Tumor Inhibitors XL.[1a] The Isolation and Structural Elucidation of Elephantin and Elephantopin, Two Novel Sesquiterpenoid Tumor Inhibitors from *Elephantopus elatus*

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Evidence is presented for the assignment of structures for elephantin (**3**) and elephantopin (**4**), two tumor-inhibitory sesquiterpene dilactones isolated from *Elephantopus elatus* Bertol. Elemental analysis and high-resolution mass spectrometry supported a $C_{20}H_{22}O_7$ molecular formula for elephantin (**3**) and a $C_{19}H_{20}O_7$ molecular formula for elephantopin (**4**). Chemical and spectral evidence indicated the presence of α,β-unsaturated lactone, α,β-unsaturated ester, and epoxide groupings in **3** and **4**. Alkaline hydrolysis of **3** gave elephantol (**5**) and dimethylacrylic acid, while, under the same conditions, **4** gave elephantol (**5**) and methacrylic acid. Catalytic hydrogenation of elephantol (**5**) gave tetrahydroelephantol (**9**). Treatment of elephantol (**5**) with *p*-bromobenzoyl chloride gave elephantol *p*-bromobenzoate (**14**). X-Ray crystallographic analysis established the structure and stereochemistry of **14** and **5**. Hydrogenation of elephantopin (**4**) gave tetrahydroelephantopin (**6**). Alkaline hydrolysis of **6** gave dihydroelephantol (**8**) and isobutyric acid. Acylation of **5** with methacrylic anhydride gave elephantol methacrylate (**11**), while acylation of **8** with isobutyric anhydride gave elephantol isobutyrate (**12**). Acid hydrolysis of **6** gave dihydroelephantolide (**13**), which on acylation with isobutyric anhydride gave **6**. Low- and high-resolution mass spectra of elephantin (**3**) and elephantopin (**4**) and a number of derivatives (**5**, **6**, **8**, **10**, **11**, **12**, and **13**) are discussed.

Elephantin and elephantopin are cytotoxic sesquiterpene lactones from *Elephantopus elatus* Bertol., and their isolation and characterization have recently been reported.[4,5] It is the purpose of this paper to present in detail the fractionation of the active extract of *E. elatus* and the isolation and structural elucidation of the active constituents, elephantin and elephantopin.[6]

Fractionation of the concentrated ethanol extract (A in Chart I), guided by assay against KB cells,[6] revealed that the active principles were concentrated (Table I) in the chloroform phase (D) of a chloroform–water partition. Partition of this residue between 10% aqueous methanol and petroleum ether concentrated the activity in the aqueous methanol layer (G). Fraction G was chromatographed on a silicic acid–Celite (3:1) column. Elution with chloroform and 2% methanol in chloroform yielded two crystalline materials (H and I) with similar R_f values on tlc. Complete resolution was achieved by rechromatography, which gave elephantin (**3**) and elephantopin (**4**), respectively.[7]

TABLE I

BIOLOGICAL ACTIVITY[a]

A. Cytotoxicity of Fractions against Eagle's KB Strain of Human Carcinoma of the Nasopharynx

Fraction	ED_{50}, μg/ml
A	26.0
B	100
C	58.0
D	5.00
E	100
F	34.0
G	1.70
H	0.28
I	0.32

B. Tumor-Inhibitory Activity against the Walker 256 Intramuscular Carcinosarcoma in Rats[a]

Compd	Dose, mg/kg	Survivors	Animal wt change difference, g T − C	Tumor wt, mg T/C	T/C × 100
3	100	4/4	−17	600/5000	12
	50	4/4	−3	2700/5000	54
	25	4/4	+1	3000/5000	60
4	100	3/4	−21	900/4000	22
	50	4/4	−18	1000/4000	25
	25	3/4	+1	3900/4000	97

[a] Reference 6. [b] T, treated animals; C, control animals.

The molecular formula, $C_{20}H_{22}O_7$, was assigned for elephantin (**3**) on the basis of elemental analysis and high-resolution mass spectrometry. The presence of bands in the infrared spectrum of **3** at 5.68 and 6.07 μ, together with the typical low-field doublets of the exocyclic methylene group at τ 3.85 and 4.22 (Table

(1) (a) University of Wisconsin. Part XXXIX: S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch, R. M. Smith, J. A. Saenz Renauld, H. K. Schnoes, A. L. Burlingame, and D. H. Smith, *J. Org. Chem.*, **34**, 3858 (1969). The investigation at the University of Wisconsin was supported in part by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275) and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551). (b) Author to whom inquiries should be directed: Department of Chemistry, University of Virginia, Charlottesville, Va. 22901.

(2) National Institutes of Health Postdoctoral Fellow, 1965–1966.

(3) University of California. This is part XXIX in the series entitled "High Resolution Mass Spectrometry in Molecular Structure Studies." Part XXVIII: A. L. Burlingame, P. C. Wzolek, and B. R. Simoneit in "Advances in Organic Geochemistry," I. Havenaar and P. A. Schenck, Ed., Pergamon Press, New York, N. Y., in press. The investigation at the University of California was supported in part by a grant from the National Aeronautics and Space Administration (NGL 05-003-003).

(4) S. M. Kupchan, Y. Aynehchi, J. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 3674 (1966).

(5) Leaves, stems, flowers, and roots were gathered in Florida, Sept 1963. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNSC).

(6) Cytotoxicity [activity against human carcinoma of the nasopharynx carried in cell culture (KB)] and *in vivo* inhibitory activity (against the WM carcinosarcoma[7]) were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962). The evaluation of the KB assay results by the CCNSC in sequential testing is such that a compound is considered active if the average ED_{50} of two tests ≦4 μg/ml and if this result is reproducible by a second screener. The evaluation of the assay results in the WM *in vivo* system on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42% or less.

(7) Elephantin and elephantopin showed significant tumor-inhibi ry activity against the Walker intramuscular carcinosarcoma 256 (WM) at 100 mg/kg (see Table I).

TABLE II

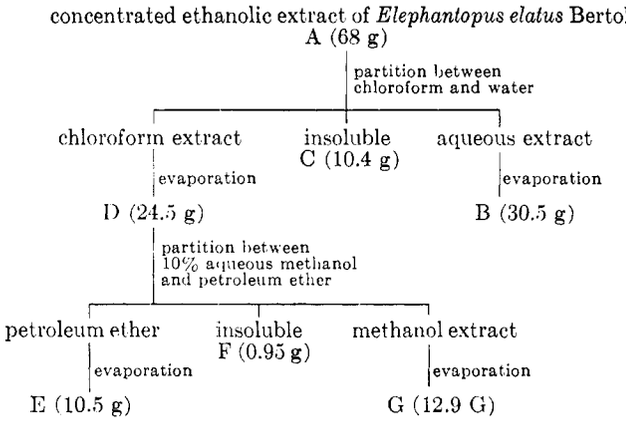
NUCLEAR MAGNETIC RESONANCE DATA[a]

Compd	C-1	C-2	C-5	C-6	C-7	C-8	C-13	C-15	OCOR	OH
3	1.94 br s	4.5 m	6.9–7.3	5.80 t (8.5)	6.3 m	5.9 m	3.85 d (3) 4.22 d (3)	8.83 s	4.25 m 7.97 d (1) 8.12 d (1)	...
4	1.93 br s	4.5 m	6.9–7.2	5.8 m	6.25 m	5.9 m	3.82 d (3) 4.19 d (3)	8.82 s	3.84 br s 4.21 br s 8.12 br s	...
5	2.03 br s	4.6 m	7.2–7.6	4.6 m	4.1–4.8	4.1–4.8	3.73 m 3.95 m	8.89 s	...	4.56 m
6	2.03 br s	4.57 br s	7.1–7.4	5.97 t (8)	7.1–7.4	5.4 m	8.76 d (6)	8.82 s	8.87 d (7)	...
8	2.10 br s	4.6 m	7.1–7.4	6.7 m	7.1–7.4	6.0 m	8.69 d (7)	8.88 s	...	4.75 d (5)
10	2.00 br s	4.6 m	7.0–7.4	5.20 t (10)	6.4 m	6.0 m	3.75 d (2) 4.14 d (2)	8.85 s	7.95 s	...
11	1.98 br s	4.55 m	6.95 d (10)	5.05 t (10)	6.3 m	6.0 m	3.88 d (3) 4.25 m	8.82 s	3.9 br s 4.25 br s 8.10 br s	...
12	2.11 br s	4.6 m	7.1–7.3	5.02 t (9)	7.1–7.6	5.85 m	8.72 d (6)	8.81 s	8.90 d (7)	...
13	2.18 br s	4.6 m	7.2–7.6	6.02 t (9)	7.2–7.6	6.7 m	8.72 d (7)	8.88 s	...	4.46 d (5)

[a] Spectra were determined on a Varian A-60 spectrometer in hexadeuterated dimethyl sulfoxide. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; m, multiplet center; br s, broad singlet. Numbers in parentheses denote coupling constants in hertz.

CHART I

FRACTIONATION OF CYTOTOXIC EXTRACTS FROM *Elephantopus elatus* Bertol.



II),[8] suggested the presence of an α,β-unsaturated γ-lactone grouping (**1**). The presence of an α,β-unsaturated ester grouping in **3** was indicated by infrared absorption at 5.84 and 8.12 μ and was supported by the presence of a peak at *m/e* 275 (M − $C_5H_7O_2$) in its mass spectrum. A third carbonyl absorption at 5.64 μ, together with a downfield signal at τ 1.94 in the nmr spectrum, assignable to a vinyl proton β to carbonyl,[9,10] was indicative of a second α,β-unsaturated γ-lactone grouping (**2**). The high-intensity absorption at 215 mμ (ϵ 25,000) for **3** was consistent with the presence of these three chromophores. The lack of any spectroscopic or chemical evidence for the presence of a hydroxyl or ketone function indicated the presence of an ether group to account for the remaining oxygen atom in **3**.

Elephantopin (**4**) was shown to have the molecular formula $C_{19}H_{20}O_7$ and functional groups similar to **3** by analysis of its spectral data. The presence of the lactone function **1** was indicated by infrared absorption at 5.73 and 6.12 μ and low-field doublets in the nmr spectrum (Table II) at τ 3.82 and 4.19; the presence of lactone **2** was indicated by infrared absorption at 5.68 μ and an nmr signal at τ 1.93. The presence of an α,β-unsaturated ester grouping was indicated by infrared absorption at 5.86 and 6.08 μ and was supported by the peak at *m/e* 274 (M − $C_4H_6O_2$) in the mass spectrum of **4**.

Alkaline hydrolysis of elephantin (**3**) gave elephantol (**5**), $C_{15}H_{16}O_5$. This compound showed diminished absorption at *ca.* 210 mμ in the ultraviolet region, absence of signals at τ 4.24 (vinyl proton) and at τ 7.97 and 8.12 (methyl on double bond) in the nmr spectrum, and absence of absorption at 5.84 μ in the infrared spectrum, indicative of cleavage of a dimethylacrylate grouping in the transformation from **3** to **5**. Hydrolysis of **4** under the same conditions gave elephantol (**5**) and methacrylic acid. Thus, **3** and **4** were assumed to differ only in the nature of the ester moiety. Catalytic hydrogenation of elephantol (**5**) gave tetrahydroelephantol (**9**).

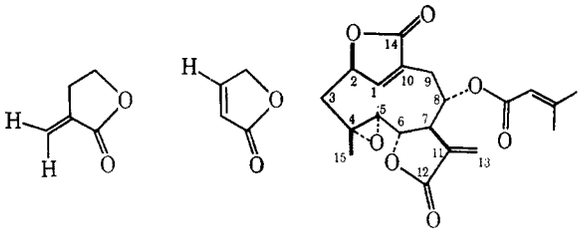
Catalytic hydrogenation of elephantopin (**4**) with palladium resulted in absorption of 2 mol equiv of hydrogen and formation of tetrahydroelephantopin (**6**), $C_{19}H_{24}O_7$. Inspection of the nmr spectrum of **6** indicated that saturation of the ester side chain had occurred, as indicated by the absence of signals at τ 3.84, 4.21, and 8.12 and the presence of a doublet at τ 8.87 for the methyl groups of the ester side chain. Saturation of lactone **1** was indicated by the absence of signals at τ 3.82 and 4.19 (exocyclic methylene) and the presence of a methyl doublet at τ 8.76 in the nmr spectrum. Complete saturation of **4** was accomplished by catalytic hydrogenation with platinum as catalyst, whereupon hexahydroelephantopin (**7**) was obtained. Catalytic hydrogenation of elephantin (**3**) with palladium gave tetrahydroelephantin (**15**).

Alkaline hydrolysis of tetrahydroelephantopin (**6**) gave dihydroelephantol (**8**), $C_{15}H_{18}O_6$. Inspection of the nmr spectrum of **8** in comparison with **5** showed

(8) *Cf.* S. M. Kupchan, J. M. Cassady, J. E. Kelsey, H. K. Schnoes, D. H. Smith, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 5292 (1966), and references cited therein.

(9) H. Immer, J. Polonsky, R. Toubiana, and H. D. An, *Tetrahedron*, **21**, 2117 (1965).

(10) W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Amer. Chem. Soc.*, **84**, 2601 (1962).



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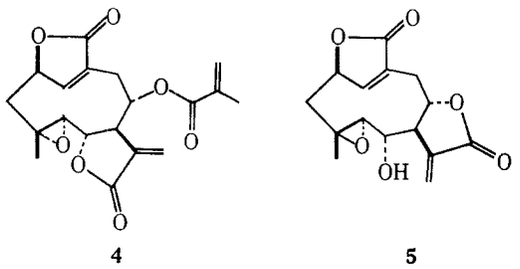

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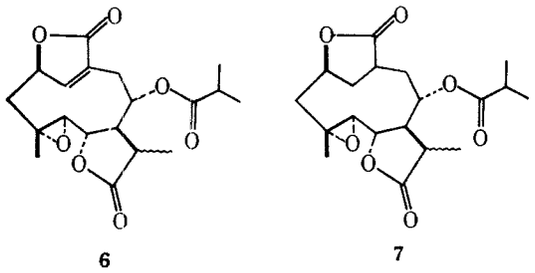

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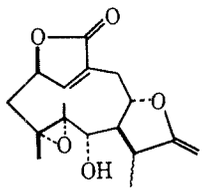

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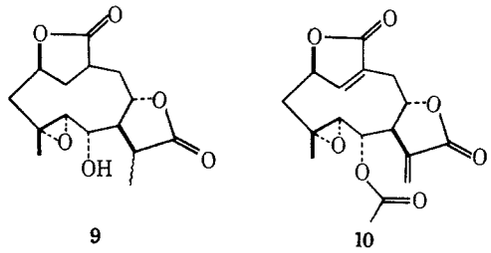
loss of the exocyclic methylene proton signals at τ 3.73 and 3.95 in **5** and appearance of a methyl doublet at τ 8.69.

Acetylation of elephantol (**5**) gave a monoacetate (**10**) and treatment of **5** with p-bromobenzoyl chloride gave elephantol *p*-bromobenzoate. X-Ray crystallographic analysis established that the *p*-bromobenzoate ester has the structure and stereochemistry[4,11] **14**. It follows that elephantol has structure **5**. In addition, related structures could be considered for elephantin and elephantopin, assuming no chemical change had occurred during hydrolysis and subsequent acylation.

In order to investigate this point, elephantol was acylated with methacrylic anhydride, and an isomer of **4**, elephantol methacrylate (**11**), $C_{19}H_{20}O_7$, was obtained. Similar treatment of dihydroelephantol (**8**) with isobutyric anhydride gave dihydroelephantol isobutyrate (**12**), $C_{19}H_{24}O_7$, an isomer of tetrahydroelephantopin (**6**). Comparison of the nmr spectra of **12** and **6** (Table II) led to the conclusion that a lactone rearrangement[12] had taken place during alkaline hydrolysis. In the nmr spectrum of **12**, the signal at τ 5.02 (t, J = 9 Hz) corresponded to the proton at ester-bearing C-6, while the multiplet at τ 5.85 corresponded to the proton (spin coupled to three protons) at lactone-bearing C-8. In contrast, the nmr spectrum of **6** showed a multiplet at τ 5.42 assigned to the proton at ester-bearing C-8, while the lactone proton signal appeared as a triplet at τ 5.97, indicative of attachment at C-6. In accord with this view, acid hydrolysis of **6** gave dihydroelephantolide (**13**), which was isomeric with **8** and therefore corresponded to the direct hydrolysis product. Accordingly, the signal for the proton at lactone-bearing C-6 in **13** appeared as a triplet at τ 6.02; the signal for the proton at lactone-bearing C-8 in **8** appeared as a complex multiplet. Furthermore, treatment of dihydroelephantolide (**13**) with



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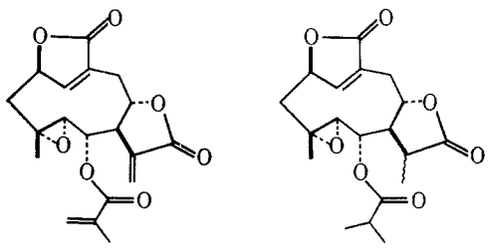

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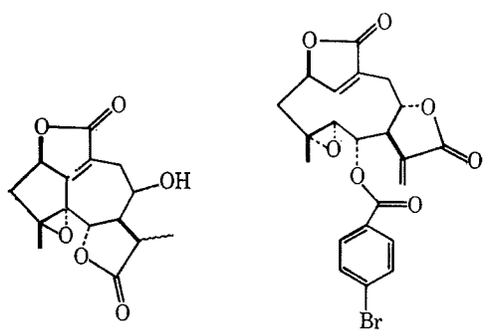
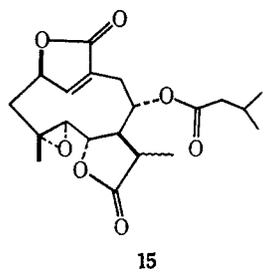


13 14



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isobutyric anhydride gave a compound shown to be **6** on the basis of comparison of nmr, infrared, and ultraviolet spectra with those of authentic tetrahydroelephantopin (**6**). Upon treatment of **13** with base under conditions identical with those used during the alkaline hydrolysis of **6**, **8** was produced. The last result substantiated the view that relactonization had occurred, and, together with the mass spectrometric studies which follow, supported assignment of **3** as the structure of

(11) A full paper describing the details of the X-ray crystallographic analysis will be published separately by Professor G. A. Sim and coworkers.

(12) *Cf.* D. H. R. Barton, O. C. Bockman, and P. de Mayo, *J. Chem. Soc.*, 2263 (1960).

elephantin and **4** as the structure of the companion lactone, elephantopin.

Mass Spectrometry.—Sesquiterpenes have not been studied extensively by mass spectrometry and it is appropriate to discuss some of the more important features of the mass spectra of elephantin and elephantopin and related derivatives, particularly since the complete high-resolution mass spectra of these compounds permit more reasonable deductions concerning their ionic decomposition. Brief discussions of the mass spectra of santonin and derivatives[13,14] and some sesquiterpene systems[15-17] have appeared and a somewhat more extensive treatment of the sesquiterpene widdrol has been presented.[18-20]

In the course of this study, we have obtained both low- and high-resolution mass spectra of elephantopin (**4**), tetrahydroelephantopin (**6**), elephantol methacrylate (**11**), and dihydroelephantol isobutyrate (**12**), and low-resolution mass spectra of elephantin (**3**), elephantol acetate (**10**), elephantol (**5**), dihydroelephantol (**8**), and dihydroelephantolide (**13**) (Figures 1–11).

The spectra of the esters are somewhat unusual, since sesquiterpenes in general seem to fragment in a relatively unspecific manner, which makes it difficult to assign structures to fragments with any degree of certainty. In this series of compounds, however, relatively minor structural modifications led to significantly different fragmentation patterns, a phenomenon which proved valuable in the assignment of the structures **3** and **4** to elephantin and elephantopin, respectively.

Discussion of Spectra.—Prominent peaks in the spectra of compounds **3**, **4**, **6**, **10**, **11**, **12**, and **15** are due to fragmentation of the ester side chain. These fragmentations are summarized in Table III.

TABLE III

FRAGMENTATION OF ESTER SIDE CHAIN RCOR′

Compd	M+	Acyl fragment, *m/e*	R′ fragment, *m/e*
3	374	83 (C_5H_7O)	274, 275
4	360	69 (C_4H_5O)	274, 275
6	364	71 (C_4H_7O)	276, 277
10	334	43 (C_2H_3O)	274, 275
11	360	69 (C_4H_5O)	274, 275
12	364	71 (C_4H_7O)	276, 277
15	378	85 (C_5H_9O)	276, 277

Fragmentations which occur following loss of the ester side chain appear to be directed by the position of attachment of the lactone oxygen (C-6, natural; C-8, rearranged) and therefore are of significance in terms of the structures assigned for elephantin (**3**) and elephantopin (**4**). In elephantopin (**4**) (Figure 2), further elimination of *m/e* 58 mass units (C_3H_6O) from the ion at *m/e* 274 gives rise to the intense peak at *m/e* 216 ($C_{12}H_8O_4$) as determined from the high-resolution heteroatomic plots[21] (Figure 8). Similarly, tetrahydroelephantopin (**6**) (Figures 5 and 9) shows an intense peak at *m/e* 218 ($C_{12}H_{10}O_4$). This elimination plays a minor role for the corresponding rearranged compounds elephantol methacrylate (**11**) (Figures 3 and 10) and dihydroelephantol isobutyrate (**12**) (Figures 7 and 11), where the loss of the ester side chain is followed by preferential elimination of 96 mass units ($C_5H_4O_2$, representing one of the lactone rings) and gives rise to the intense peaks at *m/e* 178 ($C_{10}H_{10}O_3$ for **11**) and 180 ($C_{10}H_{12}O_3$ for **12**). This difference is readily explicable in terms of rearranged structures and, although details of mechanism and structure cannot be specified from our data, the ionic decompositions can be rationalized on the basis of common fragmentation reactions, such as cleavage of ester side chain and cleavage α to heteroatoms with subsequent hydrogen transfer, which are typical for less complex model systems. A plausible mechanism for the fragmentation of elephantopin (**4**) is outlined in Scheme I. Tetrahydroelephantopin (**6**) behaves in an analogous manner, with the peak due to the acyl ion (fragment a, *m/e* 69 for **4**, Scheme I) being shifted to *m/e* 71 and the *m/e* 216 ion for **4** (fragment b, Scheme I) being shifted to *m/e* 218.

A possible mechanism for the fragmentation of one of the rearranged compounds, elephantol methacrylate (**11**), is outlined in Scheme II. Fragmentation involves loss of the side chain followed by cleavage of the C-2,3 and C-8,9 bonds to generate either the ion at *m/e* 178 or the ion at *m/e* 96 (see Figures 3 and 4). In all cases, high-resolution mass measurement supported the assigned ionic structures, since the peak at *m/e* 178 had the composition $C_{10}H_{10}O_3$ and the peak at *m/e* 96 had the composition $C_5H_4O_2$.

Experimental Section

Melting points were determined on a Thomas–Hoover capillary melting point apparatus which had been calibrated with standard samples. Values of $[\alpha]D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a Beckman Model DK2A and a Cary Model 11 spectrophotometer. Infrared absorption spectra were determined on a Perkin-Elmer Model 421 spectrophotometer and a Beckman Model 5-A recording spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer in deuterated dimethyl sulfoxide solution and deuteriochloroform solution with tetramethylsilane as internal standard. Chemical shifts are recorded in τ values. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Low-resolution mass spectra were obtained on a C.E.C.-103 instrument, using an ionizing energy of 70 eV and a current of 20 μA. All high-resolution mass spectra were determined employing a Mattauch–Herzog double-focussing instrument (C.E.C.-21-110) with photoplate recording. Spectra were run with an ionizing voltage of 70 eV and a current of 150 μA. Direct introduction of the sample was used for both instruments at minimum temperatures necessary to vaporize the sample. Petroleum ether refers to the fraction with bp 60–68°.

Elephantin (3) and Elephantopin (4).—The whole dried plant (*Elephantopus elatus* Bertol., 850 g) was extracted with 95% ethanol in a Soxhlet apparatus. The ethanol was removed under reduced pressure and the residue (A, 68 g) was partitioned between chloroform (2 l.) and water (1 l.). The chloroform

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(15) R. I. Reed in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York, N. Y., 1963, Chapter 13.
(16) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 23.
(17) S. Sasaki, Y. Itagaki, H. Moriyama, K. Nakanishi, E. Watanabe, and T. Aoyama, *Tetrahedron Lett.*, 623 (1966).
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(21) A. L. Burlingame and C. H. Smith, *Tetrahedron*, **24**, 5749 (1968).

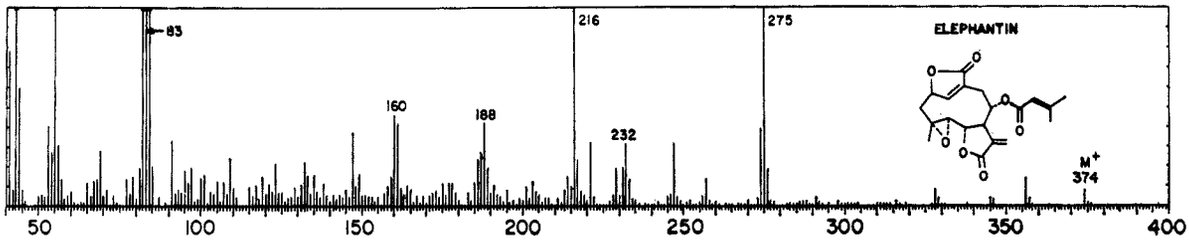


Figure 1.—Low-resolution mass spectrum of elephantin (3).

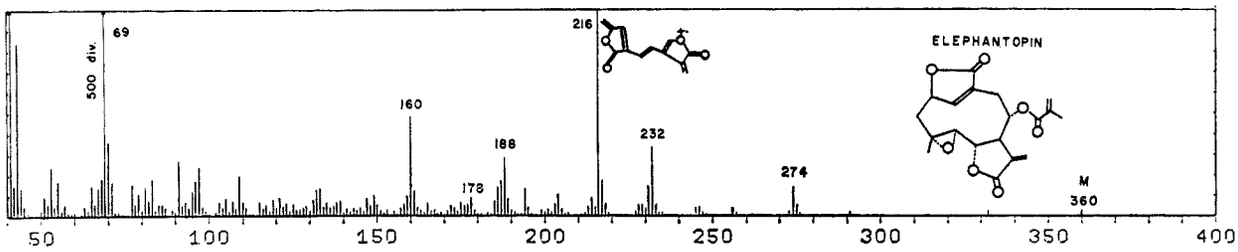


Figure 2.—Low-resolution mass spectrum of elephantopin (4).

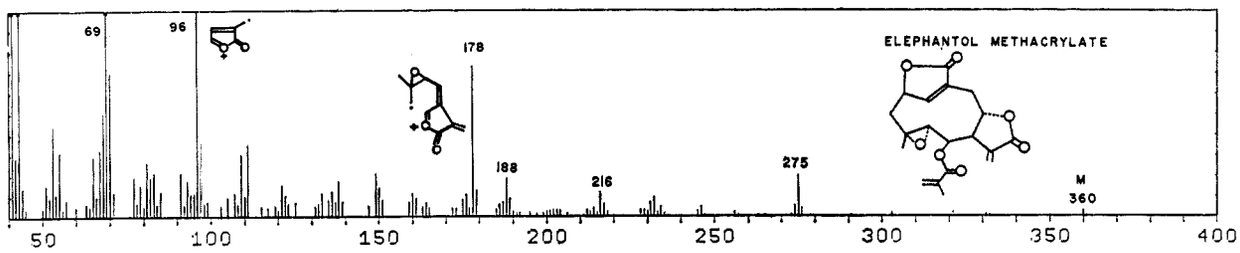


Figure 3.—Low-resolution mass spectrum of elephantol methacrylate (11).

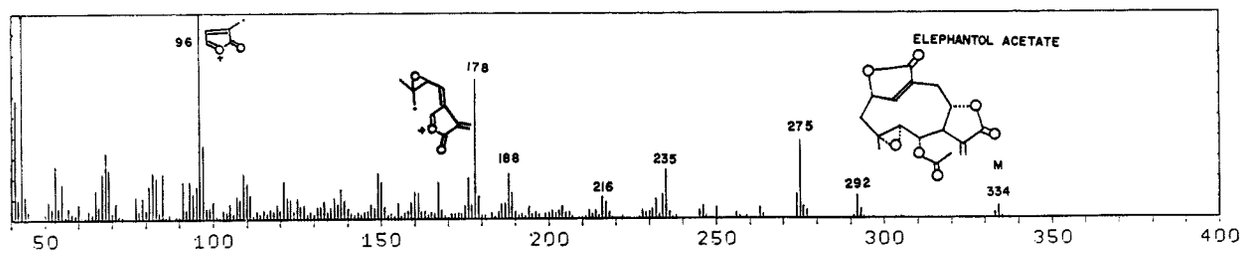


Figure 4.—Low-resolution mass spectrum of elephantol acetate (10).

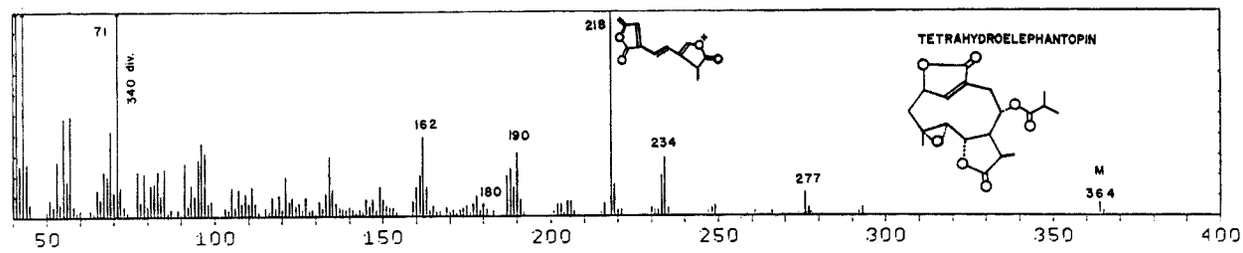


Figure 5.—Low-resolution mass spectrum of tetrahydroelephantopin (6).

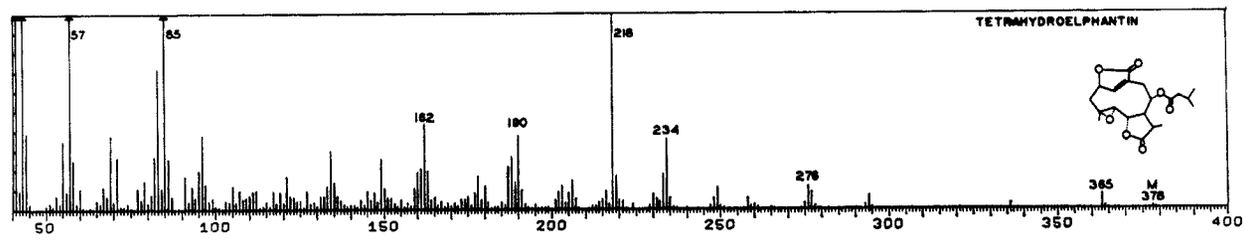


Figure 6.—Low-resolution mass spectrum of tetrahydroelephantin (15).

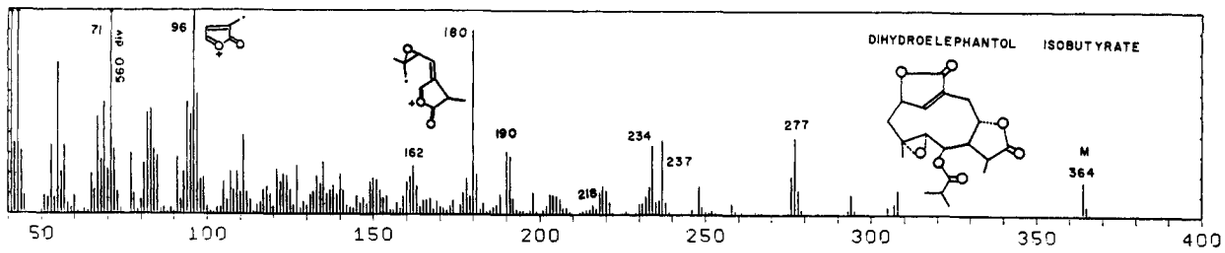


Figure 7.—Low-resolution mass spectrum of dihydroelephantol isobutyrate (12).

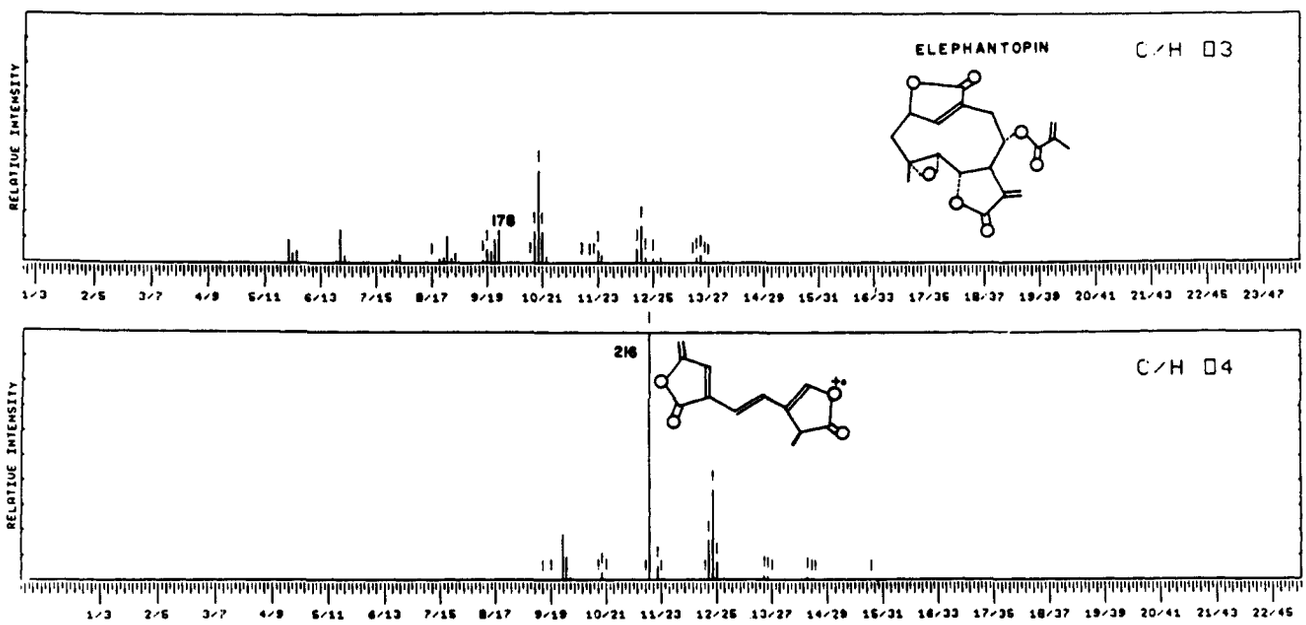


Figure 8.—High-resolution mass spectrum of elephantopin (4).

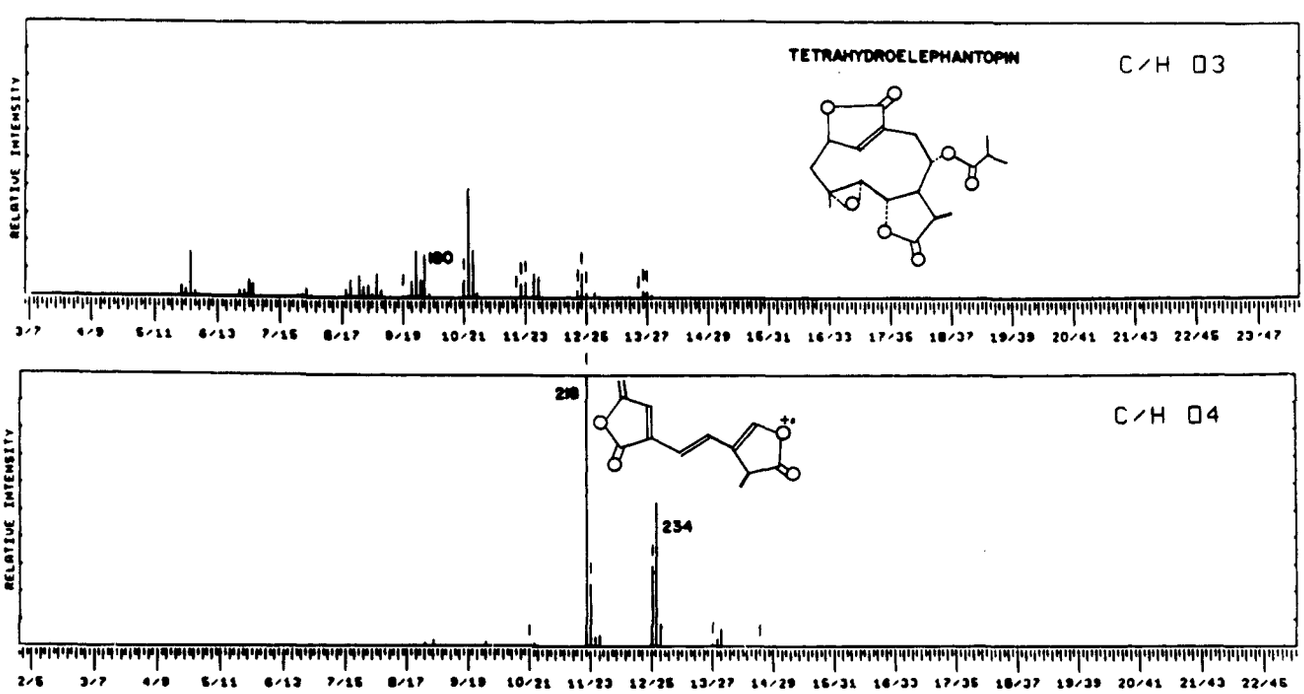


Figure 9.—High-resolution mass spectrum of tetrahydroelephantopin (6).

phase was separated and concentrated to give D (24.5 g). This residue (D) was partitioned between 10% aqueous methanol (500 ml) and petroleum ether (1 l.). The 10% aqueous methanol layer was concentrated to give fraction G (12.9 g). Fraction G was chromatographed on 400 g (4.5 × 150 cm column) of silicic acid (Mallinckrodt) and Celite (Johns-Manville) (3:1). Elephantin (3) and elephantopin (4) were eluted with 2% methanol in chloroform (fractions H and I). These fractions were rechromatographed on 300 g of silicic acid–Celite (3:1) using a fraction collector equipped with an ultraviolet intensity scanner to give two fractions which were crystallized from methanol. The first fraction gave elephantin (3, 490 mg): mp 242–244°;

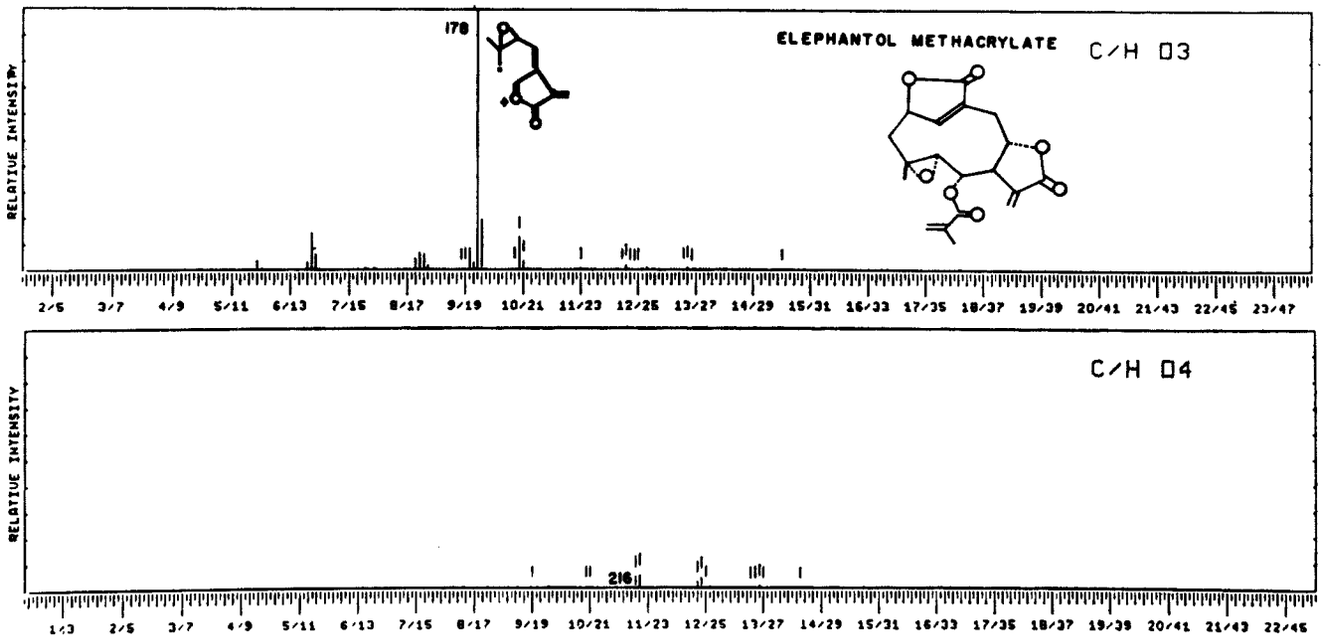


Figure 10.—High-resolution mass spectrum of elephantol methacrylate (11).

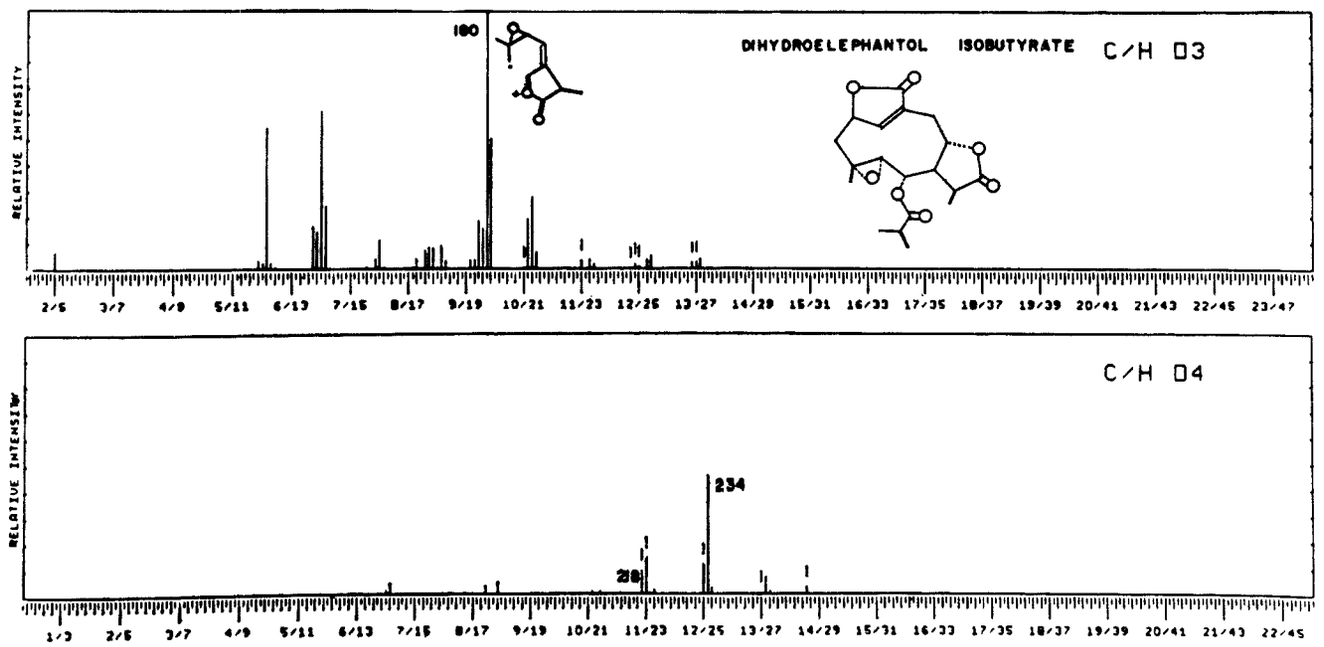


Figure 11.—High-resolution mass spectrum of dihydroelephantol isobutyrate (12).

$[\alpha]^{27}D$ −380° (*c* 0.015, MeOH); uv λ_{max}^{MeOH} 215 mμ (ε 25,000); ir λ_{max}^{KBr} 5.64, 5.68, 5.84, 6.07, and 8.12 μ; mass spectrum *m/e* 374 (M^+).

Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 63.84; H, 5.88.

The second fraction gave elephantopin (**4**, 2.15 g): mp 262–264°; $[\alpha]^{25}D$ −398° (*c* 0.016, MeOH); uv λ_{max}^{MeOH} 210 mμ (ε 27,000); ir λ_{max}^{KBr} 5.68, 5.73, 5.86, 6.08, and 6.12 μ; mass spectrum *m/e* 360 (M^+).

Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 63.34; H, 5.37.

Tetrahydroelephantopin (**6**).—Elephantopin (**4**, 100 mg) was dissolved in methanol (25 ml) and hydrogenated using prereduced 10% palladium on charcoal as catalyst at atmospheric pressure and room temperature. Uptake of hydrogen stopped after 2 mol equiv of hydrogen had been absorbed. The suspension was filtered, the solvent was removed under reduced pressure, and the residue was crystallized from methanol to give 90 mg of **6**: mp 290–292°; $[\alpha]^{25}D$ −370° (*c* 0.009, MeOH); uv λ_{max}^{MeOH} 210 mμ (ε 10,600); ir λ_{max}^{KBr} 5.64, 5.78, 5.83, 6.08, and 8.03 μ; mass spectrum *m/e* 364 (M^+).

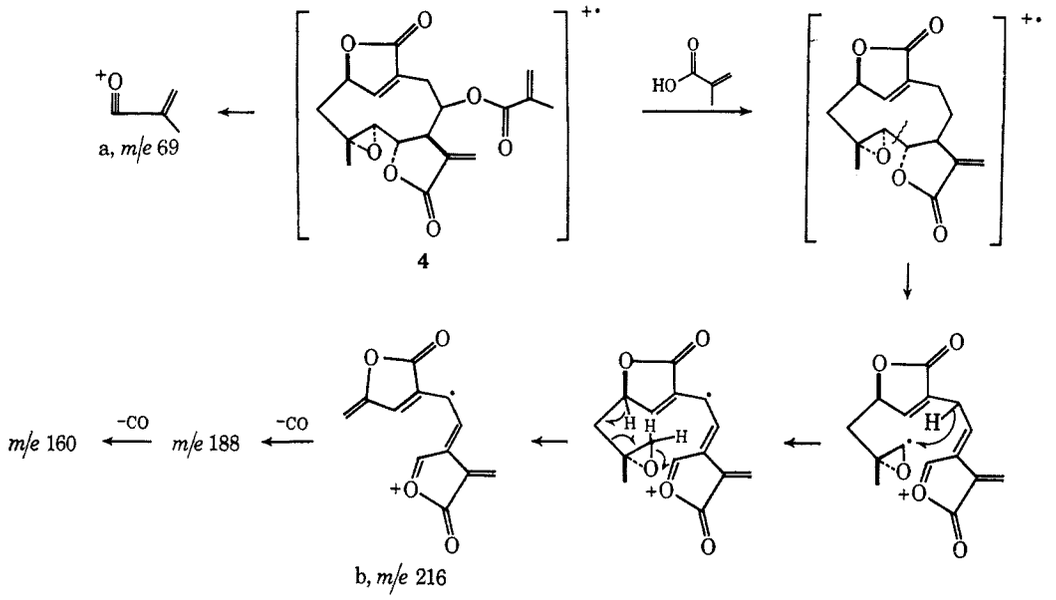
Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.62; H, 6.64. Found: C, 62.65; H, 6.51.

Hexahydroelephantopin (**7**).—Elephantopin (**4**, 100 mg) was dissolved in ethanol (50 ml) and hydrogenated using prereduced platinum as catalyst at atmospheric pressure and room temperature for 6 hr, a total of 3 mol equiv of hydrogen being absorbed. The mixture was filtered and evaporated under reduced pressure. The residue was purified by chromatography on an alumina (Woelm grade I, 14 g) column (eluent, chloroform). A material (45 mg, homogeneous by tlc) was separated and crystallized from chloroform and petroleum ether to give 35 mg of hexahydroelephantopin (**7**): mp 231–233°; $[\alpha]^{27}D$ −100° (*c* 0.108, MeOH); ir λ_{max}^{KBr} 5.65, 5.68, 5.80, and 8.12 μ.

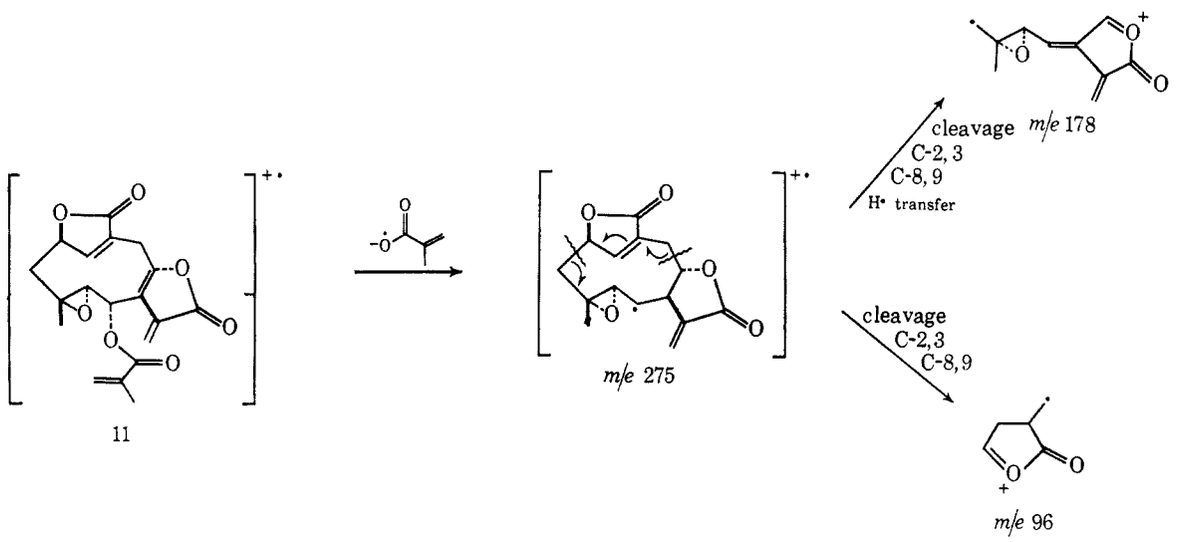
Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.94; H, 6.67.

Elephantol (**5**). **A. From Elephantopin.**—A solution of **4** (100 mg) in 1% potassium hydroxide in 20% aqueous methanol (8 ml) was allowed to stand at room temperature for 24 hr. After methanol was removed under reduced pressure, water (10 ml) was added, and the mixture was acidified with dilute sulfuric acid and distilled. The residue was extracted with ether using a

SCHEME I



SCHEME II



continuous extraction apparatus. After evaporation of the ether a solid was obtained, which was crystallized from methanol to give 32 mg of elephantol (**5**): mp 282–284°; $[\alpha]^{27}D$ +274° (*c* 0.0095, MeOH); uv λ_{max}^{MeOH} 209 mμ (ε 18,300); ir λ_{max}^{KBr} 2.94, 5.68, 5.73, and 6.06 μ; mass spectrum *m/e* 292 (M^+).

Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.45; H, 5.42.

The distillate was shaken with ether and the ethereal solution was dried (Na_2SO_4) and evaporated under reduced pressure. The nmr spectrum of the residue was identical with that of methacrylic acid. Reduction of the acid with sodium amalgam[22] gave isobutyric acid, which, after conversion to its ammonium salt, was chromatographed, using butanol saturated with 1.5 *N* ammonium hydroxide as the solvent system and an ethanolic solution of bromophenol blue as the developer. The R_f of the acid salt was the same as that of authentic ammonium isobutyrate.

B. From Elephantin.—A solution of elephantin (**3**, 100 mg) in 20% aqueous methanol (10 ml) containing KOH (100 mg) was allowed to stand at room temperature overnight. The solution was concentrated under reduced pressure and diluted with water. Upon cooling, a precipitate was obtained which was crystallized from methanol to give 3 mg of elephantol (**5**): mp 280–282°.

(22) A. I. Vogel, "Practical Organic Chemistry," Longmans Green and Co., London, 1957, p 194.

Dihydroelephantol (8).—A solution of **6** (150 mg) in 20% aqueous methanol (15 ml) containing potassium hydroxide (150 mg) was allowed to stand at room temperature overnight. The solution was acidified with dilute sulfuric acid and the precipitate of potassium sulfate obtained was removed by filtration. The filtrate was concentrated under reduced pressure and the residue was crystallized from methanol to give 35 mg of dihydroelephantol (**8**): mp 288–290°; uv λ_{max}^{MeOH} 211 mμ (ε 9,600); ir λ_{max}^{KBr} 2.90, 5.62, and 5.72 μ; mass spectrum *m/e* 294 (M^+).

Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17. Found: C, 60.59; H, 6.30.

Elephantol Acetate (10).—Elephantol (**5**, 50 mg) was treated with acetic anhydride (1 ml) in pyridine (1 ml) at room temperature overnight. Cold water was added and the mixture was extracted with ether. The ether layer, on drying over sodium sulfate and evaporation, gave a residue which on crystallization from methanol yielded 30 mg of elephantol acetate (**10**): mp 300–302°; uv λ_{max}^{MeOH} 209 mμ (ε 22,000); ir λ_{max}^{KBr} 5.65, 5.72, 6.04, and 8.20 μ; mass spectrum *m/e* 334 (M^+).

Anal. Calcd for $C_{17}H_{18}O_7$: C, 61.07; H, 5.43. Found: C, 61.29; H, 5.50.

Elephantol Methacrylate (11).—Elephantol (**5**, 75 mg) was dissolved in pyridine (1 ml) and treated with methacrylic anhydride[23] (1 ml) and the mixture was allowed to stand overnight

(23) T. K. Brotherton, J. Smith, and J. W. Lynn, *J. Org. Chem.*, **26**, 1283 (1961).

at room temperature. Cold water was added and the aqueous solution was extracted with chloroform. The combined chloroform extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The solid obtained was crystallized from methanol to give 50 mg of elephantol methacrylate (**11**): mp 238–240°; uv λ_{max}^{MeOH} 209 mμ (ε 21,400); ir λ_{max}^{KBr} 5.65, 5.78, 5.86, 6.09, 6.15, and 8.08 μ; mass spectrum *m/e* 360 (M^+).

Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.39. Found: C, 63.13; H, 5.63.

Dihydroelephantol Isobutyrate (**12**).—Dihydroelephantol (**8**, 50 mg) was dissolved in pyridine (1 ml) and treated with isobutyric anhydride (1 ml) and the mixture was allowed to stand overnight at room temperature. Cold water was added and the aqueous solution was extracted with chloroform. The combined chloroform extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The solid obtained was crystallized from methanol to give 38 mg of dihydroelephantol isobutyrate (**12**): mp 201–203°; uv λ_{max}^{MeOH} 211 mμ (ε 10,300); ir λ_{max}^{KBr} 5.64, 5.72, 5.82, 6.05, and 8.10 μ; mass spectrum *m/e* 364 (M^+).

Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.62; H, 6.64. Found: C, 62.66; H, 6.62.

Tetrahydroelephantol (**9**).—Elephantol (**5**, 62 mg) was dissolved in methanol (30 ml) and hydrogenated using prereduced platinum as catalyst at atmospheric pressure and room temperature; 2 mol equiv of hydrogen were absorbed. The mixture was filtered and evaporated under reduced pressure. The residue was purified by chromatography on a silicic acid–Celite (3:1, 15 g) column (eluent, chloroform). A homogeneous material (42 mg, tlc) was separated and crystallized from chloroform–petroleum ether to give 32 mg of tetrahydroelephantol (**9**): mp 230–232°; $[\alpha]^{27}D$ +40° (*c* 0.015, MeOH); ir λ_{max}^{KBr} 2.91, 5.68, and 5.72 μ.

Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.24; H, 6.90.

Dihydroelephantolide (**13**).—Tetrahydroelephantopin (**6**, 150 mg) was dissolved in a mixture of 3 *N* sulfuric acid in 50% aqueous methanol (35 ml) and heated under reflux for 4 hr, allowed to cool, and extracted with methylene chloride. The organic solvent was removed under reduced pressure and the residue was purified by chromatography on an alumina (Woelm grade I, 30 g) column (eluent, chloroform). A homogeneous material (70 mg, tlc) was separated and crystallized from methanol to give 41 mg of dihydroelephantolide (**13**): mp 300–302°; $[\alpha]^{25}D$ −273° (*c* 0.0177, MeOH); uv λ_{max}^{MeOH} 211 mμ (ε 8,700); ir λ_{max}^{KBr} 2.85, 5.68, 5.80, and 6.10 μ; mass spectrum *m/e* 294 (M^+).

Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17. Found: C, 61.18; H, 6.29.

Conversion of Dihydroelephantolide (**13**) **to Dihydroelephantol** (**8**).—Dihydroelephantolide (**13**, 50 mg) was dissolved in 20% aqueous methanol (5 ml) containing KOH (50 mg) and allowed to stand at room temperature overnight. The solution was concentrated under reduced pressure, diluted with cold water, acidified with 1 *N* hydrochloric acid to pH 1, and cooled. The precipitate thus obtained was filtered and crystallized from methanol to yield 40 mg of dihydroelephantol (**8**): mp 288–290°. The mixture melting point with an authentic sample of **8** was not depressed and the infrared absorption spectrum was superimposable on that of **8**.

Elephantol *p*-Bromobenzoate (**14**).—Elephantol (**5**, 150 mg) was dissolved in pyridine (2.5 ml) and was treated with 200 mg of *p*-bromobenzoyl chloride. The reaction mixture was heated at 60° for 2 min and allowed to stand overnight at room temperature. A solution of 10% sodium bicarbonate was added and the precipitate was purified by chromatography on a neutral alumina (Woelm grade I, 30 g) column (eluent, chloroform). A homogeneous solid (120 mg, tlc) was separated and crystallized from methanol to give 105 mg of elephantol *p*-bromobenzoate (**14**): mp 302–304°; $[\alpha]^{25}D$ +124° (*c* 0.0949, MeOH); uv λ_{max}^{MeOH} 246 mμ (ε 25,000); ir λ_{max}^{KBr} 5.69, 5.74, 5.78, and 6.23 μ.

Anal. Calcd for $C_{22}H_{19}BrO_7$: C, 55.57; H, 4.40; Br, 16.63. Found: C, 56.12; H, 4.23; Br, 17.83.

Tetrahydroelephantin (**15**).—Elephantin (**3**, 110 mg) was dissolved in ethanol (25 ml) and hydrogenated with prereduced 10% palladium on charcoal as catalyst at atmospheric pressure and room temperature. The mixture was filtered and evaporated under reduced pressure to give 105 mg of product, which was crystallized from methanol to give 95 mg of tetrahydroelephantin (**15**): mp 274–276°; uv λ_{max}^{MeOH} 211 mμ (ε 10,800); ir λ_{max}^{KBr} 5.62, 5.73, 5.84, and 6.04 μ; mass spectrum *m/e* 378 (M^+).

Anal. Calcd for $C_{20}H_{26}O_7$: C, 63.48; H, 6.93. Found: C, 62.99; H, 6.67.

Isovaleric Acid from Tetrahydroelephantin.—A solution of tetrahydroelephantin (**15**, 500 mg) in 20% aqueous methanol (60 ml) containing KOH (900 mg) was allowed to stand at room temperature for 24 hr. The solution was concentrated under reduced pressure, and the residue was diluted with 80 ml of water, acidified to pH 1 with sulfuric acid, and steam distilled. The distillate was extracted with ether. The ethereal solution was dried over anhydrous sodium sulfate and evaporated to afford an oil whose nmr spectrum was identical with that of isovaleric acid. This acid was converted to its *p*-bromophenacyl ester derivative, mp 66–68°, undepressed by admixture with authentic isovaleric acid *p*-bromophenacyl ester. The mother liquor remaining after steam distillation gave 70 mg of **8**.

Registry No.—**3,** 21899-50-3; **4,** 21899-51-4; **5,** 21899-52-5; **6,** 21899-53-6; **7,** 21899-54-7; **8,** 10207-68-8; **9,** 21899-55-8; **10,** 21899-56-9; **11,** 10207-70-2; **12,** 10207-71-3; **13,** 21927-73-1; **14,** 21899-59-2; **15,** 21899-60-5.