SHORT REPORT

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Scoparic acid E: A New Labdane Diterpenoid on Attenuating Palmitate Induced Viability in MIN6 Cells from *Scoparia dulcis*

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Abstract: A new labdane diterpenoid, named Scoparic acid E (**1**), was obtained from the medicinal plant of *Scoparia dulcis* L. The structure of Scoparic acid E (**1**) was elucidated and characterized through detailed physical analyses of its NMR spectroscopic data and HRMS. Scoparic acid E (**1**) was tested for its effect on attenuating palmitate-induced viability at 25 and 50 μM. The results showed that compound **1** significantly attenuated palmitate-induced viability in MIN6 cells.

Keywords: *Scoparia dulcis* L.; scoparic acid E; labdane diterpenoid; palmitate-induced viability.

1. Plant Source

The fresh plant of *Scoparia dulcis* L. was obtained from Wuzhi Mountain of Hainan Island, China, in July 2018. This medicinal plant was authenticated by Dr. Yuguang Fan, and a voucher specimen has been deposited with a documented number of No.SD201807.

2. Previous Studies

S. dulcis, belonging to the Scrophulariaceae family, is native to Hainan Island and abound in natural resources. It is commonly used as alternatives for the treatment of many illnesses [1,2]. Pharmacological investigations indicated that the extract of *S. dulcis* possessed anti-diabetic, anti-inflammatory, antioxidant, anti-tumour, and other properties [3,4]. The previous investigations showed that *S. dulcis* contains diterpenoids such as Scoparic acids A-D, alkaloids, flavonoids, and other chemical constituents [5-7].

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3. Present Study

In present study, we investigate the chemical constituents of *Scoparia dulcis* L. By this process, a new labdane diterpenoid denoted scoparic acid E (**1**) was obtained and structurally elucidated. Its biological activity on attenuating palmitate-induced viability in MIN6 cells was also reported herein.

Dried *S. dulcis* (10.0 kg) was cut into pieces and extracted by a mixture of ethanol-water (8:2) for three times to give an extract (1200 g). The extract was dissolved in H_2O and partitioned by petroleum for three times to remove the hydrophobic constituents. The mother-liquid was then partitioned by CH_2Cl_2 for three times. The CH_2Cl_2 solution was concentrated to produce the CH_2Cl_2 extract. The CH_2Cl_2 extract was coarsely isolated and purified. A gradient elution of petroleum-dichloromethane-acetone was applied to elute the chemical constitutes in the silica gel column, which gave six fractions (Fra.1-Fra.6). Fra.3 was further purified by the same step as above using petroleum-dichloromethane-acetone (5:1:1) as the eluent to afford six fractions (Subfra.1-Subfra.6). Subfra.4 (1.0g) was successively separated by a column Sephadex LH-20 and finally isolated by HPLC using a mixture of methanol-water (60:40) to afford **1** (10.0 mg). The retention time of compound **1** is 53.5 min under the above HPLC conditions.

*Scoparic acid E (**1**)*: White powder, $[\alpha]^{25}_D$ = -12 (c 0.05), UV (MeOH) λ_{max} (log ε): 228 (3.80), 276 (2.34) nm. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) = 1.74 (1H, m, H-1α), 1.20 (1H, brd, *J* = 13.2 Hz, H-1β), 1.78 (1H, m, H-2β), 1.61 (1H, m, H-2α), 1.72 (2H, m, H-3), 2.65 (1H, brs, H-5), 5.47 (1H, brs, H-6), 2.60 (1H, brd, *J* = 13.2 Hz, H-7α), 2.48 (1H, brd, *J* = 13.2 Hz, H-7β), 1.93 (1H, brs, H-9), 1.97 (1H, m, H-11a), 1.68 (1H, m, H-11b), 2.22 (1H, t, *J* = 13.2 Hz, H-12a), 2.10 (1H, m, H-12b), 5.67 (1H, s, H-16a), 6.26 (1H, s, H-16b), 4.76 (2H, H-17), 1.36 (3H, s, H-19), 1.50 (3H, s, H-20), 8.04 (2H, d, *J* =8.4 Hz, H-2', 6'), 7.56 (1H, t, *J* =8.4 Hz, H-4'), 7.46 (2H, t, *J* = 8.4 Hz, H-3', 5'). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) = 37.8 (C-1), 18.2 (C-2), 40.0 (C-3), 47.0 (C-4), 42.3 (C-5), 74.0 (C-6), 37.0 (C-7), 144.4 (C-8), 57.4 (C-9), 38.6 (C-10), 26.8 (C-11), 32.4 (C-12), 140.7 (C-13), 172.9 (C-14), 127.6 (C-16), 113.3 (C-17), 184.7 (C-18), 19.2 (C-19), 25.6 (C-20), 129.6 (C-1'), 130.7 (C-2', 6'), 128.4 (C-3', 5'), 132.8 (C-4'), 166.2 (C-7'). HRESIMS: *m/z* 439.2130 ([M - H]$^-$, calcd. $C_{26}H_{31}O_6$ for 439.2121).

Bioactivity Test-Cell Viability Assay: The testing method was applied as previous report [10]. MIN6 cells were incubated with the absence of 25 and 50 μM of Scoparic acid E and then the cell viability was measured.

The dried *S. dulcis* plant was cut into small pieces and extracted under reflux. The solvent was quickly evaporated to produce an extract, which was processed by a series of isolation procedures to afford Scoparic acid E (Figure 1).

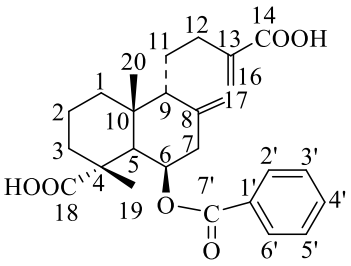

Figure 1. Structure of Scoparic acid E **(1)** isolated from *S. dulcis*

Compound **1,** scoparic acid E, has a molecular formula $C_{26}H_{32}O_6$ as established by HRESIMS at *m*/*z* 439.2130 [M - H]$^-$ (calcd. for 439.2121). The ^{1}H NMR spectral data showed resonances for two methyls [δ_H 1.36 (s, H-19), 1.50 (s, H-20)], a set of benzoyl protons [δ_H 8.04 (d, *J* =8.4 Hz, H-2', 6'),

7.56 (t, *J* =8.4 Hz, H-4'), 7.46 (t, *J* = 8.4 Hz, H-3', 5')], two exocyclic methylenes [δ_H 4.76 (H-17), 5.67 (H-16a), 6.26 (H-16b)], six methylenes [δ_H 1.74 (m, H-1α), 1.20 (brd, *J* = 13.2 Hz, H-1β), 1.78 (m, H-2β), 1.61 (m, H-2α), 1.72 (2H, m, H-3), 2.60 (brd, *J* = 13.2 Hz, H-7α), 2.48 (1H, brd, *J* = 13.2 Hz, H-7β), 1.97 (m, H-11a), 1.68 (m, H-11b), 2.22 (t, *J* = 13.2 Hz, H-12a), 2.10 (m, H-12b)], three methines [δ_H 2.65 (brs, H-5), 5.47 (brs, H-6), 1.93 (brs, H-9)]. The ^{13}C NMR spectrum, associated with HSQC experiments, resolved 26 carbon resonances attributable to one benzoyl group [129.6 (C-1'), 130.7 (C-2', 6'), 128.4 (C-3', 5'), 132.8 (C-4'), 166.2 (C-7')], two methyls [δ_C 19.2 (C-19), 25.6 (C-20)], six methylenes [δ_C 37.8 (C-1), 18.2 (C-2), 40.0 (C-3), 37.0 (C-7), 26.8 (C-11), 32.4 (C-12)], three methines [δ_C 42.3 (C-5), 74.0 (C-6), 57.4 (C-9)], two tertiary carbons [δ_C 47.0 (C-4), 38.6 (C-10)], two carboxyl groups [δ_C 172.9 (C-14), 187.4 (C-18)], and two double bonds [δ_C 146.7 (C-8), 114.3 (C-17), 140.7 (C-13), 127.6 (C-16)].

The observed ^{1}H-^{1}H COSY spectroscopic data indicated the three units of C_1-C_2-C_3, and C_5-C_6-C_7, and C_9-C_{11}-C_{12} as depicted in Figure 2. The HMBC spectrum of **1** exhibited correlations from δ_H 1.75 (H-3) to signals at δ_C 47.0 (C-4), δ_C 42.3 (C-5), and correlations from δ_H 1.36 (H-19) to carbons of C-4, C-5 and C-18 (δ_C 187.4) allowed the elucidation of connections from C-1 to C-5. HMBC connections of the methyl resonance at δ_H 1.50 (H-20) to carbons at δ_C 37.8 (C-1), 38.6 (C-10), 42.3 (C-5), and correlations from proton at δ_H 1.93 (H-9) to δ_C 146.7 (C-8), 38.6 (C-10), 26.8 (C-11), 32.4 (C-12) allowed the establishment of the carbon skeleton of **1** [8]. The HMBC connections from δ_H 2.22, 2.10 (H-12) and δ_H 6.26, 5.67 (H-16) to the carboxyl group of δ_C 172.9 (C-14) and correlation from δ_H 5.47 (H-6) to δ_C 129.6 (C-1') showed **1** have a same skeleton with Scoparic acid C, except for the aldehyde group of C-14 which changed into the carboxyl group in **1** [9].

The relative configurations of compound **1** was diagnosed according to ROESY correlations as the similar compounds [9]. H-20 (δ_H 1.50), H-19 (δ_H 1.36), and H-9 (δ_H 1.93) were determined to be β orientated, and H-5 (δ_H 2.65), H-6 (δ_H 5.47) were arranged in α-orientation. Thus, compound **1** was established with a given name Scoparic acid E as depicted in Figure 1.

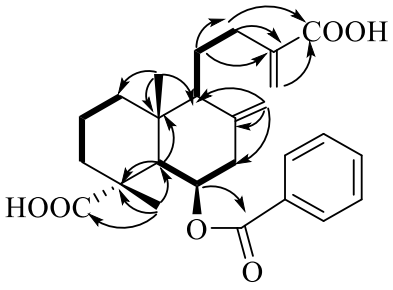

Figure 2. Key ^{1}H-^{1}H-COSY (bold) and HMBC (arrow) connections for Scoparic acid E (**1**)

Scoparic acid E was tested for its effect on attenuating palmitate-induced viability in MIN6 cells. The result showed that compound **1** significantly attenuated the viability by palmitate-induced decrease in MIN6 cells as shown in Figure 3.

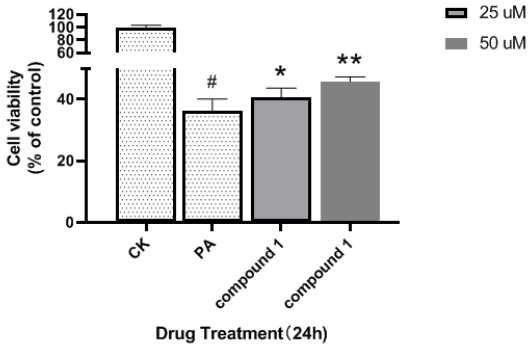

Figure 3. Cell viability treated by Scoparic acid E (**1**) in MIN6 cells. # p<0.01 compared with control, *p<0.05 compared with PA, **p<0.01 compared with PA.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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