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Phytochemistry 65 (2004) 491–496
PHYTOCHEMISTRY
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Fasciculones A and B from a mycelial culture of *Naematoloma fasciculare*

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Received 30 May 2003; received in revised form 16 September 2003

Abstract

Two sesquiterpenoids, fasciculones A and B, have been isolated from the culture broth of a poisonous mushroom, *Naematoloma fasciculare*. Their structures were determined using spectroscopic methods.

Keywords: *Naematoloma fasciculare*; Nigakuritake; Poisonous mushroom; Fasciculones A and B; Sesquiterpenoids

1. Introduction

The bitter poisonous mushroom *Naematolome fasciculare* (Japanese name: Nigakuritake) is widely distributed in northeast Japan. In a previous chemical investigation of these mushrooms, we isolated and identified six new lanostane triterpenoids, called fasciculols A–F (Ikeda et al., 1977a, b, c), some of which have been shown to inhibit the growth of Chinese cabbage seedlings and to have antimicrobial activity. It has been reported that fasciculols B, C, and F have calmodulin inhibitory activity (Kubo et al., 1985), and fasciculols E and F are the toxic principles of this mushroom (Suzuki et al., 1983). Three fasciculol esters, fasciculic acids A, B and C, having potent calmodulin antagonistic activity, were also isolated (Takahashi et al., 1989). From a liquid culture of this fungus, Ito et al. and Doi et al. isolated new ring-fused sesquiterpenes, the cytotoxic naematolin (Ito et al., 1967) and naematolins B, C (**1**), and G (**2**) (Tsuboyama et al., 1986; Doi et al., 1986, 1990). We are interested in nontoxic biologically active substances in mushrooms (Hirai et al., 1998) and have studied the biogenetic relationship between the components of the mycelium and the fruiting body of this fungus. In continuation of our investigation of the chemical constituents from the liquid culture of this fungus, we isolated two sesquiterpeneoids, fasciculones A (**3**) and B (**4**), as detailed below (Chart 1).

2. Results and discussion

The fungus was isolated from cultured tissues of fruiting bodies of mushrooms *N. fasciculare* collected in Yamagata Prefecture, Japan. The mycelium was grown in a stationary culture for 90 days at 25 °C, and the filtrate was extracted with EtOAc. The EtOAc extract was examined by TLC, and typical compound coloration was visualized by 10% vanillin in a sulfuric acid solution. Monitoring these spots by TLC, repeated use of Amberlite XAD-2, Sephadex LH-20 and silica gel column chromatography of the EtOAc extract led to the isolation of fasciculones A (**3**) and B (**4**).

The molecular formula of fasciculone A (**3**) was established to be $C_{14}H_{20}O_4$ by HREIMS analysis together with ^{1}H- and ^{13}C-NMR spectral data (Table 1), thus requiring five degrees of unsaturation. The UV spectrum showed an absorption maximum at 248 nm, suggesting the presence of a conjugated carbonyl group. The IR spectrum of **3** displayed bands ascribed to a hydroxyl group (3360 cm^{-1}) and an α,β-unsaturated ketone (1674 cm^{-1}). ^{13}C-NMR and DEPT spectra showed 14 carbon signals including characteristic signals due to three methyls [δ_C 12.7 (C-14), 25.5 (C-13)

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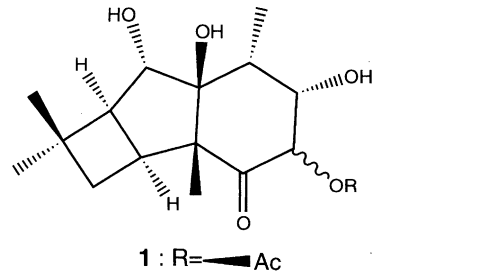
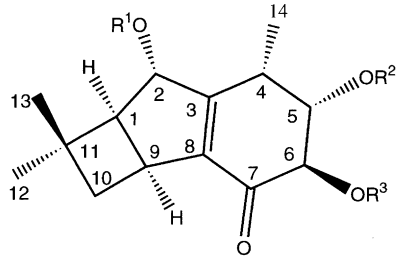
doi:10.1016/j.phytochem.2003.10.002

and 32.1 (C-12)], a carbonyl carbon [δ_C 196.3 (C-7)], one double bond [δ_C 141.4 (C-8) and 167.5 (C-3)], a methylene carbon [δ_C 38.6 (C-10)], six methines, three of which were linked to oxygen atoms [δ_C 35.1 (C-9), 36.4 (C-4), 54.5 (C-1), 74.1 (C-5), 74.2 (C-6) and 81.3 (C-2)], and a quaternary carbon [δ_C 34.2 (C-11)]. The ^{1}H-NMR spectrum, analyzed using ^{1}H–^{1}H-COSY and HMQC coupling correlations, indicated the presence of two tertiary methyls [δ_H 0.90 (3H, *s*, H_3-13) and 1.20 (3H, *s*, H_3-12)], –C<u>H</u>$_2$–C<u>H</u>–C<u>H</u>–C<u>H</u> (OH)- linkage [δ_H 1.36 (1H, *dd*, *J* = 12.2, 4.4 Hz, H-10), 2.13 (1H, *dd*, *J* = 12.2, 9.3 Hz, H-10), 3.41 (1H, *m*, H-9), 2.46 (1H, *d*, *J* = 6.3 Hz, H-1) and 4.78 (1H, *br. s*, H-2)], and a –C<u>H</u>$_3$–C<u>H</u>–C<u>H</u>(OH)–C<u>H</u>(OH)- linkage [δ_H 1.40 (3H, *d*, *J* = 6.8 Hz, H-14), 3.03 (1H, *m*, H-4), 3.97 (1H, *dd*, *J* = 11.2, 5.4 Hz, H-5), 4.28 (1H, *d*, *J* = 11.2 Hz, H-6)]. Based on these data, we assumed **3** had a tricyclic sesquiterpenoid-derived structure. Acetylation of **3** gave triacetate **3a**, for which the H-2, H-5 and H-6 signals at δ_H 4.78, 3.97, and 4.28 in the ^{1}H-NMR spectrum of **3** were shifted downfield to δ_H 5.86, 5.42, and 5.64. This clearly indicated that hydroxyl groups were located at C-2, C-5, and C-6. For the connectivity of partial structures, we conducted HMBC experiments (Table 1). The correlations between H_3-12 and C-11, and between H_3-13 and C-11, indicating that H_3-12 and H_3-13 were *geminal* methyls at C-11. The signals of H_3-12 and-13 also correlated with C-1 and C-10, the signal of H-9 with C-11, and the signal of H-1 with C-10. These data suggested the presence of a four-membered ring consisting of C-1, C-9, C-10, and C-11. The signal of H_3-14 correlated with C-3, C-4 and C-5, that of H-4 with C-8, that of H-5 with C-7, and that of H-6 with C-4 and C-8, suggesting the presence of a 2-cyclohexenone (C-3, C-4, C-5, C-6, C-7 and C-8). Correlations between H-2 and C-8, H-4 and C-2, and H-10 and C-8 allowed us to connect these partial fragments through a C–C bond between C-2 and C-3 and between C-8 and C-9 as shown in **3**. The relative stereochemistry of **3** was determined by a nuclear Overhauser effect (NOE) difference experiment (Fig. 1). NOEs from H-2 to H-4 and H_3-13 showed that these protons had β-configurations. NOEs from H-5 to H-4, but not to H_3-14 and H-6, and the coupling constant ($J_{4.5}$ = 5.4 and $J_{5.6}$ = 11.2 Hz) showed H-5 to have a β-configuration. NOEs from H-1 to H-9 and H_3-12 and from H-6 to H_3-14 indicated that these protons had α-configurations. To determine the absolute configuration at C-2, we used the modified Mosher's method. Protection of the diol group in **3** with



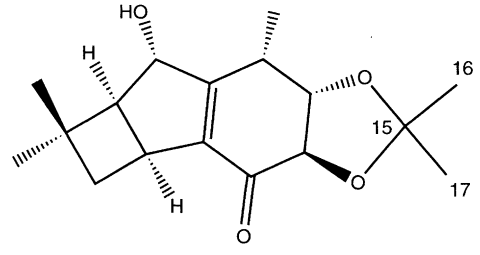
1 : R= Ac

2 : R= Ac



3 : R^1=H, R^2=H, R^3=H

3a : R^1=Ac, R^2=Ac, R^3=Ac



3b

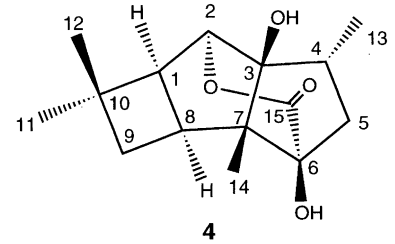


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Chart 1.

Table 1
^{1}H and ^{13}C NMR spectral data for fascicularone A (**3**) in $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C

No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	54.5 *d*	2.46 (1H, *d*, 6.3)	3, 8, 10, 12, 13
2	81.3 *d*	4.78 (1H, *br. s*)	8, 9, 11
3	167.5 *s*		
4	36.4 *d*	3.03 (1H, *m*)	2, 6, 8, 14
5	74.1 *d*	3.97 (1H, *dd*, 11.2, 5.4)	4, 7, 14
6	74.2 *d*	4.28 (1H, *d*, 11.2)	4, 5, 7, 8
7	196.3 *s*		
8	141.4 *s*		
9	35.1 *d*	3.41 (1H, *m*)	1, 3, 8, 11
10	38.6 *t*	1.36 (1H, *dd*, 12.2, 4.4)	1, 8, 12, 13
		2.13 (1H, *dd*, 12.2, 9.3)	1, 8, 12, 13
11	34.2 *s*		
12	32.1 *q*	1.20 (3H, *s*)	1, 10, 11, 13
13	25.5 *q*	0.90 (3H, *s*)	1, 10, 11, 12
14	12.7 *q*	1.40 (3H, *d*, 6.8)	3, 4, 5

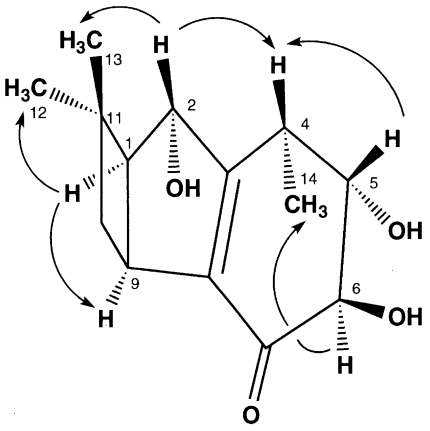
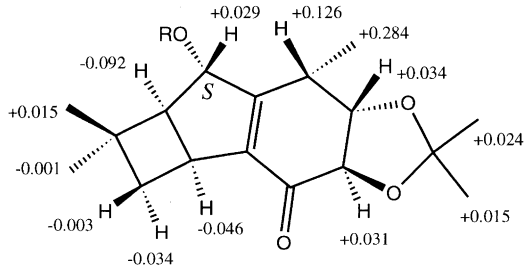


Fig. 1. NOE effects obtained from the NOE difference spectra of fascicularone A (**3**).



3c : R= (*S*)-MTPA
3d : R= (*R*)-MTPA

Fig. 2. $\Delta\delta$ values [δS-δR] for (*S*)-MTPA ester (**3c**) and (*R*)-MTPA ester (**3d**) of fascicularone A (**3**).

2,2-dimethoxylpropane gave acetonide **3b**. We used the modified Mosher's method to synthesize the MTPA ester of **3b** (Ohtani et al., 1991). Acetonide **3b** was esterified with (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetyl chloride (MTPACl) in pyridine to afford the (*S*)-MTPA ester (**3c**) and the (*R*)-MTPA ester (**3d**). The differences in chemical shift values [Δ values; δ (*S*)-δ (*R*)] for **3c** and **3d** are shown in Fig. 2. Based on the above results, we concluded that the configuration at C-2 in **3** must have a *S*-configuration. The absolute configuration at C-2 of **3** was the same as that of naematolins C and G. Consequently, we assumed the structure of **3** as (1*S*,9*S*)-4α,11,11-trimethyl -2α,5α,6β-trihydroxytricyclo[5.4.0.$0^{2,5}$]undec-3-en-7-one.

HR-EIMS of fascicularone B (**4**) showed the molecular formula $C_{15}H_{22}O_4$, indicating five degrees of unsaturation. IR absorption bands due to hydroxyls and an ester carbonyl stretches were observed at 3457 and 1727 cm^{-1}. The ^{13}C-NMR spectrum (Table 2) of **4** contained 15 carbons and its DEPT spectrum indicated the presence of four methyls [δ_C 7.7 (C-14), 14.4 (C-13), 23.9 (C-12) and 33.9 (C-11)], two methylenes [δ_C 33.5 (C-9), 42.5 (C-5)], four methines, including one oxygen-bearing methine, [δ_C 36.7 (C-8), 38.5 (C-4), 56.3 (C-1) and 88.0 (C-2)], four quaternary carbons, two of which were linked to oxygen [δ_C 33. 0 (C-10), 56.1 (C-7), 81.0 (C-6) and 89.5 (C-3)] and an ester carbon [δ_C 178.5 (C-15)]. ^{1}H- and ^{13}C-NMR data of **4** resembled that of **3**. The ^{1}H-NMR spectroscopic data of **4** showed the existence of a 4-membered ring with *gem*-dimethyl group signals [δ_H1.08 (3H, *s*, H_3-12), 1.20 (3H, *s*, H_3-11), 1.60 (1H, *ddd*, *J* = 11.7, 9.2. 2.0 Hz, H-9), 2.24 (1H, *dd*, *J* = 11.7, 6.8 Hz, H-9), 2.50 (1H, *br. d*, *J* = 7.3 Hz, H-1) and 2.83 (1H, *ddd*, *J* = 9.2, 7.3, 6.8 Hz, H-8)] observed in **3**. In the ^{1}H-NMR and ^{1}H–^{1}H COSY spectra of **4**, new signals were observed corresponding to a –CH_3–CH–CH_2– moiety [δ_H 1.29 (3H, *d*, *J* = 6.8 Hz, H_3-13), 2.32 (1H, *m*, H-4), 1.43 (1H, *dd*, *J* = 11.7, 2.9 Hz, H-5) and 2.35 (1H, *t*, *J* = 11.7 Hz, H-5)]. The connection of partial structures and remaining functional groups was determined based on HMBC correlations (Table 2). The remaining linkages of C-3 to C-2, C-4, and C-7 were deduced from HMBC correlations from H-1, H-8, H_3-13, and H_3-14 to C-3. In addition, the connectivity of C-7 to C-3, C-6 and C-8 is based on HMBC correlations from H-1, H-2, H-5, H-9 and OH-6 to C-7. HMBC correlations from H-2 and H-5 to C-15 suggested that the lactone ring was located between C-2 and C-6. To confirm the lactone ring, alkaline hydrolysis of **4** with 1 N KOH–MeOH, followed by methylation with CH_2N_2, yielded one methyl ester derivative **4a**. ^{1}H-and ^{13}C-NMR spectral data of **4a** corresponded well to that of **4**, except for the absence of the lactone ring moiety in **4** and the presence of signals due to a methyl ester (δ_H 3.77, δ_C 52.5, and δ_C 173.7). The stereochemical assignment of **4** was established by NOE experiments, the results of which are shown in Fig. 3 and denoted by arrows. NOEs between H-2 and H_3-12, H-2 and H_3-13,

Table 2
^{1}H and ^{13}C NMR spectral Data for fascicularone B (**4**) in $CDCl_3$ at 400 MHz for^{1}H and 100 MHz for ^{13}C

No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	56.3 *d*	2.50 (1H, *br. d*, 7.3)	2, 3, 7, 9, 10, 11
2	88.0 *d*	4.58 (1H, *br. s*)	3, 4, 7, 8, 10, 15
3	89.5 *s*		
4	38.5 *d*	2.32 (1H, *m*)	2, 3, 6, 13
5	42.5 *t*	1.43 (1H, *dd*, 11.7, 2.9)	3, 4, 7, 13, 15
		2.35 (1H, *t*, 11.7)	3, 7, 13, 15
6	81.0 *s*		
7	56.1 *s*		
8	36.7 *d*	2.83 (1H, *ddd*, 9.2, 7.3, 6.8)	2, 3, 6, 10, 14
9	33.5 *t*	1.60 (1H, *ddd*, 11.7, 9.2, 2.0)	1, 7, 10, 11, 12
		2.24 (1H, *dd*, 11.7, 6.8)	1, 7, 11, 12
10	33.0 *s*		
11	33.9 *q*	1.20 (3H, *s*)	1, 9, 10, 12
12	23.9 *q*	1.08 (3H, *s*)	1, 9, 10, 11
13	14.4 *q*	1.29 (3H, *d*, 6.8)	3, 4, 5
14	7.7 *q*	1.09 (3H, *s*)	3, 6, 7, 8
15	178.5 *s*		
3-OH		2.02 (1H, *br. s*)	2, 3, 7
6-OH		3.26 (1H, *br. s*)	6, 7, 15

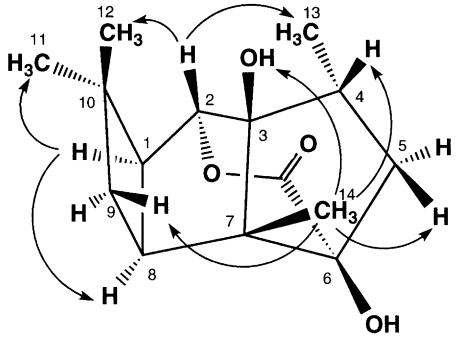


Fig. 3. NOE effects obtained from the NOE difference spectra of fascicularone B (**4**).

H_3-14 and OH-3, H_3-14 and H-4, H_3-14 and H-5β, and H_3-14 and H-9β indicated that H-2, OH-3, H-4, and H_3-14 all had β-configurations. NOEs between H-1 and H_3-11, and H-1 and H-8 implied that H-1 and H-8 had α-configurations. To determine the absolute configuration at C-2, **4** was converted into its (*S*)-and (*R*)- MTPA esters. The ^{1}H chemical-shift differences due to the 2-MTPA ester did not, however, allow us to suggest any absolute configuration at C-2. We therefore assumed that compound **4** possesses the same absolute configurations at C-1, C-2, and C-8 as those of **3** on biosynthetic grounds. Thus, the structure of **4** was determined to be (1*S*,8*S*)-4α,7β,10,10-tetramethyl-3β,6β-dihydroxy-tricyclo[5,3,0,$0^{2,5}$]decan-2α,6-olide.

3. Conclusions

We determined the structures of fascicularones A (**3**) and B (**4**) which contain *cis*-fused four-member ring moiety in their structures. As stated earlier, naematolins C (**1**) and G (**2**) were isolated together with structurally related sesquiterpenes such as naematolin and naematolin B possessing a *cis*-bycyclo [7.2.0] undecane skeleton by Doi et al. (1990). Based on these structural similarities, it would be of great interest to investigate whether *cis*-fused caryophyllene sesquiterpene would be transformed in a few steps to **3** and **4** based on the tricyclic framework.

4. Experimental

4.1. General experimental procedures

Melting points (mp) data are uncorrected. Optical rotations were measured with a Horiba model SEPA-300 polarimeter, whereas IR and UV spectra were recorded with JASCO J-20A, Shimadzu UV mini-1240 spectrophotometer, respectively. Mass spectra were obtained using a Jeol JMS-700 instrument, and ^{1}H- and ^{13}C-NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. CC was conducted on Sephadex LH-20 (Pharmacia), silica gel 60 (Kanto Chemical Co., Inc.) and Amberlite XAD-2 (ORGANO corporation). TLC was carried out using precoated silica gel plates (Merck), and spots were detected by spraying with 10% vanillin in H_2SO_4 followed by heating, or by UV irradiation.

4.2. Mushroom material

The fruiting bodies of *N. fasciculare* were collected at the foot of Mt. Gassan, Yamagata Prefecture, Japan, in autumn 1997 and identified by one of the authors (M. I.). The strain of *N. fasciculare* (No. 0701) has been deposited at our laboratory of the Faculty of Agriculture, Yamagata University, Yamagata, Japan.

4.3. Fermentation

Pieces of fruiting bodies were surface-sterilized with 100 ppm streptomycin soln. and placed on potato dextrose agar plates. Plates were incubated at 25 °C for 7 days and colonies appearing on the plates were isolated. The strain *N. fasciculare* grew on slants of potato dextrose agar. A loopful of the culture was transferred into five 1 l flasks containing 300 ml of a medium consisting of malt extract (40 g), glucose (40 g), and peptone (1 g) per 1 l of water. The inoculated flasks were incubated in the stationary phase at 25 °C for 90 days.

4.4. Extraction and isolation

The cultured broth (1.5 l) of *N. fasciculare* was filtered and the filtrate passed through an Amberlite-XAD-2 column. The column was washed thoroughly with H_2O and the organic residue removed from the resin by elution with MeOH. The MeOH solubles upon drying in vacuo (4.68 g) was applied to silica gel CC using a mixture of $CHCl_3$–MeOH (20:1) to give 6 fractions (fr.1.1-1.6). Fr. 1.2 (912 mg) was subjected to silica gel CC eluting with hexane–EtOAc (3:1, 1:1) to give 6 fractions (fr.2.1-2.6). Fr. 2.2 (116 mg) was separated by silica gel CC with hexane–EtOAc (4:1) to yield fascicularone B (**4**, 48 mg). Fr. 1.5 (436 mg) was subjected to silica gel CC eluting with hexane–EtOAc (2:3) to give 3 fractions (fr.3.1–3.3). Fr. 3.2 (223 mg) was applied to Sephadex LH-20 with MeOH to yield fascicularone A (**3**, 140 mg).

4.4.1. Fascicularone A (*3*)

Colorless needles; m.p.75–77 °C; $[\alpha]_D^{20}$ +323.8 (*c* 0.56, $CHCl_3$); UV λ_{max} (MeOH) nm (ε): 248 (8000); HREIMS: *m*/*z* (M^+): calc. for $C_{14}H_{20}O_4$, 252.1362; found, 252.1363. IR (NaCl) ν_{max} cm^{-1}: 3360, 1674, 1373, 1253, 1018, 754. For ^{1}H and ^{13}C NMR spectra, see Table 1; EIMS *m*/*z* (rel. int.,%): 252 (M^+, 27), 235

(70), 234 (17), 196 (84), 178 (78), 149 (80), 136 (72), 121 (96), 108 (100), 91 (60), 71 (72).

4.4.2. Acetylation of fascicularone A (3)

Fascicularone A (**3**, 7.2 mg) in pyridine (0.1 ml) was acetylated with acetic anhydride (0.1 ml) at room temp overnight. The reaction mixture was poured into water and extracted with EtOAc (3 × 5 ml). The organic layer was washed with 1 N HCl, satd. $NaHCO_3$, and satd. NaCl and dried over Na_2SO_4 and concentrated in vacuo to give a residue which was purified by silica gel CC to yield the triacetate (**3a**, 10.5 mg) as an amorphous powder. EIMS *m/z* (rel. int., %): 378 (M^+, 1), 354 (2), 336 (2), 318 (4), 280 (6), 258 (8), 238 (8), 220 (60), 178 (90), 160 (100), 136 (30), 119 (24), 91 (14). IR ν_{max} (NaCl) cm^{-1}: 1747, 1695, 1373, 1224, 1029, 756. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.05 (3H, *s*, H_3-13), 1.22 (3H, *s*, H_3-12), 1.29 (3H, *d*, $J = 6.8$ Hz, H_3-14), 1.45 (1H, *dd*, $J = 12.2$, 4.4 Hz, H-10), 2.07 (3H, *s*, OAc), 2.12 (3H, *s*, OAc), 2.13 (1H, *m*, H-10), 2.17 (3H, *s*, OAc), 2.42 (1H, *d*, $J = 6.8$ Hz, H-1), 3.15 (1H, *m*, H-4), 3.46 (1H, *m*, H-9), 5.42 (1H, *dd*, $J = 11.7$, 5.4 Hz, H-5), 5.64 (1H, *d*, $J = 11.7$ Hz, H-6), 5.86 (1H, *br.s*, H-2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.5 (*q*, C-14), 20.6 (*q*, $\underline{C}H_3CO$), 20.8 (*q*, $\underline{C}H_3CO$), 21.2 (*q*, $\underline{C}H_3CO$), 25.6 (*q*, C-13), 31.8 (*q*, C-12), 34.2 (*d*, C-4), 34.4 (*s*, C-11), 35.9 (*d*, C-9), 38.8 (*t*, C-10), 52.2 (*d*, C-1), 72.1 (*d*, C-5), 72.5 (*d*, C-6), 82.6 (*d*, C-2), 145.4 (*s*, C-8), 160.8 (*s*, C-3), 169.8 (*s*, $CH_3\underline{C}O$), 169.9 (*s*, $CH_3\underline{C}O$), 170.2 (*s*, $CH_3\underline{C}O$), 188.8 (*s*, C-7).

4.4.3. Acetonide of fascicularone A (3)

To fascicularone A (**3**, 9.1 mg) in 2,2-dimethoxylpropane (0.3 ml) and CH_2Cl_2 (0.3 ml), was added catalytic amounts of pyridinium *p*-toluenesulfonate, with the mixture left to stir at room temperature for 70 h. The reaction mixture was poured into satd. $NaHCO_3$ and extracted with EtOAc (3 × 10 ml). Evaporation of the organic layer yielded a residue which was purified by prep. TLC ($CHCl_3$–MeOH = 20:1) to yield 5,6-*O*-isopropylidene-fascicularone A (**3b**, 3.0 mg) as an amorphous powder. EIMS *m/z* (rel. int., %) : 292 (M^+, 3), 275 (7), 263 (2), 236 (6), 215 (7), 205 (20), 192 (93), 178 (96), 149 (96), 136 (97), 108 (100), 77 (44). IR ν_{max} (NaCl) cm^{-1}: 1701, 1591, 1377, 1232, 1089, 1022. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H_3-13), 1.21 (3H, *s*, H_3-12), 1.41 (3H, *d*, $J = 7.3$ Hz, H_3-14), 1.48 (3H, *s*, H_3-17), 1.49 (1H, *m,* H-10), 1.52 (3H, *s*, H_3-16), 2.16 (1H, *dd*, $J = 12.2$, 9.3 Hz, H-10), 2.47 (1H, *d*, $J = 6.8$ Hz, H-1), 3.19 (1H, *m*, H-4), 3.41 (1H, *m*, H-9), 4.13 (1H, *dd,* $J = 11.2$, 5.4 Hz, H-5), 4.49 (1H, *d*, $J = 11.2$ Hz, H-6), 4.78 (1H, *br. s*, H-2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 11.3 (*q*, C-14), 25.6 (*q*, C-13), 26.6 (*q*, C-16), 26.7 (*q,* C-17), 32.1 (*q,* C-12), 34.1 (*d*, C-4), 34.8 (*d*, C-9), 34.9 (*s*, C-11), 38.8 (*t*, C-10), 55.0 (*d*, C-1), 77.3 (*d*, C-6), 79.0 (*d*, C-5), 81.3 (*d*, C-2), 112.4 (*s*, C-15), 143.9 (*s*, C-8), 163.5 (*s*, C-3), 191.7 (*s*, C-7).

4.4.4. Preparation of (S)- and (R)-MTPA esters of fascicularone A (3)

To a solution of the 5,6-*O*-isopropylidene-fascicularone A (**3b**, 1.5 mg) in CCl_4 (0.1 ml) and pyridine (0.1 ml), was added (*S*)- or (*R*)- MTPACl (0.02 ml). Each mixture stood at room temperature for 20 h. After the solvent was evaporated, each residue was subjected to prep. TLC ($CHCl_3$) to yield the (*S*)- and (*R*)-MTPA esters of **3b** (**3c**, 1.0 mg and **3d**, 1.3 mg). (*S*)- MTPA ester **3c**: amorphous powder. EIMS *m/z* (rel. int., %): 508 (M^+, 6), 275 (M-OMTPA$^+$, 30). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 1.07 (3H, *s*, H_3-13), 1.10 (3H, *d*, $J = 6.8$ Hz, H_3-14), 1.24 (3H, *s*, H_3-12), 1.45 (3H, *s*, H_3-16), 1.51 (3H, *s*, H_3-17), 1.52 (1H, *m*, H-10), 2.16 (1H, *dd*, $J = 12.2$, 9.3 Hz, H-10), 2.39 (1H, *d*, $J = 6.4$ Hz, H-1), 3.06 (1H, *m*, H-4), 3.37 (1H, *m*, H-9), 3.47 (3H, MTPA-O$\underline{Me}$), 4.11 (1H, *dd*, $J = 11.2$, 5.4 Hz, H-5), 4.45 (1H, *d*, $J = 11.2$, H-6), 5.88 (1H, *br.s*, H-2), 7.44 (3H, MTPA-ArH), 7.49 (2H, MTPA-ArH). (*R*)-MTPA ester **3d**: amorphous powder. EIMS *m/z* (rel. int., %):508 (M^+, 7), 275 (M-OMTPA$^+$, 51). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, *d*, $J = 7.3$ Hz, H_3-14), 1.05 (3H, *s*, H_3-13), 1.24 (3H, *s,* H_3-12), 1.43 (3H, *s*, H_3-16), 1.49 (3H, *s*, H_3-17), 1.53 (1H, *m*, H-10), 2.20 (1H, *dd*, $J = 12.7$, 9.8 Hz, H-10), 2.48 (1H, *d,* $J = 6.4$ H, H-1), 2.94 (1H, *m,* H-4), 3.42 (1H, *m*, H-9), 3.58 (3H, MTPA-OMe), 4.08 (1H, *dd*, $J = 11.2$, 5.4 Hz, H-5), 4.42 (1H, *d*, $J = 11.2$, H-6), 5.85 (1H, *br. s*, H-2), 7.42 (3H, MTPA-ArH), 7.53 (2H, MTPA-ArH).

4.4.5. Fascicularone B (4)

Colorless needles; m.p. 127–128 °C; $[\alpha]_D^{20}$ +63.5 (*c* 0.88, $CHCl_3$). HR-EIMS *m/z* (M^+): calcd. for $C_{15}H_{22}O_4$, 266.1521; found, 266.1519. EIMS *m/z* (rel. int., %): 266 (M^+, 48), 248 (11), 238 (16), 210 (17), 192 (55), 182 (17), 164 (39), 153 (33), 139 (48), 123 (100), 122 (63), 96 (63), 83 (72). IR (NaCl) ν_{max} cm^{-1}: 3457, 1727, 1270, 1160, 1106, 989, 755. For ^{1}H- and ^{13}C-NMR spectra, see Table 2.

4.4.6. Acid hydrolysis and methylation of fascicularone B (4)

1 N KOH in MeOH (10 ml) was added to fascicularone B (**4**, 20 mg) and stirred at 50 °C for 11 h. The mixture was extracted with EtOAc and, after evaporation of the organic solvent, excess CH_2N_2 in Et_2O was added to the residue at room temperature. The mixture was stirred for 1 h, and, after evaporation, the residue was subjected to silica gel CC to yield the methyl ester (**4a**, 26 mg) of **4** as an amorphous powder. EIMS *m/z* (rel. int., %): 298 (5), 280 (35), 262 (30), 248 (3), 221 (8), 195 (20), 169 (30), 140 (43), 127 (100), 97 (58), 85 (83). IR (NaCl) ν_{max} cm^{-1}: 3430, 1724, 1445, 1254, 1100, 754. ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 0.99 (3H, *s,* H_3-12), 1.04 (3H, *s*, H_3-14), 1.22 (3H, *s*, H_3-11), 1.31 (3H, *d*, $J = 7.3$, H_3-13), 1.58 (1H, *ddd*, $J = 11.7$, 9.3, 2.9 Hz, H-9), 1.76

(1H, *dd*, $J = 11.7$, 9.3 Hz, H-9), 1.83 (1H, *m*, H-5), 1.89 (1H, *t*, $J = 12.2$ Hz, H-5), 2.11 (1H, *td*, $J = 9.3$, 2.9 Hz, H-1), 2.30 (1H, *m*, H-4), 2.57 (1H, *q*, $J = 9.3$ Hz, H-8), 3.77 (3H, *s*, OMe) 4.39 (1H, *d*, $J = 9.3$ Hz, H-2). ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 12.9 (*q*, C-14), 15.4 (*q*, C-13), 24.5 (*q*, C-12), 29.9 (*q*, C-11), 32.7 (*s*, C-10), 33.9 (*d*, C-8), 36.5 (*t*, C-9), 41.8 (*t*, C-5), 44.7 (*d*, C-4), 49.3 (*d*, C-1), 52.5 (*q*, OMe), 56.0 (*s*, C-7), 79.6 (*d,* C-2), 84.6 (*s*, C-6), 91.2 (*s*, C-3), 173.7 (*s*, C-15).

Acknowledgements

We thank Dr. Hiromasa Kiyota of the Faculty of Agriculture at Tohoku University for MS measurements.

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