The Journal of Antibiotics (2012) 65, 95–97

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NOTE

Daldinan A, a novel isoindolinone antioxidant from the ascomycete *Daldinia concentrica*

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The Journal of Antibiotics (2012) **65,** 95–97; doi:10.1038/ja.2011.109; published online 23 November 2011

Keywords: antioxidant; daldinan A; *Daldinia concentrica*; mushroom

Free radicals have been known to be involved in the pathogenesis of various diseases such as ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation and in the initiation of cancer.[1–3] Free-radical scavengers are considered to be protective agents against these diseases and thus, the demand for antioxidants having free-radical scavenging effect is gradually growing.

Mushrooms are ubiquitous in nature and are a good source of food with high nutritional attributes, as well as they produce various classes of structurally unique secondary metabolites with valuable biological activities. During the screening for natural antioxidants from the Korean native ascomycetes,[4] we found that the fruiting body of ascomycete *Daldinia concentrica*, belonging to family Xylariaceae, exhibited significant free-radical scavenging activity. *D. concentrica* is known to produce diverse bioactive entities including azaphilone derivatives daldinins,[5] diaporthin,[6] an anti-HIV agent concentricolide,[7] squalene-type triterpenoids concentriols,[8] aromatic steroids,[9] daldinones[10] and neuroprotective lignans.[11] In this study, we isolated and characterized a novel isoindolinone derivative (daldinan A, Figure 1) responsible for free-radical scavenging effect from the methanolic extract of the fruiting body of *D. concentrica*.

The fruiting body of *D. concentrica* (~320 g) collected near Muju county, Jeonbuk province, Korea, was ground and extracted with methanol at room temperature. This methanolic extract was concentrated under reduced pressure, and the aqueous resultant was partitioned consecutively between hexane, chloroform, ethyl acetate and butanol and water. The ethyl acetate-soluble portion, exhibiting potent radical-scavenging activity, was concentrated under reduced pressure, subjected to silica gel column chromatography, and eluted stepwise with chloroform:methanol (100:1 → 1:1, v/v). An antioxidant fraction was further separated by the second silica gel column chromatography eluted with chloroform:methanol (20:1, v/v). Active fractions were combined, concentrated *in vacuo* and chromatographed on a column of Sephadex LH-20 (Pharmacia, Uppsala, Sweden) eluted with methanol. Active fractions were combined and subjected to a reversed phase (C_{18}) Sep-pak cartridge eluted with 70% aqueous methanol, followed by preparative HPLC equipped with reversed phase column (150×10 mm i.d.; Cosmosil, Nacalai tesque, Japan) and eluted with 35% aqueous methanol/0.04% trifluoroacetic acid to afford daldinan A (20 mg).

The molecular weight of daldinan A was determined to be 415 by EI-mass measurement and its molecular formula was established to be $C_{22}H_{25}NO_7$ by high-resolution EI-mass measurement (*m/z* 415.1634 [M^+], Δ+3.0 m.m.u.) in combination with ^1H and ^{13}C NMR data. This molecular formula requires 11 degrees of unsaturation. The UV absorption maxima at 293 and 258 nm suggested the presence of aromatic functions in its structure. The ^1H and ^{13}C NMR peaks of daldinan A measured at room temperature was very broad and thus, two-dimensional NMR spectra did not provide enough and critical correlations to determine its chemical structure. At low temperatures such as 273, 253 and 233 K; however, the ^1H and ^{13}C NMR peaks were sharpen and well resolved. Therefore, all NMR spectra were obtained at 243 K. The ^1H NMR spectrum of daldinan A showed signals due to

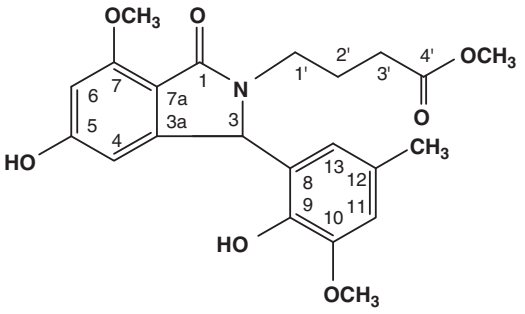

Figure 1 Chemical structure of daldinan A.

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Received 2 October 2011; revised 27 October 2011; accepted 28 October 2011; published online 23 November 2011

Table 1 ^{1}H and ^{13}C NMR spectral data for daldinan A in $CDCl_3$[a]

No.	δ_C	δ_H
1	169.0	
3	56.1	5.95 (1H, s)[b]
3a	151.1	
4	102.3	6.40 (1H, s)
5	162.7	
6	98.5	6.35 (1H, s)
7	158.1	
7-OCH_3	55.6	3.81 (3H, s)
7a	110.1	
8	122.4	
9	142.0	
10	146.7	
10-OCH_3	56.0	3.87 (3H, s)
11	111.0	6.58 (1H, s)
12	129.8	
12-CH_3	21.4	2.12 (3H, s)
13	118.2	6.05 (1H, s)
1′	39.0	3.92 (1H, m)
		2.81 (1H, m)
2′	23.5	1.87 (2H, m)
3′	31.4	2.34 (2H, m)
4′	174.6	
4′-OCH_3	52.1	3.59 (3H, s)

[a]NMR (JNM-ECA600 600 MHz FT-NMR spectrometer, JEOL, Tokyo, Japan) spectra were recorded at 600 MHz for protons and at 150 MHz for carbons at 243 K.
[b]Proton resonance integral and multiplicity are in parentheses.

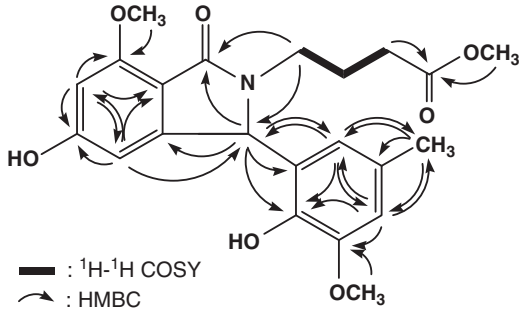

Figure 2 Two-dimensional NMR correlations for daldinan A.

four aromatic singlet methines at δ 6.58, 6.40, 6.35 and 6.05, a methine at δ 5.95, three methoxy methyls at δ 3.87, 3.81 and 3.59, three methylenes at δ 3.92/2.81, 2.34 and 1.87 and a methyl at δ 2.12. In the ^{13}C NMR spectrum, two carbonyl carbons at δ 174.6 and 169.0, four oxygenated sp^2 carbons at δ 162.7, 158.1, 146.7 and 142.0, four sp^2 methine carbons at δ 118.2, 111.0, 102.3 and 98.5, four sp^2 quaternary carbons at δ 151.1 129.8, 122.4 and 110.1, three methoxy methyl carbons at δ 56.0, 55.6 and 52.1, a methine carbon at δ 56.1, three methylene carbons at δ 39.0, 31.4 and 23.5 and a methyl carbon at δ 21.4 were evident. The proton-bearing carbons were established by HMQC spectrum, as shown in Table 1. In an ^{1}H-^{1}H COSY spectrum, the methylene protons at δ 3.92/2.81 (H-1′) showed a cross-peak with the methylene protons at δ 1.87 (H-2′), which was in turn correlated with the methylene protons at δ 2.34 (H-3′) (Figure 2). The structure of daldinan A was determined by the HMBC spectrum, as shown in Figure 2. Long-range correlations from the methine proton at δ 6.40 (H-4) to a methine carbon C-3, aromatic oxygenated carbon C-5 and aromatic carbons C-6 and C-7a, from the methine proton at δ 6.35 (H-6) to aromatic carbons C-4, C-5, C-7 and C-7a, and from the methine proton at δ 5.95 (H-3) to a carbonyl carbon C-1 and an aromatic quaternary carbon C-3a were observed in the HMBC spectrum. In addition, the molecular formula and the chemical shift (δ 56.1) of C-3 suggested the presence of an isoindolinone moiety. The methylene carbon of C-1′ at δ 39.0 was attached to nitrogen atom of isoindolinone moiety by the HMBC correlations of the methylene protons at δ 3.92/2.81 (H-1′) to C-1 and C-3. Two aromatic methine protons at δ 6.58 (H-11) and 6.05 (H-13) exhibited the long-range correlations to C-9, C-10 and C-13, and C-9 and C-11, respectively, and the long-range correlations from the methyl protons at δ 2.12 (12-CH_3) to C-11, C-12 and C-13 and from the methoxy protons at δ 3.87 (10-OCH_3) to C-10 revealed the presence of 2-hydroxy-3-methoxy-5-methylbenzene moiety, which was in turn connected to C-3 of isoindolinone moiety by the HMBC correlations from the methine proton at δ 5.95 (H-3) to C-8, C-9 and C-13. Finally, the long-range correlations from the methoxy protons at δ 3.81 (7-OCH_3) to C-7 and from the methylene protons at δ 2.34 (H-3′) and the methoxy protons at 3.59 (4′-OCH_3) to the carbonyl carbon at δ 174.6 (C-4′) were evident. Therefore, the chemical structure of daldinan A was unambiguously assigned as a new antioxidant of isoindolinone class, as shown in Figure 2. The stereochemistry of C-3 remains to be unknown.

The antioxidant effect of daldinan A was evaluated by free radical-scavenging activity and reducing power assays. Free radical-scavenging activities against the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation and the 1,1-diphenyl-2-picrylhydrazyl radical were measured using methods described in the literature.[12] Daldinan A showed no 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, but exhibited potent 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical-scavenging activity with an IC_{50} value of $\sim$10.4 μM, comparable to those of butylated hydroxyanisole (IC_{50} 10.8 μM) and trolox (IC_{50} 11.5 μM). Reducing power was evaluated using the potassium ferricyanide reduction method with minor modification.[13] In brief, sample (10 μl) was mixed with 25 μl of 200 mM potassium phosphate buffer (pH 6.6) and 25 μl of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After addition of 25 μl of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 r.p.m. for 10 min. The upper layer (50 μl) was mixed with 50 μl distilled water and 10 μl of 0.1% ferric chloride, and absorbance was measured at 700 nm. When reducing power was expressed as activity relative to trolox, daldinan A was about three times less active than trolox.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Korea Forest Service and, in part, by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0005844), Republic of Korea.

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