Isolation of Three New Azaphilones, Luteusins C, D, and E, from an Ascomycete, *Talaromyces luteus*

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Three new azaphilones, named luteusins C, D, and E, were isolated from an Ascomycete, *Talaromyces luteus*, together with luteusins A and B (previous tentative designations: TL-1 and -2, respectively), which had formerly been isolated as monoamine oxidase (MAO)-inhibitory azaphilones from the fungus. The new compounds had no MAO-inhibitory activity.

Key words fungal metabolite; Ascomycete; *Talaromyces luteus*; monoamine oxidase (MAO)-inhibitory activity; luteusin; azaphilone

During our screening project on monoamine oxidase (MAO)-inhibitory fungal metabolites, two new azaphilones named luteusins A [(8*R*)-7-deacetyl-*O*⁸,8-dihydro-7-*epi*-sclerotiorin] (**1**), having (7*S*,8*R*,13*S*) and (9*E*,11*E*) configuration, and B (**2**), the (11*Z*)-isomer of **1** (previous tentative designations: TL-1 and -2, respectively), were isolated from an Ascomycete, *Talaromyces luteus* (Zukal) C. R. BENJAMIN¹⁾ (see Chart 1). We have been searching for new metabolites related to **1** from *T. luteus* and *T. helicus*.²⁾ Now, we report the isolation of three new azaphilones named luteusins C (**3**), D (**4**), and E (**5**) from *T. luteus*, and their MAO-inhibitory activities.

Results and Discussion

The AcOEt extract of the moldy rice obtained by the cultivation of *T. luteus* on sterilized rice was partitioned with *n*-hexane–MeOH into fatty and defatted layers. The defatted layer was then treated with *n*-hexane–acetone to give *n*-hexane–acetone-soluble and -insoluble fractions. The *n*-hexane–acetone-soluble fraction was subjected to chromatography on a silica gel column to give five fractions, I–V. Fraction III was further chromatographed on a silica gel column to give luteusin E (**5**), and a mixture of luteusins A (**1**) and B (**2**), which was subjected to chromatography as described in our preceding report to give **1** and **2**.¹⁾ Fraction IV was also further chromatographed on a silica gel column and successively on a medium-pressure liquid chromatographic (MPLC) silica gel column to afford luteusins C (**3**) and D (**4**) (luteusin C was obtained as an amorphous powder containing 15% of **4** and luteusin D was also obtained as an amorphous powder containing 35% of **3**).

Luteusin C (**3**), yellow amorphous powder, C₂₅H₂₉ClO₆. The IR spectrum showed the presence of hydroxyl, ester, conjugated ketone, and conjugated C=C in **3**. The UV spectrum was quite similar to that of **1**,¹⁾ suggesting that **3** might be a similar azaphilone to **1**. These data and the ¹³C- and ¹H-NMR spectra of **3** in CDCl₃ showed that **3** was a new chlorine-containing azaphilone related to **1**.¹⁾ Comparison of the ¹³C- and ¹H-NMR spectra of **3** with those of **1** indicated that each signal in the two spectra of **3** was similar to the corresponding signal in those of **1** except that the signals of OH-7 and -8 disappeared, and those of C-7, -8 and -18 were shifted to δ 83.8 (+6.4),

47.3 (–24.9) and 24.2 (+5.2), those of H-8 and -18 were shifted to δ 3.37 (d, *J*=8.9 Hz) (–1.30) and 1.40 (3H, s) (+0.18), respectively, and those of a –CH₃ [δ 20.6 (q), 1.42 (3H, d, *J*=6.3 Hz)], a –CH₂– [44.6 (t), 1.87 (H_a, dd, *J*=13.9, 10.3) and 2.55 (H_b, d, *J*=13.9)], a >CH– [57.2 (d), 3.00 (H, d, *J*=8.9)], a >CH–O [73.6 (d), 4.67 (H, dq, *J*=10.3, 6.3)], a >C(–O)–O [102.5 (s)], and an ester carbonyl [172.6 (s)] newly appeared in the ¹³C- and ¹H-NMR spectra of **3**. These facts suggested that the newly appeared signals in the spectra of **3** were due to a side chain attached to C-7 and -8 in **3**. The ¹³C- and ¹H-NMR spectral data of **3**, including spin-decoupling, two-dimensional ¹H–¹H (¹H–¹H COSY) and ¹³C–¹H shift correlation (¹³C–¹H COSY), and ¹³C–¹H correlation spectroscopy *via* long-range coupling (COLOC) NMR data (see Table 1), suggested that **3** was composed of two partial structures, *a* (a chlorine-containing azaphilone skeleton moiety) and *b*, as shown in Chart 1.

On catalytic hydrogenation with 5% Pd–C, **3** gave a tetrahydro derivative (**6**), colorless amorphous powder, C₂₅H₃₃ClO₆, optically active. The spectral data of **6** indicated that the conjugated (9*E*,11*E*)-diene system in **3** was hydrogenated to give **6** in this reaction. If **3** is composed of the partial structures *a* and *b*, **6** should be composed of the partial structures *a'* and *b*, as shown in Chart 1. In the COLOC NMR experiment, significant cross peaks of C-2' (in *a'*, δ 57.3)/H_b-4' (*b*, 2.52) and C-3' (*b*, 102.5)/H-2' (*a'*, 2.98) were observed in the spectrum of **6**, indicating that *a'* might be linked with *b* through a [C-2'(*a'*)]–[C-3'(*b*)] bond in **6**. The presence of the signals of C-1' (*a'*, 172.6), -3' (*b*, 102.5), and -5' (*b*, 73.6) in the ¹³C-NMR spectrum of **6**, which were assigned to ester carbonyl, hemiacetal and carbinyl carbons, respectively, and the presence of the absorption at 1725 cm^{–1} assigned to an ester C=O in the IR spectrum of **6** suggested that *a'* might also be linked with *b* through [C-7(*a'*)]–O–[C-3'(*b*)] and [C-1'(*a'*)]–O–[C-5'(*b*)] bonds in **6** (see Chart 1). Therefore, the structure of tetrahydroluteusin C could be expressed as **6** (in Chart 2) without its stereochemistry. Thus, the structure of luteusin C was deduced to be **3** without its stereochemistry (see Chart 2). The absolute configurations at positions 7 and 13 in **3** were expected on biosynthetic grounds to be the same as those [(*S*) and (*S*)] in **1**,¹⁾ because **3** was isolated together with **1** from

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Table 1. ^{13}C - and ^1H -NMR Data for Luteusins A (1)—E (5), Tetrahydroluteusin C (6), and Chaetoviridine A (7), δ (ppm) from TMS in CDCl_3 [Coupling Constants (Hz) in Parentheses]

Position	1 ¹⁾		2 ¹⁾		3		4	
	^{13}C -NMR	^1H -NMR	^{13}C -NMR	^1H -NMR	^{13}C -NMR	^1H -NMR	^{13}C -NMR	^1H -NMR
1	144.3 (d)	7.47 (d, 2.0)	144.4 (d)	7.50 (d, 2.1)	146.9 (d)	7.72 (s)	147.1 (d)	7.77 (s)
3	159.2 (s)		159.0 (s)		158.4 (s)		158.3 (s)	
4	104.7 (d)	6.54 (s)	105.4 (d)	6.57 (s)	105.4 (d)	6.57 (s)	106.1 (d)	6.60 (s)
4a	142.7 (s)		142.6 (s)		140.9 (s)		141.0 (s)	
5	107.2 (s)		107.5 (s)		109.8 (s)		110.0 (s)	
6	191.5 (s)		191.6 (s)		189.3 (s)		189.6 (s)	
7	77.4 (s)		77.4 (s)		83.8 (s)		83.8 (s)	
OH-7		4.15 (br s)		4.15 (br s)				
8	72.2 (d)	4.67 (br s)	72.1 (d)	4.67 (br s)	47.3 (d)	3.37 (d, 8.9)	47.3 (d)	3.38 (d, 8.9)
OH-8		3.03 (br s)		3.03 (br s)				
8a	118.9 (s)		118.9 (s)		115.4 (s)		115.5 (s)	
9	116.1 (d)	6.08 (d, 15.8)	119.0 (d)	6.17 (d, 15.8)	116.4 (d)	6.07 (d, 15.7)	118.7 (d)	6.16 (d, 15.6)
10	142.3 (d)	7.08 (d, 15.8)	133.8 (d)	7.46 (d, 15.8)	142.1 (d)	7.05 (d, 15.7)	133.9 (d)	7.46 (d, 15.6)
11	131.9 (s)		129.9 (s)		131.9 (s)		129.9 (s)	
12	147.8 (d)	5.68 (dd, 9.9, 1.0)	145.3 (d)	5.51 (br d, 9.5)	147.8 (d)	5.66 (br d, 9.7)	145.4 (d)	5.51 (br d, 10.1)
13	35.0 (d)	2.48 (m)	34.1 (d)	2.64 (m)	35.0 (d)	2.48 (m)	34.0 (d)	2.64 (m)
14	30.1 (t)	1.32, 1.43 (each m)	30.3 (t)	1.32, 1.43 (each m)	30.1 (t)	1.32, 1.42 (each m)	30.2 (t)	1.32, 1.42 (each m)
15	11.9 (q)	0.86 (3H, t, 7.4)	12.0 (q)	0.86 (3H, t, 7.5)	11.9 (q)	0.86 (3H, t, 7.4)	11.9 (q)	0.86 (3H, t, 7.3)
16	12.3 (q)	1.84 (3H, d, 1.0)	20.1 (q)	1.91 (3H, d, 1.2)	12.3 (q)	1.84 (3H, d, 0.9)	20.0 (q)	1.91 (3H, d, 1.3)
17	20.2 (q)	1.01 (3H, d, 6.6)	21.0 (q)	1.02 (3H, d, 6.7)	20.2 (q)	1.01 (3H, d, 6.7)	20.9 (q)	1.01 (3H, d, 6.6)
18	19.0 (q)	1.22 (3H, s)	19.0 (q)	1.22 (3H, s)	24.2 (q)	1.40 (3H, s)	24.1 (q)	1.40 (3H, s)
1'					172.6 (s)		172.6 (s)	
2'					57.2 (d)	3.00 (d, 8.9)	57.2 (d)	3.00 (d, 8.9)
3'					102.5 (s)		102.5 (s)	
4'					44.6 (t)	1.87 (dd, 13.9, 10.3) 2.55 (d, 13.9)	44.6 (t)	1.84 (dd, 14.2, 11.5) 2.47 (d, 14.2)
5'					73.6 (d)	4.67 (dd, 10.3, 6.3)	73.6 (d)	4.65 (dd, 11.5, 6.4)
6'					20.6 (q)	1.42 (3H, d, 6.3)	20.5 (q)	1.42 (3H, d, 6.4)

Position	5		6		7 ³⁾	
	^{13}C -NMR	^1H -NMR	^{13}C -NMR	^1H -NMR	^{13}C -NMR	^1H -NMR
1	152.2 (d)	8.86 (s)	147.7 (d)	7.72 (s)	151.5 (d)	8.80 (s)
3	158.0 (s)		165.1 (s)		157.1 (s)	
4	105.7 (d)	6.62 (s)	104.3 (d)	6.51 (s)	105.3 (d)	6.56 (s)
4a	139.6 (s)		140.9 (s)		139.7 (s)	
5	108.6 (s)		109.3 (s)		108.9 (s)	
6	182.9 (s)		189.5 (s)		183.4 (s)	
7	87.8 (s)		83.9 (s)		87.5 (s)	
8	164.8 (s)		47.2 (d)	3.35 (d, 8.8)	162.6 (s)	
8a	110.2 (s)		115.9 (s)		110.4 (s)	
9	115.5 (d)	6.10 (d, 15.6)	44.2 (t)	2.52 (m)	119.7 (d)	6.10 (d, 15.7)
10	143.8 (d)	7.15 (d, 15.6)			148.0 (d)	6.62 (dd, 15.7, 8.3)
11	132.0 (s)				38.9 (d)	2.30 (m)
12	149.5 (d)	5.75 (br d, 10.1)	^{a)}	0.95–1.72 (8H, m)	30.1 (t)	1.45 (2H, m)
13	35.2 (d)	2.50 (m)			11.6 (q)	0.92 (t, 7.4)
14	30.0 (t)	1.33, 1.44 (each m)			19.2 (q)	1.10 (d, 6.6)
15	11.9 (q)	0.87 (3H, t, 7.5)	11.4 (q)		26.2 (q)	1.70 (s)
16	12.3 (q)	1.86 (3H, s)	^{a)}	0.83–0.93 (9H, m)		
17	20.2 (q)	1.03 (3H, d, 6.6)				
18	26.4 (q)	1.74 (3H, s)	24.2 (q)	1.38 (s)		
1'	167.9 (s)		172.6 (s)		167.9 (s)	
2'	123.2 (s)		57.3 (d)	2.98 (d, 8.8)	125.1 (s)	
3'	196.7 (s)		102.5 (s)		201.1 (s)	
4'	50.2 (t)	3.01 (dd, 17.6, 2.7) 3.28 (dd, 17.6, 9.3)	44.7 (t)	1.86 (dd, 14.4, 11.5) 2.52 (d, 14.4)	51.0 (d)	3.64 (m)
CH ₃ -4'					21.4 (q)	1.17 (3H, d, 6.6)
5'	64.6 (d)	4.25 (m)	73.6 (d)	4.65 (dq, 11.5, 6.2)	70.8 (d)	3.86 (m)
OH-5'		2.74 (br s)				
6'	22.9 (q)	1.26 (3H, d, 6.4)	20.6 (q)	1.42 (3H, d, 6.2)	13.4 (q)	1.17 (3H, d, 6.6)

^{a)} Position 10, 12, 14: 29.2 (t) or 31.5 (t) or 34.8 (t); position 11, 13: 29.9 (d) or 31.7 (d); position 16, 17: 19.0 (q) or 20.6 (q).

Chart 1

Chart 2

the same fungus. Significant nuclear Overhauser effects (NOE) were observed at $H_3-18/H-8$, $H-8/H-2'$, and $H-2'/H-5'$ in the differential NOE experiment on **6** (see Chart 2), indicating that the absolute configurations at positions 8, 2', and 5' in **3** were (*S*), (*S*), and (*R*), respectively. Accordingly, luteusin C was deduced to be an angular-type azaphilone³⁾ **3**, as shown in Chart 2. As regards similar angular-type fungal azaphilones, chaetoviridins A (**7**)—D from *Chaetomium globosum* var. *flavo-viridae*,³⁾ ochrephilone from *Penicillium multicolor*,^{4,5)} and monochaetin (**8**) from *Monochaetia compta*⁵⁾ (see Chart 2) have so far been isolated.

Luteusin D (**4**), yellow amorphous powder. All signals in the 1H -NMR spectrum of **4** were identical with those of **3**, except for the five signals of H-9 at δ 6.16 (d, $J=$

15.6 Hz), H-10 at 7.46 (d, $J=15.6$), H-12 at 5.51 (br d, $J=10.1$), H-13 at 2.64 (m), and H_3-16 at 1.91 (d, $J=1.3$). Comparison of the ^{13}C -NMR spectrum of **4** with that of **3** indicated that the signals of only two carbons at positions 10 and 16 were significantly shifted to δ 133.9 (-8.2) and 20.0 ($+7.7$). This fact suggested that the γ -effect⁶⁾ which was present between C-13 and -16 in **3** was absent in **4** and, instead, the γ -effect appeared between C-10 and -13 in **4**. Therefore, **4** was estimated not to have a (9*E*,11*E*)-diene system like that of **1**¹⁾ and **3**, but to have a (9*E*,11*Z*)-diene system like that of **2**¹⁾ in the side chain attached to C-3. Accordingly, the structure of luteusin D was deduced to be **4**, as shown in Chart 2.

Luteusin E (**5**), reddish amorphous powder, $C_{25}H_{27}ClO_6$, optically active. The IR spectrum showed the presence of

hydroxyl, ester, conjugated ketone, and conjugated C=C in **5**. The UV spectrum suggested that the conjugated system in **5** might be longer than that in **1**. The ^{13}C - and ^1H -NMR spectra suggested that **5** was also a new azaphilone related to **1**.¹⁾ Comparison of the ^{13}C - and ^1H -NMR spectra of **5** with those of **1** indicated that each signal in the two spectra of **5** was similar to the corresponding signal in those of **1** except that the signals of H-8, OH-7 and OH-8 disappeared, that of C-7 was shifted to δ 87.8 (+10.4), that of C-8, which was changed to singlet, was shifted to 164.8 (+92.6), and those of a $-\text{CH}_3$ [δ 22.9 (q), 1.26 (3H, d, $J=6.4$ Hz)], a $-\text{CH}_2-$ [50.2 (t), 3.01 (dd, $J=17.6, 2.7$) and 3.28 (dd, $J=17.6, 9.3$)], a $>\text{CH}-\text{O}$ [64.6 (d), 4.25 (m)], a $>\text{C}=\text{C}$ [123.2 (s)], an ester carbonyl [167.9 (s)], a ketone carbonyl [196.7 (s)] newly appeared in the ^{13}C - and ^1H -NMR spectra of **5**. These facts suggested that the newly appeared signals in the ^{13}C - and ^1H -NMR spectra of **5** were due to a side chain attached to C-7 and -8 in **5**. The ^{13}C - and ^1H -NMR spectral data of **5**, including spin-decoupling, $^1\text{H}-^1\text{H}$ COSY and $^{13}\text{C}-^1\text{H}$ COSY, and ^1H -detected multiple-bond heteronuclear multiple quantum coherence (HMBC) NMR spectra, indicated that **5** was composed of two partial structures, *c* (a chlorine-containing azaphilone skeleton moiety) and a side chain *d*: $-\text{O}-\text{CO}-\text{C}(=\text{C})-\text{CO}-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_3$, which is similar to the side chain attached to C-7 and -8 in chaetoviridin A (**7**): $\{(\text{C}-7)-\text{O}-\text{CO}-\text{C}(=\text{C}-8)-\text{CO}-\text{CH}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_3\}$ ³⁾ (see Table 1 and Chart 2). Comparison of the ^{13}C - and ^1H -NMR spectra of **5** with those of **7** suggested that the side chain *d* might also be attached to C-7 and -8 in *c* to construct **5** in the following mode: $(\text{C}-7)-\text{O}-\text{CO}-\text{C}(=\text{C}-8)-\text{CO}-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_3$ (see Chart 2). Accordingly, the structure of luteusin E was deduced to be **5**, as shown in Chart 2. All of the spectral data of luteusin E including its IR, UV, ^1H - and ^{13}C -NMR data were compatible with the structure **5**. The absolute configurations at positions 7 and 13 in **5** might be the same [(*S*) and (*S*)] as those in **1**,¹⁾ because **5** was isolated together with **1** from the same fungus. The CD spectrum of **5**, with $\Delta\epsilon -2.8$ at 373 nm, confirmed the (*7S*) configuration in **5**.³⁾

As regards MAO-inhibitory activity, it was previously found that the IC_{50} values of **1** and **2** were 6.6×10^{-6} and 1.1×10^{-5} M, respectively.¹⁾ The MAO-inhibitory potency of **1** decreased in the 8-acetyl (**9**) and 7,8-diacetyl derivatives (**10**), and was lost in the 7-acetyl-8-oxo derivative (7-*epi*-sclerotiorin) (**11**)⁷⁾ (see Chart 1), indicating that the presence of OH-8 in **1** is important for the appearance of MAO-inhibitory potency of **1**. On bioassay with the modified Kraml's method,⁷⁾ **3**, **4**, and **5** exhibited no inhibitory activity against mouse liver MAO even at 1.0×10^{-5} M. This result confirmed the previous findings.

Experimental

The IR spectra were recorded with a Hitachi EPI-G3, the UV spectra with a Hitachi U-3400, the high-resolution EI-MS (HREI-MS) and 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 ^{13}C - and ^1H -NMR spectra with JEOL JNM-A500, -GSX500, and -GSX400 instruments at 125.65, 125.65, and 100.40 MHz for ^{13}C -NMR, and 500, 500, and 400 MHz for ^1H -NMR, respectively.

Isolation of Luteusins C—E (3—5) *T. luteus* IFM 42239¹⁾ was cultivated on sterilized rice⁸⁾ (200 g/flask $\times$ 100) at 25 °C for 4 weeks. The moldy rice was extracted with AcOEt (36 l $\times$ 2) to give an extract (36 g), which was then partitioned with *n*-hexane–MeOH (3:1, v/v) (0.6 l) into fatty (*n*-hexane) and defatted (MeOH) layers. The defatted layer was treated with *n*-hexane–acetone (1:1) to give *n*-hexane–acetone (1:1)-soluble and -insoluble fractions. The *n*-hexane–acetone (1:1)-soluble fraction was subjected to chromatography on a silica gel column to give five fractions I—V. Fraction III, eluted with *n*-hexane–acetone (3:1) (870 mg), was further chromatographed repeatedly on silica gel columns with *n*-hexane–AcOEt and CHCl_3 to afford **5** (158 mg) and a mixture of **1** and **2** (**1**:**2** = ca. 10:1) (107 mg). Fraction IV, eluted with *n*-hexane–acetone (2:1) (600 mg), was also further chromatographed on a silica gel column with *n*-hexane–AcOEt and successively on an MPLC silica gel column (Kusano) with *n*-hexane–AcOEt (1:1) at a flow rate of 7.0 ml/min to afford **4** (35 mg) and **3** (83 mg).

Luteusin C (3): Yellow amorphous powder, HREI-MS *m/z* Calcd for $\text{C}_{25}\text{H}_{29}\text{ClO}_6$ (M^+): 460.1640. Found: 460.1654. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1730, 1620, 1550. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 251, 339 (sh), 356, 390, 408, 433 (sh), 463 (sh). **Luteusin D (4)**: Yellow amorphous powder. **Luteusin E (5)**: reddish amorphous powder, $[\alpha]_D^{27} -287^\circ$ ($c=1.00$, MeOH), HRFAB-MS *m/z* Calcd for $\text{C}_{25}\text{H}_{28}\text{ClO}_6$ [$(\text{M}+\text{H})^+$]: 459.1574. Found: 459.1562. CD ($c=0.038$, MeOH) $\Delta\epsilon$ (nm): -0.9 (212), 0 (230), $+1.5$ (246), $+0.2$ (280), $+1.5$ (315), 0 (347), -2.8 (373), 0 (429). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 1770, 1690, 1640, 1515. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 245 (4.20), 254 (sh, 4.16), 279 (4.04), 289 (sh, 4.09), 319 (sh, 4.09), 372 (sh, 4.49), 436 (sh, 4.21).

Formation of Tetrahydroluteusin C (6) A solution of **3** (43 mg) in EtOH (1 ml) was added to a suspension of activated 5% Pd–C (60 mg) in EtOH (2 ml). The mixture was shaken under hydrogen gas for 30 min, and then treated as usual to give a crude product (71 mg), which was purified on an MPLC silica gel column with *n*-hexane–AcOEt (3:1) to afford **6**, colorless amorphous powder, $[\alpha]_D^{27} -9.0^\circ$ ($c=0.82$, MeOH), HRFAB-MS *m/z* Calcd for $\text{C}_{25}\text{H}_{34}\text{ClO}_6$ [$(\text{M}+\text{H})^+$]: 465.2044. Found: 465.2034. CD ($c=0.033$, MeOH) $\Delta\epsilon$ (nm): -1.9 (222), 0 (240), $+1.5$ (251), 0 (298), -2.2 (326), 0 (345), $+1.9$ (365). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1725, 1640, 1540. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 238, 252, 367, 384.

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