

## A NEW FLAVONOID FROM *OPHIPOGON CONFERTIFOLIUS*

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### SUMMARY

From the methanolic extract of the roots of *Ophiopogon confertifolius* N. Tanaka (Convallariaceae) a new flavonoid named ophiofolius A (**1**) and two known compounds (**2**, **3**) were isolated. Their structures were elucidated as 6,8-dimethyl-4'-methoxy-5,7,3',5'-tetrahydroxyflavanone (**1**), 6,8-dimethyl-3',4'-dimethoxy-5,7,5'-trihydroxyflavanone (**2**), and 6-methoxy-5,7-dihydroxyflavanone (**3**) by the spectroscopic evidences (1D NMR, 2D NMR, ESI-MS).

### I - INTRODUCTION

“Cao cẳng” is a traditional medicinal plant of the people who lives in some mountainous areas of Bac Giang province. It has been used to treat osteocopic pain, dispel swelling and blood clotting in ecchymosis, renal failure...The scientific name of this plant has been identified as *Ophiopogon confertifolius* N. Tanaka (Convallariaceae). This is a new species of Vietnamese flora [1]. Up to date, no studies on the chemical and bioactivities of this plant were carried out. As a part of our study on this plant, we report herein the isolation and the structural elucidation of 8-dimethyl-4'-methoxy-5,7,3',5'-tetrahydroxyflavanone (**1**), 6,8-dimethyl-3',4'-dimethoxy-5,7,5'-trihydroxyflavanone (**2**), and 6-methoxy-5,7-dihydroxyflavanone (**3**) by the spectroscopic evidences (1D NMR, 2D NMR, ESI-MS). Compound **1** was isolated for the first time from nature, and compounds **2** and **3** were first isolated from the *Ophiopogon* species.

### II - EXPERIMENTAL

#### 1. Plant material

The roots of *Ophiopogon confertifolius* N. Tanaka (Convallariaceae) were collected in Yen The, Bac Giang province, Vietnam, and the plant was identified by Dr Nguyen Thi Do, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen was deposited at the National Institute of Medicinal Materials, Ministry of Health.

#### 2. General experimental procedures

Melting points were determined using an Electro thermal IA-9200. The IR spectra were obtained on a Hitachi 270-30 type spectrometer with KBr discs. Optical rotations were determined on a Jasco DIP-1000 KUY polarimeter. The electrospray ionization (ESI) mass spectra were obtained using an AGILENT 1100 LC-MSD Trap spectrometer. The <sup>1</sup>H-NMR (500 MHz) and <sup>13</sup>C-NMR (125 MHz) spectra

were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and YMC RP-18 resins.

### 3. Extraction and isolation

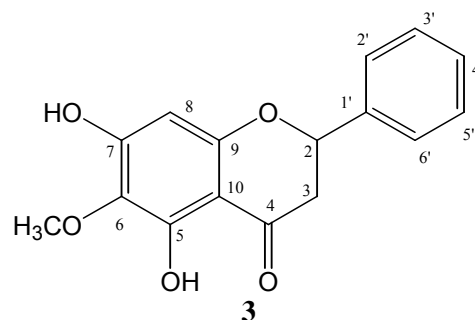
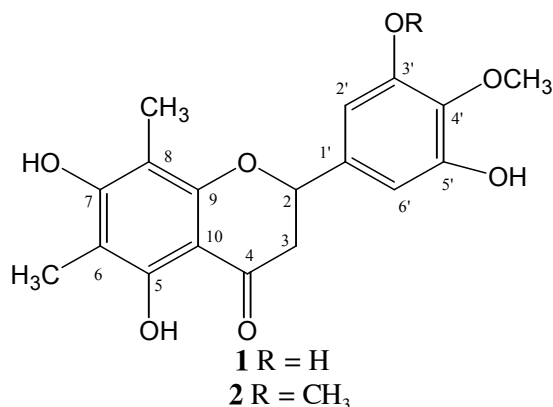
Dried roots of *O. confertifolius* were powdered and then extracted three times with MeOH. The MeOH extract (50 g) was suspended in water and partitioned in turn with *n*-hexane, chloroform, ethyl acetate, and *n*-BuOH to obtain *n*-hexane (5.8 g), chloroform (10.2 g), ethyl acetate (20 g), and *n*-BuOH fractions (13.0 g). The ethyl acetate fraction (20 g) was chromatographed on silica gel column using CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (80:20:2) and on YMC column using MeOH-H<sub>2</sub>O (3:1) to yield compounds **1** (75 mg), **2** (15 mg), and **3** (21 mg)

as yellow crystals.

**6,8-Dimethyl-4'-methoxy-5,7,3',5'-tetrahydroxyflavanone (1):** Yellow crystals; mp. 223-224°C; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3400 (OH), 1716 (C=O), 1445 (C=C); ESI-MS  $m/z$ : 347 [M+H]<sup>+</sup>; 345 [M-H]<sup>-</sup> (C<sub>18</sub>H<sub>18</sub>O<sub>7</sub>); <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>): see Table 1.

**6,8-Dimethyl-3',4'-dimethoxy-5,7,5'-trihydroxyflavanone (2):** Yellow crystals, mp. 234-235°C; ESI-MS  $m/z$ : 361 [M+H]<sup>+</sup>; 359 [M-H]<sup>-</sup> (C<sub>19</sub>H<sub>20</sub>O<sub>7</sub>); <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>), see table 1.

**6-Methoxy-5,7-dihydroxyflavanone (3):** Yellow crystals; mp. 176-177°C; ESI-MS  $m/z$ : 287 [M+H]<sup>+</sup>; 285 [M-H]<sup>-</sup> (C<sub>16</sub>H<sub>14</sub>O<sub>5</sub>); <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD), see table 1.



### III - RESULTS AND DISCUSSION

Compound **1** was isolated as yellow crystals from the ethyl acetate fraction suggesting a flavonoid compound. The <sup>1</sup>H-NMR spectrum of **1** showed only a singlet at  $\delta$  6.43 (2H) of the aromatic ring suggesting that the A ring was full substituted, and the B ring was substituted at C-1, C-3, C-4, C-5 positions. The signals at  $\delta$  5.34 (dd,  $J = 3.0, 12.5$  Hz), 2.76 (dd,  $J = 3.0, 12.5$  Hz) and 3.02 (dd,  $J = 12.5, 17.0$  Hz) confirming the presence of a flavanone compound [2], two methyl groups resonance at  $\delta$  1.98 (3H, s) and 1.95 (3H, s) suggested that they were directly

attached to the A ring. In addition, the signal at  $\delta$  12.33 displayed the hydroxyl group at C-5 [3], and a methoxyl group was at  $\delta$  3.68 (3H, s).

The <sup>13</sup>C-NMR of **1** displayed signals of 18 carbon atoms, including 15 signals of the flavonoid and 3 signals of the two methyl groups ( $\delta$  7.55 and 8.26) and of the methoxyl group at  $\delta$  59.63. The carbonyl group was assigned at  $\delta$  196.51, the flavanone compound was confirmed by the signals of the oxymethine at  $\delta$  77.58 and of the methylene group at  $\delta$  42.06. In the HSQC spectrum, protons at  $\delta$  6.43, 5.34, 3.68, 1.98 and 1.95 had cross-peaks to

carbons at  $\delta$  105.33, 77.58, 59.63, 8.26 and 7.55, respectively. While protons at  $\delta$  2.76 and 3.02 had cross-peaks to carbon at  $\delta$  42.06. In the HMBC, proton H-2' at  $\delta$  6.43 correlated with carbon C-2 ( $\delta$  77.58)/C-1' ( $\delta$  135.25)/C-3' ( $\delta$  150.70) and carbon C-4' ( $\delta$  134.36), methoxyl proton at  $\delta$  3.68 correlated with C-4' ( $\delta$  134.36), methyl proton at  $\delta$  1.98 correlated with C-7 ( $\delta$  162.39)/C-8 ( $\delta$  102.54)/C-9 ( $\delta$  157.09), methyl proton at  $\delta$  1.95 correlated with C-5 ( $\delta$  158.34)/C-6 (103.21)/C-6 (162.39). This evidence confirmed the position of the two hydroxyl groups at C-3' and C-5', and methoxyl group at -4', and two methyl groups at C-6 and C-8 of the A ring. In addition, H-C long range correlations were observed between H-2 ( $\delta$

5.34) and C-1' ( $\delta$  135.25)/C-3 ( $\delta$  42.06)/C-4 ( $\delta$  196.51), between proton H-3 ( $\delta$  2.67/3.02) and carbons C-2 ( $\delta$  77.58)/C-4 ( $\delta$  196.51) in the HMBC confirming again the flavanone skeleton of **1**. Comparing the chemical shifts ( $\delta_C$  and  $\delta_H$ ) and proton coupling-constants ( $J$ ) at C-2 and C-3 of **1** ( $J_{H-2/Ha-3} = 12.5$  Hz,  $J_{H-2/Hb-3} = 3.0$  Hz,  $J_{H-3gem} = 17.0$  Hz) with those of naringerin [4] led to determine the absolute configuration at C-2 as *R*. Furthermore, the ESI-MS of **1** exhibited the quasi ion peaks at  $m/z$  347  $[M+H]^+$  (positive) and 345  $[M-H]^-$  (negative), corresponding to the molecular formula of  $C_{18}H_{18}O_7$ . From the above data, compound **1** was determined to be new natural product 6,8-dimethyl-4'methoxy-5,7,3',5'-tetrahydroxyflavanone, which we named ophiofolius A.

Table 1: NMR data of **1** - **3**

C	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_C^{a,c}$	$\delta_H^{b,c}$	$\delta_C^{a,c}$	$\delta_H^{b,c}$	$\delta_C^{a,d}$	$\delta_H^{b,d}$
2	77.58	5.34 dd (3.0, 12.5)	77.92	5.36 dd (3.0, 12.0)	80.54	5.42 dd (3.0, 12.0)
3	42.06	2.76 dd (3.0, 17.00) 3.02 dd (12.5, 17.0)	42.19	2.70 dd (3.0, 17.00) 3.12 dd (12.5, 17.0)	44.19	2.78 dd (3.0, 17.00) 3.08 dd (12.0, 17.0)
4	196.51	-	196.56	-	198.05	-
5	158.34	-	158.46	-	160.85	-
6	103.21	-	103.37	-	130.53	-
7	162.39	-	162.47	-	159.98	-
8	102.54	-	102.62	-	96.27	6.02 s
9	157.09	-	157.15	-	140.38	-
10	101.74	-	102.62	-	103.52	-
1'	135.25	-	134.61	-	140.38	-
2'	105.33	6.43 s	107.40	6.64 s	127.32	7.38-7.50
3'	150.70	-	153.19	-	130.53	7.38-7.50
4'	134.36	-	136.2	-	129.60	7.38-7.50
5'	150.70	-	150.57	-	130.53	7.38-7.50
6'	105.33	6.43 s	101.75	6.64	127.32	7.38-7.50
6-Me	7.55	1.95 s	7.59	1.96 s		
8-Me	8.26	1.98 s	8.29	1.99 s		
4'-OMe	59.63	3.68 s	55.75	3.68 s		
3'-OMe			55.89	3.79 s		
6-OMe					60.99	3.80 s
5-OH		12.33		12.35		

<sup>a</sup>125 MHz, <sup>b</sup>500 MHz, <sup>c</sup>In DMSO-*d*<sub>6</sub>, <sup>d</sup>In CD<sub>3</sub>OD. Chemical shifts are given in ppm; coupling constant *J* (in parentheses) in Hz.

The NMR data of **2** were very similar to those of **1** except for the additional signals of one methoxyl group at  $\delta_C$  59.89/ $\delta_H$  3.79. This suggested that compound **2** was a methoxyl derivative of **1** with the suggested molecular formula as  $C_{19}H_{20}O_7$ , which was further confirmed by the appearance of the quasi ion peaks at  $m/z$  361  $[M+H]^+$  (positive) and 359  $[M-H]^-$  (negative) in the ESI-MS spectrum. To determine the position of the additional methoxyl group, the HSQC and HMBC spectra

were taken. All HMBC correlations in the HMBC spectrum were shown in Fig. 2, and the NMR data of this compound were summarized in Table 1. Consequently, the structure of **2** was determined as 6,8-dimethyl-3',4',5'-trimethoxy-5,7-dihydroxyflavanone, which was isolated from *Alluaudiposis marnieriana*. However, this is the first report of **2** from *Ophiopogon* species, and the NMR data (1D and 2D) of this compound have been reported here for the first time.

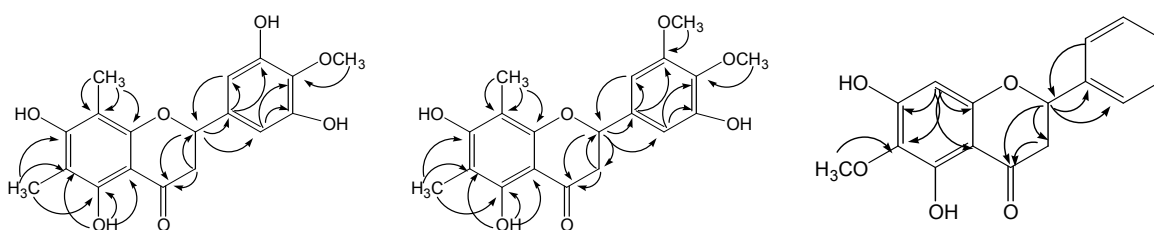


Figure 2: H-C correlations in the HMBC of **1** - **3**

The  $^{13}C$ -NMR spectrum of **3** displayed the resonances of 16 carbon atoms including a benzene ring (B ring) and a methoxyl group at  $\delta_C$  60.99/ $\delta_H$  3.80. The signals at  $\delta$  80.54 (CH) and 44.19 (CH<sub>2</sub>) suggest a flavanone compound. In the  $^1H$ -NMR, signal at  $\delta$  5.42 (1H, dd,  $J = 3.0, 12.0$  Hz), 2.78 (1H, dd,  $J = 3.0, 17.0$  Hz) and 3.08 (1H, dd,  $J = 12.0, 17.0$  Hz) suggesting the 2*R* configuration of **2** [4] as **1**. The position of the methoxyl and the two hydroxyl groups were determined from the analysis of the HSQC and HMBC as shown in table 1 and Fig. 2. In addition, the ESI-MS of **3** showed the quasi ion peaks at  $m/z$  287  $[M+H]^+$  (positive) and 285  $[M-H]^-$  (negative) corresponding to the molecular formula of  $C_{16}H_{14}O_5$ . Thus, compound **3** was determined to be 6-methoxy-5,7-dihydroxyflavanone, which was the first isolated from *Ophiopogon* species. To the best of our knowledge, the NMR data (1D and 2D) of this compound have been reported here for

the first time.

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