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# DE-*O*-METHYLDIAPORTHIN, A PHYTOTOXIN FROM *DRECHSLERA SICCANS*

YALI F. HALLOCK, JON CLARDY,* DOUG S. KENFIELD† and GARY STROBEL*†

Department of Chemistry-Baker Laboratory, Cornell University, Ithaca, NY 14853-1301 U.S.A.; †Department of Plant Pathology, Montana State University, Bozeman, MT 59717, U.S.A.

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**Key Word Index**—*Drechslera siccans*; isocoumarin; phytotoxin; diaporthin; de-*O*-methyldiaporthin.

**Abstract**—A phytotoxic metabolite has been isolated from cultures of *Drechslera siccans* and structurally characterized as 6,8-dihydroxy-3-(2′-hydroxypropyl) isocoumarin (de-*O*-methyldiaporthin).

## INTRODUCTION

*Drechslera siccans* is a pathogenic fungus on oats (*Avena sativa* 'Park'), perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* Lam.). Fungal infection causes irregular dark brown to reddish brown spots (0.5–1 mm long and 0.2–0.5 mm wide) on the leaves of these hosts within one week of inoculation [1]. Occasionally, these lesions have tan centres and assume an eyespot appearance. Our interest in new phytotoxic compounds from the genus *Drechslera* led us to study *Drechslera siccans*, and we now report the isolation and structural elucidation of a new phytotoxic isocoumarin, de-*O*-methyldiaporthin.

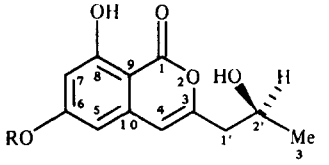
## RESULTS AND DISCUSSION

Isolates of *D. siccans* were cultured in M-1-D media using our previously reported procedure [2, 3]. Culture filtrates were extracted with ethyl acetate and the title compound was isolated by successive preparative TLC and further purified by reverse phase HPLC to yield pure **1** as a white solid.

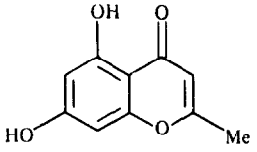
The high resolution mass spectra of **1** gave $[M]^+$ at *m/z* 236.0690, corresponding to the molecular formula $C_{12}H_{12}O_5$ (236.0685). The UV spectra (Table 1) indicated a conjugated aromatic chromophore [4]. The $^1H$ NMR also indicated that an aromatic ring was part of the structure. Two doublets at $\delta$6.38 and 6.42 with a small coupling constant, $J=2.17$ Hz, suggested two aromatic protons with a *meta* relationship. In addition, the $^1H$ NMR showed signals corresponding to a methyl group ($\delta$1.24), a methylene group ($\delta$2.60) and an olefinic proton ($\delta$6.44). A signal at $\delta$11.18 was interpreted as a hydrogen bonded phenolic proton.

The mass spectral fragmentation pattern revealed a benzopyran as the major molecular building block. Loss of 44 mass units to give the high intensity peak at *m/z* 192 in the mass spectra was consistent with the benzopyran structure, leaving the methyl and methylene groups as part of the side chain. Lack of a peak at *m/z* 152 ruled out a chromone structure, since this peak is typically ob-

*Authors to whom correspondence should be addressed.



**1** R = H de-*O*-methyldiaporthin
**2** R = Me diaporthin



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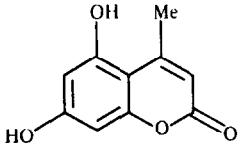

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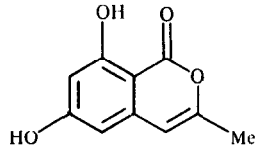

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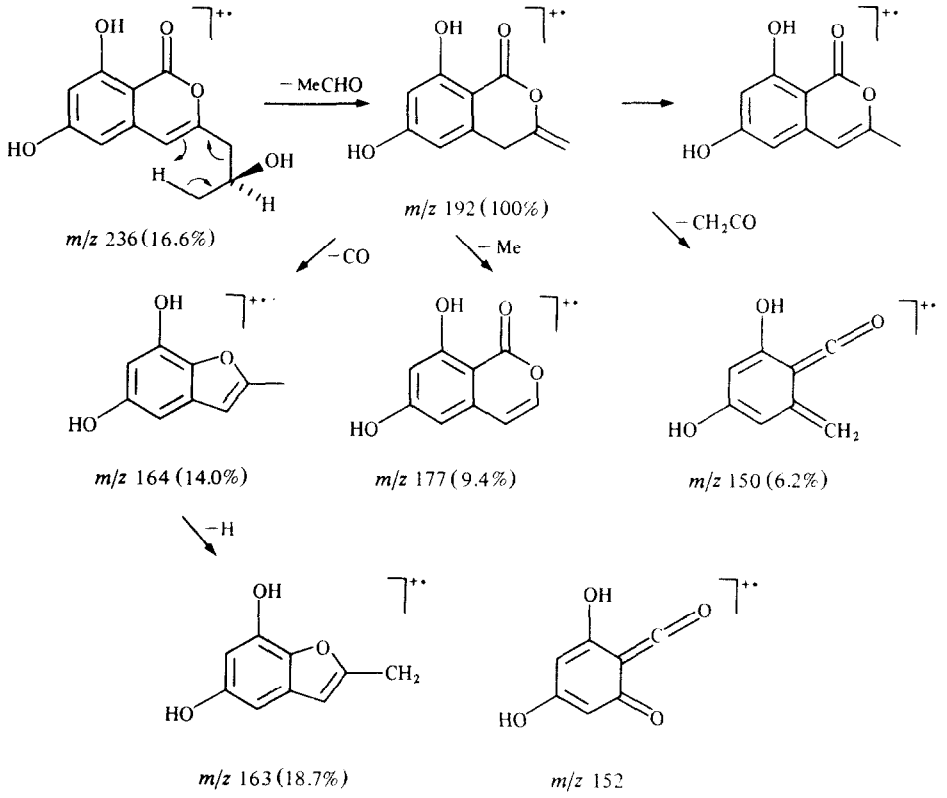


Scheme 1. Mass spectra fragments of de-*O*-methyldiaporthin.

served for γ-benzopyrans [5] (Scheme 1, bottom right). The most plausible structure for **1** appeared to be an 6,8-dihydroxy-isocoumarin (α-benzopyran) substituted at the 3-position with a 2-hydroxypropyl sidechain. The proposed structure was related to diaporthin (**2**) as de-*O*-methyldiaporthin (**1**).

This identification was supported by an examination of the UV data of 5,7-dihydroxy-2-methylchromone (**3**) [6], 5,7-dihydroxy-4-methylcoumarin (**4**) [7] and 6,8-dihydroxy-3-methylisocoumarin (**5**) [8], shown in Table 1. It was further confirmed by comparing spectroscopic data with the known compound diaporthin (**2**), a fungal metabolite from *Endothia parasitica* [9].

Bioassay data are presented in Table 2. Host plants of this pathogen showed little or no sensitivity to de-*O*-methyldiaporthin (**1**) (Table 2). However, corn, crabgrass, and soybean reacted to 1 μg (4 nmol). Barnyard grass and spiny amaranth were sensitive to 8 and 21 nmol, respectively.

Table 1. UV data of compounds **1**, **3**–**5**

| $\lambda_{max}^{MeOH}$ (nm) (log ε) | | | |
|---|---|---|---|
| **1** | **3** | **4** | **5** |
| 237 (4.31) | 227 (4.08) | — | 237 (4.62) |
| 246 (4.38) | 249 (4.13) | — | 245 (4.69) |
| 275 (3.68) | 256 (4.13) | 258 (3.82) | 278 (3.85) |
| 289 (3.53) | 295 (3.65) | — | 289 (3.72) |
| 326 (3.52) | — | 322 (4.10) | 324 (3.79) |

## EXPERIMENTAL

*Fungal isolates and cultures.* Isolates of *D. siccans* were kindly provided by Dr Richard Morrison at Northrup King Co., Woodland, California. Cultures were maintained on potato dextrose agar with 18% V-8 juice (v/v). Broth cultures were prepared in M-1-D medium and fermented for 21 days at 25° in a shaker (200 rpm).

*Isolation of de-O-Methyldiaporthin* (**1**). The fungal culture was filtered through four layers of cheese cloth. The filtrate was extracted with EtOAc (3 × 1/3 vol) and concd *in vacuo*. The residue was taken up in MeOH, applied to a Sephadex LH-20 column and eluted with MeOH. The major fraction was then separated by prep TLC (20 × 20 cm, 1000 μm) using $CHCl_3$–MeOH (9:1). The fraction with an $R_f$ of 0.38 was prep. TLCed again and further purified by reverse phase HPLC (4.6 × 250 mm Supelco column, UV detector at 254 nm, MeCN–$H_2O$, linear gradient program, 0–100%MeCN, 1 ml/min, $R_t$ 18.4 min). Evaporation from $Me_2CO$ gave de-*O*-methyldiaporthin (**1**) as a white solid. The yield was *ca* 3 mg/l of culture.

*De-O-methyldiaporthin* HREIMS: $C_{12}H_{12}O_5$, obsd 236.0690, calcd 236.0685. LREIMS (probe) 70 eV, *m/z* (rel. int.): 236 (17), 221 (3), 193 (15), 192 (100), 177 (9), 174 (8), 164 (14), 163 (19), 150 (6), 146 (24), 135 (4), 121 (21). $[\alpha]_D$ +22° (MeOH, *c* 0.09). UV (see Table 1). $^1$HNMR (400 MHz, acetone-$d_6$): δ1.24 (*d*, 3H, *J* = 6.2 Hz), 2.60 (*m*, 2H), 4.17 (*m*, 1H), 6.38 (*d*, 1H, J = 2.17 Hz), 6.42 (*d*, 1H, *J* = 2.17 Hz), 6.44 (*s*, 1H), 11.18 (*s*, 1H). $^{13}$C NMR: δ23.4 (*q*,

Table 2. Sensitivity of various plants to de-*O*-methyldiaporthin (**1**)

| Plant | | Necrotic area (square mm) 10 μg | 5 μg | 2 μg | 1 μg |
|---|---|---|---|---|---|
| Perennial rye | *Lolium perenne* L. | 0 | 0 | 0 | 0 |
| Park oat | *Avena sativa* L. | 1 | <1 | 0 | 0 |
| Corn (W64A-N) | *Zea mays* L. | — | 24 | 15 | 4 |
| Crabgrass | *Digitaria ischaemum* Schreb. | 12 | 16 | 6 | 1 |
| Barnyard grass | *Echinochloa crus-galli* L. | 12 | 9 | 2 | 0 |
| Yellow nutsedge | *Cyperus esculentus* L. | 0 | 0 | 0 | 0 |
| Soybean | *Glycine max* L. | 12 | 9 | 9 | 2 |
| Rape (Harosoy 63) | *Brassica campestris* L. | 0 | 0 | 0 | 0 |
| Spiny amaranth | *Amaranthus spinosus* L. | 3 | 1 | 0 | 0 |

Specified amounts were applied in 5% aqueous ethanol (3 μl). Necrotic area was measured after 48 hr.

C3′), 44.1 (*t*, C1′), 65.7 (*d*, C2′), 99.2 (*d*), 102.4 (*d*), 103.8 (*s*), 106.6 (*s*), 140.5 (*d*, C4), 156.3 (*s*, C3), 164.5 (*s*), 167.2 (*s*), 167.8 (*s*).

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