

A new sesquiterpene from elephantopus scaber

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Abstract

Elephantopus scaber have the effects of anti-inflammatory and anti-tumor. In the methanolic extract of the aerial part from *E. scaber*, we isolated a new sesquiterpene lactone, scabertopinol (1), and eight known compounds, including *trans*-caffeic acid (2), methyl 3,4-dicaffeoylquinic acid (3), luteolin-4'-*O*- β -D-glucoside (4), *trans-p*-coumaric acid (5), indole-3-carbaldehyde (6), methyl *trans*-caffeate (7), luteolin-7-*O*-glucuronide 6"-methyl ester (8), and luteolin (9). The structure of the new compound was identified by EI-MS, IR, and NMR spectral data. The other eight known compounds were determined by spectral methods and comparison of data reported in the literature.

Key words: *Elephantopus scaber*, sesquiterpene lactone, scabertopinol

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1. Introduction

The whole plant of *Elephantopus scaber* L. (Compositae) has been used in Taiwanese folk medicine for the treatment of pneumonia (Lin et al., 1992), nephritis (Lin et al., 1992), hepatitis (Lin et al., 1995), chronic arthritis (Tsai et al., 1999), tumor (Xu et al., 2006), and diabetes (Daisy et al., 2009). In the previous chemical studies, some sesquiterpene lactones (scabertopin, isoscabertopin, deoxyelephantopin, isodeoxyelephantopin, 11,13-dihydrodeoxyelephantopin, and elescaberin, etc.) have been isolated from *E. scaber* (But et al., 1997; de Silva et al., 1982; Liang et al., 2004 and 2008). Being interested in exploring biologically active components from Chinese herbs, we analysed the methanolic extract of this plant, isolated and characterized a new sesquiterpene lactone compound 1 and eight known compounds 2-9. This paper showed the new compound in its structure. The structures of the known compounds 2-9 were confirmed by comparison of their spectral data with those reported in the literature.

2. Materials and methods

2.1 General methods

Melting points were determined on a Yanaco MP-I3 micro melting point apparatus. Optical rotations were measured using a Jasco DIP-370 polarimeter. EI-MS spectra were taken on a Finnigan MAT GCQ Mass spectrometer (70eV). IR spectra were recorded using a Nicolet Avatar 320 FT-IR spectrophotometer. UV spectra were measured using a Hitachi U-3200

spectrophotometer. The ^1H NMR, ^{13}C NMR, DEPT, and HMBC spectra were obtained on a Varian Unity Inova-500 spectrometer. $\text{DMSO-}d_6$ was used as solvents and TMS as the internal standard. Diaion HP-20 (Mitsubishi Chemical Corporation) and Sephadex LH-20 (Pharmacia, 25-100 μm) were used for the column chromatography. Silical gel column chromatography was performed on Merck silica gel (230-400 mesh).

2.2 Plant material

The aerial part of *E. scaber* was purchased from a Chinese herbal drug store in Taipei, Taiwan and was identified by Professor Jun-Chih Ou.

2.3 Extraction and separation

Air-dried aerial parts of *E. scaber* (20 Kg) were crushed into small pieces and extracted at 50 °C with methanol (140 L \times 3) for 72 hrs. The methanol extract was evaporated to a black residue in vacuo, which was suspended in water (10 L), and separated into water-soluble and water-insoluble portions. The water-soluble portion was separated again on a Diaion HP-20 column eluting with water and methanol, respectively, to give the methanol fraction. The methanol fraction was further chromatographed with a Sephadex LH-20 column, which was eluted with methanol to four fractions (Fr. 1-4). Fr. 2 was repeatedly chromatographed with silica gel and Sephadex LH-20 to get compound 1 (22.5 mg), 2 (16.6 mg), 3 (303.0 mg), 5 (4.5mg), 6 (1.7 mg), 7 (20.4 mg) and 8 (168.0 mg). Fr. 3 was repeatedly chromatographed with silica gel and Sephadex LH-20 to get compound 4 (12.2 mg). Fr. 4 was

chromatographed with silica gel to get compound 9 (16.1 mg).

Scabertopinol (1)

Colorless prisms, mp 246-248 °C; $[\alpha]_D^{25}$ -90 ° (*c* 1.0, MeOH); UV λ_{\max} nm (MeOH) (log ϵ): 204 (4.36), 250 (3.57); IR ν_{\max} (KBr) cm^{-1} : 3479,

1766, 1742, 1457, 1073, 1041; EI-MS *m/z* (rel int): 293 $[M+1]^+$ (20), 274 (51), 178 (100), 163 (85), 150 (50); ^1H NMR (500 MHz, DMSO- d_6) and ^{13}C NMR (125 MHz, DMSO- d_6): see Table 1. The structure of compound 1: see Figure 1.

Table 1. ^1H - and ^{13}C -NMR spectral data for 1 (500 and 125 MHz, DMSO- d_6 , *J* in Hertz and δ in ppm).

Number	δ_{H}	δ_{C}
1	7.21 (s)	155.0
2	5.48 (dd, <i>J</i> = 2.4, 4.2 Hz)	81.2
3	2.54 (dd, <i>J</i> = 4.5, 13.5 Hz) 2.63 (d, <i>J</i> = 13.5 Hz)	40.7
4	-	133.8
5	4.21 (d, <i>J</i> = 10.0 Hz)	131.6
6	5.71 (d, <i>J</i> = 10.0 Hz)	78.7
7	-	168.5
8	4.23 (m)	68.1
8-OH	6.04 (d, <i>J</i> = 4.5 Hz)	-
9	2.77 (dd, <i>J</i> = 2.0, 13.0 Hz) 3.04 (dd, <i>J</i> = 10.0, 13.0 Hz)	36.1
10	-	128.7
11	-	129.5
12	-	173.7
13	4.29 and 4.40 (dd, <i>J</i> = 5.0, 13.5 Hz)	54.4
13-OH	5.16 (t, <i>J</i> = 5.0 Hz)	-
14	1.71 (s)	19.9
15	-	173.0

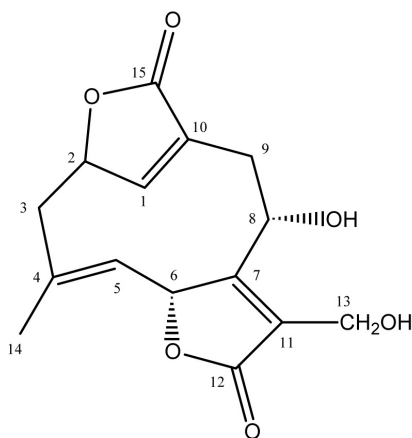


Fig. 1: Structure of compound 1.

3. Results and discussion

The methanolic extract of the dried aerial part of *E. scaber* was concentrated to a residue. The residue was suspended in water to separated into water-soluble and water-insoluble portions. The water-soluble portion was chromatographed on Diaion HP-20, silica gel and Sephadex LH-20 to get nine compounds 1-9. The compounds 2-9 had the same physical and spectral data with those in previous reports. The structures of the known compounds 2-9 have been identified as

trans-caffeic acid (2) (Lin et al., 2002), methyl 3,4-dicaffeoylquininate (3) (Lin et al., 2002), luteolin-4'-*O*- β -D-glucoside (4) (Lin et al., 2002), *trans*-*p*-coumaric acid (5) (Bergman et al., 2001), indole-3-carbaldehyde (6) (Nakajima et al., 2002; Yue et al., 2000), methyl *trans*-caffeate (7) (Silva et al., 2000), luteolin-7-*O*-glucuronide 6"-methyl ester (8) (Makino et al., 1998), and luteolin (9) (Chen et al., 2003) (Figure 2). Compound 1 is a new sesquiterpene lactone, which structure was elucidated as following.

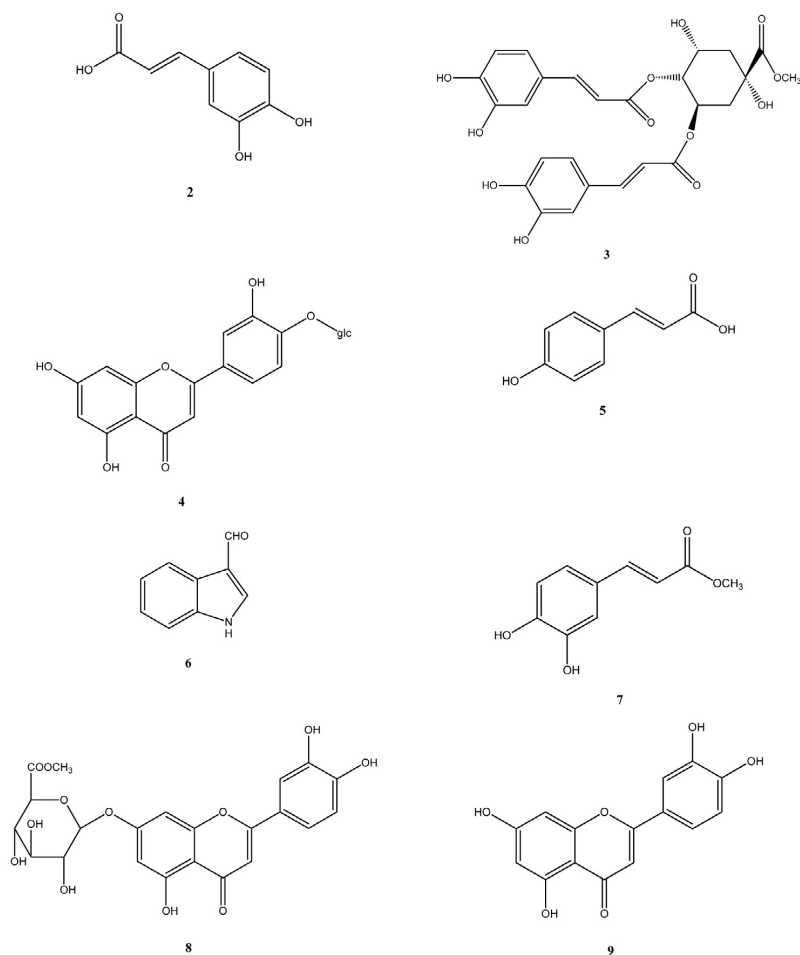


Fig. 2: Structure of compounds 2-9.

Compound 1 was obtained as a colorless prism. The molecular formula of compound 1 was established to be $C_{15}H_{16}O_6$ based on EI-MS and NMR spectra. The base peak was at m/z 178. After investigating mass spectra of compound 1, the fragment ion at m/z 274 is consecutive to a respective loss of H_2O (-18) in the C8-OH and C9-H. To analyze the fragmented ion at m/z 178, "active structure" is shown in Figure 3. The m/z 178 is consecutive to a respective loss of 114.

The IR absorption showed two carbonyl groups of lactone rings at 1766 and 1742 cm^{-1} . The ^{13}C NMR and DEPT spectra exhibited fifteen carbon signals, including two signals for the carbonyl groups at δ 173.0 and 173.7, four quaternary carbons at δ 133.8, 168.5, 128.7 and 129.5, five tertiary carbons at δ 155.0, 81.2, 131.6, 78.7 and 68.1, three secondary carbons at δ 40.7, 36.1 and 54.4, and one methyl group at δ 19.9.

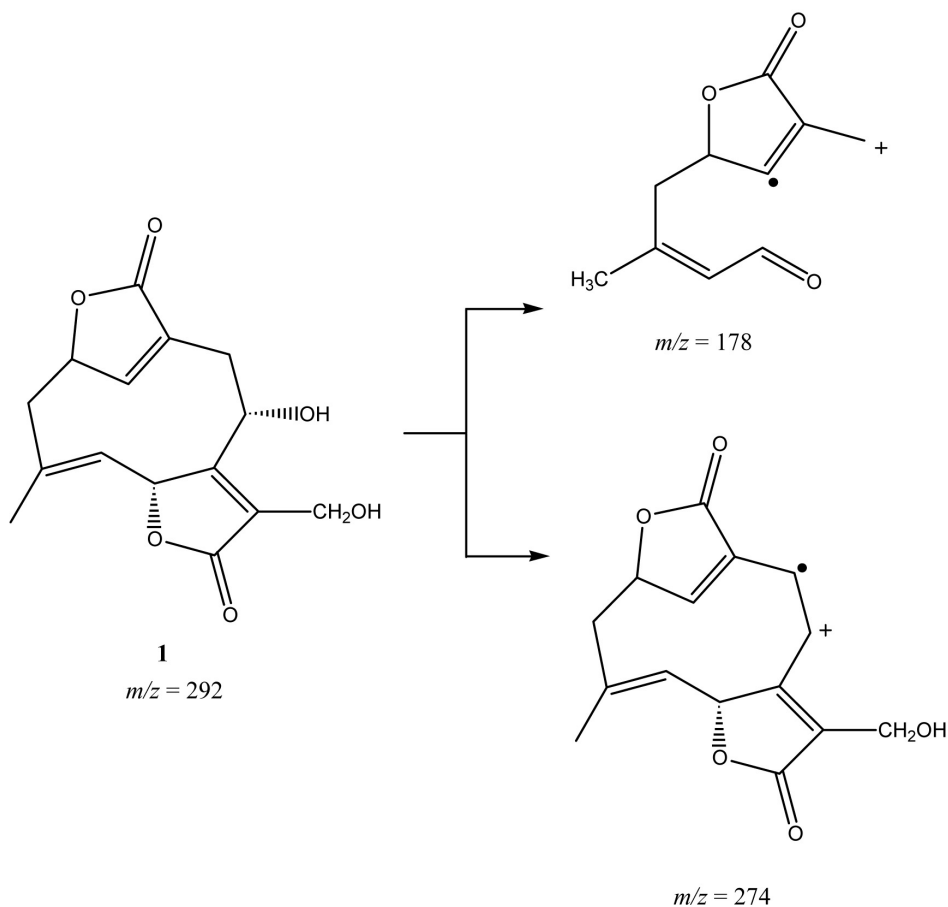


Fig. 3: Proposed EI-MS fragmentation patterns of compound 1.

The IR absorption at 1766 cm⁻¹, a ¹H NMR signal at δ 7.21 (1H, s, H-1), and ¹³C NMR signals at δ 155.0 (C-1), 128.7 (C-10) and 173.0 (C-15) suggested the presence of a α,β-unsaturated lactone in compound 1. The ¹H NMR spectrum revealed a deshielded hydroxymethyl group at δ 4.29 and 4.40 (each dd, *J* = 5.0, 13.5 Hz, H-13), and the ¹³C NMR signal was at δ 54.4 (C-13). The ¹H NMR spectrum of compound 1 revealed two coupled protons at δ 4.21 (d, *J* = 10.0 Hz, H-5) and 5.71 (d, *J* = 10.0 Hz, H-6), and a signal at δ 4.23 (m, H-8) appeared. In the HMBC spectra, proton H-1 correlated with the carbons of C-3, C-9, and C-15, and proton H-5 correlated with the carbons of C-3, C-7, and C-14. Furthermore, proton H-8 correlated with the carbons of C-6, C-10, and C-12, and proton H-13 correlated with the carbons of C-7, C-11, and C-12. Therefore, based on the previous data, compound 1 was determined to be a sesquiterpene lactone, scabertopinol (1).

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燈豎朽之新倍半萜類成分

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摘要

燈豎朽具有抗發炎與抗癌的作用，本研究自燈豎朽地上部的甲醇萃取物分離得到一個新倍半萜內酯環類scabertopinol (1)，以及八個已知化合物，包括*trans*-caffeic acid (2)、methyl 3,4-dicaffeoylquinic acid (3)、luteolin-4'-*O*- β -D-glucoside (4)、*trans-p*-coumaric acid (5)、indole-3-carbaldehyde (6)、methyl *trans*-caffeate (7)、luteolin-7-*O*-glucuronide 6''-methyl ester (8) 與 luteolin (9)。新化合物之結構經由廣泛的EI-MS、IR與NMR光譜的解析而確認，八個已知化合物皆藉由光譜方法與比對文獻中的數據而確定。

關鍵詞：燈豎朽、倍半萜內酯環類、scabertopinol

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