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GERMACRANOLIDES FROM *ELEPHANTOPUS* SPECIES

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Key Word Index—*Elephantopus angustifolius* (= *Orthopappus angustifolius*); *E. mollis*; Compositae; sesquiterpene lactones; elephantopin derivatives; germacranolide.

Abstract—The aerial parts of *Elephantopus angustifolius* (= *Orthopappus angustifolius*) afforded, in addition to elephantopin and isoelephantopin, five new lactones which were all closely related to isoelephantopin, and a germacranolide, most likely the precursor of the elephantopin derivatives. A reinvestigation of *E. mollis* gave a derivative of desoxyelephantopin. The structures were elucidated by high-field NMR spectroscopy.

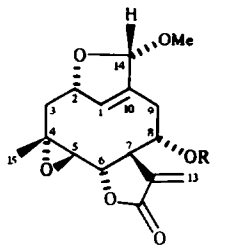
INTRODUCTION

The tropical genus *Elephantopus* (Compositae, tribe Vernonieae) is placed in the subtribe Elephantopodinae together with *Pseudoelephantopus* and *Orthopappus* [1]. So far, the aerial parts of six out of the 32 species have been studied chemically. Most species contain the unique sesquiterpene lactone elephantopin and related compounds which have been studied by several groups due to the anticancer activity of these lactones [2–15]. One species also gave guaianolides [11] while the roots contain polyacetylenes and sesquiterpenes [12]. We have now studied the constituents of *Elephantopus angustifolius* Sw. (= *Orthopappus angustifolius*) from two different locations and reinvestigated *E. mollis* H. B. K. The results are discussed in this paper.

RESULTS AND DISCUSSION

The aerial parts of *Elephantopus angustifolius* Sw. [= *Orthopappus angustifolius* (Sw.) Gleason] from two different locations gave the same compounds but in different concentrations. In addition to lupeol and its acetate, also present were elephantopin [2] and isoelephantopin (**4**), which have already been prepared by epoxidation of isodeoxyelephantopin [15]. Furthermore, two new dilactones (**5** and **6**), three closely related acetals (**1–3**) and a germacranolide (**7**) were isolated.

The molecular formula of **1** was $C_{20}H_{24}O_7$, although no molecular ion could be observed. The highest ion was m/z 345 formed by loss of a methoxy unit as the ^{13}C NMR spectrum indicated the presence of 20 carbons (see Experimental). In the ^1H NMR spectrum (Table 1) all the signals could be assigned by spin decoupling and it was in part similar to that of isoelephantopin (**4**). However, the upfield shift of the H-1 signal and additional signals at δ6.23 (*br s*, 1H) and 3.37 (*s*, 3H) indicated that most likely the carbonyl group at C-14 was reduced and that the resulting hemiacetal was transformed to a methyl acetal. This assumption agreed with the ^{13}C NMR spectrum, especially as a doublet at δ111.2 required a carbon with two oxygen functions. The complete stereochemistry



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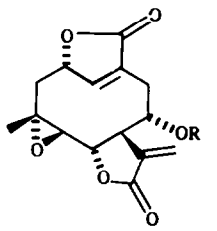
2 R = Sen
3 R = Tigl



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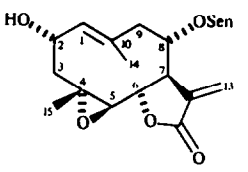

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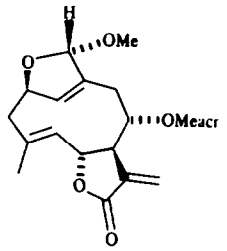

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could be determined by NOE difference spectroscopy. Saturation of H-14 gave NOEs with H-2 (6%), H-8 (5%) and H-15 (5%). Further NOEs were observed between H-1, H-7 (11%), H-5 (5%) and methoxy (8%); between H-8, H-6 (6%), H-9 (10%) and H-14 (3%); between H-7, H-1 (10%) and H-9α (3%); and between H-15, H-3β (4%), H-6 (10%), H-8 (2%) and H-14 (8%).

The ^1H NMR spectra of **2** and **3** (Table 1) clearly showed that the corresponding senecioate and tiglate were

present. We have named the desacyl derivative of **1–3** orthopappolide. Similar lactones with a hemiacetal moiety are reported from a *Mikania* species [16].

The ^{1}H NMR spectra of **5** and **6** (Table 1) were close to the spectrum of isoelephantopin (**4**). The typical signals of the ester group indicated that **5** was desacyl isoelephantopin senecioate and that **6** was the corresponding tiglate. NOE difference spectroscopy with desacyl isoelephantopin tiglate (**6**) clearly supported that the new esters belong to the 2α-olide series. Depending on the configuration at C-2, the conformations of the two series are also different [15, 16].

The structure of **7** followed from the ^{1}H NMR spectral data (Table 1). Spin decoupling allowed the assignment of all the signals, which indicated the presence of a germacranolide. As a small coupling between H-1 and H-9 and a *W*-coupling between H-15 and H-3α were visible, the whole sequence was established. The observed couplings allowed the assignment of the configuration at C-2. Most likely **7** is the precursor of **2** and **5**, which are probably formed by allylic oxidation at C-14. The corresponding germacranolides with a methacrylate or a tiglate residue were not detected.

The aerial parts of *E. mollis* H. B. K., which has been investigated previously [5, 7, 10], afforded deoxyelephantopin [13], deoxyisoelephantopin [14] and a further lactone, the cyclic acetal **8**. The structure followed from the ^{1}H NMR spectrum (Table 1), which was close to that of deoxyelephantopin. As in the case of **1**, the missing C-14 carbonyl group caused an upfield shift of H-1. The additional methoxy singlet at δ3.36 and a broadened singlet at δ5.89 indicated the presence of an acetal. NOEs between H-15, H-14 (7%), H-3 (3%) and H-6 (10%); between H-1, H-5 (5%), H-7 (11%) and OMe (5%); and between H-7 and H-1 (10%) allowed the assignment of the stereochemistry. Previous reports on this species gave different sesquiterpene lactones [5, 7, 10]. The results again showed that elephantopin derivatives are characteristic of the genus *Elephantopus* as this type of lactone has not been reported from any other genus of the tribe Vernonieae.

EXPERIMENTAL

The air-dried aerial parts grown from seeds in a greenhouse were extracted and separated as reported previously [17]. The two extracts of *Elephantopus angustifolius* (100 g, voucher 74-58, originally collected near São Paulo, Brazil; voucher Jones 22633 and 80 g voucher 76-14, original material collected near São Paulo, Brazil, voucher Jones 22627) gave on CC (silica gel) four fractions (Fr. 1: Et_2O–petrol, 1:9; Fr. 2: Et_2O–petrol, 1:3; Fr. 3: Et_2O and Fr. 4: Et_2O–MeOH, 9:1), which were separated further by preparative TLC (silica gel, PF 254) and HPLC (RP 8, *ca* 100 bar) (in parentheses the quantities obtained from the second extract). Preparative TLC of Fr. 1 gave 20 mg lupeyl acetate (50 mg) and of Fr. 2, 5 mg lupeol (80 mg). Preparative TLC of Fr. 3 ($CHCl_3$–C_6H_6–Et_2O, 1:1:1) gave 80 mg **1** (10 mg) (R_f 0.5) and a mixture (R_f 0.4) which gave upon HPLC (MeOH–H_2O, 3:2) 30 mg **2** (5 mg) and 10 mg **1**. HPLC of Fr. 4 (MeOH–H_2O, 3:2) gave a mixture (R_t 1.6 min, 4/1), 20 mg elephantopin (R_t 2.5 min) (4 mg), 18 mg **4** (R_t 2.9 min) (18 mg), 10 mg **1** (R_t 3.9 min) (10 mg), a mixture (R_t 4.1 min, 4/5), 5 mg **5** (R_t 4.4 min) (2 mg), 2 mg **6** (R_t 4.6 min) (1.7 mg) and 20 mg **2** (R_t 5.7 min) (2 mg). Repeated HPLC of Fr. 4/1 gave 2 mg **3** (1 mg) and preparative TLC of Fr. 4/5 ($CHCl_3$–C_6H_6–Et_2O, 1:1:1, 2 developments) gave 5 mg **1** and 1.5 mg **7** (0.6 mg).

The extract of the aerial parts of *E. mollis* (95 g, originally collected in Brazil, Gerais State, voucher Jones 22670) gave upon CC and HPLC of the polar fractions (RP 8, MeOH–H_2O, 3:2) 5 mg **8** (R_t 12.2 min). The most polar fraction gave upon HPLC

Table 1. ^{1}H NMR spectral data of compounds **1–3** and **5–8** ($CDCl_3$, TMS as internal standard)

	1	2	3	5	6	7	8
H-1	5.63 *d*	5.62 *d*	5.63 *d*	7.48 *br s*	7.48 *br s*	5.36 *br d*	5.58 *d*
H-2	5.24 *br dd*	5.23 *br dd*	5.24 *br dd*	5.36 *br d*	5.37 *br d*	4.72 *ddd*	5.29 *dd*
H-3α	1.88 *br d*	1.88 *br d*	1.88 *br d*	2.17 *br d*	2.17 *br d*	1.26 *dd*	2.68 *br d*
H-3β	2.36 *dd*	2.36 *dd*	2.36 *dd*	2.53 *dd*	2.53 *dd*	2.62 *dd*	2.23 *dd*
H-5	2.95 *d*	2.95 *d*	2.96 *d*	2.81 *d*	2.81 *d*	2.73 *d*	5.16 *ddq*
H-6	4.41 *t*	4.42 *t*	4.43 *t*	4.29 *t*	4.31 *t*	4.28 *dd*	5.27 *dd*
H-7	3.03 *dddd*	2.99 *dddd*	3.02 *dddd*	3.23 *dddd*	3.26 *dddd*	3.25 *dddd*	2.87 *dddd*
H-8	4.60 *ddd*	4.59 *ddd*	4.60 *ddd*	4.53 *ddd*	4.57 *ddd*	4.59 *ddd*	4.60 *ddd*
H-9α	2.63 *t*	2.56 *t*	2.62 *t*	3.03 *t*	3.09 *t*	2.56 *dd*	2.57 *dd*
H-9β	2.90 *dd*	2.84 *dd*	2.89 *dd*	2.80 *dd*	2.85 *dd*	2.46 *dd*	2.79 *dd*
H-13	6.27 *d*	6.28 *d*	6.26 *d*	6.28 *d*	6.26 *d*	6.34 *d*	6.21 *d*
H-13′	5.70 *d*	5.74 *d*	5.69 *d*	5.76 *d*	5.71 *d*	5.77 *d*	5.64 *d*
H-14	6.23 *br s*	6.22 *br s*	6.22 *br s*	—	—	1.89 *d*	5.89 *br s*
H-15	1.46 *s*	1.48 *s*	1.47 *s*	1.49 *s*	1.47 *s*	1.30 *s*	1.79 *d*
OCOR	6.12 *br s*	5.66 *qq*	6.88 *qq*	5.68 *qq*	6.90 *qq*	5.66 *qq*	6.12 *br s*
	5.64 *dq*	2.11 *d*	1.82 *dq*	2.11 *d*	1.84 *br d*	2.13 *d*	5.66 *dq*
	1.91 *t*	1.92 *d*	1.81 *dq*	1.94 *d*	1.81 *br s*	1.93 *d*	1.93 *br s*
OMe	3.37 *s*	3.39 *s*	3.39 *s*	—	—	—	3.36 *s*

J (Hz): Compounds **1–3**: 1,2 = 3.5; 2,3 = 5; 3,3′ = 15; 5,6 = 9.5; 6,7 = 9; 7,8 = 2.5; 7,13 = 3.5; 7,13′ = 3; 8,9α = 9α,9β = 12; 8,9β = 4; compounds **5** and **6**: 2,3 = 5; 3,3′ = 15; 5,6 = 9.5; 6,7 = 9; 7,8 = 8,9β = 4; 7,13 = 3.5; 7,13′ = 3; 8,9α = 9α,9β = 12.5; compound **7**: 1,2 = 10; 1,14 = 1; 2,3 = 6; 2,3′ = 11; 3,3′ = 12; 5,6 = 9; 6,7 = 7; 7,8 = 5; 7,13 = 3.5; 7,13′ = 3; 8,9α = 10.5; 8,9β = 1.5; 9α,9β = 12; compound **8**: 1,2 = 4; 2,3β = 5; 3α,3β = 14; 3α,5 = 1; 5,6 = 6,7 = 10; 5,15 = 1.5; 7,8 = 3.5; 7,13 = 3.8; 7,13′ = 3.2; 8,9α = 12.5; 8,9β = 3.5; 9α,9β = 12.5; OMeacr: 3′,3′ = 3′,4 = 1; OSen: 2′,4′ = 2′,5 = 1; OTigl: 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.

(RP 8, MeOH–H_2O, 1:1) 15 mg deoxyelephantopin (*R*, 7.5 min) and 10 mg deoxyisoelephantopin (*R*, 6.2 min). Known compounds were identified by comparison of the 400 MHz ^1H NMR spectra with those of authentic material.

Orthopappolide methacrylate (**1**). Colourless crystals, mp 85°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (γ-lactone), 1710, 1640 (C=CCO_2R); MS *m/z* (rel. int.): 345.134 $[M-OMe]^+$ (6) (cal. for $C_{19}H_{21}O_6$: 345.134), 344 $[M-MeOH]^+$ (1), 258 $[344-RCO_2H]^+$ (3), 69 $[C_3H_5CO]^+$ (100); ^{13}C NMR ($CDCl_3$, C-1–C-15): δ 133.3 *d*, 74.5 *d*, 42.9 *t*, 57.8 *s*, 59.2 *d*, 81.8 *d*, 47.3 *d*, 80.3 *d*, 31.5 *t*, 133.1 *s*, 134.3 *s*, 166.9 *s*, 126.9 *t*, 111.2 *d*, 22.2 *q*; OMe: 54.2 *q*; OCOR: 168.7 *s*, 135.4 *s*, 124.1 *t*, 18.1 *q*; CD (MeCN) $\Delta\varepsilon_{246}-1.5$.

Orthopappolide senecioate (**2**). Colourless crystals, mp 89°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1720, 1650 (C=CCO_2R); MS *m/z* (rel. int.): 359.149 $[M-OMe]^+$ (1.4) (calc. for $C_{20}H_{23}O_6$: 359.149), 358 $[M-MeOH]^+$ (0.3), 258 $[358-RCO_2H]^+$ (0.7), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (22).

Orthopappolide tiglate (**3**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1715, 1650 (C=CCO_2R); MS *m/z* (rel. int.): 359.149 $[M-OMe]^+$ (1.6) (calc. for $C_{20}H_{23}O_6$: 359.149), 83 $[C_4H_7CO]^+$ (100).

Desacylisoelephantopin senecioate (**5**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), 1715, 1640 (C=CCO_2R); MS *m/z* (rel. int.): 374.137 $[M]^+$ (0.3) (calc. for $C_{20}H_{22}O_7$: 374.137), 274 $[M-RCO_2H]^+$ (2), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (18).

Desacylisoelephantopin tiglate (**6**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), 1710, 1650 (C=CCO_2R); MS *m/z* (rel. int.): 374.137 $[M]^+$ (0.4) (calc. for $C_{20}H_{22}O_7$: 374.137), 274 $[M-RCO_2H]^+$ (3), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (45).

2α-Hydroxy-8α-senecioyloxy-4α,5β-epoxygermacra-1(10)E, 11(13)-dien-12,6α-olide (**7**). Colourless crystals, mp 195°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590 (OH), 1765 (γ-lactone), 1710, 1645 (C=CCO_2R); MS *m/z* (rel. int.): 344.162 $[M-H_2O]^+$ (0.2) (calc. for $C_{20}H_{24}O_5$: 344.162), 262 $[M-RCO_2H]^+$ (1), 244 $[262-H_2O]^+$ (1), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (32); CIMS: 363 $[M+1]^+$ (14), 345 $[363-H_2O]^+$ (20), 263 $[363-RCO_2H]^+$ (30), 245 $[263-H_2O]^+$ (41), 227 $[245-H_2O]^+$ (100), 83 $[C_4H_7CO]^+$ (57); CD (MeCN) $\Delta\varepsilon_{240}-1.9$.

2-epi-Deoxyorthopapp-4E-enolide methacrylate (**8**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 274.119 $[M-RCO_2H]^+$ (2) (calc. for $C_{16}H_{18}O_4$: 274.120), 242 $[274-MeOH]^+$ (4), 213 $[242-CHO]^+$ (8), 69 $[RCO]^+$ (100).

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