Pergamon

Tetrahedron Letters 42 (2001) 61–63

TETRAHEDRON LETTERS

# Novel aromatic substances, dictyomedin A and B, from *Dictyostelium* cellular slime molds and their inhibitory effects on *Dictyostelium* development

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Received 20 September 2000; revised 18 October 2000; accepted 20 October 2000

**Abstract**—To elucidate the diversity of secondary metabolites of *Dictyostelium* cellular slime molds and search for physiologically active substances affecting their development, we investigated the constituents of *D. medium*. From the methanol extract of its fruit body, we obtained two novel aromatic compounds named dictyomedin A (**1**) and B (**2**), and their structures were elucidated by means of physicochemical data. Biological evaluation of the compounds showed that they delayed the differentiation of *D. discoideum* cells. 

In a certain period of the life cycle of cellular slime molds, they form an animal-like feeding stage which then undergoes transition into a plant-like fruit body stage. This characteristic has led the slime molds to be classified into both the plant and the animal kingdoms. They are a very fascinating organism in the field of developmental biology, and have long been established as an excellent model organism for the study of various aspects of multicellular development, such as the structure and functions of cytoskeletal proteins, cell motility, membrane trafficking and signal transduction. Several chemical stimuli such as DIF-1,[1] discadenine,[2] and cAMP[3] are identified as physiologically active substances which act during the life cycle of cellular slime molds, and among them DIF-1 also showed strong antitumor activity.[4] Besides these chemical stimuli, no chemical study on cellular slime molds has been carried out. In recent years, we have focused on the chemistry of cellular slime molds and reported α-pyronoids with unique structures.[5] In this paper, we describe the structure elucidation of two novel aromatic substances, dictyomedin A (**1**) and B (**2**), and their inhibitory effects on *Dictyostelium* development.

*Dictyostelium* is a typical genera of cellular slime molds. Spores of *D. medium* were cultured at 22°C with *E. coli* Br on A-medium (0.5% glucose, 0.5% polypeptone, 0.05% yeast extract, 0.225% $KH_2PO_4$, 0.137% $Na_2HPO_4{\cdot}12H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$ and 1.5% agar). The cultured fruit body (wet weight 150 g), which was formed in several days, was harvested and was then lyophilized to give a dried fruit body (37 g). It was extracted twice with methanol–$H_2O$ (9:1) at room temperature to yield the extract (9.0 g), which was partitioned with ethyl acetate and water to afford ethyl acetate solubles (0.49 g). The ethyl acetate solubles were chromatographed over ODS, and the column was eluted with methanol–$H_2O$ (8:2), methanol and ethyl acetate. The methanol–$H_2O$ (8:2)-eluting fraction was further chromatographed over ODS using methanol–$H_2O$ (5:5 and 6:4) and methanol as eluent. The methanol–$H_2O$ (6:4)- and methanol-eluting fractions were put on $SiO_2$ gel column chromatography using chloroform–methanol (39:1) followed by purification with preparative $SiO_2$ gel TLC (chloroform–methanol (9:1)) to give **1** (4.9 mg) and **2** (2.1 mg).

The molecular formula $C_{21}H_{16}O_7$ indicated for **1**, a colorless powder, was assumed by a molecular ion peak at $m/z$ 380.0886 $[M]^+$ (calcd for $C_{21}H_{16}O_7$: 380.0896) in

*Keywords*: *Dictyostelium medium*; cellular slime molds; dibenzofuran; differentiation inhibitor.

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0040-4039/01/$ - see front matter 
PII: S0040-4039(00)01879-7

its HREI-MS. Its IR absorption at 1694 $cm^{-1}$ suggested the presence of a carbonyl group, and the $^1H$ NMR spectrum exhibited the presence of a 1,2,4-trisubstituted benzene ring ($\delta$ 6.96 (1H, d, $J = 8.2$ Hz), 7.40 (1H, dd, $J = 8.2$, 1.9 Hz) and 7.45 (1H, d, $J = 1.9$ Hz)), a 1,2,3,5-tetrasubstituted benzene ring ($\delta$ 8.17 and 8.49 (each 1H, d, $J = 1.5$ Hz)), a 1,2,4,5-tetrasubstituted benzene ring ($\delta$ 7.10 and 7.60 (each 1H, s)) and two methoxyl groups ($\delta$ 3.97 and 3.99 (each 3H, s)) (Table 1). The $^{13}C$ NMR spectrum of **1** displayed 19 $sp^2$-carbon signals (–O–C = ×6, >C = ×5, –CH = ×7 and >C = O×1) and two $sp^3$-carbon signals (–$OCH_3$×2). The proton signals of the two methoxyl groups of **1** at $\delta$ 3.97 and 3.99 gave distinct NOEs with H-2′ ($\delta$ 7.45) and H-9 ($\delta$ 7.60), respectively. HMBC experiment showed long-range correlation peaks between the following carbons and hydrogens: C-4a–H-1, C-4a–H-3, C-8–H-6, C-5a–H-9, C-7–H-9, C-3′–H-5′, C-4′–H-2′, C-4′–H-6′, C-9a–H-1 and C-9b–H-9. A dibenzofuran and a 4-hydroxy-3-methoxybenzene moieties were thus deduced by these NMR data.

Methylation of **1** with diazomethane afforded **3**[6] ($m/z$ 422 [$M^+$]) which was converted to **4** ($m/z$ 408 [$M^+$]) by alkali hydrolysis with sodium hydroxide (Fig. 1). These results, along with a finding that **1** was unreactive to sodium hydroxide, suggested that **1** bears two phenolic

**Table 1.** $^{13}C$ and $^1H$ NMR spectral data of dictyomedin A (**1**) and dictyomedin B (**2**)

| Positions | Dictyomedin A | | Dictyomedin B | |
|---|---|---|---|---|
| | $^{13}C$ | $^1H$ | $^{13}C$ | $^1H$ |
| 1 | 121.5 | 8.49 (d, $J = 1.5$ Hz) | 121.5 | 8.51 (d, $J = 1.5$ Hz) |
| 2 | 125.9 | | [a] | |
| 3 | 128.0 | 8.17 (d, $J = 1.5$ Hz) | 127.9 | 8.18 (d, $J = 1.5$ Hz) |
| 4 | 127.3 | | 127.3 | |
| 4a | 157.7 | | 157.8 | |
| 5a | 150.2 | | 150.3 | |
| 6 | 100.4 | 7.10 (s) | 100.4 | 7.13 (s) |
| 7 | 147.9 | | 147.9 | |
| 8 | 154.1 | | 154.1 | |
| 9 | 104.6 | 7.60 (s) | 104.7 | 7.63 (s) |
| 9a | 116.9 | | 116.8 | |
| 9b | 128.0 | | 128.1 | |
| 1′ | 129.7 | | 129.1 | |
| 2′ | 114.3 | 7.45 (d, $J = 1.9$ Hz) | 131.7 | 7.79 (d, $J = 8.6$ Hz) |
| 3′ | 149.9 | | 117.3 | 6.98 (d, $J = 8.6$ Hz) |
| 4′ | 148.7 | | 159.5 | |
| 5′ | 117.3 | 6.96 (d, $J = 8.2$ Hz) | 117.3 | 6.98 (d, $J = 8.6$ Hz) |
| 6′ | 123.7 | 7.40 (dd, $J = 8.2$, 1.9 Hz) | 131.7 | 7.79 (d, $J = 8.6$ Hz) |
| COOH | 172.0 | | [a] | |
| 8-OMe | 58.0 | 3.99 (s) | 58.0 | 4.03 (s) |
| 3′-OMe | 57.5 | 3.97 (s) | | |

[a] Not identified because of the weak intensities of signals due to a trace amount of the compound.

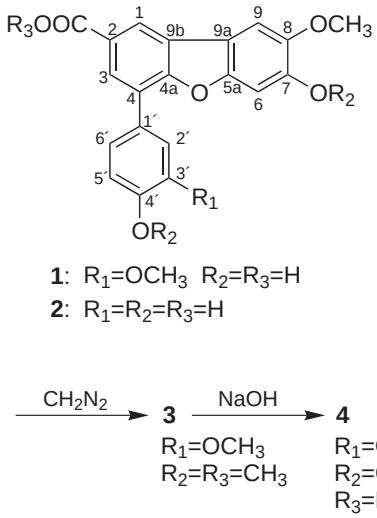


**Figure 1.** Structures of dictyomedin A (**1**) and B (**2**), and transformations of dictyomedin A (**1**) to determine the number of the methoxyl groups.

hydroxyl groups and one carboxyl group in its molecule. The presence of the C-2 carboxyl group is substantiated by the chemical shifts of the $^1H$ NMR signals of H-1 and H-3 at $\delta$ 8.49 and 8.17 which are reasonable for the *ortho* position to the carboxyl group, and by the three bond H–C couplings between the H-1 and H-3 hydrogens and the carbonyl carbon (Fig. 2). Moreover, cross peaks of C-4–H-2′ and C-1′–H-3 in the HMBC spectrum of **1** unambiguously established the linkages of the dibenzofuran and the disubstituted phenyl group, giving the structure of dictyomedin A (**1**).

Compound **2**, a colorless powder, has the molecular formula $C_{20}H_{14}O_6$ (HREI-MS: $m/z$ 350.0797 [$M]^+$, calcd for $C_{20}H_{14}O_6$: 350.0791). Its IR, $^1H$ and $^{13}C$ NMR spectral data quite resembled those of **1**. Instead of the ABX type signals due to H-2′, H-5′ and H-6′ in the $^1H$ NMR of **1**, **2** showed AA′XX′ type signals at $\delta$ 6.98 and 7.79 (each 2H, d, $J = 8.6$ Hz), demonstrating that **2** is a demethoxylated compound of **1** as indicated in the formula. The structure **2** completely satisfied its molecular formula and $^{13}C$ NMR data.

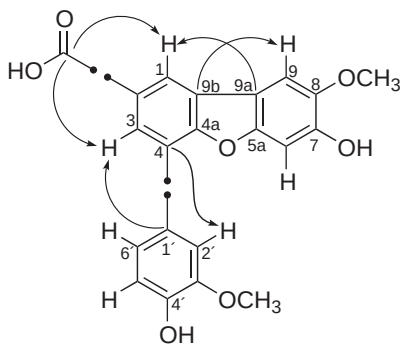


**Figure 2.** Partial structures of dictyomedin A (**1**). The arrows indicate the HMBC correlations.

Biological evaluation on the *Dictyostelium* differentiation was investigated qualitatively with *D. discoideum* Ax-2 cells. When starved cells, which were harvested at the exponential growth phase and washed in Bonner's salt solution, were incubated on 1.5% non-nutrient agar containing **1** at 22°C, a considerable number of cells still remained as non-aggregated single cells even after 24 h of incubation. Unexpectedly, however, such inhibition of differentiation was not observed on agar containing **2** (1–10 μg/ml).

Similar inhibition of morphogenesis including cell aggregation was also observed when starved Ax-2 cells were incubated with compounds **1** and **2** under submerged conditions. A majority of cells exhibited delayed differentiation at a relatively high concentration (10 μg/ml) of **1** and **2** (1–10 μg/ml), as compared with the control without these compounds, and non-aggregated single cells were found even after a prolonged period (24 h). Incidentally, the growth of Ax-2 cells in a growth medium (PS-medium) seemed to be slightly inhibited in the presence of 10 μg/ml **2**.

In addition to the α-pyrones and DIF-1, the isolation of the novel aromatics (**1** and **2**) suggests that cellular slime molds contain various types of compounds which are presumed to lead to structurally novel bioactive compounds.

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6. $^1$H NMR of **3** (500 MHz, $CDCl_3$) $\delta$ 8.51 (1H, d, $J = 1.6$ Hz; H-1), 8.22 (1H, d, $J = 1.6$ Hz; H-3), 7.50 (1H, dd, $J = 2.1$, 8.4 Hz; H–6′), 7.45 (1H, s; H–9), 7.44 (1H, d, $J = 2.1$ Hz; H–2′), 7.17 (1H, s; H-6), 7.05 (1H, d, $J = 8.4$ Hz; H-5′), 4.03 (3H, s; 8-OMe), 4.01 (3H, s; 3′-OMe), 4.00 (3H, s; COOMe), 3.99 (3H, s; 7-OMe), 3.98 (3H, s; 4′-OMe).