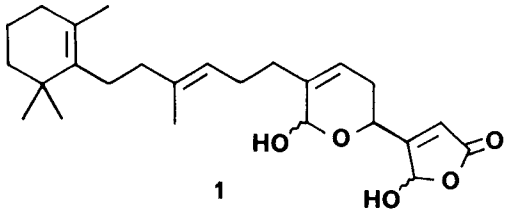

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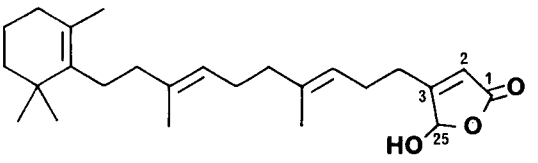

2

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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7 Luffariellolide (**2**): oil; UV (MeOH), 214 nm (ε 10,000), (MeOH/OH^-) 253 nm (ε 4400); (IR ($CHCl_3$) 3300 (br), 1760 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ0.99 (s, 6 H), 1.60 (br s, 3 H), 1.64 (br s, 6 H), 5.14 (br t, 2 H, J = 7 Hz) 5.85 (br s, 1 H), 6.01 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 171.9 (s), 169.9 (s), 136.9 (s), 136.8 (s), 136.0 (s), 126.6 (s), 123.1 (d), 121.9 (d), 119.0 (d), 117.0 (d), 99.5 (d), 40.1 (t), 39.7 (t), 39.5 (t), 34.8 (s), 32.6 (t), 28.5 (q), 27.8 (t), 27.7 (t), 26.4 (t), 24.9 (t), 19.7 (q), 19.4 (t), 16.0 (q), 15.9 (1); HRMS obsd. m/z 386.2821, $C_{25}H_{38}O_3$ requires 386.281.

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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*

calycularis Pallas, an anthozoan common in Mediterranean waters.

In this fraction, which contains aplysinopsins[8], we have now found a new pterin, 2-amino-6-[(1'R,2'S)-1',2'-dihydroxy-propyl]-3-methyl-pterin-4-one (**1**), which exhibits interesting biological activity.

Material and methods. 20 medium-sized colonies of *A. calycularis*, collected in the bay of Naples (May 1985; a voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli), were freeze-dried, chopped and extracted with MeOH for 24 h at room temperature (four times). The hydro-methanolic solution was decanted, filtered and concentrated in vacuo; the aqueous residue was clarified by centrifugation and passed through a column of Amberlite XAD-2 (eluent: H_2O) and successively on Dowex 50WX-2 (100–200 mesh, H^+; eluent: 0.5 N NH_4OH). Gel filtration of the eluate through Sephadex LH-20 with MeOH provided a crude product (100 mg) which was then subjected to PLC (SiO_2; eluent $CHCl_3$/MeOH 3:2) thus obtaining 20 mg of pure **1**, m.p. 229–231 °C (from MeOH); $[\alpha]_D = -60°$ (c = 0.03 in 0.1 N HCl).

1 was synthesized from L-biopterin (10 mg) with an excess of CH_2N_2 in MeOH-Et_2O (1:1; 20 ml) at room temperature for 30 min. The solution was taken to dryness and the residue was partitioned on TLC (SiO_2; eluent $CHCl_3$/MeOH 7:3); the band R_f 0.6 (UV light), was scraped off and eluted with MeOH to afford 5 mg of **1**, which was proved to be identical to the naturally occurring product by comparison of their chromatographic and physical properties ($[\alpha]_D$; m.p.; ^{1}H-NMR).

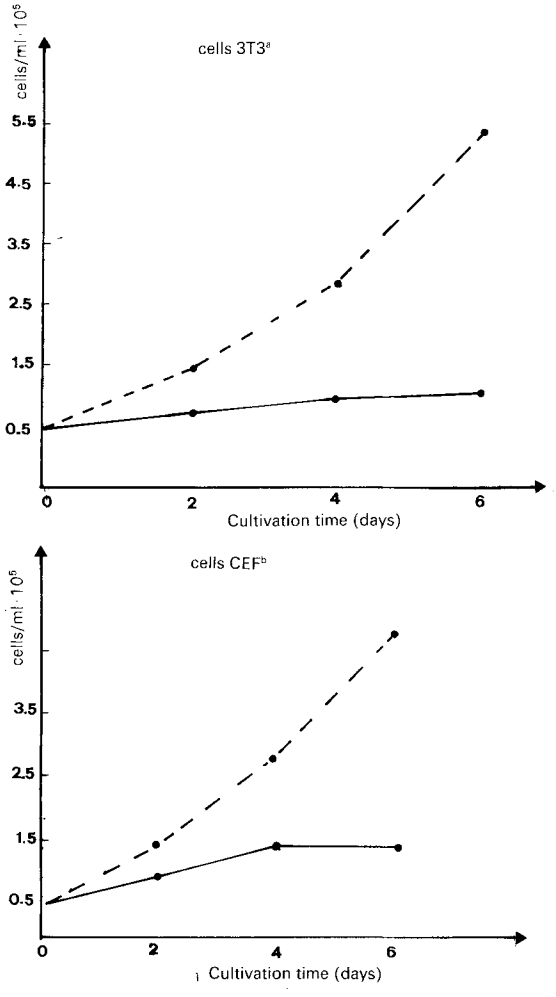
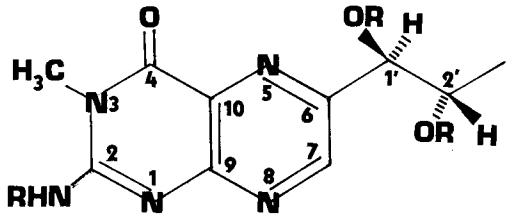


Figure 1. Growth curve of 3T3 cells and CEF cultured in presence of 1×10^{-3} mM (–). The effect of the added substance is evident compared with parallel control cultures (---). Points of each curve are the mean of 3 different determinations. The conditions used for the cell cultures are described in 'Materials and methods'.



1 R=H

2 R=Ac

Figure 2.

Acetylation of **1** (4 mg) was performed with Ac_2O (0.5 ml) in anhydrous pyridine (0.3 ml) at room temperature for 2 h; the solution was successively taken to dryness and the residue, after purification by HPLC (LiChrosorb Si-60; eluent $CHCl_3$-MeOH 13:7), gave 4 mg of pure **2** $[\alpha]_D = -67°$ (c = 0.04 in $CHCl_3$)[9].

The growth-inhibiting activity of the compound **1** is shown in figure 1. The established fibroblast line 3T3, developed from random-bred Swiss mouse embryo cells, was obtained from Flow Laboratories; primary chick embryo fibroblasts (CEF) were prepared from 10–12-day-old embryos. Both lines were cultured in Dulbecco's Modified Medium, supplemented with 10% fetal calf serum, 100 µg/ml penicillin and 100 U/ml streptomycin, seeded at 5×10^4 cells per dish and incubated at 37 °C under 95% air, 5% CO_2. 24 h after plating, 2-amino-6-[(1'R,2'S)-1',2'-dihydroxy-propyl]-3-methyl-pterin-4-one was added to the medium at a final concentration of 1×10^{-3} mM. Cell cultures were aged for 6 days and harvested by trypsinization with 0.25% trypsin EDTA and counted with a hemocytometer chamber. At the concentration used, only controls grow to confluence. The growth kinetic of the cells was determined by counting exposed and control cultures for both cellular lines every two days.

Results and discussion. Compound **1** is an optically active pale yellow crystalline powder. ^{1}H-NMR spectrum including double resonance experiments indicated the presence of an N-CH_3 group (3H, s at δ 3.39), of an CH proton belonging to a heterocyclic ring (1H, s at δ 8.73;) and of a 1,2-dihydroxy-propyl group which is most probably attached to an aromatic nucleus (δ 1.02, 3H, d, J = 6.5 Hz; δ 3.87, 1H, dq, J = 6 and 6.5 Hz; δ 4.46, 1H, d, J = 6 Hz). Treatment of **1** with Ac_2O-py at room temperature for 2 hours afforded the triacetyl derivative **2** [$C_{16}H_{19}N_5O_6$ from HRMS on the first fragment ion at $M^+ - 60$ (m/z 317.1119; $C_{14}H_{15}N_5O_4$, requires 317.1125)[10]; ^{1}H-NMR: singlets at δ 1.99, 2.15 and 2.30 3H each]. This confirmed the presence in **1** of two secondary hydroxyls, the remaining acetoxyl group being placed very probably on a nitrogen atom.

All the above data strongly suggested that the compound under investigation was an N-methyl derivative of biopterin. The following evidence clearly indicated that this function must be linked to N-3: UV spectra at various pH's [pH = 1: λ_{max} 229.5 nm (log ε 4.45), λ_{max} 318.5 nm (log ε 4.07); pH = 7: λ_{max} 242 nm (log ε 4.45), λ_{max} 275.5 nm (log ε 4.38), λ_{max} 356.0 nm (log ε 3.98)] are quite identical to those of 2-amino-3-methyl-pterin-4-one[11]; furthermore the mass spectrum of **2**

showed an intense ion at m/z 260.0901 deriving from the molecular ion by loss of CH_3COOH and $CH_3-N=C=O$. ^{13}C-NMR is fully consistent with structure **1**: [δ 161.00, (C-2), 175.64 (C-4), 151.76 (C-6), 149.27 (C-7), 154.24 (C-9), 125.20 (C-10), 29.10 (C-11), 76.53 (C-1′), 69.42 (C-2′), 18.86 (C-3′)][12]. Finally a gated decoupling experiment confirmed the proposed structure as being in complete agreement with the above assignments; particularly the multiplicity of the carbonyl signal at δ 175.64, which appeared as a quartet (J = 6.5 Hz), further supports the positioning of the CH_3 group at N-3.

Definitive proof for the structure **1** including the stereochemistry at C-1′ and C-2′ was obtained by the partial synthesis starting from L(−)-biopterin, which was treated with diazomethane in MeOH/Et_2O solution at room temperature for 30 min.

2-Amino-6-[(1′R,2′S)-1′,2′-dihydroxypropyl]-3-methyl-pterin-4-one exhibits a growth-inhibiting activity. A strong effect was observed when **1** was added (concentration 1×10^{-3} mM) to two bacterial cell cultures, as illustrated in the diagram of figure 1, where only controls grow to confluence. This result fully agrees with the behavior of many pteridine derivatives, whose effect on growth is clearly related to their structural relationship with folate derivatives which occupy key positions in cellular metabolism.

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9 ^1H-NMR of **2** in $CDCl_3$: δ 1.99 (3H, s, CH_3COO-); 2.15 (3H, s, CH_3COO-); 2.30 (3H, s, CH_3CON-); 8.69 (1H, bs, H-7); 6.01 (1H, d, J = 5.5 Hz, H-1′); 5.44 (1H, dq, J = 5.5 and 6.5 Hz, H-2′); 3.61 (3H, s, CH_3-N); 1.26 (3H, d, J = 6.5 Hz, H_3-3′).

10 High-resolution mass spectra fragments of **2**: m/z 317.119 (2%), M^+-AcOH; 302.0909 (4), M^+-AcOH-CH_3; 291.0953 (22), M^+-CH_2=CHOAc; 275.0999 (88), M^+-AcOH-CH_2=C=O; 260.0901 (20), M^+-AcOH-CH_3-N=C=O; 259.1066 (36), M^+-2AcOH; 249.0851 (100), M^+-CH_2=CHOAc-CH_2=C=O; 244.0846 (95), M^+-2AcOH-CH_3. Mass spectra were taken on AEI-902 instrument.

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12 Spectra were recorded on a Bruker WM-250 spectrometer in DMSO. The assignments of the protonated carbon atom were based on ^{13}C-^1H shift correlation 2D-NMR spectroscopy via ^1J couplings which showed interrelation of all the protonated carbons with the pertinent proton(s); the shift correlation with polarization transfer via J-coupling experiments were performed using a Bruker microprogram adjusting the fixed delays D_3 and D_4 to give maximum polarization for J_{C-H} = 135 Hz. The assignments of the remaining carbon atoms were based on comparison with the 2-amino-4-pteridinones, whose ^{13}C-NMR data were previously reported[13], and on the results of the gated decoupling experiment.

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Announcements

Friedrich Miescher-Award 1988

To commemorate the 100-year anniversary of the discovery of nucleic acids the Swiss Society for Biochemistry has created the Friedrich Miescher-Award. This prize is intended to honor young biochemists and is donated by the Friedrich Miescher-Institute of Ciba Geigy Inc. in Basel.

Excerpts from the statutes:

1. The Friedrich Miescher-Award will be awarded once every two years to a young scientist for outstanding achievements in biochemistry.
2. Preference will be given to candidates not older than 35 years. Eligibility extends only to candidates not exceeding their 40th year.
3. The scientific work must have been carried out in Switzerland or by Swiss scientists abroad.

Applications or nominations of candidates should be submitted by **November 1, 1987** to the secretary of the Swiss Society for Biochemistry:

Dr. L. Kühn, Swiss Institute for Experimental Cancer Research, 155, ch. des Boveresses, CH-1066 Epalinges s. Lausanne, Switzerland.