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Short Communication

Monilidiols, Characteristic and Bioactive Metabolites of Benomyl-resistant Strains of *Monilinia fructicola*

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In the course of an attempt to isolate a self-growth inhibitor from benomyl-resistant strains of cherry brown rot fungus *Monilinia fructicola*, we have found that the benomyl-resistant strains produced characteristic metabolites possessing antimicrobial and phytotoxic activities. In this communication, we wish to describe the isolation and structural determination of new salicylaldehyde-type octaketides, named monilidiol and dechloromonilidiol, which are structurally related to pyriculol[1)] and pyriculariol,[2)] phytotoxins from rice blast fungus *Pyricularia oryzae*.

A benomyl-resistant strain of *M. fructicola* (minimum inhibitory concentration (MIC) of an effective fungicide benomyl: 500 ppm) was shake-cultured in a malt extract-sucrose medium at 25~27°C for 9~12 days; 100 ml of the medium in a 500 ml flask, consisting of 2.0% malt extract, 2.0% commercial sucrose, 0.1% peptone and 0 or 0.2% sodium chloride, was used. The culture filtrate (15 liters, pH 4.8) was saturated with sodium chloride and extracted with ethyl acetate. The ethyl acetate extracts (0.7 g) were separated into seven fractions by silica gel column chromatography eluted with a step-wise increase of ethyl acetate in benzene to give two active fractions (fra. 2 and 6). The dark brown material of fra. 6 (65 mg), obtained as an eluate of 10% ethyl acetate in benzene, was carefully rechromatographed on silica gel with 2% ethanol in benzene to afford pale yellow needles (15 mg, mp

TABLE I. SPECTRAL DATA OF MONILIDIOL (I) AND DECHLOROMONILIDIOL (II)

	I	II
IR ν_{max}^{KBr} cm^{-1}	3300, 1642, 976	3350, 1640, 978
UV λ_{max}^{MeOH} nm (ε)	357 (4,000) 273 (7,600) 232 (18,000)	347 (5,300) 274 (11,000) 228 (24,000)
$\lambda_{max}^{MeOH-NaOH}$ nm	398, 287, 217	389, 285, 220
^{1}H-NMR δ (ppm, J(Hz))	12.32 (1H, s, OH)*	12.0 (1H, br. s, OH)**
	10.36 (1H, s)	10.45 (1H, s)
	7.59 (1H, d, 8.3)	7.51 (1H, t, 8)
	7.18 (1H, br. d, 15.6)	7.30 (1H, d, 15.5)
	7.01 (1H, d, 8.3)	7.00 and 6.82 (each 1H, d, 8)
	6.31 (1H, dd, 15.6, 5.4)	6.31 (1H, dd, 15.5, 5.5)
	4.08 (1H, br. t, *ca.* 5)	4.15 (*ca.* 1H, m)
	3.8 (2H, br. s, OH)	
	3.5 (1H, m)	3.6 (*ca.* 1H, m)
	1.7~1.05 (8H, m)	1.7~1.1 (*ca.* 8H, m)
	0.88 (3H, m)	0.87 (3H, m)

* In d^1-chloroform–d^6-DMSO (2:1) at 100 MHz.
** In d^6-acetone at 60 MHz.

133°C).[3] These were further separated into two components by HPLC using an ODS column eluted with 40% water in acetonitrile. One (**I**; t_R 10.8 min) was obtained as a pale yellow eluate (9 mg) and the other (**II**; t_R 8.4 min) as a colorless eluate (3 mg).

Monilidiol (**I**; $C_{16}H_{21}O_4Cl$, mp 136~137°C, $[\alpha]_D^{20}$ +20° (c=0.26, MeOH), FD-MS m/z 314 and 312 (1 : 3, M^+)) gave a 2,4-dinitrophenylhydrazone and a positive ferric chloride test (violet in ethanol). The IR, UV and ^{1}H-NMR (δ (ppm) 12.32, a chelated phenolic hydroxyl; 10.36, an aromatic aldehyde; 7.18 and 6.31, *E*-disubstituted olefinic protons; 7.59 and 7.01, *ortho*-coupling protons on the benzene ring) spectra (Table I) revealed a salicylaldehyde chromophore with conjugation to a double bond. Its UV spectra in alkaline methanol as well as in methanol fully resembled those of pyriculol in particular. The ^{1}H-NMR spectrum showed additional and mutually coupled signals at δ4.08 and *ca.* 3.5 ppm, one (δ 4.08 ppm) of which was coupled to the olefinic proton at δ6.31 ppm. The remaining multiplets unassigned in the ^{1}H-NMR spectrum (Table I) were clearly analyzed by ^{13}C-NMR spectrometry; seven sp^3 carbons were observed at δ74.9 (d), 73.9 (d), 32.8 (t, $C_{14\text{ or }12}$), 31.7 (t, $C_{14\text{ or }12}$), 25.3 (t, C_{13}), 22.3 (t, C_{15}) and 14.0 (q, C_{16}) ppm, demonstrating the presence of an *n*-pentyl group together with a *vicinal* glycol unit in the side chain. These spectral data suggest that monilidiol should have the structure **I** (Fig. 1). This was strongly supported in the field-desorption mass spectrum by the presence of fragment ions at m/z 213 and 211 in the ratio of approximately 1 : 3, and m/z 101, which correspond to the characteristic ions produced by the carbon-carbon bond cleavage of the *vicinal* glycol system.

The substitution pattern on the benzene ring and the structure of the side chain were determined as follows (Fig. 1). Monilidiol was reduced with sodium borohydride, followed by oxidation with potassium metaperiodate in dioxane, to yield *n*-hexanal (its 2,4-dinitrophenylhydrazone mp 108°C), a conjugated aldehyde **IV** (the minor yellow product; m/z 214 and 212 (1 : 3, M^+), ν_{max}^{KBr} cm^{-1} 3420, 1668, 1122 and 975, λ_{max}^{MeOH} nm (ε) 364 and 252 (each *ca.* 14,000)) and a heterocyclic aldehyde **V** characterized as its 2,4-dinitrophenylhydrazone (mp 170~174°C, m/z 394 and 392 (1 : 3, M^+), $\nu_{max}^{CHCl_3}$ cm^{-1} 3680, 3600, 3530 and 3310). **V** ($\nu_{C=O}^{CHCl_3}$ cm^{-1} 1723, m/z 214 and 212 (1 : 3, M^+)) showed a characteristic intense peak at m/z 169.004 ($C_8H_6O_2Cl$, $M^+ - {}^{\bullet}CH_2CHO$) in the mass spectrum, indicating the formation of a furan ring by nucleophilic attack of the primary hydroxyl group on the α,β-unsaturated aldehyde.[4] In addition, the IR spectrum in a dilute solution

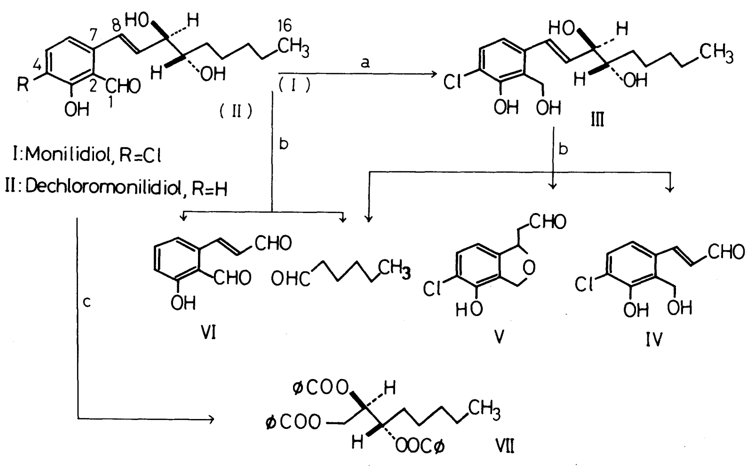

FIG. 1. Structures and Chemical Degradation of Monilidiol and Dechloromonilidiol.

a) NaBH$_4$; b) KIO$_4$; c) 1, ϕCOCl/pyridine; 2, O$_3$/O$_2$; 3, Me$_2$S/MeOH; 4, NaBH$_4$; 5, ϕCOCl/pyridine.

showed an intramolecular hydrogen bond absorption at 3535 cm^{-1} due to chelation between the phenolic hydroxyl and chlorine atom.[5] The absence of a chlorine atom at C_6 on the benzene ring was also supported by the UV spectrum of the dihydromonilidiol **III** (mp 125~127°C, λ_{max}^{MeOH} nm (ε) 295 (4,300), 254 (16,000) and 217 (29,000)).[6] The plain structure of monilidiol, therefore, was established as shown in **I**.

The absolute stereochemistry of the glycol moiety in the side chain was determined as follows (Fig. 1). Ozonolysis of the 10,11-dibenzoate of monilidiol, followed by reduction with sodium borohydride and successive benzoylation with benzoyl chloride in pyridine, gave 1,2,3-octanetriol tribenzoate **VII** (mp 90~90.5°C, $[\alpha]_D$ +51° ($CHCl_3$)), which was directly compared with both the (2*R*)-*threo*- and *erythro*-isomers stereoselectively prepared from D-glyceraldehyde[7] and found to be identical with a synthetic sample (mp 89~90°C, $[\alpha]_D$ +47° ($CHCl_3$)) of (+)-(2*R*)-*erythro*-isomer (also IR and HPLC).

Dechloromonilidiol (**II**; mp 123~125°C, $[\alpha]_D^{20}$ +20.5° (c=0.23, MeOH), EI-MS (20 eV) m/z 296 (M^++18), 278 (M^+), 178)) was quite similar to **I** in spectral properties (Table I, in which the UV spectra were superimposable on those of pyriculol), indicating the close similarity of their structural features. This was immediately oxidized with potassium metaperiodate. The volatile aldehyde obtained was identified as *n*-hexanal (its 2,4-dinitrophenylhydrazone mp 108~109°C). The nonvolatile crystalline aldehyde **VI** (mp 112~113°C) was directly compared with a periodate oxidation product of pyriculol and confirmed to be (*E*)-2-formyl-3-hydroxycinnamaldehyde (mixed mp, UV, IR and MS). Thus, the structure of dechloromonilidiol was determined as shown in **II**, whose stereochemistry must be in the same configurations as those of **I** because of the almost same molecular rotation in **I** and **II**.

These salicylaldehyde-type octaketides are the first instance of isolation from fungi. Although the absolute configurations of pyriculol[4] and pyriculariol[2] remain undetermined, it is of interest from a biooxidation point of view that all of these metabolites possess the same *erythro* configuration about the glycol moiety in the side chain.

Monilidiol was active against *Staphylococcus aureus* (MIC 6.2 ppm), but not at concentrations less than 100 ppm against some of the plant pathogenic fungi including *M. fructicola*. On the other hand, this was phytotoxic to cherry leaves to induce dark necrotic spots by application of 2~5 μg on the leaves with a pinhole injury, and inhibited the growth of rice seedlings as well as pyriculol and pyriculariol did.[2] Dechloromonilidiol was less active than monilidiol in both phytotoxic and plant growth-inhibiting activities.

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7) This stereoselective synthesis will be reported elsewhere.