

## OPHIOFOLIUS B, A NEW SESQUITERPENE FROM *OPHIOPOGON CONFERTIFOLIUS*

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### SUMMARY

From the methanolic extract of the roots of *Ophiopogon confertifolius* N. Tanaka (Convallariaceae) a new sesquiterpene named ophiofolius B (**1**) was isolated. Its structure was elucidated as 3-eudesmene-1 $\alpha$ ,11-diol by the spectroscopic experiments (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT 90°, DEPT 135°, HSQC, HMBC, <sup>1</sup>H-<sup>1</sup>H COSY, and ESI-MS).

### I - INTRODUCTION

“Cao cãng” (*Ophiopogon confertifolius* N. Tanaka (Convallariaceae)) is a new species of Vietnamese flora [1] is a traditional medicinal plant used to treat osteocopic pain, dispel swelling and blood clotting in ecchymosis, renal failure □ 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 a new sesquiterpene named ophiofolius B (**1**) from the methanolic extract of the roots of this plant. The new nature product was elucidated as 3-eudesmene-1 $\alpha$ ,11-diol by the spectroscopic experiments (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT 90°, DEPT 135°, HSQC, HMBC, <sup>1</sup>H-<sup>1</sup>H COSY, ROESY and ESI-MS).

### II - EXPERIMENT

#### 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 of specimen was deposited at 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 EI mass spectrum was obtained using a Jeol AX-505 spectrometer. The ionization voltage was 70 eV. 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 (13.0 g) fractions. The chloroform fraction (10.2 g) was combined chromatographed on silica gel column and then on YMC column to yield compounds **1** (14 mg) as colorless crystals.

**3-Eudesmene-1 $\alpha$ ,11-diol (ophiofolius B, 1):** Colorless crystals; mp. 175 - 176°C; IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3340 (OH), 2985-2890 (CH), 1445 (C=C); EI-MS (70 eV)  $m/z$  (%): 220 [ $\text{M}-\text{H}_2\text{O}]^+$  ( $\text{C}_{15}\text{H}_{26}\text{O}_2$ ), (54.6), 203 (55.4), 187 (14.2), 177 (98.4), 159 (46.6), 147 (28.3), 107 (44.3), 91 (40.1), 81 (43.7), 59 (100.0);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ), see table 1.

### III - RESULTS AND DISCUSSION

Compound **1** was isolated as colorless crystals from the chloroform fraction. The  $^1\text{H-NMR}$  spectrum of **1** showed a broad singlet signal of the three-substituted double bond at  $\delta$  5.33, four methyl singlet signals at  $\delta$  1.11, 1.18, 1.19 and 1.70, in which proton resonance at  $\delta$  1.17 suggesting that this methyl group must be attached to the double bond. A proton of the methine bearing oxygen atom was assigned at  $\delta$  3.40 as a broad singlet. Two other methine protons were assigned at  $\delta$  2.16 and 1.16, and eight protons of four methylene groups were at 2.47 (dm,  $J = 16.5$  Hz,  $\text{H}_{\text{ax}}-2$ ), 2.05 (dm,  $J = 16.5$  Hz,  $\text{H}_{\text{eq}}-2$ ), 1.95 (dd,  $J = 13.5, 2.5$  Hz,  $\text{H}_{\text{ax}}-6$ ), 1.38 (dd,  $J = 13.5, 4.5$  Hz,  $\text{H}_{\text{eq}}-6$ ), 1.61 (ddd,  $J = 12.5, 6.0, 3.0$  Hz,  $\text{H}_{\text{ax}}-8$ ), 1.29 (dd,  $J = 12.5, 3.5$  Hz,  $\text{H}_{\text{eq}}-8$ ), 1.26 (overlapped,  $\text{H}_{\text{ax}}-9$ ), and 1.40 (dd,  $J = 12.5, 3.5$  Hz,  $\text{H}_{\text{eq}}-9$ ) deduced from the DEPT, HSQC and ROESY spectra.

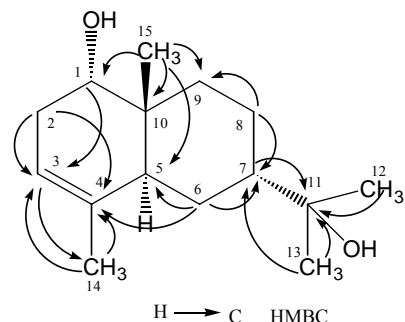


Fig. 1: The structure and HMBC correlations of **1**

The  $^{13}\text{C-NMR}$  spectrum exhibited signals of 15 carbon atoms suggesting a sesquiterpene structure, including four methyl ( $\delta$  27.05, 26.93, 22.09, and 21.66), four methylene ( $\delta$  31.32, 31.30, 24.18, and 22.32), four methine (119.10, 74.52, 44.68, and 39.57), two quaternary carbons ( $\delta$  135.40 and 36.29), and one tertiary carbon at  $\delta$  72.77, which were further deduced from the DEPT  $90^\circ$  and DEPT  $135^\circ$  spectra. In the heteronuclear single quantum coherence (HSQC) spectrum, H-3 proton at  $\delta$  5.33 had a cross peak with carbon at  $\delta$  119.10, H-1 proton at  $\delta$  3.40 had cross peak with carbon at  $\delta$  74.52. Other cross peaks between protons linking to corresponding carbons was elucidated from the HSQC as shown in table 1.

The partial structures of **1** were carefully deduced from the  $^1\text{H}-^1\text{H}$  correlation spectroscopy ( $^1\text{H}-^1\text{H}$  COSY). In the  $^1\text{H}-^1\text{H}$  COSY spectrum, H-2 ( $\delta$  2.47 and 2.05) correlated with H-1 ( $\delta$  3.40) and with H-3 ( $\delta$  5.33), H-6 ( $\delta$  1.95 and 1.38) correlated with H-5 ( $\delta$  2.16) and with H-7 ( $\delta$  1.16), as well as H-8 ( $\delta$  1.29 and 1.61) correlated with H-7 ( $\delta$  1.16) and with H-9 ( $\delta$  1.40 and 1.26). Comparing the NMR data of **1** with those of 3-eudesmene-1 $\beta$ ,11-diol and of 7-epi- $\gamma$ -eudesmol (Su, *et. al.* 1995) suggesting the 3-eudesmene skeleton with the H-7 configuration as equatorial ( $\text{H}_{\beta}-7$ ). The carbon chemical shifts at  $\delta$  119.10 (CH) and 135.4 (C) together with the results of  $^1\text{H}-^1\text{H}$  COSY spectrum confirmed the location of the double bond at C-3 and C-4 (Su, *et. al.* 1995). The suggesting chemical structure of **1** was

shown in Fig. 1. The chemical shifts at C-5, C-6, C-7, C-8, C-11, C-12, and C-13 of **1** and 3-eudesmene-1 $\alpha$ ,11-diol differed. This evidence suggested that their C-7 absolute configurations

differed with the H $_{\beta}$ -7 of **1** and H $_{\alpha}$ -7 of 3-eudesmene-1 $\alpha$ ,11-diol. This was further confirmed by the ROESY spectrum.

Table 1: NMR data of **1**

Pos.	$\delta_C$	DEPT	$\delta_H$	$^1H$ - $^1H$ COSY	ROESY	HMBC H to C
1	74.52	CH	3.40 br s, H $_{eq}$	H-2	H-15	C-3
2	31.30	CH $_2$	2.47 dm (16.5), H $_{ax}$ 2.05 dm (16.5), H $_{eq}$	H-1, H3 H-1, H-3		C-3, C-4
3	119.10	CH	5.83 br s	H-2	H-14	C-1, C-5, C-14
4	135.40	-	-			
5	39.57	CH	2.16*, H $_{ax}$	H-6		
6	24.18	CH $_2$	1.95 dd (13.5, 2.5), H $_{ax}$ 1.38 dd (13.5, 4.5), H $_{eq}$	H-5, H-7 H-5, H-7	H $_{eq}$ -7, H $_{ax}$ -8, H $_{eq}$ -9 H-13	C-4, C-5, C-7 C-4, C-5, C-7
7	44.68	CH	1.16*, H $_{eq}$	H-6, H-8	H $_{ax}$ -6, H $_{ax}$ -8	
8	22.32	CH $_2$	1.61 ddd (12.5, 6.0, 3.0), H $_{ax}$ 1.29 dd (12.5, 3.5), H $_{eq}$	H-7, H-9 H-7, H-9	H $_{ax}$ -6, H $_{eq}$ -7, H-15 H-12	C-7, C-9
9	31.32	CH $_2$	1.26*, H $_{ax}$ 1.40 dd (12.5, 3.5), H $_{eq}$	H-8 H-8	H $_{ax}$ -6	
10	36.29	-	-			
11	72.77	-	-			
12	26.93	CH $_3$	1.18 s		H $_{eq}$ -8	C-7, C-11
13	27.05	CH $_3$	1.19 s		H $_{eq}$ -6	C-7, C-11
14	21.66	CH $_3$	1.70 s		H-3	C-3, C-4, C-5
15	22.09	CH $_3$	1.11 s		H-1, H $_{ax}$ -8	C-1, C-5, C-9, C-10

<sup>a</sup>125 MHz, <sup>b</sup>500 MHz, <sup>c</sup>In CDCl $_3$ . Chemical shifts are given in ppm; coupling constant *J* (in parentheses) in Hz, \*Overlapped signals.

In the ROESY spectrum, H-15 methyl protons at  $\delta$  1.11 correlated with H-1 proton ( $\delta$  3.40) and H $_{ax}$ -8 ( $\delta$  1.61) confirming that they are  $\beta$ -configuration. H $_{ax}$ -8 proton ( $\delta$  1.61) correlated with H $_{eq}$ -7 at  $\delta$  1.16 further confirming the  $\beta$ -configuration of H-7. All the absolute

configuration of **1** was carefully deduced and shown in Fig. 2. In addition, H-C long-range correlation between H-14 ( $\delta$  1.70) and C-3 ( $\delta$  119.10)/C-4 ( $\delta$  135.40)/C-5 (39.57), between H-2 ( $\delta$  2.47/2.05) and C-1 ( $\delta$  74.52)/C-3 ( $\delta$  119.10)/C-4 (135.40)/C-10 ( $\delta$  36.29), between

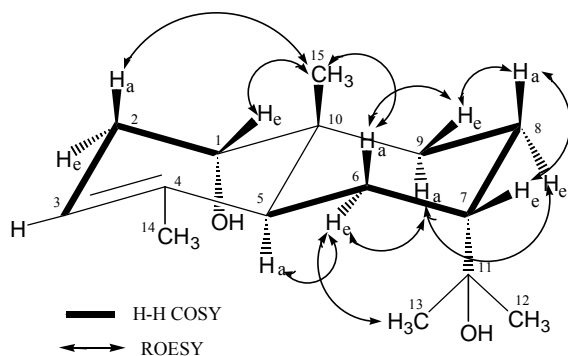


Fig. 2: The ROESY and H-H COSY correlations of **1**

H-13 ( $\delta$  1.19)/H-12 ( $\delta$  1.18) and carbon C-11 ( $\delta$  72.77)/C-7 ( $\delta$  44.68), and between H-6 ( $\delta$  1.95/1.38) and C-5 (39.57)/C-7 ( $\delta$  44.68) were observed in the HMBC spectrum of **1**. These evidence confirmed the 3-eudesmene-1 $\alpha$ ,11-diol structure of **1**. Furthermore, the exhibition

of the quasi ion peak at  $m/z$  220  $[M-H_2O]^+$  in the EI-MS spectrum of **1** correspond to the molecular formula of  $C_{15}H_{26}O_2$ . From the above data, the structure of **1** was determined to be a new nature product as 3-eudesmene-1 $\alpha$ ,11-diol and named ophiofolius B.

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