Isolation and Structure Elucidation of Colletopyrone from *Colletotrichum nicotianae*

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In the previous papers^{1~3)} we have reported the isolation from a strain of *Colletotrichum nicotianae*, a pathogenic fungus causative of tobacco anthracnose, of several orsellinaldehydes possessing a monoprenyl or diprenyl side chain at C-3 together with or without a chlorine atom at C-5. However, these compounds showed no biological activities responsible for the symptom of the disease. In continuation of the work we succeeded recently in the isolation of an α -pyrone compound named colletopyrone which is considered to be one of the fungal metabolites functioning as toxic principles. In this paper we wish to report the isolation and structure elucidation of the novel compound.

The fungus was grown for 72 hr at 26.5°C in a 1 ton-fermentation tank containing 400 liters of Czapek-Dox medium added with yeast extract (being kept around pH 7). The culture filtrate was worked up as illustrated in Fig. 1 to give 98 mg of colletopyrone (I) as colorless plates, mp >300°C. I, C₁₅H₁₆O₆ (M⁺ m/e 292), was soluble in pyridine, acetic acid, dioxane and dimethylsulfoxide, slightly soluble in methanol, ethanol, acetone, chloroform, ethyl acetate and water, and insoluble in *n*-hexane. It showed positive coloration with iodine and 2,4-dinitrophenylhydrazine and was negative to ferric chloride. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 289 (10800) and 300 (shoulder, 9930). IR ν_{max} cm⁻¹: 3440, 2940, 2720, 2600, 1682, 1620 and 1580 (KBr); 3010, 2970, 2900, 2820, 2730 and 2610 (CHCl₃).

In the NMR spectrum of I (CDCl₃, TMS)

were observed a 6H singlet due to two $\text{CH}_3\text{-}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{C-}$ at δ 1.95, a 6H singlet due to two $\text{CH}_3\text{-}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{C-}$ or $\text{CH}_3\text{-}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{C-}$ at δ 2.24 and a 2H siglet ascribable to a methylene at δ 3.53. A rather broad 2H singlet observed at δ 11.18 disappeared on the treatment with D₂O. This suggested the presence of two hydroxyls each constituting hydrogen bond with a carbonyl. Proton decouplings in ¹³C-NMR of I revealed eight signals corresponding to 15 carbon atoms at δ 169.51, 168.68, 156.83, 109.64, 101.74, 19.13, 17.26, and 9.83 ppm. These spectral data indicated that I should be a compound with an extremely symmetrical structure in which a methylene connects two α -pyrone rings (C₇H₇O₃) each containing a hydroxyl and two methyls.

- I: R₁=CH₃, R₂=CH₃
 III: R₁=H, R₂=CH₃
 IV: R₁=CH₃, R₂=C₂H₅

To elucidate the location of the hydroxyls on the rings, diazomethane-ether was added dropwise to I in ether for 4 hr with stirring. A monomethyl ether (II), C₁₆H₁₈O₆ (M⁺ m/e 306) was obtained as the main product. IR $\nu_{\text{max}}^{\text{Film}}$ cm⁻¹: 1675 and 1565. In the NMR spectrum of II, four 3H singlets appeared at δ 2.30, 2.15, 1.96 and 1.90 suggesting the dissymmetry in the structure of the ether. In addition, a 3H singlet due to a methoxyl was observed at δ 4.08. The UV spectrum of II showed an absorption maximum at 262 nm (ϵ 15000) ascribable to γ -pyrone together with a shoulder at 292 nm (ϵ 9550) ascribable to α -pyrone. This indicated the occurrence of transformation of an α -pyrone in I to a γ -pyrone in II during the methylation. Accordingly, the hydroxyl was assigned to C-4.

Then, we prepared 4,4'-dihydroxy-6,6'-dimethyl-3,3'-methylene-di-pyr-2-one (III) according to the method of Acker *et al.*⁴⁾ by the reaction of formaldehyde with 4-hydroxy-6-

FIG. 1. Procedure for the Isolation of Colletopyrone.

methyl-2-pyrone obtainable from dehydroacetic acid on the treatment with conc. sulfuric acid. The patterns in the NMR, IR and mass spectra of I were quite similar to those of III except a difference in δ values of their hydroxyls (11.18 in I and 10.74 in III). Moreover, the spectral data of I revealed similarity with those reported by Opitz and Hänsel⁵⁾ for helipyrone (IV), which was isolated from *Helichrysum italicum* G. Don (Compositae), except the splitting of carbonyl band in the IR spectrum of the latter (1688, 1678 and 1672 cm^{-1}). These suggested that the methylene in I is attached to C-3 on each pyrone ring being substituted with methyls at C-5 and C-6. The assignment of the methylene to C-3 is also favorable to explain the formation of hydrogen bond in I. Thus, the structure of colletopyrone was established as

4, 4'-dihydroxy-5, 5', 6, 6'-tetramethyl-3, 3'-methylene-di-pyr-2-one.

To examine the biological activity of colletopyrone, the compound was suspended in dimethylsulfoxide-water (5:95) at serial concentrations, and a drop (*ca.* 20 μl) of each suspension was placed on a young tobacco leaf pricked with a needle. Three days after administration, brown necrotic spots, being analogous to the symptom caused by the pathogenic fungus, appeared on the leaves treated at dosages more than 100 ppm.

This is the first report to show the occurrence of 3,3-methylene-di-pyr-2-one as a microbial metabolite. Except for I, helipyrone from a higher plant (Compositae) is the sole natural compound of this series hitherto known. The structural similarity between I and IV would be worthy of note from the bio-

genetical standpoint.

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