Short Communication

3-Hydroxy-7-oxo-sandaracopimaradiene (Oryzaalexin A),
a New Phytoalexin Isolated
from Rice Blast LeavesTadami AKATSUKA, Osamu KODAMA,
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Phytoalexins of the rice plant (*Oryza sativa*) were first observed by the drop-diffusate method, employing a conidia suspension of *Pyricularia oryzae*, 20 years ago.¹⁾ Since that time, many researchers^{2~6)} have reported the existence of phytoalexin-like substances in rice plants infected with *P. oryzae*, but the chemical characterization of these substances has not yet been reported. Recently, Cartwright *et al.*^{7~9)} have reported that momilactones A and B occur as the phytoalexins in ultraviolet-irradiated, dark-grown rice coleoptiles. These compounds had earlier been obtained as the plant growth inhibitors from rice husk.^{10,11)} The production of momilactones was increased when rice plants were pretreated with a compound WL 28325 (2,2-dichloro-3,3-dimethylcyclopropane carboxylic acid) prior to infection with blast disease and momilactone B was scarcely observed in WL 28325-untreated rice blast leaves.¹²⁾ The object of this experiment was to isolate and identify the phytoalexins produced in the host plant as a result of metabolic interaction between the host plant *O. sativa* and the parasitic fungus *P. oryzae*.

Approximately 1.5 kg fresh weight of the

lesions obtained from 50 kg of the foliar part of rice plants infected with blast disease in the paddy field were boiled in 70% methanol for 3 min, and then homogenized with an ultrahomogenizer (Physoctron). The homogenate was centrifuged at $3900 \times g$ for 10 min and the supernatant was then concentrated *in vacuo*. The concentrated supernatant was partitioned four times using petroleum ether, and the extract was concentrated *in vacuo*. This was then subjected to separation by preparative TLC (silica gel H, E. Merck, 0.5 mm, 20×20 cm) with benzene-ethyl acetate (10:1, v/v). The area between *R_f* 0.15 and 0.4 on the developed TLC was scraped off and extracted four times with methanol. The eluates were combined, concentrated, applied to a Sephadex LH-20 column (2.1×58.5 cm) and eluted with methanol. The eluted fractions were tested for antifungal activity by the spore germination method and the antifungal fractions were concentrated *in vacuo*, and further purified by precoated preparative TLC (silica gel, PLK5F, Whatman, 1 mm, 20×20 cm) with benzene-ethyl acetate (10:1). Antifungal activity was observed at *R_f* 0.22, 0.28, 0.36 and 0.61 on TLC. The *R_f* values of these four active substances on the TLC were seemingly distinguishable from those of the momilactones by a direct comparison. On the other hand, these compounds could not have been detected in the healthy area of the infected rice leaves. The major substance at *R_f* 0.22 was further purified with a Sephadex LH-20 column (2.2×92.5 cm) using methanol. The active eluates were concentrated *in vacuo* and further purified on the TLC plate (PLK5F) in the same manner as described above. The substance extracted from TLC was finally purified in a Toyopearl HW-40 column (1.4×27.0 cm) using methanol, and the final yield was 1.5 mg. The biological activity of the substance was then tested by observing its inhibitory action on the germ tube elongation and spore germination of *P. oryzae*. The major substance significantly inhibited the germ tube elongation of *P. oryzae* at 40 ppm, and completely inhibited spore germination at 200 ppm.

TABLE I. ASSIGNMENTS OF SIGNALS IN PROTON NMR

Assigned H	Coupled with—	Assigned H	Coupled with—
1H a 1.28 e 1.83	1He, 2Ha, 2He 1Ha, 2Ha, 2He	12H a 1.51 e 1.64	12He, 11Ha, 11He* 12Ha, 11Ha, 11He*, (14H)
2H a 1.65 e 1.73	2He, 1Ha, 1He, 3Ha 2Ha, 1Ha, 1He, 3Ha	14H 6.74	(9a), (12H)
3H a 3.30	2Ha, 2He	15H 5.70	16Ht, 16Hc
5H a 1.50	6Ha, 6He	16H t 5.00 c 4.98	16Hc, 15H 16Ht, 15H
6H a 2.37 e 2.55	6He, 5Ha 6Ha, 5Ha	17Me 1.10	
9H a 2.30	11Ha, 11He, (14H)	18Me 0.98 α	
11H a 1.43 e 1.74	11He, 9a, 12a, 12e* 11Ha, 9a, 12a*, 12e*	19Me 0.88 β	
		20Me 0.85	

Parentthesized H are long range coupled.

* Coupling constant was not clear (3~4 Hz).

The major substance obtained was an oily substance (hydrated crystals in aqueous methanol): $C_{20}H_{30}O_2$ (HRMS; obsd. 302.2211 calcd. 302.2244); $[\alpha]_D^{27} + 20^\circ$ ($c=0.13$, MeOH); UV λ_{max}^{MeOH} nm (ϵ): 250 (3730); IR ν_{max}^{KBr} cm^{-1} : 1685 (C=O), 3353 (OH).

Using proton NMR spectroscopic analysis (400 MHz, D_2O saturated $CDCl_3$), the authors would like to propose the chemical structure to be 3-hydroxy-7-oxo-sandaracopimaradiene (named oryzalexin A), including relative configurations, as a novel diterpene which has been isolated for the first time from a rice plant infected with *P. oryzae* as shown in Fig. 1. The assignments of signals in the proton NMR are given in Table I. The conclusive positions of a hydroxyl and an exo-vinyl function were determined using 1H - 1H NOE measurements between the 18-methyl and 3H-methine, and the 17-methyl and olefinic 15H. The relative configuration was also determined using 1H - 1H NOE techniques and simultaneously its primary structure was confirmed as indicated in Fig. 1. The details of this structural elucidation will be presented elsewhere. Isolation and characterization for the remaining three active fractions are now in progress.

Copalylpyrophosphate is known as the pre-

FIG. 1. The Structure of Oryzalexin A with Relative Configuration.

cursor of sandaracopimaradiene as well as gibberellin A_{19} .^{13,14} Therefore, the authors considered the possibility that the accumulation of 3-hydroxy-7-oxo-sandaracopimaradiene in the lesions of blast disease on rice leaves as a native phytoalexin in the rice plant might result from metabolic changes in the diterpene biosynthetic pathway *via* copalylpyrophosphate by infection of parasitic fungus *P. oryzae*. A determination of the absolute configuration of oryzalexin A will serve to elucidate this possibility.

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