PERGAMON

Phytochemistry 51 (1999) 187–192

PHYTOCHEMISTRY

12-Oxo-phytodienoic acid and indole-3-acetic acid in jasmonic acid-treated tendrils of *Bryonia dioica*

Boguslawa A. Stelmach, Axel Müller, Elmar W. Weiler*

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität, D-44780 Bochum, Germany

Received 18 November 1998; received in revised form 16 December 1998; accepted 17 December 1998

Abstract

Treatment of tendrils of *Bryonia dioica* with jasmonic acid (JA) or with the *Pseudomonas* toxin coronatine results in a several-fold and parallel increase in the levels of endogenous 12-oxo-phytodienoic acid (OPDA) and indole-3-acetic acid (IAA), both effective inducers of tendril coiling in this species. The dose–response curve for JA-induced OPDA reflects the biological activity of JA in inducing tendril coiling, suggesting that JA acts via endogenous OPDA. A survey of a number of species most frequently used in JA research revealed that JA-induced increases of endogenous OPDA are not restricted to *B. dioica*, but do also occur in *Nicotiana tabacum, Lepidium sativum, Hordeum vulgare* and *Brassica oleracea*, but not in all species analyzed. The results reveal differences in the regulation of octadecanoid levels in different plant species and show that JA-treatment may have consequences on the auxin economy of a plant. In some species JA-treatment may affect the levels of OPDA, an octadecanoid that is itself biologically active.

Keywords: *Bryonia dioica*; Cucurbitaceae; Signalling molecules; Octadecanoids; 12-Oxo-phytodienoic acid; Jasmonic acid; Coronatine; Indole-3-acetic acid; Mechanotransduction

1. Introduction

The tendril coiling response of *Bryonia dioica,* normally initiated following contact stimulation of the organ can also be triggered — in the absence of a mechanical stimulus — by octadecanoids, octadecanoid analogs or indole-3-acetic acid (IAA) (Falkenstein, Groth, Mithöfer, & Weiler, 1991; Weiler et al., 1993). When applied exogenously, methyl jasmonate (MJ) or jasmonic acid (JA) effectively induce coiling (Falkenstein et al., 1991). However, it has become clear that the endogenous signal transducer is not JA, but rather the JA-precursor 12-oxo-phytodienoic acid (OPDA) (Weiler et al., 1994; Stelmach et al., 1998; Blechert, Bockelmann, Füßlein, & Weiler, 1999). After contact stimulation, the endogenous level of OPDA in tendrils rises drastically and transiently, while that of JA is very low and does not change (Stelmach et al., 1998; Blechert et al., 1999). The OPDA analog, coronatine, is an extremely active inducer of tendril coiling, while the JA analog, coronafacic acid, is nearly inactive (Weiler et al., 1994). The kinetics of OPDA- (or coronatine)-induced tendril coiling differ from that of the JA-induced reaction in that the response sets in almost immediately, while the JA-induced response starts after a lag-phase of several hours (Weiler et al., 1993). In detailed structure–activity analyses, it became clear that structural requirements for activity of OPDA differed from those of JA in the tendril system (Blechert et al., 1999). It was hypothesized (Blechert et al., 1999) that these findings could be explained assuming JA was not acting as a mechanotransducer at all but rather stimulated the accumulation of OPDA, the signalling compound in mechanotransduction. Indeed, Laudert and Weiler (1998) have recently shown that JA-treatment of *Arabidopsis thaliana* leaves induced the accumulation of the mRNA, protein and activity of allene oxide synthase, the enzyme catalyzing the

* Corresponding author. Tel.: +49-234-700-4291; fax: +49-234-709-4187.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00017-5

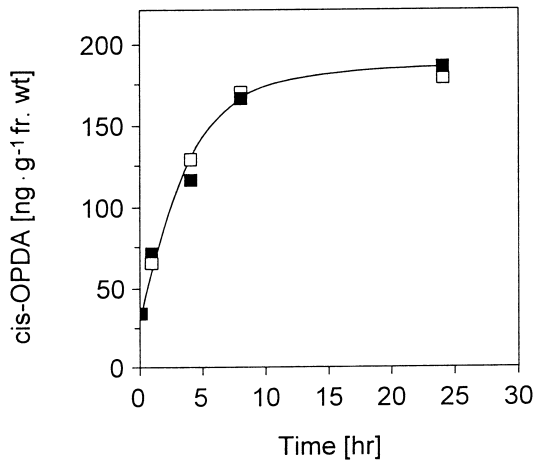

Fig. 1. Time-course of *cis*-OPDA-accumulation in detached tendrils of *Bryonia dioica* incubated, for the times indicated, in buffer without additions. The results of two independent experiments are plotted.

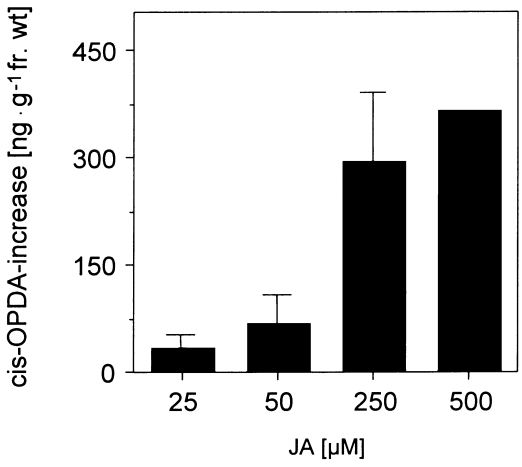

Fig. 2. Increase above controls in levels of endogenous *cis*-OPDA in detached *B. dioica* tendrils incubated in the presence of increasing concentrations of JA for 8 h. Shown are the results of $n \geq 3$ independent experiments (average ± S.D.) except for 500 μM JA (one experiment).

entrance reaction of the octadecanoid pathway. Parchmann, Gundlach, and Mueller (1998) have presented data for elicited cell cultures of several species showing fluctuations in the levels of OPDA and JA that cannot be reconciled with a simple precursor (OPDA) to product (JA) relationship.

It was thus necessary to analyze OPDA-levels in tendrils treated with exogenous JA. In the context of these experiments, IAA-levels in JA-treated tendrils and OPDA-levels in IAA-treated tendrils were also followed in order to gain insights into potential interactions between the two groups of growth regulators. Since JA (or its methyl ester) is usually applied in studies of octadecanoid biology, it was important to analyze OPDA-levels after JA-treatment in a range of species other than *B. dioica,* among them the standard objects of octadecanoid biology.

2. Results

2.1. Levels of OPDA in tendrils treated with JA

To analyze for OPDA, approximately 3 g of tendril tissue (40 tendrils) had to be used per data point. During the incubations, the organs got in contact in the incubation vessels. Additionally, the basal end of each detached tendril carried a wound site. Interorgan contact and wounding, both, were expected to affect OPDA-levels during the incubation period. This is shown in Fig. 1. Indeed, OPDA-levels increased steadily over 8–10 h of incubation and remained constant thereafter, associated with an initiation of the coiling reaction of the detached organs. Thus, for all subsequent experiments, untreated controls were included and incubated under exactly the same conditions as in all other treatments. Control OPDA-levels were subtracted pairwise and the differences from controls were evaluated.

When detached tendrils were incubated in solutions containing JA, endogenous OPDA-levels increased at all concentrations of JA tested. This increase was dependent on the dose of JA and at 250 μM JA, endogenous levels of OPDA were triple those of untreated controls incubated in parallel, reaching a level 13.5-fold higher than that of t_0-controls (Fig. 2). It is thus evident that exogenous JA clearly induces the accumulation of its precursor, OPDA, in *B. dioica* tendrils. OPDA-levels were increased above controls 1 h after the start of the experiment and continued to rise during the entire incubation period (24 h) (Fig. 3a).

Indole-3-acetic acid (IAA) is an effective inducer of tendril coiling in several species including *B. dioica* (Jaffe, 1975; Weiler et al., 1993). However, when applied at concentrations inducing a full coiling response (Weiler et al., 1993), IAA had no effect on the levels of endogenous OPDA (Fig. 3c). Vice versa, however, incubations in JA not only increased the level of OPDA, but also that of endogenous IAA (Fig. 3a), the kinetics (Fig. 3a) and the magnitude of the response (Fig. 4) being similar to that of OPDA.

Coronatine is an effective inducer of tendril coiling, but has no effect on the level of endogenous JA in tendrils (Weiler et al., 1994). However, the compound induces an accumulation of, both, OPDA and IAA (Fig. 3b and Fig. 4) with kinetics similar to that following JA-application. The concentrations used in the experiment shown in Fig. 3 for coronatine, JA and IAA were those required to give a full coiling response following comparable kinetics.

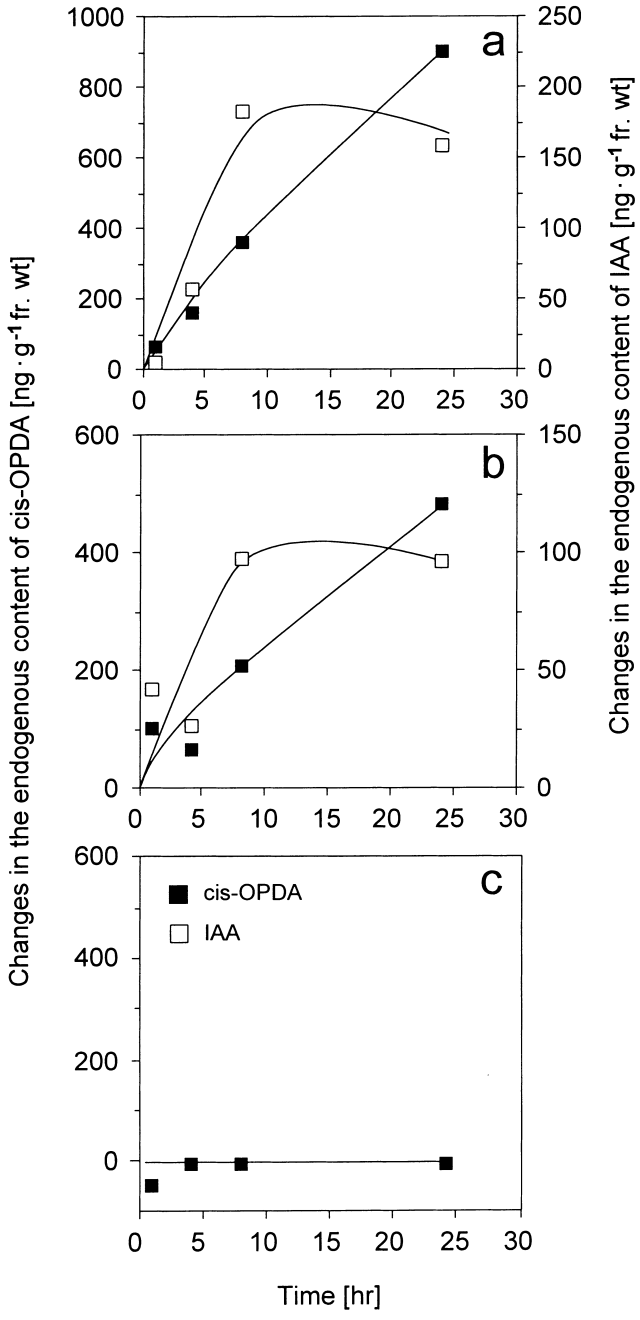

Fig. 3. Time-course of accumulation of *cis*-OPDA and IAA in detached tendrils of *B. dioica* incubated in (a) 500 μM JA, (b) 25 μM coronatine or (c) 100 μM IAA. Each value was corrected for the level of the control reaction incubated and processed in parallel and represents a separate experiment. The actual data points plotted are averages of between one and three separate experiments.

Since JA (or its methyl ester) is frequently used as an exogenous inducer of octadecanoid-dependent responses in diverse plant species, the results obtained with *B. dioica* prompted us to analyze OPDA-levels in the presence of exogenous JA in a range of species including those frequently used in jasmonate research.

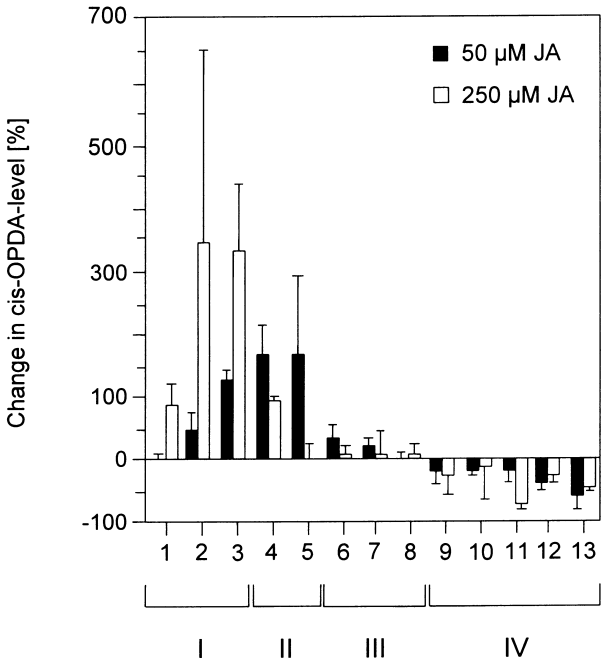

Fig. 5. Changes relative to controls in the levels of *cis*-OPDA in leaf tissue (*Bryonia dioica*, tendril tissue) of several species incubated in JA for 8 h (average ± S.D. of $n \geq 3$ separate experiments). 1, *Lepidium sativum*; 2, *Nicotiana tabacum*; 3, *B. dioica*; 4, *Hordeum vulgare*; 5, *Brassica oleracea*; 6, *Triticum aestivum*; 7, *Raphanus sativus*; 8, *Zea mays*; 9, *Arabidopsis thaliana*; 10, *Lycopersicon esculentum*; 11, *Beta vulgaris*; 12, *Phaseolus vulgaris*; 13, *Spinacia oleracea*.

Leaf tissue of young plants (see Section 4) was analyzed in all cases. The results (Fig. 5) show that plants can be divided into four groups depending on their responses. In addition to *B. dioica,* several other species reacted to JA-treatment with increasing OPDA-levels

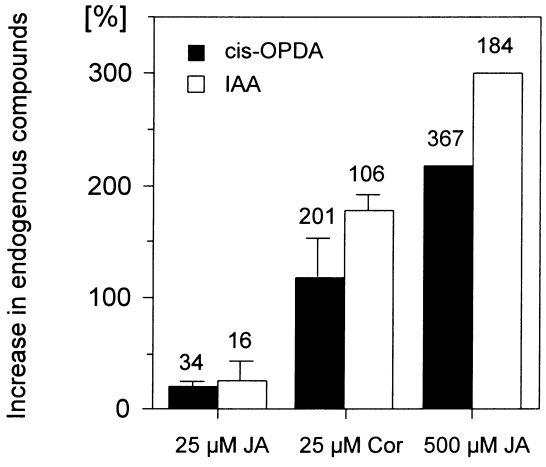

Fig. 4. Relative increase above controls (columns) and absolute differences (in ng g^{-1} fr. wt, numbers above the columns) of *cis*-OPDA and IAA in tendrils of *B. dioica* treated with JA or coronatine (Cor) for 8 h. Average ± S.D. of $n = 3$ experiments (500 μM JA, one experiment).

(groups I and II in Fig. 5). Group I plants showed a steady increase of OPDA-levels when JA-concentrations were increased, while group II plants showed less OPDA-accumulation at the higher compared to the lower dose of JA. Other species showed no (*Zea mays*) or only slight increases in OPDA-levels after JA-treatment, while group IV species including *A. thaliana* and *Lycopersicon esculentum* developed lower than control levels of OPDA when treated with JA.

3. Discussion

Results reported recently for the first enzyme specific for the JA biosynthetic pathway, AOS (Laudert, & Weiler, 1998), revealed a feed-forward regulation of the levels of AOS mRNA, polypeptide and enzymatic activity in *A. thaliana* by JA and OPDA as well as by the OPDA analogue, coronatine. JA also induces an accumulation of lipoxygenases, some of which are 13-lipoxygenases in the chloroplast (Feussner, Hause, Vörös, Parthier, & Wasternack, 1995; Vörös et al., 1998) and may be involved in JA-biosynthesis. Upregulation of the level of AOS mRNA in potato resulted in increases in JA-levels (Harms et al., 1995), however, no such increase was observed, when the experiment was repeated in *Nicotiana tabacum* or *A. thaliana* (D. Laudert and E. W. Weiler, unpublished observation). Thus, substrate availability may limit output of the octadecanoid biosynthetic pathway, even under conditions of elevated expression of octadecanoid biosynthetic genes.

The results reported here prove that exogenous application of JA (at levels required to elicit the biological response, which are higher than those required for methyl jasmonate (Weiler et al., 1993)), elevates the level of endogenous OPDA in tendrils quite substantially. This is in agreement with the hypothesis (Blechert et al., 1999) that the delayed kinetics of tendril coiling observed in JA-solutions as compared to OPDA- or coronatine-solutions reflect the time required to raise the level of endogenous OPDA. This result lends further support to the notion that not JA, but rather OPDA, is an endogenous signalling compound in mechanotransduction. The fact that several species other than *B. dioica,* but not all those tested, responded similarly to JA-treatment, necessitates to reevaluate those systems with respect to the actual role(s) of JA and/or OPDA.

In more general terms, our results suggest differences in pathway control by jasmonates in different species. In *A. thaliana*, it has been shown (Laudert, & Weiler, 1998) that JA-treatment increases AOS mRNA, polypeptide and enzymatic activity. The data in Fig. 5, however, demonstrate that these processes are not accompanied by an elevation in OPDA-levels. Thus, substrate (α-linolenic acid) limits OPDA-production in this case. In *B. dioica,* several possibilities exist to explain the increases in OPDA-levels in JA-treated tendrils. These require further attention and are not mutually exclusive:

1. JA induces a rate-limiting enzyme of the biosynthetic pathway (AOS would be a candidate for this), while substrate (α-linolenic acid and/or its 13(*S*)-hydroperoxide) is available in sufficient amounts.
2. JA induces release of substrate to the pathway's enzymes.
3. JA inhibits OPDA-release by plastids or its metabolism, while ongoing OPDA-synthesis elevates the OPDA-pool.

It is clear from the data of Fig. 3c that IAA, being an active inducer of tendril coiling (Jaffe, 1975; Weiler et al., 1993), does not act through inducing OPDA-accumulation. IAA has also previously been shown not to act through ethylene (Weiler et al., 1993) and thus, in all probability, is a directly active coiling inducer. Unexpected was our finding that JA-treatment of tendrils leads to increased (doubled to tripled) levels of endogenous IAA, suggesting that, in fact, octadecanoids produced as a result of mechanical stimulation may act via, or in concert with, IAA. An involvement of IAA may also accompany other JA-responses, such as the well-known inhibition of root elongation (Staswick, Su, & Howell, 1992). This aspect, too, needs further attention.

Finally, the results reported here shed light on the role of coronatine with respect to octadecanoid action. For the tendril system, it has been shown that coronatine is not a mimick of JA, but rather of OPDA (Weiler et al., 1994). Consequently, its activity in increasing OPDA-levels suggests that OPDA by itself may increase its own accumulation. In any case, exogenously applied coronatine may affect the levels of endogenous octadecanoids substantially. This has to be taken into account in physiological studies.

4. Experimental

4.1. Growth and treatments of plants

B. dioica Jacq. was raised as described (Weiler et al., 1993). All other species were raised in standard soil in a phytotron at 20°C and 70% relative humidity (light period 16 h, 150 μE m^{-2} s^{-1}). The following species and cultivars were used (ages in days after sowing at time of harvest in brackets following species names): *A. thaliana* (L.) Heynh. ecotype Columbia (35), *Beta vulgaris* ssp. *vulgaris* L. cv. Rote Kugel 2 (16), *Brassica*

oleracea ssp. *viridis* L. cv. Grüner Ring (16), *Hordeum vulgare* L. cv. Baronesse (10), *Lepidium sativum* L. cv. Krause (6), *L. esculentum* Mill. cv. First-in-the-Field (60), *N. tabacum* L. (60), *Phaseolus vulgaris* ssp. *vulgaris* L. cv. Neckargold (14), *Raphanus sativus* ssp. *sativus* L. cv. Eiszapfen (16), *Spinacia oleracea* L. (40), *Triticum aestivum* L. (10), *Zea mays* L. (10) (Bruno Nebelung GmbH and Co., Kiepenkerl-Pflanzenzüchtung, Everswinkel, Germany). *B. dioica* tendrils were harvested when 10–12 cm long and were not previously stimulated mechanically. *B. dioica* tendrils were cut with scissors, and the detached organs were immediately immersed in test solution [50 mM K–Pi, pH 6.5, 0.5% (w/v) glucose, 100 μM KCl, 0.02 vol% Tween 20] and incubated for 8 h in the phytotron during the light period. One set of samples was incubated with the test compounds, the control sets were treated identically except that only solvent was used. All stock solutions were prepared freshly in MeOH, and 50 μl were added to 100 ml of the test buffer (test solution as above) per glass beaker so that the MeOH concentration was kept constant at 0.05 vol%. Different stock solutions of JA were used, giving final concentrations of 25 μM, 50 μM, 250 μM and 500 μM. IAA was applied at a final concentration of 100 μM and coronatine at a final concentration of 25 μM. Controls were incubated in 0.05 vol% MeOH in the test buffer. All experiments for the species survey Fig. 5 were repeated at least three times; the others were replicated as indicated in the text.

4.2. Determination of cis-12-oxo-phytodienoic acid and indole-3-acetic acid

The incubated tissues (young leaves: 2–2.5 g fr. wt, tendrils: 40 organs, approximately 3–3.5 g fr. wt) were extracted in 50 ml MeOH at 4°C overnight. At the beginning of the extraction, an internal standard of [$^2H_5$]*cis*-OPDA (about 250 ng (Stelmach et al., 1998)) and, where appropriate, [$^{13}C_6$]-IAA (about 1.75 μg, Cambridge Isotope Laboratories, Andover, MA, isotopic enrichment 99%) was added. The extracts were filtered and taken to dryness, redissolved in 20 ml of peroxide-free Et_2O and passed through columns of 0.5 g aminopropyl material (Chromabond NH_2, Macherey-Nagel, Düren, Germany), as described in Mueller, Brodschelm, Spannagl, and Zenk (1993). Columns were washed with 10 ml $CHCl_3$:*iso*-PrOH (2:1, v/v), OPDA and IAA were eluted with 12 ml Et_2O:HOAc (49:1, v/v). Eluates were reduced to dryness under a stream of nitrogen, the residues were redissolved in 0.2 ml HPLC-solvent, and centrifuged for 2 min at $15.000 \times g$ to remove particles. Simultaneous analysis of OPDA and IAA was achieved by dividing the samples into two parts. Each then was taken up in 100 μl of HPLC-solvent (for OPDA analysis: *n*-hexane:*iso*-PrOH:HOAc (98:1.61:0.11, v/v); for IAA analysis: *n*-hexane:EtOAc (60:40, v/v) saturated with 0.5 M HCO_2H). For purification of OPDA an aliquot of 100 μl was subjected to HPLC, and isocratic chromatography (flow rate 1.5 ml min^{-1}, HPLC-solvent as above, UV detection at 221 nm) was performed on Nucleosil 100 (3 μm, 250×4 mm i.d.). The analysis of IAA was also done in an isocratic mode (flow rate 1 ml min^{-1}, HPLC-solvent as above, UV detection at 254 nm) on Zorbax Sil (5 μm, 250×4 mm i.d.). Fractions of *cis*-OPDA ($R_t = 9$ min) and IAA ($R_t = 9$ min) were collected, taken to dryness in a stream of nitrogen, redissolved in 0.1 ml MeOH and treated with etheral CH_2N_2. The dry fractions were finally redissolved in 50 μl $CHCl_3$ and analyzed by GC–MS.

4.3. Gas chromatography–mass spectrometry

Aliquots of 1 μl were subjected to GC–MS using a Finnigan-Magnum ion trap spectrometer in CI-mode with MeOH reactand gas. The GC-separation was achieved on a fused WCOT silica capillary (DB-35 MS, J&W Scientific, Folsom, USA; 30 m × 0.25 μm coat); Varian GC 3400; temperature programme: 1 min at 80°C, then at 30°C min^{-1} to 200°C, then at 5°C min^{-1} to 250°C, 10 min isothermal 250°C, R_t *cis*-OPDA = 914 s, R_t [$^2H_5$]*cis*-OPDA = 909 s; R_t IAA = 602 s, R_t [$^{13}C_6$]IAA = 602 s.

Quantitations were based on ion traces for $m/z = 307$ ($[M + H]^+$, OPDA) vs $m/z = 312$ ($[M + H]^+$, [$^2H_5$]OPDA) and $m/z = 190$ ($[M + H]^+$, IAA) vs. $m/z = 196$ ($[M + H]^+$, [$^{13}C_6$]IAA) and on the weight of the plant tissues.

Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft, Bonn and by Fonds der Chemischen Industrie, Frankfurt (literature provision).

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