

Antitumor Germacranolides from *Anvillea garcinii*

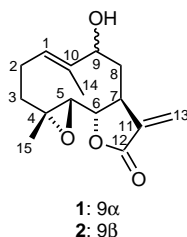
Essam Abdel Sattar,* Ahmed M. Galal, and Gaber S. Mossa

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

Received May 15, 1995[⊗]

The aerial parts of *Anvillea garcinii* yielded two new germacranolides, 9 α -hydroxy-1 β ,10 α -epoxyparthenolide (**4**) and parthenolid-9-one (**5**), in addition to the known 9 α -hydroxyparthenolide (**1**), 9 β -hydroxyparthenolide (**2**), and 9 β -hydroxy-1 β ,10 α -epoxyparthenolide (**3**). The structures of the new compounds were elucidated from their spectral data (IR, MS, ¹H- and ¹³C-NMR, ¹H–¹H COSY, and ¹H–¹³C HETCOR) and by chemical derivatization. The hitherto unreported ¹³C-NMR data and carbon atom assignments of the previously isolated lactones **1**, **2**, and **3** were given. The in-vitro antitumor and anti-HIV activities were evaluated for the isolated compounds.

Anvillea garcinii (Burm.) (S.) DC (Asteraceae) is a wild plant found in areas of the Middle East¹ and has been reported to have hypoglycemic activity.² Three germacranolides were isolated from the aerial parts of the plant: 9 α -hydroxyparthenolide (**1**),³ 9 β -hydroxyparthenolide (**2**) (epimer of **1**), and 9 β -hydroxy 1 β ,10 α -epoxyparthenolide (**3**) (epoxide of **2**).⁴ The flavones of the aerial parts of the plant were also studied by Ulubelen et al. in 1979.⁵ A phytobiological investigation of *A. garcinii* DC. ssp. *radiata*⁶ evaluated the hypoglycemic activities of compounds **1** and **2** and a humulano-lactone asteriscunolide A (IC₅₀ 9.8, 6.2, and 10 mg/kg, respectively).



In this study, we report the isolation and identification of two new germacranolides, compounds **4** and **5**, in addition to the known lactones **1**, **2** and **3**. ¹³C-NMR data are reported for lactones **1**–**5** for the first time. The in vitro antitumor and anti-HIV activities of lactones **1**–**5** were also evaluated.

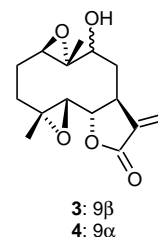
The defatted CHCl₃ extract of *A. garcinii* was chromatographed on a Si gel column using CH₂Cl₂ containing increasing amounts of CH₃CN. Further chromatography of the main fractions on Si gel columns yielded compounds **1**–**5**. The IR spectrum of compound **4** showed absorption bands corresponding to OH (3460 cm⁻¹) and α,β -unsaturated γ -lactone (1760 cm⁻¹) groups. The ¹H- and ¹³C-NMR spectral data of **4** (see Experimental Section and Table 1) showed general features similar to those of **3**. The major difference in the ¹H-NMR spectra was the presence of signals at δ 3.28 (dd, J = 10.6, 1.7 Hz) and δ 4.12 (brd, J = 7.5 Hz) in **3** and **4**, respectively. The ¹³C-NMR spectrum of compound **4**

Table 1. ¹³C NMR (75 MHz) Spectral Data for Germacranolides 1–5^a

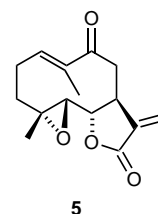
carbon	1	2	3	4	5 ^b
1	121.74 d	126.10 d	57.37 d	63.19 d	139.87 d
2	23.45 t	23.76 t	23.05 t	23.43 t	23.63 t
3	36.18 t	36.99 t	34.88 t	34.84 t	35.34 t
4	61.39 s	61.52 s	60.05 s	60.60 s	60.83 s
5	66.48 d	66.09 d	64.96 d	64.11 d	65.33 d
6	82.53 d	81.59 d	81.73 d	80.94 d	80.91 d
7	37.50 d	44.34 d	36.42 d	44.18 d	44.39 d
8	37.47 t	38.06 t	38.06 t	33.95 t	39.91 t
9	71.26 d	79.44 d	68.34 d	79.29 d	202.47 s
10	137.45 s ^c	136.65 s ^d	62.78 s	63.85 s	137.02 s ^e
11	139.68 s ^c	138.29 s ^d	139.52 s	139.94 s	138.08 s ^e
12	169.53 s	169.01 s	169.02 s	168.57 s	168.02 s
13	121.16 t	121.60 t	121.30 t	121.68 t	121.15 t
14	16.38 q	10.87 q	16.32 q	11.50 q	12.70 q
15	17.23 q	17.31 q	16.93 q	17.03 q	17.87 q

^a Multiplicities of the carbon signals were determined using APT and DEPT experiments. ^b Spectrum run using JEOL Ex-400 (100 MHz). ^{c–e} Signals with the same superscript may be interchangeable.

was shown to be very close to those of **1** and **2**, except that **4** showed the absence of the two olefinic carbons.



Comparison of ¹H- and ¹³C-NMR data of **4** to those of **1** and **3** suggested that compound **4** is the epoxide of **1** and the epimer of **3**. The structure of compound **4** was further confirmed by epoxidation of **17** to give a product indistinguishable from **4**. Therefore, compound **4** is the 1,10-epoxide of **1** and the epimer of **3**.



* Author to whom correspondence should be addressed. Telephone: (966)-1-467-77250. Fax: (966)-1-467-6383.

[⊗] Abstract published in *Advance ACS Abstracts*, February 15, 1996.

Table 2. Cytotoxicity^a of Compounds 1–5

panel/cell line	compound (ED ₅₀)				
	1	2	3	4	5
leukemia					
CCRF-CEM	0.84	0.80	2.43	1.44	0.05
MOLT-4	0.94	0.68	2.83	1.67	0.07
non-small cell lung					
HOP-92	1.32	4.57	5.74	4.82	0.63
NCI-H522	0.50	0.45	0.78	1.58	0.15
colon cancer					
HCT-116	1.66	3.83	4.65	4.96	0.45
SW-620	1.08	0.55	3.81	3.72	0.21
cns cancer					
SF-268	10.30	7.76	12.10	13.47	1.73
SNB-75	^b	8.53	22.51	20.75	4.38
melanoma					
LOX IMVI	1.66	2.46	^b	3.70	0.11
MALME-3M	1.07	1.62	3.36	3.28	0.33
ovarian cancer					
IGROV1	8.63	3.91	4.17	^b	0.94
OVCAR-8	4.07	4.75	5.32	9.72	0.78
renal cancer					
ACHN	1.91	2.21	5.07	3.84	0.30
UO-31	7.63	3.88	4.79	4.76	0.28
prostate cancer					
PC-3	11.62	^b	4.98	7.59	0.90
DU-145	6.73	5.60	9.66	10.22	3.30
breast cancer					
MDA-MB-231/ATCC	4.49	4.99	^b	5.35	0.17
BT-549	2.77	^b	3.86	^b	0.34
T-47D	1.54	^b	5.40	^b	0.45

^a ED₅₀ = Effective dose that inhibits net cell growth to 50% of control growth in $\mu\text{g/mL}$. ^b ED₅₀ value not taken.

The IR spectrum of compound **5** did not have OH absorption bands but showed instead absorption bands corresponding to two carbonyl groups at 1660 (α,β -unsaturated ketone) and 1770 cm^{-1} (α,β -unsaturated γ -lactone). The ¹H- and ¹³C-NMR spectra of **5** also suggested a structure related to **1** and **2**. The main difference in ¹³C-NMR spectra between **5** and **1** or **2** was the presence in **5** of a downfield quaternary carbon (APT and DEPT) resonating at δ 202.47 instead of signals at δ 71.26 and 79.44, as in **1** and **2**, respectively (C-9). The structure of **5** was elucidated by correlation to **1** and **2**. Oxidation of **1** or **2** using pyridinium chlorochromate⁸ gave a product indistinguishable from **5**. Therefore, compound **5** was identified as parthenolid-9-one.

Compounds **1–5** were evaluated by the National Cancer Institute (NCI) by an in vitro disease-oriented antitumor screen, which determines cytotoxic effects against a panel of approximately 60 human tumor cell lines.^{9,10} Cytotoxicity is expressed as the C₅₀ (half-maximal effective dose in $\mu\text{mol/L}$) or as ED₅₀ (effective dose that inhibits the net cell growth to 50% of the control growth in $\mu\text{g/mL}$) values. ED₅₀ values of selected cell lines are given for compounds **1–5** in Table 2. Kupchan et al.¹¹ reported that compounds showing ED₅₀ \leq 4 $\mu\text{g/mL}$ or C₅₀ \leq 15 $\mu\text{mol/L}$ are deemed significantly cytotoxic. Compound **5** showed the highest activity (ED₅₀ 0.05–4.38 $\mu\text{g/mL}$), which agrees with the observation of Kupchan et al.¹¹ that increasing the degree of unsaturation increases the activity. Compounds **1–4** showed moderate to significant activity, as indicated in Table 2. Compounds **1–5** were inactive when tested by NCI in their anti-HIV testing program.¹²

Experimental Section

General Experimental Procedures. Mps were determined on an electrothermal melting point ap-

paratus (Electrothermal Ltd., England) and are uncorrected. IR spectra were recorded on a Pye Unicam Sp3–300 and mass spectra on Varian mat 445, CIMS with NH₃. ¹H-NMR, homonuclear COSY and ¹³C-NMR, APT, DEPT, and HETCOR spectra were recorded in CDCl₃ with TMS as the internal standard, employing a Varian XL-300 and JEOL EX-400 instruments. Optical rotations were measured at ambient temperature, using a Perkin-Elmer 241 MC polarimeter. TLC was performed on Si gel 60 F₂₅₄, CHCl₃–CH₃CN (7:3); visualization was done with *p*-anisaldehyde/H₂SO₄ as spray reagent.

Plant Materials. The aerial parts of *A. garcinii* were collected from El Gassim Road, Riyadh, Saudi Arabia, in April 1992. A voucher specimen (no. 12811) was deposited in the Herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction and Isolation. The dried, ground aerial parts of *A. garcinii* (1.6 kg) were percolated with CHCl₃ (10 L) at room temperature. The CHCl₃ extract (53 g) was dissolved in 400 mL of MeOH, diluted with an equal volume of H₂O, and left for 48 h in a cold room (4 °C). The aqueous MeOH solution was filtered through a Büchner funnel, and the filtrate was shaken with *n*-hexane (3 × 150 mL) and concentrated under vacuum to give 19 g of a syrupy extract. This extract was chromatographed on a Si gel column (type 60, 500 g) using CH₂Cl₂ containing increasing amounts of CH₃CN. Three main fractions were collected; **A** (95 mg), **B** (5.1 g), and **C** (2.9 g). Fraction **A** on crystallization from CHCl₃/Et₂O gave 37 mg of **5** as needle crystals. Fraction **B** on crystallization from CHCl₃/Et₂O yielded 2.62 g of **1**. Fraction **C** on repeated column chromatography over Si gel using MeOH/CH₂Cl₂ (3:97) as eluent yielded, in the following order, compounds **4** (110 mg), **2** (455 mg), and **3** (100 mg).

Compound **4** was obtained as needles (*n*-hexane/CH₂Cl₂): mp 200–201 °C; [α]_D –70.8° (*c* 0.148, CHCl₃); IR, ν_{max} (KBr) cm^{-1} 3460 br (OH), 1760 (γ -lactone); ¹H-NMR (300 MHz) δ 6.3 (1H, d, $J_{13a,7}$ = 3.8, H-13a), 5.64 (1H, d, $J_{13b,7}$ = 3.3, 13b), 4.12 (1H, d(br), $J_{9,8\alpha}$ = 7.5, H-9 α), 3.93 (1H, t, $J_{5,6}$ = $J_{6,7}$ = 9, H-6), 3.35 (1H, m, H-7), 3.22 (1H, dd, $J_{1,2\beta}$ = 11.2, $J_{1,2\alpha}$ = 1.2, H-1), 2.82 (1H, d, $J_{5,6}$ = 9, H-5), 2.48 (1H, ddd, $J_{7,8\alpha}$ = 2, $J_{9,8\alpha}$ = 7.5, $J_{8\alpha,8\beta}$ = 15.8, H-8 α), 2.25 (1H, ddd, $J_{2\beta,3\beta}$ = 5, $J_{2\alpha,3\beta}$ = 2.3, $J_{3\alpha,3\beta}$ = 13.5, H-3 β), 2.20 (1H, m, H-2 α), 1.89 (1H, ddd, $J_{8\alpha,8\beta}$ = 15.8, $J_{7,8\beta}$ = 6.8, $J_{9,8\beta}$ = 1.3, H-8 β), 1.58 (1H, m, H-2 β), 1.36 (1H, overlapping, H-3 α), 1.37 (3H, s, H-14), and 1.36 (3H, s, H-15); ¹³C-NMR (75 MHz), see Table 1; CIMS with NH₃ *m/z* (rel int): [M]⁺ absent, 200 (46.7), 183 (12), 168 (6.5), 130 (35.9), 114 (13), 102 (100), and 88 (33.7).

Compound **5** was obtained as needles (CHCl₃/Et₂O): mp 241–243 °C; [α]_D –17.44° (*c* 0.088, CHCl₃); IR, ν_{max} (KBr) cm^{-1} 1770 (γ -lactone), 1660 (carbonyl); ¹H-NMR (400 MHz) δ 6.41 (1H, d, $J_{13a,7}$ = 3.4, H-13a), 6.31 (1H, dd, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 8.5, H-1), 5.78 (1H, d, $J_{13b,7}$ = 3.1, H-13b), 4.07 (1H, dd, $J_{5,6}$ = 8.7, $J_{7,6}$ = 9.3, H-6), 3.25 (1H, dd, $J_{8\alpha,8\beta}$ = 13.4, $J_{8\alpha,7}$ = 8.3, H-8 α), 2.86 (1H, m, H-7), 2.79 (1H, d, $J_{8\alpha,8\beta}$ = 13.4, H-8 β), 2.69 (1H, d, $J_{5,6}$ = 8.7, H-5), 2.68 (1H, overlapping, H-3 β), 2.47 (1H, m, H-2 β), 2.38 (1H, m, H-2 α), 1.93 (3H, s, H-14), 1.55 (3H, s, H-15) and 1.36 (1H, m, H-3 α); ¹³C-NMR (100 MHz), see Table 1; CIMS with NH₃ *m/z* (rel int) 280 [M + NH₄]⁺ (17.7), 236 (9.9), 200 (73.7), 183 (25), 136 (16.8), 102 (100), and 88 (20.3).

Epoxidation of 1. *m*-Chloroperbenzoic acid (*m*-CPBA) (50 mg) was added to a solution of compound **1** (100 mg) in CH₂Cl₂ (15 mL). The mixture was stirred for 20 min at room temperature and treated in the usual manner.⁷ The residue left after evaporation of the solvent was washed several times with Et₂O to remove traces of acid, then the residue was crystallized from CH₂Cl₂/*n*-hexane to give **4** (70 mg).

Oxidation of 1 or 2. Pyridinium chlorochromate (70 mg) was added to a solution of compound **1** (150 mg) in CH₂Cl₂ (10 mL), and the mixture was stirred for 3 h at room temperature. The reaction mixture was worked up in the usual manner⁸ and purified on Si gel column chromatography using 1% MeOH in CH₂Cl₂ to give 80 mg of compound **5**. Oxidation of compound **2**, using the same procedure, afforded the same product **5**.

Biological Assays. The *in vitro* antitumor activity of compounds **1–5** was evaluated according to the standard procedures of the NCI^{9,10}, on a cell line panel consisting of 60 lines against which compounds were tested at a minimum of five concentrations at 10-fold dilutions. The more significant ED₅₀ values for selected cell lines are given in Table 2. Compounds **1–5** were also evaluated for *in vitro* anti-HIV activity¹² and found to be inactive.

Acknowledgment. The authors thank Dr. C. D. Hufford, Department of Pharmacognosy, University of Mississippi, University, MS for running the 300 MHz 1D- and 2D-NMR spectra; Dr. A. Nahrstedt, Institute für Pharmazeutische Biologie und Phytochemie, Mün-

ster, Germany, for running mass spectra; and Dr. S. Ul-Abedin for identification of the plant material. The authors are also indebted to Dr. V. L. Narayanan, Drug Synthesis and Chemistry Branch, and J. P. Bader, Antiviral Evaluations Branch, NCI, Bethesda, MD, for *in vitro* antitumor and anti-HIV testing.

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NP960064G