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Antitumor Agents XV: Deoxyelephantopin, an Antitumor Principle from *Elephantopus carolinianus* Willd.

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Abstract □ The active principle responsible for the significant inhibitory activity against the Walker 256 carcinosarcoma (ascites) in rats, isolated from the chloroform extract of the whole plant of *Elephantopus carolinianus* Willd., was characterized as deoxyelephantopin, a sesquiterpene lactone.

Keyphrases □ *Elephantopus carolinianus* Willd.—isolation and structure identification of deoxyelephantopin, antitumor activity □ Deoxyelephantopin—isolation from *Elephantopus carolinianus* Willd., structure identification, antitumor activity □ Antitumor agents, potential—deoxyelephantopin, major constituent of *Elephantopus carolinianus* Willd. □ Structure–activity relationships—sesquiterpene lactones (deoxyelephantopin) as antitumor agents

As a result of the continuing search for *Elephantopus* principles having new and novel potential antitumor agents (1, 2)[1], the chloroform extract of the whole plant of *Elephantopus carolinianus* Willd. (Compositae) was found to show significant inhibitory activity against the Walker 256 carcinosarcoma (ascites) in rats[2]. The extraction of the active principles was carried out according to an exact procedure described in the literature (4). A preliminary examination of the chloroform extract revealed that the antitumor activity was associated with the presence of the terpene-like constituents. Fractionation, characterization, and screening[2] of the isolated compound led to the conclusion that this major active principle was the germacranolide dilactone, deoxyelephantopin (I).

DISCUSSION

Deoxyelephantopin (I), the antitumor principle, was isolated in 0.15% yield as colorless needles from the chloroform eluate. Deoxyelephantopin, mp >320° (sintered at 198–200°)[3], has the empirical formula $C_{19}H_{20}O_6$ as determined by high-resolution mass spectrometry[4]. It shows IR bands in chloroform[5] at 1760 (double strength) and 1650 cm^{-1} and a pair of low field doublets in the NMR spectrum ($CDCl_3$)[6] at δ 6.22 (1H, d, J = 3.5 Hz, H-13) and 5.64 (1H, d, J = 3.5 Hz, H-13)[7], characteristic of an α-methylene-γ-lactone ring system.

An IR band at 1710 cm^{-1} and a characteristic base peak in the mass spectrum of I at m/e 69 suggested the presence of a methacrylate ester side chain, and the NMR signals at δ 1.93 (3H, t, J = 1.0 Hz, H-18), 5.66 (1H, t, J = 1.0 Hz, H-19)[7], and 6.14 (1H, t, J = 1.0 Hz, H-19), which are comparable with those found in molephantin (1) and phantomolin (2), established the presence of this structural feature. The NMR spectrum of I showed, in addition to the well-defined one-proton doublet of doublets (δ 5.13, J = 10.0

[1] See K. H. Lee, T. Ibuka, H. C. Huang, and D. L. Harris, *J. Pharm. Sci.*, **64**, 1077(1975).

[2] *In vivo* antitumor activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, using a literature method (3). Deoxyelephantopin showed significant inhibitory activity against the Walker 256 (ascites) carcinosarcoma in rats at 226% T/C at the 2.5-mg/kg level. A compound is active if it exhibits a T/C of ≥125% (3).

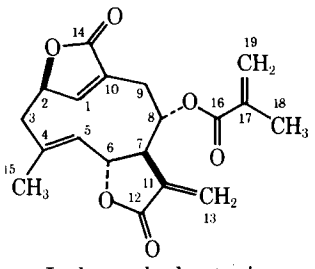
[3] Reference 5 reported mp 198–200°.

[4] The authors thank Dr. David Rosenthal and Mr. Fred Williams of the Research Triangle Center for Mass Spectrometry for mass spectral data.

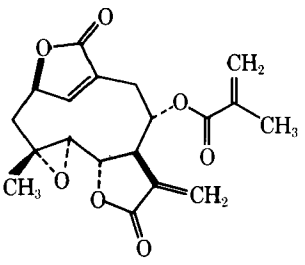
[5] This IR absorption band shifted to 1755 and 1738 cm^{-1} in mineral oil. Reference 5 reported 1766 and 1747 cm^{-1} in KBr.

[6] The authors thank Dr. David L. Harris of the Department of Chemistry, University of North Carolina at Chapel Hill, for NMR measurements. The model XL-100 used for the NMR measurements was purchased from grants from the National Science Foundation and the National Institutes of Health to the Department of Chemistry, University of North Carolina at Chapel Hill.

[7] Partially overlapped with H-19. Reference 5 reported H-13 and H-19 as a multiplet at δ 5.64.



I: deoxyelephantopin



II: elephantopin

and 8.0 Hz) for the proton at C-6 (showing the *trans*-disposition of the lactone fusion) (6), a second vinyl methyl group, seen as a doublet, slightly split by allylic coupling (J = 1.0 Hz) at δ 1.93 (3H). These observations are indicative of the relationship of the protons at C-15, C-5, C-6, and C-7 of I.

The absence of the hydroxy groups and the presence of a strong carbonyl absorption band at 1760 cm^{-1} in the IR spectrum, coupled with the fact that the NMR spectrum of I showed a broad, low field, one-proton singlet at δ 7.13 corresponding to a vinyl proton beta to carbonyl, indicates that the remaining two oxygens must form a second α,β-unsaturated γ-lactone ring.

After treatment with *m*-chloroperbenzoic acid in chloroform, deoxyelephantopin gave a 4,5-epoxide (II: $C_{19}H_{20}O_7$; mp 263–265°[8]) whose spectral data were all in accord with the assigned Structure II. The identity of this compound with elephantopin, a tumor inhibitor isolated from *Elephantopus elatus* (7), was established by comparative TLC and NMR (dimethyl sulfoxide-d_6) spectral analyses[9].

The foregoing evidence led to the conclusion that I was deoxyelephantopin, a germacranolide dilactone which had been isolated from *Elephantopus scaber* (5). To establish the identity of the germacranolide beyond doubt[10], the IR (KBr), NMR, and mass spectra of I were compared with those of deoxyelephantopin. Studies on the structure–activity relationships among deoxyelephantopin-related sesquiterpene lactones are currently in progress.

EXPERIMENTAL[11]

Extraction of *E. carolinianus* Willd.—The *E. carolinianus* (Compositae) used was from a collection made on Oct. 13, 1972, along Flat River at Rt. 1005 in Durham County, N.C.[12]. The ground, air-dried, whole plant material (735 g) was exhaustively extracted with chloroform and worked up in the usual manner (4, 8), affording 18.4 g of a dark-brown syrup.

Isolation of Deoxyelephantopin (I)—The syrup was chromatographed on silica gel (4 × 65 cm) with elution with benzene, chloroform, and acetone. Twenty-four fractions of about 100 ml each were collected and examined by TLC. The first benzene eluate (fractions 1–6) contained only traces of low melting waxes. The subsequent chloroform eluate (fractions 8–16) contained mainly material giving a single spot on TLC. Upon removal of the chloroform solvent, these fractions gave an oily residue which crystallized upon addition of anhydrous ether. One recrystallization from chloroform yielded 974 mg of I as colorless needles, mp > 320° (sintered at 198–200°)[3]. The identity of this compound with deoxyelephantopin was established by their equivalent IR, NMR, and mass spectral data as already described.

Anal.—Calc. for $C_{19}H_{20}O_6$: m/e 344.1260. Found: m/e 344.1256.

The acetone eluate (372 mg) obtained from fractions 17–24 afforded a mixture which, upon rechromatography on silica gel[13] (70–325 mesh, 1.5 × 15 cm, eluted with chloroform), yielded mainly additional deoxyelephantopin (100 mg) (total yield 0.15%) and very small amounts of polar substances whose structures are now under investigation.

Epoxidation of Deoxyelephantopin (I) to Yield Elephantopin (II)—A solution of I (40 mg) in chloroform (1 ml) was treated with a solution of *m*-chloroperbenzoic acid (50 mg) in chloroform (1 ml), and the mixture was allowed to stand at room temperature overnight. Then the solution was washed with 10% sodium sulfite, 5% sodium bicarbonate, and water, dried, and evaporated *in vacuo* to give a crystalline residue. Silica gel column chromatography (0.6 × 10 cm, eluted with chloroform), followed by recrystallization from methylene chloride–absolute ethanol, yielded II as colorless needle tufts, mp 263–265°[14]. The identity of this compound with an authentic sample of elephantopin was confirmed by TLC and superimposable NMR spectra (in dimethyl sulfoxide-d_6).

Anal.—Calc. for $C_{19}H_{20}O_7$: m/e 360.1209. Found: m/e 360.1200.

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[8] Reference 7 reported mp 262–264°.

[9] The authors thank Professor S. M. Kupchan, Department of Chemistry, University of Virginia, Charlottesville, Va., for providing the specimen as well as a copy of the NMR spectrum of elephantopin for comparison.

[10] The authors thank Professor Tadashi Kurokawa of the Department of Bacteriology, School of Medicine, Tohoku University, Sendai, Japan, for providing copies of the IR, NMR, and mass spectra of deoxyelephantopin for comparison.

[11] Unless otherwise specified, melting points were determined on a Thomas-Hoover melting-point apparatus and are corrected. IR spectra were determined in chloroform with a Perkin-Elmer 710 A grating IR spectrophotometer. NMR spectra were measured in $CDCl_3$ with a Varian XL-100 NMR spectrometer using tetramethylsilane as an internal standard. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 ev using a direct inlet system. Silica gel for column chromatography refers to Baker A.R. No. 3405, and silica gel for TLC refers to Merck silica gel G developed with chloroform–acetone (3:1) and visualized by spraying with concentrated sulfuric acid and heating.

[12] The authors thank Mr. S. W. Leonard, Costal Zone Resources Corp., Wilmington, N.C., for collecting and identifying the plant material. A voucher specimen has been placed in the herbarium of the Department of Botany, University of North Carolina at Chapel Hill.

[13] Merck.

[14] Reference 7 reported mp 262–264°.