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Four New Chlorinated Azaphilones, Helicusins A—D, Closely Related to 7-*epi*-Sclerotiorin, from an Ascomycetous Fungus, *Talaromyces helicus*

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Four new azaphilones, named helicusins A, B, C, and D, were isolated from an Ascomycete, *Talaromyces helicus*. Helicusins A and B were deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-2′*E*-pentenedioate and its 2′*Z* isomer, and helicusins C and D were deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-3′*Z*-pentenedioate and its 3′*E* isomer, respectively, on the basis of chemical and spectral data.

These four new azaphilones showed weak monoamine oxidase-inhibitory effects.

Key words fungal metabolite; azaphilone; Ascomycete; *Talaromyces helicus*; monoamine oxidase inhibitory activity; 7-*epi*-sclerotiorin derivative

Two new azaphilones named TL-1 [(8*R*)-7-deacetyl-O^8,8-dihydro-7-*epi*-sclerotiorin] and -2 (the 11*Z* isomer of TL-1), having remarkable inhibitory activity against mouse monoamine oxidase (MAO), were isolated from an Ascomycetous fungus, *Talaromyces luteus*, in our laboratory.[1] In a further survey of fungal components having MAO-inhibitory activity, the AcOEt extract of *Talaromyces helicus* (RAPER *et* FENNELL) C. R. BENJAMIN was found to inhibit MAO and to contain four new yellow pigments structurally related to TL-1 and -2.

This paper describes the isolation, structure elucidation, and MAO-inhibitory effect of these azaphilone pigments.

Results and Discussion

The AcOEt extract of *T. helicus* cultured on sterilized rice inhibited mouse liver MAO by 14% at 1.0×10^{-4} g/ml in an assay using a modification of Kraml's method.[2] The AcOEt fraction obtained from the extract by partitioning with AcOEt–H_2O was further partitioned with *n*-hexane–MeOH. The MeOH fraction was then fractionated through successive chromatography on a Diaion HP-20 column, a silica gel column and a medium-pressure liquid chromatographic (MPLC) silica gel column to furnish four new acidic pigments named helicusins A—D (**1**—**4**). Among them, **1** was obtained as a major metabolite of the fungus.

Helicusin A (**1**), a yellow amorphous powder, $C_{25}H_{27}ClO_7$, was optically active and soluble in 0.1 N $NaHCO_3$. The UV spectrum of **1** suggested the presence of long extended unsaturation systems conjugated with C=O (at 287 and 364 nm) and the IR spectrum suggested the presence of COOH and conjugated C=O (1700 cm^{-1}). The ^{13}C-NMR spectrum of **1** (Table 1) indicated that this compound is composed of five methyls including two olefinic ones, two methylenes, seven methines including six olefinic ones and eleven quaternary carbons including six olefinic and four carbonyl ones. The ^{1}H-NMR spectrum was consistent with the above findings (see Table 1). Comparison of the ^{13}C- and ^{1}H-NMR data of **1** with those of some fungal azaphilones TL-1, -2, sclerotiorin (**5**, from *Penicillium sclerotiorum*),[3] and 7-*epi*-sclerotiorin (**6**, from *Penicillium hyrayamae*)[4] suggested that the structure of **1** is similar to that of **6** except for its ester moiety at position 7 (see Chart 1). From the molecular formula of **1** the ester moiety should consist of $C_6H_7O_4$. The ^{13}C- and ^{1}H-NMR spectral data, including two-dimensional ^{13}C–^{1}H shift correlation (^{13}C–^{1}H COSY) NMR, of **1** indicated that the ester moiety of this compound was composed of one olefinic methyl [δ 19.3 (q), 2.20 (3H, d, J=0.7 Hz)], one methylene [δ 45.5 (t), 3.21 (2H, br s)], one olefinic methine [δ 118.0 (d), 6.02 (1H, br s)], one olefinic quaternary carbon [δ 153.9 (s)], and two carbonyl carbons (an ester C=O and a carboxylic C=O) [δ 164.6 and 174.5 (each, s)] (see Table 1). In the ^{13}C–^{1}H correlation spectroscopy *via* long-range coupling (COLOC) NMR spectrum of **1**, significant cross peaks due to C–H long-range coupling with J_2 and/or J_3 of C (δ 118.0, 153.9, 45.5)/H_3 (δ 2.20), C (δ 19.3, 118.0, 153.9, 174.5)/H_2 (δ 3.21) were observed. This fact suggested that the ester moiety in **1** is either –O–CO–CH=C(CH_3)–CH_2–COOH (a) or –O–CO–CH_2–C(CH_3)=CH–COOH (b) (see Fig. 1).

On methylation with CH_2N_2, **1** afforded a methyl ester (**1a**), ^{1}H-NMR: δ 3.70 (3H, s). Comparison of the ^{13}C-NMR spectrum of **1a** with that of **1** showed that all signals of **1a** were identical with those of **1** except that a signal at δ 52.1 (q) newly appeared and the signal of one of the two carbonyl carbons in the ester moiety at δ 174.5 shifted to δ 169.8 (-4.7) (see Table 1). This result and the fact that cross peaks of C (δ 169.8)/H_3 (δ 3.70) and C (δ 169.8)/H_2 (δ 3.17) were observed in the COLOC NMR spectrum of **1a** indicated that the ester moiety in **1** is –O–CO–CH=C(CH_3)–CH_2–COOH (a). Furthermore, significant nuclear Overhauser effects (NOE) were ob-

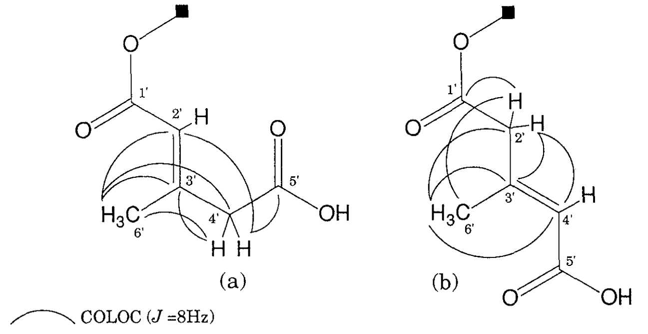

Fig. 1. Possible Partial Structures of **1**

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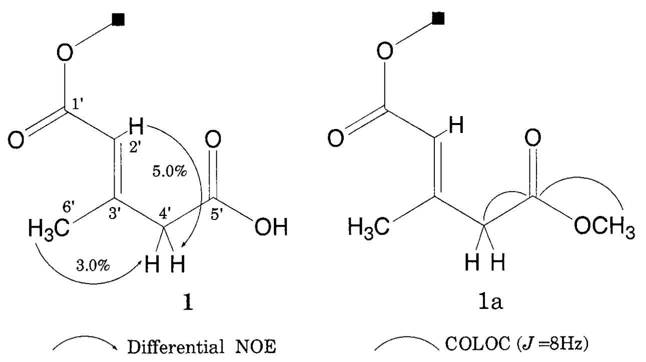

Fig. 2. Partial Structures of **1** and **1a**

served between the signal of H-2′ at δ 6.02 and H_2-4′ at δ 3.21 (5.0%), and between the signal of H_3-6′ at δ 2.20 and H_2-4′ (3.0%) in the differential NOE spectrum of **1** (see Fig. 2). These facts indicated that the configuration at 2′ in **1** is *E*. The stereochemistry at position 13 in **1** was established as (*S*), since **1** gave an optically active carboxylic acid identical with (+)-(2*E*,4*E*,6*S*)-4,6-dimethylocta-2,4-dienoic acid (**7**) on alkaline degradation. Compound **7** has already been obtained from TL-1[1] and **6**[4] by similar degradation. It is known that 7-*epi*-sclerotiorin type azaphilones afford (−)$\Delta\varepsilon$ at *ca.* 390 nm in their circular dichroism (CD) spectra.[5,6] The fact that **1** gave $\Delta\varepsilon$ −7.3 at 388 nm in the CD spectrum showed that the configuration at position 7 in **1** is (*S*). Accordingly, the structure of helicusin A (**1**) was deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-2′*E*-pentenedioate, as shown in Chart 1.

Helicusin B (**2**), $C_{25}H_{27}ClO_7$, was obtained as a yellow amorphous powder. Comparison of the ^{1}H-NMR spectrum of **2** with that of **1** suggested that **2** is an isomer of **1** which differs from **1** only in the ester moiety at position 7, and comparison of the ^{13}C-NMR spectrum of **2** with that of **1** indicated that all of the signals of **2** are similar to those of **1** except for shifting of the signals of C-1′, -4′, -5′, and -6′ to δ 165.7 (+1.1), 39.5 (−6.0), 171.9 (−2.6), and 25.9 (+6.6), respectively (see Table 1). This fact led to the conclusion that **2** is the *Z* isomer of **1** at position 2′, because the deshielding shifts of C-1′ and -6′ and shielding shifts of C-4′ and -5′ observed in the ^{13}C-NMR spectrum of **2** indicated disappearance of the γ-effect[7] between C-1′ and -6′, which existed in **1**, and new appearance of the effects between C-1′ and -4′ and between C-5′ and -6′ in **2**. This conclusion was also supported by the differential NOE, which amounted to 3.9% between H-2′ (δ 6.09) and H_3-6′ (δ 2.04) of **2**. The CD spectrum of **2** also gave (−)$\Delta\varepsilon$ at 389 nm, indicating that the configuration at position 7 in **2** is (*S*) (see Experimental). The configuration at position 13 in **2** was biogenetically expected to be (*S*), since **2** was obtained together with **1** from the same fungus. Accordingly, the structure of helicusin B was deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-2′*Z*-pentenedioate (**2**), as shown in Chart 1.

Helicusin C (**3**), $C_{25}H_{27}ClO_7$, was obtained as a yellow amorphous powder. Comparison of the ^{13}C- and ^{1}H-NMR spectra of **3** with that of **1** suggested that **3** is also an isomer of **1**, which differs from **1** in the ester moiety. The ^{13}C- and ^{1}H-NMR spectral data, including ^{13}C–^{1}H COSY NMR data, indicated that the ester moiety in **3** consisted of one olefinic methyl [δ 25.4 (q), 2.03 (3H, d, J = 1.4 Hz)], one methylene [δ 37.4 (t), 3.88 and 4.03 (each 1H, d, J = 16.2 Hz)], one olefinic methine [δ 119.0 (d), 5.88 (1H, d, J = 0.8 Hz)], one olefinic quaternary carbon [δ 152.6 (s)], and two carbonyl carbons (an ester C=O and a carboxylic C=O) [δ 169.4 and 170.3 (each, s)]. The COLOC NMR spectrum of **3** afforded significant cross peaks of C (δ 37.4, 152.6, 119.0)/H_3 (δ 2.03), C (δ 25.4, 152.6, 169.4)/H_2 (δ 3.88, 4.03), C (δ 152.6, 25.4)/H (δ 5.88), indicating that the ester moiety in **3** may be $-O-CO-CH=C(CH_3)-CH_2-COOH$ (a) or $-O-CO-CH_2-C(CH_3)=CH-COOH$ (b). On methylation with CH_2N_2, **3** afforded a methyl ester (**3a**), ^{1}H-NMR: 3.71 (3H, s). Comparison of the ^{13}C-NMR spectrum of **3a** with that of **3** showed that all of the signals of **3a** are similar to those of **3** except for the appearance of a new signal at δ 51.2 (q) and the shift of the signal of one of the two carbonyl carbons in the ester moiety (δ 170.3) to δ 166.2 (−4.1). A significant cross peak of H_3 (δ 3.71)/C (δ 166.2) was observed in the ^{1}H-detected multiple bond heteronuclear multiple quantum coherence (HMBC) NMR spectrum of **3a**. Those facts indicated that the ester moiety in **3** is $-O-CO-CH_2-C(CH_3)=CH-COOH$ (b). In the differential NOE experiment, **3** gave 2.8% NOE between H-4′ (δ 5.88) and H_3-6′ (δ 2.03), indicating that the configuration at 3′ in **3** is *Z*. The configurations at positions 7 and 13 in **3** were also estimated to be both (*S*) for the same reason as in the case of **2**. Accordingly, the structure of helicusin D was deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-3′*Z*-pentenedioate (**3**), as shown in Chart 1.

Helicusin D (**4**), $C_{25}H_{27}ClO_7$, was obtained as a yellow amorphous powder. Comparison of the ^{1}H-NMR spectrum of **4** with that of **3** suggested that **4** is an isomer of **3**, and **4** differs from **3** only in the ester moiety. The comparison of the ^{13}C-NMR spectrum of **4** with that of **3** showed that all signals of **4** are similar to those of **3** except that the signals of C-2′, -5′, and -6′ are shifted to δ 44.8 (+7.4), 168.9 (−1.4), and 19.1 (−6.3), respectively (see Table 1). Both a deshielding shift of C-2′ and shielding shifts of C-5′ and -6′ were observed in the ^{13}C-NMR spectrum of **4** indicating disappearance of the γ-effect[7] between C-2′ and -5′ which existed in **3**, and new appearance of the effect between C-5′ and -6′ in **4**. These facts indicated that **4** is the *E* isomer of **3** at position 3′. This conclusion was also supported by the difference NOE, which amounted to 5.1% between H_2-2′ (δ 3.21) and H-4′ (δ 5.87) of **4**. The configurations at positions 7 and 13 in **4** were both estimated to be (*S*), for the same reason as in the case of **2**. Accordingly, the structure of helicusin C was deduced to be (7*S*,13*S*)-7-deacetyl-7-*epi*-sclerotiorin-7-yl hydrogen 3′-methyl-3′*E*-pentenedioate (**4**), as shown in Chart 1.

The inhibition of mouse liver MAO by **1**—**4** amounted to 41, 31, 15, and 13% at 1.0×10^{-4} g/ml, respectively, in an assay using a modification of Kraml's method.[2] These data indicated that the MAO-inhibitory activity of compounds **1**—**4** is weak. TL-1 and -2 exhibited comparatively high MAO-inhibitory activity (IC_{50} TL-1:

Table 1. ^{13}C- and ^{1}H-NMR Data for Helicusins A—D (**1**—**4**), Helicusin A Methyl Ester (**1a**), Helicusin C Methyl Ester (**3a**), and 7-*epi*-Sclerotiorin (**6**) (δ ppm from TMS in $CDCl_3$, Coupling Constants (Hz) in Parentheses)

Position	**1**		**1a**		**2**		**3**	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	152.7 (d)	7.96 (s)	152.6 (d)	7.95 (s)	152.8 (d)	7.95 (s)	152.8 (d)	7.94 (s)
3	158.0 (s)		158.0 (s)		158.2 (s)		158.1 (s)	
4	106.4 (d)	6.66 (s)	106.4 (d)	6.66 (s)	106.4 (d)	6.66 (s)	106.3 (d)	6.62 (s)
4a	138.7 (s)		138.6 (s)		138.8 (s)		138.7 (s)	
5	110.8 (s)		110.9 (s)		110.6 (s)		110.7 (s)	
6	191.5 (s)		191.8 (s)		191.4 (s)		191.2 (s)	
7	84.2 (s)		84.1 (s)		84.9 (s)		85.0 (s)	
7-O$\underline{\mathrm{H}}$								
8	186.1 (s)		186.1 (s)		185.6 (s)		185.8 (s)	
8-O$\underline{\mathrm{H}}$								
8a	114.5 (s)		114.5 (s)		114.5 (s)		114.5 (s)	
9	115.6 (d)	6.08 (d, 15.6)	115.7 (d)	6.08 (d, 15.7)	115.6 (d)	6.08 (d, 15.7)	115.7 (d)	6.07 (d, 15.7)
10	142.8 (d)	7.07 (d, 15.6)	142.7 (d)	7.06 (d, 15.7)	142.9 (d)	7.07 (d, 15.7)	142.8 (d)	7.05 (d, 15.7)
11	131.9 (s)		131.9 (s)		132.0 (s)		132.0 (s)	
12	148.8 (d)	5.71 (d, 9.4)	148.7 (d)	5.71 (d, 9.9)	148.9 (d)	5.71 (d, 9.1)	148.8 (d)	5.70 (d, 9.9)
13	35.1 (d)	2.49 (m)	35.1 (d)	2.49 (m)	35.1 (d)	2.48 (m)	35.1 (d)	2.48 (m)
14	30.1 (t)	1.33 (m) 1.43 (m)	30.0 (t)	1.33 (m) 1.43 (m)	30.0 (t)	1.33 (m) 1.43 (m)	30.1 (t)	1.34 (m) 1.44 (m)
15	11.9 (q)	0.87 (3H, t, 7.2)	11.9 (q)	0.87 (3H, t, 7.4)	11.9 (q)	0.87 (3H, t, 7.5)	11.9 (q)	0.87 (3H, t, 7.4)
16	12.3 (q)	1.85 (3H, s)	12.3 (q)	1.85 (3H, d, 1.1)	12.3 (q)	1.85 (3H, d, 1.2)	12.3 (q)	1.84 (3H, d, 1.1)
17	20.2 (q)	1.01 (3H, d, 6.5)	20.2 (q)	1.01 (3H, d, 6.6)	20.2 (q)	1.01 (3H, d, 6.5)	20.2 (q)	1.01 (3H, d, 6.6)
18	22.5 (q)	1.59 (3H, s)	22.5 (q)	1.59 (3H, s)	22.7 (q)	1.60 (3H, s)	22.5 (q)	1.58 (3H, s)
O$\underline{\mathrm{C}}$OCH$_3$								
OCOC$\underline{\mathrm{H}}_3$								
1′	164.6 (s)		164.7 (s)		165.7 (s)		169.4 (s)	
2′	118.0 (d)	6.02 (br s)	117.6 (d)	5.98 (br s)	117.8 (t)	6.09 (s)	37.4 (t)	3.88 (d, 16.2) 4.03 (d, 16.2)
3′	153.9 (s)		154.5 (s)		153.7 (s)		152.6 (s)	
4′	45.5 (t)	3.21 (2H, br s)	45.9 (t)	3.17 (2H, d, 0.8)	39.5 (d)	3.53 (d, 14.9) 3.68 (d, 14.9)	119.0 (d)	5.88 (d, 0.8)
5′	174.5 (s)		169.8 (s)		171.9 (s)		170.3 (s)	
6′	19.3 (q)	2.20 (3H, d, 0.7)	19.2 (q)	2.17 (3H, d, 1.4)	25.9 (q)	2.04 (3H, d, 1.2)	25.4 (q)	2.03 (3H, d, 1.4)
COOC$\underline{\mathrm{H}}_3$			52.1 (q)	3.70 (3H, s)				

Position	**3a**		**4**		**6**	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	152.6 (d)	7.93 (s)	152.7 (d)	7.94 (s)	152.6 (d)	7.94 (s)
3	158.0 (s)		158.2 (s)		158.1 (s)	
4	106.3 (d)	6.63 (s)	106.3 (d)	6.64 (s)	106.4 (d)	6.65 (s)
4a	138.6 (s)		138.8 (s)		138.6 (s)	
5	110.7 (s)		110.6 (s)		110.9 (s)	
6	191.3 (s)		191.3 (s)		191.8 (s)	
7	84.9 (s)		85.3 (s)		84.6 (s)	
7-O$\underline{\mathrm{H}}$						
8	185.7 (s)		185.5 (s)		185.9 (s)	
8-O$\underline{\mathrm{H}}$						
8a	114.4 (s)		114.5 (s)		114.0 (s)	
9	115.6 (d)	6.07 (d, 15.8)	115.6 (d)	6.08 (d, 15.7)	115.7 (d)	6.08 (d, 15.8)
10	142.8 (d)	7.06 (d, 15.8)	143.0 (d)	7.07 (d, 15.7)	142.8 (d)	7.08 (d, 15.8)
11	131.9 (s)		132.0 (s)		132.0 (s)	
12	148.8 (d)	5.71 (d, 9.8)	149.0 (d)	5.71 (d, 9.9)	148.8 (d)	5.71 (d, 9.6)
13	35.1 (d)	2.48 (m)	35.1 (d)	2.49 (m)	35.1 (d)	2.48 (m)
14	30.0 (t)	1.34 (m) 1.44 (m)	30.0 (t)	1.34 (m) 1.42 (m)	30.1 (t)	1.32 (m) 1.42 (m)
15	11.9 (q)	0.87 (3H, t, 7.5)	11.9 (q)	0.87 (3H, t, 7.4)	11.9 (q)	0.87 (3H, t, 7.4)
16	12.3 (q)	1.84 (3H, d, 1.2)	12.3 (q)	1.85 (3H, d, 1.1)	12.4 (q)	1.85 (3H, d, 1.0)
17	20.2 (q)	1.01 (3H, d, 6.9)	20.2 (q)	1.01 (3H, d, 6.6)	20.2 (q)	1.01 (3H, d, 6.6)
18	22.5 (q)	1.58 (3H, s)	22.5 (q)	1.59 (3H, s)	22.5 (q)	1.57 (3H, s)
O$\underline{\mathrm{C}}$OCH$_3$					170.1 (s)	
OCOC$\underline{\mathrm{H}}_3$					20.2 (q)	2.18 (3H, s)
1′	169.6 (s)		170.5 (s)			
2′	37.2 (t)	3.87, 4.02 (each dd, 16.0, 0.8)	44.8 (t)	3.21 (2H, br s)		
3′	149.8 (s)		153.1 (s)			
4′	119.2 (d)	5.84 (d, 1.5)	119.3 (d)	5.87 (d, 1.3)		
5′	166.2 (s)		168.9 (s)			
6′	25.1 (q)	2.00 (3H, d, 1.5)	19.1 (q)	2.27 (3H, d, 1.3)		
COOC$\underline{\mathrm{H}}_3$	51.2 (q)	3.71 (3H, s)				

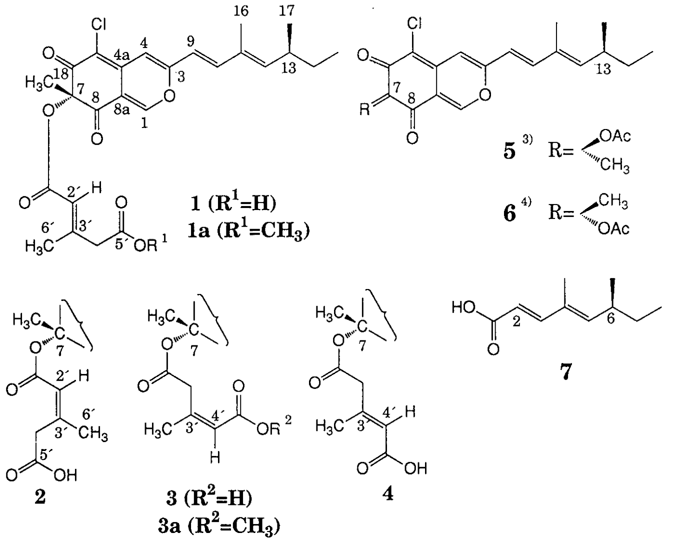

Chart 1

6.6×10^{-6} M, TL-2: 1.1×10^{-6} M).[1] These results suggest that the presence of the hydroxyl group at position 8 is indispensable for MAO inhibitory activity.

Experimental

The IR spectra were recorded with a Hitachi EPI-G3, the UV spectra with a Hitachi U-3400, the high-resolution FAB-MS (HRFAB-MS) spectra with a JEOL JMS-HX 110, the optical rotations with a JASCO DIP-140, the CD spectra with a JASCO J-20, and the ^{1}H- and ^{13}C-NMR spectra (in $CDCl_3$) with JEOL JNM-A500, -GSX500, and -GSX400 instruments at 500, 500, and 400 MHz for ^{1}H-NMR, and at 125.65, 125.65, and 100 MHz for ^{13}C-NMR, respectively.

Isolation of Helicusins A—D (1—4) *Talaromyces helicus* IFM 42241 (a voucher specimen has been deposited in the Culture Collection of the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University) was cultivated on sterilized rice[8] (200 g/flask × 250) at 25 °C for 4 weeks. The moldy rice was extracted with AcOEt (60 l × 2) with shaking at room temperature for 3 h two times to afford an extract (175 g), which was then partitioned with AcOEt–H_2O (1 : 1, v/v) (0.5 l) into AcOEt and aqueous layers. Evaporation afforded the AcOEt and aqueous fractions, respectively. The AcOEt fraction (140 g) was further partitioned with *n*-hexane–MeOH (3 : 1) (0.2 l) into a *n*-hexane fraction and a MeOH fraction. The MeOH fraction (75 g) was subjected to DIAION HP-20 (Nippon Rensui) column chromatography with MeOH to give fractions 1-A and -B, and with acetone to give fraction 1-C. Fraction 1-B (29 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (1500 : 20 : 1—500 : 20 : 1) to afford a fraction (6 g), which was further chromatographed on a medium-pressure liquid chromatographic (MPLC) silica gel column (Kusano) with *n*-hexane–AcOEt (3 : 1) to furnish **3** (0.7 g), **4** (0.3 g), **2** (0.3 g), and **1** (3.3 g).

Helicusin A (**1**): Yellow amorphous powder, $[\alpha]_D^{24}$ $-519°$ ($c=1.29$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{25}H_{28}ClO_7$ $[(M+H)^+]$: 475.1524. Found: 475.1523. CD ($c=0.046$, MeOH) $\Delta\varepsilon$ (nm): 0 (250), -2.0 (263), -2.0 (285), 0 (304), $+0.8$ (325), 0 (343), -7.3 (388). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 2900, 2850, 1700, 1620, 1600, 1520. UV λ_{max}^{MeOH} nm (log ε): 224 (4.35), 269 (sh, 3.98), 287 (4.09), 298 (sh, 4.05), 364 (4.45), 392 (sh, 4.37), 422 (sh, 4.22), 450 (sh, 4.07), 481 (sh, 3.69).

Helicusin B (**2**): Yellow amorphous powder, $[\alpha]_D^{27}$ $-630°$ ($c=0.87$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{25}H_{28}ClO_7$ $[(M+H)^+]$: 475.1524. Found: 475.1523. CD ($c=0.048$, MeOH) $\Delta\varepsilon$ (nm): 0 (251), -1.6 (264), -1.8 (285), 0 (309), $+0.2$ (302), 0 (327), -7.4 (389). IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 2900, 2850, 1715, 1640, 1600, 1530. UV λ_{max}^{MeOH} nm (log ε): 223 (4.37), 272 (sh, 4.03), 286 (4.10), 297 (sh, 4.06), 365 (4.43), 394 (sh, 4.35), 421 (sh, 4.20), 450 (sh, 4.04), 482 (sh, 3.65).

Helicusin C (**3**): Yellow amorphous powder. $[\alpha]_D^{27}$ $-380°$ ($c=1.04$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{25}H_{28}ClO_7$ $[(M+H)^+]$: 475.1524. Found: 475.1523. CD ($c=0.032$, MeOH) $\Delta\varepsilon$ (nm): 0 (250), -1.8 (265), 0 (302), $+2.0$ (345), 0 (356), -4.7 (410). IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 2900, 2850, 1710, 1640, 1520. UV λ_{max}^{MeOH} nm (log ε): 213 (4.34), 266 (sh, 3.96), 287 (4.08), 298 (sh, 4.04), 365 (4.43), 391 (sh, 4.36), 422 (sh, 4.20), 450 (sh, 4.05), 482 (sh, 3.69).

Helicusin D (**4**): Yellow amorphous powder, $[\alpha]_D^{27}$ $-414°$ ($c=0.65$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{25}H_{28}ClO_7$ $[(M+H)^+]$: 475.1524. Found: 475.1523. CD ($c=0.043$, MeOH) $\Delta\varepsilon$ (nm): 0 (250), -1.0 (263), -1.3 (285), 0 (304), $+0.8$ (326), 0 (348), -4.5 (390). IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 2900, 2850, 1720, 1640, 1600, 1530. UV λ_{max}^{MeOH} nm (log ε): 215 (4.35), 264 (sh, 3.96), 286 (4.07), 298 (sh, 4.04), 366 (4.36), 393 (sh, 4.21), 423 (sh, 4.04), 450 (sh, 4.16), 484 (sh, 3.65).

Methylation of Helicusin A A solution of **1** (50 mg) in Et_2O (1.0 ml) was added to a solution of CH_2N_2 [prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (0.5 mg)] in Et_2O (3.0 ml) and the mixture was allowed to stand at room temperature for 17 h. It was evapolated *in vacuo* to give a crude product (62 mg), which was chromatographed on an MPLC silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (3 : 1) at a flow rate of 5 ml/min to afford **1a** (21 mg), yellow amorphous powder, $[\alpha]_D^{20}$ $-565°$ ($c=0.69$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{26}H_{30}ClO_7$ $[(M+H)^+]$: 489.1680. Found 489.1679. IR ν_{max}^{KBr} cm^{-1}: 2940, 2900, 2850, 1700, 1620, 1600, 1520. UV λ_{max}^{MeOH} nm (log ε): 221 (4.33), 272 (sh, 3.98), 286 (3.97), 297 (sh, 3.94), 371 (4.38), 392 (sh, 4.34), 4.18 (sh, 4.17), 450 (sh, 3.94), 484 (sh, 3.60).

Methylation of Helicusin C A solution of **3** (50 mg) in Et_2O (1.0 ml) was added to a solution of CH_2N_2 [prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (1.0 g)] in EtOH (3 ml) and the mixture was treated in the same way as described for methylation of **1** to give **3a** (21 mg), yellow amorphous powder, $[\alpha]_D^{22}$ $-389°$ ($c=0.55$, $CHCl_3$). HRFAB-MS *m/z* Calcd for $C_{26}H_{30}ClO_7$ $[(M+H)^+]$: 489.1680. Found: 489.1689. IR ν_{max}^{KBr} cm^{-1}: 2960, 2900, 2850, 1720, 1640, 1530. UV λ_{max}^{MeOH} nm (log ε): 215 (4.29), 266 (sh, 3.88), 287 (3.96), 298 (sh, 3.93), 363 (4.29), 399 (sh, 4.14),422 (sh, 4.04), 450 (sh, 3.88), 482 (sh, 3.59).

Alkaline degradation of Helicusin A A solution of **1** (20 mg) in 1% KOH (1 ml) was stirred at room temperature for 90 min. After neutralization with Amberlite MB-3, the reaction mixture was evaporated to give a crude product (10 mg), which was chromatographed on an MPLC silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (3 : 1) at a flow rate of 5 ml/min to afford **7** (2.4 mg), white amorphous powder (lit.[4] mp 91—92 °C), $[\alpha]_D^{20}$ $+63.8°$ ($c=0.30$, EtOH) (lit.[4] $+66.3°$). 1H-NMR: 0.85 (3H, t, $J=7.4$ Hz), 1.00 (3H, d, $J=6.7$ Hz), 1.32 (1H, m), 1.42 (1H, m), 1.80 (3H, d, $J=1.2$ Hz), 2.46 (1H, m), 5.72 (1H, d, $J=9.8$ Hz), 5.79 (1H, d, $J=15.6$ Hz), 7.40 (1H, d, $J=15.6$ Hz). ^{13}C-NMR: 11.9 (q), 12.3 (q), 20.1 (q), 29.9 (t), 35.0 (d), 114.4 (d), 131.6 (s), 150.0 (d), 52.3 (d), 172.1 (s). UV λ_{max}^{EtOH} nm (log ε): 254 (4.38) (lit.[4] 261 (4.44)).

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