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DEHYDROFULIGOIC ACID, A NEW YELLOW PIGMENT ISOLATED FROM THE MYXOMYCETE *FULIGO SEPTICA* F. *FLAVA*

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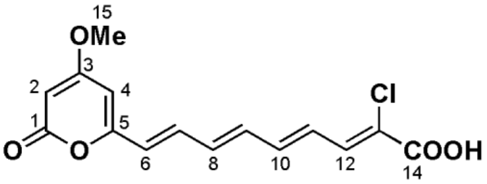
Abstract – Dehydrofuligoic acid (**1**), a new yellow pigment with a chlorinated polyene-pyrone acid structure, was isolated from field-collected fruit bodies of the myxomycete *Fuligo septica* f. *flava*, and its structure was elucidated from spectral data.

During our search for bioactive natural products from myxomycetes,[1] we have reported the isolation and structural elucidation of a new chlorinated polyene-pyrone acid compound, fuligoic acid (**2**),[2] from field-collected materials of fruit bodies of *Fuligo septica* f. *flava* from Kochi prefecture in Japan. Recently, we further investigated the extract of this myxomycete, resulting in the isolation of a new yellow pigment, and here we describe the isolation and structure elucidation of the new compound, named dehydrofuligoic acid (**1**).



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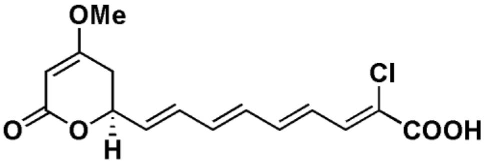

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Fruit bodies of the myxomycete *Fuligo septica* f. *flava*, collected in Kochi Prefecture, Japan, were extracted with MeOH and acetone. The combined extracts were subjected to ODS column chromatography, followed by purification with a Sephadex LH-20 column to give a new yellow pigment, dehydrofuligoic acid (**1**), in 0.022% yield.

Dedicated to Professor Dr. Albert Eschenmoser on the occasion of his 85th birthday.

The negative ESIMS spectrum of **1** showed a quasi-molecular ion peak at *m*/*z* 307 $(M-H)^-$ together with its isotopic peak at *m*/*z* 309 in a ratio of *ca.* 3:1. This isotopic pattern of **1** suggested the presence of one chlorine atom, and its molecular formula was revealed as $C_{15}H_{13}O_5Cl$ by HRESIMS data at *m*/*z* 307.0338 [calcd for $C_{15}H_{12}O_5{}^{35}Cl$, $(M-H)^-$, 307.0373] and at *m*/*z* 309.0360 [calcd for $C_{15}H_{12}O_5{}^{37}Cl$, $(M-H)^-$, 309.0344]. Negative HRESIMS showed significant fragment ion peaks at *m*/*z* 265.0462 [calcd for $C_{14}H_{12}O_3{}^{37}Cl$, 265.0445] and 263.0491 [calcd for $C_{14}H_{12}O_3{}^{35}Cl$, 263.0475], which corresponded to isotopic ions for the $(M-H-CO_2)^-$ ion, and at *m/z* 227.0719 [calcd for $C_{14}H_{11}O_3$, 227.0708], which was assignable to the $(M-H-CO_2-HCl)^-$ ion. Observation of these ions implied the presence of a carboxylic acid and one chlorine atom. The IR absorption bands observed at 3420 (broad), 1680, and 1600 cm^{-1} were suggestive of the presence of a carboxyl group and a conjugated carbonyl group. The ^1H NMR spectrum of **1** in DMSO-d_6 (Table 1) showed signals for a methoxy group at δ_H 3.81 (3H, s) and many olefinic protons, and the ^{13}C NMR spectrum revealed the presence of fourteen sp^2 carbons, including one ester or lactone (δ_C 163.0) and one acid carbonyl carbon (δ_C 163.0), thus accounting for 8 of 9 unsaturations. The remaining unsaturation was therefore ascribable to one ring. These ^1H and ^{13}C NMR data were almost identical to those of fuligoic acid (**2**),[2] and the molecular formula suggested that **1** had two hydrogen atoms fewer than **2**. The ^1H and ^{13}C NMR spectra of **1** did not show the signals for an sp^3 methylene and an sp^3 oxymethine carbon due to the C-3 and C-4 positions of **2**, respectively. The UV absorption maxima of **1** were observed at longer wavelengths (λ_{max} 406, 386, and 303 nm) than those of **2** (λ_{max} 340, 325, and 233 nm), implying that **1** had a longer conjugation system than **2**. Analysis of the 2D NMR data (Figure 1) suggested that **1** had an unsaturated bond at the C-4/C-5 position (H-4, δ_C 6.27; C-4, δ_C 101.4; C-5, δ_C 158.3), forming an α-pyrone ring, from the ^1H-^1H COSY cross peak for H-2/H-4 and HMBC correlations for H-2/C-1, H-2/C-3, H-2/C-4, H-4/C-2, H-4/C-3, H-4/C-5. A methoxy group [δ_H 3.81 (3H, s); δ_C 56.6] was assigned to the C-3 position from the HMBC correlation from methoxy protons [δ_H 3.81 (3H, s)] to C-3 (δ_C 170.9). A polyene side-chain was shown to be attached to the C-5 position from the HMBC correlations for H-4/C-6, H-6/C-5, and H-7/C-5, and this side-chain was assigned as a tetraene (C-6 to C-13) with a carboxyl group attached to the terminal (C-14) from ^{13}C NMR chemical shift data (Table 1). A doublet with low-field resonance (δ_H 7.17) in the ^1H NMR spectrum of **1** was assigned to the β-position (H-12) from the carboxyl group (C-14), which was consistent with the HMBC correlation observed from H-12 to C-14. A chlorine atom was located on the remaining sp^2 quaternary carbon at δ_C 137.5, which was assigned to the α-position (C-13) of the carboxyl group (C-14) from the HMBC correlations for H-12/C-13 and H-12/C-14. From all of these results, the structure of compound **1**, named dehydrofurigoic acid, was revealed as a 4,5-dehydroderivative of fuligoic acid (**2**).[2]

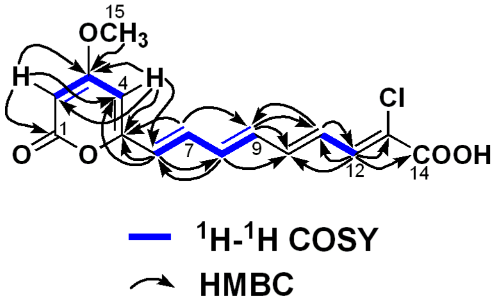

Figure 1. Key ^{1}H-^{1}H COSY and HMBC correlations observed for **1**.

Table 1. ^{1}H and ^{13}C NMR spectral data of dehydrofuligoic acid (**1**) in DMSO-d_6

position	δ_H (*J* in Hz)	δ_C	HMBC correlations (^{1}H to ^{13}C)
1		163.0	
2	5.61 d (2.3)	88.9	170.9, 163.0, 101.4
3		170.9	
4	6.27 d (2.3)	101.4	170.9, 158.3, 123.1, 88.9
5		158.3	
6	6.37 d (15.7)	123.1	158.3, 132.9, 101.4
7	7.04 dd (15.7, 11.3)	134.3	158.3, 138.6
8	6.58 m	132.9	135.8, 123.1
9	6.80 m	138.6	135.8, 131.7
10	6.48 m	135.8	
11	6.62 m	131.7	138.6, 128.5
12	7.17 d (10.5)	128.5	163.0, 137.5, 135.8, 131.7
13		137.5	
14		163.0	
15	3.81 s (3H)	56.6	170.9

EXPERIMENTAL

General Procedures UV spectra were obtained on a Shimazu UV mini-1240 spectrometer. IR spectra were measured on ATR on a JASCO FT-IR 230 spectrophotometer. The NMR spectra were recorded on a JEOL JNM ECP 600 spectrometer with deuterated solvents, the chemical shift of which was used as an internal standard. Negative ESIMS were measured on an Exactive (Thermo Scientific).

Organism The fruit bodies of *Fuligo septica* f. *flava* were collected in Konan-shi, Kochi Prefecture, Japan in July 2008 and identified by Y.Y. A voucher specimen (#31365) is maintained by Y.Y.

Extraction and isolation The wild fruit bodies (17.4 g) were extracted with 90% MeOH (200 mL x 2) and 90% acetone (100 mL x 2). The combined MeOH and acetone extracts (743 mg) were subjected to ODS column chromatography (20 x 200 mm) eluted with 0–100% MeOH in water, and the fraction (15 mg) eluted with 25% MeOH in H_2O was further separated by LH-20 column chromatography twice (1st, 15 x 220 mm, MeOH; 2nd, 10 x 220 mm, MeOH) to afford compound **1** (3.8 mg).

Dehydrofuligolic acid (1): Yellow powder; UV(MeOH) λ_{max} 406 (ε 10000), 386 (13000), and 303 nm (6600); IR (ATR) ν_{max} 3420 (broad), 1680, 1600, 1460, and 1370 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); (−)-ESIMS *m*/*z* 307 and 309 $(M-H)^-$; (−)-HRESIMS *m*/*z* 307.0338 [calcd for $C_{15}H_{12}O_5{}^{35}Cl$, $(M-H)^-$ 307.0373], *m*/*z* 309.0360 [calcd for $C_{15}H_{12}O_5{}^{37}Cl$, $(M-H)^-$ 309.0344], *m*/*z* 265.0462 [calcd for $C_{14}H_{12}O_3{}^{37}Cl$, $(M-H-CO_2)^-$ 265.0445], *m*/*z* 263.0491 [calcd for $C_{14}H_{12}O_3{}^{35}Cl$, $(M-H-CO_2)^-$ 263.0475], and *m*/*z* 227.0719 [calcd for $C_{14}H_{11}O_3$, $(M-H-CO_2-HCl)^-$ 227.0708].

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1. M. Ishibashi, *Yakugaku Zasshi*, 2007, **127**, 1369.
2. A. Shintani, T. Ohtsuki, Y. Yamamoto, T. Hakamatsuka, N. Kawahara, Y. Goda, and M. Ishibashi, *Tetrahedron Lett*., 2009, **50**, 3189.
3. The ^{1}H NMR spectrum of **1** showed the coupling constant for $J_{6,7}$ to be 15.7 Hz, implying 6*E*-configuration, while coupling constants of other double bonds unfortunately remained unassigned due to overlapping signals, and their geometry was tentatively drawn here as analogous to compound **2**.