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Tomenphantadenine, an unprecedented germacranolide-adenine hybrid heterodimer from the medicinal plant *Elephantopus tomentosus* L.

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ABSTRACT

An unusual adenine-substituted germacrane sesquiterpene lactone, tomenphantadenine (**1**), has been isolated from the whole plant of *Elephantopus tomentosus* L. The structure of this compound was established by comprehensive spectroscopic analysis including high resolution (HR) ESI-MS, 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data. This compound features novel hybrid pattern of germacrane sesquiterpene with adenine through C-N linkage, and a possible biosynthetic pathway for it was proposed. Compound **1** showed potent antibacterial activity against the gram-positive *Staphylococcus aureus* and weak acetylcholinesterase (AChE) inhibitory activity.

1. Introduction

Species of the genus *Elephantopus* (Asteraceae), mainly distributed in the regions of America, are sources of abundant sesquiterpene lactones with significant antileishmanial and cytotoxic activity [1–3]. In China, there are two species including *Elephantopus scaber* L. and *Elephantopus tomentosus* L. Only *Elephantopus tomentosus* have long been used as a folk traditional medicine in south of China and for the treatment of various diseases such as fever, bronchitis, hepatitis, and the cough associated with pneumonia, and arthralgia [4]. As part of a program to investigate the chemical and biological diversity of the ethnic Li folk medicinal plants in Hainan Province [5–9], we phytochemically investigated the ethanol extracts of the medicinal plant *Elephantopus tomentosus*. From the EtOAc portion of the ethanol extract of the whole plants, six triterpenes with antibacterial activity and five new sesquiterpene lactones tomenphantopin C–F, H with antibacterial and cytotoxic activities have been isolated from this species for the first time [10–13]. Subsequent investigation on the fractions of the EtOAc portion by ^{1}H NMR and LC-MS revealed the presence of a sesquiterpene derivative with $[M + H]^+$ ion at m/z 500, which did not match the previously isolated compounds, indicative of a new germacranolide derivative. Eventually it was purified, identified and designated as tomenphantadenine (**1**) (Fig. 1). To our knowledge, this is the first example of germacranolide derivatives coupled with adenine. Herein, we report details of the isolation, structure determination, and hypothetical biosynthetic pathway of this novel compound, as well as its antibacterial and AChE inhibitory activity.

2. Results and discussion

Tomenphantadenine (**1**) was obtained as a white powder. The molecular formula of **1** was found to be $C_{24}H_{29}N_5O_7$ derived from the HR-ESI-MS, giving an $[M + H]^+$ ion at m/z 500.2141 (calculated 500.2140) and an $[M - H]^-$ ion at 498.2002 (calculated 498.1994). The IR spectrum displayed the presence of hydroxyl (3450 cm^{-1}) and carbonyl (1773, 1721 cm^{-1}). The complete assignments of all proton and carbon resonances, as shown in Table 1, were deduced by comprehensive analysis of various 2D experiments including HSQC, ^{1}H-^{1}H COSY, HMBC, and ROESY, confirmed the structure of **1** as shown in Fig. 1. The ^{1}H NMR spectrum of **1** (acquired in DMSO-d_6) showed signals of two methyls [δ_H 1.54 (3H, s, H-14) and 1.86 (3H, s, H-3″)], four olefinic protons [δ_H 5.13 (2H, m, H-15), 5.90 (1H, br s, H$_\alpha$-4″), and 5.55 (1H, br s, H$_\beta$-4″)], two aromatic protons [δ_H 8.00 (1H, s, H-2′) and 7.84 (1H, s, H-8′)], four methylenes [δ_H 2.44 (1H, d, J = 13.5 Hz, H$_\alpha$-1) and 2.29 (1H, d, J = 13.5 Hz, H$_\beta$-1); δ_H 2.57 (1H, d, J = 13.5 Hz, H$_\alpha$-3) and 1.80 (1H, d, J = 13.5 Hz, H$_\beta$-3); δ_H 2.57 (2H, m, overlap, H-9), and 4.37 (2H, br d, J = 6.5 Hz, H-13)], two aliphatic methines [δ_H 3.22 (2H, m, overlap, H-7 and H-11)], three oxygenated methines [δ_H 4.07

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(1H, d, J = 6.5 Hz, H-5), 4.30 (1H, dd, J = 6.5, 3.6 Hz, H-6), and 5.20 (1H, m, H-8)], two hydroxyl groups [δ_H 6.05 (1H, br s, 2-OH) and 5.31 (1H, br s, 4-OH)], and one NH_2 group [δ_H 7.15 (2H, br s, 6-NH_2)]. The ^{13}C NMR and DEPT135 spectra of **1** (in DMSO-d_6) exhibited twenty four carbons resonances, including two methyls [δ_C 31.9 (q, C-14) and 18.2 (q, C-3″)], two olefinic methylene carbons [δ_C 119.8 (t, C-15) and 125.7 (t, C-4″)], two olefinic methine carbons [δ_C 152.1 (d, C-2′), 141.4 (d, C-8′)], and five non-protonated olefinic carbons [149.6 (s, C-4′), 118.8 (s, C-5′), 155.9 (s, C-6′), 135.8 (s, C-2″), and 141.1 (s, C-10)], four aliphatic methylene carbons [δ_C 50.9 (t, C-1), 49.1 (t, C-3), 33.9 (t, C-9), and 44.2 (t, C-13)], two aliphatic methine carbons [δ_C 43.4 (d, C-11) and 40.1 (d, C-7)], and seven oxygenated carbons [δ_C 105.9 (s, C-2), 77.6 (s, C-4), 83.9 (d, C-5), 79.3 (d, C-6), 76.9 (d, C-8), 175.4 (s, C-12) and 165.9 (s, C-1″)]. Comprehensive interpretation of ^1H-^1H COSY correlations from H-6 to H-5 and H-7, from H-7 to H-6, H-8, and H-11, from H-8 to H-7 and H_2-9, and from H-11 to H-7 and H_2-13 revealed the partial linkage of C-5-C-6-C-7(-C-11-C-13)-C-8-C-9. HMBC correlations from H_2-1 and H_2-9 to C-10 and C-15, and from H_2-1 to C-9 assigned the connectivity of C-1-C-10(-C-15)-C-9, while the HMBC correlations from H_3-14 to C-3, C-4, and C-5, from H_2-3 to C-1 and C-2 revealed the fragment of C-1-C-2-C-3-C-4(-C-14)-C-5, which was also supported by the HMBC correlations from OH-2 to C-2 and C-3 and from 4-OH to C-3, C-4 and C-5. The lactone fragment was deduced from the HMBC correlations from H-7 and H_2-13 to C-12. Therefore the germacrane sesquiterpene skeleton was determined. The position of the methacrylate ester side chain was suggested by the analysis of HMBC correlations from H-3″ and H-4″ to C-1″ and C-2″, from H-3″ to C-4″, and from H-4″ to C-3″. The key HMBC correlations from H_2-13 to C-4′ and C-8′ indicated that C-13 was linked to the N-9′ on the adenine, which was also secured by the observation of NOE correlations of H-8′ with H_2-13 and H-11. The relative configuration of **1** was determined by ROESY and NOESY experiments in DMSO-d_6 and $CDCl_3$ + methanol-d_4, respectively. Both of the spectra displayed correlations of H-8 with H-6 and H-11, of H-5 with H-6 and H_3-14, and of H_3-14 with OH-2, demonstrating that OH-2, H_3-14, H-5, H-6, H-8 and H-11 were on the same plane, while the NOE correlation of H-7 with H_2-13 proved them in the opposite plane. Thus the structure of compound **1** was assigned as shown in Fig. 2 and named as tomenphantadenine, representing a new type of germacrane sesquiterpene lactone coupled with adenine through C-N linkage in the germacranolide family.

A plausible biosynthetic pathway for **1** is proposed in Scheme 1. The germacrane skeleton is formed from farnesyl diphosphate (FPP) and undergoes a series of cyclization, oxidation and esterification reactions to an known intermediate tomenphantopin D (**4**) [13], then two hydrogens could be transferred by a dehydrogenase, using FAD (flavin adenine dinucleotide) as an electron acceptor, to form a terminal double bond in the lactonic ring of a proposed intermediate **5**. The construction of the unprecedented hybrid **1** involves the initial step of hydrogen atom abstraction from the 9-NH of a primary metabolite adenine by a key catalytic basic amino acid (here we showed a histidine

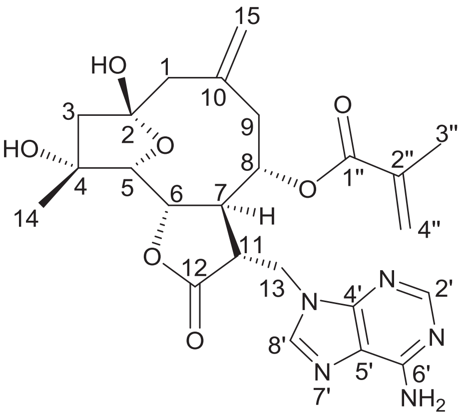

Fig. 1. Structure of tomenphantadenine (**1**).

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for tomenphantadenine (**1**).

Position	δ_H[a] (mult, J in Hz)	δ_C[a]	δ_H[b] (mult, J in Hz)	δ_C[b]
1	2.44, d (13.5, H_α); 2.29, d (13.5, H_β)	50.9, CH_2	2.51, d (13.5, H_α); 2.41, d (13.5, H_β)	51.1
2		105.9, C		106.7
2-OH	6.05, br s			
3	2.57, d (13.5, H_α); 1.80, d (13.5, H_β)	49.1, CH_2	2.51, d (13.7, H_α); 1.85, d (13.7, H_β)	48.9
4		77.6, C		78.5
4-OH	5.31, br s			
5	4.07, d (6.5)	83.9, CH	4.24, d (3.5)	84.6
6	4.30, dd (6.5, 3.6)	79.3, CH	4.28, dd (7.0, 3.5)	80.8
7	3.22, m (overlap)	40.1, CH	3.34, m (overlap)	40.4
8	5.20, m	76.9, CH	5.23, dt (11.2, 3.0)	78.4
9	2.57, m (overlap)	33.9, CH_2	2.71, dd (16.0, 3.0, H_α); 2.65, d (16.0, H_β)	34.9
10		141.1, C		140.7
11	3.22, m (overlap)	43.4, CH	3.20, m	45.5
12		175.4, C		176.9
13	4.37, br d (6.5)	44.2, CH_2	4.56, m	44.3
14	1.54, s	31.9, CH_3	1.62, s	31.6
15	5.13, m	119.8, CH_2	5.40, s-like; 5.19, br s	121.4
2′	8.00, s	152.1, CH	8.15, s	152.8
4′		149.6, C		150.6
5′		118.8, C		119.0
6′		155.9, C		155.8
6-NH_2	7.15, br s			
8′	7.84, s	141.4, CH	7.90, s	142.5
1″		165.9, C		167.4
2″		135.8, C		136.7
3″	1.86, s	18.2, CH_3	2.02, s	18.7
4″	5.90, br s; 5.55, br s	125.7, CH_2	6.29, s-like; 5.67, t-like (1.4)	127.0

[a] Acquired in DMSO-d_6.
[b] Acquired in $CDCl_3$ + methanol-d_4 (v/v, 1:1).

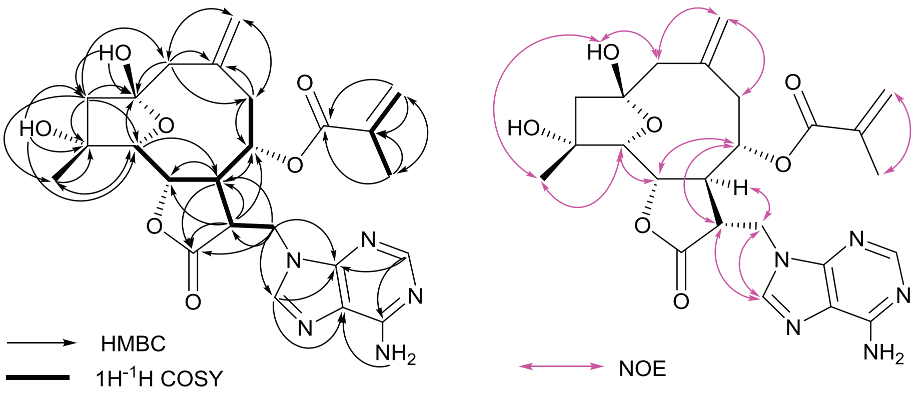

Fig. 2. The key ^1H-^1H COSY, HMBC and NOESY correlations supporting the structure of tomenphantadenine (**1**).