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

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Abstract

Five new phenolic glycosides, liparisglycoside 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, while α-glucosidase inhibition is critical for the early treatment of diabetes mellitus [7]. Therefore, effective inhibition of both enzymes is 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 were evaluated for their potential activity and Anti-diabetes effect.

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 to pH 10.0 and then extracted three times with chloroform, EtOAc and n-BuOH, respectively. The n-BuOH fraction (100.0 g) was subjected to macroporous resin CC (PRP-512A, ∅ 10 × 50 cm) and eluted with a gradient of EtOH in water (30-95% EtOH). The 70% EtOH elution (100.0 mg) was subjected to reversed-phase chromatography using a C18 silica gel column (∅ 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 (∅ 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 C (50.0 mg) was chromatographed on a silica gel column (∅ 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 C (150 mg) was chromatographed using a Sephadex LH-20 column (∅ 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 1 (105.0 mg) and compound 4 (6.0 mg). Subfraction C (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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Received July 06, 2016; Accepted July 14, 2016; Published July 20, 2016

Citation: Liu H, Li B, Jiang P, Zhong Y, Zhang D, et al. (2016) Anti-diabetes and Anti-inflammatory Activities of Phenolic Glycosides from Liparis odorata. Med chem (Los Angeles) 6: 500-505. doi:10.4172/2161-0444.1000390

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(21.0 mg), and subfraction C, (79.0 mg) was purified by preparative HPLC with MeOH-H$_2$O (60:40, 8.0 mL/min) to yield compound 3 (2.5 mg). Finally, subfraction C, (82 mg) was subjected to a reversed-phase C18 silica gel column and eluted with MeOH-H$_2$O (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).

**Liparis glycoside K (1):** Colorless oil; $[\alpha]_20$ D: +23.6 (c 1.0, MeOH); UV(MeOH) $\lambda_{max}$ (log$e$): 209(4.31), 242(3.97) nm; IR(KBr) $\nu_{max}$: 3376, 2973, 2916, 1681, 1426, 1378, 1190, 1042, 954 cm$^{-1}$; $^1$H 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 610.2632 [M]$^+$ (Calcd for $C_{30}H_{42}O_{13}$, 610.2625).

**Liparis glycoside L (2):** White amorphous powder; $[\alpha]_20$ D: +10.2 (c 1.10, MeOH); UV(MeOH) $\lambda_{max}$ (log$e$): 208(4.34), 241(3.79) nm; IR(KBr) $\nu_{max}$: 3370, 2969, 2928, 1714, 1601, 1553, 1424, 1250, 1095, 961 cm$^{-1}$; $^1$H (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 640.2729 [M]$^+$ (Calcd for $C_{31}H_{44}O_{14}$, 640.2731).

**Liparis glycoside M (3):** Colorless oil; $[\alpha]_20$ D: +23.6 (c 1.0, MeOH); UV(MeOH) $\lambda_{max}$ (log$e$): 209(4.31), 242(3.97) nm; IR(KBr) $\nu_{max}$ 3359, 2969, 2926, 1679, 1424, 1378, 1250, 1135, 1080, 936 cm$^{-1}$;
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 ± 0.5°C for 15 min and stopped with 4 mL of 0.1 M Na2CO3. 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 a 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 C26H30O12, determined by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) of its quasi-molecular ion peak at m/z 610.2632 [M]+ (calcd. for C26H30O12, 610.2625). The 1H NMR spectrum (Table 1) displays the signals attributable to two aromatic protons at δH 7.54 (s, H-2, 6), two olefinic protons at δH 5.24 (t, J=8.5 Hz, H-8/13), two methylene protons at δH 3.45 (m, H-7/12), and four methyl protons at δH 1.05-1.40 (s, 10H). The 13C NMR spectrum (Table 2) and HSQC spectra exhibited signals for two aromatic methenyl carbons at δC 126.5 (C-1), all indicating a meta-tetrasubstituted benzene ring.

HMBC spectroscopy corre...
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        | Gluc       |
| 1*       | 102.6      | 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       | 63.0       | 63.0       | 63.0       | 63.0       |
| CH₃COH   | 20.5       | 21.0       | 20.5       | 21.0       | 20.5       |

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

| Position | Compound 1 | Compound 2 | Compound 3 | Compound 4 | Compound 5 |
|----------|------------|------------|------------|------------|------------|
| 1        | 104.8      | 103.3      | 104.2      | 104.2      | 104.2      |
| 2        | 76.2       | 76.5       | 76.5       | 76.5       | 76.5       |
| 3        | 74.5       | 73.3       | 74.5       | 74.5       | 74.5       |
| 4        | 70.1       | 70.1       | 70.1       | 70.1       | 70.1       |
| 5        | 73.9       | 77.0       | 77.0       | 77.0       | 77.0       |
| 6        | 63.9       | 61.1       | 61.2       | 61.2       | 61.2       |
| CH₃COH   | 20.0       | 20.0       | 20.0       | 20.0       | 20.0       |

*Date were measured in DMSO-d6 (125 MHz, δ in ppm)
The 2D-NMR spectra (Figure 2) showed the presence of an acetyl group at δ 1.62 (3H, s), δ 1.702 and δ 20.0, this group was assigned to C-6′- (δ 63.9) from the HMBC cross-peak of H-6′ (δ 4.15 and δ 3.93) with the acetyl group (δ 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→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→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_{23}H_{21}O_{18} from HR-ESI-MS at m/z 663.2622 [M + Na]^+ (calcd. for C_{23}H_{22}O_{18}Na, 663.2518). 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 4.69) with C-4(δ 156.0), and Glc H-1′ (δ 4.25) with Glc C-2′ (δ 80.4), and the carbonyl carbons of the acetyl δ 170.0 with Glc H-6′ (δ 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, the structure of compound 2 was determined to be 4-O-[6′-O-acetyl-β-D-glucopyranosyl-(1→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 a colorless oil. Its molecular formula was established as C_{24}H_{22}O_{19} by analysis of the HR-ESI-MS spectrum at m/z 633.2522 [M + Na]^+ (calcd. for C_{24}H_{23}O_{19}Na, 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 acetoxyl (OAc) group. Connectivity of the OAc group was established from the HMBC spectrum, which showed a correlation between Ara H-4′ (δ 4.16) and the carboxyl carbon of the acetyl unit (δ 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→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_{22}H_{19}O_{16} by HR-ESI-MS at m/z 501.2094 [M + Na]^+ (calcd. for C_{22}H_{20}O_{16}Na, 501.2095). The ^1H (Table 1) and ^13C NMR data (Table 2) of 4 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 1.89 (3H, s); δ 170.2, 20.5] was located at C-6′, determined by the HMBC correlation of H-6′ (δ 4.15, 4.05) with C=O (δ 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_{26}H_{19}O_{16} via HR-ESI-MS at m/z 429.1885 [M+Na]^+ (calcd. for C_{26}H_{20}O_{16}Na, 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 61.1% 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 acetoxyl (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.
Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (No.81260629), the Jiangxi province young scientist training fund (Jing gang star item, 2008-222), the Jiangxi province major fund of Education Ministry (GJJ12515), and the Jiangxi province fund of Medical Ministry (2009A056).

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