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ANTITUMOR AGENTS, 71.[1] NUDAPHANTIN, A NEW CYTOTOXIC GERMACRANOLIDE, AND ELEPHANTOPIN FROM *ELEPHANTOPUS NUDATUS*

MITSUMASA HARUNA, GREGORY RICE, and KUO-HSIUNG LEE*

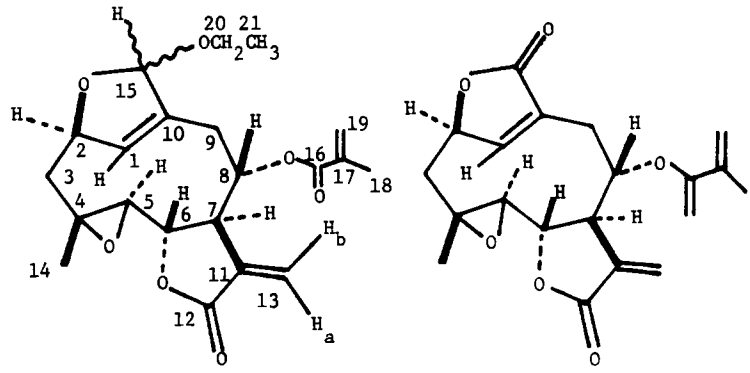
Natural Products Laboratory, Division of Medical Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

ABSTRACT.—Nudaphantin (**1**), a new cytotoxic germacranolide, and the known elephantopin (**2**) were isolated from *Elephantopus nudatus*, and their structures elucidated on the basis of physicochemical data, spectral evidence, and direct comparison with authentic samples.

Certain *Elephantopus* species of the Compositae family are known to elaborate a number of novel sesquiterpene lactones as cytotoxic antitumor agents. Examples are elephantin and elephantopin from *Elephantopus elatus* (1, 2), deoxyelephantopin from *Elephantopus scaber* (3) and *Elephantopus carolinianus* (4), and molephantin, molephantinin, and phantomolin from *Elephantopus mollis* (5-8). As part of our continuing study of *Elephantopus* species for further novel cytotoxic antitumor agents, we have examined *Elephantopus nudatus* Grey, a hitherto uninvestigated species.

The KB-active (9) $CHCl_3$ extract of *E. nudatus* was chromatographed on silica gel. Elution of the column by $CHCl_3$ and $CHCl_3$-Me_2CO (3:1) followed by preparative tlc separation yielded two cytotoxic principles: the new germacranolide nudaphantin [**1**, ED_{50} (KB)=0.31 μg/ml] and the known elephantopin [**2**, ED_{50} (KB)=0.32 μg][2] which was identified by direct comparison with an authentic sample, prepared by epoxidation of deoxyelephantopin (4).

Nudaphantin (**1**), $[\alpha]D$ −16° (c 0.5, $CHCl_3$), was isolated in 0.1% yield as a colorless oil. Compound **1** showed a molecular ion peak at *m/z* 390, which corresponds to $C_{21}H_{26}O_7$, in the mass spectrum. The presence of an α-methylene-γ-lactone moiety in **1** was indicated by the presence of ir bands ($CHCl_3$) at 1770, 1672, and 1630 cm^{-1} and was substantiated by the appearance in the nmr ($CDCl_3$) spectrum of a characteristic



1 **2**

[1]For Part 70, see K.H. Lee, "Chinese Plant Antitumor Agents," *Proceedings of the International Symposium on Chinese Medicinal Materials Research, June 12-14, 1984, Hong Kong*, in press.

[2]Elephantopin showed potent antileukemic activity in vivo against P-388 lymphocytic leukemia growth at T/C=160% (10 mg/kg), and 171% (40 mg/kg) (10).

pair of low-field doublets at δ6.29 (1H, $J_{Ha\text{-}13/H\text{-}7}$=3.5 Hz, Ha-13) and δ5.75 (1H, $J_{Hb\text{-}13/H\text{-}7}$=3.5 Hz, Hb-13). Double resonance experiments involving Ha and Hb established the location of the H-7 multiplet at 3.06. The *trans*-axial relationships between H-5, H-6, and H-7, that is, H-5α, H-6β, and H-7α,[3] were seen in the signal for H-6 which occurred as a well-defined one-proton triplet at δ4.44 (J=9.5 Hz), a feature common to this class of compounds, for example, eupahyssopin (11).

Irradiation at the frequency of H-6 (δ4.44) collapsed a sharp doublet at δ2.99 (H-5, J=9.5 Hz) to a singlet. The proton responsible for this signal (H-5) was assumed to be associated with an oxirane function because of its chemical shift and was suggestive that C-4 was fully substituted. This assignment was also in agreement with the presence in the ^{13}C-nmr spectrum of a doublet at δ59.17 for the -CH(O)- linkage at C-5 and a singlet at δ57.95 for >C(O)-C moiety at the C-4 position.

The one-proton eight-line pattern which appeared at δ4.65 (J=2.5, 4.5 and 12.0 Hz) was assigned to H-8 as it was adjacent to two protons at C-9 [δ2.96 (dd, J=4.5 and 13.0 Hz, partially overlapped); δ2.65 (dd, J=12.0 and 13.0 Hz)] which was in turn adjacent to a completely substituted C-10.

The ^{13}C-nmr spectrum of **1** displayed two carbonyl signals at δ166.68 and 168.78. One of these was due to the lactonic carbonyl; the other was from an ester which was also indicated by an ir band at 1715 cm^{-1}. The comparable nmr peaks and splitting pattern between **1** and the two C-8 α-substituted methylacrylate ester side chain bearing **2** (1)[4] and molephantin (8)[3] led to the assignment of this same ester side chain for **1**. Thus, the broad three proton singlet at δ1.94, and two low-field multiplets at δ5.69 and 6.16 were assigned to H-18, H-19, and H-19, respectively. Further confirmation for these assignments was obtained by the diagnostically important mass peak at *m/z* 304 (*M*-86) (**1b**, Scheme 1) which was due to the loss of a methylacrylate in **1**.

The one-proton multiplet at δ5.27 was assigned to H-2 since irradiation at H-1 (δ5.73, partially overlapped) caused the signal for H-2 to collapse to a broad doublet (J=5.5 Hz). Further irradiation at the frequency of H-2 (δ5.27) sharpened the singlet at δ5.73 (H-1) and collapsed the well-separated doublet of doublets at δ2.38 (J=5.5 and 15.0 Hz) and broad doublet at δ1.88 (J=15.0 Hz, partially overlapped) into two doublets. The above experiment suggested that the methylene protons of C-3 were adjacent to a quaternary carbon at C-4. The remaining three-proton singlet at δ1.48 was assigned to an epoxy methyl group attached to C-4.

The presence of an ethyl ether group of a hemiacetal moiety was first suggested by a strong ir band at 1150 cm^{-1}, by a characteristic three-proton triplet at δ1.25 (J=6.5 Hz, H-21) and a two-proton multiplet[5] at 3.68 (H-20), and confirmed by a high resolution mass peak at *m/z* 345 ($C_{19}H_{21}O_6$) which was due to the cleavage of an ethoxy linkage and corresponded to structure **1c**. The appearance of ^{13}C-nmr peaks at δ63.04 (t), which was also supportive of the presence of an OCH_2CH_3 group, and δ110.52 (d, C-15), coupled with the co-occurrence of **1** and **2** led to the location of this OCH_2CH_3 group at C-15, exclusive of its stereochemistry.

The foregoing evidence supported assignment of **1** as the structure of nudaphantin, which differs from **2** only in its C-15 substituents. This was further confirmed by a characteristic mass peak at *m/z* 246 (**1d**, $C_{14}H_{14}O_4$), which was due to an identical fragmentation pattern (**1a**→**1b**→**1d**) of **2**(1).

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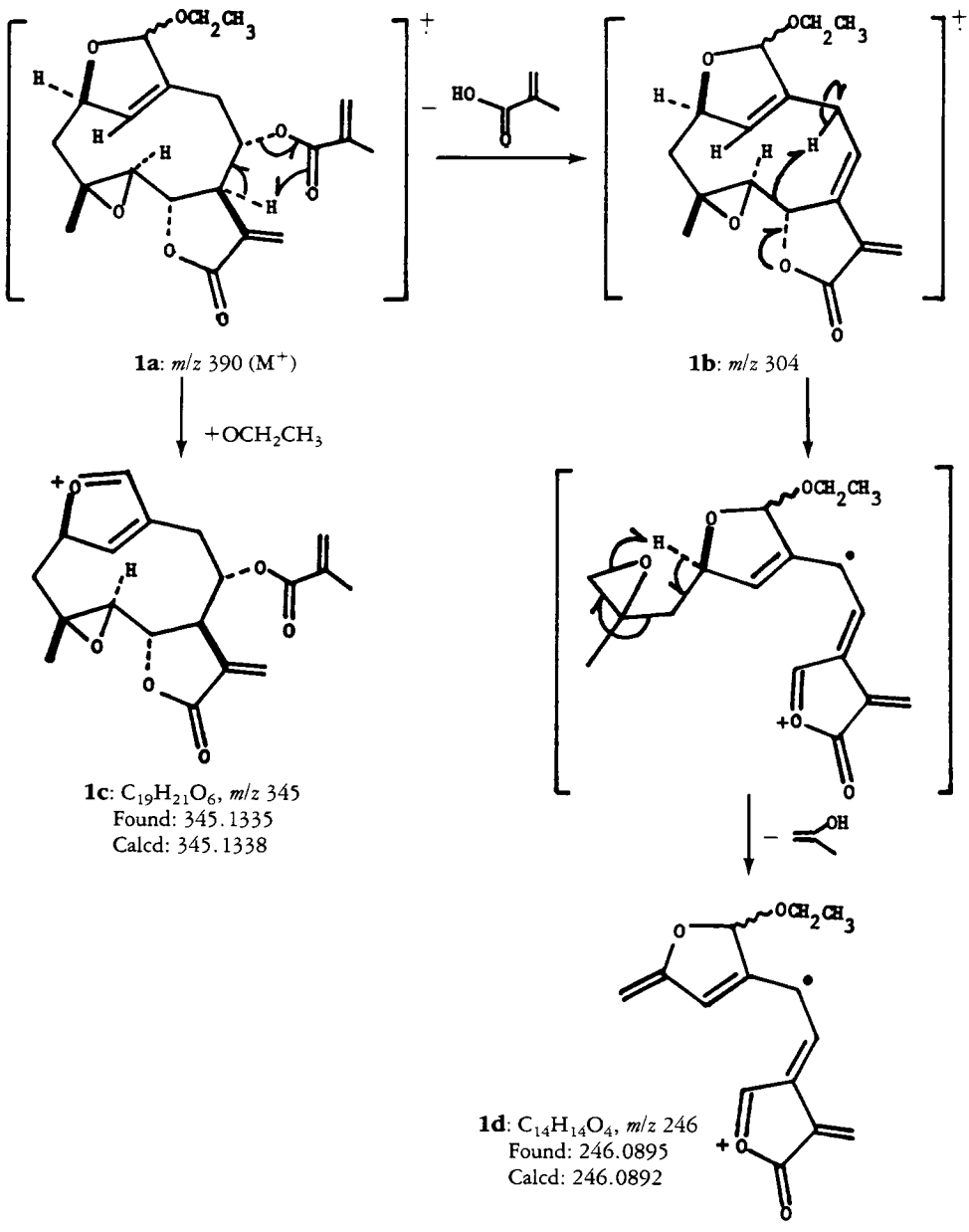
[3]Assuming that the H-7 is α-oriented as in all known naturally occurring germacranolides from higher plants.

[4]Elephantopin [measured in DMSO-d_6 (1)] and molephantin ($CDCl_3$) displayed corresponding peaks at δ1.88 (3H, br.s. H-18), 5.79 (1H, br.s, H-19), and 6.16 (1H, br.s, H-19); and 1.96 (3H, m, H-18), 5.68 (1H, m, H-19), and 6.15 (1H, m, H-19), respectively.

[5]The motion of the methylene protons of the ethoxy group is apparently restricted by steric factors.



SCHEME 1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Specific rotations were obtained on a Rudolph Autopol III automatic polarimeter (l=0.5 dm). Ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-nmr spectra were recorded on a Varian XL-100 spectrometer and are given in parts per million (δ) downfield from an internal TMS standard. ^{13}C-nmr spectra were recorded on a Jeolco FX-100 spectrometer functioning at 25.20 MHz. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. 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 Merck silica gel 60 (70-230 mesh). Silica gel for preparative tlc refers to Analtech silica gel G (1000 microns). Compounds were visualized by uv light or spraying with 1% $Ce(SO_4)_2$-10% H_2SO_4 solution followed by heating.

PLANT MATERIAL.—The *E. nudatus* used was from a collection made in December, 1975, in coastal Georgia, by Professor Wayne R. Faircloth of Valdosta State College. A voucher specimen is available for inspection at the herbarium of the Department of Biology, University of North Carolina, Chapel Hill.

PRELIMINARY EXTRACTION.—The ground, air-dried whole plant (1.2 kg) was exhaustively extracted with hexane, $CHCl_3$, and MeOH. The $CHCl_3$ extract was purified according to an exact procedure described in the literature (12) to give 20.8 g of a dark-green syrup, which showed potent cytotoxicity [ED_{50} (KB) <4 μg/ml].

ISOLATION OF NUDAPHANTIN (**1**) AND ELEPHANTOPIN (**2**).—The $CHCl_3$ extract (20.8 g) was chromatographed on silica gel (5.5 x 65.5 cm) and eluted with $CHCl_3$, $CHCl_3$-Me_2CO, and Me_2CO. The first eight fractions were 1000 ml each and all others were 500 ml each. Fractions 10-16, eluted from $CHCl_3$-Me_2CO (9:1), yielded 307 mg of crude product which, after preparative tlc purification, gave 140 mg of nudaphantin (**1**). Elephantopin (**2**, 223 mg) was isolated from Fractions 54-56 eluted with $CHCl_3$-Me_2CO (3:1).

NUDAPHANTIN (**1**).—Nudaphantin was isolated as a colorless oil. Its [α]D, ir ^{1}H-nmr and mass spectral data have been described in the text. Its ^{13}C-nmr spectrum ($CDCl_3$) exhibited peaks at δ168.78 (s), 166.68 (s) (C-16 and C-12), 135.51 (s), 134.61 (s), 133.27 (s) (C-10, C-11 and C-17), 133.15 (d) (C-1), 126.78 (t), 123.88 (t) (C-13 and C-19), 110.52 (d) (C-15), 81.59 (d), 80.38 (d), 74.61 (d) (C-6, C-8 and C-2), 63.04 (t) (C-20), 59.17 (d) (C)5), 57.95 (s) (C-4), 47.25 (d) (C-7), 42.97 (t), 31.57 (t) (C-3 and C-9), 22.13 (q), 18.10 (q), and 15.40 (q) C-14, C-18, and C-21).

ELEPHANTOPIN (**2**).—This was isolated as colorless crystals: mp 262-263°; Lit. (1) reported mp 262-264°. Compound **2** was identical by tlc, mmp, and ir and nmr spectra to that obtained from the epoxidation of deoxyelephantopin (4).

EPOXIDATION OF DEOXYELEPHANTOPIN.—Deoxyelephantopin (100 mg), obtained from an extraction of *E. carolinianus* (4), was epoxidized with *m*-chloroperbenzolic acid (150 mg) in dry CH_2Cl_2 (20 ml) in the usual way to yield 86 mg of elephantopin as colorless needle tufts, mp 263-265°, after one recrystallization from CH_2Cl_2-absolute EtOH.

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