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.

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

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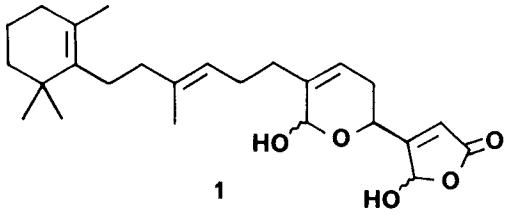
30 December 1986

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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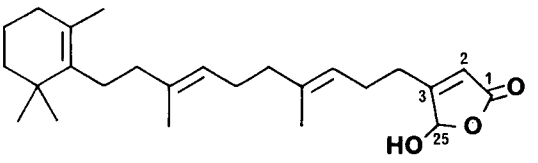

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the sponge *Megalopastas* sp.[8]. Luffariellolide (**2**) has also been found in a sponge of the genus *Fascaplysinopsis*[9].

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].

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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*