

FOUR FLAVONOIDS FROM *HEDYOTIS NIGRICANS*

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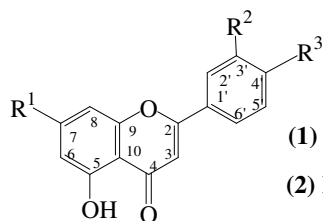
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ABSTRACT

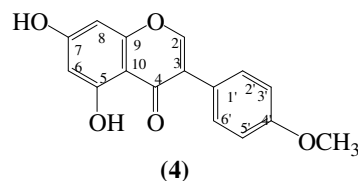
Four flavonoids, 5-hydroxyflavone (**1**), 5,7-dihydroxyflavone (**2**), 5,7,3',4'-tetrahydroxyflavone (**3**), and 5,7-dihydroxy-4'-methoxyisoflavone (**4**) were isolated from the ethyl acetate extract of whole plant of *Hedyotis nigricans*. These compounds were previously also isolated from other plants. However, they were the first time known to be present in *Hedyotis* genus from *H. nigricans*. The present paper reports the spectroscopic analyses which lead to the elucidation of their structures.



(1) $R^1 = R^2 = R^3 = H$

(2) $R^1 = OH, R^2 = R^3 = H$

(3) $R^1 = R^2 = R^3 = OH$



Key words: Rubiaceae, *Hedyotis nigricans*, flavone, isoflavone.

I - INTRODUCTION

In previous paper, we had reported the isolation and structural determination of triterpenoids and glycosides such as ursolic and oleanolic acids, α -amyirin, stigmasterol and arbutin from the whole plant of *H. nigricans* [1]. In the continuation of this work, we isolated four flavonoids, 5-hydroxyflavone (**1**), 5,7-dihydroxyflavone (**2**), 5,7,3',4'-tetrahydroxyflavone (**3**) and 5,7-dihydroxy-4'-methoxyisoflavone (**4**). All these compounds were the first time known to be present in *Hedyotis* genus from *H. nigricans*. Their structures were elucidated by spectroscopic methods.

II - EXPERIMENTAL

1. General

Silica gel 60F₂₅₄ and 60H (Merck) were used for column and thin-layer chromatographies. ¹H- and ¹³C-NMR were recorded on Bruker Avance at 500 MHz and 125 MHz, respectively, in DMSO-d₆ or CD₃OD solution.

2. Plant material

Whole fresh plants of *H. nigricans* were collected in September 2005 in Binh Phuoc province and identified by Prof. Dr. Le Cong Kiet, Department of Botany, University of

Natural Sciences, National University of Ho Chi Minh City. A voucher specimen (No US B-004) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, University of Science, HCM City.

3. Extraction and Isolation

Dried and powdered whole plant of *H. nigricans* (1400 g) was exhaustively extracted with ethanol at room temperature to yield the crude ethanolic extract (102 g). It was subjected successively to silica gel solid phase extraction using petroleum ether, chloroform, ethyl acetate and methanol to give corresponding extracts.

The ethyl acetate extract (28.47 g) was performed on silica gel column chromatography eluting with CHCl_3 -MeOH (9:1) to yield 12 fractions. Fraction 4 (2.62 g) was rechromatographed and preparative TLC using CHCl_3 -MeOH (92:7) to give **1** (34 mg) and **2** (36 mg). Fraction 6 (3.29 g) was rechromatographed using CHCl_3 -MeOH (90:10) to give 8 subfractions. Subfraction 3 (250 mg) was preparative TLC using CHCl_3 -MeOH (90:10) to afford **3** (41 mg) and **4** (42 mg).

Furthermore, in this study we recognized that arbutin, reported in previous work [1] presented in high yield in the fraction 2 of the ethyl acetate extract and was easily purified by washing this fraction with cold chloroform and then recrystallized in ethyl acetate.

5-Hydroxyflavone (1) [8, 9]. Yellow powder. Mp. 158 - 160°C. ^1H and ^{13}C -NMR ($\text{DMSO}-d_6$, δ ppm), table 1.

5,7-Dihydroxyflavone (2) [6 - 8]. Yellow powder. Mp. 283 - 285°C. ^1H and ^{13}C -NMR ($\text{DMSO}-d_6$, δ ppm): Table 1.

5,7,3',4'-Tetrahydroxyflavone (3) [4, 5, 8]. Yellow solid. Mp. 300 - 303°C. ^1H and ^{13}C -NMR (CD_3OD , δ ppm): table 2. HMBC: figure 2.

5,7-Dihydroxy-4'-methoxyisoflavone (4) [2, 3]. Colourless needles (MeOH). Mp. 215 - 216°C. ^1H and ^{13}C -NMR ($\text{DMSO}-d_6$, δ ppm): table 2. HMBC: figure 3.

III - RESULTS AND DISCUSSION

Compound **4** was isolated as colourless needles (MeOH). Its ^1H -NMR spectrum showed the signals of two *meta* protons of the A ring at δ 6.23 ppm (1H, d, J = 2.5 Hz, H-6) and δ 6.38 ppm (1H, d, J = 2.0 Hz, H-8) together with the presence of four aromatic protons of a 1,4-di-substituted B ring, two doublets at δ 6.99 ppm and δ 7.49 ppm (each 2H, J = 9.0 Hz). Besides, there were two hydroxyl groups at δ 12.92 ppm (1H, s, OH-5), δ 10.87 ppm (1H, s, OH-7) and a methoxyl group connecting to benzene ring at δ 3.78 ppm (3H, s, OCH_3 -4'). The ^{13}C -NMR spectrum displayed 16 signals, including one carbonyl carbon (δ 180.60 ppm, C-4), one methoxyl carbon (δ 55.12 ppm, OCH_3 -4') and fourteen olefinic carbons in the low field. These data proved that **4** should be a flavonoid. Furthermore, in HMBC spectrum, the ^1H peak at δ 8.34 ppm (H-2) showed four long-range couplings with the ^{13}C peak at δ 180.60 ppm (C-4), δ 157.55 ppm (C-9), δ 121.92 ppm (C-3) and δ 122.90 ppm (C-1') and in HSQC it attached directly to the ^{13}C peak at δ 154.18 ppm (C-2). From these data, the structure of compound **4** was 5, 7-dihydroxy-4'-methoxyisoflavone (biochanin A), also isolated from *Myristica malabarica*.^[2,3]

Compound **3** was isolated as a yellow solid. Its ^1H -NMR spectrum showed the signals of two *meta* protons of the A ring at δ 6.22 ppm (1H, d, J = 1.5 Hz, H-6) and δ 6.44 ppm (1H, d, J = 1.0 Hz, H-8) together with the presence of three aromatic protons of the B ring at δ 6.91 ppm (1H, d, J = 9.0 Hz, H-5') and δ 7.38 ppm (2H, m, H-2',6'). The ^{13}C -NMR spectrum displayed 15 signals in the low field. These data proved that **3** could be a flavonoid. Furthermore, in HMBC, the ^1H peak at δ 6.54 ppm (H-3) showed four long-range couplings with the ^{13}C peak at δ 183.85 ppm (C-4), δ 166.33 ppm (C-2), δ 123.70 ppm (C-1') and δ 105.30 ppm (C-10) and in the HSQC spectrum this proton was directly stucked to the ^{13}C peak at δ 103.86 ppm (C-3). Therefore, the structure of compound **3** was consistent with 5,7,3',4'-

tetrahydroxyflavone (luteolin), found also in *Lonicera japonica* [4, 5].

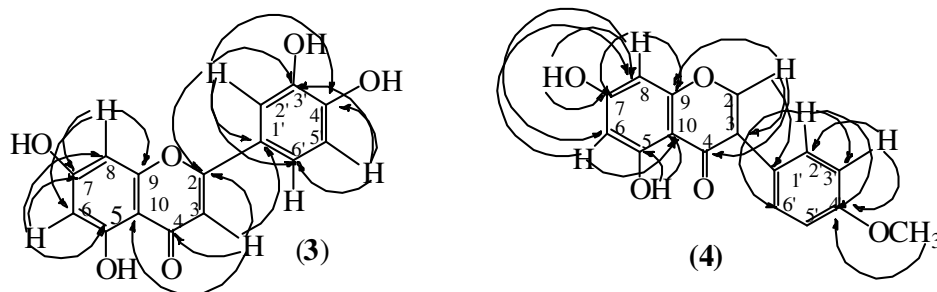


Figure 1: HMBC correlation of compounds 3 and 4

Compound 2 was shown to be closely related to compound 3 when analyzed by 1D and 2D-NMR spectra. The only difference was the absence of two hydroxyl groups at C-3' and C-4' in the B ring. Thus, the structure of 2 could be 5,7-dihydroxyflavone (chrysin), isolated from species in *Pelargonium* genus [6, 7].

Compound 1 had NMR spectra analogous to the ones of 2. The only difference was the absence of one hydroxyl group at C-7 in the A ring. Thus, the structure of 1 should be 5-hydroxyflavone, also known as primuletin, isolated from *P. denticulate* [8, 9].

Table 1: ^1H - and ^{13}C -NMR together with HMBC correlations of compounds 1 and 2

Compound 1 (in DMSO- d_6)				Compound 2 (in DMSO- d_6)		
N ^o	^1H (δ)	^{13}C (δ)	HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$)	^1H (δ)	^{13}C (δ)	HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$)
2		164.08			164.39	
3	7.11 (s)	105.64	1', 2, 4, 10	6.93 (s)	105.13	1', 2, 4, 10
4		183.85			181.80	
5		159.80			161.44	
6	6.82 (d, 8.0)	110.96	5, 7, 8, 10	6.22 (d, 2.0)	98.98	5, 7, 8, 10
7	7.69 (dd, 8.0; 8.5)	135.90	5, 6, 8, 9		163.10	
8	7.21 (d, 8.5)	107.51	6, 7, 9, 10	6.50 (d, 2.0)	94.07	6, 7, 9, 10
9		155.90			157.40	
10		110.12			103.94	
1'		130.52			130.68	
2', 6'	8.11 (d, 8.5)	126.59	2, 1', 2', 6'	8.04 (d, 9.5)	126.33	2, 1', 2', 6'
3', 5'	7.66 - 7.56, m	129.14		8.03, m	129.05	
4'	7.66 - 7.56, m	132.29		8.03, m	131.91	
5-OH	12.65 (s)		5, 6, 10	12.81 (s)		5, 6, 10
7-OH				10.88 (s)		6, 7, 8

Table 2: ^1H - and ^{13}C -NMR together with HMBC correlations of compounds **3** and **4**

Compound 3 (in CD_3OD)				Compound 4 (in DMSO-d_6)		
N^0	^1H (δ)	^{13}C (δ)	HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$)	^1H (δ)	^{13}C (δ)	HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$)
2		166.33		8,34 (s)	154.18	1', 3, 4, 9
3	6.54 (s)	103.86	1', 2, 4, 10		121.92	
4		183.85			180.60	
5		163.18			161.96	
6	6.22 (d, 1.5)	100.13	5, 7, 8, 10	6.23 (d, 2.0)	98.98	5, 7, 8, 10
7		165.99			164.29	
8	6.44 (d, 1.0)	95.09	6, 7, 9, 10	6.38 (d, 2.5)	93.67	6, 7, 9, 10
9		159.39			157.55	
10		105.30			104.43	
1'		123.70			122.90	
2'	7.38 (m)	114.17	2, 1', 6'	7.49 (d, 9.0)	130.10	3, 1', 3', 2', 6'
3'		147.08		6.99 (d, 9.0)	113.66	1', 2', 4', 6'
4'		150.97			159.12	
5'	6.91 (d, 9.0)	116.77	1', 6'	7.49 (d, 9.0)	113.66	3, 1', 3', 2', 6'
6'	7.38 (m)	120.30	2, 1', 2'	6.99 (d, 9.0)	130.10	1', 2', 4', 6'
5-OH	12.59 (s)		5, 6, 10	12.59 (s)		5, 6, 10
7-OH	10.87 (s)		6, 7, 8	10.87 (s)		6, 7, 8
OCH_3				3.78 (s)	55.12	4'

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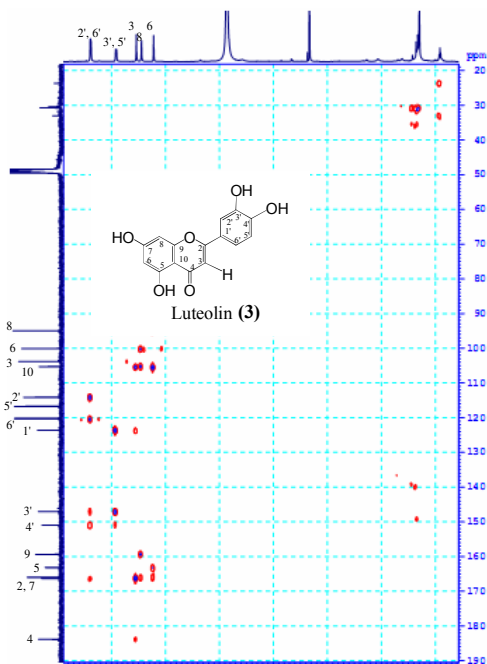


Figure 2: The HMBC spectrum of **3**

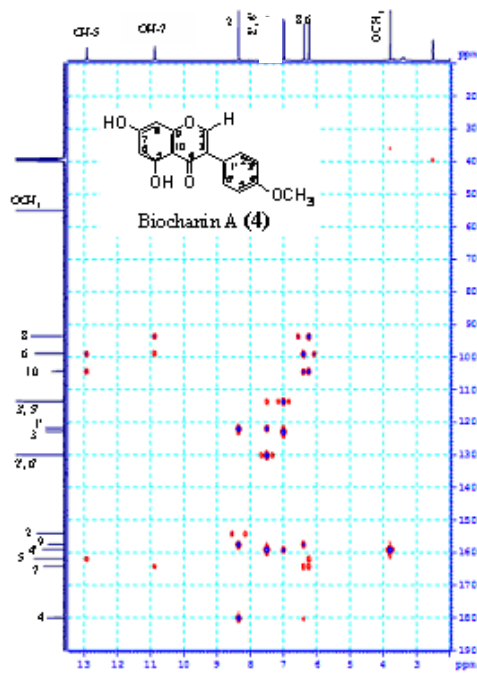


Figure 3: The HMBC spectrum of **4**

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**BÓN FLAVON TỪ CÂY HOA KIM CƯƠNG *HEDYOTIS NIGRICANS*,
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TÓM TẮT

Bốn hợp chất flavon, 5-hydroxyflavon (1), 5,7-dihydroxyflavon (2), 5,7,3',4'-tetrahydroxyflavon (3) và 5,7-dihydroxy-4'-methoxyisoflavon (4) đã được cô lập từ cao etyl acetat của cây Hoa Kim Cương Hedyotis nigricans L. Tất cả các hợp chất này lần đầu tiên biết có hiện diện trong chi Hedyotis từ loài cây H. nigricans. Cấu trúc của các hợp chất được xác định bằng các phổ cộng hưởng từ hạt nhân (1D và 2D-NMR) và so sánh với các số liệu trong tài liệu tham khảo.

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