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PREMNAODOROSIDE A AND 10-O-*TRANS-P*-METHOXYCINNAMOYLCATALPOL, TWO IRIDOID GLYCOSIDE DERIVATIVES FROM THE LEAVES OF *PREMNA INTEGRIFOLIA* L.

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ABSTRACT

*From the leaves of Premna integrifolia L. two iridoid glycoside derivatives premnaodoroside A (**1**) and 10-O-trans-p-methoxycinnamoylcatalpol (**2**) were isolated. Their chemical structures were elucidated by means of ESI-mass MS, ^{1}H-NMR, ^{13}C-NMR (CPD and DEPT), HSQC, and HMBC spectra in comparison with the literature. This is the first report of **1** and **2** from P. integrifolia species.*

I - INTRODUCTION

Premna integrifolia L. (Verbenaceae) widely distributed in Vietnam, Laos, and Cambodia. In Vietnamese traditional medicine, the leaves of this plant are used to treat indigestion, dysuria, and dysentery. The roots are also employed against indigestion, stomachache and fever [1, 2]. Previous phytochemical investigations on the species of the genus *Premna* reported the isolation and structural identification of a number of iridoid glycosides [3-6]. Iridoid glycosides occupy an important position in the field of natural product chemistry and biology [7]. From *P. integrifolia* some alkaloids as aphelandrine, premnine, and premnazole; two diterpenes 12,16-epoxy-11,14-dihydroxy-5,8,11,13-abietatetraen-7-one and 11,12,16-trihydroxy-5,8,11,13-abietatetraen-7-one; a sesquiterpene premnaspirodiene, and a flavonoid luteolin were isolated [8, 9]. This paper reports on the isolation and structure elucidations of two iridoid glycoside derivatives premnaodoroside A (**1**) and 10-*O-trans-p*-methoxycinnamoylcatalpol (**2**) isolated from the leaves of *P. integrifolia*.

II - EXPERIMENTAL

1. Plant material

The leaves of *Premna integrifolia Roxb.* were collected in Hanam province during July 2007 and was identified by Prof. Vu Van Chuyen, Hanoi University of Pharmacy. A voucher of specimen was deposited at the herbarium of Hanoi University of Pharmacy.

2. General experimental procedures

Melting points were determined using an Electro thermal IA-9200. The IR spectra were obtained on a Hitachi 270-30 type spectrometer with KBr discs. Optical rotations were determined on a Jasco DIP-1000 KUY polarimeter. The electrospray ionization (ESI) mass spectra were obtained using an AGILENT 1100 LC-MSD Trap spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR

spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230 - 400 mesh, Merck) and YMC RP-18 resins.

3. Extraction and Isolation

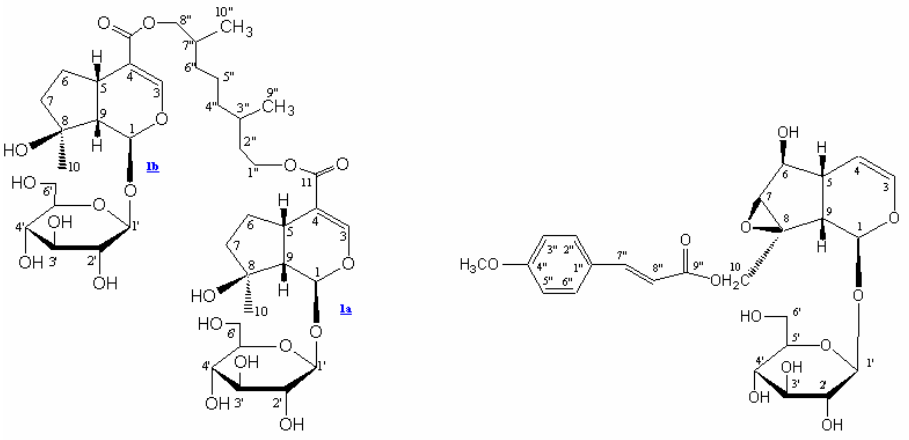
Dried leaves of *P. integrifolia* (1.0 kg) were powdered and then extracted three times with MeOH. The MeOH extract (50 g) was suspended in water and partitioned in turn with chloroform and ethyl acetate to obtain chloroform (12.0 g) and ethyl acetate (15.0 g) fractions. The ethyl acetate fraction (15.0 g) was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (gradient from 1:1.5:0.05 to 1:5:0.25) as eluent to give six fractions (F1-F6). The fraction F5 (0.7 g) was chromatographed on an YMC column using MeOH-H_2O (3:1) to yield compounds **1** (5 mg) as an amorphous powder. The fraction F6 (1.0 g) was chromatographed on an YMC column using MeOH-H_2O (4:1) to yield compounds **2** (7 mg) as a yellow amorphous powder.

Premnaodoroside A (1): Amorphous powder, mp. 190-191°C; $[\alpha]^{25}_D$ +84° (c, 0.5 in MeOH); ESI-MS *m/z*: 891 $[M+H]^+$; 889 $[M-H]^-$ ($C_{42}H_{66}O_{20}$, M=890); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): see table 1.

10-O-*trans-p*-methoxycinnamoylcatalpol (2): A yellow amorphous powder, mp. 186-187°C; $[\alpha]^{25}_D$ -63° (c, 0.5 in MeOH); ESI-MS *m/z*: 523 $[M+H]^+$; 521 $[M-H]^-$ ($C_{25}H_{30}O_{12}$, M=522); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): see Table 1.

III - RESULTS AND DISSCUSSION

Compound **1** was isolated as an amorphous powder from the methanolic extract of the leaves of *P. integrifolia* by a combination of YMC RP-18 column chromatography and silica gel column chromatography. The ESI-mass spectrum showed the quasi molecular ion peaks at *m/z* 891 $[M+H]^+$ (positive ion mode) and *m/z* 889 $[M-H]^-$ (negative ion mode), corresponding to the molecular formula of $C_{42}H_{66}O_{20}$. In the ^{1}H-NMR spectrum, two olefinic protons were observed at δ 7.41 and two protons of the oxymethine carbons at δ 5.47 as a doublet (*J* = 4.5 Hz), which were characteristic for H-3 and H-1 in iridoids, respectively [10].



1 **2**

Figure 1: Structures of **1** and **2**

In addition, two glucose units were confirmed by the observation of two anomeric protons at δ 4.69 (d, *J* = 7.5 Hz), two oxymethylene group at δ 3.66 (dd, *J* = 12.0, 6.0 Hz) and 3.92 (d, *J* = 12.0 Hz), and of the other oxymethine proton signals at the region from δ 3.23 to 3.36. Besides, two

secondary methyl groups at δ 0.92 (d, *J* = 6.5 Hz) and δ 0.98 (d, *J* = 6.5 Hz), two other oxymethylene groups at δ 4.18 (t, *J* = 6.5 Hz) and 3.92 (m)/ 4.00 (m), together with signals at the region of δ 1.22-1.85 suggested the appearance of a monoterpene portion [10].

Table 1: NMR data of **1** and of the literature

C	δ_C #[10]		δ_C a,b		Dept	δ_H a,c, *J* (Hz)	HMBC
	1a	1b	1a	1b			
Iridoid aglycon							
1	95.43	95.47	95.43	95.47	CH	5.47 d (4.5)	C-1′, 3, 5
3	151.91	151.94	151.90	151.90	CH	7.41 d (1.0)	C-4, 5,11a
4	113.59	113.62	113.57	113.60	C	-	
5	32.07	32.11	32.06	32.10	CH	3.20 m	C-6, 7, 8, 9
6	30.93	31.01	30.91	31.00	CH_2	1.62 m/1.74 m	
7	40.68	40.71	40.65	40.95	CH_2	1.77 m	
8	80.55	80.58	80.54	80.57	C	-	
9	52.30		52.29		CH	2.24 dd (1.0, 6.0)	C-1, 5, 6, 8
10	24.70		24.67		CH_3	1.34 s	C-7, 8, 9
11	169.03	169.07	169.04	169.09	C	-	
Glucose							
1′	99.86		99.84		CH	4.69 d (7.5)	C-1, 3′
2′	74.76		74.75		CH	3.23 dd (7.5, 9.0)	
3′	78.40		78.37		CH	3.23 m	
4′	71.75		71.73		CH	3.26 t (9.0)	
5′	78.02		77.99		CH	3.36 t (9.0)	
6′	62.98		62.96		CH_2	3.66 dd (6.0,12.0) 3.92 d (12.0)	C-5′
Monoterpene portion							
1″	63.5		63.50		CH_2	4.18 t (6.5)	C-11a
2″	36.8		36.77		CH_2	1.50 m/1.73 m	
3″	31.0		30.95		CH	1.45 m	
4″	38.2		38.15		CH_2	1.22 m/1.34 m	
5″	25.3		25.22		CH_2	1.33 m	
6″	34.8		34.71		CH_2	1.22 m/1.40 m	
7″	34.0		33.96		CH	1.85 m	
8″	69.9		69.89		CH_2	3.92 m/4.00 m	C-6,10,11b
9″	20.0		19.93		CH_3	0.92 d (6.5)	C-2, 3
10″	17.5		17.49		CH_3	0.98 d (6.5)	C-6, 8

[a]Measured in CD_3OD [b]125 MHz, [c]500 MHz, Chemical shift (δ) in ppm, #δ_C of premnaodoroside A [10].

The ^{13}C-NMR and DEPT spectra (table 1) revealed the presence of 42 carbon atoms (12×CH_2, 20×CH, 6×C and 4×CH_3). Analysis of the NMR spectra, the presence of two β-glucopyranosyl moiety were confirmed by typical signals at 99.84 (2 x CH), 78.37 (2 x CH), 77.99 (2 x CH), 74.75 (2 x CH), 71.73 (2 x CH), and 62.96 (2 x CH_2), two iridoids were characterized at δ 95.43/95.47 (2 x CH), 151.90 (2 x CH), 113.57/113.60 (2 x C), 32.06/32.10 (2 x CH), 30.09/31.00 (2 x CH_2), 40.65/40.95 (2 x CH_2), 80.54/80.57 (2 x C), 52.29 (2 x CH), 24.67 (2 x CH_2) and 169.07/169.09 (2 x C) [10]. Of the remaining signals, ester carbonyl (169.07

and 169.09), double bond [151.90 (2 x CH), 113.57/113.60 (2 x C)] and acetal (95.43 and 95.47) carbons were characterized. The monoterpene portion was further confirmed by the carbon chemical shifts as shown in Table 1. The H-C connections in the structure of **1** were deduced from the HSQC spectrum and the partial structure of **1** were deduced from the results of HMBC spectrum in comparison with the NMR data of premnaodoroside A, which was isolated from *Premna odorata* [10]. All the NMR and the other physical data of **1** were compared to those of premnaodoroside A and found to match well (table 1). This evidence led us to conclude that compound **1** was premnaodoroside A, which was first isolated from *P. integrifolia*.

Table 2: NMR data of **2** and of the literature

C	δ_C [1]	δ_C [a,b]	Dept	δ_H [a,c] *J* (Hz)	HMBC
1	95.6	95.60	CH	5.09*	C-1′, 3, 9
3	141.8	141.8	CH	6.37 d (2.0, 6.5)	C-1, 4
4	103.7	103.71	CH	5.08*	C-1, 3, 5, 9
5	39.0	39.01	CH	2.32 m	C-1
6	79.5	79.45	CH	3.97 dd (1.0,8.5)	
7	62.8	62.78	CH	3.51 d (1.0)	
8	63.6	63.60	C	-	
9	43.6	43.62	CH	2.67 dd (8.0, 9.0)	C-1, 6, 7, 8
10	63.0	64.26	CH_2	4.29 d (12.5) 4.99 d (12.5)	C-8, 9, 9″
Glucose					
1′	100.3	100.30	CH	4.77d (8.0)	C-1
2′	74.8	74.78	CH	3.21 t (9.0)	
3′	78.4	78.42	CH	3.30*	
4′	71.4	71.42	CH	3.32 m	
5′	77.8	77.82	CH	3.40 m	
6′	63.0	62.98	CH_2	3.69 dd (6.0, 12.0) 3.92 dd (2.0,12.0)	
p-Methoxycinnamoyl					
1″	128.3	128.32	C	-	
2″	131.1	131.08	CH	7.57 d (8.5)	
3″	115.4	115.40	CH	6.96 d (8.5)	
4″	163.2	163.18	C	-	
5″	115.4	115.40	CH	6.96 d (8.5)	C-4″
6″	131.1	131.08	CH	7.57 d (8.5)	C-4″
7″	146.6	146.51	CH	7.68 d (16.0)	C-1″, 9″
8″	115.9	115.85	CH	6.41 d (16.0)	C-9″
9″	168.9	168.82	C	-	
OMe	55.9	55.88	CH_3O	3.84 brs	

[a]Measured in CD_3OD [b]125 MHz, [c]500 MHz, Chemical shift (δ) in ppm,*Overlapped signals,
[#]δ_C of 10-O-*trans*-*p*-methoxycinnamoylcatalpol [6].

Compound **2** was also isolated as a yellow amorphous powder from the methanolic extract of the leaves of *P. integrifolia* by a combination of YMC RP-18 column chromatography and silica gel column chromatography. The ^{13}C-NMR spectrum of **2** showed signals of 25 carbons, including 18 CH, 2 CH_2, 4 C, and 1 methoxyl carbon determining from the DEPT 90 and DEPT 135 spectra. Analysis of the NMR spectra suggested the presence of an iridoid, a β-glucopyranosyl, and *p*-methoxycinnamoyl moiety. The double bond signals at δ 141.8 (CH), 103.71 (CH), the acetal signal at δ 95.60, the epoxi carbons at δ 62.78 and 63.60, the methine carbon bearing to oxygen atom at δ 79.45, together with two methine signals at δ 39.01 and 43.62 were characterized for the presence of an iridoid [6]. The *p*-methoxycinnamoyl moiety was confirmed by the ester carbonyl signal at δ 168.82, double bond signal at δ 146.51 and 115.85, methoxyl signal at δ 55.88, and *p*-substituted aromatic ring at δ 128.32 (C), 163.18 (C), 131.08 (2 x CH), 115.40 (2 x CH). The β-glucopyranosyl moiety was confirmed at δ 100.30, 74.78, 78.42, 71.42, 77.82 (5 x CH) and 62.98 (CH_2). Proton resonance at δ 6.41 and 7.68 (1H, each, J = 16.0 Hz) were attributable to *trans*-olefinic protons and two doublet at δ 7.57 and 6.96 (each 2H, J = 8.5 Hz) in the ^{1}H-NMR spectrum of **2** clearly confirmed the presence of a *trans-p*-methoxycinnamoyl. A double signal with large coupling constant at δ 4.74 (1H, J = 7.5 Hz) indicated a *β*-configuration anomeric proton of the sugar moiety (vicinal H-1″/2″ in *cis* position). The H-C connections in the structure of **2** were deduced from the HSQC, and the partial structure of **2** were deduced from the results of HMBC spectrum in comparison with the NMR data of 10-O-*trans-p*-methoxycinnamoylcatalpol [6] (Table 2) and found to match well. Moreover, in the HMBC spectrum, proton H-1′ (δ 4.77) correlated with C-1 (δ 95.60), proton H-10 (δ 4.29/4.99) correlated with C-8 (δ 63.60) and with carbonyl carbon at δ 168.82) confirming that the *p*-methoxycinnamoyl unit linked to C-8, and the *β*-D-glucopyranose attached to C-1 of the aglycon. From the above data, the structure of **2** was determined to be 10-O-*trans-p*-methoxycinnamoylcatalpol [6], whose molecular formula is $C_{25}H_{30}O_{12}$, which was further confirmed by the exhibition of the quasi molecular ion peaks at m/z 523 $[M+H]^+$ (positive ion mode) and m/z 521 $[M-H]^-$ (negative ion mode) in the ESI-mass spectrum. Compound **2** was isolated from *Premna subscandens* [6]. However, this is first report of this compound from *P. integrifolia*.

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