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Structural and biosynthetic studies on eremophilenols related to the phytoalexin capsidiol, produced by *Botrytis cinerea*

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ABSTRACT

A thorough study of the fermentation broth of three strains of *Botrytis cinerea* which were grown on a modified Czapek-Dox medium supplemented with 5 ppm copper sulphate, yielded five undescribed metabolites. These metabolites possessed a sesquiterpenoid (+)-4-*epi*-eremophil-9-ene carbon skeleton which was enantiomeric to that of the phytoalexin, capsidiol. The isolation of these metabolites when the fungus was stressed, suggests that they may be potential effectors used by *B. cinerea* to circumvent plant chemical defences against phytopathogenic fungi. The biosynthesis of these compounds has been studied using ^{2}H and ^{13}C labelled acetate.

1. Introduction

The ascomycete *Botrytis cinerea* Pers. Fr, classified within the family Sclerotiniaceae, is the causative agent of a grey mould disease which infects at least 1400 plant species (Elad et al., 2016), including crops of economic importance such as grapes and strawberries (Dean et al., 2012). The fungus is a typical necrotroph whose infective cycle includes the induction of plant cell death followed by the maceration of the plant tissue and then reproduction by forming asexual spores on the decomposing plant material. The emergence of the disease symptoms depends on the host plant, the infected part of the plant and the weather conditions (van Kan, 2006; Williamson et al., 2007). It has recently been reported that *B. cinerea* can also systemically colonize plants without causing disease symptoms (van Kan et al., 2014). In general, *B. cinerea* is responsible for severe economic losses that are either due to the damage to the growing plants in the field or to the subsequent decay of the harvested fruits, flowers and vegetables during storage under cold and humid conditions (van Kan, 2006; Williamson et al., 2007).

The wide host range of *B. cinerea* in contrast to other *Botrytis* species that are specialized on a single host species, e.g. *B. elliptica* on lilies, may be due to the formation of phytotoxic metabolites and proteins with necrotizing activities that are effective against a range of plant species. Thus *B. cinerea* produces specific terpenoid and polyketide metabolites such as the sesquiterpene botrydial (**1**) and related compounds (Colmenares et al., 2002), botrylactone (**2**) and the botcinic (**3**) and botcineric acids (**4**) as well as their cyclic relatives, the botcinins (**5**, **6**) (Tani et al., 2005, 2006). The sesquiterpenoid abscisic acid (**7**) (Marumo et al., 1982) which mediates leaf fall and the polyketide, cinbotolide (**8**) have also been isolated from the fungus (Fig. 1) (Botubol et al., 2014; Collado and Viaud, 2016).

It is now clear that the development of pathogenesis by *B. cinerea* is much more complex than previously believed. Disease progression is tightly regulated throughout the infection process and the fungus undergoes developmental adaptions that coincide with the different stages of the infection. The interactions between the fungus and the plant are mediated by compounds which affect both the fungus and the plant (González et al., 2016). One class of such compounds are the effectors. These compounds are secreted by a microbial pathogen in order to inhibit the plant defensive system (Maffei et al., 2012). In many plant-pathogen interactions, effectors are key pathogenicity determinants that modulate the plant's intrinsic immunity to facilitate the development of the parasitic infection (Oliva et al., 2010). Thus the discovery of the fungal effectors produced by *B. cinerea* and the identification of

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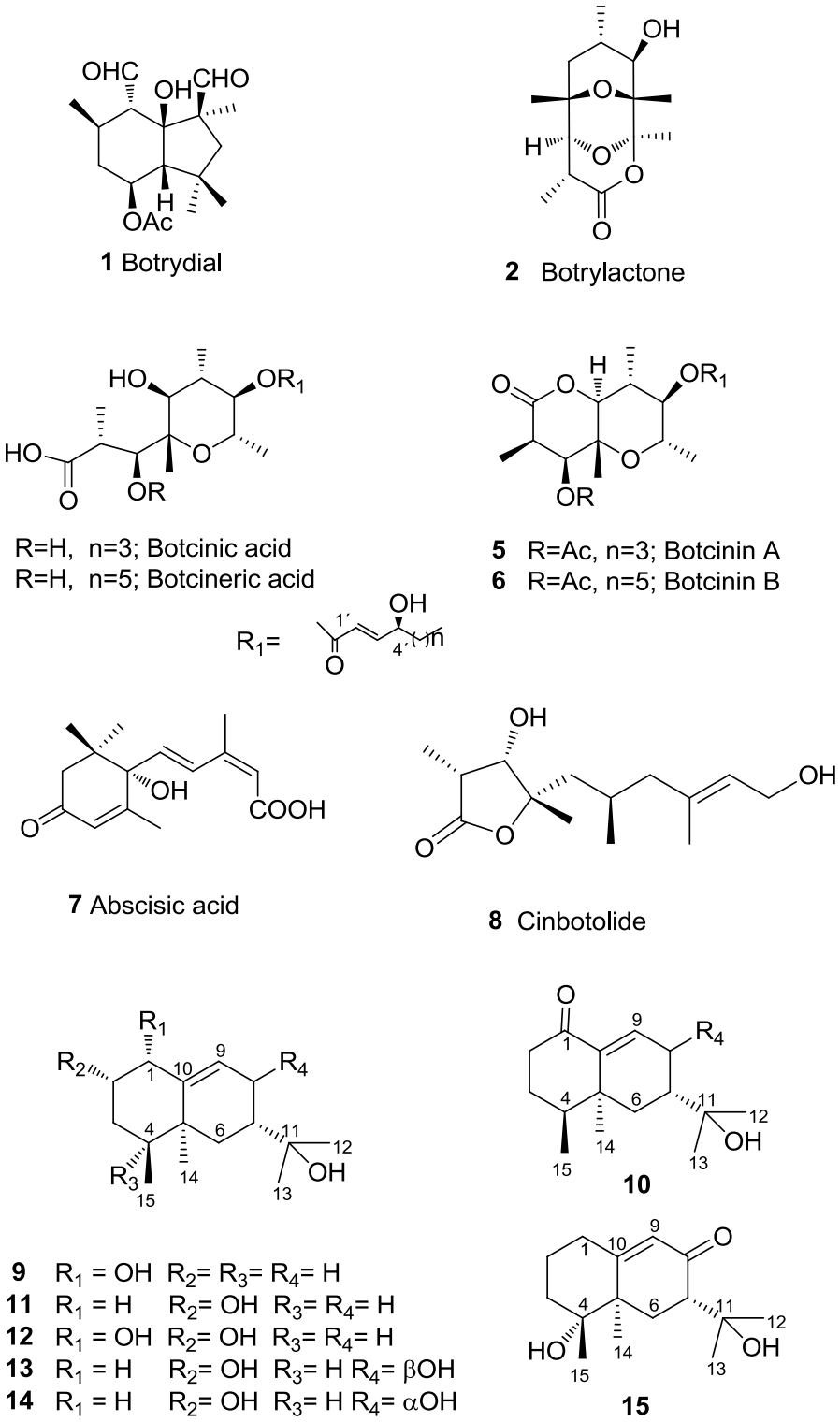

Fig. 1. Some metabolites isolated from *B. cinerea*.

their plant targets holds promise for the improvement of plant tolerance to this serious pathogen.

The recent sequencing of the *B. cinerea* genome has provided an opportunity to discover novel biosynthetic gene clusters involved in the biosynthesis of novel fungal metabolites. Forty-four genes that encode key enzymes have been identified including those involved in the biosynthesis of terpenes, polyketides and peptides that are specific to this phytopathogen (Amselem et al., 2011). The genome has shown that the biosynthetic potential of this microorganism has been barely explored and, as a result, a significant number of metabolites remain to be discovered. Consequently *B. cinerea* is a valuable organism with which to study the role of orphan biosynthetic genes and cryptic metabolites (Scherlach and Hertweck, 2009). In this context we have recently described the induction of a silent eremophilene biosynthetic pathway using sub-lethal doses of copper ions and characterized an undescribed family of eremophilenols (**9**–**15**) as cryptic metabolites (Pinedo et al., 2016).

These metabolites, which have sporogenic activity, possess a (+)-5-