23) Alfonso, D.; Johnson, H. A.; Colman-Saizarbitoria, T.; Preslay, C. P.; McCabe, G. P.; McLaughlin, J. L. *Nat. Toxins* **1996**, *4*, 181−188.

(24) Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427−438.
(25) Ahammadsahib, K. I.; Hollingworth, R. M.; McGroven, J. P.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113−1120.
(26) Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K.; McLaughlin, J. L. *Pesticide Biochem. Physiol.* **1993**, *45*, 15−23.
(27) Degli Esposti, M.; Ghelli, A.; Ratta, M.; Cortes, D.; Estornell, E. *Biochem. J.* **1994**, *301*, 161−167.
(28) Morré, J. D.; Decabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343−348.
(29) Wolvetang, E. J.; Johnson, K. L.; Krauer, K.; Ralph, S. J.; Linnane, A. W. *FEBS Lett.* **1994**, *339*, 40−44.
(30) Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; McLaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55−62.
(31) Oberlies, N. H.; Croy, V. L.; Chang, J-.C.; McLaughlin, J. L. *Cancer Lett.* **1997**, *115*, 73−79.
(32) Oberlies, N. H.; Chang, C.-J.; McLaughlin, J. L. *J. Med. Chem.* **1997**, *40*, 2102−2106.
(33) Alali, F.; Kaakeh, W.; Bennett, G. W.; McLaughlin, J. L. *J. Econ. Entomol.* **1998**, *91*, 641−649.
(34) Flora of North America Editorial Committee. *Flora of North America*; Oxford University Press: New York, 1993; Vol. 3, pp 11−20.

NP980552J