STRUCTURES OF DALDININS A~C, THREE NOVEL AZAPHILONE DERIVATIVES FROM ASCOMYCETOUS FUNGUS *DALDINIA CONCENTRICA*

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Three novel azaphilone derivatives, daldinins A~C, have been isolated from the fungus (Ascomycetes) *Daldinia concentrica*. Their structures including the absolute configuration have been established by a combination of high resolution NMR and CD spectra, X-ray crystallographic analysis, and chemical degradation.

KEYWORDS daldinin A; daldinin B; daldinin C; Ascomyceteous fungus; *Daldinia concentrica* ; azaphilone derivative

In our previous paper, we reported the isolation and structure elucidation of a novel binaphthyl and three novel benzophenone derivatives having plant-growth inhibitory activity from the fungus *Daldinia concentrica* belonging to the Xylariaceae.¹⁾ In the course of our reinvestigation of the biologically active substances such as anti-microbial and plant-growth inhibitory activities from the same fungus, we isolated three novel azaphilone derivatives named daldinins A-C (**1-3**). Here we wish to report the structure elucidation of **1-3**.

The AcOEt extract (26.7g) of the dry material (424 g) of *D. concentrica* collected in Tokushima in 1993 was subjected repeatedly to column chromatography on Sephadex LH-20 (CHCl₃: MeOH = 1:1) and on silica gel (CHCl₃-AcOEt gradient) to afford daldinins A (**1**)²⁾ (1.366 g), B (**2**)³⁾ (37 mg), and C (**3**)⁴⁾ (1.662 g).

The IR and UV spectra of daldinin A (**1**) indicated the presence of an ester [1718 cm⁻¹] and an α , β , γ , δ -conjugated carbonyl [1657 cm⁻¹; $\lambda_{\max}$ 330 nm] group. The ¹H and ¹³C NMR spectra of **1** showed the presence of two tertiary methyl groups [δ_{H} 1.52(s, 7-Me), 2.17 (s, 3-Me)], three olefinic protons [δ_{H} 5.50 (s, 5-H), 6.12 (s, 4-H), 7.86 (s, 1-H)], linear long chain fatty acid ester [δ_{H} 0.88 (t, J=7.3 Hz), 1.25 (br s, -(CH₂)_n-); δ_{C} 172.9] and two carbonyl carbons (δ_{C} 192.7, 193.3), which were similar to those of a known azaphilone (**4**)⁵⁾ [δ_{H} 1.49 (s, 7-Me), 2.17 (s, 3-Me), 6.17 (s, 4-H), 7.76 (s, 1-H)]. **1** was hydrolyzed with lipase (porcine pancreas) at 37° C, followed by methylation with CH₂N₂ to afford azaphilone (**5**) and methyl esters, the latter of which consisted of **6** (37%), **7** (8%), **8** (29%) and **9** (25%) judging from GC-MS analysis. **1** was a mixtures of fatty acid esters, which were very difficult to separate by any separation procedure. The planar structure of **1** was derived from a careful analysis of the 2D NMR spectra including HMBC (Fig. 1) of **1** and **5**. The absolute configuration of C-7 of **1** was confirmed by comparison of the CD spectrum of **1** with that of **4**, whose absolute configuration of C-7 was determined as *S*. The CD spectrum of **1** showed negative first (350 nm) and positive second Cotton effects (271 nm), while that of **4** showed positive first (357 nm) and negative second Cotton effects (270 nm), indicating that the C-7 of **1**

was represented as *R*.

The spectral data (^1H and ^{13}C NMR, and IR) of daldinin B (**2**) were similar to those of daldinin A (**1**), except for the presence of signals of one secondary methyl [δ 1.51 (d, $J=6.4$ Hz)], one methylene [δ 2.75 (m)] and one methine [δ 4.38 (m)] bearing an oxygen function. Compound **2** was heated with DDQ in benzene to afford **1**. Thus, the structure of daldinin B (**2**) was determined as 3,4-dihydro derivative of daldinin A (**1**) from the above results and careful analysis of the HMBC spectrum (Fig. 1) except for the absolute configuration of C-3.

Fig. 1

The IR and UV spectra of daldinin C (**3**, $\text{C}_{22}\text{H}_{26}\text{O}_9$) indicated the presence of a hydroxyl group (3522 cm^{-1}), an α, β -conjugated ester (1715 cm^{-1} ; λ_{max} 216 nm) and an $\alpha, \beta, \gamma, \delta$ -conjugated ketone (1678 cm^{-1} ; λ_{max} 303 nm). The ^1H and ^{13}C NMR of **3** indicated a secondary methyl [δ_{H} 1.13 (d, $J=7.3$ Hz)], two olefinic methyls [δ_{H} 1.53 (s), 1.78 (d, $J=5.3$ Hz)], an acetoxy [δ_{H} 2.17 (s)], two methines [δ_{H} 3.31 (br. s), 3.84 (m)] bearing oxygen functions and an acetal (δ_{C} 102.5) groups. Acetylation (Ac_2O , Py) of **3** afforded a monoacetate **10**. Hydrolysis (KOH/MeOH , rt., 24hr) of **3** gave a diol **11** and tiglic acid. The structure of **3** was deduced from careful analysis of the 2D NMR spectra including DQF-COSY, HMQC, HMBC (Fig. 2) and NOESY, and finally established by X-ray crystallography⁶) as shown in Fig. 3. The absolute configuration of **3** was deduced by comparison of the CD spectrum of daldinin B (**2**) with that of the diol **11**, and was further confirmed by the CD spectrum of dibenzoate (**12**) derived from **11** with *p*-Br-BzCl/Py/DMAP. The CD spectrum of **2** showed positive first (351 nm) and negative second Cotton effects (304 nm)

Fig. 2 The HMBC Correlation of 3

Fig. 3 ORTEP Drawing of 3

nm), while that of **11** showed negative first (350 nm) and positive second Cotton effects (302 nm). The CD spectrum of **12** exhibited negative (254 nm) and positive Cotton effects (237 nm). Thus, the whole structure of daldinin C including the absolute configuration was established as depicted in formula **3**. Daldinin C (**3**) is the first example from nature of an azaphilone derivative with spiro-acetal structure between the B and C rings. It is very interesting that two enantiomers at C-7 (daldinins A, B: *R* configuration; daldinin C: *S* configuration) were isolated from the same fungus. Bioassays of new compounds are in progress.

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REFERENCES AND NOTES

- 1) T. Hashimoto, S. Tahara, S. Takaoka, M. Tori, Y. Asakawa, *Chem. Pharm. Bull.*, **42**, 1528 (1994).
- 2) $[\alpha]_D^{20}$ -126.5° (c 0.34, CHCl₃); FT-IR (KBr) cm⁻¹: 1718 (O-C=O), 1657 (C=O), 1224; UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 219 (4.18), 330 (3.72); CD (EtOH) $\lambda_{\max}$ nm ($\Delta\epsilon$): 350 (-3.38), 271 (+1.63), 249 (-0.37); ¹H NMR (CDCl₃): δ 1.52 (s, 7-Me), 2.17 (s, 3-Me), 5.50 (s, 5-H), 6.12 (s, 4-H), 7.86 (s, 1-H); ¹³C NMR (CDCl₃): δ 172.9(-OCO-), 192.7, 193.3 (C=O).
- 3) $[\alpha]_D^{20}$ -108.3° (c 0.12, CHCl₃); FT-IR (KBr) cm⁻¹: 1720 (-OCO-), 1703, 1657 (C=O), 1232; UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 202 (3.86), 222 (3.43), 308 (3.80); CD (EtOH) $\lambda_{\max}$ nm ($\Delta\epsilon$): 351 (+2.29), 304 (-13.72); ¹H NMR (CDCl₃): δ 1.51 (d, J=6.4 Hz, 3-Me), 1.54 (s, 7-Me), 5.77 (s, 5-H), 7.89 (s, 1-H).
- 4) mp 171-172°, $[\alpha]_D^{20}$ +437.5° (c 0.48, CHCl₃); HR-MS: *m/z* 434.1582, C₂₂H₂₆O₉ requires 434.1577; EI-MS: *m/z* 434 (M⁺), 392, 292, 83 (100), FT-IR (KBr) cm⁻¹: 3522 (OH), 1715 (-OCO-), 1678 (CO); UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 216 (4.13), 303 (4.06); CD (EtOH) $\lambda_{\max}$ nm ($\Delta\epsilon$): 350 (-1.55), 304 (-13.92), 234 (-12.37); ¹H NMR (CDCl₃): δ 1.13 (d, J=6.2 Hz, 12-Me), 1.53 (s, 7-Me), 1.78 (d, J=5.3 Hz, 3'-Me), 1.82 (s, 2'-Me).
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- 6) The crystal data for **3** are as follows: Orthorhombic; space group P 2₁2₁2₁ with a=16.058 (8), b=23.64 (1), c=5.968 (3) Å, V=2265 (2) Å³, Z=4, and Cu K- α (λ =1.54178) by Mac Science MXC 18 instrument. Final R value was 0.078 for 2090 reflections.

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