Note

(3*R*,5*Z*)-(-)-3-Hydroxy-5-dodecenoic Acid,
A Phytotoxic Metabolite
of *Pythium ultimum*Akitami ICHIHARA, Masaaki HASHIMOTO
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In the previous papers,^{1~3)} we reported the isolation and structural elucidation of the phytotoxins from *Phoma betae*, which is one of the phytopathogenic fungi causing black root disease in sugar beet (*Beta vulgaris* L. var. *rubra* Moq). The present paper reports a continuation of our study of phytotoxins related to the disease, and describes the isolation and characterization of a phytotoxin, (3*R*,5*Z*)-(-)-hydroxy-5-dodecenoic acid (**1**), from the culture filtrate of *Pythium ultimum* 73-2, another incitant of black root disease in sugar beet. The phytotoxic compound was isolated from the culture broth as described in EXPERIMENTAL.

- 1 $R^1, R^2 = H$
 2 $R^1 = H, R^2 = Ac$
 3 $R^1 = CH_3, R^2 = H$

The phytotoxin (**1**), a yellow oil, $[\alpha]_D^{22} - 10.7^\circ$ ($c = 1.55$, $CHCl_3$), has the molecular formula $C_{12}H_{22}O_3$ from the high resolution and field desorption mass spectra (HR- and FD-MS). The IR spectrum showed absorption bands at $3100\sim 3500\text{ cm}^{-1}$ and 1729 cm^{-1} due to a carboxylic acid. Since the ^1H NMR spectrum exhibited characteristic signals at δ 5.37 (1H, dt, $J = 10.7, 7.3\text{ Hz}$) and 5.56 (1H, dt, $J = 10.7, 7.3\text{ Hz}$) due to two vinylic protons, two unsaturations deduced from the molecular formula are ascribable to one carboxylic acid and one *cis* double bond in **1**. Acetylation of **1** with acetic anhydride-pyridine gave a monoacetate **2**, whose ^1H NMR spectrum showed a signal at δ 5.17~5.31 (1H, m) due to an α methine to the acetoxy group. From this result, the presence of a secondary hydroxyl group in **1** was deduced. Methylation of **1** with diazomethane yielded a monomethyl ester, whose decoupling experiments in the ^1H NMR spectrum lead to the

structure **3**. Thus, irradiation of the signal at δ 2.03 due to 7- CH_2 transformed the double-triplet at δ 5.56 (6-CH) to the doublet ($J = 11.2\text{ Hz}$), whose coupling constant is compatible with a *cis* double bond, and then slightly modified the signals due to neighboring methylene protons (8- CH_2). Irradiation of the signals at δ 2.20~2.36 (4- CH_2) changed the signals at δ 5.38 (dt, $J = 11.2, 7.3\text{ Hz}$, 5-CH) to a doublet ($J = 11.2\text{ Hz}$), and effected the signals at δ 4.05 (m) due to an α proton to the hydroxyl group. Double resonance showed that 2- CH_{ax} was coupled with 2- CH_{eq} ($J = 16.1\text{ Hz}$) and with 3-CH ($J = 8.7\text{ Hz}$), and 2- CH_{eq} was also coupled with 3-CH ($J = 3.4\text{ Hz}$). These results indicate the formation of a hydrogen-bonded six-membered ring as illustrated in **1a**. Further irradiation of the signal at δ 5.38 due to 5-CH modified the signals ascribable to 4- CH_2 . These results elucidate the structure as **1** for the phyto-

toxin. Although 3-hydroxy-5-dodecenoic acid had been isolated as a metabolite of *Serratia marcescens*,⁴⁾ its stereochemistry has not been mentioned. The absolute configuration of **1** was determined as follows: Catalytic hydrogenation of **1** over palladium-carbon yielded 3-hydroxy-dodecanoic acid (**4**), whose spectral data were identical with an authentic sample obtained by fungal hydration of *trans*-2-decenoic acid.⁵⁾ In addition, the optical rotation $[\alpha]_D^{22} - 15.7^\circ$ ($c = 0.74$, $CHCl_3$) of the hydroxy acid **4** was the same as that of the sample $[\alpha]_D^{22} - 15.8^\circ$ ($c = 1.6$, $CHCl_3$), whose stereochemistry has been unequivocally established.⁶⁾ Therefore the structure of **1** was determined as (3*R*,5*Z*)-(-)-3-hydroxy-5-dodecenoic acid.

The phytotoxic effects of **1** on lettuce seedlings, sugar beet seedlings and rice seedlings were examined by growth tests. The toxin **1** darkened the root and shoot of sugar beet at 250 ppm, and darkened only the root at 100 ppm. Interestingly, the toxin promoted growth of the root and shoot of sugar beet seedlings at 50 ppm. To the lettuce seedlings, the toxin showed a 97.3% inhibition to growth of the shoot and 98.1% inhibition to growth of the root at 250 ppm. However, the toxin showed 6.7% inhibition to shoot growth and 17.2% promotion to growth of the root at 100 ppm. In the growth test on rice seedlings, the toxin inhibited growth of the root and shoot at 500 ppm to 52.0% and 31.0% respectively. Even at 25 ppm, the toxin showed a 14.0% inhibition to the growth of the shoot and an 8.8% inhibition to the growth of the root.

EXPERIMENTAL

Melting points (mp) were measured on a Yanaco MP-35

Micro Melting Point Apparatus and are uncorrected. IR spectra were taken on a Hitachi 285 spectrometer. NMR spectra were recorded on JEOL 100 and JEOL 400 spectrometers. MS were run on a JEOL JMS-D 300 Mass Spectrometer and a JEOL JMS-DISG-2 Mass Spectrometer. Optical rotations were determined with a JASCO DIP-4 Digital Polarimeter.

Cultures and isolation. The fungus was grown in 500 ml flasks containing 150 ml of potato-sucrose medium at 25°C for 15 days. The culture filtrates (15 liters from 100 flasks) were concentrated *in vacuo* to 4 liters below 40°C, and the concentrate (pH 5.0) was extracted three times with ether (2 liters each), and additionally three times with ethyl acetate (2 liters each). Concentration of the combined extracts gave a dark brown residue (1.109 g), which was chromatographed on a silicic acid column (110 g) eluting with chloroform-methanol (98:2) to give fractions A (137 mg) and B (187.5 mg), with chloroform-methanol (95:5) to give fractions C (81 mg), D (69 mg) and E (195 mg), and with methanol to give fraction F (325 mg). Phytotoxic activities on the growth of lettuce seedlings were observed with fractions D and E. The fraction D (69 mg) was chromatographed on Sephadex LH 20 (80 g) eluting with chloroform-methanol (1:1) to give an active compound **1** (16 mg). Fraction E (189 mg) was chromatographed on a silicic acid column (20 g), which was eluted with chloroform-methanol (93:7) to give an active fraction (64 mg). This fraction was further purified with a Sephadex LH 20 column (80 g) eluting with chloroform-methanol to yield more of active compound **1** (14 mg) as a yellow syrup, $[\alpha]_D^{22} = -10.7^\circ$ ($c = 1.55$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2915, 2860, 1720, 1415, 1280, 1180, 1050. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 0.88 (3H, t, $J = 6.6$ Hz, CH_3), 1.29~1.36 (8H, m, CH_2), 2.04 (2H, q, $J = 6.8$ Hz, CH_2), 2.20~2.37 (2H, m, CH_2), 2.48 (1H, dd, $J = 16.6$, 8.9 Hz, CH), 2.58 (1H, dd, $J = 16.6$, 2.7 Hz, CH), 4.07~4.09 (1H, m, CH), 5.37 (1H, dt, $J = 10.7$, 7.3 Hz, =CH), 5.56 (1H, dt, $J = 10.7$, 7.3 Hz, =CH), 6.85 (1H, br. s, COOH). High resolution MS (HR-MS) found m/z 196.1469 ($\text{M}^+ - \text{H}_2\text{O}$), with m/z 196.1436 calcd. for $\text{C}_{12}\text{H}_{22}\text{O}_2$.

(3*R*, 5*Z*)-(-)-3-Acetoxy-5-dodecenoic acid (**2**). A solution of **1** (1 mg) and acetic anhydride (0.2 ml) in pyridine (0.4 ml) was allowed to stand overnight. The reaction mixture was extracted with ethyl acetate and the extracts were evaporated *in vacuo* to give an acetate **2** as an oil. IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 3100, 3500, 1720, 1220. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 0.88 (3H, t, $J = 5.3$, CH_3), 1.26 (8H, s, CH_2), 2.04 (3H, s, COCH_3), 2.40 (2H, dd, $J = 6.8$, 6.3 Hz, CH_2), 2.61 (2H, m, CH_2), 5.17~5.31 (1H, m, CH), 5.37 (2H, m, =CH).

Methyl (3*R*, 5*Z*)-(-)-3-hydroxy-5-dodecenoate (3). A mixture of **1** (3 mg) in methanol (2 ml) and an ethereal solution (2 ml) of diazometane was allowed to stand for 1 hr at room temperature to give a methyl ester **3** as an oil. IR $\delta_{\text{max}}^{\text{neat}}$ cm^{-1} : 3100~3600, 1710. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 0.88

(3H, t, $J = 6.9$ Hz, CH_3), 1.26~1.38 (8H, m, CH_2), 2.03 (2H, q, $J = 6.8$ Hz, CH_2), 2.20~2.36 (2H, m, CH_2), 2.31 (1H, dd, $J = 16.4$, 8.7 Hz, CH), 2.54 (1H, dd, $J = 16.4$, 3.4 Hz, CH), 3.71 (3H, s, OCH_3), 4.05 (1H, m, CH), 5.38 (1H, dt, $J = 11.2$, 7.3 Hz, =CH), 5.56 (1H, dt, $J = 11.2$, 7.3 Hz, =CH). EI-MS m/z 210 ($\text{M}^+ - \text{H}_2\text{O}$). HR-MS m/z 228.1764 (M^+), with m/z 228.1760 calcd. for $\text{C}_{16}\text{H}_{24}\text{O}_3$.

(3*R*)-(-)-3-Hydroxydodecanoic acid (**4**). A solution of **1** (10 mg) in ethanol (0.5 ml) was mixed with Pd-C (5 mg) in a stainless vessel for catalytic hydrogenation, and hydrogenated with stirring under a pressure of 5 kg/cm² at 60°C for 3 hr. The reaction mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was crystallized from *n*-hexane to give 1 mg of product **4**, mp 63.0~64.0°C, $[\alpha]_D^{22} = -15.7^\circ$ ($c = 0.74$, CHCl_3). IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 3500, 3100, 1740, 1680. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 0.88 (3H, t, $J = 5.4$ Hz, CH_3), 1.26 (16H, s, CH_2), 2.55 (2H, m, CH_2), 4.04 (1H, m, CH), EI-MS m/z 198 ($\text{M}^+ - \text{H}_2\text{O}$). FD-MS m/z 217 (MH^+). HR-MS m/z 198.1640 ($\text{M}^+ - \text{H}_2\text{O}$), with m/z 198.1620 calcd. for $\text{C}_{12}\text{H}_{22}\text{O}_2$.

Bioassay

a. Growth inhibition of lettuce (Great Lakes 366) and rice (Kitahikari) seedlings. A methanol solution containing a sample at a definite concentration (500, 250, 100, 50, 25 ppm, etc.) was poured on to a sheet of filter paper in a Petri dish. After removing the solvent, 3 ml of distilled water was added to the dish. Lettuce or rice seedlings of uniform shape and size were placed on the filter paper and grown in the dark at 25°C. Inhibitory effects were observed after 72 hr for the lettuce seedlings and 48 hr for the rice seedlings.

b. Growth inhibition of sugar beet (Monohope) seedlings. Uniformly grown seedlings of sugar beet were placed in a Petri dish (3 cm diameter) and to the dish was added 0.5 ml of a sample at a definite concentration (500, 250, 100 and 50 ppm) in methanol. The seedlings were grown in the dark at 25°C for 24 hr.

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