

Anti-diabetes and Anti-inflammatory Activities of Phenolic Glycosides from *Liparis odorata*

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Abstract

Five new phenolic glycosides, *lipariglycoside* K-O (**1-5**) and one known compound, 4-allyl-2,6-dimethoxyphenol glucoside (**6**) were isolated from the whole plant of *Liparis odorata*. Compound **6** was isolated and identified from this genus for the first time. The structures of all compounds were elucidated through extensive spectroscopic methods including UV, IR, MS, 1D- and 2D-NMR. All compounds from *Liparis odorata* were evaluated for their ability to inhibit LPS-induced NO production on the BV2 microglial cell line *in vitro*, as well as their inhibitory effects on PTP1B and α -glucosidase enzyme assays.

Keywords: *Liparis odorata*; Orchidaceae; Phenolic glycosides; Anti-inflammatory activity; Anti-diabetes effect

Introduction

Liparis odorata (Willd.) Lindl., belonging to the Orchid family [1], is an herbaceous plant widely distributed in southern China, and usually used to inhibit inflammation and reduce lipid in Jiangxi province folk medicine in China. Through our continuous interest in the chemical and biologically active constituents of this plant [2-4], five new phenolic glycosides (Figure 1) were isolated and their structures elucidated through extensive spectroscopic analyses, as well as literature comparisons. In addition, one known compound was isolated and identified as 4-allyl-2,6-dimethoxyphenol glucoside [5]. To the best of our knowledge, obesity therapy using phenolic glycoside derivatives has not been studied yet, and we here report the anti-diabetes effects against protein tyrosine phosphatase 1B (PTP1B) and α -glucosidase enzymes for all the isolated compounds. PTP1B plays a critical role as a key negative regulator of the insulin and leptin signaling pathways, thereby regulating glucose homeostasis and body weight, respectively [6], while α -glucosidase inhibition is critical for the early treatment of diabetes mellitus [7]. Therefore, effective inhibition of both enzymesis a potential therapy for both type 2 diabetes mellitus and obesity.

Materials and Methods

General experimental procedures

Ultraviolet (UV) spectra were recorded using a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a transmission microscope method. HR-ESI-MS results were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Optical rotations were measured on a Perkin Elmer 241 automatic digital polarimeter. The 1D- and 2D-NMR spectra were recorded using INOVA 500 and Mercury-400 spectrometers in dimethyl sulfoxide-*d*₆ (DMSO-*d*₆). GC was conducted on an Agilent Technologies 7890A instrument. Preparative highpressure liquid chromatography (HPLC) was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μ m). Column chromatography (CC) was performed using silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), ODS gel (50 μ m, YMC, Japan) and PRP-512A macroporous resin (100-200 mesh). Thin layer chromatography (TLC) was performed with glass pre-coated silica gel (GF₂₅₄) plates. Spots were visualized by UV light (254 nm) or spraying with 10% H₂SO₄ in ethanol (EtOH) followed by heating.

Plant material

L. odorata was collected in the Jiangxi province of China in August 2012. The plant materials were identified by professor Lai Xuewen, Jiangxi University of Traditional Chinese Medicine in China, where a voucher specimen (No. 002017) was deposited.

Extraction and isolation

The whole air-dried plant of *L. odorata* (30.0 kg) was extracted three times under reflux with 95% EtOH at ambient temperature. After removing the organic solvent under reduced pressure, the 95% EtOH extract of *L. odorata* was dissolved in 0.2M HCl. The HCl-soluble fraction was basified by NH₃·H₂O to pH 10.0 and then extracted three times in succession with chloroform, EtOAc and n-BuOH, respectively. The n-BuOH fraction (100.0 g) was subjected to macroporous resin CC (PRP-512A, \varnothing 10 × 50 cm) and eluted with a gradient of EtOH in water (30-95% EtOH). The 70% EtOH eluate (1.8 g) was further subjected to reversed-phase chromatography using a C18 silica gel column (\varnothing 2.0 × 60 cm) with gradient mixtures of CH₃OH-H₂O (30:100-100:0) as eluents to yield five fractions (A-E). Fraction C (800.0 mg) was applied to a silica gel column (\varnothing 2.0 × 60 cm) and eluted with CHCl₃-MeOH (50:1, 25:1, 15:1, 10:1, 5:1, 2:1, 1:1, 0:1) to yield 8 subfractions (C₁-C₈) based on TLC analysis.

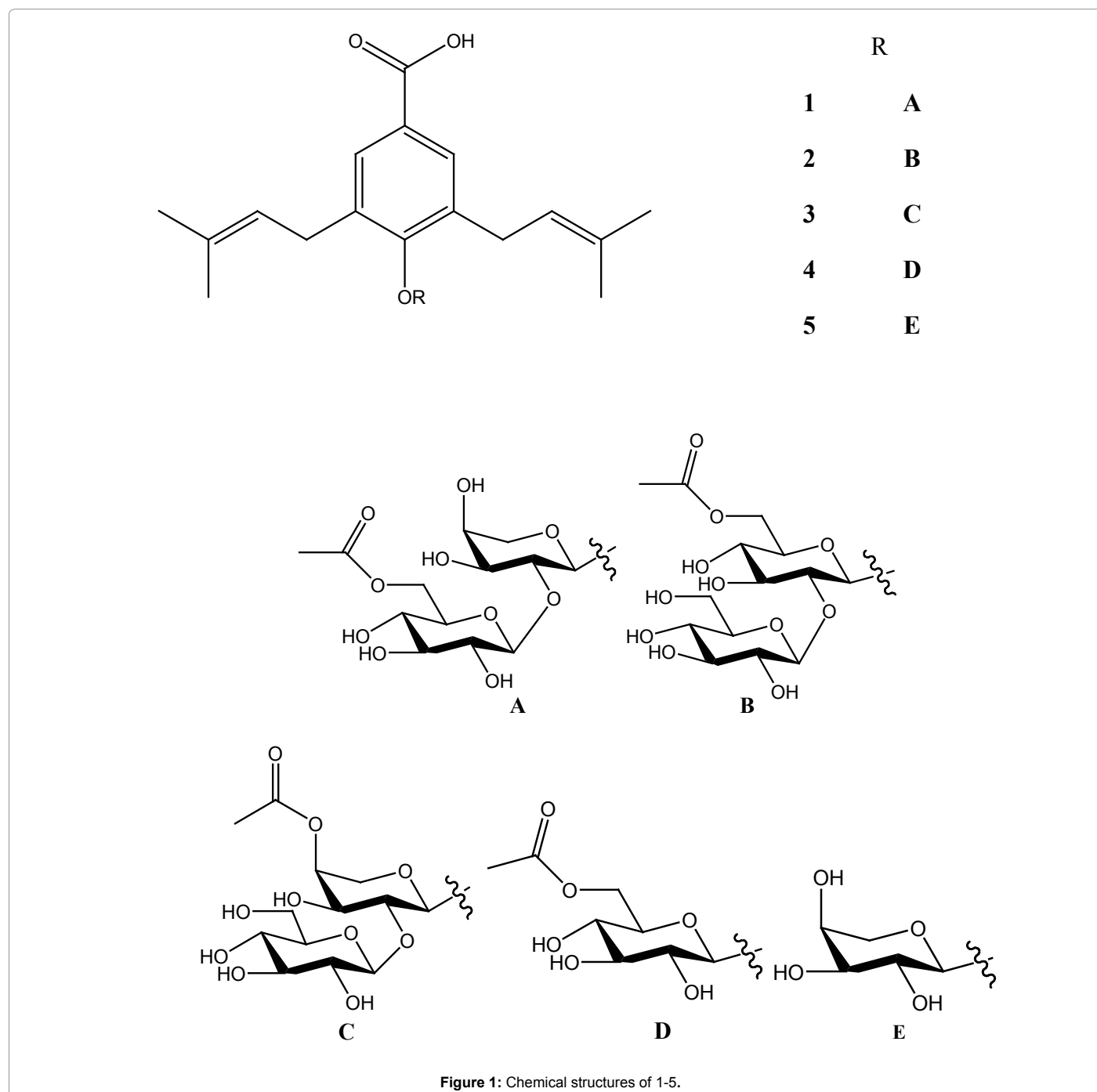
Subfraction C₂ (50.0 mg) was chromatographed on a silica gel column (\varnothing 1.2 × 50 cm) using CHCl₃-MeOH (15:1) and purified by preparative HPLC with MeOH-H₂O (45:55, 8.0 mL/min) to give compound **6** (11.0 mg). Subfraction C₃ (150 mg) was chromatographed using a Sephadex LH-20 column (\varnothing 1.5 × 200 cm) eluting with MeOH, then further purified by preparative HPLC and eluted with MeOH-H₂O (68:32, 8.0 mL/min) to yield compound **5** (105.0 mg) and compound **4** (6.0 mg). Subfraction C₆ (62.0 mg) was purified with preparative HPLC eluting with MeOH-H₂O (60:40, 8.0 mL/min) to yield compound **1**

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(21.0 mg), and subfraction C₇ (79.0 mg) was purified by preparative HPLC with MeOH-H₂O (60:40, 8.0 mL/min) to yield compound 3 (2.5 mg). Finally, subfraction C₈ (82 mg) was subjected to a reversed-phase C18 silica gel column and eluted with MeOH-H₂O (15:85, 30:70, 50:50, 75:25, 100:0). Then, the 75% eluate was further separated by repeated preparative HPLC with 55% MeOH at a flow rate of 8 mL/min to yield compound 2 (8.0 mg).

Liparisglycoside K (1): Colorless oil; [α]₂₀ D: +23.6 (c 1.0, MeOH); UV(MeOH) λ_{max} (logε): 209(4.31), 242(3.97) nm; IR(KBr) ν_{max}: 3376, 2973, 2916, 1681, 1426, 1378, 1190, 1042, 954 cm⁻¹; ¹H NMR (DMSO-d₆, 500 MHz) and ¹³C NMR (DMSO-d₆, 125 MHz) data (see

Tables 1 and 2); HR-ESI-MS m/z 610.2632 [M]⁺ (Calcd for C₃₀H₄₂O₁₃, 610.2625).

Liparisglycoside L (2): White amorphous powder; [α]₂₀ D: +10.2 (c 1.10, MeOH); UV(MeOH) λ_{max} (logε): 208(4.34), 241(3.79) nm; IR(KBr) ν_{max}: 3370, 2969, 2928, 1714, 1601, 1553, 1424, 1250, 1095, 961 cm⁻¹; ¹H (DMSO-d₆, 500 MHz) and ¹³C NMR (DMSO-d₆, 125 MHz) data (see Tables 1 and 2); HR-ESI-MS m/z 640.2729 [M]⁺ (Calcd. for C₃₁H₄₄O₁₄, 640.2731).

Liparisglycoside M (3): Colorless oil; [α]₂₀ D: +23.6 (c 1.0, MeOH); UV(MeOH) λ_{max} (logε): 209(4.31), 242(3.97) nm; IR(KBr) ν_{max}: 3359, 2969, 2926, 1679, 1424, 1378, 1250, 1135, 1080, 936 cm⁻¹;

^1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data (see Tables 1 and 2); HR-ESI-MS m/z 610.2630 $[\text{M}]^+$ (Calcd. for $\text{C}_{30}\text{H}_{42}\text{O}_{13}$, 610.2625).

Liparisglycoside N (4): White amorphous powder; $[\alpha]_{20}^{\text{D}}$: -20.8 (c 1.0, MeOH); UV(MeOH) λ_{max} (log ϵ): 208 (4.24), 240 (3.81) nm; IR(KBr) ν_{max} : 3362, 2971, 2927, 1715, 1601, 1428, 1380, 1271, 1190, 910 cm^{-1} ; ^1H NMR (DMSO- d_6 , 400 MHz) and ^{13}C NMR(DMSO- d_6 , 100 MHz) data (see Tables 1 and 2); HR-ESI-MS m/z 478.2202 $[\text{M}]^+$ (Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_9$, 478.2203).

Liparisglycoside O (5): White amorphous powder; $[\alpha]_{20}^{\text{D}}$: +63.8 (c 0.95, MeOH); UV(MeOH) λ_{max} (log ϵ): 208 (4.39), 242 (3.87) nm; IR(KBr) ν_{max} : 3358, 2974, 2924, 1691, 1545, 1424, 1382, 1189, 1150, 953 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data (see Tables 1 and 2); HR-ESI-MS m/z 406.1993 $[\text{M}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{O}_7$, 406.1992).

Anti-diabetes and anti-inflammatory assays: As previous studies showed that phenolic glycosides compounds from *Liparis odorata* possessed anti-inflammatory activities [3], so, these new phenolic glycosides in this paper were also evaluated activities to inhibit inflammation. Also because of new compounds, widespread screening on activities were looked forward to, thus evaluation of these compounds for their protein tyrosine phosphatase 1B inhibition and α -glucosidase inhibition activities were undertaken in our experiments, seeking new potential drugs for the clinic.

Protein tyrosine phosphatase 1B inhibition: The assay was carried out as previously described [8]. Briefly, all samples were dissolved in 100% DMSO. *p*-Nitrophenyl phosphate (*p*-NPP, 2 mM) and PTP1B (0.05-0.1 μg) were added to a buffer containing 50 mM citrate (pH 6.0), 1mM EDTA, 0.1M NaCl, and 1mM dithiothreitol, with or without test sample. Following incubation at 37°C for 30 min, the reaction was terminated by adding 10M NaOH (10 μL). The amount of released produced *p*-nitrophenol (*p*-NP) was estimated by measuring the absorbance at 405 nm. The measured values were corrected for non-enzymatic hydrolysis of 2mM *p*-NP by measuring the increase in absorbance at 405 nm in the absence of the PTP1B enzyme.

α -Glucosidase inhibition: α -Glucosidase inhibitory activity was determined according to a previously reported method [9]. Briefly,

for each compound, the extract was premixed with *p*-nitrophenyl glucopyranoside (*p*-NPG) (2 mM) as a substrate in 2 mL 0.1 M phosphate buffer (pH=6.86). Then, α -glucosidase (0.05 units) was added to the mixture to start the reaction. The reaction was incubated at $37 \pm 0.5^\circ\text{C}$ for 15 min and stopped with 4 mL of 0.1 M Na_2CO_3 . The α -glucosidase inhibitory activity was determined by measuring the absorbance at 400 nm as an indication for *p*-NP produced from *p*-NPG.

Anti-inflammatory activity: The murine microglial BV2 cell lines were purchased from the Cell Culture Centre at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. LPS (from *Escherichia coli* 055: B5), were obtained from Sigma-Aldrich. The inhibitory activity of extracted compounds on LPS-stimulated NO production in BV2 cells was measured as described previously [3].

Results and Discussion

Liparis odorata is widely used as a folk medicine to inhibit inflammation and reduce lipid in China, through our continuous interests in the bioactive constituents of this plant [2-4], on the basis of pharmacological action tracking method, systematically studies on the chemical compositions and bioactivities of *Liparis odorata* were carried out, looking for new biological compounds.

Compound **1**, a colorless solid, was assigned a molecular formula of $\text{C}_{30}\text{H}_{42}\text{O}_{13}$ determined by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) of its quasi-molecular ion peak at m/z 610.2632 $[\text{M}]^+$ (calcd. for 610.2625). The ^1H NMR spectrum (Table 1) displays the signals attributable to two aromatic protons at δ_{H} 7.54 (s, H-2, 6), two olefinic protons at δ_{H} 5.24 (t, $J=8.5$ Hz, H-8/13), two methylene protons at δ_{H} 3.45 (m, H-7/12), and four methyl protons at δ_{H} 1.68 (s, H-10/15) and 1.71 (s, H-11/16). The ^{13}C NMR (Table 2) and HSQC spectra exhibited signals for two aromatic methenyl carbons at δ_{C} 128.5 (C-2, 6), and four quaternary carbons at δ_{C} 156.6 (C-4), δ_{C} 135.2 (C-3/5) and δ_{C} 126.5 (C-1), all indicating a meta-tetrasubstituted benzene ring. HMBC spectroscopy correlations were observed from H-2/6 (δ_{H} 7.54) with carbonyl carbon (δ_{C} 167.2, C-17), suggesting that the carbonyl group was attached to C-1 (δ 126.5). The HMBC spectrum exhibited long-range correlations of H-7/12 (δ_{H} 3.45) with C-2/6 (δ 128.5), C-3/5 (δ 135.2), C-9/14 (δ 132.2) and C-4 (δ 156.6), indicating that there were two prenyl groups attached to the benzene

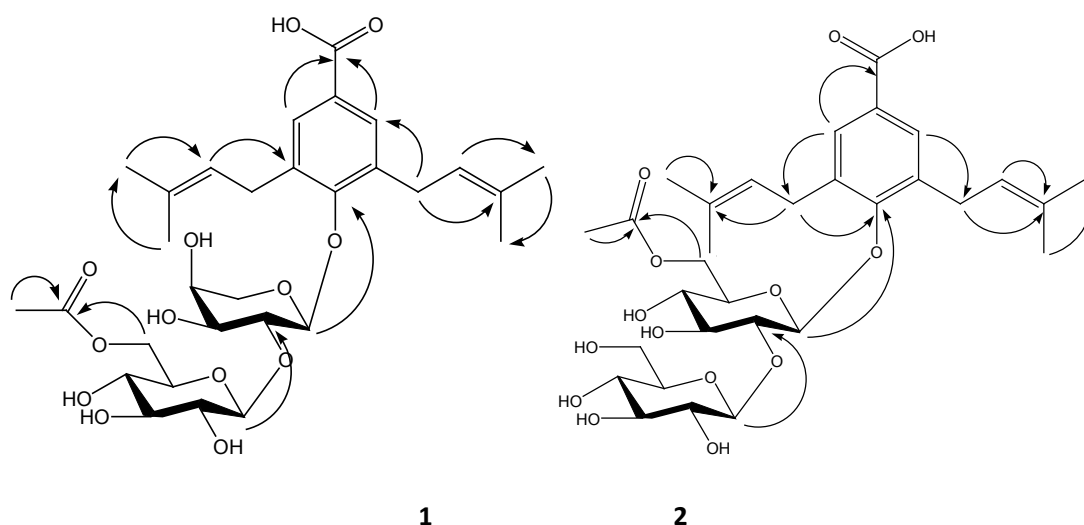


Figure 2: HMBC and key correlations of compounds 1 and 2.

H	1 ^a	2 ^a	3 ^a	4 ^b	5 ^a
2/6	7.54 s	7.55 s	7.55 s	7.55 s	7.55 s
7/12	3.45 m	3.43 m	3.46 d (7.5)	3.48 m	3.49 m
8/13	5.24 t (8.5)	5.22 t (7.0)	5.26 t (7.5)	5.24 m	5.24 t (7.5)
10/15	1.68 s	1.68 s	1.69 s	1.68 s	1.69 s
11/16	1.71 s	1.71 s	1.71 s	1.71 s	1.72 s
	Ara	Gluc	Ara	Gluc	Ara
1'	4.72 d (6.0)	4.69 d (7.5)	4.81d (6.0)	4.62d (7.6)	4.50 d (7.5)
2'	3.95 m	3.43 m	3.94 m	3.33 m	3.66 m
3'	3.73 m	3.49 m	3.81 m	3.24 m	3.40 m
4'	3.73 m	3.43 m	4.16 m	3.19 m	3.64 m
5'	3.77 m 3.36 m	3.55 m	3.61 m 3.94 m	3.45 m	3.16 m 3.69 m
6'	-	4.25 overlapped 4.25 overlapped	-	4.05 m 4.15 m	
CH ₃ CO-	-	1.89 s	2.08 s	1.89 s	
	Gluc	Gluc	Gluc		
1''	4.46 d (7.5)	4.25 d (6.5)	4.53d (8.0)		
2''	3.18 m	3.13 m	3.09 m		
3''	3.05 m	2.96 m	3.04 m		
4''	3.05 m	3.03 m	3.09 m		
5''	3.33 m	3.22 m	3.17 m		
6''	3.93 m 4.15 m	3.70 m 3.39 m	3.41 m 3.52 m		
CH ₃ CO-	1.62 s	-	-		

^a-Date in DMSO-d6 (500 MHz, δ in ppm, J in Hz); ^b-Date in DMSO-d6 (400 MHz, δ in ppm, J in Hz)

Table 1: ¹H NMR Spectroscopic Data of Compounds 1-5.

Position	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
1	126.5	126.9	126.7	126.8	126.6
2/6	128.5	128.5	128.5	128.5	128.6
3/5	135.2	135.5	135.3	135.4	135.4
4	156.6	156.0	156.2	156.0	156.3
7/12	28.1	27.9	28.2	27.8	28.1
8/13	122.7	122.8	122.7	122.8	122.8
9/14	132.2	132.2	132.1	132.2	132.2
10/15	17.9	17.8	17.8	17.8	17.9
11/16	25.7	25.6	25.6	25.6	25.6
17	167.2	167.1	167.1	167.2	167.2
	Ara	Gluc	Ara	Gluc	Ara
1'	102.8	104.0	102.6	104.4	105.5
2'	80.5	80.4	79.8	73.4	71.2
3'	71.2	74.5	70.1	76.1	72.6
4'	66.5	73.8	69.8	69.8	67.8
5'	64.9	71.6	62.4	74.0	66.5
6'		63.0		63.4	
CH ₃ CO		20.5	21.0	20.5	
CH ₃ CO		170.0	170.0	170.2	
	Gluc	Gluc	Gluc		
1''	104.8	103.3	104.2		
2''	76.2	76.5	76.5		
3''	74.5	73.3	74.5		
4''	70.1	70.1	70.1		
5''	73.9	77.0	77.0		
6''	63.9	61.1	61.2		
CH ₃ CO	20.0				
CH ₃ CO	170.2				

^aDate were measured in DMSO-d6 (125 MHz, δ in ppm)

Table 2: ¹³C NMR data for compounds 1-5 a.

ring at C-3 and C-5 [2-3]. The 2D-NMR spectra (Figure 2) showed the presence of an acetyl group at δ_H 1.62 (3H, s), δ_C 170.2 and δ_C 20.0, this group was assigned to C-6" (δ_C 63.9) from the HMBC cross-peak of H-6" (δ_H 4.15 and δ_H 3.93) with the acetyl group (δ_C 170.2). Next, the proton resonances of the sugar units were observed, and their hydrolyzed products were identified as α -L-arabinose and β -D-glucose by gas chromatography. In the HMBC spectrum, long-range correlations were observed of Ara H-1' (δ 4.72) with C-4 (δ 156.6), and Glc H-1" (δ 4.46) with Ara C-2' (δ 80.5), indicating that the sugar moiety was located at C-4 of the aglycone unit. The spectral data were similar to the known compound methyl-3,5-bis(3-methyl-2-butenyl)-4-O-[β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl] benzoate [10], except for the major difference in the presence of an additional acetyl group assigned to C-6". Consequently, the structure of compound 1 was confirmed as 4-O-[α -L-arabinopyranosyl-(1 \rightarrow 2)-6"-O-acetyl- β -D-glucopyranosyl]-3,5-bis(3-methyl-2-butenyl) benzoic acid (Figure 1) and named liparisglycoside K(1).

Compound 2 was obtained as a white amorphous powder. Its molecular formula was deduced as $C_{31}H_{44}NaO_{14}$ from HR-ESI-MS at m/z 663.2622 [M + Na]⁺ (calcd. for $C_{31}H_{44}NaO_{14}$, 633.2518). The ¹H (Table 1) and ¹³C NMR data (Table 2) of 2 showed a close structural similarity to the aglycone moiety of compound 1, indicating that the major differences were in their sugar moieties. Aided by 2D-NMR analysis (Figure 2) of 2, one acetyl and two glucopyranosyl groups were confirmed. In HMBC data, long-range correlations were observed from Gluc H-1' (δ_H 4.69) with C-4 (δ_C 156.0), and Gluc H-1" (δ_H 4.25) with Gluc C-2' (δ_C 80.4), and the carbonyl carbons of the acetyl δ_C 170.0 with Gluc H-6" (δ_H 4.25), indicating that the acetyl unit was located at C-6' of the first Glc unit. The sugar residues were identified as two β -D-glucopyranosyl groups by GC of the hydrolyzed product. Thus, structure 2 was determined to be 4-O-[6'-O-acetyl- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-3,5-bis(3-methyl-2-butenyl) benzoic acid, and the compound was named liparisglycoside L(2).

Compound 3 was isolated as colorless oil. Its molecular formula was established as $C_{30}H_{42}O_{13}$ by analysis of the HR-ESI-MS spectrum at m/z 633.2522 [M+Na]⁺ (calcd. for $C_{30}H_{42}O_{13}$, 633.2518). The ¹H (Table 1) and ¹³C NMR data (Table 2) of 3 were comparable to those of 1 and 2, showing that the main differences were in the sugar part and the location of the acetoxy (OAc) group. Connectivity of the OAc group was established from the HMBC spectrum, which showed a correlation between Ara H-4' (δ_H 4.16) and the carboxyl carbon of the acetyl unit (δ_C 170.0). Hence, the OAc group was located at C-4' of the Ara. Moreover, the sugar residues were identified as α -L-arabinose and β -D-glucose by GC of the hydrolyzed product. So the structure of 3 was established as 4-O-[4'-O-acetyl- α -L-arabinopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-3,5-bis(3-methyl-2-butenyl) benzoic acid, which was named liparisglycoside M(3).

Compound 4 was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{25}H_{34}O_9$ by HR-ESI-MS at m/z 501.2094 [M+Na]⁺ (calcd. for $C_{25}H_{34}NaO_9$, 501.2095). The ¹H (Table 1) and ¹³C NMR data (Table 2) revealed that compound 4 was structurally very similar to compound 1, but the molecular weight of compound 4 was 132 less than compound 1 due to the absence of an arabinose. The acetyl group [δ_H 1.89 (3H, s); δ_C 170.2, 20.5] was located at C-6', determined by the HMBC correlation of H-6' (δ_H 4.15, 4.05) with C=O (δ_C 170.2) (Figure 2), while the sugar residue was identified as β -D-glucopyranose by GC of the hydrolyzed product. Therefore compound 4 was established as 4-O-(6'-O-acetyl- β -D-glucopyranosyl)-3,5-bis(3-methyl-2-butenyl) benzoic acid, named liparisglycoside N(4).

Compound 5, obtained as a white amorphous powder, was assigned the molecular formula of $C_{22}H_{30}O_7$ via HR-ESI-MS at m/z 429.1885 [M+Na]⁺ (calcd. for $C_{22}H_{30}O_7$, 429.1884). Analysis of the ¹H (Table 1) and ¹³C-NMR spectra (Table 2) indicated that compound 5 also possessed a structure similar to compound 1 and that the major differences between them were the absence of the acetyl group and glucose. The sugar residue was identified as α -L-arabinose by GC of the hydrolyzed product. Therefore, the structure of compound 5 was determined to be 4-O-(α -L-arabinopyranosyl)-3,5-bis(3-methyl-2-butenyl) benzoic acid, named liparisglycoside O (5).

The biological activity of the above compounds 1-6, isolated from *Liparis odorata*, was tested by individual evaluation of their *in vitro* hypolipidemic activity against α -glucosidase and PTP1B enzymes. The results are summarized in Table 3. Only compound 3 showed inhibitory activity (9.7% of PTP1B and 6.1% of α -glucosidase), other compounds didn't have significant effects. As the structure of compound 3 is different from other compounds by the existence of an acetoxy (OAc) group linking to the C-4 of Arabinose, and maybe it was the reason to have such bioactivities. In addition, the compounds were evaluated *in vitro* for their inhibition (%) of lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in BV2 microglial cells using the Griess reagent. As shown in Table 4, all compounds were found to possess weak inhibitory activity.

Conclusions

In summary, five new phenolic glycosides (1-5), along with one known compounds (6) were isolated from *L. odorata*. We found only compound 3 showed weak inhibitory activity against α -glucosidase and PTP1B enzymes and all the compounds possessed anti-inflammatory effects by inhibition the NO production in LPS-activated BV2 microglial cells. Further studies on the action mechanism of phenolic glycosides compounds of *Liparis odorata* were taken in our laboratory, it was better to expand the usage of this ancient and effective folk medicine.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Compound ^a	Concentration (μ M)	Inhibition (%) of PTP1B	Inhibition (%) of α -Glucosidase
1	10	0.2	-5.1
2	10	2.5	-8.2
3	10	9.7	6.1
4	10	3.2	5.5
5	10	-0.2	8.0
6	10	9.5	-6.3

^aThe purities of compounds for assay were purified by HPLC over 95%

Table 3: Inhibitory effects of isolated compounds 1-6 on PTP1B enzyme and α -Glucosidase.

Compounds	Concentration (Mol/L)	Inhibition (%)
1	10 ⁻⁵	20.43
2	10 ⁻⁵	1.11
3	10 ⁻⁵	20.30
4	10 ⁻⁵	15.05
5	10 ⁻⁵	19.89
6	10 ⁻⁵	19.56
Curcumin ^a	10 ⁻⁵	62.16

^aPositive control

Table 4: Inhibitory activities on LPS-induced NO production in BV2 of compounds 1-6.

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