Tetrahedron Letters No. 1, pp 13 - 16, 1972. **Pergamon Press. Printed in Great Britain.**

ISOLATION OF 3,4-DIHYDRO-3,4,8-TRIHYDROXY-1(2H)-NAPHTHALENONE AND TENUAZONIC ACID FROM *PYRICULARIA ORYZAE* CAVARA

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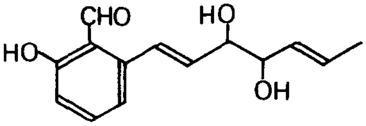
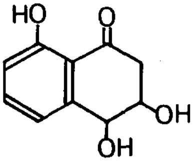
(Received in Japan 19 November 1971; received in UK for publication 1 December 1971)

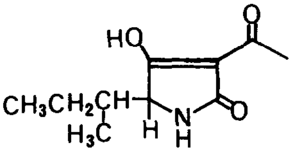
In the course of our study on phytotoxic substances produced by *Pyricularia oryzae* Cavara we have reported the isolation and structural elucidation of pyriculol (I)[1] which is considered to be responsible for the symptoms of rice blast. This paper reports the isolation of a new phytotoxic substance, 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone (II), and tenuazonic acid (III) from *P. oryzae*.



I



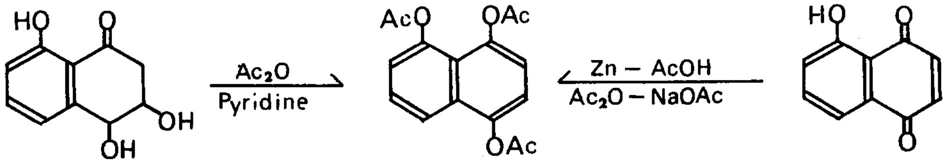
II



III

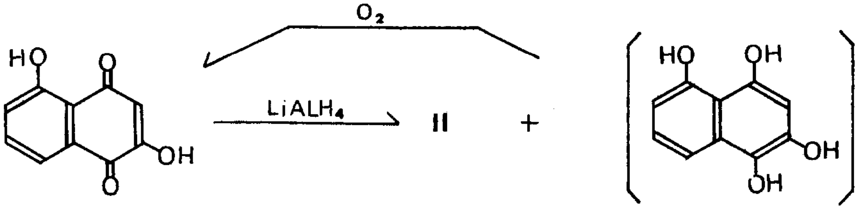
The ethylacetate extract from the culture broth of *P. oryzae* (Ken 53-33) was subjected to a silica gel column chromatography. The compound II was eluted with a mixture of benzene and ethyl acetate (6:4). Repeated chromatographies of the crude crystalline substance and a subsequent crystallization from acetone afforded colorless crystals. This compound reduced growth of rice seedlings when applied to them in high concentrations, but it was observed that the growth of rice seedlings treated with solutions containing 100 p.p.m. of this compound for 24 hours before they were transferred to culture solution was slightly stimulated.

The compound II, $C_{10}H_{10}O_4$, m.p. 191-2°, $[\alpha]_D$ -36.0° (MeOH), showed its uv absorption maxima (EtOH) at 254.8, 260.8, 335.0 nm (ε: 10,500, 10,500, ca 4,000), and ir bands (KBr) at 3600-2400, 1640, 1580, 1080, 965 cm^{-1}. These spectral data indicate the presence of hydroxy groups (3600-2400 cm^{-1}) and carbonyl group conjugated with an aromatic ring (1540, 1580 cm^{-1} and 335.0 nm) in the molecule. The nmr spectrum in acetone containing D_2O exhibits the signals due to three aromatic protons at 7.54 (triplet, J=7.5 Hz), 7.15 (doublet, J=7.5 Hz) and 6.73 (doublet, J=7.5 Hz), two protons on the carbons bearing hydroxy groups at 4.66 (doublet, J=8.0 Hz) and 4.07 (multiplet), and two geminal methylene protons at 3.06 (quartet, Js=17.5 and 5.0 Hz) and 2.70 (quartet, Js=17.5 and 8.0 Hz). Upon acetylation with acetic anhydride in pyridine compound II gave rise to 1,4,5-trihydroxynaphthalenetriacetate (IV), which was identified with the authentic sample synthesized by a reductive acetylation of juglone (V) with zinc in acetic acid in the presence of acetic anhydride and sodium acetate[2]. Since these data could acount for the structure II, the compound was prepared in 27 % yield by a



II IV V

reduction of 2-hydroxyjuglone (VI)[3] with large excess amount of $LiAlH_4$ in tetrahydrofurane under cooling in ice water, accompanying about 50 % of recovery of the starting material. The compound thus synthesized was completely identical with the natural product in all respects except optical rotation. The apparent recovery of the starting material would probably be due to the formation of unstable 1,2,4,5-tetrahydroxynaphthalene (VII) as the primary product, which could be converted to VI by an air oxidation in the work-up procedure.



VI VII

The stereochemistry of the glycol moiety is not determined yet, however the coupling constant (J=8.0 Hz) between the two protons on the particular carbons would suggest that the two hydroxy groups are oriented to *trans*[4]. The assignment of the nmr signals of the compound II are shown in Fig. 1, the relative arrangement of the protons were verified by the spin-spin decoupling technique. The compound has been isolated also from other strains Hoku 373 and Hoku 373-61 of *P. oryzae*.

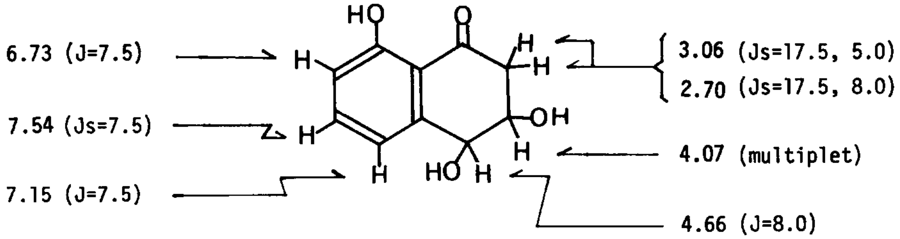


Figure 1

Another phytotoxic substance was first isolated from the culture broth of the strain Hoku 373-61 which is a non-pathogenic mutant from Hoku 373, and turned out to be produced also by pathogenic strains of *P. oryzae*. The substance was purified by a silica gel column chromatography in which the silica gel was treated with diluted hydrochloric acid and air-dried[3]. The compound $C_{10}H_{15}NO_3$ was viscous gummy substance, and was identified with tenuazonic acid (III) by a synthesis according to the known procedure condensing L-isoleucine with diketene[5].

Tenuazonic acid inhibited growth of shoots and roots of rice seedlings by 72.5 % at a concentration of 200 p.p.m., whereas stimulation of root elongation occurred at 5 p.p.m.. It is of interest that the germination of conidia of *P. oryzae* was completely prevented at a concentration of 40 p.p.m. of tenuazonic acid as well as of its congeners prepared by the condensations of leucine and norleucine with diketene.

The isolations of tenuazonic acid from the culture filtrates of several fungi have been reported[6], and the anti-neoplastic activities of the acid and of the related derivatives as well as their metabolic activities have been investigated intensively[7]. Recently this compound was reported as a vivotoxin of *alternaria longipes*, a fungus causative of the tobacco brown-spot disease[8]. Our finding demonstrates the second example of the phytotoxic nature of the compound.

REFERENCES AND FOOTNOTE

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4. *Trans*-1-acetoxy-2-phenyltetralin has a coupling constant of 8.0 Hz between the particular protons, whereas the *cis* isomer has 3.5 Hz [K. Hanaya, *J. of Chem. Soc. Japan*, 87 745 (1966)]. The similar feature are also shown for *trans*-(6.5 Hz) and *cis*-1-hydroxy-2-methyltetralin (3.0 Hz) [K. Hanaya, *ibid.*, 87, 991, (1966)].
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