Fascicularones H–K, Four New Sesquiterpenoids from the Cultured Mycelia of the Fungus *Hypholoma fasciculare*

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The AcOEt extract of *Hypholoma fasciculare* yielded four new congeners, fascicularones H (**4**), I (**5**), J (**6**), and K (**7**). All structures were unambiguously established by 1D and 2D NMR and MS data. In a lettuce seedling assay, compounds **4**–**7** showed radicle elongation.

Introduction. – *Hypholoma fasciculare*[2]) is known as a bitter poisonous mushroom distributed in northeast Japan. Phytochemical investigation of the dried fruiting bodies of this mushroom revealed fasciculols A–F which inhibit plant growth [1–3]. Some have also been shown to induce calmodulin inhibition [4]. We previously reported the isolation and structure determination of fascicularones A, B (**1**), C, D (**2**), E, F (**3**), and G, which contain a *cis*-fused four-membered ring moiety, from the mycelial culture of *H. fasciculare* [5–7]. These compounds promoted radicle elongation in lettuce seedlings. In a further study of the same source, we found new fascicularone derivatives, *i.e.*, fascicularones H (**4**), I (**5**), J (**6**), and K (**7**). Here, we discuss the structure determination of these new compounds.[3])
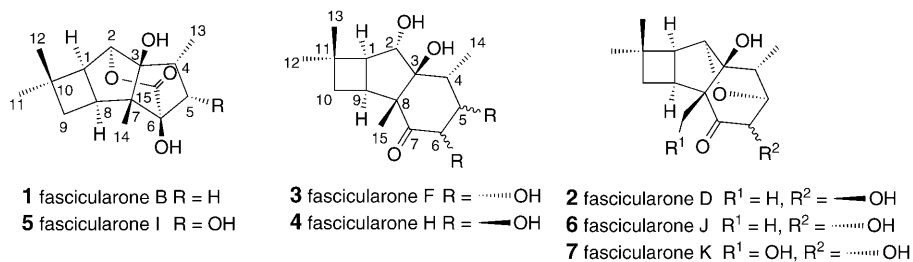

1 fascicularone B R = H
5 fascicularone I R = OH

3 fascicularone F R = ⋯⋯OH
4 fascicularone H R = ▬OH

2 fascicularone D R¹ = H, R² = ▬OH
6 fascicularone J R¹ = H, R² = ⋯⋯OH
7 fascicularone K R¹ = OH, R² = ⋯⋯OH

Fig. 1. *Structures of fascicularones B* (**1**), *D* (**2**), *F* (**3**), *H* (**4**), *I* (**5**), *J* (**6**), *and K* (**7**)[3])
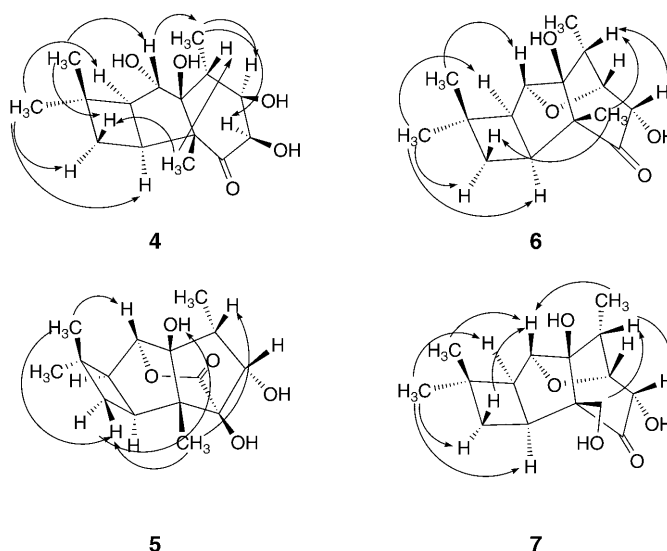
Fig. 2. *Selected NOE correlations for fascicularones H* (**4**)*, I* (**5**)*, J* (**6**) *and K* (**7**)

Results and Discussion. – The culture broth (5.0 l) of *H. fasciculare* was filtered and the filtrate extracted with AcOEt. The AcOEt extract was separated by repeated chromatography yielding fascicularones H (**4**), I (**5**), J (**6**), and K (**7**) (see *Exper. Part*).

The molecular formula of fascicularone H (**4**) is $C_{15}H_{24}O_5$ according to HR-FAB-MS data, suggesting the presence of four degrees of unsaturation or rings. The IR spectrum of **4** showed absorption due to OH (3388 cm^{-1}) and C=O (1700 cm^{-1}) groups. The NMR data of fascicularone H (**4**) (*Table 1*) suggested that it had a structure similar to that of fascicularone F (**3**) [7]. The proposed structure of **4** and its relative configuration was confirmed by the NOE data (*Fig. 2*).

The ^{1}H-NMR spectrum of **4** in CD$_3$OD showed signals due to 3 OCH, 3 CH, 1 CH$_2$, and 4 Me (3s and 1d) groups, which were confirmed by the ^{13}C-NMR data, including those from the DEPT experiments, which gave 15 signals assignable to 1 C=O, 3 sp^3 OCH, 3 nonoxygenated sp^3 CH, 1 sp^3 CH$_2$, and 4 Me groups and to 3 sp^3 quaternary C-atoms. The ^{1}H,^{1}H COSY plot of **4** revealed connectivities for two different proton networks: CH$_2$(10)/ H−C(9)/H−C(1)/H−C(2) and Me(14)/H−C(4)/H−C(5)/H−C(6). The Me groups at C(11) correlated with C(1), C(10), and C(11), CH$_2$(10) correlated with C(1) and C(12), and H−C(9) correlated with C(11), as established by the HMBC data (*Table 1*), suggesting the presence of a substituted cyclobutane ring. HMBC correlations of Me(14) with C(3) and C(5), of H−C(5) with C(3), C(7), and C(14), of H−C(6) with C(4) and C(7), and of Me(15) with C(3) and C(7) were detected, implying a substituted cyclohexanone ring. Correlations between H−C(2) and C(8), H−C(4) and C(2), and H−C(10) and C(8) enabled us to connect these partial structures through a C−C bond between C(2) and C(3) and between C(8) and C(9) as shown in **4**. The molecular skeleton of **4**, deduced from these results as shown in *Fig. 1* is reminiscent of that of fascicularone F (**3**) [7]. The relative configuration of **4** was deduced from NOE experiments (*Fig. 2*). Observation of the NOEs Me(13)/H$_β$−C(10), Me(13)/H−C(2), Me(15)/H−C(4), and Me(15)/H$_β$−C(10) indicated that Me(13), Me(15), H−C(2), and H−C(4) were all β-oriented. Compound **4** also showed NOEs from Me(14) to H−C(5) and Me(14) to H−C(6) which were not observed in **3**, implying that these protons are all α-oriented.

Fascicularone I (**5**) showed spectral characteristics quite similar to those of fascicularone B (**1**) [5]. The molecular formula of **5**, $C_{15}H_{22}O_5$, was determined by HR-FAB-

Table 1. *NMR Data for Fascicularone H* (**4**)[a]). In CD_3OD; δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC		δ(C)	δ(H)	HMBC
H–C(1)	58.2	2.12[b])	C(8), C(10), C(11), C(13)	H–C(9)	39.9	3.10 (*q*, *J*=8.3)	C(1), C(7), C(8), C(10), C(11)
H–C(2)	82.2	4.21 (*d*, *J*=2.4)	C(8), C(9), C(11)	H$_\alpha$–C(10)	36.3	1.53 (*ddd*, *J*=10.5, 8.3, 2.4)	C(1), C(8), C(9), C(11), C(12)
C(3)	93.0			H$_\beta$–C(10)		2.12[b])	C(1), C(8), C(9), C(11), C(12)
H–C(4)	41.8	2.40 (*m*)	C(2), C(6), C(8), C(14)	C(11)	34.4		
H–C(5)	76.4	3.74 (*t*, *J*=4.9)	C(3), C(4), C(6), C(7), C(14)	Me(12)	25.8	1.05 (*s*)	C(1), C(10), C(11), C(13)
H–C(6)	80.6	4.00 (*d*, *J*=4.9)	C(4), C(5), C(7)	Me(13)	33.7	1.21 (*s*)	C(1), C(10), C(11), C(12)
C(7)	215.5			Me(14)	11.7	1.05 (*d*, *J*=7.3)	C(3), C(4), C(5)
C(8)	61.7			Me(15)	16.8	1.20 (*s*)	C(3), C(7), C(8), C(9)

[a]) Assignments were made based on 2D ^1H,^1H-COSY, HMQC, and HMBC experiments. [b]) Multiplicity patterns were unclear due to overlapping signals.

Table 2. *NMR Data for Fascicularone I* (**5**)[a]). In $CDCl_3$; δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC		δ(C)	δ(H)	HMBC
H–C(1)	56.4	2.55 (br. *d*, *J*=7.8)	C(2), C(3), C(8), C(9), C(11)	H$_\alpha$–C(9)	33.8	1.62 (*ddd*, *J*=11.7, 7.8, 2.0)	C(1), C(8), C(11)
H–C(2)	87.7	4.57 (br. *s*)	C(3), C(7), C(8), C(15)	H$_\beta$–C(9)		2.20 (*dd*, *J*=11.7, 7.8)	C(7), C(8), C(11), C(12)
C(3)	88.7			C(10)	33.2		
H–C(4)	42.2	2.49 (*m*)	C(13)	Me(11)	33.8	1.20 (*s*)	C(1), C(9), C(10), C(12)
H–C(5)	75.6	4.31 (*d*, *J*=11.7)	C(13), C(15)	Me(12)	24.1	1.08 (*s*)	C(1), C(9), C(10), C(11)
C(6)	86.7			Me(13)	8.0	1.26 (*d*, *J*=7.3)	C(3), C(4), C(5)
C(7)	55.0			Me(14)	8.5	1.09 (*s*)	C(3), C(6), C(7), C(8)
H–C(8)	37.0	2.81 (*q*, *J*=7.8)	C(1), C(2), C(3), C(6), C(14)	C(15)	174.9		

[a]) Multiplicity patterns were unclear due to overlapping signals.

MS, and implies the presence of one O-atom more than in **1**. The IR spectrum of **5** showed absorption bands for OH (3416 cm^{-1}) and C=O groups (1725 cm^{-1}). ^1H- and ^{13}C-NMR data (*Table 2*) for **5** correspond well to those of **1**, except for the presence of an OCH signal in **5**. An HMBC experiment (*Table 2*) established that the supposed supplementary OH group of **5** was positioned at C(5) (correlations H−C(5)/C(13) and C(15)). Unambiguous signal assignments in the ^1H- and ^{13}C-NMR spectra of **5** were based on HMBC experiments (*Table 2*). The relative configurations at C(1), C(2),

Table 3. *NMR Data for Fascicularone J* (**6**)[a]). In CDCl$_3$; δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC		δ(C)	δ(H)	HMBC
H–C(1)	52.3	2.21–2.25[b])	C(8), C(9), C(12), C(13)	H–C(9)	45.3	3.08 (*q*, *J* = 7.3)	C(1), C(2), C(3), C(7), C(10), C(11)
H–C(2)	86.9	4.45 (*s*)	C(1), C(3), C(5), C(8), C(9), C(11)	H$_\alpha$–C(10)	34.5	1.63 (*ddd*, *J* = 11.7, 7.3, 2.9)	C(1), C(8), C(9), C(12), C(13)
C(3)	90.9			H$_\beta$–C(10)		2.21–2.25[b])	C(1), C(8), C(9), C(12), C(13)
H–C(4)	41.5	2.16 (*q*, *J* = 7.8)	C(2), C(3), C(6), C(8), C(14)	C(11)	32.9		
H–C(5)	81.3	4.11 (*s*)	C(2), C(3), C(6), C(7), C(14)	Me(12)	32.1	1.16 (*s*)	C(1), C(10), C(11), C(13)
H–C(6)	77.6	3.76 (*s*)	C(4), C(7), C(8)	Me(13)	24.5	0.97 (*s*)	C(1), C(10), C(11), C(12)
C(7)	217.3			Me(14)	13.8	1.35 (*d*, *J* = 7.8)	C(3), C(4), C(5)
C(8)	64.0			Me(15)	12.6	1.14 (*s*)	C(3), C(7), C(8), C(9)

[a]) Assignments were made based on 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Multiplicity patterns were unclear due to overlapping signals.

C(3), C(4), C(6), C(7), and C(8) were determined to be the same as those of **1** based on NOE experiments (*Fig. 2*), and OH−C(5) was α-positioned as established by the NOE Me(14)/H−C(5) and the ^{1}H,^{1}H coupling constants.

The molecular formula of fascicularone J (**6**) was established as C$_{15}$H$_{22}$O$_4$ by HR-FAB-MS, indicating that **6** is an isomer of fascicularone D (**2**) [6]. The ^{1}H-NMR spectrum of **6** (*Table 3*) resembled that of **2**, except for the signals of H−C(4) (δ 2.16 and 1.73, resp.) and H−C(6) (δ 3.76 and 2.69, resp.). The ^{1}H- and ^{13}C-NMR data of **6** were fully assigned by 2D NMR spectra, including HMBC (*Table 3*). The NOE experiment (*Fig. 2*; NOEs H−C(6)/H−C(4) and Me(15)/H−C(4)) confirmed that **6** was the epimer of **2** at C(6).

The molecular formula C$_{15}$H$_{22}$O$_5$ of fascicularone K (**7**) was established by HR-FAB-MS. The ^{1}H-NMR spectrum (*Table 4*) of **7** resembled that of **6**, except for the absence of the Me(15) signal, which was replaced by a CH$_2$OH group (δ(H) 3.81 and 4.02 each (*d*, *J* = 10.6 Hz, CH$_2$(15)), indicating that **7** was 15-hydroxyfascicularone J. The HMBC experiment confirmed this tentative structure proposal, and the relative configuration of **7** was deduced to be the same as that of **6** by the NOE experiments (*Fig. 2*).

The absolute configurations of fascicularones H (**4**), I (**5**), J (**6**), and K (**7**) were not established independently, but are assumed to be the same as in fascicularone A, whose absolute configuration has been determined by using a modification of *Mosher*'s method.

Fascicularones H (**4**), I (**5**), J (**6**), and K (**7**) showed radicle elongation of 180, 173, 165, and 184% of controls at a concentration of 100 ppm with lettuce seedlings.
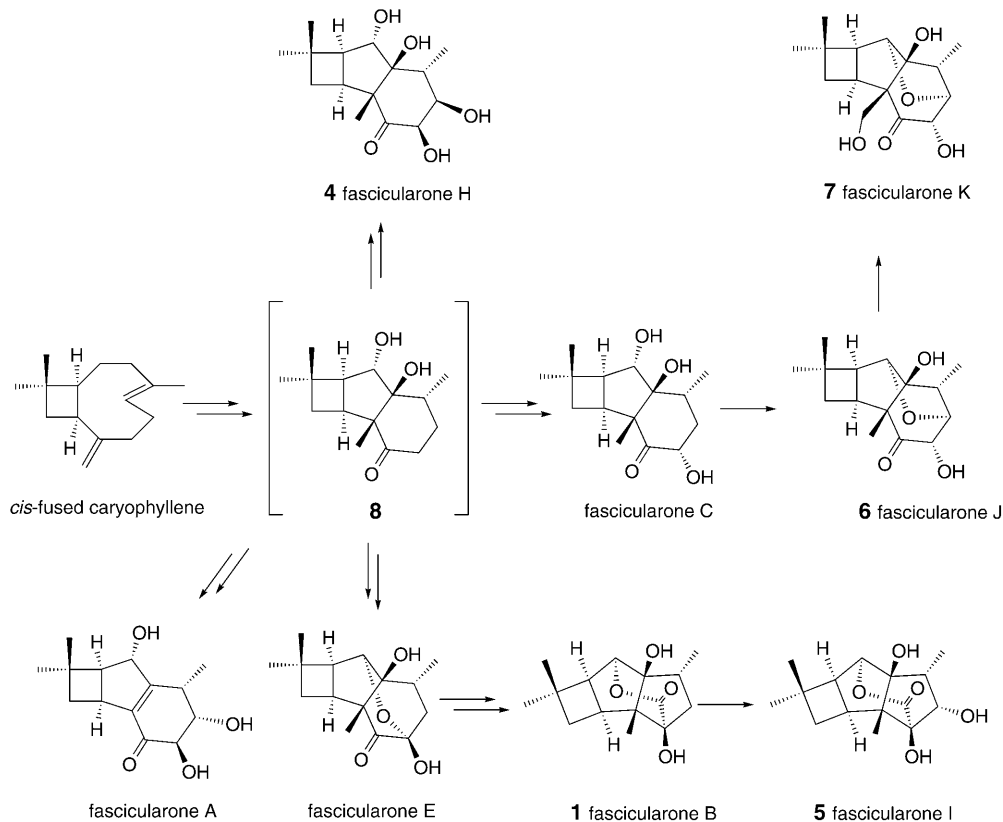
Based on the structures of the fascicularones isolated so far, we propose a common biosynthetic pathway, outlined in the *Scheme*. According to our proposal, compound **8** represents the biogenetic precursor of the fascicularones. Compound **8** would be derived from the *cis*-fused caryophyllene by allylic oxidations, reduction of the C=C

Table 4. *NMR Data for Fascicularone K* (**7**)[a]). In CD₃OD; δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC		δ(C)	δ(H)	HMBC
H–C(1)	54.5	2.18 (br. *d*, $J = 7.1$)	C(9), C(10), C(12), C(13)	H$_a$–C(10)	35.9	1.50 (*td*, $J = 10.3, 2.8$)	C(1), C(8), C(9), C(12), C(13)
H–C(2)	89.3	4.44 (*s*)	C(1), C(3), C(5), C(8), C(9), C(11)	H$_\beta$–C(10)		2.40 (*t*, $J = 10.3$)	C(1), C(9), C(12), C(13)
C(3)	91.9			C(11)	35.4		
H–C(4)	45.6	2.63 (*q*, $J = 7.1$)	C(2), C(3), C(6), C(8), C(14)	Me(12)	33.6	1.13 (*s*)	C(1), C(10), C(11), C(13)
H–C(5)	85.4	4.00 (*d*, $J = 1.9$)	C(2), C(3), C(6), C(14)	Me(13)	25.4	0.99 (*s*)	C(1), C(10), C(11), C(12)
H–C(6)	80.8	3.70 (*d*, $J = 1.9$)	C(4), C(5), C(7), C(8)	Me(14)	15.2	1.30 (*d*, $J = 7.1$)	C(3), C(4), C(5)
C(7)	219.6			H–C(15)	63.3	3.81 (*d*, $J = 10.6$)	C(3), C(7), C(8), C(9)
C(8)	71.9			H–C(15)		4.02 (*d*, $J = 10.6$)	C(3), C(7), C(8), C(9)
H–C(9)	45.2	2.89 (*m*)	C(1), C(2), C(3), C(7), C(10), C(11)				

[a]) Assigments were made based on 2D ¹H,¹H-COSY, HMQC, and HMBC experiments.

Scheme. *Plausible Biosynthetic Pathway of Fascicularones*

4 fascicularone H

7 fascicularone K

cis-fused caryophyllene

8

fascicularone C

6 fascicularone J

fascicularone A

fascicularone E

1 fascicularone B

5 fascicularone I

bond and a subsequent transannular aldol reaction [8]. Fascicularone H (**4**) would, in turn, be formed from **8** by oxidation. The formations of fascicularone J (**6**) and K (**7**) are thought to occur *via* **8**, followed by oxidation and intramolecular cyclization. The formation of fascicularones B (**1**) and I (**5**), which have a tricyclo[5.3.0.0^{2,5}]decane skeleton, would be due to a lactonization reaction of fascicularone E [7].

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Kanto Chemical Co., Inc.*), and *ODS* (*RP-18*; *Fuji Silysia Chemical Ltd.*). TLC: silica gel F_{254} (0.5 mm; *Merck*); detection by spraying with 10% vanillin in H_2SO_4, followed by heating at 120°. Optical rotations: *Horiba SEPA-300* polarimeter. M.p.: *Yanagimoto* melting-point apparatus; uncorrected. IR Spectra: *Jasco J-20A-FT-IR* spectrometer, KBr pellets; $\tilde{v}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Jeol EX-400* spectrometer, at 400/100 MHz, resp.; δ in ppm rel. to Me$_4$Si as internal standard; coupling constants J in Hz. EI- and FAB-MS: *Jeol JMS-700* spectrometer; in m/z (rel.%).

Mushroom Material and Fermentation. The producing fungal strain was isolated from cultured tissue of the fruiting bodies of *H. fasciculare* [5] and was deposited in the Faculty of Agriculture, Yamagata University, Yamagata, Japan. The mycelium was grown in fifty 500-ml *Sakaguchi* flasks containing 100 ml of a medium consisting of glucose (40 g), and pepone (1.0 g) per 1 l of H_2O at 25° for 30 days on a rotary shaker at 120 rpm.

Extraction and Isolation. After the incubation period, 5.0 l of culture broth were separated from the mycelium by filtration. The resulting filtrate was extracted with AcOEt. The AcOEt extract was evaporated and the residue (9.9 g) subjected to CC (silica gel, 10% stepwise elution with hexane/AcOEt, AcOEt/MeOH 1:1, and MeOH): (*Fractions 1.1–1.13*). (TLC monitoring by the characteristic intense blue spots with 10% vanillin in H_2SO_4). *Fr. 1.7* (1.3 g) was resubjected to CC (silica gel, CHCl$_3$/AcOEt gradient); the 40% MeOH eluate was further subjected to CC (silica gel, CHCl$_3$/MeOH 95:5): **6** (10.7 mg). *Fr. 1.9* (340 mg) was resubjected to CC (silica gel, CHCl$_3$/AcOEt gradient; then *ODS*, H_2O/MeOH gradient); the 80% MeOH eluate was further subjected to CC (silica gel, CHCl$_3$/MeOH 95:5): **4** (5.5 mg), **5** (3.6 mg), and **7** (5.5 mg).

Fascicularone H (=rel-*(2aR,2bS,4S,5S,6S,6aR,7R,7aR)-Decahydro-4,5,6a,7-tetrahydroxy-1,1,2b,6-tetramethyl-3*H-*cyclobut[a]inden-3-one*; **4**): Colorless needles. M.p. 131–134°. $[\alpha]_D^{20} = +17.7$ ($c = 0.53$, MeOH). IR (KBr): 3388, 2945, 2860, 1700. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 307 ([M+Na]$^+$). HR-FAB-MS: 307.1524 ([*M*+Na]$^+$, C$_{15}$H$_{24}$O$_5$Na$^+$; calc. 307.1521).

Fascicularone I (=rel-*(2aR,2bS,3R,4R,5S,5aR,6R,6aR)-Decahydro-3,4,5a-trihydroxy-1,1,2b,5-trimethyl-6, 3-(epoxymethano)cyclobuta[a]pentalen-8-one*; **5**): Colorless oil. $[\alpha]_D^{20} = +54.0$ ($c = 0.06$, MeOH). IR (KBr): 3401, 2942, 2863, 1716. ^{1}H- and ^{13}C-NMR: *Table 2*. FAB-MS: 283 ([*M*+H]$^+$). HR-FAB-MS: 283.1549 ([*M*+H]$^+$, C$_{15}$H$_{23}$O$_5^+$, calc. 283.1545).

Fascicularone J (=rel-*(2aR,2bS,4R,5R,6S,6aR,7R,7aR)-Decahydro-4,6a-dihydroxy-1,1,2b,6-tetramethyl-5, 7-epoxy-3*H-*cyclobut[a]inden-3-one*; **6**). Colorless oil. $[\alpha]_D^{20} = -120$ ($c = 0.8$, CHCl$_3$). IR (KBr): 3409, 2942, 2861, 1706, 1001. ^{1}H- and ^{13}C-NMR: *Table 3*. EI-MS: 266 (59, *M*$^+$), 248 (6), 210 (59), 192 (100), 164 (14), 152 (64), 124 (100), 85 (28). HR-FAB-MS: 267.1600 ([*M*+H]$^+$, C$_{15}$H$_{23}$O$_4^+$, calc. 267.1598).

Fascicularone K (=rel-*(2aR,2bR,4R,5R,6S,6aR,7R,7aR)-Decahydro-4,6a-dihydroxy-2b-(hydroxymethyl)-1,1,6-trimethyl-5,7-epoxy-3*H-*cyclobut[a]inden-3-one*; **7***). Colorless oil. $[\alpha]_D^{20} = -82.0$ ($c = 0.41$, MeOH). IR (KBr): 3370, 2942, 2931, 1711, 1022. ^{1}H- and ^{13}C-NMR: *Table 4*. FAB-MS: 283 ([*M*+H]$^+$). HR-FAB-MS: 283.1557 ([*M*+H]$^+$, C$_{15}$H$_{23}$O$_5^+$, calc. 283.1545).

Lettuce Seedling Assay. Lettuce seeds (*Lactuca sativa* L.) were used for the bioassay: 15 seeds were sown in filter paper containing a defined concentration of the test compound in a *Petri* dish (5 cm i.d.). Dist. H_2O (1 ml, containing 100 ppm (*w/v*) of *Tween 80*) was added to the *Petri* dish, and incubation was carried out at 25° under continuous light for 7 days. The control experiments were conducted in dist. H_2O. The elongation of roots and shoots were measured and compared to those of the control.

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