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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Cytotoxic lipid esters from *Peucedanum ledebourielloides*

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Abstract Two cancer cell growth inhibitory esters, 1,2-dipalmitoyl-3-glucosyl glycerol (**1**) and 1,6-dihydroxy-hexane-bis-palmitoyl ester (**2**), together with arachidic acid-2-hydroxy-glycerol ester, daucosterol, and oleanolic acid, were isolated from the roots of *Peucedanum ledebourielloides* (Apiaceae family). The structures were determined by spectroscopic analyses. The esters **1** and **2** displayed significant activity against the SGC-7901, HT-29, and HL-60 cancer cell lines.

Keywords Apiaceae family · *Peucedanum ledebourielloides* · Ester · Anticancer

Introduction

Terrestrial plants continue to be a well-established source of medically useful drugs, and the currently important anticancer drugs provide ample illustration (Newman

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et al., 2007; Jiangsu 1991). In the present study, we investigated *Peucedanum ledebourielloides* (Apiaceae family) as a potential source of cancer cell growth inhitbitors. *P. ledebourielloides* is a shrub that grows in Huaying County (Shaanxi Province), People's Republic of China. The root of this plant is a well-known traditional medicine in China and has essentially replaced *Saposhnikovia divaricata* to treat arthritis pain as well as other uses (Cragg *et al*., 2005). Various compounds, including coumarins, flavones, lignans, and volatile oils, have been isolated from many species of *Peucedanum* (Cisowski 1983; Duh *et al*., 1991; Kong *et al*., 2003; Li *et al*. 2003; Rao *et al*., 1997; Zhang *et al*., 2003). Some have been reported to show anticancer activity (Duh *et al*., 1991; Schillaci *et al*., 2003; Zhang *et al*., 2003). However, the chemical constituents of *P. ledebourielloides* have not been previously investigated.

Continuation of our studies concerning new sources of naturally occurring antineoplastic agents (Pettit *et al*., 2007; Zheng *et al*., 2003a, 2003b), two cancer cell growth inhibitors, 1,2-dipalmitoyl-3-glucosyl glycerol (**1**), and 1,6-dihydroxy-hexane-bis-palmitoyl ester (**2**), as well as arachidic acid-2-hydroxy-glycerol ester, daucosterol, and oleanolic acid, were isolated from *Peucedanum ledebourielloides.* Esters from many higher plants exhibit a wide variety of biological activities, including antineoplastic (Boeryd *et al*., 1986; Graca *et al*., 2002). We report the isolation, structure, and cancer cell growth inhibitory activities of two esters, 1,2-dipalmitoyl-3-glucosyl glycerol (**1**) and 1,6-dihydroxy-hexane-bis-palmitoyl ester (**2**).

Materials and methods

General

Melting points were measured using a Kofler-type melting point apparatus and are uncorrected. IR spectra were recorded with a PE-577 spectrometer (in cm^{-1}; KBr pellets). The ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker Am-400 unit with TMS as an internal standard (chemical shifts (δ) in ppm). The mass spectra were recorded by using a VG Austospec-3000 spectrometer; in *m/z* (rel. int. in % of the base peak). Column chromatography (CC) was performed with silica gel (200-300 mesh, Qingdao Marine Chemical Co., China). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Plant material

The roots of *P. ledebourielloides* were collected in the Huaying County of Shaanxi Province, People's Republic of China, and were identified by Prof. L. R. Xu. A voucher specimen was deposited at the Xibei Institute of Botany (No. K. T. Fu 17188).

Extraction and isolation

The dry powdered roots of *P. ledebourilloide* (1 kg) were extracted with MeOH (3 × 4 L) at room temperature for a week. Removal of the organic solvent under

reduced pressure provided a combined MeOH extract (58 g), which was subjected to separation by column chromatography (2 kg silica gel, 5 × 100 cm) using gradient elution (10–60%) with a n-hexane-ethyl acetate. Five major fractions (I–V) were obtained on the basis of TLC monitoring of the individual fractions. Fraction III (0.4 g) was further separated by column chromatography over silica gel (4 × 75 cm, 300 g) eluted with n-hexane-ethyl acetate [2:98 (4 L), 5:95 (4 L), 10:90 (5 L), 15:85 (5 L), V:V] to obtain four subfractions (*Fr.* 3.1, *Fr.* 3.2, *Fr.* 3.3, *Fr.* 3.4). *Fr.* 3.2 was further purified by repeated column chromatography over silica gel (4 × 75 cm, 300 g) and elution with n-hexane-ethyl acetate [2:98 (4 L), 5:95 (4 L), 10:90 (4 L), V:V] to yield diester **2** (18 mg), arachidic acid-2-hydroxy-glycerol ester (12 mg), daucosterol (17 mg), and oleanolic acid (13 mg). *Fr.* 3.4 provided glycerine ester **1** (22 mg) following purification chromatography over silica gel (3 × 45 cm, 160 g) and with n-hexane-ethyl acetate [10:90 (5 L), V:V].

Identification of compounds

Glucoside **1** was obtained as amorphous colorless powder that gave an $[M\text{-}H]^+$ ion in the HRFABMS at m/z 729.5510, corresponding to molecular formula $C_{41}H_{78}O_{10}$. The IR absorption at 3100–3700 cm^{-1} and 1735 cm^{-1} indicated the presence of one or more hydroxyl groups and ester carboxyls. The ^{13}C-NMR and DEPT spectral data suggested two long-chain aliphatic ester groups corresponding to δ173.6 (s), 173.9 (s), 14.3 (q), 23.0 (t), 25.3 (t), 29.9 (t, strong), 32.2 (t), and 34.4 (t). Furthermore, the height of every peak between 14–35 ppm was nearly twice that of the others, which served to confirm the presence of two long-chain esters. The existence of 1-β-D-glucoside also was confirmed by ^{13}C- and DEPT spectra signals at δ 100.4(d), 75.2(d), 75.1(d), 69.8(d), and 63.6(t). Those assignments left only three unaccounted for signals: δ 66.7(t), 71.1(d), and 55(t) generated by a glycerol ester. Thus, the structure of glycerol ester **1** was deduced to be 1,2-dipalmitoyl-3-glucosyl glycerol and further suggested by results of EIMS spectroscopy (Fig. 1).

Diester **2** also was obtained as a colorless powder, which yielded a molecular ion in its HRFABMS spectra at $[M\text{- }H]^+$ at *m/z* 593.5583, pointing to molecular formula $C_{38}H_{74}O_4$. The ^{1}H-NMR spectrum of **2** showed one methyl at δ 0.86 (t, $J = 6.8$ Hz), a methane bonded to a oxygen at δ 4.03 (t, $J = 6.8$ Hz), another methane connected to a carbonyl at δ 2.26 (t, $J = 6.8$ Hz), and a number of methane signals at 1–1.96 on the ^{13}C-NMR spectrum of diester **2** indicated long aliphatic ester chains. Further analysis of the NMR data and molecular formula gave results consistent with a symmetrical compound. The prominent palmityl mass fragment in the EIMS spectrum completed the spectral data necessary to assign the new diester **2** as 1,6- dihydroxy-hexane-bis-palmityl ester (Fig. 2).

Arachidic acid-2-hydroxy-glycerol ester, daucosterol, and oleanolic acid were identified by agreement between their spectroscopic data and that published in the literature (Giron *et al.*, 1992; Rawat *et al.*, 1988; Wandji *et al.*, 2003).

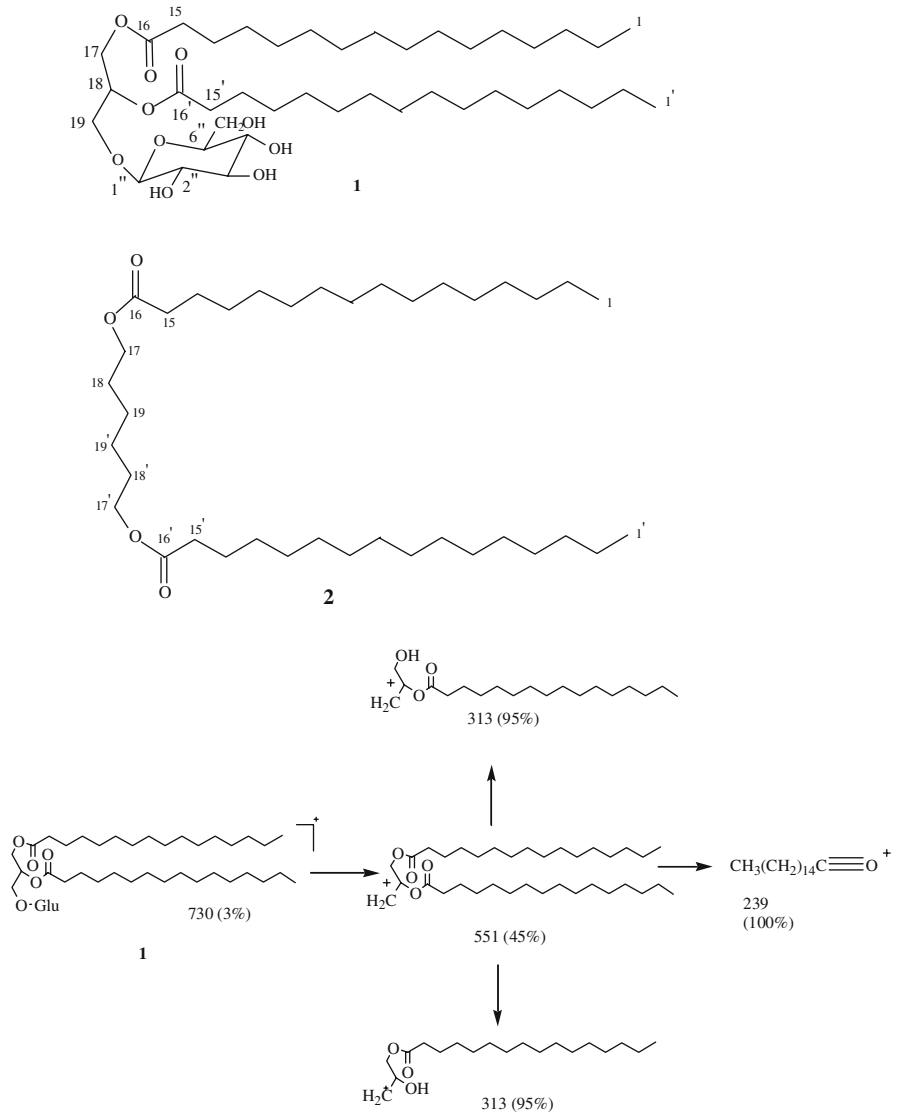
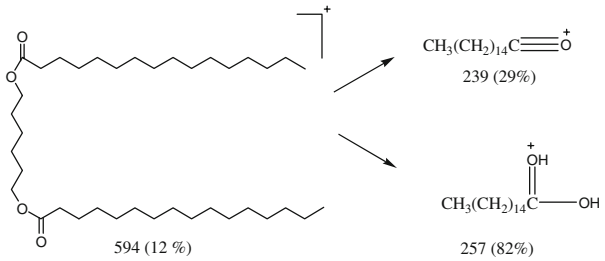


Fig. 1 Mass special fragmentation of glucoside **1**

Results and discussion

The active compounds were identified as 1,2-dipalmitoyl-3-glucosyl glycerol and 1,6-dihydroxy-hexane-bis-palmitoyl ester. The structures of the compounds are shown in Figs. 1 and 2.



2

Fig. 2 Diester **2** mass special fragmentation

Table 1 Human cancer cell line growth inhibition values (IC_{50} μg/ml) for 1,2-dipalmitoyl-3-glucosyl glycerol (**1**) and 1,6-dihydroxy-hexane-bis-palmitoyl ester (**2**)[a]

Cancer cell lines[b]			
	SGC-7901	HT-29	HL-60
Compound			
1	4.9	0.21	27.7
2	1.6	34.2	0.49
5-FU[c]	0.58	10.26	25.4

[a] In DMSO

[b] Cancer type: SGC-7901(gastric carcinoma), HT-29 (colon), HL-60 (promyelocytic leukemia)

[c] 5-fluorouracil (99%) from Sigma Aldrich Co., USA as positive control

1,2-Dipalmitoyl-3-glucosyl glycerol (**1**)

Colorless powder amorphous, mp 203–206°C; IR ν_{max} (log ε) 3700–3100, 2920, 2860, 1735, 1455, 1060, and 1040 cm^{-1}; ^{1}H-NMR (C_5D_5N, 400 MHz) δ 4.73 (d, $J = 7.6$ Hz, H-1″), 3.41 (m, H-2″), 3.44 (m, H-3″), 3.39 (m, H-4″), 3.43 (m, H-5″), 3.69 (m, H-6″). ^{13}C-NMR (C_5D_5N, 100 MHz) δ 173.9 (C-16), 173.6 (C-16′), 100.4 (C-1″), 75.2 (C-3″), 75.1 (C-2″), 71.1 (C-18), 69.8 (C-4″), 66.7 (C-17), 63.6 (C-6″), 55.0 (C-19), 32.2 (C-15′), 23.0 (C-2 and C-2′), 14.3 (C-1 and C-1′), 25.1, 29.9 (C-3-C-14 and C-3′-C-14′); and EIMS *m/z* (%): 730 (3), 551 (45), 311 (95), 239 (100).

Diester **2**

Colorless powder (amorphous), mp 65-68°C. ^{1}H-NMR (C_5D_5N, 400 MHz) δ 4.03 (4H, t, $J = 6.8$ Hz, H-17 and H-17′), 2.26 (4H, t, $J = 6.8$ Hz, H-15 and H-15′), 0.86 (6H, t, $J = 6.8$ Hz, H-1 and H-1′); ^{13}C-NMR (C_5D_5N, 100 MHz) δ 173.9 (C-16 and C-16′), 64.4 (C-17 and C-17′), 34.5 (C-15 and C-15′), 22.7 (C-2 and C-2′), 14.1 (C-1 and C-1′), 25.1, 29.3 (C3-C14 and C-3′-C14′); EIMS *m/z* (%): 594 (12), 257 (82), 239 (29).

Cytotoxicity assay

Aliphatic ester **1** and **2** were evaluated for in vitro anticancer activity against the SGC-7901, HT-29, and HL-60 cell lines by MTT-based assay. These experiments were performed in 96-well plates essentially as described by Mosmann (1983). The IC_{50} concentrations listed represent the concentration that results in a 50% decrease in cell growth during 6-day incubation. Both 1,2-dipalmitoyl-3-glucosyl glycerol (**1**) and 1,6-dihydroxy-hexane-bis-palmitoyl ester (**2**) exhibited significant cancer cell growth inhibitory activities against human gastric carcinoma SGC-7901, human colon cancer HT-29, and human promyelocytic leukemia HL-60 cell lines (Table 1).

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