Antitumor Agents. 190.[1] Absolute Stereochemistry of the Cytotoxic Germacranolides, Tomenphantins A and B, from *Elephantopus tomentosus*

Toshimitsu Hayashi,[†] Tatsuhiko Nakano,[†] Mutsuo Kozuka,[†] Donald R. McPhail,[‡] Andrew T. McPhail,[‡] and Kuo-Hsiung Lee*,[†]

Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, and Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708

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The structures and absolute stereochemistries of tomenphantins A (**1**) and B (**2**), cytotoxic germacranolides isolated from *Elephantopus tomentosus*, are reported herein. ^{1}H and ^{13}C NMR spectroscopic data, chemical transformation, and single-crystal X-ray analysis were used in these determinations.

Elephantopus species of the family Compositae are known to be an abundant source of novel cytotoxic antitumor sesquiterpene lactones.[2] We previously reported on the isolation and structure determination of two new cytotoxic germacranolides, tomenphantopins A (**3**) and B (**4**) from *Elephantopus tomentosus* L.[3] Further detailed examination of the bioactive fractions of this same plant has led to the isolation of two additional germacranolides, tomenphantins A (**1**) and B (**2**).[4] This paper describes the isolation, structural characterization, and absolute stereochemical assignments of these two compounds.

The extraction and separation of the active principles of *E. tomentosus* were carried out as described previously.[2] Four germacranolides, tomenphantins A (**1**) (0.0083%) and B (**2**) (0.0138%), and the previously reported tomenphantopins A (**3**)[3] (0.0083%) and B (**4**)[3] (0.0113%) were isolated. After our initial report,[2] compound **1** was isolated from *Zexmenia aspilioides* and reported by Silva et al.[5] with the relative stereochemistry and conformation assignments based on ^{1}H NMR analysis using computational methods.

Tomenphantin A (**1**), $C_{19}H_{24}O_6$, mp 161−162 °C, $[\alpha]_D$ −151.9°, showed IR bands at 3530 (OH), 1765 (γ-lactone CO), 1695 (CO), and 1635 (C=C) cm^{-1}. The ^{1}H NMR spectral data were assigned on the basis of double resonance experiments and agreed with those reported previously.[5] All spectroscopic data were compatible with structure **1**. The ^{13}C NMR spectral data are given in the Experimental Section.

The complete structure and absolute stereochemistry of **1** were established unequivocally by single-crystal X-ray analysis of its *p*-bromobenzoate derivative (**5**). The crystal structure was solved by the heavy-atom approach. Full-matrix least-squares refinement of nonhydrogen atom positional and anisotropic thermal parameters, with hydrogen atoms incorporated at their calculated positions, converged at $R = 0.042$ ($R_w = 0.058$; GOF = 1.41) {$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma_w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$; GOF = $[\Sigma w\Delta^2/(N_{obsvns} - N_{param})]^{1/2}$); $\Sigma w\Delta^2[w = 1/\sigma^2|F_o|)$; $\Delta = (|F_o| - |F_c|$ was minimized} over 1737 reflections with $I > 3.0\sigma(I)$. The absolute stereochemistry represented by structure **5** was established by use of the anomalous scattering of Cu Kα X-radiation (see Experimental Section). Final nonhydrogen atom positional parameters are in Table 1. Although bond lengths in general lie close to expected values,[6] bond strain in the ten-membered ring is reflected in the somewhat elongated value of the C-2−C-3 bond [1.564 (9) Å]. A view of the solid-state conformation, with the atom numbering scheme, is provided in Figure 1. In contrast to the situation in **3** and **4**, where C-14 and C-15 are oriented anti, the methyl at C-4 and the derivatized hydroxymethyl group at C-10 in **5** are syn-oriented on the β-face of the molecule. The ten-membered ring in **5** adopts a chair−chair conformation similar to that found in eupahyssopin diacetate (**6**)[7] [endocyclic torsion angles (ω_{ij}, σ 0.5−0.6°) characterizing the ten-membered ring conformation in **5**, with corresponding values for **6** (σ 1−2°) in parentheses, follow: $\omega_{1,2}$ −115.5 (−98), $\omega_{2,3}$ 45.6 (52), $\omega_{3,4}$ −98.7 (−83), $\omega_{4,5}$ 147.6 (146), $\omega_{5,6}$ −100.5 (−133), $\omega_{6,7}$ 95.2 (98), $\omega_{7,8}$ −121.0 (−78), $\omega_{8,9}$ 88.2 (58), $\omega_{9,10}$ −87.1 (−111), $\omega_{10,1}$

Table 1. Nonhydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for Tomenphantin A *p*-Bromobenzoate (5)[a]

atom	*x*	*y*	*z*	B_{eq}(Å^2)
C-1	0.4771 (4)	0.5398 (2)	−0.772 (7)	4.9 (1)
C-2	0.4932 (4)	0.5883 (2)	0.0381 (9)	5.7 (1)
C-3	0.4252 (4)	0.5882 (2)	0.2166 (9)	5.8 (1)
C-4	0.4262 (3)	0.5361 (2)	0.3151 (7)	4.6 (1)
C-5	0.3474 (3)	0.5003 (2)	0.2661 (7)	4.6 (1)
C-6	0.3576 (3)	0.4423 (2)	0.2604 (8)	4.7 (1)
C-7	0.3692 (3)	0.4234 (2)	0.0546 (8)	4.4 (1)
C-8	0.4755 (3)	0.4160 (2)	−0.0076 (8)	4.3 (1)
C-9	0.5023 (4)	0.4493 (2)	−0.1821 (7)	4.6 (1)
C-10	0.5360 (3)	0.5020 (2)	−0.1101 (7)	4.1 (1)
C-11	0.3038 (3)	0.3780 (2)	0.0486 (10)	5.9 (1)
C-12	0.2419 (4)	0.3805 (2)	0.2192 (12)	7.3 (2)
C-13	0.2917 (4)	0.3420 (2)	−0.0833 (13)	8.4 (2)
C-14	0.6424 (4)	0.5059 (2)	−0.0792 (8)	5.1 (1)
C-15	0.5224 (3)	0.5186 (2)	0.3879 (8)	5.3 (1)
O-16	0.3484 (2)	0.5268 (1)	0.4499 (6)	6.04 (8)
O-17	0.2705 (2)	0.4195 (1)	0.3352 (6)	6.57 (9)
O-18	0.1744 (3)	0.3538 (2)	0.2572 (11)	11.1 (2)
O-19	0.4982 (2)	0.3635 (1)	−0.0608 (6)	5.34 (8)
O-20	0.6769 (2)	0.4635 (1)	0.0406 (5)	4.45 (6)
C-1′	0.5099 (3)	0.3302 (2)	0.0863 (11)	6.4 (1)
C-2′	0.5447 (4)	0.2783 (2)	0.0176 (14)	8.7 (2)
C-3′	0.5651 (5)	0.2710 (3)	−0.1739 (16)	11.6 (3)
C-4′	0.5577 (5)	0.2401 (2)	0.1637 (20)	12.9 (3)
O-5′	0.4911 (3)	0.3411 (1)	0.2504 (8)	7.5 (1)
C-1″	0.7516 (3)	0.3846 (2)	0.0996 (9)	5.0 (1)
C-2″	0.7186 (4)	0.3854 (2)	0.2881 (8)	5.2 (1)
C-3″	0.7426 (5)	0.3464 (2)	0.4149 (9)	6.4 (1)
C-4″	0.7962 (4)	0.3058 (2)	0.3494 (10)	7.1 (1)
C-5″	0.8318 (5)	0.3034 (2)	0.1646 (12)	8.4 (2)
C-6″	0.8093 (4)	0.3436 (2)	0.0422 (10)	6.9 (1)
C-7″	0.7274 (3)	0.4255 (2)	−0.0387 (8)	4.7 (1)
O-8″	0.7519 (3)	0.4247 (1)	−0.2068 (6)	6.62 (9)
Br″	0.82473 (9)	0.25131 (3)	0.5232 (2)	12.04 (3)

[a] Estimated standard deviations are in parentheses.

* To whom correspondence should be addressed. Tel.: (919) 962-0066. Fax: (919) 966-3893. E-mail: khlee@email.unc.edu.
[†] University of North Carolina at Chapel Hill.
[‡] Duke University.

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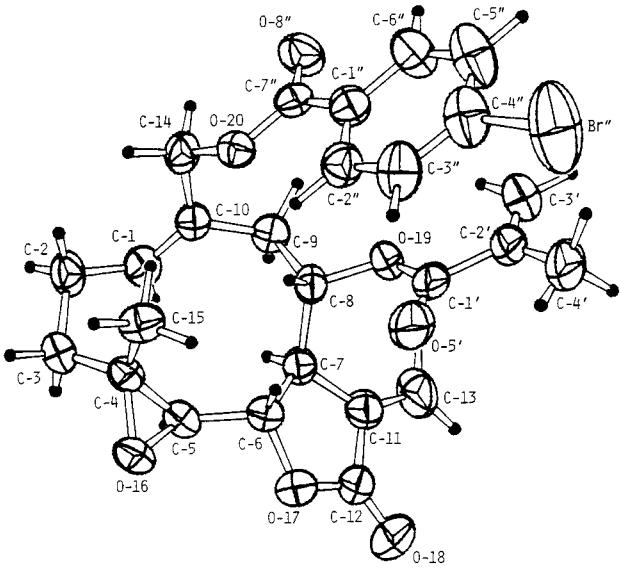
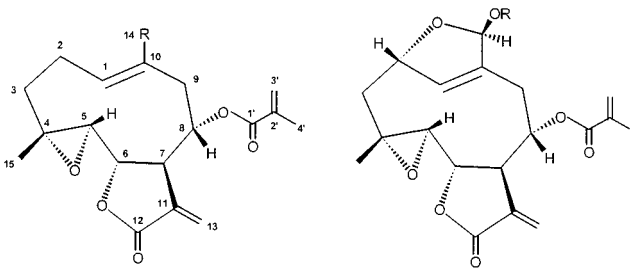
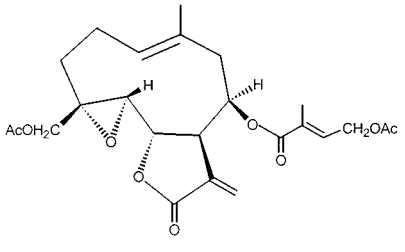


Figure 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of tomenphantin A-*p*-bromobenzoate (**5**); small filled circles represent hydrogen atoms.

162.9 (173)°]. The latter also has β-oriented C-4 and C-10 substituents, but the α- vs β-configuration of the ester substituents at C-8 in **5** and **6**, respectively, allows for a greater degree of ring puckering around this center in the former. That the strain at the trans C-1−C-10 double bond, manifested in the large departure of the C-2−C-1−C-10−C-9 torsion angle [162.9(5)°] from the unstrained value of 180°, may be ascribed entirely to a true twisting around the double bond is reflected in the exactly planar bonding geometry at C-10. Endocyclic torsion angles (ω_{ij}, +0.5−0.6°) in the trans-fused γ-lactone ring of **5** ($\omega_{6,7}$ −17.4, $\omega_{7,11}$ 14.3, $\omega_{11,12}$ −6.3, $\omega_{12,17}$ −5.3, $\omega_{17,6}$ 14.6°) are related by an approximate C_2-symmetry axis passing through C-12 and the midpoint of the C-6−C-7 bond; accordingly, this ring has a half-chair form. In common with many other germacranolides having a C-6−C-7 trans-fused γ-lactone ring, the exocyclic O=C−C=C torsion angle [−3.1 (10°)] is paired in sign with the endocyclic torsion angle [−17.4 (5)°][3,7−9] about the C-6−C-7 bond.





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Tomenphantin B (**2**), $C_{19}H_{22}O_6$, $[\alpha]_D$ −102.1°, was isolated as colorless needles, mp 178−179 °C (dec). Its spectroscopic data were similar to those of **1**. The presence of an α-methylene γ-lactone ring and a methacrylate ester group was clearly indicated by the IR absorptions [1765 (γ-lactone CO), 1675 (ester CO), and 1635 (C=C) cm^{-1}] and ^1H NMR signals [δ 5.75, 6.31 (1H each, d, J = 3.4 Hz, H-13a,b′); δ 5.62, 6.12 (each 1H, s, H-3′a,b); δ 1.91 (3H, s, H-4′)]. Thus, this compound was closely related to tomenphantin A (**1**), but lacked the primary allylic OH group, as indicated by the IR and ^1H NMR spectra. An IR band at 1715 cm^{-1} and a ^1H NMR singlet at δ 10.12 (1H, s) suggested that this compound possessed an aldehyde group at C-10 instead of a primary hydroxy group. Oxidation of **1** with pyridinium chlorochromate[10] in CH_2Cl_2 afforded tomenphantin B, confirming the structure of the latter as **2**. In initial screening, **1** and **2** showed significant and equivalent cytotoxicity in the KB cell line, with ED_{50} values of 3.0 μg/mL and 2.7 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were taken on a Fisher−Johns melting point apparatus and are uncorrected. Optical rotations were obtained in $CHCl_3$ solutions on an Autopol III automatic polarimeter. IR spectra were recorded on a Perkin−Elmer model 257 grating spectrometer. UV spectra were measured on a Varian 2290 spectrophotometer in EtOH solution. ^1H and ^{13}C NMR spectra were determined in $CDCl_3$ on Bruker WM 250 and JEOL FX-60 instruments. X-ray measurements were performed on an Enraf−Nonius CAD-4 diffractometer (Cu Kα radiation, graphite monochromator). Si gel (60, 230−400 mesh, Merck; 60 F_{254}, Merck; GF, 500 and 1000 μm, Analtech) was used for column chromatography, TLC, and preparative TLC, respectively. Elemental analyses were carried out by Mic Anal, Tucson, Arizona.

Plant Material. *Elephantopus tomentosus* L. was collected in the summer of 1981, at Colony Woods, Chapel Hill, North Carolina. A voucher specimen is deposited in the herbarium of the Department of Biology, University of North Carolina at Chapel Hill.

Extraction and Isolation. The air-dried, powdered, whole plant material (600 g) was exhaustively extracted with $CHCl_3$. Workup as described previously[11] afforded a dark brown syrup (4.5 g), which was chromatographed on Si gel (200 g). The column was eluted successively with C_6H_6−EtOAc (9:1 to 7:3) and $CHCl_3$−Me_2CO (9:1 to 4:1).

Elution with C_6H_6−EtOAc (4:1) afforded a gummy fraction **a** and a crystalline solid fraction **b**. Purification of fraction **a** by preparative TLC [Si gel, $CHCl_3$−MeOH (9:1)] yielded tomenphantopin A (**3**) as a colorless powder (50 mg).[3] Fraction **b** was treated with Et_2O, and the insoluble portion was recrystallized from MeOH to give tomenphantin B (**2**) as colorless needles, mp 178−179 °C (dec) (83 mg); $[\alpha]^{26}_D$ −102.1° (*c* 0.35, $CHCl_3$); IR ($CHCl_3$) 1765, 1715, 1675, and 1635 cm^{-1}; UV λ_{max} 209 (log ϵ 4.31), 232 (log ϵ 4.12) nm; ^1H NMR δ 1.28 (3H, s, H-15), 1.45 (1H, m, H-3a), 1.92 (3H, s, H-4′), 2.40 (2H, m, H-2a, 3b), 2.65 (2H, m, H-5, 9a), 3.18 (3H, m, H-2b, 7, 9b), 4.25 (1H, dd, J = 9.0, 7.0 Hz, H-6), 4.44 (1H, d, J = 12.0 Hz, H-8), 5.62 (1H, s, H-3′a), 5.66 (1H, d, J = 3.4 Hz, H-13a), 6.12 (1H, s, H-3′b), 6.25 (1H, d, J = 3.4 Hz, H-13b), 6.76 (1H, dd, J = 12.0 Hz, H-1); *anal.* C 65.60%, H 6.39%, calcd for $C_{19}H_{22}O_6$, C 65.88%, H 6.40%.

Elution with $CHCl_3$−Me_2CO (9:1) yielded fraction **c** from which, after chromatography by preparative TLC [Si gel, $CHCl_3$−MeOH (9:1)], tomenphantin A (**1**) was obtained as colorless prisms (50 mg), mp 161−162 °C; $[\alpha]^{26}_D$ −151.9° (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) 3530, 1765, 1695, and 1635 cm^{-1}; ^{13}C NMR δ 17.1 (q, C-15), 18.3 (q, C-4′), 23.7 (t, C-3), 35.8 (t, C-2), 42.8 (t, C-9), 49.0 (d, C-7), 60.4 (t, C-14), 66.2 (d, C-5), 73.7 (d, C-8), 79.9 (d, C-6), 125.4 (t, C-13), 126.5 (t, C-3′), 130.5 (d, C-1),

133.4, 133.9, 135.8 (each s, C-10,-11,-2′); *anal.* C 65.32%, H 6.90%, calcd. for $C_{19}H_{24}O_6$, C 65.50%, H 6.94%.

Further elution with $CHCl_3$−Me_2CO (9:1) afforded fraction **d**, which was chromatographed on Si gel eluting with $CHCl_3$ to give tomenphantopin B (**4**) as colorless needles (68 mg), mp 114−116 °C (from $CHCl_3$).[3]

Oxidation of Tomenphantin A (1). Compound **1** (20 mg) in dry CH_2Cl_2 (0.5 mL) was treated with pyridinium chlorochromate (20 mg) in dry CH_2Cl_2 (0.5 mL) at room temperature. After stirring for 2 h, the reaction mixture was diluted with dry Et_2O, and the resulting solid was filtered and discarded. The filtrate was evaporated to dryness, and the residue was purified by preparative TLC (Si gel, $CHCl_3$−MeOH) to afford an aldehyde identical with tomenphantin B (**2**).

Tomenphantin A *p*-Bromobenzoate. Compound **1** was treated with *p*-bromobenzoyl chloride−pyridine using standard methodology. The *p*-bromobenzoate thus obtained was recrystallized from $CHCl_3$−MeOH and had a mp of 180−181 °C.

X-ray Crystal Structure Analysis of Tomenphantin A *p*-Bromobenzoate (5).[12] Crystal data: $C_{26}H_{27}BrO_7$; mol wt = 531.41, orthorhombic, space group $P2_12_12_1$ ($D_2$4)-No.19, a = 13.978 (2) Å, b = 25.868 (2) Å, c = 6.958 (1) Å, V = 2515.9 (9) Å^3, Z = 4, D_{calcd} = 1.403 g·cm^{-3}, μ (Cu Kα radiation, λ = 1.5418 Å) = 25.8 cm^{-1}; crystal dimensions: 0.10 × 0.10 × 0.50 mm.

Preliminary unit cell parameters and space group information were derived from oscillation and Weissenberg photographs. Refined unit-cell parameters were calculated from the diffractometer setting angles for 25 reflections ($35° < \theta < 40°$) widely separated in reciprocal space. The space group was defined uniquely by the Laue symmetry and systematic absences: h00, when $h \neq 2n$; 0k0, when $k \neq 2n$; 00l, when $l \neq 2n$. One octant of intensity data was recorded on an Enraf−Nonius CAD-4 diffractometer [Cu Kα radiation, incident beam graphite monochromator; ω-2θ scans; scanwidth (1.00 + 0.14tan θ)°, θ_{max} = 75°; 2963 reflections]. The intensities of four reference reflections, monitored every 2 h during data collection, showed no significant variation (<2% overall). The data were corrected for the usual Lorentz and polarization corrections; an empirical absorption correction [T_{max}:T_{min} (relative) 1.00:0.95], based on the ϕ-dependency of the intensities of several reflections with χ ca. 90°, was also applied. Those 1737 reflections with $I > 3.0\sigma(I)$ were retained for the analysis.

The crystal structure was solved by the heavy-atom approach. Initial bromine atom coordinates were derived from a Patterson map. Coordinates for the other nonhydrogen atoms were obtained from a weighted F_o Fourier synthesis phased by the bromine atom. Nonhydrogen atom positional and temperature factor parameters (first isotropic and then anisotropic), with hydrogen atoms incorporated at their calculated positions in the later cycles, were adjusted by means of several rounds of full-matrix least-squares calculations. An extinction coefficient (g) was included as a variable during the later iterations, and the parameter refinement converged at R = 0.0437 (R_w = 0.0604). The absolute stereochemistry was established at this stage by introducing the imaginary contributions to the anomalous dispersion corrections into the structure−factor calculations. For parameters corresponding to the absolute stereochemistry represented by **5**, R was 0.0424, while R_w was 0.0588, whereas when those of the mirror image were used, the values were 0.0476 and 0.0668, respectively. These differences are significant at the 0.005 level[13] when R'_w/R_w (0.0668/0.0588 = 1.136) equals or exceeds 1.003 and indicate that structure **5** correctly represents the absolute stereochemistry. Confirmation of this assignment was derived by comparison of the magnitudes of the relative intensities for Friedel pairs of 22 enantiomer-sensitive reflections with $I > 10\sigma(I)$ and $\Delta I > 20\%$; in all cases the measured differences were in the same sense as those calculated. Continuation of the least-squares parameter refinement for the correct enantiomer led to convergence (max shift:esd = 0.01) at R = 0.042 [R_w = 0.058, GOF = 1.42, $g = 7(1) \times 10^{-7}$]. A final difference Fourier contained no unusual features [$\Delta\rho$(e.Å^{-3}) = 0.34 (max), −0.27 (min)].

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf−Nonius Structure Determination Package (SDP). For structure−factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from *Internatinal Tables*.[14]

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