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GLYCOSIDES ISOLATED FROM THE AERIAL PARTS OF *PREMNA INTEGRIFOLIA* GROWING IN THAI BINH

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Abstract. From the aerial parts of *Premna integrifolia* L., three glycosides: acteoside (**1**), premnaodoroside A (**2**), and premnaodoroside B (**3**) were isolated. Their chemical structures were elucidated by means of ESI-MS, ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC spectra and compared with the previous literature. To our best knowledge, this is the first report of **1** and **3** from *P. integrifolia*.

Keywords: Premna integrifolia, acteoside, premnaodoroside A, premnaodoroside B.

Classification numbers: 1.1.1; 1.1.6

1. INTRODUCTION

Premna intergrifolia L. (Verbenaceae), a herb known as "vong cach", "cach" or "bong cach" in Vietnamese, is widely distributed in Papua New Guinea, Australia, and South-East Asian countries [1]. The aerial parts of this plant have been used in folk medicines to treat cirrhosis and dysentery [2]. The phytochemical investigations of *P. intergrifolia* confirmed the presence of alkaloids [2], flavonoids [2-4], lignans [5, 6], iridoids [3, 7], and terpenoids [8]. In addition, biological activities of methanol extracts and isolated compounds from *P. intergrifolia* have been studied, such as anti-bacterial [6, 9, 10], anti-inflammatory [11, 12], anti-oxidant [6, 13-16], anti-diabetic [13, 17] anti-tumor [18] and anti-atherosclerotic activities [19]. Herein, we reported the isolation and structure elucidation of three glycosides from the aerial parts of *P. integrifolia*.

2. MATERIAL AND METHODS

2.1. Plant materials

The aerial parts of *P. integrifolia* L. were collected in Dong Hoang, Tien Hai, Thai Binh province, Viet Nam, in September 2017, and authenticated by Dr. Do Thanh Tuan, Thai Binh University of Medicine and Pharmacy. A voucher specimen (VMMU-2017-17) was deposited at the Herbarium of Viet Nam Military Medical University.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). ESI-MS spectra were recorded on Agilent 1260 Series Single Quadrupole LC/MS Systems. Optical rotations, Jasco P-2000 digital polarimeter. Plant sample was extracted on a JP. Selecta 300867 sonicator. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried powder of the aerial parts of *P. integrifolia* (1.8 kg) was sonicated in methanol (three times × 4L each) to obtain 220 g of crude extract, which was then suspended in water and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate to give corresponding extracts: *n*-hexane (PIH, 30 g), dichloromethane (PID, 9 g), and ethyl acetate (PIE, 20 g) and water layer (PIW). The water layer was subsequently chromatographed over Diaion HP-20 column and then eluted in turn with mixture of methanol/water (0 % to 100 % methanol, respectively) to obtain four fractions, PIW1-PIW4. The PIW1 fraction (2.25 g) was chromatographed on a silica gel column, eluted with ethyl acetate/methanol (10/1, v/v) to yield two sub-fractions, PIW1A and PIW1B. The PIW1A sub-fraction (350 mg) was further separated in a RP-18 column eluting with methanol/water (2/1, v/v) to give compound **1** (30 mg). The PIW3 fraction (12.0 g) was continued to be chromatographed on a silica gel column, eluted with dichloromethane/methanol/water (5/1/0.1, v/v/v) to obtain five sub-fractions, PIW3A-PIW3E. Compound **3** (10 mg) was obtained from PIW3B (240 mg) fraction through chromatography on reverse RP-18 column using methanol/water (1/1, v/v) as eluent. The PIW3C sub-fraction (280 mg) was further separated on a reverse RP-18 column and eluted with methanol/water (1/1, v/v) to yield compound **2** (10 mg).

Acteoside (**1**): Amorphous powder; ESI-MS: *m/z* 625 $[M+H]^+$ ($C_{29}H_{36}O_{15}$, M = 624); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1.

Premnaodoroside A (**2**): Amorphous powder; ESI-MS: *m/z* 891 $[M+H]^+$ ($C_{42}H_{66}O_{20}$, M=890); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 2.

Premnaodoroside B (**3**): Amorphous powder; ESI-MS: *m/z* 891 $[M+H]^+$ ($C_{42}H_{66}O_{20}$, M = 890); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder. The molecular formula of **1** was determined to be $C_{29}H_{36}O_{15}$ by the combination of ESI-MS ion at *m/z* 625 $[M + H]^+$ and ^{13}C-NMR spectral data. The ^{1}H-NMR spectrum of **1** showed the signals of six protons of two aromatic ABX systems at δ_H 6.72 (1H, d, *J* = 2.0 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.70 (1H, d, *J* = 8.0 Hz), 6.81 (1H, d, *J* = 8.0 Hz), 6.59 (1H, dd, *J* = 2.0, 8. 0 Hz), 6.98 (1H, dd, *J* = 2.0, 8.0 Hz),

which are characteristic of two 1,3,4-substituted aromatic rings; two doublet protons at δ_H 6.30 (1H, d, J = 16.0 Hz) and 7.62 (1H, d, J = 16.0 Hz) suggesting the presence of a double bond with *E* configuration; one anomeric proton δ_H 5.21 (1H, d, J = 1.5 Hz) together with a methyl signal at δ_H 1.12 (3H, d, J = 6.5 Hz) revealing the presence of a rhamnopyranosyl moiety with α-configuration. Besides, the other anomeric proton at δ_H 4.40 (1H, d, J = 8.0 Hz) suggested a sugar unit with β-configuration. The ^{13}C-NMR and HSQC spectra of **1** showed the signals of 26 carbons including one carbonyl carbon signal at δ_C 168.3; six other non-protonated carbon signals; seventeen methine carbon signals; three methylene carbon signals at δ_C 36.5, 62.3, and 72.2 and one methyl signal at δ_C 18.4. The ^{1}H- and ^{13}C-NMR spectral data of **1** were similar to those of acteoside (Table 1) [20].

Table 1. The ^{1}H- and ^{13}C-NMR spectral data of **1** and reference compound.

C	$\delta_C^{\#}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ **(mult., *J* in Hz)**	**C**	$\delta_C^{\#}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ **(mult., *J* in Hz)**
1	131.6	131.5	-	8′	114,8	114,7	6.30 (d, 16.0)
2	116.4	116.3	6.72 (d, 2.0)	9′	168.4	168.3	-
3	144.6	144.6	-	1″	104.2	104.2	4.40 (d, 8.0)
4	146.1	146.1	-	2″	76.0	76.0	3.55 (m)
5	117.2	117.1	6.70 (d, 8.0)	3″	81.7	81.6	3.84 (t. 9.0)
6	121.3	121.3	6.59 (dd, 2.0, 8.0)	4″	70.7	70.6	4.94 (m)
7	36.5	36.5	2.81 (m)	5″	76.2	76.2	3.42 (m)
8	72.3	72.2	3.73 (m) 4.06 (m)	6	62.5	62.3	3.54 (m) 3.64 (m)
1′	127.7	127.7	-	1‴	102.9	103.0	5.21 (d, 1.5)
2′	115.4	115.3	7.08 (d, 2.0)	2‴	72.1	72.0	3.60 (m)
3′	146.8	146.8	-	3‴	72.3	72.3	3.94 (m)
4′	149.7	149.7	-	4‴	73.8	73.8	3.32 (m)
5′	116.4	116.5	6.81 (d, 8.0)	5‴	70.7	70.4	3.88 (m)
6′	123.2	123.2	6.98 (dd, 2.0, 8.0)	6‴	18.4	18.4	1.12 (d, 6.5)
7′	148.0	148.0	7.62 (d, 16.0)				

[a] measured in MeOD, [b] 125 MHz, [c] 500 MHz, [#] δ_C of acteoside [20].

The HMBC correlations between proton H-2 (δ_H 6.72) and carbons C-4 (δ_C 146.1)/ C-6 (δ_C 121.3)/ C-7 (δ_C 36.5); proton H-5 (δ_H 6.70) and carbons C-1 (δ_C 131.5)/ C-3 (δ_C 144.6); proton H-6 (δ_H 6.59) and carbons C-2 (δ_C 116.3)/ C-4 (δ_C 146.1)/ C-7 (δ_C 36.5); proton H-7 (δ_H 2.81) and carbons C-2 (δ_C 116.3) /C-6 (δ_C 121.3)/C-8 (δ_C 72.2); protons H-8 (δ_H 3.73, 4.06) and carbons C-1′ (δ_C 131.5)/ C-7 (δ_C 36.5) suggested the presence of a hydroxytorosol moiety. The position of this moiety at C-1″ of glucopyranoside was confirmed by HMBC correlations between protons H-8 (δ_H 3.73 and 4.06) and carbon C-1″ (δ_C 103.0); between the anomeric proton H-1″ (δ_H 4.40)

and carbon C-8 (δ_C 72.2). The presence of *E*-caffeoyl moiety was determined by the HMBC cross peaks from proton H-2′ (δ_H 7.08) to carbons C-4′ (δ_C 149.7)/ C-6′ (δ_C 123.2)/ C-7′ (δ_C 148.0); proton H-5′ (δ_H 6.81) to carbons C-1′ (δ_C 127.7)/ C-3′ (δ_C 146.8); proton H-6′ (δ_H 6.98) to carbons C-2′ (δ_C 115.3)/ C-4′ (δ_C 149.7)/ C-7′ (δ_C 148.0); proton H-7′ (δ_H 7.62) to carbons C-2′ (δ_C 115.3)/ C-6′ (δ_C 123.2)/ C-9′ (δ_C 168.3); and proton H-8′ (δ_H 6.30) to carbons C-1′ (δ_C 127.7) /C-9′ (δ_C 168.3). In addition, the HMBC correlation between proton H-4″ (δ_H 4.94) and carbon C-9′ (168.3) indicated the ester linkages between *E*-caffeoyl moiety and glucopyranoside at C-9′ and C-4″. Finally, the attachment of rhamnopyranosyl moiety at C-3″ was confirmed by the HMBC correlation between anomeric proton H-1‴ (δ_H 5.21) and C-3″ (δ_C 81.6) (Figure 1). Based on the above evidence, compound **1** was determined to be acteoside [20]. This compound has been reported from genus *Premna* (*P. subscandens* [21], *P. japonica* [22], and *P. corymbosa* [23]). However, this is the first report of acteoside from *P. integrifolia.*

Compound **2** was obtained as an amorphous powder. The molecular formula of **2** was determined to be $C_{42}H_{66}O_{20}$ by the combination of ESI-MS ion at m/z 891 $[M + H]^+$ and ^{13}C-NMR spectral data. The ^{1}H-NMR spectrum of **2** revealed the signals of two oxygenated methine protons at δ_H 5.47 (2H, d, J = 4.0 Hz) and two singlet olefinic protons at δ_H 7.41 (2H, s), which are characteristic of protons H-1 and H-3 of iridoid skeletons [24]. Besides, the signals of two methyl groups at δ_H 0.95 (3H, d, J = 6.5 Hz) and 0.98 (3H, d, J = 6.5 Hz); four oxygenated methylene protons at δ_H 4.18 (2H, t, J = 6.5 Hz), 3.94 (1H, dd, J = 6.0, 10.5 Hz), 4.04 (1H, dd, J = 6.0, 10.5 Hz) and other protons at δ_H 1.20 – 1.83 ppm suggested the presence of a monoterpene fragment [24]. In addition, the signals of two anomeric protons at δ_H 4.70 (2H, d, J = 7.5 Hz) were confirmed the presence of two sugar units. The ^{13}C-NMR and HSQC spectra of **2** showed the presence of 42 carbons including six non-protonate carbons, 20 methine carbons, 12 methylene carbons and four methyl carbons (Table 2). The ^{1}H- and ^{13}C-NMR spectral data of **2** were similar to those of premnaodoroside A [24]. The presence of the monoterpene fragment were confirmed by the HMBC correlations from protons H-9″ (δ_H 0.95) to carbons C-2″ (δ_C 36.7)/ C-3″ (δ_C 31.0)/ C-4″ (δ_C 38.1); protons H-10″ (δ_H 0.98) to carbons C-6″ (δ_C 34.7)/ C-7″ (δ_C 34.0)/ C-8″ (δ_C 69.9) together the shielded carbon signals (δ_C 63.5, 36.7, 31.0, 38.1, 25.2, 34.7, 34.0, 69.9, 19.9, 17.5) and their corresponding proton splitting pattern [δ_H 4.18 (t), 1.48 (m)/1.71 (m), 1.45 (m), 1.20 (m)/1.37 (m), 1.33 (m)/1.45 (m), 1.20 (m)/1.44 (m), 1.83 (m), 3.94 (dd, 6.0, 10.5)/4.04 (dd, 6.0, 10.5), 0.95 (d), 0.98 (d)]. The HMBC cross peaks between proton H-3a (δ_H 7.41) and carbons C-4a (δ_C 113.6)/ C-11a (δ_C 169.0), proton H-8″ (δ_H 3.94 and 4.04) and carbon C-11a (δ_C 169.0) revealed the presence of the ester linkage between iridoid 1a group and the monotepene fragment at C-4a/C-8″ and the carbonyl carbon of iridoid 1a group. The HMBC correlations between proton H-3b (δ_H 7.41) and carbons C-4b (δ_C 113.6)/ C-11b (δ_C 169.0); protons H-1″ (δ_H 4.18) and carbon C-11b (δ_C 169.1) confirmed the attachment of iridoid 1b group to the monoterpene fragment at C-1″. Moreover, the position of two sugar units was assigned with the aid of the HMBC correlations from two anomeric protons H-1′a and H-1′b (δ_H 4.70) to carbons C-1a (δ_C 95.4)/C-1b (δ_C 95.5) (Figure 1). Consequently, compound **2** was identified as premnaodoroside A [7, 24].

Similarly, detailed analysis of the NMR data as well as comparison of them with the literature values led to identification of compound **3** as premnaodoroside B [24]. To our best knowledge, this is the first report of premnaodoroside B from *P. integrifolia.*

Compound **1** has been reported with various activities as hepatoprotective, anti-inflammation, anti-oxidant and cytotoxic activities [20]. Currently, biological activities of compounds **2** and **3** have not been reported, however, several studies of the pharmacological effects have shown that iridoids exhibits neuroprotective, anti-tumor, anti-inflammatory, anti-

oxidant, anti-diabetic, anti-viral, anti-microbial, immunomodulator, antiallergic, anti-leishmanial and molluscicidal activities [25]. Therefore, the presence of these compounds in chemical component of *P. intergrifolia* demonstrate the therapeutic effects of this plant in traditional Vietnamese medicine.

Table 2. The ^{1}H- and ^{13}C-NMR spectral data of **2**-**3** and reference compounds.

C	δ_C [#]	Compound 2		δ_C [$]	Compound 3	
		δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)		δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)
1a	95.43	95.4	5.47 (d, 4.0)	95.5	95.5	5.47 (d, 4.5)
3a	151.91	151.9	7.41 (s)	151.9	151.9	7.41 (s)
4a	113.59	113.6	-	113.6	113.6	-
5a	32.07	32.1	3.19 (m)	32.1	32.1	3.20 (m)
6a	30.93	30.9	1.45 (m)/ 2.30 (m)	31.0	31.0	1.45 (m)/ 2.30 (m)
7a	40.68	40.7	1.74 (m)	40.7	40.7	3.93 (m)
8a	80.55	80.5	-	80.6	80.6	-
9a	52.30	52.3	2.24 (dd, 4.0, 9.0)	52.3	52.3	2.24 (dd, 4.0, 9.0)
10a	24.70	24.7	1.34 (s)	24.7	24.7	1.07 (d, 7.0)
11a	169.03	169.0	-	169.0	169.1	-
1b	95.47	95.5	5.47 (d, 4.0)	96.2	95.3	5.47 (d, 4.5)
3b	151.94	151.9	7.41 (s)	152.3	152.3	7.41 (s)
4b	113.62	113.6	-	114.2	114.2	-
5b	32.11	32.1	3.19 (m)	31.1	31.1	3.20 (m)
6b	31.01	31.0	1.45 (m)/ 2.30 (m)	41.3	41.3	1.45 (m)/ 2.30 (m)
7b	40.71	40.7	1.74 (m)	79.3	79.2	1.74 (m)
8b	80.58	80.6	-	45.3	45.2	2.62 (m)
9b	52.30	52.3	2.24 (dd, 4.0, 9.0)	43.0	43.0	2.24 (dd, 4.0, 9.0)
10b	24.70	24.7	1.34 (s)	14.4	14.4	1.35 (s)
11b	169.07	169.1	-	169.0	169.1	-
1′a/1′b	99.86	99.9	4.70 (d, 7.5)	99.9	99.9	4.70 (d, 7.5)
2′a/2′b	74.76	74.8	3.21 (dd, 7.5, 9.0)	74.8	74.8	3.21 (dd, 7.5, 9.0)
3′a/3′b	78.40	78.4	3.30 (m)	78.4	78.4	3.33 (m)
4′a/4′b	71.75	71.7	3.27 (m)	71.8	71.7	3.27 (m)
5′a/5′b	78.02	78.0	3.40 (m)	78.0	78.0	3.40 (m)
6′a/6′b	62.98	63.0	3.66 (dd, 6.5, 12.0)	63.0	63.0	3.67 (dd, 6.5, 12.0)

			3.92 (dd, 2.0, 12.0)			3.93 (dd, 2.0, 12.0)
1″	63.5	63.5	4.18 (t, 6.5)	63.5	63.5	4.18 (t, 6.5)
2″	36.8	36.7	1.48 (m)/ 1.71 (m)	36.8	36.7	1.48 (m)/ 1.71 (m)
3″	31.0	31.0	1.45 (m)	31.0	31.0	1.45 (m)
4″	38.2	38.1	1.20 (m)/ 1.37 (m)	38.2	38.1	1.20 (m)/ 1.37 (m)
5″	25.3	25.2	1.33 (m)/ 1.45 (m)	25.3	25.2	1.33 (m)
6″	34.8	34.7	1.20 (m)/ 1.44 (m)	34.8	34.7	1.20 (m)/ 1.44 (m)
7″	34.0	34.0	1.83 (m)	34.0	34.0	1.84 (m)
8″	69.9	69.9	3.94 (dd, 6.0, 10.5) 4.04 (dd, 6.0, 10.5)	69.9	69.9	3.94 (dd, 6.0, 10.5) 4.04 (dd, 6.0, 10.5)
9″	20.0	19.9	0.95 (d, 6.5)	20.0	19.9	0.96 (d, 6.5)
10″	17.5	17.5	0.98 (d, 6.5)	17.5	17.5	0.99 (d, 6.5)

[a]measured in MeOD, [b]125 MHz, [c]500 MHz, [#]δ_C of premnaodoroside A [24], [$]$\delta_C$ of premnaodoroside B [24].

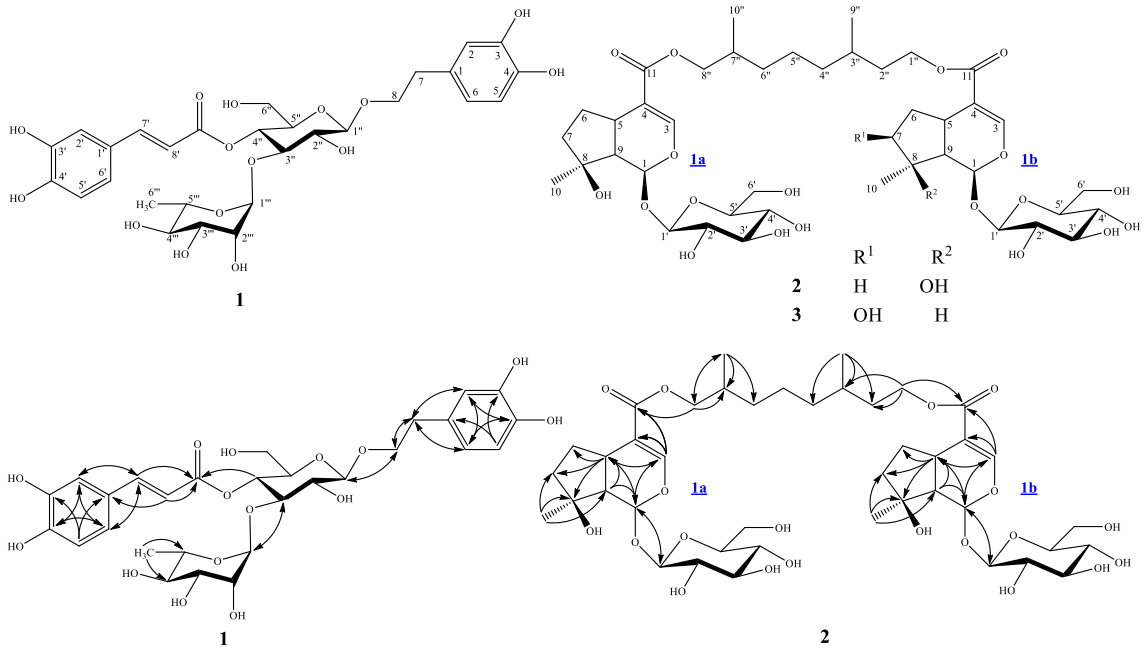

Figure 1. Chemical structures of compounds **1-3** and key HMBC correlations of compounds **1-2**.

4. CONCLUSIONS

From the aerial parts of *P. integrifolia* L. collected in Thai Binh, three glycosides, acteoside (**1**), premnaodoroside A (**2**) and premnaodoroside B (**3**) were isolated and structurally elucidated. To our best knowledge, this is the first report of isolation of compounds **1** and **3** from this species. It would be of interest to determine biological activity of these compounds to discover new medicines from medicinal plants in Vietnam.

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