A New Alkaloid from *Bombycis Feculae* and Its α-Glucosidase Inhibitory Activity

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Abstract: **Objective** To study the chemical constituents of *Bombycis Feculae*. **Methods** Chemical constituents were isolated by HPLC-ELSD. The structures of the isolated compounds were determined by spectral means. **Results** Two compounds were isolated and identified as 1-deoxynojirimycin (**1**) and (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol, named as 1,3-dideoxygalatonojirimycin (**2**). **Conclusion** Compound **2** is a new alkaloid. The extract of *Bombycis Feculae*, compound **1** and compound **2** show inhibitory activities against α-glucosidase.

Key words: alkaloid; *Bombycis Feculae*; 1-deoxynojirimycin; 1,3-dideoxygalatonojirimycin; α-glucosidase inhibitor

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Introduction

Bombycis Feculae (*Cansha* in Chinese) is the excret of *Bombyx mori* L., which is mainly composed of crude proteins and carbohydrates. *Bombycis Feculae* is composed of 15.4% crude proteins, 3.88% crude fat, 19.6% crude fiber, 36.2% nitrogen free extract, alkaloids, and microelements (Jiangsu New Medical College, 1995). The excretion of baby silkworm generally contains higher amount of organic chemical gradients. Interestingly, *Bombycis Feculae* has served as a component in prescription for the treatment of influenza, infantile convulsions, and epilepsy, *etc*. in traditional Chinese medicine (Ye and Su, 1990). It is important to investigate the chemical constituents of *Bombycis Feculae* for understanding its biological activities. Chen, Xiao, and Chen (2003; 2004) characterized alkaloid 1-deoxynojirimycin (DNJ) as one of the chemical components in *Bombycis Feculae*. Here we described the alkaloids isolated from *Bombycis Feculae* by preparative HPLC. Their structures were characterized as (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol and DNJ. To our best knowledge, it is the first report on (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol and its presence in *Bombycis Feculae*. Moreover, we present the biological activities of alkaloids extracted from *Bombycis Feculae* against α-glucosidase.

Materials and methods

Methods

Bombycis Feculae was purchased from Tianjin Zhongxin Pharmaceuticals and identified by Prof. ZHANG Tie-jun in Tianjin Institute of Pharmaceutical Research. DNJ standard sample was purchased from Sigma Inc. ^{1}H-NMR and ^{13}C-NMR spectra were measured in D_2O on a Bruker 300 spectrometer. Chemical shifts relative to TMS, and *J* is expressed in Hz. NOESY was performed using standard pulse sequences of the spectrometer on a Bruker 600 spectrometer. Optical rotations were obtained on an Autopol IV polarimeter. ESI-MS was determined in the positive-ion mode using an LCQ Advantage from Thermofinnigan Company. The HPLC was carried out on Waters 1525 coupled with a Waters 2424-ELSD.

Extraction and isolation

The dried *Bombycis Feculae* (2 kg) was extracted with ionized water (pH was adjusted to 3.0 with PHS-3C acidimeter) at 40－50 ℃, shaking for 3 h. The extraction procedure was repeated for three times. The

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aqueous solution was concentrated to 2.4 L. A portion of the above solution (0.1 L) was evaporated to give a residue (9 g). Correspondingly, 2.4 L of the solution should contain a total of 216 g of extract, which indicated the extraction efficiency was 10.8%. The rest of solution (2.3 L) was mixed with ethanol (3 L) and centrifuged at 10 000 r/min for 20 min. The supernatant was subjected to ion exchange chromatography on ion exchange resin (001 × 7), 50% ethanol and deionized water were used sequentially to remove the unbounded constituents, followed by aqueous ammonia to elute the desired constituents. The gradients of interest were determined by their activities against α-glucosidase. The collections were evaporated to give dark brownish residue (9.7 g, 0.87%). The above residue was subjected to 70 g of silica gel, followed by elution with ethanol to give desired extract I (3.2 g, 0.3%) of *Bombycis Feculae*. The above extract was separated by preparative HPLC on Waters 1525 coupled with a Waters 2424-ELSD. Hypersil SCX (250 mm × 4.0 mm, 5 μm) with elution of actonitrile-glacial acetic acid (80:20) at a flow rate of 0.8 mL/min was utilized for separation. Two fractions were collected with the t_R of 28.6 min (II) and 35.9 min (III), respectively. The two fractions were dried to achieve pure compounds **1** (52.4 mg) and **2** (22.1 mg) which were thoroughly for structure elucidation and bioassayed upon α-glucosidase.

Bioassay

The bioactivity against α-glucosidase was measured by the production of glucose which was determined by standard GOD-PAP enzymatic colorimetric method (Pierre *et al*, 1978). The sample of 200 μL (10 mg/mL) was incubated with 100 μL α-glucosidase solution (6 IU/mL α-glucosidase in 20 mmol/L NaH_2PO_4-Na_2HPO_4, pH 7.0, containing 150 mmol/L NaCl and 1% BSA) for 15 min at 37 ℃, followed by addition of 100 μL maltose solution (10 mg/mL). The reaction was left for 20 min at 37 ℃ before heated at reflux for 2 min. Glucose GOD-PAP kit was used to determine the amount of glucose produced in the reaction. The absorption intensity at wavelength of 490 nm, which is correlated to the concentration of glucose, was obtained on a Bioelisa Reader ELX800. Acarbose was used as standard in the assay.

Results and discussion

Structure characterization

Compound **1** with t_R at 28.6 min. ESI-MS *m*/*z*: 164.0919 $[M + H]^+$. ^{1}H-NMR (D_2O, 300 MHz) *δ*: 3.86—3.63 (3H, m, 2H-6, H-3), 3.52—3.36 (3H, m, H-2, H-4, H-1e), 3.09 (1H, dd, H-1a), 2.86 (1H, t, H-5). ^{13}C-NMR (D_2O, 75 MHz) *δ*: 45.8 (C-1), 57.7 (C-5), 59.9 (C-6), 66.9 (C-2), 67.7 (C-4), 76.2 (C-3).

Compound **2** with a t_R at 35.9 min. ESI-MS *m*/*z*: 148.0969 $[M + H]^+$; $[\alpha]_D^{20} = -16.1$ (*c* 2.43 × 10^{-3} g/mL, H_2O), mp 178—179 ℃. ^{1}H-NMR (D_2O, 300 MHz) *δ*: 1.53 (1H, ddt, *J* = 14.0, 11.4, 4.5 Hz, H-4ax), 2.02 (1H, dddd, *J* = 14.0, 5.0, 2.4 Hz, H-4eq), 2.91 (1H, m, H-6ax), 2.98 (1H, m, H-5), 3.24 (1H, ddd, *J* = 13.1, 4.5, 2.4 Hz, H-6eq), 3.28 (1H, t, *J* = 10.4 Hz, H-2), 3.54 (1H, ddd, *J* = 11.4, 9.1, 4.8 Hz, H-3), 3.74 (2H, m, H-7). ^{13}C-NMR (D_2O, 75 MHz) *δ*: 28.7 (C-4), 42.0 (C-6), 57.8 (C-7), 60.2 (C-5), 69.7 (C-2), 70.6 (C-3).

Compound **1** was characterized as DNJ based on the literature data (Asano *et al*, 1994). Experimental data showed that compound **2** belonged to 1,3-deoxynojirimycin class. However, compound **2** isolated from *Bombycis Feculae* has a different stereochemistry from the known ones (**3**: 2-(hydroxylmethyl) piperidin-3,5-diol and **4**: 1,3-dideoxynojirimycin) (Ostrowski *et al*, 2003; Johnson *et al*, 1994) (Fig. 1).

The diastereomeric assignment for compound **2** was made by ^{1}H-NMR spectroscopy. The coupling constant (9.1 Hz) between H3 and H2 protons indicates axial: equatorial interaction. Furthermore, the coupling constants (4.5, 2.4 Hz) between H3 and H4ax, H4eq confirm that H3 is on the equatorial position.

Therefore, compound **2** was characterized as (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol, which is reported from *Bombycis Feculae* for the first time (Fig. 1).

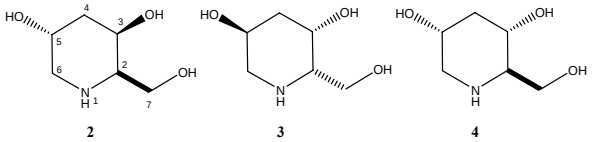

Fig. 1 Chemical structures of compounds 2, 3, and 4

Inhibitory activities against α-glucosidase

The extract I of *Bombycis Feculae* and two isolated compounds **1** and **2** together with acarbose as reference were assayed for the activity against α-glucosidase.

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The extract I (IC_{50}=0.07906 mg/mL) and compound **1** (IC_{50}=0.07906 mg/mL) showed similar inhibitory effects on α-glucosidase as acarbose (IC_{50}=0.07337 mg/mL) which is widely used in clinic for the treatment of diabetes, while compounds **2** (IC_{50}=0.87980 mg/mL) had less activity.

Conclusion

(2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol (**2**) is a new alkaloid isolated from *Bombycis Feculae*, and the inhibitory activity of the extract of *Bombycis Faceces*, compounds **1** and **2**, against α-glucosidase has been first reported.

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