was assigned to H-2α based on a coupling constant of 13 Hz with H-1. Irradiation at the frequency of H-1 also affected the signal at $\delta = 2.25$ which was assigned to H-2β. Irradiation at the frequency of the C-4-Me and the multiplet beneath it ($\delta \approx 1.28$) partially collapsed the multiplet at $\delta = 2.16$ resulting in the assignment of H-3β($\delta = 2.16$) and H-3α($\delta = 1.28$). The single irradiations of H-6 and H-7 combined with the observed couplings ($J_{5,6} = 9.0, J_{6,7} = 8.5$ Hz) showed that the γ-lactone ring was trans-fused and delineated the stereochemical relationship of the epoxide ring. These assignments agreed favorably with those of Herz and Sharma[5] and Lee and coworkers[6] for a series of germacranolides isolated from *Eupatorium hyssopifolium* L. Further irradiation at 4.32 ppm permitted us to intercorrelate the resonance signals of H-9, H-8, H-7, H-6 and H-5. Thus, we could assign the hydroxy group to position C-9. This assignment was further substantiated by the large downfield shift of the H-1 signal upon MnO_2 oxidation ($\Delta\delta = 0.78$ ppm).

The PMR-spectrum of the acetate displayed the upfield shift of the H-7 (α-configuration) ($\Delta\delta = 0.2$ ppm) and H-1 ($\Delta\delta = 0.17$ ppm) signals, indicating the syn relationship between the H-7 and the acetate group.

The 360 MHz PMR spectrum of **1** recorded in d_5-pyridine demonstrated a substantial solvent effect for H-7 ($\Delta\delta = 0.43$) and H-1 ($\Delta\delta = 0.38$). A study of molecular models showed that such a solvent effect would be expected only if the hydroxyl group at C-9 was in an α-configuration.

If the C-9 hydroxyl were β, a downfield shift would be expected for the C-10-Me, and no such shift was observed. Therefore, we propose that this crystalline compound is 9α-hydroxyparthenolide (**1**). This compound showed cytotoxicity ($ED_{50} = 10^{\circ} - 10^{-1}$ µg/ml in 9KB and $ED_{50} = 10^{\circ} - 10^{-2}$ µg/ml in P388 in vitro) as well as significant in vivo activity in P388 (T/C = 150% at 80–90 mg/kg, best test).

^{1}H-NMR. spectral data of 9α-hydroxyparthenolide

Chemical shift (ppm)	Assignment	Multiplicity[4]	Coupling constants (Hz)
6.30	H-13a	d	3.7 (H-7)
5.63	H-13b	d	3.4 (H-7)
5.60	H-1	br, dd	13.0 (H-2α), 1.8 (H-2β)
4.32	H-9β	br, d	6.0 (H-8α)
3.84	H-6	t	8.5 (H-5, H-7)
3.39	H-7	m	
2.74	H-5	d	9.0 (H-6)
2.48	H-2α	qd	13.0 (H-2β, H-3α, H-1), 5.4 (H-3β)
2.35	H-8α	ddd	15.2 (H-8β), 6.0 (H-9), 1.5 (H-7)
2.25	H-2β	br, d	13.0 (H-2α)
2.16	H-3β	ddd	13.0 (H-3α), 5.4 (H-2α), 1.8 (H-2β)
1.92	H-8β	dd	15.2 (H-8α), 8.5 (H-7), 1.0 (H-9)
1.84*	–OH	br, s	
1.69	3H-14	br, s	
1.28	3H-15	s	
1.28	H-3α	td	13.0 (H-3β, H-2α), 5.0 (H-2β)

* Lost with D_2O shake-out; [4]br = broad.

1 To whom correspondence should be addressed. The authors acknowledge the support of contract No. N01-CM-97296 from the National Cancer Institute and the use of the Purdue University Biochemical Magnetic Resonance Laboratory (NIH grant No. RR01077). This is paper 16 in the series 'Potential Antitumor Agents'.

2 College of Pharmacy, University of Tehran, Tehran, Iran.
3 Collected in April 1976 near Hajiabad, 100 km north of the Persian Gulf and air dried in the shade. Voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, University of Tehran, under No. 408 and were identified by Professor Karl Humel, Tübingen University, and Mr C.H. Amin, University of Tehran.
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Linderazulene, a new naturally occurring pigment from the gorgonian *Paramuricea chamaeleon*

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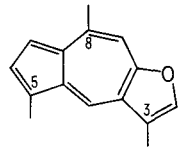
Summary. The purple pigment of the gorgonian *Paramuricea chamaeleon* has been isolated and identified as linderazulene (**I**).

The first azulenofuran, linderazulene (**I**), was obtained by zinc-dust distillation of linderene isolated from the roots of *Lindera strychnifolia*[2], and its structure was confirmed by synthesis[3]. In the course of our investigations on the chemistry of marine organisms in Turkish waters we have isolated the same dark purple pigment from the purple parts of the gorgonian *Paramuricea chamaeleon.*

The corals, collected in the Marmara Sea near Istanbul, were preserved immediately in acetone, and extracted with the same solvent (dry wt after extraction, 600 g). The combined extracts were concentrated in vacuo and the remaining aqueous mixture was extracted with ether. The oily residue (10 g) from the ether extract was chromatographed on a silica gel column. The petrol (b.p. 50–70 °C) eluate, containing mainly the purple pigment, was purified by PLC on silica gel in petrol (50–70 °C), and the pigment was crystallized from aqueous alcohol as dark purple plates, m.p. 103–105 °C (106–107 °C)[3] (320 mg).

High resolution mass spectrometry gave the molecular formula $C_{15}H_{14}O$ (found: M^+, 210.1042 (100%); calculated 210.1044) and the following main fragments were observed at low resolution: *m/e* 209(94), 195(9.5), 165(19), and 152(4.5). The IR spectrum (CS_2) showed that the pigment contains neither hydroxyl nor carbonyl groups while the

electronic spectrum was typically azulenic; λ_{max} (hexane) 280, 287, 292, 300, 315, 321, 361, 372, 380, 391, 563, 601, 612, 644sh, and 673 nm. Both UV/VIS and IR spectra are in excellent agreement with those of authentic linderazulene. The ^{1}H-NMR spectrum[4] (220 MHz, CCl_4) showed singlets for three aromatic methyl groups at δ 2.33(C-3),



I

2.64(C-5) and 2.75(C-8), 3 isolated aromatic protons at 7.13(H-2), 7.28(H-9), and 8.10(H-4), and 2 coupled aromatic protons at 7.04 and 7.25 (each 1H, d, J 4Hz). Thus all the evidence agrees with structure (**I**). Furthermore the pigment formed a 1,3,5-trinitrobenzene complex, violet-black needles, m.p. 154–155 °C (154–155 °C)[3].

Naturally occurring azulenes have been found previously only in the liverwort *Calypogeia trichomanis*[5], the fungus *Lactarius indigo*[6], and possibly in the alga *Laurencia obtusa*[7].

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Studies on the inactivation of glyceraldehyde-3-phosphate dehydrogenase by methylglyoxal[1]

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Summary. Glyceraldehyde-3-P dehydrogenase (E.C. 1.2.1.12) from rabbit muscle is rapidly inactivated by methylglyoxal following pseudo first-order kinetics. Substrate, as well as inorganic phosphate, affords protection, whereas NAD^+ is ineffective. The arginine residue implicated in the reaction is probably the anion binding site of the phosphate group of the substrate.

Several studies of the action of methylglyoxal (MG) on different cell functions have been carried out. Kun[2,3] has demonstrated that the ketoaldehyde inactivates succinate dehydrogenase, hexokinase and triose phosphate dehydrogenase; Kikuchi et al.[4] have studied the inhibition of L-glutamine: D-fructose 6-phosphate aminotransferase and recently, in our laboratory, the inactivation of fructose 1,6-P_2 aldolase from rabbit muscle was examined[5].

Materials and methods. Substrates, enzymes and coenzymes were purchased from Sigma Chemicals Co. Methylglyoxal (Fluka), distilled every time before use, was tested according to Racker's procedure[6].

Glyceraldehyde-3-P dehydrogenase (Sigma) from rabbit muscle, dialyzed overnight at 2 °C before use aganist large volumes of 40 mM triethanolamine (TEA) buffer (pH 7.6), was assayed in 50 mM pyrophosphate buffer pH 8.5, 2.5 mM EDTA, 7.5 mM sodium arsenate, 0.5 mM NAD^+ and 0.5 mM D-glyceraldehyde-3-phosphate. Protein concentration was determined according to Fox and Dandliker[7] and thiol groups according to Ellman[8]. The amino acid composition of the native and modified enzyme was determined after hydrolysis for 24 h in 6 N HCl containing 20 µl of mercaptoacetic acid at 105 °C. The hydrolyzates were taken to dryness and finally analyzed on a Spinco amino acid analyzer mod. 120 B, equipped with a high sensitivity cuvette[9].

Results and discussion. Glyceraldehyde-3-phosphate dehydrogenase is rapidly inactivated by methylglyoxal, following first-order kinetics. Saturation kinetics are observed if the first-order rate constant of inactivation is measured as a function of methylglyoxal concentration: when τ (the half-time of inactivation) is plotted vs 1/[MG], values of 2.6 mM and 0.075 min^{-1} were found for K_i and k_3 respectively: K_i is the dissociation constant for the initial reversible complex and k_3 is the rate constant for the conversion of the reversible complex to the irreversibly inhibited enzyme[10] (fig. 1). A double logarithmic plot of the reciprocal of the half-time of inactivation against inhibitor concentration[11,12] yields the reaction order of 0.94 with respect to inhibitor.

The effect of pH on the rate of methylglyoxal inactivation was examined in the pH range from 6.6 to 9.9. No maximal

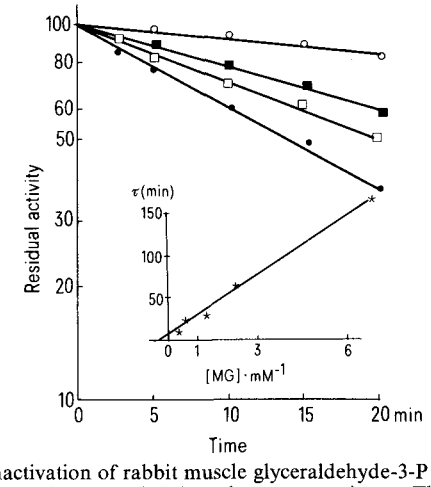

Fig. 1. Inactivation of rabbit muscle glyceraldehyde-3-P dehydrogenase at various methylglyoxal concentrations. The enzyme (0.015 mM) was incubated in 50 mM TEA buffer pH 7.5 with either 0.45 mM (○), 0.75 mM (■), 1.5 mM (□), or 2.25 mM (●) methylglyoxal. A control in absence of methylglyoxal was carried out under the same conditions. Insert: plot of the half-inactivation time (τ) vs the reciprocal of the inhibitor concentration.