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ISOLATION AND STRUCTURAL ELUCIDATION OF A PHYTOTOXIC SUBSTANCE PRODUCED BY PYRICULARIA ORYZAE CAVARA

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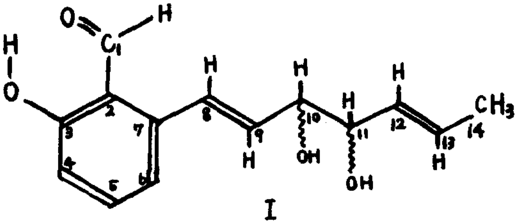
Blast caused by Pyricularia oryzae is the most destructive disease of the rice plant in Japan and other Asian countries. Piricularin ($C_{18}H_{14}N_2O_3$) and α-picolinic acid were isolated from this fungus as the causative substances of the disease by Tamari et al[1], but the structure of the former remains unknown.

We wish to report the isolation of a substance from P. oryzae which is considered to be responsible for the symptoms of rice blast, and the elucidation of the chemical structure.

The ethyl acetate extract from the culture broth of P. oryzae (Ken. 53-33) was subjected to a silica gel column chromatography. The compound I eluted with a mixture of benzene and ethyl acetate (7:3) was obtained as a pale yellow needle after repeated crystallization. This compound causes a dark-coloured necrotic spot on a rice leaf which closely resembles that caused by the parasite. Growth of shoots and roots of rice seedlings is greatly reduced when plants are placed in solutions containing 50 to 500 p.p.m. of the compound.

The compound I, $C_{14}H_{16}O_4$[*1], m.p. 96-7°C, $[\alpha]_D$-54.3°($CHCl_3$), which is now designated as Pyriculol, showed uv absorption maxma (EtOH) at 232.5, 280.0, 358.0 nm, (ε, 15600, 6000, 2800), and ir bands (KBr) at 3600 - 2400, 1642, 1612, 1560, 1550, 970, 795 cm^{-1}. The molecular formula and the spectral data of Pyriculol (I)

[*1] Compound I had a satisfactory elemental analysis. Its mass spectrum showed no M^+ peak but m/e 178 which corresponds to the ion cleaved at C_{10}-C_{11} bond.



I

Table 1

Proton Magnetic Resonances*2 of Pyriculol (I) in $CDCl_3$

Proton Position (Proton Number)	Chemical Shift (ppm)		Spin-spin Coupling (Hz)
C_1-H (1H)	10.27	s	
C_3-OH (1H)*a	11.32	s	
C_4,C_6-H (2H)	6,85, 6.88	two d	7.5, 8.0
C_5-H (1H)	7.41	t	8.0, 7.5
C_8-H (1H)	7.13	d	16.3
C_9-H (1H)	6.10	q	16.3, 5.5
C_{10}-H (1H)*b	4.37	dif. q	5.5, 4.0 l.r.c.*c with C_8-H
C_{11}-H (1H)*b	4.22	dif. q	4.0, 5.7 l.r.c. with C_{13}-H
C_{12}-H (1H)	5.53	dif. q	5.7, 15.5 l.r.c with C_{14}-H
C_{13}-H (1H)	5.79	o	15.5, 6.0
C_{14}-H (1H)	1.73	d	6.0 l.r.c. with C_{12}-H
C_{10},C_{11}-OH (1H)*a	2.00 - 3.00	broad s	chemical shifts are depending on the conditions.

*a Dissapear on addition of D_2O.

*b Upon acetylation shift to down field by ca 1 p.p.m.

*c l.r.c. = long range coupling.

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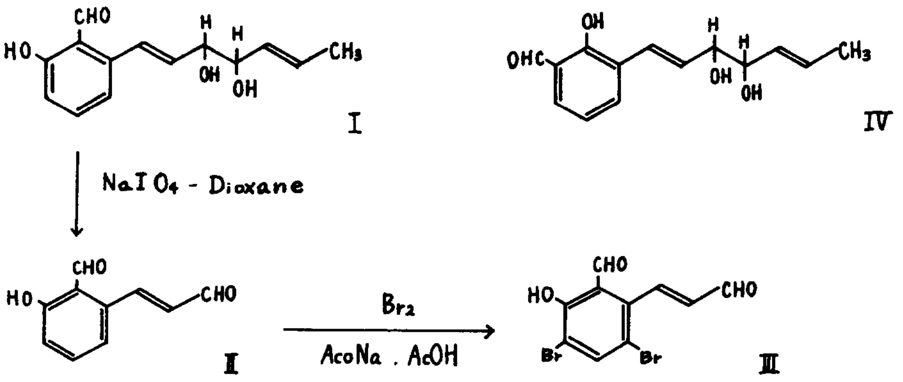
*2 The nmr data are given as follows: Signal positions are given in δ-value and the coupling constants are given with Hz. Abbreviations: s(singlet), d(doublet), t(triplet), q(qualtet), o(octet) and dif.(difuse).

indicate a highly unsaturated chemical structure possessing an aromatic ring in the molecule. The phenolic property of the compound was shown by solubilities in aqueous alkaline solutions as well as the deep colouration in a fèrric chloride test. Upon acetylation I gave a diacetate in which phenolic hydroxy group was still remained unacetylated. The remaining one oxygen function was determined to be an aldehyde conjugated to the aromatic ring according to the ir band at 1642 cm^{-1} and the nmr signal at 10.27 which did not dissapear by an addition of D_2O in a $CDCl_3$ solution. A detailed nmr study in $CDCl_3$ solution provided the complete assignment of all the proton signals of the molecule as shown in Table 1 and the relative arrangements of protons on the C_3-, C_4-, and C_5-, and the protons on the C_8-, C_9-, C_{10}-, C_{11}-, C_{12}-, C_{13}- and C_{14}-carbon atoms were established by spin-spin decoupling technique. The trans configurations of C_8-C_9 and C_{12}-C_{13} double bonds with respect to the longer carbon chain were apparent from respective large coupling constants between C_8-H and C_9-H (16.3 Hz) and C_{12}-H and C_{13}-H (15.5 Hz). A prominent downfield shift of the phenolic proton of I (δ 11.82 p.p.m.) compared with that of phenol itself (δ 4.20 p.p.m.) suggest that the phenolic proton of I is strongly hydrogen-bonded with the aldehyde group indicating the vicinity of the two groups, as demonstrated in the case of salicyl aldehyde. Periodate oxidation of I afforded a dialdehyde (II) as a yellow crystal m.p. 102-3° (benzene-hexane), uvλ_{max}^{EtOH} nm (ε), 247.5 (17100), 291.0 (11800), 361.0 (5900), nmr, C_1-H 10.51(s), C_3-OH 11.94(s), C_4-H and/or C_6-H 7.08, 7.12 (two d 7.5, 8.5), C_5-H 7.57 (t, 7.5, 8.5), C_8-H 8.09 (d, 15.0), C_9-H 6.67 (q, 8.0, 15.0), C_{10}-H 9.85 (d, 8.0).



The assignment of the position of the alkyl substituent rest on the observation that a bromination of II with bromine in acetic acid with the presence of sodium acetate proceeded analogously to that of salycyl aldehyde to give 3.5-dibromo salycyl aldehyde, affording a dibromide (III), m.p. 147-50° (benzene-hexane), ms M^+ 232, nmr, C_1-H 9.98 (s), C_3-OH 12.52 (s), C_5-H 8.00 (s), C_8-H 6.27 (d, 16.5), C_9-H 7.62 (q, 7.5, 16.5), C_{10}-H 9.81 (d, 7.5). The fact indicate that one of the two *ortho* positions of hydroxy group is not substituted and, therefore, an alternative structure IV could be excluded. Thus the structure of Pyriculol I was determined except the stereochemistry at C_{10} and C_{11} carbon atoms.

References

1. a) K. Tamari and J. Kaji: Nippon Nougei-kagaku Kaishi, 28 254 (1954).
   b) K. Tamari and J. Kaji: Nippon Nougei-kagaku Kaishi, 31 387 (1957).