Annonacin, a novel, biologically active polyketide from *Annona densicoma*

T. G. McCloud, D. L. Smith, C.-J. Chang and J. M. Cassady*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 25 September 1986

Summary. A new linear polyketide, Annonacin (**I**), has been isolated from active extracts of the stembark of *Annona densicoma* Mart. Annonacin (**I**) is highly cytotoxic and is active in an assay designed to detect antimitotic agents. The structure of (**I**) was determined by analysis of spectroscopic data.
Key words. Annonacin; polyketide; *Annona densicoma;* Annonaceae; biological activity.

In preliminary screening, extracts of *Annona densicoma* Mart. (Annonaceae) were highly cytotoxic to KB cells (human nasopharyngeal carcinoma) and P388 cells (mouse leukemia) in vitro, and gave an active response in the astrocytoma reversal assay[1–5]. A large scale ethanol extraction of stembark from *Annona densicoma* followed by solvent partitioning, chromatography on silica and C-18 phase bonded silica, and preparative thinlayer chromatography on RP-2 silica yielded a low melting, waxy solid which was active in several cell culture systems (1×10^{-3} µg/ml in KB, 1×10^{-5} µg/ml in P388 and 51% reversal in the astrocytoma assay).

The molecular weight of **I** was determined to be 596 by both field desorption (FD) and fast atom bombardment (FAB) mass spectrometry. Chemical ionization mass spectra of both the trimethylsilyl (TMS) (**III**), obtained from reacting **I** with bis(trimethylsilyl)acetamide in pyridine, and acetyl derivative (**II**), obtained from reacting with acetic anhydride in pyridine, exhibited large MH^+ ions for the tetra-TMS derivative and tetracetate, suggesting that **I** had four hydroxyl groups. Exact mass measurements on the MH-CH_4^+ and MH^+-TMSOH ions showed that underivatized **I** had an elemental composition of $C_{35}H_{64}O_7$. The infrared spectrum contained prominent absorption at 3440

Table 1. ^{13}C NMR (50 MHz, $CDCl_3$) of (**I**) and ^1H NMR (470 MHz, $CDCl_3$) of (**I**) and tetraacetate (**II**). All signals are given in ppm downfield from reference TMS

	^{13}C	^1H (**I**)	^1H (**II**)
1	174.6	–	–
2	131.1	–	–
3a	22–38 #	2.38 dddd J_{3a-3b} = 14 Hz, J_{3a-4} = 8 Hz, J_{3a-4OH} = 1 Hz, J_{3a-33} = 0.5 Hz	2.48 m
3b	22–38 #	2.51 dddd J_{3a-3b} = 14 Hz, J_{3b-4} = 3.4 Hz, J_{3b-4OH} = 0.5 Hz, J_{3b-33} = 0.5 Hz	2.52 m
4	71.6	3.81 tt J_{3-4} = 8 Hz, J_{4-5} = 4.5 Hz	5.06 tt J_{3-4} = 8 Hz, J_{4-5} = 4.7 Hz
5–9	29.5 #	*	*
10	69.8	3.56 m	4.82 ▲m
11–14	29.5 #	*	*
15	73.9△	3.38 dt J_{15-16} = 11.6 Hz, J_{14-15} = 5.8 Hz	4.82 ▲m
16	82.6●	3.77 dt J_{15-16} = 11.6 Hz, J_{16-17} = 6.9 Hz	3.94 ●m
17–18	22–38 #	1.67 m and 1.97 m	
19	82.7●	3.77 dt J_{19-20} = 11.6 Hz, J_{18-19} = 6.9 Hz	3.94 ●m
20	74.1△	3.38 dt J_{19-20} = 11.6 Hz, J_{20-21} = 5.8 Hz	4.82 ▲m
21–31	29.5 #	*	*
32	14.1	0.85 t J_{32-31} = 6.9 Hz	0.85 t J_{31-32} = 7.0 Hz
33	151.8	7.16 d J_{33-34} = 1.4 Hz, J_{33-3} = 0.5 Hz	7.06 d J_{33-34} = 1.4 Hz
34	77.9	5.04 qd J_{34-33} = 1.4 Hz, J_{34-35} = 6.8 Hz	4.98 qd J_{33-34} = 1.4 Hz, J_{34-35} = 6.4 Hz
35	19.0	1.40 d J_{34-35} = 6.8 Hz	1.37 d J_{34-35} = 6.6 Hz

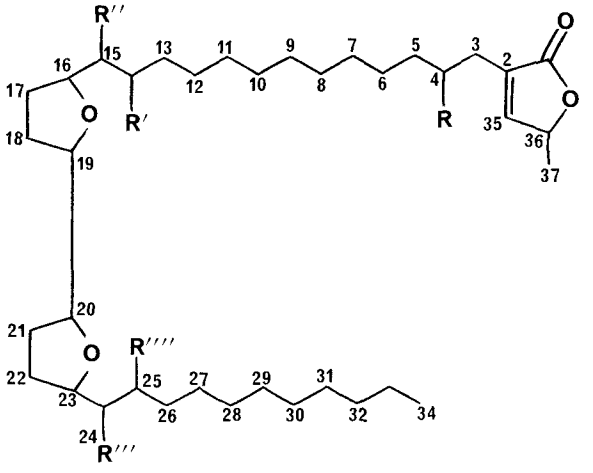
△, ●, ▲ may be interchanged within the group. # 23 methylene signals occur between δ 22 and 38, with considerable overlap, at approximately the following positions: 22.6(1), 25.5 (3), 28.7 (2 or 3), 29.5 (many), 31.8 (1), 33.3 (3), 37.2 (2). * Signals lie within the methylene envelop at δ 1.2–1.7.

Table 2. Exact mass and elemental composition of TMS derivatives

m/z of fragments from derivative **III**	Composition	△MASS (amu) for homologous fragment of derivative **IV**	△MASS (amu) for homologous fragment of derivative **V**
213.0961	$C_7H_8O_3(TMS)_1$	2	9
271.2446	$C_{13}H_{26}O(TMS)_1$	0	9
341.2859[b]	$C_{17}H_{32}O_2(TMS)_1$	0	9
385.2228[b]	$C_{13}H_{19}O_4(TMS)_2$	2	18
543.3346	$C_{18}H_{28}O_5(TMS)_3$	2	28
614.3796[b]	$C_{22}H_{35}O_6(TMS)_3$	2	27
885[a]	$C_{35}H_{61}O_7(TMS)_4$	2	36

[a] Chemical ionization. [b] The homologous ion is found in the mass spectrum of **II**.

cm^{-1} consistent with the presence of hydroxyl groups. An IR absorption at 1750 cm^{-1} and a UV maximum at 207 nm (ε 15,390 in isopropanol) suggested the presence of an α,β-unsaturated γ-lactone. A positive Kedde test supported the presence of this functionality.



			R	R′	R″	R‴	R⁗
Uvaricin	$C_{39}H_{68}O_7$	648	H	H	OH	OAc	H
Uvaricinone	$C_{39}H_{66}O_7$	646	H	H	=O	OAc	H
Desacetyluvaricin	$C_{37}H_{66}O_6$	606	H	H	OH	OH	H
Rollinicin	$C_{37}H_{66}O_7$	622	H	H	OH	OH	OH
Rollinone (2,35-dihydro)	$C_{37}H_{66}O_7$	622	H	=O	OH	OH	H
Asimicin	$C_{37}H_{66}O_7$	622	OH	H	OH	OH	H

Figure 1. Substitution patterns of known acetogenins.

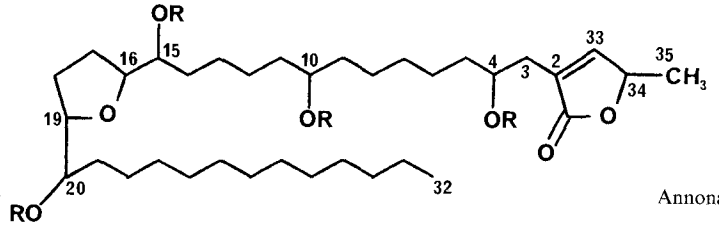
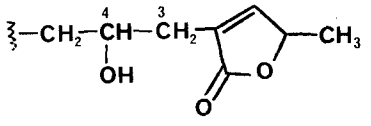


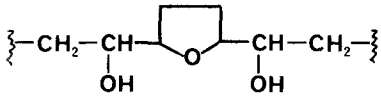
Figure 2. Derivatives of annonacin analyzed by MS.

The structure of fragment A, which contained the lactone and one of the hydroxyl functions, was elucidated by high resolution ^1H NMR analysis; [7.16 (dt, 1.4 and 0.5 Hz), 5.04 (dq, 1.4 and 6.8 Hz) and 1.40 (d, 6.8 Hz) ppm], and substantiated by ^{13}C NMR analysis (174.6, 151.8, 131.1, 77.9 and 19.0 ppm). Further selective ^1H-^1H decoupling experiments linked H-33, H3a and 3b and H-4 and therefore established the presence of fragment A in **I** (see table 1).

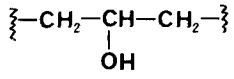


A

Attempts to chemically cleave **I** by treatment with periodate or lead tetraacetate were unsuccessful as were attempts to obtain a boronate derivative, indicating the absence of a 1,2 diol system. The position of the other three isolated hydroxyl groups was disclosed by a one-proton signal at 3.56 ppm (multiplet) and a two-proton signal at 3.38 ppm (doublet of triplets, 11.6 and 5.8 Hz), shifted to 4.82 ppm (multiplet, three protons) upon the formation of acetate **II**. This two-proton signal was linked to another two-proton signal at 3.77 ppm (doublet of triplets, 11.6 and 6.9 Hz). From further comparisons with the ^1H and ^{13}C NMR data of other known polyketides which have been reported from other species of Annonaceae[6–10] (fig. 1), the structures of the fragments B and C could be established.



B



C

Annonacin (**I**)

I R = H
II R = Ac
III R = TMS
IV R = TMS, 2,33 dihydro
V R = TMS-d_9

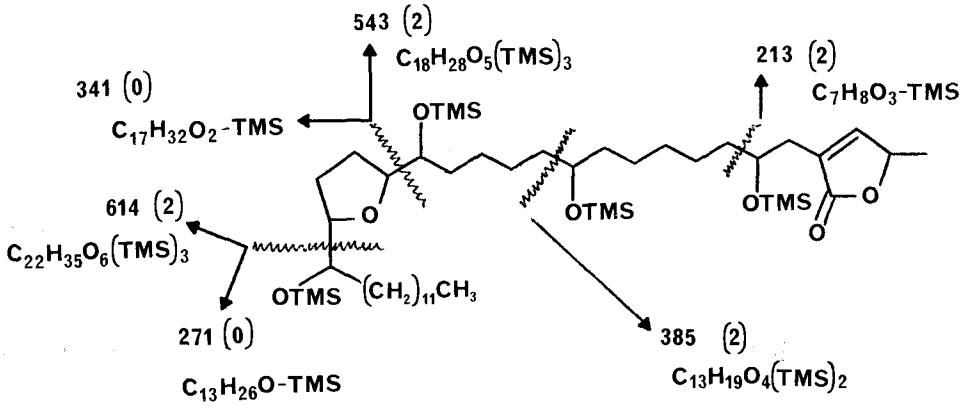


Figure 3. Diagnostic fragment ions of **III** and **IV**. Numbers in parentheses indicated mass shifts observed for derivative **IV**.

To elucidate the placement of these subunits along the hydrocarbon chain mass spectral studies were undertaken. Several derivatives of **I** were prepared and utilized in lieu of the underivatized **I** because their mass spectra were more reproducible. Figure 2 and table 2 list these derivatives. The measured exact masses and corresponding elemental composition of several key fragments and ions listed in table 2 are assigned to specific parts of **I** in figure 3. The ion at m/z 213 in the EI spectrum of **III** increased by 2 μm in the spectrum of **IV**, the 2,33-dihydro-derivative produced by catalytic hydrogenation of **I** by 10% Pd/C in EtOH, providing further evidence that this fragment contained the lactone ring of subunit A, and that bond rupture had occurred adjacent to the hydroxyl group at C-4. The ion at m/z 385, which also shifts by 2 μm in the spectrum of **IV**, indicates that a hydroxyl is located at C-10. The number of carbons between the two rings is established by the ions at m/z 543 and 614, both of which contain the unsaturated lactone ring. The length of the hydrocarbon chain attached to the tetrahydrofuran ring is indicated by the ions at m/z 271 and 341, which do not increase by 2 μm in the mass spectrum of **IV**. Other ions in the mass spectrum of the TMS derivative of **I** not listed in table 2, as well as in homologous ions observed in the EI spectra of **II** and **V**, the perdeuteriotrimethylsilyl derivative of **I** obtained from treating **I** with bis(perdeuterotrimethylsilyl)trifluoracetamide in pyridine, support these assignments.

Annonacin is the first representative of a new class of C_{35} polyketides in contrast to the C_{34} series previously found in the Annonaceae[6–10]. Also **I** is the first member of this group with a single tetrahydrofuran ring system. Compounds of this type have shown significant cytotoxicity and are currently under evaluation as potential anticancer agents. Annonacin (**I**) is unique among this series in producing a reversal of differentiation of ASK (rat brain glioma) cells at sub-cytotoxic doses. This activity is associated with agents which bind to tubulin and in turn produce antimitosis. Therefore **I** may represent the first member of a new class of antimitotic agents. Further studies are underway on the chemistry and pharmacology of **I** and related compounds.

Acknowledgments. This investigation was partially supported by Grant No. CA33326 awarded by the Division of Cancer Treatment of the National Cancer Institute, Public Health Service, Bethesda, MD, to Purdue University. The cytotoxicity testing was provided by Dr Linda Jacobsen, 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. All NMR spectra were obtained at the Purdue Biological Magnetic Resonance Center, supported in part by National Institutes of Health Research No. RR01077, from the Division of Research Resources. 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, Head. It was authenticated by the Economic Botany Laboratory, United States Dept of Agriculture, Beltsville, MD, where a voucher specimen is on deposit. We wish to thank Mr J.K. Rupprecht for his useful discussions during the progress of this investigation.

1 Geran, R.I., Greenberg, N.H., MacDonald, M.M., Schumacher, A.M., and Abbott, B.J., Cancer Chemotherapy Reports, vol. 3 (1972) protocols 1.6 and 13.
2 Shoemaker, R.H., Abbott, B.J., MacDonald, M.M., Mayo, J.G., Venditti, J.M., and Wolpert-DeFilippes, M.K., Cancer Treatm. Reps *67* (1983) 1.
3 Abbott, B.J., National Institutes of Health, Instruction 275 (1978) protocols 1.8 and 14.
4 Igarashi, K., Ikegama, S., Takeuchi, M., and Sugino, Y., Cell Struct. Funct. *3* (1978) 103.
5 Abbot, B.J., National Institutes of Health, Instruction 314 (1980).
6 Jolad, S.D., Hoffmann, J.J., Schram, K.H., Cole, J.R., Tempesta, M.S., Kriek, G.R., and Bates, R.B., J. org. Chem. *47* (1982) 1351.
7 Dabrah, T.T., and Sneden, A.T., Phytochemistry *23* (1984) 2013.
8 Dabrah, T.T., and Sneden, A.T., J. nat. Prod. *47* (1984) 652.
9 Rupprecht, J.J., Chang, C.-j., Cassady, J.M., McLaughlin, J.L., Mikolajczak, K.L., and Weisleder, D., Heterocycles *24* (1986) 1197.
10 Cortes, D., Rios, J.L., Villar, A., and Valverde, S., Tetrahedron Lett. *25* (1984) 3199.

0014-4754/87/080947-03$1.50 + 0.20/0

Luffariellolide, an anti-inflammatory sesterterpene from the marine sponge *Luffariella* sp.

K.F. Albizati, T. Holman, D.J. Faulkner[1,2], K.B. Glaser* and R.S. Jacobs*

*Scripps Institution of Oceanography (A-012F), University of California, San Diego, La Jolla (California 92093, USA), and *Department of Biological Sciences, University of California, Santa Barbara, Santa Barbara (California 93106, USA),*
30 December 1986

Summary. Luffariellolide (**2**) is a sesterterpene from the Palauan sponge *Luffariella* sp. that has useful anti-inflammatory properties. In contrast with the irreversible action of manoalide (**1**) on phospholipase A_2, luffariellolide (**2**) is a slightly less potent but partially reversible PLA_2 inhibitor.
Key words. Marine sponge; *Luffariella* sp.; sesterterpene; phospholipase A_2 inhibitor; anti-inflammatory.

Manoalide (**1**) is a sesterterpene from the marine sponge *Luffariella variabilis*[3] that significantly reduces chemically-induced inflammation in vivo and irreversibly inhibits the in vitro hydrolysis of phosphotidyl choline by purified bee venom phospholipase A_2 (PLA_2)[4]. Although manoalide (**1**) can be obtained in good yield from the natural source and has been synthesized[5], we have nonetheless continued the search for related anti-inflammatory agents, particularly those that reversibly inhibit phospholipases. Luffariellolide (**2**), isolated from a Palauan sponge *Luffariella* sp., is a less potent but partially reversible inhibitor of bee venom PLA_2. The hexane extract (15.4% dry weight) of *Luffariella* sp. (85–027) contained > 90% luffariellolide (**2**), that was easily purified by medium pressure chromatography on a Lobar LiChroprep Si 60 column using 20% ethyl acetate in hexane as eluant. Luffariellolide (**2**) is an optically inactive oil of molecular formula $C_{25}H_{38}O_3$. The broad infrared bands at 3300 and 1760 cm^{-1}, ^1H NMR signals at δ 6.01 (br s, 1 H, H-25) and 5.85 (br s, 1 H, H-2) and ^{13}C NMR signals at δ 171.9 (s, C-1), 117.0 (d, C-2), 169.9 (s, C-3) and 99.5 (d, C-25) define the γ-hydroxybutenolide moiety, which has previously been encountered in several sponge metabolites[6]. The 2,6,6-trimethylcyclohexene terminus gave rise to the expected ^{13}C NMR signals at δ 136.9 (s), 126.6 (s), 32.6 (t), 19.4 (t), 39.5 (t), 34.8 (s), 19.7 (q), 28.5 (2xq)[5]. The *E*-geometry of the two trisubstituted olefinic bonds was defined by the ^{13}C NMR signals at δ 16.0 (q) and 15.9 (q) assigned to the olefinic methyl groups. The remaining spectral data[7] all support the proposed structure for luffariellolide (**2**) which is a sesterterpenoid analog of hydroxymokupalide, a hexaprenoid from