



Mode of Action of Jasmonates

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Wasternack, C.; Hause, B.; BFP1: One of 700 Arabidopsis F-box proteins mediates degradation of JA oxidases to promote plant immunity *Mol. Plant* **17**, 375-376, (2024) DOI: [10.1016/j.molp.2024.02.008](https://doi.org/10.1016/j.molp.2024.02.008)

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Mik, V.; Poslíšil, T.; Brunoni, F.; Grúz, J.; Nožková, V.; Wasternack, C.; Miersch, O.; Strnad, M.; Floková, K.; Novák, O.; Šíroká, J.; Synthetic and analytical routes to the L-amino acid conjugates of cis-OPDA and their identification and quantification in plants *ChemRxiv* (2023) DOI: [10.26434/chemrxiv-2023-qlzj4](https://doi.org/10.26434/chemrxiv-2023-qlzj4)

Abstract
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Cis-(+)-12-oxophytodienoic acid (cis-(+)-OPDA) is a bioactive jasmonate, a precursor of jasmonic acid, which also displays signaling activity on its own. Modulation of cis-(+)-OPDA actions may be carried out via biotransformation leading to metabolites of various functions, similar to other phytohormones. This work introduces a methodology for the synthesis of racemic cis-OPDA conjugates with amino acids (OPDA-aa) and their deuterium-labeled analogs, which enables the identification and accurate quantification of these compounds in plants. We have developed a highly sensitive liquid chromatography-tandem mass spectrometry-based method for the reliable determination of seven OPDA-aa (OPDA-Alanine, OPDA-Aspartate, OPDA-Glutamate, OPDA-Glycine, OPDA-Isoleucine, OPDA-Phenylalanine, and OPDA-Valine) from minute amount of plant material. The extraction from 10 mg of fresh plant tissue by 10% aqueous methanol followed by single-step sample clean-up on hydrophilic-lipophilic balanced columns prior to final analysis was optimized. The method was validated in terms of accuracy and precision, and the method parameters such as process efficiency, recovery and matrix effects were evaluated. In mechanically wounded 30-day-

old *Arabidopsis thaliana* leaves, five endogenous (+)-OPDA-aa were identified and their endogenous levels reached a maximum of pmol/g. The time-course accumulation revealed a peak 60 min after the wounding, roughly corresponding to the accumulation of cis-(+)-OPDA. Current synthetic and analytical methodologies support studies on cis-(+)-OPDA conjugation with amino acids and research into the biological significance of these metabolites in plants.

Brunoni, F.; Šíroká, J.; Mik, V.; Pospíšil, T.; Kralová, M.; Ament, A.; Pernisová, M.; Karady, M.; Htitich, M.; Ueda, M.; Floková, K.; Wasternack, C.; Strnad, M.; Novák, O.;

Conjugation of cis-OPDA with amino acids is a conserved pathway affecting cis-OPDA homeostasis upon stress responses (2023) DOI:

[10.1101/2023.07.18.549545](https://doi.org/10.1101/2023.07.18.549545)

Abstract
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Jasmonates (JAs) are a family of oxylipin phytohormones regulating plant development and growth and mediating 'defense versus growth' responses. The upstream JA biosynthetic precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA) has been reported to act independently of the COI1-mediated JA signaling in several stress-induced and developmental processes. However, its means of perception and metabolism are only partially understood. Furthermore, cis-OPDA, but not JA, occurs in non-vascular plant species, such as bryophytes, exhibiting specific functions in defense and development. A few years ago, a low abundant isoleucine analog of the biologically active JA-Ile, OPDA-Ile, was detected in wounded leaves of flowering plants, opening up to the possibility that conjugation of cis-OPDA to amino acids might be a relevant mechanism for cis-OPDA regulation. Here, we extended the analysis of amino acid conjugates of cis-OPDA and identified naturally occurring OPDA-Val, OPDA-Phe, OPDA-Ala, OPDA-Glu, and OPDA-Asp in response to biotic and abiotic stress in *Arabidopsis*. The newly identified OPDA-amino acid conjugates show cis-OPDA-related plant responses in a JAR1-dependent manner. We also discovered that the synthesis and hydrolysis of cis-OPDA amino acid conjugates are regulated by members of the amidosynthetase GH3 and the amidohydrolase ILR1/ILL families. Finally, we found that the cis-OPDA conjugative pathway already functions in non-vascular plants and gymnosperms. Thus, one level of regulation by which plants modulate cis-OPDA homeostasis is the synthesis and hydrolysis of OPDA-amino acid conjugates, which temporarily store cis-OPDA in stress responses.

Mik, V.; Pospíšil, T.; Brunoni, F.; Grúz, J.; Nožková, V.; Wasternack, C.; Miersch, O.; Strnad, M.; Floková, K.; Novák, O.; Šíroká, J.; Synthetic and analytical routes to the L-amino acid conjugates of cis-OPDA and their identification and quantification in plants *Phytochemistry* **215**, 113855, (2023) DOI: [10.1016/j.phytochem.2023.113855](https://doi.org/10.1016/j.phytochem.2023.113855)

Abstract
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Cis-(+)-12-oxophytodienoic acid (cis-(+)-OPDA) is a bioactive jasmonate, a precursor of jasmonic acid, which also displays signaling activity on its own. Modulation of cis-(+)-OPDA actions may be carried out via biotransformation leading to metabolites of various functions. This work introduces a methodology for the synthesis of racemic cis-OPDA conjugates with amino acids (OPDA-aa) and their deuterium-labeled analogs, which enables the unambiguous identification and accurate quantification of these compounds in plants. We have developed a highly sensitive liquid chromatography-tandem mass spectrometry-based method for the reliable determination of seven OPDA-aa (OPDA-Alanine, OPDA-Aspartate, OPDA-Glutamate, OPDA-Glycine, OPDA-Isoleucine, OPDA-Phenylalanine, and OPDA-Valine) from minute amount of plant material. The extraction from 10 mg of fresh plant tissue by 10% aqueous methanol followed by single-step sample clean-up on hydrophilic-lipophilic balanced columns prior to final analysis was optimized. The method was validated in terms of accuracy and precision, and the method parameters such as process efficiency, recovery and matrix effects were evaluated. In mechanically wounded 30-day-old *Arabidopsis thaliana* leaves, five endogenous (+)-OPDA-aa were identified and their endogenous levels were estimated. The time-course accumulation revealed a peak 60 min after the wounding, roughly corresponding to the accumulation of cis-(+)-OPDA. Our synthetic and analytical methodologies will support studies on cis-(+)-OPDA conjugation with amino acids and research into the biological significance of these metabolites in plants.

Wasternack, C.; Deciphering the oxylipin signatures of necrotrophic infection in plants. A commentary on: Differential modulation of the lipoxygenase cascade during typical and latent *Pectobacterium atrosepticum* infections *Ann. Bot.* **129**, i-iii, (2022) DOI: [10.1093/aob/mcab142](https://doi.org/10.1093/aob/mcab142)

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Wasternack, C.; Sulfation switch in the shade *Nat. Plants* **6**, 186-187, (2020) DOI: [10.1038/s41477-020-0620-8](https://doi.org/10.1038/s41477-020-0620-8)

Abstract
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Plants adjust the balance between growth and defence using photoreceptors and jasmonates. Levels of active jasmonates are reduced in a phytochrome B-dependent manner by upregulation of a 12-hydroxyjasmonate sulfotransferase, leading to increase in shade avoidance and decrease in defence.

Wasternack, C.; Determination of sex by jasmonate *J. Integr. Plant Biol.* **62**, 162-164, (2020) DOI: [10.1111/jipb.12840](https://doi.org/10.1111/jipb.12840)

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Wasternack, C.; Hause, B.; The missing link in jasmonic acid biosynthesis *Nat. Plants* **5**, 776-777, (2019) DOI: [10.1038/s41477-019-0492-y](https://doi.org/10.1038/s41477-019-0492-y)

Abstract
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Jasmonic acid biosynthesis starts in chloroplasts and is finalized in peroxisomes. The required export of a crucial intermediate out of the chloroplast is now shown to be mediated by a protein from the outer envelope called JASSY.

Wasternack, C.; Termination in Jasmonate Signaling by MYC2 and MTBs *Trends Plant Sci.* **24**, 667-669, (2019) DOI: [10.1016/j.tplants.2019.06.001](https://doi.org/10.1016/j.tplants.2019.06.001)

Abstract
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Jasmonic acid (JA) signaling can be switched off by metabolism of JA. The master regulator MYC2, interacting with MED25, has been shown to be deactivated by the bHLH transcription factors MTB1, MTB2, and MTB3. An autoregulatory negative feedback loop has been proposed for this termination in JA signaling.

Wasternack, C.; New Light on Local and Systemic Wound Signaling *Trends Plant Sci.* **24**, 102-105, (2019) DOI: [10.1016/j.tplants.2018.11.009](https://doi.org/10.1016/j.tplants.2018.11.009)

Abstract
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Electric signaling and Ca²⁺ waves were discussed to occur in systemic wound responses. Two new overlapping scenarios were identified: (i) membrane depolarization in two special cell types followed by an increase in systemic cytoplasmic Ca²⁺ concentration ([Ca²⁺]_{cyt}), and (ii) glutamate sensed by GLUTAMATE RECEPTOR LIKE proteins and followed by Ca²⁺-based defense in distal leaves.

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