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Alkylbenzoquinone Involved in Development of Cellular Slime Molds

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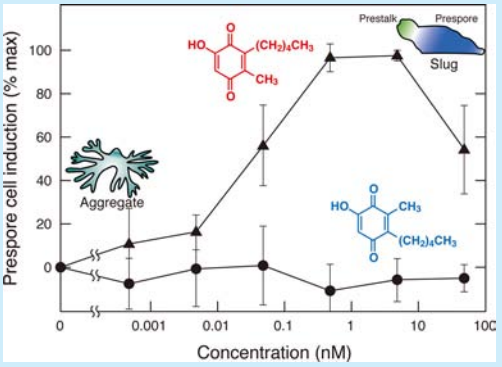
Supporting Information

ABSTRACT: The structure of the prespore-cell-promoting factor from *Dictyostelium discoideum* was determined to be 2-hydroxy-5-methyl-6-pentylbenzoquinone. The synthetic compound has prespore-cell-promoting activity similar to the natural one, with half-maximal induction at a concentration as low as 40 pM. It was also found that the factor induces aggregation in an aggregation-deficient mutant of a related species, *Polysphodilium violaceum*. Both these activities are sensitive to positional isomerism with the 6-methyl-5-pentyl isomer showing no detectable activity.



The life cycle of *Dictyostelium discoideum* consists of growth and developmental phases. In the growth phase, amoebae of this organism multiply by binary fission, and they initiate development when the food source is exhausted. In the developmental stage, they aggregate and form multicellular structures called slugs composed of prespore and prestalk cells. At fruiting body construction, these cells differentiate into spores and stalk cells, respectively.

The 34 Mb-genome of *D. discoideum* has more than 40 genes of putative polyketide synthase.[1−3] They are expressed in a variety of patterns, suggesting their diverse roles in the life cycle of the organism,[4,5] but only several polyketides have so far been identified.[6−9] Of them, (1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-1-hexanone (differentiation-inducing factor-1, DIF-1) (**2**) and 4-methyl-5-pentylbenzene-1,3-diol (MPBD) (**3**) have been isolated from conditioned medium (CM) and shown to be involved in cell differentiation (Figure 1).[4,8,10−13] Here we report on the identification of a unique alkylbenzoquinone that plays significant roles in the multicellular development of *D. discoideum* and its related species.

In our previous paper, we described a polyketide-like factor (PLF) with a molecular weight of 208 obtained from CM of *D. discoideum*, which plays a key role in the acquisition of differentiation commitment in early stages of development.[14] In this paper, we describe the structure of the factor (2-hydroxy-5-methyl-6-pentylbenzoquinone), as dictyoquinone (**1**), which was determined by spectral analysis and chemical synthesis, and by their prespore-cell inducing activity.

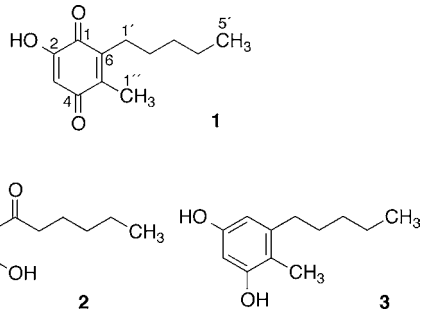


Figure 1. Structures of dictyoquinone (**1**), DIF-1 (**2**), and MPDB (**3**).

The molecular formula of **1** was established as $C_{12}H_{16}O_3$ by HR-ESI-MS (*m*/*z* 208.1084 $[M]^-$, calcd *m*/*z* 208.1099). The ^{1}H NMR spectrum of **1** showed only aliphatic signals, i.e., two methyl signals at δ 0.899 (3H, t) and 1.972 (3H, s), a methylene signal at δ 2.413 (2H, t), and a signal corresponding to three methylenes at δ 1.27−1.41 (6H, m). From these signals, only an *n*-pentyl and a methyl groups are suggested on the basis of the DQF-COSY spectrum, and those groups should be attached to sp^2 carbons according to their chemical shifts. On the other hand, six sp^3 carbon signals (δ 32.9, 29.3, 26.7, 23.3, 14.1, and 12.7) were estimated from cross peaks on a HSQC spectrum. In the heteronuclear

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multiplebond correlation (HMBC) spectrum, the methyl group at δ 1.972 (5-CH_3) and the methylene signal at δ 2.413 (H-1′) showed cross peaks at δ_C 189.2, and they also correlated with signals around δ_C 140.6 and δ_C 143.9 (C-5 and -6). As we reported previously,[14] dictyoquinone (**1**) showed the absorption at λ_{max} 491 nm, suggesting the presence of a quinone moiety in this molecule. These pieces of evidence demonstrated two possible structures (**A** and **B**) for **1**, with a pentyl and a methyl groups on one side of the quinone ring, and a hydroxyl group on the other side (Figure 2). If the correct structure of **1** is one of these suggested,

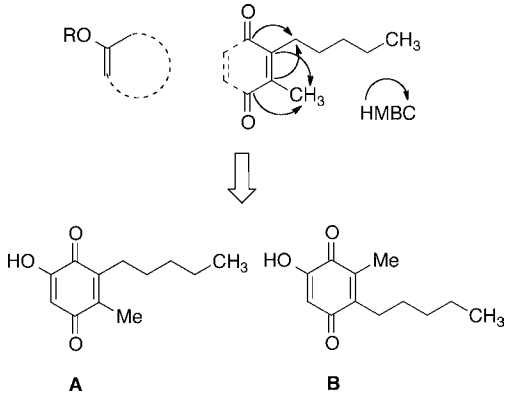
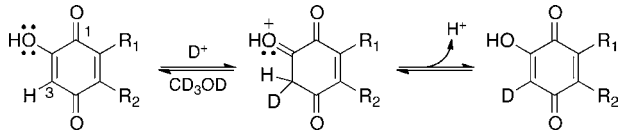


Figure 2. HMBC correlations and possible structures (**A** and **B**) for dictyoquinone (**1**).

another proton signal corresponding to a proton on benzoquinone ring (H-3) should be observed on the ^{1}H NMR spectrum, though no other signal was detected. This is accounted for by H−D exchange on C-3 position of the compound in methanol-d_4, which was used as a solvent to measure NMR spectra since we used aqueous methanol to purify this compound (**1**) without any information on the structure (Scheme 1).

Scheme 1. H−D Exchange on C-3

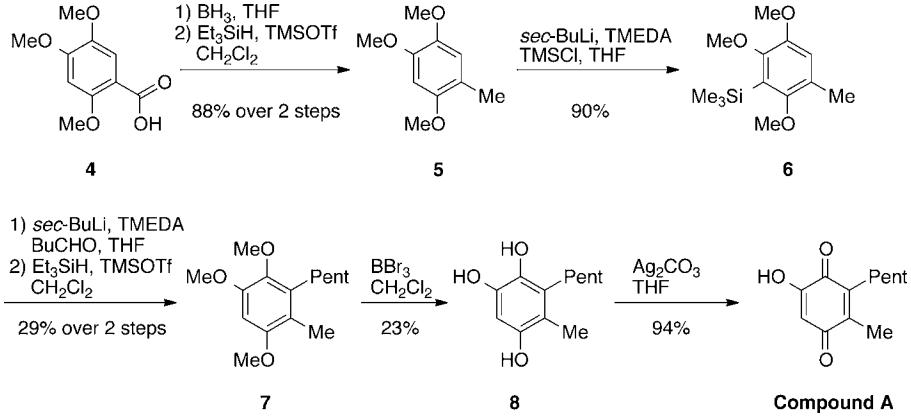


To determine which of the two structures **A** and **B** is correct, we synthesized both compounds for evaluation of their biological activities. As shown in Scheme 2, 2,4,5-trimethoxybenzoic acid (**4**) was used for starting material for both compounds. The carboxyl group in **4** was transformed to a methyl group, followed by introduction of C_5 unit to C−6 position to afford compound **7**. Boron tribromide treatment of compound **7** transformed the methoxyl groups to hydroxyl groups,[15] and the consequent phenol **8** was oxidated by Ag_2CO_3 to give compound **A**.[16] On the other hand, the compound **B** was prepared as follows. The carboxyl group in **4** was reduced to an aldehyde **9**, and then it was transformed to a pentyl group to give compound **10**. A methyl group was incorporated into **10** to give compound **12**. Subsequently, compound **B** was obtained in a manner similar to that for compound **A** (Scheme 3). However, as the ^{1}H NMR spectra of the synthetic **A** and **B** are almost identical, comparison of the NMR data alone was not sufficient for identification of the natural compound (**1**).

Dictyoquinone (**1**) promotes prespore cell differentiation in submerged culture conditions at low cell densities in the presence of the prespore-inducing PsiA glycoprotein[17,18] and also promotes DIF-dependent stalk cell differentiation in vitro.[14] The synthesized compounds **A** and **B** were tested for their in vitro prespore- and stalk cell-promoting activities under respective assay conditions. As shown in Figure 3, compound **A** showed prespore-promoting activity, with half-maximal induction at a concentration as low as 40 pM, whereas compound **B** had no detectable activity even at the highest concentration tested. In addition, compounds **A** but not **B** promoted stalk cell differentiation in vitro (Figure SI-1 in SI).

We also examined whether compounds **A** and **B** have the D-factor activity. D-factor is a small molecule required for the initiation of aggregation in a related species *Polysphodilium violaceum*; starved cells of the D-factor nonproducing *aggA* mutants of *P. violaceum* aggregate and form fruiting bodies normally if supplied with D-factor.[19] Its structure is unresolved but reported to have structural features very similar to those of dictyoquinone (**1**).[20] We used the *aggA* mutant strain A586 of *P. violaceum* to test compounds **A** and **B**, as well as crude dictyoquinone preparations from *D. discoideum*, for their D-factor activity. As shown in Figure 4, only compound **A** rescued the mutant phenotype. Crude

Scheme 2. Synthesis of Compound A



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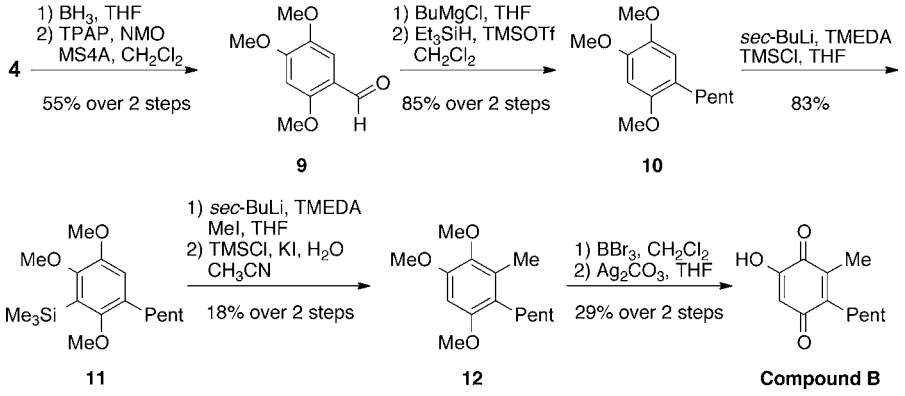
Scheme 3. Synthesis of Compound B



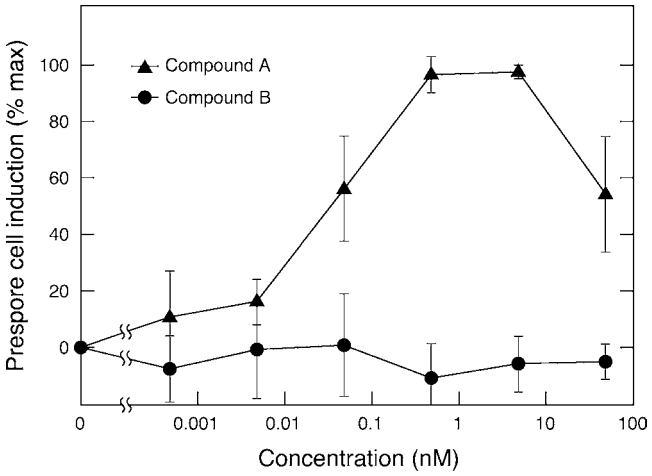


Figure 3. Dose–response curves for the effects of compounds **A** and **B** on prespore cell differentiation. The results shown are the means and SDs of three independent experiments.

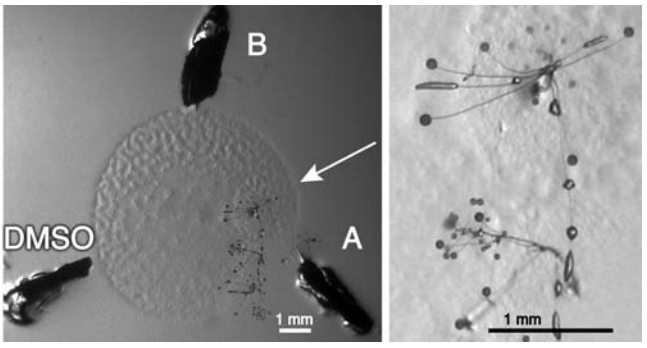


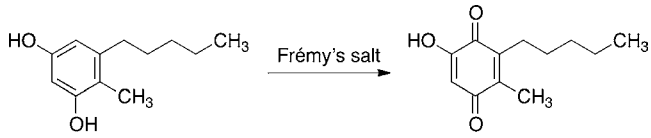
Figure 4. Effects of compounds **A** and **B** on the development of the aggregation-defective mutant A586 of *P. violaceum*. Small pieces of filter paper containing 4.8 pmol of compound A, compound B, or DMSO as a solvent control were placed around the starved A586 cells spotted on agar plate. Cells responded to compound A alone to form fruiting bodies (arrow). Higher magnification image of the fruiting bodies is shown to the right. Aggregation was never observed around the filter paper of compound B or DMSO.

dictyoquinone preparations also had the D-factor activity (data not shown).

From these results, the molecular structure of dictyoquinone (**1**) was determined to be 2-hydroxy-5-methyl-6-pentylbenzoquinone (structure **A**). The isomeric specificity and the extremely low concentrations required of dictyoquinone (**1**) for its activities suggested that its action is mediated by binding to a specific receptor.

Structural comparison of dictyoquinone (**1**) with MPBD (**3**) suggests that they are metabolites of the same biosynthetic pathway, possibly the former being an oxidation product of the latter. In support of this possibility, synthetic MPBD (**3**) treated with Frémy's salt[21] gave dictyoquinone (**1**) in good yield (Scheme 4). Further studies are required to determine the biosynthetic pathways of these compounds and their relationship.

Scheme 4. Transformation of MPBD (3) into Dictyoquinone (1) by Frémy's Salt



In conclusion, we have determined the structure of an alkylbenzoquinone, dictyoquinone (**1**), which plays significant roles in the multicellular development of *D. discoideum* and possibly in other species of the Dictyostelid slime mold. In multicellular organisms, besides ubiquinone and plastoquinone, naturally occurring benzoquinone compounds have been shown to have various biological functions: e.g., antioxidation,[22] antimicrobial defense,[23] signal transmission,[24,25] and allelopathic interaction.[26] Our results point to the possibility of benzoquinone compounds playing a role as signaling molecules in the control of development.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details of the syntheses of compounds **A** and **B** and the bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Eichinger, L.; Pachebat, J. A.; Glöckner, G.; Rajandream, M.-A.; Sucgang, R.; Berriman, M.; Song, J.; Olsen, R.; Szafranski, K.; Xu, Q.; Tunggal, B.; Kummerfeld, S.; Madera, M.; Konfortov, B. A.; Rivero, F.; Bankier, A. T.; Lehmann, R.; Hamlin, N.; Davies, R.; Gaudet, P.; Fey, P.; Pilcher, K.; Chen, G.; Saunders, D.; Sodergren, E.; Davis, P.; Kerhornou, A.; Nie, X.; Hall, N.; Anjard, C.; Hemphill, L.; Bason, N.; Farbrother, P.; Desany, B.; Just, E.; Morio, T.; Rost, R.; Churcher, C.; Cooper, J.; Haydock, S.; van Driessche, N.; Cronin, A.; Goodhead, I.; Muzny, D.; Mourier, T.; Pain, A.; Lu, M.; Harper, D.; Lindsay, R.; Hauser, H.; James, K.; Quiles, M.; Madan Babu, M.; Saito, T.; Buchrieser, C.; Wardroper, A.; Felder, M.; Thangavelu, M.; Johnson, D.; Knights, A.; Loulseged, H.; Mungall, K.; Oliver, K.; Price, C.; Quail, M. A.; Urushihara, H.; Hernandez, J.; Rabbinowitsch, E.; Steffen, D.; Sanders, M.; Ma, J.; Kohara, Y.; Sharp, S.; Simmonds, M.; Spiegler, S.; Tivey, A.; Sugano, S.; White, B.; Walker, D.; Woodward, J.; Winckler, T.; Tanaka, Y.; Shaulsky, G.; Schleicher, M.; Weinstock, G.; Rosenthal, A.; Cox, E. C.; Chisholm, R. L.; Gibbs, R.; Loom/is, W. F.; Platzer, M.; Kay, R. R.; Williams, J.; Dear, P. H.; Noegel, A. A.; Barrell, B.; Kuspa, A. *Nature* **2005**, *435*, 43−57.

(2) Heidel, A. J.; Lawal, H. M.; Felder, M.; Schilde, C.; Helps, N. R.; Tunggal, B.; Rivero, F.; John, U.; Schleicher, M.; Eichinger, L.; Platzer, M.; Noegel, A. A.; Schaap, P.; Glöckner, G. *Genome Res.* **2011**, *21*, 1882−1892.

(3) Zucko, J.; Skunca, N.; Curk, T.; Zupan, B.; Long, P. E.; Cullum, J.; Kessin, R. H.; Hranueli, D. *Bioinformatics* **2007**, *23*, 2543−2549.

(4) Austin, M. B.; Saito, T.; Bowman, M. E.; Haydock, A.; Kato, B. S.; Moore, K. L.; Kay, R. R.; Noel, J. P. *Nat. Chem. Biol.* **2006**, *2*, 494−502.

(5) Ghosh, R.; Chhabra, A.; Phatale, P. *J. Biol. Chem.* **2008**, *283*, 11348−11354.

(6) Morris, H. R.; Taylor, G. W.; Jermyn, K. A.; Kay, R. R. *Nature* **1987**, *328*, 811−814.

(7) Morris, H. R.; Masento, M. S.; Taylor, G. W.; Jermyn, K. A.; Kay, R. R. *Biochem. J.* **1988**, *249*, 903−906.

(8) Saito, T.; Taylor, W. G.; Yang, J. C.; Neuhaus, D.; Stetsenko, D.; Kato, A.; Kay, R. R. *Biochim. Biophys. Acta* **2006**, *1760*, 754−761.

(9) Takaya, Y.; Kikuchi, H.; Terui, Y.; Komiya, J.; Furukawa, K.; Seya, K.; Motomura, S.; Ito, A.; Oshima, Y. *J. Org. Chem.* **2000**, *65*, 985−989.

(10) Anjard, C.; Su, Y.; Loomis, W. F. *Eukaryot. Cell* **2011**, *10*, 956−963.

(11) Narita, T. B.; Koide, K.; Morita, N.; Saito, T. *FEMS Microbiol. Lett.* **2011**, *319*, 82−87.

(12) Saito, T.; Kato, A.; Kay, R. R. *Dev. Biol.* **2008**, *317*, 444−453.

(13) Thompson, C. R. L.; Kay, R. R. *Mol. Cell* **2000**, *6*, 1509−1514.

(14) Oohata, A. A.; Fukuzawa, M.; Hotta, R.; Nakagawa, M.; Niwa, M.; Takaya, Y. *Dev. Growth Differ.* **2009**, *51*, 743−752.

(15) Saito, S.; Kawabata, J. *Helv. Chim. Acta* **2006**, *89*, 1395−1407.

(16) Ling, K.-Q.; Lee, Y.; Macikenas, D.; Protasiewicz, J. D.; Sayre, L. M. *J. Org. Chem.* **2003**, *68*, 1358−1366.

(17) Kawata, T.; Nakagawa, M.; Shimada, N.; Fujii, S.; Oohata, A. A. *Dev. Growth Differ.* **2004**, *46*, 383−392.

(18) Oohata, A. A.; Nakagawa, M.; Tasaka, M.; Fujii, S. *Development* **1997**, *124*, 2781−2787.

(19) Hanna, M. H.; Cox, E. C. *Dev. Biol.* **1978**, *62*, 206−214.

(20) Hanna, M. H.; Fatone, M.; Newth-Clark, C.; Salerno, J.; Clemans, S. *Dev. Genet.* **1988**, *9*, 653−662.

(21) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1985**, *107*, 479−485.

(22) Dandawate, P. R.; Vyas, A. C.; Padhye, S. B.; Singh, M. W.; Baruah, J. B. *Mini Rev. Med. Chem.* **2010**, *10*, 436−454.

(23) Yezerski, A.; Ciccone, C.; Rozitski, J.; Volingavage, B. *J. Chem. Ecol.* **2007**, *33*, 1217−1225.

(24) Nojima, S.; Schal, C.; Webster, F. X.; Santangelo, R. G.; Roelofs, W. L. *Science* **2005**, *307*, 1104−1106.

(25) Verheggen, F.; Ryne, C.; Olsson, P.-O. C.; Arnaud, L.; Lognay, G.; Högberg, H.-E.; Persson, D.; Haubruge, E.; Löfstedt, C. *J. Chem. Ecol.* **2007**, *33*, 525−539.

(26) Dayan, F. E.; Rimando, A. M.; Pan, Z.; Baerson, S. R.; Gimsing, A. L.; Duke, S. O. *Phytochemistry* **2010**, *71*, 1032−1039.