## A New Fungal Metabolite and Root Growth-stimulating Activity of Its Methyl Ester

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Recently, radiclonic acid has been isolated from a fungus as a new root-growth stimulant and its structure has been elucidated.<sup>1)</sup> In continuation of our work, we have isolated a new fungal metabolite from an unidentified fungus\* and have found that its methyl ester markedly stimulates the root growth of Chinese cabbage seedlings.

The fungus was grown in a medium containing corn steep liquor by shaking. A strongly acidic portion obtained from the culture filtrate by ethyl acetate extraction was treated with ethereal diazomethane. The methylated products were subjected to elution chromatography on silica gel with a mixed solvent (ethyl acetate: hexane=1:4). A crystalline substance, which gave positive color tests to FeCl<sub>3</sub> in ethanol (dark purple color) and 2,4-dinitrophenylhydrazine in 2 N HCl, was separated and was recrystallized from ethyl acetate-hexane to give pale yellow needles (II), mp  $172 \sim 3^{\circ}$ C; IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3300, 1674, 1634, UV  $\lambda_{\max}^{MeOH}$  nm ( $\varepsilon$ ): 237 (20,000), 252 (shoulder, 14,000), 299 (13,000), 333 (8300), NMR  $\delta^{**}$ : 2.36, 3.77 (each 3H, s), 7.29 (1H, s), 11.65 (1H, s), ca. 14 (2H, OH), MS m/e: 210 (M<sup>+</sup>), Found: C, 57.28; H, 4.65. Calcd. for C<sub>10</sub>H<sub>10</sub>O<sub>5</sub>: C, 57.14; H, 4.80%. Hydrolysis of II with 1 N NaOH gave an acid (I), mp  $255 \sim 60^{\circ}$ C (decomp.), which was also obtained from the culture filtrate by preparative TLC using kieselgel GF254 plates and ethyl acetate-chloroform-acetic acid=5: 20: 1.

On acetylation, II gave a monoacetate (III), mp 100°C; MS m/e: 252 (M<sup>+</sup>), FeCl<sub>3</sub>: dark purple color. Its NMR spectrum in deuteriochloroform showed an acetoxyl signal at  $\delta$  2.34 and a hydrogen-bonded hydroxyl signal at  $\delta$  12.80. Long range coupling (J=ca. 0.3 Hz) between an aromatic proton ( $\delta$  7.27) and a methyl group ( $\delta$  2.10) was observed. Treatment of II with NaBH<sub>4</sub> in methanol yielded a phthalide (IV), mp  $242 \sim 4^{\circ}C$ ; IR  $\nu_{c=0}^{\text{KBr}}$  cm<sup>-1</sup>: 1703, UV  $\lambda_{\max}^{\text{MeOH}}$  nm ( $\varepsilon$ ): 261 (9900), 308 (4000),  $\lambda_{max}^{MeOH-OH^{-}}$  nm: 234, 282, 332, MS m/e: 180 (M<sup>+</sup>), FeCl<sub>3</sub>: no color. In the NMR spectrum, a methylene signal newly appeared at  $\delta$  5.33 (2H, single peak) and the methoxyl signal observed in the spectrum of II disappeared. The UV spectrum of IV was in good agreement with that of a-resorcylic acid methyl ester (V).

From the above facts, the partial structure of I is represented as A. The position of a methyl group in the partial structure (A) was determined at 4 by the following evidence; (a) in the NMR spectrum, aromatic protons of 3,5-dihydroxyphthalaldehydic acid methyl ester (VI) prepared from V<sup>2</sup> appeared at  $\partial 6.82$  and 7.25 and the latter, which was assigned to the proton at 6 in VI,<sup>3</sup> agreed closely with that of II ( $\partial$  7.29), and (b) a positive Gibb's test of IV\* indicated the absence of a substituent at 7 in IV.

In the solution of phthalaldehydic acid, an equilibrium between the open-chain form and the lactol form is observed.<sup>4)</sup> The NMR spectrum of 3,5-dihydroxyphthalaldehydic acid (VII)<sup>2)</sup> in  $d^4$ -methanol showed broad signals at  $\partial$  6.46, 6.89 (each 1H, b.d, J=3 Hz) and 10.1~10.5 (1H, diffused signal), suggesting that an equilibrium also exists in the solution of VII and I (B).

Phenolic carboxylic acids such as orsellinic acid are common metabolites in fungi and several of these products are derivatives of a phthalaldehydic acid with hydroxyl groups

<sup>\*</sup> Strain 480-3, which is believed to be a *Penicillium* sp.

<sup>\*\*</sup> Unless otherwise stated, NMR spectra were measured in  $d^{\delta}$ -pyridine at 60 MHz.

<sup>\*</sup> The color reactions to I, II, III and VI were negative.



at 4 and/or  $6.5^{\circ}$  From the point of the biosynthesis, the substitution pattern may result from the cyclization of a tetraketide precursor with retention of oxygen atoms during aromatization. Isolation of I, being a derivative of VII, is the first instance and such a product with the unusual substitution pattern is only a few among fungal metabolites.<sup>6</sup>

Since II had been found to accelerate markedly root elongation of Chinese cabbage seedlings at a low concentration,\* the biological activities of several compounds structurally related to II (I–VII and  $\beta$ -resorcylaldehyde (VIII)) were examined. As shown in Table I, compounds II, III, VI and VIII

Table I. Effects of II, III, VI and VIII on Root Growth of Chinese Cabbage Seedlings (6 Days) $^{\alpha_1}$ 

Control=100; 28 mm

Compound	Concentration (ppm)				
	100	50	10	5	
II	86	189	164	129	
III	214	157	118	114	
VI	86	161	139	111	
VIII	43	168	143	125	

<sup>*a*</sup>) See Reference 1a).

promoted the root growth of Chinese cabbage seedlings. On the other hand, compounds I, IV, V and VII showed almost no activity. The maximum activity of II, VI and VIII, except III, appeared at around 50 ppm where the degrees of their activity were almost equal. However, the maximum activity of III appeared at a concentration more than 100 ppm, whereas II, VI and VIII inhibited root growth at 100 ppm. From the above results, it is concluded that  $\beta$ -resorcylaldehyde or salicylaldehyde moiety in the molecule plays an important part in root growth-stimulating activities of II, III and VI\*\* To the best of our knowledge, this is the first example which demonstrates an active chromophore for root growth-stimulating activity.<sup>7</sup>

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<sup>\*</sup> II showed little stimulation of root growth of rice seedlings at concentrations between 5 ppm and 50 ppm.

<sup>\*\*</sup> While a solution of II in methanol-water (1:1) was treated under the same condition as that for bioassay, the decomposition product could not be detected by NMR spectrometry.