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Pregnane glycosides from *Dregea volubilis* and their α-glucosidase inhibitory activity

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ABSTRACT

Three new pregnane glycosides namely volubilosides D-F (**1**–**3**) along with three known, dregeoside Da1 (**4**), volubiloside A (**5**), and drevoluoside N (**6**) were isolated from the methanol extract of the leaves of *Dregea volubilis* using combined chromatographic methods. Their structures were elucidated by 1D-, 2D-NMR, and HR-ESI-MS spectra and comparing with those reported in the literature. Compounds **5** and **6** showed the most significant α-glucosidase inhibitory activity at the concentration of 40 μM with inhibition of 51.3 ± 3.2% and 50.4 ± 3.1%, respectively, compared to acarbose (59.8 ± 1.6%).

1. Introduction

Dregea volubilis (L.f.) Benth. ex Hook. f. (Apocynaceae) has been used in oriental medicine for the treatment of inflammation, rheumatic pain, stomach ache, fever, cough, severe cold, and snake bite (Chi, 2012). The phytochemical investigation of this plant showed pregnanes, pregnane glycosides (Sahu et al., 2002; Yoshimura et al., 1985), and flavonoids as main components. The extract and compounds from this plant have exhibited anti-diabetic, antioxidant (Das et al., 2017), and anti-inflammatory activities (Hossain et al., 2010). As a part of our ongoing investigation on anti-diabetic compounds from various Vietnamese medicinal plants (Nhiem et al., 2010), we report herein the isolation and structural elucidation of pregnane-*type* glycosides and their α-glucosidase inhibitory activity Fig. 1.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder and its molecular formula was determined to be $C_{28}H_{46}O_8$ by HR-ESI-MS at m/z 545.2887 $[M + Cl]^-$ (calcd for $[C_{28}H_{46}O_8Cl]^-$, 545.2887). The ^{1}H-NMR spectrum of **1** (CD_3OD) showed the signals of one olefinic proton at δ_H 5.49 (br d, J = 5.5 Hz), one secondary methyl group at δ_H 1.22 (d, J = 6.5 Hz), and two tertiary methyl groups at δ_H 1.12 (s) and 1.20 (s), suggested the presence of a pregnane aglycone. In addition, one anomeric proton at δ_H 4.86 (br d, J = 7.0 Hz), one secondary methyl group at δ_H 1.24 (d, J = 6.0 Hz), and one methoxy group at δ_H 3.45 (s) suggested the appearance of one sugar unit. The ^{13}C-NMR and HSQC spectra (Table 1.) revealed the signals of 28 carbons, including 4 non-protonated carbons, 12 methines, 7 methylenes, and 5 methyl carbons. The analysis of ^{1}H- and ^{13}C-NMR data indicated the structure of **1** was similar to those of dregeoside Da1 (**4**) (Yoshimura et al., 1985) with the difference of a monosaccharide moiety at C-3 of aglycone. The position of the double bond at C-5/C-6 was confirmed by HMBC (Fig. 2) correlations between H-19 (δ_H 1.20) and C-1 (δ_C 40.2)/C-5 (δ_C 141.3)/C-9 (δ_C 50.5)/C-10 (δ_C 40.2). The HMBC correlations from H-9 (δ_H 1.25) to C-11 (δ_C 72.1)/C-12 (δ_C 80.9); from H-18 (δ_H 1.12) to C-12 (δ_C 80.9)/C-13 (δ_C 54.4)/C-14 (δ_C 85.7)/C-17 (δ_C 54.7); and from H-21 (δ_H 1.22) to C-17 (δ_C 54.7)/C-20 (δ_C 71.4) confirmed the positions of hydroxyl groups at C-11, C-12, C-14, and C-20. The large coupling constants of H-9 and H-11, J = 10.5 Hz; H-11 and H-12, J = 10.0 Hz and NOESY correlations of H-11 (δ_H 3.67) and H-19 (δ_H 1.20); H-12 (δ_H 3.04) and H-17 (δ_H 2.18) suggested the configurations of hydroxyl groups at C-11 and C-12 to be α and β, respectively. The

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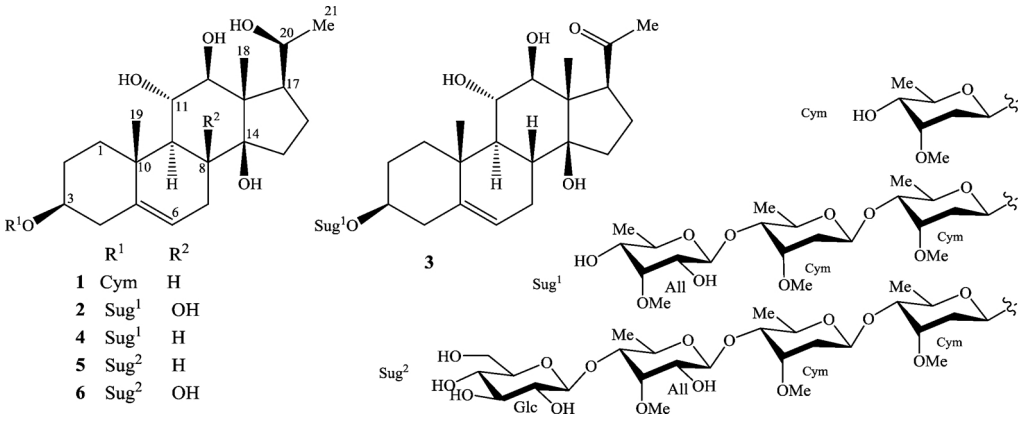

Fig. 1. Chemical structures of compounds **1–6.**

Table 1
^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectroscopic data for compounds **1–3** in CD_3OD.

C	1		2		3	
	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
Aglycon						
1	40.2	1.15 (m)	41.2	1.09 (ddd, 3.5, 10.0, 13.5)	40.4	1.15 (ddd, 3.5, 10.5, 13.5)
		2.68 (dt, 3.5, 14.0)		2.67 (br d, 13.5)		2.68 (br d, 13.5)
2	30.8	1.58 (m)/1.84 (m)	30.4	1.65 (m)/1.80 (m)	30.8	1.57 (m)/1.83 (m)
3	79.2	3.51 (m)	79.6	3.53 (m)	79.2	3.50 (m)
4	40.3	2.22 (m)	40.2	2.31 (br d, 13.5)	40.3	2.20 (m)
		2.35 (dd, 2.5, 12.0)		2.37 (ddd, 1.5, 5.0, 13.5)		2.35[a]
5	141.3	–	142.0	–	141.2	–
6	122.9	5.49 (br d, 5.5)	118.8	5.34 (br d, 5.5)	122.9	5.48 (br d, 5.5)
7	28.7	1.83 (m)/2.25 (m)	36.1	1.65 (m)	28.7	1.80 (m)/2.27 (m)
				2.17 (dd, 2.0, 12.5)		
8	38.5	1.76 (m)	76.9	–	37.8	1.69 (ddd, 4.5, 7.0, 11.5)
9	50.5	1.25[a]	51.6	1.44 (d, 10.5)	50.4	1.27[a]
10	40.2	–	40.2	–	40.0	–
11	72.1	3.67 (dd, 10.0, 10.5)	70.8	4.02 (dd, 10.0, 10.5)	72.3	3.65 (dd, 10.0, 10.5)
12	80.9	3.04 (d, 10.0)	82.6	3.17 (d, 10.0)	78.8	3.08 (d, 10.0)
13	54.4	–	54.0	–	56.0	–
14	85.7	–	86.0	–	85.9	–
15	33.9	1.63 (m)/1.75 (m)	36.0	1.75 (m)/2.15 (m)	35.4	1.80 (m)/2.00 (m)
16	27.0	1.62 (m) /1.92 (m)	27.3	1.61 (m)/1.84 (m)	24.9	1.96 (m)/2.03 (m)
17	54.7	2.18 (m)	56.9	2.08 (m)	59.0	3.57 (dd, 4.0, 9.0)
18	11.0	1.12 (s)	11.5	1.28 (s)	10.4	0.93 (s)
19	19.2	1.20 (s)	18.0	1.40 (s)	19.2	1.18 (s)
20	71.4	3.79 (dq, 6.0, 7.5)	70.5	3.75 (dq, 6.0, 7.5)	218.9	–
21	23.0	1.22 (d, 6.5)	22.6	1.20 (d, 6.0)	32.6	2.27 (s)
Cym I						
1	97.1	4.86 (br d, 7.0)	97.1	4.88 (dd, 2.0, 9.5)	97.1	4.87 (dd, 2.0, 9.5)
2	35.9	1.54 (m)/2.16 (m)	36.6	1.57 (m)/2.09 (m)	36.7	1.56 (m)/2.07 (m)
3	79.1	3.61 (br dd, 3.0, 3.0)	78.6	3.86[a]	78.6	3.85[a]
4	74.4	3.19 (dd, 3.5, 9.5)	83.9	3.24 (dd, 2.0, 6.0)	83.8	3.25[a]
5	71.4	3.75 (m)	70.0	3.83 (dq, 6.5, 9.5)	69.9	3.82 (dq, 6.5, 9.5)
6	18.7	1.24 (d, 6.0)	18.5	1.21 (*d*, 6.5)	18.5	1.19 (d, 6.5)
3-OMe	58.1	3.45 (s)	58.5	3.45 (s)	58.5	3.45 (s)
Cym II						
1			101.1	4.82 (dd, 2.0, 11.0)	101.1	4.81 (dd, 2.0, 11.0)
2			36.4	1.61 (m)/2.16 (m)	36.3	1.62 (m)/2.15 (m)
3			78.7	3.86[a]	78.7	3.85[a]
4			84.0	3.25 (dd, 2.0, 6.0)	84.0	3.25[a]
5			70.1	3.89 (dq, 6.5, 9.5)	70.1	3.88 (dq, 6.5, 9.5)
6			18.8	1.31 (d, 6.5)	18.8	1.31 (d, 6.5)
3-OMe			58.5	3.46 (s)	58.5	3.46 (s)
All						
1			104.0	4.60 (d, 8.0)	104.0	4.60 (d, 8.0)
2			73.3	3.38 (dd, 3.0, 8.0)	73.3	3.38 (dd, 3.0, 8.0)
3			83.9	3.65 (t, 3.0)	83.8	3.64 (t, 3.0)
4			74.9	3.20 (dd, 3.0, 9.5)	74.9	3.20 (dd, 3.0, 9.5)
5			70.9	3.69 (dq, 6.5, 9.5)	70.9	3.68 (dq, 6.5, 9.5)
6			18.3	1.24 (d, 6.5)	18.3	1.24 (d, 6.5)
3-OMe			62.5	3.62 (s)	62.6	3.62 (s)

Assignments were done by HSQC, HMBC, and COSY experiments.
[a] Overlapped signal; Cym, *β*-D-cymaropyranosyl; All, 6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl.

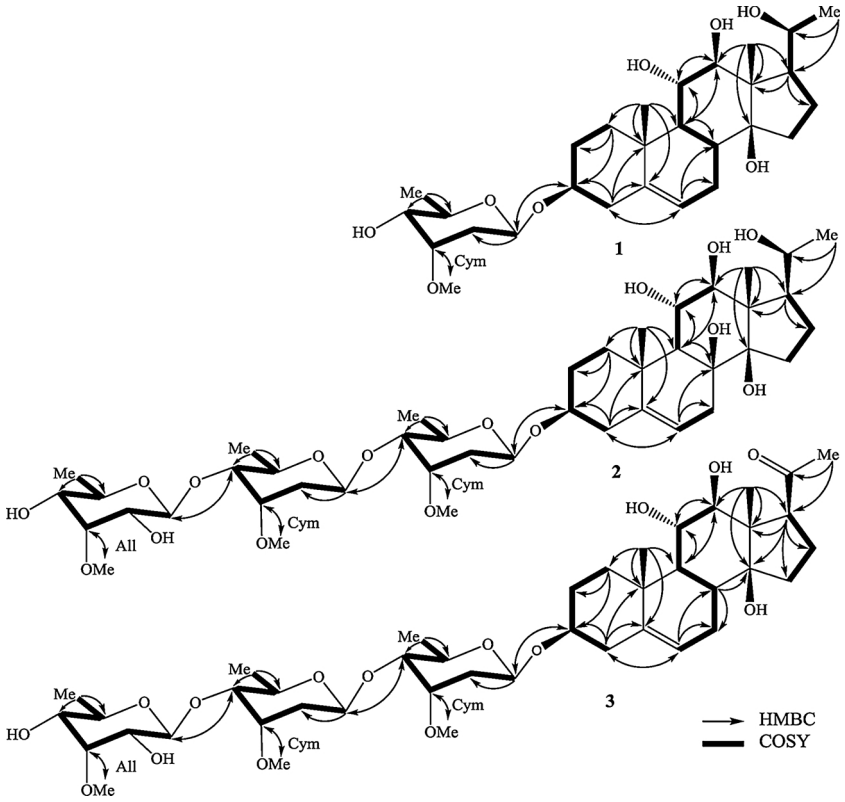

Fig. 2. The key HMBC and COSY correlations of compounds **1–2.**

configuration at C-20 was proved as *R* by comparing their ^{13}C-NMR chemical shifts at C-17 (δ_C 54.7), C-20 (δ_C 71.4), and C-21 (δ_C 23.0) to those of (20*R*)-3*β*,12*β*-diacetoxy-8*β*,14*β*,20-trihydroxypregn-5-ene [C-17 (δ_C 53.1), C-20 (δ_C 70.6), and C-21 (δ_C 23.4)] and (20*S*)-3*β*,12*β*-diacetoxy-8*β*,14*β*,20-trihydroxypregn-5-ene [C-17 (δ_C 51.9), C-20 (δ_C 65.3), and C-21 (δ_C 22.4)] (Kimura et al., 1982). The acid hydrolysis of **1** gave D-cymarose which identified by comparing its specific rotation with those reported (Warashina and Noro, 2000). The HMBC correlations between Cym I H-1 (δ_H 4.86) and C-3 (δ_C 79.2) determined the position of *O*-*β*-D-cymaropyranosyl at C-3 of aglycone. Thus, the structure of **1** was elucidated as (20*R*)-3*β*,11*α*,12*β*,14*β*,20-pentahydroxy-pregn-5-ene 3-*O*-*β*-D-cymaropyranoside and named volubiloside D.

Compound **2** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{42}H_{70}O_{16}$ by HR-ESI-MS at m/z 853.4561 $[M + Na]^+$ (calcd for $[C_{42}H_{70}O_{16}Na]^+$, 853.4556). The ^{1}H-NMR spectrum of **2** (CD_3OD) showed the specific signals of one pregnane aglycone: one olefinic proton, one secondary methyl group, and two tertiary methyl groups; three sugar units [three anomeric protons at δ_H 4.60 (d, J = 8.0 Hz), 4.82 (dd, J = 2.0, 11.0 Hz), and 4.88 (dd, J = 2.0, 9.5 Hz), three secondary methyl groups at δ_H 1.21 (d, J = 6.5 Hz), 1.24 (d, J = 6.5 Hz), and 1.31 (d, J = 6.5 Hz), three methoxy groups at δ_H 3.45 (s), 3.46 (s), and 3.62 (s)]. The ^{1}H- and ^{13}C-NMR data of **2** (Table 1) were found to be similar to those of drevoluoside N (Zhang et al., 2013) except for the absence of one sugar moiety. The HMBC correlations from H-6 (δ_H 5.34)/H-9 (δ_H 1.44) to C-8 (δ_C 76.9); H-9 (δ_H 1.44) to C-11 (δ_C 70.8)/C-12 (δ_C 82.6); H-18 (δ_H 1.28) to C-12 (δ_C 82.6)/C-13 (δ_C 54.0)/C-14 (δ_C 86.0)/C-17 (δ_C 56.9); and from H-21 (δ_H 1.20) to C-17 (δ_C 56.9)/C-20 (δ_C 70.5) confirmed the positions of hydroxyl groups at C-8, C-11, C-12, C-14, and C-20. The ^{13}C-NMR chemical shifts at C-17 (δ_C 56.9), C-20 (δ_C 70.5), and C-21 (δ_C 22.6) were identical to **2** confirmed the configuration at C-20 as *R*. The acid hydrolysis of **2** gave monosaccharides, which were recognized as D-cymarose, and 6-deoxy-3-*O*-methyl-D-allose based on specific rotations (Abe et al., 1999; Warashina and Noro, 2000). The HMBC correlations between All H-1 (δ_H 4.60) and Cym II C-4 (δ_C 84.0); 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 4.88) and C-3 (δ_C 79.6) determined the sugar linkages as 6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl and at C-3 of aglycone. These sugar linkages previously reported by Yoshimura S et al. from *Dregea* genus (Yoshimura et al., 1985). Thus, the structure of **2** was determined as (20*R*)-3*β*,8*β*,11*α*,12*β*,14*β*,20-hexahydroxy-pregn-5-ene 3-*O*-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside and named as volubiloside E.

Compound **3** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{42}H_{68}O_{15}$ by HR-ESI-MS at m/z 813.4614 $[M + H]^+$ (calcd for $[C_{42}H_{69}O_{15}]^+$, 813.4631). The ^{1}H-NMR spectrum of **3** exhibited one pregnane aglycone and three sugar units. The ^{13}C-NMR and HSQC spectra indicated that **3** contained one carbonyl, four non-protonated carbons, twenty methines, eight methylenes, and nine methyl carbons. The analysis of ^{1}H- and ^{13}C-NMR data indicated the structure of **3** was similar to those of dregeoside Da1 (**4**) (Yoshimura et al., 1985) with the appearance of a ketone group at C-20. The result of hydroxymethine oxidative process at C-20 to form the ketone group. This was confirmed by HMBC correlations from H-21 (δ_H 2.27) to C-17 (δ_C 59.0)/C-20 (δ_C 218.9). The configurations of functional groups of aglycone **3** were determined by the analysis of coupling constants and the observation on NOESY spectrum. Acid hydrolysis of **3** confirmed the existence of D-cymarose and 6-deoxy-3-*O*-methyl-D-allose. In addition, the HMBC correlations from All H-1 (δ_H 4.60) to Cym II C-4 (δ_C 84.0); Cym II H-1 (δ_H 4.81) to Cym I C-4 (δ_C 83.8); Cym I H-1 (δ_H 4.87) to C-3 (δ_C 79.2) proved the sugar linkages as 3-*O*-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl. Based on the above evidence, the structure of **3** was elucidated as 3*β*,11*α*,12*β*,14*β*-tetrahydroxy-pregn-5-ene-20-one 3-*O*-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside and named as volubiloside F.

Table 2
α-Glucosidase activity of compounds **1–6** at the concentration of 40 μM.

Compounds	α-glucosidase inhibition (%)
1	11.8 ± 0.5
2	16.9 ± 0.7
3	< 10
4	14.7 ± 0.3
5	51.3 ± 3.2
6	50.4 ± 3.1
Acarbose[a]	59.8 ± 1.6

[a] Acarbose was used as positive control at the concentration of 40 μM.

The known compounds were identified as dregeoside Da1 (**4**) (Yoshimura et al., 1985), volubiloside A (**5**) (Sahu et al., 2002), and drevoluoside N (**6**) (Zhang et al., 2013) by analysis of 1D and 2D NMR spectra and by comparison with those reported in the literature.

All compounds were screened for the α-glucosidase inhibitory activity at the concentration of 40 μM. Acarbose, an α-glucosidase inhbitor was used as a positive control. As shown in Table 2, compounds **5** and **6** showed the most significant α-glucosidase inhibitory activity with inhibition of 51.3 ± 3.2% and 50.4 ± 3.1%, compared to those of acarbose (59.8 ± 1.6%). This is the first report of α-glucosidase inhibitory activity of compounds from *D. volubilis*.

3. Experimental

3.1. General

All NMR spectra were recorded on a Bruker 500 MHz AVANCE III HD NMR spectrometer. HR-ESI-MS spectra were obtained using AGILENT Q-TOF system (Agilent HPLC 1290 infinity, Agilent Q-TOF 6550 iFunnel, dual AJS ESI source). HPLC was carried out using an AGILENT 1200 HPLC system (J'sphere ODS M-80 column, 150 mm length × 20 mm ID with the flow rate of 3 mL/min, detector DAD). Column chromatography (CC) was performed on silica-gel (Kieselgel 60, 230–400 mesh, Merck) or RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd.). For thin layer chromatography (TLC), pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates were used.

3.2. Plant material

The *Dregea volubilis* leaves were collected at Lang Son, Vietnam in September 2017 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P75) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of *D. volubilis* leaves (5.0 kg) was sonicated three times with hot methanol and then removed solvent to yield dark solid extract (630 g). The extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate giving *n*-hexane (DV1, 90 g), dichloromethane (DV2, 200 g), ethyl acetate extracts (DV3, 23 g) and residual water layer (DV4). The DV2 fraction was chromatographed on a silica gel column eluting with *n*-hexane/acetone (100:0 → 0:100, v/v) to give six sub-fractions, DV2A-DV2F. DV2D was chromatographed on a RP-18 column eluting with methanol/water (2/1, v/v) to give six sub-fractions, DV2D1-DV2D6. DV2D1 was chromatographed on a RP-18 column eluting with acetone/water (1.2/1, v/v) to give two smaller fractions, DV2D1A-DV2D1B. DV2D1A was further chromatographed on HPLC column eluting with ACN in H_2O (35%) to yield compound **3** (40.6 mg). Compounds **2** (19.1 mg) and **4** (88.4 mg) were obtained from DV2D1B on HPLC column eluting with ACN in H_2O (35%, v/v). DV2F was chromatographed on a RP-18 column eluting with acetone/water (1/1.8, v/v) to give three smaller fractions, DV2F1-DV2F3. DV2F1 fraction was chromatographed on a RP-18 column eluting with methanol/water (1/1, v/v) to give two smaller fractions, DV2F1A- DV2F1B. The DV2F1A was purified on HPLC column eluting with 20% acetonitrile to yield **1** (40.0 mg). Compounds **6** (7.3 mg) and **5** (151.0 mg) were obtained from DV2F1B chromatographed on HPLC column eluting with ACN in H_2O (24%).

3.3.1. Volubiloside D (1)

White amorphous powder; $[\alpha]_D^{25}$ — 14.1 (*c* 0.1, MeOH); $C_{28}H_{46}O_8$, HR ESI MS m/z: 545.2887 $[M + Cl]^-$ (calcd for $[C_{28}H_{46}O_8Cl]^-$, 545.2887); ^{1}H- (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) data, see Table 1.

3.3.2. Volubiloside E (2)

White amorphous powder; $[\alpha]_D^{25}$ — 27.5 (*c* 0.1, MeOH); $C_{42}H_{70}O_{16}$, HR ESI MS m/z: 853.4561 $[M + Na]^+$ (calcd for $[C_{42}H_{70}O_{16}Na]^+$, 853.4556); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1.

3.3.3. Volubiloside F (3)

White amorphous powder; $[\alpha]_D^{25}$ — 34.9 (*c* 0.1, MeOH); $C_{42}H_{68}O_{15}$, HR ESI MS m/z: 813.4614 $[M + H]^+$ (calcd for $[C_{42}H_{69}O_{15}]^+$, 813.4631); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1.

3.4. Acid hydrolysis

Each compound (**1–3**, 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); 6-deoxy-3-*O*-methyl-D-allose: found + 11.0 (*c* 0.4, H_2O); lit + 10.0 (Abe et al., 1999). Based on the above evidence and experiments, sugar components were found in compound **1**: D-cymarose; compounds **2** and **3**: D-cymarose and 6-deoxy-3-*O*-methyl-D-allose.

3.5. α-Glucosidase inhibitory assay

The α-glucosidase (Sigma–Aldrich, St. Louis, MO) enzyme inhibition assay was performed as the previously described method (Hanh et al., 2014). The sample solution (2.0 mL) dissolved in dimethyl sulfoxide) and 0.5 U/mL α-glucosidase (40 μL) were mixed in 0.1 M phosphate buffer (pH 7.0, 120 μL). After 5 min pre-incubation, *p*-nitrophenyl-α-D-glucopyranoside solution (5.10^{-3} M, 40 μL) 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).

Declaration of Competing Interest

The authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2020.04.013.

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