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Dihydrodictyopyrone A and C: new members of dictyopyrone family isolated from *Dictyostelium* cellular slime molds

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Abstract—We have explored the diversity of secondary metabolites produced by cellular slime molds to evaluate if they are valuable resources for biologically potential substances. From the methanol extract of fruiting bodies of *Dicyostelium firmibasis*, we obtained new α-pyranoids, dihydrodictyopyrone A (**1**) and C (**2**). Their structures including absolute configurations were determined by spectral means and asymmetric total synthesis. Compounds **1** and **2** are new members of the dictyopyrone family, which are characteristic secondary metabolites of various species of *Dictyostelium* cellular slime molds.

The cellular slime mold *Dictyostelium discoideum* is thought to be an excellent model organism for the study of cell and developmental biology because of its simple pattern of development.[1] Vegetative amoeba of *D. discoideum* grow by eating bacteria. When starved, they initiate a developmental program of morphogenesis and gather to form a slug-shaped multicellular aggregate. This aggregate then differentiates into two distinct cell types, prespore and prestalk cells, which are precursors of spores and stalk cells, respectively. Eventually, they form a fruiting body consisting of spores and a multicellular stalk.

Several small molecules including DIF-1,[2] discadenine,[3] and cAMP[4] have been reported as development-regulating substances of cellular slime molds. However, few other small molecules have been reported except an antibacterial substance AB0022A[5] and a resorcinol derivative MPBD.[6] We have focused on the utility of cellular slime molds as a resource for novel drug development, and have studied the diversity of secondary metabolites of cellular slime molds.[7a–e] We have recently isolated α-pyronoids, named dictyopyrone A–D (**3**–**6**), from various species of *Dictyostelium* cellular slime molds.[7a] It was shown that they inhibit cell growth, enhance morphogenesis and promote stalk cell differentiation of *D. discoideum*.[8a–c] In this study, we describe the structure elucidation and syntheses of dihydrodictyopyrone A (**1**) and C (**2**), new members of the dictyopyrone family, isolated from *Dictyostelium firmibasis* (Fig. 1).

Fruiting bodies (wet weight 346 g) of the cellular slime mold, *D. firmibasis*, were cultured on plates and extracted three times with methanol at room temperature to yield an extract (10.7 g), which was partitioned with ethyl acetate and water. The ethyl acetate solubles (2.28 g) were separated by repeated column chromatography over SiO_2 and ODS to yield dihydrodictyopyrone A (**1**) (10.1 mg) and C (**2**) (8.6 mg), respectively.

The ^{13}C and ^{1}H NMR spectra of **1**, including an enolic hydroxyl proton at δ_H 14.17 (1H, br s), showed that **1** is an equilibrium mixture of keto-enol tautomers in a ratio of 2:3 (Table 1). The molecular formula $C_{19}H_{32}O_3$ indicated for **1** was established by HREI-MS (*m*/*z* 308.2263). We first analyzed the structure of the keto tautomer (**1a**). The ^{13}C NMR spectrum of **1a** showed

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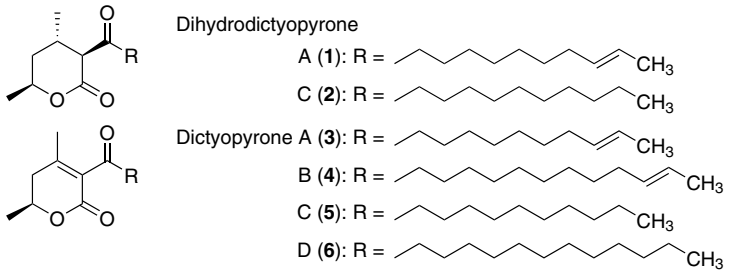


Figure 1. Structures of dihydrodictyopyrones and dictyopyrones.

Table 1. ^{13}C and ^{1}H NMR spectral data of dihydrodictyopyrone A (**1**)[a]

	Keto form		Enol form	
	^{13}C	^{1}H	^{13}C	^{1}H
2	169.3		173.2	
3	59.3	3.22 (1H, d, $J = 8.3$ Hz)	97.8	
4	25.8	2.55–2.62 (1H, m)	26.2	2.77–2.83 (1H, m)
5	35.6	1.57–1.60 (1H, m)	36.8	1.67–1.72 (2H, m)
		1.84 (1H, ddd, $J = 14.3$, 9.0, 6.8 Hz)		
6	74.0	4.53–4.59 (1H, m)	71.4	4.55–4.61 (1H, m)
7	20.9	1.02 (3H, d, $J = 6.8$ Hz)	21.7	1.12 (3H, d, $J = 7.1$ Hz)
8	21.2	1.37 (3H, d, $J = 6.6$ Hz)	21.7	1.36 (3H, d, $J = 6.4$ Hz)
1′	205.2		179.5	
2′	43.7	2.72 (1H, dt, $J = 17.5$, 7.7 Hz)	31.5	2.22–2.28 (1H, m)
		2.51 (1H, dt, $J = 17.5$, 7.2 Hz)		2.29–2.36 (1H, m)
3′	23.4	1.57–1.62 (2H, m)	26.6	1.57–1.62 (2H, m)
4′–8′	29.6	1.23–1.34 (10H, m)	29.6	1.23–1.34 (10H, m)
	29.5		29.5	
	29.4		29.4	
	29.3		29.3	
	29.1		29.1	
9′	32.6	1.91–1.96 (2H, m)	32.6	1.91–1.96 (2H, m)
10′	131.6	5.37–5.41 (2H, m) (H10′ and H11′)	131.6	5.37–5.41 (2H, m) (H10′ and H11′)
11′	124.6		124.6	
12′	17.9	1.61–1.62 (3H, m)	17.9	1.61–1.62 (3H, m)
1′-OH				14.17 (1H, br s)

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.

the presence of a keto carbonyl, an ester carbonyl, two olefinic, an oxymethine, two methine, nine methylene, and three methyl carbons. ^{1}H ^{1}H COSY revealed that C-3–C-4(–C-7)–C-5–C-6–C-8, C-2′–C-3′, and C-9′–C-10′–C-11′–C-12′ were connected. The correlations of H-3–C-2, H-3–C-1′, H-4–C-2, H-4–C-1′, H-2′–C-1′, and H-3′–C-1′ in the HMBC spectrum indicated the partial structure A, in which the ester group was attached to C-6, an oxymethine carbon (δ_C 74.0) (Fig. 2). In the partial structure B, the *E* configuration was assigned to the C-10′–C-11′ double bond by comparison of the chemical shifts of C-9′–C-12′ of **1a** with those of dictyopyrone A (**3**). All signals of remaining five methylene carbons in NMR spectra were observed in the aliphatic region, δ_H 1.23–1.34 and δ_C 29.1–29.6, indicating that the partial structures A and B were connected with the methylene chain. These findings revealed the planar structure of **1a**, which had a saturated ring instead of α-dihydropyrone ring in dictyopyrone A (**3**).[7a] Thus, the keto-enol tautomerism of **1** was caused by its β-ketoester moiety. The structure of the enol tautomer (**1b**) was also confirmed by ^{1}H ^{1}H COSY and HMBC spectrum (Fig. 2). NOESY spectrum exhibited

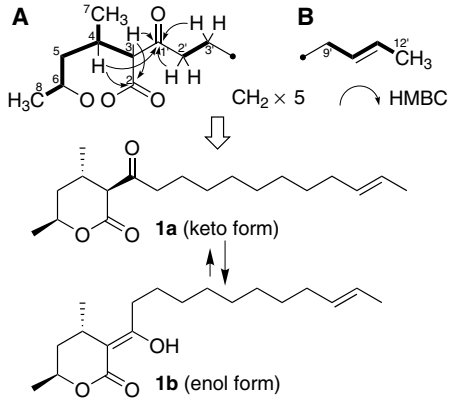


Figure 2. Planar structure of dihydrodictyopyrone A (**1**).

cross peaks for the keto form (**1a**) as follows: H-3–H-6, H-3–H-7, H-5α–H-6, H-6–H-7, and indicated that the relative configuration of **1a** was 3*S**, 4*S**, and 6*S** with the boat conformer bearing three equatorial substituents (Fig. 3). For the enol form (**1b**), the correlation of H_3-7–H-6 and H-4–H-2′ in NOESY revealed the *Z* con-

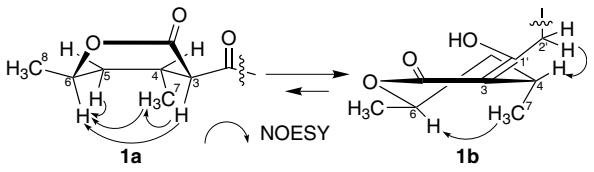


Figure 3. Relative structure of dihydrodictyopyrone A (**1**).

figuration of the C-3–C-1′ double bond with the pseudo-chair conformer.

The HREI-MS (*m*/*z* 310.2502) of dihydrodictyopyrone C (**2**) showed a molecular formula $C_{19}H_{34}O_3$, which differs from that of **1** by two hydrogen atoms. Although the ^{1}H NMR spectra of **1** and **2** were very similar, the signals of two olefinic protons (δ 5.37–5.41) disappeared in the case of **2**. It was thus suggested that the structure of dihydrodictyopyrone C (**2**) had a saturated side chain, which corresponded to dictyopyrone C (**5**) (Table 2).

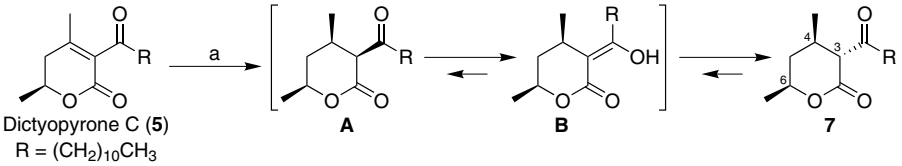
To determine absolute configuration of **1** and **2**, conversion of dictyopyrone C (**5**) into **2** was intended. Compound **5** was synthesized by the method of our previous work.[8b] Catalytic hydrogenation of **5**, however, did not afford dihydrodictyopyrone C (**2**), but compound **7** as a sole product via the intermediates **A** and **B** (Scheme 1). Reduction of **5** by $NaBH_4$ also gave only **7**. Since compound **7** has the same relative configuration with the C-6 epimer of dihydrodictyopyrone C (**2**), we planed to convert *ent*-dictyopyrone C (*ent*-**5**) into dihydrodictyopyrone C (**2**) by the reduction of C-3–C-4 double bond and the epimerization at C-6 (Scheme 2).

Catalytic hydrogenation of *ent*-dictyopyrone C[8b] (*ent*-**5**) gave *ent*-**7**. Treatment of *ent*-**7** with $NaBH_4$ at 70 °C induced reduction of both of the ketone and lactone. Acetonidation of resulting triol produced **8** as diastereomixture. After tosylation of **8**, S_N2 reaction with sodium acetate in DMF–HMPA (1:1) afforded the C-6 epimerized product **9**. The acidic hydrolysis of acetonide and acetate provided triol **10**, which was oxidized and lactonidized by TEMPO to give lactone **11**. Finally, Dess–Martin oxidation of **11** allowed us to complete the synthesis of dihydrodictyopyrone C (**2**). All of the spectral data on synthetic dihydrodictyopyrone C (**2**), including its specific rotation (natural $[\alpha]_D$ −19.2 (*c* 0.359, $CHCl_3$), synthetic $[\alpha]_D$ −21.1 (*c* 0.356, $CHCl_3$)), were identical with those of its natural compound. From this result, the absolute configuration of dihydrodictyopyrone C (**2**) was determined to be 3*S*, 4*S*, and 6*S*. The wave length and intensity of the Cotton effect in the CD

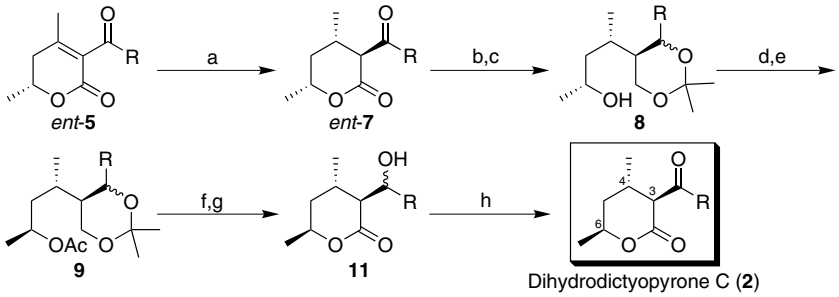
Table 2. ^{13}C and ^{1}H NMR spectral data of dihydrodictyopyrone C (**2**)[a]

	Keto form		Enol form	
	^{13}C	^{1}H	^{13}C	^{1}H
2	169.3		173.2	
3	59.3	3.22 (1H, d, J = 8.3 Hz)	97.8	
4	25.8	2.55–2.62 (1H, m)	26.2	2.78–2.83 (1H, m)
5	35.6	1.58–1.63 (1H, m)	36.8	1.67–1.71 (2H, m)
		1.84 (1H, ddd, J = 14.3, 9.0, 6.8 Hz)		
6	74.0	4.54–4.59 (1H, m)	71.4	4.55–4.60 (1H, m)
7	20.9	1.02 (3H, d, J = 6.8 Hz)	21.7	1.12 (3H, d, J = 7.1 Hz)
8	21.2	1.37 (3H, d, J = 6.8 Hz)	21.7	1.37 (3H, d, J = 6.8 Hz)
1′	205.2		179.5	
2′	43.7	2.73 (1H, dt, J = 17.3, 7.1 Hz)	31.5	2.30–2.36 (1H, m)
		2.51 (1H, dt, J = 17.3, 7.1 Hz)		2.23–2.29 (1H, m)
3′	23.4	1.58–1.63 (2H, m)	26.6	1.58–1.63 (2H, m)
4′–9′	29.6	1.21–1.32 (16H, m) (H4′–H11′)	29.6	1.21–1.32 (16H, m) (H4′–H11′)
	29.5		29.5	
	29.5		29.5	
	29.4		29.4	
	29.4		29.4	
	29.3		29.3	
10′	31.9		31.9	
11′	22.7		22.7	
12′	14.1	0.86 (3H, t, J = 7.1 Hz)	14.1	0.86 (3H, t, J = 7.1 Hz)
1′-OH				14.17 (1H, *t*, J = 0.8 Hz)

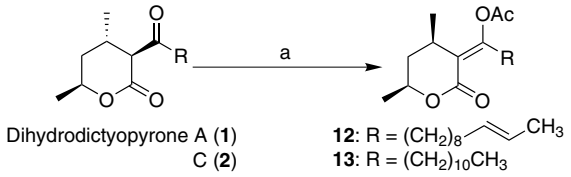
[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.



Scheme 1. Reduction of dictyopyrone C (**5**). Reagents and conditions: (a) H_2 (1 atm), $Pd(OH)_2$/C, EtOAc, rt (74%).



Scheme 2. Synthesis of dihydrodictyopyrone C (**2**). Reagents and conditions: (a) H_2 (1 atm), $Pd(OH)_2$/C, EtOAc, rt (84%); (b) $NaBH_4$, EtOH, 60 °C (85%); (c) 2,2-dimethoxymethane, *p*-TsOH, DMF, rt (68%); (d) *p*-TsCl, pyridine, DMAP, rt; (e) AcONa, DMF–HMPA (1:1), 70 °C (50% (two steps)); (f) 10% HCl–MeOH, rt (78%); (g) TEMPO, NCS, TBACl, CH_2Cl_2, H_2O, rt (87%); (h) Dess–Martin periodinane, CH_2Cl_2, rt (74%).



Scheme 3. Acetylations of dihydrodictyopyrone A (**1**) and C (**2**). Reagents and conditions: (a) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt (47% for **1** and 55% for **2**).

spectra of **12** and **13** (λ ($\Delta\varepsilon$) 226.0 nm (2.46) (MeOH) and 226.2 nm (2.78) (MeOH), respectively), which were the acetate of dihydrodictyopyrone A (**1**) and C (**2**) (Scheme 3), respectively, were almost the same, suggesting that the absolute configuration of **1** was the same of **2**.

As already mentioned, it was revealed that dictyopyrones showed several biological effects on the life cycle of *D. discoideum* such as inhibition against cell growth, enhancement of morphogenesis[8a,b] and promotion of stalk cell differentiation.[8c] Thus, we examined these activities of dihydrodictyopyrone C (**2**) and its stereoisomer **7**. These compounds at up to 60 μM showed, however, no effect on cell growth and morphogenesis. In order to assess the effect on stalk cell differentiation, we utilized *D. discoideum* HM44 cells,[9] which is a differentiation-inducing factor (DIF)-deficient strain, and can differentiate in vitro into stalk cells, only if DIF-1[2] is supplied into the culture. As shown in Figure 4, all compounds except dictyopyrone A (**3**) hardly induced stalk cell formation in HM44 cells (black bars). In the presence of 0.2 nM DIF-1 (white bars), stalk cell formation was clearly promoted by **3** and **5** as previously reported,[8c] and dihydrodictyopyrone A (**1**) also showed weak effect. While, other compounds had no activities. These facts suggested that stereochemistry and the double bond between C-3 and C-4 in 2*H*-pyrane moiety were important for the biological activity.

Dihydrodictyopyrone A (**1**) and C (**2**) are isolated from various species of *Dictyostelium* cellular slime molds,[7a,8c] although they did not have biological activities on *D. discoideum* cells. The isolation of novel class compounds such as dictyopyrones and DIF-1 shows that cellular slime molds are promising resources for natural product

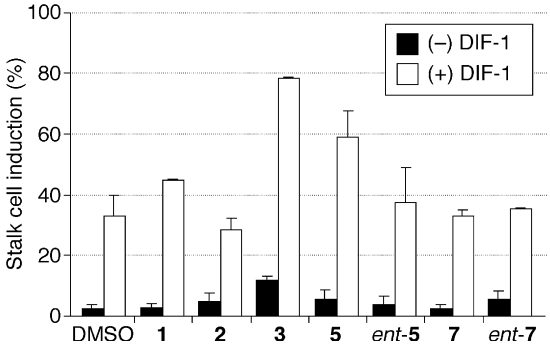


Figure 4. Effects of **1**–**3**, **5**, *ent*-**5**, **7**, and *ent*-**7** on stalk differentiation in *D. discoideum* HM44 cells. HM44 cells were incubated in vitro with cAMP (5 mM) and **1**–**3**, **5**, *ent*-**5**, **7**, and *ent*-**7** (each 10 μM), or vehicle (0.2% DMSO) in the presence or absence of 0.2 nM DIF-1. The stalk cell population was assessed using a phase-contrast microscope on day 2. Data are the mean values ±SD of two independent experiments.

chemistry. In addition, dictyopyrones may be chemotaxonomic indicators of cellular slime molds.

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