PRODUCTION OF A HOST-SPECIFIC PHYTOTOXIN BY ALTERNARIA BRASSICAE

W.A. Ayer*, P.S. Bains, L.M. Pena-Rodriguez*
and J.P. Tewari
Departments of Chemistry* and Plant Science,
University of Alberta, Edmonton, Alberta,
Canada T6G 2P5

INTRODUCTION

Alternaria brassicae causes the blackspot disease of rapeseed (Brassica campestris and B. napus) which is economically important in Canada and many other parts of the world. Despite the importance of this disease, little information on the mode of pathogenesis is available. Several species of Alternaria Nees ex Fr. are reported to produce toxins. There are at least six host-parasite combinations, involving species of Alternaria, where the toxins produced are host-specific (Nishimura and Kohmoto, 1983).

Toxic effect of the culture filtrate of A. brassicae on B. campestris var. yellow sarson seedlings was reported by Husain and Thakur (1966). Later, two groups of non-specific toxins (in semi-purified preparations) produced by the fungus and capable of reproducing the disease symptoms on leaves were reported (Degenhardt, 1978). This paper reports on the isolation and identification of a host-specific toxin (AB-toxin) and several other interesting metabolites produced by A. brassicae in culture.

MATERIALS AND METHODS

Two methods were developed to isolate the AB-toxin produced by A. brassicae in V8 juice culture broth supplemented with glucose. The first method included extraction with ethyl acetate, dissolution of the crude extract in water-methanol, successive partitioning with hexane, ethyl acetate and n-butanol, dry flash column chromatography (Pena-Rodriguez, 1986), and thin layer chromatography. The second method included gel filtration using Sephadex G-50 and G-25 columns, adsorption on activated charcoal, successive desorption with ethyl acetate and water saturated

n-butanol followed by silica gel column and Sep-Pak C_{18} cartridge chromatography, respectively, and high pressure liquid chromatography (HPLC).

All fractions at each step were assayed for biological activity on B. napus cv. Altex leaves. Various chemical (ninhydrin reaction and derivatization) and physical techniques (infra-red spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, high resolution mass spectroscopy and fast atom bombardment mass spectroscopy) were used to identify the AB-toxin and some other metabolites.

Host-specificity of the AB-toxin was studied by applying the toxin both on the hosts and non-hosts of A. brassicae. The hosts included B. nigra, B. campestris var. yellow sarson, var. toria and cv. Candle, B. juncea cv. Lethbridge 22A, B. napus cv. Altex, B. hirta cv. Gisilba and B. rapa and the non-hosts included barley, corn, cowpea, cucumber, flax, oats, rye, tomato and wheat.

RESULTS AND DISCUSSION

Using the first method of AB-toxin isolation, extraction of the concentrated aqueous broth with ethyl acetate provided a complex mixture of compounds, including steroidal glycosides and glycerides, which proved difficult to separate. However, when a solution of the metabolites in aqueous methanol was extracted successively with hexane, ethyl acetate, and n-butanol, it was found that the active components were concentrated in the ethyl acetate extract. This "medium polarity" fraction was separated into its components by dry flash chromatography followed by thin layer chromatography.

Using the second method of toxin isolation, toxic activity was observed both in ethyl acetate and n-butanol extracts of activated charcoal on which the active component was previously adsorbed. Silica gel column and Sep-Pak C_{18} cartridge chromatography of ethyl acetate and n-butanol fractions, respectively, and HPLC of both the fractions led to the isolation of AB-toxin in pure state.

Application of AB-toxin on rapeseed leaf resulted in the development of necrosis and chlorosis, characteristic of the blackspot disease (Figs. 1, 2).

The most active component (AB-toxin), mp 225 - 227 degrees, $[\alpha]_D$-237 degrees, was shown by high resolution mass spectrometry to possess the molecular formula $C_{30}H_{51}N_5O_7$. Analysis of the mass spectrum and the ^1H NMR and ^{13}C NMR spectra of the compound and its methanolysis product indicated that it was destruxin B (Fig. 3a), a cyclodepsipeptide previously isolated from Metarhizium anisopliae, a fungus which is pathogenic to silkworms (Tamura et al., 1964). Comparison with an authentic sample confirmed the identity. Two other cyclodepsipeptides, the known desmethyldestruxin B (Fig. 3b) and the previously unknown homodestruxin B (Fig. 3c), were also isolated and identified. The location of the extra methylene group in homodestruxin B was determined by mass spectrometry.

During the course of separation of the biologically active cyclodepsipeptides depicted as a, b, and c in Figure 3, we also isolated and determined the structures of three new sesquiterpenes, albrassitriol (Fig. 3d), isoalbrassitriol (Fig. 3e), and deoxyuvidin (Fig. 3f). A biogenetically interesting new compound which we have called brassicadiol was also obtained and shown to possess the structure shown in Figure 3g. The details of the isolation and structure elucidation of the metabolites have been described (Ayer et al., 1987a, b; Bains and Tewari, 1987).

The order of sensitivity of Brassicas to AB-toxin was similar to their order of susceptibility to A. brassicae. In a decreasing order of sensitivity/susceptibility it was B. nigra, B. campestris var. yellow sarson and var. toria > B. juncea cv. Lethbridge 22A > B. campestris cv. Candle, B. napus cv. Altex and B. hirta cv. Gisilba > B. rapa. On the most susceptible host, the minimum concentration of AB-toxin required to cause symptoms was between 15 and 30 $\mu g\ ml^{-1}$. On the non-hosts, even toxin concentration of 300 $\mu g\ ml^{-1}$ did not cause any symptoms. These results indicated that the toxin isolated is host-specific (Bains and Tewari, 1987).

This is the first report on production of a host-specific toxin by a pathogen of rapeseed. The AB-toxin can be used for host resistance selection using conventional and tissue culture methods.

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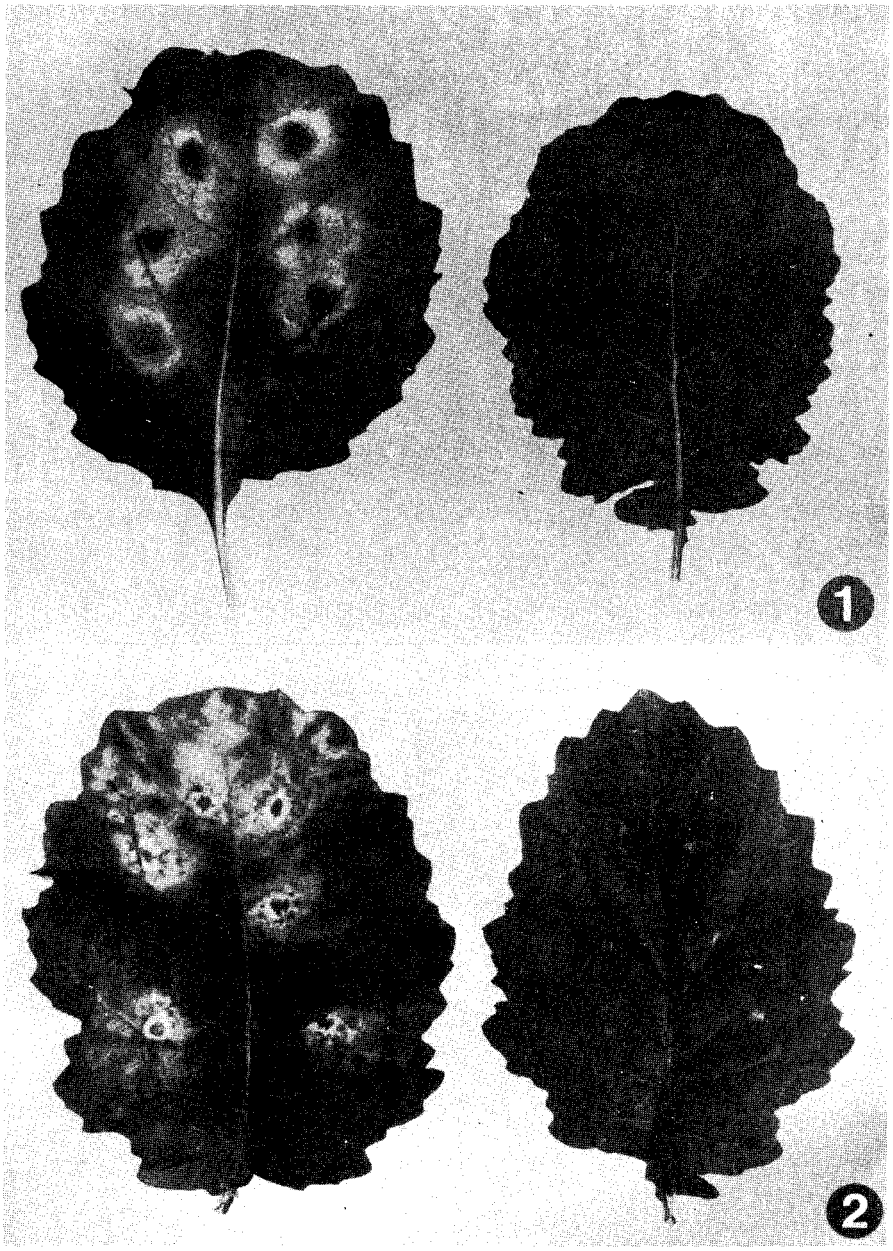
LEGEND

Fig. 1. Symptoms caused by A. brassicae on B. napus cv. Altex (left leaf). Right leaf-control.

Fig. 2. Symptoms caused by AB-toxin on B. napus cv. Altex. (left leaf). Right leaf-control.

Fig. 3. Structural formulae of A. brassicae metabolites. a. Destruxin B (AB-toxin). b. Desmethyldestruxin B. c. Homodestruxin B. d. Albrassitriol. e. Isoalbrassitriol. f. Deoxyuvidin. g. Brassicadiol.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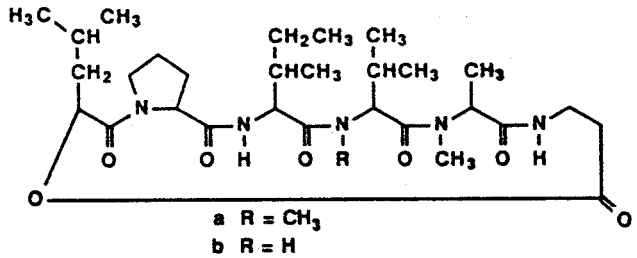

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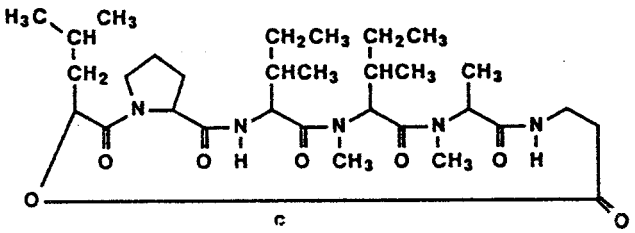
b R = H



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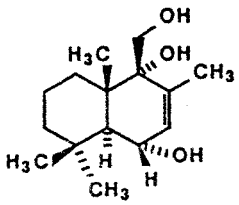

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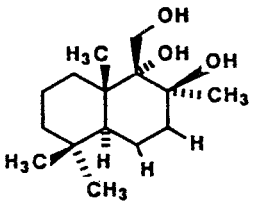

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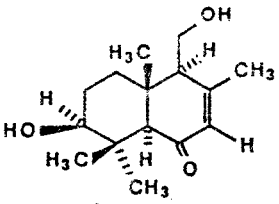

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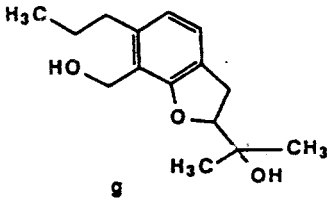

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Fig. 3