64. Biosynthesis of *cis*-Jasmone: a Pathway for the Inactivation and the Disposal of the Plant Stress Hormone Jasmonic Acid to the Gas Phase?

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Dedicated to Prof. Dr. *E. Winterfeldt* on the occasion of his 65th birthday

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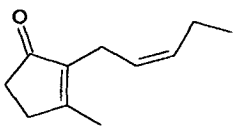
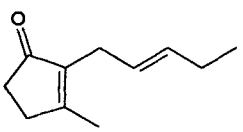
Flowers of *Jasminum rincospernum* convert deuterium-labeled jasmonic acid [$^2H_5$]-**4a** and methyl 1,2-didehydrojasmonate [$^2H_4$]-**8** into labeled *cis*-jasmone [$^2H_4$]-**1**. The labeling pattern of the resulting *cis*-jasmone (**1**) is consistent with a *Grob* fragmentation of the didehydrojasmonic acid **8a** by decarboxylation after protonation of the keto group. The pathway is also operative in leaves of several higher plants, including mono- and dicotylodonous specimens. In Lima beans besides *cis*-jasmone (**1**) an equimolar mixture of *trans*- and *cis*-isomers of methyl jasmonate (**4**) and *epi*-**4**, is emitted after treatment with jasmonic acid (**4a**). The relative ratio of **1** and **4**/*epi*-**4** is critically dependent on the concentration of the administered jasmonic acid (**4a**) and the ambient temperature of the plant. Unlike **4a**, the 1,2-didehydrojasmonic acid (**8a**) is not able to induce volatile biosynthesis. Therefore, the transformation of **4a** *via* **8a** into **1** appears to have a special importance for the irreversible inactivation and disposal of the plant stress hormone jasmonic acid to the gas phase and may serve, besides other modes of inactivation, as a shunt in case of high internal of the stress hormone **4a**. The conversion of the biologically active jasmonic acid (**4a**) into the inactive and volatile *cis*-jasmone (**1**) is the first example of a disposal of an inactive metabolite of a phytohormone to the gas phase as an infinite sink.

Introduction. – The exquisite, warm, and highly diffusing fragrance of jasmine scents has been praised in virtually all centuries [1]. Today, jasmine flower oil and its synthetic substitutes are key ingredients universally employed in the manufacture of high-grade perfumes. The 'jasmine chemistry' started about 90 years ago, when benzyl alcohol, benzyl acetate, linalool, indole, and methyl anthranilate were discovered together with a ketone of unknown structure called jasmone (**1**) [2]. Forty years later, *Ruzicka* and *Pfeiffer* [3] were the first to solve the structure of **1**, which represents *ca.* 2% in jasmine absolute. A number of trace or minor constituents such as *trans*-jasmone (**2**) and dihydrojasmone (**3**), structurally related to **1** but of lower odor quality, were found more recently in jasmine flower and bergamot oil [4]. Other important sources of **1** are the absolute of jonquil (*Narcissus jonquilla* L.) [5], peppermint oil (*Mentha piperita* L.) [6] and tea flavor (*Thea chinensis* Sims.) [7]. Interestingly, *cis*-jasmone (**1**) has been also found as a constituent of the pheromone cocktail of the danaid butterfly *Amauris ochlea* [8].

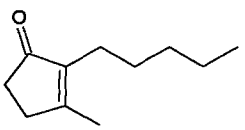
Besides *cis*-jasmone (**1**), also the ester **4**, present only in *ca.* 0.8% in jasmine absolute, contributes significantly to the 'jasmine-type' odor. Of the four possible stereoisomers, the *cis*-ester (+)-*epi*-**4** has the strongest odor activity [9]. The odorless, bicyclic lactone **6** and the lactone **7** appear to be biosynthetically related to **4** by oxidative or reductive processes followed by internal esterification. Although **5** is well-known as 'jasmine lactone' (0.5–1.5% of jasmine absolute), the lactone is a rather widespread fragrance compound in nature.



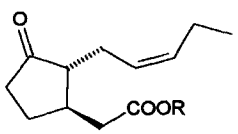
1, *cis*-jasmone



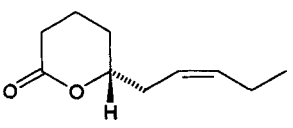
2, *trans*-jasmone



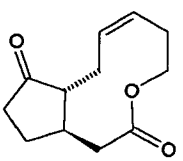
3, dihydrojasmone



4 R = Me, methyl jasmonate
4a R = H, jasmonic acid



5, jasmine lactone



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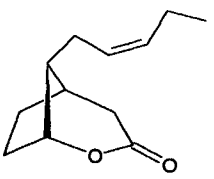

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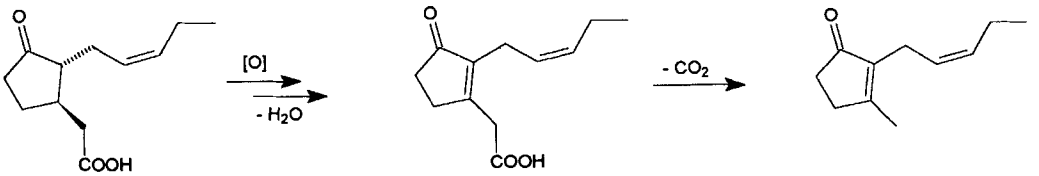
In recent years, methyl jasmonate (**4**) and, more important, jasmonic acid (**4a**), and, in particular, the *cis*-isomer *epi*-**4a** have been recognized as plant hormones of outstanding importance which, among many other effects, induce the production of secondary metabolites in response to wounding (for reviews, see [10–12]) and osmotic stress [13]. Other, well-established defenses which are under the control of the jasmonate family are the production of high-molecular-weight proteinase inhibitors [14], the biosynthesis of proteinaceous antifungals (*e.g.*, the pathotoxic thionines [15]) and some hydroxyproline-rich proteins for cell-wall strengthening [16], the induction of the key enzyme of the phenylpropanoid pathway, the phenylalanine ammonia lyase (PAL) [17], as well as the stimulation of the biosynthesis of low-molecular phytoalexins like certain alkaloids [18], terpenoids, and, in addition, the emission of ethylene [19] and many others volatiles [20]. Even the mechanoreceptors of *Bryonia dioica* rely on jasmonic acid for signal transduction [21].

According to the high impact of jasmonic acid (**4a**) on the plants secondary metabolism, correspondingly effective mechanisms of their inactivation must exist. Pathways leading to the formation of conjugates with, for example, glucose and amino acids are likely candidates and have been discussed previously [22]. However, the conventional pathways have to be considered with special care or even disregarded, since the conjugates of **4a** are not necessarily inactive compounds. For example, a glycoside of **6** (conjugation with the C(5′)–O-atom of the side chain; *cf.* **6**) is the tuber-inducing factor of potato plants [23], and certain amino-acid conjugates of **4a** with, especially, leucine and isoleucine appear to be even more active signal compounds than the free acid **4a** [24] [25]. It was this background which prompted us to investigate, whether or not the pathway leading to *cis*-jasmone (**1**) (*Scheme 1*) may be considered as alternate pathway for an irreversible inactivation and disposal of jasmonic acid (**4a**) to the gas phase as an infinite sink.

Although there is no rigorous experimental proof for the biosynthesis of *cis*-jasmone (**1**) from jasmonic acid (**4a**) in the literature, it is generally believed that **1** is formed for

Scheme 1



4a **8a** **1**

4a by an oxidative degradation pathway. The sequence is outlined in *Scheme 1* and follows an earlier proposal of *Demole* [26].

Accordingly, **4a** should be first converted into 1,2-didehydrojasmonic acid (**8a**). Subsequent protonation of the carbonyl O-atom of **8a** is assumed to induce a *Grob*-type fragmentation of the molecule yielding CO_2 and *cis*-jasmone (**1**) which then leaves the plant as a volatile.

The essence of the current work is twofold: it will be shown by using D labeled jasmonic acid [$^2H_5$]-**4a** and methyl didehydrojasmonate [$^2H_2$]-**8** that *i*) the biosynthesis of *cis*-jasmone (**1**) proceeds, indeed, along the line of *Scheme 1* and that *ii*) already an early intermediate of the sequence, namely the 1,2-didehydrojasmonic acid (**8a**), is a biologically inactive compound that no longer induces plant defense reactions like, for example, volatile production. The results qualify this degradative pathway as a novel and effective route for the inactivation of jasmonic acid (**4a**). This is the first report of a disposal of a metabolite of a plant hormone to the gas phase.

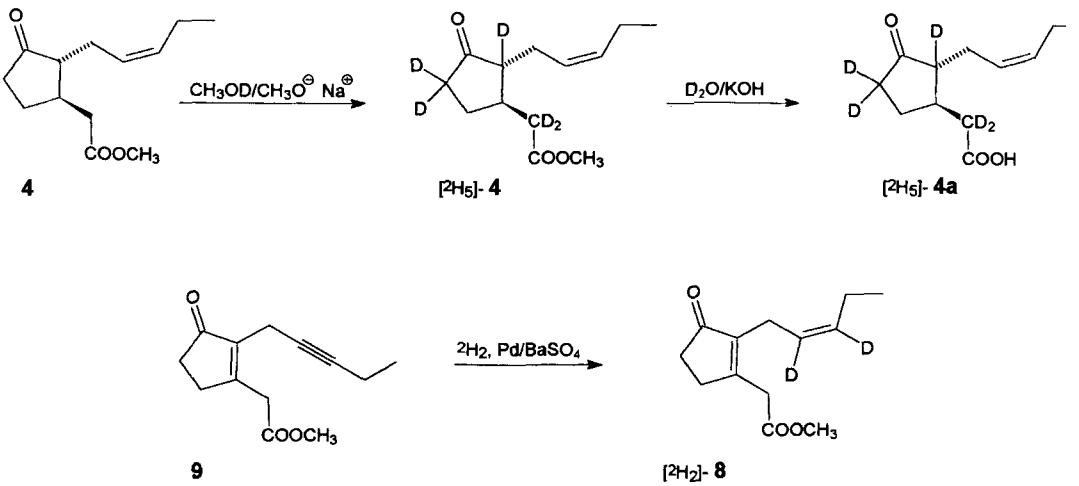
Results. – *Synthesis of D-Labeled Precursors.* To establish the biosynthetic sequence outlined in *Scheme 1*, isotopically labeled jasmonic acid [$^2H_5$]-**4a** and methyl didehydrojasmonate [$^2H_2$]-**8** were required to follow the metabolic flux of the compounds by mass spectrometry. D-Labeled methyl jasmonate [$^2H_5$]-**4** was readily obtained by exchange of the acidic α-protons next to the keto group and next to the ester function by stirring unlabeled **4** in MeO^{2}H/MeONa at room temperature. Saponification of the labeled ester in ^2H_2O/KOH gave the free acid [$^2H_5$]-**4a**. D-Labeled methyl 1,2-didehydrojasmonate [$^2H_2$]-**8** was prepared from the acetylenic precursor **9** by semihydrogenation using $^2H_2$ gas and *Lindlar*'s catalysts as outlined in *Scheme 2*.

The acetylene **9** was available in three steps from cyclopentane-1,3-dione and 1-bromopent-2-yne following the protocol of *Oberhänsli* [27].

Treatment of [$^2H_2$]-**8** in phosphate buffer (pH 7.0) with pig liver esterase yielded a solution of the corresponding unstable free acid [$^2H_2$]-**8a** which, after acidification to pH 3.0 (oxalic acid), spontaneously decomposed to give *cis*-jasmone [$^2H_4$]-**1** and CO_2. Owing to the limited stability of the free acid, only the methyl ester [$^2H_2$]-**8** was used for incubation experiments.

Administration Experiments. Jasmonic acid [$^2H_5$]-**4a** and methyl 1,2-didehydrojasmonate [$^2H_2$]-**8** were dispersed in tap water by sonication (1.0 to 5.0 μmol/ml^{-1}). All compounds gave stable emulsions which could be used for the time of administration experiments (max. 3 days) without noticeable decomposition. Freshly detached small plantlets or single leaves were placed into the emulsion, and the whole set-up was

Scheme 2



enclosed in a small desiccator. After 24 h, the emitted volatiles were collected by absorption onto charcoal traps as described in [28], or, more conveniently, by absorption to SPME fibres (solid-phase micro-extraction, commercialised by *Supelco Inc.* [29]) for 10 min. The adsorbed volatiles were immediately analyzed by GLC/MS. Most experiments were conducted with the Lima bean *Phaseolus lunatus, cv. Sieva* as the standard system.

Fig. 1, a, shows the profile of the volatiles observed in a typical administration experiment with [$^2H_5$]-**4a** (at 5.0 μmol/ml). Besides of the major component, identified as labeled *cis*-jasmone [$^2H_4$]-**1**, the leaf of the Lima bean emitted a number of other compounds which were produced in response to the phytohormone jasmonic acid [20] [30]. Virtually none of the compounds was released from a freshly detached leaf of the Lima bean kept in pure tap water under the same conditions. If the ester [$^2H_2$]-**8** was administered to the Lima bean, no induction of volatile biosynthesis occurred, but D-labeled *cis*-jasmone [$^2H_2$]-**1** was still produced in high amounts (*Fig. 1, b*). Control experiments monitoring the gas phase above aqueous solutions (at pH 7.0) containing the ester [$^2H_2$]-**8** and pig liver esterase indicated that, at least at this pH, no spontaneous decarboxylation of the free acid, liberated from **4** by phytogenic esterases, could have led to the formation of *cis*-jasmone **1** in the previous experiment. Only, if the aqueous solution was adjusted to pH 3.5 prior to the collection of volatiles, significant amounts of **1** were found in the gas phase.

The mass spectra of natural *cis*-jasmone (**1** *m/z* 164, *Fig. 2, a*) and those of the labeled metabolites [$^2H_2$]-**1** and [$^2H_4$]-**1**, derived from [$^2H_2$]-**8** and [$^2H_5$]-**4a**, are depicted in *Fig. 2, b* and *c*, respectively. According to the molecular ion at *m/z* 166 in the mass spectrum of [$^2H_2$]-**1** (*Fig. 2, b*) resulting from the administration of [$^2H_2$]-**8** no loss of D-atoms had occurred during the transformation of [$^2H_2$]-**8** into *cis*-jasmone [$^2H_2$]-**1**.

The mass spectrum of D-labeled the *cis*-jasmone **1**, derived from administration of the D-labeled jasmonic acid [$^2H_5$]-**4** (*Fig. 2, c*) exhibited a cluster of isotopomers with molecular ions at *m/z* 168, corresponding to [$^2H_4$]-**1** (48%), *m/z* 167, (41%), and *m/z* 166

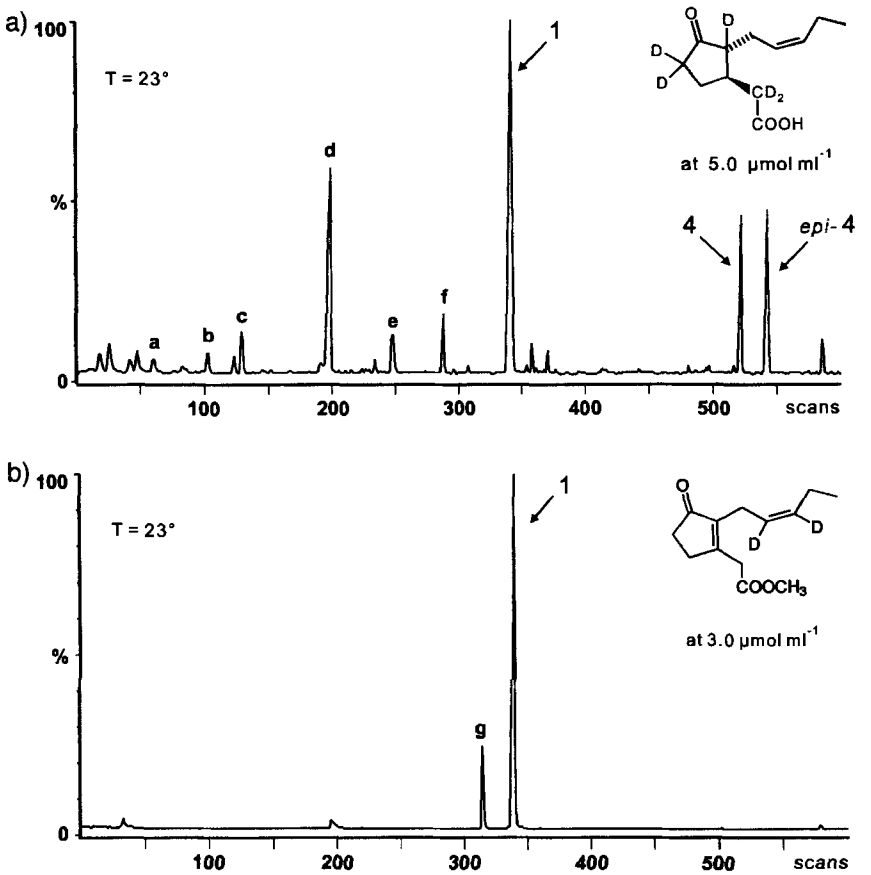


Fig. 1. *Profile of the volatiles from the Lima bean after administration of [$^2H_5$]-4a and [$^2H_2$]-8.* Profile of the volatiles resulting from administration of labeled jasmonic acid [$^2H_5$]-**4a** (*a*) and labeled methyl didehydrojasmonate [$^2H_2$]-**8** (*b*) to freshly cut plantlets of the Lima bean (*Phaseolus lunatus*). Volatiles were collected 24 h after administration of the rest substances to the leaves. The separation of compounds was achieved on a fused silica column coated with *DB1* (15 m × 0.25 mm) under programmed conditions (50° for 2 min, then to 200° at 10° per min followed by 30° per min to 280°). Injected amount: 1.0 µl. Detection and identification of compounds: quadrupole mass spectrometer, *Fisons MD 800*. Scan range: 35–350 Da s^{-1}, interface at 270°. Identified compounds: (*a*) β-ocimene, (*b*) linalool, (*c*) $C_{10}H_{14}$, (*d*) $C_{10}H_{16}O$, (*e*) indole, (*f*) methyl anthranilate, isotopomers of labeled *cis*-jasmone (**1**), isotopomers of labeled methyl jasmonate **4**, isotopomers of labeled methyl jasmonate *epi*-**4**, (*g*) 1-bromodecane as internal standard.

(11%). The pattern of the isotopomers was, however, largely in agreement with the pattern of the isotopomers of the administered precursor [$^2H_5$]-**4a**. Owing to the highest mass at *m/z* 168, this corresponded to a loss of only a single D-atom. In combination with the date from *Fig. 2, a* and *b*, this H-atom had been obviously removed from C(2′) of the cyclopentanone. The mass spectra shown in *Fig. 2, a* and *b*, exhibited a distinct loss of a Me group (*m/z* 164 → *m/z* 149 and *m/z* 166 → 151) as the first fragment. In agreement with the labeling pattern of the precursor [$^2H_5$]-**4a**, the corresponding fragment of the metabolite [$^2H_4$]-**1** (*Fig. 2, c*) carried two D-atoms (C^2H_2H, *m/z* 168 → *m/z* 151). Thus,

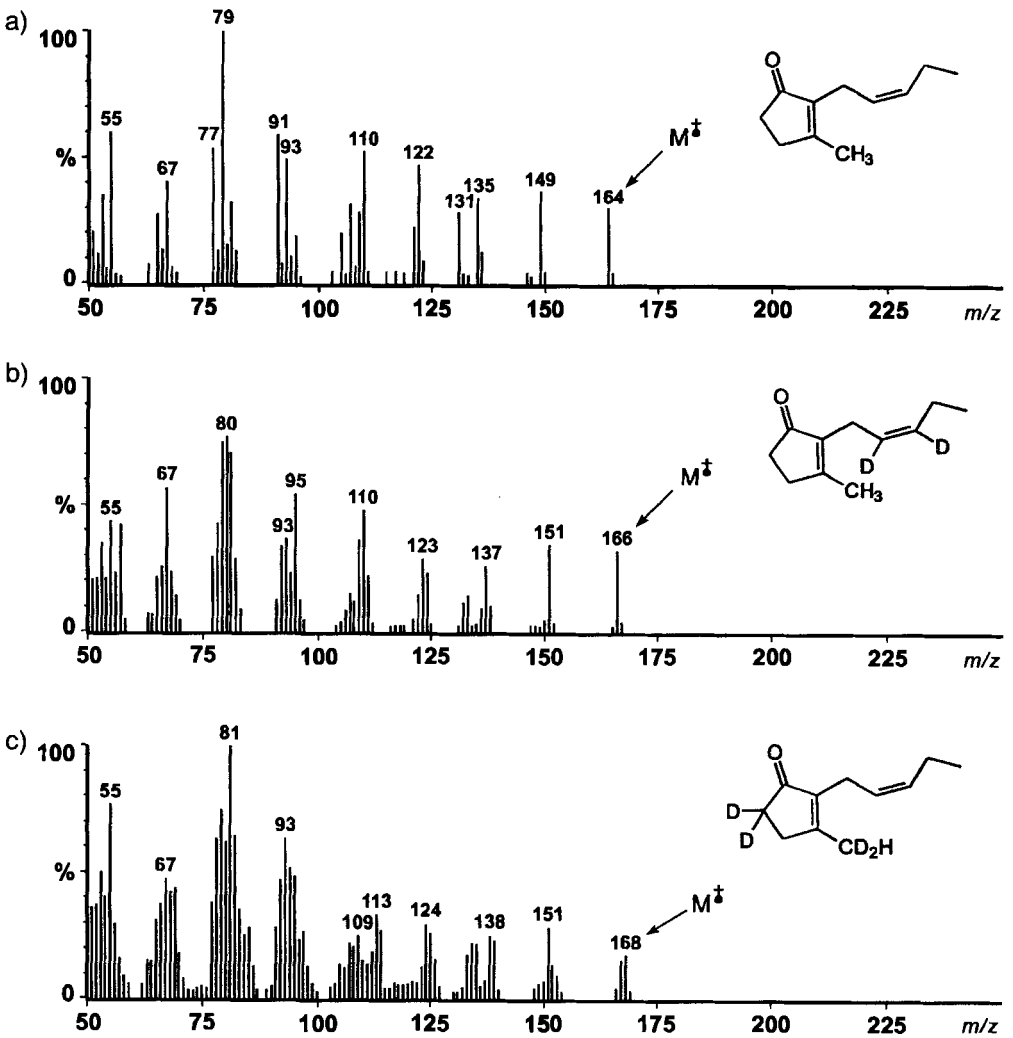


Fig. 2. *Mass spectra* (70 eV) of *natural and labeled* cis-*jasmone*. The mass spectra of natural (*a*) and labeled *cis*-jasmone (**1**) were from the headspace of leaves of precursor-treated Lima beans. *b*) [$^2H_2$]-**1** from administration of [$^2H_2$]-**8**. *c*) [$^2H_4$]-**1** from administration of [$^2H_5$]-**4a**. Separation and mass spectrometry: *cf. Fig. 1*.

two of the four remaining D-atoms were located in the exocyclic Me group, two others on the ring; most likely at C(4) as indicated in *Fig. 2, c*. While the number of D-atoms within the exocyclic Me group of the metabolite **1** proved to be constant throughout all administration experiments, the number of the remaining isotopes in the cyclopentanone moiety of **1** showed considerable variation (0–2 D-atoms).

Besides [$^2H_4$]-**1**, almost equimolar amounts of [$^2H_5$]-**4** and *epi*-[$^2H_5$]-**4** (*Fig. 1, a*) were emitted from the leaf. The ratio of the two stereoisomers was remarkably different from the equilibrium mixture (94% *trans*) of the administered D-labeled racemic *trans*-jasmonic acid [$^2H_5$]-**4a**. Moreover, mass spectrometry evidenced both isomers as D-labeled,

indicating that, indeed, only the externally added jasmonic acid served as the source for this transformation. Further characterization of the emitted methyl jasmonates **4** and *epi*-**4** on a chiral GLC column (6-methyl-2,3-di-*O*-pentyl-γ-cyclodextrin) identified the dominating *trans*-metabolite as (−)-(1*R*,2*R*)-**4** (55%). The ester *epi*-**4** could not be separated into the enantiomers [31]. Moreover, the relative ratio of the emitted *cis*-jasmone **1** and **4**/*epi*-**4** proved to be depended on the amount of the administered jasmonic acid. For example, treatment of the Lima bean with jasmonic acid at 1.0 to 5.0 μmol/ml^{-1} resulted in the emission of *cis*-jasmone (**1**) as the major product (31%), accompanied by only small amounts of **4**/*epi*-**4** (*ca.* 5–10% each isomer). Higher concentrations (> 5.0 μmol/ml^{-1}) of **4a** resulted in an enhanced production of equimolar quantities of **4**/*epi*-**4**, while at the same time the amount of *cis*-jasmone (**1**) was reduced.

Moreover, at least in the Lima bean, *cv. Sieva*, the regulation of the pathway leading to *cis*-jasmone (**1**) appeared to be critically dependend on the temperature. At 20°, only the induction of a limited number of volatiles was observed (*Fig. 3, a*). At 26°, the emission of volatiles was generally much more pronounced, and **1** became the major compound.

If the pathway leading to *cis*-jasmone (**1**) in the Lima bean were, indeed, a general pathway for the inactivation of the phytohormone jasmonic acid (**4a**), as suggested by *Fig. 1, b*, many plants should possess the capability to transform jasmonic acid (**4a**) into *cis*-jasmone (**1**) or methyl jasmonate (**4**). To verify this hypothesis, the ester [$^2H_2$]-**8** was administered to several other plants, and the emitted volatiles were analyzed for **1**. The results are compiled in the *Table*.

In most plants, the treatment with [$^2H_2$]-**8** resulted in the emission of *cis*-jasmone (**1**), but, unlike jasmonic acid (**4a**), this compound failed to induce volatile production. Interestingly, the archetype *Gingko biloba* proved to be an exception. Leaves of this plant were not able to convert [$^2H_2$]-**8** into **1**. Instead, a significant induction of volatile biosynthesis was observed with most compounds being identical to those emitted after jasmonic acid (**4a**) treatment.

Since [$^2H_5$]-**4a** and [$^2H_2$]-**8** were also converted by the inflorescence of *Jasminum rincospernum* to labeled *cis*-jasmone, it is reasonable to assume that the biosynthesis of this compound follows the same general pathway in the two different plant organs.

Discussion. – *cis*-Jasmone (**1**) is a well appreciated flower fragrance and valuable perfume ingredient known since the ancient days [1]. Flowers of *Jasminum spp.* evaporate a complex bouquet of more than hundred volatiles containing small amounts of *cis*-jasmone (**1**) together with methyl jasmonate (**4**), jasmolactone (**5**), and leaf alcohol. All of these compounds have to be considered as late metabolites of the same lipid peroxidation process which begins with the conversion of linoleic- and/or linolenic acid into their corresponding (13*S*)-hydroperoxides. While the pathway leading to jasmonic acid has been well studied by *Vick* and *Zimmermann* [32], no rigorous proof of the biosynthesis of *cis*-jasmone (**1**) from jasmonic acid (**4a**) has been, as yet, published. The current work principally confirms the biosynthetic sequence proposed by *Demole* [26], who was the first to postulate didehydrojasmonic acid (**8a**) as an intermediate *en route* to *cis*-jasmone (**1**). Accordingly, both, D-labeled jasmonic acid [$^2H_5$]-**4a**, as well as the methyl didehydrojasmonate [$^2H_2$]-**8** were converted by the flowers of *J. rincospernum* and leaves of the plants listed in the *Table* (except of *G. biloba*) into *cis*-jasmone (**1**). The retention of up

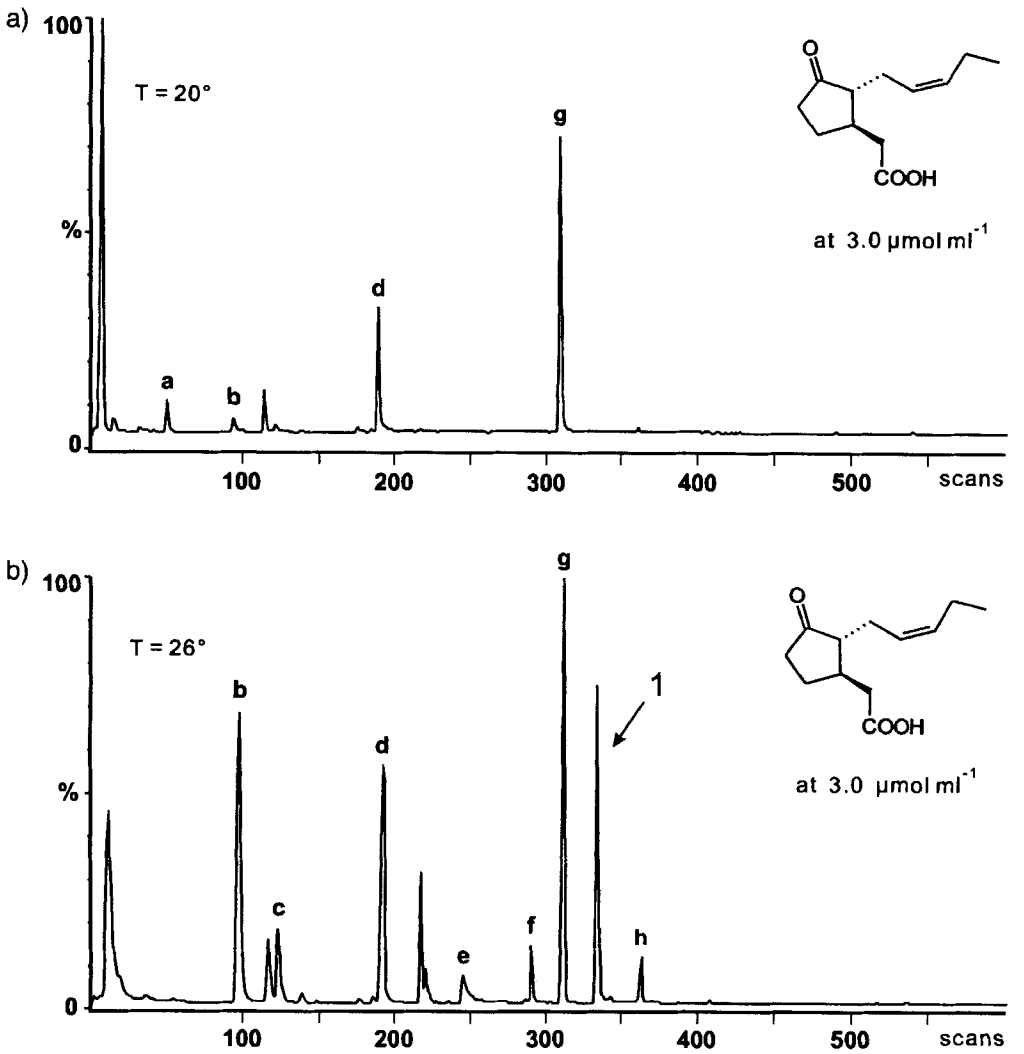


Fig. 3. *Influence of the temperature on the production of* cis-*jasmone* **1** *in jasmonic-acid-treated Lima beans.* Profile of the volatiles collected from the headspace of jasmonic-acid-treated Lima bean leaves at 20° (*a*) and 26° (*b*). Volatiles were collected 24 h after administration of jasmonic acid to the leaves. The separation of compounds: *cf. Fig. 1.* Identified compounds: (*a*) β-ocimene, (*b*) linalool, (*c*) $C_{10}H_{14}$, (*d*) $C_{10}H_{16}O$, (*e*) indole, (*f*) methyl anthranilate, *cis*-jasmone (**1**), (*g*) 1-bromodecane as internal standard, (*h*) caryophyllene.

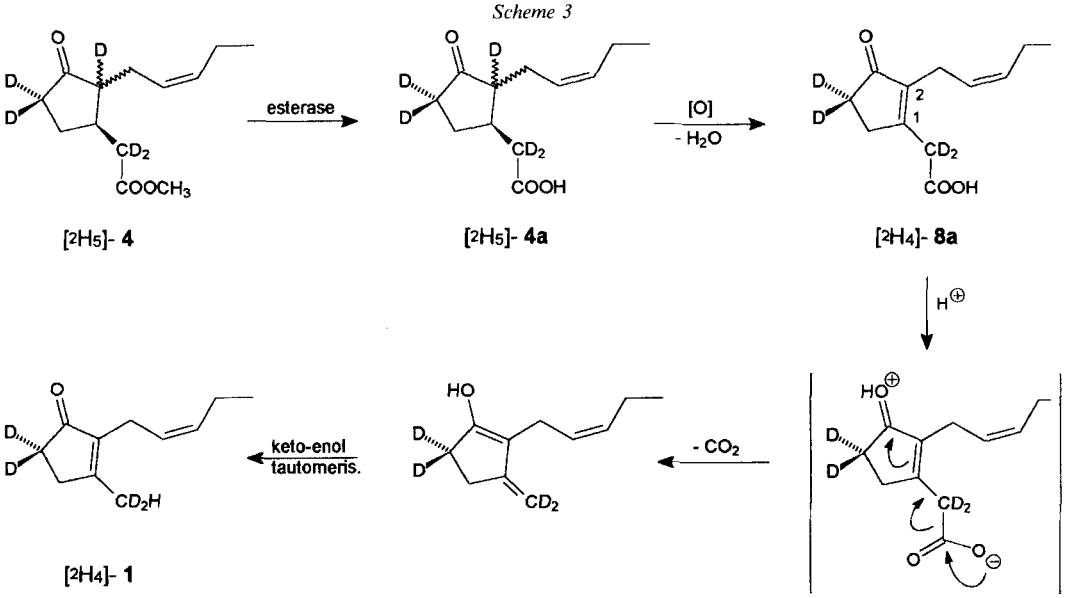
to four of the originally five D-atoms in the product **1** is in agreement with the protonation-decarboxylation mechanism outlined in *Scheme 3* and rules out further oxidative manipulations with discrete intermediates at the labeled C-atoms of **4a**. As already pointed out, two D-atoms were resistant against a further exchange with protons and could be attributed those of the exocyclic Me group of **1**.

Table. cis-*Jasmone* (**1**) *Biosynthesis in Selected Plants.* Plants were tested for the induction of volatile biosynthesis and the emission of *cis*-jasmone (**1**) after administration of labeled jasmonic acid [$^2H_5$]-**4a** and methyl didehydrojasmonate [$^2H_2$]-**8**. The isomerization of racemic **4a** (*ca.* 94% *trans*, corresponding to the thermodynamic equilibrium) into an equimolar mixture of the esters **4** and *epi*-**4** was only observed with the Lima bean (*P. lunatus*).

Plant species	Production of *cis*-jasmone (**1**)		Induction of volatile biosynthesis		Formation of methyl jasmonate **4**/*epi*-**4** [%]
	4a	**8**	**4a**	**8**	
Gingko biloba	–	–	+	+	–
Gossypium hirsutum	–	+	+	–	+[a])
Lycopersicon lycopersicum	–	+	+	–	–[a])
Nicotiana tabacum	–	+	+	–	+[a])
Phaseolus lunatus, cv. Sieva	+	+	+	–	(52/48)
Salix alba	+	+	+	–	+
Zea mays	–	+	+	–	+[a])
Jasminum rincospernum	+	+	n.d.[b])		+

[a]) At 10.0 μmol/ml^{-1}. [b]) n.d. = not determined.

Scheme 3

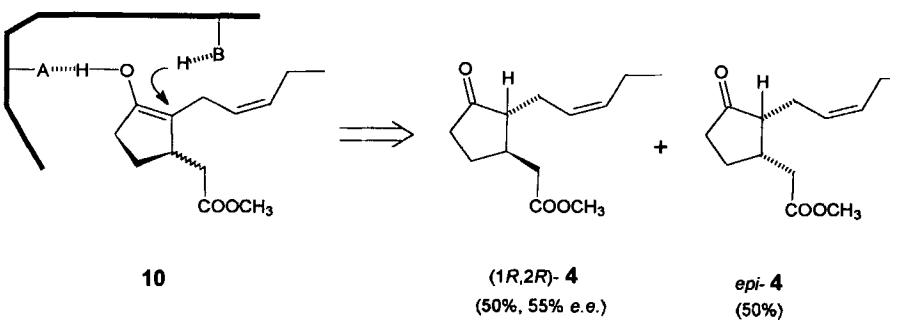


The mode of the introduction of the C=C bond into jasmonic acid (**4a**) is, as yet, not known. Besides of a direct dehydrogenation the C=C bond may also result from oxygenation at C(1′) followed by elimination of H_2O.

If the concentration of the administered jasmonic acid (**4a**) was increased to more than 5.0 μmol/ml^{-1}, the capacity of the pathway leading from **4a** to *cis*-jasmone (**1**) became apparently exhausted. Instead of being desaturated, the acid was methylated, and the emission of methyl jasmonate became more dominant. In the Lima bean, this process was accompanied by an isomerization of the administered equilibrium mixture

of [$^2H_5$]-**4a** (*trans*/*cis* 94:6) into a 1:1 mixture of (1*R*,2*R*)-**4** (55% *e.e.*) and *epi*-**4** (*cf.* also *Table*). The isomerization may be rationalized by transformation of the racemic *trans*-acid into a planar enolate. Reprotonation of both enantiomers of the enolate from the same direction could easily account for the production of the equimolar ratio of both isomers (*Scheme 4*). The sequence of the transformation, enolization prior to esterification of the administered acid or *vice versa* is, as yet, not known.

Scheme 4



More important than the confirmation of the previously proposed pathway [26] is the observation that leaves from plants which generally do not emit *cis*-jasmone (**1**), such as *Zea mays* or *Salix alba*, obviously possess all the required enzymes to convert jasmonic acid and/or didehydrojasmonic acid into *cis*-jasmone (**1**). Moreover, unlike jasmonic acid (**4a**), the didehydro derivative **8** is obviously not able to induce the volatile biosynthesis in the examined species. Only the archetype *Gingko biloba* responded with an emission of volatiles after treatment with **8**, but failed to convert **8** into **1** (*Table*). Distinct differences on the molecular level of the signal transduction and signal metabolism between the archetype and the modern plants may be the reason for this contrasting behaviour. The above differences in the biological activity of jasmonic acid (**4a**) and didehydrojasmonic acid (**8a**) appear to be general, since similar results were observed with barley leaves by measuring a highly sensitive response on jasmonates on the expression of a gene coding for a 23 kDa protein (*C. Wasternack et al.*, unpublished).

Taking into account an apparently broad occurrence of *cis*-jasmone biosynthesis in plants, and considering the lack of biological activity of the ester **8**, it is reasonable to assume that the pathway from jasmonic acid (**4a**) to *cis*-jasmone (**1**) may have some importance as a pathway for the inactivation of the plant stress hormone **4a**. Although already the unsaturated ester **8** is no longer able to induce volatile biosynthesis, it is the final transformation into the volatile *cis*-jasmone (**1**) which irreversibly removes the oxo-cyclopentanoid from the plant by emission to the gas phase as an infinite sink.

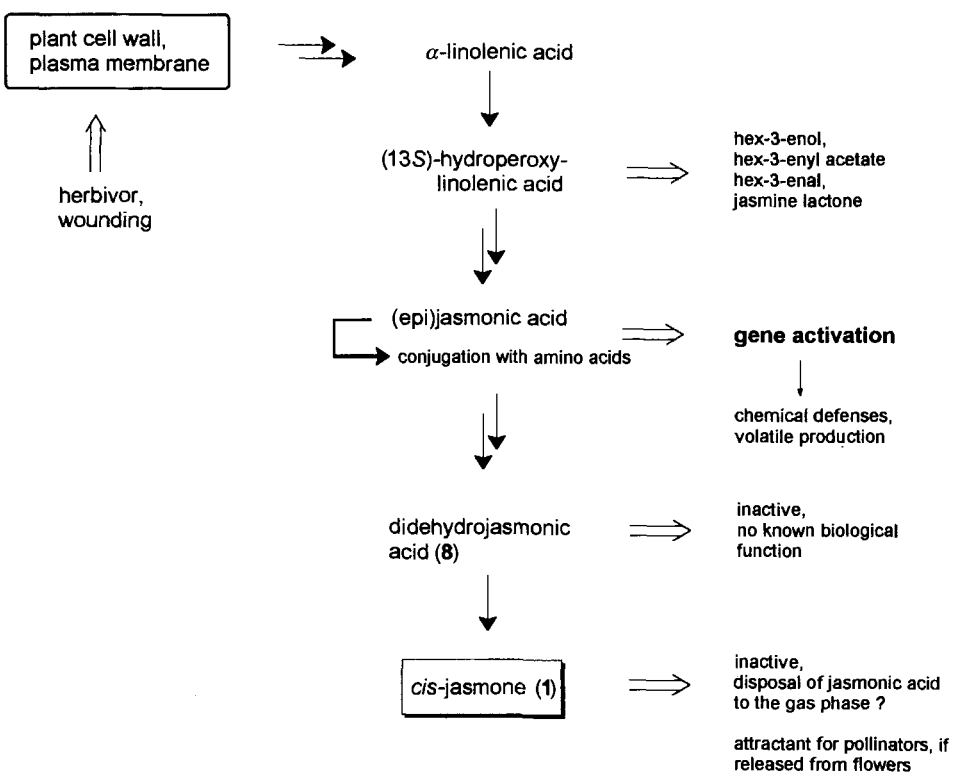
The putative function of *cis*-jasmone biosynthesis as a pathway for the disposal of jasmonic acid (**4a**) is independently supported by the emission of trace amounts of *cis*-jasmone (**1**) from several herbivore damaged plants. As outlined in *Scheme 5*, jasmonic acid plays a central role in the activation of the plants defense strategies. For example, *Loughrin et al.* [33] reported the emission of **1** from damaged leaves of cotton after beet armyworm-feeding (*Spodoptora exigua*). Intact plants did not emanate *cis*-jasmone, but wounded species released the maximum amount of **1** on day two after the onset of the

damage followed by a slow decline on day three. Such a time course coincides with the generally observed production of high endogenous levels of jasmonic acid as an early response to wounding (30–120 min) [34]. If the Lima bean (*cv. Sieva*, reared at 25°) was infested by spider mites, strongly enhanced endogenous levels of jasmonic acid (unpublished results) were followed by an emission of small amounts of **1** after 24 h. Also, if plantlets of the same cultivar, were placed into solutions containing certain cellulases of microbial origin (*J. Piel* and *W. Boland*, to be published), this pre-treatment induced a massive volatile biosynthesis including the emission of **1**. *H. Meyer* observed *cis*-jasmone (**1**) among the volatiles collected from female bark beetles drilling in pine cones [35]; however, in this case the origin of the volatiles remained to be established.

The emission of methyl jasmonate may have a dual function: *i*) the release of the volatile ester may serve as a shunt for the irreversible disposal of high endogenous levels of jasmonic acid from the plant, and *ii*) methyl jasmonate may act as a volatile signal which induced a prophylactic build-up of chemical defenses in neighboured, but undamaged plants [14] [36].

Finally it must be emphasised that in the Lima bean the ratio of the two volatile metabolites of jasmonic acid (**4a**), namely methyl jasmonate (**4**) and *cis*-jasmone (**1**), is critically depended on the ambient temperature (*Fig. 3*). Apparently, the regulation of the pathways is linked to a number of environmental factors allowing a greater plasticity

Scheme 5



of the plants defense responses. Further studies on these regulatory aspects as well as on the mechanistic details of *cis*-jasmone (**1**) production are urgently needed and are expected to enhance our understanding concerning the quantitative significance of individual inactivation pathways of compounds from the octadecanoid signalling cascade in plants.

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Experimental Part

General. Reactions were performed under Ar; solvents were dried according to standard methods. GLC: *Carlo Erba, Series 4100*, equipped with a fused silica capillaries coated with *SE30* (10 m × 0.31 mm) or 6-methyl-2,3-di-*O*-pentyl-γ-cyclodextrin (50 m × 0.31 mm) from *Macherey & Nagel* (Düren, Germany). Silica gel, *Si 60* (0.200–0.063 mm, *E. Merck*, Darmstadt, Germany) was used for chromatography. TLC: silica-gel plates *Polygram Sil* G_{F254}, from *Merck*. SPME (solid phase micro extraction; stationary phase: 100 μm polydimethylsiloxane) from *Supelco, Inc.* IR: *Perkin-Elmer Series 1600* FTIR spectrophotometer. ^{1}H- und ^{13}C-NMR: *Bruker AC 250* or *Bruker AC 400* spectrometer; $CDCl_3$ as solvent. Chemical shifts in ppm (δ) downfield relative to TMS. GC-MS (70 eV): *Finnigan ITD 800* coupled with a *Carlo Erba GC 6000*, model *Vega* or *Fisons MD 800* GLC-MS system. HR-MS: *Kratos MS 50*.

2-[3'-Oxo-2'-(pent-2''-enyl)[2',4',4'-$^2H_3$]cyclopentyl][2,2-$^2H_2$]acetic Acid ([$^2H_5$]-**4a**). A chilled and well-stirred soln. of methyl jasmonate (**4**, 1.00 g, 4,5 mmol) in dry CH_3OD (5.0 ml) was treated with Na metal (5.00 mg, 0.2 mmol) and stirred for 24 h at r.t. Then, the solvent was removed, and the procedure was repeated with fresh CH_3OD, to ensure a high incorporation of D-atoms. Et_2O was added, and the soln. was filtered through *Celite*. Following removal of solvents, the residue was treated with D_2O (30.0 ml; 1.68 mol) and KOH (1.00 g, 17.9 mmol). Stirring was continued for 5 h at r.t. and unreacted ester was extracted with Et_2O (2 × 10 ml). The soln. was adjusted to pH 4.5 by acidification with dil. H_2SO_4 (12 N), and the acid was extracted with Et_2O (3 × 10 ml). Drying and removal of solvent afforded 0.85 g (82%) of pure [$^2H_5$]-**4a**. Faint-yellow oil. IR (KBr, film): 3600–2400 (br.), 3009, 2965, 2934, 2876, 1738, 1709, 1462, 1409, 1296, 1209, 1088, 1070, 1045, 941, 864, 799, 719. ^{1}H-NMR ($CDCl_3$): 5.39 (*m*, 1H–C(3'')); 5.20 (*m*, 1H–C(2'')); 2.69 (*m*, 0.6H–C(2)); 2.31–2.23 (*m*, 4.4H–C(2,1',5'1'')); 1.99 (*quint.*, 2H–C(4'')); 1.45 (*m*, 1H–C(5')); 0.89 (*t*, 3H–C(5'')). ^{13}C-NMR ($CDCl_3$): 219 (C(3')); 178 (C(1)); 134 (C(3'')); 125 (C(2'')); 54 (C(2')); 39 (C(4')); 38 (C(2)); 27 (C(5')); 25 (C(1'')); 21 (C(4'')); 14 (C(5'')). MS (70 eV): 215 (9, [$^2H_5$]-$M^{+\cdot}$), 213 (13, [$^2H_5$]-$M^{+\cdot}$), 196 (2), 185 (3), 167 (2), 154 (39), 146 (19), 145 (18), 135 (8), 123 (5), 112 (10), 111 (11), 98 (23), 86 (100), 85 (54), 80 (12), 69 (19), 68 (18), 55 (14). According to the rel. abundance of the molecular ions at *m/z* 215, 214, and 213, the distribution of the isotopomers of the free acid **4a**: [$^2H_5$]-**4a** (21%), [$^2H_4$]-**4a** (49%), and [$^2H_3$]-**4a** (30%).

Methyl-2-[3'-Oxo-2'-([2'',3''-$^2H_2$]pent-2''-enyl)cyclopent-1'-enyl]acetate ([$^2H_2$]-**8**). *Lindlar*'s catalyst (7.9 mg, *Fluka AG*, Switzerland) was placed in a two-necked round-bottomed flask, connected to a gas burette. The catalyst was dried *in vacuo*, and D_2 gas was introduced. Then, MeOH (2 ml) was injected with stirring through a rubber septum, followed by quinoline (6.9 μl) to deactivate the catalyst. After injection of a soln. of **9** (0.05 g, 0.2 mmol) in MeOH (0.5 *m*) the hydrogenation started and ceased after the uptake of 1 equiv. of D_2. The progress of the reaction was monitored by GLC. The product was isolated by filtration through *Celite*. The solvent was removed *in vacuo*. Chromatography of the crude residue on silica gel using pentane/Et_2O 60:40 (*v/v*) afforded 0.039 g (88%) of **8**. Colourless liquid. IR (KBr, film): 2963, 2934, 2874, 1742, 1701, 1647, 1437, 1356, 1329, 1306, 1260, 1194, 1175, 1123, 1057, 1013, 889, 845, 818, 793, 721. ^{1}H NMR ($CDCl_3$): 3.65 (*s*, COOMe); 3.41 (*s*, 2H–C(2)); 2.89 (*s*, 2H–C(1'')); 2.55 (*m*, 2H–C(4')); 2.35 (*m*, 2H–C(5')); 2.07 (*q*, 2H–C(4'')); 0.91 (*t*, 3H–C(5'')). ^{13}C-NMR ($CDCl_3$): 208.6 (C(3')); 169.5 (C(1)); 164.0 (C(3'')); 141.8 (C(2'')); 52.3 (COOMe); 36.6 (C(2)); 34.3 (C(5')); 29.9 (C(4')); 21.1 (C(1'')); 20.4 (C(4'')); 14.0 (C(5'')). MS (70 eV): 224 (54, $M^{+\cdot}$), 206 (6), 195 (98), 193 (19), 168 (17), 167 (17), 165 (24), 151 (100), 146 (11), 139 (11), 138 (11), 137 (17), 135 (41), 122 (21), 121 (20), 109 (28), 107 (36), 93 (35), 79 (34), 67 (7), 59 (13), 57 (18). HR-MS: calc. for $C_{13}H_{16}{}^2H_2O_3$: 224.1412; found: 224.1374.

Administration of D-Labeled Precursors and Analysis of Volatiles; General Procedure. Labeled jasmonic acid [$^2H_5$]-**4a**, methyl jasmonate (**4**), or methyl didehydrojasmonate ([$^2H_5$]-**8**, at 3.0 μmol ml^{-1}, if not specified otherwise) were solicited in tap water (35 kHz) without additives for *ca.* 3 min until a milky suspension resulted. Freshly detached leaves, small plantlets, or small twigs with blossoms (*J. rincospernum*) were immediately placed into the

suspension (2 ml in small screw capped vial). The vial was then enclosed in a small desiccator (250 ml), and, after 24 h, the volatiles were collected on charcoal (1.5 mg, *CLSA Filter*, Winterthur, Switzerland) as described in [28]. Following desorption of the carbon traps (2×20 µl CH_2Cl_2) the volatiles were analyzed by GLC/MS for identification. Alternatively, an SPME-fibre (*Supelco Inc.*) [29], coated with 100-µm polydimethylsiloxane was introduced into the desiccator through a tightly fitting whole in a *Teflon* stopper, and the fibre was exposed to the atmosphere for *ca.* 15 min. Then, the fibre was inserted into the injection system of the gas chromatograph, and, after evaporation of the absorbed volatiles, the compounds were separated under temp.-programmed conditions and identified by their mass spectra (*Fisons MD 800* quadruple mass spectrometer). GLC Column: fused silica, coated with *DB 5* (15 m × 0.32 mm). Carrier gas: He. Temp. program: 50° for 2 min, then at 10° min^{-1} to 200°, followed by 30° min^{-1} to 280°. Transfer line: 260°. Ion source. 220°.

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