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CONTRIBUTION TO THE STUDY ON CHEMICAL CONSTITUENTS OF *HEDYOTIS CRASSIFOLIA* L., (RUBIACEAE)

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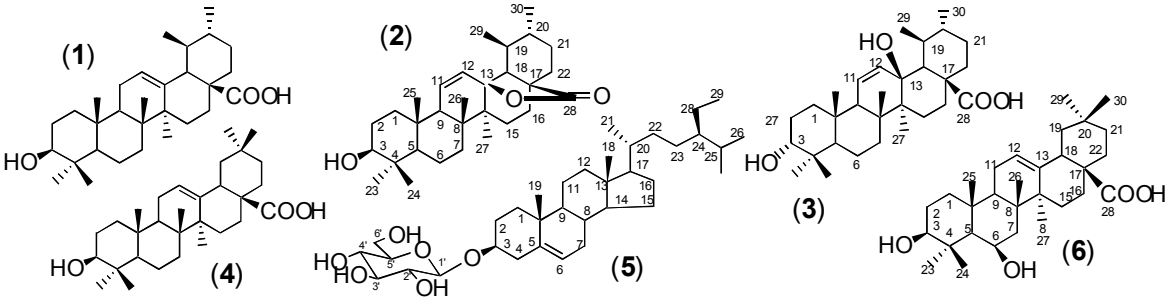
SUMMARY

*Six compounds as ursolic acid (**1**); 3β-hydroxyurs-11-ene-28(13)-lactone (**2**); 3α,13β-dihydroxyurs-11-ene-28-oic acid (**3**); oleanolic acid (**4**); 3β-D-glucopyranosyl-β-sitosterol (**5**) and 3β,6β-dihydroxyolean-12-ene-28-oic acid (**6**) were isolated from Hedyotis crassifolia L. (Rubiaceae). Among them, (**3**) was a new triterpen.*



I - INTRODUCTION

Hedyotis corymbosa L., *Hedyotis diffusa* Willd, *Hedyotis heynii* R. Br. have widely used in the Asian traditional medicine to cure the inflammation of the liver [1], infected injury [2], snake-bite [3] and especially to cure cancer [4]. Their chemical constituents and pharmaceutical properties have already been reported.

Hedyotis crassifolia L. (Vietnamese name: An điềnlá dầy), the plant that is the same genus with these above-mentioned ones but has not yet been studied. In this paper, we report a preliminary result about chemical constituents of this plants growing in Vietnam.

Figure 1: Part of *Hedyotis crassifoli*

Table 1: The comparison (δppm) of ^{1}H-NMR and ^{13}C-NMR spectra of compounds **2** and **3** with the authentic sample of 3β,13β-dihydroxyurs-11-ene-28-oic acid (**X**) [5]

C	(**X**) (C_5D_5N) (δ ppm)	**2** (DMSO) (δ ppm)	**3** ($CDCl_3$ + CD_3OD)	^{1}H-NMR (δ ppm)		
				(**X**) (C_5D_5N)	**2** (DMSO)	**3** ($CDCl_3$ + CD_3OD)
1	38.7	38.42	38.20			
2	23.2	22.12	22.67			
3	78.0	76.58	78.53	3.44 (dd; J = 9.0; 7.3 Hz)	3.01 (dd; J = 10; 5.5 Hz)	3.61 (s)
4	39.6	38.94	38.78			
5	55.1	53.84	54.66			
6	18.0	17.30	17.66			
7	31.0	30.90	29.57			
8	42.1	41.06	41.59			
9	53.4	52.25	52.94			
10	36.7	37.03	36.23			
11	133.7	133.02	133.64	5.65 (dd; J = 10.4; 3.0 Hz)	5.49 (dd; J = 10.0; 2.5 Hz)	5.49 (dd; J = 10.0; 3.0 Hz)
12	129.4	128.49	128.50	6.01 (d; J = 10.4 Hz)	5.98 (d; J = 10.0 Hz)	5.93 (d; J = 10.0 Hz)
13	89.4	88.90	90.20			
14	42.3	41.33	41.83			
15	27.9	26.69	26.57			
16	25.9	24.95	25.41			
17	45.2	44.28	45.15			
18	60.6	59.35	60.47			
19	38.7	37.55	38.01			
20	40.4	39.95	40.12			
21	31.6	30.08	30.64			
22	32.0	30.90	31.12			
23	28.4	27.68	27.56	0.84 (s)	0.83 (s)	0.87 (s)
24	16.0	15.24	14.78	1.01 (s)	0.95 (s)	1.00 (s)
25	16.2	15.43	15.97	1.19 (s)	1.12 (s)	1.13 (s)
26	19.2	18.57	18.68	1.22 (s)	1.23 (s)	1.22 (s)
27	18.3	17.60	17.75	1.24 (s)	1.25 (s)	1.22 (s)
28	179.3	178.64	180.64			
29	18.2	17.21	17.57	0.97 (d, J = 6 Hz)	0.93 (d, J = 6 Hz)	0.95 (d., J = 6 Hz)
30	19.4	18.72	18.96	0.83 (d, J = 6 Hz)	0.89 (d, J = 6 Hz)	0.90 (d. J = 6 Hz)

Table 2: The ^{1}H-,^{13}C-, DEPT-NMR, HMQC and HMBC (DMSO, 500 MHz) of **6**

C	^{13}C-NMR (δppm)	DEPT	^{1}H-NMR	HMBC ($^{2}J_{CH}$ and $^{3}J_{CH}$)
1	40.10	$-CH_2-$		
2	27.07	$-CH_2-$		
3	77.14	>CH-OH	2.92 (dd, J = 11.5; 6.5 Hz)	
4	40.05	>C<		
5	55.09	>CH-		C_6-O**H**/C-5 ($^{3}J_{CH}$)
6	66.19	>CH-OH	4.34 (br.s)	C_6-O**H**/C-6 ($^{2}J_{CH}$)
7	38.96	$-CH_2-$		
8	37.96	>C<		
9	47.34	>CH-		
10	36.02	>C<		
11	22.76	$-CH_2-$		
12	121.67	-CH=	5.18 (t, J = 0.2 Hz)	H-12/C-9; H-12/C-14; H-12/C-18
13	143.12	=C<		
14	41.65	>C<		
15	27.07	$-CH_2-$		
16	22.54	$-CH_2-$		
17	45.61	>C<		
18	40.75	>CH-	2.75 (dd, J = 14; 4 Hz)	H-18/C-19 ($^{2}J_{CH}$)
19	45.37	$>CH_2$		
20	30.30	>C<		
21	33.25	$-CH_2-$		
22	32.05	$-CH_2-$		
23	27.77	$-CH_3$	0.94 (s)	
24	17.13	$-CH_3$	1.05 (s)	
25	16.29	$-CH_3$	1.21 (s)	
26	17.67	$-CH_3$	0.97 (s)	
27	25.55	$-CH_3$	1.05 (s)	
28	178.46	-COOH		
29	32.73	$-CH_3$	0.87 (s)	
30	23.26	$-CH_3$	0.87 (s)	

II - EXPERIMENTAL

1. Plant material

Plants were collected in Long An province in October 2002 and was identified by Dr. Tran Hop, Department of Biology, University of Natural Sciences, National University - Ho Chi Minh City.

2. Extraction and isolation

Plants were washed, dried, ground into powder and exhaustedly extracted by 95° ethanol at room temperature. After evaporating, the ethanolic solution gave crude extract. The crude extract was subjected to silica gel solid

phase extraction [7], and then successively eluted with petroleum ether, chloroform A, chloroform B and methanol. Each fraction of chloroform A, chloroform B and methanol were rechromatographied to afford six compounds.

Compound **1** was isolated from chloroform A. Compounds **2**, **3** and **4** were isolated from chloroform B and compounds **5** and **6** from methanol fraction. Their yield (%) comparing to the dried powder was 0.27, 0.008, 0.001, 0.17, 0.034 and 0.035, respectively.

III - RESULTS AND DISCUSSION

Using modern methods (IR, NMR, MS) and comparing with references, the chemical structures of these compounds were elucidated.

1. Compound 2

White powder. M.p.: 161 - 163°C (methanol). Silica gel TLC with eluent of benzene : chloroform : methanol (1 : 9 : 0.1) revealed by concentrated sulfuric acid gave one lotus-red spot with Rf = 0.70. *LC-MS*, ESI spectrum showed the $[M]^+$ ion peak at m/z = 454 corresponded to $C_{30}H_{46}O_3$. IR(KBr) ν_{max} cm^{-1}: 3447 (O-H); 1767 (strong, C=O lactone); 1638 (C=C); 1090 (C-O). *^{1}H-, ^{13}C-NMR* (DMSO, 500 MHz) were presented in table 1.

2. Compound 3

White powder. M.p.: 231 - 232°C (recrystallized in methanol). Silica gel TLC. with eluent of benzene : chloroform : methanol (1 : 9 : 0.1) revealed by concentrated sulfuric acid gave one lotus-red spot with Rf = 0.56. IR(KBr) ν_{max} cm^{-1}: 3443 (O-H); 1695 (medium, C=O acid); 1639 (C=C); 1217 (C-O). ^{1}H-,^{13}C-NMR ($CDCl_3$+CD_3OD, 500 MHz) were presented in table 1. There was a little difference from the standard compound (3β,13β-dihydroxyurs-11-ene-28-oic acid [5]) that compound **3** had a pointed resonant peak of H3 at δppm = 3.61, showed that this proton occupied β-position so the hydroxyl group occupied the α-position. These findings substantiated that **3** is 3α,13β-dihydroxyurs-11-ene-28-oic acid and is a new triterpen.

3. Compound 6

White powder. M.p.: 211 - 212°C (methanol). Silica gel TLC. with eluent of hexane: ethyl acetate (1:1) revealed by concentrated sulfuric acid gave one dark violet spot with Rf = 0.50. LC-MS, ESI spectrum showed the $[M]^+$ peak at m/z = 472, suited to the formular $C_{30}H_{48}O_4$ or $C_{30}H_{47}O_3(OH)$. The spectra also had peaks at m/z =248 (100), 203 (90) that were characteristic peaks of oleanolic acid, so **6** perhaps was an oleanolic acid that contained one more hydroxyl group. ^{1}H-, ^{13}C-, DEPT-NMR, HMQC and HMBC (DMSO, 500 MHz) were presented in table 2 and figure 2. The ^{1}H-NMR and HMQC showed that the second hydroxyl group is at C-6 (–C**H**$_6$-OH at δppm = 4.34). The signal was a broad singlet that meaned it had the little J, so the hydrogen -C_6**H**-OH was at the position α and the hydroxyl group –C_6H-O**H** was at the position β. In conclusion, **6** was 3β,6β-dihydroxyolean-12-ene-28-oic acid (acid sumaresinolic) [6].

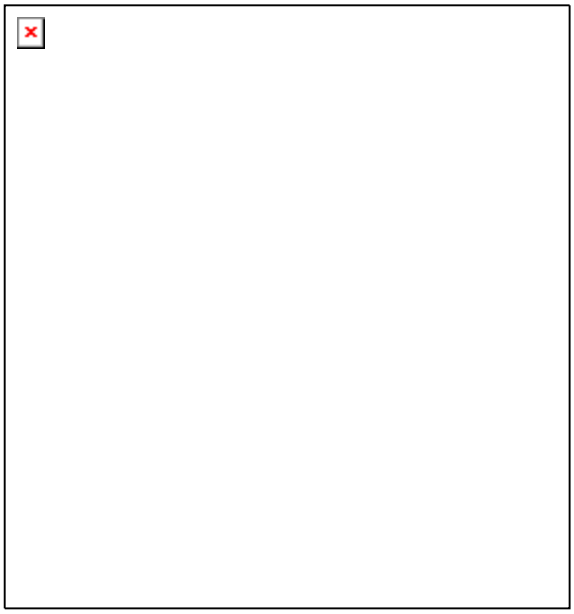


Figure 2: HMBC spectrum of **6**

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