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Phomenins and fatty acids from *Alternaria infectoria*

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ABSTRACT

Taxa of the *Alternaria infectoria* species group are the predominant *Alternaria* spp. found in cereals in Northern Europe. While several pyrones have been isolated from *A. infectoria* and described as taxonomical markers for species identification, information about the bioactivity of metabolites from the fungus is missing. Bioassay-guided fractionation of rice culture extracts from several strains of *A. infectoria* linked the observed toxicity of the extracts in MRC-5 cells to free fatty acids, i.e. linoleic acid and α-linolenic acid. The fungus also produced a cytotoxic pyrone, which upon isolation and NMR spectroscopic analysis was identified as a mixture of phomenins A and B (approximately 10:1), which have not previously been isolated from an *Alternaria* species.

1. Introduction

The genus *Alternaria* was originally described by Ness von Esenbeck's in 1816 and includes some of the most common fungi which can be found in soil, seed and agricultural commodities (Panigrahi, 1997; Bottalico and Logrieco, 1998). It includes both plant pathogenic and saprophytic species that may damage crops in the field or cause post-harvest decay of plant products in storage. Historically, the taxonomic status of the species within the genus has been under continuous debate with diverging views of their classification (Chelkowski and Visconti, 1992; Simmons, 1992). Approximately 400–450 species of *Alternaria* are currently reported in the literature based primarily on conidial morphology and host specificity (Labuda et al., 2008). Confusion has especially been related to small-spored species, which often have been misidentified due to the use of non-standardized growth conditions (Andersen and Thrane, 1996; Andersen et al., 2002; Christensen et al., 2005). This might have been the reason for the lack of information about taxa within the *Alternaria infectoria* species group, which has recently been reported as the most common species group in Norwegian grains (Kosiak et al., 2004).

It has been reported that species of *Alternaria* produce approximately 125 metabolites, many of them with phytotoxic activity playing an important role in the pathogenesis of plants (Chelkowski and Visconti, 1992; Panigrahi, 1997). In addition, it is well known that several toxic metabolites produced by *Alternaria* species may contaminate food products and feedstuffs, and thus elicit adverse effects in animals (King and Schade, 1984). Despite this, the mycochemistry of *A. infectoria* has so far poorly been investigated. Studies by Andersen et al. demonstrated the possibility to use the metabolite profile of *A. infectoria* for chemotaxonomic characterization (Andersen and Thrane, 1996; Andersen et al., 2002). During the last years several secondary metabolites have been purified from strains of *A. infectoria*, and infectopyrone, 4Z-infectopyrone, novae-zelandin A and B have been chemically characterized (Larsen et al., 2003; Christensen et al., 2005) (Fig. 1). It should be noted that there is practically no information available concerning the bioactivity of individual compounds or their bio-production under natural

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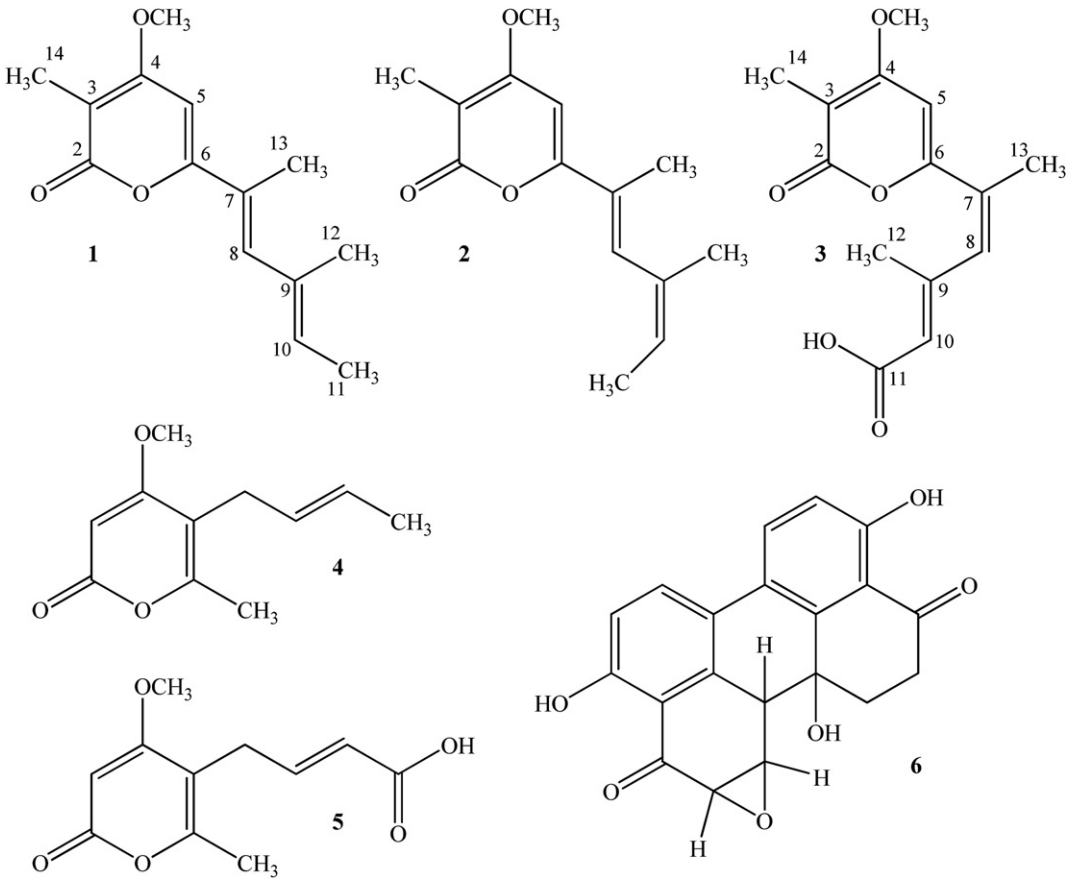

Fig. 1. Chemical structures of phomenin A (1), B (2), infectopyrone (3), novae-zelandin A (4), B (5) and altertoxin II (6).

conditions. Because of their close structural relationship to other known biologically active compounds (pyrenocines, α-pyrones) it has been suggested that they could be phytotoxins and potential mycotoxins (Larsen et al., 2003; Christensen et al., 2005).

The aim of the present work was to investigate the cytotoxicity of *A. infectoria* rice culture extracts in a cell-line of human origin, and to identify major metabolites, which contribute to the biological activity of the extracts. This would be the first step in assessing if the high prevalence of *A. infectoria* in grain is of concern for feed and food safety.

2. Materials and methods

2.1. General experimental protocols

NMR spectra of linoleic and α-linolenic acid, phomenins A and B and infectopyrone were obtained from a solution in 500 μL of acetone-*d*6 ($(CD_3)_2C(O)$, 99.9%D, Aldrich, Milwaukee, WI) in WG5Economy 5 mm tubes (Wilmad Labglass, Buena, NJ, USA). NMR spectra of infectopyrone were in addition obtained from a solution in 500 μL of DMSO-*d*6 ($(CD_3)_2S(O)$, 99.96%D, Aldrich). The spectra were acquired on an Avance AVII 600 MHz NMR spectrometer (Bruker BioSpin, Silberstreifen, Germany) with a 5 mm CP-TCI ($1H/^{13}C$, 15 N–^{2}H) triple-resonance inverse cryo probe, equipped with a Z-gradient coil. NMR assignments of phomenins were obtained from examination of ^{1}H- and ^{13}C spectra, attached proton test (APT), distortionless enhancement by proton transfer (DEPT135), correlated spectroscopy (COSY45), total correlation spectroscopy (TOCSY), pulsed field gradient heteronuclear single quantum coherence (g-HSQC), pulsed field gradient heteronuclear multiple bond correlation (g-HMBC), pulsed field gradient heteronuclear two-bond correlation (H2BC) and nuclear Overhauser spectroscopy (NOESY). NMR assignments for infectopyrone were obtained from ^{1}H, APT, g-HSQC, g-HMBC and NOESY spectra. Linoleic acid was identified from examination of ^{1}H, ^{13}C, APT, COSY45, TOCSY, g-HSQC, g-HMBC, g-H2BC and NOESY data, while only ^{1}H and COSY45 spectra were recorded for α-linolenic acid. The data were processed using the Bruker TOPSPIN (version 1.3) software. Chemical shifts, determined at 298 K, are reported relative to either internal $CHD_2C(O)CD_3$ (2.04 ppm), $CHCl_3$ (7.26 ppm) or $CHD_2S(O)CD_3$ (2.49 ppm), $CDCl_3$ (77.2 ppm) and $(CD_3)_2C(O)$ (27.8 ppm) or $(CD_3)2S(O)$ (39.5 ppm), respectively. Metabolite screening of culture extracts was performed using HPLC coupled to a Surveyor PDA Plus detector and a Finnigan LTQ linear ion trap mass spectrometer (all Thermo Electron, San Jose, CA). Ions were generated upon electrospray ionisation in the positive or negative mode. The MS was operated in the full-scan mode (*m/z* 200–1000), while UV–VIS spectra were recorded in the range 190–600 nm. Separation was achieved on