VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 127-130 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-030

IRIDOID CONSTITUENTS FROM THE ANT PLANT *HYDNOPHYTUM FORMICARUM*

Nguyen Phuong Hanh[1], Nguyen Huu Toan Phan[2*], Nguyen Thi Dieu Thuan[2], Le Thi Vien[3], Tran Thi Hong Hanh[3], Nguyen Van Thanh[3], Nguyen Xuan Cuong[3], Nguyen Hoai Nam[3], Chau Van Minh[3]

[1]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST)*

[2]*Tay Nguyen Institute of Scientific Research, VAST*

[3]*Institute of Marine Biochemistry, VAST*

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Using various chromatographic methods, four iridoids namely asperulosidic acid (**1**), deacetylasperulosidic acid (**2**), 6α-hydroxygeniposide (**3**), and 10-hydroxyloganin (**4**), were isolated from the methanol extract of the ant plant *Hydnophytum formicarum*. The structural elucidation was done using 1D and 2D-NMR experiments and comparison of the NMR data with reported values. This is the first report of these compounds from *H. formicarum*.

Keywords. *Hydnophytum formicarum*, Rubiaceae, ant plant, iridoid.

1. INTRODUCTION

The ant plant - *Hydnophytum formicarum* (Vietnamese names: Ổ kiến, bí kỳ nam) is a herb of the Rubiaceae. This plant forms a symbiotic relationship with ants and mainly distributed a long spring sides at altitudes above 600 m. The plant was used as folk medicine against liver, alimentary tract, and bone related diseases by some local populations in Tay Nguyen. This species was found to have cytotoxic, antioxidant, and anti-microbial activities [1-3]. As part of our ongoing investigations to find bioactive compounds from Vietnamese medicinal plants, we have reported 3-hydroxyphenyl *β*-D-glucopyranoside, butin, catechin, adenosine, and daucosterol from *H. formicarum* [4]. The current paper addresses the isolation and structural elucidation of four iridoids (Figure 1) namely asperulosidic acid (**1**), deacetylasperulosidic acid (**2**), 6α-hydroxygeniposide (**3**), and 10-hydroxyl-oganin (**4**) from the same species.

2. EXPERIMENTAL

2.1. General experimental procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer, TMS was used as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 minutes.

2.2. Plant materials

The sample of the ant plant *H. formicarum* Jack was collected in Dam Rong, Lam Dong, Vietnam, in August 2013 and identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST. A voucher specimen (TN3/325) was deposited at the Tay Nguyen Institute of Scientific Research, VAST, Vietnam.

2.3. Isolation

The samples of *H. formicarum* were cut into pieces, dried, and powdered. The dried powder (0.6 kg) were extracted three times with methanol in ultrasonic condition. The resulted solutions were

filtered, combined, and concentrated under reduced pressure to obtain methanol residue (50 g). This was suspended in water (1 L) and partitioned in turn with *n*-hexane, dichloromethane, and ethyl acetate to give the corresponding partitions, *n*-hexane (4.0 g), CH_2Cl_2 (2.0 g), ethyl acetate (15 g), and water layer.

The water layer was passed through Diaion HP-20 CC and eluted first with water and then with MeOH-H_2O (25:75, 50:50, 75:25, and 100:0) to obtain four fractions, W_1-W_4. Fraction W_2 (1.9 g) was separated by silica gel CC eluting with CH_2Cl_2-MeOH-H_2O (5:1:0.1) to give seven subfractions, $W_2A \rightarrow W_2G$. Subfraction W_2C (60 mg) afforded compounds **3** (3 mg) and **4** (6 mg) after subjecting it on an YMC RP-18 CC eluted with MeOH-H_2O (1:4), followed by silica gel CC with ethyl acetate-MeOH-H_2O (5:1:0.01). Compound **1** (8 mg) was purified from subfraction W_2F (280 mg) by using Sephadex LH-20 CC with MeOH-H_2O (1:1), followed by YMC RP-18 CC eluted with MeOH-H_2O (1:4). Finally, separation of subfraction W_2G (440 mg) by YMC RP-18 CC eluted with MeOH-H_2O (1:4) and silica gel CC with CH_2Cl_2-MeOH (4:1) to give compound **2** (6 mg).

Asperulosidic acid (**1**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1; ESI-MS: *m/z* 455 $[M+Na]^+$ ($C_{18}H_{24}O_{12}$, M= 432).

Deacetylasperulosidic acid (**2**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 413 $[M+Na]^+$ ($C_{16}H_{22}O_{11}$, M = 390).

6α-Hydroxygeniposide (**3**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 405 $[M+H]^+$ ($C_{17}H_{24}O_{11}$, M = 404).

10-Hydroxyloganin (**4**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 407 $[M+H]^+$ ($C_{17}H_{26}O_{11}$, M= 406).

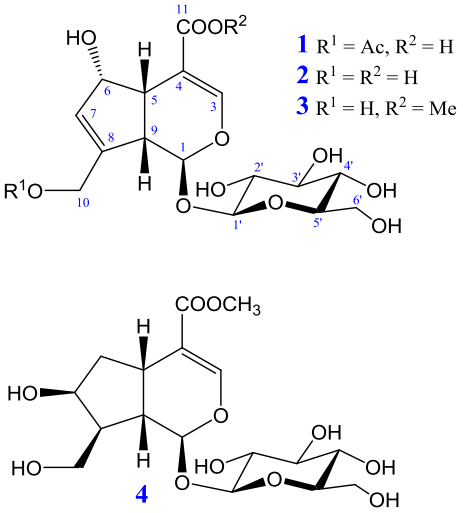

Figure 1: Chemical structures of **1–4**

Table 1: ^{1}H-NMR (500MHz) and ^{13}C-NMR (125 MHz) data in CD_3OD of **1**, **2** and reported compounds

C	[a]δ_C	**1**		[b]δ_C	**2**	
		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	100.97	101.27	5.08 d (9.0)	101.30	101.40	5.06 d (9.0)
3	153.90	155.33	7.67 s	154.66	154.54	7.62 s
4	109.18	108.60	-	109.53	109.30	-
5	43.00	42.50	3.04 t (6.5)	42.97	43.04	3.04 t (6.5)
6	75.61	75.39	4.81[c]	75.49	75.57	4.87[c]
7	131.77	131.96	6.04 s	129.78	129.88	6.04 d (1.5)
8	146.00	145.93	-	151.54	151.48	-
9	46.46	46.27	2.65 t (8.0)	45.91	46.03	2.57 t (9.0)
10	63.88	63.79	4.80[c]	61.75	61.74	4.23 br d (11.0)
			4.96 dd (2.0, 12.0)			4.47 br d (11.0)
11	170.18	171.00	-	171.86	171.65	-
C=O	172.57	172.56	-			
CO<u>CH$_3$</u>	20.73	20.73	2.11 s			
1′	100.46	100.60	4.74 d (8.0)	100.34	100.44	4.74 d (8.0)
2′	74.95	74.93	3.28 dd (8.0, 9.0)	74.98	75.00	3.28 dd (8.0, 9.0)
3′	78.55	77.90	3.40 t (9.0)	78.51	77.84	3.42 t (9.0)
4′	71.56	71.59	3.30[c]	71.64	71.66	3.30[c]
5′	77.85	78.55	3.31[c]	77.81	78.48	3.30[c]
6′	62.96	62.99	3.64 dd (5.0,12.5)	62.81	62.84	3.65 dd (5.0, 11.0)
			3.87 dd (2.0, 12.5)			3.87 dd (1.5, 11.0)

[a]δ_C of asperulosidic acid [5], [b]δ_C of deacetylasperulosidic acid [5], [c]overlapped signals.

Table 2: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data in CD_3OD of **3**, **4** and reported compounds

C	[a]δ_C	3		[b]δ_C	4	
		δ_C	δ_H mult. (*J*= Hz)		δ_C	δ_H mult. (*J*= Hz)
1	101.6	101.56	5.08 d (9.0)	98.80	99.02	5.18 d (5.5)
3	155.4	155.38	7.67 d (1.5)	153.12	153.01	7.48 br s
4	108.3	108.27	-	112.76	112.99	-
5	42.7	42.69	3.04 td (7.5, 1.5)	32.57	33.43	3.16 m
6	75.4	75.40	4.81[c]	42.16	43.05	1.56 m/2.28 m
7	130.0	129.82	6.04 d (2.0)	72.70	73.25	4.32 m
8	151.5	151.50	-	41.98	42.18	2.11 m
9	45.9	45.86	2.58 t (8.0)	49.04	49.75	2.11 m
10	61.7	61.67	4.23 d (16.0)	61.82	62.29	3.82 m
			4.47 dd (1.5, 16.0)			
11	169.5	169.45	-	170.50	169.50	-
OMe	51.6	51.81	3.67 s	52.49	51.69	3.82 s
1′	100.5	100.49	4.74 d (8.0)	100.05	100.54	4.68 d (8.0)
2′	75.0	74.97	3.27 dd (8.0, 9.0)	73.99	74.72	3.24 dd (8.0, 9.0)
3′	77.8	77.84	3.40 t (9.0)	77.48	77.93	3.40 t (9.0)
4′	71.6	71.65	3.30[c]	70.75	71.50	3.31[c]
5′	78.5	78.52	3.30[c]	77.04	78.27	3.32[c]
6′	62.8	62.84	3.64 dd (5.5, 12.0)	61.64	62.64	3.68 dd (5.5, 12.0)
			3.87 dd (2.0, 12.0)			3.89 dd (1.5, 12.0)

[a]δ_C of 6α-hydroxygeniposide [6], [b]δ_C of 10-hydroxyloganin [5], [c]overlapped signals.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Its ^{13}C-NMR spectrum exhibit 18 carbon atoms including typical signals of an acetyl groups [δ_C 172.56 (s) and 20.73 (q)] and a glucose moiety [δ_C 100.60 (C-1′), 74.93 (C-2′), 77.90 (C-3′), 71.59 (C-4′), 78.55 (C-5′), and 62.99 (C-6′)]. The 10 remaining carbon signals be long to the aglycon suggesting that **1** is an acetylated iridoid glucoside. The ^{1}H and ^{13}C-NMR of the aglycon part confirmed the presence of a dioxygenated methine group [δ_C 101.27 (d, C-1)], two trisubstituted double bonds [δ_C 155.33 (d, C-3), 108.60 (s, C-4), 131.96 (d, C-7), and 145.93 (s, C-8)/δ_H 7.67 (1H, s, H-3) and 6.04 (1H, s, H-7)], an oxymethine [δ_C 75.39 (d, C-6)/δ_H 4.81 (H-6)], an oxymethylene [δ_C 63.79 (t, C-10)/δ_H 4.80 (H-10) and 4.96 (1H, dd, *J* = 2.0, 12.0 Hz, H-10)], a carboxylic [δ_C 171.00 (s, C-11)], and two methine groups [δ_C 42.50 (d, C-5) and 46.27 (d, C-9)/δ_H 3.04 (1H, t, *J* = 6.5 Hz, H-5) and 2.65 (1H, t, *J* = 8.0 Hz, H-9)].

From the obtained evidence, the ^{13}C-NMR data of **1** (table 1) were found to be similar to those of asperulosidic acid [5]. The structure of **1** was further confirmed by HMBC experiment. In which, the HMBC correlation of H-10 (δ_H 4.80 and 4.96) with the acetyl carbonyl carbon (δ_C 172.5) and that of H-1′ (δ_H 4.74) with C-1 (δ_C 101.27) confirmed locations of the acetyl group and glucose moiety at C-10 and C-1, respectively. Detailed analysis of the other HMBC cross-peaks (figure 2) confirmed the structure of **1** as asperulosidic acid [5].

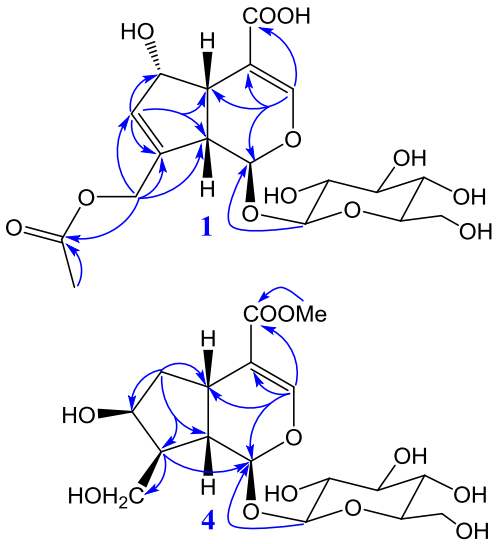

Figure 2: Key HMBC correlations of **1** and **4**

The ^{1}H and ^{13}C-NMR data of **2** were similar to those of **1** (table 1) except for an absence of the acetyl group, which was confirmed by the strong upfield shift of the carbon signal of the oxymethylene group in **2** at δ_C 61.74 (C-10) relative to that in **1** at δ_C 63.79 (C-10). A good agreement of

the ^{13}C-NMR data of **2** with those reported in the literature (table 1) and combination with 2D-NMR data led to elucidation of **2** as deacetylasperulosidic acid [5].

Compound **3** was also obtained as a white powder. Its ^{1}H- and ^{13}C-NMR data (table 2) were similar to those of **2** except for an additional presence of a methoxyl group at δ_C 51.81 and δ_H 3.67 (3H, s). The carbon signal of the methoxyl group was strongly shifted upfield suggesting for its esterification at the carboxylic group C-11. This was confirmed by HMBC correlation of the methoxyl proton (δ_H 3.67) with C-11 (δ_C 169.45). Consequently, compound **3** was identified as 6α-hydroxygeniposide [6].

The ^{1}H- and ^{13}C-NMR data of **4** were similar to those of **3** (table 2). The most easily visible change is the absence of one trisubstituted double bond in **4** relative to **3**. Location of the remaining double bond was identified at C-3/C-4 by a strong HMBC correlation of the olefinic proton at δ_H 7.48 (H-3) with C-1 (δ_C 99.02). In addition, the placement of the oxymethine group at C-7 was confirmed by HMBC cross-peak (Figure 2) of the oxymethylene protons H-10 (δ_H 3.82) with C-7 (δ_C 73.25). Thus, compound **4** was assigned as 10-hydroxyloganin [5].

4. CONCLUSION

Using combined chromatographic methods, four iridoids namely asperulosidic acid (**1**), deacetylasperulosidic acid (**2**), 6α-hydroxy-geniposide (**3**), and 10-hydroxyloganin (**4**) were isolated from the methanol extract of the ant plant *Hydnophytum formicarum*. Their structures were elucidated by spectroscopic methods. This is the first report of these compounds from *H. formicarum*.

Acknowledgements. *This work was financially supported by a Vietnam national project of the Tay Nguyen 3 Program, code: TN3/T14.*

REFERENCES

1. J. Y. Ueda, Y. Tezuka, A. H. Banskota, Q. Le Tran, Q. K. Tran, Y. Harimaya, I. Saiki, S. Kadota. *Antiproliferative activity of Vietnamese medicinal plants*, Biological & Pharmaceutical Bulletin, **25(6)**, 753-760 (2002).
2. S. Prachayasittikul, P. Buraparuangsang, A. Worachartcheewan, C. Isarankura-Na-Ayudhya, S. Ruchirawat, V. Prachayasittikul. *Antimicrobial and antioxidative activities of bioactive constituents from Hydnophytum formicarum Jack*, Molecules, **13(4)**, 904-921 (2008).
3. T. Senawong, S. Misuna, S. Khaopha, S. Nuchadomrong, P. Sawatsitang, C. Phaosiri, A. Surapaitoon, B. Sripa. *Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of Hydnophytum formicarum Jack.: sinapinic acid acts as HDAC inhibitor*, BMC Complement Altern Med., **13** 232 (2013).
4. N. P. Hanh, N. H. T. Phan, N. D. Thuan, L. T. Vien, T. T. H. Hanh, N. V. Thanh, N. X. Cuong, N. H. Nam, C. V. Minh. *Premilary chemical study of Hydnophytum formicarum*, Vietnam Journal of Science and Tecnology, **52(5A)**, 76-81 (2014).
5. O. Tzakou, P. Mylonas, C. Vagias, P. V. Petrakis. *Iridoid glucosides with insecticidal activity from Galium melanantherum*, Z Naturforsch C, **62(7-8)**, 597-602 (2007).
6. M. Miyagoshi, S. Amagaya, Y. Ogihara. *The structural transformation of gardenoside and its related iridoid compounds by acid and β-glucosidase*, Planta Medica, **53(5)**, 462-464 (1987).

Corresponding author: **Nguyen Huu Toan Phan**
Tay Nguyen Institute of Scientific Research,
Vietnam Academy of Science and Technology
116 Xo Viet Nghe Tinh Street, 7th Ward, Dalat, Viet Nam
E-mail: nhtphan@gmail.com.