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Lasiojasmonates A–C, three jasmonic acid esters produced by *Lasiodiplodia* sp., a grapevine pathogen

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ABSTRACT

In this study, a strain (BL 101) of a species of *Lasiodiplodia*, not yet formally described, which was isolated from declining grapevine plants showing wedge-shaped cankers, was investigated for its ability to produce *in vitro* bioactive secondary metabolites. From culture filtrates of this strain three jasmonic acid esters, named lasiojasmonates A–C and 16-*O*-acetylbotryosphaerilactones A and C were isolated together with (1*R*,2*R*)-jasmonic acid, its methyl ester, botryosphaerilactone A, (3*S*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone and (3*R*,4*S*)-botryodiplodin. The structures of lasiojasmonates A–C were established by spectroscopic methods as (1*R**,2*R**,3′*S**,4′*R**,5′*R**)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone, (1*R**,2*R**,3′*S**,4′*R**,5′*R**,10′*R**,12′*R**,13′*R**,14′*S**) and (1*R**,2*R**,3′*S**,4′*R**,5′*R**,10′*S**,12′*R**,13′*R**,14′*S**)-4-(4-hydroxymethyl-3,5-dimethyltetrahydro-furan-2-yloxymethyl)-3,5-dimethyldihydro-2-furanones jasmonates (**1**, **4** and **5**). The structures of 16-*O*-acetylbotryosphaerilactones A and C were determined by comparison of their spectral data with those of the corresponding acetyl derivatives obtained by acetylation of botryosphaerilactone A. The metabolites isolated, except **4** and **5**, were tested at 1 mg/mL on leaves of grapevine cv. Cannonau and cork oak using the leaf puncture assay. They were also tested on detached grapevine leaves at 0.5 mg/mL and tomato cuttings at 0.1 mg/mL. In all phytotoxic assays only jasmonic acid was found to be active. All metabolites were inactive in the zootoxic assay at 50 μg/mL.

1. Introduction

The Botryosphaeriaceae represent a well-known family of plant pathogenic fungi associated with fruit rot, leaf spots, dieback, cankers and root rot of Angiosperms and Gymnosperms worldwide (Phillips et al., 2013). Over the past decades, several species of the Botryosphaeriaceae have been recognized as important pathogens of grapevine in the many growing areas (Larignon et al., 2001; Phillips, 2002; van Niekerk et al., 2006; Pitt et al., 2010; Úrbez-Torres, 2011). In particular, to date, 21 different *taxa* of *Botryosphaeriaceae* have been reported as weak or aggressive pathogens on grapevine. Among these, four species belonging to the *Lasiodiplodia* genus namely *Lasiodiplodia crassispora* T.I. Burgess & Barber, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Lasiodiplodia missouriana* Úrbez-Torres, Peduto & Gubler and *Lasiodiplodia viticola* Úrbez-Torres, Peduto & Gubler have been recognized to be highly virulent on grapevine (Úrbez-Torres, 2011). The main disease symptoms caused by *Lasiodiplodia* spp. on grapevine consist of sunken cankers associated with wedge-shaped lesions of the vascular tissues.

Results of a recent study conducted in several Sardinian vineyards and aimed at clarifying the aetiology of grapevine canker and dieback, have led to the identification and characterization of 9 different species of *Botryosphaeriaceae* from symptomatic grapevine tissues (Linaldeddu et al., unpublished data). Among these, a *Lasiodiplodia* species morphologically (shape and size of conidia) and phylogenetically (ITS and EF1-α sequence data) distinct from all known species was isolated. Its formal description will be addressed in a future publication currently in preparation.

At present, 18 species are recognized in *Lasiodiplodia* (Phillips et al., 2013) and recent studies, based on ITS and EF-1α sequence data, have led to the clarification of systematics of this genus and to the identification of cryptic species within the *L. theobromae* species complex (Alves et al., 2008; Abdollahzadeh et al., 2010; Begoude et al., 2010; Úrbez-Torres et al., 2012).

The nature of symptoms (vascular necrosis) caused by this new species of *Lasiodiplodia* on grapevine suggests that phytotoxic

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metabolites may be involved in the host-pathogen interaction. On the other hand, it is well-known that *L. theobromae*, which is considered the type species of the *Lasiodiplodia* genus, biosynthesizes a variety of lipophilic and hydrophilic metabolites that exhibit interesting biological activities (Aldridge et al., 1971; Husain et al., 1993; Nakamori et al., 1994; Matsuura et al., 1998; Yang et al., 2000; He et al., 2004; Miranda et al., 2008; Kitaoka et al., 2009; Abdou et al., 2010; Pandi et al., 2010; da Cunha et al., 2012).

Therefore, the main aims of this work were: (1) to isolate and identify the main secondary metabolites produced *in vitro* by a selected strain (BL 101) of this new *Lasiodiplodia* species; (2) to evaluate their biological activities such as phytotoxicity and zootoxicity.

2. Result and discussion

The fungal culture filtrates were exhaustively extracted with EtOAc at pH 2, and the corresponding organic extract was purified by combined column and TLC chromatography as detailed reported in the experimental section, yielding 10 metabolites (**1**–**10**, Fig. 1). The preliminary ^{1}H NMR investigation showed that the metabolites belong to different classes of natural compounds. The structures of the known compounds were confirmed by physical and spectroscopic methods (OR, IR, UV, ^{1}H and ^{13}C NMR, ESI and/or APCI MS) and by comparison of the obtained data with those reported in the literature for (1*R*,2*R*)-jasmonic acid and its methyl ester (**6** and **7**) (Husain et al., 1993; Yukimune et al., 2000), botryosphaerilactone A (**8**) (Rukachaisirikul et al., 2009), (3*S*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone (**9**) (Ravi et al., 1979), and (3*R*,4*S*)-botryodiplodin (**10**) (Ramezani et al., 2007). The identification of jasmonic acid and its methyl ester was confirmed by comparison by their behavior TLC (silica gel eluent A *Rf* 0.43, and on reversed phase eluent C *Rf* 0.50; silica gel eluents A *Rf* 0.8 and B *Rf* 0.13, respectively) and ^{1}H NMR with those of commercial samples of (±)-jasmonic acid and its methyl ester. Some other new spectroscopic data as ESI and/or APCIMS spectra and in particular the ^{13}C NMR data for the trisubstituted dihydrofuranone **9** were detailed reported in the corresponding paragraphs of the experimental section. In addition, the identification of **8** was also supported by data from its COSY, HSQC and HMBC spectra. Finally, as (3*R*,4*S*)-botryodiplodin (**10**) was obtained as an inseparable anomeric mixture (α/β, 65:35), its structure was confirmed by acetylation carried out in the usual conditions. In fact, this reaction yielded only the 2,3-*trans*-botryodiplodin acetate, whose physic and spectroscopic data were very similar to those previously reported (Arsenault and Althaus, 1969).

Furthermore, the preliminary ^{1}H and ^{13}C NMR investigation of **1** showed its close correlation with both jasmonic acid and 3,4,5-trisubstituted dihydrofuranone **9** and, being a new compound as described below, it was named lasiojasmonate A. These suggestions were also confirmed by the band typical of ketone ester and olefinic groups (Nakanishi and Solomon, 1977) and the end absorption maximum (Scott, 1964) observed in the IR and UV spectra, respectively. It showed a molecular weight of 336 associated with the molecular formula of $C_{19}H_{28}O_5$ with six hydrogen deficiens as deduced from the HRESIMS spectrum. The ^{1}H NMR spectrum, also compared to that of jasmonic acid (Husain et al., 1993) and of standard commercial sample (Table 1), showed the signal patterns of the jasmonyl residue. For the 2-pentenyl moiety the multiplets of the protons (H-10 and H-9) of the *cis* double bond, the two allylic methylene groups (H_2-8 and H_2-11) and the triplet (J = 7.4 Hz) of the terminal methyl group (Me-12) were observed at δ 5.45, 5.26, 2.37, 2.04 and 0.95, respectively. For the 2,3-disubstituted cyclopentanone residue the multiplets of H-1 and H-2, the double doublet (J = 20.0 and 8.2 Hz) and the multiplet, and the multiplets of H_2-4 and H_2-5 were observed at δ 2.10, 1.90, 2.74 and 2.37, and 2.33 and 1.50

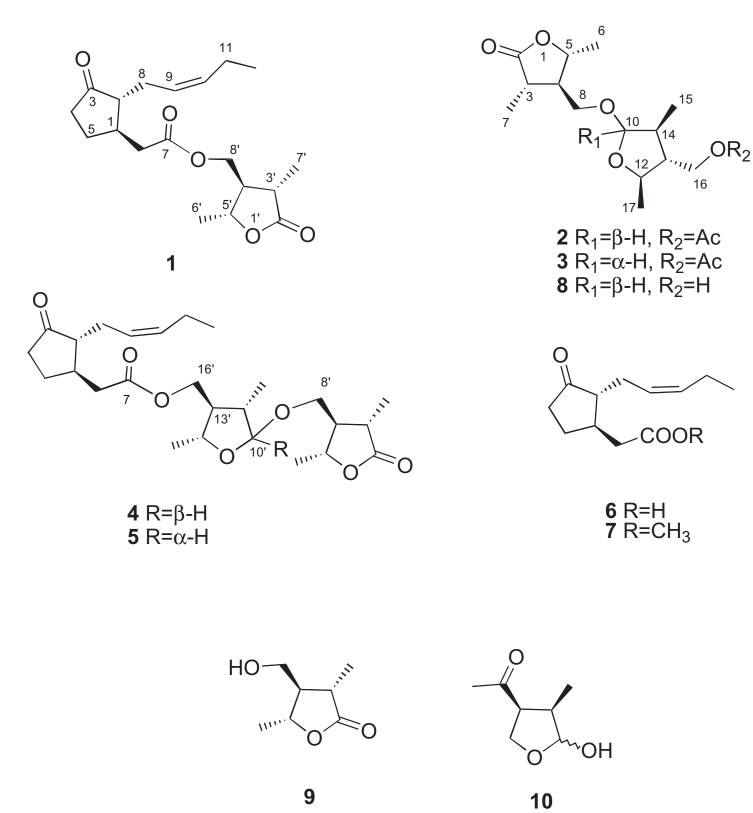

Fig. 1. Structures of lasiojasmonate, 16-*O*-acetylbotryosphaerilactone A and C, (−)-(1*R*,2*R*)-jasmonic acid and its methyl ester, botryosphaerilactone A, (3*R*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone and (−)-(3*R*,4*S*)-botryodiplodin (**1, 4, 5, 2, 3, 6**–**10**) produced by *Lasiodiplodia* sp.