Three Sesquiterpenoids, Fascicularones E, F, and G Produced by the Fungus *Hypholoma fasciculare*

Yoshihito Shiono, Hironari Akasaka, Fuminori Hiramatsu, Kozue Sato, Tetsuya Murayama, and Michimasa Ikeda

Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan

Reprint requests to Dr. Y. Shiono. Fax: +81-235-28-2820. E-mail: yshiono@tds1.tr.yamagata-u.ac.jp

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Three new sesquiterpenoids, fascicularones E, F, and G, were isolated from the liquid culture extract of the fungus *Hypholoma fasciculare*. Their structures were established based on spectroscopic data.

Key words: Fascicularones E, F, and G, *Hypholoma fasciculare*

Introduction

As part of our ongoing investigation of biologically active compounds from fungi, we have studied the liquid culture of the poisonous mushroom *Hypholoma fasciculare* (*nigakuritake* in Japanese [1 – 3]), which belongs to the family Strophariaceae. From culture filtrates of this fungal source, we isolated several tricyclo[5.4.0.0^{2,5}]undecane skeletons, fascicularones A, C (**1**), and D (**2**), and a tricyclo[5.3.0.0^{2,5}]decane skeletons, fascicularone B (**3**) [4, 5]. The tricarbocyclic skeleton of fascicularones has been shown to be biosynthesized from *trans-trans* farnesyl pyrophosphate through the intermediation of a *cis*-caryophyllene derivative. The structural features and the interesting biosynthetic pathway aroused our interest in these compounds. In our ongoing search for new fascicularone derivatives from the liquid culture of this strain, we isolated three new fascicularones E (**4**), F (**5**), and G (**6**). Here, we discuss the isolation and structural clarification of these compounds.

Results and Discussion

A filamentous fungus strain that produced fascicularones was isolated from cultured tissues of fruiting bodies of the mushroom *Hypholoma fasciculare* [4]. The fungus was cultured at 25 °C for 30 days in a medium containing 4% glucose, 0.1% peptone, and 4% malt extract in distilled water. The EtOAc extract of the culture filtrate was purified by fractionation using a combination of silica gel and ODS column chromatography to yield fascicularones E (**4**), F (**5**), and G (**6**).

Fascicularone E (**4**) had the molecular formula $C_{15}H_{22}O_4$ according to HRFABMS and thus five degrees of unsaturation. The IR spectrum showed hydroxyls absorption at 3455 cm^{-1}, a carbonyl absorption at 1733 cm^{-1}, and a C-O-C group at 1016 cm^{-1}. ^{13}C NMR together with DEPT spectra of **4** showed one carbonyl carbon, three methines, two methylenes, four methyls, and four quaternary carbons. The ^{1}H NMR spectrum of **4** indicated the presence of four methine protons, one of which was linked to an oxygen [$\delta =$ 2.07 (m, 1H, 4-H), 2.20 (dd, $J = 7.3$, 1.5 Hz, 1H, 1-H), 2.56 (dt, $J = 9.3$, 7.3 Hz, 1H, 9-H), 4.56 (s, 1H, 2-H)], two methylenes [$\delta = 1.60$ (ddd, $J = 11.7$, 9.3, 2.0 Hz, 1H, 10 α-H), 1.83 (dd, $J = 14.2$, 3.9 Hz, 1H, 5 β-H), 1.88 (dd, $J = 14.2$, 10.3 Hz, 1H, 5 α-H), 2.26 (dd, $J = 11.7$, 7.3 Hz, 1H, 10 β-H)], three singlet methyls [$\delta = 1.06$ (s, 3H, 13-H$_3$) 1.17 (s, 3H, 15-H$_3$), 1.19 (s, 3H, 12-H$_3$)], and one secondary methyl [$\delta = 1.33$ (d, $J = 6.8$ Hz, 3H, 14-H$_3$)]. The ^{1}H and ^{13}C NMR spectra of **4** generally closely resembled those of **2** except for the absence of methine signals assigned to C-5 in **2**, the appearance of proton and carbon signals for a methylene (C-5) and a quarternary carbon (C-6), and chemical shift differences in some proton and carbon signals. ^{1}H and ^{13}C NMR shifts of **4** were fully assigned by 2D NMR spectra including HMBC. The HMBC correlation of 2-H with C-6 suggested that the ether bridge was located between C-2 and C-6. The gross structure of fascicularone E was thus clarified to be **4**.

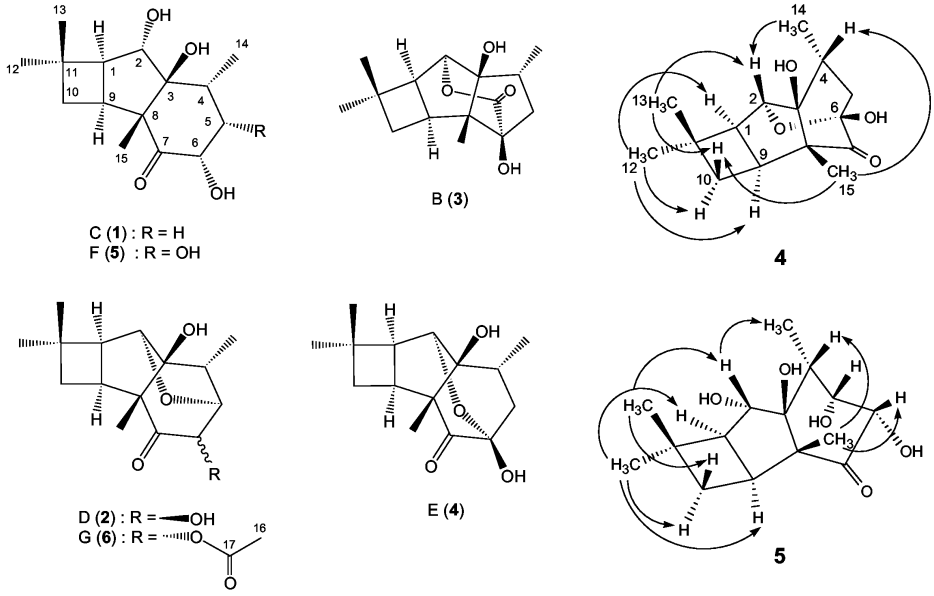

Fig. 1. The structures of fascicularones B (**3**), C (**1**), D (**2**), E (**4**), F (**5**) and G (**6**).

The stereostructure of **4** was ascertained by NOE experiments (Fig. 2). 12-H_3 was found to exhibit significant NOE interaction with 1-H and 9-H, implying the α-orientation of 1-H and 9-H, and simultaneously establishing the *cis*-fusion of the four- and five-member rings. The NOE correlation from 13-H_3 with 2-H, 13-H_3 with 10 β-H, 14-H_3 with 2-H, and 15-H_3 with 10 β-H showed that 2-H, 4-H, 13-H_3, and 15-H_3 all had a β-orientation, unambiguously establishing the relative structure of fascicularone E as **4** in Fig. 1.

The molecular formula of fascicularone F (**5**), $C_{15}H_{24}O_5$, was determined by HRFABMS, corresponding to one more oxygen atom than that of **1**. The IR spectrum of **5** showed the presence of a hydroxyl (3401 cm^{-1}) and a carbonyl (1716 cm^{-1}) absorption. ^{1}H and ^{13}C NMR spectra of **5** correspond well with those of **1**, but are characterized by the disappearance of the methylene signals of **1** and the appearance of characteristic signals due to an oxymethine signal [$\delta = 4.17$ (t, $J = 4.9$ Hz, 1H, 5-H)]. An HMBC experiment was conducted on **5** to determined the connectivity of the hydroxyl group. Observation of long-range ^{1}H and ^{13}C correlations among 5-H and C-3, 5-H and C-7, and 14-H_3 and C-5 showed that the hydroxyl group was at C-5. Based on these considerations, we clarified the planar structure of **5**. The stereochemical assignment of **5** was established by NOE experiments (Fig. 2). NOEs from 12-H_3 to 1-H and 9-H showed these protons to be on same side. NOEs from 13-H_3 to 2-H, 2-H to 14-H_3, 15-H_3 to 4-H, and 15-H_3 to 6-H were also observed in **5**, implying that 2-H, 4-H, 6-H, 13-H_3, and 15-H_3 all had β-orientation. The α-orientation of hydroxyl group at C-5 of **5** was deduced from the multiplicity of 5-H. Based on these results, the relative stereostructure of **5** was clarified as shown in Fig. 1.

Fig. 2. Selected NOE correlations for fascicularones E (**4**), F (**5**) and G (**6**).

The molecular formula of fascicularone G (**6**) was established to be $C_{17}H_{24}O_5$ by HRFABMS. IR absorption for **6** implied the presence of ester carbonyl (1745 cm^{-1}), carbonyl (1714 cm^{-1}), and ether (1024 cm^{-1}). The ^{1}H NMR spectrum (Table 3) of **6** analyzed by ^{1}H-^{1}H COSY and HMQC experiments showed signals for four methyls including one doublet methyl at $\delta = 0.98$ (s, 3H, 13-H_3), 1.15 (s, 3H, 15-H_3), 1.18 (s, 3H, 12-H_3) and 1.35 (d, $J = 7.3$, 3H, 14-H_3), a methylene proton at $\delta = 1.65$ (ddd, $J = 11.7$, 9.8, 2.0 Hz, 1H, 10 α-H) and 2.18 (m, 1H, 10 β-H), and six methine protons at $\delta = 2.24$ (m, 1H, 4-H), 2.31

(d, $J = 7.3$ Hz, 1H, 1-H), 3.05 (m, 1H, 9-H), 4.09 (d, $J = 2.0$ Hz, 1H, 5-H), 4.46 (s, 1H, 2-H), 5.04 (d, $J = 2.0$, 1H, 6-H), together with a singlet at $\delta = 2.16$ (s, 3H, 16-H_3) for a methyl of acetyl group (Table 3). ^{13}C NMR (Table 3) and DEPT spectra demonstrated signals characteristic of five methyls, one methylene, six methines including two oxymethines, and five quaternary carbons including two carbonyls. These data suggested that **6** was structurally related to **2**. The mass spectrum displayed prominent fragment ions at *m/z* 248 corresponding to the loss of an acetoxyl group (as acetic acid) from the molecule. The precise connectivity of **6** was established by interpretating HMBC data summarized in Table 3. In its HMBC spectrum, the correlation of H-6 with C-17 showed the connectivity of the acetoxyl group at C-6. HMBC correlations between H-2 and C-5, and 5-H and C-2 indicated that the ether bridge was located between C-2 and C-5. Based on the above evidence, planar structure of **6** was elucidated. The relative stereochemistry of fascicularone G was found to be **6** from the following NOEs: 12-H_3/ 1-H, 12-H_3/9-H, 13-H_3/2-H, 15-H_3/4-H, 15-H_3/10 β-H and 6-H/4-H (Fig. 2).

Fascicularones E (**4**), F (**5**), and G (**6**) are tricyclo[5.4.0.0^{2,5}]undecane skeleton sesquiterpenoids. The absolute configurations at C-1, C-4, and C-9 of **4**, **5**, and **6** may be biogenetically the same as those of fascicularone A.

In a lettuce seedling assay, fascicularones E (**4**), F (**5**), and G (**6**) showed radicle elongation of 180%, 160%, and 164% of controls at a concentration of 100 ppm.

Fascicularones A, C (**1**)-E (**4**), and F (**5**) and naematolins [6] C and G possessing the tricyclo[5.4.0.0^{2,5}]undecane skeleton are rare as naturally occurring microbial products. A similar sesquiterpene metabolite has been reported from cultured mycelia of *Hebeloma longicaudum* [7]. Biogenetically, fascicularones C (**1**) and E (**4**) would be derived from *cis*-fused caryophyllene by intramolecular cyclization and further oxidation. Fascicularone F (**5**) would in turn be formed by oxidation at C-5 from **1**. The formation of fascicularone G (**6**) would be derived from **1** by forming an ether bridge between C-2 and C-5 and further acetylation. When dissolved in $CHCl_3$ or MeOH, only a small amount of **4** slowly changed to fascicularone B (**3**), but $>$ 70% of **4** was left unchanged after 24 h. To explain this interesting conversion from **4** to **3**, we propose the reaction shown in Fig. 3. The possibility of **3** being an artifact produced during separation was excluded because **3** is present in amounts relatively larger than that of **4** in a fresh crude extract of this fungus. This reaction is thus catalyzed *in vivo* and **4** is considered to be a plausible biogenetic precursor of **3**. To clarify the biosynthetic pathway, we are now currently studying these new fascicularone analogs further.

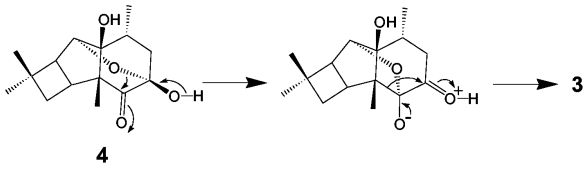

Fig. 3. Plausible synthesis and biosynthetic rearrangement of facicularone E (**4**) to facicularone B (**3**).

Experimental Section

General experimental procedures

Optical rotation was measured with a Horiba model SEPA-300 polarimeter, IR spectra were recorded with a JASCO J-20A spectrophotometer. Mass spectra were recorded with a JEOL JMS-700 instrument, and ^{1}H and ^{13}C NMR spectra were obtained with a JEOL EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc.) and ODS (Fuji Silysia). TLC was done on a precoated silica gel plate (Merck), and spots were detected by spraying 10% vanillin in H_2SO_4 followed by heating.

Mushroom material and fermentation

The producing fungal strain was isolated from cultured tissue of the fruiting bodies of *H. fasciculare* [1] and has been deposited in the Faculty of Agriculture, Yamagata University, Yamagata, Japan. The mycelium was grown in ten 500 ml Sakaguchi flasks containing 100 ml of a medium consisting of 40 g of glucose, and 1.0 g pepone per 1 l of water at 25 °C for 30 d on a rotary shaker at 120 rpm.

Extraction and isolation

Culture broth (5.0 l) of *H. fasciculare* was filtered under suction and the resulting filtrate was extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* to give a residue (9.9 g), which was subject to silica gel chromatography. Stepwise elution with *n*-hexane, *n*-hexane-EtOAc (9:1 – 1:9), EtOAc, EtOAc-MeOH (1:1) and MeOH give fractions 1 through 13 (Fr. 1-1 $\sim$ Fr. 1-13). Purification of the eluates was monitored by the characteristic intense blue coloration with 10% vanillin in H_2SO_4 on TLC plates.

Fr. 1-5 (620 mg), Fr. 1-7 (1.3 g) and Fr. 1-9 (344 mg) were separately chromatographed on silica gel column by eluting with $CHCl_3$ and increasing volume of EtOAc, suc-

Table 1. ^{1}H and ^{13}C NMR data for fascicularone E (**4**).

No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	55.1 d	2.20 (1H, dd, 7.3, 1.5)	2, 3, 12, 13
2	81.8 d	4.56 (1H, s)	3, 6, 8, 9
3	91.2 s		
4	40.8 d	2.07 (1H, m)	2
5	37.4 t	β 1.83 (1H, dd, 14.2, 3.9)	3, 4, 6, 7, 14
		α 1.88 (1H, dd, 14.2, 10.3)	3, 4, 6, 7, 14
6	93.0 s		
7	210.1 s		
8	59.6 s		
9	41.7 d	2.56 (1H, dt, 9.3, 7.3)	1, 2, 3, 7, 10
10	34.3 t	α 1.60 (1H, ddd,	
		11.7, 9.3, 2.0)	1, 8, 9, 12, 13
		β 2.26(1H, dd, 11.7, 7.3)	1, 8, 9, 12, 13
11	33.0 s		
12	33.7 q	1.19 (3H, s)	1, 10 11, 13
13	24.2 q	1.06 (3H, s)	1, 10, 11, 12
14	18.2 q	1.33 (3H, d, 6.8)	3, 4, 5
15	8.4 q	1.17 (3H, s)	3, 7, 8, 9

Taken in $CDCl_3$ at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

cessively subjected to column chromatography on ODS with H_2O-MeOH (10% stepwise gradient).

50% MeOH eluate from Fr. 1-5 was further flash-chromatographed on silica gel column using $CHCl_3$-MeOH (95:5, v/v) to obtain fascicularone G (**6**, 6.4 mg). 50% MeOH eluate from Fr. 1-7 was subject to silica gel column chromatography with a mixture *n*-hexane-EtOAc (2:1, v/v) to obtain fascicularone E (**4**, 3.8 mg). 40% MeOH eluate from Fr. 1-9 was flash-chromatographed on silica gel column using $CHCl_3$-MeOH (95:5) to obtain fascicularone F (**5**, 10.9 mg).

*Fascicularone E (**4**)*

Colorless oil – $[\alpha]_D^{20}$ −72.0 (*c* 0.23, $CHCl_3$). – IR (KBr): ν = 3455 (OH), 2948, 2863, 1733 (C=O), 1016 (C-O-C) cm^{-1}. – ^{1}H (400 MHz, $CDCl_3$) and ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) data see Table 1. – HRMS (positive mode, FAB): m/z = 267.1601 [M+H$^+$] (calcd. for $C_{15}H_{23}O_4$, 267.1598). – MS (positive mode, FAB): m/z = 267 [M+H$^+$].

*Fascicularone F (**5**)*

Colorless oil – $[\alpha]_D^{20}$ +84.2 (*c* 0.93, CH_3OH). – IR (KBr): ν = 3401 (OH), 2942, 2863, 1716 (C=O) cm^{-1}. – ^{1}H (400 MHz, $CDCl_3$) and ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) data see Table 2. – HRMS (positive mode, FAB): m/z = 285.1707 [M+H$^+$] (calcd. for $C_{15}H_{25}O_5$, 285.1702). – MS (positive mode, FAB): m/z = 285 [M+H$^+$].

*Fascicularone G (**6**)*

Colorless oil – $[\alpha]_D^{20}$ −106 (*c* 0.36, $CHCl_3$). – IR (KBr): ν = 3450 (OH), 2932, 1745 (COO), 1714 (C=O), 1024 (C-O-C) cm^{-1}. – ^{1}H (400 MHz, $CDCl_3$) and ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) data see Table 3. – HRMS (positive mode, FAB): m/z = 309.1705 [M+H$^+$] (calcd. for $C_{17}H_{25}O_5$, 309.1702). – MS (positive mode, FAB): m/z = 309 [M+H$^+$]. – MS (EI): m/z(%) = 308 (5) [M$^+$], 266 (43), 248 (15) [M$^+$-CH_3COOH], 218 (61), 132 (92), 123 (100), 85 (78).

Table 2. ^{1}H and ^{13}C NMR data for fascicularone F (**5**).

No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	57.5 d	2.12 (1H, dd, 7.8, 1.5)	2, 8, 9, 10, 11, 13
2	79.0 d	4.01 (1H, s)	8, 9, 11
3	92.5 s		
4	41.0 d	2.39 (1H, m)	3, 6, 14
5	72.6 d	4.17 (1H, t, 4.9)	3, 4, 6, 7, 14
6	73.1 d	4.51 (1H, d, 4.9)	4, 8
7	210.5 s		
8	59.0 s		
9	35.9 d	3.57 (1H, m)	1, 7, 8, 10
10	34.4 t	α 1.60 (1H, ddd,	
		11.7, 9.8, 2.4)	1, 9, 11, 12
		β 2.15(1H, dd, 11.7, 7.8)	1, 8, 9, 11, 12
11	32.8 s		
12	24.9 q	1.03 (3H, s)	1, 10 11, 13
13	33.2 q	1.26 (3H, s)	1, 10, 11, 12
14	11.2 q	1.37 (3H, d, 7.3)	3, 4, 5
15	15.6 q	1.23 (3H, s)	3, 7, 9

Taken in $CDCl_3$ at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

Table 3. ^{1}H and ^{13}C NMR data for fascicularone G (**6**).

No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	52.4 d	2.31 (1H, d, 7.3)	2, 3, 10, 12
2	87.2 d	4.46 (1H, s)	3, 5, 8, 9, 11
3	90.6 s		
4	41.9 d	2.24 (1H, m)	2, 3, 6, 8, 14
5	80.2 d	4.09 (1H, d, 2.0)	2, 3, 4, 6, 7, 14
6	77.2 d	5.04 (1H, d, 2.0)	4, 7, 8, 17
7	211.4 s		
8	64.9 s		
9	45.7 d	3.05 (1H, m)	1, 2, 3, 7, 10
10	34.7 t	α 1.65 (1H, ddd,	
		11.7, 9.8, 2.0)	1, 8, 9, 12
		β 2.18 (1H, m)	8, 9, 12, 13
11	32.9 s		
12	32.0 q	1.18 (3H, s)	1, 10, 13
13	24.6 q	0.98 (3H, s)	1, 10, 11
14	13.7 q	1.35 (3H, d, 7.3)	3, 4, 5
15	12.9 q	1.15 (3H, s)	3, 7, 8, 9
16 (CH_3CO)	20.8 q	2.16 (3H, s)	17
17 (CH_3CO)	170.4 s		

Taken in $CDCl_3$ at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

Lettuce seedling assay

This assay was performed as reported [8].

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