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STRUCTURE AND ABSOLUTE STEREOCHEMISTRY OF TOMENPHANTOPIN-A AND -B, TWO CYTOTOXIC SESQUITERPENE LACTONES FROM *ELEPHANTOPUS TOMENTOSUS**

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Key Word Index—*Elephantopus tomentosus*; Compositae; cytotoxic activity; sesquiterpene lactones; germacranolides; tomenphantopin-A; tomenphantopin-B.

Abstract—The structure and absolute stereochemistry of the new cytotoxic sesquiterpene lactones, tomenphantopin-A and -B, isolated from *Elephantopus tomentosus*, were determined from spectral data, chemical transformation and single-crystal X-ray analysis. Tomenphantopin-A and -B are two elephantopin-type germacranolides which possess an α-orientation of the oxygen substituent at C-2.

INTRODUCTION

Elephantopus species of the Compositae are known to be rich in novel cytotoxic antitumour sesquiterpene lactones [1]. *E. tomentosus* was previously reported to contain dihydroelephantopin [2]. As part of our continuing studies of *Elephantopus* species for further novel cytotoxic antitumour agents, we have examined the whole plant of *E. tomentosus* which showed significant cytotoxicity against *in vitro* growth of KB tissue culture cells [3]. Bioassay-directed fractionation of the active $CHCl_3$ extract, followed by successive silica gel chromatography, led to the isolation of two new cytotoxic principles, tomenphantopin-A [**1**; ED_{50} (KB) = 2.5 μg/ml] and -B [**2**; ED_{50} (KB) = 5.0 μg/ml]. We report herein the isolation and structural characterization of **1** and **2**.

RESULTS AND DISCUSSION

Tomenphantopin-A (**1**) was isolated in 0.0083% yield as a colourless powder which analysed for $C_{20}H_{24}O_7$. An α-methylene-γ-lactone moiety and a methacrylate ester side chain in **1** were revealed by the appearance of IR bands at 1770, 1715, 1660 and 1635 cm^{-1}, and substantiated by characteristic ^1H NMR signals at δ5.64 (1H, *d*, *J* = 3.0 Hz, H_a-13), 6.20 (1H, *d*, *J* = 3.0 Hz, H_b-13), 1.85 (3H, *s*, H-19), 5.60 (1H, *s*, H-18) and 6.06 (1H, *s*, H-18). Double resonance experiments led to the assignment of resonances to protons at C-6, C-7, C-8 and C-9. Thus, irradiation of a one-proton multiplet at δ2.99 (H-7) collapsed a pair of doublets at δ5.64 and 6.20 to two singlets, converted a one-proton triplet at δ4.35 (*J* = 11.0 Hz, H-6) into a doublet and transformed a doublet of triplets at δ4.54 (1H, *J* = 12.4 and 3.8 Hz, H-8) into a doublet of doublets. Irradiation of a triplet at δ2.56 (1H, *J* = 12.4 Hz, H-9) converted a doublet of doublets at δ2.86 (1H, *J* = 12.4 and 3.8 Hz, H-9) into a doublet (*J* = 12.4 Hz) and also simplified the doublet of triplets at δ4.54 (H-8). The *trans*-diaxial relationships between $H_β$-5, $H_β$-6, and $H_α$-7, with large dihedral angles of > 130° between H-5 and H-6 as well as between H-6 and H-7, were suggested by the presence of the well-defined one-proton triplet at δ4.35 (*J* = 11.0 Hz) for H-6 (*vide supra*). A doublet at δ2.90 (1H, J = 11.0 Hz), which was coupled with H-6, was assigned to an epoxidic hydrogen (H-5) and it suggested that C-4 was fully substituted. Further support for this assignment was derived from the presence of two carbon signals at δ56.8 and 55.6 in the ^{13}C NMR spectrum. The foregoing observations established the relationships between the protons in the C-4–C-9 region, with a methacrylate ester side chain at C-8, to be as shown in partial structure **3**.

In addition to low-field signals at δ5.57 (1H, *d*, *J* = 3.0 Hz, H-1) and 6.17 (1H, *s*, H-15), the ^1H NMR spectrum of **1** contained signals corresponding to one methyl group at δ1.41 (3H, *s*, H-14), one methoxy group at δ3.32 (3H, *s*), one *O*-bonded methine proton at δ5.18 (1H, *m*, H-2) and methylene protons at δ1.82 (1H, *d*, *J* = 15.0 Hz, H-3) and 2.31 (1H, *dd*, *J* = 15.0 and 5.6 Hz, H-3); spin-decoupling experiments involving H-1 and H-2 established the assignments for H-1–H-3. ^{13}C NMR spectral evidence pointing to the presence of an acetal carbon [δ109.0 (C-15)] and three *O*-bonded carbons [δ79.6 and 78.0 (C-6 and C-8) and 72.2 (C-2)] led to the formulation of structure **1**, exclusive of stereochemistry, for tomenphantopin-A.

Tomenphantopin-B (**2**) was isolated as colourless needles in 0.0113% yield. The ^1H NMR spectrum of **2** was very similar to that of **1**. Absence of a three-proton singlet due to a methoxy group and the appearance of an IR band at 3450 cm^{-1} suggested that **2** was a demethyl derivative of **1**, a fact which was readily verified by methylation of **2** with MeI–Ag_2O in MeOH, according to the method of

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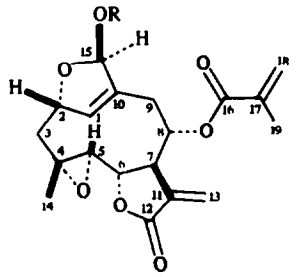
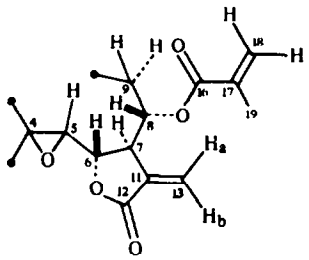


1 R = Me
2 R = H



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Anderson *et al.* [4], to yield an *O*-methyl ether identical with **1** (TLC and NMR data).

Unequivocal proof of the complete structure and stereochemistry of **2**, and hence of **1**, was provided by a single-crystal X-ray analysis of the chloroform solvate. The crystal structure was solved by direct methods.* Full-matrix least-squares refinement of non-hydrogen atom positional and anisotropic thermal parameters, with hydrogen atoms included at their calculated positions, converged to $R = 0.066$ ($R_W = 0.083$)† over 1220 reflections. The absolute configuration, represented by **2**, was established by use of the anomalous scattering effect of the chlorine atoms. Final non-hydrogen atom positional parameters are in Table 1. Bond lengths and angles agree with expected values. A view of the solid-state conformation is provided in Fig. 1. In the crystal, chloroform molecules are hydrogen-bonded (C-27 . . . O-25 3.14 Å) to molecules of **2** which are associated *via* a bifurcated O–H . . . O hydrogen bond (O-26 . . . O-22 3.03 Å; O-26 . . . O-23 3.13 Å) involving molecules related by the 2_1 screw axis along *a*.

Endocyclic torsion angles (deg.) characterizing the 10-membered ring conformation ($\omega_{1,2}$ 115, $\omega_{2,3}$ −30, $\omega_{3,4}$ −64, $\omega_{4,5}$ 149, $\omega_{5,6}$ −107, $\omega_{6,7}$ 87, $\omega_{7,8}$ −120, $\omega_{8,9}$ 62, $\omega_{9,10}$ 70, $\omega_{1,10}$ −167) are, not surprisingly, quite similar to corresponding values in the *trans,trans*-cyclodecadiene ring of the α-dimethylamine adduct of isodeoxyelephantopin [5] (106, −33, −67, 159, −105, 77, −115, 64, 76, −163) which also has an α-oriented oxygen substituent at C-2 and an anti C-14/C-15 relationship. In accord with previous results from X-ray studies on like systems, the C-2–C-1–C-10–C-9 torsion angle at −167° departs significantly from the ideal unstrained value of 180° and, with the C-1, C-10, C-9/C-1, C-10, C-15 dihedral angle being only 4°, the major contribution (9°) to the distortion arises from true twisting about the C-1=C-10 double bond rather than out-of-plane bending at C-10.

Both the substituted 2,5-dihydrofuran ring and the γ-lactone ring approximate to half-chair forms. In the former, C-10 and C-15 lie 0.073 Å and 0.103 Å, respectively, to opposite sides of the C-1, C-2, O-20 plane, whereas in the latter, slightly more puckered ring, corresponding C-6 and C-7 displacements from the C-11, C-12, O-22 plane are 0.204 and 0.234 Å. In agreement with earlier observations on sesquiterpenoid α-methylene-γ-lactones [5–7], the signs of the C-13–C-11–C-12–O-23 and O-22–C-6–C-7–C-11 torsion angles (−10° and −26°, respectively) are paired.

Table 1. Non-hydrogen atom fractional coordinates (x10⁴) with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
C(1)	1191 (8)	2766 (3)	4076 (9)
C(2)	1340 (9)	2145 (3)	3888 (10)
C(3)	2629 (10)	1980 (4)	3145 (10)
C(4)	3087 (9)	2436 (4)	2171 (11)
C(5)	2212 (9)	2572 (3)	1003 (9)
C(6)	2147 (8)	3156 (3)	384 (8)
C(7)	824 (8)	3465 (3)	731 (8)
C(8)	888 (8)	3799 (3)	2099 (8)
C(9)	−143 (9)	3583 (3)	3106 (8)
C(10)	178 (8)	2969 (3)	3400 (8)
C(11)	492 (9)	3767 (3)	−576 (9)
C(12)	1221 (10)	3459 (4)	−1641 (10)
C(13)	−309 (11)	4192 (4)	−837 (10)
C(14)	4124 (9)	2848 (4)	2725 (12)
C(15)	−601 (9)	2476 (3)	2863 (9)
C(16)	1628 (10)	4706 (3)	1374 (10)
C(17)	1300 (12)	5313 (3)	1202 (12)
C(18)	281 (19)	5534 (5)	1831 (21)
C(19)	2192 (17)	5627 (5)	290 (15)
O(20)	220 (6)	1996 (2)	3066 (6)
O(21)	3435 (7)	2250 (2)	798 (7)
O(22)	2063 (7)	3084 (3)	−1078 (6)
O(23)	1104 (9)	3484 (3)	−2849 (6)
O(24)	637 (6)	4399 (2)	1908 (6)
O(25)	2662 (6)	4485 (3)	1084 (9)
O(26)	−1777 (6)	2434 (3)	3626 (7)
C(27)	5775 (13)	4430 (5)	1187 (16)
Cl(1)	6475 (5)	5054 (2)	558 (7)
Cl(2)	6288 (6)	3854 (2)	275 (5)
Cl(3)	6112 (7)	4339 (2)	2843 (6)

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 250 MHz and 62.89 MHz, respectively. MS were determined at

*Crystallographic calculations were performed on a PDP11/44 computer by use of the Enraf-Nonius SDP suite of programs. The direct methods program MULTAN 11/82 was employed.

†$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$.

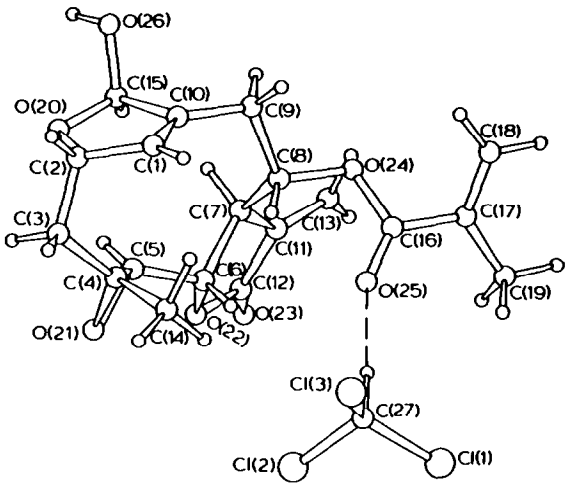

Fig. 1. Structure and solid-state conformation of tomenphantopin-B **2** in crystals of the chloroform solvate; small circles denote hydrogen atoms and the broken line indicates a C–H . . . O hydrogen bond.

70 eV using a direct inlet system. Silica gel (Merck silica gel 60, 70–230 mesh) was used for CC and precoated silica gel (Merck silica gel 60 F 254) was used for TLC. Detection of components was made by either spraying with 1% $Ce_2(SO_4)_3$ or 10% H_2SO_4 soln, followed by heating or by UV.

Extraction. E. tomentosus was from a collection made in October, 1981, at Chapel Hill, NC. A voucher specimen is available for inspection at the herbarium of the Department of Biology, University of North Carolina, Chapel Hill. The ground, air-dried whole plant material (600 g) was exhaustively extracted with $CHCl_3$ and worked up in the usual manner [8], affording 4.5 g of a dark brown syrup.

Isolation of tomenphantopin-A (**1**) *and -B* (**2**). The syrup was subjected to CC on silica gel (200 g) by elution with C_6H_6–EtOAc (9:1 → 7:3) and $CHCl_3$–Me_2CO (9:1 → 4:1). The C_6H_6–EtOAc (8:2) eluate yielded a gummy residue which was further purified by prep. TLC [silica gel, $CHCl_3$–MeOH (9:1)] to yield tomenphantopin-A (**1**, 50 mg, 0.0083%) as a colourless powder: $[\alpha]_D$ −8.6° (*c* = 0.35, $CHCl_3$). Calc. for $C_{20}H_{24}O_7 \cdot H_2O$: C, 60.90; H, 6.64; found: C, 60.69; H. 6.04%. IR and NMR data for **1** are described in the text.

The $CHCl_3$–Me_2CO eluate (9:1) furnished a residue which on CC on silica gel (15 g) and elution with $CHCl_3$ gave tomenphantopin-B (**2**, 68 mg, 0.0113%) as colourless needles after one recrystallization from $CHCl_3$. Compound **2**: mp 114–116°; $[\alpha]_D$ −11.1° (*c* = 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3450, 1765, 1710, 1655, 1630 and 1605 (sh); ^1H NMR (CD_3OD, 250 MHz): δ1.45 (3H, *s*, H-14), 1.70 (1H, *d*, *J* = 15.0 Hz, H-3), 1.92 (3H, *s*, H-19), 2.41 (1H, *dd*, *J* = 15.0 and 5.6 Hz, H-3), 2.68 (1H, *t*, *J* = 12.4 Hz, H-9), 2.93 (1H, *dd*, *J* = 12.4 and 3.8 Hz, H-9), 3.04 (1H, *d*, *J* = 11.0 Hz, H-5), 3.30 (1H, *m*, H-7), 4.42 (1H, *t*, *J* = 11.0 Hz, H-6), 4.62 (1H, *ddd*, *J* = 12.4, 4.3, and 3.8 Hz, H-8), 5.21 (1H, *m*, H-2), 5.71 (1H, *s*, H-18), 5.75 (1H, *d*, *J* = 3.0 Hz, H-13), 5.98 (1H, *d*, *J* = 3.0 Hz, H-1), 6.15 (1H, *d*, *J* = 3.0 Hz, H-13), 6.16 (1H, *s*, H-18), and 6.39 (1H, *s*, H-15).

Methylation of tomenphantopin-B (**2**) *to yield tomenphantopin-A* (**1**). Methylation of **2** (2 mg) with MeI and Ag_2O in MeOH for 2 hr according to the method of ref. [4] yielded an *O*-methyl ether which was identical with **1** [TLC and NMR (250 MHz) data].

X-Ray analysis of tomenphantopin-B (**2**) *as its chloroform solvate.* Crystal data: $C_{19}H_{22}O_7.CHCl_3$, M_r = 481.76, orthorhombic, *a* = 10.061(1) Å, *b* = 23.492(3) Å, *c* = 9.902(1) Å, *V* = 2340.4 Å^3, *Z* = 4, D_{calc} = 1.367 g cm^{-3}, μ(Cu-*K*α radiation, λ = 1.5418 Å) = 39.4 cm^{-1}. Space group $P2_12_12_1$ (D_2^4) uniquely from the systematic absences: *h*00 and *h* ≠ 2*n*, O*K*O when *k* ≠ 2*n*, 00*l* when *l* ≠ 2*n*. Sample dimensions: 0.10 × 0.20 × 0.34 mm.

Preliminary unit-cell parameters and space group information were obtained from oscillation, Weissenberg and precession photographs. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-*K*α radiation, incident-beam graphite monochromator; ω-2θ scans, θ_{max} = 67°). From a total of 2383 independent measurements, those 1220 reflections with $I > 3.0\sigma(I)$ were retained for the structure analysis and corrected for the usual Lorentz and polarization effects. Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 25 reflections (29° < θ < 47°) widely separated in reciprocal space.

The crystal structure was solved by direct methods. Full-matrix least squares adjustment of non-hydrogen atom positional and anisotropic thermal parameters, with hydrogen atoms included at their calculated positions, reduced *R* to 0.069. Inclusion of the imaginary contribution for the anomalous scattering of chlorine into the structure-factor calculations yielded *R* = 0.068 for parameters corresponding to the enantiomer shown whereas *R* for the mirror image was 0.071, thus establishing the absolute stereochemistry to be as represented. Continuation of the refinement of parameters for this enantiomer led to convergence at *R* = 0.066 (R_w = 0.083). Final non-hydrogen atom coordinates are in Table 1. A view of the solid state conformation, with the atom numbering scheme, is provided in Fig. 1. Anisotropic temperature factor parameters, hydrogen atom positional and isotropic thermal parameters, bond lengths and angles, torsion angles and a list of observed and calculated structure amplitudes have been deposited with the Cambridge Crystallographic Data Centre.

Neutral atom scattering factors used in the structure factor calculations, and their anomalous scattering corrections, were taken from lit. [9]. In the least-squares iterations, $\sum W\Delta^2$ ($\Delta = ||F_o| - |F_c||$) was minimized with weights, *w*, assigned according to the scheme: $\sqrt{w} = 1$ for $|F_o| \leq 21.0$ and $\sqrt{w} = 21.0/|F_o|$ for $|F_o| > 21.0$ to ensure no systematic dependence of $<W\Delta^2>$ when analysed in ranges of $|F_o|$.

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REFERENCES

1. Haruna, M., Rice, G. and Lee, K. H. (1985) *J. Nat. Prod.* **48**, 93 and references cited therein.
2. Rustaiyan, A., Niknejad, F. and Nazarians, L. (1978) *Pazhoohandeh* (*Tehran*) **21**, 183; (1980) *Chem. Abstr.* **92**, 143262X.
3. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J. (1972) *Cancer Chemother. Rep.* (*Part* 3) **3**, 1.
4. Anderson, A. S., Barker, G. R., Gulland, J. M. and Lock, M. V. (1952) *J. Chem. Soc.* 369.
5. McPhail, A. T. and Onan, K. D. (1975) *J. Chem. Soc. Perkin Trans.* 2 1798.

6. McPhail, A. T. and Sim, G. A. (1973) *Tetrahedron* **29**, 1751.
7. McPhail, A. T. and Onan, K. D. (1976) *J. Chem. Soc. Perkin Trans.* 2 578.
8. Lee, K. H. and Geissman, T. A. (1970) *Phytochemistry* **9**, 403.
9. *International Tables for X-Ray Crystallography* (1974) Vol. 4. Kynoch Press, Birmingham.