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DRECHSLEROL-B, A HOST-SELECTIVE PHYTOTOXIN PRODUCED BY *DRECHSLERA MAYDIS**

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Key Word Index—*Drechslera maydis*; *Cochliobolus*; *Costus speciosus*; phytotoxin; drechslerol-B; 3-hydroxy-eicos-11(*Z*)-enyl eicos-4(*Z*)-enoate.

Abstract—A new host-selective phytotoxin, drechslerol-B, isolated from the culture filtrate of *Drechslera maydis*, the organism which causes leaf blight of *Costus speciosus*, was characterized as 3-hydroxy-eicos-11(*Z*)-enyl eicos-4(*Z*)-enoate on the basis of spectral analysis.

INTRODUCTION

In a continuation of our earlier studies [1] on the toxic metabolites of *Drechslera maydis* (*Helminthosporium maydis* = *Cochliobolus heterostrophus* CMI No. 256411), the causal agent of leaf blight disease of *Costus speciosus* [2], we have now isolated a host-selective phytotoxin drechslerol-B, from the toxin complex obtained from the culture filtrate of *D. maydis*. The present communication deals with isolation, characterization and phytotoxicity of this toxin.

RESULTS AND DISCUSSION

Drechslerol B, $[M]^+$ *m/z* 604, was assigned the formula $C_{40}H_{76}O_3$. It had an ester group (^{13}C NMR: δ173.02; IR: 1735 cm^{-1}), a secondary hydroxyl group (^1H NMR: δ4.16 *m*; ^{13}C NMR: δ68.92; IR 3400 cm^{-1}), two disubstituted olefinic unsaturations (^1H NMR: δ5.36 *br t*, J = 5.5 Hz (4H); ^{13}C NMR: δ129.93 × 2, 129.60 × 2) and an oxymethylene (^1H NMR: δ4.30, *t*; ^{13}C NMR: δ62.04). Its long chain character was shown by IR absorption bands at 730 and 720 cm^{-1} which was in full accordance with the presence of a broad signal at δ1.27 in its ^1H NMR spectrum and several signals in between δ29.71 to 22.50 in its ^{13}C NMR spectrum. The triplet (J = 7 Hz) at δ0.89 (6H) in its ^1H NMR corresponded to two terminal methyls (^{13}C NMR: δ13.99) whereas a methylene adjacent to a CO function was seen at δ2.33. On acetylation, **1** yielded a monoacetate (IR: 1738, 1732 cm^{-1}; ^1H NMR: δ2.01, 3H, *s*, OAc). Its ^1H NMR displayed acetoxy methine at δ4.95 thus confirming the presence of a secondary hydroxyl group.

Alkaline hydrolysis of **1**, afforded an acid (IR: 1710 cm^{-1}). Its MS exhibited $[M]^+$ at *m/z* 310 and other characteristic fragment ions at *m/z* 251, 197, 113 and 59 and at *m/z* 237, 211, 99 and 73 due to β- and α-fission ions respectively [3], hence revealing the presence of a disubstituted olefinic bond at C-4. These data suggested the structure of the acid as eicos-4(*Z*)-enoic acid. The ^1H NMR of the alcohol displayed a triplet at δ3.71 (2H) and multiplets at δ4.01 (1H) and δ5.32 (2H) corresponding to hydroxymethylene, hydroxymethine and a disubstituted oelfinic unsaturation, respectively. A multiplet at δ2.33 (2H) was assigned to a CH_2OH–CH_2–CHOH methylene. Its MS exhibited M^+ at *m/z* 312 and prominent fragment ions at *m/z* 75 and 237 hence inferring the site of secondary hydroxyl group at C-3. The presence of fragment ions at *m/z* 199, 173, 137 and 113 supported the occurrence of the olefinic bond at C-11. Thus, the alcohol was identified as 11(*Z*)-eicosen-1,3-diol.

On the basis of its hydrolysis products, **1**, should therefore be 3-hydroxy-eicos-11(*Z*)-enyl eicos-4(*Z*)-enoate. The mass spectral fragmentation (Fig. 1) was consistent with the proposed structure. The ions at *m/z* 339, 311, 309, 295 and 293, and at *m/z* 353 and 251 were due to α- and β-fissions respectively of the ester group [4] and other fragments (see Experimental) were consistent with the proposed structure. The geometry of both olefinic bonds was *cis* (*Z*) as shown by the appearance of olefinic methines as a broad triplet (J = 5.5 Hz) [5] in the ^1H NMR spectrum and allylic methylene resonances at δ27.16 and 27.11 in the ^{13}C NMR spectrum. The allylic methylenes were expected to resonate relatively, *ca* 6 ppm, downfield in *trans* (*E*) geometry [3, 6]. ^{13}C shielding data (see Experimental) was analysed by analogy with the literature values for related compounds [3, 7–9].

Drechslerol-B was also isolated from tissues infected with *D. maydis* implicating it in the disease syndrome. The amount of **1** found in the infected tissues, 48 hr after infection, was *ca* 0.1 mg/g. It was not present in healthy uninfected tissues. Its early appearance and possible disappearance in 72 and 96 hr samples, suggest that it is metabolized into other non-phytotoxic products.

It is well known that several nonspecific toxin-producing pathogens also produce host specific toxins, viz *H. carbonum* [10], *H. maydis* race T [11] and *H. oryzae* [12]. The presence of a host selective phytotoxin, 6-epiophiobolin-A, in *D. maydis*, the causal agent of Southern corn leaf blight has been reported by Sugawara *et al.* This toxin was selectively toxic to corn bearing Tms cytoplasm [13]. 6-Epiophiobolin A and 3-anhydro-6-epiophiobolin A have recently been characterized from *D.* maydis race T and were shown to inhibit of malate oxidation in Tms

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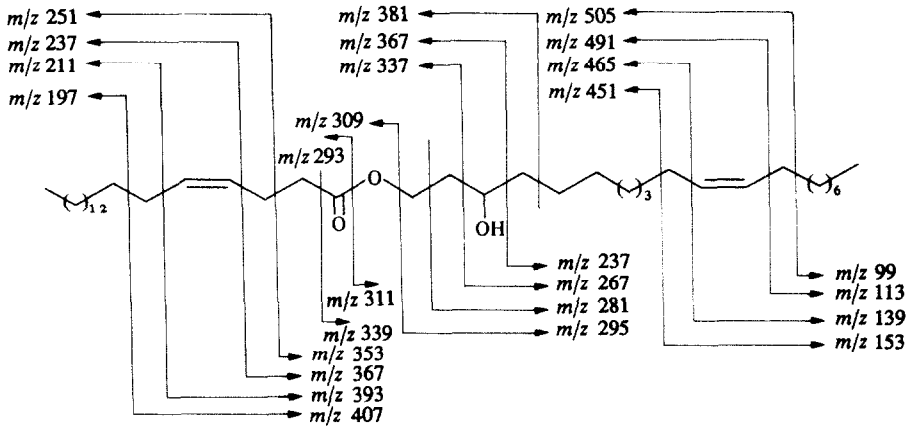

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Fig. 1. Mass spectral fragmentation of drechslerol-B.

corn [14]. Both of the compounds belong to the ophiobiolin family of sesterpenoids. Interestingly, an ophiobiolin type of phytotoxin could not be isolated from the *Costus* strain of *D. maydis*, however drechslerol-B shows selective phytotoxicity and induces severe chlorosis surrounded by yellowing on the leaves of *C. speciosus* (Fig. 2) and other host plants (Table 1) whereas drechslerol-A [1] is a non-specific phytotoxin. Thus, drechslerol-B has the same plant selectivity as the fungus itself. The degree of toxin sensitivity of all plants tested was correlated with their degree of susceptibility to *D. maydis* suggesting that the activity of toxin is host-selective.

EXPERIMENTAL

IR: neat ^{1}H NMR: 400 MHz ($CDCl_3$) TMS as int. standard. ^{13}C NMR: 100 MHz. TLC: silica gel-G, the spots were visualized by I_2.

Extraction of toxin complex. Drechslera maydis, freshly isolated from the leaf lesion, was inoculated into Roux bottles containing 150 ml modified Fries medium and incubated at 24 ± 2° for 21 days [15]. Mycelium, obtained by filtering the culture through four layers of cheese cloth, was homogenized in 70% aq. Me_2CO (15 min). The culture filtrates were treated with 3% (w/v) Norit A, stirred for 2 hr and centrifuged at 18 000 rpm (20 min). Norit A, so obtained after washing with H_2O, was added to the Me_2CO homogenate of the mycelium and stirred (2 hr). The recovered Norit-A was washed with Me_2CO. The toxin complex was desorbed by extraction with $CHCl_3$–MeOH (9:1) for 16 hr. The extractive was concd at 42–45° under vacuum to 20 ml, which on keeping at 2–4° yielded a light yellow oil (toxin complex).

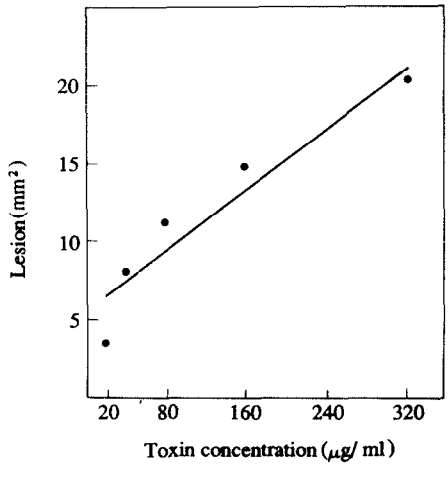

Fig. 2. Dosage response to drechslerol-B, as measured by chlorotic and necrotic lesion development after 48 hr in leaves of *Costus speciosus* (12 observations). The regression curve is $Y = 5.546 + 0.048x$, $r = 0.948$, $p < 0.05$.

Table 1. Symptoms produced by *D. maydis* and its toxin on the hosts and non-hosts of the fungus

	Symptom intensity*	
Hosts/non-hosts	Toxin†	Fungus
Costus speciosus	+ + +	+ + +
Datura innoxia	+ + +	+ + +
Hyoscyamus muticus	+ + +	+ + +
Beta vulgaris	+ +	+ +
Zea mays	+ +	+ +
Phaseolus mungo	+	+
Triticum aestivum	+	+
Oryza sativa	+	+
Catharanthus roseus	—	—
Rauvolfia serpentina	—	—
Vigna unguiculata	—	—
Zingiber officinale	—	—

* + + +, Very severe; + +, moderate; +, mild; —, no symptoms.

†Based upon severity of symptoms expressed on the host and non-hosts after 48 hr when treated with 25 μl of 100 μg/ml of the toxin required for visible chlorosis development.

Isolation of drechslerol-B. The toxin complex was fractionated by prep. TLC on silica gel-G developed with C_6H_6–$CHCl_3$ (4:1). A band at R_f 0.41 was scraped off and eluted with hot $CHCl_3$ to afford drechslerol-B (35 mg) which was homogenous in several

solvent systems and to various indicator treatments. The purification was further confirmed by HPLC which showed a single peak (column μ porosil 3.9 mm × 30 cm, flow 1 ml/min, solvent $CHCl_3$, 270 nm, R_t 1.6 min.).

Drechslerol-B (**1**), yellow viscous mass, IR ν_{max}^{neat} cm^{-1}: 3400, 2900, 1735, 1640, 1050, 900, 730, 720; ^1H NMR: δ0.89 (6H, *t*, *J* = 7 Hz, 2 × Me), 1.27 [*br s*, $(CH_2)_n$], 1.92 (2H, *t*, *J* = 7 Hz), 2.33 (2H, *t*, *J* = 7 Hz), 4.16 (1H, *m*), 4.30 (2H, *t*, *J* = 7 Hz), 5.36 (4H, *br t*, *J* = 5.5 Hz); MS *m/z* (rel. int.): 604 (18), 575 (34), 533 (2.2), 505 (2.8), 491 (2.2), 465 (3.4), 451 (3.3), 437 (2.2), 423 (6.7), 409 (2.8), 395 (9.0), 381 (4.5), 367 (7.8), 353 (3.9), 339 (3.3), 337 (9.0), 330 (5.6), 312 (14.6), 283 (4.5), 281 (3.4), 267 (2.2), 265 (9.0), 262 (28.1), 251 (2.2), 237 (18.5), 211 (4.5), 197 (3.7), 183 (7.9), 153 (3.3), 139 (2.8), 113 (6.7), 99 (3.9), 85 (2.2), 71 (23.6), 57 (36.0), 43 (100); ^{13}C NMR: δ173.9 (CO), 129.93 (CH × 2), 129.60 (CH × 2), 68.90 (CH), 62.02 (CH_2), 34.12, 33.97, 31.87 × 2, 29.71 × 2, 29.63, 29.55 × 3, 29.46 × 2, 29.41 × 2, 29.27 × 2, 29.20 × 2, 29.12 × 2, 29.05 × 3, 28.99, 27.16, 27.11 × 2, 24.81 × 2, 22.61 × 2, (all CH_2), 13.99 × 2 (Me).

Acetylation of **1**. To **1** (5 mg) was added pyridine and Ac_2O (0.5 ml each) and the mixture left overnight at room temp. It was then dil. with H_2O (5 ml) and extracted with Et_2O (4 × 5 ml). The Et_2O extract was washed successively with dil. HCl, H_2O, $NaHCO_3$ and H_2O and then dried (Na_2SO_4). Removal of solvent gave a viscid mass (4 mg), IR ν_{max}^{neat} cm^{-1}: 2900, 1738, 1732, 1640, 730, 720; ^1H NMR: δ0.88 (6H, *t*, *J* = 7 Hz, 2 × Me), 1.27 [*br s* $(CH_2)_n$], 1.94 (2H, *t*, *J* = 7 Hz), 2.01 (3H, *s*, OAc), 2.35 (2H, *t*, *J* = 7 Hz), 4.28 (2H, *m*), 4.95 (1H, *m*), 5.36 (4H, *br t*, *J* = 5.5 Hz).

Hydrolysis of **1**. Compound **1** (15 mg) was refluxed with 5% alcoholic KOH (5 ml). The vol. was reduced and the reaction mix. diluted with H_2O (10 ml) and extracted with Et_2O (4 × 10 ml). The Et_2O phases were washed with H_2O (2 × 10 ml) and dried (Na_2SO_4). Removal of solvent gave an alcohol (4 mg) IR ν_{max}^{neat} cm^{-1} 3400, 2900, 1640, 730, 720; ^1H NMR: δ0.89 (3H, *t*, *J* = 6.5 Hz, Me), 1.28 [*br s*, $(CH_2)_n$], 2.33 (2H, *m*), 3.71 (2H, *t*), 4.01 (1H, *t*), 5.32 (2H, *m*); MS *m/z*: 312 $[M]^+$, 294, 282, 281, 267, 230, 223, 139, 113, 99, 89, 85, 75, 71, 57. The aq. layer was acidified with dil. HCl and extd with Et_2O (4 × 10 ml) washed with H_2O (2 × 10 ml) and dried (Na_2SO_4). Removal of solvent gave an acid (5 mg), IR ν_{max}^{neat} cm^{-1}: 2920, 2850, 1710, 720; ^1H NMR: δ0.87 (3H, *t*, *J* = 6.0 Hz), 1.25 [*br s* $(CH_2)_n$], 2.28 (2H, *t*), 5.32 (2H, *br t*, *J* = 5.5 Hz). MS *m/z*: 310 $[M]^+$, 266, 265, 251, 237, 213, 211, 197, 99, 73, 60, 58, 57.

Isolation of **1** *from infected tissues*. Healthy potted plants were inoculated with *D. maydis* and incubated in a humidity chamber for 24 hr. Infected leaves of different stages (24, 48, 72 and 96 hr) were collected and processed separately following the method described by Karr *et al.* [16] with some modification [2]. The toxin complex was isolated from the supernatant liquid (infected tissues) by the method described earlier for the isolation of toxin from the culture filtrate. The identity of the isolate as **1** was confirmed by co-TLC and by superimposibility of the IR spectra.

Assay for biological activity. Attached third or fourth leaves from 8-week-old plants of *C. speciosus* were inoculated with the toxin. An area of *ca* 2 mm^2 on the upper leaf surface was gently scratched with the tip of a pasteur pipette, and 25 μl of toxin soln applied. Three leaves from each plant were used for toxin/spore inoculations and each leaf was inoculated at four points. The toxin obtained after purification was diluted to 320, 160, 80, 40, 20 and 10 μg/ml with H_2O. The corresponding undiluted fraction from medium was used as a control. The biological activity was based on the maximum dilution factor of the toxin required to cause chlorosis and yellowing on the host leaves after 48 hr of incubation [2]. A dosage-response curve for the toxin is shown (Fig. 2) to illustrate a correlation between toxin concentration and lesion development. Visible symptoms could be observed at toxin concentration as low as 20 μg/ml. Toxic activity was bioassayed after each purification step. All assays and all experiments were repeated three times.

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REFERENCES

1. Shukla, R. S., Agrawal, P. K. and Husain, A. (1987) *Plant Sci.* **48**, 159.
2. Shukla, R. S. (1983) Ph.D. Thesis. Kanpur University, Kanpur (India).
3. Singh, B., Agrawal, P. K. and Thakur, R. S. (1989) *J. Nat. Prod.* **52**, 180.
4. Gupta, M. M., Verma, R. K. and Akhila, A. (1986) *Phytochemistry* **25**, 1899.
5. Rollin, O. and Pauguy, J. R. (1986) *Tetrahedron* **42**, 3479.
6. Rossi, R., Carpita, A., Quirici, M. C. and Veracini, C. A. (1982) *Tetrahedron* **38**, 639.
7. De Haan, J. W. and Van de Van, L. J. M. (1973) *Org. Magn. Reson.* **5**, 147.
8. Couperus, P. A., Clague, A. D. H. and Dougen, J. P. C. M. (1976) *Org. Magn. Reson.* **8**, 426.
9. Schneider, H. J. and Agrawal, P. K. (1986) *Magn. Reson. Chem.* **24**, 718.
10. Pringle, R. B. and Scheffer, R. P. (1967) *Phytopathology* **57**, 1169.
11. Tipton, C. L., Paulsen, P. V. and Bets, R. E. (1971) *Plant Physiol.* **59**, 907.
12. Vidhyasekaran, P., Borromeo, E. S. and Mew, T. W. (1986) *Phytopathology* **76**, 261.
13. Sugawara, F., Strobel, G., Strange, R. N., Siedow, J. N., van Dyne, G. D. and Clardy, J. (1987) *Proc. Natl Acad. Sci.* U.S.A. **84**, 3081.
14. Canales, M. W. and Gray, G. R. (1988) *Phytochemistry* **27**, 1653.
15. Shukla, R. S. and Husain, A. (1987) *J. Phytopathol.* **118**, 187.
16. Karr, A. L., Karr, D. B. and Strobel, G. A. (1974) *Plant Physiol.* **53**, 250.