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Novel prenylated and geranylated aromatic compounds isolated from *Polysphondylium* cellular slime molds

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ABSTRACT

We have studied the diversity of secondary metabolites of cellular slime molds to utilize them as new biological resources for natural product chemistry. From the methanol extract of fruiting bodies of *Polysphondylium tenuissimum*, we obtained five prenylated and geranylated aromatic compounds, Pt-1–5 (**1**–**5**). An additional aromatic compound, Ppc-1 (**6**), was isolated from *Polysphondylium pseudo-candidum*. The structures of these compounds were determined by spectral analysis, and synthetic routes to **4**, **5**, and **6** were developed. Compound **5** showed the glucose consumption-promotive activity on 3T3-L1 cells.

1. Introduction

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 cells of *D. discoideum* grow as single amoebae 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. At the end of its development, the aggregate forms a fruiting body consisting of spores and a multicellular stalk.

Several small molecules including DIFs (Differentiation-Inducing Factors),[2] discadenine,[3] and cAMP[4] have been reported as development-regulating substances of cellular slime molds. DIFs and their derivatives also exhibit many biological effects in mammalian cells, such as suppression of cell growth,[5–7] induction/promotion of cell differentiation,[5a,d] promotion of glucose consumption[7,8] and regulation of IL-2 production.[9] A chlorine-substituted dibenzofuran derivative AB0022A[10] and a resorcinol derivative MPBD[11] were also isolated from cellular slime molds, although no other reports have, to date, described the secondary metabolites of cellular slime molds.

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.[12] Cellular slime molds are classified into three genera, *Dictyostelium*, *Polysphondylium*, and *Acytostelium*, according to their morphology.[13] We have recently isolated α-pyronoids,[12a,f] amino sugar derivatives,[12c,d] and aromatics[12b,e] with unique structures from several species of *Dictyostelium*. This paper reports the isolation, structure elucidation and synthesis of new prenylated and geranylated aromatic compounds Pt-1–5 (**1**–**5**) and Ppc-1 (**6**) from *Polysphondylium* cellular slime molds (Fig. 1). The antiproliferative and glucose consumption–promotive activities of these compounds in mammalian cells are also described.

2. Results and discussion

2.1. Isolation and structure elucidation

Fruiting bodies (dry weight 73.6 g) of the cellular slime mold, *Polysphondylium tenuissimum*, were cultured on plates and extracted three times with methanol at room temperature to yield an extract (18.2 g) that was subsequently partitioned between ethyl acetate and water. The ethyl acetate solubles (5.49 g) were separated by repeated column chromatography over SiO_2 and ODS to

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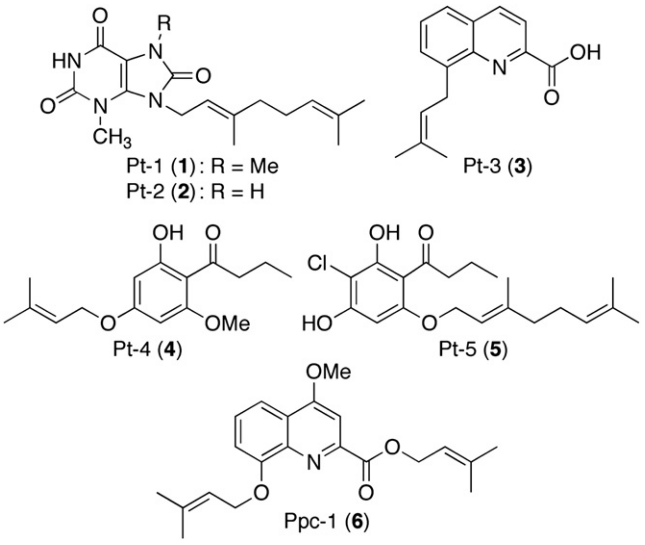

Figure 1. Structures of Pt-1–5 (**1**–**5**) and Ppc-1 (**6**).

yield Pt-1 (**1**) (1.8 mg), Pt-2 (**2**) (9.7 mg), Pt-3 (**3**) (0.8 mg), Pt-4 (**4**) (2.6 mg), and Pt-5 (**5**) (4.7 mg). In the same manner, Ppc-1 (**6**) (1.8 mg) was obtained from the fruiting bodies (dry weight 57.3 g) of *Polysphondylium pseudo-candidum*.

HREIMS (*m*/*z* 332.1835 $[M]^+$), ^1H and ^{13}C NMR spectra indicated that the molecular formula of **1** was $C_{17}H_{24}N_4O_3$. The ^{13}C NMR spectrum of **1** showed the presence of seven quaternary sp^2 carbons, two tertiary sp^2 carbons, three methylene carbons and five methyl carbons (Table 1). ^1H–^1H COSY revealed that C-1′–C-2′ and C-4′–C-5′–C-6′ were connected. The correlations of H_3-9′–C-2′, C-3′, and C-4′; and H_3-10′–C-6′, C-7′, and C-8′ in the HMBC spectrum indicated a geranyl group. The molecular formula of the structure with neither the geranyl group ($C_{10}H_{17}$) nor the two methyl groups was $C_5HN_4O_3$, suggesting the presence of a trisubstituted uric acid moiety. This was supported by the observation that the chemical shifts of the five remaining sp^2 carbons (δ 152.6 (C-6), 151.8 (C-8), 149.7 (C-2), 137.1 (C-4), and 99.7 (C-5)) were consistent with those of uric acid[14] (δ 153.4, 152.6, 150.1, 136.6, and 97.1). The correlations between the three sets of peaks: H_3-11 with C-2 and C-4; H_3-12 with C-5 and C-8; and H_2-1′ with C-4 and C-8, in the HMBC spectrum, indicated that two methyl groups (C-11 and C-12) and a geranyl group were attached to N-3, N-7, and N-9, yielding the complete structure of **1** (Fig. 2).

Table 1
^{13}C and ^1H NMR spectral data of Pt-1 (**1**), Pt-2 (**2**) and **7**

	Pt-1 (**1**)[a]		Pt-2 (**2**)[b]		**7**[a]
	^{13}C	^1H	^{13}C	^1H	^{13}C
2	149.7		151.5		150.8
4	137.1		137.6		135.4
5	99.7		99.8		99.5
6	152.6		154.4		153.5
8	151.8		153.2		151.9
10					28.2
11	30.5	3.61 (3H, s)	30.0	3.68 (3H, s)	30.5
12	29.3	3.56 (3H, s)			29.3
1′	41.8	4.66 (2H, d, *J*=6.0 Hz)	41.2	4.82 (2H, d, *J*=5.7 Hz)	41.8
2′	119.4	5.08–5.12 (1H, m)	121.4	5.36–5.40 (1H, m)	119.6
3′	140.7		139.5		140.5
4′	39.2	2.03 (2H, t, *J*=7.2 Hz)	39.4	2.05 (2H, t, *J*=7.0 Hz)	39.2
5′	26.1	2.07 (2H, q, *J*=7.2 Hz)	26.4	2.11 (2H, q, *J*=7.0 Hz)	26.1
6′	123.3	4.98–5.03 (1H, m)	124.3	5.07–5.12 (1H, m)	123.3
7′	132.3		131.8		132.3
8′	25.7	1.64 (3H, d, *J*=1.0 Hz)	25.7	1.63 (3H, d, *J*=0.8 Hz)	25.7
9′	16.7	1.72 (3H, d, *J*=1.0 Hz)	16.4	1.71 (3H, d, *J*=1.0 Hz)	16.7
10′	17.7	1.56 (3H, s)	17.7	1.52 (3H, s)	17.7
1-NH		8.08 (1H, br s)		13.40 (1H, br s)[c]	
9-NH				13.29 (1H, br s)[c]	

[a] ^1H (600 MHz) and 150 MHz for ^{13}C in $CDCl_3$.
[b] ^1H (600 MHz) and 150 MHz for ^{13}C in pyridine-d_5.
[c] These signals were indistinguishable.

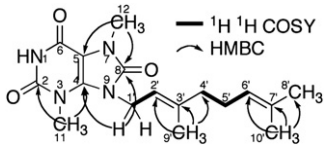

Figure 2. Structural elucidation of Pt-1 (**1**).

HREIMS of **2** (*m*/*z* 318.1688 $[M]^+$) indicated a molecular formula, $C_{16}H_{22}N_4O_3$, which differed from that of **1** by a methylene unit. The ^1H and ^{13}C NMR spectra of **2** were nearly identical to those of **1** (Table 1), suggesting that the methyl group and geranyl group were attached to the uric acid moiety in the structure of **2**. In the HMBC spectrum of **2**, the two sets of correlation peaks: H_3-11 to the signals at δ_c 137.6 (C-4) and 151.5; and H_2-1′ to the signals at δ_c 137.6 (C-4) and 153.2, indicated that these groups were bonded to N-3 and N-9 (Fig. 3). However, the positions of each substituent could not be determined because the chemical shifts of the signals at δ_c 151.5 and 153.2 were too close to assign them to C-2 and C-8, respectively. To resolve this issue, compound **2** was methylated. The HMBC spectrum of the product **7** showed two sets of correlation peaks: between H_3-12 and the signals at δ_c 99.5 and 151.9; and between H_2-1′ and the signals at δ_c 135.4 and 151.9. Because the signals at δ_c 99.5 and 135.4 could be readily assigned to C-5 and C-4, respectively, the position of the geranyl group was assigned to N-9.

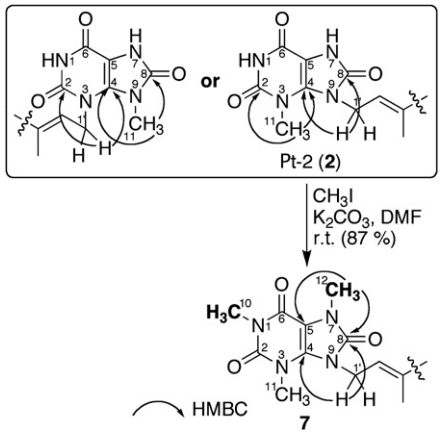

Figure 3. Structural elucidation of Pt-2 (**2**).

Compound **3** showed a molecular ion peak at *m*/*z* 241.1091 in HREIMS, suggesting that its molecular formula was $C_{15}H_{15}NO_2$. The ^{13}C NMR spectrum of **3** showed the presence of one carbonyl, five sp^2 quaternary, six sp^2 tertiary, one methylene, and two methyl carbons (Table 2). The ^1H NMR spectrum showed the presence of a 1,2,3-trisubstituted benzene ring (δ 7.78 (1H, dd, *J*=8.1, 1.2 Hz, H-5), 7.62 (1H, dd, *J*=8.1, 7.1 Hz, H-6), and 7.65 (1H, dd, *J*=7.1, 1.2 Hz, H-7)). Other low field-shifted proton signals at δ 8.26 (1H, d, *J*=8.3 Hz, H-3) and 8.39 (1H, d, *J*=8.3 Hz, H-4) implied the presence of a nitrogen-containing heterocycle, and the HMBC correlations shown in Figure 4 gave 2,8-disubstituted quinoline moiety. Moreover, the presence of a prenyl group at C-8 was revealed by the correlation of H_2-1′–H-2′ in the ^1H–^1H COSY and the correlations of H_3-5′–C-2′, C-3′, and C-4′; and H_2-1′–C-7, C-8, and C-8a in the HMBC spectrum. Therefore, the remaining moiety, CHO_2, was assigned as a carboxyl