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

Toshihiro HASHIMOTO, Shigenobu TAHARA, Shigeru TAKAOKA, Motoo TORI, and Yoshinori ASAKAWA*
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770, Japan

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 educidation 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 ($CHCl_3$: MeOH = 1:1) and on silica gel ($CHCl_3$-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 $\alpha, \beta, \gamma, \delta$-conjugated carbonyl [1657 cm^{-1} ; λ_{max} 330 nm] group. The ^1H and ^{13}C 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, -$(CH_2)_n$-) ; 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 CH_2N_2 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 acidesters, 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 (^{1}H 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. Compond **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.

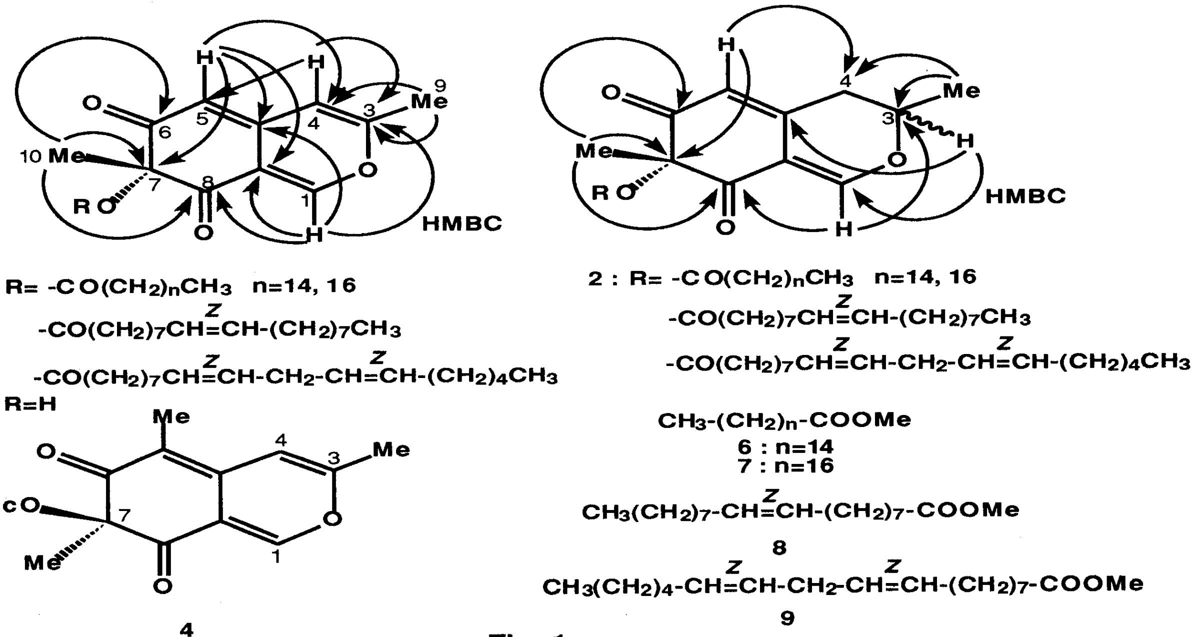
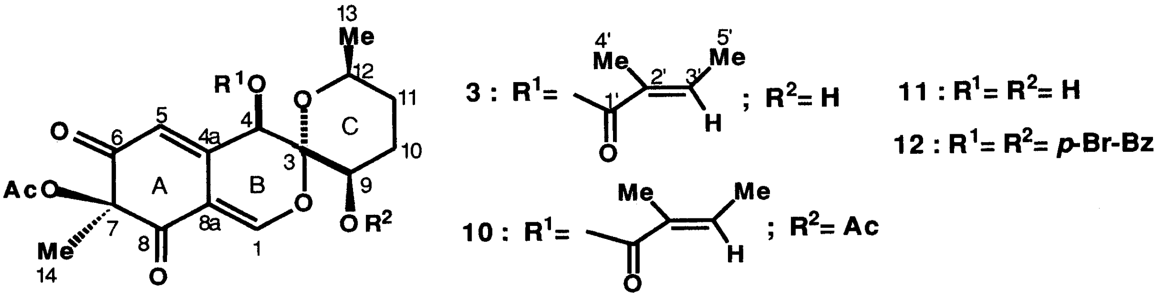


Fig. 1

The IR and UV spectra of daldinin C (**3**, $C_{22}H_{26}O_9$) indicated the presence of a hydroxyl group (3522 cm^{-1}), an α, β-conjugated ester (1715 cm^{-1} ; λ_{max} 216 nm) and an α, β, γ, δ-conjugated ketone (1678 cm^{-1} ; λ_{max} 303 nm). The ^{1}H 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)] beaing 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 crystallography[6] 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)



11 : R^1= R^2= H

12 : R^1= R^2= *p*-Br-Bz

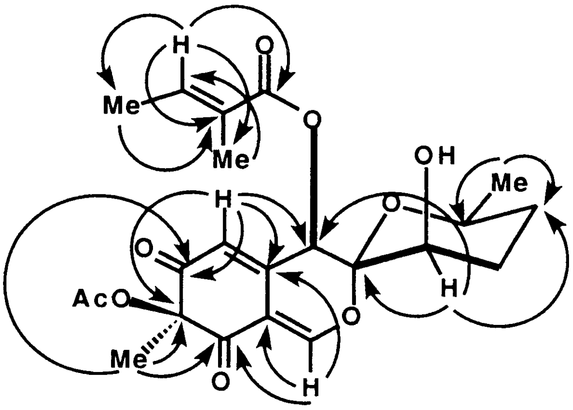


Fig. 2 The HMBC Correlation of 3

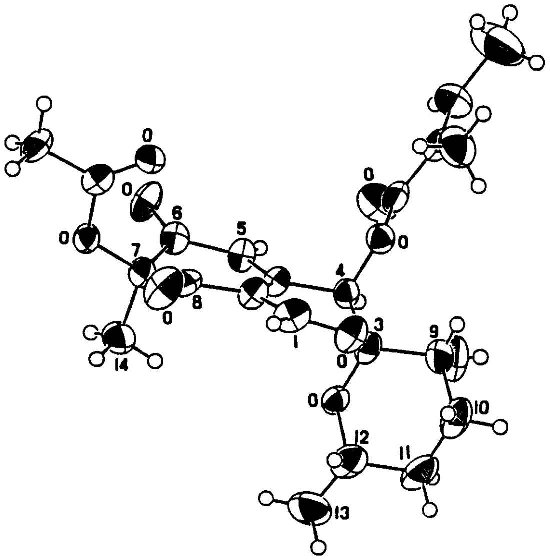


Fig. 3 ORTEP Drawing of 3

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, $CHCl_3$) ; FT-IR (KBr) cm^{-1} : 1718 (O-C=O), 1657 (C=O), 1224; UV (EtOH) λ_{max} nm (logε) : 219 (4.18), 330 (3.72) ; CD (EtOH) λ_{max} nm (Δε) : 350 (-3.38), 271 (+1.63), 249 (-0.37) ; ^1H NMR ($CDCl_3$) : 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); ^{13}C NMR ($CDCl_3$) : δ 172.9(-OCO-), 192.7, 193.3 (C=O).

3) $[\alpha]_D^{20}$-108.3° (c 0.12, $CHCl_3$); FT-IR (KBr) cm^{-1}: 1720 (-OCO-), 1703, 1657 (C=O), 1232; UV (EtOH) λ_{max} nm (logε): 202 (3.86), 222 (3.43), 308 (3.80) ; CD (EtOH) λ_{max}nm (Δε) : 351 (+2.29), 304 (-13.72); ^1H NMR ($CDCl_3$) : 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, $CHCl_3$); HR-MS: *m/z* 434.1582, $C_{22}H_{26}O_9$ 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) λ_{max} nm (logε) : 216 (4.13), 303 (4.06) ; CD (EtOH) λ_{max}nm (Δε) : 350 (-1.55), 304 (-13.92), 234 (-12.37); ^1H NMR ($CDCl_3$) : 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).

5) P. S. Steyn, R. Vleggaar, *J. Chem. Soc., Perkin Trans.* I, **1976**, 204.

6) The crystal data for **3** are as follows : Orthorhombic ; space group P $2_12_12_1$ with a=16.058 (8), b=23.64 (1), c=5.968 (3) Å, V=2265 (2)$Å^3$, 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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