1, 143034-09-7; 13 isomer 2, 143773-90-4; 14, 143746-76-3; 15, 143746-71-8; 16, 143746-77-4; 2-iodothiophene, 3437-95-4; 1,4-dihydroxynaphthalene, 571-60-8; 2,3-thiophenedicarboxaldehyde, 932-41-2; dithieno[2,3-*b*][6,7-*b*]-9,10-anthraquinone, 143746-72-9; 1,4-cyclohexanedione, 637-88-7; malononitrile, 109-77-3.

Supplementary Material Available: X-ray data for $C_{14}N_4S_1H_6$ and $C_{15}N_2O_1S_1H_6$ (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Cytotoxic Polyketides from *Annona densicoma* (Annonaceae): 10,13-*trans*-13,14-*erythro*-Densicomacin, 10,13-*trans*-13,14-*threo*-Densicomacin, and 8-Hydroxyannonacin

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Three new linear polyketides, 10,13-*trans*-13,14-*erythro*-densicomacin (1), 10,13-*trans*-13,14-*threo*-densicomacin (2), and 8-hydroxyannonacin (3), and a known polyketide goniothalamicin were isolated from the stem bark of the Peruvian plant *Annona densicoma* Mart (Annonaceae). Their structures were elucidated on the basis of UV, IR, ^1H and ^{13}C NMR, and mass spectrometry data of the natural compounds and their derivatives. These polyketides are cytotoxic against human tumor cells in culture. In particular, densicomacins (1 and 2) were significantly active against the lung carcinoma (A-549) and the colon adenocarcinoma (HT-29) cell lines with ED_{50} at 4×10^{-4} and 1×10^{-5} μg/mL, respectively.

In earlier studies we reported four cytotoxic polyketides, annonacin,[1] annonacin-10-one, isoannonacin, and isoannonacin-10-one,[2] from the Peruvian plant *Annona densicoma* Mart. These polyketides possess a C_{35} skeleton with one tetrahydrofuran ring, one lactone moiety, and several hydroxyl groups. In this paper[3] we report the discovery of three novel cytotoxic polyketides from the stem bark of *A. densicoma*: 10,13-*trans*-13,14-*erythro*-densicomacin (1), 10,13-*trans*-13,14-*threo*-densicomacin (2), and 8-hydroxyannonacin (3) as well as a known polyketide (goniothalamicin (4)) which was first isolated from *Goniothalamus giganteus* Hook, f., Thomas (Annonaceae) by Alkofahi et al.,[4] (Chart I). Compounds 1 and 2 represented the first two examples of C_{35} polyketides with the tetrahydrofuran ring located between C-10 and C-13.

Results and Discussion

Densicomacin was isolated as white crystals, mp 83–4 °C, $[\alpha]_D$ +26° (*c* 0.05, MeOH). The molecular formula was established to be $C_{35}H_{64}O_7$ by high resolution CI-MS: obsd 597.4716 (MH^+), calcd 597.4730. The IR absorption band at 1748 cm^{-1}, the UV absorption at λ_{max} (MeOH) 209.5 nm (log ϵ, 3.85), the proton signals at δ 7.19, 5.05, and 1.43, and the carbon signals at δ 174.59, 151.85, 131.11, and 19.06 were characteristic for the α,β-unsaturated lactone moiety typical of the annonacin-type[1] of polyketides (Table I). The EI-MS ions of 227, 239, 281, 297, 333, 351, and others suggested the position of the tetrahydrofuran ring to be at C-10 and C-13 and those of the hydroxyl groups at C-4, C-14, C-17, and C-18 as shown (Scheme I, Table II). The conversion of densicomacin to its isomer[5] with KOH/*t*-BuOH confirmed the presence of a hydroxyl group at C-4. By ^1H–^1H 2D-COSY, H-13 of densicomacin was found to be coupled to H-14 while H-10 was not coupled to any hydroxyl methine proton. This pattern was also observed in the ^1H NMR spectra of the tetraacetate (5), acetonide (6), and acetonide diacetate (7) derivatives. The assignment of the last two hydroxyl groups at C-17 and C-18 was substantiated by the formation of the acetonide 6, the acetonide diacetate 7, and pentadecanoic acid by treatment with sodium periodate. In compound 6, the *gem*-dimethyl signal appeared as a singlet at δ 1.38 (6 H). Downfield shifts were observed for the H-17 (from δ 3.43 to 3.58) and H-18 (from δ 3.40 to 3.58) protons. The conversion of 6 to 7 induced downfield shifts at H-4 (from δ 3.85 to 5.10) and H-14 (From δ 3.41 to 4.89) but not at H-17 or H-18).

From the ^{13}C and ^1H NMR data (Table I), densicomacin was observed as a mixture of two stereoisomers. These isomers were resolvable by preparative TLC as the mesitoates 8 and 9. The H-13 and H-14 signals of the me-

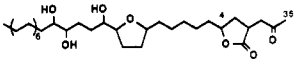
(1) McCloud, T. G.; Smith, D. L.; Chang, C.-j.; Cassady, J. M. *Experientia* **1987**, *43*, 947.

(2) Xu, L.; Chang, C.-j.; Yu, J. G.; Cassady, J. M. *J. Org. Chem.* **1989**, *54*, 5418.

(3) A portion of the results was presented at the 1989 International Chemical Congress of Pacific Basin Societies, Honolulu, Haiwaii, Organic Chemistry Division, Abstract 182. The structure of annonadencin as reported has been revised and renamed as densicomacin.

(4) Alkofahi, A.; Rupprecht, J. K.; Smith, D. L.; Chang, C.-j.; McLaughlin, J. L. *Experientia* **1987**, *44*, 83.

(5) Densicomacin (4 mg) was treated with 2% KOH in *t*-BuOH (0.5 mL) at room temperature for 24 h. The reaction mixture was acidified with dilute HCl and extracted with CH_2Cl_2. The extract was purified by preparative TLC to give isodensicomacin (1 mg): CI-MS *m/z* (rel int) 597 (MH^+, 100), 579 (12), 561 (30); EI-MS *m/z* (rel int) 351 (2), 333 (2), 315 (0.3), 297 (3), 281 (55), 263 (5), 245 (8), 239 (50), 221 (15), 141 (15), 123 (30); ^1H NMR (250 MHz in $CDCl_3$) δ 4.39 (m, H-4 of 2,4-cis isomer), 4.54 (m, H-4 of 2,4-trans isomer), 2.20 (s, H-35).



0022-3263/92/1957-6198$03.00/0

Table I. ^{13}C and ^{1}H NMR Data of 10,13-*trans*-13,14-*erythro*- and 10,13-*trans*-13,14-*threo*-Densicomacin (1, 2), Densicomacin Tetraacetates (5), Densicomacin Acetonides (6), and Densicomacin Acetonide Diacetate (7)

C/H	^{13}C(1 and 2)[a,b] (125.8 MHz, $CDCl_3$)	^{1}H(1 and 2)[a,c] (500 MHz, $CDCl_3$)	^{1}H(5)[a,c] (500 MHz, $CDCl_3$)	^{1}H(6)[c] (250 MHz, $CDCl_3$)	^{1}H(7)[a,c] (500 MHz, $CDCl_3$)
1	174.59 s				
2	131.11 s				
3a	33.32 t	2.39 dd (14, 8.2)	2.51 dd (14, 8.0)	2.38 ddt (14, 8.2, 1.2)	2.51 dd (14, 7.8)
3b		2.51 br d (14)	2.56 dd (14, 4.4)	2.52 ddt (14, 3.4, 1.6)	2.56 ddt (14, 4.4, 1.7)
4	69.85 d	3.83 m	5.10 m	3.85 m	5.10 m
10	79.31 d	3.88 m	3.85 m	3.85 m	3.87 m
11	32.36 t	1.50, 2.02 m	1.50, 2.02 m	1.52, 2.02 m	1.47, 2.00 m
12	28.39 t	1.58, 1.97 m	1.60, 1.97 m	1.58, 1.98 m	1.62, 1.98 m
13	81.81, 81.76 d	3.79 m	3.94 m	3.78 m	3.98 m
14	74.49, 74.37 d[d]	3.42 m	4.86 m, 4.81 m	3.41 m	4.89 m
17	74.35, 74.25 d[d]	3.43 m	4.97 m	3.58 m	3.58 m
18	74.72 d[d]	3.40 m	4.97 m	3.58 m	3.55 m
32	14.06 q	0.88 t (6.8)	0.88 t (6.8)	0.88 t (6.8)	0.88 t (7.0)
33	151.85 d	7.19 d (1.4)	7.07 d (1.4)	7.19 d (1.3)	7.07 d (1.3)
34	77.97 d	5.05 qd (6.8, 1.4)	5.00 m	5.04 qd (6.8, 1.3)	5.00 qd (6.8, 1.3)
35	19.06 q	1.43 d (6.8)	1.40 d (6.8)	1.44 d (6.8)	1.39 d (6.8)
ketal Me				1.38 s (6 H)	1.36 s (6 H)
OAc			2.020 s (3 H)		2.02 s (3 H)
			2.069 s (3 H)		
			2.075 s (3 H)		
			2.079, 2.084 s (3 H)		2.085, 2.088 s (3 H)

[a] According to ^{1}H–^{13}C NMR HETCOR and COSY (500–125.8 MHz) in $CDCl_3$, TMS as the standard. [b] Signals of other methylene carbons occur between δ 22 and 38 with considerable overlap at approximately the following positions: δ 37.24, 35.40, 33.66, 33.48, 31.88, 30.38, 29.91, 29.71, 29.65, 29.61, 29.47, 29.31, 29.11, 26.07, 25.73, 25.70, 25.46, and 22.64. [c] Signals of other protons occur between δ 1.26 and 1.65 m. [d] Signals may be interchanged.

Table II. Exact Mass Measurement and Elemental Composition of 10,13-*cis*-Densicomacin (1), 10,13-*trans*-Densicomacin (2), 8-Hydroxyannonacin (3), Goniothalamicin (4)

1, 2			3			4		
m/z of fragments	calcd	compstn	*m/z* of fragments	calcd	compstn	*m/z* of fragments	calcd	compstn
351.2161	351.2171	$C_{20}H_{31}O_5$	631.3718	631.3702	$C_{30}H_{63}O_6Si_4$	333.2059	333.2066	$C_{20}H_{29}O_4$
333.2053	333.2066	$C_{20}H_{29}O_4$	357.1915	357.1917	$C_{17}H_{33}O_4Si_2$	315.1951	315.1960	$C_{20}H_{27}O_3$
297.2766	297.2793	$C_{19}H_{37}O_2$	271.2456	271.2457	$C_{16}H_{35}OSi$	297.2784	297.2793	$C_{19}H_{37}O_2$
279.2676	279.2688	$C_{19}H_{35}O$	213.0946	213.0947	$C_{10}H_{17}O_3Si$	279.2686	279.2688	$C_{19}H_{35}O$
281.1752	281.1753	$C_{16}H_{25}O_4$				281.1767	281.1753	$C_{16}H_{25}O_4$
263.1643	263.1647	$C_{16}H_{23}O_3$				263.1649	263.1647	$C_{16}H_{23}O_3$
245.1528	245.1542	$C_{16}H_{21}O_2$				245.1545	245.1542	$C_{16}H_{21}O_2$
257.2478	257.2480	$C_{16}H_{33}O_2$				241.1476	241.1440	$C_{13}H_{21}O_4$
239.2377	239.2377	$C_{16}H_{31}O$				223.1364	223.1334	$C_{13}H_{19}O_3$
227.2368	227.2375	$C_{15}H_{31}O$				141.0588	141.0552	$C_7H_9O_3$
193.1225	193.1229	$C_{12}H_{17}O_2$						
175.1117	175.1123	$C_{12}H_{15}O$						
123.0438	123.0446	$C_7H_7O_2$						

sitoates in C_6D_6 were 4.12 and 5.54 in **8** and 4.09 and 5.36 in **9**. Applying an NMR method developed in our laboratories,[6] the relative stereochemistry of C-10, C-13/C-14 was assigned as trans-erythro in **8** and trans-threo in **9** (Table III). Therefore *A. densicoma* contained both 10,13-*trans*-13,14-*erythro*-densicomacin (**1**) and 10,13-*trans*-13,14-*threo*-densicomacin (**2**).

Compound **3** was isolated as a waxy solid, $[\alpha]_D$ +6.1° (*c* 0.12, MeOH). It showed the characteristic IR spectral absorption at 1749 cm^{-1} and UV absorption at λ_{max} (MeOH) 209.5 nm (log ε, 4.22) for an α,β-unsaturated lactone. The typical proton signals for the lactone moiety were found at δ 7.18, 5.05, and 1.44 (Table IV). The molecular formula was determined to be $C_{35}H_{64}O_8$ by FAB-MS (obsd 613.4772 (MH^+), calcd 613.4679), which was one oxygen more than that of annonacin ($C_{35}H_{64}O_7$). The ^{1}H NMR of its acetate (**10**) showed five acetoxyl groups (δ 2.022, 2.077, 2.088 each 3 H; 2.067 6 H), suggesting that **3** contained a total of five hydroxyl groups. The MS fragment ions from cleavage adjacent to the tetrahydrofuran ring in **3** were 269, 325, and 395. Also, the exact mass of selected fragments of the TMS derivative of **3** were at 213.0946 (calcd for $C_{10}H_{17}O_3Si$ 213.0947), 271.2456 (calcd for $C_{16}H_{35}OSi$ 271.2457), 357.1915 (calcd for $C_{17}H_{33}O_4Si_2$ 357.1917), and 631.3718 (calcd for $C_{30}H_{63}O_6Si_4$ 631.3702) (Table II). These characteristic fragment ions led to the conclusion that **3** was 8-hydroxyannonacin.

(6) Gale, J. B.; Yu, J. G.; Ho, D. K.; Cassady, J. M. Results to be published. A series of six model compounds (1–6) representing all the possible relative stereochemical relationships between the dihydroxyl groups and the tetrahydrofuranyl ring were synthesized. The chemical shift pattern of the ring junction methine and the carbinol methine signals in C_6D_6 was utilized to assign stereochemistry. The shifts of these two protons were 3.97 and 5.29 for the threo-cis model, 3.93 and 5.45 for the erythro-cis model, 4.09 and 5.35 for the threo-trans model, and 4.05 and 5.48 for the erythro-trans model. These values were compared to the corresponding signals of the mesitoylated natural polyketides. The model that gave the smallest sum of the absolute values of the chemical shift differences would have the same stereochemistry as the natural products.

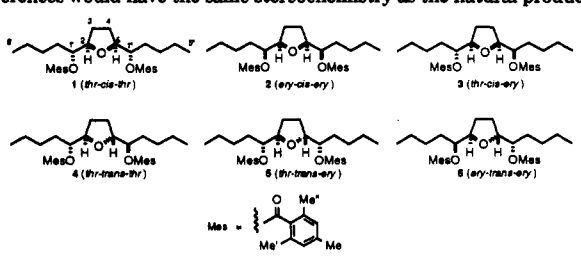


Table III. ^{1}H NMR (500 MHz) Data of 10,13-*trans*-13,15-*erythro*-Densicomacin Tetramesitoate (8) and 10,13-*trans*-13,14-*threo*-Desicomacin Tetramesitoate (9) in $CDCl_3$ and C_6D_6

H	compound 8		compound 9	
	$CDCl_3$	C_6D_6[a]	$CDCl_3$	C_6D_6[a]
3a	2.70 dd (14, 7.6)	2.64 dd (14, 7.7)	2.70 dd (14, 7.6)	2.64 dd (14, 7.7)
3b	2.61 ddt (14, 4.7, 1.7)	2.48 m	2.61 ddt (14, 4.6, 2.0)	2.48 m
4	5.41 m	5.52 m	5.41 m	5.53 m
10	3.92 m	3.93 m	3.91 m	3.90 m
11	1.64, 2.02 m	1.53, 1.77 m	1.52, 2.02 m	1.53, 1.73 m
12	1.64, 1.93 m	1.68, 1.75 m	1.65, 2.00 m	1.46, 1.73 m
13	4.05 dd (11, 7.1)	4.12 dd (11, 7.1)	4.07 dd (11, 7.3)	4.09 dd (11, 7.1)
14	5.17 m	5.54 m	5.12 m	5.36 dt (11, 3.7)
17	5.39 m	5.83 m	5.33 m	5.68 m
18	5.33 m	5.71 m	5.30 m	5.65 m
32	0.88 t (6.9)	0.91 t (6.9)	0.88 t (6.9)	0.91 t (6.9)
33	7.17 d (1.3)	6.44 d (1.4)	7.17 d (1.3)	6.44 d (1.4)
34	4.99 qd (6.8, 1.3)	4.25 qd (6.8, 1.4)	4.99 qd (6.8, 1.3)	4.25 qd (6.8, 1.4)
35	1.39 d (6.8)	0.81 d (6.8)	1.39 d (6.8)	0.81 d (6.8)
Ph-CH_3	2.235 s (2 × CH_3)	2.284 s (3 × CH_3)	2.230 s (3 × CH_3)	2.284 s (3 × CH_3)
	2.243 s (2 × CH_3)	2.369 s (3 × CH_3)	2.242 s (3 × CH_3)	2.334 s (3 × CH_3)
	2.249 s (2 × CH_3)	2.381 s (3 × CH_3)	2.255 s (3 × CH_3)	2.384 s (3 × CH_3)
	2.264 s (2 × CH_3)	2.541 s (3 × CH_3)	2.270 s (3 × CH_3)	2.485 s (3 × CH_3)
	2.269 s (2 × CH_3)			
	2.291 s (2 × CH_3)			
Ph-H	6.799 s (2 H)	6.601 s (4 H)	6.749 s (2 H)	6.567 s (2 H)
	6.810 s (4 H)	6.637 s (2 H)	6.770 s (2 H)	6.626 s (2 H)
	6.832 s (2 H)	6.667 s (2 H)	6.826 s (2 H)	6.636 s (2 H)
			6.831 s (2 H)	6.658 s (2 H)

[a] According to 2D-COSY (500 MHz).

Table IV. ^{13}C NMR (125.8 MHz) and ^{1}H NMR (500 MHz) Data ($CDCl_3$) of 8-Hydroxyannonacin (3), Goniothalamicin (4), and Their Acetates 10 and 11

C/H	^{1}H(3)[a] (500 MHz, $CDCl_3$)	^{1}H(10)[a] (500 MHz, $CDCl_3$)	^{1}H(4)[a,b] (500 MHz, $CDCl_3$)	^{13}C(4)[b,c] (125.8 MHz, $CDCl_3$)	^{1}H(11)[a] (500 MHz, $CDCl_3$)
1				174.58 s	
2				131.17 s	
3a	2.39 dd (14, 7.5)	2.51 dd (14, 7.8)	2.40 ddt (14, 8.3, 1.2)	33.36 t	2.51 ddt (14, 7.8, 1.2)
3b	2.50 d (14)	2.56 d (14)	2.52 ddt (14, 3.5, 1.7)		2.56 ddt (14, 4.4, 1.7)
4	3.84 m	5.08 m	3.84 m	69.88 d	5.09 m
8	3.84 m	4.96 m	1.26–1.65 m	22–38 t	1.26–1.65 m
10	3.61 m	4.96 m	3.63 m	71.49 d	4.84 m
13	1.26–1.65 m	1.26–1.65 m	3.46 m	74.34 d	4.83 m
14	1.26–1.65 m	1.26–1.65 m	3.83 m	82.53 d	3.97 dd (10.4, 6.5)
15	3.38 m	4.84 m	1.67, 2.00 m	28.79 t	1.62, 1.97 m
16	3.77 dd (12, 6.5)	3.96 m	1.67, 2.00 m	28.79 t	1.62, 1.97 m
17	1.54, 1.97 m	1.58, 1.96 m	3.80 m	82.69 d	3.97 dd (10.4, 6.5)
18	1.54, 1.97 m	1.58, 1.96 m	3.41 dd (10.3, 6.6)	74.09 d	4.83 m
19	3.77 dd (12, 6.5)	3.96 m	1.26–1.65 m	22–38 t	1.26–1.65 m
20	3.38 m	4.84 m	1.26–1.65 m	22–38 t	1.26–1.65 m
32	0.88 t (6.8)	0.88 t (7.0)	0.88 t (7.0)	14.09 q	0.88 t (7.0)
33	7.18 d (1.4)	7.08 d (1.4)	7.19 d (1.3)	151.83 s	7.07 d (1.4)
34	5.05 qdd (6.8, 2.5, 1.2)	5.01 qd (6.8, 1.4)	5.05 qd (6.8, 1.3)	77.96 d	5.01 qd (6.8, 1.3)
35	1.44 d (6.8)	1.40 d (6.8)	1.43 d (6.8)	19.09 q	1.40 d (6.8)
OAc		2.022 s (3 H)			2.022 s (3 H)
		2.067 s (6 H)			2.037 s (3 H)
		2.077 s (3 H)			2.068 s (3 H)
		2.088 s (3 H)			2.073 s (3 H)

[a] Signals of other protons occur between δ 1.26 and 1.65. [b] According to ^{1}H–^{13}C NMR HETCOR and COSY (500–125.8 MHz) in $CDCl_3$, TMS as the standard. [c] Signals of other occur between δ 22 and 38 at the following positions: δ 37.27, 33.53, 33.47, 31.91, 29.67, 29.45, 29.34, 25.57, 25.47, 22.67.

Compound 4 was isolated as white crystals, mp 91–2 °C, $[\alpha]_D$ +10.4° (*c*, 0.08, MeOH). Comparison of spectroscopic data led to the conclusion that 4 and goniothalamicin[4] were identical. The ring junction and the carbinol methines of the mesitoate derivative of 4 in C_6D_6 were at δ 4.11 and 5.31. By comparison to model compounds,[6] 4 was determined to possess threo-trans-threo relative stereochemistry across the tetrahydrofuranyl ring.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The IR spectra were measured on a Laser Precision Analytical RFX-40 FT-IR spectrometer. The UV spectra were obtained on a Beckman DU-7 spectrometer. The optical rotations were determined on a Perkin-Elmer 241 digital polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as standard on a Bruker AM-500 NMR spectrometer. Mass spectra were measured on a Finnigan 4023 GC/MS with INCOS 2000 data system. High resolution mass spectra were measured on a VG 70-250S or a Kratos MS-50 mass spectrometer.

Plant Material. Stem bark of *A. densicoma* Mart. (Annonaceae) was collected in 1981 in Peru by the Natural Products Branch of the National Cancer Institute. A voucher specimen is deposited in the Economic Botany Laboratory, United States Department of Agriculture, Beltsville, MD.

Extraction and Isolation. Dry stem bark of *A. densicoma* (10 kg) was ground and exhaustively percolated with CH_2Cl_2 to

Chart I. Structures of Compounds 1–11

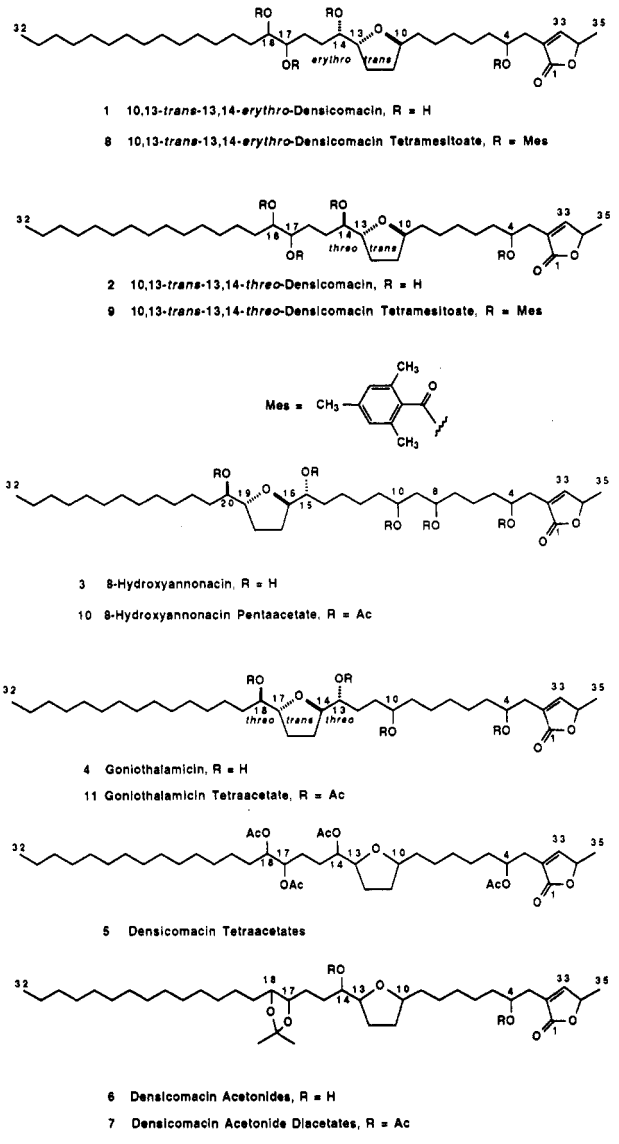


Table V. Cytotoxicities (ED_{50} in μg/mL) in Tumor Cell Cultures[a]

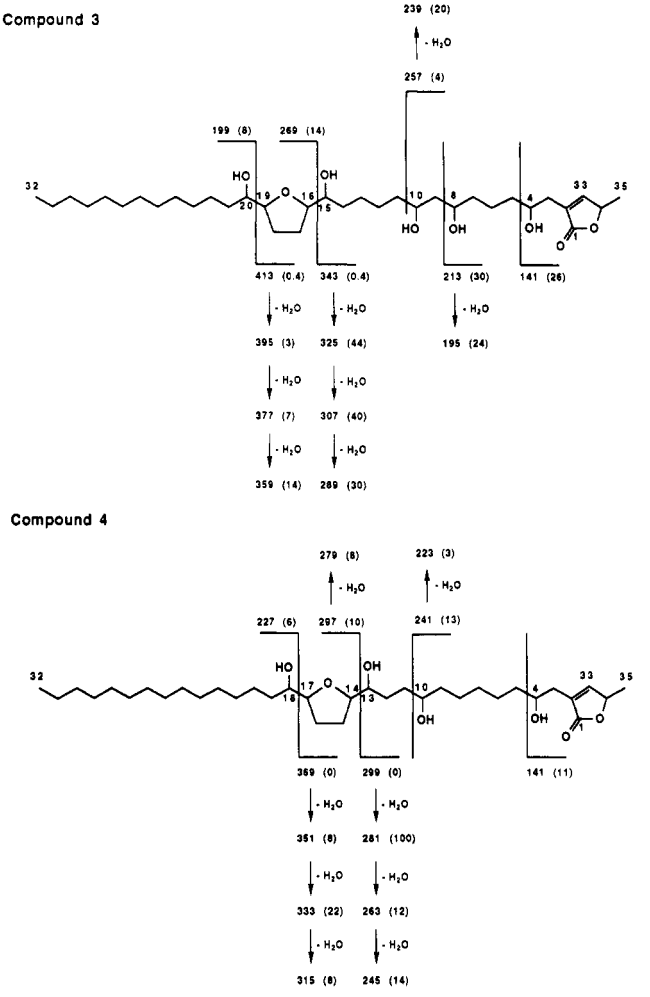
compounds	P388	A549	HT-29
annonacin	1×10^{-5}	1×10^{-3}	3
densicomacin	5×10^{-5}	4×10^{-4}	1×10^{-5}
8-hydroxyannonacin	2×10^{-1}	5×10^{-2}	3×10^{-1}
goniothalamicin	2×10^{-1}	8×10^{-2}	2

[a] P388, mouse leukemia; A549, human lung carcinoma; HT29, human colon adenocarcinoma.

give a brown solid (132 g). This fraction was partitioned between 10% aqueous MeOH and hexane. The 10% aqueous MeOH fraction (29.6 g), which showed significant cytotoxicity in mouse lymphocytic leukemia (P-388) (ED_{50}: 5×10^{-9} μg/mL) (Table V) was subjected to silica gel column chromatography and eluted with $CHCl_3$ containing increasing amounts of MeOH. The eluants were combined to five fractions on the basis of TLC and biological activity. Further purification by recrystallization resulted in annonacin (1 g), annonacin-10-one (210 mg), isoannonacin (140 mg), and isoannonacin-10-one (10 mg).[1,2] From the mother liquor of annonacin, densicomacins (1 and 2, 210 mg) and goniothalamicin (4, 30 mg) were isolated by preparative TLC on silica (250 μm, 20 cm × 20 cm, EtOAc/MeOH 97:3). 8-Hydroxyannonacin (3, 4 mg) was isolated preparative TLC on RP-2 (250 μm, 20 cm × 20 cm, CH_3CN/H_2O/MeOH 40:50:10).

Densicomacins (1 and 2): white crystals, mp 83–4 °C, $[\alpha]_D$ +26° (*c*, 0.05, MeOH); UV λ_{max} (MeOH) 209.5 nm (log ε, 3.85),

Scheme I



IR ν_{max} (KBr) 3420, 1748, 1740, 1470, 1471, 1373, 1119, 1064 cm^{-1}, CI-MS obsd 597.4716 (MH^+), calcd for $C_{35}H_{65}O_7$, 597.4730, EI-MS: see Table II. ^1H and ^{13}C NMR: see Table I.

Densicomacin Tetraacetate (5). Acetylation of densicomacin was carried out with Ac_2O–pyridine and yielded the tetraacetate 5: CI-MS, *m/z* (rel int) 765 (MH^+, 41) 705 ($MH^+ - 60$, 100), 645 ($MH^+ - 2 \times 60$, 8); EI-MS, *m/z* (rel int) 435 (7) 375 (5), 323 (77), 315 (3), 281 (4), 263 (68), 245 (50), 221 (3). ^1H NMR: see Table I.

Densicomacin trimethylsilyl ether derivative: CI-MS, *m/z* 885 (MH^+, 5), 795 ($MH^+ - 90$, 30), 705 ($MH^+ - 2 \times 90$, 12); EI-MS, *m/z* (rel int) 585 (18), 531 (1), 495 (19), 441 (11), 405 (45), 353 (15), 351 (14), 311 (5), 299 (13), 263 (5), 245 (7), 221 (4), 213 (13).

Densicomacin Acetonide (6). Densicomacin (10 mg) was reacted with acetone (5 mL) in the presence of *p*-toluenesulfonic acid (1 mg) at room temperature overnight. The acetonide 6 (6 mg) was purified by preparative TLC on silica (250 μm, 20 cm × 20 cm) with hexane–EtOAc (1:1): CI-MS, *m/z* (rel int) 637 (MH^+) (42), 579 ($MH^+ - 58$) (36), 561 (100); EI-MS, *m/z* (rel int) 637 (MH^+) (0.3), 579 (0.6), 367 (0.3), 351 (2), 333 (3), 297 (28), 281 (25), 279 (5), 263 (8), 245 (9), 239 (52), 221 (6), 141 (10), 123 (17). ^1H NMR: see Table I.

Densicomacin Acetonide Diacetate (7). The acetonide acetate 7 was prepared from 6 using Ac_2O in pyridine and purified with preparative TLC plates. ^1H NMR: see Table I.

10,13-*trans*-13,13-*erythro*-Densicomacin Tetramesitoate (8) and 10,13-*trans*-13,14-*threo*-Densicomacin Tetramesitoate (9). Densicomacin (5 mg) was treated with 2,4,6-trimethylbenzoyl chloride (20 μL) and *N,N*-diisopropylethylamine (5 μL) in $CHCl_3$ (0.2 mL) at room temperature overnight, and the mixtures was separated by TLC on silica gel (250 μm, 20 cm × 20 cm) with hexane–EtOAc (80:20). 10,13-*cis*-Densicomacin tetramesitoate (8, 2 mg) (higher R_f) and 10,13-*trans*-densicomacin tetramesitoate (9, 2 mg) (lower R_f) were obtained in separate fractions. ^1H NMR: see Table III. FAB-MS of compound 8: *m/z* (rel int) 1181 (MH^+,

10), 1180 (M^+, 13), 1018 (70), 1017 ($MH^+ - 164$, 100), 854 (17), 853 ($MH^+ - 2 \times 164$, 28), 690 (13), 689 ($MH^+ - 3 \times 164$, 28), 525 ($MH^+ - 4 \times 164$, 10), 427 (18). FAB-MS of compound **9**: m/z (rel int) 1181 (MH^+, 11), 1180 (M^+, 12), 1018 (70), 1017 ($MH^+ - 164$, 100), 854 (16), 853 ($MH^+ - 2 \times 164$, 27), 690 (13), 689 ($MH^+ - 3 \times 164$, 27), 525 ($MH^+ - 4 \times 164$, 9), 427 (18).

Oxidation of Densicomacin with Sodium Periodate. Densicomacin (10 mg) was treated with $NaIO_4$ (100 mg) in dioxane–water (3:1) (4 mL) for 170 h at room temperature. The reaction product was isolated by preparative TLC (250 μm, 20 cm × 20 cm) with with $CHCl_3$–MeOH (97:3). The major product was pentadecanoic acid (2 mg): HR-EI-MS 242.2239, calcd for $C_{15}H_{30}O_2$ 242.2246; ^1H NMR (500 MHz, $CDCl_3$–CD_3OD), δ 0.79 (t, J = 6.8 Hz, 3 H, H-15), 1.51 (m, 2 H, H-14), 2.14 (t, J = 7.5 Hz, 2 H, H-2). Pentadecanoic acid was reacted with CH_2N_2 to give methyl pentadecanoate: FAB-MS 257 (MH^+, 100); ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3 H, H-15), 1.62 (m, 2 H, H-14), 2.30 (t, J = 7.6 Hz, 2 H, H-2), 3.67 (s, 3 H, RCO_2CH_3).

8-Hydroxyannonacin (3): waxy solid, $[\alpha]_D$ +6.1° (c, 0.12, MeOH); UV(MeOH) λ_{max} 209.5 nm (log ε, 4.22); IR ν_{max} (film) 3421, 1749, 1465, 1404, 1216, 1120, 1080 cm^{-1}; FAB-MS, m/z 613.4772 $(MH)^+$ calcd 613.4679 for $C_{35}H_{64}O_8$+H. EI-MS: see Table II and Scheme I.

8-Hydroxyannonacin trimethylsilyl ether derivative: EI-MS, m/z (rel int) 701 (1), 631 (4), 611 (4), 541 (4), 521 (5), 451 (9), 473 (12), 371 (15), 357 (44), 341 (20), 267 (10), 271 (52), 213 (30).

8-Hydroxyannonacin pentaacetate (10): CI-MS, m/z (rel int) 823 (MH^+, 3), 763 (20), 703 (30); EI-MS, m/z (rel int) 581 (1), 521 (3), 511 (0.1), 461 (2), 451 (0.2), 401 (2), 391 (1), 383 (3), 331 (3), 323 (3), 311 (30), 297 (3), 263 (7), 251 (20), 237 (10), 191 (12), 183 (8), 123 (27). ^1H NMR: see Table IV.

Goniothalamicin (4): white crystals, mp 91–2 °C, $[\alpha]_D$ +10.4° (c, 0.08, MeOH); UV λ_{max}, (MeOH) 209.5 nm (log ε, 3.90); IR ν_{max} (KBr) 3463, 1749, 1479, 1430, 1332, 1119, 1081 cm^{-1}; FAB-MS, m/z 619.4512 (MNa^+), calcd 619.4550 for $C_{35}H_{64}NaO_7$; EI-MS: see Scheme I and Table II. ^1H NMR and ^{13}C NMR: see Table IV.

Goniothalamicin trimethylsilyl ether derivative: EI-MS, m/z (rel int) 585 (2), 515 (6), 495 (7), 425 (64), 405 (4), 369 (6), 385 (20), 335 (8), 299 (34), 213 (40).

Goniothalamicin Tetraacetate (11). Acetylation was carried out with Ac_2O–pyridine and yielded tetraacetate 11: CI-MS, m/z (rel int) 765 (0.2), 705 ($MH^+ - 60$, 19), 645 ($MH^+ - 2 \times 60$, 52), 523 ($MH^+ - 4 \times 60$, 10), 385 ($MH^+ - 3 \times 60$, 34); EI-MS, m/z (rel int) 495 (28), 435 (14), 375 (4), 365 (1.4), 339 (20), 325 (1), 315 (5), 305 (2), 279 (6), 265 (2), 253 (2), 205 (3), 183 (2), 123 (15).

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Supplementary Material Available: ^1H, ^{13}C, and 2D NMR spectra of 1–11 (42 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

$C_5H_9O_2^+$ Ions: The Correlation between Their Thermochemistry in Acidic Solution and Their Chemistry in the Gas Phase

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Each of a series of $C_5H_8O_2$ isomeric carboxylic acids and lactones (1–9) was protonated in both concentrated sulfuric acid and trifluoromethanesulfonic acid. The thermally induced transformations of the protonated species were then studied over the temperature range −40 to +160 °C. As a general rule, all the initially generated cations were eventually converted to protonated γ-valerolactone ($1H_O^+$) and, finally, to protonated cyclopentenone ($10H_O^+$). The cations derived from the cyclopropanecarboxylic acids 7 and 8 both underwent ring opening to the unsaturated cation $6H_O^+$, which then rearranged to a protonated α-lactone. In concentrated sulfuric acid the latter species loses carbon monoxide to afford protonated 2-butanone $11H_O^+$. The CIMS spectra of compounds 1–9 were recorded, allowing a correlation between the fragmentation routes in the gas phase and the transformations observed in solution. In this way, the data obtained in strong acids are used to assign reasonable structures to the gas-phase ions.

Introduction

In previous papers[1,2] we described the protonation of carboxylic acid derivatives in concentrated sulfuric acid, the thermally induced transformations of the resulting ionic species, and finally, the correlation between these transformations and the fragmentation patterns found for the same ions in the gas phase by recording the chemical ionization mass spectra (CIMS) of the corresponding precursors. This work established the feasibility of

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