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TWO NEW ALKALOIDS AND A CARBOHYDRATE COMPOUND FROM THE SPECIES *Hydrocotyle bonariensis* COMM. EX LAM., FAMILY APIACEAE

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ABSTRACT

*Continuing to the chemical study on Hydrocotyle bonariensis Comm. ex Lam. we now focus on the method for extracting alkaloid compound. From chloroform extract of Hydrocotyle bonariensis Comm. ex Lam., two isoquinoline alkaloids: tetrahydropalmatine (**1**), (-)-(S)-xylopinine (**2**) and a carbohydrate: ethyl 2-O-α-fructofuranoside (**3**) were isolated and identified. The structures of these new compounds were elucidated based on the data of NMR, ESI–MS spectra and compared with the reported documents. This is the first report about these compounds from the genus Hydrocotyle.*

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1 INTRODUCTION

Hydrocotyle bonariensis, a penny large leaf medicinal herb belongs to the *Hydrocotyle* genus, (family Apiaceae), widely distributed in the Mekong-delta of Vietnam, Thailand and some regions in the USA. *Hydrocotyle asiatica* and *Hydrocotyle sibthorpioides* were used as vegetable and also were medicinal remedies. From *Hydrocotyle asiatica*, an alkaloid was known as hydrocotyline, but its structure was not showed in the reference (Loi, 2004). The essential oil and the methanol crude extract of fresh whole plant *Hydrocotyle bonariensis* possessed anti-bacterial activity and effective inhibition on some cancer cells as RD and Hep-G2 cells (Huong *et al.*, 2009).

On the plants collected in Tien Giang province, our recent study showed that the chloroform extract of *H. bonariensis* possessed a weak toxicity on MCF-7 cell lines and a good lethality on *Artemia salina* L. in the brine-shrimp test, besides, the polar extracts have wound healing on rats, *in vivo* assay (Huong *et al.*, 2012). This paper informs the isolation and characterization of two isoquinoline alkaloids (**1** and **2**) along with ethyl 2-*O*-*α*–fructofuranoside (**3**), from the chloroform extract of *H. bonariensis* after using the alkaloid extraction method.

2 EXPERIMENTAL

2.1 Material

Hydrocotyle bonariensis (20 kg) were collected from Ben Tre province in June 2014.

2.2 Method for isolation and structural analysis

The pure solvent (Chemsol, Vietnam): petroleum ether, *n*-hexane, chloroform, ethyl acetate, acetone and methanol. Thin layer chromatography was performed on precoated TLC F_{254} 60 G (Merck, Germany) and detection was achieved by spraying the mix of vanilin-10% H_2SO_4 following by heating. Column chromatographies were carried out on silica gel 60G (Merck, for flash chromatography) and silica gel 60 (Merck).

2.3 Extraction

Dry powder of *Hydrocotyle bonariensis* (2.5 kg) was macerated in ethanol (acidification to pH 2.0) at room temperature. After each 24 h, the solution was filtrated and neutralysed by $Ca(OH)_2$ to pH 9.0, then concentrated by evaporator. The extract was partitioned between chloroform and water. The chloroform layer (90 g) was subjected repeatedly to a flash column chromatography over silica gel using the mixture of petroleum and ethyl acetate with increasing polarity to yield 9 fractions, C.1 to C.9. After continuous chromatography on the fraction C.4 (2.5 g) and the sub-fraction C.4.3 (0.017 g), compound **3** was isolated (10 mg). In addition, the fractions C.8 (4.5 g) and C.9 (6.3 g) were chromatographed several times by reversed phase silica gel columns to afford the compounds **1** (16 mg) and **2** (35 mg).

2.4 Structural analysis

The ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker 500 MHz equipment and the chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The Agilent Mass spectrometer was used for MS analysis, with ESI method, at Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi.

The NMR and MS data of these compounds were showed on the Tables 1 and 2.

3 RESULT AND DISCUSSION

* Compound **1** was obtained as pale yellow needles (acetone). Its HR-ESI-MS showed a pseudomolecular ion peak at *m/z* 356.1857 $[M+H]^+$, corresponding to the molecular formula $C_{21}H_{26}NO_4$ (ref.[5] MW: 356.1861). The ^{1}H-NMR (Table 1) showed the mutual coupling aliphatic proton signals of the methylene groups at δ_H 3.57 (1H, *dd,* 11,5/5.0 Hz, H-8a) and 4.22 (1H, *d,* 15.5 Hz, H-8b), and at δ_H 2.67 (1H, *dd,* 11.5/3.5 Hz,H-6a), 3.26 (1H, *dd,*11.0/4.0 Hz, H-6b), and a methine proton signal at δ_H 3.62 (1H, *dd,* 11.0/2.5 Hz,H-14); base on the HSQC correlations of these proton signals with their carbon signals, the structure of a methine and two methylene groups linked with nitrogene on the heterocyclic skeleton, >***N*H-**, was confirmed. In addition, a pair of *ortho*-coupled at δ_H 6.92, (1H, *d*, *J*=8.5 Hz, H-11) and 6.96, (1H, *d*, *J*=8.5 Hz, H-12) and a pair of *para* aromatic proton signals at δ_H 6.73 (1H, *s*, H-1), 6.89 (1H, *s,* H-4) presented for two different aromatic rings of this compound.

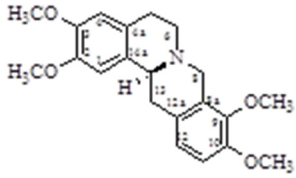
Furthermore, the presence of four methoxy groups were also identified. The carbon signals were assigned based on HSQC and HMBC spectra (Table 1).

All the above signals showed characteristics of the skeleton structure of 2,3,9,10-tetrahydroprotoberberine alkaloid. Compound **1** was finally identified as tetrahydropalmatine, (Qu *et al*., 2007).

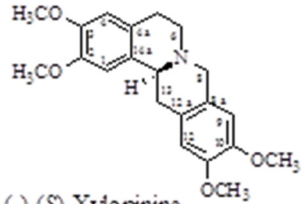
* Compound **2** was obtained as light yellow needles (methanol). Its HR-ESI-MS showed a pseudomolecular ion peak at *m/z* 356.1824 $[M+H]^+$. Comparison with compound **1**, the ^{1}H-NMR spectrum had the same of coupling aliphatic signals, the four methoxy groups with no *metha* aromatic proton signals, so its structure was suitable with 2,3,10,11-tetrahydroprotoberberine alkaloid. The ^{13}C-NMR signals of **2** were also assigned by HSQC and HMBC data spectra. This compound has the polarization, [α_D = - 262 (*c* = 0.10, $CHCl_3$)].

Comparison data of **2** with the authentic sample showed the coincident result, (Mujahidine and Doye, 2005). Accordingly, compound **2** was identified as (-)-(*S*) xylopinine.

This is the first report about the presence of these isoquinoloine skeleton alkaloids as compounds **1** and **2** from this plant. Previously, the presence of an alkaloid named Hydrocotyline was reminded, isolated from *Hydrocotyle asitica,* but its structure was not described.



Tetrahydropalmatine
(1)



(-)-(*S*)-Xylopinine
(2)

* Compound **3** (10 mg)**:** colourless needles, good solubility in methanol. ESI-MS gave a pseudo-molecular ion at *m/z* = 209.1 $[M+H]^+$, suitable to $C_8H_{16}O_6$ (molecular ion mass as 208 amu).

The ^{1}H-NMR spectrum showed 3 signals of protons form > CHOH; 3 signals of protons as $–CH_2OH$ and 3 signals of the terminate protons, at δ 1.20 (*t*, 8.0 Hz).

Table 1: The comparison of NMR data of compounds 1, 2 with the literature values data δ(ppm)

No	Compound 1 CD_3OD			THP. $CDCl_3$	Compound 2 CD_3OD		Xyl. DMSO-d_6
	δ_C	**δ_H (*J*, Hz)**	**HMBC**	**δ_C**	**δ_C**	**δ_H (*J*, Hz)**	**δ_C**
1	110.7	6.73 *s*	2,3,4a	108.4	109.3	6.87 *s*	108.6
2	149.3	-		147.3	147.1	-	147.7
3	149.4	-		147.3	147.1	-	147.5
4	113.1	6.89 *s*	2,3,5,14a	111.2	111.6	6.68 *s*	111.4
4a	127.8	-		126.7	126.3	-	126.4
5	29.3	2.78 *dd* (15.5/8.5) 3.15 *m*	4a, 4a, 6	29.3	28.5	2.61 *d* (16.0) 2.91 *dd* (11.5/5.0)	29.1
6	52.6	2.67 *dd* (11.5/3.5) 3.26 *dd* (11.0/4.0)	4 4a,5,14	51.4	50.9	2.46 *d* (3.5) 3.10 *m*	51.4
8	54.8	3.57 *dd* (11.5/5.0) 4.22*d* (15.5)	6,9,13,14 9,14a	53.9	53.4	3.40 *m* 4.07 *dd* (16.0/11)	58.3
8a	128.6	-		127.6	127.6	-	126.8
9	146.3	-		144.9	111.1	6.88 *s*	109.1
10	151.8	-		150.2	144.4	-	147.4
11	112.8	6.92 *d* (8.5)	9,124,14	110.8	149.8	-	147.7
12	125.2	6.96 *d* (8.5)	10,12a,13	123.8	123.6	6.88 *s*	126.3
12a	128.7	-		129.6	128.3	-	129.8
13	36.5	2.78 *dd* (15.5/8.5) 3.47 *dd* (16.0/4.0)	11,12a,14 6,12a	36.3	35.6	2.55 *dd* (13.5/4.0) 3.40 *dd* (16.0/4.0)	36.4
14	60.6	2.50*d* (11.0)	8	60.1	59.5	3.74 *dd* (11.0/3.5)	59.6
14a	128.7	-		128.6	128.3	-	126.8
MeO	60.8	3.86 *s*	9	59.2	55.7	3.86 *s*	56.0
MeO	56.8	3.85 *s*	2	56.0	55.7	3.85 *s*	56.0
MeO	56.5	3.85 *s*	3	55.7	55.6	3.85 *s*	55.8
MeO	56.4	3.83 *s*	10	55.7	55.6	3.85 *s*	55.9

THP.: Tetrahydropalmatine; Xyl.: Xylopinine

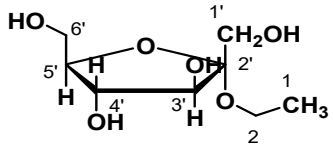
Herein, the appearance of a hydroxymethine proton at δ 3.97 (*dd*, J_1=5.0, J_2=11.5 Hz, H-4′) between a proton at δ 3.87 (*m*, H-5′) and a proton at δ 4.07 (*d*, *J* = 5.0 Hz, H-3′) which are closed to a carbinol group, that all confirmed the formation of a furanoside ring.

Table 2: The comparison of NMR data of Compound 3 and Ethyl 2-*O*-α-fructofuranoside

No	Compound 3		Ethyl 2-*O*-α-fructofuranoside
	^{1}H-NMR, δ ppm, *J* (Hz)	**^{13}C-NMR, δ ppm**	**^{13}C-NMR, δ ppm**
1	1.20 (*t*, 8.0 Hz)	16.0	16.0
2	3.75 (2H, *dd*, J_1=3.5, J_2=11.0 Hz)	57.5	57.6
1′	3.79 (2H, *dd*, J_1=3.5, J_2=12.0 Hz)	61.5	61.5
2′	-	108.9	108.9
3′	4.07 (*d*, *J* = 5.0 Hz)	83.0	83.0
4′	3.97 (*dd*, J_1=5.0, J_2=11.5 Hz)	78.6	78.6
5′	3.87 (*m*)	84.0	84.0
6′	3.57 (2H, *dd*, J_1=3.5, J_2=11.5 Hz)	62.7	62.7

The ^{13}C- and DEPT-NMR of compound (**3**) showed 8 carbon signals: 3 methine carbons at δ 78.6, 83.0 and 84.0 ppm; 3 methylene carbons at δ 57.5, 71.5 and 62.7; a methyl carbon at δ 16.0 and a carbon quaternary at δ 108.9. The upfield shift of a methylene group (C-2, δ 57.5) can be explained by the efficiency of the furanose ring, and so on this structure was determined as ethyl 2-*O*-α-fructofuranoside. The compatibility of data spec-

trum with the reference was showed on the Table 2, (Anh *et al.*, 2004).



4 CONCLUSION

This is the first report about the isolation of the two isoquinoline alkaloids and a carbohydrate from the species *Hydrocotyle bonariensis.* These were identified as tetrahydropalmatine (**1**), (*S*)-(-) xylopinine (**2**) and ethyl 2-*O*-α-fructofuranoside (**3**). The presence of these alkaloids contributed for the phytochemical constituents of the species *Hydrocotyle bonariensis* and the genus *Hydrocotyle*.

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