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Antidiabetic Coumarin and Cyclitol Compounds from *Peucedanum japonicum*

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The antidiabetic activity-guided fractionation and isolation of the 80% EtOH extracts from Peucedani Radix (*Peucedanum japonicum*, Umbelliferae) led to the isolation and characterization of a coumarin and a cyclitol as active principles, that is, peucedanol 7-*O*-β-D-glucopyranoside (**1**) and *myo*-inositol (**2**). Their structures were identified by spectroscopic methods. Compound **1** showed 39% inhibition of postprandial hyperglycemia at 5.8 mg/kg dose, and compound **2** also significantly inhibited postprandial hyperglycemia by 34% ($P<0.05$).

Key words: *Peucedanum japonicum*, Umbelliferae, Coumarin, Inositol

INTRODUCTION

Diabetes mellitus can be classified into two major categories: type 1 and type 2 diabetes. Of the patients who have diabetes mellitus, 85-95% suffers from type 2 diabetes (Attels *et al.*, 2002). Currently available pharmacological agents for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure. Due to these factors, diabetic patients and healthcare professionals are increasingly considering complementary and alternative approaches, including the use of medicinal herbs with anti-hyperglycemic activities (Xie *et al.*, 2002). *Peucedanum japonicum* (Umbelliferae, PJ) is widely distributed in southern and eastern Asia including Korea. The root (Peucedani Radix) was used as a folk medicine in the treatment of cough, headache and anodyne in Korean folk medicine (Bae, 2001). In our preliminary study, *Peucedanum japonicum* showed a significant antidiabetic activity (unpublished data), further study was undertaken to isolate active ingredients from 80% ethanol extract of *Peucedanum japonicum* using bioassay-guided isolation of antidiabetic agent(s). Literature survey revealed that various coumarin derivatives, (-)-*trans*-3'-acetyl-4'-senecioylkhellactone, (±)-*cis*-3'-acetyl-4'-senecioylkhellactone, (±)-*cis*-4'-tigloylkhellactone, (+)-*trans*-4'-tigloylkhellactone (Chen *et al.* 1996; Ikeshiro *et al.*, 1994) were reported from the roots of *Peucedanum japonicum*. Repeated column chromatographic separation guided by diabetic activity using oral glucose tolerance test led to the isolation of a coumarin and a cyclitol, peucedanol 7-*O*-D-glucopytanoside (**1**) and *myo*-inositol (**2**). This paper describes the isolation, structural characterization and antidiabetic activities of the compounds.

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MATERIALS AND METHODS

General experimental procedure

Mps: uncorr. NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl_4, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography : Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh), Licroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm).

Plant materials

Cultivated Korean Peucedani Radix (*Peucedanum japonicum*, Umbelliferae) were purchased from a market in Seoul, Korea in July, 2003. A voucher specimen (SKK-03-011) was deposited at the College of Pharmacy in

SungKyunKwan University.

Extraction, separation and purification of compounds

The dried material (3.2 kg) was extracted with 80% aqueous EtOH (10 L×3) at room temperature for one week and at 60-70°C for two days. The resultant 80% EtOH extract (1.2 kg) was subjected to successive solvent partitioning to give $CHCl_3$ (PE-HC, 10 g), *n*-BuOH (PE-B, 20 g) and H_2O (PE-W, 850 g) soluble fractions. The *n*-BuOH fraction showed significant antihyperglycemic activity (Fig. 1). Thus, the *n*-BuOH extract (20 g) was chromatographed on a silica gel column using a gradient solvent system of EtOAc:MeOH:H_2O (9:2:0.5~0:1:0) to give four fractions (P1~P4). The P2 fraction (4 g) showed significant antidiabetic activity (Fig. 2) and was chromatographed on a silica gel column eluted with EtOAc:MeOH:H_2O (9:2:0.3) to give three subfractions (P2-1~P2-3). The hypoglycemic active P2-1 and P2-2 fraction (Fig. 3) were further chromatographed. The subfraction P2-1 (230 mg) was purified with RP Lobar®-A column (50% MeOH) and HPLC (reverse phase; RP, 45% MeOH) to yield **1** (3 mg). The subfraction P2-2 (1.6 g) was purified with Sephadex LH-20 (MeOH) to give four subfractions (P2-21-P2-24). The second subfraction P2-22 (910 mg) was purified with RP Lobar®-A column (45% MeOH) to yield **2** (7 mg).

Peucedanol 7-*O*-D-glucopyranoside (1)

White powder, mp. 207°C; FAB-MS *m/z* : 427 ($[M+H]^+$); ^{1}H-NMR (500 MHz, CD_3OD) : aglycone moiety δ 6.31 (1H, d, *J*=9.5 Hz, H-3), 7.89 (1H, d, *J*=9.5 Hz, H-4), 7.48 (1H, s, H-5), 7.20 (1H, s, H-8), 2.37 (1H, dd, *J*=14.0 and 10.0 Hz, H-9a), 3.95 (1H, dd, *J*=14.0 and 2.0 Hz, H-9b), 1.26 (3H, s, CH_3), 1.24 (3H, s, CH_3), glucose moiety: 4.99 (1H, d, *J*=7.0 Hz, H-1'), 3.52-3.57 (3H, m, overlap H-2', H-5' and H-10), 3.50 (1H, d, *J*=9.0 Hz, H-3'), 3.42 (1H, t, *J*=9.0 Hz, H-4'), 3.73 (1H, dd, *J*=12.5 and 5.5 Hz, H-6'a), 3.95 (1H, dd, *J*=12.5 and 2.0 Hz, H-6'b); ^{13}C-NMR (125 MHz, CD_3OD) : δ 162.3 (C-2), 159.0 (C-7), 154.3 (C-8a), 144.6 (C-4), 130.0 (C-5), 127.7 (C-6), 113.6 (C-4a), 113.0 (C-3), 102.7 (C-8), 101.5 (C-1'), 77.9 (C-5'), 77.3 (C-10), 76.6 (C-3'), 73.7 (C-2'), 73.0 (C-11), 70.2 (C-4'), 61.3 (C-6'), 32.1 (C-9), 26.1 (Me), 22.2 (Me)

myo-Inositol (2)

Amorphous powder, mp 212°C; FAB-MS *m/z*: 181 ($[M+H]^+$); ^{1}H-NMR (500 MHz, CD_3OD) : δ 3.32 (1H), 3.53 (2H), 3.61 (2H), 3.66 (1H); ^{13}C-NMR (125 MHz, CD_3OD) : δ 75.1 (C-5), 72.6 (C-1, 3), 72.2 (C-2), 71.5 (C-4, 6).

Oral glucose tolerance test (OGTT)

ICR mice(n=8 for each group) were fasted for 10 h followed by oral administrations of glucose (1.5 g/kg) and subfractions or pure compounds. The doses administered are described in each figure legend. The blood samples were collected at 0 (prior to glucose administration), 30, 60 and 120 min after glucose administration. Blood was withdrawn from the orbital venous plexus using a heparinized capillary tube. The blood samples were placed on ice and centrifuged at 5000 rpm, 4 for 15 min and plasma was stored at -20°C until assay. Plasma glucose concentration was determined by glucose oxidase method (Trinder, 1969).

Statistical analysis

All data were expressed as a mean±S.E.M. For multiple comparisons, an analysis of variance(ANOVA) was carried out, followed by Fishers protected least significant difference test as a post hoc test (Statview, SAS Institute, Carry, USA). A value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

80% ethanol extract of PJ was subject to solvent fractionation and *n*-butanol subfraction showed the most potent antidiabetic activity when each subfraction was administered with corresponding yield calculated from 1000 mg/kg of PJ ethanol extract. *n*-Butanol subfraction at 28 mg/kg dose inhibited postprandial hyperglycemia by 46% (Fig. 1). Antidiabetic activity was then effectively enriched by silica gel chromatography into P2 fraction, as shown in Fig. 2. P2 fraction was further fractionated using silica gel column to give P2-1, P2-2 and P2-3. As shown in Fig. 3, P2-1 and P2-2 significantly inhibited postprandial hyperglycemia by 39% and 25%, respectively. P2-1 fraction was finally purified using HPLC to yield compound **1**, P2-2

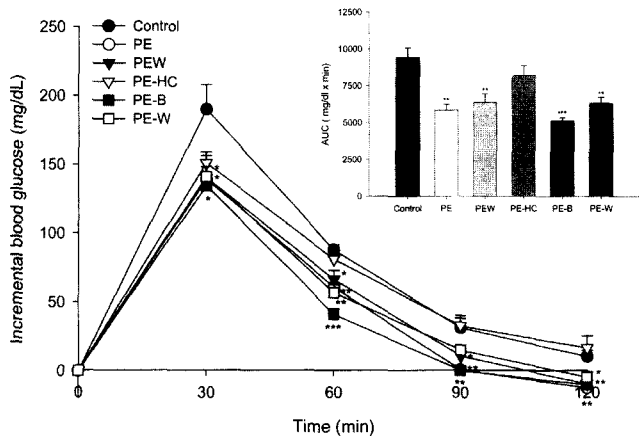


Fig. 1. Effect of solvent fractions of the 80% EtOH extract of *Peucedanum japonicum* on oral glucose tolerance test in ICR mice. The doses administered for PE, PEW, PE-HC, PE-B and PE-W were 1000, 104, 157, 28 and 681 mg/kg, respectively. ***P<0.001, **P<0.01, *P<0.05 compared to control.

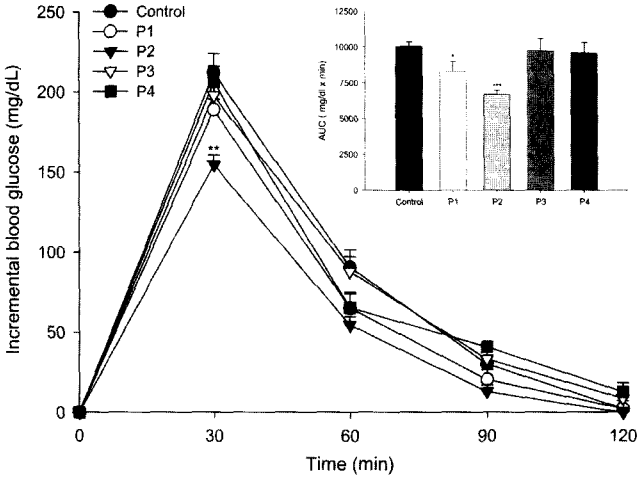


Fig. 2. Effect of P1P4 subfractions of the *n*-BuOH fraction on oral glucose tolerance test in ICR mice. The doses administered for P1, P2, P3 and P4 15.8, 21.1, 12.1 and 47.4 mg/kg, respectively. ***P<0.001, **P<0.01, *P<0.05 compared to control.

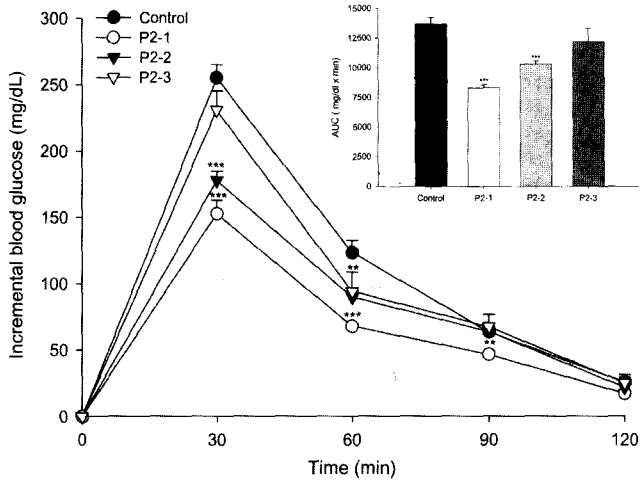


Fig. 3. Effect of P2-1P2-3 of the P-2 subfraction on oral glucose tolerance test in ICR mice. The doses administered for P2-1, P2-2 and p2-3 were 5.8, 40 and 40 mg/kg, respectively. ***P<0.001, **P<0.01 compared to control.

fraction compound **2**.

Compound **1** was obtained as a white powder. The FAB-MS spectrum gave a pseudo-molecular formula ion peak ($[M+H]^+$) at m/z 427 ($C_{20}H_{26}O_{10}$). The ^{1}H-NMR spectrum showed AB system at δ 6.31(1H, d, *J*=9.5 Hz, H-3), 7.89 (1H, d, *J*=9.5 Hz, H-4) and two aromatic protons at δ 7.48 (each 1H, s, H-5), 7.20 (1H, s, H-8), a glucosyl anomeric proton at δ 4.99 (1H, d, *J*=7.0 Hz, H-1'), two methyl proton at δ 1.26 (3H, s, CH_3), 1.24 (3H, s, CH_3) and an oxygenated protons at δ 3.52-3.57 (3H, m, H-2', H-5' and H-10). The ^{13}C-NMR spectrum exhibited the presence of 20 carbon signals, consisting of eight olefinic carbon signals at δ 102.7, 113.0, 113.6, 127.7, 130.0,

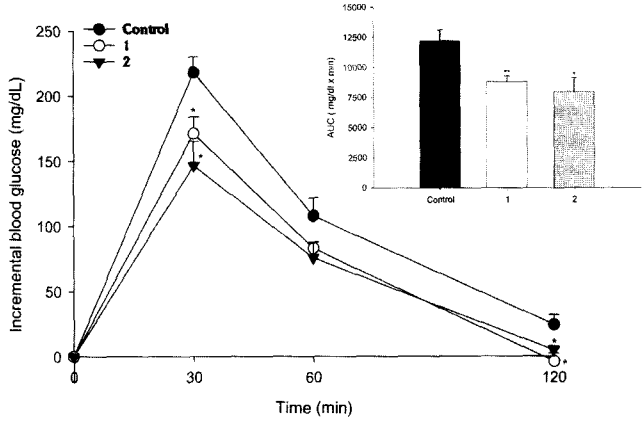
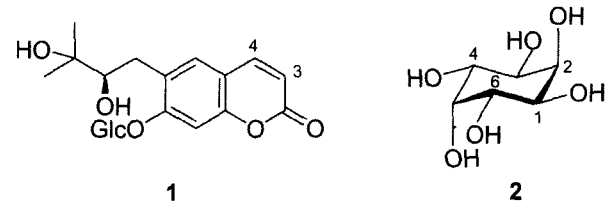


Fig. 4. Effect of compounds **1** and **2** on oral glucose tolerance test in ICR mice. The doses administered for compounds **1** and **2** were 0.127 and 1.182 mg/kg, respectively. **P<0.01, *P<0.05 compared to control.



1 **2**

Fig. 5. Structures of compounds **1** and **2**

144.6, 154.3, and 159.0, a carbonyl carbon signal at δ 162.3 and two oxygenated carbon signals at δ 73.0 and 77.3. These data indicated that the compound **1** was supposed to be coumarin derivative. The NMR spectral and physical data of the compound **1** were in good agreement with those reported in the previous paper (Ikeshiro *et al.*, 1994), thus, the structure of **1** was determined to be peucedanol 7-*O*-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous powder. The FAB-MS spectrum gave a pseudo-molecular formula ion peak ($[M+H]^+$) at m/z 181 ($C_6H_{12}O_6$). The ^{1}H-NMR data showed six oxygenated protons at δ 3.32 (1H), 3.53 (2H), 3.61 (2H) and 3.66 (1H). The ^{13}C-NMR data exhibited the presence of six carbon signals, consisting of oxygenated carbon signal at δ 71.5, 72.2, 72.6 and 75.1. These spectral data suggested that **2** was the cyclic alcohol (cyclitol) derivative (Loewus and Murthy, 2000). The NMR spectral and physical data of the compound **2** were in good agreement with those reported in the previous paper (Yoo *et al.*, 2002), thus, the structure of **2** was determined to *myo*-inositol.

Although there has been well known that PJ was utilized to treat stroke, headache and diarrhea, PJ significantly reduced the body weight and plasma glucose levels in our previous study using db/db mice. Overexpression of glucose transporter-4 (GLUT-4) in skeletal muscle and amelioration of insulin resistance were found to be re-

sponsible for plasma glucose lowering activity (unpublished data). Until now there were no known active ingredients responsible for antihyperglycemic activity of PJ. In this experiment we isolated two pure compounds, coumarin and cyclitol, by using the hypoglycemic activity-guided fractionation. Among six subfractions, *n*-BuOH subfraction obtained from 80% ethanol extract of PJ showed the most active hypoglycemic activity at a dose of 28 mg/kg (Fig. 1), then through the two consecutive purification steps P2-1 and P2-2 gave rise to compounds **1** and **2**, respectively, which showed more than 20% hypoglycemic activities in OGTT assay.

We are now trying to determine the hypoglycemic mechanism(s) of isolated two compounds using several *in vitro* experiments, such as hepatic glucose production assay using H4IIE hepatoma cell line, insulin secretion assay using primary pancreatic beta cell, and insulin resistance related assay using differentiated adipocytes.

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