

## Two New C<sub>21</sub> Steroidal Glycosides from the Leaves of *Hoya parasitica*

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**Abstract:** Two new pregnane glycosides, parasiticoside A (1) and parasiticoside B (2), were isolated from the leaves of *Hoya parasitica*. Their structures were determined by spectroscopic analysis 1D and 2D NMR, and mass spectrometry (HR-ESI-MS in positive mode) techniques as 3 $\beta$ ,20-dihydroxy-pregn-5-ene-3-*O*- $\beta$ -D-fucopyranoside-20-*O*- $\beta$ -D-glucopyranoside (1), and 3 $\beta$ ,20-dihydroxy-pregn-5-ene-3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fucopyranoside-20-*O*- $\beta$ -D-glucopyranoside (2). These compounds are believed to be the first example of pregnane glycosides from a plant of the genus *Hoya*.

**Keywords:** *Hoya parasitica*; Apocynaceae; pregnane glycoside; parasiticoside A; parasiticoside B; NMR.  
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### 1. Introduction

The *Hoya* genus, belonging to the Apocynaceae plant family, contains about 350 species, some of which are planted for ornamental purposes. Majority of species of this genus are distributed in tropical and subtropical South and Southeast Asia [1]. Some species of the genus such as *H. kerrii*, *H. parasitica*, were reported to possess biological activities including antibacterial, anticancer, antinociceptive, antioxidant, and anti-inflammatory activities [2–6]. Previous phytochemical studies on the genus revealed the presence of pregnanes, triterpenoids, sesquiterpenoids, pregnane glycosides, androstanes, phenolic compounds, and oligosaccharides [2,3,7–12].

*H. parasitica* (synonym: *H. acuta*) of the *Hoya* genus is commonly known in Viet Nam as waxvine, waxflower, or “Lan cam cu”. This species is a parasite creeper with a fragrant flower, whose aerial part is used to cover wounds, increase the milk supply, and treat melasma in children [13]. A previous phytochemical investigation on this plant reported the isolation of triterpenes, triterpenoids, pregnanes, phenolic compounds, triterpenoids [3,11,14]. As part of our ongoing studies for investigation of phytotoxic natural products from Vietnamese medicinal plants, the leaves of *H. parasitica* were further investigated in the present study, led to the isolation of two new pregnane glycosides, named as parasiticoside A (1) and parasiticoside B (2). This paper reports the isolation and structural determination of these new compounds.

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## 2. Materials and Methods

### 2.1. General Experimental Procedures

An Elmasonic S10H ultrasonicator (Elma, Switzerland) was used for the extraction of the leaves of species. The purification of the compounds was performed on a vacuum liquid chromatography (VLC) system on silica gel RP-18 (75-200  $\mu$ m, Silicycle, Canada), and silica gel 60 (63-200  $\mu$ m, Sigma-Aldrich, France). The isolation and further purification of saponins from the subfractions were carried out on medium-pressure liquid chromatography (MPLC) system by using stationary phase on Merck silica gel 60, particle size 15-40  $\mu$ m (Germany) with a Gilson M 305 pump and a fraction collector (Biopharmacia). A Büchi glass columns (460 mm x 25 mm, 110 mm x 15 mm) were used in the MPLC system. Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were performed on Merck silica gel 60F<sub>254</sub> (Germany), development with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O-AcOH (70:30:5:1). TLC and HPTLC spots were visualized by spraying the plates with a mixture of EtOH-H<sub>2</sub>SO<sub>4</sub> (50:1), heated at 100 °C for 10 min. Optical rotation was determined on an AA-10R automatic polarimeter (Optical Activity LTD, England). High resolution electrospray ionization mass spectrum (HR-ESI-MS) was carried out on a Bruker microTOF II mass spectrometer (Bruker, Germany) in positive-ion mode. Gas chromatography (GC) was carried out on a ThermoQuest Trace Gas Chromatograph apparatus (Thermo Scientific, USA) using an OV-17 on Silanox (0.3 mm x 50 m) column. 1D and 2D NMR were recorded on an NMR Inova 600 MHz spectrometer (Agilent Technologies, USA) in pyridine-*d*<sub>5</sub>.

### 2.2. Plant Material

*Hoya parasitica* was collected from at Hop Thanh, Son Duong, Tuyen Quang, Viet Nam in February 2023 at geographic coordinates of 21°41'04" N, 105°29'03" E, and authenticated by Prof. Thuong Danh Sy, Department of Botany. The voucher specimen (N° HOYP202302TQ1) was deposited in the Department of Biology, Thai Nguyen University of Education, Thai Nguyen, Viet Nam.

### 2.3. Extraction and Isolation

The extraction of the dried powdered leaves (53.6 g) of *H. parasitica* was performed by the ultrasound-assisted method using the solvent EtOH/H<sub>2</sub>O (75:35) at the condition following: 200 W, 60 °C, 45 min, 500 mL each x 2 times. After filtration and evaporation, 6.18 g of crude of extract was yielded and further separated by a VLC silica gel RP-18 and eluted respectively with H<sub>2</sub>O 100%, EtOH/H<sub>2</sub>O 50:50, EtOH 100%, respectively resulting 3 fractions (A-C) based on a TLC analysis. Fraction B (519.6 mg) was chromatographed on a MPLC silica gel 60 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 80:20:2, 75:25:3, v/v/v) resulting 5 subfractions (B1-B5). Subfraction B2 (32.5 mg) was submitted again on a successive MPLC silica gel 60 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 80:20:2, v/v/v) affording **1** (3.3 mg). Compound **2** (3.1 mg) was obtained from subfraction B3 (64.5 mg) by a successive MPLC silica gel 60 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 75:25:3, v/v/v).

*Parasiticoside A (1)*: Amorphous white powder,  $[\alpha]_D^{25} = -38^\circ$  (MeOH, *c* 0.30). HR-ESI-MS: *m/z* 649.3563 [M+Na]<sup>+</sup> (calcd. for C<sub>33</sub>H<sub>54</sub>NaO<sub>11</sub>, 649.3558). <sup>1</sup>H and <sup>13</sup>C NMR data are given in Table 1.

*Parasiticoside B (2)*: Amorphous white powder,  $[\alpha]_D^{25} = -45^\circ$  (MeOH, *c* 0.22). HR-ESI-MS: *m/z* 795.4142 [M+Na]<sup>+</sup> (calcd. for C<sub>39</sub>H<sub>64</sub>NaO<sub>15</sub>, 795.4137). <sup>1</sup>H and <sup>13</sup>C NMR data are given in Table 1;

### 2.4. Acid Hydrolysis and GC Analysis

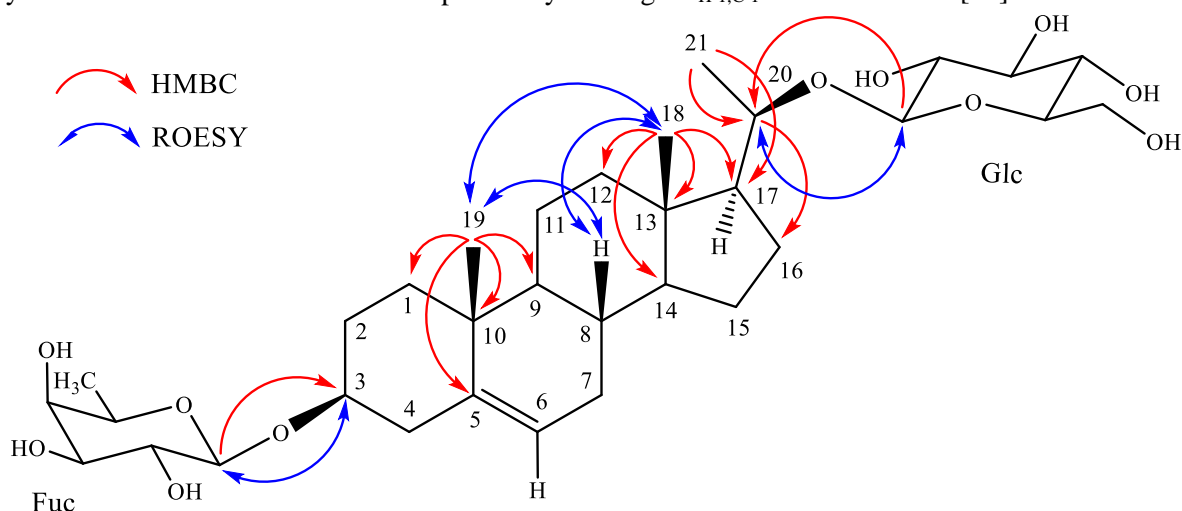
Hydrolysis of 3 mg of each pregnane glycoside was performed with 5 mL of 2N aq. CF<sub>3</sub>COOH (4 mL) and water (1 mL) for 3h at 95 °C. The reaction mixture was then diluted in water (10 mL) and extracted with 5 mL of CH<sub>2</sub>Cl<sub>2</sub> (3 times). The CH<sub>2</sub>Cl<sub>2</sub> layer was evaporated with MeOH until the solvent became neutral to give the sugar residue, and further chromatographed by TLC over silica gel using

eluent system of  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  8:5:1 by comparison with authentic samples. Furthermore, the monosaccharide mixture was diluted with 100  $\mu\text{L}$  of anhydrous pyridine, and treated with 0.06 mol/L of  $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOCH}_3\cdot\text{HCl}$  at 60  $^\circ\text{C}$  for 1 h, followed by adding 150  $\mu\text{L}$  of HMDS-TMCS (3:1) at 60  $^\circ\text{C}$  for 30 min. After stopping the reaction by addition of 2 mL of  $\text{H}_2\text{O}$ , the sugar residues were extracted with 0.1 mL *n*-hexane. The *n*-hexane layer was collected, and then analyzed (1  $\mu\text{L}$ ) by GC. The identification of sugars was performed by comparison of their retention times with those of standard sugars (Sigma-Aldrich) [15]. The retention times (min): 11.07 (rhamnose), 13.82 (fucose), and 17.51 (glucose).

### 3. Results and Discussion

#### 3.1. Structure Elucidation

The performance on the aqueous ethanol extract of leaves of *H. parasitica* led to the isolation and structure elucidation of two new pregnane glycosides **1** and **2**. Acid hydrolysis and extensive 2D NMR spectra of these compounds gave sugar moieties identified as D-fucopyranosyl (Fuc), D-glucopyranosyl (Glc) for **1**, and D-fucopyranosyl (Fuc), D-glucopyranosyl (Glc), and  $\alpha$ -rhamnopyranosyl (Rha) for **2**. The monosaccharides, including their absolute configurations, were identified by GC analysis as D for Fuc and Glc for **1**, and D for Fuc and Glc, and L for Rha for **2** (the retention times at 11.07 min for Rha, 13.82 min for Fuc and 17.51 min for Glc [15]). A  $\beta$  anomeric orientation of Fuc and Glc units was determined by calculating the relatively large  $^3J_{\text{H-1,H-2}}$  values at 7.6 Hz [16], [17]. An equatorial  $\alpha$ -pyranoid form was set to the Rha unit proved by the large  $^3J_{\text{H-1,C-1}}$  value at 166 Hz [18].



**Figure 1.** The structure of compound **1** with keys of HMBC and ROESY correlations

Compound **1** displayed a pseudo-molecular ion peak at  $m/z$  649.3563  $[\text{M}+\text{Na}]^+$  (calcd. 649.3558) in the HR-ESI-MS spectrum, thus the molecular formula was assigned as  $\text{C}_{33}\text{H}_{54}\text{O}_{11}$  with a molecular weight of 626. The  $^1\text{H}$  NMR spectrum of **1** showed the signals of three methyl groups at  $\delta_{\text{H}}$  0.85 (s, H-18), 1.28 (s, H-19), and 1.12 (d,  $J = 6.0$  Hz, H-21), a trisubstituted olefinic proton at  $\delta_{\text{H}}$  5.67 (br s, H-6), and two anomeric protons at  $\delta_{\text{H}}$  4.78 (d,  $J = 7.6$  Hz) and 4.90 (d,  $J = 7.6$  Hz). The  $^{13}\text{C}$  NMR spectrum of **1** displayed resonances of thirty-three carbon resonances that was classified as four methyls at  $\delta_{\text{C}}$  16.9 (Me-18), 16.0 (Me-19), 23.5 (Me-21), and 17.0 ( $\text{C}'$ -6), nine methylenes (37.5, 31.3, 39.3, 32.1, 21.0, 39.1, 28.0, 26.4, 62.4), seventeen methines (78.1, 125.0, 31.8, 50.3, 56.7, 62.4, 81.3, 99.8, 74.2, 76.6, 72.9, 70.8, 103.4, 74.7, 78.0, 72.2, 78.1), and three quaternary carbons (140.9, 36.9, 40.5). These signals were in good agreement with those of pregnane diglycoside as described in the literature data [19]. The ROESY cross peaks of H-8 with H<sub>3</sub>-19 and H<sub>3</sub>-18 disclosed these groups as co-facial and defined as an  $\beta$ -orientation. The absence of ROESY correlations between H-8 and H-9/H-14/H-17 established an  $\alpha$ -orientation of these protons. The configuration of C-20 could not be determined on the ROESY spectrum due to the free rotation of the C-17 – C-20 bond [20].

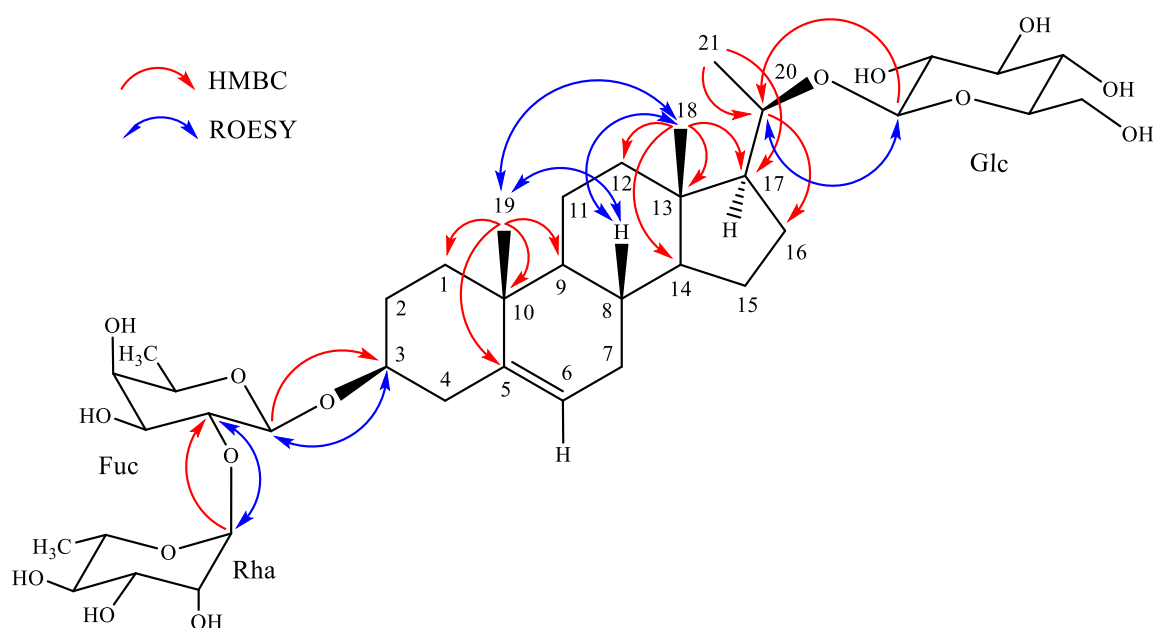
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In the sugar region, the signals of two cross-peaks in the HSQC spectrum at  $\delta_{\text{H}}/\delta_{\text{C}}$  4.78 (d,  $J = 7.6$  Hz)/99.8, and 4.90 (d,  $J = 7.6$  Hz)/103.4, indicated the presence of two sugar units, Fuc and Glc, respectively (Table 1). The Fuc unit was determined to be linked to the C-3 position of the aglycone by the HMBC correlation at  $\delta_{\text{H}}/\delta_{\text{C}}$  4.78 (d,  $J = 7.6$  Hz, Fuc H-1)/78.1 (C-3), together with the ROESY cross-peak between  $\delta_{\text{H}}$  4.78 (d,  $J = 7.6$  Hz, Fuc H-1) and  $\delta_{\text{H}}$  3.93 (m, H-3). The linkage of the Glc unit to the C-20 position of the aglycone was proved by the HMBC correlation at  $\delta_{\text{H}}/\delta_{\text{C}}$  4.90 (d,  $J = 7.6$  Hz, Glc H-1)/81.3 (C-20), and the ROESY correlation at  $\delta_{\text{H}}/\delta_{\text{H}}$  4.90 (d,  $J = 7.6$  Hz, Glc H-1)/3.98 (m, H-20). Therefore, the structure of **1** was assigned as 3 $\beta$ ,20-dihydroxy-pregn-5-ene-3-*O*- $\beta$ -D-fucopyranoside-20-*O*- $\beta$ -D-glucopyranoside, and trivially named as parasiticoside A (Figure 1).

**Table 1.** <sup>13</sup>C and <sup>1</sup>H NMR spectral data of compounds **1** and **2** (pyridine-*d*<sub>5</sub>,  $\delta$  in ppm,  $J$  in Hz)

	<b>1</b>		<b>2</b>	
Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	37.5	1.06 m, 1.62 m	37.4	1.08 m, 1.66 m
2	31.3	1.70 m, 2.14 m	32.3	1.75 m, 2.18 m
3	78.1	3.93 ov	77.9	3.97 ov
4	39.3	2.28 m, 2.72 m	39.2	2.40 m, 2.82 m
5	140.9	-	140.9	-
6	125.0	5.67 br s	125.0	5.72 br s
7	32.1	1.40 m, 1.95 m	32.7	1.43 m, 1.97 m
8	31.8	1.30 ov	32.2	1.35 ov
9	50.3	0.80 m	50.3	0.87 m
10	36.9	-	36.3	-
11	21.0	1.27 m, 1.45 m	23.9	1.18 m, 1.48 m
12	39.1	1.00 m, 1.82 m	39.3	1.11 m, 1.83 m
13	40.5	-	40.6	-
14	56.7	1.08 ov	56.7	1.09 ov
15	28.0	1.91 m, 2.40 m	28.0	1.92 m, 2.48 m
16	26.4	1.05 m, 1.53 m	26.8	1.09 m, 1.54 m
17	62.4	1.60 ov	62.3	1.64 ov
18	16.9	0.85 s	16.9	0.91 s
19	16.0	1.28 s	15.9	1.26 s
20	81.3	3.98 ov	81.2	4.01 ov
21	23.5	1.12 d (6.0)	23.6	1.28 d (6.0)
Fuc-1	99.8	4.78 d (7.6)	99.9	4.80 d (7.6)
2	74.2	4.52 dd (9.4, 7.6)	74.4	4.53 dd (9.4, 7.6)
3	76.6	4.08 dd (9.4, 2.9)	76.4	4.12 dd (9.4, 2.9)
4	72.9	3.93 br d (2.9)	73.1	3.96 br d (2.9)
5	70.8	3.62 br q (6.4)	70.9	3.68 br q (6.4)
6	17.0	1.46 d (6.4)	16.8	1.49 d (6.4)
Glc-1	103.4	4.90 d (7.6)	103.5	4.88 d (7.6)
2	74.7	4.05 ov	74.6	4.06 ov
3	78.0	4.26 ov	77.9	4.28 ov
4	72.2	4.18 ov	72.3	4.18 ov
5	78.1	3.96 m	78.1	3.96 m
6	62.4	4.33 m, 4.54 m	62.3	4.30 m, 4.52 m
Rha-1			101.2	6.35 br s
2			72.1	4.79 br s
3			72.2	4.61 dd (9.4, 3.5)
4			73.8	4.35 dd (9.9, 9.4)
5			69.0	4.83 dq (9.9, 5.9)
6			18.7	1.78 d (5.9)

\*Overlapped proton signals (ov) are reported without designated multiplicity



**Figure 2.** The structure of compound **2** with keys of HMBC and ROESY correlations

The HR-ESI-MS spectrum of compound **2** gave an  $[M+Na]^+$  pseudo-molecular ion peak at  $m/z$  795.4142 (calcd. 795.4137), thus the molecular formula was assigned as  $C_{39}H_{64}O_{15}$  with a molecular weight of 772. The difference between molecular weight of **2** and **1** is 146 amu, which could correspond to a supplementary 6-deoxyhexosyl group. On comparison of the  $^{13}C$  NMR signal of **2** (Table 1), a set of additional signals, corresponding to a terminal  $\alpha$ -L-rhamnopyranosyl unit appeared. The glycosidic linkage between the Fuc unit and the C-3 position of the aglycone was determined by the HMBC correlation at  $\delta_H/\delta_C$  4.78 (d,  $J = 7.6$  Hz, Fuc H-1)/78.1 (C-3), and the ROESY correlation at  $\delta_H/\delta_H$  4.80 (d,  $J = 7.6$  Hz, Fuc H-1)/3.97 (m, H-3). The Rha unit was proved to be attached at the C-2 position of the Fuc unit by the HMBC correlation between  $\delta_H$  6.35 (br s, Rha H-1) and  $\delta_C$  74.4 (Fuc C-2), and the ROESY correlation between  $\delta_H$  6.35 (br s, Rha H-1) and  $\delta_H$  4.53 (dd,  $J = 9.4, 7.6$  Hz, Fuc H-2). Finally, the Glc was proved to be linked to the C-20 position of the aglycone by the HMBC correlation at  $\delta_H/\delta_C$  4.88 (d,  $J = 7.6$  Hz, Glc H-1)/81.2 (C-20), and the ROESY correlation at  $\delta_H/\delta_H$  4.88 (d,  $J = 7.6$  Hz, Glc H-1)/4.01 (m, H-20). All the evidences above led to the elucidation of **2** as 3 $\beta$ ,20-dihydroxy-pregn-5-ene-3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fucopyranoside-20-*O*- $\beta$ -D-glucopyranoside, trivially named as parasiticoside B (Figure 2).

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## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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