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STEROIDS FROM *HEDYOTIS LEPTONEURA*

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Abstract

Four metabolites, 7*β*,19*α*-dihydroxy-*3β*-isoarborinol, 3*β*-isoarborinol, stigmasta-5,22-diene-3*β*-ol and daucosterol, were isolated from the aerial parts of *Hedyotis leptoneura* plant, collected in Bach Ma National Park (Thua Thien – Hue Province). Their structures were elucidated by various spectroscopic analyses.

Keywords. *Hedyotis leptoneura*, *7β,19α*-dihydroxy-*3β*-isoarborinol, *3β*-isoarborinol.

1. INTRODUCTION

Hedyotis plants, which are popularly called "Bòi ngòi" [1] or "An điền" [2], have widely used in the Asian and Vietnamese traditional medicine for curing the inflammation of the liver, infected injury, snake-bite… [1-6]. Chemical investigations on *Hedyotis* genus have resulted in the isolation of some flavonoids, anthraquinones, triterpenoids, iridoids glycosides… [7-9]. Herein, we describe the isolation and structural elucidation of some steroids from *Hedyotis leptoneura* plant, growing in Bach Ma National Park (Thu Thien – Hue Province).

2. EXPERIMENTAL

2.1. General experimental procedures

IR spectra were recorded on Shimadzu-FTIR 8101M spectrophotometer, using KBr disk, NMR (^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC) spectra were recorded on a Bruker Avance 500MHz. The chemical shift (δ) values are given in ppm with TMS as internal standard, coupling constant *J* (by Hz). ESI-LC-MS spectra were recorded on an Agilent LC mass spectrometer. Silica gel (Merck Co., Germany) was used for flash chromatography. TLC was carried out on precoated Si gel GF_{254} (Merck Co., Germany) and TLC spots were viewed at 254, 302 and 366 nm and visualized by spraying with vanillin - 10% H_2SO_4 solution.

2.2. Plant material

The aerial parts of *H. leptoneura* were collected in Bach Ma National Park (Thua Thien - Hue Province) in June, 2013. The plant material was identified by MSc. Nguyen The Anh, Institute of Chemistry, Vietnam Academy of Science and Technology, Vietnam.

2.3. Extraction and isolation

The air-dried aerial parts of *H. leptoneura* (2.0 kg) were ground into a powder and extracted with 80 % MeOH (10L x 3) at room temperature to give a crude extract (250 g) and white precipitate matter (12 g). Purifying the matter afforded **1** (10 g). The crude extract which was subjected to fractional extraction and then vacuum evaporation, giving *n*-hexane (87 g), ethyl acetate (32 g), methanol (115 g) extracts, respectively. The *n*-hexane extract was subjected to column chromatography over silica gel and eluted with *n*-hexane–ethyl acetate gradient from 10:1 to 1:1. Eight fractions were successively obtained. Fraction 2 (1.20 g) was precipitated as white crystals, recrystallized afford **2** (25 mg). Fraction 5 (520 mg) was separated by column chromatography (SiO_2) to afford **3** (20 mg). From the ethyl acetate extract, by CC over Si gel, eluted with *n*-hexane–ethyl acetate gradient from 4:1 to 1:4., were obtained six fractions. From fraction 2 (830 mg) white powder was precipitated. After purifying the powder, **4** was obtained (22 mg).

Compound 1. (7*β*,19*α*-Dihydroxy-3*β*-isoarborinol): White powders; mp. 294-295 ^{o}C. ESI-LC-MS (m/z): 459 $[M+H]^+$; IR (KBr, ν cm^{-1}): 3334 (OH), 2933, 1759 (C=O), 1445, 1381, 1094, 1052. ^{1}H NMR (500 MHz, $CDCl_3$+MeOD, TMS), ^{13}C NMR (125 MHz, $CDCl_3$+MeOD, TMS), see the table 1.

Compound 2. (3*β*-Isoarborinol): white powder,

m.p.: 139.5-140.5 ^{o}C; R_f = 0.33 (*n*-hexane:EtOAc =1:4), well dissolved in *n*-hexane, ethyl acetate, chloroform. ESI-LC-MS (m/z) 427 $[M+H]^+$; ^{1}H NMR (500 MHz, TMS, $CDCl_3$, δ, ppm): 5.35 (1H, m, H-6), 3.49 (1H, m, H-3), 0.82 (3H, d, *J* = 6.5 Hz, H-29), 0.77 (3H, s, H-19), 0.76 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$), see the table 2.

Compound 3. (Stigmasta-5,22-diene-3*β*-ol): white needles; ESI-LC-MS: m/z 413 $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$ + MeOD), δ (ppm): 5.35 (1H, brd m, H-6), 5.16 (1H, dd, *J* = 8.5 15.0 Hz, H-23), 5.03 (1H, dd, *J* = 8.5 10.0, H-22), 3.35 (1H, m, H-3), 1.04 (3H, s, H-19), 0.93 (3H, d, *J* = 5.5 Hz, H-21), 0.85 (3H, d, *J* = 7.5, H-29), 0.82 (3H, d, *J* = 6.5 Hz, H-26), 0.81 (3H, d, *J* = 6.5 Hz, H-27), 0.68 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$+MeOD, TMS), see the table 2.

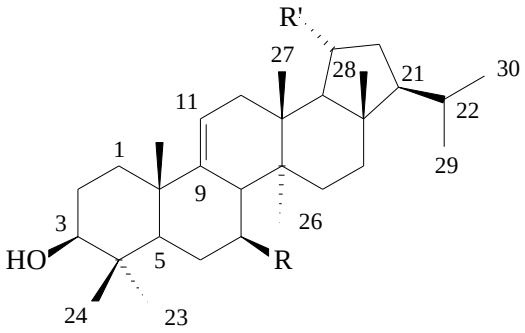
Compound 4 (Daucosterol). White powders, dissolved in DMSO, methanol, mp. 287-289 ^{o}C, FT-IR ν_{max} (cm^{-1}): 3390 (strong, OH), 2934; 1644; 1461; 1373; 1073; 1026. EI-MS (*m/z*): 396 $[M-C_6H_{12}O_6]^+$. ^{1}H NMR (500 MHz, $CDCl_3$ + MeOD), δ (ppm): 5.37 (1H, brd, H-6), 3.24 (1H, t 7.5, H-3), 1.01 (3H, s, H-19), 0.92 (3H, d 5.5, H-21), 0.85 (3H, t 6.5, H-29), 0.84 (1H, d 7.5, H-25), 0.83 (3H, d 6.5, H-27), 0.68 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$ + MeOD, TMS), see the table 2.

Table 1: ^{1}H NMR and ^{13}C NMR spectral data of compounds **1** and **2**

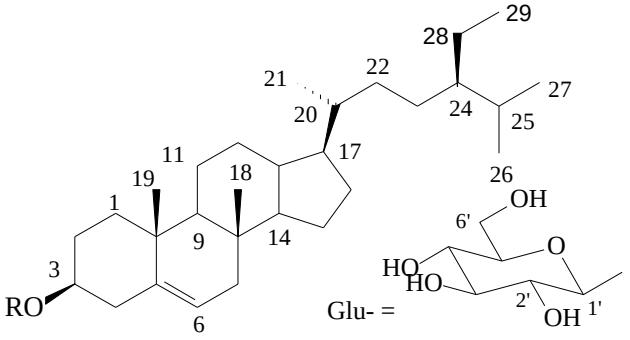
No.	**^{1}H NMR** (δ_H ppm, *J* Hz)		**^{13}C NMR** (δ_C ppm)	
	1 ($CDCl_3$&CD_3OD)	**2** ($CDCl_3$)	**1** ($CDCl_3$&CD_3OD)	**2** ($CDCl_3$)
1	1.68 m/1.87 m		36.8t	36.0t
2	1.72 m		27.5t	27.8t
3	3.19 m	3.21 m	78.7d	78.9d
4	-	-	39.4s	39.1s
5	0.86 m	0.86 m	48.3d	52.3d
6	1.63 m/ 1.95 m	1.76 m	32.8t	21.4t
7	3.71 m	1.21 m	72.0d	26.7t
8	2.06 d 11.0 Hz	2.03 m	49.1d	41.0d
9	-	-	146.5s	148.9s
10	-	-	39.5s	39.6s
11	5.31 d 6.0 Hz	5.23 d 6.5 Hz	117.2d	114.3d
12	1.40 m/ 1.69 m	1.72 m	36.6t	36.0t
13	-	-	37.8s	36.8s
14	-	-	38.8s	38.2
15	1.61 m/ 2.01 m	1.48 m	31.8t	29.6t
16	1.57 m/1.81 m	1.65 m	36.6t	35.9t
17	-	-	43.9s	42.8s
18	1.61 m	1.59 m	59.1d	52.1d
19	4.17 td 9.0 3.0 Hz	1.33 m	71.1d	20.2t
20	1.6 m/ 1.81 m	1.38 m	40.8t	28.2t
21	1.29 m	0.95 m	57.4d	59.6d
22	1.42 m	1.26 m	30.5d	30.8d
23	0.98 s	0.98 s	28.1q	28.2q
24	0.82 s	1.26s	15.6q	15.6q
25	1.06 s	1.03 s	21.8q	22.1q
26	0.98 s	0.80 s	16.8q	17.0q
27	0.92 s	0.77 s	16.7q	15.3q
28	0.81 s	0.76 s	15.5q	14.0q
29	0.83 s d 6.5 Hz	0.82 d 6.5Hz	22.0q	22.1q
30	0.88 d 6.5 Hz	0.87 d 6.0 Hz	23.0q	23.0q

3. RESULTS AND DISCUSSION

Compound 1 was obtained as colourless amorphous powder. Its molecular formula was deduced to be $C_{30}H_{50}O_3$ on the basic of the *pseudo*-molecular ion $[M+H]^+$ peak at *m/z* 459.2 in ESI-LC-MS spectrum, with six degrees of unsaturation. Its IR spectrum (KBr) showed the presence of a free hydroxyl group (3334 cm^{-1}), a double bond >C=C< (1445 and 1381 cm^{-1}). The ^1H NMR of **1** in $CDCl_3$/MeOD is typical for triperpenoids, including a multiplet (1H) at δ_H 5.31 for the olefinic proton H-11; two other multiplets (1H each) at δ_H 3.19 3.71 and one doublet triplet δ_H at 4.17 (td, 9.0 3.0 Hz) for three carbinol C-3, C-7, C-19 protons. The ^{13}C NMR, DEPT and HSQC spectra of **1** presented 30 carbon signals including eight methyl carbons, seven methylene carbons, nine methine carbons and six quaternary carbons (table 1). Among them, two carbons at δ_C 146.5 (C-9) and 117.2 (C-11) were assigned to a double bond >C=C< of pentacyclic triterpenoids; three methine carbons δ_C 78.7 (C-3), 72.0 (C-7) and 71.1 (C-19) were assigned to carbinol carbons. From above analyses of NMR, MS spectra, melting point of **1**, in comparison with those in [10], we made a conclusion that **1** is *7β,19α*-dihydroxy-*3β*-isoarborinol. This metabolite presented in some plants, such as *G. arborea, I. cylindrical, Hedyotis acutangula, Rubia cordifolia*… [11].



1 R,R'=OH 7*β*,19*α*-Dihydroxy-3*β*-isoarborinol
2 R,R'=H 3*β*-Isoarborinol



3 (R=H, △22, stigmasta-5,22-diene-3*β*-ol)
4 (R= Glu-, Daucosterol)

Figure 1: Structures of compounds **1-4**

Table 2: The ^{13}C NMR (δ_C ppm) spectral data of **3** and **4** compounds

No.	3	4	No.	3	4
1	36.9t	36.4t	**19**	18.9q	19.4q
2	31.5t	27.9t	**20**	40.2 d	33.7d
3	70.9d	76.2d	**21**	18.5q	19.4q
4	41.9t	38.4t	**22**	138.0d	31.6t
5	140.6s	140.1s	**23**	129.0d	28.8t
6	121.1d	121.8d	**24**	50.9d	49.9d
7	31.5t	31.6t	**25**	30.8d	25.8d
8	31.5s	31.6s	**26**	20.5q	18.9q
9	49.9d	51.0d	**27**	18.9q	20.7q
10	36.2s	35.9s	**28**	23.9t	22.7t
11	20.7t	20.8t	**29**	11.7q	15.0q
12	39.5 t	36.9t	**1'**	_	100.9d
13	41.9d	45.6d	**2'**	-	73.3d
14	56.6d	56.6d	**3'**	-	78.8d
15	25.0t	23.9t	**4'**	-	70.0d
16	28.5t	29.3t	**5'**	-	76.2d
17	55.6d	55.8d	**6'**	-	61.6t
18	11.96q	11.8q			

Compound 2. Its molecular formula was deduced to be $C_{30}H_{50}O$ based on the *pseudo*-molecular ion $[M+H]^+$ peak at *m/z* 427.2 in ESI-LC-MS spectrum. Its ^1H NMR spectrum is similar to that of *β*-sitosterol so at first sight, that may cause a confusion. By using ^{13}C NMR evidence it became clearly that **2** must be a pentacyclic triterpenoid. So, **2** has signals at 3.21 ppm (1H, m, H-3), 5.23 ppm (1H, brd m, H-11) suggested the presence of OH (at C-3) and C=C groups at C9-C11. Especially six methyl signals at δ_H 0.68-1.03 ppm are typical for protons of pentacyclic triterpenes. The ^{13}C NMR data of **2** (Table 2) revealed 30 carbon signals, including two olefinic carbons at δ_C 148.9 and 114.3 ppm very typical for double bonds at C9-C11, one carbinol carbon at δ_C 79.0 ppm. The mass, ^1H and ^{13}C NMR spectral data identified **2** as 3*β*-isoarborinol [12].

Compound 3. Its molecular formula was deduced to be $C_{29}H_{48}O$ based on the *pseudo*-molecular ion $[M+H]^+$ peak at *m/z* 413.2 in ESI-LC-MS spectrum. The ^1H NMR signals indicate the presence of three olefinic protons at δ_H 5.35, 5.16, 5.03 ppm and six methyl signals at δ_H 0.68-1.06 ppm. The ^{13}C NMR spectrum of **3** (table 2) revealed 29 carbon signals with four olefinic carbons at δ_C 140.5, 138.0, 128.9 and 121.2. It means that **3** has two double carbon-carbon bonds. The MS, ^1H and ^{13}C NMR spectral data identified **3** as stigmast-5,22-diene-3*β*-ol [13].

Compound 4. Its molecular formula was deduced to be $C_{35}H_{60}O_6$ based on the *pseudo*-molecular ion $[M-C_6H_{12}O_6]^+$ peak at *m/z* 396 in EI-MS spectrum. The ^1H-NMR data of **4** are similar to those of **3**, except a group of six multiplet proton signals with δ_H from 3.24 to 4.41 ppm, assigned for glucose protons, and the absence of two olefinic protons at δ_H 5.16 and 5.03 ppm. The ^{13}C NMR and HSQC spectra of **4** (Table 2) revealed 35 carbon signals with two olefinic carbon at δ_C 140.1, 121.8; six glucoside carbons at δ_C 61.5-100.8 ppm. The MS, ^1H and ^{13}C NMR spectral data identified **4** as daucosterol [13].

4. CONCLUSION

From the aerial parts of *Hedyotis leptoneura* plant, collected in Bach Ma National Park (Thua Thien – Hue Province) in June, 2013 we isolated four compounds. By various spectral methods, these compounds were elucidated as 7*β,*19*α*-dihydroxy-3*β*-isoarborinol, 3*β*-isoarborinol, stigmasta-5,22-diene-3*β*-ol and daucosterol.

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REFERENCES

1. Vo Van Chi. *A dictionary of Traditional Vietnamese Medicinal plants.* Medicine Publishing House, HCM city, Hanoi, 102-105 (2001).
2. Pham Hoang Ho. *Vietnamese medicinal plants*, Youth Publishing House, 512-514 (2001).
3. Yoshida Y., Wang M., Shan B. E., Yamashita U. *Interl*., J. Immunopharmacology, **19(7)**, 359-370 (1997).
4. Lai Quang Long, Phan Thanh Ky, Vu Van Tien, Chu Dinh Kinh. Vietnam J. Pharmacy, **9**, 9-11 (2001).
5. Le Tran Duc. *Medicinal plant of Vietnam: cultivated, processed, initially treatment*, Agric. Publishing House, 1259 (1965).
6. Huang Xiao Jian. Zhongcaoyao, **27(7)**, 408 (1996) (C.A., 125, 257321w).
7. Bhakuni D. S. et al. J. Sci. Ind. Res., **18**, 445-446 (1959).
8. Hamzah A. S., Ami N., Lajis N. H. J. *Constituents of Hedyotis herbacea (Rubiaceae),* Bio. Chem. Sys. Ecol., **24**, 273-278 (1996).
9. Bui Chi Huu, Nguyen Phi Kim Phung. *Chemical constituents from Hedyotis crassifolia,* Vietnam J. Chemistry, **45(3)**, 363-367 (2007).
10. Itokawa H., Qiao Y and Takeya K. Chem. Pharm. Bull., **38**, 1435 (1990).
11. Buckingham J. Dictionary of Organic compounds, **8**, 485 (2001).
12. Melissa L. Flagg. *Bioprospecting, chemical investigation and drug discovery from Chilean plants*, PhD dissertation, The Univ. of Arizona, USA (2000).
13. Goad L. J. and Akihisa T. *Analysis of sterols*, Chapman & Hall, 324-333 (1997).

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