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Pregnane glycosides from *Gymnema inodorum* and their α-glucosidase inhibitory activity

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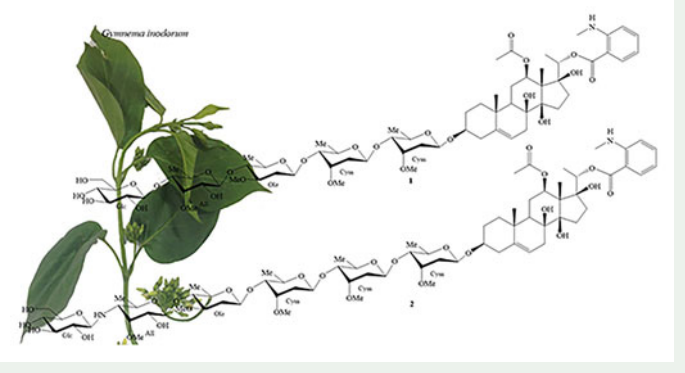
ABSTRACT

Two new pregnane glycosides, gyminosides A and B (**1** and **2**) and three known, tinctoroside B (**3**), tinctoroside C (**4**), and gymnepregoside F (**5**) were isolated from the leaves of *Gymnema inodorum* (Lour.) Decne. Their structures were elucidated by physical and chemical methods and comparing with those reported in the literature. All these compounds were evaluated for α-glucosidase assay. Compound **5** exhibited the most anti α-glucosidase activity with inhibitory percentage of 63.7 ± 3.9% at the concentration of 200 μM. Compounds **1**–**4** showed moderate anti α-glucosidase activity with inhibitory percentage ranging from 40.0 to 52.1%.



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Gymnema inodorum; Asclepiadacaea; pregnane; α-glucosidase; gyminoside

1. Introduction

The leaves of *Gymnema sylvestre* and *Gymnema inodorum* (Asclepiadacaea) have been known in the folk medicine for the treatment of diabetes mellitus, rheumatic arthritis, and gout. *G. inodorum* suppresses the intestinal glucose absorption. A few phytochemical

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studies on *G. inodorum* have been investigated (Atsuchi et al. 1998; Wang et al. 2008). In addition, the compounds and extracts of this plant showed antidiabetic activity and suppression of glucose absorption (Atsuji et al. 1993; Shimizu et al. 1997). As a part of our ongoing investigation on new anti-diabetic compounds from Vietnamese plants (Nhiem et al. 2010), we report herein the isolation, structural elucidation of pregnane saponins from *Gymnema inodorum* and their anti α-glucosidase activity.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{65}H_{101}O_{26}N$ by HR ESI MS ion at *m/z* 1310.6512 $[M-H]^-$ (calcd for $[C_{65}H_{100}O_{26}N]^-$, 1310.6539). The ^{1}H NMR spectrum of **1** showed the proton signals of three methyl groups at δ_H 1.13 (3 H, s), 1.53 (3 H, s), and 1.32 (3 H, d, $J = 6.0$ Hz), one olefinic proton at δ_H 5.32 (1 H, br s) suggesting the presence of a pregnane aglycone; one methyl group at δ_H 1.85 (3 H, s) suggested the appearance of an acetyl moiety; one *N*-methyl group at δ_H 2.91 (3 H, s) together with four aromatic protons at δ_H 6.62 (ddd, $J = 1.5$, 8.0, 8.0 Hz), 6.74 (d, $J = 8.0$ Hz), 7.41 (ddd, $J = 1.5$, 8.0, 8.0 Hz), and 8.08 (dd, $J = 1.5$, 8.0 Hz), indicated the presence of a *N*-methylanthraniloyl moiety; five anomeric protons at δ_H 4.87 (dd, $J = 1.5$, 9.5 Hz), 4.82 (dd, $J = 1.5$, 9.0 Hz), 4.60 (dd, $J = 1.5$, 9.5 Hz), and 4.75 (d, $J = 8.0$ Hz), 4.38 (d, $J = 7.5$ Hz), four methoxy groups at δ_H 3.44, 3.45 × 2, 3.62 along with four secondary methyl groups at δ_H 1.20 (3 H, d, $J = 6.5$ Hz), 1.23 (d, $J = 6.0$ Hz), 1.33 (d, $J = 6.5$ Hz), and 1.38 (d, $J = 6.0$ Hz)] confirming the presence of five sugar units. The ^{13}C NMR and DEPT spectra showed the following carbon signals: two carbonyls, eight non-protonateds (three oxygenateds), thirty-one methines (twenty-five oxygentateds), eleven methylenes, and thirteen methyl carbons (four methoxys and one *N*-methyl), suggested that **1** was a pregnane glycoside. Of these, 21 carbons were assigned to the pregnane aglycone, 2 to the acetyl group, 8 to the *N*-methylanthraniloyl moiety, and 34 to five sugar units. The analysis of ^{1}H and ^{13}C NMR spectra indicated the structure of **1** was similar to those of tinctoroside B (**3**) (Gao et al. 2009) except for the addition of a glucose unit at All C-4. The position of the double bond at C-5/C-6 was confirmed by the HMBC correlations (Figure S1) between H-19 (δ_H 1.13) and C-1 (δ_C 39.8)/C-5 (δ_C 140.3)/C-9 (δ_C 44.8)/C-10 (δ_C 38.0). The positions of hydroxyl/oxygenated groups at C-8, C-12, C-14, C-17, and C-20 were proved by HMBC correlations between H-18 (δ_H 1.53) and C-12 (δ_C 75.4)/C-13 (δ_C 57.6)/C-14 (δ_C 89.5)/C-17 (δ_C 88.6); between H-21 (δ_H 1.32) and C-17 (δ_C 88.6)/C-20 (δ_C 75.2); and between H-6 (δ_H 5.35)/H-7 (δ_H 2.18 and 2.22)/H-9 (δ_H 1.51) and C-8 (δ_C 75.1). The HMBC correlations from H-12 (δ_H 4.74) to Ac carbonyl (δ_C 173.1) and from H-20 (δ_H 4.76) to Ant carbonyl (δ_C 169.1) proved the positions of the acetoxy and *N*-methylanthraniloyloxy moieties at C-12 and C-20, respectively. The constitutions of **1** were proven by the analysis of NOESY observation as well as by similar biogenetic pregnane from *Gymnema* genus. The aglycone of **1** was supposed to have the same configurations as those of known compounds **3**-**5**. In addition, the alkaline hydrolysis of **1** gave sarcostin, [(20*S*)-3β,8β,12β,14β,17β,20-hexahydroxypregn-6-ene] (Seto et al. 1976). The acid hydrolysis of **1** gave four monosaccharides, which were identified as D-cymarose, D-oleandrose, 6-deoxy-3-*O*-methyl-β-D-allose, and D-glucose by

comparing its specific rotation with those reported (Abe et al. 1999; Warashina and Noro 2000). The multiplicity of H-1 of monosaccharide units: Cym I δ_H 4.87 (dd, $J = 1.5$, 9.5 Hz), Cym II δ_H 4.82 (dd, $J = 1.5$, 9.0 Hz), Ole δ_H 4.60 (dd, $J = 1.5$, 9.5 Hz), All δ_H 4.75 (d, $J = 8.0$ Hz), and Glc δ_H 4.38 (d, $J = 7.5$ Hz) suggested the configurations of monosaccharides as β-D-cymaropyranosyl, β-D-oleandropyranosyl, 6-deoxy-3-*O*-methyl-β-D-allopyranosyl, and β-D-glucopyranosyl. Moreover, the HMBC correlations from Glc H-1 (δ_H 4.38) to All C-4 (δ_C 83.8), from All H-1 (δ_H 4.75) to Ole C-4 (δ_C 83.7), from Ole H-1 (δ_H 4.60) to Cym II C-4 (δ_C 83.9), from Cym II H-1 (δ_H 4.82) to Cym I C-4 (δ_C 83.9), from Cym I H-1 (δ_H 4.87) to aglycone C-3 (δ_C 79.3) as well as from aglycone H-3 (δ_H 3.53) to Cym I C-1 (δ_C 97.3), from Cym I H-4 (δ_H 3.24) to Cym II C-1 (δ_C 101.1), from Cym II H-4 (δ_H 3.24) to Ole C-1 (δ_C 102.6), from Ole H-4 (δ_H 3.28) to All C-1 (δ_C 102.0), and from All H-4 (δ_H 3.35) to Glc C-1 (δ_C 106.4) indicated the sugar linkages as β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and the sugar moiety linked at C-3 of aglycone. Based on the above evidence, the structure of **1** was elucidated as (20*S*)-12β-acetyloxy,20-*N*-methylanthraniloyl 3β,8β,14β,17β-tetrahydroxypregn-6-ene 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, a new compound named gyminoside A.

The molecular formula of **2** was determined as $C_{72}H_{113}O_{29}N$ by the HR ESI MS ion at *m/z* 1454.7345 $[M-H]^-$ (calcd for $[C_{72}H_{112}O_{29}N]^-$, 1454.7326). The ^1H and ^{13}C NMR spectra of **2** exhibited a pregnane aglycone and six sugar units (Table S1). In addition, the NMR data of **2** were similar to those of gyminoside A (**1**) except for the addition of a cymaropyranosyl unit in the saccharide linkages. Similar to those of **1**, the positions of functional groups and the aglycone (sarcostin) were proven by the analysis of 1 D- and 2 D-NMR spectra and further confirmed by the alkaline hydrolysis of **2** (Seto et al. 1976). Acid hydrolysis of **2** confirmed the presence of D-cymarose, D-oleandrose, 6-deoxy-3-*O*-methyl-β-D-allose, and D-glucose as sugar components. Furthermore, the ^1H and ^{13}C NMR data of **2** showed the sugar units as β-D-cymaropyranosyl, β-D-oleandropyranosyl, 6-deoxy-3-*O*-methyl-β-D-allopyranosyl, and β-D-glucopyranosyl. The HMBC correlations between Glc H-1 (δ_H 4.37 and All C-4 (δ_C 83.8), between All H-1 (δ_H 4.72) and Ole C-4 (δ_C 83.8), between Ole H-1 (δ_H 4.60) and Cym III C-4 (δ_C 83.9), between Cym III H-1 (δ_H 4.80) and Cym II C-4 (δ_C 83.9), between Cym II H-1 (δ_H 4.82) and Cym I C-4 (δ_C 83.9), and between Cym I H-1 (δ_H 4.87) and aglycone C-3 (δ_C 79.2) indicated the sugar linkages to be 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, which connected to C-3 of the aglycone. Consequently, the structure of **2** was elucidated to be (20*S*)-12β-acetyloxy,20-*N*-methylanthraniloyl 3β,8β,14β,17β-tetrahydroxypregn-6-ene 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, a new compound named gyminoside A.

The known compounds were identified as tinctoroside B (**3**), tinctoroside C (**4**) (Gao et al. 2009), gymnepregoside F (**5**) (Yoshikawa et al. 1998) by analysis their MS, 1 D

and 2 D NMR spectra and by comparison with those reported in the literature (Figure 1).

All isolated compounds were evaluated for the α-glucosidase effect at the concentration of 200 μM. Acarbose, an antidiabetic drug was used as a positive control. As shown in Figure S22, compound **5** showed the most significant α-glucosidase inhibitory activity (63.7 ± 4.9%), comparing with those of acarbose (93.1 ± 4.5%). In the structure-activity relationship of compounds **1**-**4** with same aglycone structure, compounds contain the shorter saccharides chain showed stronger α-glucosidase inhibitory activity than compounds contain the longer saccharide chains. Previous studies have indicated that pregnane glycosides from G. *griffithii* showed moderate α-glucosidase inhibitory activity (Srisurichan et al. 2014). However, this is the first report of α-glucosidase inhibitory activity of compounds from *G. inodorum*.

3. Experimental

3.1. General

Chemical shifts are reported in parts per million from TMS. All NMR spectra were recorded on a Bruker AM500 NMR spectrometer operated at 500 and 125 MHz for hydrogen and carbon, respectively. Data processing was carried out with the MestReNova ver.12.0 program. HR ESI MS spectra were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Preparative HPLC was carried out using an AGILENT 1100 HPLC system. Column chromatography was performed on silica-gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) or RP-18 resins (30–50 μm, Fujisilisa Chemical Ltd.). For thin layer chromatography (TLC), a pre-coated silica-gel 60 F254 (0.25 mm, Merck) and RP-18 F254S plates (0.25 mm, Merck) were used.

3.2. Plant material

The leaves of *Gymnema inodorum* (Lour.) Decne were collected in Yen Vuong, Huu Lung, Lang Son, Vietnam in April, 2017, and identified by one of the authors, Dr. Nguyen The Cuong. A voucher specimen (NCCT-P74) was deposited at Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powders of *G. inodorum* leaves (0.7 kg) were sonicated with hot methanol (3 times × 20 L, each 3 h) to give MeOH extract (70 g) after evaporation of the solvent. The MeOH extract was suspended in water and successively partitioned with *n*-hexane and then EtOAc to obtain the *n*-hexane (GI1, 9.0 g) and EtOAc extracts (GI2, 19.0 g), and H_2O fraction (GI3). Continuously, the water fraction was removed organic solvent then chromatographed on a Diaion HP-20 column eluting with water to remove sugar components, then increase concentration of methanol in water (25, 50, 75 and 100%, v/v) to obtain four fractions, GI3A, GI3B, GI3C and GI3D, respectively. The GI3A fraction was chromatographed on a silica gel column eluting with dichloromethane/methanol

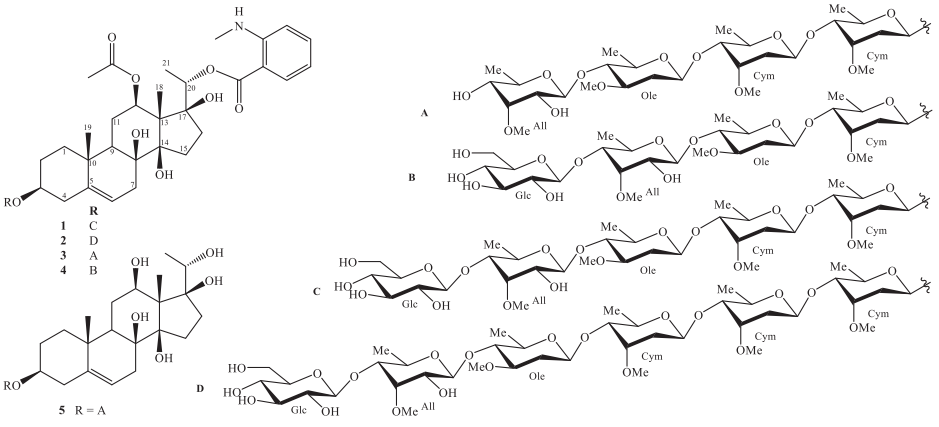

Figure 1. Chemical structures of compounds of **1**–**5**.

(12/1, 6/1, 3/1, 1/1, v/v) to give four sub-fractions, GI3A1-GI3A4. GI3A1 was chromatographed on a RP-18 column using acetone:H_2O (1.8:1, v/v) as solvent eluent to give four fractions, GI3A1A-GI3A1D. Compounds **3** (30.0 mg), **4** (8.0 mg), and **5** (12.0 mg) were yielded from the GI3A1B fraction using HPLC system: J'sphere H-80 column (150 × 20 mm I. D.), solvent condition of 40% acetonitrile. Compound **1** (25.0 mg) was yielded from the GI3A1C fraction on J'sphere H-80 column (150 × 20 mm I. D.), solvent condition of 35% acetonitrile. The GI3A1D fraction was separated on J'sphere H-80 column (150 × 20 mm I. D.) with solvent condition of 35% acetonitrile to yield compound **2** (32.0 mg).

3.3.1. Gyminoside A (1)

White amorphous powder; $[\alpha]_D^{25}$ − 7.0 (*c* 0.1, MeOH); $C_{65}H_{101}O_{26}N$, HR ESI MS *m/z*: 1310.6512 $[M-H]^-$ (calcd for $[C_{65}H_{100}O_{26}N]^-$, 1310.6539); ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S1.

3.3.2. Gyminoside B (2)

White amorphous powder; $[\alpha]_D^{25}$ − 5.0 (*c* 0.1, MeOH); $C_{72}H_{113}O_{29}N$, HR ESI MS *m/z*: 1454.7345 $[M-H]^-$ (calcd for $[C_{72}H_{112}O_{29}N]^-$, 1454.7326); ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S1.

3.4. Acid hydrolysis

Each compound (**1** and **2**, 10.0 mg) was separately dissolved in 1.0 N HCl (dioxane–H_2O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 to give aqueous residue (A). The aqueous residue (A) was separated by silica gel CC eluting with CH_2Cl_2–MeOH (10:1, v/v) and then further fractionated by RP-18 CC using a stepwise gradient of MeOH–H_2O (6:4, 7:3, and 8:2, v/v) to give the saccharides. The specific rotation of these sugars was determined. The specific rotation ($[\alpha]_D^{25}$) of sugars was determined after dissolving in H_2O for 24 h and

compared to the literature (lit):D-cymarose: found +50.0 (*c* 0.4, H_2O), lit +51.8 (Warashina and Noro 2000); D-oleandrose: found -12.0 (*c* 0.4, H_2O), lit +11.7 (Warashina and Noro 2000); 6-deoxy-3-*O*-methyl-D-allose: found + 11.0 (*c* 0.4, H_2O); lit +10.0 (Abe et al. 1999); D-glucose: found + 50.0 (*c* 0.4, H_2O); lit +48.0 (Abe et al. 1999). Based on the above evidence and experiments, sugar components were found in compounds **1** and **2**: D-cymarose, D-oleandrose, 6-deoxy-3-*O*-methyl-D-allose, and D-glucose.

3.5. Alkaline hydrolysis

A solution of compound **1** (8.0 mg) in 1.0 ml of 5% potassium hydroxide in methanol was heated at 40 °C four 4 hours and then neutralized with HCl 0.1 M. After that, the solution was partitioned with $CHCl_3$ to give $CHCl_3$ layer. $CHCl_3$ layer was separated on HPLC system: J'sphere H-80 column (150 × 20 mm I. D.), solvent condition of 55% acetonitrile to yield sarcostin. Similar way, sarcostin was found as aglycone of **2**.

3.6. α-Glucosidase inhibitory assay

The α-glucosidase (G0660-750UN, Sigma-Aldrich, St. Louis, MO) enzyme inhibition assay was performed according to the previously described method (Hanh et al. 2014). The sample solution (2 ml dissolved in dimethyl sulfoxide; DMSO) and 0.5 U/ml α-glucosidase (40 ml) were mixed in 120 ml of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mM p-nitrophenyl-a-D-glucopyranoside solution (40 ml) was added, and the solution was incubated at 37 °C for 30 min. The absorbance of released 4-nitrophenol was measured at 405 nm by using a microplate reader (Molecular Devices, Sunnyvale, CA). Acarbose was used as positive control.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Abe F, Okabe H, Yamauchi T, Honda K, Hayashi N. 1999. Pregnane glycosides from *Marsdenia tomentosa*. Chem Pharm Bull. 47(6):869–875.

Atsuchi M, Hirao Y, Iwasaki Y, Inventors; Kowa Chemical Industries Co., Ltd., Japan. assignee. 1998. Isolation of 28-trihydroxyolean-12-ene-3-yl-β-D-glucopyranuronic acids from Gymnema inodorum leaves as glucose absorption inhibiting agents. United States patent US5843909A.

Atsuji M, Hikimoto K, Yamashita C, Iwasaki Y, Inventors; Kowa Chem Ind Ltd, Japan. assignee. 1993. Antidiabetic triterpene glycoside and its manufacture from Gymnema inodorum. Japan patent JP05247086A.

Gao Z-L, He H-P, Dl Y-T, Fang X, Li C-S, Zhang Q, Zhao P-J, Li S-L, Hao X-J. 2009. Three new pregnane glycosides from *Marsdenia tinctoria*. Helv Chim Acta. 92(9):1775–1781.

Hanh TTH, Chau NM, Tram LH, Luyen NT, Binh PT, Minh CV, Nam NH, Dat NT. 2014. Inhibitors of alpha-glucosidase and alpha-amylase from *Cyperus rotundus*. Pharm Biol. 52(1):74–77.

Nhiem NX, Kiem PV, Minh CV, Ban NK, Cuong NX, Tung NH, Minh HL, Ha DT, Tai BH, Quang TH, et al. 2010. Α-glucosidase inhibition properties of cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia*. Chem Pharm Bull. 58(5):720–724.

Seto H, Sasaki T, Hayashi K, Mitsuhashi H. 1976. Studies on the constituents of asclepiadaceae plants. XXXIX. Components of *Marsdenia tomentosa* Decne: Structure of deacetyldehydrotomentodin, 20-*O*-acetylpenupogenin, deacetylkidjoladinin, and kidjoladinin. Chem Pharm Bull. 24(9):2185–2190.

Shimizu K, Ozeki M, Tanaka K, Itoh K, Nakajyo S, Urakawa N, Atsuchi M. 1997. Suppression of glucose absorption by extracts from the leaves of *Gymnema inodorum*. J Vet Med Sci. 59(9): 753–757.

Srisurichan S, Puthong S, Pornpakakul S. 2014. Pregnane-type steroidal glycosides from *Gymnema Griffithii* Craib. Phytochem. 106:197–206.

Wang D, Li G, Feng Y, Xu S. 2008. Two new oleanane triterpene glycosides from *Gymnema inodorum*. J Chem Res (S). 2008(11):655–657.

Warashina T, Noro T. 2000. Steroidal glycosides from the aerial part of *Asclepias incarnata*. Phytochemistry. 53(4):485–498.

Yoshikawa K, Matsuchika K, Arihara S, Chang HC, Wang JD. 1998. Pregnane glycosides, gymnepregosides A-F from the roots of *Gymnema alternifolium*. Chem Pharm Bull. 46(8):1239–1243.