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ISOLATIONS OF PHYTOTOXIC SUBSTANCES PRODUCED BY *PYRICULARIA ORYZAE* CAVARA

Shigeo Iwasaki, Hideki Muro, Keizo Sasaki, Shigeo Nozoe and Shigenobu Okuda

Institute of Applied Microbiology, University of Tokyo

Zenji Sato

National Institute of Agricultural Sciences

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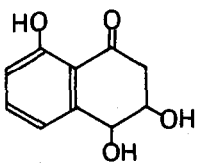
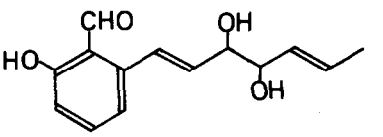
In our continuing work to search for phytotoxic substances produced by *Pyricularia oryzae* Cavara we have isolated additional five new compounds related to 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone (I)[1] and pyriculol (II)[2]. This paper reports the isolations and structural elucidations of these compounds.



I

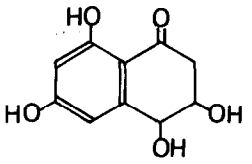


II

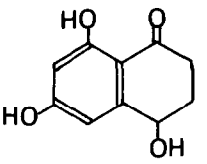
Repeated silica-gel column chromatographies of ethyl acetate extract from culture broth of *P. oryzae* (Ken 53-33) afforded new phytotoxic substances, 3,4-dihydro-3,4,6,8-tetrahydroxy-1(2H)-naphthalenone (III) and 3,4-dihydro-4,6,8-trihydroxy-1(2H)-naphthalenone (IV) and three new compounds V, VIa and VIb related to pyriculol (II). In addition to these compounds we have isolated known compounds, 2-carboxy-3,5-dihydroxybenzylmethyl ketone (VII)[3], 3,4-dihydro-6,8-dihydroxy-3-methyl isocoumarin (VIII)[4] and prolylleucyl anhydride (IX)[5] whose isolations from other fungi were already reported.

The compound III, $C_{10}H_{10}O_5$, mp 166°, $[\alpha]_{400}^{23°}$ -75° (EtOH), showed its uv absorption maxima (EtOH) at 216, 221, 233.5, 239, 284 nm (ε: 16500, 14900, 9600, 8500, 13900, 6200), and ir bands (KBr) at 3360, 1610, 1485 cm^{-1}. The compound IV, $C_{10}H_{10}O_4$, mp 194-5°, $[\alpha]_{400}^{23°}$ +40°

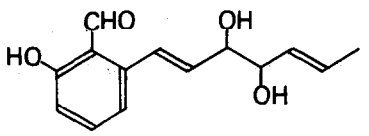
(EtOH), showed its uv absorption maxima (EtOH) at 210, 221, 233.5, 239, 284, 313 nm (ε: 15600, 14600, 9600, 8700, 14800, 6900), and ir bands (KBr) at 3280, 3200, 1630, 1610, 1590, 1570 cm^{-1}.



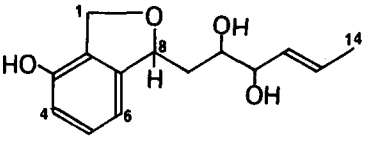
III



IV



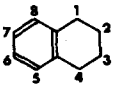
V



VIa,b

Their molecular formulas and the spectral data indicate that these compounds have the structures close to that of I whose structure was already determined[1], and comparisons of the

Table I



	I	III	IV
C_2-H	3.06 (1H, q, 17.5, 5.0)	3.03 (1H, q, 17.5, 5.0)	2.5-5.9 (2H, m)
	2.70 (1H, q, 17.5, 8.0)	2.68 (1H, q, 17.5, 8.0)	
C_3-H	4.07 (1H, m)	4.01 (1H, m)	1.8-2.5 (2H, m)
C_4-H	4.66 (1H, d, 8.0)	4.54 (1H, d, 8.0)	4.76 (1H, q, 8.0, 3.0)
C_5-H	7.15 (1H, q, 7.5, 2.5)	6.70 (1H, d, 2.5)	6.60 (1H, d, 2.5)
C_6-H	7.54 (1H, t, 7.5, 7.5)	- - - - -	- - - - -
C_7-H	6.73 (1H, q, 7.5, 2.5)	6.26 (1H, d, 2.5)	6.24 (1H, d, 2.5)

The chemical shifts are given in δ-value and coupling constants are with Hz.

nmr data (Table I) of III and IV with that of I could lead to the structural assignments of these compounds. The configuration of the α-glycol moiety of III was assigned to be *trans* based on the same argument for compound I[1]. The relative arrangements of the protons on C_2, C_3, C_4 and C_5 of the compound III and the protons on C_3, C_4 and C_5 of IV were verified by spin-spin decoupling technique.

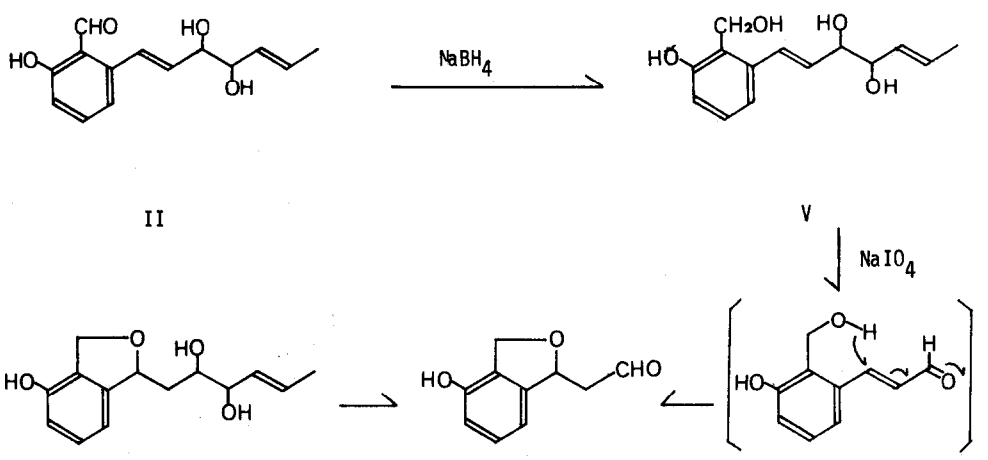
The compound V, $C_{14}H_{18}O_4$, gummy substance, $[\alpha]_{400}^{20°}$ +100° (EtOH), showed its uv absorption maxima (EtOH) at 219, 254, 293 nm (ε: 29500, 9400, 3300), and its ir bands ($CHCl_3$) showed the presence of OH groups (3600-3510 cm^{-1}) and aromatic group (1600 cm^{-1}) but no carbonyl group. The nmr spectrum in CD_3COCD_3 exhibited the signals due to C_{14}-H, δ 1.67 (3H, d, J=6.0), C_{10}-H, 4.02 (1H, q, Js=12.5, 6.5), C_{11}-H, 4.20 (1H, q Js=12.5, 6.0), C_1-H, 4.84 (2H, s), C_{13}-H, 5.75 (1H, o, Js=15.0, 6.0), C_{12}-H, 6.10 (1H, q, Js=15.0, 6.0), C_9-H, 6.15 (1H, q, Js=16.0, 6.5), C_8-H, 6.95 (1H, d, J=16.0), C_4-, C_6-H, 6.75, 6.90 (2H, 2d, Js=7.0) and C_5-H, 7.05 (1H, t, Js=7.0, 7.0). These data suggested the structure V, and it was further confirmed by an identification with the product derived by a sodium borohydride reduction of pyriculol (II).

Table II

	VIa	VIb
C_{14}-H	1.73 (3H, d, J=6.0)	1.70 (3H, d, J=5.0)
C_9-H	1.80 (2H, q, Js=6.0, 4.0)	1.77 (1H)*
		1.97 (1H, s, J=13.0, 4.0, 4.0)
C_{10}-, C_{11}-H	3.7-4.1 (2H, m)	3.7 (2H, m)
C_1-H	5.02 (2H, br, s)	4.97 (1H, d, J=12.5)
		5.10 (1H, d, J=12.5)
C_8-H	5.42 (1H, t, J=6.0)	5.20-5.50 (1H, difused q)
C_{12}-H	5.52 (1H, q, Js=15.0, 6.0)	5.55 (1H, q, Js=15.0, 5.0)
C_{13}-H	5.75 (1H, o, Js=15.0, 6.0)	5.77 (1H, o, Js=15.0, 5.0)
C_4, C_6-H	6.68 (2H, 2d, Js=8.0)	6.67 (1H, d, J=8.0)
		6.90 (1H, d, J=8.0)
C_5-H	7.12 (1H, t, J=8.0)	7.10 (1H, t, J=8.0)

* Splittings of the signal were not observable because of the overlapping of C_{14}-H signal.

Two isomeric compounds VIa [$C_{14}H_{18}O_4$, mp 189°, $[\alpha]_{400}^{26°}$ +61.1° (EtOH)] and VIb [$C_{14}H_{18}O_4$, gummy substance, $[\alpha]_{400}^{26°}$ -4.8° (EtOH)] possess the identical uv and ir spectral data [λ_{max}^{EtOH} 270, 278 nm (ε: 2600, 2370), $\nu_{max}^{CHCl_3}$ 3400-2800, 1605, 1470, 1380, 1290, 1100-950 cm^{-1}]. The nmr signals of VIa and VIb in CD_3OD are also very similar to each other indicating the same grouping (Table II), and they could be stereoisomeric compounds possessing a ether linkage between C_1 and C_8. Periodate oxidation of VIa and b afforded aldehydes Xa and Xb, respectively, which were completely identical in all respects except their optical rotations ($[\alpha]_{400}^{23°}$ +49.5° for Xa and -39.1° for Xb in EtOH), and they, therefore, should be stereoisomeric at original C_8 position. Consequently the compounds VIa and b should also differ in stereochemistry at this position. The structures of Xa and b were supported by formation of the racemic aldehyde X by a periodate oxidation of V to give hydroxy aldehyde XI followed by a facile ether ring closure. Thus the partial structures containing C_1 to C_{10} of the compounds II, V, VIa and VIb were correlated chemically.

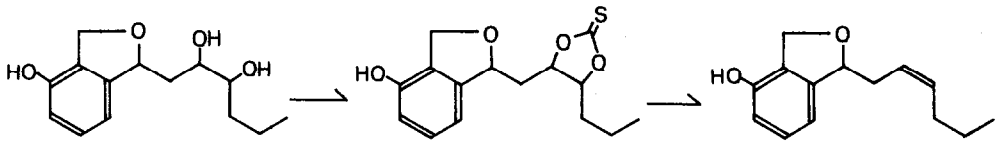


VIa,b Xa,b XI

On the other hand, a microbial transformation of pyriculol to V, VIa and VIb was carried out using *P. oryzae* (Ken 53-33) and pyriculol was proved to be transformed by this microorganism to compound V, VIa and b. This result provided not only biosynthetic evidences but also the structural proof for the stereochemical identity of C_{10}-C_{11} α-glycol moieties of

these compounds, since no structural change of this part might be involved during these biological processes.

Configurational assignment of the α-glycol moiety has not been achieved so far, and the elucidation of the relative configuration was attempted by an elimination of the hydroxy groups of compound XII to give an olefin XIV according to the Corey's procedure[6] *via* a cyclic thiacarbonate X which undergo *cis* elimination by affecting triethyl phosphite. The olefin XIV exhibited the nmr signals at δ 4.9 (1H, m) and 5.2 (1H, m) due to olefinic protons, and a double decoupling technique proved the coupling constant between them to be 7.5 Hz which assigned a *cis* configuration of the double bond. Accordingly the α-glycol moiety of pyriculol and the related compounds should possess an *erythro* configuration.



XII XIII XIV

The structures of the compounds VII [$C_{10}H_{10}O_5$, mp 156°, uv λ_{max}^{EtOH} 216, 268, 301 nm (ε: 27900, 10900, 6000), ir ν_{max}^{KBr} 3180, 1640, 1635, 1600, 1580, 1500 cm^{-1}, nmr (CD_3COCD_3 + H_2O) δ 6.28 (1H, d, J=2.5), 6.33 (1H, d, J=2.5), 3.18 (2H, s), 1.70 (3H, s)], VIII [$C_{10}H_{10}O_4$, mp 2.4-5°, uv λ_{max}^{EtOH} 270, 305 nm (ε: 13200, 5980), ir ν_{max}^{KBr} 3440, 1635, 1480, 1260 cm^{-1}, nmr (CD_3COCD_3) δ 6.28 (2H, s), 2.80 (1H, q, Js=15.0, 11.0), 3.00 (1H, q, 15.0, 5.0), 4.68 (1H, m), 1.45 (3H, d, J=7.5)] and prolylleucyl anhydride IX [$C_{11}H_{18}N_2O_2$, mp 159°, $[\alpha]_D^{20°}$ · -123°, ir ν_{max}^{KBr} 3385, 2960, 2880, 1670, 1475, 1435 cm^{-1}] were confirmed through comparisons of their spectral data with those in literatures[3, 4, 5]. Co-occurrence of the compounds I, III and IV together with VII would suggest that the compound VII might be the biogenetic precursor of the other compounds.

The compound III showed no significant activity on the growth of rice seedlings. The compounds IV and IX exhibited slightly promoted growths of the second leaves of rice seedlings at a concentration of 500 p.p.m. The compounds VII and VIII inhibited growth of roots

of rice seedlings at high concentration of 500 p.p.m., whereas stimulation of root elongation occurred at 60 p.p.m. The compounds V, VIa and VIb promoted root elongation of rice seedlings at a concentration of 250 p.p.m.

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