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ZINNIMIDINE AND 5-(3′,3′-DIMETHYLALLYLOXY)-7-METHOXY-6-METHYLPHTHALIDE FROM *ALTERNARIA PORRI*

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Key Word Index—*Alternaria porri*; Deuteromycotina; fungus; metabolite; imidine; phthalide.

Abstract—The structures of two metabolites, isolated from the culture liquid of *Alternaria porri*, have been identified as zinnimidine and 5-(3′,3′-dimethylallyloxy)-7-methoxy-6-methylphthalide. The latter is the first reported isolation as a naturally occurring product.

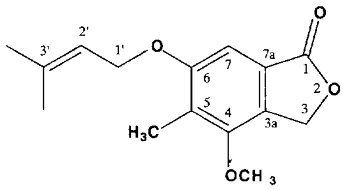
INTRODUCTION

The fungus *Alternaria porri* (Ellis) Ciferri, the causal fungus of black spot disease in stone-leek and onion, produces phytotoxins such as tentoxin [1], porritoxin [2], silvaticol [3] and porritoxinol [4]. In addition, we reported 6-(3′,3′-dimethylallyloxy)-4-methoxy-5-methylphthalide (**1**) [5], although no particular inhibitory activity was apparent toward lettuce and stone-leek seedlings. This paper deals with the isolation and structural elucidation of further metabolites, zinnimidine (**2**) and 5-(3′,3′-dimethylallyloxy)-7-methoxy-6-methylphthalide (**3**), from *Alternaria porri*.

RESULTS AND DISCUSSION

Silica gel chromatography of adsorbates with Amberlite XAD-7 led to the isolation of two compounds, **A** and **B**, in order of increasing R_f values. High resolution mass spectral analysis gave $C_{15}H_{19}NO_3$ for **A** and IR absorption was indicative of amides at 3456 and 1695, ether oxygen at 1115, a methoxy group at 1452, together with benzene rings at 1473 and 665 cm^{-1}. The UV spectral data showed the existence of a benzoyl group at 213 (4.73), 252 (4.04), 292 (3.67). The ^1H and ^{13}C NMR data are given in Table 1. Comparing the spectral data of **A** with those of zinnimidine (**2**), which was isolated from *Alternaria cichorii* [6], gave essentially the same signals. Consequently, **A** was identified as zinnimidine (**2**).

High resolution mass spectral analysis gave $C_{15}H_{18}O_4$ for compound **B**. IR absorption was indicative of a γ-lactone at 1758, together with benzene rings at 1610 and 695 cm^{-1}. The UV spectral data gave signals at 215.8 (4.49) and 258.5 (4.17). The ^1H and ^{13}C NMR data are shown in Table 1. In order to compare them with those of **1**, spectral data of **A** are also shown in Table 1. Comparing the spectral data of **B** with those of **1**, the former closely resembles the latter, except for the chemical shift of the aromatic proton at δ6.62, while the aromatic



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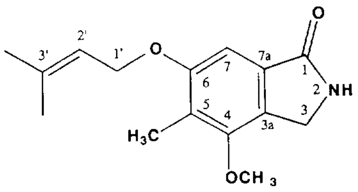

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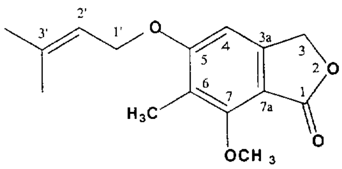

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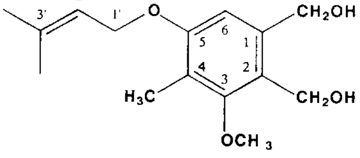

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Table 1. Comparison of ^{1}H and ^{13}C NMR spectral data of 6-(3′,3′-dimethylallyloxy)-4-methoxy-5-methylphthalide, compounds A and B.

6-(3′,3′-dimethylallyloxy)-4-methoxy-5-methylphthalide (**1**)				**A** (zinnimidine)				**B** (**3**)			
H		C		H		C		H		C	
		1	171.3			1	171.2			1	169.0
3	5.83 *s*	3	68.3	3	4.59 *s*	3	43.9	3	5.18 *s*	3	68.7
		3a	124.7			3a	126.2			7a	148.1
		4	159.2			4	158.7			7	163.6
		5	125.8			5	123.9			6	120.8
		6	152.9			6	153.7			5	157.8
7	7.08 *s*	7	101.9	7	7.11 *s*	7	101.2	4	6.62 *s*	4	99.5
		7a	128.1			7a	130.8			3a	109.5
1′	4.57 *d*	1′	65.8	1′	4.58 *d*	1′	65.7	1′	4.59 *d*	1′	65.8
	(6.72)				(6.72)				(6.71)		
2′	5.47 *t*	2′	119.2	2′	5.18 *t*	2′	119.6	2′	5.49 *t*	2′	118.9
	(6.72)				(6.72)				(6.71)		
		3′	138.2			3′	137.8			3′	138.6
OMe-4	3.89 *s*	OMe-4	59.3	OMe-4	3.87 *s*	OMe-4	59.8	OMe-7	4.03 *s*	OMe-7	62.1
Me-5	2.21 *s*	Me-5	9.7	Me-5	2.22 *s*	Me-5	9.6	Me-6	2.15 *s*	Me-6	8.7
Me-3′-1	1.75 *s*	Me-3′-1	18.3	Me-3′-1	1.74 *s*	Me-3′-1	18.3	Me-3′-1	1.75 *s*	Me-3′-1	18.3
Me-3′-2	1.80 *s*	Me-3′-2	25.8	Me-3′-2	1.80 *s*	Me-3′-2	25.8	Me-3′-2	1.81 *s*	Me-3′-2	25.8

proton in **1** is at δ7.08 (Table 1). The strikingly lower shift of the aromatic proton in **1** can be accommodated by the anisotropy of the carbonyl group. Starratt has reported that two phthalides, **1** (mp 85–86°) and **3** (mp 106–108°), were obtained on chromic oxidation of zinniol (**4**) [7]. Comparing the UV, IR and ^{1}H NMR spectral data of **B** with those of **3**, the former closely resembles the latter. Consequently, based on those spectral data and the coincidence of melting point, **B** was identified as **3**. Compound **3** is the first example found naturally occurring in fungi.

EXPERIMENTAL

Fungus. The strain of *A. porri* used in this experiment was purchased from IFO (Institute for Fermentation, Osaka), strain no. 9762.

Extraction and isolation of zinnimidine and 5-(3′,3′-*dimethylallyloxy*)-7-*methoxy*-6-*methylphthalide.* The culture conditions using Richards medium were the same as those previously reported [5] and the isolation method was the same as that previously reported [3]. The Me_2CO extract of adsorbents obtained from Amberlite XAD-7 was subjected to prep. TLC (Merck Silica gel 60) in C_6H_6–Me_2CO–HOAc (60:40 : 1) to obtain fraction A (R_f 0.82–0.95) and B (R_f 0.33–0.60). Fraction A was further subjected to TLC in C_6H_{14}–EtOAc (2:1) and finally the fr. at R_f 0.58–0.64 was chromatographed in $CHCl_3$–MeOH (97:3). The fr. at R_f 0.52 gave needles of mp 107–109° in a yield of 5 mg from 300 l of culture medium. Fraction B was subjected to prep. TLC in $CHCl_3$–MeOH (9:1) and the fr. at R_f 0.55–0.73 further chromatographed in C_6H_{14}–EtOAc (5:1). Finally, the fr. at R_f 0.38–0.53 was purified by HPLC using YMC S-343 (Yamamura Chemical Labs) with 40% MeCN and gave needles of mp 136–138° in a yield of 6 mg from 300 l of the culture medium.

5-(3′,3′-*dimethylallyloxy*)-7-*methoxy*-6-*methylphthalide* (**3**). Needles, mp 107–109° (lit. mp 106–108° [7]); IR ν_{max}^{KBr} cm^{-1}: 1758 (γ-lactone, lit. 1743 [6]), 1610, 695 (phenyl); UV λ_{max}^{MeOH} nm (log. ε): 215.8 (4.49), 258.5 (4.17) (lit. 259 (4.18) [7]). EI-MS *m/z*: 262.1221 $[M]^+$ corresponding to $C_{15}H_{18}O_4$ (262.1204), 194 $(M - C_5H_8]^+$, 165 $[194 - CHO]^+$, 69 $[C_5H_9]^+$. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR spectra: Table 1.

Zinnimidine (**2**). Needles, mp 136–138°; IR ν_{max}^{KBr} cm^{-1}: 3456 (NH), 1695 (CONH), 1473, 665 (phenyl), 1115 (ether); UV λ_{max}^{MeOH} nm (log ε): 213 (4.73), 252 (4.04), 292 (3.67). EI-MS *m/z*: 261.1329 $[M]^+$ corresponding to $C_{15}H_{19}NO_3$ (261.1364), 193 $[M - C_5H_8]^+$. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR spectra: Table 1.

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