Chemistry and Selective Cytotoxicity of Annonacin-10-one, Isoannonacin, and Isoannonacin-10-one. Novel Polyketides from *Annona densicoma* (Annonaceae)

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Summary: Using cytotoxicity assays as a guide, three novel polyketides were isolated from active extracts of the stem bark of the Peruvian plant *Annona densicoma* Mart. (Annonaceae).

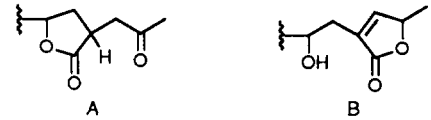
Sir: Further studies on the active extracts of the stem bark of *Annona densicoma* Mart. have led to the isolation of several novel cytotoxic polyketides. These compounds have shown significant cytotoxicity and are currently under evaluation as potential anticancer agents. Prior studies on *Annona densicoma* resulted in the elucidation of annonacin (**1**), which was the first member of the C_{35} polyketide series.[1] This series was found to possess a single tetrahydrofuran ring in contrast to the more common C_{37} polyketide group, which has two adjacent tetrahydrofuran rings.[2-7] Recently McLaughlin and co-workers have isolated goniothalamicin, which is related to **1** and which has potential as an insecticide.[8]

The ethanol extract of *A. densicoma* was highly cytotoxic to KB (human nasopharyngeal carcinoma), P388 (mouse leukemia, 9PS), and ASK (brain glioma) cells in culture.[9-13] Using the 9PS system as a guide, the crude extract was fractionated by solvent partitioning, column chromatography on silica gel and C-18 phase-bonded silica gel, and preparative TLC on silica gel leading to three novel polyketides, annonacin-10-one (**2**), isoannonacin (**3**), and isoannonacin-10-one (**4**) (Figure 1). The pure compounds were shown to be cytotoxic in several systems and then characterized.

Annonacin-10-one (**2**) was isolated as white crystals, mp 73-75 °C, $[\alpha]^{20}_D$ +31.1° (*c*, 0.06, CH_3OH). CIMS gave an MH^+ at 595. The molecular formula of **2** was established to be $C_{35}H_{62}O_7$ on the basis of analysis of the triacetate **2a**, which gave a molecular ion ($M + H^+$) at 721.4868 (calcd for $C_{35}H_{60}O_7 \cdot 3CH_3CO$, 721.4890). The formation of the triacetate indicated the presence of three hydroxyl groups in contrast to annonacin, which had a formula of $C_{35}H_{64}O_7$ and four hydroxyls (as evidenced by its conversion to tetraacetate **1a**).[1] The presence of an additional carbonyl at 1700 cm^{-1} in the IR of **2** suggested that **2** was a keto analogue of **1**. The location of the keto group was determined on the basis of mass spectral fragmentation patterns and the exact mass measurements of the key fragments (Figure 2). The other spectral properties of **1** and **2** were very similar. The presence of an α,β-unsaturated γ-lactone was confirmed by the carbonyl absorption at 1749 cm^{-1} in the IR and a UV (CH_3OH) λ_{max} at 210 nm (log ϵ, 4.03). Typical resonances in the ^1H NMR at δ 7.18 (d, 1.4 Hz), 5.06 (qd, 6.8 and 1.4 Hz), and 1.44 (d, 6.8 Hz) supported this conclusion (see Table I).

The ^1H NMR data also supported the presence of the ketone at C-10 since the signal at δ 3.60 in **1**, which was assigned to the proton on the carbon attached to the 10-OH group of annonacin, was missing in **2**. Two additional two-proton triplets (J = 7.5 Hz) were seen in the spectrum of **2** at δ 2.39 and 2.42, consistent with two methylene groups flanking the keto group at C-9 and C-11. Confirmation of the structure was achieved by reduction of **2** with $NaBH_4$, which yielded **1** in 40% yield (Scheme I).

Isoannonacin (**3**) was separated as white crystals, mp 96-98 °C, $[\alpha]^{20}_D$ +24.8 (*c*, 0.12, CH_3OH). The molecular weight of 596 was established by high resolution CIMS ($M + H^+$, 597.4720; calcd for $C_{35}H_{65}O_7$, 597.4730) and based on this **3** was isomeric with **1**, $C_{35}H_{64}O_7$. The IR spectrum showed absorptions at 1700 cm^{-1} for a ketone and 1760 cm^{-1} for a γ-lactone. This lactone was not α,β-unsaturated as in **1** since the compound was transparent in the UV at 208 nm. The signals for the butenolide ring were also missing in the ^{13}C and ^1H NMR of **3** (Table I). Signals at δ 4.54 (tdd; 7.0, 4.3, and 3.6 Hz), consistent with a proton on a carbon attached to a lactone oxygen, and δ 2.20 (s), assigned to a methyl group adjacent to a ketone, suggested partial structure A, which is isomeric with the butenolide structure (B) in the normal series. This isomeric relationship was confirmed by conversion of **1** to **3** by base-catalyzed isomerization.[14] The major product from treatment of **1** with KOH in *tert*-butyl alcohol was identical with **3** by comparison of IR, MS, and NMR data. A minor product was detected by NMR, which was isomeric with **3** at position 2. This isomeric lactone was also de-



A B

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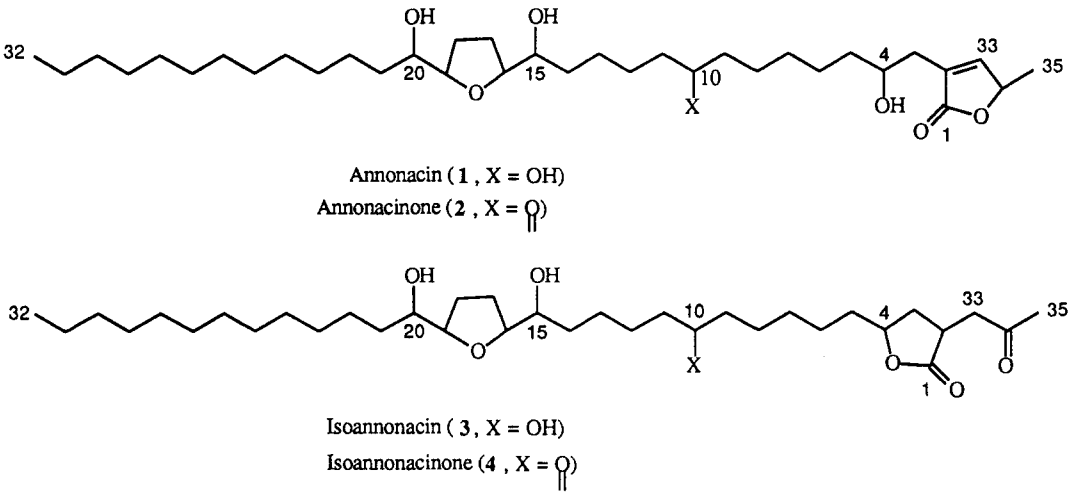


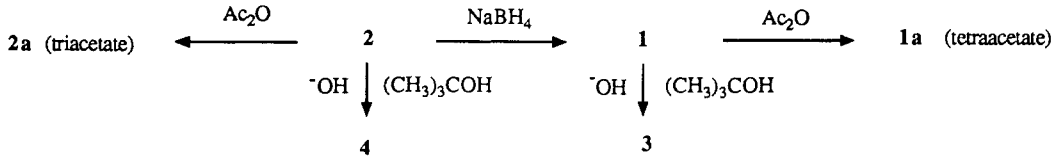
Figure 1. Structures of annonacin, annonacin-10-one, isoannonacin, and isoannonacin-10-one.

Table I. ^{1}H NMR Spectral Data (470 MHz, $CDCl_3$) of 2, 3, and 4 and Their Acetates 2a, 3a, and 4a

	^{1}H (2)	^{1}H (2a)	^{1}H (3)[a]	^{1}H (3a)	^{13}C (3)[b]	^{1}H (4)	^{1}H (4a)
1	C=O	C=O	C=O	C=O	178.65, s	C=O	C=O
2			3.02, dddd (17, 9.5, 9.0, 3.6)	3.03, ddd (17, 9.5, 9.0, 3.6)	34.52, d	3.03, dddd (17, 9.5, 9.0, 3.6)	3.03, dddd (17, 9.5, 9.0, 3.6)
3a	2.41, dd (14, 7.5)	2.52, ddt (14, 7.5, 1.0)	2.23, ddd (9.5, 9.0, 3.6)	2.23, ddd (9.5, 9.0, 3.6)	33.31, t	2.23, ddd (9.5, 9.0, 3.6)	2.23, ddd (9.5, 9.0, 3.6)
3b	2.52, ddt (14, 3.2, 1.0)	2.57 dd (14, 3.2, 1.0)	1.99, m	1.96, m		1.99, m	1.96, m
4	3.83, m	5.10, ddd (7.5, 5.0, 3.2)	4.54, tdd (7.0, 4.3, 3.6)	4.55, tdd (7.0, 4.3, 3.6)	78.81, d	4.55, tdd (7.0, 4.3, 3.6)	4.54, m
5–8	1.26–1.60, m	1.26–1.61, m	1.25–1.60, m	1.25–1.60, m	22–38,[c] t	1.25–1.60, m	1.25–1.60
9	2.42, t (7.5)	2.39, t (7.5)	1.43, m	1.25–1.60, m	22–38,[c] t	2.42, t (7.5)	2.39, t (7.5)
10	C=O	C=O	3.59, m	4.85, m	71.73, d	C=O	C=O
11	2.39, t (7.5)	2.37, t (7.5)	1.43, m	1.25–1.60, m	22–38,[c] t	2.41, t (7.5)	2.39, t (7.5)
12–14	1.26–1.60, m	1.26–1.60, m	1.25–1.60, m	1.25–1.60, m	22–38,[c] t	1.25–1.60, m	1.25–1.60, m
15, 20	3.40, m	4.86, dt (9.0, 5.5)	3.41, ddd (12.2, 6.4, 5.2)	4.85, m	73.97, 74.03, d	3.41, m	4.85, m
16, 19	3.79, dd (12, 6.5)	3.97, dd (9.0, 5.5)	3.80, dd (12.2, 6.4)	3.97, dd (12.2, 6.4)	82.64, 82.70, d	3.79, dd (12, 6.5)	3.97, m
17, 18	1.68, 1.99, m	1.69, 1.96, m	1.66, 1.98, m	1.66, 1.98, m	22–38,[c] t	1.68, 1.99, m	1.70, 1.99, m
21–31	1.26–1.60, m	1.26–1.61, m	1.25–1.60, m	1.25–1.60, m	22–38,[c] t	1.28–1.60, m	1.25–1.60, m
32	0.88, t (6.8)	0.91, t (7.0)	0.88, t (6.8)	0.88, t (6.8)	14.04, q	0.89, t (7.0)	0.89, t (7.0)
33a	7.18, d (1.4)	7.09, d (1.4)	2.66, dd (17, 9.5)	2.68, dd (17, 9.5)	44.25, t	2.68, dd (17, 9.0)	2.68, dd (17, 9.0)
33b			3.01, dd (9.5, 9.0)	3.01, dd (9.5, 9.0)		3.01, dd (9.5, 9.0)	3.01, dd, (9.5, 9.0)
34	5.06, qd (6.8, 1.4)	5.02, qd (7.0, 1.4)	C=O	C=O	205.38, s	C=O	C=O
35	1.44, d (6.8)	1.41, d (7.0)	2.20, s	2.20, s	29.86, q	2.20, s	2.20, s
OAc		2.03, s (3 H) 2.08, s (6 H)		2.04, s (3 H) 2.08, s (6 H)			2.08, s (6 H)

[a] According to 2D-COSY (500 MHz). [b] According to ^{1}H–^{13}C HETCOR (270–67.5 MHz). [c] In ^{13}C NMR no. 23 methylene signals occur between δ 22 and 38, with considerable overlap at approximately the following positions: 22.64 (2), 25.27 (1), 25.44 (2), 25.59 (1), 25.44 (1), 25.67 (1), 29.31 (3), 29.61 (4), 29.70 (2), 31.90 (2), 33.41 (1), 33.53 (1), 37.31 (1) and 37.43 (1).

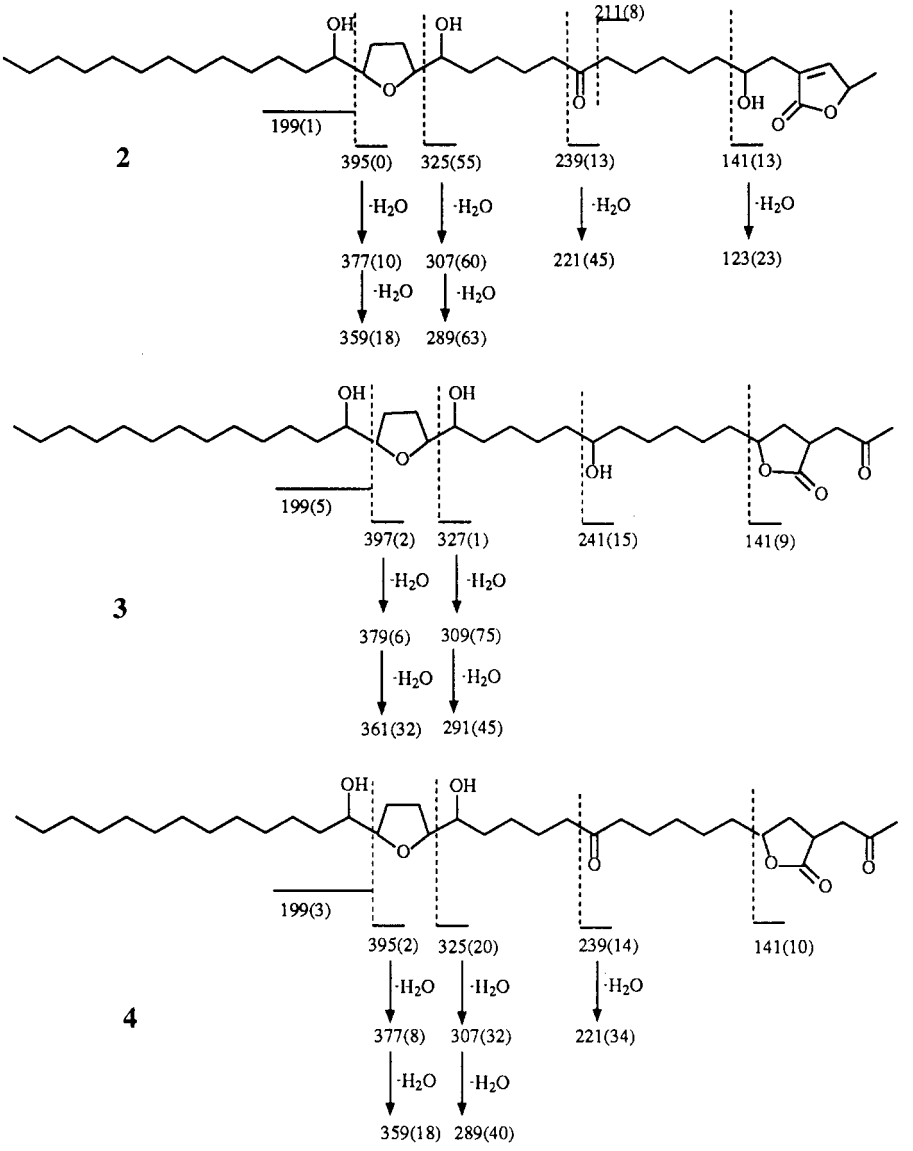
Scheme I. Chemical Interrelationship among Annonacins



tected in impure samples of natural 3 by NMR.

A similar relationship was established between annonacin-10-one (2) and its isomer 4. Isoannonacin-10-one was isolated as white crystals, mp 103.5 °C, $[\alpha]^{20}_D$ +19.8° (*c*, 0.05, CH_3OH). The molecular formula and weight were established by high resolution CIMS (M + H$^+$, 595.4573; calcd for $C_{35}H_{63}O_7$ 595.4573) and were consistent with an isomer of annonacin-10-one, $C_{35}H_{62}O_7$, 594. The IR and NMR confirmed the presence of partial structure A, γ-lactone, 1740 cm^{-1}, two ketone carbonyls at 1715 and 1695 and signals at δ 2.20 (s) and 4.55 (tdd; 7.0, 4.3, and 3.6 Hz) for the methyl ketone and γ-lactone. The UV was transparent in the region from 208 nm. Treatment of annonacin-10-one (2) with KOH in *tert*-butyl alcohol gave 4 as a major product (36% yield). The chemical interrelationships of 1, 2, 3, and 4 are outlined in Scheme I. Diagnostic MS fragmentation patterns are shown in Figure 2.

Compounds 3 and 4 are the first reported members of the iso series of C_{35} polyketides. These compounds have demonstrated very interesting selective activity in our human tumor cell lines (HTCL, see Table II). The dis-



EXACT MASS MEASUREMENT AND ELEMENTAL COMPOSITION

m/z of fragments from annonacinone	composition	m/z of fragments from isoannonacin -TMS	composition	m/z of fragments from isoannonacinone -TMS	composition
141.0558	$C_7H_9O_3$	141.0553	$C_7H_9O_3$	141.0552	$C_7H_9O_3$
211.1333	$C_{12}H_{19}O_3$	271.1702	$C_{13}H_{26}O(TMS)_1$	271.2456	$C_{13}H_{26}O(TMS)_1$
239.1285	$C_{13}H_{19}O_4$	313.2565	$C_{13}H_{20}O_4(TMS)_1$	397.2456	$C_{18}H_{28}O_5(TMS)_1$
325.2005	$C_{18}H_{29}O_5$	471.2966	$C_{18}H_{29}O_5(TMS)_2$	467.2394	$C_{22}H_{34}O_6(TMS)_1$
377.2325	$C_{22}H_{33}O_5$	541.3377	$C_{22}H_{35}O_6(TMS)_2$		

Figure 2. Diagnostic fragment ions of **2**, **3**, and **4**.

Table II. Selective Cytotoxicity in the Annonacin Series

compd	ED_{50} in μg/mL in 9PS[a]	A549[a]	HT29[a]
annonacin (**1**)	1×10^{-5}	1×10^{-3}	3×10^{0}
annonacin-10-one (**2**)	1×10^{-6}	1×10^{-1}	1×10^{0}
isoannonacin (**3**)	3×10^{0}	2×10^{-2}	2×10^{-3}
isoannonacin-10-one (**4**)	5×10^{-1}	7×10^{-2}	9×10^{-3}

[a] 9PS, mouse leukemia; A549, human non-small cell lung carcinoma; HT29, human colon adenocarcinoma.

covery of these compounds and the chemical process to convert the normal to the iso series gains significance based on preliminary cytotoxicity data. The members of the iso series (**3**, **4**) are 10000× less active against leukemia cells (9PS) and 1000× more active against colon tumor cells (HT29) than the normal butenolide (**1**, **2**) series. On the basis of this, further research is underway to establish the stereochemistry, mechanism of action, and in vivo antitumor activity of this series.

Acknowledgment. This investigation was supported by Grant No. CA 33326 awarded by the Division of Cancer Treatment of the National Cancer Intitute, Public Health Service, Bethesda, MD, to The Ohio State University (J.M.C.). The cytotoxicity testing was provided by Dr.

Peggy Criswell, Purdue Cell Culture Laboratory, Purdue Cancer Center, partially supported by National Cancer Institute Core Grant No. 5P30CA23168. Dr. John L. Occolowitz of Eli Lilly Laboratories, Indianapolis, IN, provided FDMS. High resolution (470 MHz) proton spectra were recorded at the Purdue University Magnetic Resonance Laboratory (NIH grant RR01077). The collection of *Annona densicoma* Mart. (Annonaceae) was made in 1981 in Peru under a program of the National Cancer Institute, Natural Products Branch, Dr. Matthew Suffness, Chief. The plant material was authenticated by the Economic Botany Laboratory, United States Department of Agriculture, Beltsville, MD, where a voucher specimen is on deposit.

Group Recognition by an Octamethoxy-Substituted Cyclophane Host As Studied by Electron Spin Resonance

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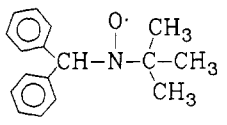
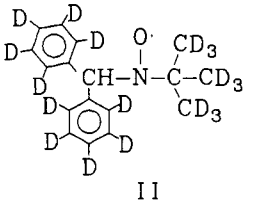
Summary: Inclusion complexation of α-substituted benzyl *tert*-butyl nitroxide spin probes is used to investigate the group recognition ability of a novel water-soluble octamethoxy-*p*-cyclophane by ESR spectroscopy. It is found that the phenyl or substituted-phenyl group is included but alkyl or cycloalkyl groups are not. Evidence for different spectra is obtained in the aggregated *p*-cyclophane at high concentration of the host.

Sir: Recent investigations have demonstrated that multiparameter nitroxide probes can provide information about bimodal inclusion in cyclodextrins. Thus for diphenylmethyl *tert*-butyl aminoxyl I, two different inclusion

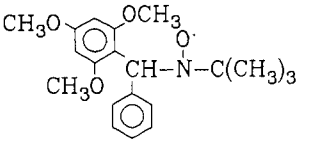


I

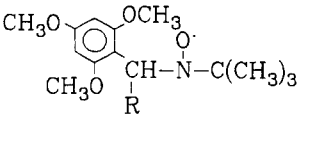


II

complexes of β-cyclodextrin in water can be detected by ESR spectroscopy.[1] These were assigned to "phenyl-in" and "*tert*-butyl-in" on the basis of the relative magnitudes of the nitrogen and β-hydrogen hyperfine splitting constants (N- and β-H hfsc's) and a comparison with the same parameters of the "free" spin probe. This assignment was confirmed by the finding that the phenyl-in complex actually consists of an equimolar mixture of two species as determined by ENDOR spectroscopy.[2] The components of this mixture are believed to be due to a diastereomeric pair of complexes since the β-cyclodextrin is pure D+. Similar results can be obtained with α-cyclodextrin but γ-cyclodextrin gives only one type of inclusion complex.[3] These observations are consistent with the larger aperture for the latter cyclodextrin. In these studies II was used for the first time, which is perdeuterated I except for the β-hydrogen. With this spin probe extremely sharp lines can be obtained that greatly facilitate analysis of multicomponent ESR spectra.[3] Another method for separating the component spectra more completely is to use a spin probe that gives a larger β-H hfsc. This occurs in spin adducts of *C*-(2,4,6-trimethoxyphenyl)-*N*-*tert*-butylnitrone.[4] Thus III also gives two spectra with β-cyclodextrin assigned to phenyl-in and *tert*-butyl-in since the trimethoxyphenyl group is assumed to be too large to be included. The complete separation found for the spectra



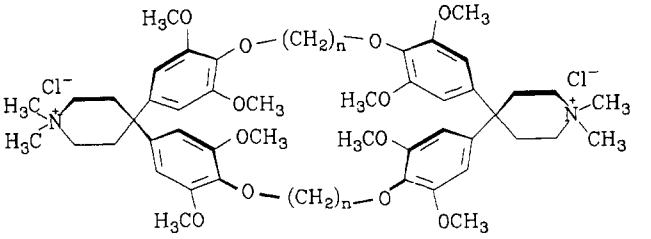
III



IV

of the two inclusion complexes of III has prompted a detailed study on the relative tendency of the alkyl group in IV to be included in γ-cyclodextrin.[5] The magnitudes of the association constants for inclusion increase in the sequence *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl (K = 50, 60, 70, 150 M^{-1}). However, the largest values are found for cyclohexyl (K = 750 M^{-1}). Complexation with the methyl or ethyl group could not be detected.

In this communication we would like to report on a comparison of these results with a novel water-soluble synthetic cyclophane host,[6] V, named here octamethoxyparacyclophane-3, OMCP-3, in order to give emphasis to the portion of the molecule most influential in determining the hydrophobicity of the cavity. It has been shown by



n = 3

V

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