Phytochemistry, Vol. 28, No. 1, pp. 73–75, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

PHYTOTOXINS FROM *ALTERNARIA CASSIAE*

CYNTHIA M. HRADIL, 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.

(*Received* 3 *May* 1988)

Key Word Index—*Alternaria cassiae*; phytotoxins; stemphyperylenol; stemphyltoxin II; alteichin; altertoxin II; dihydroalterperylenol; altertoxin I; alterperylenol.

Abstract—The isolation, structure determination, and phytotoxicity of stemphyperylenol, stemphyltoxin II, alterperylenol, and altertoxin I from *Alternaria cassiae* are reported.

INTRODUCTION

Some fungi of the genus *Alternaria* cause diseases of food and other plants. As part of a programme to explore the chemistry and biology of phytopathogenic fungi, we investigated *Alternaria cassiae*. Our interest in this species was aroused by reports that the mycelia of *A. cassiae* had been incorporated into herbicides used to treat sicklepod in soybean [1]. This note reports the four phytotoxic compounds we isolated from *A. cassiae*.

Alternaria species produce a variety of partially reduced perylenequinone derivatives which have also been isolated from the genus *Stemphylium* [2]. The literature on these metabolies is confused by having several different names for the same structure [2].

RESULTS AND DISCUSSION

Alternaria cassiae was cultured using a modified Czapek Dox media and our previously reported procedure [3]. Compounds **1–4** were isolated by preparative thin layer chromatography of the ethyl acetate extract of the culture filtrate. The structures of compounds **1–4** were established either by direct or literature comparison with previously reported compounds.

Stemphyperylenol (**1**) had been previously isolated by Arnone and co-workers from *Stemphylium botryosum*, a fungus which caused leaf spot disease of lettuce [4]. Compound **1** was identified as stemphyperylenol by comparison of MS, UV, and ^{1}H NMR data with the published values.

Stemphyltoxin II (**2**) was also first reported by Arnone and co-workers from *Stemphylium botryosum* [4]. Independently, Stack and co-workers isolated a compound from *Alternaria alternata* to which they assigned structure **2** but named altertoxin II [5]. Comparison of UV and ^{1}H NMR data with the published data identified **2** as stemphyltoxin II.

Alterperylenol (**3**) was previously isolated from an *Alternaria* species [6]. The same structure was independently named alteichin, although there was a slight disagreement between the $[\alpha]_D$ measurements [7].‡ Comparison of MS, UV, and ^{1}H NMR data with the published data for alterperylenol identified **3** as alterperylenol.

Altertoxin I (**4**) had been previously isolated from a number of *Alternaria* species [5, 8]. Previously the same structure was reported as dihydroalterperylenol by Okuno and co-workers [6]. Comparison of published MS and UV data and direct comparison of ^{1}H NMR spectra identified **4** as altertoxin I.

Stack and co-workers tested altertoxin I (**4**) and altertoxin II (**2**) for mutagenicity in the Ames test, but no studies of the phytotoxicity of these compounds had been reported [9]. Phytotoxicity levels, as measured by the size of necrotic lesions 72 hr after application of 1 μg (3 μl drop) of compounds **1–4** are shown in Fig. 1.

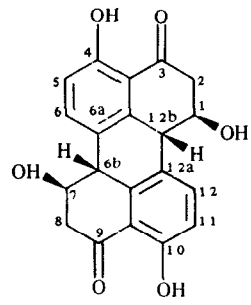
Some host specificity is clearly observed; stemphyperylenol (**1**) is a selective toxin for crabgrass, while altertoxin I (**4**) is quite selective for B73 corn. Alterperylenol (**3**) shows a low level of phytotoxicity toward B73 corn and soybean without strong selectivity, and stemphyltoxin II (**2**) shows some nonspecific phytotoxicity toward B73 corn. Ironically, only a low nonspecific level of phytotoxicity is observed in sicklepod.

EXPERIMENTAL

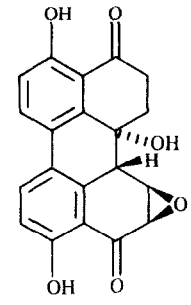
Culturing of fungus and isolation procedures. The fungus was maintained on potato dextrose agar containing 15% v/v V-8 juice. Modified Czapek Dox media was inoculated with the fungus (5 mm^2/l). The culture was incubated at 25° for 21 days with continuous shaking at 200 rpm or at *ca* 25° or for 28 days without shaking. Shake culture conditions seemed to favour stemphyperylenol (**1**) while compounds **2–4** were more prevalent in the still cultures. The culture was filtered through 4 layers of cheesecloth and the filtrate was extracted with ethyl acetate (3 × 1/3 filtrate vol.). The crude extract was concd and subjected to prep. TLC using silica plates (1000 μm, 20 × 20 cm) and $CHCl_3$–MeOH (9:1). The bands of interest were detected by irradiation with UV light and the compounds recovered with Me_2CO extraction. Further purification was obtained by using prep. TLC (250 μm, 20 × 20 cm) with $CHCl_3$–MeOH (9:1) or toluene–EtOAc (1:1). Typical yields were 1–2 mg/l for each compound.

*Authors to whom correspondence should be addressed.

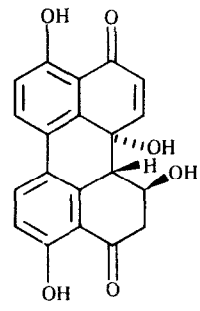
‡Altertoxin II was incorrectly reported as having the same structure as alterperylenol and alteichin in ref. [1].



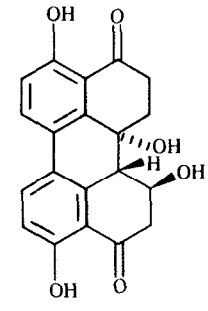
1



2



3



4

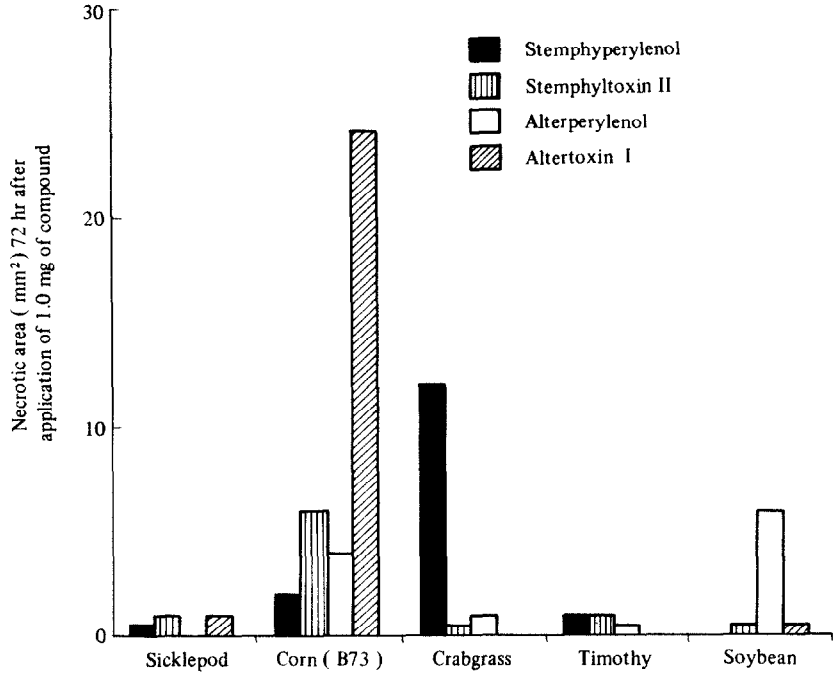


Fig. 1. Phytotoxicity data for compounds **1–4**.

Stemphyperylenol (**1**). HREIMS $C_{20}H_{16}O_6$, obsd 352.0941, calcd 352.0946. UV λ_{max}^{MeOH} nm (log ε) 206 (4.08), 260 (3.91), 340 (3.51). ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 3.07 (2H, *dd*, *J* = 4.8, 15.5 Hz, H-2α, H-8α), 3.19 (2H, *dd*, *J* = 11.8, 15.5 Hz, H-2β, H-8β), 3.78 (2H, *d*, *J* = 9.0 Hz, H-6b, H-12b), 4.64 (2H, *s*, OH-1, OH-7), 4.78 (2H, *ddd*, *J* = 4.8, 9.1, 11.8 Hz, H-1, H-7), 6.84 (2H, *d*, *J* = 8.8 Hz, H-6, H-12), 8.17 (2H, *d*, *J* = 8.8 Hz, H-5, H-11), 12.10 (2H, *s*, OH-4, OH-10).

Stemphyltoxin II, altertoxin II (**2**). UV λ_{max}^{MeOH} nm (log ε) 204 (3.88), 218 (3.85), 260 (3.90), 360 (3.06). ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 2.59 (1H, *m*, H-1β), 2.77 (1H, *m*, H-2β), 2.97 (1H, *ddd*, *J* = 2.6, 5.0, 13.4 Hz, H-1α), 3.26 (1H, *ddd*, *J* = 5.0, 14.0, 17.6 Hz, H-2α), 3.64 (1H, *d*, *J* = 0.6 Hz, H-12a), 3.73 (1H, *dd*, *J* = 0.5, 3.6 Hz, H-11), 4.45 (1H, *dd*, *J* = 0.6, 3.6 Hz, H-12), 7.00 1H, *dd*, *J* = 0.5, 8.8 Hz, H-8), 7.08 (1H, *d*, *J* = 8.8 Hz, H-5), 8.06 (1H, *d*, *J* = 8.8 Hz, H-7), 8.15 (1H, *d*, *J* = 8.8 Hz, H-6).

Alterperylenol, alteichin (**3**). HREIMS $C_{20}H_{14}O_6$, obsd 350.0794, calcd 350.0790. UV λ_{max}^{MeOH} nm (log ε) 256 (4.27), 288 (4.07), 364 (3.47). ^{1}H NMR (300 MHz, CD_3CN) δ 2.99 (1H, *dd*, *J* = 5.0, 16.0 Hz, H-11α), 2.85 (1H, *dd*, *J* = 12.0, 16.0 Hz, H-11β), 3.15 (1H, *dd*, *J* = 0.5, 9.8 Hz, H-12a), 4.60 (1H, *ddd*, *J* = 5.0, 9.8, 12.0 Hz, H-12), 6.32 (1H, *d*, *J* = 10.4 Hz, H-2), 6.96 (1H, *dd*, *J* = 0.5, 8.8 Hz, H-8), 7.05 (1H, *d*, *J* = 8.8 Hz, H-5), 7.84 (1H, *d*, *J* = 10.4 Hz, H-1), 7.92 (1H, *d*, *J* = 8.8 Hz, H-7), 7.97 (1H, *d*, *J* = 8.8 Hz, H-6).

Altertoxin, I, Dihydroalterperylenol (**4**). HREIMS $C_{20}H_{16}O_6$ obsd 352.0946, calcd 352.0946. UV λ_{max}^{MeOH} nm (log ε) 216 (3.94), 258 (4.04), 286 (3.72), 356 (3.23). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (1H, *m*, H-2β), 2.70 (1H, *m*, H-1β), 2.92 (1H, *dd*, *J* = 12.0, 16.1 Hz, H-11β), 3.02 (1H, *m*, H-2α), 3.07 (1H, *m*, H-12a), 3.08 (1H, *m*, H-11α), 3.18 (1H, *m*, H-1α), 4.75 (1H, *ddd*, *J* = 4.8, 9.1, 11.8 Hz, H-12), 7.03 (1H, *dd*, *J* = 0.9, 8.8 Hz, H-8), 7.09 (1H, *d*, *J* = 8.8 Hz, H-5), 7.83 (2H, *d*, *J* = 8.8 Hz, H-6, H-7).

Acknowledgements—The authors wish to thank Dr Michael Stack for generously providing a sample of altertoxin I (**4**). The authors gratefully acknowledge NSF grant DMB-8607347 and the Montana Agricultural Experiment Station for financial support.

REFERENCES

1. Walker, H. L. and Connick, W. J., Jr. (1984) *Chem. Abstr.* **100**, 169956k.
2. Weiss, U., Merlini, L. and Nasini, G. (1987) *Prog. Chem. Org. Nat. Prod.* **52**, 1.
3. Robeson, D. J., Gray, G. R. and Strobel, G. A. (1982) *Phytochemistry* **21**, 2359.
4. Arnone, A., Nasini, G., Merlini, L. and Assante, G. (1986) *J. Chem. Soc. Perkin Trans. I* 525.
5. Stack, M. E., Mazzola, E. P., Page, S. W., Pohland, A. E., Highet, R. J., Tempesta, M. S. and Corley, D. G. (1986) *J. Nat. Prod.* **49**, 866.
6. Okuno, T., Natsume, I., Sawai, K., Sawamura, K., Furusaki, A., and Matsumoto, T., (1983) *Tetrahedron Letters* **24**, 5653.
7. Robeson, D., Strobel, G., Matsumoto, G. K., Fisher, L. E., Chen, M. H. and Clardy, J. (1984) *Experientia* **40**, 1248.
8. Stinson, E. E., Osman, S. F. and Pfeffer, P. E. (1982) *J. Org. Chem.* **47**, 4110.
9. Stack, M. E. and Prival, M. J. (1986) *Appl. Environ. Microbiol.* **52**, 718.