



DICTYOPHORINES A AND B, TWO STIMULATORS OF NGF-SYNTHESIS FROM THE MUSHROOM *DICTYOPHORA INDUSIATA*

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Abstract—Two novel eudesmane-type sesquiterpenes, dictyophorines A and B, and a known compound, teucrone, were isolated from the mushroom *Dictyophora indusiata*. Dictyophorines A and B promoted nerve growth factor (NGF)-synthesis by astroglial cells. © 1997 Elsevier Science Ltd. All rights reserved

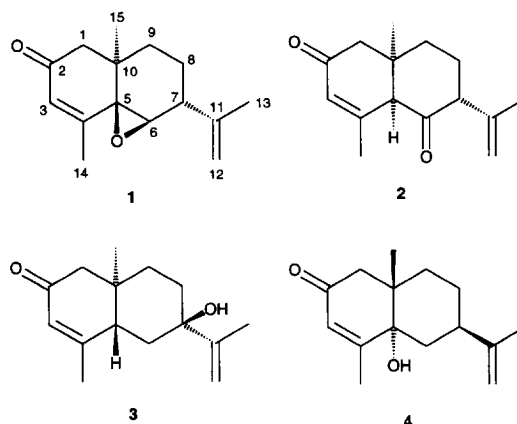
INTRODUCTION

In our research for biologically active compounds from various mushrooms [1–25], two new sesquiterpenes were isolated from the mushroom *Dictyophora indusiata* (Kinugasatake in Japanese), which is an edible mushroom used in Chinese food and medicine. We describe the isolation, structure determination and biological activity of the compounds.

RESULTS AND DISCUSSION

Air dried fruiting bodies of the fungus were extracted with 85% ethanol. The extract after concentration was partitioned between ethyl acetate and water. Repeated silica gel chromatography and HPLC of the ethyl acetate extract gave **1** and **2**.

Dictyophorine A (**1**) showed ion peaks at m/z 233 $[M + H]^+$ (FABMS) and at m/z 232 $[M]^+$ (EIMS). The molecular formula $C_{15}H_{20}O_2$ was determined by HR-EIMS of the peak ion at m/z 232.1477 (calcd 232.1464). The 1H NMR, ^{13}C NMR (Table 1) and IR data (1674 cm^{-1}) showed that **1** has a β -substituted- α,β -unsaturated carbonyl group [δ_H 6.06 (s); δ_C 198.4, 131.8 and 154.3]. Furthermore, **1** possessed an epoxide ring [δ 3.51 (s); δ 63.3, 61.2] and an exo-methylene group [δ 4.79 (s), 4.85 (s); δ 111.2]. The complete planar structure was determined by interpretation of the HMBC cross peaks as follows; H-1 and C-2, H-1



and C-5, H-1 and C-15, H-3 and C-5, H-3 and C-14, H-12 and C-11, H-12 and C-13, H-13 and C-7, H-13 and C-12, H-14 and C-3, H-14 and C-4, H-14 and C-5, H-15 and C-1, H-15 and C-5, H-15 and C-9, and H-15 and C-10. Thus, **1** was determined as 5,6-epoxy-3,11-eudesmadien-2-one. The relative stereochemistry of **1** was confirmed by a NOESY experiment; cross peaks between H-6 and H-13, H-6 and H-14, H-6 and H-15, H-15 and H-13, and H-15 and H-12 were observed. The absolute configuration of **1** was deduced by comparison of the CD spectrum of **1** with that of α -rotunol (**4**); **1** showed a positive Cotton effect for an $n-\pi^*$ transition ($[\Theta]_{358} + 2400$), while **4** exhibited a negative one ($[\Theta]_{361} - 530$) [26, 27]. In addition, teucrone (**3**) was also isolated from this mushroom and its CD spectrum coincided with that of the authentic **3** which was isolated from *Teucrium het-*

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Table 1. ^{13}C and ^1H NMR data for compounds **1** and **2** (in CDCl_3)*

Position	δ ppm (multiplicity, J in Hz)			
	1		2	
1	49.5	2.59(<i>d</i> , 16.2), 2.26(<i>d</i> , 16.2)	49.1	2.34(<i>d</i> , 16.2), 2.18(<i>d</i> , 16.2)
2	198.4		198.0	
3	131.8	6.06(<i>s</i>)	127.6	6.04(<i>br. s</i>)
4	154.3		155.3	
5	63.3		62.4	3.10(<i>br. s</i>)
6	61.2	3.51(<i>s</i>)	209.7	
7	41.6	2.75(<i>dd</i> , 10.2, 8.9)	55.7	2.96(<i>dd</i> , 5.9, 9.6)
8	22.0	1.47(<i>m</i>), 1.66 (<i>m</i>)	26.4	1.8-2.2(<i>m</i>)
9	31.5	1.12(<i>m</i>), 1.80 (<i>m</i>)	32.4	1.8-2.2(<i>m</i>)
10	35.0		41.4	
11	147.3		141.8	
12	111.2	4.85(<i>s</i>), 4.79(<i>s</i>)	113.2	5.03(<i>br. s</i>), 4.79(<i>br. s</i>)
13	21.3	1.82(<i>s</i>)	21.7	1.79(<i>s</i>)
14	18.8	1.82(<i>s</i>)	22.6	1.85(<i>s</i>)
15	22.0	1.17(<i>s</i>)	26.9	1.12(<i>s</i>)

* These assignments were established by the decoupling, COSY, NOESY, NOE-difference, HMQC, and/or HMBC experiments.

erophyllum and whose absolute configuration has been determined (data not shown) [28].

The molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_2$ (HR-MS, $[\text{M}]^+ = m/z$ 232.1472, calcd 232.1464) of dictyophorine **B** (**2**), was the same as that of **1**, and the NMR data (Table 1) were similar to those of **1**. However, this compound had a saturated carbonyl group (δ_{C} 209.7) and an allylic methine one [δ_{H} 3.10 (*br. s*)] showing a long range-coupling with an olefinic proton [δ_{H} 6.04 (*br. s*)] instead of an epoxide ring in **1**. These results indicated that the structure of **2** was 3,11-eudesmadiene-2,6-dione. This assumption was confirmed by the following reaction; treatment of **1** with HClO_4 in THF afforded **2** through cleavage of the epoxide ring of **1** by hydration with the reagent and then dehydration of the resulting diol followed by keto-enol tautomerization. Since a NOE was observed between H-5 and H-15 and the CD spectrum of **2** formed from **1** was identical with that of natural **2** isolated from the mushroom, the stereochemistry including the absolute configuration was deduced as proposed.

Many eudesmane-type sesquiterpene have been isolated from natural sources. However, to our knowledge, this is the first time that they have been isolated from mushrooms.

Dictyophorine **A** (**1**) promoted the synthesis of NGF in every experiment using astroglial cells. [11, 14, 16, 19, 25, 29–31]. A typical result showed that the amounts (45.1 ± 1.1 pg ml^{-1}) of NGF secreted into the medium in the presence of 3.3 mM of **1** was four times higher than those in the absence of the compound. **2** caused a slight but significant increase in NGF secretion level. The detail for the activity of these compounds will be reported elsewhere.

EXPERIMENTAL

Mp: uncorr. ^1H NMR: 400 MHz as int. standard. ^{13}C NMR: 100 MHz. *Dictyophora indusiata* (Vent.: Pers.) Fisch was collected from bamboo groves in Japan and China.

Extraction and isolation. Air dried fruiting bodies of *D. indusiata* (1.2 kg) were extracted with 85% EtOH (15 l, $\times 5$) and the solvent was concd under red. pres. and partitioned between EtOAc and H_2O . The residue (20.2 g) obtained after removing the EtOAc was fractionated by repeated silica gel CC and HPLC to give **1** (246.0 mg, mp 39–40°) as crystals, and **2** (8.0 mg) amorphous.

Dictyophorine A (1). $[\alpha]_{\text{D}}^{25} + 28.1$ ($c = 1.00$, MeOH); IR $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 1675, 1440, 1413, 1378.

Dictyophorine B (2). $[\alpha]_{\text{D}}^{25} + 27$ ($c = 0.20$, MeOH); IR $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 1716, 1671, 1380.

Estimation of NGF synthesis stimulating activity. Quiescent rat astroglial cells, which were prepd as described previously [29], were used for estimation of NGF synthesis stimulating activity. Test samples were dissolved in Dulbecco's MEM medium containing 0.5% BSA and added to the culture. Media conditioned for 24 hr by the cells were collected and used for NGF measurement. NGF content was determined by a sensitive enzyme immunoassay as described previously [31]. Fold-increase of NGF content in the media was considered as the index of stimulating activity of NGF synthesis.

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