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Annomocherin, Annonacin and Annomontacin: A Novel and Two Known Bioactive Mono-Tetrahydrofuran Annonaceous Acetogenins from *Annona cherimolia* Seeds

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A novel and two known bioactive mono-tetrahydrofuran (THF) annonaceous acetogenins, annomocherin (**1**), annonacin (**2**) and annomontacin (**3**), have been isolated from the fractionated ethanolic extracts of the seeds of *Annona cherimolia*, guided by the brine shrimp lethality test (BST). Their structures were elucidated on the basis of spectroscopic and chemical methods. All compounds have a relative stereochemistry of *threo/trans/threo* for the mono-THF ring with two flanking hydroxyls. Compound **1** has a double bond at C-23/24 of aliphatic chain. Compound **1** was isolated from natural sources for the first time, and was named annomocherin. Two known Compounds **2** and **3** which have never been isolated from this species before, were obtained. Compound **1** exhibited potent and selective cytotoxicities against the breast carcinoma (MCF-7) and kidney carcinoma (A-498) cell lines with 100 to 1,000 times the potency of adriamycin. In brine shrimp lethality test (BST), **1-3** exhibited cytotoxicity.

Key words: Annonaceous acetogenins, *Annona cherimolia*, Annomocherin, Annonacin, Annomontacin, Cytotoxicities

INTRODUCTION

Annonaceous acetogenins are a relatively new class of natural polyketides which have promising anticancer, antiinfective, and pesticidal properties (Morre *et al.*, 1995; Zeng *et al.*, 1996). *Annona cherimolia* Mill. (Annonaceae) is the only species cultivated in Europe and is grown principally in the south of Spain, between Almuñecar and La Herradura (Granada coast) (Fies, 1959). *Annona cherimolia* is used in traditional medicine as an insecticide and parasiticide. In our previous studies of *Annona cherimolia* seeds, we isolated five new mono-THF acetogenins (annocherine, (2,4)-*cis*- and *trans*-annocherinones (Woo *et al.*, 1999), annomolin, and annocherimolin (Kim *et al.*, 2001) and five known acetogenins (*cis*-annonacin, (2,4)-*cis*- and *trans*-isoannonacins (Woo *et al.*, 1999), corrosolin, and compound-2 (Kim *et al.*, 1999)). Our continuing search for other members of the same class in the seeds of the *Annona cherimolia* has led to the isolation of three acetogenins, one of which is a novel mono-THF acetogenin and was named annomocherin (**1**). The other two are the already known annonacin (**2**) and annomontacin (**3**). Their occurrence in *Annona cherimolia* is reported here for the first time.

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MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a JASCO FT/IR-300E spectrophotometer. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. NMR spectroscopy was taken on a Varian VXR 500S spectrophotometer in $CDCl_3$ using TMS as an internal standard. Mass spectra was recorded on a Quattro spectrometer. For TLC, silica gel 60 F_{254} (EM 5717) glass plates (0.25 mm) were

used and visualized by spraying with 5% phosphomolybdic acid in MeOH and subsequent heating. HPLC was performed on a Waters 600 apparatus equipped with a Waters 486 UV detector at 225 nm using the Autochrowin software system (Waters Korea, Seoul, Korea). A μ Bondapak C_{18} columns (19 × 300 mm and 7.8 × 300 mm) were used for preparative purpose. For preparation of tetra-TMSi derivative, *N,O-bis*-(trimethylsilyl)acetamide (BSA) and pyridine in silylation grade were purchased from the Aldrich Company.

Plant material

The seeds of *Annona cherimolia* were obtained in 1996 from fruits grown commercially in plantations in southern California and purchased from Hurov Botanicals and Seeds located in Chula Vista, California.

Extraction and isolation

The dried seeds of *Annona cherimolia* (8 kg) were repeatedly percolated with 95% EtOH to yield 700 g of an extract (F001), on removal of solvent. F001 was partitioned between CH_2Cl_2-H_2O (1:1) to yield the H_2O-soluble fraction (F002, 300 g) and the CH_2Cl_2-soluble fraction (F003, 400 g). F003 was then partitioned between 90% aqueous MeOH-hexane (1:1) to yield a hexane-soluble fraction (F006, 150 g) and an aqueous MeOH-soluble fraction (F005, 250 g). All fractions were subjected to the brine shrimp lethality (BST), with the most active fraction being F005 (BST $LC_{50}=1.13\times10^{-2}$ μg/mL). 250 g of F005 was subjected to open column chromatography over Si gel (2.8 kg) eluted with a gradient of hexane/$CHCl_3$/MeOH. Fractions (F_1-1 to F_1-18) were collected and pooled according to their similar TLC patterns. The BST active pool, F_1-12 (130 g), was further resolved on another Si gel (1.5 kg) open column, eluted with a gradient of hexane/$CHCl_3$/MeOH. Fractions were collected into 13 pools (F_2-1 to F_2-13) on the basis of similar TLC patterns. Further purification of the most bioactive BST fractions (F_2-6, BST $LC_{50}=2.00\times10^{-4}$ μg/mL) were carried out by HPLC eluted with 85% acetonitrile in water to afford compounds **1** (15 mg), **2** (34 mg) and **3** (20 mg).

Annomocherin (1)

white amorphous powder, mp 74.6-75.3°C, $[\alpha]_D^{25}$: +9.2° (*c* 0.02, CH_2Cl_2), UV λ_{max} nm (log ε): 220 (3.7), IR ν (cm^{-1}): 3467 (OH), 1734 (C=O of α, β-unsaturated γ-lactone), ^{1}H NMR see Table I.

Table I. ^{1}H and ^{13}C NMR spectral data of **1**, **2**, and **3** ($CDCl_3$, δ)

No.	^{1}H NMR (300 MHz)			^{13}C NMR (75.5 MHz)		
	1	**2**	**3**	**1**	**2**	**3**
1	-	-	-	174.68	174.62	174.581
2	-	-	-	131.16	130.16	131.18
3a	2.40 dd (15.0, 8.2)	2.39 dd (15.0, 8.2)	2.39 dd (15.0, 8.2)	33.34	33.16	33.48
3b	2.52 dt (15.0, 1.5)	2.52 dt (15.0, 1.5)	2.53 dt (15.0, 1.5)			
4	3.84 m	3.80 m	3.80 m	69.93	69.66	69.91
5-9	1.26-1.57 m	1.25-1.59 m	1.25-1.59 m	22.69-37.35	22.57-37.24	22.67-37.43
10	3.59 m	3.59 m	3.58 m	71.77	71.45	71.86
11-14	1.26-1.57 m	1.25-1.59 m	1.25-1.59 m	22.69-37.35	22.57-37.24	22.67-37.43
15	3.43 m	3.40 m	1.25-1.59 m	74.02	73.98	22.67-37.43
16	3.80 m	3.80 m	1.25-1.59 m	82.66	82.64	22.67-37.43
17a	1.67 m	1.67 m	3.40 m	28.76	28.73	74.02
17b	1.98 m	1.98 m				
18a	1.67 m	1.67 m	3.80 m	28.79	28.73	82.62
18b	1.98 m	1.98 m				
19a	3.80 m	3.80 m	1.67 m	82.63	82.64	28.74
19b			1.98 m			
20a	3.43 m	3.40 m	1.67 m	73.58	73.98	28.74
20b			1.98 m			
21	1.48 m	1.25-1.59 m	3.80 m	33.38	22.57-37.24	82.62
22	2.20 m	1.25-1.59 m		23.33	22.57-37.24	74.02
23	5.37 m	1.25-1.59 m	3.40 m	128.95	22.57-37.24	23.14-37.85
24	5.37 m	1.25-1.59 m	1.25-1.59 m	130.85	22.57-37.24	23.14-37.85
25	2.04 m	1.25-1.59 m	1.25-1.59 m	27.25	22.57-37.24	23.14-37.85
26-31	1.26-1.57 m	1.25-1.59 m	1.25-1.59 m	22.69-37.35	22.57-37.24	23.14-37.85
32	0.88 t (7.0)	0.88 t (7.0)	1.25-1.59 m	14.12	14.02	23.14-37.85
33	7.19 q (1.5)	7.18 q (1.5)	1.25-1.59 m	151.91	151.85	23.14-37.85
34	5.06 dq (7.0,1.5)	5.06 dq (7.0, 1.5)	0.88 t (7.0)	78.03	77.94	14.10
35	1.43 d (6.5)	1.43 d (7.0)	7.18 q (1.5)	19.11	18.99	151.81
36			5.06 dq (7.0, 1.5)			77.97
37			1.43 d (7.0)			19.10

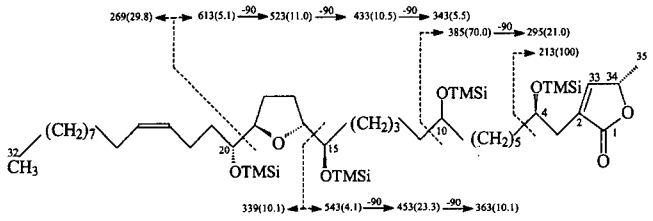


Fig. 1. Diagnostic EIMS fragmentations of tetra-TMSi derivative of **1** (intensities are indicated in parentheses).

Tetra-TMSi derivative (1a) of annomocherin
Mass (EI) *m/z*: Fig. 1.

Annonacin (2)
white amorphous powder, mp 59.3-61.7°C, $[\alpha]_D^{22}$: +8.6° (*c* 0.07, CH_2Cl_2), UV λ_{max} nm (log ε): 205 (4.0), IR ν (cm^{-1}): 3348 (OH), 1751 (C=O of α, β-unsaturated γ-lactone), ^1H NMR see Table I.

Tetra-TMSi derivative (2a) of annonacin
Mass (EI) *m/z*: Fig. 2.

Annomontacin (3)
white amorphous powder, mp 65.2-67.4 °C, $[\alpha]_D^{25}$: -7.5° (*c* 0.06, CH_2Cl_2), UV λ_{max} nm (log ε): 205 (3.8), IR ν (cm^{-1}): 3567 (OH), 1736 (C=O of α, β-unsaturated γ-lactone), ^1H NMR see Table I.

Tetra-TMSi derivative (3a) of annomontacin
Mass (EI) *m/z*: Fig. 3.

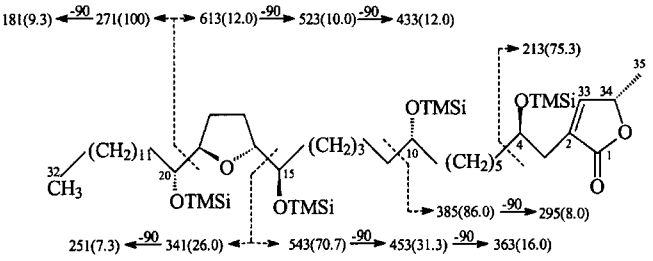


Fig. 2. Diagnostic EIMS fragmentations of tetra-TMSi derivative of **2** (intensities are indicated in parentheses).

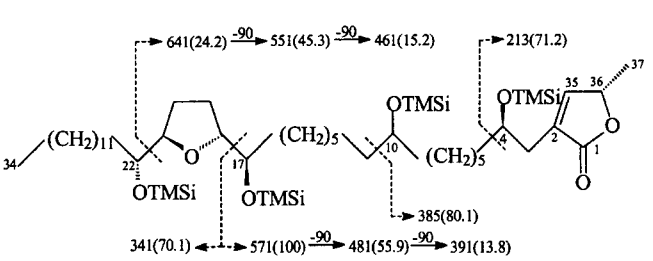
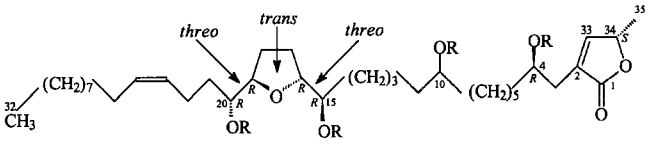


Fig. 3. Diagnostic EIMS fragmentations of tetra-TMSi derivative of **3** (intensities are indicated in parentheses).

Table II. Characteristic ^1H NMR data of Mosher esters of **1s** and **1r** for determinations of stereochemistries

Position	**1s** δ s	**1r** δ r	Δδ s-r
5	1.59	1.58	+ 0.01
4	5.32	5.36	R
3	2.58	2.65	- 0.07
33	6.73	6.97	- 0.24
34	4.85	4.92	- 0.07
35	1.29	1.32	- 0.03
14	1.62	1.56	+ 0.06
15	4.98	5.01	R
16	3.93	4.05	- 0.12
17	1.57	1.63	- 0.06
	1.94	1.95	- 0.01
18	1.57	1.63	- 0.06
	1.94	1.95	- 0.01
19	3.93	4.05	- 0.12
20	4.98	5.01	R
21	1.62	1.56	+ 0.06



1 R=H, 1a R=TMSiOH, 1s R=(*S*)-MTPA, 1r R=(*R*)-MTPA

Preparation of TMSi-derivatives

Approximately 10 μg of **1**, **2**, and **3** were separately treated with 0.2 μl of pyridine and 2 μl of *N,O-bis*-(trimethylsilyl) acetamide (BSA) for 5 h to give a **1a** , **2a** and **3a**, respectively (Laprevote *et al.*, 1992).

Preparation of Mosher esters

1 mg of **1** in 0.5 mL of CH_2Cl_2, were added sequentially 0.2 mL pyridine, 0.5 mg 4-(dimethylamino)-pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride. The mixture was left at room temperature overnight and purified over a micro-column (0.6 × 6 cm) of silica gel (230-400 mesh) eluted with 3-4 mL of hexane-CH_2Cl_2 (1:2); the eluate was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 mL × 3) and H_2O (5 mL × 2); the washed eluate was dried *in vacuo* to give the *S* Mosher ester of **1**. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride afforded the *R* Mosher ester (Mosher *et al.*, 1973; Rieser *et al.*, 1992; Gu *et al.*, 1994). Their pertinent ^1H NMR chemical shifts are given in Table II.

Bioassay

The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp lethality test (BST) (Meyer *et al.*, 1982; McLaughlin, 1991).

MTT cytotoxicity tests

Seven-day *in vitro* MTT cytotoxicity tests against human tumor cell lines were carried out at the Cell Culture Laboratory, Purdue Cancer Center, Purdue University, using standard protocols for A-549 (human lung carcinoma) (Giard *et al.*, 1973), MCF-7 (human breast carcinoma) (Soule *et al.*, 1973), HT-29 (human colon adenocarcinoma) (Fogh *et al.*, 1975), A-498 (human kidney carcinoma) (Giard *et al.*, 1973), PC-3 (human prostate adenocarcinoma) (Kaighn *et al.*, 1979), and MIA PaCa-2 (human pancreatic carcinoma) (Yunis *et al.*, 1977) with adriamycin as a positive control.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. The molecular weight of **1** was indicated by a peak at *m/z* 595 $[MH]^+$ in the FABMS spectrum. The HRFABMS spectrum gave *m/z* 595.4574 for the $[MH]^+$ (*calcd 595.4580*) *corresponding to the molecular formula* $C_{35}H_{63}O_7$. The presence of four hydroxyl groups was indicated by the losses of four molecules of H_2O from $[M+H]^+$ in the FABMS spectrum and by a broad absorption in the IR spectrum at 3467 cm^{-1}. This is confirmed by preparation of the tetra-TMSi derivative (**1a**). The EIMS of **1a** showed successive losses (*m/z* 792, 702, 612, 522) of four TMSiOH (*m/z* 90) units from the M^+ (*m/z* 882) of the tetra-TMSi. The spectral data of **1** provided the characteristic features for the usual α,β-unsaturated γ-lactone moiety with a 4-OH, including an IR carbonyl absorption at 1734 cm^{-1}, a UV (MeOH) λ_{max} at 220 nm, six proton resonances at δ 7.19 (H-33), 5.06 (H-34), 3.84 (H-4), 2.52 (H-3b), 2.40 (H-3a), and 1.43 (H-35) in the ^1H NMR, and seven carbon resonances at δ 174.68 (C-1), 151.91 (C-33), 131.16 (C-2), 78.03 (C-34), 69.93 (C-4), 33.34 (C-3), and 19.11 (C-35) in the ^{13}C NMR spectra (Table I) (Rupprecht *et al.*, 1990). The presence of isolated double bond in **1** was suggested by two-proton multiplet at δ 5.35-5.43 and two carbon resonances at δ 128.95 and 130.85 in the ^1H and ^{13}C NMR spectra (Colman-Saizarbitoria *et al.*, 1998). The position of the double bond was determined at C-23/24 from the double-relayed COSY spectrum, which showed correlation cross peaks between H-23 (δ 5.37) and H-21 (δ 1.48). The configuration of the double bond was assigned as *cis* by comparing the NMR spectra with other double bond containing acetogenins of known configuration (Woo *et al.*, 1995; Hopp *et al.*, 1997).

The presence of a mono-THF ring in **1**, with two OH groups adjacent to the ring, was suggested by ^1H NMR resonances at δ 3.43 (H-15/20) and 3.80 (H-16/19), and ^{13}C NMR peaks at δ 74.02 (C-15), 82.66 (C-16), 82.63 (C-19), and 73.58 (C-20); similar peaks are characteristic of other mono-THF acetogenins having two OH groups adjacent to the ring, such as corrosolin (Corter *et al.*, 1991) and xylomaticin (Colman-Saizarbitoria *et al.*, 1994). Determination of the relative stereochemistry around the mono-THF moiety was done by Borns technique (Born *et al.*, 1990) and by comparison with reported spectral data of a series of synthetic mono-THF diol compounds of known relative configuration (Harmange *et al.*, 1992). The arrangement was assigned as *threo* between C-15/16 and C-19/20 because of the proton signals of compound **1** at δ 3.43 for H-15/20 and carbon signals at δ 74.02 for C-15 and 73.58 for C-20. The ^1H NMR signals at δ 1.98 and 1.67 corresponding to H-17 and H-18 are typical methylene proton signals of a *trans*-THF ring, since the methylene proton signals for *cis*-THF rings are δ 1.94 and 1.82 (Gu *et al.*, 1994b). The presence of one free hydroxyl along the hydrocarbon chain was suggested by a proton signal at δ 3.59 (H-10) and a carbon signal at δ 71.77 (C-10) (Jossang *et al.*, 1990). By analysis of the EIMS fragmentations of **1a** (Fig. 1), the placement of the mono-THF ring with its two flanking OH groups were determined to be at C-15 to C-20, and the free OH group was located along the hydrocarbon chain at C-10. The absolute stereochemistries of the carbinol stereocenters in **1** have been determined using advanced Mosher ester methodology which analyzes differences between the ^1H NMR chemical shifts of *S*- and *R*-MTPA [methoxy (trifluoromethyl) phenyl acetate] ester derivatives on both sides of the stereogenic carbinol centers (Mosher *et al.*, 1973; Rieser *et al.*, 1992; Gu *et al.*, 1994). The ^1H NMR data for **1s** and **1r** derivatives are summarized in Table II. Based on Moshers arguments, C-15 and C-20 were assigned to have the *R* absolute configuration, since the signs of Δδs-r were negative for H-16 and H-19 showing relatively less shielding for this side in the *S*-MTPA ester. As the relative stereocheimstry form C-15 to C-20 of compound **1** was known to be *threo/trans/threo*, the absolute configurations of C-15 *R*, C-16 *R*, C-19 *R* and C-20 *R* were readily concluded. Analysis of differences between the *S*- and *R*-Mosher derivatives (**1s** and **1r**) around the γ-lactone ring moiety exhibited negative results for H-3, H-4, H-33, H-34, and H-35 and positive results for H-5, thus suggesting *R* configuration for C-4 (Table II). The absolute configuration at C-34 in the butenolide ring has been directly determined as *S* only for uvaricin (Jolad *et al.*, 1985) and squamocin (Born *et al.*, 1990; Araya *et al.*, 1994; Sahai *et al.*, 1994). However, all acetogenins are predicted as having the 34 *S* (or 36 *S* in the thirty-seven carbon acetogenins) configuration by comparisons of NMR data of the diagnostic protons and carbons with those of uvaricin and squamocin. Recently, a novel use of Mosher ester data for determining the relative configuration between C-4 and C-34 of the 4-hydroxylated annonaceous acetogenins has been described by Hoye *et al.* (Hoye, *et al.*, 1994a, 1994b). Analysis of the ^1H NMR data of **1s** and **1r**, using Hoye's method, indicated that

Table III. Brine Shrimp Lethality for **1**, **2** and **3**, and Cytotoxicities in Human Solid Tumor Cell Lines for **1**

Compound	BST LC_{50} (μg/mL)	Human cancer cell line ED_{50} (μg/mL)					
		A-549	MCF-7	HT-29	A-498	PC-3	MIA PaCa-2
1	1.62×10^{-2} NT	4.41×10^{-1}	8.51×10^{-4}	1.83	1.78×10^{-6}	4.61×10^{-2}	1.48×10^{-1}
Adriamycin		2.08×10^{-3}	1.91×10^{-2}	3.39×10^{-2}	2.78×10^{-3}	2.36×10^{-2}	1.51×10^{-3}

BST (brine shrimp lethality test): **2** (BST $LC_{50} = 1.02 \times 10^{-2}$ μg/mL), **3** (BST $LC_{50} = 6.30 \times 10^{-4}$ μg/mL), A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), MIA PaCa-2 (human pancreatic carcinoma), adriamycin (positive control standard), NT (not tested).

the $|\Delta\delta|$ values for H-33 and H-34 were 0.24 ppm and 0.07 ppm, respectively (Table II), and strongly suggested "unlike" relative configuration between C-4 and C-34 for **1**. Since C-4 in **1** has the *R* configuration, C-34 must possess the *S* configuration, as is usual. Compound **1** is a novel C_{35} mono-THF acetogenin with double bond at C-23/24; it was named annomocherin.

Compound **2** was a white amorphous powder. The FABMS gave $[MH]^+$ at *m/z* 597 indicating a $[M]^+$ of 596. The presence of four OH moieties was suggested by successive losses of four H_2O molecules (*m/z* 18) in the FABMS as well as by the formation of a tetra-TMSi derivative, **2a**. The IR carbonyl absorption band in **2** at 1751 cm^{-1}, the UV absorption λmax (MeOH) at 205 nm (log ε 4.0), the proton resonances at δ 7.18 (H-33), 5.06 (H-34), 3.80 (H-4), 2.39 (H-3a), 2.52 (H-3b), and 1.43 (H-35), and the carbon signals at δ 174.62 (C-1), 151.85 (C-33), 130.16 (C-2), 77.94 (C-34), 69.66 (C-4), and 18.99 (C-35) provided characteristic spectral features for an α,β-unsaturated γ-lactone fragment with a 4-OH. The existence of a mono-THF ring with two flanking hydroxyls in **2** was indicated by the ^1H NMR signals at δ 3.40 (H-15/20) and 3.80 (H-16/19) and ^{13}C signals at δ 73.98 (C-15/20) and 82.64 (C-16/19). The presence of a free hydroxyl along the hydrocarbon chain was suggested by a proton signal at δ 3.59 (H-10) and a carbon signal at δ 71.45 (C-10). The position of free hydroxyl group, as well as the total structure of the molecule, was established by close examination of the EIMS spectral fragmentation pattern of **2a** (Fig. 2). The peaks at *m/z* 613, 543, 341, and 271 in the EIMS spectrum of **2a** allowed placement of a mono-THF ring with two flanking hydroxyls between C-15 and C-20, whereas the ion fragments at *m/z* 385 and 295 suggested that the free hydroxyl group was on C-10. The relative stereochemistry C-15/16 and C-19/20 was defined by comparing the ^{13}C NMR signals of the hydroxylated carbons at C-15/20 (δ 73.98) and C-16/19 (δ 82.64) and the ^1H NMR signals of H-15/20 (δ 3.40) and H-16/19 (δ 3.80) in **2**, with those of model compounds of known relative stereochemistry (Born *et al.*, 1990). These data suggested that the relative configurations between C-15/ 16 and C-19/20 were both *threo*. Mono-THF ring of **2** possesses a *trans* configuration based on the observation that the methylene proton signals resonated at δ 1.98 (H-17b/18b) and 1.67 (H-17a/18a) (Harmange *et al.*, 1992). Thus, the relative configuration for these four chiral centers in **2** was assigned as *threo/trans/threo*. So, this compound was identified as anno-nacin (Alkofahi *et al.*, 1988).

Compound **3** was isolated as a white powder. The FABMS of **3** gave an $[MH]^+$ at 625. Sequential losses of four molecules of H_2O from the $[MH]^+$ in the FABMS as well as the formation of the tetra-TMSi derivative **3a** confirmed the existence of four hydroxyl groups in **3**. The presence of an α,β-unsaturated γ-lactone with a hydroxyl group at C-4 in **3** was suggested by the ^1H NMR resonances at δ 7.18 (H-35), 5.06 (H-36), 3.80 (H-4), 2.39 (H-3a), 2.53 (H-3a), and 1.43 (H-37) corresponding in the ^{13}C NMR spectrum to the resonances at δ 174.58 (C-1), 151.81 (C-35), 131.18 (C-2), 77.97 (C-36), 69.91 (C-4), 33.48 (C-3), and 19.10 (C-37) (Table I). The THF ring was placed between C-17 and C-22 based on peaks at *m/z* 543 and 613 in the EIMS fragmentation of **3a** (Fig. 3). A free hydroxyl attached somewhere along the aliphatic chain could be identified by a multiplet at δ 3.58 in the ^1H NMR and a signal at δ 71.86 in the ^{13}C NMR spectra. The peaks in the EIMS fragment of **3a** at *m/z* 385 indicated that this hydroxyl was located along the hydro-carbon chain at C-10. Resonances at δ 3.40 (H-17/22) and 3.80 (H-18/21) in the ^1H NMR spectrum and δ 74.02 (C-17/22), and 82.62 (C-18/21) in the ^{13}C NMR spectrum indicated the presence of a mono-THF ring and two flanking hydroxyls with a *threo/trans/threo* relative stereocheimstry. Finally this compound was identified as annomontacin with C_{37} (Jossang *et al.*, 1991).

Table III summarizes the bioactivities for compounds **1**-**3**. **1** was very toxic to the brine shrimp lethality test (BST) and showed potent and selective cytotoxicities to the human solid tumor cell lines, breast carcinoma (MCF-7) and kidney carcinoma (A-498). **1** exhibited potent and selective cytotoxicities against breast carcinoma (MCF-7) and kidney carcinoma (A-498) cell lines with 100 1,000 times the potency of adriamycin. Compounds **2** and **3** are very active in the brine shrimp lethality test. The acetogenins exert their biological effects, at least in part, through inhibition of mitochondrial electron transport (complex I) and the inhibition of the plasma membrane

NADH oxidase of tumor cells (Zeng *et al.*, 1996; Alali *et al.*, 1999).

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