Cadinane-Type Sesquiterpenoids, Strobilols E – K, from the Liquid Culture of *Strobilurus ohshimae*

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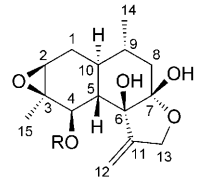
New cadinane sequiterpenoids, strobilols E – K (**5** – **11**, resp.) have been isolated from a liquid culture of *Strobilurus ohshimae*. Their structures have been established on the basis of spectral analyses.

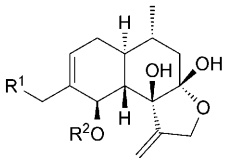
Introduction. – In recent years, mushrooms have become attractive objects as functional food and as source for biologically active compounds due to their diverse nutritional, medicinal, and pharmacological properties. In addition, the biological importance of the edible mushrooms arises from their chemical components, including especially various biologically active polysaccharides and polyphenolic compounds [1][2].

Strobilurus ohshimae, a wild edible mushroom, which belongs to Tricholomataceae family, is widely distributed over the cedar forest area of Japan. The Japanese name for *S. ohshimae* is sugiedatake. This wild mushroom has traditionally been eaten by specific groups of local people and seasonally in Japan. However, little is known about the chemical composition and nutritional and pharmacological values of *S. ohshimae*. During our search for bioactive natural products from edible wild mushrooms, we have investigated the chemical constituents of the fresh fruiting bodies of *S. ohshimae* collected in Japan, and recently isolated cadinane-sesquiterpenes, strobilols A (**1**), B (**2**), C (**3**), and D (**4**) [3]. Strobilol A showed moderate activity against *Altemia salina* (LD_{50} value of 100 μM). Subsequently, we investigated the possibility of growing *S. ohshimae* in liquid fermentation, comparing the chemical compositions of mycelium with those of fresh fruiting bodies. Two new drimane sesquiterpenes, strobilactones A and B were isolated from the liquid cultured of *S. ohshimae*, which are not produced in the fruiting bodies of this fungus [4]. In our ongoing investigation of chemical compositions of the liquid culture of *S. ohshimae*, we have isolated seven new cadinane-sesquiterpenes, strobilols E – K (**5** – **11**, resp.), together with the known compounds **1** – **4**. In this report, we describe the isolation and structure elucidation of **5** – **11**.

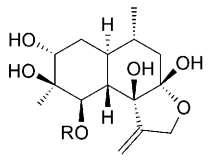
Results and Discussion. – The culture broth (5.0 l) of *Strobilurus ohshimae* was filtered, and the cultured filtrate was extracted with AcOEt. The AcOEt extract was chromatographed on silica gel and separated into 13 fractions. Further purification by column chromatography yielded strobilol J (**10**) from *Fr. 5*, and strobilols H (**8**) and I (**9**) from *Fr. 6*, as well as strobilols E (**5**), F (**6**), G (**7**), and K (**11**) from *Fr. 8* and *9*.



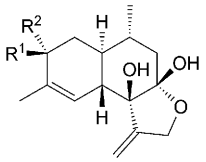
1 R = H
9 R = Me–(CH$_2$)$_8$–C(=O)



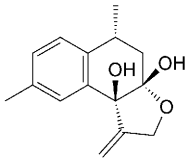
2 R^1 = H, R^2 = H
4 R^1 = OH, R^2 = H
10 R^1 = H, R^2 = Me–(CH$_2$)$_8$–C(=O)



3 R = H
11 R = Me–(CH$_2$)$_8$–C(=O)



5 R^1 = OH, R^2 = H
6 R^1 = H, R^2 = H
7 R^1, R^2 = O



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The molecular formula of strobilol E (**5**) was determined as $C_{15}H_{22}O_4$ by HR-EI-MS. The ^{13}C-NMR spectrum of **5** gave 15 signals assignable to two sp^3 quaternary C-atoms, two sp^2 quaternary C-atoms, four sp^3 CH groups, one sp^2 CH group, three sp^3 CH_2 groups, one sp^2 CH_2 group, and two Me groups. The ^{1}H-NMR (CD_3OD) spectrum (*Table 1*) of **5** revealed signals due to a vinyl Me group (δ(H) 1.77 (*s*, Me(15)), a Me group (δ(H) 0.90 (*d*, *J* = 7.1, Me(14)), an olefinic H-atom (δ(H) 5.86 (br. *s*, H–C(4)), an *exo*-CH_2 group (δ(H) 5.03 (*t*, *J* = 2.4, CH_2(12)), 5.37 (*t*, *J* = 2.4, CH_2(12))), an oxygenated CH_2 group (δ(H) 4.13 (*dd*, *J* = 13.2, 2.4, CH_2(13)), 4.50 (*dd*, *J* = 13.2, 2.4, CH_2(13))), and an oxygenated CH group (δ(H) 4.07 (br. *s*, H–C(2))). In addition, it showed also signals of three CH groups (δ(H) 1.10–1.19 (*m*), 2.25–2.80 (overlapped signal), 1.42–1.46 (*m*)), and two CH_2 groups (δ(H) 1.02–1.11 (*m*), 1.21–1.32 (*m*), 1.80 (*dd*, *J* = 13.8, 3.3), 2.25–2.80 (overlapped signal)). Since two out of five unsaturation degrees were accounted for by the presence of the two C=C bonds, compound **5** was inferred to be a tricyclic system. Compound **5** was assumed to be a cadinane-type sesquitrpenoids, because the ^{1}H- and ^{13}C-NMR spectral data of **5** were similar to those of strobilols A (**1**)–D (**4**) co-occurring in this species. The ^{1}H,^{1}H-COSY spectrum of **5** indicated the two partial structures **a** and **b** (*Fig. 1*). Moreover, the ^{13}C,^{1}H-COSY and HMBC spectra of **5** clarified the connectivity of each partial structure. HMBC Correlations of Me(15) with C(2) and C(4), H–C(2) with C(4), C(10), and C(15), H–C(4) with C(6) and C(10), and CH_2(8) with C(6) were detected, implying a bicyclo[4.4.0]decane moiety. HMBC correlations from the *exo*-CH_2 group (CH_2(12)) to C(6) and C(13) and from CH_2(13) to C(6) and C(7) defined the locations of tetrahydrofuran ring with the *exo*-CH_2 group (fragment **b**). Accordingly, the constitutional formula of **5** was deduced as shown in the *Formulae* above. The relative configuration of **5** was elucidated by the analysis of NOE difference experiments and coupling constants. In the ^{1}H-NMR spectrum (C_5D_5N) of **5**, the large coupling constant

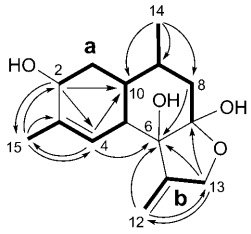

Fig. 1. *Important* $^1H,^1H$*-COSY* (bold lines) *and HMBC* (arrows) *correlations observed for* **5**

of H–C(5) ($J(5,10) = 11.1$), characteristic for a diaxial relationship with H–C(10), demonstrated the *trans*-juncture of the decalin skeleton. NOEs from H–C(9) to H–C(5) suggested that the Me group is equatorial. The β-orientation of the OH group at C(2) was deduced from an NOE from H–C(10) to H–C(2). Furthermore, NOEs from CH_2(12) to H–C(2) and H–C(10) pointed to a *cis* fusion between the five- and six-membered ring. A chair-like conformation of the cyclohexane ring consistent with the results from NOEs experiments is shown in *Fig. 2*.

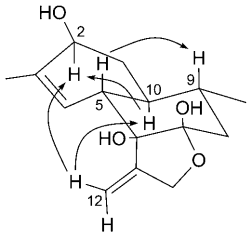

Fig. 2. *Selected NOE correlations observed for* **5**

Table 1. ^1H*-NMR Data of Strobilols E* (**5**) *and F* (**6**). At 400 MHz; δ in ppm, J in Hz.

	5[a])	**5**[b])	**6**[c])	**6**[b])
CH_2(1)	1.02–1.11 (*m*), 2.25–2.80[d])	1.45–1.63[d]), 2.59–2.64 (*m*)	2.06 (*dd*, *J* = 16.1, 13.4), 2.74 (*dd*, *J* = 16.1, 6.4)	2.11 (*dd*, *J* = 16.1, 13.8), 2.82 (*dd*, *J* = 16.1, 3.8)
H–C(2) or CH_2(2)	4.07 (br. *s*)	4.51–4.56 (*m*)	1.23–1.27 (*m*), 1.58–1.67[d])	1.48–1.56[d]), 1.70–1.83[d])
H–C(4)	5.86 (br. *s*)	6.42 (br. *s*)	7.15 (br. *s*)	7.23 (br. *s*)
H–C(5)	2.25–2.80[d])	2.84 (br. *d*, *J* = 11.1)	2.65 (*dt*, *J* = 9.7, 2.4)	2.98 (*dt*, *J* = 10.6, 2.4)
CH_2(8)	1.21–1.32 (*m*), 1.80 (*dd*, *J* = 13.8, 3.3)	1.45–1.63[d]), 2.25 (br. *d*, *J* = 13.7)	1.31–1.40[d]), 1.97 (*dd*, *J* = 14.5, 3.3)	1.48–1.56[d]), 2.20 (*dd*, *J* = 13.7, 2.5)
H–C(9)	1.42–1.46 (*m*)	1.72–1.77 (*m*)	1.58–1.67[d])	1.70–1.83[d])
H–C(10)	1.10–1.19 (*m*)	1.45–1.63[d])	1.31–1.40[d])	1.70–1.83[d])
CH_2(12)	5.03 (*t*, *J* = 2.4), 5.37 (*t*, *J* = 2.4)	5.12 (br. *s*), 5.71 (br. *s*)	5.16 (*t*, *J* = 2.4), 5.34 (*t*, *J* = 2.4)	5.10 (*t*, *J* = 2.5), 5.40 (*t*, *J* = 2.5)
CH_2(13)	4.13 (*dd*, *J* = 13.2, 2.4), 4.50 (*dd*, *J* = 13.2, 2.4)	4.35 (*dd*, *J* = 13.2, 2.3), 4.69 (*dd*, *J* = 13.2, 2.3)	4.24 (*dt*, *J* = 13.3, 2.4), 4.60 (*dt*, *J* = 13.3, 2.4)	4.32 (*dt*, *J* = 13.4, 2.2), 4.67 (*dt*, *J* = 13.4, 2.2)
Me(14)	0.90 (*d*, *J* = 7.1)	0.90 (*d*, *J* = 6.3)	0.90 (*d*, *J* = 6.5)	0.80 (*d*, *J* = 6.5)
Me(15)	1.77 (br. *s*)	2.09 (br. *s*)	1.85 (br. *d*, *J* = 2.4)	1.92 (*dd*, *J* = 2.4, 1.2)

[a]) In CD_3OD. [b]) In C_5D_5N. [c]) $CDCl_3$. [d]) Overlapped signals.

The absolute configuration of **5** was determined by the CD excitation chirality method for the allylic benzoate **5a** as shown in *Fig. 3* [5]. Thus, treatment of **5** with benzoyl chloride in the presence of 4-(dimethylamino)pyridine (4-DMAP) afforded the 2-*O*-benzoate (**5a**). The 2-*O*-benzoate (**5a**) showed a negative *Cotton* effect (245 nm ($\Delta\varepsilon$ −3.0)), which indicated the absolute configuration (*S*) for C(2).

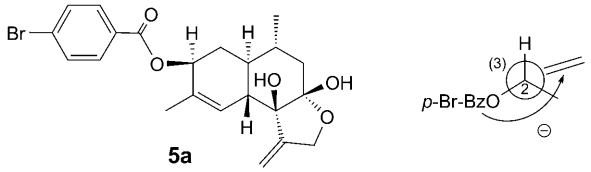

Fig. 3. *Sign of* Cotton *effect of* p-*bromobenzoate derivative* **5a**

The molecular formula of strobilol F (**6**) was determined to be $C_{15}H_{22}O_3$ by HR-EI-MS, and implied the presence of one O-atom less than in **5**. The ^{1}H- and ^{13}C-NMR spectral data of **6** (*Tables 1* and *2*) indicated many common signals, which were attributable to two Me groups, an *exo*-CH_2 moiety, and an isolated CH_2 group as those of **5**. However, the data lacked the oxygenated CH group at C(2) which had been observed in **5**, and they were accompanied by the appearance of an additional CH_2 group (δ(H) 1.23–1.27 (*m*, 1 H), 1.58–1.67 (*m*, 1 H), δ(C) 28.5 (*t*)). In the HMBC experiments of **6**, correlations between Me(15) and C(2) and between H–C(4) and C(2) indicated that **6** was a 2-deoxy-derivative of **5**.

Table 2. *^{13}C-NMR Spectral Data of Strobilols E* (**5**), *F* (**6**), *G* (**7**), *and H* (**8**). At 100 MHz; δ in ppm.

	5[a])	**6**[b])	**7**[a])	**8**[b])
H–C(1) or CH_2(1)	38.6 (*t*)	41.9 (*t*)	43.7 (*t*)	127.9 (*d*)
H–C(2) or CH_2(2)	72.0 (*d*)	28.5 (*t*)	202.3 (*s*)	129.3 (*d*)
C(3)	139.3 (*s*)	135.9 (*s*)	137.0 (*s*)	135.9 (*s*)
H–C(4)	125.9 (*d*)	145.0 (*d*)	149.3 (*d*)	128.6 (*d*)
H–C(5) or C(5)	48.6 (*d*)	45.9 (*d*)	48.8 (*d*)	134.4 (*s*)
C(6)	78.1 (*s*)	77.0 (*s*)	77.8 (*s*)	77.5 (*s*)
C(7)	107.2 (*s*)	104.8 (*s*)	106.9 (*s*)	103.1 (*s*)
CH_2(8)	43.6 (*t*)	40.3 (*t*)	43.2 (*t*)	37.1 (*t*)
H–C(9)	35.1 (*d*)	33.6 (*d*)	35.7 (*d*)	31.4 (*d*)
H–C(10) or C(10)	43.6 (*d*)	42.9 (*d*)	45.2 (*d*)	137.6 (*s*)
C(11)	149.9 (*s*)	146.7 (*s*)	149.6 (*s*)	150.7 (*s*)
CH_2(12)	111.5 (*t*)	112.1 (*t*)	111.7 (*t*)	108.7 (*t*)
CH_2(13)	68.6 (*t*)	66.5 (*t*)	68.6 (*t*)	67.3 (*t*)
Me(14)	19.9 (*q*)	18.0 (*q*)	19.3 (*q*)	22.9 (*q*)
Me(15)	20.1 (*q*)	15.7 (*q*)	16.5 (*q*)	21.0 (*q*)

[a]) In CD_3OD. [b]) In $CDCl_3$.

The molecular formula $C_{15}H_{20}O_4$ for strobilol G (**7**) was established by HR-EI-MS, indicating that **7** had two H-atoms less than **5**. The ^{1}H-NMR spectrum of **7** was similar to that of **5**, but it lacked the H–C(2) group present in **5**. In the ^{13}C-NMR spectrum of **7**, the signal for C(2) that had been observed in **5** also disappeared, and new signals

assigned to an α,β-unsaturated C=O C-atom system were observed (δ(C) 202.3 (*s*), 137.0 (*s*), 149.3 (*d*)). The presence of the α,β-unsaturated ketone was also supported by the 1670 cm^{-1} absorption in the IR spectrum, and by the 234 nm ($\log \varepsilon = 3.6$) band in the UV spectrum. From these results, the structural difference between **5** and **7** was explainable by replacement of the HO–CH(2) group of **5** with a O=C(2) group for **7**. Furthermore, these assumptions were confirmed by $^{13}C,^{1}H$-COSY and HMBC correlations between Me(15) and C(2) and between H–C(4) and C(2).

The molecular formula of strobilol H (**8**) was determined to be $C_{15}H_{18}O_3$ by HR-EI-MS. The IR spectrum of **8** revealed the presence of OH (3430 cm^{-1}) and aromatic groups (1506 cm^{-1}). The UV spectrum of **8** also showed an aromatic maximum at 239 nm ($\log \varepsilon = 3.2$), indicating the presence of an aromatic system. The ^{1}H-NMR spectrum ($CDCl_3$) of **8** showed three new aromatic signals at δ(H) 7.10 (*dd*, $J = 7.9, 2.1$, H–C(2)), 7.11 (*d*, $J = 7.9$, H–C(1)), and 7.38 (br. *s*, H–C(4)), suggesting a 1,2,4-trisubstituted aromatic spin system. The downfield shift observed for Me(15) (δ(H) 2.33) was indicative of an aromatization of the cyclohexene ring in compound **8**. Furthermore, signals at δ(H) 3.12–3.17 (*m*, H–C(9)), 1.93 (*dd*, $J = 13.9, 5.0$, CH_2(8)), 2.24 (*dd*, $J = 13.9, 6.6$, CH_2(8)), 4.29 (*dt*, $J = 12.8, 2.4$, CH_2(13)), 4.54 (*dt*, $J = 12.8, 2.4$, CH_2(13)), 5.24 (*t*, $J = 2.4$, CH_2(12)), and 5.58 (*t*, $J = 2.4$, CH_2(12)) revealed the presence of one benzylic CH group, two CH_2 groups and one *exo*-CH_2 group. ^{13}C-NMR and DEPT data of **8** showed six aromatic C-atoms, two Me groups: one benzylic and one aliphatic; one benzylic CH group, three quaternary C-atoms, two CH_2 groups, one *exo*-CH_2 group. These data suggested a strobilol derivative. The complete attribution for the observed signals was accomplished based on the $^{13}C,^{1}H$-COSY and HMBC correlations (*Fig. 4*). HMBC correlations between Me(15) and C(2), C(3), and C(4), between H–C(1) and C(3) and C(5), and between H–C(4) and C(2) and C(6) allowed us to fully determine the aromatic moiety. The benzylic function linked at C(5) and C(10) was supported by the correlations between Me(14) and C(10), between H–C(9) and C(5). The linkage of tetrahydrofurane moiety was determined through the correlations from CH_2(12) to C(6), from CH_2(13) to C(6) and C(7). These HMBC correlations allowed us to determine the structure of **8**.

The HR-FAB-MS data of strobilol I (**9**) indicated that the molecular formula of **9** was $C_{25}H_{40}O_6$. The ^{1}H- (*Table 3*) and ^{13}C-NMR (*Table 4*) spectra of **9** were similar to those of strobilol A (**1**), except for the presence of additional signals at δ(H) 0.87 (*t*, $J = 6.4$, Me(10′)), 1.25–1.29 (overlapped signal), 1.54–1.67 (overlapped signal), 2.44–2.49 (*m*, CH_2(2′)), δ(C) 22.6 (*t*), 24.7 (*t*), 29.0 (*t*), 29.2 (*t*), 29.4 (*t*), 31.8 (*t*), 34.6 (*t*), 14.1 (*q*), 172.6 (*s*). The signals assignable to a H–C(4) group, which were observed at δ(H) 4.60 (*d*, $J = 10.8$) in **1**, were observed at δ(H) 6.00 (*d*, $J = 10.9$) in **9**. The presence of the

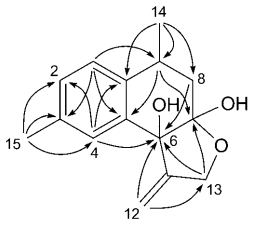

Fig. 4. *HMBC* (arrows) *Correlations observed for* **8**

C=O resonance in the ^{13}C-NMR spectrum could be accounted for a saturated C_{10} fatty acid ester. A combination of HMQC and HMBC experiments thus permitted the assignment of a cadinane sesquiterpene skeleton esterified with decanoic acid. Furthermore, the HMBC correlation between H–C(4) and C(1′) and the substantial downfield shift for H–C(4) revealed the location of the decanoyloxy moiety was at C(4) (*Fig. 5*). Thus, the structure of **9** was determined to be the 4-decanoyl ester derivative of strobilol A (**1**).

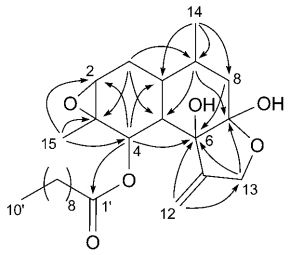

Fig. 5. *HMBC* (arrows) *Correlations observed for* **9**

The molecular formula of strobilol J (**10**), $C_{25}H_{40}O_5$, was determined by HR-FAB-MS measurement. The ^{1}H- (*Table 3*) and ^{13}C-NMR (*Table 4*) data for **10** were very similar to those of strobilol B (**2**), except for the lower H–C(4) chemical shift (δ(H) 6.01 of **10** and δ(H) 4.90 of **2**). The NMR spectral data of **10** indicated additional signals due to a decanoyl moiety (δ(H) 0.87 (*t*, *J* = 7.0, Me(10′)), 1.20–1.34 (overlapped signal), 1.59–1.64 (overlapped signal), 2.35 (*t*, *J* = 7.6, CH_2(2′)) and δ(C) 14.1 (*q*), 22.6 (*t*), 24.9 (*t*), 29.2 (*t*), 29.4 (*t*), 31.8 (*t*), 34.8 (*t*), 175.5 (*s*)). The identification of the 4-

Table 3. *^{1}H-NMR Data of Strobilols I* (**9**)*, J* (**10**)*, and K* (**11**). At 400 MHz; δ in ppm, *J* in Hz.

	9[a])	**10**[a])	**11**[b])
CH_2(1)	1.54–1.67[c]), 2.21 (*ddd*, *J* = 15.8, 7.5, 4.6)	1.68–1.73 (*m*), 2.26–2.29 (*m*)	1.58–1.65[c]), 1.78–1.84[c])
H–C(2)	3.07 (*d*, *J* = 4.6)	5.82 (br. *d*, *J* = 6.6)	3.59 (*t*, *J* = 3.0)
H–C(4)	6.00 (*d*, *J* = 10.9)	6.01 (*d*, *J* = 6.6)	5.72 (*d*, *J* = 12.3)
H–C(5)	2.40 (*dd*, *J* = 12.0, 10.9)	2.05 (*dd*, *J* = 12.5, 6.6)	2.27 (*t*, *J* = 12.3)
CH_2(8)	1.10–1.22[c]), 1.80 (*dd*, *J* = 12.5, 3.8)	1.20–1.35[c]), 1.89 (*dd*, *J* = 13.9, 3.8)	1.35–1.39 (*m*), 1.78–1.84[c])
H–C(9)	1.48–1.52 (*m*)	1.50–1.54 (*m*)	1.47–1.53[c])
H–C(10)	1.10–1.22[c])	1.20–1.35[c])	1.47–1.53[c])
CH_2(12)	5.06 (br. *s*), 5.44 (br. *s*)	5.14 (*t*, *J* = 2.3), 5.22 (*t*, *J* = 2.3)	5.11 (*t*, *J* = 2.7), 5.56 (*t*, *J* = 2.7)
CH_2(13)	4.13 (*dt*, *J* = 12.5, 2.2), 4.55 (*dt*, *J* = 12.5, 2.2)	4.19 (*dt*, *J* = 12.5, 2.3), 4.62 (*dt*, *J* = 12.5, 2.3)	4.17 (*dt*, *J* = 12.5, 2.7), 4.46 (*dt*, *J* = 12.5, 2.7)
Me(14)	0.88 (*d*, *J* = 6.4)	0.88 (*d*, *J* = 6.6)	0.88 (*d*, *J* = 7.2)
Me(15)	1.25 (*s*)	1.64 (br. *s*)	1.15 (*s*)
CH_2(2′)	2.44–2.49 (*m*)	2.35 (*t*, *J* = 7.6)	2.35–2.39 (*m*)
CH_2(3′)	1.54–1.67[c])	1.59–1.64[c])	1.58–1.65[c])
CH_2(4′)–CH_2(9′)	1.25–1.29[c])	1.20–1.34[c])	1.24–1.32[c])
Me(10′)	0.87 (*t*, *J* = 6.4)	0.87 (*t*, *J* = 7.0)	0.90 (*t*, *J* = 6.0)

[a]) In $CDCl_3$. [b]) In CD_3OD. [c]) Overlapped signals.

Table 4. *^{13}C-NMR Data of Strobilols I* (**9**), *J* (**10**), *and K* (**11**). At 100 MHz; δ in ppm.

	9[a])	**10**[a])	**11**[b])
CH_2(1)	29.0 (*t*)	28.6 (*t*)	34.4 (*t*)
H–C(2)	60.5 (*d*)	128.5 (*d*)	75.6 (*d*)
C(3)	59.7 (*s*)	131.5 (*s*)	75.5 (*s*)
H–C(4)	74.3 (*d*)	72.2 (*d*)	75.5 (*d*)
H–C(5)	42.2 (*d*)	48.8 (*d*)	46.1 (*d*)
C(6)	76.9 (*s*)	77.2 (*s*)	79.8 (*s*)
C(7)	104.4 (*s*)	104.7 (*s*)	107.0 (*s*)
CH_2(8)	39.4 (*t*)	40.7 (*t*)	42.3 (*t*)
H–C(9)	35.5 (*d*)	34.7 (*d*)	36.5 (*d*)
H–C(10)	39.4 (*d*)	41.6 (*d*)	38.6 (*d*)
C(11)	147.2 (*s*)	147.2 (*s*)	149.2 (*s*)
CH_2(12)	109.5 (*t*)	109.8 (*t*)	112.9 (*t*)
CH_2(13)	68.0 (*t*)	67.7 (*t*)	69.4 (*t*)
Me(14)	19.1 (*q*)	18.6 (*q*)	20.9 (*q*)
Me(15)	19.6 (*q*)	19.7 (*q*)	26.5 (*q*)
C(1′)	172.6 (*s*)	175.5 (*s*)	175.8 (*s*)
CH_2(2′)	34.6 (*t*)	34.8 (*t*)	36.5 (*t*)
CH_2(3′)	24.7 (*t*)	24.9 (*t*)	33.1 (*t*)
CH_2(4′) – CH_2(8′)	[c])	[d])	[e])
CH_2(9′)	[c])	22.6 (*t*)	24.5 (*t*)
Me(10′)	14.1 (*q*)	14.1 (*q*)	15.2 (*q*)

[a]) $CDCl_3$. [b]) CD_3OD. [c]) δ(C) 22.6 (*t*), 29.0 (*t*), 29.2 (*t*), 29.4 (*t*), 31.8 (*t*). [d]) δ(C) 29.2 (*t*), 29.4 (*t*), 31.8 (*t*). [e]) δ(C) 31.1 (*t*), 31.2 (*t*), 31.4 (*t*), 33.8 (*t*).

substituent in **10** as the 4-decanoyloxy group was deduced from data of HMBC correlations between H–C(4) and C(1′). Thus, the structure of **10** was determined to be 4-decanoyloxystrobilol B.

The molecular formula of strobilol K (**11**), $C_{25}H_{42}O_7$, was determined by HR-FAB-MS measurement, suggesting a decanoyl ($C_{10}H_{19}O$) derivative of strobilol C (**3**). The ^{1}H- (*Table 3*) and ^{13}C-NMR (*Table 4*) data for **11** were very similar to those of **3**, except for the signals of a decanoyl moiety (δ(H) 0.90 (*t*, *J* = 6.0, Me(10′)), 1.24–1.32 (overlapped signal), 1.58–1.65 (overlapped signal), 2.35–2.39 (*m*, CH_2(2′)), and δ(C) 15.2 (*q*), 24.5 (*t*), 31.1 (*t*), 31.2 (*t*), 31.4 (*t*), 33.1 (*t*), 33.8 (*t*), 36.5 (*t*), 175.8 (*s*)). The esterification site was determined by a HMBC correlation between H–C(4) to the ester C=O group at δ(C) 175.8. From these results, the structure of **11** was determined to be 4-decanoyloxystrobilol C.

The absolute configurations of strobilols F–K (**6**–**11**, resp.) are considered to be the same as that of **5** lead on the biosynthetic considerations.

Strobilols A–K (**1**–**11**, resp.) were screened for cytotoxicity and found weakly active or inactive.

Strobilols are members of a novel series of fungal metabolites consisting of cadinane sesquiterpenoids possessing 6,6,5-fused tricycles. It is noteworthy that there has been only one report of a cadinane sesquiterpenoid possessing a 6,6,5-fused tricycle: a hydrated panal derivative which was considered to be formed from cadinane sesquiterpene, panal, in wet $CHCl_3$. Panal was originally isolated from luminous

mushroom *Panellus stipticus*. In addition, the panal derivative has not been reported as an isolated natural product [6].

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh), and *ODS* (*RP-18*). TLC: *Kieselgel* F_{254} (0.25 mm; *Merck*); detection by splaying with 10% vanillin in H_2SO_4, follwed by heating at 120°. Optical rotations: *Horiba* model *SEPA-300* polarimeter. UV Spectra: *Shimadzu UV mini-1240* spectrophotometer. CD Spectra: *Jasco J-20A* spectrophotometer. IR spectra: *JASCO J-20A* spectrophotometer, KBr pellets; ν in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *JEOL EX-400* spectrometer, at 400/100 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard; coupling constants J in Hz. EI-MS: *JEOL JMS-700* instrument spectrometer; in m/z (rel. %).

Fungus and Cultivation. The producing strain *Strobilurus ohshimae* NBRC 30370 was purchased from biological resource center, National Institute of Technology and Evaluation, Chiba, Japan. For fermentation, the fungal strain was grown in three 500 ml-*Sakaguchi* flasks containing 100 ml of a medium consisting of 40 g of malt extract, 40 g of glucose, and 1.0 g peptone per 1 l of H_2O. The inoculated flask was incubated at 25° for 4 weeks on a rotary shaker. The culture broth (5.0 l) was separated from the mycelia by filtration. The filtrate was extracted with AcOEt. The org. layer was concentrated *in vacuo* to give an oily residue (2.6 g). The residue was subjected to SiO_2 CC with mixture of hexane/AcOEt, and mixture of AcOEt/MeOH to give *Fr. 1–13*. *Fr. 5* (40% AcOEt eluate, 165.2 mg) was further chromatographed on SiO_2 by eluting with $CHCl_3$ and increasing volume of AcOEt to afford 40–50% AcOEt eluates (99 mg).

These fractions were combined and rechromatographed on *ODS* with H_2O/MeOH (10% stepwise gradient) to yield crude strobilol J (**10**), which was finally purified by SiO_2 flash-CC with hexane/AcOEt (1:1, *v*/*v*) to obtain 6.9 mg of strobilol J (**10**, 11.2 mg). *Fr. 6* (50% AcOEt eluate, 177.9 mg) was further chromatographed on SiO_2 with mixture of $CHCl_3$/AcOEt (10% stepwise gradient). The 30% AcOEt eluate (107 mg) was further purified by *ODS* CC with mixture of H_2O/MeOH (10% stepwise gradient), followed by SiO_2 CC with mixture of $CHCl_3$/AcOEt to obtain strobilols H (**8**, 4.3 mg) and I (**9**, 10.4 mg). *Fr. 8* and *9* (70–80% AcOEt eluates, 221.5 mg) were combined and chromatographed (SiO_2, $CHCl_3$/AcOEt stepwise; then *ODS*, H_2O/MeOH, stepwise). 50–80% MeOH eluates were combined and further subjected to CC (SiO_2, $CHCl_3$/MeOH 20:1) to obtain strobilols E (**5**, 21.3 mg), F (**6**, 22.8 mg), G (**7**, 6.6 mg), and K (**11**, 12.2 mg).

Strobilol E (=*(3a*S,*5*R,*5a*S,*7*S,*9a*S,*9b*R*)-1,2,4,5,5a,6,7,9a-Octahydro-5,8-dimethyl-1-methylidene-naphtho[2,1-*b*]furan-3a,7,9b-triol*; **5**). Colorless oil. $[\alpha]_D^{20} = +19.0$ ($c = 0.47$, MeOH). IR (KBr): 3443, 2955, 1031. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 266 (5, M^+), 230 (11), 167 (16), 149 (64), 119 (26), 85 (82), 83 (100). HR-EI-MS: 266.1519 (M^+, $C_{15}H_{22}O_4^+$; calc. 266.1518).

Strobilol F (=*(3a*S,*5*R,*5a*S,*9a*S,*9b*R*)-1,2,4,5,5a,6,7,9a-Octahydro-5,8-dimethyl-1-methylidenenaph-tho[2,1-*b*]furan-3a,9b-diol*; **6**). Colorless oil. $[\alpha]_D^{20} = -10.6$ ($c = 0.24$, MeOH). IR (KBr): 3409, 2929, 1031. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 250 (12, M^+), 246 (71), 177 (92), 159 (100), 135 (95), 121 (43), 109 (80). HR-EI-MS: 250.1573 (M^+, $C_{15}H_{22}O_3^+$; calc.250.1569).

Strobilol G (=*(3a*S,*5*R,*5a*S,*9a*S,*9b*R*)-1,2,4,5,5a,6,9a,9b-Octahydro-3a,9b-dihydroxy-5,8-dimethyl-1-methylidenenaphtho[2,1-*b*]furan-7(3a*H*)-one*; **7**). Colorless oil. $[\alpha]_D^{20} = +29.0$ ($c = 0.10$, MeOH). UV (MeOH): 234 (3.6). IR (KBr): 3443, 2983, 1670, 1031. ^1H- and ^{13}C-NMR: see *Tables 2* and *5*. EI-MS: 264 (48, M^+), 246 (50), 216 (29), 191 (31), 172 (67), 171 (63), 143 (58), 109 (63), 91 (100). HR-EI-MS: 264.1316 (M^+, $C_{15}H_{20}O_4^+$; calc. 264.1362).

Strobilol H (=*(3a*S,*5*R*)-1,2,4,5-Tetrahydro-5,8-dimethyl-1-methylidenenaphtho[2,1-*b*]furan-3a,9b-diol*; **8**). Colorless oil. $[\alpha]_D^{20} = +39.3$ ($c = 0.22$, MeOH). UV (MeOH): 239 (3.2). IR (KBr): 3430, 2935, 1506. ^1H- and ^{13}C-NMR: see *Tables 2* and *5*. EI-MS: 246 (29, M^+), 228 (18), 210 (19), 186 (39), 172 (72), 159 (100), 128 (40), 115 (41), 91 (95). HR-EI-MS: 246.1256 (M^+, $C_{15}H_{18}O_3^+$; calc. 246.1256).

Strobilol I (=*(3a*S,*5*R,*5a*S,*6a*S,*7a*S,*8*R,*8a*S,*8b*R*)-Dodecahydro-3a,8b-dihydroxy-5,7a-dimethyl-1-methylideneoxireno[6,7]naphtho[2,1-*b*]furan-8-yl Decanoate*; **9**). Colorless oil. $[\alpha]_D^{20} = +54.0$ ($c = 0.05$,

Table 5. *^{1}H-NMR Data of Strobilols G* (**7**) *and H* (**8**). At 400 MHz; δ in ppm, J in Hz.

	7[a])	**7**[b])	**8**[c])	**8**[b])
CH_2(1)	2.09 (*dd*, *J* = 16.4, 13.9),	2.11 (*dd*, *J* = 15.9, 13.5),	7.11 (*d*, *J* = 7.9)	7.20 (*d*, *J* = 8.0)
or H–C(1)	2.58–2.67[d])	2.82 (*dd*, *J* = 15.9, 3.9)		
C(2)			7.10 (*dd*, *J* = 7.9, 2.1)	7.13 (*dd*, *J* = 8.0, 2.4)
or H–C(2)				
H–C(4)	7.28 (br. *s*)	7.58 (br. *s*)	7.38 (br. *s*)	7.88 (br. *s*)
H–C(5)	2.58–2.67[d])	2.98 (*dd*, *J* = 10.7, 2.4)		
CH_2(8)	1.34 (*dd*, *J* = 14.0, 11.6),	1.51 (*dd*, *J* = 13.7, 11.9),	1.93 (*dd*, *J* = 13.9, 5.0),	2.25 (*dd*, *J* = 13.9, 6.7),
	1.84 (*dd*, *J* = 14.0, 3.2)	2.19 (*dd*, *J* = 13.7, 3.5)	2.24 (*dd*, *J* = 13.9, 6.6)	2.61 (*dd*, *J* = 13.9, 5.0)
H–C(9)	1.58–1.65[d])	1.69–1.89[d])	3.12–3.17 (*m*)	3.22–3.28 (*m*)
H–C(10)	1.58–1.65[d])	1.69–1.89[d])		
CH_2(12)	5.10 (*t*, *J* = 2.6),	5.11 (*t*, *J* = 2.5),	5.24 (*t*, *J* = 2.4),	5.24 (*t*, *J* = 2.5),
	5.22 (*t*, *J* = 2.6)	5.40 (*t*, *J* = 2.5)	5.58 (*t*, *J* = 2.4)	5.84 (*t*, *J* = 2.5)
CH_2(13)	4.18 (*dt*, *J* = 13.5, 2.6),	4.32 (*dt*, *J* = 13.4, 2.4),	4.29 (*dt*, *J* = 12.8, 2.4),	4.40 (*dt*, *J* = 12.8, 2.2),
	4.55 (*dt*, *J* = 13.5, 2.6)	4.67 (*dt*, *J* = 13.4, 2.4)	4.54 (*dt*, *J* = 12.8, 2.4)	4.65 (*dt*, *J* = 12.8, 2.2)
Me(14)	0.89 (*d*, *J* = 6.2)	0.81 (*d*, *J* = 7.2)	1.36 (*d*, *J* = 7.2)	1.48 (*d*, *J* = 7.2)
Me(15)	1.78 (br. *s*)	1.49 (br. *d*, *J* = 2.4)	2.33 (*s*)	2.27 (*s*)

[a]) In CD_3OD. [b]) In C_5D_5N. [c]) In $CDCl_3$. [d]) Overlapped signals.

MeOH). IR (KBr): 3444, 2923, 1733, 1000. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *4*. FAB-MS (pos.): 459 ($[M+Na]^+$). HR-FAB-MS (pos.): 459.2719 ($[M+Na]^+$, $C_{25}H_{40}NaO_6^+$; calc. 459.2723).

Strobilol J (=*(3a*S,*5*R,*5a*S,*9*R,*9a*S,*9b*R*)-1,2,3a,4,5,5a,6,9,9a,9b-Decahydro-3a,9b-dihydroxy-5,8-dimethyl-1-methylidenenaphtho[2,1-*b*]furan-9-yl Decanoate*; **10**). Colorless oil. $[\alpha]_D^{20} = -49.3$ ($c = 0.15$, MeOH). IR (KBr): 3467, 2854, 1733, 1033. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *4*. FAB-MS (neg.): 419 ($[M-H]^-$). HR-FAB-MS (neg.): 419.2796 ($[M-H]^-$, $C_{25}H_{39}O_5^-$; calc. 419.2797).

Strobilol K (=*(3a*S,*5*R,*5a*S,*7*R,*8*S,*9*R,*9a*S,*9b*R*)-Dodecahydro-3a,7,8,9b-tetrahydroxy-5,8-dimethyl-1-methylidenenaphtho[2,1-*b*]furan-9-yl Decanoate*; **11**). Colorless oil. $[\alpha]_D^{20} = +5.35$ ($c = 0.64$, MeOH). IR (KBr): 3400, 2854, 1733, 1049. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *4*. FAB-MS (neg.): 453 ($[M-H]^-$). HR-FAB-MS (neg.): 453.2860 ($[M-H]^-$, $C_{25}H_{41}O_7^-$; calc. 453.2852).

Esterification of Strobilol E (**5**). To a suspension of **5** (3 mg) in pyridine (5 ml) was added *p*-bromobenzoylchloride (10 mg) and 4-dimethylaminopyridine (3 mg), and this mixture was stirred overnight at r.t. The mixture was filtered and purified on SiO_2 CC to afford the benzoate **5a** (1.5 mg). UV (MeOH): 245 (2.9). CD: $\Delta\varepsilon_{245}$ −3.0 ($c = 0.2$, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.5, Me(14)); 1.24–1.43 (*m*, 3 H, CH_2(1), CH_2(8), H–C(10)); 1.50–1.53 (*m*, H–C(9)); 1.75 (br. *s*, Me(15)); 1.98 (*dd*, *J* = 14.1, 3.9, CH_2(8)); 2.43–2.47 (*m*, 2 H, CH_2(1), H–C(5)); 4.23 (*dt*, *J* = 13.2, 2.3, CH_2(13)); 4.54 (*dt*, *J* = 13.2, 2.3, CH_2(13)); 5.13 (*t*, *J* = 2.5, CH_2(12)); 5.49 (*t*, *J* = 2.5, CH_2(12)); 5.63–5.69 (*m*, H–C(2)); 6.00 (br. *s*, H–C(4)); 7.59 (*d*, *J* = 8.5, 2 arom. H); 7.91 (*d*, *J* = 8.5, 2 arom. H). EI-MS: 448 (10, M^+), 436 (10), 264 (20), 248 (56), 230 (62), 185 (98), 161 (93), 119 (100).

REFERENCES

[1] G. Chihara, *Dev. Biol. Stand.* **1992**, *77*, 191.
[2] L. Barros, M.-J. Ferreira, B. Queirós, I. C. F. R. Ferreira, P. Baptista, *Food Chem.* **2007**, *103*, 413.
[3] F. Hiramatsu, T. Murayama, T. Koseki, Y. Shiono, *Phytochemistry* **2007**, *68*, 1267.
[4] Y. Shiono, F. Hiramatsu, T. Murayama, T. Koseki, T. Funakoshi, K. Ueda, H. Yasuda, *Z. Naturforsch., B* **2007**, *62*, 1585.
[5] N. C. Gonnella, K. Nakanishi, V. S. Martin, K. B. Sharpless, *J. Am. Chem. Soc.* **1982**, *104*, 3775.
[6] H. Nakamura, Y. Kishi, O. Shimomura, *Tetrahedron* **1988**, *44*, 1597.

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