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Biosynthesis of jasmonic acid in a plant pathogenic fungus, *Lasiodiplodia theobromae*

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ABSTRACT

Jasmonic acid (JA) is a plant hormone that plays an important role in a wide variety of plant physiological processes. The plant pathogenic fungus, *Lasiodiplodia theobromae* also produces JA; however, its biosynthesis in this fungus has yet to be explored. Administration of [1-^{13}C] and [2-^{13}C] NaOAc into *L. theobromae* established that JA in this fungus originates from a fatty acid synthetic pathway. The methyl ester of 12-oxo-phytodienoic acid (OPDA) was detected in the culture extracts of *L. theobromae* by GC–MS analysis. This finding indicates the presence of OPDA (a known intermediate of JA biosynthesis in plants) in *L. theobromae*. ^{2}H NMR spectroscopic data of JA produced by *L. theobromae* with the incorporation of [9,10,12,13,15,16-$^{2}H_6$] linolenic acid showed that five deuterium atoms remained intact. In plants, this is speculated to arise from JA being produced by the octadecanoid pathway. However, the observed stereoselectivity of the cyclopentenone olefin reduction in *L. theobromae* was opposite to that observed in plants. These data suggest that JA biosynthesis in *L. theobromae* is similar to that in plants, but differing in the facial selectivity of the enone reduction.

1. Introduction

Lasiodiplodia theobromae is a pathogenic fungus that infects plants in tropical and subtropical regions of the world, causing considerable damage to crops during storage. This fungus is known to produce a variety of bioactive compounds (Aldridge et al., 1971). Previous studies in our laboratory have demonstrated the presence of various potato-tuber-inducing substances in a culture of *L. theobromae*: (−)-JA (**7**) (see Fig. 1), theobroxide (and related compounds), and lasiodiplodins (Nakamori et al., 1994; Matsuura et al., 1998a,b; Yang et al., 2000; Li et al., 2006; Takei et al., 2006). Among these compounds, theobroxide exhibits the most interesting biological activity, which effects not only potato-tuber-inducing activity in potato (*Solanum tuberosum*), but also induces flower bud formation in morning glory (*Pharbitis nil*) (Yoshihara et al., 2000). Encouraged by the unique biological activities that these compounds display, the biosynthesis of theobroxide (Li et al., 2006) and lasiodiplodins (Kashima et al., 2009a,b) were elucidated. Among the compounds described above, (−)-JA (**7**) was first isolated from the culture of *L. theobromae* as a substance used to accelerate plant senescence in the 1970s (Aldridge et al., 1971). It has since been established that JA (**7**) is a plant hormone that controls responses to environmental stresses and developmental events in flowering plants. It plays an important role in coordinating plant defense responses with physiological stresses associated with herbivores and microbial pathogens. JA (**7**) biosynthesis and its regulatory mechanism, has been thoroughly investigated (Wasternack, 2007). Recently, the presence of JA (**7**) and the activity of allene oxide synthase (AOS), with respect to JA (**7**) biosynthesis, was demonstrated in a model moss *Physcomitrella patens* (Oliver et al., 2009; Bandara et al., 2009).

In plants, JA (**7**) is biosynthetically produced by the octadecanoid pathway as shown in Fig. 1 (Schaller and Stinzi, 2009). The first step in the octadecanoid pathway is the lipoxygenase (LOX)-catalyzed oxygenation of α-linolenic acid (**1**). In the specific case of JA (**7**) biosynthesis, hydroperoxidation to intermediated (**2**) takes place at C-13 of α-linolenic acid (**1**), and is effected by 13-LOX. The resulting hydroperoxide [13(*S*)-hydroperoxyoctadecatrienoic acid (13-HPOT)] (**2**) can be metabolized by AOS into an unstable allene oxide [12,13(*S*)-epoxyoctadecatrienoic acid (12,13-EOT) **3**], in which cyclization is facilitated by allene oxide cyclase (AOC) to provide 12-oxo-phytodienoic acid (OPDA) (**4**). The later is reduced by OPDA reductase 3 (OPR3) to yield 3-oxo-2-[(*Z*)-pent-2-enyl]-cyclopentane-1-octanoic acid (OPC-8:0) (**5**). Three subsequent β-oxidation steps afford (+)-*iso*-JA (**6**) [(3*R*,7*R*)-configuration], which is further epimerized at C-7 to provide (−)-JA (**7**) [(3*R*,7*S*)-configuration]. Due to keto-enol interconversion, the *cis*-isomer, (+)-*iso*-JA (**6**), is readily converted into the more stable *trans*-isomer, (−)-JA (**7**), for steric reasons.

As referred to above, the detailed mechanism of JA (**7**) signaling and biosynthesis in plants has been elucidated. However, there is a little information about the biosynthetic pathway and functions of JA (**7**) in *L. theobromae*. Thakkar et al. (2004) reported that development of systemic acquired resistance (SAR) was restricted in the plants infected with *L. theobormae* due to deficiency of salicylic

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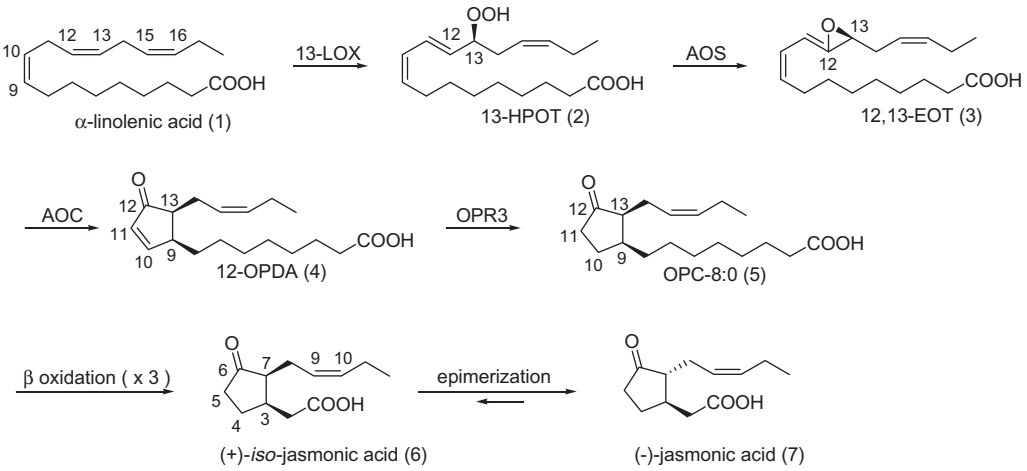

Fig. 1. The octadecanoid pathway.

acid (SA). Inhibition of SA biosynthesis in the infected plants, which was caused by JA (**7**) released from *L. theobromae*, might contribute to the infection of this fungus to plants. In the case of *Pseudomonas syringae*, coronatine, a phytotoxin functioning like JA (**7**), was shown to induce suppression of SA-mediated defense system, and disease development in tomato (Uppalapati et al., 2007).

In this work, it is demonstrated that JA (**7**) is synthesized *via* a fatty acid synthetic pathway in *L. theobromae*, which is supported by ^{13}C labeling experiments. The incorporation of a synthetic ^{2}H-labeled linolenic acid (**1**) into *iso*-JA indicates that JA (**7**) biosynthesis in *L. theobromae* is similar to that of plants, differing only in the facial selectivity of the cyclopentenone reduction (i.e., α- vs. β-hydride attack); the facial selectivity observed in plants is posited on the basis of the X-ray crystal structure data of tomato OPR3 (Breithaupt et al., 2006).

2. Results and discussion

L. theobromae was statically incubated in 1% potato-glucose medium at 29 °C for 7 days. ^{13}C-labeled sodium acetate ([1-^{13}C] and [2-^{13}C]) was administered to the culture at a concentration of 10 mM. The culture was filtered to separate the mycelia and supernatant after an additional 10 days of incubation. The aqueous layer was extracted with ethyl acetate (150 ml) and subsequently purified by chromatography to provide 3.9 mg/150 ml of [1-^{13}C]acetate-derived JA, and 2.8 mg/150 ml of [2-^{13}C]acetate-derived JA, respectively.

The $^{13}C\{^{1}H\}$-NMR spectrum of [1-^{13}C]acetate-derived JA (**7**) showed enhanced signals at C-1, -3, -5, -7, -9, and -11. Meanwhile, the $^{13}C\{^{1}H\}$ NMR spectrum of JA (**7**) derived from [2-^{13}C]acetate had intensified signals at C-2, -4, -6, -8, -10, and -12 (Table 1 and Fig. 2). Biosynthetically ^{13}C-labeled JA (**7**) was converted into the methyl ester with ethereal diazomethane, and then its specific incorporation ratio was calculated by the relative intensity of the methyl ester signal normalized to 1.11% (natural abundance). The specific incorporation of ^{13}C generally observed was 1.3–5.7 atom% for [1-^{13}C]acetate-derived carbons and 4.5–7.8 atom% for [2-^{13}C]acetate-derived carbons. These data provide clear evidence that JA (**7**) is produced through a fatty acid biosynthetic pathway in *L. theobromae*.

In plants, α-linolenic acid (**1**) is transformed into JA (**7**) through the octadecanoid pathway. We have been interested in whether OPDA (**4**)—a key intermediate of the octadecanoid pathway—is present in the culture of *L. theobromae*; however, the presence of OPDA (**4**) in this fungus has not been reported. In an attempt to test this hypothesis, the acetone extract of mycelia of *L. theobromae* was partially purified by preparative TLC, and subsequently subjected to GC–MS analysis. In conclusion, OPDA (**4**) was not detected in free form; however, the OPDA methyl ester was observed in the selected ion mode monitored at *m*/*z* 306 ($[M]^+$), 275 ($[M-OCH_3]^+$), and 238 (Fig. 3) (Laudert et al., 1996). The retention time associated with all of the selected ion peaks in this analysis was 87.2 min, which was the same as that of the molecular ion peaks of an authentic (+)-*trans*-OPDA methyl ester prepared (i.e., CH_2N_2, Et_2O) from natural OPDA (**4**). These data strongly suggested that *L. theobromae* produces natural occurring OPDA (**4**). JA (**7**) production by way of a fatty acid synthetic pathway, and the presence of OPDA (**4**) in *L. theobromae*, suggests that this fungus produces JA (**7**) *via* OPDA (**4**). As previously stated, OPDA (**4**) is an intermediate

Table 1
Incorporation of ^{13}C-labeled sodium acetate ([1-^{13}C] and [2-^{13}C]) into jasmonic acid (7).

Position	δ_c (ppm)	^{13}C-atom%	
		[1-^{13}C]acetate	[2-^{13}C]acetate
1	172.4	2.41	1.80
2	38.8	0.69	5.49
3	38.0	2.63	1.73
4	25.5	0.86	4.62
5	37.7	3.00	1.19
6	219.0	0.65	7.81
7	54.0	1.30	1.17
8	27.2	1.06	5.89
9	124.9	2.16	0.73
10	134.1	0.63	4.45
11	20.6	5.68	1.79
12	14.1	1.27	5.70
OMe	51.6	1.11	1.11

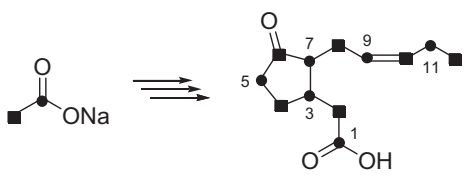

Fig. 2. Labeling patterns of jasmonic acid (**7**) following incorporation of ^{13}C-labeled sodium acetate into *L. theobromae*. Filled square and circle symbols represent [1-^{13}C] and [2-^{13}C] in ^{13}C-labeled sodium acetate, respectively.