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Hydroquinone and terpene glucosides from *Leontopodium leontopodioides* and their lipase inhibitory activity

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ABSTRACT

Three new glucosides of hydroquinone, monoterpene, and megastigmane, benzyl 2,5-dihydroxybenzoate 5-*O*-β-D-glucopyranoside (isotrichocarpin, **1**), (2*S*,3*R*)-3,7-dimethyl-6-octene-1,2,3-triol 2-*O*-β-D-glucopyranoside (leontopodioside D, **4**), and (6*R*,7*R*,8*R*,9*S*)-6,9-epoxy-7,8-dihydroxymegastigman-4-en-3-one 8-*O*-β-D-glucopyranoside (leontopodioside E, **7**) were isolated from the whole herbs of *Leontopodium leontopodioides* (Willd.) Beauv. (Asteraceae), along with nebrodenside A (**2**), pungenin (**3**), betulalbuside A (**5**), geranyl *O*-β-D-glucopyranoside (**6**), and 3β-hydroxy-β-ionone 3-*O*-β-D-glucopyranoside (**8**). Their structure were determined by spectroscopic and chemical methods. All the known compounds were reported from this species for the first time. Compounds **2**–**6** showed potent *in vitro* pancreatic lipase inhibitory activity, suggesting their participation in the reductive effect of the herbs on triglyceride absorption.

1. Introduction

Leontopodium leontopodioides (Willd.) Beauv. (Asteraceae) is perennial herbs growing at the altitudes of 100–3800 m in northwest and northeast regions of China, Mongolia, Russia, Korea, and Japan [1]. The herbs have been used in Chinese medicinal formulae to treat influenza, acute or chronic nephritis, urinary tract infection, hematuria, and trauma hemorrhage [2]. Previous pharmacological studies revealed that the herbs possess anti-inflammatory, antibacterial, and hepatoprotective activities. Chemical studies clarified diverse structural types of compounds, including flavone, flavonol, flavanonol, phenylpropanoid, isobenzofuranone, and *ent*-kaurene diterpenoid [2–6]. Liu et al. (2014) clarified that the decoction of the herbs could significantly reduce the levels of blood glucose, triglyceride, and cholesterol, and increase serum insulin level in type 2 diabetic rats [7]. Our pharmacological study showed that the ethanol extract of the whole herbs could remarkably reduce the blood glucose level of hyperglycemia mice [8]. That inspired us to investigate the chemical compounds present in the herbs with hypoglycemic or lipid disorder improving activities. Alpha-glucosidase breaks down starch and disaccharides to glucose and other monosaccharides, and pancreatic lipase rapidly converts a triglyceride to a 2-monoglyceride and two fatty acids. They are the major enzymes involved in the hydrolysis of carbohydrates and fat. Therefore, α-glucosidase and pancreatic lipase become a conventional approach for the search of natural inhibitors of carbohydrates and triglyceride absorption [9]. As a result, three new acyl flavone and lignan glucosides with potent α-glucosidase inhibitory activity were previously obtained from the whole herbs [10]. Further study led to the isolation of eight glucosides of hydroquinone (**1** and **2**), acetophenone (**3**), monoterpene (**4**–**6**), and megastigmane (**7** and **8**), of which compounds **1**, **4**, and **7** were new structures and the others were obtained from this species for the first time (Fig. 1).

2. Experimental

2.1. General procedures

Silica gel (100–200 mesh, Qingdao Haiyang Chemical Co., Shandong, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography (CC). Thin layer chromatography (TLC) was conducted on pre-coated silica gel $HSGF_{254}$ plates (Jiangyou Silica Gel Development Co., Yantai,

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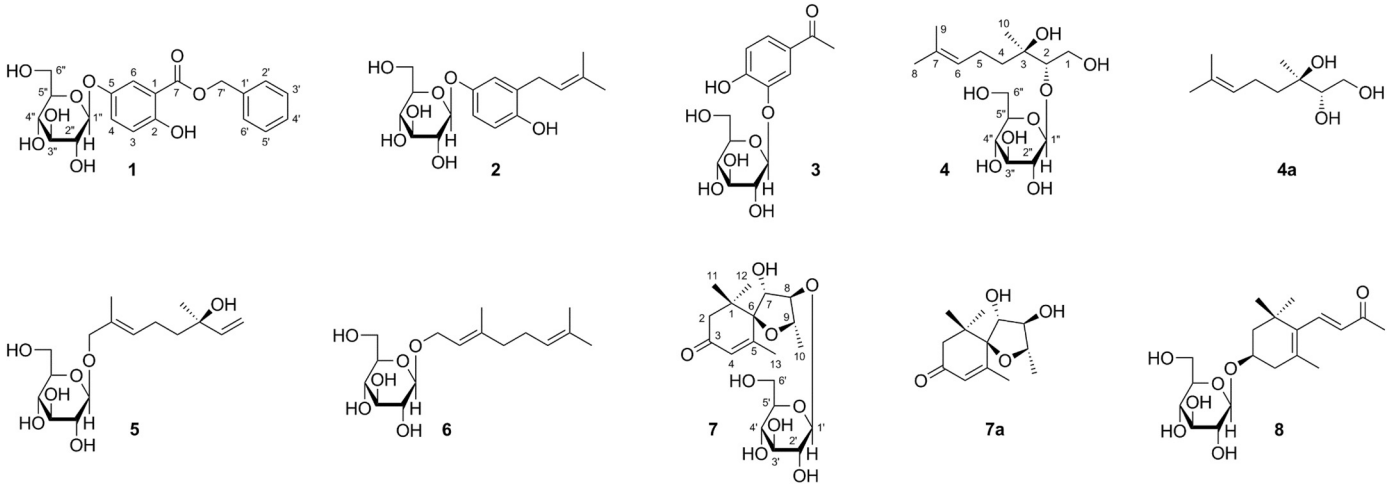

Fig. 1. Structures of compounds **1**–**8**, **4a**, and **7a.**

China) and visualized under UV light (λ = 254 nm) and then by heating after sprayed 10% sulfuric acid in ethanol (*v*/v). Medium pressure liquid chromatography (MPLC) was performed on an EZ Purifier (Lisure Science, Suzhou, China) and the column used was 400 mm × 25 mm i.d., Chromatorex RP-18 SMB100, particle size 20–45 μm (Shanghai Lisui *E*-Tech Co., Shanghai, China). HPLC was run on a LC-6AD liquid chromatograph (Shimadzu, Kyoto, Japan) connected to a RID-10A refractive index detector (Shimadzu) and the columns used were 250 mm × 4.6 mm i.d., Cosmosil 5C18-MS-II, 5 μm (Nacalai Tesque, Inc., Kyoto, Japan) for analysis and 250 mm × 20 mm i.d., YMC-Pack ODS-A, 5 μm (YMC Co., Kyoto, Japan) for preparation. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Ascend-500 spectrometer in CD_3OD or acetone-d_6 (Sigma-Aldrich, St. Louis, MO, USA) using solvent residual peaks as references or in $CDCl_3$ using tetramethylsilane (TMS) as reference. ^{1}H—^{1}H COSY, HSQC, HMBC, and NOESY spectra were measured on the same spectrometer as necessary. HR-ESI-MS and ESI-MS spectra were performed on Bruker maXis mass spectrometer and MDS SCIEX API 2000 LC/MS/MS apparatus (Applied Biosystems Inc., Forster, CA, USA). Optical rotations (α_D) and UV spectra were acquired on a Perkin-Elmer 343 polarimeter and a Lambda 650 UV/Vis spectrometer (Perkin-Elmer, Waltham, MA, USA). IR spectra were measured on an FT-IR Affinity-1 spectrometer (Shimadzu). CD spectra were recorded on a Chirascan circular dichroism spectrometer (Applied Photophysics Ltd., Surrey, UK).

2.2. Plant material

Fresh whole herbs of *Leontopodium leontopodioides* (Willd.) Beauv. (Asteraceae) were collected from Miaoergou village (87°44′20"–87°44′41" E and 43°44′13"–43°44′16" N) at the altitudes of 1760–1800 m, Shuixi Town, Urumqi County, Xinjiang Uygur Autonomous Region in late June of 2013 and botanically authenticated by associate professor F.Y. Suo and laboratory technician Y.M. Lu, College of Life Science and Technology, Xinjiang University. A voucher specimen (No. 13062) was deposited at the Herbarium of Xinjiang University.

2.3. Extraction and isolation

Air-dried herbs were ground to powder. The powder (7270 g) was immersed in 95% ethanol (60 L and 50 L) twice and 50% ethanol (50 L) once for 2 d per time. The combined filtrate was condensed under vacuum to yield an ethanol extract (1414.50 g). The extract was dissolved in 3 L of distilled water and fractionated with $CHCl_3$ for four times (3 L × 4) and *n*-butanol for five times (3 L × 5) to give $CHCl_3$ soluble (115.87 g) and *n*-butanol soluble (198.68 g) fractions. The *n*-butanol soluble fraction was separated by silica gel CC eluted with $CHCl_3$/MeOH (95:5–6:4, *v*/v) to furnish fractions 1–12 according to their TLC profiles. Fraction 5 (2.24 g) was separated by MPLC eluted with MeOH/H_2O (1:9–10:0, v/v) to yield fractions 5–1–5-12. Fraction 5–6 was purified by HPLC eluted with MeOH/H_2O (33:67, *v*/v) at the flow rate of 6 mL/min to provide compound **8** [retention time (t_R) = 75 min, 7 mg]. Fraction 5–10 was purified by HPLC eluted with MeOH/H_2O (48:52, *v*/v) at 6 mL/min to yield compound **6** (t_R = 88 min, 4 mg). Fraction 6 (4.92 g) was separated by MPLC eluted with MeOH/H_2O (1:9–10:0, *v*/v) to afford fractions 6–1–6-13. Fraction 6–9 was purified by HPLC eluted with MeOH/H_2O (50:50, v/v) at 6 mL/min to furnish compound **1** (t_R = 76 min, 9 mg). Fraction 7 (11.03 g) was separated by MPLC eluted with MeOH/H_2O (1:9–7:3, *v*/v) to give fractions 7–1–7-15. Fraction 7–4 was separated by Sephadex LH-20 CC eluted with MeOH to provide compound **5** (6 mg). Fraction 7–5 was purified by HPLC eluted with MeOH/H_2O (30:70, *v*/v) at 6 mL/min to yield compound **2** (t_R = 95 min, 24 mg). Fraction 7–6 was purified by HPLC eluted with MeOH/H_2O (34:66, *v*/v) at 6 mL/min to furnish compound **7** (t_R = 70 min, 3 mg). Fraction 7–7 was purified by HPLC eluted with MeOH/H_2O (34:66, *v*/v) at 6 mL/min to obtain compound **4** (t_R = 88 min, 4 mg). Fraction 9 (9.63 g) was separated by MPLC eluted with MeOH/H_2O (1:9–10:0, v/v) to afford fractions 9–1–9-12. Fraction 9–3 was purified by HPLC eluted with MeOH/H_2O (10:90, v/v) at 6 mL/min to give compound **3** (t_R = 58 min, 10 mg).

2.4. Spectroscopic data of compounds *1, 4, 7,* and *8*

2.4.1. Isotrichocarpin *(1)*

White amorphous powder (MeOH); $[\alpha]_D^{20}$–25.6 (*c* 0.22, MeOH); UV (MeOH) λ_{max} nm (log ε) 211 (4.27), 237 (3.70), and 323 (3.40); IR (MeOH) ν_{max} 3377, 2924, 1684, 1489, 1456, 1213, 1072, 1018, 730, and 692 cm^{-1}; ESI-MS *m/z* 429.4 $[M + Na]^+$, 404.8 $[M - H]^-$, and 441.1 $[M + Cl]^-$; HR-ESI-MS *m/z* 441.0969 $[M + Cl]^-$ (calcd for $C_{20}H_{22}O_9Cl^-$ 441.0958, error − 1.1 mDa); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD and acetone-d_6, see Table 1.

2.4.2. Leontopodioside D *(4)*

White amorphous powder (MeOH); $[\alpha]_D^{20}$–19.0 (*c* 0.30, MeOH); UV (MeOH) λ_{max} nm (log ε) 210 (3.47); IR (MeOH) ν_{max} 3277, 2924, 1645, 1072, and 1028 cm^{-1}; ESI-MS *m/z* 373.2 $[M + Na]^+$, 349.3 $[M - H]^-$, and 385.3 $[M + Cl]^-$; HR-ESI-MS *m/z* 373.1837 $[M + Na]^+$ (calcd for $C_{16}H_{30}O_8Na^+$ 373.1833, error − 0.4 mDa); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD, see Table 2.

Table 1
^{1}H and ^{13}C NMR data of compound **1.**

No.	δ_H (mult., J in Hz) [a]	δ_C [a]	δ_H (mult., J in Hz) [b]	δ_C [b]
1		113.4, C		117.9
2		158.4, C		157.9
3	6.89 (d, 9.1)	119.1, CH	6.91 (d, 9.0)	118.9
4	7.32 (dd, 9.1, 3.1)	127.3, CH	7.34 (dd, 9.0, 3.0)	126.9
5		151.5, C		151.2
6	7.62 (d, 3.1)	118.4, CH	7.58 (d, 3.0)	118.9
7		170.7, C		170.4
1′		137.0, C		136.6
2′	7.48 (dt, 7.4, 1.8)	129.4, CH	7.55 (br d, 7.1)	129.2
3′	7.40 (tt, 7.4, 1.8)	129.7, CH	7.44 (br t, 7.1)	129.5
4′	7.36 (tt, 7.4, 1.8)	129.5, CH	7.38 (br t, 7.1)	129.3
5′	7.40 (tt, 7.4, 1.8)	129.7, CH	7.44 (br t, 7.1)	129.5
6′	7.48 (dt, 7.4, 1.8)	129.4, CH	7.55 (br t, 7.1)	129.2
7′	5.41 (d, 12.3)	68.2, CH_2	5.46 (d, 12.6)	67.9
	5.37 (d, 12.3)		5.42 (d, 12.6)	
1″	4.75 (d, 7.0)	103.7, CH	4.83 (d, 7.5)	103.2
2″	3.42 (m)	74.9, CH	3.46 (m)	74.6
3″	3.42 (m)	77.9, CH	3.46 (m)	77.8
4″	3.41 (m)	71.1, CH	3.46 (m)	71.2
5″	3.35 (m)	78.1, CH	3.42 (m)	77.8
6″	3.80 (dd, 12.1, 2.3)	62.3, CH_2	3.84 (br d, 11.6)	62.5
	3.69 (dd, 12.1, 5.0)		3.70 (br d, 11.6)	

[a] In CD_3OD.
[b] In acetone-d_6.

Table 2
^{1}H and ^{13}C NMR data of compounds **4** and **7** in CD_3OD.

	4		**7**	
No.	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1	3.77 (dd, 12.0, 2.6)	63.4, CH_2		41.6, C
	3.63 (dd, 12.0, 7.4)			
2	3.52 (dd, 7.4, 2.6)	90.9, CH	1.97 (d, 17.9, 1.3)	49.7, CH_2
			3.40 (d, 17.9)	
3		75.0, C		202.3, C
4	1.63 (m)	38.8, CH_2	5.83 (br t, 1.3)	126.5, CH
	1.45 (ddd,13.9, 11.7, 5.4)			
5	2.12 (m)	23.0, CH_2		169.4, C
	2.05 (m)			
6	5.12 (tt, 7.2, 1.5)	125.8, CH		86.3, C
7		132.1, C	4.36 (d, 8.5)	85.0, CH
8	1.67 (3H, s)	25.9, CH_3	4.08 (t, 8.5)	88.5, CH
9	1.62 (3H, s)	17.7, CH_3	3.91 (dq, 8.5, 6.0)	76.3, CH
10	1.19 (3H, s)	22.9, CH_3	1.40 (3H, d, 6.0)	18.7, CH_3
11			1.24 (3H, s)	25.9, CH_3
12			0.97 (3H, s)	26.1, CH_3
13			2.04 (3H, d, 1.3)	18.7, CH_3
1′	4.38 (d, 7.8)	106.0, CH	4.58 (d, 7.8)	103.7, CH
2′	3.27 (dd, 7.8, 9.0)	75.5, CH	3.19 (dd, 7.8, 8.8)	75.2, CH
3′	3.37 (t, 9.0)	78.1, CH	3.35 (t, 8.8)	78.0, CH
4′	3.29 (m)	71.6, CH	3.33 (overlap)	71.4, CH
5′	3.32 (m)	78.0, CH	3.28 (m)	77.9, CH
6′	3.88 (dd, 11.8, 2.2)	62.6, CH_2	3.87 (dd, 12.0, 2.2)	62.6, CH_2
	3.64 (dd, 11.8, 5.6)		3.70 (dd, 12.0, 5.1)	

2.4.3. Leontopodioside E (7)

White amorphous powder (MeOH); $[\alpha]_D^{20}$–7.3 (*c* 0.30, MeOH); UV (MeOH) λ_{max} nm (log ε) 242 (3.99); IR (MeOH) ν_{max} 3288, 2924, 1771, 1645, 1033, and 665 cm^{-1}; CD (MeOH) $\Delta\varepsilon$ 221 (−5.11) and 266 (+0.31); ESI-MS *m/z* 425.4 $[M + Na]^+$, 401.4 $[M - H]^-$, and 439.5 $[M + Cl]^-$; HR-ESI-MS *m/z* 425.1768 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_9Na^+$ 425.1782, error + 1.4 mDa); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data in CD_3OD, see Table 2.

2.4.4. 3β-Hydroxy-β-ionone 3-O-β-D-glucopyranoside (8)

White amorphous powder (MeOH); $[\alpha]_D^{20}$–22.4 (*c* 0.29, MeOH); ESI-MS *m/z* 393.6 $[M + Na]^+$, 409.6 $[M + K]^+$, and 405.1 $[M + Cl]^-$; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.33 (1H, d, J = 16.4 Hz, H-7), 6.14 (1H, d, J = 16.4 Hz, H-8), 4.44 (1H, d, J = 7.8 Hz, H-1′), 4.10 (1H, m, H-3), 3.87 (1H, dd, J = 11.9, 2.0 Hz, H-6′), 3.68 (1H, dd, J = 11.9, 5.1 Hz, H-6′), 3.16 (1H, dd, J = 9.2, 7.8 Hz, H-2′), 2.56 (1H, dd, J = 17.8, 5.6 Hz, H-4), 2.30 (3H, s, H_3–10), 2.17 (1H, dd, J = 17.8, 5.6 Hz, H-4), 1.81 (3H, s, H_3–13), 1.15 (3H, s, H_3–12), and 1.12 (3H, s, H_3–11); ^{13}C NMR (CD_3OD, 125 MHz) δ 37.7 (C-1), 47.3 (C-2), 72.5 (C-3), 40.4 (C-4), 133.2 (C-5), 137.1 (C-6), 144.4 (C-7), 134.0 (C-8), 201.2 (C-9), 27.2 (C-10), 30.5 (C-11), 28.7 (C-12), 21.8 (C-13), 102.5 (C-1′), 75.2 (C-2′), 78.1 (C-3′), 71.7 (C-4′), 77.9 (C-5′), and 62.7 (C-6′). This was the first report of its NMR data measured in CD_3OD.

2.5. β-Glucosidase hydrolysis of compounds 4 and 7

β-Glucosidase hydrolysis was conducted following our previous procedures [11]. Briefly, the aqueous solution of compound **4** (2.8 mg in 1.0 mL of distilled water) was added 2 mg of β-glucosidase (from Almond, Sigma-Aldrich Co., St. Louis, MO, USA), stirred at 37 °C for 4 d, and then fractionated with EtOAc thrice (1 mL per time). After dehydrated with anhydrous sodium sulfate and filtrated, the combined EtOAc solution was concentrated under vacuum to give **4a** (1.4 mg, 93% yield). Through the same procedures, compound **7a** (1.1 mg, 92% yield) was obtained from **7** (2.0 mg).

2.5.1. (2S,3R)-3,7-Dimethyl-6-octene-1,2,3-triol (4a)

White amorphous powder ($CHCl_3$); $[\alpha]_D^{20}$–12.5 (*c* 0.12, $CHCl_3$); ESI-MS *m/z* 211.1 $[M + Na]^+$, 227.1 $[M + K]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.12 (1H, tt, J = 6.8, 1.4 Hz, H-6), 3.78 (2H, d, J = 4.8 Hz, H_2–1), 3.49 (1H, t, J = 4.8 Hz, H-2), 2.14, 2.05 (each 1H, tt, J = 12.4, 6.8 Hz, H_2–5), 1.69 (3H, s, H_3–8),1.64 (1H, m, H-4), 1.63 (3H, s, H_3–9), 1.43 (1H, ddd, J = 14.0, 10.9, 5.6 Hz, H-4), and 1.25 (3H, s, H_3–10); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 63.2 (C-1), 76.2 (C-2), 74.7 (C-3), 37.9 (C-4), 22.2 (C-5), 124.0 (C-6), 132.3 (C-7), 25.7 (C-8), 17.7 (C-9), and 23.5 (C-10).

2.5.2. (6R,7R,8R,9S)-6,9-Epoxy-7,8-dihydromegastigman-4-en-3-one (7a)

White amorphous powder ($CHCl_3$); $[\alpha]_D^{20}$ + 12.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} nm (log ε) 231 (3.82); ESI-MS *m/z* 241.3 $[M + H]^+$, 263.3 $[M + Na]^+$, 279.0 $[M + K]^+$, and 275.1 $[M + Cl]^-$; CD (MeOH) $\Delta\varepsilon$ 231 (−0.90) and 263 (+1.52); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.83 (1H, t, J = 1.4 Hz, H-4), 4.21 (H, d, J = 8.7 Hz, H-7), 4.00 (1H, t, J = 8.7 Hz, H-8), 3.80 (1H, dq, J = 8.7, 6.1 Hz, H-9), 3.24 (1H, d, J = 18.1 Hz, H-2), 2.06 (1H, d, J = 18.1 Hz, H-2), 2.02 (3H, s, H_3–13), 1.37 (1H, d, J = 6.0 Hz, H_3–10), 1.19 (3H, s, H_3–11), 0.99 (3H, s, H_3–12); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 40.4 (C-1), 48.7 (C-2), 199.9 (C-3), 126.0 (C-4), 166.4 (C-5), 85.0 (C-6), 84.9 (C-7), 80.1 (C-8), 75.6 (C-9), 18.2 (C-10), 25.8 (C-11), 25.5 (C-12), and 18.5 (C-13).

2.6. Determination of glucose absolute configuration

Glucose absolute configuration was determined following our previous procedures [10]. In brief, compound **1** (1 mg) was dissolved in 5 mL of 2 M aqueous HCl and refluxed at 90 °C for 4 h. After removal of the solution under vacuum, the residue was dissolved in 5 mL of water, and partitioned with 5 mL of EtOAc thrice. The aqueous layers of compound **1** from acid hydrolysis and of compounds **4** and **7** from β-glucosidase hydrolysis were condensed, and each residue was dissolved in 1 mL of pyridine containing 1 mg/mL L-cystein methyl ester hydrochloride (Shanghai Macklin Biochemical Co., China). After the solution was heated at 60 °C for 1 h, 2 μL of *O*-tolylisothiocyanate (Tokyo Chemical Industry Co., Japan) was added and kept at 60 °C for 1 h, and then condensed under vacuum. Each residue was dissolved in 1 mL of MeOH and analyzed by HPLC at 40 °C on a Prominence LC-20AT connected to a SPD-M20A diode array detector and a CTO-20A column oven (Shimadzu) at 254 nm, and the column used was Cosmosil 5C18-MS-II using CH_3CN/H_2O/acetic acid (22:78:0.1, *v*/v/v) as mobile phase at the flow

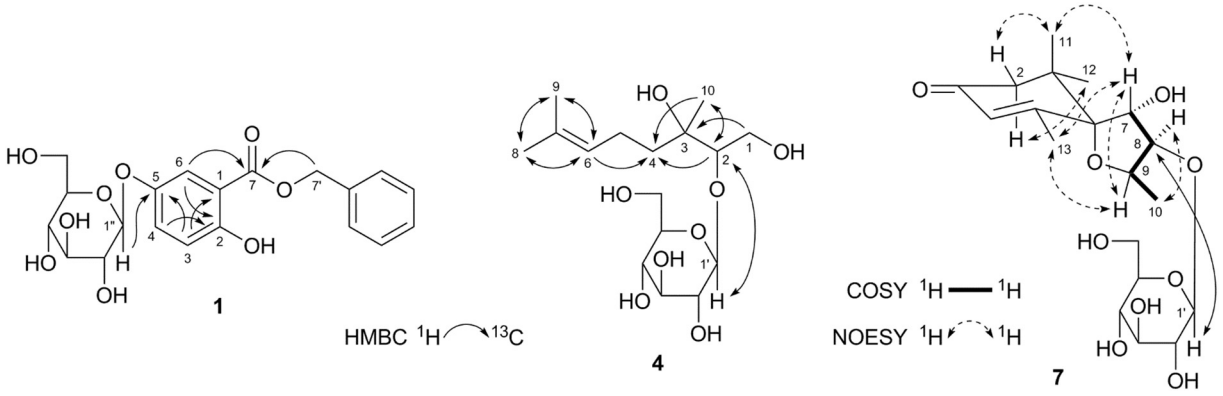

Fig. 2. Key 2D-NMR correlations of compounds **1**, **4**, and **7**.

rate of 0.8 mL/min for 60 min, and then washed with 90% aqueous CH_3CN. Authentic D-glucose and L-glucose (Aladdin Industrial Corp., Shanghai, China) were treated in the same procedures.

2.7. Assays for enzyme inhibitory activities

Assays for α-glucosidase and pancreatic lipase inhibitory activities were conducted following our previous methods [10,12].

3. Results and discussion

Compound **1** was determined to have the molecular formula $C_{20}H_{22}O_9$ based on its HRESIMS and NMR data. The ^{1}H and ^{13}C NMR spectra (Table 1) exhibited signals of three ABX-coupled aromatic protons (H-3, H-4, and H-6), five aromatic carbons of a mono-substituted phenyl (H-2′–6′), twelve aromatic carbons (C-1–6 and C-1′–6′), a carboxy (C-7), an isolated oxygenated methylene (CH_2–7′), and a β-glucosyl moiety. With the aid of the HSQC spectrum, direct connections of protons to carbons were assigned. The HMBC correlation (Fig. 2) from H_2–7′ to C-7 revealed the presence of a benzyl benzoate skeleton [13]. However, comparison of its NMR data with those of trichocarpin (both in acetone-d_6) found that there were obvious differences in H-1–6 and C-1–6 [14]. Further analyses of the HMBC spectrum clarified the connection of glucosyl moiety to C-5 instead of to C-2 in trichocarpin. Finally, acid hydrolysis of **1** liberated D-glucose, which was identified by comparison of its t_R value with those of authentic D-glucose ($t_R \approx 26$ min) and L-glucose ($t_R \approx 24$ min) (Fig. S30). Therefore, compound **1** was identified as benzyl 2,5-dihydroxybenzoate 5-*O*-β-D-glucopyranoside, *i.e.*, isotrichocarpin.

Compound **4** was assigned the molecular formula $C_{16}H_{30}O_8$ from its *m/z* peak of $[M + Na]^+$ in the HR-ESI-MS spectrum. The ^{1}H and ^{13}C NMR and HSQC spectra (Table 2) showed signals of three methyl groups (CH_3–8–10), an olefinic bond (CH-6 and C-7), and a β-glucosyl moiety, which suggested a monoterpene aglycone. The HMBC correlations (Fig. 2) from H_2–1 to C-3 and C-2, H-2 to C-4, C-10, and C-1, H_3–10 to C-2, C-4, and C-3, H_3–8 and H_3–9 to C-6 and C-7, H-6 to C-4, in combination with the δ values of C-1, C-2, and C-3, led us to elucidate the aglycone of 3,7-dimethyl-6-octene-1,2,3-triol [15]. In addition, the HMBC correlations from H-2 to C-1′ and H-1′ to C-2 clarified the connection of glucosyl moiety to C-2. Further, β-glucosidase hydrolysis of **4** yielded its aglycone **4a** with negative optical rotation ($[\alpha]_D^{20} = -12.5$ in $CHCl_3$), which indicated 2*S*,3*S* or 2*S*,3*R* absolute configurations [15–17]. Comparison of the $[\alpha]_D$, ^{1}H, and ^{13}C NMR data of **4a** with those of (2*S*,3*S*)- and (2*S*,3*R*)-3,7-dimethyl-6-octene-1,2,3-triol led us to determine the 2*S*,3*R* absolute configurations. Finally, from the aqueous layer of β-glucosidase hydrolysis of **4**, D-glucose was identified (Fig. S30). Thus, compound **4** was identified as (2*S*,3*R*)-3,7-dimethyl-6-octene-1,2,3-triol 2-*O*-β-D-glucopyranoside, and trivially named leontopodioside D.

The molecular formula $C_{19}H_{30}O_9$ of compound **7** was deduced from its HR-ESI-MS and NMR data. Except for the signals of six carbons readily recognized for a β-glucosyl moiety in the ^{1}H and ^{13}C NMR spectra (Table 2), the remaining thirteen carbons including four methyl groups (CH_3–10–13) were typical of a megastigmane skeleton [18]. In combination with the HSQC spectrum, the presence of a carbonyl (C-3), an olefinic bond (CH-4 and C-5), an oxygenated quaternary carbon (C-6), and three oxygenated methines (C-7–9) were established. Analyses of the HMBC and COSY spectra (Fig. 2) elucidated its planar structure of 6,9-epoxy-7,8-dihydromegastigman-4-en-3-one, which was previously reported from *Kalanchoe tubiflora* (Crassulaceae) [19] and *Sonneratia ovata* (Sonneratiaceae) [18]. The HMBC correlations from the anomeric proton (H-1′) to C-8 and H-8 to C-1′ ascertained the connection of β-glucosyl moiety to C-8. Moreover, The NOESY correlations between H-7 and H-9, H-7 and H_3–11, H-7 and H_3–13, H-8 and H_3–10, H-9 and H_3–13 indicated that H-7 and H-9 were in β-orientation and H-8 was in α-orientation. That is to say, the relative configurations of H-7, H-8, and H-9 of **7** are the same as tubiflorone, which was also confirmed by the consistence of their *J* values [19]. Further, β-glucosidase hydrolysis of **7** afforded its aglycone **7a**, which showed a positive Cotton effect at 263 nm and so C-6 was assigned the *R* absolute configuration [20]. Hence, compound **7** was identified as (6*R*,7*R*,8*R*,9*S*)-6,9-epoxy-7,8-dihydromegastigman-4-en-3-one 8-*O*-β-D-glucopyranoside, and trivially named leontopodioside E.

The known compounds were identified as nebrodenside A (**2**) [21], pungenin (**3**) [22], betulalbuside A (**5**) [23], geranyl *O*-β-D-glucopyranoside (**6**) [24], and 3β-hydroxy-β-ionone 3-*O*-β-D-glucopyranoside (**8**) [25] by analyses of their spectroscopic data and comparison of the data with the reported values.

All the compounds except **7** (little quantity after enzymatic hydrolysis) were tested for *in vitro* inhibitory activities against α-glucosidase from *Saccharomyces cerevisiae* Type I, lyophilized powder, ≥10 units/mg protein (using *p*-nitrophenyl α-D-glucoside as substrate (Sigma-Aldrich) and lipase from porcine pancreas Type II, 100–500 units/mg protein (using olive oil (30 min incubation)), 30–90 units/mg protein (using triacetin) (Sigma-Aldrich). As shown in Table 3, their inhibitory rates against α-glucosidase were very low at the concentration of 100 μM, *i.e.*, they did not exhibit α-glucosidase inhibitory activity. Whereas compounds **2–6** demonstrated potent lipase inhibitory activity with the IC_{50} values of 12.3 ± 0.9, 10.9 ± 1.6, 41.2 ± 4.7, 12.2 ± 0.8, and 75.7 ± 4.9 μM, respectively.

4. Conclusion

This study clarified the structures of three new and five known glucosides of hydroquinone, acetophenone, monoterpene, and megastigmane in the whole herbs of *Leontopodium leontopodioides* (Asteraceae), and all the known compounds were previously undescribed from this species. Potent *in vitro* pancreatic lipase inhibitory

Table 3
Pancreatic lipase and α-glucosidase inhibitory activities of compounds **1–6** and **8.**

Compound	IC_{50} (μM)	Inhibitory rate (%) at 100 μM
	Lipase	α-Glucosidase
1	> 100	4.0 ± 0.0
2	12.3 ± 0.9	6.1 ± 0.0
3	10.9 ± 1.6	6.0 ± 0.0
4	41.2 ± 4.7	1.9 ± 0.0
5	12.2 ± 0.8	11.4 ± 0.1
6	75.7 ± 4.9	2.4 ± 0.0
8	> 100	4.0 ± 0.0
Orlistat	0.28 ± 0.03	
Corosolic acid		IC_{50} = 35.6 ± 0.6 μM

Value represents mean ± SD (n = 3).

activity of these compounds suggests their participation in the reductive effect of the herbs on triglyceride absorption.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2018.08.010.

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