

## CONTRIBUTION TO THE STUDY ON CHEMICAL CONSTITUENTS OF *HEDYOTIS AURICULARIA* L. (RUBIACEAE)

Received 30 May 2006

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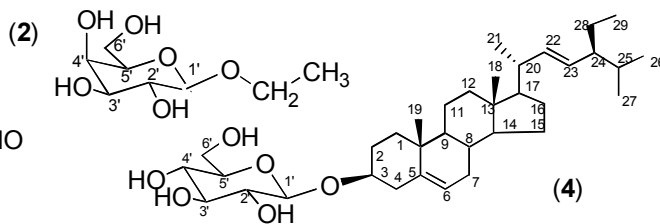
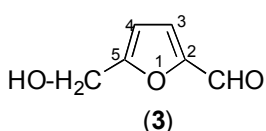
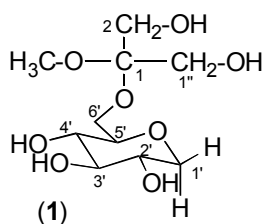
### SUMMARY

From the whole plant of *Hedyotis auricularia* L., a new glycoside, 1'-deoxy-6'-O-(1-hydroxymethyl-2-hydroxy-1-methoxy)ethylglucopyranoside (**1**) was isolated along with 1'-O-ethyl-β-D-galactopyranoside (**2**), 2-formyl-5-hydroxymethylfuran (**3**), stigmasta-5,22-diene-3-O-β-D-glucopyranoside (**4**), ursolic acid (**5**) and oleanolic acid (**6**). Among them (**1**), (**2**), (**3**), (**4**) were the first time known to be present in this plant.

### I - INTRODUCTION

Other authors had proved that *Hedyotis auricularia* L. contained auricularine, stigmaterol, β-sitosterol, ursolic and oleanolic acids [1, 2]. However, this plant has not yet been studied in Vietnam. In our research, we isolated 1'-deoxy-6'-O-(1-hydroxymethyl-2-

hydroxy-1-methoxy)ethyl glucopyranoside (**1**), together with five other compounds: 1'-O-ethyl-β-D-galactopyranoside (**2**), 2-formyl-5-hydroxymethylfuran (**3**), stigmasta-5,22-diene-3-O-β-D-glucopyranoside (**4**), ursolic acid (**5**) and oleanolic acid (**6**). Their chemical structures were elucidated by spectroscopic means.



### II - EXPERIMENTAL

#### 1. General

IR spectra were measured in KBr. <sup>1</sup>H- and <sup>13</sup>C-NMR were recorded on Bruker Avance 500 MHz and 125 MHz, respectively, in pyridine-d<sub>5</sub> or DMSO-d<sub>6</sub> solutions. MS spectra were conducted on Agilent-MSD-Trap-SL.

#### 2. Plant material

Whole fresh plants of *H. auricularia* were

collected in September 2003 in Binh Phuoc province. Identification of the plant specimen was performed by pharmacist Phan Duc Binh, Magazine of Health and Medicine, Ho Chi Minh City.

#### 3. Extraction and isolation

Dried and powdered whole plant of *H. auricularia* (1157 g) was exhaustively extracted with ethanol at room temperature to yield the crude ethanolic extract (118 g). This extract was

subjected successively to silica gel solid phase extraction [4] using petroleum ether, chloroform, ethyl acetate and methanol to give corresponding extracts.

The chloroform fraction (14.86 g) was chromatographed on silica gel eluting with mixtures of benzene, chloroform and methanol sequentially increasing polarity to yield 11 fractions. Fraction 4 (2.42 g, eluted by benzene/chloroform 1:1) was rechromatographed on silica gel eluting with 1% methanol in chloroform to afford 2-formyl-5-hydroxymethylfuran (**4**; 32 mg). Fraction 6 (3.28g, eluted by chloroform), was rechromatographed eluting with 3% methanol in chloroform afforded ursolic acid (**5**; 1034 mg). Fraction 7 (1.78 g, eluted by 1% methanol in chloroform), was rechromatographed eluting with 10% methanol in chloroform afforded oleanolic acid (**6**; 726 mg). The ethyl acetate fraction (21.43 g) was performed by silica gel

chromatography with CHCl<sub>3</sub>/MeOH (9 : 1) to yield 9 fractions. Fraction 4 (2.715 g) and 6 (1,723 g) were rechromatographed with CHCl<sub>3</sub>/MeOH (9 : 1, for the former fraction and 8:2, for the latter one) to afford 1'-O-ethyl-β-D-galactopyranoside (**2**; 37 mg) and 1'-deoxy-6'-O-(1-hydroxymethyl-2-hydroxy-1-methoxy)-thylucopyranoside (**1**; 29 mg), respectively. The methanol fraction (43.27 g) was chromatographed over silica gel eluting with CHCl<sub>3</sub>-MeOH (8:2) to yield 6 fractions. Fraction 3 (1.08 g) was rechromatographed to give stigmasta-5, 22-diene-3-O-β-D-glucopyranoside (**3**, 28 mg).

#### 4. 1'-Deoxy-6'-O-(1-hydroxymethyl-2-hydroxy-1-methoxy)ethylglucopyranoside (**1**)

Pale yellow prisms. *IR*  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3446 (O-H); 1062 (C-O). *LC-MS* MSD-Trap-SL [M]<sup>+</sup> *m/z* 268.8. <sup>1</sup>H, <sup>13</sup>C-NMR assignment and *HMBC* correlation of compound (**1**) were presented in table 1 and appendix 1.

Table 1: <sup>1</sup>H-, <sup>13</sup>C-NMR assignment and *HMBC* correlation of compound (**1**)

Position	<sup>1</sup> H (δ)	<sup>13</sup> C (δ)	<i>HMBC</i>
C-1		100.05	OH/C-1
C-2	3.54	61.58	H-2/C-1
C-1''	3.46 (brs)	61.58	H-1''/C-1
OCH <sub>3</sub>	3.14 (s)	47.84	OCH <sub>3</sub> /C-1
OH (to C-1'')	3.25 (s)*		OH/C-1
OH (to C-2)	3.16 (s)*		OH/C-1
C-1'	3.48 (brs)	63.75	H-1'/C-2'
C-2'	3.72 (brs)	68.83	H-2'/C-3'
C-3'	3.58 (dd)	69.27	H-3'/C-2'
C-4'	3.53 (brs)	69.36	
C-5'	3.41 (br)	72.48	OH/C-5'
C-6'	3.50 (br)	63.05	H-6'/C-4'; H-6'/C-1
OH (to C-2')	3.66 (br)**		OH/C-3'
OH (to C-3')	3.68 (br)**		OH/C-2'
OH (to C-4')	3.75 (br)**		OH/C-3'

Note: (\* ; \*\*) = exchangeable.

### 5. 1'-O-ethyl-β-D-galactopyranoside (2)

White powder. IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3457 (O-H) and 1081 (C-O).  $^1\text{H}$ ,  $^{13}\text{C}$ -NMR assignment and HMBC correlation of compound (2) were presented in table 2 and appendix 2.

### 6. 2-formyl-5-hydroxymethylfuran (4)

Yellow powder. IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3408 (O-H); 1666 (C=O conjugated to C=C); 1024 (C-O).

LC-MS MSD-Trap-SL:  $[\text{M}]^+$  ion peak at  $m/z = 126$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ),  $\delta$  ppm: 9.59 (1H, s,  $\text{CH}=\text{O}$ ), 7.22 (1H, d,  $J = 3.5$  Hz, H-3), 6.52 (1H, d,  $J = 3.5$  Hz, H-4) and 4.72 (2H, s,  $\text{CH}_2\text{-OH}$ ).  $^{13}\text{C}$ -NMR displayed a conjugated aldehyde group at 177.69 ( $\text{CH}=\text{O}$ ); four aromatic carbons corresponding to a furan ring at 160.65 (C-2), 152.45 (C-5), 122.68 (C-3), 109.99 (C-4) and a methylene carbon at 57.66 ( $\text{CH}_2\text{-OH}$ ).

Table 2:  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR assignment and HMBC correlation of compound (2)

Position	$^1\text{H}$ ( $\delta$ )	$^{13}\text{C}$ ( $\delta$ )	HMBC
1	3.80, 3.50 (2H, q, $J = 7.0$ Hz)	63.81	H-1/C-2; H-1/C-1'
2	1.15 (3H, t, $J = 7.0$ Hz)	15.15	H-2/C-1
1'	4.11 (1H, d, $J = 8.0$ Hz)	102.61	H-1'/C-1, H-1'/C-5'
2'	2.93 (1H, dd, $J = 8.0$ Hz)	73.43	H-2'/C-4', H-2'/C-1'
3'	3.16 (1H, dd, $J = 8.0$ Hz)	76.77	H-3'/C-5', H-3'/C-2'
4'	3.08 (1H, dd, $J = 5.5, 2.0$ Hz)	76.81	H-4'/C-6', H-4'/C-3'
5'	3.03 (1H, dt, $J = 8.0, 2.0$ Hz)	70.09	H-5'/C-6', H-5'/C-4'
6'	3.65, 3.40 (1H, d, $J = 9.0$ Hz)	61.09	

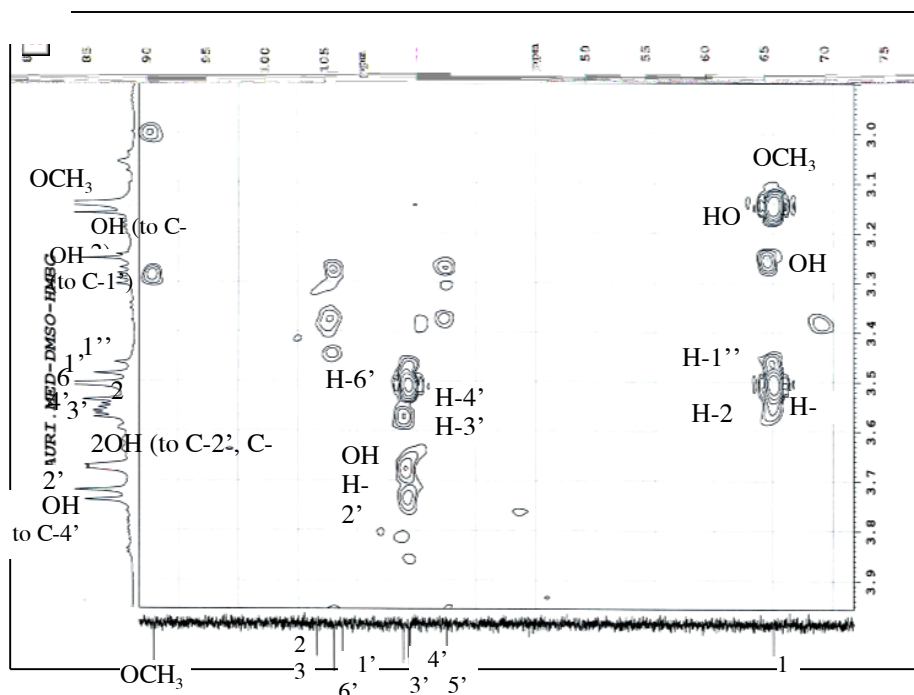
## III - RESULTS AND DISCUSSION

The methanolic fraction of an ethanolic extract of the dried and powdered whole plant of *H. auricularia* was separated by chromatographic methods to afford six compounds: 1'-deoxy-6'-O-(1-hydroxymethyl-2-hydroxy-1-methoxy)ethylglucopyranoside (1), together with five other compounds: 1'-O-ethyl-β-D-galactopyranoside (2), 2-formyl-5-hydroxymethylfuran (3), stigmasta-5,22-diene-3-O-β-D-glucopyranoside (4), ursolic acid (5) and oleanolic acid (6).

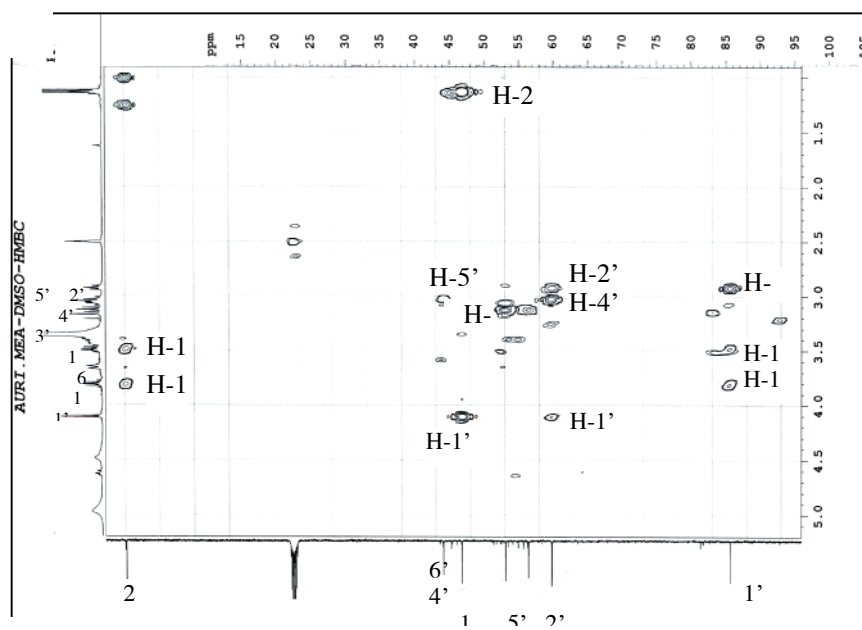
Compounds (2), (3) and (4) have been isolated and identified for the first time in *H. auricularia* and (1) is a new natural product which has not been reported so far. The compounds (5), (6) were determined by their IR,  $^1\text{H}$ -,  $^{13}\text{C}$ -DEPT-NMR spectra and comparison with those in literature [3].

Compound (1) was isolated as pale yellow prisms. Its LC-MS spectrum showed a molecular ion peak at  $m/z$  268.8, demonstrating its molecular

formula to be  $\text{C}_{10}\text{H}_{20}\text{O}_8$ . The IR spectrum of (1) indicated the presence of a hydroxyl group at  $3446 \text{ cm}^{-1}$ . Its  $^1\text{H}$ -NMR spectrum displayed signals resembled to those of a glucopyranose, *i.e.*  $\delta$ ppm 3.72 (1H, brs, H-2'), 3.58 (1H, dd, H-3'), 3.53 (1H, brs, H-4'), 3.41 (1H, brs, H-5') and 3.50 (2H, brs, H-6'). However, the signal due to the anomeric proton was absent, instead of that there appeared a signal of an oxygenated methylene at 3.48 (2H, brs, H-1'). The data suggested (1) to be a 1'-deoxyglucose. Furthermore, the proton spectrum also displayed signals of two isolated oxymethylene at 3.54 (2H, s, H-2) and 3.46 (2H, brs, H-1'') and a methoxy groups at 3.14 (3H, s, Me).  $^{13}\text{C}$ -NMR spectrum fully supported our suggestion by showing signals of a 1'-deoxyglucopyranose at 63.75 (C-1'), 68.83 (C-2'), 69.27 (C-3'), 69.36 (C-4'), 72.48 (C-5') and 63.05 (C-6').  $^{13}\text{C}$ -NMR and HMBC displayed a quaternary cetal carbon at 100.05 (C-1), this carbon connected to two oxymethylenes, one methoxyl group, as well as the sugar part. Therefore, the structure of (1) was suggested as shown.



Appendix 1: HMBC of 1'-deoxy-6'-O-(1-hydroxymethyl-2-hydroxy-1-methoxy)ethylglucopyranoside

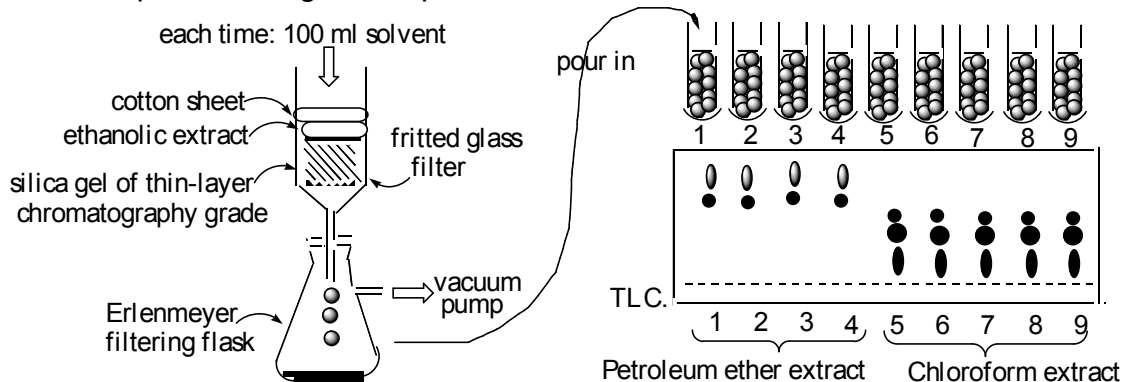


Appendix 2: HMBC-NMR spectrum of 1'-O-ethyl- $\beta$ -D-galactopyranoside (2)

#### REFERENCES

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2. K. K. Purushothaman, A. Sarada. *Phytochemistry*, 20, P. 351 - 352 (1981).
3. B. Shashi Mahato, P. Asish Kundu. *Phytochemistry*, 37, P. 1517 - 1575 (1994).
4. J. P. Richard Cannell. *Natural products isolation*. Humana Press, P. 68 - 74 (1998).

### An example of silica gel solid phase extraction



In a fritted glass filter, silica gel of thin-layer chromatography grade was loaded into a layer of 5 cm height. The ethanolic extract was loaded onto the surface of the silica gel. The fritted glass filter was fitted onto an Erlenmeyer filtering flask that was jointed to a vacuum pump. Each time, a portion of 100 ml solvent was pour into the fritted glass filter, and the pump was on to suck dried. The eluted solvent was poured into a tube that was numbered. The tubes that had the same result of the thin-layer chromatography were grouped together, evaporated and gave a kind of fraction. The solvent was changed from petroleum ether, chloroform, ethyl acetate and methanol to give corresponding extracts.

### GOÙP PHAÀN TÈM HIEAU THAIÑH PHAÀN HOÙA HOÃC CUÙA CAÂY AN ỆIEÀN TAI *HEDYOTIS AURICULARIA* L., HOÃ CAI PHEÃ (RUBIACEAE)

#### TOÙM TAÃT

Từ cây An ệieàn tai *Hedyotis auricularia* L., ủai coõ laọp ủai ùiuc caực hụp chaỏt 1'-deoxy-6'-O-(1-hydroxymetyl-2-hydroxy-1-metoxetyl)glucopyranosid (1), 1'-O-etyl-β-D-galactopyranosid (2), 2-formyl-5-hydroxymetylfuran (3), stigmasta-5,22-dien-3-O-β-D-glucopyranosid (4), axit ursolic (5) vaít axit oleanolic (6). Trong ủaọ (1), (2), (3), (4) laàn ủaàu tieõn bieỏt ủaỏn coự trong caõy.