Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 8, pp 1271–1279, 1996

PII: S0968-0896(96)00111-3
0968-0896/96 $15.00+0.00

cis-Gigantrionenin and 4-Acetyl Gigantetrocin A, Two New Bioactive Annonaceous Acetogenins from *Goniothalamus giganteus*, and the Stereochemistries of Acetogenin 1,2,5-Triols

Lu Zeng, Yan Zhang, Qing Ye, Gouen Shi, Kan He and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

Abstract—Using activity-directed fractionation, two new bioactive acetogenins, *cis*-gigantrionenin (**1**) and 4-acetyl gigantetrocin A (**2**), have been isolated from the bark of *Goniothalamus giganteus* (Annonaceae). Compound **1** has a *cis*-mono-THF ring with one flanking hydroxyl and possesses a *cis*-double bond at C-21/22 of the aliphatic chain; it represents only the second example of the *cis*-mono-THF ring annonaceous acetogenins having one flanking hydroxyl. Compound **2** has a *trans*-mono-THF ring with one flanking hydroxyl, but it possesses a mono-acetyl group at the 4-OH position; it represents only the second natural example of the acetylated annonaceous acetogenins; the first acetogenin reported, uvaricin, was mono-acetylated at the 24-OH. The stereochemistries of **1** and **2** were determined by the advanced Mosher ester method. In addition, the absolute stereochemistries of gigantriocin (**3**), gigantrionenin (**4**), and giganenin (**5**) were determined by the advanced Mosher ester method and by circular dichroism (CD). The stereochemistries of the 7,8-diols in murihexocins A (**6**) and B (**7**) were determined to have the *S,S*-configurations, respectively.

Introduction

Goniothalamus giganteus Hook. f. & Thomas (Annonaceae) is a tropical tree distributed in southeast Asia. It has a great reputation as a drug among the Malays.[1] The bark of this plant, obtained from Thailand, showed toxicities in the brine shrimp lethality test (BST) and murine toxicities in the 3PS (P388) leukemia bioassay.[2] Fifteen bioactive annonaceous acetogenins have been previously isolated from the bark, including the first nonring acetogenin, giganin,[3] mono-THF ring acetogenins, goniothalamicin, annonacin, gigantriocin, gigantetrocin (or gigantetrocin A), gigantrionenin, gigantetronenin, giganenin, and gonionenin,[4–9] adjacent bis-THF acetogenins, goniodenin and asimilobin,[10] the first nonadjacent bis-THF ring acetogenin, gigantecin,[5] as well as bullatalicin and bullatalicinone,[11] and the first tri-THF ring acetogenin, goniocin.[12] In our further bioactivity-directed search for antitumor compounds, two additional bioactive acetogenins, *cis*-gigantrionenin (**1**) and 4-acetyl gigantetrocin A (**2**), have now been isolated. Both have mono-THF rings with only one flanking hydroxyl group. Prior to this work, only one *cis*-mono-THF ring with one flanking hydroxyl acetogenin, muricatetrocin A,[13,14] and only one acetylated acetogenin, uvaricin,[14,15] had been reported. The stereochemistries of **1** and **2** were determined by the advanced Mosher ester method using methoxy-α-(trifluoromethyl)phenylacetate (MTPA). Also, the stereochemistries of gigantriocin (**3**), gigantrionenin (**4**), and giganenin (**5**), isolated previously from the same plant materials, were determined by the advanced Mosher ester method and circular dichroism (CD). Based on the summarized ^{1}H NMR data of the tri-MTPA esters of several acetogenins containing 1,2,5-triols, a chemical shift pattern was proven to be useful for the determination of the absolute stereochemistries of this type of compounds, when they are *R,R-S* (*R*) or *S,S-S* (*R*) 1,2,5-triols. Thus, the stereochemistries of the 7,8-diol moieties in murihexocins A (**6**) and B (**7**) were both determined to have *S,S* configurations.

Key words: *Goniothalamus giganteus*, Annonaceae, acetogenin, *cis*-gigantrionenin, 4-acetyl gigantetrocin A.

Results and Discussions

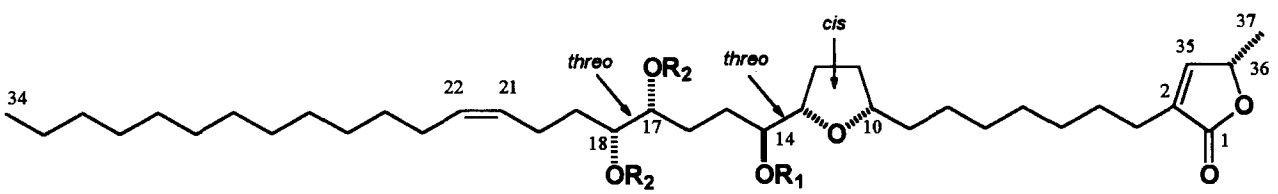
Compound **1** (Fig. 1) was isolated as a colorless oil, $[\alpha]_D$ +8.5° (*c* 0.18, MeOH). The molecular formula of **1** was established to be $C_{37}H_{66}O_6$ by HRFABMS (glycerol) which gave *m/z* 607.4928 for the MH^+ (calcd 607.4938). Spectral characteristics of **1** and its MTPA and TMS derivatives, including ^{1}H and ^{13}C NMR (Table 1) and MS (Fig. 2) data, suggested that **1** is a mono-THF annonaceous acetogenin with one flanking hydroxyl and a double bond along the aliphatic chain. The IR spectrum of **1** contained an absorption peak for hydroxyls at 3440 cm^{-1}; this peak and sequential losses of three molecules of H_2O (*m/z* 18) from the MH^+ in the CIMS indicated that **1** has three hydroxyl groups. The IR carbonyl absorption band in **1** at 1750 cm^{-1}, the UV absorption λ_{max}(EtOH) 207 nm (log ε 3.1), the proton resonances at δ 6.99, 5.00, 2.26, and 1.41, and the carbon signals at δ 174.0, 148.9, 134.3, 77.2, and 19.2 provided characteristic spectral features for an α,β-unsaturated γ-lactone moiety without a 4-hydroxyl group.[14]

The mono-THF ring with one flanking hydroxyl was indicated by the proton signals in **1** at δ 3.87 (1H, H-10), 1.48 and 1.98 (each 1H, H-11), 1.64 and 1.92 (each 1H, H-12), 3.73 (1H, H-13), 3.41 (1H, H-13); and carbon signals at δ 26.2 (C-8), 36.0 (C-9), 80.0 (C-10), 31.4 (C-11), 27.8 (C-12), 80.2 (C-13), and 74.9 (C-14). These data also indicated that the relative stereochemistry of the carbon centers C-13/14 was *threo* and the configuration across the THF ring was *cis*, by comparisons with series of model compounds of known relative stereochemistries.[16] The *cis*-THF ring configuration was also confirmed by a NOESY experiment, in which the cross peak was observed between H-10 (δ 3.87) and H-13 (δ 3.73). The carbon skeleton and the placement of the THF ring were determined on the basis of the EIMS fragmentation of the TMS derivative (**1a**) of **1** (Fig. 2).

The formation of the acetonide derivative **1b** from **1** provided information that a 1,2-vicinal diol was present

Table 1. ^{13}C NMR and ^{1}H data of **1**, **1b**, **2**, and **2b**

	1 δ_C	**1** δ_H	**1b** δ_H	**2** δ_C	**2** δ_H	**2b** δ_H
1	174.0			173.8		
2	134.3			130.2		
3	25.2	2.26, br t, 7.0	2.26, br t, 7.0	34.0	2.51, ddd, 2.57, ddt	2.51, ddd, 2.57, ddt
4	27.2	1.25–1.30, m	1.25–1.30, m	71.9	5.10, ddt	5.10, ddt
5	29.1–29.7	1.25–1.30, m	1.25–1.30, m	37.3	1.65, m	1.65, m
6–7	29.1–29.7	1.25–1.30, m	1.25–1.30, m	29.1–29.6	1.25–1.30, m	1.25–1.30, m
8	26.2	1.25–1.30, m	1.25–1.30, m	26.0	1.25–1.30, m	1.25–1.30, m
9	36.0	1.43, m	1.43, m	35.4	1.43, m	1.43, m
10	80.0	3.87, m	3.88, m	79.2	3.88, m	3.88, m
11	31.4	1.48, m, 1.98, m	1.48, m, 1.99, m	32.4	1.53, m, 2.02, m	1.53, m, 2.02, m
12	27.8	1.64, m, 1.92, m	1.65, m, 1.92, m	28.4	1.62, m, 1.96, m	1.62, m, 1.97, m
13	82.0	3.73, q, 7.5	3.74, q, 7.5	81.8	3.81, q, 7.5	3.81, q, 7.5
14	74.9	3.41, m	3.41 ddd, 2.0, 5.0, 7.0	74.6	3.42, m	3.40, ddd, 2.0, 5.0, 7.0
15	31.9	1.42–1.60, m	1.38, m	31.9	1.42, m	1.42, m
16	29.9	1.42–1.60, m	1.48–1.55, m	29.9	1.42–1.60, m	1.50, m
17	74.2	3.45, m	3.59, m	74.3	3.44, m	3.57, m
18	74.2	3.45, m	3.61, m	74.4	3.44, m	3.60, m
19	33.4	1.42–1.60, m	1.48–1.55, m	33.5	1.42–1.60, m	1.50, m
20	23.5	2.02, m	1.45–1.55, m	25.6	1.25–1.30, m	1.25–1.30, m
21	129.0	5.40, dt, 5.0, 9.0	5.39, dt, 5.0, 9.0	29.1–29.6	1.25–1.30, m	1.25–1.30, m
22	130.8	5.36, dt, 5.0, 9.0	5.33, dt, 5.0, 9.0	29.1–29.6	1.25–1.30, m	1.25–1.30, m
23	27.4	2.20, m	2.02, m	29.1–29.6	1.25–1.30, m	1.25–1.30, m
24–31	29.1–29.7	1.25–1.30, m	1.25–1.30, m	22.7	1.26, m	1.26, m
32	29.1–29.7	1.25–1.30, m	1.25–1.30, m	14.1	0.88, t, 7.0	0.88, t, 7.0
33	22.7	1.26, m	1.26, m	150.9	7.08, q, 1.5	7.08, q, 1.5
34	14.1	0.88, t, 7.0	0.88, t, 7.0	77.5	5.01, qq, 2.0, 7.0	5.01, qq, 2.0, 7.0
35	148.9	6.99, q, 1.5	6.99, q, 1.5	19.0	1.41, d, 7.0	1.41, d, 7.0
36	77.4	5.00, qq, 2.0, 7.0	5.00, qq, 2.0, 7.0			
37	19.2	1.41, d, 7.0	1.41, d, 7.0			
Me			1.38, s, 1.38, s			1.38, s, 1.38, s
AcO				170.6		
				21.1	2.03, s	2.03, s



	1	**1a**	**1b**	**1s**	**1r**
R_1	H	TMSi	H	*S*-MTPA	*R*-MTPA
R_2	H	TMSi	Acetonyl	*S*-MTPA	*R*-MTPA

Figure 1. The structures of **1**, **1a**, **1b**, **1s**, and **1r**.

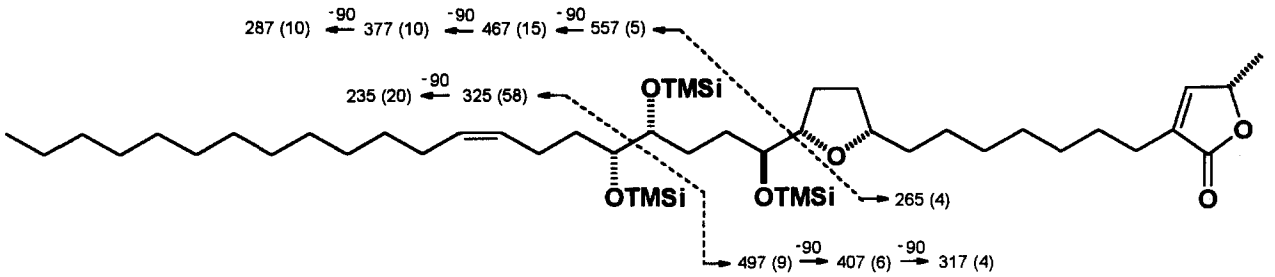


Figure 2. EIMS fragmentation of **1a**.

and was *threo*, since the acetonyl methyls appeared at δ 1.38 as a singlet and the dioxolane ring protons appeared at δ 3.59 and 3.61.[17] The position of this 1,2-diol was determined to be at C-17/18 from the double-relayed COSY[18] experiment of **1b**, which showed the expected cross peaks between H-17 (δ 3.59) and H-14 (δ 3.42). The presence of an isolated double bond in **1** was determined in the ^{1}H NMR by the proton signals at δ 5.40 and 5.36 and the carbon signals at δ 130.8 and 129.0. When the protons at δ 2.20 (H-23) were selectively irradiated, they showed correlation with the proton at δ 5.36 (H-22) in the COSY spectrum, and the latter became a doublet (J = 11.0 Hz); when the protons at δ 2.02 (H-20) were selectively irradiated, they showed a correlation with the proton at δ 5.40 (H-21) in the COSY spectrum, and the latter became a doublet (J = 11.0 Hz). These observations also indicated that the double bond has a *cis* configuration. The position of the double bond was determined at C-21/22 from the double-relayed COSY experiment of **1**, which showed the expected cross peaks between H-21 (δ 5.40) and H-18 (δ 3.45).

The absolute configurations of the 1,2,5-triol group, at the C-18, 17 and 14 positions, were determined first by inspection of the acetonide derivative, **1b**. The third hydroxyl methine proton (H-14 of **1b**) showed a doublet of double doublet pattern, which indicated the *R,R*-configurations for the 1,2-diol; the *S,S*-configurations give a quartet pattern.[13,19] Also, in the advanced Mosher ester experiments, $\Delta\delta H_{S,R}$ showed positive values along the double bond side (Table 2) and confirmed that C-18 has the *R* configuration. The tri-MTPA esters of **1** (**1s** and **1r**) also provided information about the absolute stereochemistries across the THF ring from which C-14 could be concluded to have the *S* configuration; therefore, **1** has C-13*S* and C-10*S* configurations.

The absolute configuration at C-36 in the butenolide ring has been directly determined as *S* only for uvaricin[20] and squamocin.[21-23] Recently a synthetic model butenolide has supported the previous conclusions.[24] In the CD spectra of squamocin and the synthetic model butenolide, both showed a negative Cotton effect at ca. 236 nm, which is attributable to the 36*S* configuration in the γ-lactone moiety and provides a useful method for the determination of the C-36 configuration in those annonaceous acetogenins which lack a C-4 hydroxyl. Compound **1** showed a negative CD curve at 238 nm, and it was, thus, concluded that **1** has a 36*S* configuration. The structure and the absolute configurations of **1** were concluded to be as illustrated, and this compound was named *cis*-gigantrionenin (**1**) being identical to gigantrionenin except for the *cis*-THF ring at C-10/13.[7]

Compound **2** (Fig. 3) was obtained as a colorless oil, $[\alpha]_D$ +13.5° (*c* 0.11, MeOH). The molecular formula of **2** was established to be $C_{37}H_{66}O_8$ by HRFABMS

Table 2. Characteristic ^{1}H NMR data of Mosher esters of **1s**, **1r**, **2s**, and **2r** for determinations of stereochemistries

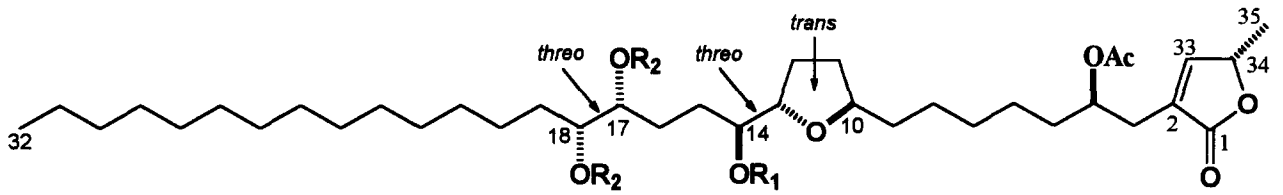
	1s δ*S*	**1r** δ*R*	δ*S*-*R*	δ*S*	**2s** δ*R*	**2r** δ*S*-*R*
10	3.79, dd, 7.0	3.72, m	+0.07	3.84, dd, 7.0	3.73, m	+0.11
11	1.42, m	1.35, m	+0.07	1.38, m	1.34, m	+0.04
	1.90, m	1.78, m	+0.12	1.96, m	1.84, m	+0.12
12	1.40, m	1.25, m	+0.15	1.35, m	1.34, m	+0.01
	1.76, m	1.67, m	+0.09	1.85, m	1.78, m	+0.07
13	3.79, dd, 7.0	3.78, dd, 7.0	+0.01	3.88, q, 7.0	3.86, q, 7.0	+0.02
14	4.88, m	4.91, m	*S*	4.88, m	4.91, m	*S*
15	1.41, m	1.57, m	−0.16	1.37, m	1.54, m	−0.17
17	5.05, m	5.20, m		5.04, m	5.15, m	
18	4.99, m	5.16, m	*R*	4.99, m	5.20, m	
20	1.92, m	1.90, m	+0.02			
21	5.40, dd, 7.0, 13.5	5.38, dd, 7.0, 13.5	+0.02			
22	5.16, dd, 7.0, 13.5	5.16, dd, 7.0, 13.5				

(glycerol), which gave *m/z* 639.4849 for the MH^+ (calcd 639.4836). ^1H and ^{13}C NMR spectra of **2** clearly showed an acetyl group (δ 2.03 1H, s, and δ 21.1, 170.6) (Table 1) among the other characteristic annonaceous acetogenin signals, and the MS fragmentation data (Fig. 4) suggested that **2** is a mono-THF annonaceous acetogenin with one flanking hydroxyl and a mono-acetoxyl group. The proton resonances at δ 5.10, for the acetoxyl methine (H-4), and at δ 2.51, 2.57, for its adjacent methylene (H-3a ,3b), indicated that the acetoxyl group is located at the C-4 position.

The ^1H NMR spectra of the acetonide derivative **2b** from **2** showed the acetonyl methyls at δ 1.38 and the dioxolane ring protons at δ 3.57 and 3.60, providing evidence for a *threo* 1,2-vicinal diol. The position of the 1,2-diol was determined at C-17/18 from the double-relayed COSY experiment of **2b**, which showed the expected cross peaks between H-17 (δ 3.57) and H-14 (δ 3.40) and also from the MS fragmentation data (Fig. 4). The absolute configurations of the 1,2,5-triol moiety, at the C-18, 17 and 14 positions, were determined again by inspection of the acetonide derivative, **2b**. The methine proton of H-14 showed a pattern of a doublet of double doublet, which was consistent with the *R,R*-configurations for a 1,2-diol. Also in the advanced Mosher ester experiments, the $\Delta\delta H_{S\text{-}R}$ showed positive values for the right side of THF ring and a negative value for the left side (Table 2); and, thus, indicated that C-14 has the *S* configuration. A CD experiment with **2** showed a negative peak at 243 nm ([θ]-1353.8), which supported the conclusion that C-34 has the *S* configuration. The per-acetate derivative of **2** (**2c**) gave exactly the same CD absorption pattern and exactly the same ^1H NMR data as those of the tetra-acetate of gigantetrocin A[6] and demonstrated that these two compounds are the same; this observation proved that C-4 in **2** has the *R* configuration.[13] Thus, the structure and the absolute configurations of **2** were concluded to be as illustrated, and **2** was named 4-acetyl gigantetrocin A after the parent compound.[13]

Compounds **1** and **2** showed potent respective bioactivities in the BST[2] (LC_{50} 2.52 and 6.78 μg/mL), and significant cytotoxicities among six human solid tumor cell lines (Table 7).[25-29] The acetogenins act as inhibitors of complex I in mitochondrial electron transport systems[30] and as inhibitors of the plasma membrane NADH oxidase of tumor cells.[31]

Gigantriocin (**3**) and gigantrionenin (**4**) are mono-THF acetogenins, possessing one flanking hydroxyl group and no 4-hydroxy group, that have been isolated from the same plant.[6,7] The proton resonances of H-14 in their acetonide derivatives (**3a**, **4a**) gave a pattern of doublet of double doublets, and this information, combined with their per-Mosher ester data (Table 3) and their CD data (both showed negative curves at 238 nm [θ]-1645.5, 238 nm [θ]-1920.3), provided evidence for their absolute stereostructures as illustrated in Figures 5 and 6. Giganenin (**5**), also from this plant, is also a mono-THF acetogenin, but with two flanking hydroxyl groups and without a 4-hydroxyl group. The position of its double bond has been revised from C-9/10 to C-21/22.[14] The negative CD curve at 238 nm



	2	2a	2b	2s	2r
R_1	H	TMSi	H	*S*-MTPA	*R*-MTPA
R_2	H	TMSi	Acetonyl	*S*-MTPA	*R*-MTPA

Figure 3. The structures of **2**, **2a**, **2b**, **2s**, and **2r**.

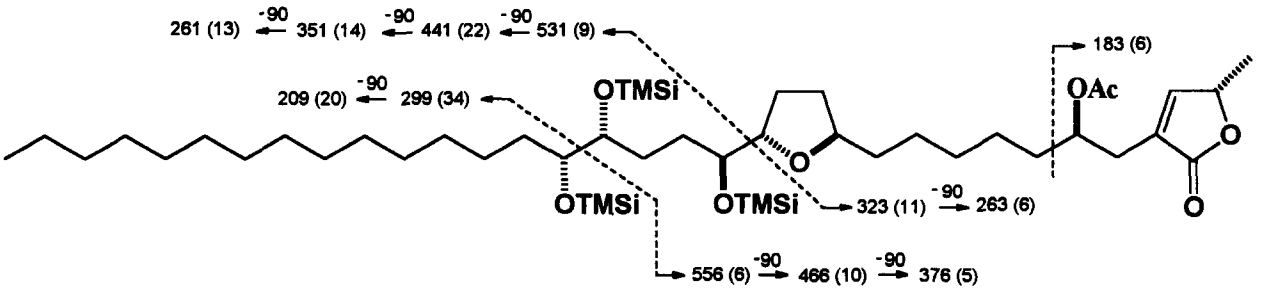


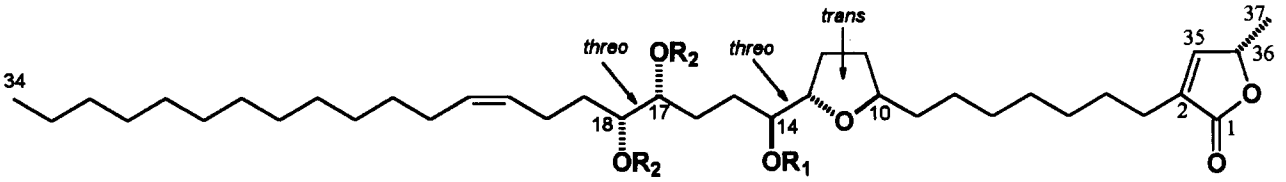
Figure 4. EIMS fragmentation of **2a**.

Table 3. Characteristic ^{1}H NMR data of Mosher esters of **3s**, **3r**, **4s**, and **4r** for determinations of stereochemistries

	3s δ*S*	**3r** δ*R*	δ*S*-*R*	δ*S*	**4s** δ*R*	**4r** δ*S*-*R*
10	3.84, dd, 7.0	3.72, m	+0.12	3.84, dd, 7.0	3.73, m	+0.11
11	1.44, m	1.40, m	+0.04	1.37, m	1.34, m	+0.03
	1.96, m	1.83, m	+0.13	1.96, m	1.84, m	+0.12
12	1.33, m	1.28, m	+0.05	1.35, m	1.34, m	+0.01
	1.83, m	1.78, m	+0.05	1.86, m	1.78, m	+0.08
13	3.88, dd, 7.0	3.86, q, 7.0	+0.02	3.88, q, 7.0	3.86, q, 7.0	+0.02
14	4.84, m	4.88, m	*S*	4.85, m	4.90, m	*S*
15	1.38, m	1.54, m	−0.16	1.38, m	1.54, m	−0.16
17	5.04, m	5.20, m		5.03, m	5.14, m	
18	4.98, m	5.16, m	*R*	4.94, m	5.18, m	
20	1.92, m	1.90, m	+0.02			
21	5.40, dd, 7.0, 13.5	5.38, dd, 7.0, 13.5	+0.02			
22	5.16, dd, 7.0, 13.5	5.16, dd, 7.0, 13.5				

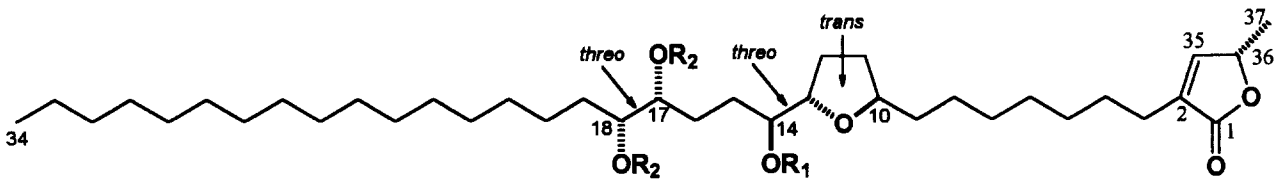
[θ]-1766.2 and the per-Mosher ester data (Table 4) suggested the absolute structure of **5** as illustrated in Figure 7.

The 1,2,5-triol group is becoming a common structural feature among the annonaceous acetogenins, including several mono-THF ring compounds with one flanking hydroxyl group, such as gigantrionenin (**3**), gigantriocin (**4**), gigantetronenin, gigantetrocins A and B, muricatetrocins A and B,[13] and murihexocins A (**6**) and B (**7**).[19] All of these 1,2,5-triol groups have a *threo* configuration for the vicinal 1,2-diol, and the absolute stereochemistries may either be *R,R* or *S,S*. By formation of the formaldehyde acetal to connect the two hydroxyl groups at C-14 and C-17 in gigantetrocin A and subsequent Mosher esterification at C-18, the stereochemistries of the 1,2-diol at C-17,18 of gigantetrocin A were determined as *R,R* by Gu et al.[23] By using mono-Mosher esterifications at C-18 in both gigantetrocins A and B, the stereochemistries of their 1,2-diols were proven to be *R,R* and *S,S*, respectively, by Rieser et al.[13] By analysis of the ^{1}H NMR spectra of the acetonides of gigantetrocins A and B, the hydroxy methine protons in the 1,2,5-triol (C-18,17,14) moiety, gave a different pattern, i.e., a doublet of double doublet for gigantetrocin A, which has an *R,R*-1,2-diol; and a



	3	**3a**	**3s**	**3r**
R_1	H	H	*S*-MTPA	*R*-MTPA
R_2	H	Acetonyl	*S*-MTPA	*R*-MTPA

Figure 5. The structures of **3**, **3a**, **3s**, and **3r**.



	4	**4a**	**4s**	**4r**
R_1	H	H	*S*-MTPA	*R*-MTPA
R_2	H	Acetonyl	*S*-MTPA	*R*-MTPA

Figure 6. The structures of **4**, **4a**, **4s**, and **4r**.

quartet for gigantetrocin B, which has an *S,S*-1,2-diol. Using this acetonide derivative method, Rieser et al. also determined the stereochemistries of the 1,2-diol (C-19,20) in muricatetrocins A and B to have the *R,R* configuration.[13] Zeng et al. later demonstrated that the 1,2-diol (at C-19,20) in murihexocins A and B have *R,R* and *S,S* configurations, respectively.[19] As described above *cis*-gigantrionenin (**1**), 4-acetyl gigantetrocin A (**2**), gigantriocin (**3**) and gigantrionenin (**4**) have *R,R* configurations of their 1,2-diols. Analyses of the tri-Mosher ester data of the 1,2,5-triol moieties in all of these acetogenins can now be compared (Tables 5 and 6). The chemical shifts of the esterified methine proton signals appear at ca. δ 4.91–4.94 and δ 5.01–5.03 (δ 4.91–4.94 and δ 5.05 in cases where another double bond is located two carbons away) for the *S*-Mosher esters in the *R,R*-1,2-diols, and at ca. δ 5.10–5.15 and δ 5.16–5.19 (δ 5.16 and δ 5.18–5.20 in cases where another double bond is located two carbons away) for the *R*-Mosher esters in *R,R*-1,2-diols; whereas these signals are located at ca. δ 5.03–5.06 and δ 5.10–5.16 for the *S*-Mosher esters in the *S,S*-1,2-diols and at ca. δ 5.03–5.04 and δ 5.17 for the *R*-Mosher esters in the *S,S*-1,2-diols. By using this comparative chemical shift pattern data, we have reanalyzed and reassigned the chemical shifts of murihexocins A (**6**) and B (**7**).[19] The chemical shifts of the 1,2-diol at C-7,8 appeared at δ 5.03 and 5.10 (in both **6** and **7**) in the *S*-Mosher ester, and at δ 5.03 and 5.17 (**6**) and δ 5.03 and 5.20 (**7**), in the *R*-Mosher ester. These values indicated that **6** and **7** both have *S,S*-1,2-diols at C-7,8. Thus, the absolute stereochemistries of murihexocins A (**6**) and B (**7**) can now be completely illustrated as in Figure 8.

Table 4. Characteristic ^{1}H NMR data for Mosher esters of **5a** and **5r** for determinations of stereochemistries

	5s δ_S	**5r** δ_R	δ_{S-R}
3	2.256, t, 7.5	2.25, t, 7.5	+0.006
4	1.43, m	1.42, m	+0.01
9	1.38–1.50, m	1.38–1.50, m	
10	5.00, m	4.98, m	*R*
11	1.38–1.50, m	1.38–1.50, m	
12	1.64, m	1.58, m	+0.06
13	4.95, m	5.04, m	*R*
14	3.95, dt, 3.5, 7.5	4.00, dd, 6.5	−0.05
15	1.37, m	1.56, m	−0.19
	1.67, m	1.88, m	−0.21
16	1.26, m	1.46, m	−0.20
	1.51, m	1.83, m	−0.32
17	3.77, dd, 6.5	3.93, dd, 6.5	−0.16
18	4.88, dd, 1.5, 5.5	4.92, m	*R*
19	1.44, m	1.40, m	+0.02
20	1.94, m	1.88, m	+0.06
21	5.39, dd, 7.0, 13.5	5.35, dd, 7.0, 13.5	+0.04
22	4.28, dd, 7.0, 13.5	4.21, dd, 7.0, 13.5	+0.07
23	2.02, m	1.90, m	+0.12

Experimental

Instrumentation

Optical rotations were determined on a Perkin–Elmer 241 polarimeter. IR spectra (film) were measured on a Perkin–Elmer 1600 FTIR spectrometer. UV spectra were taken in EtOH on a Beckman DU-7 UV spectrophotometer. CD spectra were recorded on a JASCO Model J600 circular dichroism spectrometer. ^{1}H NMR, ^{1}H–^{1}H COSY, and ^{13}C NMR spectra were obtained on a Varian VXR-500S spectrometer. Low-resolution MS data were collected on a Finnigan 4000 spectrometer. Low-resolution CIMS and EIMS for TMS derivatives and high-resolution FABMS were performed on a Kratos MS50. HPLC separations were performed with a Rainin Dynamax solvent delivery system (model SD-200) using a Dynamax software system and a silica gel column (Dynamax 60-A 250 × 21 mm) equipped with a Dynamax absorbance detector (model UV-1) set at 225 nm. Analytical TLC was carried out on silica gel plates (0.25 mm) developed with hexane:acetone (3:2) and $CHCl_3$:MeOH (9.5:0.5), respectively, and visualized with 5% phosphomolybdic acid in EtOH.

Bioassays

The bioactivities of extracts, fractions and pure compounds were routinely assayed using a test for lethality to brine shrimp larvae (BST).[2] In vitro cytotoxicities, against human tumor cell lines, were carried out at the Purdue Cancer Center, Cell Culture Laboratory, using standard 7 day MTT assays for A-549 (human lung carcinoma),[25] MCF-7 (human breast carcinoma),[26] HT-29 (human colon adenocarcinoma),[27] A-498 (human kidney carcinoma),[25] PC-3 (human prostate adenocarcinoma),[28] and PACA-2 (human pancreatic carcinoma).[29] Adriamycin is always used as a positive control in the same runs.

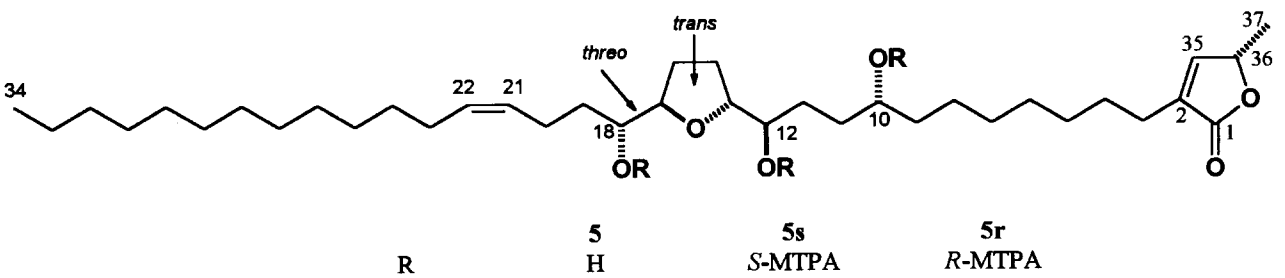


Figure 7. The structures of **5**, **5s**, and **5r**.

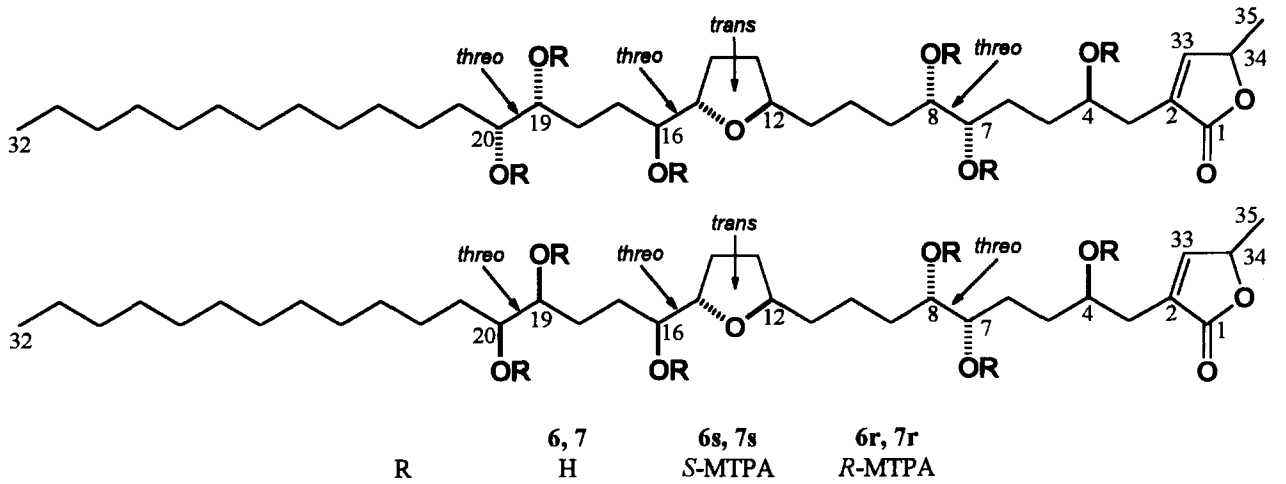


Figure 8. The structures of **6**, **7**, **6s**, **7s**, **6r**, and **7r**.

Plant material

The stem bark of *G. giganteus* (B-826538, PR-50604) was collected in Thailand in September 1978 under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, MD, where voucher specimens are maintained.

Extraction and isolation

The stem bark (10.7 kg) was ground into powder and percolated with 95% ethanol. The dry extract (900 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 (430 g, F003) was parti-

Table 5. Characteristic ^1H NMR data for Mosher esters of 1,2,5-triols for determinations of stereochemistries

		Muricatetrocin A			Muricatetrocin B	
Configuration	H-20	H-19	H-16	H-20	H-19	H-16
δ_S	4.94	5.01	4.86	4.91	5.01	4.83
δ_R	5.12	5.17	4.90	5.12	5.16	4.88
		Murihexocin A (**7s**, **7r**)			Gigantetrocin A	
Configuration	H-20	H-19	H-16	H-18	H-17	H-14
δ_S	4.96	5.02	4.82	4.91	5.01	4.82
δ_R	5.10	5.17	4.87	5.11	5.16	4.87
		4-Acetyl gigantetrocin A (**2s**, **2r**)			Gigantriocin (**4s**, **4r**)	
Configuration	H-18	H-17	H-14	H-18	H-17	H-14
δ_S	4.94	5.02	4.85	4.94	5.02	4.85
δ_R	5.14	5.18	4.90	5.14	5.18	4.90
		Gigantetronenin (**3s**, **3r**)			*cis*-Gigantetronenin (**1s**, **1r**)	
Configuration	H-18	H-17	H-14	H-20	H-19	H-16
δ_S	4.97	5.05	4.84	4.99	5.05	4.88
δ_R	5.16	5.18	4.89	5.16	5.20	4.91

Table 6. Characteristic ^1H NMR data of Mosher esters of 1,2,5-triols for determinations of stereochemistries

		Gigantetrocin B			Murihexocin B (**7s**, **7r**)	
Configuration	H-18	H-17	H-14	H-20	H-19	H-16
δ_S	5.06	5.16	4.91	5.03	5.10	4.82
δ_R	5.04	5.17	4.92	5.03	5.17	4.87
		Murihexocin A (**6s**, **6r**)			Murihexocin B (**7s**, **7r**)	
Configuration	H-8	H-7	H-4	H-8	H-7	H-4
δ_S	5.03	5.10	4.83	5.03	5.10	4.83
δ_R	5.03	5.17	4.89	5.03	5.20	4.88

Table 7. Bioactivities of compounds **1** and **2** (ED_{50} μg/mL)

	BST[a]	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	MIA PaCa-2[g]
1	2.52	5.99×10^{-2}	2.68×10^{-1}	6.94×10^{-6}	1.39×10^{-2}	1.11×10^{-1}	1.15×10^{-1}
2	6.78	$<10^{-2}$	8.50×10^{-1}	$<10^{-2}$	1.55×10^{-1}	1.02	$<10^{-2}$
Adr.[h]		4.28×10^{-3}	4.95×10^{-1}	4.98×10^{-2}	4.62×10^{-2}	5.83×10^{-2}	4.23×10^{-3}

[a]Brine shrimp lethality test.[2]
[b]Human lung carcinoma.[25]
[c]Human breast carcinoma.[26]
[d]Human colon adenocarcinoma.[27]
[e]Human kidney carcinoma.[25]
[f]Human prostate adenocarcinoma.[28]
[g]Human pancreatic carcinoma.[29]
[h]Adriamycin—standard positive control.

tioned between 90% MeOH and hexane, giving a MeOH layer (400 g, F005) and a hexane layer (30 g, F006). The MeOH layer (F005) was the most active fraction in the BST (LC_{50} 1.02 μg/mL). Thus, a portion (190 g) of F005 was repeatedly chromatographed over silica gel columns directed by the BST test, using gradients of hexane:acetone, hexane:EtOAc and $CHCl_3$:MeOH and purified by normal phase HPLC eluted with 10% THF in MeOH:hexane (4–6%) to give the colorless oils (**1** and **2**, as well as **3–5**).

Preparation of Mosher esters

To an acetogenin (0.5–1 mg, in 0.5 mL of CH_2Cl_2) were sequentially added pyridine (0.1 mL), 4-(dimethylamino)pyridine (0.1 mg), and 15 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The mixture was stirred at rt from 4 h to overnight and passed through a disposable pipette (0.6 × 4 cm) containing silica gel (60–200 mesh) and eluted with 3 mL of CH_2Cl_2. The CH_2Cl_2 residue, dried in vacuo, was redissolved in CH_2Cl_2 and washed in 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5mL); the CH_2Cl_2 layer was dried in vacuo to give the (*S*)-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%.

Preparation of TMS derivatives

Compounds **1** and **2** (ca. 0.3 mg of each) were treated with *N,O*-bis(trimethylsilyl) acetamide (20 μL) and pyridine (2 μL) and heated at 70 °C for 30 min to yield the respective tri-TMS derivatives, **1a** and **2a**. EIMS fragmentations are shown in Figures 2 and 4.

***cis*-Gigantrionenin (1).** Colorless oil, $[\alpha]_D + 8.5°$ (*c* 0.18, MeOH); UV λ_{max} (EtOH): 207 nm (log ε, 3.1); IR ν_{max} (film): 3440, 2928, 2860, 1750, 1455, 1325 cm^{-1}; HRFABMS (glycerol): obsd *m/z* 607.4928, for $C_{37}H_{66}O_6$ calcd 607.4938; ^1H and ^{13}C NMR, see Table 1; for pertinent ^1H NMR signals of the Mosher esters (**1s**, **1r**), see Table 2.

4-Acetyl gigantetrocin A (2). Colorless oil, $[\alpha]_D + 13.5°$ (*c* 0.11, MeOH), UV λ_{max} (EtOH): 208 nm (log ε, 3.2); IR ν_{max} (film): 3445, 2930, 2867, 1750, 1745, 1430, 1347 cm^{-1}; HRFABMS (glycerol): obsd *m/z* 639.4849, for $C_{37}H_{66}O_8$ calcd 639.4836. ^1H and ^{13}C NMR, see Table 1; for pertinent ^1H NMR signals of the Mosher esters (**2s**, **2r**), see Table 2.

Acknowledgments

This investigation was supported by R01 grant No. CA30909 from the National Cancer Institute, National Institutes of Health. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing.

References

1. Perry, L. M.; Metzger, J. *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses*; MIT: Cambridge, 1980; p 18.

2. (a) McLaughlin, J. L. *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic: London, 1991, Vol. 6, p 1; (b) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. *J. Cancer Chemother. Rep.* **1972**, *3*, 1.

3. Fang, X.-P.; Song, R.; Gu, Z.-M.; Rieser, M. J.; Miesbauer, L. R.; Smith, D. L.; Wood, K. V; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1153.

4. Alkofahi, A.; Rupprecht, J. K.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *Experientia* **1988**, *44*, 83.

5. Alkofahi, A.; Rupprecht, J. K.; Liu, Y.-M.; Chang, C.-J.; Smith, D. L; McLaughlin, J. L. *Experientia* **1990**, *46*, 539.

6. Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu, Y.-M.; Smith, D. L.; Wood, K. V; McLaughlin, J. L. *Heterocycles* **1991**, *32*, 11.

7. Fang, X.-P.; Anderson, J. E.; Smith, D. L.; Wood, K. V; McLaughlin, J. L. *J. Nat. Prod.* **1992**, *55*, 1655.

8. Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu, Y.-M.; Smith, D. L.; Wood, K. V; McLaughlin, J. L. *Heterocycles* **1992**, *34*, 1075.

9. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Song, R.; Ng, J. H.; Wood, K. V.; Smith, D. L.; McLaughlin, J. L. *J. Org. Chem.* **1994**, *59*, 3472.

10. Zhang, Z.; Zeng, L.; Woo, M.-H.; Gu, Z.-M.; Ye, Q.; Wu, F.-E; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1743.

11. Fang, X.-P.; Gu, Z.-M.; Rieser, M. J.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1993**, *56*, 1095.

12. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; McLaughlin, J. L. *Tetrahedron Lett.* **1994**, *35*, 5367.

13. Rieser, M. J.; Fang, X.-P.; Anderson, J. E.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1993**, *76*, 2433; erratum **1994**, *77*, 882.

14. (a) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237; (b) Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27; (c) Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Romeo, J. T., Ed.; Plenum: New York, 1995; Vol. 29, p 217; (d) Cavé, A.; Cortes, D.; Figadere, B.; Hocquemiller, R.; Laprevote, O.; Laurens, A.; Leboeuf, M. In *Recent Advances In Phytochemistry*; Downum, K. R.; Romeo, J.; Stafford, H. A., Eds.; Plenum: New York, 1993; Vol. 27, p 167.

15. Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Kreik, G. R.; Bates, R. B. *J. Org. Chem.* **1982**, *47*, 3151.

16. (a) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175; (b) Harmange, J. C.; Figadere, B.; Cavé, A. *Tetrahedron Lett.* **1992**, *33*, 5749.

17. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Kozlowski, J. F.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1993**, *4*, 473.

18. (a) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731; (b) Bax, A.; Drobny, A. *J. Magn. Reson.* **1985**, *61*, 306.

19. Zeng, L.; Wu, F.-E.; Gu, Z.-M.; McLaughlin, J. L. *Tetrahedron Lett.* **1995**, *36*, 5291.

20. Jolad, S. D.; Hoffmann, J. J.; Cole, J. R.; Barry, C. E.; Bates, R. B.; Linz, G. S.; Konig, W. A. *J. Nat. Prod.* **1985**, *48*, 644.

21. 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.

22. Born, L.; Lieb, F.; Lorentzen, J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312.

23. Araya, H.; Hara, N.; Fujimoto, Y.; Srivastava, A.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 388.

24. Naito, H.; Kawahara, E.; Maruta, K.; Maeda, M.; Sasaki, S. *J. Org. Chem.* **1995**, *60*, 4419.

25. Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.

26. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.

27. Fogh, J.; Trempe, G. In *Human Tumor Cells*; Fogh, J. Ed.; Plenum: New York, 1975; p 115.

28. Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16.

29. Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128.

30. Ahammadsahib, K. I.; Hollingworth, R. M.; McGovren, J. P.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113.

31. Morre, D. J.; de Cabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

(*Received in U.S.A.* 21 *December* 1995; *accepted* 15 *April* 1996)