Dechlorogeodin and Its New Dihydro Derivatives, Fungal Metabolites with Herbicidal Activity

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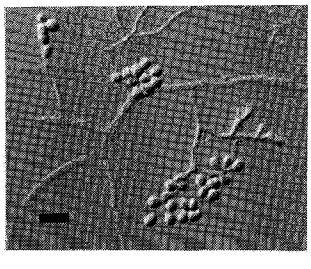
During our search for new bioactive microbial metabolites, a fungal soil isolate, *Chrysosporium* sp. FO-4712, was found to produce anti-*Bacillus subtilis* substances detectable in Davis defined medium. The anti-*Bacillus* activity, extracted into an ethyl acetate layer, was antagonized by casamino acids and was associated with herbicidal activity. The active principles isolated and characterized were dihydrobisdechlorogeodin (**1**), a new compound, and bis-dechlorogeodin (**2**) and sulochrin, both known fungal metabolites. Here we describe the fermentation, isolation, characterization and biological activities of **1** and **2**.

Taxonomy of Producing Organism

Strain FO-4712 was originally isolated from a soil sample collected at the Lake Mendota, Madison, U.S.A. Colonies of the fungus grew rather restrictedly on peptone yeast extract (PYE) agar, attaining a diameter of 20~25 mm with a color in pastel yellow after incubation at 25°C for 14 days. Reverse of colonies was reddish yellow. No soluble pigment was produced. Microscopic observations of the morphology of FO-4712 grown on PYE agar revealed conidiophores born from substrate hyphae and branched verticillatelly (Fig. 1). The aleuriospores were born singly at the tips, and were typically subglobose to pyriform with smooth or roughened hyaline walls. They were $2\sim4\times3\sim5\,\mu m$ in size. From these characteristics, strain FO-4712 was identified as *Chrysosporium* sp.[1]

Fig. 1. Photomicrograph of strain FO-4712 on PYE agar (scale: 10 μm).



Fermentation

Chrysosporium sp. FO-4712 was grown in 500-ml SAKAGUCHI flasks at 27°C for 7 days with reciprocal shaking (110 rpm). The flasks contained 100 ml of a fermentation medium (soluble starch 3%, glycerol 1%, soybean meals 2%, dry yeast 0.3%, KCl 0.3%, $CaCO_3$ 0.2%, $MgSO_4\cdot7H_2O$ 0.05%, pH 6.5), to which allophane 0.5% was supplemented. Allophane is an alumino-silicic mineral with phosphate-trapping potential; is often able to enhance antibiotic biosynthesis, when it is susceptible to phosphate regulation[2]. Under the above fermentation conditions with added allophane, antibiotic activity against *Bacillus* sp. reached 400 units/ml (arbitrary units) at day 7, while it was less than 10 units/ml without allophane.

Isolation

The isolation procedures for active substances are summarized in Fig. 2. The culture broth of FO-4712 was centrifuged and the supernatant (7.4 liters) was extracted with an equal volume of ethyl acetate. The acetone (5 liters) extract of the mycelial cake was concentrated *in vacuo* and the resulting aqueous solution was extracted twice with an equal volume of ethyl acetate. The ethyl acetate layers were combined and concentrated to dryness. The extract (1.0 g) was partitioned between chloroform and water. The chloroform layer was concentrated under reduced pressure to give crude material (755 mg). The organic extract was chromatographed on a silica gel column, eluting stepwise with mixtures of chloroform-acetone. Active fractions 1 (100 mg) and 2 (85 mg) were purified by HPLC (column; Cosmosil ODS MS, 10 mm i.d. × 250 mm, mobile phase; acetonitrile-THF-water (22 : 3 : 75), flow rate; 3.0 ml/minute, detection; UV at 250 nm) to give FO-4712 I (**1**, 23 mg), FO-4712 II (**2**, 47 mg), and FO-4712 III (8.6 mg). FO-4712 III was identified as sulochrin[3,4].

Fig. 2. Isolation procedures FO-4712 for substances.

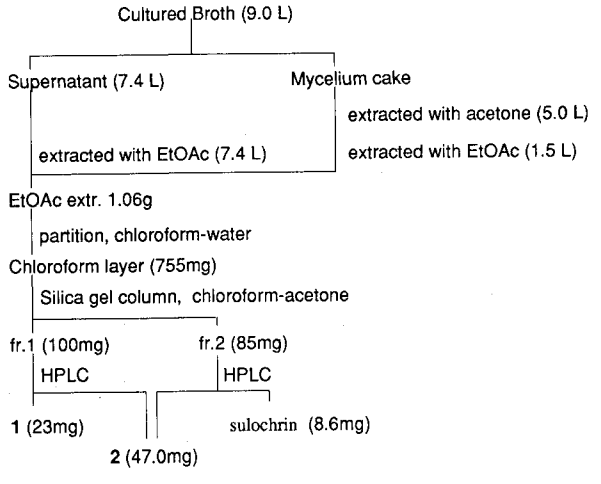


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Table 1. Physico-chemical properties of **1** and **2**.

	1	**2**
Appearance	White powder	White powder
$[\alpha]_D$ (in EtOH)	+255° (*c* 0.1)	−107° (*c* 0.1)
EI-MS (*m/z*)	332 $(M)^+$	330 $(M)^+$
FAB-MS (*m/z*)	333 $(M+H)^+$	331 $(M+H)^+$
HR-FAB-MS Found.	333.0892	331.0827
Calcd.	333.0974 for $C_{17}H_{17}O_7$	331.0818 for $C_{17}H_{15}O_7$
Molecular formula	$C_{17}H_{16}O_7$	$C_{17}H_{14}O_7$
IR ν_{max}^{KBr} cm^{-1}	2950, 1740, 1720, 1690, 1625	2955, 1715, 1660, 1625
UV λ_{max}^{EtOH} (ε)	205 (19,900), 263 (15,500), 341 (2,800)	205 (30,000), 285 (11,100)

Table 2. ^{13}C and ^1H NMR spectral data of **1** and **2** δ=ppm in $CDCl_3$.

No.	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
2	85.4 s		83.9 s	
3	194.2 s		194.2 s	
3a	107.0 s		107.3 s	
4	171.8 s		172.1 s	
5	104.1 d	6.42 1H s	104.7 d	6.47 1H s
6	153.2 s		153.3 s	
7	109.8 d	6.37 1H s	109.9 d	6.41 1H s
7a	155.7 s		156.0 s	
1′	47.4 d	3.70 1H dd, *J*=13.7, 4.9 Hz	137.9 s	
2′	35.5 t	2.79 1H dd, *J*=16.7, 4.9 Hz 3.32 1H dd, *J*=16.7, 13.7 Hz	137.0 d	7.11 1H d, *J*=1.0 Hz
3′	196.7 s		185.4 s	
4′	104.2 d	5.56 1H d, *J*=1.0 Hz	104.0 d	5.80 1H d, *J*=1.0 Hz
5′	169.5 s		168.9 s	
6-CH_3	23.0 q	2.37 3H s	23.0 q	2.40 3H s
1′-CO	170.1 s		163.3 s	
1′-COOCH_3	52.5 q	3.60 3H s	52.9 q	3.70 3H s
5′-OCH_3	56.9 q	3.64 3H s	56.8 q	3.77 3H s

Structure Elucidation

The physico-chemical properties of **1** and **2** are summarized in Table 1. The molecular formulae $C_{17}H_{16}O_7$ and $C_{17}H_{14}O_7$, respectively, were established by HR-FAB-MS. The ^1H and ^{13}C NMR chemical shifts of **1** and **2** are as shown in Table 2. The characteristic resonances in the ^1H NMR of these compounds arose from two oxymethyl proton signals and one signal for a methyl proton on an aromatic ring.

Compound **1** was obtained as white powder. The molecular formula of **1** indicates 10 degrees of unsaturation. The ^{13}C NMR of **1** indicated the presence of three carbonyl groups and eight olefinic carbons, suggesting a tricyclic system. In the ^1H NMR spectrum of **1**, signals for one methyl, two oxymethyls, three olefinic protons, an aliphatic methine proton and one methylene proton were observed. The ^1H-^1H COSY spectrum revealed the presence of a pair of meta coupled olefinic protons (δ 6.42 and δ 6.37) and a partial structure $-CH-CH_2-$. The ^{13}C NMR data of **1** showed seventeen carbon signals including nine quaternary carbons. The connectivities through quaternary carbons in **1** were deduced by HMBC experiments. A pair of two olefinic protons (δ 6.42 and δ 6.37, 5-H, 7-H, respectively) were correlated with a quaternary carbon signal at δ 107.0 (C-3a) and a methyl carbon signal at δ 23.0 (6-CH_3). 5-H was also correlated with an oxygenated carbon signals at δ 171.8 (C-4). 7-H was correlated with another oxygenated carbon signal at δ 155.7 (C-7a). Additionally, a methyl proton signal at δ 2.37 (6-CH_3) was correlated with C-5, 6 and 7. These results indicated a tetra-substituted benzene ring as in the partial structure shown in Fig. 3(a). Other HMBC correlations are as follows: an olefinic proton signal at δ 5.56 (4′-H) was correlated with a carbonyl carbon (δ 196.7, C-3′), an oxygenated olefinic carbon (δ 169.5, C-5′) and an oxygenated quaternary sp^3 carbon (δ 85.4, C-2). A methine proton signal at δ 3.70 (1′-H) and methylene proton signals at δ 2.79 and 3.32 (2′-H) were correlated with C-2 and C-5′, respectively, by three bonded coupling. The methine proton was also correlated with a carbonyl carbon signal at δ 192.4. Two oxymethyl proton signals at δ 3.64 and

Fig. 3. HMBC correlations of compound **1**.

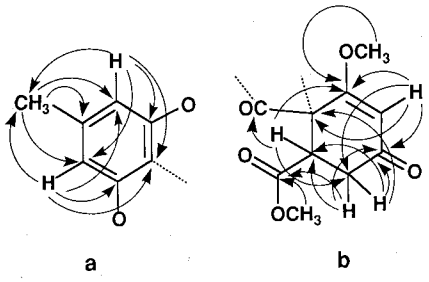
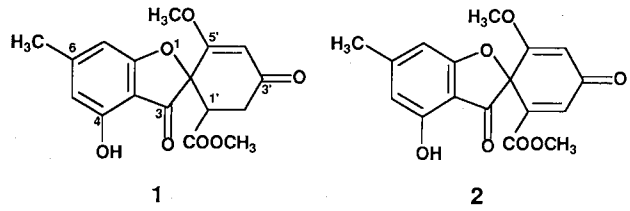


Fig. 4. Structures of dihydro-bisdechlorogeodin (**1**) and (−)-bisdechlorogeodin (**2**).



3.60 were correlated with an oxygenated olefinic carbon (C-5′) and a carbonyl carbon (δ 170.1), respectively. In addition, a methylene proton signal at δ 2.79 was correlated with the same carbonyl carbon on C-1′. These data indicated that the former signal of oxymethyl proton is a methoxy group at C-5′, the latter is a methyl ester group at C-1′. From these HMBC correlation, the presence of a cyclohexenone ring shown in Fig. 3(b) was deduced. The connectivity of the partial structures (a) and (b) is as follows; from its molecular formula and partial structures, a quaternary oxygenated olefinic carbon (C-7a) in (a) and an oxygenated olefinic carbon (C-2) in (b) were connected through an oxygen. The carbonyl carbon at C-3 must be attached at C-3a as indicated by the chemical shift of the latter, forming the third ring. These results revealed the structure of **1** 1′,2′-dihydro-bisdechlorogeodin (Fig. 4).

Compound **2** was obtained as white powder. Its molecular weight was two mass units less than that of **1**. The ^{1}H NMR spectrum of **2** was very similar to that of **1**. However, an aliphatic methine proton signal and one methylene proton signals were not observed. Instead, an olefinic proton signal appeared at δ 7.11. The ^{13}C NMR data for **2** showed two olefinic carbon signals appearing at δ 137.9 and δ 137.0, but the signals of one methine carbon and one methylene carbon observed in **1** were lacking. Fifteen other carbon signals of **2** agreed with those of **1**. The HMBC correlations were observed between an olefinic proton signal at δ 7.11 (2′-H) and C-2 and 2′-*C*OOCH$_3$. The results suggested that **2** was 1′,2′ didehydro derivative of **1**. Based on the above data and the other spectral data of **2**, the structure of **2** was determined to be (−)-bisdechlorogeodin[3~7] (Fig. 4).

Biological Activity

In a conventional paper disc assay (8 mm i.d. thick disc, containing 50 μl of a sample solution), **2** (100 μg/ml-acetone) gave inhibition zones against *Bacillus subtilis* of 23.5 mm in DAVIS' defined agar, and 17.2 mm in nutrient agar. It was weakly active against *Staphylococcus aureus* (13.5 mm) and *Micrococcus luteus* (12.5 mm), but, was inactive against yeasts and filamentous fungi tested. **1** (1000 μg/ml) was inactive as antibiotic.

The anti-*Bacillus* activity of **2** was antagonized by amino acids. Namely, the inhibition zone (23.5 mm) was reduced to 12.0, 13.5, 13.5 and 12.0 (hazy zone), when L-alanine, L-aspartic acid, L-glutamic acid, and casamino acids, respectively, were supplemented to DAVIS' agar. It is suggested that **2** is an amino acid-metabolic antagonist, rather than an antibacterial compound with a non-specific action mechanism.

Herbicidal activity was examined using radish (*Raphanus sativus*) and sorghum (*Sorghum bicoler*) as test plants in small test tubes by the method described previously[8]. **1** and **2** (each at 1000 μg/tube) inhibited the germination and proliferation of sorghum by 60 and 70%, respectively, but were inactive against radish.

The herbicidal activity of **2** is attributed to its amino acid antimetabolic activity. We propose that the herbicidal activity of **1** arises from transport inside the plant cells, followed by transformation into **2**. This hypothesis needs to be verified.

Both **1** and **2** are lipophilic compounds and bear no structural similarity to amino acids. This does not necessarily make the above interpretation unlikely. The chemically-synthesized, lipophilic herbicide chlorosulfuron is not a structural analogue of amino acids, but blocks isoleucine-valine biosynthesis by inhibiting acetolactate synthase[9].

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