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Synthesis and mode of action studies of *N*-[(-)-jasmonyl]-*S*-tyrosin and ester seiridin jasmonate

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ABSTRACT

Recent analyses on fungal jasmonic acid (JA)-containing metabolites suggest a mode-of-action of these naturally occurring compounds as inactive storage pools of JA. Plants and/or fungi can catabolize JA into the bioactive jasmonyl-isoleucine (JA-Ile) that in turn activates the JA-Ile-pathway *in planta*. To extend our knowledge on JA-derivates related to natural occurring JA conjugates, *N*-[(-)-jasmonyl]-*S*-tyrosin (JA-Tyr) and the ester JA-Sei between JA and seiridin, a fungal disubstituted furanone, were synthesized. The classical procedures for ester synthesis were applied for compound JA-Sei, while *N*-[(-)-jasmonyl]-*S*-tyrosin was synthesized with an optimized procedure. JA-Tyr and JA-Sei were characterized by spectroscopic method (essentially 1D and 2D NMR spectroscopy and ESI-MS) and their stereochemical composition was determined by means of HPLC and circular dichroism analysis. Finally, the activity of these JA-derivates was analyzed *in planta*. JA-Tyr and JA-Sei trigger JA-regulated plant responses, such as protein degradation and growth inhibition. These effects require the conversion of JA into JA-Ile and its recognition by the plant JA-Ile perception complex COI1-JAZ. Overall, these data suggest a mode-of-action of JA-Tyr and JA-Sei as inactive pool of JA that can be transformed into the bioactive JA-Ile to induce the canonical JA-Ile-pathway.

1. Introduction

Jasmonic acid (**1**, Fig. 1) is the basic compound of a naturally occurring family of substances named jasmonates which regulate many aspects of growth, development and defense responses in plants (Dewick, 2009; Osbourn and Lanzotti, 2009; Wasternack and Hause, 2013; Chini et al., 2016). In particular, JA is a signaling compound in plant defense, activating responses to wounding, herbivores and necrotrophic pathogens. JA and related compounds are also produced by fungi (Wasternack and Hause, 2013; Goossens et al., 2016; Andolfi et al., 2014). The JA-Ile biosynthetic pathway has been extensively studied in plants (Schaller and Stinzi, 2009; Wasternack and Hause, 2013; Chini et al., 2018; Wasternack and Song, 2017). The bioactive plant hormone JA-Ile [(+)−7-*iso*-JA-Ile] is generated by the conjugation of JA to Ile by the JA-amido synthetase JAR1 (Staswick and Tiryaki, 2004; Fonseca et al., 2009). JA-Ile is the ligand of the receptor complex and it acts as "molecular glue" to induce the formation of the COI1-JAZ co-receptor complex (Chini et al., 2007; Thines et al., 2007; Sheard et al., 2010). JA-Ile-promoted COI1-JAZ interaction results in ubiquitination and proteasome degradation of the JAZ repressors, which in turn activates several transcription factors that regulate specific physiological responses (Chini et al., 2007, 2016; Thines et al., 2007).

Fungi are well-known producers of secondary metabolites belonging to different class of natural compounds (Turner and Aldridge, 1983; Cole et al., 2003; Lo Presti et al., 2015; Fonseca et al., 2017). Phytopathogenic fungi are one of the main causal agents of serious diseases of agrarian and forest plants with heavy consequential economic losses (Ballio and Graniti, 1991; Evidente and Motta, 2001; Evidente et al., 2010, 2011, 2013; Cimmino et al., 2014, 2015).

Lasiodiplodia strain, recently classified as *Lasiodiplodia mediterranea* sp. nov. (Linaldeddu et al., 2015), produced *in vitro* (1*R*,2*R*)-

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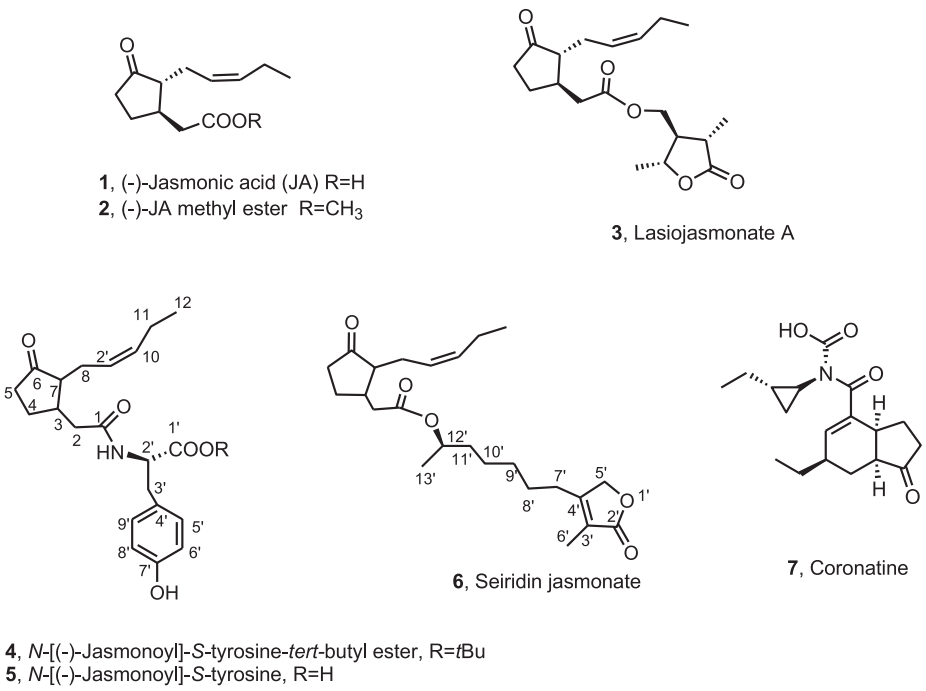

Fig. 1. Structures of jasmonic acid, its methyl ester and lasiojasmonate A (**1**-**3**), *N*-[(-)-jasmonoyl]-*S*-tyrosine and its tert-butyl ester (**4** and **5**), seiridin jasmonate ester (**6**) and coronatine (**7**).

(-)-jasmonic acid (**1**, Fig. 1), as the main phytotoxin, its methyl ester (JA-Me, **2**, Fig. 1), and three furanonenoyl esters, named lasiojasmonates A (LasA, **3**, Fig. 1) B and C, 16-*O*-acetylbotryosphaerilactones A and C, botryosphaerilactone A, (3*S*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone and (3*R*,4*S*)-botryodiplodin (Andolfi et al., 2014). A mode of action of LasA (**3**) was recently proposed (Chini et al., under review). The results show that LasA activates the plant JA-pathway and that its activity required the conjugation of JA to Ile by JAR1, the last biosynthetic step that generates the bioactive JA-Ile [(+)–7-*iso*-JA-Ile] (Staswick and Tiryaki, 2004; Fonseca et al., 2009). The activity of LasA also depends on the plant hormone receptor complex COI1-JAZ (Chini et al., 2007; Thines et al., 2007; Sheard et al., 2010). Because this fungal metabolite contains JA as a moiety of its structure, it has been proposed that plants and/or fungi can process these compounds and release free JA. Therefore, LasA might serve as inactive conjugated JA that can be converted into JA and subsequently into the bioactive JA-Ile in specific conditions (Chini et al., under review).

Considering that amino acid conjugates of JA occur naturally (Cross and Webster, 1970; Bohlmann et al., 1984; Brückner et al., 1988; Yan et al., 2016; Staswick et al., 2016; Wasternack and Song, 2017), and that the cylopentanone moiety has an important role in the direct binding of JA-Ile to its receptor COI1-JAZ, we proceeded with the synthesis of *N*-[(-)-jasmonoyl]-*S*-tyrosine-*tert*-butyl ester (**4**), the amide tyrosine-JA (JA-Tyr, **5**, Fig. 1) and the ester of JA with seiridin (JA-Sei, **6**, Fig. 1). Seiridin is produced as the main phytotoxin from *Seiridum cardinale*, the causal agent of cypress canker disease (Evidente et al., 1986), and it is a disubstituted furanone closely related to the trisubstituted didihydrofuranone **3**. The stereochemical composition of the prepared JA derivatives **5** and **6** could not be assigned by NMR, and was determined by means of HPLC separations and electronic circular dichroism (CD) measurements.

Finally, we studied the effect of JA-Tyr and JA-Sei on plant JA-regulated responses to understand their activity *in planta*. JA-Tyr and JA-Sei induce JA-regulated plant responses, including JAZ degradation and growth inhibition. These effects are dependent on JAR1 and COI1, suggesting that the activity of JA-Tyr and JA-Sei requires plant conversion of JA into JA-Ile and its consequent recognition by the COI1-JAZ receptor.

2. Results and discussion

2.1. Synthesis and characterization of N-[(-)-jasmonyl]-S-tyrosin and the ester seiridin jasmonate

JA is naturally conjugated with several amino acids and additional molecules (Wasternack and Song, 2017), and the cyclopentanone moiety has an important role in the binding of the ligand to its COI1-JAZ receptor complex (Sheard et al., 2010; Monte et al., 2014). Therefore, we synthesized the amide tyrosine-JA (JA-Tyr, **5**, Fig. 1) and the ester of JA with seridin (JA-Sei, **6,** Fig. 1), containing an alcoholic moiety related to 4-hydroxymethyl-3,5-dimethyldihydrofuran-2-one (LasA, **3**), reasoning that these JA-derivates might sterically affect the interaction with the COI1-JAZ complex (Monte et al., 2014). *N*-[(-)jasmonyl]-*S*-tyrosin (**5**) was synthesized starting from the commercially available racemic (±)-jasmonic acid and L-tyrosine *tert*-butyl ester according a previously reported procedure (Ulijn et al., 2015) as illustrated in Scheme 1A. The crude product obtained from reaction work-up was purified as reported in the Experimental Section yielding *N*-[(-)-jasmonoyl]-*S*-tyrosine-*tert*-butyl ester (**4**, Fig. 1**)** as colorless oil (67% yield). The ^{1}H NMR spectrum of **4** (Table 1) showed both the typical pattern system of L-tyrosine and JA in addition to the signal of the amidic proton (NH) observed as a doublet ($J = 7.7$ Hz) at δ 5.94 being coupled in the COSY spectrum (Berger and Braun, 2004) with the α-proton (HC-2′) resonating as a quartet ($J = 7.7$ Hz) at δ 4.75. This latter hydrogen, the X part of an ABX system, in turn coupled with the proton of the adjacent methylene group (H_2C-3′, the AB part) observed as two doublets of double doublets ($J = 10.0$, 7.7, 2.8 and $J = 10.0$, 7.7 and 2.8 Hz) at δ 3.05 and