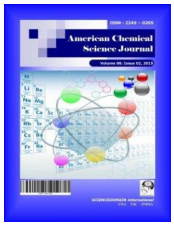

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Sugar-mimic Alkaloids from *Faeces bombycis* and Their Pronounced α-Glucosidase Inhibitory Activities

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Authors' contributions

This work was carried out in collaboration between all authors. Author XH designed the study. Authors JL and YW performed the experiments and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A new sugar-mimic alkaloid, (2R,3R,4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine- N-glyoxylamide **(1)** and a novel natural sugar-mimic alkaloid, (2R,3R,4R)-2-[(S)-1,2-dihydroxyethyl]piperidine-3,4-diol **(2)**, as well as nine known compounds, were isolated and identified from *Faeces bombycis*. Compounds 1 and 2 showed remarkable inhibitory activity against α-glucosidase with the IC_{50} value of 1.9 and 6.2 μM, which were 93.4 and 28.6 potent folds than that of acarbose. The results showed that the sugar-mimic alkaloids from *Faeces bombycis* exhibit significant and reasonably broad range α-glucosidase inhibitory activity, which may be useful in the prevention of the postprandial hyperglycemia and provide new candidates for the treatment of diabetes mellitus.

Keywords: Sugar-mimic alkaloids; Faeces bombycis; structure elucidation; α-Glucosidase inhibitory activity; diabetes mellitus.

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1. INTRODUCTION

Silkworm (*Bombyx mori* L) is an economically important insect, being a primary producer of silk, which exclusive food is mulberry leaves (monophagous). *Faeces bombycis* is the larvae of silkworm's dried dejecta (Cansha in Chinese), which is recorded in ComPendium of Materia Medica, a famous ancient Chinese medicinal work written by Li Shizhen in 1590. In traditional Chinese medicine, *Faeces bombycis* is regarded as slightly bitter in taste, mild in nature, and attributive to the liver meridian. Its pharmacological effects are to expel wind, dredge the meridians, and ease joint pains. Also, it is often contributed in folk medicine for treatment of diabetes mellitus. Additionally, *Faeces bombycis* have been shown to lower blood pressure and cholesterol levels, as well as anti-cancer effects [1,2]. Owing to monophagous characteristic and simple digestive system of silkworm, *Faeces bombycis* mainly compose of crude proteins and carbohydrates. Moreover, sugar-mimic alkaloids (also called polyhydroxylated alkaloids) are its main secondary metabolites.

Sugar-mimic alkaloids are those compounds in which a nitrogen containing ring carries a number of hydroxyl groups and have good solubility in water. In some case, sugar-mimic alkaloids almost could competitively inhibit α-glucosidase because of a structural resemblance to the sugar moiety of the natural substrate. Therefore the peak blood sugar content after meal could significantly decrease by using the α-glucosidase inhibitor before meal. This effect is therapeutic benefit to diabetes. *Morus* L. is the main source plant of sugar-mimic alkaloids, and 1-deoxynojirimycin (DNJ, a sugar-mimic alkaloid) is the highest content compound in it, which has been used for the treatment of diabetes in clinic [3-5].

Previous phytochemical studies have reported the isolation of flavonoids, lignans and megastigmane sesquiterpenes from *Faeces bombycis* [6-9]. To date, only four sugar-mimic alkaloids have been isolated [10-12]. In the current study, A new sugar-mimic alkaloid, (2R,3R,4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine- N-glyoxylamide and a novel natural sugar-mimic alkaloid, (2R,3R,4R)-2-[(S)-1,2-dihydroxyethyl]piperidine-3,4-diol, were isolated and elucidated from *Faeces bombycis*. The nine known constituents, which include four sugar-mimic alkaloids, were identified by comparing the spectroscopic data obtained with those previously reported in the literature. The bioactive results show that all of the compounds from *Faeces bombycis* exhibit the inhibitory effects on α-glucosidase. All of the six sugar-mimic alkaloids possessed significant inhibition against α-glucosidase. Especially, the new compounds were observed to exhibit remarkable inhibitory activity against α-glucosidase, with the IC_{50} values were 1.9 and 6.2 μM, respectively, which were 93.4 and 28.6 potent fold than that of acarbose (177.35 μM, a clinical drug for diabetes mellitus).

2. MATERIALS AND METHODS

2.1 Chemicals and Materials

All analytical chemicals were products of Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The deuteriated solvent for NMR measurement was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Faeces bombycis was purchased from Bozhou medicinal materials market (Bozhou, China) in 2013, and identified by Prof. X. J. He, School of Pharmacy, Guangdong Pharmaceutical University. A voucher specimen was deposited in the School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, China.

2.2 General Methods

The sugar-mimic alkaloids were tracked and colorized by TLC on silica gel GF254 using the solvent system n-PrOH-AcOH-H_2O (4:1:1), nPrOH-AcOH-H_2O (4:1:1), nPrOH-AcOH-nBuOH-AcOH-H_2O (3:1:1), EtOAca-AcOH-H_2O (3:1:1) and a chlorine-o-tolidine reagent for detection. IR spectra (4000–450 cm^{-1}) were recorded in KBr pellets on a PerkinElmer 100 spectrometer (Waltham, MA, USA). NMR spectra were measured on a Bruker AV spectrometer (Bruker Inc., Fällanden, Switzerland) for ^{1}H, ^{13}C, HSQC, HMBC and NOESY spectra in D_2O. High-resolution electrospray ionization mass spectrometer data (HR-ESI-MS) were acquired using a Waters Q-TOF Ultima mass spectrometer (Milford, MA, USA).

2.3 Extraction and Purification

Faeces bombycis (30 kg) was macerated with 70% aqueous MeOH (30 L) for 72 hours for three times. The combined filtrate was concentrated

under vacuum at 55°C to afford 70% methanol extract. The extract (1374.3 g) was applied to a 732 cation exchange resin column (1500×80 mm, H^+ form). The 0.5 M NH_4OH eluate was concentrated to get a brown residue (150 g), which was chromatographed over a 717 anion exchange resin column (1000 × 80 mm, OH^- form) eluted with H_2O to remove brown pigments and anionic compounds. The H_2O eluate was concentrated to get a yellowish residue (92 g). The yellowish residue was applied to a D152 cation exchange column (900 × 60 mm, NH_4^+ form) with H_2O as eluant. The H_2O eluate was divided into five pools: A (fractions 1-7), B (fractions 8-11), C (fraction 12) and D (fractions 13-16). The 0.5 M NH_4OH eluate from the same column was designated pool F. Total yields of pools A, B, C, D and E were 60.27, 7.90, 1.18, 1.81 and 10.19 g, respectively. Repeated chromatography of pool A with 717 anion exchange resin column (840 × 50 mm, OH^- form), D152 cation exchange column (1000 × 40 mm, NH_4^+ form), Sephadex LH-20 column (740 × 15 mm), Dowex 1-X2 column (340 × 25 mm, OH^- form) using H_2O as eluant to get compounds **8** (betaine, 25.6 g), **5** (2-O-α-D-galactopyranosyl-1-deoxynojirimycin, 6.3mg), **4** (1,5-dideoxy-1,5-imino-D-glucitol hydrochloride,12.5 mg), and **7** (trigonelline, 16.6 mg), respectively. Pool B was similarly chromatographed with HD-2 cation exchange column (NH_4^+ form) and Dowex 1-X2 column using H_2O as eluant to get compound **2** {(2R,3R,4R)-2-[(S)-1,2-Dihydroxyethyl] piperidine-3,4-diol, 3.2 mg}. Pool C was chromatographed with D152 cation exchange column, Sephadex LH-20 and Dowex 1-X2 column with H_2O as eluant to obtain compounds **9** {N-[(carboxyamino)methyl]-N,N-dimethyl ethanaminium chloride, 19.2 g}, **10**{N,N-bis(2-chloroethy1)ethylenedia, 4.3 mg} and **11** (meso-erythritol, 11.3 mg). Pool F was applied to an HD-2 cation exchange column (1000 × 25 mm, NH_4^+ form) and eluted with H_2O to get compounds **3** (1-deoxymannojirimycin, 880.0 mg) and **1** [(2R,3R,4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-N-glyoxylamide, 6.0 mg]. The 0.5 M NH_4OH eluate from the same column was chromatographed with Dowex 1-X2 column (340 × 25 mm, OH^- form) using H_2O as eluant to get compound **5** (2-O-α-D-galactopyranosyl-1-deoxynojirimycin, 8.3 mg).

2.4 Structural Elucidation

Ultraviolet spectroscopy (UV) yields no useful information, due to the absence of chromophores in the molecule of sugar-mimic alkaloids. Infrared spectroscopy (IR) shows the expected -OH and -NH bands. The structural elucidation of the sugar-mimic alkaloids is therefore primarily dependent upon mass spectrometry (MS) and 1D and 2D nuclear magnetic resonance spectroscopy (NMR).

(2R,3R,4R)-2-Hydroxymethyl-3,4-dihydroxypyrrolidine-N-glyoxylamide (**1**). White amorphous powder; IR (KBr) ν_{max} 3459, 1683, 1384. HRESIMS *m/z* 205.0896 $[M+H]^+$ (calcd for $C_7H_{13}N_2O_5$, 205.0824). For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

(2R,3R,4R)-2-[(S)-1,2-Dihydroxyethyl]piperidine-3,4-diol (**2**). White amorphous powder; IR (KBr) ν_{max} 3435, 1638, 1384, 1070, 632; ESIMS *m/z* 216.92 $[M+K]^+$. For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

Table 1. ^1H and ^{13}C NMR data (δ in ppm) of compounds 1 and 2 in D_2O (J, in parentheses, in Hz) [a,b]

Position	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	65.2	3.18-3.21, m	61.3	2.73-2.81, m
3	77.7	4.02, t (3.6)	73.2	1.48-1.65, m 2.04-2.06, m
4	74.1	4.10-4.13, m	72.5	3.59-3.67, m
5	52.1	3.65, dd (11.6,4.3) 3.72, d (5.4)	32.3	3.24-3.34, m
6	62.7	3.75, d (5.4) 3.80, dd (11.6,5.4)	43.2	3.12-3.18, m
7	164.6		72.9	3.69-3.81, m
8	163.6		61.5	3.86-3.92, m

[a] δ_H in ppm, recorded at 400 MHz for ^1H.

[b] Assignments of ^1H-NMR data are based on the HSQC and HMBC

1-Deoxynojirimycin (**3**). White amorphous powder; IR (KBr) ν_{max} 3445, 2920, 1640; ESIMS *m/z* 163.98 $[M+H]^+$; ^{1}H NMR (400 MHz, D_2O) δ_H 2.41-2.51 (1H, m, H-2a), 2.55 (1H, m, H-6), 3.06-3.18 (1H, m, H-2e), 3.24 (1H, td, *J* = 9.4, 2.1 Hz, H-5), 3.32 (1H, td, *J* = 9.0, 2.2 Hz, H-4), 3.49 (1H, ddd, *J* =10.9, 9.4, 3.6Hz, H-3), 3.64 (1H, ddd, *J* =8.8, 6.2, 2.0Hz, H-7a), 3.78-3.90 (m, 1H, H-7b); ^{13}C NMR (100 MHz, D_2O) δ_C48.7 (C-2), 60.5 (C-6), 61.4 (C-7), 70.9 (C-3), 71.6 (C-5), 78.4 (C-4).

1,5-Dideoxy-1,5-imino-D-glucitol hydrochloride (**4**). White amorphous powder; IR (KBr) ν_{max} 3390, 2473, 1774, 1635, 1405, 1094, 1045, 895, 598; ESIMS *m/z* 200.09 $[M+H]^+$; ^{1}HNMR (D_2O, 400 MHz) δ_H 3.05 (1H, tt, *J* = 8.3, 4.1 Hz, H-6), 3.22-3.35 (1H, m, H-2a), 3.53-3.64 (2H, m, H-2e, H-5), 3.67 (1H, tt, *J* = 10.5, 5.2 Hz,H-3), 3.87 (1H, ddd, *J* = 11.5, 9.2, 5.1 Hz, H-4), 3.91-3.99 (1H, m, H-7a), 4.01 (1H,dd, *J* = 12.7, 2.9 Hz,H-7b);^{13}CNMR(D_2O, 100 MHz) δ_C45.8 (C-2), 57.7 (C-6), 59.9 (C-7),66.9 (C-3), 67.7 (C-5),76.2 (C-4).

2-O-α-D-Galactopyranosyl-1-deoxynojirimycin (**5**). White amorphous powder; IR (KBr) ν_{max} 3445, 2926, 1636; ESIMS *m/z* 326.00 $[M+H]^+$; ^{1}HNMR (400 MHz, D_2O) δ_H 2.47 (1H, dd, *J* = 12.3, 10.8 Hz, H-2a), 2.51-2.64 (1H, m, H-6), 3.33 (2H, dt, *J* = 19.1, 7.3 Hz, H-5, H-2e), 3.49(1H, t, *J* = 9.1Hz, H-4), 3.59 (1H, m, H-3), 3.64-3.69 (H, m, H-7a), 3.72-3.79 (2H, m, H-6′a, H-6′b), 3.79-3.90 (2H, m, H-2′, H-7b), 3.89-3.97 (1H, m, H-3′),4.03 (1H, d, *J* = 2.9Hz, H-4′), 4.21 (1H, t, *J* = 6.3Hz, H-5′), 5.11 (1H, d, *J* = 3.9 Hz, H-1′); ^{13}CNMR (100 MHz, D_2O) δ_C 45.6 (C-2), 60.5 (C-6), 61.0 (C-6′), 61.4 (C-7), 68.1 (C-2′), 69.2 (C-4′), 69.3 (C-3′), 70.8 (C-5′), 71.6 (C-5), 75.5 (C-3), 76.6 (C-4), 95.9 (C-1′).

Fagomine (**6**). White amorphous powder; IR (KBr) ν_{max} 3390, 2473, 1774, 1635, 1405, 1094, 1045, 895, 598; ESIMS *m/z* 147.95$[M+H]^+$; ^{1}H NMR (400 MHz, D_2O) δ_H 1.54(1H, s, H-3a), 2.05 (1H, s, H-3e), 2.63 (1H, m, H-6), 2.72 (1H, m, H-2a), 3.10 (1H, s, H-2e), 3.26 (1H,s,H-5), 3.61 (1H, s, H-4), 3.71 (1H,s,H-7a), 3.91(1H, d, *J* = 11.7 Hz, H-7b); ^{13}CNMR (100M Hz, D_2O) δ_C 31.8 (C-3), 42.4 (C-2), 60.7 (C-6), 60.9 (C-7), 72.5, 72.7 (C-4,5).

Trigonelline (**7**). White amorphous powder; IR (KBr) ν_{max} 3424, 1644, 1615, 1269, 770, 675; ESIMS *m/z* 138.10 $[M+H]^+$; ^{1}HNMR (400 MHz, D_2O) δ_H 4.35 (3H, s, N-Me), 7.90-8.11 (1H, m, H-5), 8.74 (d, *J* = 7.5 Hz, 2H,H-4,H-6), 9.03 (1H,s,H-2); ^{13}CNMR (100 MHz, D_2O) δ_C 48.2 (N-Me), 127.6 (C-2), 136.9(C-3), 144.7(C-4), 145.8(C-5), 146.0(C-6), 167.7 (COO).

Betaine (**8**). White amorphous powder; IR (KBr) ν_{max} 3430, 1626, 1451, 1396, 1337, 590; ESIMS *m/z* 118.11 $[M+H]^+$; ^{1}HNMR (400 MHz, D_2O) δ_H 3.89 (2H,s,CH_2), 3.26 (9H,3CH_3); ^{13}CNMR (100 MHz, D_2O) δ_C53.3 (3CH_3), 169.1(COO), 66.2(CH_2).

N-[(Carboxyamino)methyl]-N,N-dimethylethanaminium chloride (**9**). White amorphous powder; IR (KBr) ν_{max} 3422, 1747, 1628, 1477, 1403, 1219; ESIMS *m/z* 166.18 $[M+H]^+$;^{1}H NMR (400 MHz, D_2O) δ_H 4.13 (s, 1H), 3.14 (s, 3CH_3).^{13}C NMR (100 MHz, D_2O) δ_C 53.9 (9H, s, 3CH_3), 63.5 (2H, s, CH_2), 166.8 (1H, s, COO).

N,N-Bis (2-chloroethy1)ethylenedia (**10**). White amorphous powder; IR (KBr) ν_{max} 3444, 1635; ESIMS *m/z* 183.05 $[M–H]^–$; ^{1}HNMR (400 MHz, D_2O) δ_H 3.55 (t, *J*=7.7 Hz, 2H, CH_2NH_2), 3.74 (t, *J* = 7.7 Hz, 2H, CH_2N), 3.81 (t, *J* = 5.7 Hz, 4H, CH_2CH_2Cl), 4.03 (t, *J* = 5.7Hz, 4H, CH_2Cl); ^{13}CNMR (100 MHz, D_2O) δ_C 40.8 (CH_2Cl), 52.9 (CH_2N), 57.2 (CH_2CH_2Cl).

meso-Erythritol (**11**). White amorphous powder; IR (KBr) ν_{max} 3410, 2929, 1639, 1385, 1045; ESIMS *m/z* 123.05 $[M+H]^+$; ^{1}HNMR (400MHz, D_2O) δ_H 3.43-3.52(1H, m), 3.55 (1H, d, *J* = 4.3 Hz); ^{13}CNMR (100 MHz, D_2O) δ_C 62.5, 72.0.

2.5 Inhibitory Activity of α-glucosidase

The inhibitory activity on α-glucosidase was determined of all compounds isolated from *Faeces bombycis*. The α-glucosidase inhibitory rate was performed spectrophotometrically on 96-well microplate reader according to a reported method [13]. Briefly, 20 *μ*L of phosphate buffer (0.1 M pH 7.0), 20 *μ*L of enzyme solution (0.2 U/mL α-glucosidase in 0.01 M phosphate buffer containing 0.2% of BSA) and 20 *μ*L of test sample in methanol were mixed well. The mixture was pre-incubated at 37°C for 5 min. Then the reaction was started by adding 20 *μ*L of substrate solution (2.5 mM PNPG in 0.1 M phosphate buffer). After incubation at 37°C for 15 min, 80 *μ*L of 0.2 M sodium carbonate ($NaCO_3$) was added to stop the reaction. Then the absorbance was measured at 405 nm using Microplate reader. Acarbose was dissolved in double-distilled water and diluted to various concentrations with methanol used as positive control. The control was the same mixture except

the solvent was added instead of the test sample. The sample and control blanks were the mixtures of sample and control, respectively, except α-glucosidase was instead with equivalent volume of buffer, respectively. The inhibition (%) of test sample on the enzyme could be calculated as follows,

$$\text{Inhibition (\%)} = [(A_C-A_{CB})-(A_S-A_{SB})]/(A_C - A_{CB})] \times 100$$

where A_S, A_{SB}, A_C, and A_{CB} are the absorbance of sample, sample blank, control, and control blank, respectively. The assay was performed in triplicate. The results were expressed as the sample concentration required inhibiting 50% of the enzyme activity (IC_{50}).

3. RESULTS AND DISCUSSION

Phytochemical investigations were conducted to isolate bioactive sugar-mimic alkaloids from *Faeces bombycis*. The alkaloids were purified and achieved via repeated column chromatography over ion-exchange resins and Sephadex LH-20. These efforts led to the isolation of eleven compounds. The structure identification was carried out by spectroscopic analyses and by comparisons with reported data, and the chemical structure was shown in Fig. 1.

3.1 Structural Elucidation of the Sugar-mimic Alkaloids

Compound **1**, obtained as white amorphous powder, showed the molecular formula of $C_7H_{12}N_2O_5$ as determined by HRESIMS at *m/z* 205.0896 $[M+H]^+$ (calcd for $C_7H_{13}N_2O_5$ $[M+H]^+$, 205.0824). The ^{13}C NMR spectra of **1** revealed the presence of two carbonyls (δ_C 163.6, 164.6), two methylenes (δC 52.1, 62.6), and three methines (δC 65.2, 74.1, 77.7). The complete connectivity of the carbon and hydrogen was determined from analysis of decoupling experiments, HSQC and HMBC spectral data. These experiments elucidated a carbon chain of C^5H_2-C^4H-C^3H-C^2H-C^6H_2-OH. The C-2 (δ_C 65.2) methine carbon bearing a hydroxymethyl group C-6 (δ_C 62.6), and C-5 (δ_C 52.1) methylene carbon should be bonded to the nitrogen of the heterocyclic ring. The methine carbons at δ_C 74.1 and 77.7 were assigned to C-4 and C-3, respectively, bearing OH groups. The remaining two carbonyls at δ_C 163.6 and 164.6 were attributed to two amide carbons. The stereogenic centers of the pyrrolidine ring protons were determined by extensive NOE experiments. The definite NOE effects between the C-6 (CH_2OH) proton and H-3, and between H-2 and H-4, suggest that H-2, H-3, and H-4 are in the β-, α-, and β-orientations, respectively. Thus, compound **1** was determined to be (2R,3R,4R)-3,4-dihydroxypyrrolidine-N-glyoxylamide or its enantiomer, which was a novel sugar-mimic alkaloid as far as we knew. Its ^1H and ^{13}C NMR spectroscopic data were given in Table 1.

Compound **2** showed the molecular formula $C_7H_{15}NO_4$ as determined by ESIMS at *m/z* 216.92 $[M+K]^+$. The ^{13}C NMR spectra of **2** revealed the presence of three methylenes (δ_C 32.3, 43.2, 61.5) and four methines (δ_C 61.3, 72.5, 72.9, 73.2). The C-2 (δ_C 61.3) methine carbon bearing a dihydroxyethyl group C-7, 8 (δ_C 75.7, 64.5) and C-6 (δ_C 43.2) methylene carbon must be bonded to the nitrogen of the heterocyclic ring. The methine carbons bearing OH groups at δ_C 72.5 and 73.2 were assigned to C-4 and C-5, respectively. Thus, compound **2** was determined to be (2R,3R,4R)-2-[(S)-1,2-dihydroxyethyl]piperidine-3,4-diol [14]. Its ^1H and ^{13}C NMR spectroscopic data were given in Table 1.

Compound **3** was obtained as white amorphous powder and its molecular formula was determined as $C_6H_{13}NO_4$ by ESIMS with the ion of *m/z* 163.98 $[M+H]^+$. The ^{13}C NMR spectra revealed the presence of two methylene (δ_C 48.7, 60.5), and four methine (δ_C61.4, 70.9, 71.6, 78.4) carbon atoms. The C-2 (δ_C61.4) methine carbon bearing a hydroxymethyl group C-7 (δ_C 60.5) and the C-6 (δ_C 48.7) methylene carbon bonded to the nitrogen of a heterocyclic ring could be deduced from their chemical shifts. The oxygen-bearing methines at δ_C 70.9, 71.6and 78.4 were assigned to C-3, C-5 and C-4, respectively. Thus, compound **3** was determined to be 1-deoxynojirimycin [4].

Compound **4**, obtained as white amorphous powder, showed the molecular formula $C_7H_{14}ClNO_4$ as determined by ESIMS with the ion of *m/z* 200.1 $[M+H]^+$. The ^{13}C NMR spectra revealed the presence of two methylenes (δ_C 45.8, 57.7) and four methines (δ_C 59.9, 66.9, 67.7, 76.2). The C-2 (δ_C 59.9) methine carbon bearing a hydroxymethyl group C-7 (δ_C 57.7) and the C-6 (δ_C 45.8) methylene carbon attached to the nitrogen of the heterocyclic ring could be deduced. The methine carbons at δ_C 66.9, 67.7 and 76.2 were assigned to C-3, C-5 and C-4, respectively, bearing OH groups. The ^1H and ^{13}C NMR chemical shifts of compound **4** were

consistent with those of **3**. As comparison of these two compounds, hydrochloride tends to shift all the hydrogen (δ_H 3.05-4.01 vs 2.41-3.90) and carbon (δ_C 45.8-76.2 vs 48.7-78.4) to lower field. Thus, compound **4** was determined to be 1,5-dideoxy-1,5-imino-D- glucitol hydrochloride [15].

The ESI-MS of compound **5** gave an $[M+H]^+$ ion at *m/z* 326.0, corresponding to molecular formula of $C_{12}H_{23}NO_9$. The characteristic anomeric proton (H-1′, δ_H5.11, $J_{1',2'}$ = 3.9 Hz) and carbon (C-1′, δ_C 95.9) signals in the NMR suggested that compound **5** was a glucoside. From the chemical shift and the coupling constant of the anomeric proton, the type of glycosidic linkage was determined to be α-configuration (anomeric carbon). The trans-axial orientations of the protons at positions 3, 4, 5, 6, 2′, and 3′ are all clearly indicated by the large vicinal *J* values. For piperidine alkaloids, glycosylation tends to shift the α-carbon to lower field (δ_C7-9 ppm) and β-carbon to higher field (δ_C 2-3 ppm) [16]. The glycosidic shifts for compound **3** produced a 5.7 ppm downfield shift for C-3, and 3.1 and 6.8 ppm upfield shifts for C-2 and C-4, respectively, in the ^{13}C NMR spectrum. Thus, the structure of compound **5** was determined to be 2-O-α-D-galactopyranosyl-1-deoxynojirimycin [4].

Compound **6** showed the molecular formula $C_6H_{13}NO_3$ as determined by ESIMS at the ion of *m/z* 147.95 $[M+H]^+$. The ^{13}C NMR spectra (100 MHz in D_2O) of **6** revealed the presence of three methylenes (δ_C 31.8, 60.9) and three methines (δ_C 60.7, 72.5, 72.7). It could be deduced that the C-2 (δ_C 42.4) methine carbon bearing a hydroxymethyl group C-7 (δ_C 60.9) and the C-6 (δ_C 60.7) methylene carbon bonded to the nitrogen of the heterocyclic ring. The methine carbons bearing hydroxyl at δ_C 72.5, 72.7 were assigned to C-3 and C-4, respectively. Thus, compound **6** was determined to be fagomine [17].

Compounds **7** to **11** were identified to be trigonelline (**7**) [18], betaine (**8**) [19], N-[(carboxyamino)methyl]-N,N-dimethylethanaminium chloride (**9**) [20], N,N-bis (2-chloroethyl) ethylenedia (**10**) [21], meso-erythritol (**11**) [22], respectively, according to their ^1H , ^{13}C NMR and MS data.

3.2 Inhibitory Activity of α-Glucosidase

Preventing carbohydrate absorption in the intestine is a reasonable way to decrease postprandial hyperglycemia in diabetes mellitus, since only monosaccharides could be absorbed in the intestinal lumen and transported into blood circulation. α-Glucosidase inhibitors reduce diet-induced hyperglycemia and endogenous insulin secretion by inhibiting intestinal α-glucosidase, which is considered to be an important factor for glucose homeostasis in diabetic subjects [23,24]. Acarbose, an α-glucosidase inhibitor and used as anti-diabetic drug to treat diabetes mellitus and prediabetes in clinic, is a good example.

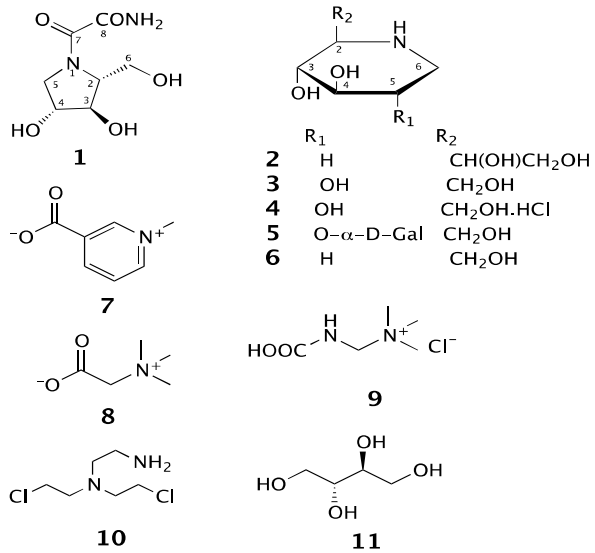

Fig. 1. Structures of the alkaloids isolated from *Faeces bombycis*

Table 2. α-Glucosidase inhibitory activity of the compounds isolated from *Faeces bombycis* [a]

Compound	α-glucosidase activity IC_{50} (μM)	Compound	α-glucosidase activityIC_{50} (μM)
1	1.9±0.1	**7**	2528.3±28.9
2	6.2±0.1	**8**	5146.9±478.7
3	109.8±9.4	**9**	438.5±32.2
4	95.4±0.9	**10**	1474.1±129.3
5	48.6±4.9	**11**	532.5±4.4
6	140.1±8.5	**acarbose** [b]	177.4±14.1

Note: [a] The results are presented as mean ± SD, n=3.
[b] Positive control

As shown in Table 2 above, all of the eleven isolated compounds from *Faeces bombycis* exhibited potent α-glucosidase inhibition activity. Especially, Compounds **1** and **2** exhibited remarkable inhibitory activity against α-glucosidase with the IC_{50} values of 1.9±0.1 and 6.2±0.1 μM, respectively. Other four sugar-mimic alkaloids also exhibited obvious inhibitory activity.

Taken as an ensemble, the following general features of the sugar-mimic alkaloids can be deduced from the current data. 1,4-Dideoxy-1,4-imino-D-arabinitol is a potent inhibitor of yeast α-glucosidase [25]. It is known that the N-alkylation of 1,4-dideoxy-1,4-imino-D-arabinitol markedly lowers or abolishes its inhibition toward all glycosidases tested [26]. However, Its N-glyoxylamide derivative (Compound **1**) also showed strong inhibition activity against α-glucosidase described above. 1-Deoxynojirimycin (Compound **3**) is present in high concentrations in all parts of the mulberry tree. Silkworms feed exclusively on its leaves and appear to accumulate it in their bodies, as the alkaloids content in silkworms is 2.7-fold more than that in the mulberry leaves [5]. The N-alkyl derivatives were most effective and this led to the development of N-hydroxyethyl-deoxynojirimycin as a drug candidate. A naturally-occurring glycoside of 1-deoxynojirimycin (compound **5**) and fagomine (compound **6**) have both been shown to have potent anti-hyperglycemic effects in isolated perfused pancreases from streptozotocin-induced diabetic mice [27,28]. A comparison of the five piperidine alkaloids including 1-deoxynojirimycin, the presence of C2-dihydroxyethyl (Compound **2**), a component of the C5-α-D-Gal (Compound **5**), is important for α-glucosidase inhibitory activity (IC_{50} = 6.2 vs 48.6 μM, respectively).

In conclusion, although the number and spatial arrangement of the hydroxyl groups of sugar-mimic alkaloids serves as a means of recognition by α-glucosidase, it is the influence of other substituent groups that is important for inhibition of enzyme activity.

4. CONCLUSION

The phytochemical investigation of *Faeces bombycis* revealed the presence of A new sugar-mimic alkaloid and a novel natural sugar-mimic alkaloid, as well as nine known compounds. Most of the sugar-mimic alkaloids have been isolated from *Faeces bombycis* for the first time. All of the isolated compounds were evaluated for their inhibitory activity against α-glucosidase. The sugar-mimic alkaloids isolated from *Faeces bombycis* exhibit significant and reasonably broad range α-glucosidase inhibitory activities, which may be useful in the prevention of the postprandial hyperglycemia. This study provide some new candidates for the treatment of diabetes mellitus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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