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Nobel Compounds from the Culture Broth of *Phyllosticta* sp.

Sir:

In previous papers, we reported the isolation and characterization of phytotoxic substances, phyllosinol (epoxydon, I),¹⁾ phyllostine²⁾ (II), 3-chloro-2,5-dihydroxybenzyl alcohol,²⁾ 6-methylsalicylic acid and gentisyl alcohol³⁾ from the culture broth of *Phyllosticta* sp. These substances were all detectable with Folin-Ciocalteu reagent. The present communication describes structural and stereochemical elucidation of three newly isolated compounds which were assigned as III, IV and V on the basis of their chemical and spectroscopic data. The extraction of the compounds was carried out by the same procedure as used for I and II.^{1,2)} Silica gel column chromatography of the extracts with an ethyl acetate-chloroform system containing a small amount of acetic acid gave a mixture of I and III, and pure IV and V. The mixture of I and III was further separated on a silica gel column with chloroform-methanol (95:5).

Compound III possessed the following properties, $C_7H_8O_4Cl$,⁴⁾ mp 126~128°C m/e 174

(M^+-H_2O), $[\alpha]_D = -158$ ($c=1.0$, EtOH); CD (dioxane, $c=0.04$) $[\theta]_{335} + 34000$; IR ν_{max}^{KBr} 3270 (OH), 1687 cm^{-1} (C=O); UV, λ_{max}^{EtOH} 226 nm (ϵ 5600) ($\alpha\beta$ -unsaturated ketone); NMR⁵⁾ τd_6 -acetone+D₂O. 5.87 (1H, *m*, 5-H), 5.57 (2H, *m*, -CH₂-), 5.53 (1H, *dJ*=6.3 Hz, 6-H), 5.27 (1H, *m*, 4-H). 3.09 (1H, broad, $W_{1/2}$ =7.4 Hz, 3-H). The position of the chlorine atom was determined by the fact that III was converted to IV as described later. A fairly large coupling (J =6.3 Hz) of the signal at τ 5.53 due to vicinal two protons, 5-H and 6-H, indicates that the chlorine atom at C-6 and the hydroxyl group at C-5 occupy two equatorial positions in the half chair conformation. Finally, the absolute configuration of III was determined by the correlation of III with phyllosinol (I) whose stereochemistry had been established by CD measurement.¹⁾ Thus, phyllosinol (I) was treated with conc. hydrochloric acid in ethanol at room temperature. The reaction proceeded through stereospecific *trans* opening of the epoxide ring⁶⁾ to an unstable conformer (a)

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2) S. Sakamura, J. Ito and R. Sakai, *Agr. Biol. Chem.*, **34**, 153 (1970); S. Sakamura, J. Ito and R. Sakai, *ibid.*, **35**, 105 (1971); M. Sequin-Frey and Ch. Tamm, *Helv. Chim. Acta*, **54**, 851 (1971); A. Ichihara, K. Oda and S. Sakamura, *Agr. Biol. Chem.*, **35**, 445 (1971).

3) S. Sakamura, T. Chida, J. Ito and R. Sakai, *Agr. Biol. Chem.*, in press.

and then immediately converted to a stable conformer (III) as illustrated below, and gave a crystalline compound which was identical

4) All the new compounds gave satisfactory elemental analyses.

5) Abbreviations s, singlet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; 1H, one-proton intensity; 5-H, proton attached to C-5; $W_{1/2}$ =7.4 Hz half-band width of 7.4 Hz.

6) E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, "Conformational Analysis," p. 358, John Wiley & Sons, Inc. London (1965).

with III in all respects. Therefore, the structure and stereochemistry including the conformation must be depicted as III and designated as 6(S)-chloro-4(R), 5(R)-dihydroxy-2-hydroxymethyl-2-cyclohexen-1-one.

The physical properties of the second compound IV are as follows; $C_7H_9O_4Cl$, mp 142~144°C, m/e 174 ($M^+ - H_2O$), $[\alpha]_D = -121$ ($c=0.65$,

EtOH); CD (dioxane, $c=0.11$) $[\theta]_{310} +11000$ UV, $\lambda_{\text{max}}^{\text{EtOH}}$ 232 nm (ϵ 8400), IR $\nu_{\text{max}}^{\text{KBr}}$ 3380 (OH) 3280 (OH) 1700 cm^{-1} (C=O); NMR⁷⁾ $\tau_{\text{d}_6\text{-acetone}}$ 5.76 (2H, $\text{CH}_2\text{-O-}$), 5.50 (1H, broad $W_{\frac{1}{2}}=9.4$ H 5-H), 5.22 (1H, broad $W_{\frac{1}{2}}=9.4$ H 4-H), 4.99 (1H, broad $W_{\frac{1}{2}}=4.8$ H, 6-H), 3.23 (1H, broad $W_{\frac{1}{2}}=6.8$ H 3-H). This compound is an isomer of compound III and the position of the chlorine atom was established by the fact that IV was easily transformed to 3-chloro-2,5-dihydroxybenzyl alcohol. Compound IV was correlated with compound III through enolization. Namely, compound III was treated with aqueous sodium bicarbonate (10%) in tetrahydrofuran for 3 min at 30°C to yield compound IV. The stereochemical structure should be

7) Broadening of signals in compound IV might be due to the presence of an equilibrium between two conformers, a and b, since double resonance experiments showed still unresolvable signals.

6(R)-chloro-4(R), 5(R)-dihydroxy-2-hydroxymethyl-2-cyclohexen-1-one.

The third compound, V has the following physical properties: $C_9H_{12}O_6$ mp 166~168°C, m/e 156 ($M^+ - \text{CH}_3\text{COOH}$), $[\alpha]_D = -181$ ($c=1$, EtOH); CD (dioxane, $c=0.10$) $[\theta]_{335} +26600$; UV $\lambda_{\text{max}}^{\text{EtOH}}$ 221 nm (ϵ 4400); IR $\nu_{\text{max}}^{\text{KBr}}$ 3430 (OH), 1740 (ester $c=0$), 1682 ($c=0$) NMR $\tau_{\text{d}_6\text{-acetone}}$ 7.90 (3H, s, OCOCH_3), 5.99 (1H, dd, $J=10.8$ Hz, $J=5.4$ Hz 5-H), 5.79 (2H, $\text{CH}_2\text{-O-}$), 5.46 (1H, t, $J_{4\text{H},5\text{H}}=5.4$ Hz, $J_{3\text{H},4\text{H}}=5.4$ Hz 4-H), 4.46 (1H, d, $J=10.8$ Hz 6-H), 2.99 (1H, dt, $J_{3\text{H},4\text{H}}=5.4$ Hz, $J_{3\text{H},\text{CH}_2}+2$ Hz 3-H). A large coupling constant ($J=10.8$ Hz) of the signal at τ 4.46 due to 6-H indicates that the acetoxyl group and vicinal hydroxyl group are equatorially oriented in the half-chair conformation. The absolute configuration shown below was determined on the basis of the fact that phyllosinol (I) was converted to V with boron trifluoride etherate in acetic acid, and compound V was designated as 6(S)-acetoxo-4(R), 5(R)-dihydroxy-2-hydroxymethyl-2-cyclohexen-1-one. It is suggested that compound IV is a precursor of 3-chloro-2,5-dihydroxybenzyl alcohol.²⁾ It was

further confirmed that IV was able to be detected in the ethyl acetate extract of the culture broth without any other separating treatments, though the other two were obscure. This may indicate at least IV is a substantial metabolite of the fungus.

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