cis-Parthenolid-9-one from *Anvillea garcinii*

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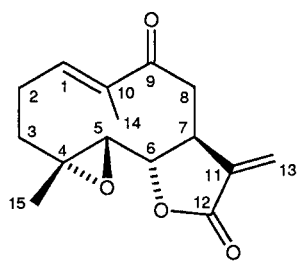
Received May 31, 2000

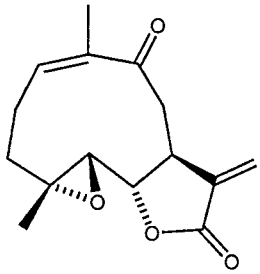
The *cis*-isomer (**2**) of the previously isolated parthenolid-9-one (**1**) was isolated from *Anvillea garcinii* and the structures and relative stereochemistries of both were determined from NMR data in combination with single-crystal X-ray analysis. In vitro cytotoxicity and in vivo antitumor activity for both compounds are reported.

Anvillea garcinii (Burm.) (S.) DC. (Asteraceae) is a wild plant found in areas of the Middle East.[1] Previous examination of the aerial parts of the plant afforded five germacranolides—9α-hydroxyparthenolide,[2] 9β-hydroxyparthenolide,[3] 9β-hydroxy-1β,10α-epoxyparthenolide,[3] 9α-hydroxy-1β,10α-epoxyparthenolide,[4] and parthenolid-9-one (**1**)[4]—and two guaianolides,[5] as well as flavonoidal components.[6] The cytotoxic effects of the isolated germacranolides against a panel of approximately 60 human tumor cell lines were reported.[4] We describe herein the isolation and structure determination of a new geometrical isomer of parthenolid-9-one and report the results of in vitro cytotoxicity and in vivo antineoplastic studies.

Compound **2** was isolated by chromatography of the partially purified $CHCl_3$ extract of *A. garcinii* on a Si gel column and further purified using a Chromatotron apparatus. Compound **1**, previously reported from the same plant,[4] was isolated along with **2**. ^1H and ^{13}C NMR spectral data for **2** were strikingly similar to those of **1**, with only small differences in their chemical shifts, indicating that **2** also has a germacranolide skeleton. Furthermore, UV irradiation (λ 254 nm) of **1** yielded **2** as a major product, suggesting that **2** was the geometrical isomer of **1**.



1



2

Single-crystal X-ray analysis established unambiguously the complete structure and relative stereochemistry of **2**. For comparison purposes, an X-ray crystallographic study of **1** was also performed. Views of the solid-state conformations of **1** and **2** are presented in Figure 1. In general, corresponding bond lengths agree well and are in accord with normal values.[7] Exceptions to this are C-2−C-3 [1.567(6) Å (**1**) > 1.505(6) Å (**2**)] and C-7−C-8 [1.557(5) Å (**1**) > 1.520(5) Å (**2**)], where the differences reflect the greater bond strain in **1** versus **2**. Moreover, whereas C-10 lies in the C-1, C-9, C-14 plane [Δ 0.011 Å (**1**), 0.015 Å (**2**)] in both compounds, the strained nature of the trans double

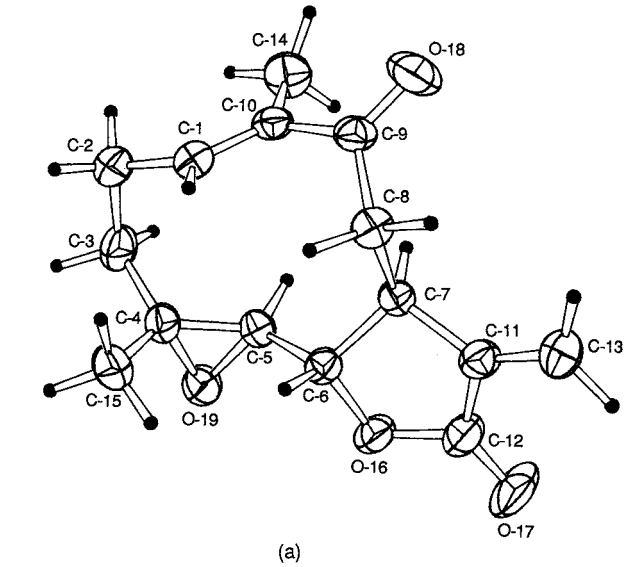


Figure 1. ORTEP diagrams (40% probability ellipsoids) showing the crystallographic atom numbering schemes and solid-state conformations of (**a**) parthenolid-9-one (**1**) and (**b**) *cis*-parthenolid-9-one (**2**). Small filled circles represent hydrogen atoms.

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10.1021/np000269w CCC: $19.00
Published on Web 10/21/2000

Table 1. Cytotoxicity of Compounds **1** and **2**

	C_{50}[a]		ED_{50}[b]	
subpanel	**1**	**2**	**1**	**2**
leukemia	0.08	1.94	0.02	0.51
nonsmall cell lung cancer	1.8	13.4	0.47	3.51
colon cancer	0.32	7.3	0.08	1.91
cns cancer	2.9	17.1	0.76	4.47
melanoma	0.57	10.6	0.15	2.77
ovarian cancer	1.4	11.9	0.37	3.13
renal cancer	0.41	8.05	0.11	2.11
prostate cancer	2.2	14.4	0.57	3.77
breast cancer	0.6	9.81	0.16	2.57
mean value	0.67	10.5	0.17	2.75

[a] C_{50} = Half-maximal effective dose in μmol/L. [b] ED_{50} = Effective dose that inhibits net cell growth to 50% of control growth in μg/mL.

bond in **1** is manifested in twisting about the C-1═C-10 bond as reflected in the angle of 7.5° between the C-1, C-2, C-10 and C-1, C-9, C-10, C-14 planes; the corresponding interplanar angle in **2** is 0.7°.

The conformation of the ten-membered ring in **1** is defined by the following endocyclic torsion angles ω_{ij} (σ 0.3−0.5°) about bonds between atoms *i* and *j*: $\omega_{1,2}$ 87.0, $\omega_{2,3}$ 33.3, $\omega_{3,4}$ −89.4, $\omega_{4,5}$ 146.1, $\omega_{5,6}$ −119.3, $\omega_{6,7}$ 83.8, $\omega_{7,8}$ -107.3, $\omega_{8,9}$ 70.2, $\omega_{9,10}$ 38.6, $\omega_{10,1}$ −171.8°; C-14 and C-15 lie respectively on the α- and β-faces of the macrocycle. This ring has a conformation that resembles those in isodeoxyelephantopin[8] and tomenphantopin B,[9] rather than the chair−chair form as in elephantol,[10] eupatolide,[11] costunolide,[12] ovatifolin,[13] alatolide,[14] eupahyssopin,[15] and tomenphantin B[16] with their syn-oriented methyl groups or their equivalents. In **2**, the endocyclic torsion angles (σ 0.3−0.5°) of the ten-membered ring are $\omega_{1,2}$ −95.3, $\omega_{2,3}$ 73.7, $\omega_{3,4}$ −87.8, $\omega_{4,5}$ 147.9, $\omega_{5,6}$ −109.5, $\omega_{6,7}$ 87.9, $\omega_{7,8}$ −70.0, $\omega_{8,9}$ −44.9, $\omega_{9,10}$ 131.9, $\omega_{10,1}$ 0.9°; C-14 and C-15 lie on the α- and β-faces, respectively, as in **1**. The conformation is similiar to those in enhydrin,[17,18] schkuhriolide,[19] alloschkuhriolide,[20] and longipilin.[21]

Endocyclic torsion angles (σ 0.3−0.4°) in the C-6−C-7 trans-fused α-methylene γ-lactone ring of **1** ($\omega_{6,7}$ −31.4, $\omega_{7,11}$ 29.3, $\omega_{11,12}$ −16.9, $\omega_{12,16}$ −4.3, $\omega_{16,6}$ 23.0°) are related by an approximate C_s plane of symmetry passing through C-7 and the midpoint of the C-12−O-16 bond and, therefore, this ring is best decribed as an envelope form with C-7 as the out-of-plane atom. In contrast, an approximate C_2 symmetry axis passing through C-12 and the midpoint of the C-6−C-7 bond relates endocyclic torsion angles (σ 0.3−0.4°) of the corresponding ring in **2** ($\omega_{6,7}$ −28.7, $\omega_{7,11}$ 23.8, $\omega_{11,12}$ −10.2, $\omega_{12,16}$ −9.2, $\omega_{16,6}$ 24.7°), and thus this ring has a half-chair form. Despite the considerable overall conformational differences of the ten-membered rings in **1** and **2**, their C-5−C-6−C-7−C-8 torsion angles are quite similar [83.8(4)° (**1**), 87.9(3)° (**2**)], and the endocyclic γ-lactone ring torsion angles [−31.4(3)° (**1**), −28.7(3)° (**2**)] about the C-6−C-7 bonds are paired in sign with the exocyclic O═C−C═C torsion angles [−14.5(7)° (**1**), −12.5-(7)° (**2**)] in accord with earlier observations.[8,9,11−13,15−22]

The in vitro cytotoxicity of compound **2** was evaluated by the National Cancer Institute (NCI).[23,24] Cytotoxicity is expressed as C_{50} (half-maximal effective dose in μmol/L) or as ED_{50} (effective dose that inhibits the net cell growth to 50% of the control growth in μg/mL). Table 1 lists average C_{50} and ED_{50} values for compounds **1** and **2** for each subpanel (selected individual cell-line values for **1** were reported earlier[4]). According to the criteria of Kupchan et al.,[25] compounds showing $C_{50} \leq 15$ μmol/L or $ED_{50} \leq 4$ μg/mL are considered to be significantly cytotoxic; for **1** and **2**, the mean ED_{50} values are 0.17 and 2.75 μg/mL, respectively. The relative sensitivity of each cell line compared with the average sensitivity of all cell lines may be represented in graphic form. Log GI_{50} data, as well as bar-graph representations for compounds **1** (NSC 672120) and **2** (NSC 687011) are available through the NCI web site, http://dtp.nci.nih.gov/docs/cancer/searches/cancer_open_compounds.html. Compounds **1** and **2** showed significant activities against leukemia, colon, and renal subpanels. Further testing by in vivo hollow fiber assays, performed by the Developmental Therapeutics Program,[26] revealed that both compounds had only marginal activity as they scored 4 and 2 for **1** and **2**, respectively, with nil cell killing for each. Compounds with a combined subcutaneous and intraperitoneal score of 20, a subcutaneous score of 8 or net cell kill of 1 or more are judged active and referred for xenograft testing (mitomycin C, a standard agent has a total score of 38).[27] The NCI Biological Evaluation Committee decided that no further action was merited for either compound.

Experimental Section

General Experimental Procedures. Melting points were determined in open capillaries on an electrothermal melting point apparatus (Electrothermal Ltd., Southend-on-Sea, Essex, UK) and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 MC polarimeter. IR spectra were recorded in KBr disks on a Pye Unicam SP3−300 instrument. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker AM-400 spectrometer (400 and 100 MHz, respectively), with the chemical shifts (δ ppm) expressed relative to TMS as internal standard. Mass spectral data were obtained by use of a Hewlett-Packard 5988A GC-MS: 1% NH_3 in CH_4 gas. Centrifugally accelerated radial TLC was performed on a Chromatotron 7924 apparatus (Harrison Research Inc., Palo Alto, CA).

Plant Material. The aerial parts of *A. garcinii* were collected from El-Kaseem Road, Riyadh, Saudi Arabia, in April 1997. The plant material was identified by Dr. Sultan Ul-Abedin, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. 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 (800 g) of *A. garcinii* were extracted with $CHCl_3$ following the previously reported procedure.[4] A 4-g sample of the partially purified $CHCl_3$ extract was chromatographed on a flash column (Si gel 60, 4 × 16 cm) eluted with CH_2Cl_2, and 50-mL fractions were collected. Fractions eluted between 500 and 1450 mL, which showed two major spots, were pooled together. This fraction (300 mg) was subjected to rechromatography on a Chromatotron apparatus (2 mm) using CH_2Cl_2 as solvent, and 4-mL fractions were collected. Compounds **2** and **1** were isolated from fractions eluted between 72 and 84 mL (32 mg) and between 100−120 mL (55 mg), respectively. Recrystallization of both compounds from $CHCl_3$/Et_2O gave 26 mg of **1** and 12 mg of **2** as colorless needles.

Parthenolid-9-one (1): colorless needles, mp 241−243 °C ($CHCl_3$/Et_2O); $[\alpha]^{25}_D$ +17.44° (*c* 0.088, $CHCl_3$) (incorrectly reported as negative sign by Abdel-Sattar et al.[4]); IR, ^{1}H NMR, ^{13}C NMR, MS, as previously reported.[4]

***cis*-Parthenolid-9-one (2):** colorless needles, mp 245−246 °C ($CHCl_3$/Et_2O); $[\alpha]^{25}_D$ +59.4° (*c* 0.18, $CHCl_3$); IR (KBr) ν_{max} 1760 (γ-lactone), 1680 (carbonyl) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.31 (1H, d, $J_{7,13a}$ = 3.4 Hz, H-13a), 5.71 (1H, br t, $J_{1,2\alpha}$ = 9.9 Hz, $J_{1,2\beta}$ = 12 Hz, H-1), 5.55 (1H, d, $J_{7,13b}$ = 3.4 Hz, H-13b), 3.81 (1H, t, $J_{5,6}$ = $J_{6,7}$ = 9.5 Hz, H-6), 3.34 (1H, m, H-7), 3.23 (1H, dd, $J_{8\alpha,8\beta}$ = 15.5 Hz, $J_{7,8\alpha}$ = 2.7 Hz, H-8α), 3.01 (1H, dd, $J_{2\alpha,2\beta}$ = 13 Hz, $J_{2\beta,3\alpha}$ = 12 Hz, H-2β), 2.79 (1H, d, $J_{5,6}$ = 9.5 Hz, H-5), 2.35 (1H, dd, $J_{8\alpha,8\beta}$ = 15.5 Hz, $J_{7,8\beta}$ = 12 Hz, H-8β), 2.09 (1H, m, H-2α), 2.07 (1H, m, H-3β), 2.01 (3H, s,

H-14), 1.31 (3H, s, H-15), 1.02 (1H, m, H-3α); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.47 (d, C-1), 22.46 (t, C-2), 36.62 (t, C-3), 59.57 (s, C-4), 62.70 (d, C-5), 81.21 (d, C-6), 41.14 (d, C-7), 42.56 (t, C-8), 203.49 (s, C-9), 136.89 (s, C-10), 138.07 (s, C-11), 168.38 (s, C-12), 120.41 (t, C-13), 20.72 (q, C-14), 17.53 (q, C-15); CIMS with NH_3 *m/z* 280 [$M^+ + NH_4$] (68), 263 [$M^+ + H$] (100), 245 [$M^+ + H - H_2O$] (61), 217 (34), 193 (19), 165 (14), 139 (14), 99 (16).

UV Irradiation of 1. Compound **1** (50 mg) in 20 mL of MeOH was subjected to UV irradiation ($\lambda = 254$ nm) for 4 h. The reaction product after evaporation of the solvent was subjected to chromatography on a Si gel column and using the Chromatotron apparatus (see above for separation of **2**) to give pure **2** (15 mg).

X-ray Crystal Structure Analysis of Compounds 1 and 2. Crystal data for **1**: $C_{15}H_{18}O_4$; MW 262.31, orthorhombic, space group $P2_12_12_1(D_2^4)$ from the Laue symmetry and systematic absences $h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$, $00l$ when $l \neq 2n$; $a = 10.869(2)$, $b = 16.490(2)$, $c = 7.700(1)$ Å, $V = 1380.1(6)$ Å^3, $Z = 4$, $D_c = 1.262$ g cm^{-3}, μ(Cu Kα radiation, $\lambda = 1.5418$ Å) = 7.1 cm^{-1}; crystal dimensions: 0.04 × 0.09 × 0.46 mm. Crystal data for **2**: $C_{15}H_{18}O_4$; MW 262.31, orthorhombic, space group $P2_12_12_1(D_2^4)$ as for **1**, $a = 7.517(1)$, $b = 27.442(4)$, $c = 6.776(1)$ Å, $V = 1397.8(6)$ Å^3, $Z = 4$, $D_c = 1.246$ g cm^{-3}, μ(Cu Kα radiation, $\lambda = 1.5418$ Å) = 7.0 cm^{-1}; crystal dimensions: 0.04 × 0.09 × 0.56 mm.

Intensity data (1650 and 1704 independent $+h+k+l$ reflections for **1** and **2**, respectively) were recorded at 298 K on an Enraf-Nonius CAD-4 diffractometer. Both crystal structures were solved by direct methods. The enantiomer was selected in each case to yield an α-configuration for H-7. Positional and thermal parameters of the carbon and oxygen atoms were adjusted by means of several rounds of full-matrix least-squares calculations in which hydrogen atoms were incorporated at calculated positions; an extinction correction (g) was also refined during the later iterations for **2**. The parameter refinements converged at $R = 0.042$ ($R_w = 0.057$, GOF = 1.28) for **1** and $R = 0.045$ [$R_w = 0.059$, GOF = 1.39, $g = 2.0(4) \times 10^{-6}$] for **2** over 1091 reflections with $I > 2.0\sigma(I)$. Final difference Fourier syntheses contained no unusual features.

Crystallographic calculations were performed by use of the Enraf-Nonius Structure Determination Package (SDP 3.0). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from *International Tables*.[28]

Acknowledgment. The authors thank Dr. Sultan Ul-Abedin, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, for identification of the plant material.

Supporting Information Available: Tables of crystallographic data and data collection parameters, fractional atomic coordinates and temperature factor parameters, bond lengths, bond angles, and torsion angles for **1** and **2**. This material is available free of charge via the Internet at http:/pubs.acs.org.

References and Notes

(1) Collenette, S. *An Illustrated Guide to the Flowers of Saudi Arabia*; Scorpion: London, 1985; pp 276−278.
(2) Tyson, R. L.; Chang, C.-J.; McLaughlin, J. L.; Aynehchi, Y.; Cassady, J. M. *Experientia* **1981**, *37*, 441−442.
(3) Rustaiyan, A.; Dabiri, M.; Jakupovic, J. *Phytochemistry* **1986**, *25*, 1229−1230.
(4) Abdel-Sattar, E.; Galal, A. M.; Mossa, G. S. *J. Nat. Prod.* **1996**, *59*, 403−405.
(5) Galal, A. M. *Al-Azhar J. Pharm. Sci.* **1997**, *19*, 30−33.
(6) Ulubelen, A.; Mabry, T. J.; Aynehchi, Y. *J. Nat. Prod.* **1979**, *42*, 624−626.
(7) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1.
(8) Zhang, D.; Haruna, M.; McPhail, A. T.; Lee, K.-H. *Phytochemistry* **1986**, *25*, 899−904.
(9) Hayashi, T.; Koyama, J.; McPhail, A. T.; Lee, K.-H. *Phytochemistry* **1987**, *26*, 1065−1068.
(10) McPhail, A. T.; Sim, G. A. *J. Chem. Soc,. Perkin Trans. 2* **1972**, 1313−1316.
(11) McPhail, A. T.; Onan, K. D. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1798−1975.
(12) Bovill, M. J.; Cox, P. J.; Cradwick, P. D.; Guy, M. H. P.; Sim, G. A.; White, D. N. J. *Acta Crystallogr.* **1976**, *B32*, 3203−3209.
(13) Gopalakrishna, E. M.; Watson, W. H.; Hoeneisen, M.; Silva, M. *J. Cryst. Mol. Struct.* **1977**, *7*, 49−57.
(14) Cox, P. J.; Sim, G. A. *J. Chem. Soc., Perkin Trans 2* **1977**, 255−258.
(15) Onan, K. D.; McPhail, A. T. *J. Chem. Res.* **1978** (*S*) 12−13; (*M*) 201−238.
(16) Hayashi, T.; Nakano, T.; Kozuka, M.; McPhail, D. R.; McPhail, A. T.; Lee, K.-H. *J. Nat. Prod.* **1999**, *62*, 302−304.
(17) Kartha, G.; Go, K. T. *J. Cryst. Mol. Struct.* **1976**, *6*, 31−42.
(18) Tak, H. Y.; Fronczek, F. R.; Vargas, D.; Fischer, N. H. *Spectrosc. Lett.* **1994**, *27*, 1481−1488.
(19) Rychlewska, U. *J. Chem. Soc., Perkin Trans. 2*, **1982**, 1641−1644.
(20) Rychlewska, U. *Acta Crystallogr.* **1983**, *C39*, 1303−1305.
(21) Fronczek, F. R.; Vargas, D.; Fischer, N. H. *Acta Crystallogr.* **1986**, *C42*, 1061−1063.
(22) McPhail, A. T.; Onan, K. D. *J. Chem. Soc., Perkin Trans. 2* **1976**, 578−582.
(23) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 662−638.
(24) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Viagro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(25) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *14*, 1147−1152.
(26) Hollingshead, M. G.; Alley, M. C.; Camalier, R. F.; Abbott, B. J.; Mayo, J. G.; Malspeis, L.; Grever, M. R. *Life Sci.* **1995**, *57*, 131−141.
(27) Criteria established by the National Cancer Institute, Division of Cancer Treatment, Developmental Therapeutics Program, Biological Testing Branch.
(28) Ibers, J. A.; Hamilton, W. C., Eds. *International Tables for X-ray Crystallography*; Kynoch: Birmingham, UK, 1974; Vol. IV.

NP000269W