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Chemical examination on the ethyl acetate extract of the bark of *Oroxylum indicum* (L.) Vent

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ABSTRACT

This paper presents the results of the study about the bark of Oroxylum indicum (L.) Vent, collected in An Giang province, Vietnam. Three compounds consisting of oroxylin A 7-O-β-D-glucopyranoside (1), baicalein 7-O-β -D-glucopyranoside (2) and verbascoside (3) were isolated from the ethyl acetate extract. The structures of these compounds have been elucidated by modern spectroscopic methods: NMR and MS.

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1 INTRODUCTION

Oroxylum indicum (L.) Vent belongs to the family Bignoniaceae, locally known as "Núc nác", is widely found in tropical regions. It is also named such as Tatelo, Karamkanda, Saune tatal (Nepali); Ka-pa, Sonapatha (India). *O. indicum* is an important traditional herbal medicine of some regions such as Vietnam, Japan, Thailand, India and China. In the Indian Ayurvedic system, it is used as Rasayana drug for treatment of various disorders as well as used as a tonic (Dev *et al.*, 2010). In Vietnamese traditional medicine, its bark, called "Nam hoàng bá", has been used in treating skin diseases, allergic diseases and hepatitis (Đỗ Tất Lợi, 2004). There were also many reports in mainstream scientific journals describing the nutritional, medicinal properties and the chemical constituents of different parts of this plant (Lalou *et al.*, 2013; Singh *et al.*, 2013), which illustrated its medicinal value.

This is our third report about phytochemical examination of "Núc nác" from An Giang, (Nguyen Dang Khoa *et al.*, 2015; Tôn Nữ Liên Hương and Lê Minh Thịnh, 2016). This paper focused on verbascoside, a phenylethanoid glycoside compound which was first isolated from *O. indicum* together with two other glycosides as oroxylin A 7-*O*-*β*-D-glucopyranoside and baicalein 7-*O*-*β*-D-glucopyranoside.

2 MATERIALS AND METHOD

2.1 Plant material

The bark of *O. indicum* was collected from Thoai Son district, An Giang provine in December 2014. The plant was identified by Department of Biology, College of Natural science, Can Tho University. The specimen was stored in Laboratory of Organic Chemistry with the number 2014-02. The material was dried in shade, ground to fine powder and stored for further study.

2.2 General experimental procedure

Silica gel 60 (0.063-0.200 mm, Merck) was used for column chromatography. TLC F_{254} plate

(Merck) was used for thin layer chromatography. The NMR spectra were measured on a Bruker Avance 500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, HSQC, HMBC), ESI-MS was recorded with a VG 7070 Mass spectrometer operating at 70 eV. All spectra were recorded at Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi.

2.3 Extraction and isolation

Dried powder of the stem bark (2 kg) was exhaustively extracted with ethanol (EtOH). The filtrated solution was concentrated *in vacuum* to obtain EtOH extract (308.48 g). Then, this extract was suspended in distilled water and partitioned with petroleum ether (PE), dichloromethane (DC), ethyl acetate (EA), and *n*-butanol (Bu), respectively. The partitioned solutions were removed solvent to give five extracts: PE (14.00 g), DC (8.95 g), EA (14.43 g), *n*-BuOH (14.53 g) and Me/H_2O (53.30 g).

The EA extract was subjected to silica gel column, eluted with EA:Me (100% EA to the mixture in the ratio of 8:2). Fractions with the similar characteristic on TLC were combined to afford 7 fractions (P1-P7), in which the fraction P3 (7.08 g) was continued chromatographed, eluted by the mixture DC:Me (in the ratio of 99:1 to 7:3) to afford 6 subfractions. After recrystallization (DC 100%) on the subfraction P3.5 (0.18 g), compound 1 (15 mg) was achieved. The subfraction P3.7 (1.01 g) was further purified by column chromatography to obtain compound 2 (18 mg) and 3 (50 mg).

Compound 1, amorphous crystal, ESI-MS (negative): *m/z* 445.0 $[M - H]^-$, 326.9; 282.9 $[M\text{-}H\text{-}glc]^-$. The NMR spectra data of 1 were compared with those of 2 and showed in the Table 1 (500 MHz, DMSO-d_6). The correlative signals among the protons and carbon signals at δ_H 3.71 and δ_C 133.1; δ_H 5.13 and 157.2 ppm; δ_H 7.07 with δ_C 106.5 and 152.9 were showed on HMBC spectra.

Compound 2, yellow crystal, ESI-MS (negative): *m/z* 431.0 $[M - H]^-$, 288.9.

Table 1: Comparison of NMR data between the compounds 1 (δ) and 2 ($\delta^{\#}$) ppm

No	δ_H, J (Hz)	$\delta^{\#}_H$, J (Hz)	δ_C	$\delta^{\#}_C$
2			164.2	163.4
3	7.05 (*s*)	7.00 (1H; *s*)	105.4	104.7
4			182.9	182.5
5			152.8	146.5
6				130.8
7				156.1
8	7.07 (*s*)	7.06 (*s*)	94.9	94.2
9			152.9	149.2
10			106.5	106.1
1'			131.1	130.6
2', 6'	8.09 (*d*; *J* = 7.5)	8.07 (*dd*; *J* = 1.5; *J* = 8.0)	126.9	126.3
3', 5'	7.58 – 7.64 (*m*)	7.57 – 7.76 (*m*)	129.7	129.1
4'			132.7	132.0
1"	5.13 (*d*; *J* = 7.0)	5.02 (*d*; *J* = 7.0)	100.6	100.9
2"			73.6	73.1
3"			77.8	77.3
4"	3.17 – 3.75	3.17 – 3.77	70.0	69.6
5"			77.2	75.8
6"			61.1	61.2
5-OH	12.56 (*s*)	12.56 (*s*)		
6-OH	-	8.59 (*s*)		
6-OCH_3	3.78 (*s*)			60.8

Compound 3, an amorphous powder, the NMR data were showed on the Table 2.

3 RESULTS AND DISCUSSION

Compound 1: The ESI-MS (negative) of 1 showed a pseudo ion peak at *m/z* 445.0 $[M\text{-}H]^-$ and 282.9 $[M\text{-}H\text{-}glc]^-$; correspondingly to the molecular formula $C_{22}H_{22}O_{10}$ (M = 446 amu).

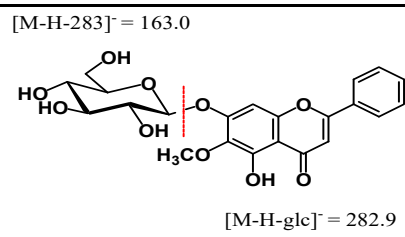
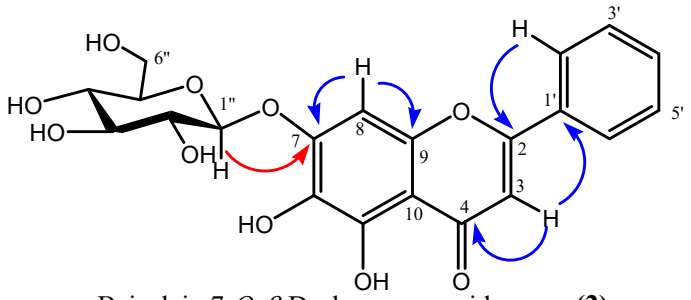


Fig. 1: The mass fraction of 1

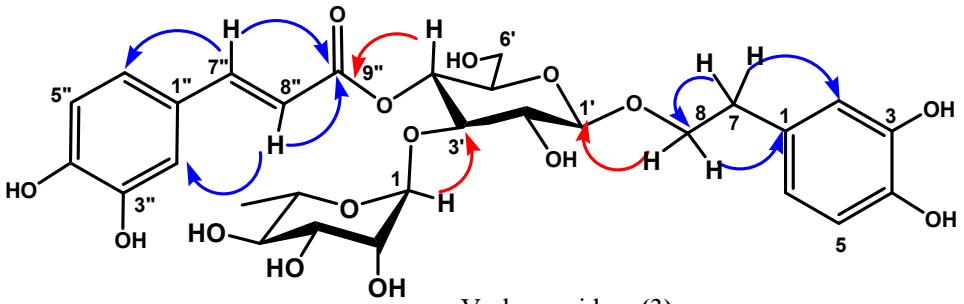
The presence of a *β*-configuration glucose moiety was confirmed via the anomeric proton signal at δ_H 5.13 (1H, *d, J*=7.0; H-1") and the other protons. The correlative signal between anomeric proton at δ_H 5.13 and δ_C 157.2 (C-7 of aglycon), δ_H 3.78 and δ_C 133.1(C-6) illustrated that the moiety was located at C-7, and the methoxylated of aglycon was posited at C-6. Thus, the structure of 1 was identified as oroxylin A 7-*O*-*β*-D-glucopyranoside. These data spectra were suitable with the authentic data of oroxylin A (Mouffok *et al*., 2012) and the data of *β*-glucoside (Andersen and Markham, 2006).

Compound 2 was obtained as yellow needles. The ^{1}H NMR spectrum indicated the presence of a hydrogen-bonded hydroxyl group at δ_H 12.56 (HO-5). Two single signals at δ_H 7.00 and 7.06 were referred to H-3 and H-8, respectively. The ring B of flavone was assigned to 1-substitued benzene by the signal of five other protons at δ_H 8.07 (2H, *dd*, *J* = 1.5, 8.0; H-2', H-6') and δ_H 7.57-7.62 (3H, *m*; H-3', H-4', H-5'). In comparison with 1D-NMR data of 1, by the lack of proton signal at δ_H 3.78 (3H; *s*) and carbon signal at δ_C 60.8 (-OCH_3), the aglycon of compound 2 was identified as baicalein.

The presence of a *β*-configuration glucose moiety was confirmed via the anomeric proton signal at δ_H 5.02 (1H, *d, J*=7.0; H-1") and the other protons. The ^{13}C and DEPT NMR spectra displayed the signals of an anomeric carbon δ_C 100.9 (C-1"), a hydroxymethylene δ_C 60.6 (C-6"), four hydroxymethine δ_C 69.6-77.3, a carbonyl group δ_C 182.5 (C-4); besides seven quaternary carbons at δ_C 163.4, 151.6, 149.2, 146.5, 130.8, 106.1 and 130.6 (C-2, C-9, C-7, C-6, C-5, C-10 and C-1') and seven other aromatic carbon signals. All data suggested the presence of a flavone skeleton connected with a pyranosyl moiety. In the HMBC, the correlative signal between the anomeric proton and the carbon at δ_C 151.6 (C-7) confirmed the connection of the glucose moiety at C-7 of the flavone. Therefore, compound 2 was identified as baicalein 7-*O*-*β*-D-glucopyranoside, in comparison with published data (Chen *et al.*, 2003).



Baicalein 7-*O*-*β*-D-glucopyranoside **(2)**



Verbascoside **(3)**

Fig. 2: The HMBC correlations of baicalein 7-*O*-glucoside (2) and verbascoside (3)

Compound 3 was obtained as an amorphous powder. ^{1}H-NMR spectrum showed the presence of six anomeric proton signals including two *meta*- spin signals at δ_H 6.72 (1H, *d*, *J* = 2.0); 7.07 (1H, *d*, *J* = 2.0), two *ortho*- spin signals at δ_H 6.70 (1H, *d*, *J* = 8.0); 6.80 (1H, *d*, *J* = 8.0) and two proton signals at δ_H 6.59 (1H, *dd*, J_1 = 2.0; J_2 = 8.0); 6.98 (1H, *dd*, J_1 = 2.0; J_2 = 8.0) which created two ABX phenyl systems, herein there is a 3,4-dihydroxyphenylethyl moiety. The presence of two *trans*- olefinic protons at δ_H 7.61 (1H, *d*, *J* = 16.0); 6.29 (1H, *d*, *J* = 16.0) was consistently with a caffeoyl group, and one signal shifted *para*-magnetically at δ 4.95 (1H, *t*, *J* = 9.5) indicating the same acylation site. The presence of α-rhamnose were demonstrated in the signals of an anomeric proton at δ_H 5.21 (1H, *J* = 1.5),

the special proton at δ_H 1.12 (*d*; *J* = 6.5) and the other protons (Table 2). Moreover, a *β*-glucose moiety was presented with a *doublet* signal at δ_H 4.40 (1H, *J* = 8.0) besides the other protons and carbon signals. The ^{13}C and DEPT NMR spectra displayed six methines and six quaternary carbon signals with four oxygenated quaternary carbons at δ_C 144.7; 146.1; 149.8; 146.8 ppm which were appropriated to two ABX systems. In addition, characteristic signals arising from two anomeric carbons at δ_C 104.2 and 103.1 ppm and one carbonyl at δ_C 168.6 ppm were consistently with disaccharide structure and caffeoyl moiety.

The HMBC correlation observed between the carbonyl carbon (δ_C 168.6) of the caffeoyl moiety and the H-4' (δ_H 4.95) of the glucose revealed that the caffeoyl group occupied the C-4' position of the glucose moiety. A prominent HMBC coupling from C-3' (δ_C 81.6) of the glucose to the H-1" (δ_H 5.21) of the rhamnose unit indicated the linkage of the rhamnose unit at the C-3' position of the glucose moiety. All these data suggested that the structure of **3** was established as 3,4-dihydroxy phenethyl-*O*-α-rhamnopyranosyl-4-*O*-caffeoyl-β-glucopyranoside (other name as verbascoside), in comparison its NMR data with those given in the previous report (Tayfun *et al.*, 2002).

Table 2: NMR data of compound 3

Position		δ_H ppm (*J* Hz)	δ_C ppm	HMBC
Aglycone	1		131.5	
	2	6.72 (*d*; *J* = 2.0)	116.5	C-6, C-1, C-3, C-4, C-7
	3		144.7	
	4		146.1	
	5	6.70 (*d*; *J* = 8.0)	117.1	C-6, C-1, C-3, C-4
	6	6.59 (*dd*; J_1 = 2.0; J_2 = 8.0)	121.3	C-7, C-5, C-3
	7	2.81 (*m*)	36.6	C-8, C-6, C-2, C-1, C-5
	8a	4.07 (*m*)	72.3	C-7, C-1, C-1'
	8b	3.75 (*m*)		
Caffeoyl	1"		127.6	
	2"	7.07 (*d*; *J* = 2.0)	114.7	C-7", C-6", C-4", C-3"
	3"		149.8	
	4"		146.8	
	5"	6.80 (*d*; *J* = 8.0)	116.3	C-4", C-3", C-1"
	6"	6.98 (*dd*; J_1 = 2.0; J_2 = 8.0)	123.2	C-7", C-8", C-3"
	7"	7.61 (*d*; *J* = 16.0)	148.0	C-9", C-8", C-6", C-1"
	8"	6.29 (*d*; *J* = 16.0)	115.2	C-9", C-1"
	9"		168.3	
Glucose	1'	4.40 (*d*; *J* = 8.0)	104.2	C-8
	2'	3.41 (*dd*; J_1 = 8.0; J_2 =9.0)	76.1	
	3'	3.83 (*t*; *J* = 9.5)	81.6	C-5', C-4', C-2', C-1'"
	4'	4.95 (*t*; *J* = 9.5)	70.4	C-6', C-5', C-3', C-2', C-9"
	5'	3.55 (*m*)	76.2	
	6'	3.62 (*dd*; J_1 = 12.0; J_2 =2.0) 3.52 (*dd*; J_1 = 12.0; J_2 =6.0)	62.4	
Rhamnose	1'"	5.21 (*d*; *J* = 1.5)	103.0	C-3', C-4', C-5'", C-3'", C-2'"
	2'"	(3.60-4.00)	72.4	
	3'"	(3.60-4.00)	72.1	
	4'"	(3.60-4.00)	73.8	
	5'"	(3.60-4.00)	70.6	
	6'"	1.12 (*d*; *J* = 6.5)	18.4	C-4'", C-5'"

4 CONCLUSIONS

The study on chemical constituents of the ethyl acetate extract of the stem bark of *Oroxylum indicum* (L.) resulted in the isolation of oroxylin A 7-*O*-*β*-D-glucopyranoside (1), baicalein 7-*O*-*β*-D-glucopyranoside (2) and verbascoside (3). Compound (3) was isolated for the first time from the genus *Oroxylum*.

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