174. Muricatetrocins A and B and Gigantetrocin B: Three New Cytotoxic Monotetrahydrofuran-Ring Acetogenins from *Annona muricata*

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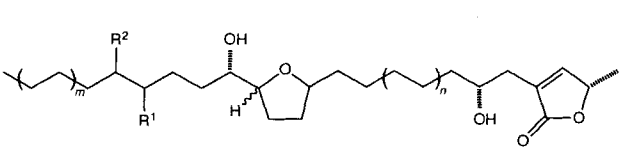
Extracts from the seeds of *Annona muricata* yielded three new Annonaceous acetogenins: muricatetrocin A (= (5*S*)-3-{(2*R*)-2-hydroxy-9-{(2*R*,5*S*)-tetrahydro-5-[(1*S*,4*S*,5*S*)-1,4,5-trihydroxyheptadecyl]furan-2-yl}nonyl}-5-methylfuran-2(5*H*)-one; **1**), muricatetrocin B (= (5*S*)-{(2*R*)-2-hydroxy-9-{(2*S*,5*S*)-tetrahydro-5-[(1*S*,4*S*,5*S*)-1,4,5-trihydroxyheptadecyl]furan-2-yl}nonyl}-5-methylfuran-2(5*H*)-one; **2**), and gigantetrocin B (= (5*S*)-3-{(2*R*)-2-hydroxy-7-{(2*S*,5*S*)-tetrahydro-5-[(1*S*,4*R*,5*R*)-1,4,5-trihydroxynonadecyl]furan-2-yl}heptyl}-5-methylfuran-2(5*H*)-one; **3**). Their C-skeletons were deduced by mass spectrometry. Configurations were determined by ^{1}H-NMR of ketal derivatives and 2D-NMR experiments utilizing *Mosher* esters. A previously described compound, gigantetrocin A (= (5*S*)-3-{(2*R*)-2-hydroxy-7-{(2*S*,5*S*)-tetrahydro-5-[(1*S*,4*S*,5*S*)-1,4,5-trihydroxynonadecyl]furan-2-yl}heptyl}-5-methylfuran-2-(5*H*)one; **4**), was also isolated and is new to this species. Compounds **1**–**4** were all selectively cytotoxic for the HT-29 human colon-tumor cell line with potencies at least 10 times that of adriamycin.

Introduction. – In recent years, an increased interest in the phytochemistry of the Annonaceae has been sparked by the discovery of the acetogenin uvaricin [1] [2], which showed potent *in vivo* antileukemic activity. Recent work has centered on the discovery of additional, new, bioactive Annonaceous acetogenins. *Annona muricata* L. (Annonaceae), also known as sour sop or guanabana, is a popular table fruit cultivated throughout the tropical regions of the world [3]. This fruit also forms the basis of a well-developed juice industry in both Latin America and the countries of tropical Asia. The seeds of *A. muricata* comprise *ca.* 7% of the weight of the fresh fruit, and thousands of metric tons of the seeds are produced as a by-product of commerical activity each year. This large biomass of seeds is presently being discarded because these seeds are toxic and, therefore, are unsuitable for uses in animal feed or as a source for vegetable oil.

Our initial screening of the defatted seeds of *Annona muricata* showed the EtOH extracts to be highly toxic to brine shrimp [4] [5] (BST). Bioactivity-directed fractionation of this plant material led to the isolation of the five mono-tetrahydrofuran (THF)-ring acetogenins of the annonacin series [6–9] and muricatacin [10], a possible breakdown product from annonacin. *Cave* and coworkers also previously reported the isolation, structural elucidation, and biological activities [11] of four new mono-THF-ring acetogenins, murisolin [12], corossolin, corossolone [13], and solamin [14], from the seeds of this fruit. We present, herein, the isolation and structural elucidation of three new, potent, cytotoxic vicinal-diol acetogenins: muricatetrocin A (**1**), muricatetrocin B (**2**), and gigantetrocin B (**3**). These new compounds are mono-THF-ring analogs of the structurally



	R^1	R^2	m	n	~~H
1	—OH	—OH	2	5	axial
2	—OH	—OH	2	5	equatorial
3	⋯OH	⋯OH	1	6	equatorial
4	—OH	—OH	1	6	equatorial

related nonadjacent bis-THF-ring compounds, gigantecin [15] and bullatalicin [16]. We also report the first isolation, from this plant source, and the absolute configurations of the known vicinal-diol acetogenin gigantetrocin A[1]) (**4**) [17].

Results and Discussion. – The dried seeds of *A. muricata* were ground, defatted in hexane by *Soxhlet* extraction, and extracted again with 95% EtOH. The EtOH extract was partitioned through a standard extraction scheme (see *Exper. Part*), and the partitions were evaluated for toxicity to brine shrimp (BST) [4] [5]. The most active fraction (*FOO5*, LC_{50} 0.8 ppm) was subjected to flash chromatography (FC; silica gel, gradient $CHCl_3$/MeOH). Fractions were combined according to similar TLC appearance and biological activity in the BST. The active pools were further subjected to repeated centrifugally accelerated radial chromatography (*Chromatotron*) to yield a mixture of compounds **1** and **2** (TLC (silica gel, CH_2Cl_2/AcOEt/EtOH 6:3:1) R_f 0.47). This mixture was resolved by reversed-phase HPLC. Similarly, compounds **3** and **4** (TLC (same system): R_f 0.40) were also isolated as a mixture which was subsequently resolved by HPLC (see *Exper. Part*). The constitutions of **1**–**4** were established by spectroscopic means.

Compounds **1**–**4** all showed a strong IR absorption band in the region 1730–1740 cm^{-1} and no appreciable UV absorption above 240 nm. A broad OH stretching absorption in the IR region at 3400 cm^{-1} indicated the presence in each compound of one or more OH groups. Each compound had identical ^{1}H-NMR spectra (500 MHz, $CDCl_3$) in the region δ 2.38–2.51 for CH_2(3) (δ 2.38 (*ddt*, J = 15.1, 8.0, 1.4 Hz) and 2.51 (*ddt*, J = 15.1, 4.0, 1.4 Hz); see *Table 1*)[2]). Additionally, all compounds had each of the following four resonances: δ 3.82 for H–C(4) (*m*), δ 7.17 for H–C(33) (*d*, J = 1.4 Hz), δ 5.04 for H–C(34) (*qd*, J = 7.1, 1.4 Hz), and δ 1.41 for Me(35) (*d*, J = 7.1 Hz). Experiments utilizing the HETCOR pulse sequence determined that these resonances correlated to ^{13}C-NMR resonances (125 MHz, $CDCl_3$) at δ 33.4 (C(3)), 69.88 (C(4)), 151.81 (C(33)), 77.99 (C(34)), and 19.14 (C(35); see *Table 1*)[2]). Two additional resonances at δ 174.53 (C(1)), and 131.05 (C(2)) showed no proton correlations. These data and previous experience with these compounds suggested the presence of an α,β-unsaturated γ-lactone with an OH group at the C(4) position [18]. The presence of this structural unit was confirmed by high-resolution EI-MS of the trimethylsilyl (Me_3Si) derivative (m/z 213) of the parent compounds [19].

High-resolution FAB-MS of **1**–**4** produced MH^+ ions at m/z 597.4720, 597.4724, 597.4724, and 597.4720, respectively, corresponding to the same molecular formula $C_{35}H_{64}O_7$ (calc. 597.4730) for each of the four compounds. Each compound showed four losses of H_2O (m/z 18) from m/z 597 in the CI-MS suggesting the presence of 4 OH moieties. Compounds **1** and **2** produced MS which were identical in every respect, indicating that these compounds differed only in their configurations. Low-resolution EI-MS of the Me_3Si derivatives of **1** and **2** each produced diagnostic fragments which were used to determine their contiguous-C-skeletons. The diagnostic fragments were confirmed by high-resolution peak matching (see *Scheme 1*). The C-skeletons of compounds **3** and **4** were also determined by EI-MS of their Me_3Si derivatives (*Scheme 2*). In addition to the ^{1}H-NMR resonances described above, compounds **1**–**4** each had a terminal Me resonance at δ 0.85, overlapping aliphatic resonances in the region δ 1.2–1.6, and 3 overlapping resonances of OH-substituted CH groups centered at δ 3.40 which differed only in their overlapped splitting patterns. All spectra contained a THF-ether CH resonance at δ 3.85 which showed COSY correlations only to protons in the aliphatic region. The ^{1}H-NMR spectrum of **1** differed from those of **2**–**4** primarily at the resonance for the THF-ether CH at C(15) and its associated ring CH_2 groups. This difference in the proton shielding allowed the ring configurations of the compounds to be deduced.

[1]) This compound was previously called gigantetrocin [17].

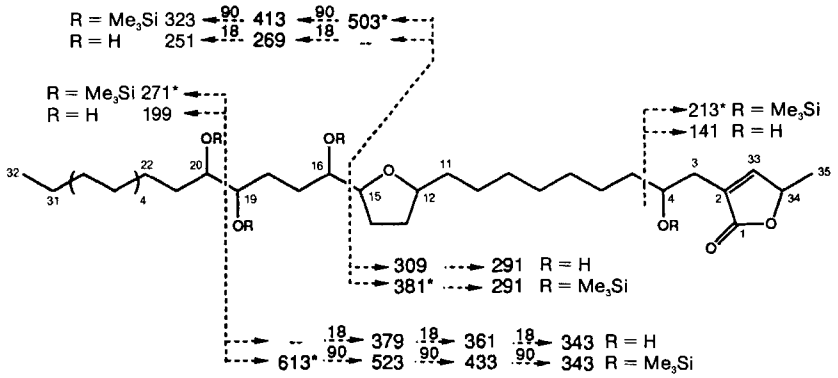
[2]) Arbitrary numbering according to *Schemes 1* and *2*; systematic names are given in the *Exper. Part*.

Table 1. *^{1}H- and ^{13}C-NMR Resonances* (500 and 125 MHz, resp.; $CDCl_3$) *and Assignments for Compounds* **1**–**3**[2]). δ in ppm rel. to residual $CHCl_3$ (δ(H) 7.24, δ(C) 77.00 ppm).

	Muricatetrocin A (**1**)		Muricatetrocin B (**2**)		Gigantetrocin B (**3**)	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	174.53	–	174.53	–	174.54
C(2)	–	131.10	–	131.04	–	131.05
H_a–C(3)	2.38 (*ddt*, *J* = 15.1, 8, 1.4)	33.39	2.38 (*ddt*, *J* = 15.1, 8, 1.4)	33.39	2.38 (*ddt*, *J* = 15.1, 8, 1.4)	33.39
H_b–C(3)	2.51 (*ddd*, *J* = 15.1, 4.0, 1.4)	–	2.51 (*ddd*, *J* = 15.1, 4.0, 1.4)	–	2.51 (*ddd*, *J* = 15.1, 4.0, 1.4)	–
H–C(4)	3.82 (*m*)	69.94	3.82 (*m*)	69.88	3.82 (*m*)	69.88
CH_2(5)	1.45 (*m*)	37.41	1.45 (*m*)	37.28	1.45 (*m*)	37.29
CH_2(6) to CH_2(11) (**1**, **2**) or CH_2(9) (**3**)	1.2–1.5	25–29	1.2–1.5	25–29	1.2–1.5	25–29
H–C(12) (**1**, **2**) or H–C(10) (**3**)	3.85 (*dt*, *J* = 5.9, 6.7)	80.03	3.86 (*dt*, *J* = 5.7, 6.7)	79.27	3.86 (*dt*, *J* = 5.7, 6.7)	79.29
H_e–C(13) (**1**, **2**) or H_e–C(11) (**3**)	1.96 (*m*)	28.43	2.00 (*m*)	28.43	2.00 (*m*)	32.45
H_a–C(13) (**1**, **2**) or H_a–C(11) (**3**)	1.65 (*m*)	–	1.63 (*m*)	–	1.64 (*m*)	–
H_e–C(14) (**1**, **2**) or H_e–C(12) (**3**)	1.91 (*m*)	32.43	1.97 (*m*)	32.43	1.97 (*m*)	28.43
H_a–C(14) (**1**, **2**) or H_a–C(12) (**3**)	1.72 (*m*)	–	1.71 (*m*)	–	1.71 (*m*)	–
H–C(15) (**1**, **2**) or H–C(13) (**3**)	3.70 (*q*, *J* = 7.0)	82.01	3.78 (*q*, *J* = 7.0)	81.73	3.77 (*q*, *J* = 7.0)	81.77
H–C(16) (**1**, **2**) or H–C(14) (**3**)	3.39 (*m*)	74.90	3.40 (*m*)	74.43	3.41 (*m*)	74.36
CH_2(17) (**1**, **2**) or CH_2(15) (**3**)	1.54 (*m*)	35.99	1.40 (*m*)	35.43	1.40 (*m*)	35.43
CH_2(18) (**1**, **2**) or CH_2(16) (**3**)	1.56 (*m*)	33.48	1.57 (*m*)	33.48	1.57 (*m*)	33.71
H–C(19) (**1**, **2**) or H–C(17) (**3**)	3.42 (*m*)	74.62	3.41 (*m*)	74.56	3.40 (*m*)	74.76
H–C(20) (**1**, **2**) or H–C(18) (**3**)	3.39 (*m*)	74.39	3.40 (*m*)	74.23	3.37 (*m*)	74.56
CH_2(21) (**1**, **2**) or CH_2(19) (**3**)	1.57 (*m*)	29.95	1.57 (*m*)	29.95	1.57 (*m*)	30.52
CH_2(22) to CH_2(30) (**1**, **2**) or CH_2(20) to CH_2(30) (**3**)	1.2–1.5	25–29	1.2–1.5	25–29	1.2–1.5	25–29
CH_2(31)	1.2–1.5	22.73	1.2–1.5	22.72	1.2–1.5	22.72
Me(32)	0.85 (*t*, *J* = 7.0)	14.18	0.85 (*t*, *J* = 7.0)	14.17	0.85 (*t*, *J* = 7.0)	14.17
H–C(33)	7.17 (*d*, *J* = 1.4)	151.81	7.17 (*d*, *J* = 1.4)	151.81	7.17 (*d*, *J* = 1.4)	151.81
H–C(34)	5.04 (*dq*, *J* = 7.1, 1.4)	77.99	5.04 (*dq*, *J* = 7.1, 1.4)	77.99	5.04 (*dq*, *J* = 7.1, 1.4)	78.01
Me(35)	1.41 (*d*, *J* = 7.1)	19.16	1.41 (*d*, *J* = 7.1)	19.14	1.41 (*d*, *J* = 7.1)	19.14

The ether proton at C(15) of **1** resonated at δ 3.70, which was *ca.* 0.07 upfield compared to the corresponding ether-proton signal of **2** (δ 3.77). This suggested that H–C(15) of **1** is in a more anisotropically shielded environment and, therefore, resides in a more axial position. The assignment of H–C(15) of **2**, which is more deshielded, must, therefore, be equatorial. In contrast to the boat and chair conformations of six-membered rings, which lie in deep energy wells, the conformations of five-membered rings are known to be very mobile, pseudorotating between the envelope (C_s symmetry) and the

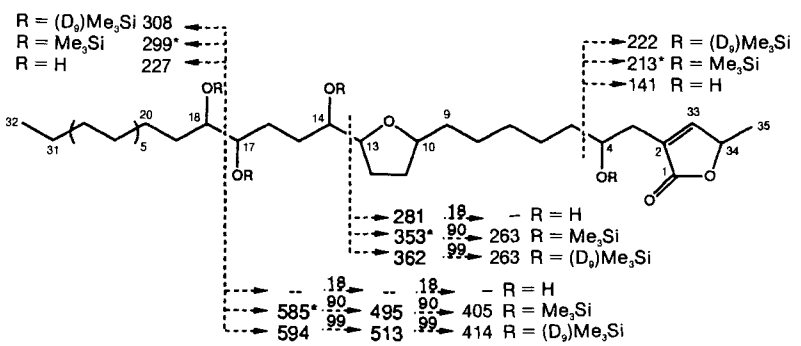
Scheme 1. *Diagnostic EI-MS Fragment Ions (m/z) of* **1** *and* **2**[a])[2])



1, 2 R = H

[a]) Losses of *m/z* 90 indicate the loss of neutral Me_3SiOH; the elemental compositions of starred (*) fragments were confirmed by high-resolution peak-matching MS.

Scheme 2. *Diagnostic EI-MS Fragment Ions (m/z) of* **3** *and* **4**[a])[2])



3, 4 R = H

[a]) Losses of *m/z* 90 indicate the loss of neutral Me_3SiOH; the elemental compositions of starred (*) fragments were confirmed by high-resolution peak-matching MS.

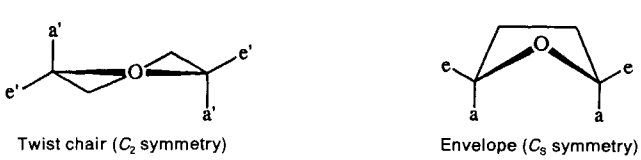
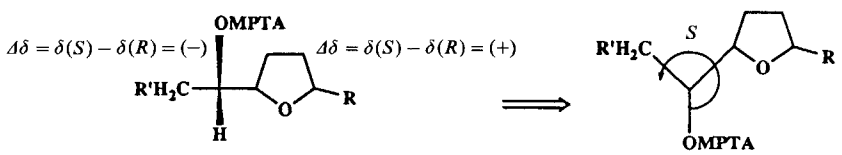


Fig. 1. *Twist-chair conformation* (C_2) *with pseudoaxial* (a') *and pseudoequatorial* (e') *exocyclic bonds and envelope conformation* (C_s) *with axial* (a) *and equatorial* (e) *exocyclic bonds*

twist-chair (C_2 symmetry) conformations [20] (*Fig. 1*). THF rings, both with and without substitutions, tend to adopt the C_2 symmetry, but it must be remembered that the lowest-energy well favoring this conformation is shallow, only of the order of 1.0 kcal/mol [21]. If the ring resides with the expected C_2 symmetry, the axial-equatorial protons of **1** suggest that the ring substituents are bound to the ring in the *cis*-configuration while those in **2–4** are *trans*.

The waxy nature of the Annonaceous acetogenins makes them unsuitable for direct X-ray crystallographic studies [19]. Many of the compounds isolated to date are diastereoisomeric, with subtle differences in configuration being responsible for appreciable differences in biological activity [19]. Acetates of these compounds were used for some time to separate the ^{1}H-NMR signals of OH-substituted CH groups in COSY analysis and to determine the relative configuration of the rings. However, acetates of these compounds do not always adequately separate the overlapping signals of OH-substituted CH centers, and, in any event, do not provide data with which one might deduce the absolute configuration of the compounds. Thus, we endorse the recommendation of *Fujimoto et al.* [22] that α-methoxy-α-(trifluoromethyl)phenylacetates (= 3,3,3-trifluoro-2-methoxy-2-phenylpropanotes; MPTA esters) be used to distinguish OH-substituted CH centers of newly isolated acetogenins. Prior to this recommendation, we embarked upon a collaborative effort with *Hoye* and coworkers [23] and determined the absolute configuration of the OH-substituted CH centers of nine natural acetogenins and two synthetic model compounds by the modified *Mosher* [24] [25] method of *Ohtani* and coworkers [26] (*Scheme 3*).

Scheme 3. *Assignment of Absolute Configurations by the* Mosher *Method*[a])



[a]) 1. Make the individual esters and assign as many ^{1}H-NMR signals as possible. 2. Obtain $\Delta\delta$ values for the protons. 3. Place the MPTAO at the top, the H on the bottom, the protons with a negative $\Delta\delta$ on the left, and those with a positive $\Delta\delta$ on the right of the projection given above. 4. Confirm the configurational assignments by confirming that all protons with positive and negative $\Delta\delta$'s actually reside on the expected side of the MPTA plane. 5. Apply *Cahn-Ingold-Prelog* rules to assign the absolute configuration.

Since it was obvious that the structures of compounds **3** and **4** differed only in their configurations at OH-substituted centers, MPTA esters of these two compounds, as well as compounds **1** and **2**, were prepared to assign their absolute configurations. The results from the COSY ^{1}H-NMR analysis of the experiments are given in *Tables 2–4*. Thus, the absolute configuration at C(4) of **1–4** was determined to be (*R*) (*Table 2*). This result is consistant with those obtained from a model (*S,S*)-β-hydroxybutenolide, synthesized by *Hoye* and coworkers [23], and is in agreement with the assignment of the (4*R*)-configuration to all natural 4-hydroxylated acetogenins that we have so far examined [23]. Similarly, data reported in *Table 3* allowed the assignment of the (*S*)-configuration to the OH-bearing center adjacent to the THF ring (C(16) of **1** and **2** and C(14) of **3** and **4**) in all four compounds.

Table 2. *^{1}H-NMR Chemical-Shift Data*[a]) *for the Determination of the Absolute Configuration at C(4) of the Tetra[(S)- and (R)-MPTA] Esters of* **1**–**4**[b])[2])

MPTA Ester of		CH_2(5)	H–C(4)	CH_2(3)	H–C(33)	H–C(34)	Me(35)	Deduced configuration
1	$\delta(S)$	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	(4*R*)
	$\delta(R)$	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	
	$\Delta\delta$	+0.02	–	−0.08, −0.04	−0.24	−0.05	−0.02	
2	$\delta(S)$	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	(4*R*)
	$\delta(R)$	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	
	$\Delta\delta$	+0.02	–	−0.08, −0.04	−0.24	−0.05	−0.02	
3	$\delta(S)$	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	(4*R*)
	$\delta(R)$	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	
	$\Delta\delta$	+0.02	–	−0.08, −0.04	−0.24	−0.05	−0.02	
4	$\delta(S)$	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	(4*R*)
	$\delta(R)$	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	
	$\Delta\delta$	+0.02	–	−0.08, −0.04	−0.24	−0.05	−0.02	

[a]) All $\delta > 2.0$ were determined directly from the 1D H-NMR spectra. All $\delta < 2.00$ were determined from the center of the relevant COSY off-diagonal peaks because of the large degree of overlap among these resonances.

[b]) (*S*) and (*R*) in $\delta(S)$ and $\delta(R)$ refer to the absolute configuration of MPTA. $\Delta\delta = \delta(S) - \delta(R)$.

Table 3. *^{1}H-NMR Chemical-Shift Data*[a]) *for the Determination of the Absolute Configuration at the OH-Substituted CH Center Flanking the THF Ring of the Tetra[(S)- and (R)-MPTA] Esters of* **1**–**4**[b])[2])

MPTA Ester of		H–C(12)	CH_2(13)	CH_2(14)	H–C(15)	H–C(16)	CH_2(17)	Deduced configuration
1	$\delta(S)$	3.72	1.87, 1.37	1.74, 1.40	3.76	4.86	1.54, 1.40	(16*S*)
	$\delta(R)$	3.68	1.74, 1.20	1.65, 1.34	3.75	4.90	1.55, 1.55	
	$\Delta\delta$	+0.04	+0.13, 0.17	+0.09, +0.06	+0.01	–	−0.01, −0.15	
2	$\delta(S)$	3.81	1.93, 1.34	1.81, 1.32	3.86	4.83	1.35, 1.20	(16*S*)
	$\delta(R)$	3.70	1.81, 1.32	1.75, 1.32	3.84	4.88	1.53, 1.46	
	$\Delta\delta$	+0.11	+0.12, +0.02	+0.06, 0.00	+0.02	–	−0.18, −0.26	
		H–C(10)	CH_2(11)	CH_2(12)	H–C(13)	H–C(14)	CH_2(15)	
3	$\delta(S)$	3.80	1.92, 1.34	1.82, 1.34	3.87	4.91	1.36, 1.31	(14*S*)
	$\delta(R)$	3.71	1.79, 1.28	1.80, 1.31	3.84	4.92	1.53, 1.51	
	$\Delta\delta$	+0.09	+0.13, +0.06	+0.08, +0.03	+0.02	–	−0.17, −0.20	
4	$\delta(S)$	3.80	1.94, 1.34	1.82, 1.32	3.86	4.82	1.35, 1.23	(14*S*)
	$\delta(R)$	3.68	1.80, 1.33	1.74, 1.31	3.83	4.87	1.54, 1.51	
	$\Delta\delta$	+0.12	+0.14, +0.01	+0.08, +0.01	+0.03	–	−0.19, −0.28	

[a])[b]) See *Footnotes a* and *b* in *Table 2*.

The assignment of the vicinal asymmetric centers C(19)/C(20) of **1** and **2** and of C(17)/C(18) of **3** and **4** could not be achieved by direct application of the MPTA-ester method without additional information. Thus, the relative configuration of the vicinal-diol moiety of **1**–**4** was determined first using their acetonide derivatives. The latter displayed an *s* at δ 1.35 (6 H) for the geminal Me groups and overlapping *m'* at δ 3.59 in **1**, **2**, and **4** and at δ 3.62 in **3** for the dioxolane CH protons. These observations allowed the assignment of the *threo*-configuration to all of the vicinal diol moieties [27] [28].

Table 4. *^{1}H-NMR Chemical-Shift Data*[a]) *for the Vicinal-Diol Centers of the Tetra[(S)- and (R)-MPTA] Esters of* **1**–**4**[b])[2])

MPTA Ester of		CH_2(18)	H–C(19)	H–C(20)	CH_2(21)	Deduced configuration at C(19), C(20)
1	$\delta(S)$	1.40, 1.27	5.01	4.94	1.37, 1.28	type A[c])
	$\delta(R)$	1.57, 1.54	5.17	5.12	1.44, 1.39	
	$\Delta\delta$	−0.17, −0.27	−0.16	−0.18	−0.07, −0.10	
2	$\delta(S)$	1.38, 1.27	5.01	4.91	1.39, 1.28	type A[c])
	$\delta(R)$	1.57, 1.54	5.16	5.12	1.44, 1.38	
	$\Delta\delta$	−0.19, −0.27	−0.15	−0.19	−0.05, −0.10	
		CH_2(16)	H–C(17)	H–C(18)	CH_2(19)	Deduced configuration at C(17), C(18)
3	$\delta(S)$	1.48, 1.31	5.16	5.06	1.37, 1.28	type B[c])
	$\delta(R)$	1.57, 1.39	5.17	5.04	1.44, 1.31	
	$\Delta\delta$	−0.09, −0.03	−0.01	+0.02	−0.07, −0.03	
4	$\delta(S)$	1.38, 1.27	5.01	4.91	1.38, 1.28	type A[c])
	$\delta(R)$	1.57, 1.54	5.16	5.11	1.44, 1.38	
	$\Delta\delta$	−0.19, −0.27	−0.15	−0.20	−0.04, −0.11	

[a])[b]) See *Footnotes a* and *b* in *Table 2*. [c]) See text.

Close inspection of the ^{1}H-NMR spectra of the acetonides (*Fig. 2*) and of the tetra-MPTA esters (*Table 4*) of **1**, **2**, and **4** suggested that these compounds have the same relative configuration (type A) at their vicinal-diol moieties when compared to **3** (type B). A partial *Mosher* esterification of **3** and **4** was carried out, and the (*R*)- and (*S*)-mono-MPTA esters at C(18) were isolated by HPLC. The ester position was established by the presence of peaks at *m/z* 585 in the EI-MS of the Me_3Si derivatives of the mono esters. ^{1}H-COSY and single-relay ^{1}H-COSY spectra of the four mono-MPTA esters were performed to assign shifts to the relevant protons (*Table 5*). Application of this method allowed assignment of the (18*S*)- and (18*R*)-configuration to **3** and **4**, respectively, and thus of the (*S*,*S*)- and (*R*,*R*)-configuration to their *threo*-diol moiety. By extrapolation, the vicinal-diol moieties of **1** and **2** were also assigned (*R*,*R*)-configuration.

Table 6 summarizes the biological activities of **1**–**4**. All compounds showed significant bioactivities in the BST and inhibited the growth of crown gall tumors on potato discs [5] [29] which is indicative of *in vivo* anti-tumor activity [30]. All compounds showed good activity against human solid tumor cells in culture [31–33]. However, the impressive selectivities to the human colon adenocarcinoma cell line (HT-29) from three repeated runs for compound **4** was not in good agreement with the previously reported data [17]. In an attempt to resolve this apparent discrepancy, the raw data in question was reviewed by the authors and the workers at the *Purdue Cell Culture Laboratory*. It was discovered that in all cases, the cytotoxicity curve hovered just over or just under the ED_{50} value; there was no complete killing of the cells, even at doses as high as 10 μg/ml, and low doses of the drugs ($< 10^{-8}$ μg/ml) continued to show inhibition, never reaching non-inhibitory concentrations found in the control samples. The reported ED_{50} values reflect the concentration where the cytotoxicities of the compounds under investigation began to reach their plateau. Interpretation of these results lead the authors to conclude that these compounds

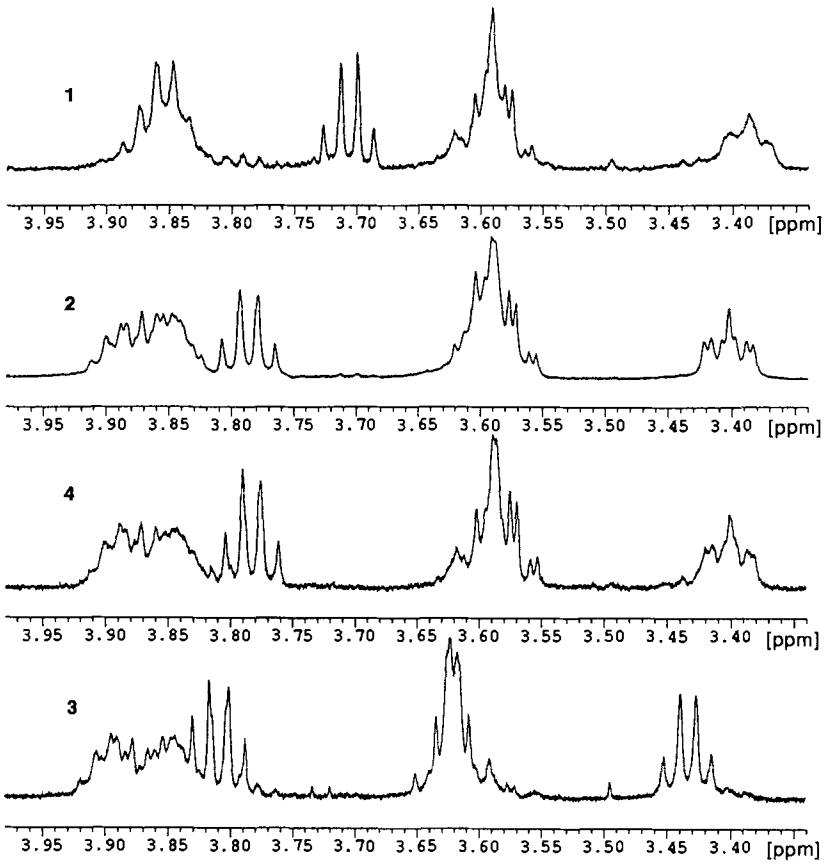


Fig. 2. *^{1}H-NMR Spectra of the acetonides of* **1**–**4**: *region of the dioxolane methine protons*

Table 5. *^{1}H-NMR Chemical-Shift Data*[a]) *for the Determination of the Absolute Configuration at C(18) of the Mono[(S)- and (R)-MPTA] Esters* **3** *and* **4**[b])[2])

MPTA Ester of		H–C(13)	H–C(14)	CH_2(15)	CH_2(16)	H–C(17)	CH_2(19)	CH_2(20)	Deduced configuration
3	$\delta(S)$	3.77	3.39	1.48	1.55	3.69	1.62, 1.55	1.20	(18*S*)
	$\delta(R)$	3.74	3.32	1.41	1.43	3.66	1.74, 1.65	1.30	
	$\Delta\delta$	+0.03	+0.07	+0.07	+0.13	+0.03	−0.12, −0.10	−0.10	
4	$\delta(S)$	3.74	3.34	1.42	1.41	3.65	1.67, 1.74	1.31	(18*R*)
	$\delta(R)$	3.78	3.40	1.50	1.54	3.70	1.52	1.18	
	$\Delta\delta$	−0.04	−0.06	−0.08	−0.13	−0.05	+0.15, +0.22	+0.13	

[a])[b]) See *Footnotes a* and *b* in *Table 2*.

Table 6. *Biological Activities of* **1**–**4**

	BST LC_{50} in μg/ml	PD[a]) % tumor inhibition	A-549[b]) ED_{50} in μg/ml (lung)	MCF-7[c]) ED_{50} in μg/ml (breast)	HT-29[d]) ED_{50} in μg/ml (colon)
1	1.4	76	$1.4 \cdot 10^{-1}$	1.03	$< 10^{-8}$
2	1.8	53	$4.9 \cdot 10^{-1}$	1.86	$2.8 \cdot 10^{-5}$
3	2.6	47	$2.5 \cdot 10^{-1}$	$6.3 \cdot 10^{-1}$	$4.1 \cdot 10^{-5}$
4	1.3	60	$8.1 \cdot 10^{-3}$	$5.3 \cdot 10^{-1}$	$< 10^{-8}$
4[e])	0.6	66	$3.5 \cdot 10^{-3}$	$6.5 \cdot 10^{-3}$	1.24
adriamycin	–	–	$1.9 \cdot 10^{-4}$	$4.6 \cdot 10^{-2}$	$8.6 \cdot 10^{-4}$

[a]) Potato disc assay. [b]) Human lung carcinoma cell line. [c]) Human breast carcinoma cell line. [d]) Human colon adenocarcinoma cell line. [e]) Values from [17].

may be more cytostatic than cytotoxic in nature; this is in good agreement with the recent findings that the Annonaceous acetogenins are inhibitors of mitochondrial NADH-ubiquinone reductase [34–36] and thereby reduce the cells energy charge; substrate level ATP production is believed to be sufficient for a certain level of cell survival, even though the electron-transport system is effectively blocked. An updated review, covering about sixty Annonaceous acetogenins discovered since 1990, was recently published [37].

Experimental Part

Bioassays. The extracts, fractions, and isolated compounds were routinely tested for lethality in the brine shrimp lethality test (BST) [4] [5]. The isolated compounds were evaluated by their ability to inhibit the growth of crown gall tumors on potato discs (PD) innoculated with *Agrobacterium tumefaciens* carrying a tumor-inducing plasmid [5] [29]. Cytotoxicity tests were carried out at the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols for A-549 (lung) [31], MCF-7 (breast) [32], and HT-29 (colon) [33] human solid tumor cell lines.

Instrumentation. M.p.: *Scientific-Instruments-Mel-Temp* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-241* polarimeter. FT-IR Spectra (thin film on NaCl; in cm^{-1}): *Perkin-Elmer-1420* instrument; $CHCl_3$ solns. ^{1}H-NMR, 2D COSY, NOESY, ^{13}C-NMR, and HETCOR Spectra: *Varian-VXR-500S* instrument; δ in ppm rel. to residual $CHCl_3$ (δ(H) 7.24, δ(C) 77.00) in $CDCl_3$. MS: low-resolution EI- and CI-MS on a *Finnigan 4000*, low-resolution EI-MS of Me_3Si derivatives on a *Kratos MS50*; high-resolution (HR) MS by peak matching on the *Kratos MS50*.

Extraction and Isolation of **1**–**4**. The dried seeds (2.2 kg) of *Annona muricata* were obtained from the Dominican Republic through the Possum Trot Tropical Fruit Nursery, 14955 S. W. 214 St., Miami, FL 33187. The seeds were ground and defatted by *Soxhlet* extraction with hexane for 72 h. The defatted plant material was repeatedly percolated with 95% EtOH to yield 100 g of extract *FOO1*, which was partitioned between $CHCl_3/H_2O$ 1:1 to yield the H_2O-soluble fraction *FOO2* (34 g) and the $CHCl_3$-soluble fraction *FOO3* (63 g). *FOO3* was then partitioned between 90% aq. MeOH/hexane 1:1 to yield a hexane-soluble fraction, *FOO6* (35 g), and an aq. MeOH-soluble fraction, *FOO5* (26 g). All fractions were subjected to the brine shrimp lethality test (BST), with the most active fraction being *FOO5* (BST, $LC_{50} = 0.8$ ppm). Repeated chromatography as described in the text resulted in the isolation of two unresolvable mixtures. The mixture **3**/**4** was separated by prep. reversed-phase high-performance liquid chromatography (HPLC; *Rainin* column (21.4 mm i.d.) packed with *Dynamax-60A* 8 mm *C18* support, isocratic elution with 15% aq. MeOH). Anal. reversed-phase HPLC of the mixture **1**/**2** failed to resolve the two components. Normal-phase anal. HPLC (*Rainin Dynamax-60A*) 8 mm silica support, gradient elution with 10% THF in MeOH (from 5–7% within 40 min/hexane) gave near-baseline separation of **1** and **2** in a 70-min run.

Muricatetrocin A (= *(5S)-3-{(2R)-2-Hydroxy-9-{tetrahydro-5-[(1S,4S,5S)-1,4,5-trihydroxyheptadecyl]-furan-2-yl}nonyl}-5-methylfuran-2-(5H)-one*; **1**): 10 mg from 2.1 g of extract. M.p. 102°. $[\alpha]_D^{25} = +10.3$ ($c = 0.15$,

$CHCl_3$). IR: 3442 (OH), 2919 and 2849 (CH), 1731 (C=O, lactone), 1469 (CH). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-MS: 213.0942 ($Me_3Si-C_7H_8O_3$, calc. 213.0947), 271.2452 ($Me_3Si-C_{13}H_{26}O$, calc. 271.2458), 381.2454 ($Me_3Si-C_{18}H_{28}O_4$, calc. 381.2462). HR-FAB-MS: 597.4724 ($C_{35}H_{61}O_7$, calc. 597.4730).

Muricatetrocin B (= *(5S)-3-{(2R)-2-Hydroxy-9-{tetrahydro-5-[(1S,4S,5S)-1,4,5-trihydroxyheptadecyl]-furan-2-yl}nonyl}-5-methylfuran-2(5H)-one*; **2**): 15 mg from 2.1 g of extract. M.p. 89–90°. $[\alpha]_D^{25} = +15.0$ ($c = 0.43$, $CHCl_3$). IR: 3442 (OH), 2919 and 2848 (CH), 1745 (C=O, lactone), 1468 (CH). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-MS: 213.0945 ($Me_3Si-C_7H_8O_3$, calc. 213.0947), 271.2452 ($Me_3Si-C_{13}H_{26}O$, calc. 271.2458), 381.2454 ($Me_3Si-C_{18}H_{28}O_4$, calc. 381.2462). HR-FAB-MS: 597.4718 ($C_{35}H_{61}O_7$, calc. 597.4730).

Gigantetrocin B (= *(5S)-3-{(2R)-2-Hydroxy-7-{tetrahydro-5-[(1S,4R,5R)-1,4,5-trihydroxynonadecyl]-furan-2-yl}heptyl}-5-methylfuran-2(5H)-one*; **3**): 30 mg from 2.1 g of extract. M.p. 91–92°. $[\alpha]_D^{25} = +4.1$ ($c = 0.41$, $CHCl_3$). IR: 3417 (OH), 2916 and 2848 (CH), 1728 (C=O, lactone), 1469 (CH). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-MS: 213.0945 ($Me_3Si-C_7H_8O_3$, calc. 213.0947), 299.2768 ($Me_3Si-C_{15}H_{30}O$, calc. 299.2772), 353.2142 ($Me_3Si-C_{16}H_{24}O_4$, calc. 353.2149), 585.3452 ($(Me_3Si)_3C_{20}H_{30}O_6$, calc. 585.3452). HR-FAB-MS: 597.4718 ($C_{35}H_{61}O_7$, calc. 597.4730).

Gigantetrocin A (**4**) was isolated as a white, amorphous powder from hexane: 40 mg from 2.1 g of extract. M.p. 93–94°. $[\alpha]_D^{25} = +14.3$ ($c = 0.45$, $CHCl_3$). IR: 3361 (OH), 2916 and 2847 (CH), 1731 (C=O, lactone), 1469 (CH). ^{1}H- and ^{13}C-NMR Spectra: identical to those described previously and virtually identical to those of **2** and **3** (see *Table 1*).

Trimethylsilyl Derivatives. Ca. 1 mg each of **1**–**4** were treated with 20 µl of *N,O*-bis(trimethylsilyl)acetamide/pyridine 10:1 and heated at 70° for 30 min.

α-Methoxy-α-(trifluoromethyl)phenylacetates. A soln. of 1 g of (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MPTA) in $SOCl_2$ was refluxed at 78° for 72 h. The unreacted $SOCl_2$ and HCl were removed by evaporation. The product MPTA-Cl was dissolved in 10 ml of dry CH_2Cl_2. *Ca.* 0.1 ml of each of these reagent solns. were added separately to a 4-mg sample of each **1**–**4** dissolved in dry CH_2Cl_2 containing a few crystals of 4-(dimethylamino)pyridine and 1 drop of dry pyridine, and allowed to react overnight. The reaction was quenched with sat. $NaHCO_3$ soln. and then partitioned into CH_2Cl_2, followed by partition between sat. $NaHSO_3$ soln. and CH_2Cl_2. The resulting MPTA esters were isolated by normal-phase HPLC and their δ(H) determined by 1D and COSY ^{1}H-NMR.

Mono-MPTA esters were obtained by monitoring by TLC (silica-gel-*60*-precoated glass plates, *Merck*, no. 5724; acetone/hexane 1:1) the dropwise addition of a 20-fold dilution of the stock MPTA-Cl soln. to a 5-mg sample of **3** or **4**. The mono esters were isolated as described above.

18-[(R)-MPTA] Ester of **3**: ^{1}H-NMR: 0.85 (*dd*, $J = 7.0$, Me(32)); 1.2–1.5 (*m*, CH_2(5) to CH_2(9), CH_2(21) to CH_2(31)); 1.41 (*d*, $J = 7.1$, Me(35)); 2.38 (*ddt*, $J = 15, 8, 1.6$, H_a–C(3)); 2.51 (*dddd*, $J = 15, 4, 1.4, 1.1$, H_b–C(3)); 3.82 (*m*, H–C(4)); 3.88 (*m*, H–C(10)); 2.03 (*m*, H_a–C(11)); 1.51 (*m*, H_b–C(11)); 1.98 (*m*, H_a–C(12)); 1.59 (*m*, H_b–C(12)); for H–C(13) to CH_2(20), see *Table 5*; 5.04 (*dq*, $J = 7.1, 1.4$, H–C(34)); 7.17 (*d*, $J = 1.4$, H–C(33)); 3.54 (*s*, MeO); 7.36 (*m*, 3 arom. H); 7.45 (*m*, 2 arom. H).

18-[(S)-MPTA] Ester of **3**: ^{1}H-NMR: 0.85 (*dd*, $J = 7.0$, Me(32)); 1.2–1.5 (*m*, CH_2(5) to CH_2(9), CH_2(21) to CH_2(31)); 1.41 (*d*, $J = 7.1$, Me(35)); 2.38 (*ddt*, $J = 15, 8, 1.6$, H_a–C(3)); 2.51 (*dddd*, $J = 15, 4, 1.4, 1.1$, H_b–C(3)); 3.82 (*m*, H–C(4)); 3.87 (*m*, H–C(10)); 2.02 (*m*, H_a–C(11)); 1.50 (*m*, H_b–C(11)); 1.94 (*m*, H_a–C(12)); 1.57 (*m*, H_b–C(12)); for H–C(13) to CH_2(20), see *Table 5*; 5.04 (*dq*, $J = 7.1, 1.4$, H–C(34)); 7.17 (*d*, $J = 1.4$, H–C(33)); 3.56 (*s*, MeO); 7.38 (*m*, 3 arom. H); 7.57 (*m*, 2 arom. H).

18-[(R)-MPTA] Ester of **4**: ^{1}H-NMR: 0.85 (*dd*, $J = 7.0$, Me(32)); 1.2–1.5 (*m*, CH_2(5) to CH_2(9), CH_2(21) to CH_2(31)); 1.41 (*d*, $J = 7.1$, Me(35)); 2.38 (*ddt*, $J = 15, 8, 1.6$, H_a–C(3)); 2.51 (*dddd*, $J = 15, 4, 1.4, 1.1$, H_b–C(3)); 3.82 (*m*, H–C(4)); 3.86 (*m*, H–C(10)); 2.11 (*m*, H_a–C(11)); 1.49 (*m*, H_b–C(11)); 1.94 (*m*, H_a–C(12)); 1.56 (*m*, H_b–C(12)); for H–C(13) to CH_2(20), see *Table 5*; 5.04 (*dq*, $J = 7.1, 1.4$, H–C(34)); 7.17 (*d*, $J = 1.4$, H–C(33)); 3.56 (*s*, MeO); 7.37 (*m*, 3 arom. H); 7.57 (*m*, 2 arom. H).

18-[(S)-MPTA] Ester of **4**: ^{1}H-NMR: 0.85 (*dd*, $J = 7.0$, Me(32)); 1.2–1.5 (*m*, CH_2(5) to CH_2(9), CH_2(21) to CH_2(31)); 1.41 (*d*, $J = 7.1$, Me(35)); 2.38 (*ddt*, $J = 15, 8, 1.6$, H_a–C(3)); 2.51 (*dddd*, $J = 15, 4, 1.4, 1.1$, H_b–C(3)); 3.82 (*m*, H–C(4)); 3.88 (*m*, H–C(10)); 2.02 (*m*, H_a–C(11)); 1.51 (*m*, H_b–C(11)); 1.97 (*m*, H_a–C(12)); 1.56 (*m*, H_b–C(12)); for H–C(13) to CH_2(20), see *Table 5*; 5.04 (*dq*, $J = 7.1, 1.4$, H–C(34)); 7.17 (*d*, $J = 1.4$, H–C(33)); 3.55 (*s*, MeO); 7.38 (*m*, 3 arom. H); 7.55 (*m*, 2 arom. H).

Acetonides. To a soln. of 3 mg each of **1**–**4** in 5 ml of acetone were added a few crystals of TsOH. The mixture was stirred at r.t. and monitored by TLC until the reaction was complete. The products were precipitated with hexane and purified by normal-phase HPLC.

19,20-O-Isopropylidenemuricatetrocin A (from **1**): ^{1}H-NMR: 0.85 (*dd*, $J = 7.0$, Me(32)); 1.351 (*s*, 3 H, Me_2C); 1.355 (*s*, 3 H, Me_2C); 1.41 (*d*, $J = 7.1$, Me(35)); 1.2–1.5 (*m*, CH_2(5) to CH_2(11), CH_2(21) to CH_2(31)); 1.54–7 (*m*,

CH_2(17), CH_2(18)); 1.65–1.72 (*m*, H_a–C(13), H_a–C(14)); 1.91–1.96 (*m*, H_b–C(13), H_b–C(14)); 2.38 (*ddt*, *J* = 15, 8, 1.6, H_a–C(3a)); 2.51 (*dddd*, *J* = 15, 4, 1.4, 1.1, H_b–C(3)); 3.38 (*m*, H–C(16)); 3.51–3.64 (*m*, H–C(19), H–C(20)); 3.70 (*q*, *J* = 7.0, H–C(15)); 3.82 (*m*, H–C(4)); 3.85 (*dt*, *J* = 5.9, 6.7, H–C(12)); 5.04 (*dq*, *J* = 7.1, 1.4, H–C(34)); 7.17 (*d*, *J* = 1.4, H–C(33)).

*19,20-*O*-Isopropylidenemuricatetrocin B* (from **2**): ^{1}H-NMR: 0.85 (*dd*, *J* = 7.0, Me(32)); 1.350 (*s*, 3 H, Me_2C); 1.352 (*s*, 3 H, Me_2C); 1.41 (*d*, *J* = 7.1, Me(35)); 1.2–1.5 (*m*, CH_2(5) to CH_2(11), CH_2(21) to CH_2(31)); 1.54–7 (*m*, CH_2(17), CH_2(18)); 1.65–1.72 (*m*, H_a–C(13), H_a–C(14)); 1.91–1.96 (*m*, H_b–C(13), H_b–C(14)); 2.38 (*ddt*, *J* = 15, 8, 1.6, H_a–C(3)); 2.51 (*dddd*, *J* = 15, 4, 1.4, 1.1, H_b–C(3)); 3.39 (*m*, H–C(16)); 3.54–3.60 (*m*, H–C(19), H–C(20)); 3.78 (*q*, *J* = 7.0, H–C(15)); 3.82 (*m*, H–C(4)); 3.85 (*dt*, *J* = 5.9, 6.7, H–C(12)); 5.04 (*dq*, *J* = 7.1, 1.4, H–C(34)); 7.17 (*d*, *J* = 1.4, H–C(33)).

*17,18-*O*-Isopropylidenegigantetrocin B* (from **3**): ^{1}H-NMR: 0.85 (*dd*, *J* = 7.0, Me(32)); 1.352 (*s*, Me_2C); 1.41 (*d*, *J* = 7.1, Me(35)); 1.2–1.5 (*m*, CH_2(5) to CH_2(11), CH_2(21) to CH_2(31)); 1.54–7 (*m*, CH_2(17), CH_2(18)); 1.65–1.72 (*m*, H_a–C(11), H_a–C(12)); 1.91–1.96 (*m*, H_a–C(11), H_a–C(12)); 2.38 (*ddt*, *J* = 15, 8, 1.6, H_a–C(3)); 2.51 (*dddd*, *J* = 15, 4, 1.4, 1.1, H_b–C(3)); 3.41 (*m*, H–C(14)); 3.54–3.61 (*m*, H–C(17), H–C(18)); 3.78 (*q*, *J* = 7.0, H–C(13)); 3.82 (*m*, H–C(4)); 3.85 (*dt*, *J* = 5.9, 6.7, H–C(10)); 5.04 (*dq*, *J* = 7.1, 1.4, H–C(34)); 7.17 (*d*, *J* = 1.4, H–C(33)).

*17,18-*O*-Isopropylidenegigantetrocin A* (from **4**): ^{1}H-NMR: 0.85 (*dd*, *J* = 7.0, Me(32)); 1.351 (*s*, 3 H, Me_2C); 1.352 (*s*, 3 H, Me_2C); 1.41 (*d*, *J* = 7.1, Me(35)); 1.2–1.5 (*m*, CH_2(5) to CH_2(11), CH_2(21) to CH_2(31)); 1.54–7 (*m*, CH_2(15), CH_2(16)); 1.65–1.72 (*m*, H_a–C(11), H_a–C(12)); 1.91–1.96 (*m*, H_e–C(11), H_e–C(12)); 2.38 (*ddt*, *J* = 15, 8, 1.6, H_a–C(3)); 2.51 (*dddd*, *J* = 15, 4, 1.4, 1.1, H_b–C(3)); 3.38 (*m*, H–C(14)); 3.53–3.61 (*m*, H–C(17), H–C(18)); 3.78 (*q*, *J* = 7.0, H–C(13)); 3.82 (*m*, H–C(4)); 3.85 (*dt*, *J* = 5.9, 6.7, H–C(10)); 5.04 (*dq*, *J* = 7.1, 1.4, H–C(34)); 7.17 (*d*, *J* = 1.4, H–C(33).

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REFERENCES

[1] S. D. Jolad, J. J. Hoffmann, K. H. Schram, J. R. Cole, *J. Org. Chem.* **1982,** *47*, 3151.
[2] S. D. Jolad, J. J. Hoffmann, J. R. Cole, C. E. Barry, R. B. Bates, G. S. Linz, *J. Nat. Prod.* **1985,** *48*, 644.
[3] J. Morton, 'Fruits of Warm Climates', Media Inc., Miami, FL, 1973, p. 65.
[4] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobson, D. E. Nichols, J. L. McLaughlin, *Planta Med.* **1982,** *45*, 31.
[5] J. L. McLaughlin, 'Methods in Plant Biochemistry, Ed. K. Hostettman, Academic Press, London, 1991, Vol. 6, p. 1.
[6] T. G. McCloud, D. L. Smith, C.-J. Chang, J. M. Cassady, *Experientia* **1987,** *43*, 947.
[7] J. M. Cassady, C.-J. Chang, R. G. Cooks, 'Natural Products Chemistry', Eds. Atta-ur-Rahman and P. W. LeQuesne, Springer Verlag, Vienna, 1988, Vol. III, p. 291.
[8] L.-Z. Xu, C.-J. Chang, J. G. Yu, J. M. Cassady, *J. Org. Chem.* **1989,** *54*, 5418.
[9] M. J. Rieser, X. P. Fang, J. K. Rupprecht, Y.-H. Hui, D. L. Smith, J. L. McLaughlin, *Planta Med.* **1993,** *59*, 91.
[10] M. J. Rieser, J. F. Kozlowski, K. V. Wood, J. L. McLaughlin, *Tetrahedron Lett.* **1991,** *32*, 1137.
[11] C. Bories, P. Loiseau, D. Cortes, S. H. Myint, A. Laurens, R. Hocquemiller, P. Gayral, A. Cave, A. Laurens, *Planta Med.* **1991,** *57*, 434.
[12] S. H. Myint, A. Laurens, R. Hocquemiller, A. Cave, D. Davoust, D. Cortes, *Heterocycles* **1990,** *31*, 861.
[13] D. Cortes, S. H. Myint, A. Laurens, R. Hocquemiller, M. Leboeuf, A. Cave, *Can. J. Chem.* **1991,** *69*, 8.
[14] S. H. Myint, D. Cortes, A. Laurens, R. Hocquemiller, M. Leboeuf, A. Cave, J. Cotte, A.-M. Queros, *Phytochemistry* **1991,** *30*, 3335.
[15] A. Alkofahi, J. K. Rupprecht, Y.-M. Liu, C.-J. Chang, D. L. Smith, J. L. McLaughlin, *Experientia* **1990,** *46*, 53.
[16] Y.-H. Hui, J. K. Rupprecht, J. E. Anderson, Y.-M. Liu, C.-J. Chang, D. L. Smith, J. L. McLaughlin, *Tetrahedron* **1989,** *45*, 6941.
[17] X. P. Fang, J. K. Rupprecht, A. Alkofahi, Y.-H. Hui, Y.-M. Liu, D. L. Smith, K. V. Wood, J. L. McLaughlin, *Heterocycles* **1991,** *32*, 11.

[18] J. K. Rupprecht, C.-J. Chang, J. M. Cassady, J. L. McLaughlin, K. L. Mikolajczak, D. Weisleder, *Heterocycles* **1986,** *24,* 1197.
[19] J. K. Rupprecht, Y.-H. Hui, J. L. McLaughlin, *J. Nat. Prod.* **1990,** *53,* 237.
[20] B. Fuchs, *Topics Stereochem.* **1978,** *10,* 1.
[21] B. Fuchs, P. S. Wechsler, *Tetrahedron* **1977,** *33,* 57.
[22] Y. Fujimoto, C. Murasaki, K. Kakinuma, T. Eguchi, N. Ikekawa, M. Furuya, K. Hirayama, T. Ikekawa, M. Sahai, Y. K. Gupta, A. B. Ray, *Tetrahedron Lett.* **1990,** *31,* 535.
[23] M. J. Rieser, Y.-H. Hui, J. K. Rupprecht, J. F. Kozlowski, K. V. Wood, J. L. McLaughlin, P. R. Hanson, Z. Zhuang, T. R. Hoye, *J. Am. Chem. Soc.* **1992,** *114,* 10203.
[24] J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973,** *95,* 512.
[25] G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem.* **1973,** *38,* 2143.
[26] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991,** *113,* 4092.
[27] E. Kurosawa, A. Fukuzawa, T. Irie, *Tetrahedron Lett.* **1972,** *21,* 2121.
[28] A. Fukuzawa, E. Kurosawa, T. Irie, *Tetrahedron Lett.* **1972,** *3,* 851.
[29] N. R. Ferrigini, J. E. Putnam, L. B. Jacobson, D. E. Nichols, J. L. Moore, J. L. McLaughlin, R. G. Powell, C. R. Smith, *J. Nat. Prod.* **1982,** *45,* 679.
[30] J. E. Anderson, C. M. Goetz, J. L. McLaughlin, M. Suffness, *Phytochem. Anal.* **1991,** *2,* 107.
[31] D. J. Giard, S. A. Aaronson, G. J. Todaro, P. Arnstein, J. H. Kersey, H. Dosik, W. P. Parks, *J. Natl. Cancer Inst.* **1973,** *55,* 1417.
[32] H. D. Soule, J. Vazquez, A. Long, S. Albert, M. Brennan, *J. Natl. Cancer Inst.* **1973,** *51,* 1409.
[33] 'Human Tumor Cells, In Vitro', Ed. J. Fogh, Plenum Press, New York, 1975, p. 115.
[34] M. Londerhausen, W. Leicht, F. Leib, H. Moeschler, *Pestic. Sci.* **1991,** *33,* 427.
[35] M. A. Lewis, J. T. Arnason, B. J. Philogene, J. K. Rupprecht, J. L. McLaughlin, *Pestic. Biochem. Physiol.* **1993,** *45,* 15.
[36] K. Ahammadsahib, R. M. Hollingworth, P. J. McGovern, Y.-H. Hui, J. L. McLaughlin, *Life Sci.* **1993,** *53,* 1113.
[37] X. P. Fang, M. J. Rieser, Z. M. Gu, G. X. Zhao, *Phytochem. Anal.* **1993,** *4,* 27, 49.