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Isolation of New Chlorosis-inducing Substances Produced by Fungus

Sir:

To the best of our knowledge, there were only two reports that phytopathogenic fungi, *Puccinia graminis*¹⁾ and *Alternaria tenuis*,²⁾ produced chlorosis-inducing substances, but the active principle is not isolated yet. We have found that the culture filtrate of the fungus No. 501-7Y (genus and species unidentified) induced chlorosis of plants, and isolated two active substances in crystalline state. We now wish to report the first instance of the isolation of chlorosis-inducing metabolites of fungus and their physicochemical properties and biological activities.

The fungus was shaken at 29°C for 6 days in a natural medium containing peptone and corn steep liquor. The culture filtrate was acidified with sulfuric acid to pH 3, and extracted with ethyl acetate. The extract was fractionated into strongly acidic and weakly acidic-neutral fraction. The former was analysed by thin-layer chromatography (silicic acid developed in chloroform/ethanol 8:1) and the active components were located at *R_f* 0.35 and 0.50. By repeated chromatography with a column of silica gel (benzene/ethanol and chloroform/ethanol) of the strongly acidic fraction, one active component (*R_f* 0.50) was isolated as colorless powder and the other (*R_f* 0.35) as colorless needle (acetone-hexane, mp 168~171°C). We proposed the name 'viridominic acids' A and C (VA-A and -C) for these compounds, respectively.

VA-A and -C are carboxylic acid and were

methyated with diazomethane in ether to give monomethyl ester, VA-A methyl ester: mp 110~112°C (acetone-hexane) and VA-C methyl ester: mp 186.5~187.5°C (ether-hexane). These compounds are optically active and the infrared (Fig. 1) and ultraviolet spectra are

FIG. 1. Infrared Spectra of Viridominic Acids A and C (KBr disk).

a, viridominic acid A; b, viridominic acid C.

almost superimposable, VA-V methyl ester: $[\alpha]_D^{25} + 23^\circ$ ($c=0.1$, MeOH), $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3500, 1710(br.), 1245, 1020 and 900, $\lambda_{\max}^{\text{EtOH}} \text{ m}\mu(\epsilon)$: 217 (8,100), VA-C methyl ester: $[\alpha]_D^{25} + 42^\circ$ ($c=0.1$, MeOH), $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3450, 1715(br.), 1216, 1017 and 915, $\lambda_{\max}^{\text{EtOH}} \text{ m}\mu(\epsilon)$: 216 (10,100). The molecular formula of VA-A methyl ester, $\text{C}_{34}\text{H}_{50}\text{O}_9$, and VA-C methyl ester, $\text{C}_{34}\text{H}_{52}\text{O}_9$, were determined by high resolution mass spectrometries and elemental analyses, VA-A methyl ester: m/e 510.3022 ($\text{M}^+-60-32$), Calcd. for $\text{C}_{31}\text{H}_{42}\text{O}_6$: 510.2981, Anal. Found: C, 67.67; H, 8.54%, Calcd. for $\text{C}_{34}\text{H}_{50}\text{O}_6$: C, 67.75; H, 8.36%. VA-C: m/e 530.3306 (M^+-60), Calcd. for $\text{C}_{31}\text{H}_{46}\text{O}_7$: 530.3244, methyl ester. Anal. Found:

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TABLE I. EFFECT OF VA-A, -C AND CCC ON THE CHLOROPHYLL SYNTHESIS IN THE COTYLEDONS OF 7 DAYS OLD SEEDLINGS

<i>Brassica campestris</i> L.						
Treatment (ppm)	Water dist.	VA-A		VA-C		CCC
		1.0	10.0	100	500	1,000
Chlorophyll a+b (mg/g fresh weight)	1.25	0.145	0.0135	0.762	0.236	0.906
<i>Panicum Crus-galli</i> L.						
Treatment (ppm)	Water dist.	VA-A		CCC		
		5.0	25.0	1,000		
Chlorophyll a+b (mg/g fresh weight)	1.43	0.953	0.125	0.918		

Seeds of Chinese' cabbage (*Brassica campestris* L. var. *pekinensis*) or barnyard grass (*Panicum Crus-galli* L. var. *frumentaceum* Trin.) were placed in Petri dishes on filter paper wetted with distilled water or solutions of the compounds to be tested, and incubated for 7 days in continuous fluorescent light at 28°C. Chlorophyll content was determined by the method of J. Bruinsma.³⁾

C, 67.89; H, 8.68%, Calcd. for $C_{34}H_{52}O_9$: C, 67.52; H, 8.67%.

The cotyledons of Chinese, cabbage (*Brassica campestris* L. var. *pekinensis*) seedling treated with VA-A and -C were yellow or pale green but these compounds had no effect on the growth of these plants at the tested concentrations for ten days. The content of chlorophyll in the cotyledons was reduced 80~90% at the concentrations of 1 ppm and 500 ppm by VA-A and -C, respectively (Table I). VA-A also reduced the content of chlorophyll in the coleoptiles and the leaves of barnyard grass (*Panicum Crus-galli* L. var. *frumentaceum* Trin.) seedling by about 90% at the concentration of 25 ppm (Table I). (2-Chloroethyl)-trimethylammonium chloride (CCC), known as chlorosis-inducing substance, reduced the content of chlorophyll in the cotyledons of Chinese' cabbage and barnyard grass by about only 10% at the concentration of 1000 ppm (Table I). Chlorosis induced by VA-A and -C was not reversed by the addition of magnesium or iron ions and also by the treatment with gibberellic acid, kinetin and potassium ion which was known to reverse the chlorosis

induced by some retardants and coumarin.⁴⁾

As naturally occurring chlorosis-inducing substances, some antibiotics produced by *Actinomycetes*, e.g. streptomycin,⁵⁾ carbomycin,⁶⁾ kanamycin⁷⁾ etc. are known to induce chlorosis of some green algae. VA-A and -C are quite different from these compounds in their physicochemical and biological properties. VA-A and -C are new compounds having strong chlorosis activity.

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