**New benzene derivatives from cultures of ascomycete**

*Daldinia concentrica*

Tao FENG, a Zheng-Hui Li, a Xia YIN, a,b Ze-Jun DONG, a Gang-Qiang WANG, c Xing-Yao LI, a Yan Li, a and Ji-Kai LIU a,*

a State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

b University of Chinese Academy of Sciences, Beijing 100049, China

c School of Chemistry and Chemical Engineering of Hunan University, Changsha 410082, China

Received 4 July 2013; Accepted 1 August 2013

© The Author(s) 2013. This article is published with open access at Springerlink.com

**Abstract:** Three new benzene derivatives, named daldins A–C (1–3), together with a known analogue, 2-hydroxymethyl-3-(1-hydroxypropyl) phenol (4) have been isolated from cultures of ascomycete *Daldinia concentrica*. The structures of 1–4 with absolute configuration were established by means of spectroscopic methods and X-ray diffraction. All compounds showed no significant inhibition on five human cancer cell lines with IC₅₀ values > 40 μmol.

**Keywords:** *Daldinia concentrica*, benzene derivatives, absolute configuration

**Introduction**

Ascomycete fungus *Daldinia concentrica* can be considered as a talent strain, while a large number of natural products with diverse structures have been reported from both the fruiting bodies and fermentation broth. The initial chemical investigation of the fruiting bodies of *D. concentrica* reported two new 4:5:4′:5′-tetrahydroxy-1:1′-binaphthyl and dihydroxyperylenedione in 1958, more than 40 natural products have been so far isolated, including benzene derivatives, azaphilone derivatives, sesquiterpenoids, squalene-type triterpenoids, steroids, cytochalasins, etc. Of these, a substantial number possess significant bioactivities. For instance, concentricolide, a benzofuran lactone isolated from fruiting bodies of *D. concentrica*, exhibited the blockage (EC₅₀ 0.83 mg/mL) of syncytium formation between HIV-1 infected cells and normal cells. Additionally, the structure of concentricolide has been fully synthesized in 2011. As our continuous search for novel secondary metabolites from higher fungi continued, we investigated the cultures of *D. concentrica*, which produced three new benzene derivatives, namely daldins A–C (1–3), together with a known analogue 2-hydroxymethyl-3-(1-hydroxypropyl)phenol (4). In this paper we report the structure, elucidation and cytotoxicity of these isolates.

**Results and Discussion**

Compound 1 was isolated as colorless crystals (MeOH). The UV absorption at λₘₐₓ 281 nm suggested the existence of a conjugated system. While the positive HRESIMS displayed an [M + Na]⁺ peak at m/z 219.0993 (calcd. 219.0997 for C₁₁H₁₆O₄Na) indicating a molecular formula C₁₁H₁₆O₄Na. The ¹H NMR displayed three aromatic protons at δH 6.81 (1H, d, J = 8.0 Hz, H-6), 6.95 (1H, d, J = 8.0 Hz, H-4), and 7.18 (1H, t, J = 8.0 Hz, H-5), corresponding to a 1,2,3-tri-substituted benzene ring. In addition, two methyls at δH 3.47 (3H, s, OMe) and 0.94 (3H, t, J = 7.6 Hz, Me-10) were clearly identified.
Compound 2 was isolated as a colorless oil. Preliminary analysis of NMR data suggested that 2 possessed a structure closely related to that of 4. However, the HRESIMS showed an [M + Na]$^+$ peak at m/z 369.1675 (calcd for C$_{26}$H$_{26}$O$_4$Na, 369.1677), corresponding to a molecular formula C$_{26}$H$_{26}$O$_4$. This important information suggested that compound 2 might be a dimer of 4, possessing two completely symmetrical units. Further evidence was detected from $^{13}$C NMR spectrum, in which the signal of the oxymethylene was presented as a downfield shift at $\delta_c$ 63.6 (t, C-7) ($\delta_c$ 58.3 in 4), suggesting that the two units were connected according to the ether bond at C-7. Detailed analysis of NMR and MS data confirmed that the structure of 2 was a dimer derivative of 4. The negative OR data ($[\alpha]_D^{20}$ = -18.4 (c 0.26, MeOH)) also suggested the $S$ form of C-8 in 2. Compound 2 was, therefore identified as daldin B.

Compound 3 was also isolated as a colorless oil. The HRESIMS identified the molecular formula C$_{26}$H$_{26}$O$_3$ (measured: m/z 369.1673; calcd for C$_{26}$H$_{26}$O$_3$Na, 369.1677), the same to that of 2. The patterns of 1D NMR spectrum suggested that compound 3 was also a dimer of 4. In the $^{13}$C NMR spectrum, two significant downfield shifts at $\delta_c$ 64.4 (t, C-7) and 82.0 (d, C-8) suggested that two units were attached with bond of C-7-O-C-8', which was further supported by the HMBC correlations from $\delta_h$ 4.52 (2H, br. s, H-7') to C-8' and from $\delta_h$ 4.50 (1H, t, J = 7.2 Hz, H-8') to C-7'. Further analysis of 2D NMR data suggested that the other parts of two units in 3 were the same to those of 4. Therefore, compound 3 was established as daldin C.

Compounds 1-4 were evaluated for their inhibitory activities on five human cancer cell lines using the MTT method as reported. Unfortunately no compound exhibited significant cytotoxicity with IC$_{50}$ values > 40 $\mu$mol.

Experimental Section

**General Experimental Procedures.** Optical Rotations (OR) were measured with a Horiba SEPA-300 polarimeter. Ultraviolet (UV) spectra were obtained using a Shimadzu UV-2401A spectrophotometer. Infrared (IR) spectra were obtained on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were run on a Bruker AV-400 MHz
or a Bruker AV600 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to solvent signals. Mass spectra were recorded on an API QSTAR Pulsar I spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China). An APEX DUO (Bruker) instrument was used for the single crystal X-ray diffraction. An Agilent 1100 series instrument equipped with Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 150 mm) was used for high-performance liquid chromatography (HPLC) analysis, and a semi-preparative Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 150 mm) was used for the sample preparation. Fractions were monitored by thin layer chromatography (TLC) (GF 254, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized by 10% H2SO4 in ethanol.

Fungal Material and Cultivation Condition. Strain (S 0318) was isolated from tissue culture of the fruiting bodies of D. concentrica collected at Laoshunjian, Yunnan Province, China, in July 2003 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (HKAS 40992) was deposited at the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The mycelial cultures were derived from tissue plugs. Culture PDA medium: potato (peeled), 200 g, glucose, 20 g, KH2PO4, 3 g, MgSO4, 1.5 g, citric acid, 0.1 g, and thiamin hydrochloride, 10 mg, in 1 L of deionized H2O. The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25 °C and 150 rpm for 20 days.

Extraction and Isolation. The culture broth (20 L) was extracted three times with EtOAc. The EtOAc layer was evaporated in vacuo to yield an extract (10 g). The latter was subjected to a silica gel column eluted with petroleum ether–acetone (1:0 to 0:1) to afford fractions 1–6. Fraction 2 (1.2 g) was separated by HPLC (acetonitrile–H2O, 81:19) to afford two subfractions a and b. Fraction a (240 mg) was separated by HPLC (acetonitrile–H2O, 48:52) to afford (4.5 mg), 2 (1.1 mg), 3 (1.8 mg), and 4 (48 mg).

Daldin A (1): colorless crystals (MeOH); [α]D 20 = 20.6 (c 0.11, MeOH); UV (MeOH) λmax (log ε) 281 (1.42), 202 (2.41) nm; IR (KBr) νmax 3384, 3169, 2966, 1587, 1469, 1280, 1200, 987, 799 cm−1; 1H (400 MHz) and 13C (100 MHz) NMR data (CDCl3), see Table 1; positive ion HRESIMS m/z 219.0993 (calcd for C11H10O4Na, 219.0997).

Daldin B (2): colorless oil; [α]D 20 = 18.4 (c 0.26, MeOH); UV (MeOH) λmax (log ε) 282 (1.48), 202 (2.61) nm; IR (KBr) νmax 3386, 3146, 2963, 1590, 1469, 1272, 1014, 958, 793 cm−1; 1H (400 MHz) and 13C (150 MHz) NMR data (CDCl3 + methanol-d4), see Table 1; positive ion HRESIMS m/z 369.1675 (calcd for C23H12O6Na, 369.1677).

Daldin C (3): colorless oil; [α]D 20 = 16.0 (c 0.19, MeOH); UV (MeOH) λmax (log ε) 281 (1.65), 202 (2.33) nm; IR (KBr) νmax 3387, 3175, 2966, 1588, 1468, 1280, 987, 799 cm−1; 1H (400 MHz) and 13C (150 MHz) NMR data (CDCl3), see Table 2; positive ion HRESIMS m/z 369.1673 (calcd for C23H12O6Na, 369.1677).

Crystallographic data of daldin A (1): C11H10O4Na, MW = 196.24; a = 5.01570(10) Å, b = 7.8428(2) Å, c = 26.9837(6) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1061.46(4) Å3, T = 100(2) K, space group P212121, Z = 4, μ(CuKa) = 0.720 mm−1, 5136 reflections measured, 1825 independent reflections (Rint = 0.0477). The final R values were 0.0509 (I > 2σ(I)). The final wR(F2) values were 0.1405 (all data). The final R(F2) values were 0.1405 (all data). The goodness of fit on F2 was 1.052. Flack parameter = 0.0(3). The Hooft parameter is –0.07(13) for 645 Bijvoet pairs. The crystal structure of compound 1 was solved by direct method SHELXS-97 and expanded using the difference Fourier techniques, refined by the program SHXLXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure of compound 1 have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 951553). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-013-0048-1 and is accessible for authorized users.

Acknowledgments

This project was supported by the National Basic Research Program of China (973 Program, 2009CB522300), the National Natural Sciences Foundation of China (30830113, 81102346), and Youth Innovation Promotion Association CAS.

Open Access

This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

[1] Dai, Y. C.; Cui, B. K. Mycosystema 2008, 27, 510.
[2] Robbins, W. J.; Kavanagh, F.; Hervey, A. Proc. Natl. Acad. Sci. U.S.A. 1947, 33, 176–182.
[3] Grabley, S.; Thiericke, R.; Zecek, A. The chemical screening approach. In: Grabley, S.; Thiericke, R. (eds.) Drug discovery from nature. Springer, Verlag Berlin Heidelberg 2000, 124–148.
[4] Allport, D. C.; Bu’Lock, J. D. J. Chem. Soc. 1958, 4090–4094.
[5] (a) Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M.; Asakawa, Y. Chem. Pharm. Bull. 1994, 42, 1528–1530. (b) Quan, D. N.; Hashimoto, T.; Tanaka, M.; Baugnattner, M.; Stadler, M.; Asakawa, Y. J. Nat. Prod. 2002, 65, 1869–1874. (c) Qin, X. D.; Dong, Z. J.; Liu, J. K.; Yang, L. M.; Wang, R. R.; Zheng, Y. T.; Lu, Y.; Wu, Y. S.; Zheng, Q. T. Helv. Chim. Acta 2006, 89, 127–133. (d) Qin, X. D.; Dong, Z. J.; Liu, J. K. Helv. Chim. Acta 2006, 89, 450–455. (e) Shao, H. J.; Qin, X. D.; Dong, Z. J.; Zhang, H. B.; Liu, J. K. J. Antibiot. 2008, 61, 115–119.
[6] Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M.; Asakawa, Y. Chem. Pharm. Bull. 1994, 42, 2397–2399.
[5] Qin, X. D.; Shao, H. J.; Dong, Z. J.; Liu, J. K. J. Antibiot. 2008, 61, 556–562.

[6] (a) Stadler, M.; Baumgartner, M.; Grothe, T.; Mahlbauer, A.; Seip, S.; Wollweber, H. Phytochemistry 2001, 56, 787–793. (b) Quang, D. N.; Hashimoto, T.; Tanaka, M.; Baumgartner, M.; Stadler, M.; Asakawa, Y. Phytochemistry 2002, 61, 345–353.

[7] (a) Qin, X. D.; Liu, J. K. J. Nat. Prod. 2004, 67, 2133–2135. (b) Quang, D. N.; Lam, D. M.; Hanh, N. T. H.; Que, D. D. Nat. Prod. Res. 2013, 27, 486–490.

[8] Chang, C. W.; Chein, R. J. J. Org. Chem. 2011, 76, 4154–4157.

[9] Weber, D.; Gorzalezny, S.; Martino, V.; Acevedo, C.; Sterner, O.; Anke, T. Z. Naturforsch. 2005, 60c, 467–477.

[10] Asai, T.; Luo, D.; Obara, Y.; Taniguchi, T.; Monde, K.; Yamashita, K.; Oshima, Y. Tetrahedron Lett. 2012, 53, 2239–2243.

[11] (a) Mosmann, T. J. Immunol. Methods 1983, 65, 55–63. (b) Feng, T.; Li, Z. H.; Dong, Z. J.; Su, J.; Li, Y.; Liu, J. K. Nat. Prod. Bioprospect. 2011, 1, 29–32.