Pergamon

PII: S0031-9422(96)00470-0

Phytochemistry, Vol. 44, No. 1, pp. 113–116, 1997

0031-9422/97 $17.00 + 0.00

SESQUITERPENE LACTONES FROM *ELEPHANTOPUS SCABER*

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(*Received* 28 *May* 1996)

Key Word Index—*Elephantopus scaber*; Compositae; sesquiterpene lactone; germacranolide; deoxyelephantopin; isodeoxyelephantopin; scabertopin.

Abstract—The whole plant of *Elephantopus scaber* afforded the known deoxyelephantopin and isodeoxyelephantopin, and a new germacranolide sesquiterpene lactone named scabertopin, whose structure and stereochemistry were determined by spectroscopic methods and single-crystal X-ray analysis. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The whole plant of *Elephantopus scaber* L. is known as 'Didancao' in Chinese medicine. It is used as a diuretic and as an antifebrile, an antiviral and an antibacterial agent, as well as in the treatment of hepatitis, bronchitis, the cough associated with pneumonia, and arthralgia [1]. Both alcohol and chloroform extracts of *E. scaber* have been reported to contain cytotoxic germacranolide-type sesquiterpene lactones [2].

Considering that both *E. scaber* and *E. mollis* H.B.K. are used in Chinese medicine for similar purposes, we have recently investigated their chemical constituents and reported on the sesquiterpene lactones isolated from *E. mollis* [3]. The present report describes the isolation and structure characterization of three germacranolide sesquiterpene lactones from *E. scaber*.

RESULTS AND DISCUSSION

Three sesquiterpene lactones, including the known deoxyelephantopin (**1**) and isodeoxyelephantopin (**2**) as well as a new structure scabertopin (**3**) were isolated and identified from the whole plant.

Scabertopin (**3**) has a molecular formula of $C_{20}H_{22}O_6$ as indicated by its high resolution L-SIMS-mass spectrum. The presence of an α-methylene-γ-lactone moiety was revealed by the IR bands at 1764 and 1652 cm^{-1} and substantiated by a pair of characteristic low field ^1H NMR signals at δ 5.63 (1 H, *d*, $J = 3.2$ Hz, H-13$_a$) and 6.20 (1 H, *d*, $J = 4$ Hz, H-13$_b$) (Table 1). In addition, an α,β-unsaturated lactone was indicated by the IR band at 1742 cm^{-1} and by ^1H NMR signals at δ 7.17 (1 H, *s*, H-1) and 5.38 (1 H, *d*, $J = 4.5$ Hz, H-2). In the mass spectrum of **3**, the signal at *m/z* 259 can be attributed to the loss of a $C_5H_8O_2$ neutral fragment from the protonated molecular ion. This is consistent with the presence of either an angelate or tiglate side chain. Indeed, the ^1H NMR spectrum showed two vinyl methyls at δ 1.90 (3H, *dd*, $J = 1.5$ Hz, H-18) and 1.97 (3H, *dq*, $J = 1.5$, 7.3 Hz, H-20) and an olefinic proton at δ 6.18 (1H, *m*, H-19), which are typical signals for an angelate moiety. For the *E*-form, i.e. tiglate, the chemical shift of H-19 should have occurred at δ 6.90 [4], due to a deshielding effect of the neighbouring carboxyl group.

The ^{13}C NMR spectrum of **3** showed the presence of 20 carbons (Table 2), of which three were carbonyl carbons at δ 174.3, 166.9 and 169.4. Two of them were assigned to the lactone carbonyls, whereas the remaining one belonged to an ester. In the ^1H NMR spectrum, a doublet at δ 5.13 ($J = 10$ Hz) was attributed to H-5 which coupled with H-6. The signal of H-6 (δ 5.17) appeared as a well-defined one-proton doublet of doublet with large coupling constants ($J_{5,6} = 10$ Hz; $J_{6,7} = 8$ Hz). This indicated a *trans*-axial relationship between H-5, H-6 and H-7, i.e. H-5 α, H-6 β, and H-7 α-oriented. These assignments were based on the fact that H-7 has been assumed to be α-oriented as in all other naturally occurring germacranolides. Moreover, a consideration of the relationship between the J value and the dihedral angle [5] indicated that a 4 Hz coupling constant between H-7 and H-8 was suggestive of an approximate dihedral angle of 122°. This allowed the assignment of H-8 as 8β.

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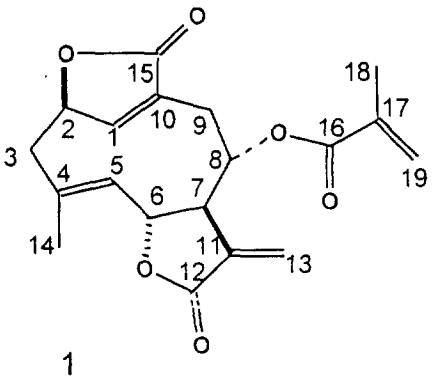

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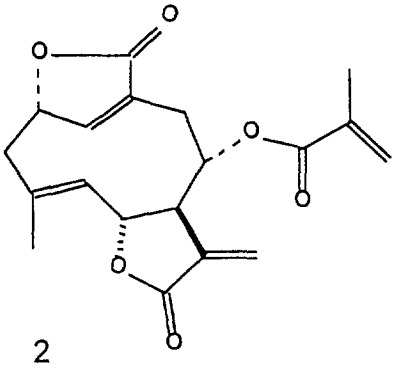

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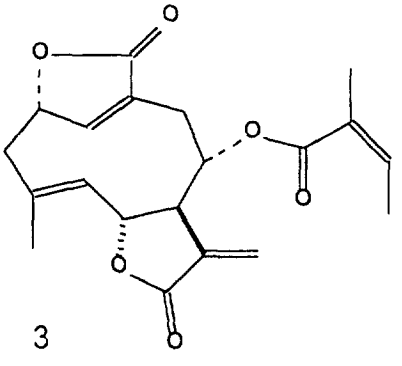

3

A comparison between the ^{1}H NMR spectra of **3** and **1** showed that the chemical shifts of H-5 and H-7 in **3** were downfield from those in **1** (H-5: δ 5.13 in **3** cf. 4.77 in **1**; and H-7: δ 3.14 in **3** cf. 2.94 in **1**). It is evident that the carboxyl group of the α,β-unsaturated lactone in **3** is in close proximity to both H-5 and H-7 thereby causing a paramagnetic effect. In agreement with this notion, the oxygen atom at C-2 should be α-oriented in **3**.

The above evidence allowed the assignment of structure **3** to scabertopin. However, in order to remove the ambiguity concerning the stereochemistry, the compound was subjected to single-crystal X-ray analysis. A perspective view of the solid-state conformation is shown in Fig. 1. Details of the analysis are given in the Experimental.

Careful examination of the ^{1}H and ^{13}C NMR spectra (including the HMQC and COLOC data) has led to unambiguous assignments of all signals (Tables 1 and 2). Thus, the carbonyl carbons were assigned with the aid of COLOC correlation peaks between C-12/H-13 and C-15/H-1. The quaternary olefinic carbons were also confirmed by the following COLOC cross signals: C-4/14-Me, C-10/H-9, C-11/H-7 and C-17/18-Me.

Table 1. ^{1}H NMR data of compounds **1–3** (δ values from internal TMS, J (Hz) in parentheses)

H	1	2	3
1	7.14 *s*	7.16 *s*	7.17 *s*
2	5.46 *dd* (2, 4)	5.38 *d* (4.5)	5.38 *d* (4.5)
3	2.69 *dd* (2, 14)	2.39 *dd* (4.5, 14)	2.40 *dd* (4.5, 14)
	2.85 *m*	2.94 *d* (14)	2.92 *d* (14)
5	4.77 *d* (10)	5.13 *d* (10)	5.13 *d* (10)
6	5.13 *dd* (8, 10)	5.17 *d* (10)	5.17 *dd* (8, 10)
7	2.94 *m*	3.15 *m*	3.14 *m*
8	4.65 *ddd* (2, 4, 12)	4.53 *ddd* (4, 4, 12)	4.53 *ddd* (4, 4, 12)
9_a	2.80 *m*	2.74 *dd* (4, 12)	2.75 *dd* (4, 12)
9_b	2.99 *m*	3.06 *dd* (12, 12)	3.06 *dd* (12, 12)
13_a	5.64 *d* (3.2)	5.65 *d* (3.2)	5.63 *d* (3.2)
13_b	6.21 *d* (4)	6.20 *d* (4)	6.20 *d* (4)
14	1.84 *d* (1)	1.78 *s*	1.80 *d* (1.3)
18	1.92 *s*	1.93 *s*	1.90 *dd* (1.5, 1.5)
19	5.66 *s*	5.67 *s*	6.18 *m*
	6.14 *s*	6.15 *s*	—
20	—	—	1.97 *dq* (7.5, 1.5)

Deoxyelephantopin (**1**) has a molecular formula of $C_{19}H_{20}O_6$ as indicated by its exact mass measurement. The IR, ^{1}H NMR and MS of **1** indicated the presence of an α-methylene-γ-lactone [IR: 1765 and 1645 cm^{-1}; ^{1}H NMR: δ 5.64 (1H, *d*, J = 3.2 Hz, H-13_a) and δ 6.21 (1 H, *d*, J = 4 Hz, H-13_b)], an α,β-unsaturated lactone [IR: 1742 cm^{-1}; ^{1}H NMR: δ 7.14 (1 H, *s*, H-1) and 5.46 (1 H, *dd*, J = 2,4 Hz, H-2)] and a methacrylate

Table 2. ^{13}C NMR data of compounds **1–3**

C	1	2	3
1	153.4 *d*	150.0 *d*	149.3 *d*
2	71.9 *d*	79.4 *d*	79.4 *d*
3	41.2 *t*	40.0 *t*	40.0 *t*
4	135.6 *s*	135.4 *s*	135.4 *s*
5	133.5 *d*	125.2 *d*	125.3 *d*
6	81.3 *d*	78.7 *d*	78.7 *d*
7	52.2 *d*	49.8 *d*	49.6 *d*
8	71.5 *d*	74.0 *d*	73.6 *d*
9	33.4 *t*	30.0 *t*	30.0 *t*
10	128.4 *s*	131.4 *s*	131.4 *s*
11	134.0 *s*	134.0 *s*	134.2 *s*
12	169.3 *s*	169.4 *s*	169.4 *s*
13	123.5 *t*	123.0 *t*	123.0 *t*
14	20.0 *q*	21.5 *q*	21.5 *q*
15	172.4 *s*	174.3 *s*	174.3 *s*
16	166.3 *s*	166.5 *s*	166.9 *s*
17	135.9 *s*	135.4 *s*	126.6 *s*
18	18.1 *q*	18.1 *q*	20.3 *q*
19	126.6 *t*	126.8 *t*	140.5 *d*
20			15.8 *q*

Signals for compound **3** were assigned by HMQC and COLOC spectra.

Assignments for **1** and **2** were made by comparison with **3**.

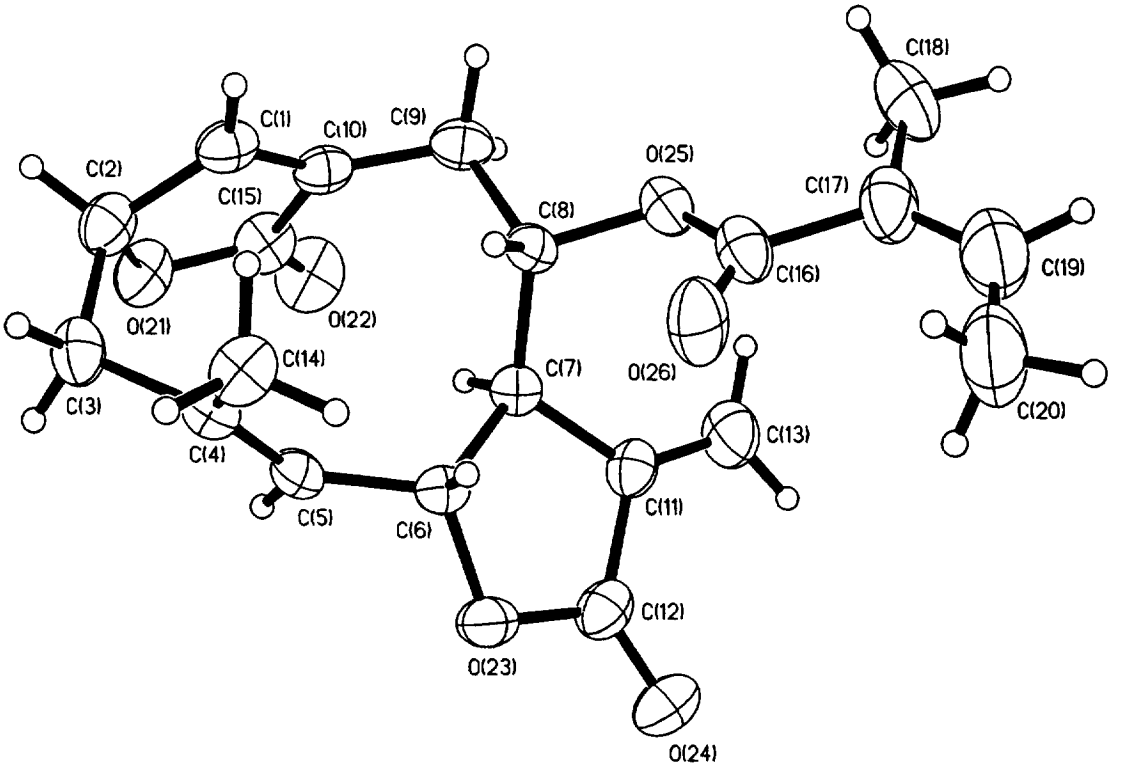

Fig. 1. Structure and solid-state conformation of scabertopin (**3**).

ester side chain [IR: 1705 and 1636 cm^{-1}; ^1H NMR: δ 1.92 (3H, s, H-18), and δ 5.66 *and* δ 6.14 (each 1H, *s*, H-19_a and H-19_b); MS: *m/z* 259 $[M + H - CH_2 = C(CH_3)\text{-}COOH]^+$]. The ^{13}C NMR spectrum (Table 2) confirmed all of the above assignments, and **1** was identified as deoxyelephentopin [6, 7].

Isodeoxyelephantopin (**2**), a stereoisomer of **1** [7], also has a molecular formula of $C_{19}H_{20}O_6$. Its IR and mass spectra were almost identical to that of **1**, whereas the ^1H NMR spectrum of **2** showed a downfield shift of H-5 and H-7 (Table 1) due to the effect of the α-oriented oxygen atom at C-2. The ^1H NMR spectrum of **2** was found to be similar to that of **3**. The major difference is the presence of the signals for a methacrylate group (δ 1.93, 3 H, *s*, H-18; δ 5.67 and δ 6.15, each 1 H, *s*, H-19_a and H-19_b) instead of an angelate.

EXPERIMENTAL

General. Mps: uncorr; ^1H NMR and ^{13}C NMR: 270 MHz and 67.80 MHz, respectively; 2-D NMR data (HMQC and COLOC): 300 MHz using standard pulse sequences. $CDCl_3$ was used as solvent, with TMS as int. standard. L-SIMS MS: 4.7 Tesla FT-ICR with 3-nitro-benzylalcohol as matrix; IR: $CHCl_3$; CC: silica gel (Merck, silica gel 60); TLC: Precoated silica gel plates (Merck, silica gel 60 F_{254}) detection of spots by spraying with vanillin–sulphuric acid soln followed by heating at 105° for 3 min.

Plant material. The whole plant of *E. scaber* L. was collected on the campus of the Chinese University of Hong Kong in September 1994. A voucher specimen (Cao 1008) is deposited in the Herbarium of the Department of Biology.

Extraction and isolation of sesquiterpene lactones. The air-dried whole plant materials (500 g) were extracted (×2) with hot (60°) 95% EtOH. After filtration, the dark green soln was evapd to dryness under red. pres. at 40°. The residue (6 g) was partitioned between H_2O and $CHCl_3$. The organic layer was sepd and concd to yield a dark green syrup (5.5 g). The $CHCl_3$ extract was then partitioned between hexane and 10% aq. MeOH. The aq. MeOH extract (2.5 g) was chromatographed on a silica gel column and eluted successively with hexane–EtOAc (1:1), (1:2) and (1:4). The eluates were monitored by TLC and grouped into 7 frs.

Frs. 5 eluted with hexane–EtOAc (1:2) was rechromatographed on a silica gel column using hexane–EtOAc (1:1.5) as eluate, yielding a lactone mixture. The mixt. was further separated on silica gel eluted by Et_2O–hexane (3:1) to yield two main frs. These frs were then purified by recrystallization using $CHCl_3$–hexane to give compounds **2** (15 mg) and **3** (20 mg).

Fr. 7 eluted with hexane–EtOAc (1:4) was recrystallized from $CHCl_3$–hexane to give pure compound **1** (150 mg).

Deoxyelephantopin (**1**), fine needle crystals from $CHCl_3$–hexane. Mp 196–199°; UV λ_{max}^{MeOH} nm: 207; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1742, 1705, 1645, 1636 and 1160; ^1H NMR (270 MHz, $CDCl_3$): Table 1; ^{13}C NMR (67.80 MHz, $CDCl_3$): Table 2; positive-ion L-SIMS

m/z: 345.1332 $[M+H]^+$ ($C_{19}H_{20}O_6$.H requires 345.1338), 259 $[M+H-CH_2=C(CH_3)\text{-}COOH]^+$.

Isodeoxyelephantopin (**2**), needle crystals from $CHCl_3$–hexane. Mp 158–160°; UV λ_{max}^{EtOH} nm: 206; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1747, 1710, 1635 and 1633; ^1H NMR (270 MHz, $CDCl_3$): Table 1; ^{13}C NMR (67.80 MHz, $CDCl_3$): Table 2; positive-ion L-SIMS *m/z*: 345.1339 $[M+H]^+$ ($C_{19}H_{20}O_6$.H requires 345.1338), 259 $[M+H-CH_2=C(CH_3)\text{-}COOH]^+$.

Scabertopin (**3**), needle crystals from $CHCl_3$–hexane. Mp 138–139°; UV λ_{max}^{EtOH} nm: 210; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1764, 1742, 1705, 1652 and 1635; ^1H NMR (270 MHz, $CDCl_3$): Table 1; ^{13}C NMR (67.80 MHz, $CDCl_3$): Table 2; positive-ion L-SIMS *m/z*: 359.1481 $[M+H]^+$ ($C_{20}H_{22}O_6$.H requires 359.1494), 259 $[M+H-CH_3\text{-}CH=C(CH_3)\text{-}COOH]^+$

X-ray analysis of scabertopin (**3**). Compound **3** crystallized from $CHCl_3$–hexane with half a molecule of $CHCl_3$; $C_{20}H_{22}O_6 \cdot 1/2\ CHCl_3$ $M_r = 418.1$, needle; orthorhombic system; space group $P2_12_12_1$, unit cell dimensions: $a = 7.057$ (1) Å, $b = 12.123$ (1) Å, $c = 27.917$ (1) Å; $V = 2388.4$ (12) Å^3; $Z = 4$; $D_{calc} = 1.163$ g cm^{-1}, μ (MoK_α radiation, $\lambda = 0.71073$ Å) = 0.245 mm^{-1}; $F(000) = 876$, temperature (K) 294; $R = 0.074$, $R_w = 0.085$ for 2796 observed reflections.

A crystal $0.20 \times 0.40 \times 1.0$ mm was oriented on the top of a thin glass fibre to rotate with its long dimension parallel to the fibre axis. The diffraction maxima were collected on a Rigaka RAXIS IIc image plate detector, using graphite-monochromated MoK_α radiation and taking oscillation IP photos: 60 frames in total, $\phi = 0$–180°, $\Delta\phi = 3.0°$ and 10 min per frame. A total of 7887 reflections were collected, of which 2796 with $|F| \geq 6\ \sigma\ (|F|)$ were used for structure determination and refinement. The crystal structure and absolute configuration were solved using the Siemens SHELXTL-PC package. All hydrogen atoms were located satisfactorily. A final weighted anisotropic full-matrix refinement gave $R = 0.074$ and $R_w = 0.085$ with weighting scheme $W = [\sigma^2|F| + 0.0005F^2]^{-1}$, goodness-of-fit $S = 1.46$, largest and mean Δ/σ 0.019 and 0.002, largest difference peak 0.63 e Å^3, largest difference hole −0.39 e Å^3.

Final non-hydrogen atom positional parameters are given in Table 3. A perspective view of the solid-state conformation is shown in Fig. 1. Anisotropic temperature factors, hydrogen atom positional and isotropic thermal parameters, bond lengths and angles of **3** are deposited as supplementary material at the Cambridge Crystallographic Data Centre.

Table 3. Atomic coordinates (×10) for compound **3**.

	x	*y*	*z*
Scabertopin molecule			
C (1)	5862 (3)	4500 (2)	7113 (1)
C (2)	7060 (3)	5319 (2)	6859 (1)
C (3)	9225 (3)	5070 (2)	6903 (1)
C (4)	9662 (2)	4501 (2)	7371 (1)
C (5)	9593 (2)	5038 (2)	7785 (1)
C (6)	9311 (2)	4516 (2)	8261 (1)
C (7)	7274 (2)	4702 (2)	8449 (1)
C (8)	5854 (2)	3867 (2)	8242 (1)
C (9)	4275 (3)	4395 (2)	7936 (2)
C (10)	5142 (2)	4932 (5)	7512 (1)
C (11)	7582 (3)	4778 (2)	8983 (1)
C (12)	9629 (3)	5007 (2)	9055 (1)
C (13)	6394 (3)	4778 (2)	9341 (2)
C (14)	9894 (3)	3248 (2)	7323 (2)
C (15)	5580 (3)	6131 (2)	7493 (2)
C (16)	5851 (3)	2413 (2)	8806 (1)
C (17)	4764 (3)	1875 (2)	9203 (2)
C (18)	2589 (3)	2289 (3)	9262 (2)
C (19)	5490 (3)	1188 (3)	9493 (2)
C (20)	7344 (3)	772 (3)	9476 (2)
O (21)	6684 (2)	6350 (2)	7108 (1)
O (22)	5118 (3)	6855 (2)	7768 (1)
O (23)	10 532 (2)	4982 (2)	8627 (1)
O (24)	10 459 (3)	5196 (2)	9423 (1)
O (25)	4894 (2)	3253 (2)	8613 (1)
O (26)	7494 (3)	2167 (3)	8664 (1)
$CHCl_3$ solvate molecule*			
C (27)	2659 (3)	7500 (3)	9328 (2)
Cl (1)	1431 (3)	8787 (3)	9328 (2)
Cl (2)	4185 (3)	7798 (3)	9788 (2)
Cl (3)	3305 (3)	7219 (2)	8821 (1)
Cl (1′)	116 (3)	7909 (3)	9130 (2)
Cl (2′)	3570 (3)	8399 (3)	9705 (2)
Cl (3′)	3880 (3)	7266 (2)	8739 (1)

* Atom C (27) has a site occupancy factor of 1/2, and atoms Cl (1) to Cl (3′) have the same site occupancy factor of 1/4.

Acknowledgements—Partial support was received with gratitude from the University Grants Committee (Hong Kong) under the 1992–1995 Central Allocation Vote, from the Chinese Medicinal Material Research Centre, Chinese University of Hong Kong, and also from CUHK A/C 242510400. Dr C. Y. Shen (HKUST) is thanked for performing the 2D NMR experiments.

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