Title

Luffariellolide, an anti-inflammatory sesterterpene from the marine spongeLuffariella sp.

Journal

Cellular and Molecular Life Sciences, 43(8)

ISSN

1420-682X

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Publication Date

1987-08-01

DOI

10.1007/bf01951682

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.

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

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

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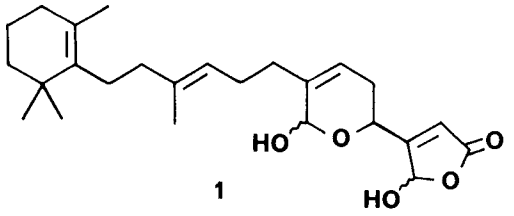
30 December 1986

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



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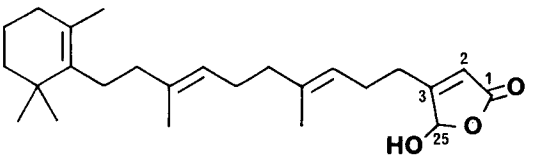

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the sponge *Megalopastas* sp.[8]. Luffariellolide (**2**) has also been found in a sponge of the genus *Fascaplysinopsis*[9].

Luffariellolide (**2**) is a potent antagonist of topical phorbol myristate acetate (PMA) induced inflammation in the mouse ear: PMA alone, (T/C-1) = 0.929 ± 0.200; PMA+luffariellolide (50 µg/ear), (T/C-1) = 0.221 ± 0.068 (n = 10)[10,11]. Subcutaneous administration of luffariellolide at concentrations of 50 mg/kg and 100 mg/kg significantly reduced the incidence of abdominal spasms in response to intraperitoneal administration of phenylquinone (2.0 mg/kg) in mice[11]. Luffariellolide inhibited in vitro hydrolysis of phosphatidyl choline by purified bee venom phospholipase A_2 ($IC_{50} = 2.3 \times 10^{-7}$ M). The maximum inhibition obtainable with luffariellolide was only 80% as compared to complete inactivation of PLA_2 by manoalide. Inhibition by luffariellolide was partially (approx. 30%) reversed by dialysis whereas manoalide inhibition is completely irreversible under dialysis conditions. Classical kinetic analysis of the luffariellolide reaction with PLA_2 demonstrated noncompetitive type inhibition with an apparent $K_i = 1.6 \times 10^{-7}$ M. In contrast with observations on manoalide (**1**)[12], pretreatment of luffariellolide with oligomers of lysine does not prevent inhibition of PLA_2 by luffariellolide. Luffariellolide is a partially reversible inhibitor of purified bee venom PLA_2 that lacks one of the two masked aldehyde groups that appears to be responsible for the irreversible reaction of manoalide with lysine residues on PLA_2[13].

1 Acknowledgment. We thank Edward Luedtke, Elise Clason and Ellen Snideman for performing some of the assays reported above. The sponge was identified by Dr. Klaus Rützler, Smithsonian Institution, Washington, D.C. The research was supported by grants from Allergan Pharmaceuticals and the California Sea Grant College Program (Projects R/MP-30 and R/MP-31).

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0014-4754/87/080949-02$1.50 + 0.20/0

2-Amino-6-[(1'R,2'S)-1',2'-dihydroxypropyl]-3-methyl-pterin-4-one, a biologically active metabolite from the anthozoan *Astroides calycularis* Pallas[1]

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Summary. 2-Amino-6-[(1'R,2'S)-1',2'-dihydroxypropyl]-3-methyl-pterin-4-one (**1**) has been isolated from the marine anthozoan *Astroides calycularis;* its structure was illustrated by spectral analyses including 2D-NMR and by partial synthesis. **1** appears to possess cell-growth inhibiting activity.

Key words. 3-Methyl-L-*erythro*-biopterin; 2-amino-6-[(1'R,2'S)-1',2'-dihydroxypropyl]-3-methyl-pterin-4-one; *Astroides calycularis* Pallas; anthozoan.

Pteridines are widely distributed in the animal kingdom, especially among insects and poikilothermic vertebrates such as fishes, amphibians and reptiles[2]. Little is known about pteridines in marine invertebrates. In 1944 xanthopterin was isolated from the crab *Cancer pagurus*[3], while Momzikoff and his co-workers have reported the presence of several previously known pteridines in diatoms[4], copepods[5] and tunicates[6].

In 1981 leucettine, a 6-(1-hydroxypropyl)-3-methyl-pteridine-2,4(1H)-dione, was found in an extract of the calcareous sponge *Leucetta microraphis*[7], but it was not ascertained if this compound was synthesized de novo by the sponge as a secondary metabolite or if it was of dietary origin.

In connection with our interest in marine chemical products we are now examining the water-soluble extract of *Astroides*