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POLYHYDROXYPREGNANE GLYCOSIDES FROM *DREGEA VOLUBILIS*

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Abstract. Four known polyhydroxypregnane glycosides, dregeoside Da1 (**1**), volubiloside A (**2**), drevoluoside N (**3**), and volubiloside C (**4**) were isolated from the methanol extract of the leaves of *Dregea volubilis* (L.f.) Benth. ex Hook. f. Their structures were elucidated by 1D-, 2D-NMR, spectra and compared with those reported in the literature. At concentration of 30 µM, compounds **1-4** did not exhibit cytotoxic activity against human colorectal adenocarcinoma cells (HT-29) with cell viability percentages ranging from 100.83 ± 1.50% to 105.45 ± 1.57% versus control. This is a new contribution to phytochemical study of *D. volubilis* in Viet Nam.

Keywords: Dregea volubilis, Apocynaceae, polyhydroxypregnane glycoside.

Classification numbers: 1.1.1, 1.2.1.

1. INTRODUCTION

Dregea volubilis (L.f.) Benth. ex Hook. f. (Apocynaceae) is a woody climbing plant that can be up to 12 m tall. It is used for treating inflammation, rheumatic pain, fever, cough, and severe cold [1]. Phytochemical screening indicated ethanol and water extracts of *D. volubilis* leaves having antibacterial activity against several microorganism such as *Bacillus subtilis*, *Staphylococcus aureus*, *S. warneri*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas putida*, and *P. aeruginosa* [2]. Ethanol extract of *D. volubilis* flowers has remarkable inhibitory effects on α-glucosidase and α-amylase activities [3]. At both oral doses of 100 and 200 mg/kgP/day during 15 days of treatment, petroleum ether extract of *D. volubilis* fruits dose dependently normalized blood glucose levels in streptozotocin induced hyperglycemic rats [4]. The chemical constituents of this plant have been then studied and showed to contain a lot of polyhydroxypregnanes and polyhydroxypregnane glycosides [5, 6], pentacyclic triterpenes [7], and flavonoids [8]. In our previous study, three new pregnane glycosides from the leaves of *D. volubilis* and their α-glucosidase inhibitory activity were reported [9]. Chemical structure of pregnane glycosides from *D. volubilis* contained interesting sugar units such as 6-

deoxy-3-*O*-methyl-D-allose, D-cymarose, D-digitoxose, and D-oleandrose which are rarely found in natural occurring compounds [5, 6]. In this paper, we continue to report detailed structural elucidation of four known polyhydroxypregnane-*type* glycosides from the leaves of *D. volubilis*. The cytotoxic activity of isolated compounds on human colorectal adenocarcinoma cells (HT-29) were also evaluated by MTS assay.

2. MATERIALS AND METHODS

2.1. Plant materials

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

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker 500 MHz. HPLC was carried out using an AGILENT 1100 HPLC system. 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.).

2.3. Extraction and isolation

The dried leaves of *D. volubilis* (5.0 kg) were sonicated with hot methanol then removed from solvent to yield solid extract (630 g). The extract was suspended in water and successively partitioned with *n*-hexane, and dichloromethane to give *n*-hexane (DV1, 90 g) and dichloromethane (DV2, 200 g) fraction and water layer. DV2 was chromatographed on a silica gel column eluting with *n*-hexane:acetone (100:0 → 0:1, v/v) to give fractions (DV2A-DV2F). DV2D was chromatographed on a RP-18 column eluting with methanol:water (2:1, v/v) to give smaller fractions (DV2D1-DV2D6). DV2D1 was chromatographed on a RP-18 column eluting with acetone:water (1.2:1, v/v) to give smaller fractions (DV2D1A and DV2D1B). Compound **1** (88.4 mg) was obtained from DV2D1B fraction on HPLC J'sphere ODS M-80 column (150 mm length×20 mm ID), 35 % ACN in H_2O, and a flow rate of 3 mL/min. DV2F was chromatographed on a RP-18 column eluting with acetone:water (1:1.8, v/v) to give smaller fractions (DV2F1-DV2F3). DV2F1 was chromatographed on a RP-18 column eluting with methanol:water (1:1, v/v) to give fractions (DV2F1A and DV2F1B). Compounds **2** (151.0 mg) and **3** (7.3 mg) were obtained from DV2F1B on HPLC column using J'sphere ODS M-80 (150 mm length×20 mm ID), 24 % ACN in H_2O, and a flow rate of 3 mL/min. DV2F3 was chromatographed on a RP-18 column eluting with methanol:water (1:1, v/v) to give two fractions (DV2F3A and DV2F3B). Compound **4** (24.0 mg) was obtained from DV2F3B by chromatography on HPLC using J'sphere ODS M-80 column (150 mm length ×20 mm ID), eluting with 24 % ACN in H_2O and a flow rate of 3 mL/min.

Dregeoside Da1 (1): White amorphous powder; $[\alpha]_D^{25}$ +10.5 (*c* 0.1, MeOH); MF $C_{42}H_{70}O_{15}$; HR-ESI-MS: *m/z* 859.4680 $[M+HCOO]^-$ (calcd for $C_{43}H_{71}O_{17}$, 859.4691); ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Volubiloside A (2): White amorphous powder; $[\alpha]_D^{25}$ –15.7 (*c* 0.1, MeOH); MF $C_{48}H_{80}O_{20}$; HR-ESI-MS: *m/z* 1021.5191 $[M+HCOO]^-$ (calcd for $C_{49}H_{81}O_{22}$, 1021.5219); ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Drevoluoside N (3): White amorphous powder; $[\alpha]_D^{25}$ +21.6 (*c* 0.1, MeOH); MF $C_{48}H_{80}O_{21}$; HR-ESI-MS: *m/z* 993.5248 $[M+H]^+$ (calcd for $C_{48}H_{81}O_{21}$, 993.5270); ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

Volubiloside C (4): White amorphous powder; $[\alpha]_D^{25}$ +24.4 (*c* 0.1, MeOH); MF $C_{48}H_{78}O_{20}$; HR-ESI-MS: 973.5022 $[M-H]^-$ (calcd for $C_{48}H_{77}O_{20}$, 973.5008); ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

2.4. Cytotoxic evaluation

HT29 cells were cultured in supplemented RPMI 1640 medium containing 10 % fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were seeded in a 96 well plate and incubated at 37 °C in humidified atmosphere (95 % air and 5 CO_2). After 24 h of incubation, the cells were treated with/without the compounds (final concentration of 30 µM) and incubated for additional 48 h. Culture medium was carefully removed and MTS solution was added for reaction in 1 h. The produced formazan by cellular reduction of MTS was quantified by measuring the absorbance at 490 nm with an Infinite M200 microplate reader (Tecan, Grodig, Austria). MTS assay was conducted using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, Madison, WI, USA). Experiments were performed in triplicate. Cell viability is expressed as the percentage of absorbance in sample wells compared to the vehicle.

3. RESULTS AND DISCUSSION

The dried powder of *D. volubilis* was extracted with methanol. Crude extract was then fractionated into low-polarity, mid-polarity, and high polarity fractions by successive separation with n-hexane and dichloromethane. Low-polarity fraction (n-hexane extract) contained oil and fatty compounds which were not subjected to chemical studies. After TLC analysis, dichloromethane extract was firstly selected for purification of compounds. Using combination of chromatographic methods, four compounds **1**-**4** were isolated from dichloromethane extract of *D. volubilis* leaves.

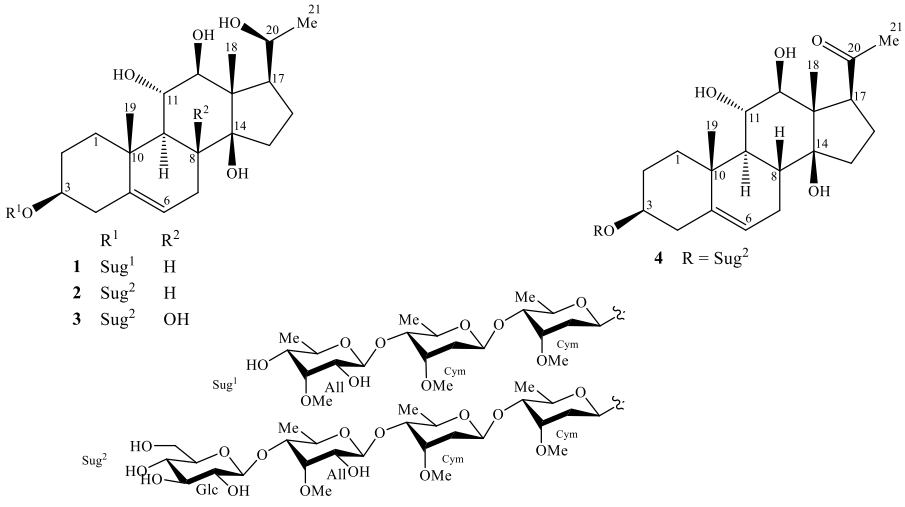

Figure 1. Chemical structures of compounds **1**-**4**.

Table 1. ^{1}H- and ^{13}C-NMR spectroscopic data for compounds **1** and **2** in CD_3OD.

C	**1**			**2**		
	δ_C [#]	δ_C	δ_H (mult, *J* in Hz)	δ_C [$]	δ_C	δ_H (mult, *J* in Hz)
1	39.7	40.2	1.13 (m)/2.68 (m)	39.8	40.2	1.14 (m)/2.68 (m)
2	30.6	30.8	1.58 (m)/1.84(m)	3.6	30.8	1.58 (m)/1.83 (m)
3	78.0	79.3	3.50 (m)	77.8	79.2	3.50 (m)
4	40.0	40.3	2.20 (m)/2.35 (m)	39.4	40.3	2.23 (m)/2.35 (m)
5	140.7	141.2	-	140.7	141.2	-
6	122.2	122.9	5.49 (br d, 5.5)	122.3	122.9	5.49 (br d, 5.5)
7	28.2	28.6	1.85 (m)/2.24 (m)	28.3	28.6	1.84 (m)/2.24 (m)
8	38.2	38.5	1.76 (m)	38.2	38.5	1.76 (m)
9	50.0	50.6	1.25 (d, 10.5)	49.9	50.5	1.25 (d, 10.5)
10	39.4	40.1	-	39.5	40.1	-
11	71.6	72.1	3.67 (dd, 10.0, 10.5)	71.6	72.1	3.66 (dd, 10.0, 10.5)
12	80.5	81.0	3.04 (d, 10.0)	80.5	80.9	3.04 (d, 10.0)
13	54.0	54.7	-	54.1	54.4	-
14	84.3	85.7	-	84.3	85.7	-
15	34.1	33.9	1.63 (m)/1.75 (m)	34.1	33.9	1.63 (m)/1.75 (m)
16	27.1	26.9	1.63 (m)/1.92 (m)	27.1	26.9	1.62 (m)/1.91 (m)
17	54.7	54.4	2.18 (m)	54.7	54.7	2.17 (m)
18	11.4	11.0	1.12 (s)	11.5	10.9	1.12 (s)
19	18.9	19.2	1.19 (s)	19.0	19.2	1.19 (s)
20	70.4	71.4	3.77 (dq, 6.5, 7.0)	70.5	71.4	3.78 (dq, 6.5, 7.0)
21	23.5	23.0	1.23 (d, 6.5)	23.7	23.0	1.23 (d, 6.5)
Cym I						
1	96.3	97.1	4.87 (br d, 10.0)	96.3	97.1	4.87 (dd, 2.0, 9.5)
2	36.9	36.6	1.57 (m)/2.07 (m)	37.3	36.6	1.58 (m)/2.08 (m)
3	78.1	78.5	3.86 (m)	78.0	78.6	3.86 (m)
4	83.8	83.8	3.25 (m)	83.2	83.8	3.24 (m)
5	69.0	69.9	3.83 (m)	69.0	69.8	3.86 (m)
6	18.5	18.5	1.20 (d, 6.5)	18.6	18.5	1.21 (d, 6.5)
3-OMe	58.8	58.5	3.46 (s)	59.0	58.5	3.45 (s)
Cym II						
1	100.3	101.1	4.80*	100.4	101.1	4.80*
2	36.9	36.2	1.62 (m)/2.16 (m)	37.1	36.3	1.64 (m)/2.15 (m)
3	77.8	78.6	3.86 (m)	78.1	78.5	3.86 (m)
4	83.3	83.9	3.25 (m)	83.4	84.0	3.24 (m)
5	69.3	70.1	3.87 (m)	69.2	69.9	3.86 (m)
6	18.5	18.3	1.31 (d, 6.5)	18.5	18.2	1.31 (d, 6.5)
3-OMe	58.8	58.4	3.45 (s)	58.9	58.5	3.45 (s)
All						
1	104.1	104.0	4.60 (d, 8.0)	104.0	103.8	4.60 (d, 8.0)
2	73.1	73.2	3.38 (dd, 3.0, 8.0)	72.5	72.6	3.40 (dd, 3.0, 8.0)
3	83.2	83.7	3.65 (t, 3.0)	83.0	83.1	3.98 (t, 3.0)
4	74.4	74.9	3.20 (dd, 3.0, 9.5)	83.0	83.8	3.36 (m)
5	70.7	70.9	3.69 (m)	68.8	70.0	3.86 (m)
6	18.5	18.8	1.24 (d, 6.5)	18.3	18.8	1.31 (d, 6.5)
3-OMe	62.0	62.6	3.62 (s)	61.8	62.0	3.62 (s)
Glc						
1				106.5	106.1	4.37 (d, 8.0)
2				75.5	75.4	3.21 (dd, 8.0, 9.0)
3				78.4	77.9	3.37 (m)
4				71.9	71.8	3.27 (m)
5				78.4	77.9	3.31 (m)
6				63.0	63.0	3.68 (dd, 5.5, 11.5)/3.92 (dd, 2.0, 11.5)

[#]δ_C of dregeoside Da1 in pyridine-d_5 [5]; [$]$\delta_C$ of volubiloside A in pyridine-d_5 [6]; Cym, β-D-cymaropyranosyl; All, 6-deoxy-3-O-methyl-β-D-allopyranosyl; Glc, glucopyranosyl; [*)]Overlapped signals.

Table 2. ^{1}H- and ^{13}C-NMR spectroscopic data for compounds **3** and **4** in CD_3OD.

C	**3**			**4**	
	δ_C	δ_H (mult, *J* in Hz)	δ_C[$]	δ_C	δ_H (mult, *J* in Hz)
1	41.4	1.09 (m)/2.67 (m)	40.0	40.4	1.15 (m)/2.67 (m)
2	30.4	1.66 (m)/1.81 (m)	30.7	30.8	1.57 (m)/1.84 (m)
3	79.6	3.54 (m)	77.9	79.3	3.50 (m)
4	40.2	2.31 (m)/2.37 (m)	40.1	40.3	2.20 (m)/2.35 (m)
5	142.0	-	140.7	141.2	-
6	118.8	5.34 (t, 3.5)	122.4	122.9	5.48 (d, 5.5)
7	36.1	1.64 (m)/2.17 (m)	28.4	28.7	1.81(m)/2.29 (m)
8	76.9	-	37.5	37.8	-
9	51.6	1.44 (d, 10.5)	49.9	50.5	1.27 (d, 12.0)
10	40.2	-	39.5	40.1	-
11	70.8	4.02 (dd, 10.0, 10.5)	71.9	72.3	3.65 (dd, 10.0, 10.5)
12	82.6	3.17 (d, 10.0)	78.5	78.8	3.07 (d, 9.5)
13	54.6	-	55.7	56.0	-
14	86.2	-	84.9	86.0	-
15	36.0	1.75 (m)/2.15 (m)	35.3	35.4	1.80 (m)/1.99 (m)
16	27.3	1.62 (m)/1.84 (m)	24.5	24.9	2.00 (m)
17	56.9	2.08 (m)	58.8	59.0	3.57 (m)
18	11.5	1.28 (s)	11.0	10.4	0.93 (s)
19	18.0	1.40 (s)	19.1	19.2	1.18 (s)
20	70.5	3.75 (dq, 6.5, 7.0)	216.7	218.9	-
21	22.6	1.20 (d, 6.5)	32.6	32.6	2.26 (s)
Cym I					
1	97.1	4.89 (dd, 2.0, 9.5)	96.4	97.2	4.87 (dd, 1.5, 9.5)
2	36.6	1.59 (m)/2.09 (m)	37.3	36.4	1.55 (m)/2.15 (m)
3	78.6	3.87 (m)	78.2	78.6	3.86 (m)
4	83.8	3.25 (m)	83.5	83.8	3.24 (m)
5	69.9	3.83 (m)	69.2	69.9	3.82 (m)
6	18.5	1.21 (d, 6.5)	18.7	18.5	1.21 (d, 6.5)
3-OMe	58.5	3.45 (s)	59.2	58.5	3.45 (s)
Cym II					
1	101.1	4.81*	100.5	101.1	4.81*
2	36.4	1.65 (m)/2.15(m)	37.5	36.6	1.55(m)/2.07(m)
3	78.7	3.87 (m)	78.3	78.7	3.86 (m)
4	84.1	3.25 (m)	83.5	84.1	3.24 (m)
5	70.0	3.87 (m)	69.4	70.0	3.85 (m)
6	18.2	1.30 (d, 6.5)	18.4	18.2	1.31 (d, 6.5)
3-OMe	58.4	3.45 (s)	59.0	58.4	3.45 (s)
All					
1	103.9	4.60 (d, 8.0)	104.2	103.9	4.60 (d, 8.5)
2	72.7	3.39 (dd, 3.0, 8.0)	72.7	72.6	3.39 (dd, 3.0, 8.0)
3	83.2	3.98 (t, 3.0)	83.4	83.2	3.97 (t, 2.0)
4	83.8	3.36 (m)	83.4	83.8	3.36 (m)
5	70.1	3.86 (m)	69.4	70.1	3.87 (m)
6	18.7	1.31 (d, 6.5)	18.7	18.8	1.30 (d, 6.5)
3-OMe	62.0	3.62 (s)	61.9	62.0	3.62 (s)
Glc					
1	106.2	4.37 (d, 8.0)	106.7	106.2	4.37 (d, 7.5)
2	75.5	3.21 (dd, 8.0, 9.0)	75.6	75.5	3.20 (m)
3	78.0	3.37 (m)	78.5	78.0	3.37 (m)
4	71.9	3.27 (m)	72.1	71.9	3.26 (m)
5	78.1	3.31 (m)	78.0	77.9	3.37 (m)
6	63.1	3.68 (dd, 6.0, 11.5) 3.92 (dd, 2.0, 11.5)	63.2	63.1	3.67 (dd, 6.0, 11.5) 3.92 (dd, 2.0, 11.5)

[#&] δ_C of volubiloside C in pyridine-d_5 [6]; Cym, β-D-cymaropyranosyl; All, 6-deoxy-3-O-methyl-β-D-allopyranosyl; Glc, glucopyranosyl. [*)] Overlapped signals.

Compound **1** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of compound **1** showed the signals of one olefinic proton [δ_H 5.49 (1H, br d, J = 5.5 Hz)], one secondary methyl group [δ_H 1.23 (1H, d, J = 6.5 Hz)], and two tertiary methyl groups [δ_H 1.12 (3H, s) and 1.20 (3H, s)], suggesting the appearance of a pregnane aglycone; three anomeric protons [δ_H 4.87 (1H, br d, J = 10.0 Hz), 4.80 (overlapped signal), and 4.60 (1H, d, J = 8.0 Hz)], two secondary methyl groups [δ_H 1.20 (1H, d, J = 6.5 Hz), 1.24 (1H, d, J = 6.5 Hz), and 1.31 (d, J = 6.5 Hz)], and three methoxy groups [δ_H 3.45, 3.46, and 3.60 (each 3H, s)] suggesting the appearance of three sugar units.

The ^{13}C-NMR and HSQC spectra of compound **1** (Table 1) exhibited the signals of 42 carbons, including 4 non-protonated carbons, 21 methines, 8 methylenes, and 9 methyl carbons. The ^{1}H- and ^{13}C-NMR data was found to be identical to dregeoside Da1 (**1**) [5]. The double bond at C-5/C-6 was indicated by HMBC (Figure 2) correlations from H-19 (δ_H 1.19) to C-1 (δ_C 40.2)/C-5 (δ_C 141.2)/C-9 (δ_C 50.6)/C-10 (δ_C 40.1). The hydroxyl groups were at C-11, C-12, C-14, and C-20 was confirmed by the HMBC correlations between H-9 (δ_H 1.25) and C-11 (δ_C 72.1)/C-12 (δ_C 81.0); H-18 (δ_H 1.12) and C-12 (δ_C 81.0)/C-13 (δ_C 54.7)/C-14 (δ_C 85.7)/C-17 (δ_C 54.4); and between H-21 (δ_H 1.22) and C-17 (δ_C 54.4)/C-20 (δ_C 71.4). The ^{13}C-NMR spectra of three sugar moieties (δ_C 97.1, 36.6, 78.5, 83.8, 69.9, 18.5, and 58.5; 101.1, 36.2, 78.6, 83.9, 70.1, 18.3, and 58.4; 104.0, 73.2, 83.7, 74.9, 70.9, 18.8, and 62.6) as well as multiplicity of anomeric protons (δ_H Cym I: 4.87 (br d, J = 10.0 Hz) and 4.60 (d, J = 8.0 Hz) indicated the sugar moieties as *β*-D-cymaropyranosyl and *β*-D-allopyranosyl. The HMBC correlations between All H-1 (δ_H 4.60) and Cym II C-4 (δ_C 83.9); Cym II H-1 (δ_H 4.80) and Cym I C-4 (δ_C 83.8); and between Cym I H-1 (δ_H 4.87) and C-3 (δ_C 79.3) 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. Furthermore, careful examination of J coupling at related protons in ^{1}H-NMR spectra also supported their relative configurations at C-3, C-11, and C-12. Particularly, signal of H-11 appeared as double doublet with both large J values (10.5 Hz) indicated both *trans axial* orientations of H-9/H-11 and H-11/H-12. In bio-synthesis partway steroid, H-9 always locates at *α-axial* position. Therefore, the large coupling constant of $J_{H\text{-}9/H\text{-}11}$ and $J_{H\text{-}11/H\text{-}12}$ indicated *β-axial* position of H-11 and *α-axial* position of H-12 which were corresponding to *α*-orientation of 11-OH and *β*-orientation of 12-OH.

Although signal of H-3 appeared as multiplet and made it difficult to calculate J value, carbon chemical shift value of C-3 (δ_C: 79~80 ppm) supported for *β*-configuration at C-3 [5]. Thus, the structure of **1** was identified as dregeoside Da1 [5], and this compound was reported from *D. volubilis*.

The ^{1}H-NMR of **2** showed the signals of one olefinic proton at δ_H 5.49 (1H, br d, J = 5.5 Hz), three methyl groups at δ_H 1.12 (3H, s), 1.19 (3H, s) and 1.23 (3H, d, J = 6.5 Hz) suggesting the presence of a pregnane. In addition, the 1H-NMR spectrum also exhibited four anomeric protons at δ_H 4.37 (1H, d, J = 8.0 Hz), 4.60 (1H, d, J = 8.0 Hz), 4.80 (1H, overlapped signal), and 4.87 (1H, dd, J = 2.0, 9.5 Hz) suggesting the presence of four sugar units. The ^{13}C-NMR of **2** exhibited the signals of 48 carbons, including 21 carbons of pregnane aglycone and 27 carbons of sugar units. Analysis of ^{1}H- and ^{13}C-NMR indicated the structure of **2** was similar to that of **1** with the addition of a glucopyranosyl unit. The sugar linkage was determined as *β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allomethylpyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside by HMBC correlations between Glc H-1 (δ_H 4.37) and All C-4 (δ_C 83.8); All H-1 (δ_H 4.60) and Cym II C-4 (δ_C 84.0); Cym II H-1 (δ_H 4.81) and Cym I C-4 (δ_C 83.8). The position of sugar linkage at C-3 of aglycone was confirmed by the

HMBC correlation between Cym I H-1 (δ_H 4.86) and C-3 (δ_C 78.4). Thus, the structure of **2** was defined as volubiloside A [6].

Compound **3** was obtained as a white amorphous powder. Analysis of ^{1}H- and ^{13}C-NMR also indicated the structure of **3** to be drevoluoside N [10]. Similar to **2**, the sugar linkage was determined as *β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allomethylpyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside by the observation on HMBC spectra. The HMBC correlations from H-19 to C-1/C-5/C-9/C-10; from H-6/H-9/H-11 to C-8; from H-18 to C-12/C-14/C-17; from H-21 to C-17/C-20 indicated the location of hydroxyl groups at C-8, C-11, C-12, C-14, and C-20.. Thus, the structure of **3** was elucidated as drevoluoside N.

The ^{1}H-NMR of **4** exhibited one olefinic proton at δ_H 5.48 (1H, d, J = 5.5 Hz), three methyl groups at δ_H 0.93 (3H, s), 1.18 (3H, s), and 2.26 (3H, s), assigned to a pregame aglycone; four anomeric protons at δ_H 4.37 (1H, d, J = 7.5 Hz), 4.60 (1H, d, J = 8.5 Hz), 4.81 (overlapped), and 4.87 (1H, dd, J = 1.5, 9.5 Hz). The ^{13}C-NMR and HSQC of **4** showed one carbonyl, five non-protonated carbons, 25 methines, and 9 methyl carbons. The ^{1}H- and ^{13}C-NMR data of **4** was identical to that of volubiloside C [6]. In addition, the position of functional groups was also reconfirmed by the analysis of HSQC and HMBC spectra. Thus, the structure of **4** was elucidated as volubiloside C.

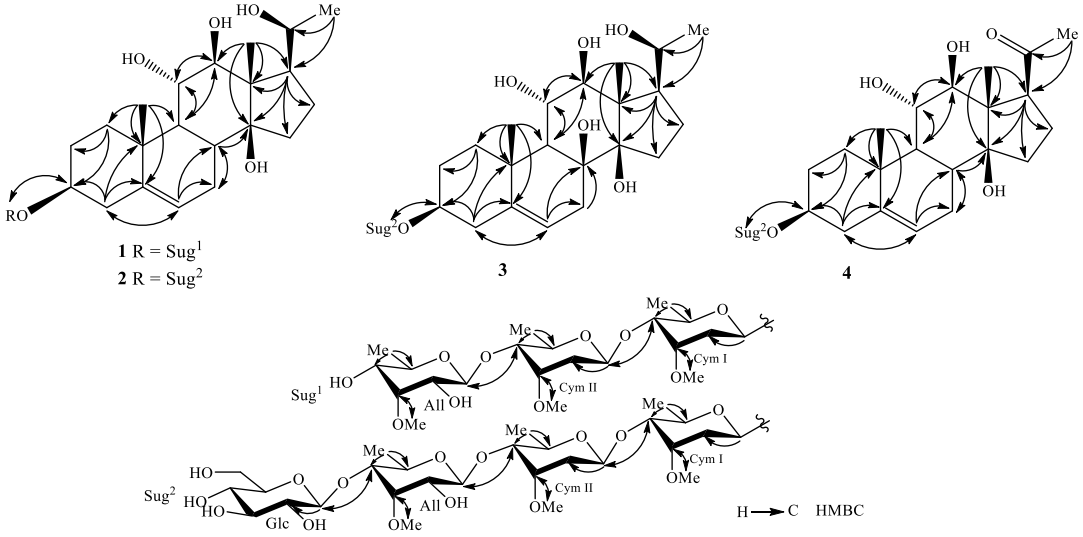

Figure 2. The key HMBC correlations of compounds **1** – **4**.

Compounds **1**-**4** were evaluated for their cytotoxic effects on HT-29 cell using MTS assay. At concentration of 30 µM, compounds **1**-**4** did not significantly inhibit HT-29 cell proliferation. The percentages of cell viability were obtained to be 101.15 ± 1.50 %, 105.45 ± 1.57 %, 100.83 ± 1.50 %, and 102.86 ± 1.53 % in the presence of compounds **1**-**4** (30 µM), respectively.

4. CONCLUSIONS

The present article reports on phytochemical study of the *Dregea volubilis*. From the methanol extract of the leaves, four polyhydroxypregnane glycosides as dregeoside Da1 (**1**), volubiloside A (**2**), drevoluoside N (**3**), and volubiloside C (**4**) were isolated and structurally

elucidated. Their chemical structures were elucidated by 1D, 2D NMR spectra and compared with those reported in the literature. At a concentration of 30 µM, all of the compounds **1**-**4** did not show significant cytotoxic activity on HT-29 cells.

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