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Fuligoic acid, a new yellow pigment with a chlorinated polyene–pyrone acid structure isolated from the myxomycete *Fuligo septica* f. *flava*

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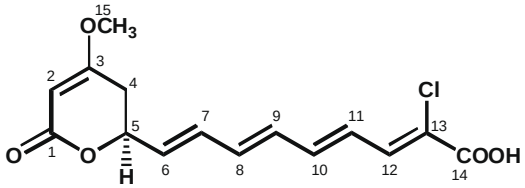
ABSTRACT

Fuligoic 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 by spectral data, including its absolute configuration based on CD data.

1. Introduction

Myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes, and chemical studies of the secondary metabolites of myxomycetes are limited so far.[1] During our search for bioactive natural products from myxomycetes,[2] we recently investigated field-collected fruit bodies of *Fuligo septica* f. *flava*. Here, we describe the isolation and structure elucidation of a new polyene–pyrone compound (**1**), named fuligoic acid.



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2. Results and discussion

Fruit bodies of the myxomycete *Fuligo septica* f. *flava*,[3] collected in Kochi Prefecture, Japan, were extracted with MeOH and acetone. The combined extracts were subjected to repeated ODS column chromatography to give a new compound (**1**) in 0.025% yield.[4,5]

The positive ESIMS spectrum of **1** showed a quasi-molecular ion peak at *m*/*z* 333 $(M+Na)^+$ and its isotopic ion peak at *m*/*z* 335 in a ratio of ca. 3:1. The negative ESIMS spectrum of **1** showed a quasi-molecular ion peak at *m*/*z* 309 $(M-H)^-$ together with its isotopic peak at *m*/*z* 311 in a ratio of ca. 3:1. These ESIMS isotopic patterns of **1** suggested the presence of one chlorine atom, and its molecular formula was revealed as $C_{15}H_{15}O_5Cl$ by negative HRESIMS data[5,6] [*m*/*z* 309.0556 $(M-H)^-$, Δ +2.6 mmu]. Negative ESI-MS/MS analysis with a precursor ion at *m*/*z* 309 $(M-H)^-$ showed daughter ions at *m*/*z* 265 and 229, which corresponded to $(M-H-CO_2)^-$ and $(M-H-CO_2-HCl)^-$ ions, respectively. These results were redolent of the presence of a carboxylic acid and one chlorine atom. The presence of a carboxylic acid group was also supported by the finding that the methylation of **1** with TMS-diazomethane gave a methyl ester of **1,** which showed an M^+ ion at *m*/*z* 324 in its EIMS spectrum. The UV spectrum of **1** showed absorption maxima at λ_{max} 340, 325, and 233 nm, indicating the presence of a conjugated system, and IR absorption bands at 3385, 1680, and 1620 cm^{-1} implied 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 due to one methoxy group at δ_H 3.73 (3H, s), one sp^3 methine (δ_H 5.01), one sp^3 methylene group (δ_H 2.56 and 2.60), and many sp^2 protons. The ^{13}C NMR spectrum revealed signals of 12 sp^2 carbons, including one ester or lactone carbonyl group (δ_C 166.1) and one acid moiety (δ_C 163.4), thus accounting for 7 of 8 unsaturation degrees. The remaining one was therefore ascribable to one ring.

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

Position	δ_H (J in Hz)	δ_C	HMBC correlations (^{1}H to ^{13}C)
1		166.1	
2	5.19 s	90.2	172.9, 166.1, 32.4
3		172.9	
4	(α) 2.56 dd (17.1, 5.1)	32.4	172.9, 131.1, 90.2, 75.2
	(β) 2.60 dd (17.1, 10.2)		172.9, 131.1, 90.2, 75.2
5	5.01 ddd (10.2, 5.7, 5.1)	75.2	172.9, 132.7, 131.1, 32.4
6	5.88 dd (14.3, 5.7)[a]	131.1	133.3, 75.2, 32.4
7	6.42 m[a]	132.7	134.7, 131.1, 75.2
8	6.40 m[a]	133.3	136.1, 134.7, 131.1
9	6.50 m[a]	134.7	132.7
10	6.58 m[a]	136.1	134.7, 133.3, 129.8, 128.8
11	6.54 m[a]	129.8	136.1, 134.7, 128.8
12	7.15 d (9.6)	128.8	163.4, 136.1, 129.8
13		135.9	
14		163.4	
15	(3H) 3.73 s	56.5	172.9

[a] Coupling constants for olefinic protons ($J_{6,7}$ = 15.1 Hz, $J_{8,9}$ = 14.7 Hz, and $J_{10,11}$ = 14.7 Hz) were determined by the ^{1}H NMR spectrum of **1** in CD_3OD at 800 MHz.[5,6]

Analysis of the 2D NMR data of **1** (Fig. 1) showed the presence of an α-pyrone ring [δ_H 5.19 (s; H-2), 2.56 (dd, J = 17.1 and 5.1 Hz; H-4α), 2.60 (dd, J = 17.1 and 10.2 Hz; H-4β), and 5.01 (ddd, J = 10.2, 5.7, and 5.1 Hz; H-5); ^{1}H–^{1}H COSY cross peak: H_2-4/H-5; HMBC correlations: H-2/C-1, H-2/C-3, H-2/C-4, H_2-4/C-2, H_2-4/C-3, H-5/C-3, H-5/C-4], to which a polyene side chain residue was attached at the C-5 position [δ_C 75.2 (C-5); ^{1}H–^{1}H COSY cross peaks: H-5/H-6, H-6/H-7, and H-7/H-8; HMBC correlations: H-5/C-6 and H-5/C-7]. A methoxy group [δ_H 3.73 (3H, s); δ_C 56.5] was shown to be attached at the C-3 position from HMBC correlation of the methoxy protons [δ_H 3.73 (3H, s)] to C-3 (δ_C 172.9).

According to ^{13}C NMR chemical shift data, the polyene side chain at the C-5 position was deduced to be a tetraene (C-6 to C-13) with a carboxyl group attached at the terminal position (C-14). A doublet signal resonating in a fairly low field (δ_H 7.15) in the ^{1}H NMR spectrum of **1** was assignable to the β-position (H-12) from the carboxyl group (C-14); this assignment was also supported by the observation of the HMBC correlation from H-12 to C-14. The 800 MHz ^{1}H NMR spectrum of **1** in CD_3OD[5,6] showed coupling constants for olefinic protons ($J_{6,7}$ = 15.1 Hz, $J_{8,9}$ = 14.7 Hz, and $J_{10,11}$ = 14.7 Hz), suggesting all-*E* configurations of three double bonds (C-6 to C-11). One remaining sp^2 quaternary carbon at δ_C 135.9 was assigned to the α-position (C-13) of the carboxyl group, and a chlorine atom had to be attached at this position (C-13) by a process of elimination. The geometry of C-12–C-13 double bond was suggested as *Z* on the basis of the $^3J_{C-H}$ value between H-12 and C-14, which was revealed to be 2.8 Hz by ^{1}H-nondecoupling ^{13}C NMR spectrum of **1**.[7] The absolute configuration of the C-5 position of **1** was revealed by comparing the CD data with that of a related known compound, kawain,[8] which bore a styrene side chain attached at C-5 in place of the polyene side chain of compound **1**. Kawain was reported to have a 5*R* configuration and showed a positive Cotton effect at 250 nm ($\Delta\varepsilon$ +10.5, in dioxane) in its CD spectrum, while the CD spectrum of compound **1** showed a negative Cotton effect at 244 nm ($\Delta\varepsilon$ −5.3, in MeOH), thus suggesting the absolute configuration of C-5 of **1** to be *S*. From these results, the structure of fuligoic acid was concluded to be **1**.

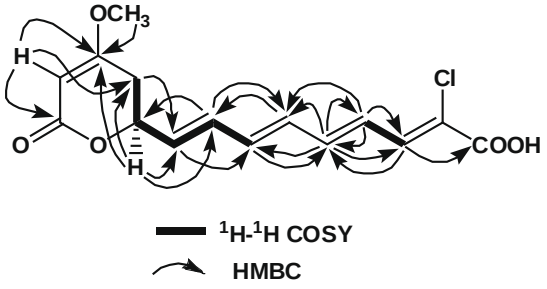

Figure 1. Key ^{1}H–^{1}H COSY and HMBC correlations observed for **1**.

Fuligoic acid (**1**) possesses a pyrone ring and a tetraene unit with a chlorine atom and a carboxylic acid group attached at the terminal position. This structure may have a resemblance with that of fuligorubin A,[9] and ceratiopyrons,[10] isolated from plasomodia of *Fuligo septica* and *Ceratiomyca fruticulosa*, respectively. We examined the bioactivity of fuligoic acid (**1**) using cell-based assay systems constructed in our laboratories targeting Wnt,[11] hedgehog,[12] and TRAIL[13] signaling pathways. Compound **1**, however, proved to be inactive in these assay systems.[14]

Acknowledgments

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1. Steglich, W. *Pure Appl. Chem.* **1989**, *61*, 281–288.
2. Ishibashi, M. *Yakugaku Zasshi* **2007**, *127*, 1369–1381.
3. Fruit bodies of *Fuligo septica* f. *flava* were collected and identified by Y.Y. in Konan-shi, Kochi Prefecture, Japan, in July 2008. A voucher specimen (#31365) is maintained by Y.Y. (Ohtsu-ko, Kochi).
4. Wild fruit bodies (17.4 g) were extracted with MeOH (200 mL × 2) and acetone (100 mL × 2). The combined MeOH and acetone extracts (743 mg) were subjected to ODS column chromatography (20 × 200 mm) eluted with 0–100% methanol in water, and the fraction (25 mg) eluting with 25% MeOH in H_2O was further separated by second ODS column chromatography (15 × 220 mm; 50%MeOH) to afford compound **1** (4.4 mg).
5. Fuligoic acid (**1**): Yellow powder; $[\alpha]_D^{17}$ −31 (*c* 0.33, MeOH); UV (MeOH) λ_{max} 340 (ε 12,820), 325 (15,100), and 233 nm (6600); IR (ATR) ν_{max} 3390, 1680, 1620, 1580, and 1380 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) in DMSO-d_6 (Table 1); ^{1}H NMR (800 MHz, CD_3OD) δ_H 2.56 (1H, dd, J = 17.0 and 4.6 Hz; H-4α), 2.63 (1H, dd, J = 17.0, 10.0 Hz; H-4β), 3.79 (3H, s; H_3-15), 5.03 (1H, ddd, J = 10.0, 6.0, 4.6 Hz; H-5), 5.20 (1H, s; H-2), 5.90 (1H, dd, J = 15.1, 6.0; H-6), 6.44 (1H, dd, J = 14.7, 10.1; H-8), 6.47 (1H, dd, J = 15.1, 10.1; H-7), 6.50 (1H, dd, J = 14.7, 10.1; H-9), 6.62 (1H, dd, J = 14.7, 10.1; H-10), 6.66 (1H, dd, J = 14.7, 10.6; H-11), and 7.30 (1H, d, J = 10.6; H-12); (+)-ESIMS *m/z* 333 and 335 $(M+Na)^+$; (−)-ESIMS *m/z* 309 and 311 $(M-H)^-$; (−)-HRESIMS *m/z* 309.0556 [calcd for $C_{15}H_{14}O_5{}^{35}Cl$, $(M-H)^-$ 309.0530], *m/z* 311.0521 [calcd for $C_{15}H_{14}O_5{}^{37}Cl$, $(M-H)^-$ 311.0500], *m/z* 265.0634 [calcd for $C_{14}H_{14}O_3{}^{35}Cl$, $(M-H-CO_2)^-$ 265.0631], and *m/z* 229.0878 [calcd for $C_{14}H_{13}O_3$, $(M-H-CO_2-HCl)^-$ 229.0865]; CD (MeOH, 0.47 mM) λ_{max} ($\Delta\varepsilon$) 224 (+1.6) and 244 (−5.3) nm.
6. Negative ESIMS and ESI-MS/MS spectra were measured on a Shimazu LCMS-IT-TOF spectrometer, and 800 MHz ^{1}H NMR spectra were recorded on a JEOL ECA 800 spectrometer.
7. The 12*Z*-configuration means that H-12 and C-14 have a *cis*-relationship. In case of methyl 2-butenoate ($CH_3CH{=}CH{-}CO_2CH_3$), the $^3J_{C,H}$-values between C-1 and H-3 of *cis*- and *trans*-relationships showed 6.8 and 14.5 Hz, respectively, and the $^3J_{C,H}$-value of the corresponding positions of (*Z*)-α-chlorocinnamic acid was 5.0 Hz: Kingsbury, C. A.; Draney, D.; Sopchik, A.; Rissler, W.; Durham, D. *J. Org. Chem.* **1976**, *41*, 3863–3868.
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14. Compound **1** was also inactive in cytotoxicity test[15] as well as TRAIL-resistant overcoming activity test[16] against TRAIL-resistant human gastric adenocarcinoma (AGS) cells, and also did not show antimicrobial activity at 50 μg/disc against *Bacillus subtilis* and *Staphyrococcus aureus*.
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