Structure of Patulodin, a New Azaphilone Epoxide, Produced by *Penicillium urticae*

SHOHEI SAKUDA, YUKIHIKO OTSUBO and YASUHIRO YAMADA

Department of Biotechnology, Faculty of Engineering, Osaka University, Yamada-oka 2-1, Suita-shi, Osaka 565, Japan

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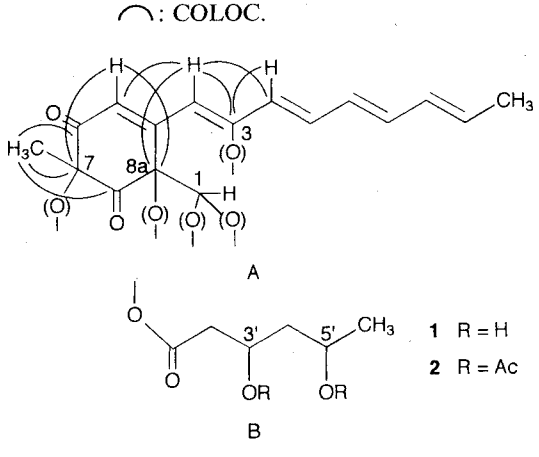
Penicillium urticae is known as a producer of a variety of secondary metabolites, such as patulin, griseofulvin or patulolides[1]. During biosynthetic studies of patulin by SEKIGUCHI *et al.*, *P. urticae* P3 was isolated as one of the patulin minus mutants[2]. Recently, we found that a spontaneous mutant from strain P3, named P3Y, started producing at the same time a yellow compound and patulolides, which are not detected in the culture broth of strain P3. This finding prompted us to investigate the regulatory mechanism of secondary metabolite production in *P. urticae* using the P3 and P3Y strains[3]. In this paper, we describe the isolation and structural elucidation of the yellow compound produced by strain P3Y, named patulodin (**1**).

P. urticae P3Y was cultured in glucose-yeast extract medium according to the method previously described[4]. A rapid isolation procedure was necessary to purify the yellow compound because of its instability. The culture filtrate obtained (25 liters) was applied onto an HP-20 column (5.5 × 32 cm) and the yellow compound was eluted from the column with methanol (4 liters). After evaporating the methanol solution, the residue was extracted with ethyl acetate (50 ml). Ten mililiters of the ethyl acetate solution was applied to a silica gel column (2.6 × 10 cm) and the column was developed with ethyl acetate. This silica gel column chromatography was repeated 5 times, and all the yellow colored fractions were pooled and evaporated to obtain crude **1** (293 mg). Crystallization from hexane-ethyl acetate afforded a yellow crystalline powder of **1** (149 mg); **1**: MP 129~133°C; HREI-MS *m/z* 430.1646 (M^+) (Calcd for $C_{23}H_{26}O_8$: 430.1644); IR ν_{max} ($CHCl_3$) cm^{-1} 3500, 1732, 1670, 1578; UV $\lambda_{max}^{CHCl_3}$ nm (ε) 414 (48,000); $[\alpha]_D^{13}$ −419° (*c* 0.21, $CHCl_3$).

Fig. 1. Partial structures A and B.



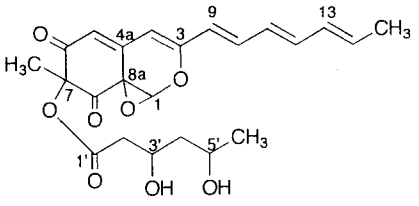
The ^{13}C NMR spectrum of **1** showed the presence of 23 carbons having following functional groups: $CH_3 \times 3$, $CH_2 \times 2$, CH–O × 2, C–O × 2, (O)-CH–O × 1, CH= × 8, C= × 2, O–C=O × 1, C=O × 2. By conventional 2D NMR experiments, two partial structures, A and B, which include all 23 carbons of **1**, were established (Figure). The positions of quaternary carbons were deduced from the COLOC (Correlation Spectroscopy *via* Long-Range Couplings) spectrum of **1** as shown in the Figure. Unfortunately, no long distance correlation with H-1 could be observed in the spectrum, but C-8a was the only possible position to which C-1 could be connected by a C-C bond. The location of the two hydroxyl groups were determined to be C-3′ and C-5′ by acetylation shifts in the ^1H NMR spectrum of the diacetate of **1** (**2**) (δ_H 5.32 and 4.95 for H-3′ and H-5′, respectively, in **2**). Assignments of protons and carbons in the NMR spectra of **1** are summarized in the Table.

From the molecular formula of **1**, the partial structure A should have another two ring systems which are formed by two ether linkages among C-1, C-3, C-7 and C-8a, and the remaining carbon is then linked to the partial structure B by forming an ester. The δ_C values of

Table 1. ^1H and ^{13}C NMR Assignments of **1**[a].

C-No.	δ_C	δ_H
1	81.0	5.75 s
3	154.5	
4	104.2	5.79 s
4a	146.0	
5	119.5	6.21 s
6	192.2[b]	
7	85.1	
7-CH_3	22.0	1.60 s
8	196.8[b]	
8a	54.4	
9	122.0	5.93 d (15)
10	137.2	6.93 dd (15, 11)
11	128.6	6.17 dd (15, 11)
12	139.9	6.47 dd (15, 11)
13	131.5	6.15 ddq (15, 11, 2)
14	134.8	5.92 dq (15, 7)
15	18.6	1.82 dd (7, 2)
1′	171.1	
2′	41.7	2.63 dd (15, 9), 2.51 dd (15, 4)
3′	69.6	4.25 m
4′	44.1	1.6 m
5′	68.1	4.05 m
6′	23.6	1.18 d (6)

[a] Spectra were obtained in $CDCl_3$ on Bruker AM-600. Coupling constants in Hertz are given in parentheses.
[b] May be interchanged.



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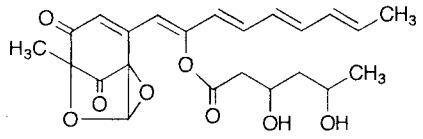

3

C-1 (δ_C 81.0) and C-8a (δ_C 54.4) which bear two and one oxygen atoms, respectively, and the large $^1J_{C\text{-}1,H\text{-}1}$ value (227 Hz) indicated the presence of an epoxide between C-1 and C-8a[5]. Since a structure having an ether linkage between C-3 and C-7 was excluded by BREDT's rule, two possible structures, **1** and **3**, remained. The epoxide function at the bridgehead in **3** may be extremely strained, and the ester carbonyl of the yellow compound absorbs at 1732 cm^{-1} in its IR spectrum, which does not agree with the enol ester present in structure **3**[6]. Therefore, the structure of patulodin is assigned as **1**.

A series of fungal secondary metabolites possessing a pyrano-quinone structure has been isolated and called the azaphilones[7]. The skeleton of patulodin basically belongs to azaphilones, but the presence of an epoxide ring between C-1 and C-8a is novel. Patulodin showed antifungal activity against *Pyricuralia oryzae* at the MIC value of 50 μg/ml. The search for other biological activities of patulodin is now in progress.

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