NOTES

F390B and C, New Antitumor Dihydroxanthone Derivatives Isolated from *Penicillium* sp.

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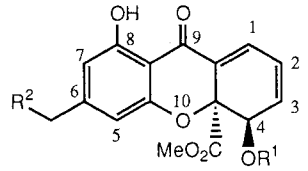
In our search for new antitumor substances, cytotoxic dihydroxanthone derivatives, F390 (**3**) and F390B (**1**) were isolated from the mycelium extract of *Penicillium* sp. AJ117292, and F390C (**2**) was isolated from *Penicillium* sp. AJ117291, respectively. F390 was identical to the known compound, nidulalin A[1,2] (Structures shown in Fig. 1). In this communication, we describe fermentation, isolation, structure elucidation and antitumor activities of F390B, C and nidulalin A.

Culture, AJ117292, was isolated from a soil sample collected at Kanagawa Prefecture, Japan and the fungus, AJ117291 was isolated from a soil sample collected at Ehime Prefecture, Japan. Both of the strains were identified as *Penicillium* sp. by morphological characteristics, and AJ117292 was deposited at the National Institute of Bioscience and Human-Technology, Ibaraki Prefecture, Japan with accession number FERM P-14419. The production media used for the cultivation of AJ117292 and AJ117291 were medium M and medium P, respectively. Medium M contained malt extract (Difco) 20 g, oatmeal powder 10 g, peptone (Difco) 5 g, glucose 10 g, soluble starch 10 g in distilled water (1 liter), pH 6.0. Medium P contained bact potato dextrose broth (Difco) 24 g in distilled water (1 liter), pH 6.0. Both strains were cultivated at 25°C for 4 days in a 500-ml Erlenmeyer flasks containing 100 ml of production media on a rotary shaker (180 rpm).

The culture broth of *Penicillium* sp. AJ117292 (1 liter) was centrifuged at 4000 rpm for 20 minutes to obtain mycelium, which was extracted with acetone (2 liters). The extract was concentrated *in vacuo* to an aqueous suspension, which was extracted with EtOAc (400 ml × 2). The organic layer was dried *in vacuo*. The residue was applied to a silica gel column (5 i.d. × 37 cm, Kieselgel 60 Merck), and eluted with $CHCl_3$ and $CHCl_3$-EtOAc (95 : 5). Two active fractions were obtained. The fraction eluted with $CHCl_3$-EtOAc (95 : 5) was subjected to a silica gel column (3 i.d. × 40 cm), and eluted with $CHCl_3$-EtOAc (98 : 2). The active fraction was chromatographed on Sephadex LH-20 column (2.5 i.d. × 40 cm) with $CHCl_3$-MeOH (1 : 1) followed by crystallization from *n*-hexane-ether to give F390 (**3**, 600 mg). Purification of the other fraction eluted with $CHCl_3$ was achieved on a column of reverse phase silica gel (2 i.d. × 20 cm, Cosmosil 140C_{18}-OPN, nacalai tesque) using aqueous 50%

Fig. 1. Structures of F390 (nidulalin A, **3**), B (**1**) and C (**2**).



	R^1	R^2
F390B(1)	Ac	H
F390C(2)	H	OH
F390(3) (Nidulalin A)	H	H

Table 1. Physico-chemical properties of F390 (**3**), B (**1**), and C (**2**).

	F390B (**1**)	F390C (**2**)	F390 (Nidulalin A) (**3**)
Appearance	Yellow powder	Yellow powder	Orange needle
Molecular formula	$C_{18}H_{16}O_7$	$C_{16}H_{14}O_7$	$C_{16}H_{14}O_6$
HRFAB-MS (*m/z*)			
Found $(M+H)^+$:	345.0981	319.0810	303.0894
Calcd:	345.0974	319.0818	303.0869
UV λ_{max}^{MeOH} nm (ε)	338 (10500)	339 (8500)	340 (11500)
Specific rotation	—[a]	$[\alpha]_D^{23} = -327°$ (*c* 0.03, MeOH)	$[\alpha]_D^{25} = -463°$ (*c* 0.28, $CHCl_3$)

[a] Not measured due to limited sample quantity.

Table 2. ^{1}H and ^{13}C NMR spectral data of F390 (**3**), B (**1**), C (**2**), and diacetate of F390 (**4**).

Position	F390B (**1**)[a]	F390C (**2**)[a]		F390 (**3**)[b] (Nidulalin A)		Diacetate (**4**)[b]	
	^{1}H	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	7.35 (d) $J=6.8$[c]	7.38 (dd) $J=1.5, 4.8$	133.5	7.33 (d) $J=6.8$	131.7	7.23 (d) $J=5.6$	130.6
2	6.43~6.47 (m)	6.40~6.46[d]	126.2	6.37~6.39[d]	126.4	6.40 (dd) $J=5.6, 9.5$	128.0
3	6.32~6.38 (m)	6.40~6.46[d]	134.3	6.37~6.39[d]	131.7	6.28 (dd) $J=5.6, 9.5$	128.3
4	5.94 (d) $J=5.4$	4.62 (br d) $J=5.4$	65.8	4.67 (d) $J=5.4$	65.2	5.91 (d) $J=5.6$	64.6
4a			84.3		82.9		81.3
5	6.30 (br s)	6.53 (br s)	105.8	6.32 (br s)	111.4	6.74 (br s)	116.6
6			156.1		151.0		148.0
7	6.40 (br s)	6.55 (br s)	108.0	6.37~6.39[d]	108.3	6.55 (br s)	118.6
8			163.8		162.8		150.3
8a			107.0		105.6		111.8
9			184.5		182.8		176.5
9a			127.9		127.1		128.6
10a			159.9		158.0		159.7
6-Me	2.29 (s)	—	—	2.26 (s)	22.6	2.32 (s)	21.9
6-CH_2OH	—	4.57 (br s)	64.0	—	—	—	—
4a-CO_2Me			170.3		168.8		168.1
4a-CO_2*Me*	3.68 (s)	3.64 (s)	54.0	3.63 (s)	53.4	3.63 (s)	53.6
8-OH	12.15 (br s)	12.24 (br s)		12.11 (br s)		—	—
4-O*C*OMe		—	—	—	—		169.8
4-OCO*Me*	2.05 (s)	—	—	—	—	2.39 (s)	21.9
8-O*C*OMe	—	—	—	—	—		170.0
8-OCO*Me*	—	—	—	—	—	2.04 (s)	20.8

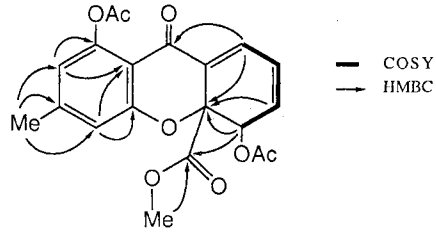
[a] Recorded in CD_3CN, [b] Recorded in $CDCl_3$, [c] Multiplicity, J in Hz, [d] Overlapping signals.

acetonitrile. The active fraction was finally purified on preparative HPLC (column; YMC AM312, 0.6 i.d. × 15 cm, mobile phase; aqueous 50% acetonitrile, flow rate; 1.0 ml/minute) to yield F390B (**1**, 0.5 mg).

The culture broth of *Penicillium* sp. AJ117291 (2.8 liters) was centrifuged at 4000 rpm for 20 minutes and the resulting supernatant was applied to a column of Diaion HP-20 (150 ml). After washing with water and aqueous 50% MeOH, the column was eluted with MeOH. The fraction eluted with MeOH was concentrated *in vacuo*. The concentrate was then adjusted to pH 2.0 with 5% HCl and extracted with EtOAc (150 ml × 2). The organic layer was evaporated to dryness. The residue was subjected to a column of reverse phase silica gel (1.6 i.d. × 14 cm), and eluted with aqueous 30% acetonitrile. The active fraction was chromatographed on a Sephadex LH-20 column (1.6 i.d. × 27 cm) with aqueous 70% MeOH. Further purification was achieved by preparative TLC (Kieselgel 60 Art 13794 Merck) developed with *n*-hexane - EtOAc - MeOH (10 : 10 : 1) to give F390C (**2**, 1.9 mg). This fungus also produced F390.

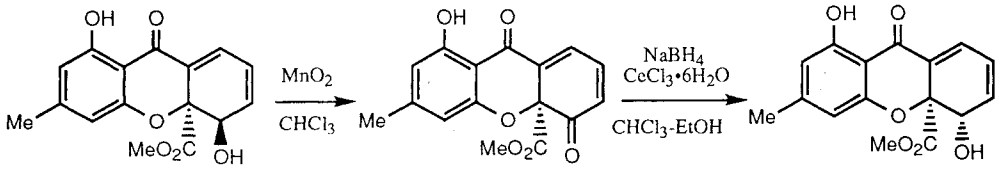
The physico-chemical properties of F390, F390B and F390C are summarized in Table 1, and ^{1}H and ^{13}C NMR

Fig. 2. ^{1}H-^{1}H correlation in the COSY spectrum and ^{1}H-^{13}C correlation in the HMBC sprctrum of **4**.



spectral data are in Table 2. They had same adsorption maxima at 340 nm in the UV spectra, and showed negative Cotton effect at 335 nm in the CD spectra. The molecular formula of the major component, F390 (**3**) was determined as $C_{16}H_{14}O_6$ by HR FAB-MS and ^{13}C NMR spectrum. Acetylation of **3** with acetic anhydride/pyridine gave the diacetate (**4**, 67%) (also see Table 2 for NMR spectra). The structure of **3** was elucidated as shown in Fig. 1 by analyses of 2D NMR spectra (^{1}H-^{1}H COSY, HMQC and HMBC) of diacetate (**4**) to avoid complexity in analyses of the ^{1}H and ^{13}C NMR spectra

Scheme



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of **3** due to overlapping the signals. The correlations in the ^1H-^1H COSY and HMBC spectra are shown in Fig. 2. F390 was identified as nidulalin A by comparison with an authentic specimen[1].

The molecular formula of F390B (**1**) was deduced as $C_{18}H_{16}O_7$ from the HR FAB-MS. The ^1H NMR spectrum of F390B was similar to that of nidulalin A. The compound (**1**) contained an additional methyl group observed at δ 2.05. The oxymethine proton of **1** was shifted downfield (δ 5.94) compared with that of **3**. These observation indicated that **1** was monoacetate of hydroxyl group at C-4 position of nidulalin A (**3**). This was confirmed by comparing **1** with ^1H NMR and CD spectra and by identity with monoacetate prepared from **3**.

The molecular formula of F390C (**2**) was determined by HR FAB-MS and ^{13}C NMR spectrum as $C_{16}H_{14}O_7$. The ^1H and ^{13}C NMR spectra of F390C were also similar to those of nidulalin A. The methyl signal on the aromatic ring was not detected and oxymethylene signal (δ_H 4.57 and δ_C 64.0) was newly observed. These results suggested that the structure of F390C was the hydroxymethyl analog at C-6 position of nidulalin A. Absolute configuration was deduced from the CD spectra and the coexistence of both compounds in the same fungus, since they are probably derived from anthraquinone derivative by the same biosynthetic pathway[3].

To evaluate the cytotoxic activities of related compounds of these xanthones, **3** was oxidized with MnO_2 to afford unsaturated ketone (**5**, 96%), from which the ketone was reduced with $NaBH_4/CeCl_3 \cdot 6H_2O$ to give 4*S* isomer (**6**, 62%) (Scheme). The ^1H NMR spectrum (300 MHz, in acetone-d_6) of **5** is as follows: δ 2.33 (3H, s), 3.68 (3H, s), 6.42~6.44 (2H, m), 6.54 (1H, m), 7.51 (1H, d, J=6.3 Hz), 7.52 (1H, d, J=4.8 Hz). The ^1H NMR spectrum (300 MHz, in $CDCl_3$) of **6** is as follows: δ 2.32 (3H, s), 2.91 (1H, d, J=5.4 Hz), 3.68 (3H, s), 5.29 (1H, m), 6.22~6.26 (2H, m), 6.41 (1H, br s), 6.43 (1H, br s), 7.30 (1H, dd, J=1.7, 5.1 Hz), 12.07 (1H, s).

The cytotoxic activities of these dihydroxanthone derivatives *in vitro* are shown in Table 3. Except for **5**, they exhibited potent cytotoxic activities against both human and murine tumor cell lines *in vitro*. Furthermore, **3** was also effective against adriamycin-resistant FM3A mouse mammary carcinoma cells (FM3A/ADR) and its parent cells (FM3A/S) with IC_{50} values of 0.13 and 0.09 μg/ml, respectively. Cytotoxic activity of nidulalin A has not been reported[2]. Further evaluation of the antitumor activities of these dihydroxanthone derivatives is under investigation.

Table 3. Cytotoxic activities of dihydroxanthone derivatives *in vitro*.

	IC_{50} (μg/ml)		
	HCT-116	K562	P388
1	0.086	0.06	0.024
2	0.25	0.47	0.22
3	0.042	0.096	0.0072
4	0.19	0.18	0.023
5	>20	>20	>20
6	0.6	0.26	0.46

Cells were treated with each drug for 72 hours. The proliferation was evaluated by MTT assay.

Acknowledgments

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