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9-α-Hydroxyparthenolide, a novel antitumor sesquiterpene lactone from *Anvillea garcini* (Burm.) DC[1]

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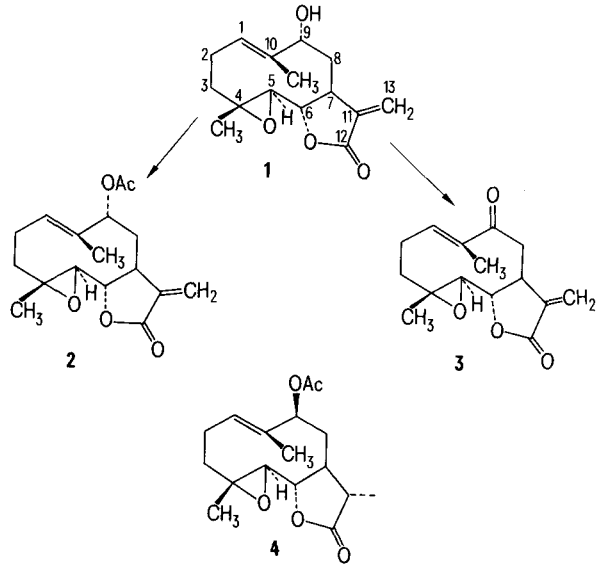
Summary. A novel sesquiterpene lactone, 9-α-hydroxyparthenolide (**1**) was isolated from a chloroform extract of *Anvillea garcini* that was collected in Iran. The structure of **1** was assigned on the basis of high field NMR decoupling experiments and other spectroscopic evidence. This α-methylene lactone has shown significant activity in both the 9KB cell culture and P388 mouse leukemia test systems.

The plant *Anvillea garcini* is found in southern Iran approximately 100 km north of the Persian Gulf. It is a member of the family Compositae which has previously produced a variety of cytotoxic and antitumor sesquiterpenes. The ground plant material[3] was extracted with chloroform, and the resulting residue was partitioned between petroleum ether and 90% methanol. The aqueous methanol soluble fraction was treated with 4% lead acetate, and the chloroform extract of the resulting aqueous layer was chromatographed on silica gel. Elution with chloroform followed by 10% ethyl acetate-chloroform gave a fraction that readily crystallized from chloroform-petroleum ether to give compound (**1**), m.p. 141-143 °C, NSC 290497.

The high resolution mass spectral data revealed the molecular formula, $C_{15}H_{20}O_4$ (M^+ 264.139). The UV-absorption at λ_{max}^{MeOH} 205 nm ($\varepsilon = 10{,}700$) and IR-absorption band at 1755 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone. This was further supported by the pair of characteristic proton signals at δ 6.30 (d, 3.7 Hz) and δ 5.63 (d, 3.4 Hz), and the carbon signals at 169.5 ($-CO_2-$) and 121.0 ($=CH_2$) ppm. The CMR-spectrum of **1** showed a total of 15 carbon atoms, 1 in the carbonyl region, 4 in the olefin region, and 2 in the methyl region. An unusually large ^{13}C-^1H one-bond coupling (180.6 Hz) of a methine carbon at 66.3 ppm indicated the existance of a trisubstituted epoxide. Further comparison of the PMR-spectrum of **1** with literature data provided strong evidence that **1** was an epoxy germacranolide. The hydroxy group was displayed by an IR-absorption band at 3450 cm^{-1}, and 2 proton signals at 4.32 ($-CH-OH$) and 1.84 (OH) ppm. This 4.32 ppm signal was shifted to 5.43 ppm upon acetylation to **2** with acetic anhydride in pyridine and disappeared upon oxidation with MnO_2 to **3**. Herbolide C (**4**) which is structurally related to **2** shows a signal for H-9 (9-β-acetoxy) at 5.05 ppm[4].

The proposed structure of **1** was then assigned primarily on the basis of the complete 360 MHz PMR-spectral analysis (table) which was achieved by extensive decoupling experiments. At high field the methylene signals (δ1.2-2.6) are well resolved to allow chemical shift and multiplicity assignments to be made. The quartet of doublets at δ = 2.48



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