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ISOLATION AND IDENTIFICATION OF AN ANTIFUNGAL NAPHTHOPYRAN DERIVATIVE FROM *RHINACANTHUS NASUTUS*

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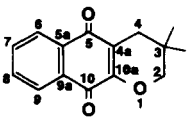
ABSTRACT.—*Rhinacanthus nasutus* (Acanthaceae) gave a new antifungal naphthopyran derivative. The structure of the compound was elucidated by spectroscopic methods as 3,4-dihydro-3,3-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione [**1**].

Rhinacanthus nasutus (L.) Kurz (Acanthaceae) is shrub that is widely distributed in Thailand, South China, and India. Extracts from seeds, roots, leaves, and the stem of this plant are often used to cure ringworm. We speculated that *R. nasutus* contains antifungal compounds, because it is a useful remedy for ringworm and other skin disease caused by fungi (1–3). Subramanian and Nagarajan (4) reported that fresh flowers of *R. nasutus* contained quercetin. Recently, Wu *et al.* (5) reported the existence of two novel naphthoquinones, rhinacanthins A and B, as the cytotoxic principles in the roots of *R. nasutus*. However, there have been no reports about antifungal compounds obtained from *R. nasutus*. In this paper, we describe the isolation and structural elucidation of a new antifungal napththopyran derivative from the leaves and stems of *R. nasutus* which shows strongly antifungal activity against *Pyricularia oryzae*, the pathogen of rice blast disease.

The presence of two carbonyl groups was suggested by the band at 1698 and 1647 cm^{-1} and the ^{13}C signals at δ 179.1 and 179.7 ppm. The ^{1}H-nmr spectrum showed two methyl groups (δ 1.08, 6H), two methylene groups (δ 2.35, 2H, δ 3.99, 2H), and methine groups (δ 7.52, 7.66, 7.81, 8.08). The red color of **1** was removed by reduction using $NaBH_4$, and the maxima at 255, 333.5, and 427 nm indicated that **1** possessed a naphthoquinone skeleton. In the ^{1}H-^{1}H correlated 2D nmr spectrum (COSY), interactions between H-6 and H-7, H-7 and H-8, and H-8 and H-9 were observed. The chemical shifts of ^{13}C, the multiplicities, and the relations of the ^{1}H-^{13}C COSY nmr spectrum are shown in Table 1.



1

On the basis of above results, **1** was identified as 3,4-dihydro-3,3-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione.

Recently, Kuwahara *et al.* (6) succeeded in the synthesis of **1** from methyl 1-methoxy-2-naphthoate in seven reaction steps, and the ^{1}H-nmr and eims spectra agreed with those of the naturally occurring sample: $\delta_H$values of the synthetic compound are 1.07, 2.34, 3.97, 7.52, 7.65, 7.81, and 8.07, respectively.

The structure of this compound was similar to that of α-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (7). However, *gem-*

TABLE 1. ^{1}H- and ^{13}C-nmr Spectral Data of **1** (δ, ppm, in $CDCl_3$).

Position	δ_H (MHz)	δ_C (MHz)
2	3.99 (2H, s)	77.3 (t)[a]
3		28.1 (s)
3-Me	1.08 (6H, s)	24.9 (q)
3-Me	1.08	24.9 (q)
4	2.35 (2H, s)	32.1 (t)
4a		113.4 (s)
5		179.1[b] (s)
5a		132.0 (s)
6	7.81 (1H, dd, J=1, 7.5 Hz)	124.2 (d)
7	7.66 (1H, dt, J=1, 7.5 Hz)	134.9 (d)
8	7.52 (1H, dt, J=1, 7.5 Hz)	130.8 (d)
9	8.08 (1H, dd, J=1, 7.5 Hz)	128.8 (d)
9a		130.0 (s)
10		179.7[b] (s)
10a		162.1 (s)

[a]Multiplicities were determined by the DEPT method.
[b]Interchangeable.

dimethyl groups of the pyran ring in this compound were flanked by two methylene groups. So far as we know, this is the first report to show the orientation. It is also of interest to clarify the biosynthetic pathway.

The antifungal activity was determined by observing its inhibitory action on spore germination of *P. oryzae*. The ED_{50} value was 0.4 ppm, and the inhibitory value of **1** for rice blast disease was 82.3% at 100 ppm. Investigations of other antifungal compounds are currently in progress.

EXPERIMENTAL

Stem and leaf dry powder (ca. 420 g) from *R. nasutus* (obtained at Prajeanbure Province in Thailand in 1990 and deposited as a voucher specimen in the reference collection, Faculty of Science, Kasetsart University) was extracted with 70% MeOH for 5 h using a shaker at room temperature. After centrifugation, the extract was concentrated in vacuo to remove the MeOH. The concentrate was further extracted with Et_2O three times. The combined extract was evaporated in vacuo. The residue was dissolved in a small volume of MeOH and diluted with 5 volumes of distilled H_2O. The resultant mixture was loaded onto a Bond Elut C_{18} cartridge under a vacuum. After the sample had been completely loaded, the cartridge was washed with distilled H_2O. The antifungal fraction was then eluted with 80% MeOH. The MeOH eluate was applied to a preparative Si gel plate (Whatman, PLK5F 20×20 cm) and was developed with C_6H_6-EtOAc (3:1). The areas corresponding to R_f 0.71 and 0.85 showed strong antifungal activity, and one of the fractons (R_f 0.71) was scraped off and extracted with EtOAc. The eluate was applied to a preparative C_{18} plate (Whatman, PLKC18F 20×20 cm), and the chromatogram was developed with 80% MeOH. The antifungal fraction (R_f 0.29) extracted with EtOAc was concentrated and applied to a column of Chromatorex SI (Fuji-Davison Chemical Ltd., 10×250 mm). The column was developed with iPrOH-hexane (5:95) at 3 ml/min of flow rate. The antifungal fraction (retention time 12 min) was collected and concentrated to dryness, and an antifungal compound **1** was obtained as a red powder (9 mg): mp 151.5–152.0°; hrms *m/z* 242.09366 (calcd 242.09424 for $C_{15}H_{14}O_3$); λ max (MeOH) nm 255 (26,055), 262 (22,642, sh), 279 (7556), 333 (1485), 429 (1713); ν max cm^{-1} 2955, 2910, 2875, 1698, 1647, 1605, 1570, 1520, 1480, 1455, 1390, 1370, 1300; ^{1}H nmr and ^{13}C nmr see Table 1; ms *m/z* 242, 214, 199, 159 (base).

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