J Plant Growth Regul (1986) 5:91–100

Journal of
Plant
Growth
Regulation

Occurrence of (+)-7-iso-Jasmonic Acid in *Vicia faba* L. and Its Biological Activity

O. Miersch, A. Meyer, S. Vorkefeld, and G. Sembdner

Institute of Plant Biochemistry, Academy of Sciences of the GDR, 4020 Halle (Saale), Weinberg 3, German Democratic Republic

Received February 12, 1986; accepted April 17, 1986

Abstract. The plant growth regulator (−)-jasmonic acid (JA) and its stereoisomer (+)-7-iso-jasmonic acid (7-iso-JA) have been isolated from young fruits of *Vicia faba* L. and identified by TLC, GC, GC-MS, and chemical transformation. Different isolation procedures gave different ratios of JA/7-iso-JA because of partial isomerization of (+)-7-iso-JA to (−)-JA. Optimal conditions, excluding artificial isomerization, were checked by the addition of [U-^{14}C]-7-iso-JA. The naturally occurring isomer ratio was determined to be 65% (−)-JA: 35% (+)-7-iso-JA in immature broad bean fruits. The biological activities of both isomers of JA have been studied using several bioassays. Growth of wheat and GA_3-stimulated dwarf rice seedlings is inhibited more effectively by (+)-7-iso-JA than by (−)-JA. In growing seedlings of barley and oat, both isomers caused a senescence-like bleaching effect characterized by chlorophyll and carotenoid decrease. The free acids are more active than their methyl esters in intact barley plants in contrast to results obtained in leaf segment tests. Highest activity was obtained with (+)-7-iso-JA.

Jasmonic acid (JA) (Fig. 1) was first isolated from the fungus *Lasiodiplodia theobromae* Griff. & Maubl. as a plant growth inhibitor (Aldridge et al. 1971). During the last several years JA and its methyl ester (JA-Me) have been found to be new plant growth regulators widely distributed in higher plants (Yamane et al. 1981a, Dathe et al. 1981, Ueda and Kato 1982a, Meyer et al. 1984) possessing different biological activities when exogenously applied to plants (Ueda and Kato 1981, 1982b; Yamane et al. 1981a,b, 1982; Sembdner and Klose 1985). Studying the endogenous plant hormones of *Vicia faba* L., Dathe et al. (1981) reported the occurrence of (−)-JA in the pericarp of young fruits. Recent investigations of Vick and Zimmerman (1983, 1984) on the biosynthesis of JA showed the existence of a stereoisomer in *Vicia faba* L. and other higher

R=H Jasmonic acid R=H 7-iso-Jasmonic acid
$R=CH_3$ Methyl jasmonate $R=CH_3$ Methyl 7-iso-jasmonate

Fig. 1. Structures of naturally occurring jasmonic acid (JA) isomers.

plants described as 2-epi-jasmonic acid or 2-iso-jasmonic acid, which we now call 7-iso-jasmonic acid (7-iso-JA) (Fig. 1). Miersch et al. (1986) identified the stereoisomer 7-iso-JA, instead of JA, in the fungus *Botryodiplodia theobromae* Pat. (synonym: *Lasiodiplodia theobromae* Griff. & Maubl.). During pheromone studies with the oriental fruit moth *Grapholitha molesta* Busck. the presence of the methyl ester of 7-iso-JA (7-iso-JA-Me) in higher plants has been assumed (Nishida et al. 1982). More recently the compound has been isolated from lemon fruits (Nishida and Acree 1984). Natural (−)-JA and synthetic (±)-JA are known to occur as mixtures consisting of 90% JA and 10% 7-iso-JA (Gerlach and Künzler 1978, Quinkert et al. 1982). On treatment with acidic or alkaline solutions or during TLC and GC procedures the 7-iso-JA can be partly isomerized giving different ratios of isomers. Our studies were performed to answer questions concerning the natural occurrence of JA and/or 7-iso-JA in *Vicia faba* L. and to determine the biological activities of both isomers.

Materials and Methods

Plant Material

Plants of *Vicia faba* L. var. minor cv. 'Fribo' were cultivated in a greenhouse in 1983. Immature fruits were harvested when 2 to 3 cm and either freshly extracted, or extracted after lyophilization or freezing in liquid nitrogen.

Standard Isolation of JA/7-iso-JA-Mixture

One kg fresh fruits were homogenized in 1 l ethyl acetate at 4°C and filtered through cellulose powder. The organic phase was partitioned with saturated $NaHCO_3$ solution (3 × 20 ml). Subsequently, the bicarbonate phase was acidified with HCl to pH 3.5 and extracted with chloroform (3 × 20 ml). Desiccation with anhydrous Na_2SO_4 and evaporation of the chloroform extract gave a crude gum which was purified by column chromatography (50 × 2 cm) on silica gel (silanized with trimethylchlorosilane) and a discontinuous gradient of ethyl acetate in chloroform (200 ml chloroform:0 ml ethyl acetate; 190:10; 180:20). Fractions (25 ml) eluted with chloroform-ethyl acetate (180:20) gave 2.5 mg of a crude mixture containing 2 mg of JA/7-iso-JA.

Alternative Isolation Procedures

Ten g plant material (fr. wt.) was homogenized at 4°C either freshly or after freezing in liquid nitrogen or lyophilization, with 2 × 50 ml of different sol-

vents (methanol, ethyl acetate, diethyl ether, or chloroform). After filtration through cellulose powder the methanol extract was evaporated to dryness and was solved in 50 ml ethyl acetate. The other organic extracts were separated from their aqueous layers. The organic phases were partitioned with 30 ml saturated $NaHCO_3$ solution and the bicarbonate extracts acidified with HCl to pH 3.5. Subsequently, they were partitioned with 10 ml chloroform and dried with anhydrous Na_2SO_4. Half of each extract was applied to a TLC plate and chromatographed in chloroform-methanol-water (140:20:1 by vol.) with (±)-JA as a standard. Detection with anisaldehyde reagent (heating 5–10 min at 120°C) showed JA spots at Rf = 0.28 and 7-iso-JA at Rf = 0.22. The other part of the extract was separated by reverse phase HPLC. The fraction containing JA/7-iso-JA was collected, diluted with water, and extracted with chloroform. After evaporation of the chloroform, the sample was methylated with diazomethane for GC analysis.

Isomerization of JA/7-iso-JA Mixture

A mixture of 100 μg JA/7-iso-JA and 10 μl 1 N KOH were heated 1 h at 60°C. Then, 200 μl 1 N HCl were added and the solution extracted with diethyl ether. Treatment with diazomethane revealed the methyl esters for GC analysis.

Reduction of JA/7-iso-JA Mixture

A mixture of 200 μg JA/7-iso-JA was dissolved in 200 μl H_2O containing 2 mg $NaHCO_3$ and treated with 1 mg $NaBH_4$ for 30 min. The aqueous solution was adjusted with HCl to pH 3 and extracted with diethyl ether. Using TLC and the solvent system n-hexane-ethyl acetate-acetic acid (60:40:1 by vol.) four compounds (I to IV) (see Fig. 3) could be separated (Rf values: I = 0.42, II = 0.27, III = 0.38, IV = 0.32). For GC analysis the eluted substances were methylated with diazomethane.

Gas-Liquid Chromatography and Combined Gas Chromatography-Mass Spectrometry

GC analysis was carried out on a Chromatron GCHF 18.3 using the following conditions: stainless steel column (2 m × 4 mm), 3% OV 225 on Gas Chrom Q (100 to 120 mesh), carrier gas N_2, 85 ml/min, isothermal column temperature 190°C, hydrogen flame ionizing detector. Retention time of JA-Me is 3.4 min; 7-iso-JA-Me is 4.0 min.

For detection of substances I to IV, a flow rate of 110 ml/min and an isothermal column temperature of 150°C were used. Retention times of the methylester were I = 18.3 min, II = 21.1 min, III = 15.5 min, and IV = 15.7 min. Combined GC-MS was performed on a VARIAN MAT 111 with 80 eV-mass spectrometer and glass column (1.80 m × 2 mm), 10% EG SS-X on Gas Chrom P (100–120 μm), column temperature 170°C; Rt (JA-Me) = 10.7 min, and Rt (7-iso-JA-Me) = 12.9 min. Fragmentation patterns were compared with those reported (Nishida et al. 1982).

High-Performance Liquid Chromatography

For reverse phase HPLC, a Pye UNICAM PU 4020 was used, fitted with a steel column (250 mm × 4.6 mm), and packed with Polyol RP 18 on Si 100. The column was eluted with 55% methanol containing 0.1% phosphoric acid at a flow rate of 1 ml/min and monitored at 228 nm. Retention time of JA/7-iso-JA was 9.5 min.

Authentic Substances

The authentic substances consisted of: (±)-JA-Me (Firmenich, Swiss); (±)-JA from (±)-JA-Me by alkaline hydrolysis; (+)7-iso-JA from *Botryodiplodia theobromae* Pat. (Miersch et al. 1986); (−)-JA by chemical isomerization of (+)-7-iso-JA; substances I and II from (+)-7-iso-JA, III and IV from (±)-JA by reduction with $NaBH_4$; methyl esters were obtained generally by reaction with diazomethane; [U-^{14}C]-(+)-7-iso-JA (1.7×10^7 Bq/mmol) from [2-^{14}C]-sodium acetate (analogous to Miersch et al. 1986).

Bioassays

Inhibitor activities were determined by measuring growth inhibition of wheat seedlings, *Triticum aestivum* L. cv. 'Hatri' (Dathe et al. 1978) and gibberellin-stimulated (10^{-6} M GA_3) dwarf rice seedlings, *Oryza sativa* L. cv. 'Tangin-bozu' (Sembdner et al. 1976) after root application. For bioassays with oat seedlings (*Hordeum vulgare* L. cv. 'Trumpf') 30 plants were grown at 18°C with 14 h of light daily in plastic boxes containing 350 g sand and 40 ml H_2O containing 113 mg NH_4NO_3, 118 mg KCl, 51 mg $MgSO_4 \times 7\ H_2O$ and 11 mg $CaHPO_4 \times H_2O$. After unfolding of the first leaf, the plants were sprayed with inhibitors solubilized in acetone-H_2O (1:1). Nine days after application the length of the seedlings was measured. Total chlorophyll and carotenoid contents of the primary leaves of barley plants, 1 to 5 days after treatment, were determined according to Arnon (1949) and Wettstein (1957). The pigment contents were calculated in relation to the acetone control.

Results

Occurrence of 7-iso-JA and JA

Using extraction and purification procedures described in the literature resulted in the isolation of the two isomers JA and 7-iso-JA in a ratio of 90:10%. We developed a modified method resulting in a higher portion of 7-iso-JA and this standard procedure (*vide*, Materials and Methods), after silica gel column chromatography, yielded a crude mixture of JA/7-iso-JA. Separation by TLC and visual estimation of the two spots after detection with anisaldehyde reagent showed a ratio of about 70% JA and 30% 7-iso-JA. GC analysis of the methylated mixture gave two peaks corresponding to JA-Me and 7-iso-JA-Me

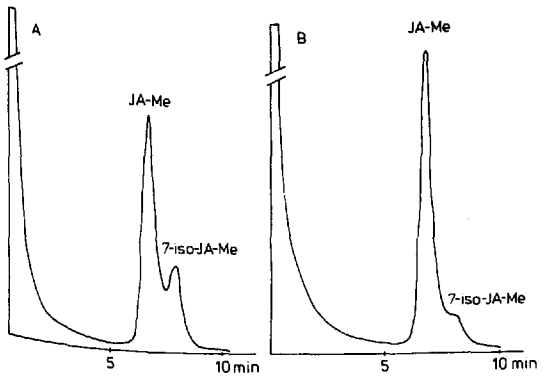

Fig. 2. GC analysis of the methylated isomer mixture isolated from *Vicia faba* L. (A); after treatment with alkaline solution (identical with synthetic JA-Me) (B).

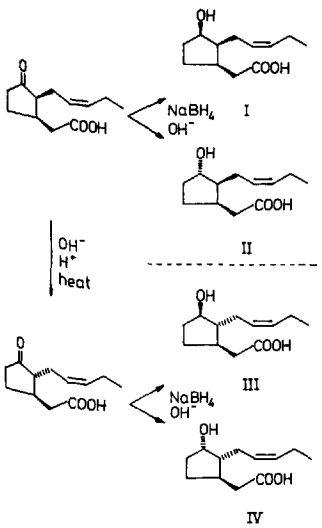

Fig. 3. Substances received by sodium borohydride reduction of jasmonic acid isomers.

(Fig. 2A) with a significantly higher content of 7-iso-JA-Me in comparison with synthetic JA-Me samples (Fig. 2B). The total amount of JA and 7-iso-JA could be calculated to be 2 μg/g fresh weight. GC-MS data of the methylated mixture were identical with those of the authentic compounds and agreed with the literature (Nishida et al. 1982). On heating with potassium hydroxide solution, the JA/7-iso-JA ratio changed to values of synthetic JA (Quinkert et al. 1982) without changing the total isomer content. These data demonstrate that in *Vicia faba* L. 7-iso-JA occurs naturally in a higher proportion than in synthetic JA or material normally obtained by the usual extraction procedures.

Furthermore, the isomer mixture isolated was reduced with sodium borohydride in sodium bicarbonate solution. The resulting 3-hydroxy-products, I to IV (Fig. 3), were methylated and analysed by GC and GC-MS. The reduction products I and II, each obtained in an amount of about 15%, confirm the orig-

inal presence of 7-iso-JA. By reduction of synthetic JA only about 5% of each I and II are obtained. Products III and IV are formed from JA; their methyl esters do not isomerize at carbon-7. GC and GC-MS data were identical with authentic material and literature data (Tanaka and Torii 1975). It is known that naturally isolated JA from *Vicia faba* L. is (−)-JA (Dathe et al. 1981) and (+)-7-iso-JA can be transformed to (−)-JA by isomerization (Nishida et al. 1985). Therefore, the optical rotation of 7-iso-JA from *Vicia faba* L. can be deduced to be dextrorotatory.

To determine whether JA is naturally occurring in *Vicia faba* L. or artificially formed even during our isolation method, [U-^{14}C]-7-iso-JA was added before homogenization. During the extraction the radiolabeled 7-iso-JA did not show any isomerization to JA confirming both the natural occurrence of JA and 7-iso-JA and the usefulness of this method. Furthermore, alternative isolation procedures were compared by extracting samples (10 g) of immature fruits under varying conditions with different organic solvents (*vide*, Materials and Methods). In one aliquot of the acidic fraction the two isomers were separated by TLC and semi-quantitatively calculated after detection with anisaldehyde reagent. Another aliquot was further purified by HPLC, and the re-extracted acids were methylated for GC analysis. During GC, isomerization of 7-iso-JA-Me to JA-Me takes place to some extent. Therefore, the quantity of the isomers was determined on the basis of a calibration curve obtained from authentic mixtures. The curve is linear and depends on column length, temperature, stationary phase, and separation time. The quantities of 7-iso-JA obtained by TLC and GC were in good agreement. The different extraction procedures resulted in different isomer ratios of JA/7-iso-JA varying between 90:10 and 65:35%. The best result (65:35) was obtained with fresh fruits immediately homogenized in cold ethyl acetate.

Biological activity of 7-iso-JA

7-iso-JA isolated from the fungus *Botryodiplodia theobromae* Pat. (Miersch et al. 1986) was tested in four bioassays and compared to JA. In wheat seedling bioassays (Fig. 4), (+)-7-iso-JA showed a significantly higher activity than (−)-JA; threshold concentrations producing significant inhibition of longitudinal growth were 1×10^{-4} and 5×10^{-4} M, respectively. Dwarf rice seedlings, stimulated by 10^{-6} M GA_3, responded more sensitively to both substances (Fig. 4). At a concentration of 1×10^{-4} M, (+)-7-iso-JA showed a higher potency than (−)-JA (86 and 65% inhibition).

The growth of oat seedlings was effectively inhibited by both the (+)-7-iso-JA and (±)-JA, and no difference in their activities could be observed at any concentration tested (Fig. 5). In barley seedlings the (±)-JA-Me was ineffective and (±)-JA gave only a small inhibition at 1×10^{-2} M. (+)-7-iso-JA was more active, having a threshold concentration of 1×10^{-3} M. The (+)-7-iso-JA-Me produced strong growth inhibition only at 1×10^{-2} M.

The senescence promoting activities of (+)-7-iso-JA, (±)-JA, and their methyl esters were compared. The results obtained with barley are shown in Figs. 6 and 7; oat seedlings responded less sensitively. Two days after applica-

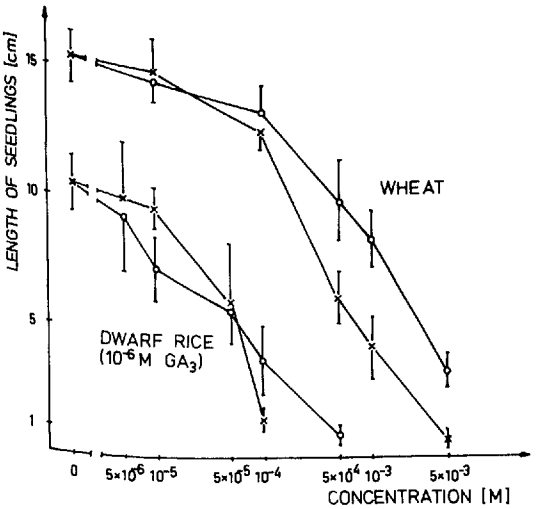

Fig. 4. Growth inhibition in wheat and GA_3-stimulated (10^{-6} M) dwarf rice seedlings by (+)-7-iso-JA (x——x) and (−)-JA (O——O).

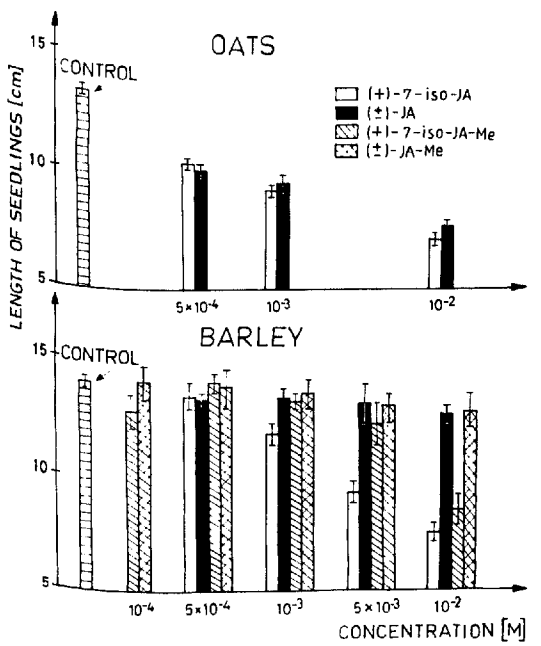

Fig. 5. Growth inhibition in oat and barley seedlings by (+)-7-iso-JA, (±)-JA, (+)-7-iso-JA-Me, and (±)-JA-Me.

tion bleaching effects in the primary leaves became obvious and remarkable losses of chlorophyll and carotenoids could be detected at concentrations $\geq 1 \times 10^{-5}$ M. At 5×10^{-3} M (+)-7-iso-JA and its methyl ester were more active than (±)-JA and (±)-JA-Me. In general the free acids showed a higher potency than their methyl esters in inducing senescence-like effects. After 3 days the chlorophyll and carotenoid contents increased slightly (Figs. 6 and 7).

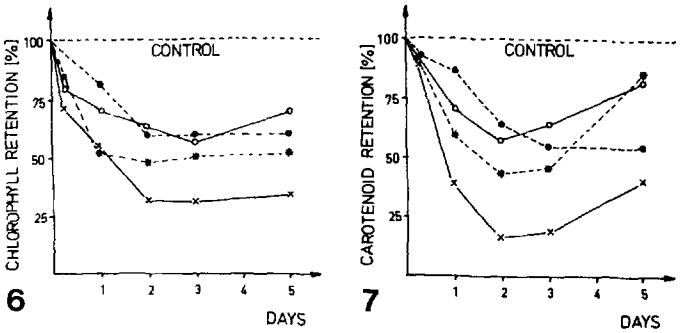

Fig. 6. Chlorophyll retention in growing barley seedlings after leaf treatment with 5×10^{-3} M (+)-7-iso-JA (x——x), (±)-JA (O——O), (+)-7-iso-JA-Me (*——*), and (±)-JA-Me (●——●).

Fig. 7. Carotenoid retention in growing barley seedlings with 5×10^{-3} M (+)-7-iso-JA (x——x), (±)-JA, (O——O), (+)-7-iso-JA-Me (*——*), and (±)-JA-Me (●——●).

Discussion

The investigations clearly demonstrate the natural occurrence of the two stereoisomers JA and 7-iso-JA (Fig. 1) in *Vicia faba* L. Thus , it must be concluded that JA isolated from broad bean fruits in earlier studies (Dathe et al. 1981, Knöfel et al. 1984) was partially formed by isomerization of 7-iso-JA during isolation procedures. 7-iso-JA is isomerized to a chemically defined mixture of JA/7-iso-JA = 90:10%.

The present studies show the occurrence of both isomers in young fruits of the broad bean with a ratio of 65% JA: 35% 7-iso-JA by use of a modified isolation procedure. It can be considered that this mixture represents the endogenous isomer ratio at the stage of isolation. Possibly this ratio changes with fruit development. In studies on the biosynthesis of JA using sections of pericarp tissue of *Vicia faba* L. and seeds of different plant species, Vick and Zimmerman (1983, 1984) assumed the cis-form of JA (7-iso-JA) to be the natural occurring stereoisomer. Miersch et al. (1986) isolated 7-iso-JA as the only isomer from *Botryodiplodia theobromae* Pat. and thus confirmed this proposition for a fungal system. Considering the proposed biosynthetic steps, all precursors of JA, beginning with 12-oxo-phytodienoic acid, can be isomerized under the influence of acids, bases, and heat (Vick and Zimmerman 1979). It remains to be determined if this also happens in plant systems. Vick and Zimmerman (1984) demonstrated that some plant enzymes involved in the biosynthesis do not distinguish between the cis- and trans-precursors. Our studies do not deal with possible isomerization of precursors or of 7-iso-JA before extraction started. However, we clearly show that in the immature pericarp of the broad bean both isomers do exist at least in one developmental stage.

JA is considered to be a plant growth regulator that is able to act in a hormone like manner (Sembdner and Klose 1985). The existence of a mixture of isomers in *Vicia faba* L. leads to a discussion about the role of 7-iso-JA. Are both isomers endogenous regulators of plant growth and development or is

only one of them of physiological significance? The singular occurrence of 7-iso-JA in the fungus and its position in the biosynthetic pathway in *Vicia faba* L. emphasizes the possible importance of this isomer.

Both natural isomers of JA inhibit plant growth when applied exogenously. It is interesting to note that the (+)-7-iso-JA proved to be more active than (−)-JA in GA_3-stimulated dwarf rice and wheat seedlings. However, in oats no significant differences in the activities of (+)-7-iso-JA and (±)-JA could be found. With respect to growth inhibition, young plants of oat and barley are not very sensitive to leaf application of (+)-7-iso-JA. Nevertheless, its activity was found to be higher than that of the synthetic retardant Ethephon (unpublished, 1984).

According to Ueda and Kato (1980, 1981) and Satler and Thimann (1981), JA-Me possesses senescence-promoting properties in leaf segments of oat. We obtained similar effects in intact seedlings of oat and barley by leaf treatment with (+)-7-iso-JA, (±)-JA, (+)-7-iso-JA-Me, and (±)-JA-Me. Barley seedlings were more sensitive than oat. In leaf segment tests the JA-Me is known to be much more effective than JA in promoting senescence (Ueda et al. 1981). However, in young growing plants (leaf application) we found the free acids to be more active than their methyl esters in inducing senescence (chlorophyll and carotenoid decrease). In barley seedlings the (+)-7-iso-JA was the most active compound. Greatest effects were obtained 2 days after treatment. The increase in pigment content, which begins after 3 days, is possibly due to continued lead growth and newly started pigment formation, but this requires further study. Effects of JA-Me on carontenoid formation and accumulation in ripening tomato fruits have been described by Saniewski and Czapski (1983) and Czapski and Saniewski (1985).

Acknowledgments. The authors are grateful to S. Jänicke and C. Kuhnt for GC and GC-MS analyses, I. Dey for help in bioassaying, and G. Geiseler for HPLC.

References

Aldridge DC, Galt S, Giles D, Turner WB (1971) Metabolites of *Lasiodiplodia theobromae*. J Chem Soc 1623–1627

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Phenoloxydase in *Beta vulgaris*. Plant Physiol 24:1–15

Czapski J, Saniewski M (1985) Effect of methyl jasmonate on carotenoids in tomato *Lycopersicon esculentum* fruits. Gartenbauwissenschaft 50:35–37

Dathe W, Rönsch H, Preiss A, Schade W, Sembdner G, Schreiber K (1981) Endogenous plant hormones of the broad bean, *Vicia faba* L. (−)-Jasmonic acid, a plant growth inhibitor in pericarp. Planta 155:530–535

Dathe W, Schneider G, Sembdner G (1978) Endogenous gibberellins and inhibitors in caryopses of rye. Phytochemistry 17:963–966

Gerlach H, Künzler P (1978) Michael-Addition von Thicarbonsäureestern. Anwendung bei der Synthese von (±)-Jasmin-ketolacton. Helv Chim Acta 61:2503–2509

Knöfel HD, Brückner C, Kramell R, Sembdner G, Schreiber K (1984) A radioimmunoassay for jasmonic acid. Biochem Physiol Pflanzen 179:317–325

Meyer A, Miersch O, Büttner C, Dathe W, Sembdner G (1984) Occurrence of the plant growth regulator jasmonic acid in plants. J Plant Growth Regul 3:1–8

Miersch O, Preiss A, Sembdner G, Schreiber K (1986) (+)-7-iso-Jasmonic acid and related compounds in *Botryodiplodia theobromae*. Phytochemistry (in press)

Nishida R, Acree TE (1984) Isolation and characterization of methyl epijasmonate from lemon (*Citrus limon* Burm.) J Agric Food Chem 32:1001–1003

Nishida R, Acree TE, Fukami H (1985) Optical resolution of methyl jasmonate and methyl epijasmonate. Agric Biol Chem 49:769–772

Nishida R, Baker TC, Roelofs WL (1982) Hairpencil pheromone components of male oriental fruit moth *Grapholitha molesta*. J Chem Ecol 8:947–959

Quinkert G, Adam F, Dürner G (1982) Asymmetrische Synthese von Methyljasmonat. Angew Chem 94:866–867

Saniewski M, Czapski J (1983) The effect of methyl jasmonate on lycopene and β-carotene accumulation in ripening red tomatoes. Experientia 39:1373–1374

Satler SO, Thimann KV (1981) Le jasmonate de méthyle: nouveau et puissant promoteur de la senescence des feuilles. CR Acad Sci Paris 293:735–740

Sembdner G, Borgmann E, Schneider G, Liebisch HW, Miersch O, Adam G, Lischewski M, Schreiber K (1976) Biological activity of some conjugated gibberellins. Gibberellins XLVIII. Planta 132:249–257

Sembdner G, Klose C (1985) (−)-Jasmonsäure—ein neues Phytohormon? Biol Rdsch 23:25–40

Tanaka H, Torii S (1975) Syntheses of methyl dl-jasmonate and methyl dl-2-epi-jasmonate. J Org Chem 40:462–465

Ueda J, Kato J (1980) Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.) Plant Physiol 66:246–249

Ueda J, Kato J (1981) Promoting effect of methyl jasmonate on oat leaf senescence in the light. Z Pflanzenphysiol 103:357–359

Ueda J, Kato J (1982a) Identification of jasmonic acid and abscisic acid as senescence-promoting substances from *Cleyera ochnacea* DC. Agric Biol Chem 46:1975–1976

Ueda J, Kato J (1982b) Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiol Plant 54:249–252

Ueda J, Kato J, Yamane H, Takahashi N (1981) Inhibitory effect of methyl jasmonate and its related compounds on kinetin-induced retardation of oat leaf senescence. Physiol Plant 52:305–309

Vick BA, Zimmerman DC (1979) Thermal alteration of a cyclic fatty acid produced by a flaxseed extract. Lipids 14:734–740

Vick BA, Zimmerman DC (1983) The biosynthesis of jasmonic acid. A physiological role for plant lipoxygenase. Biochem Biophys Research Comm 111:470–477

Vick BA, Zimmerman DC (1984) Biosynthesis of jasmonic acid by several plant species. Plant Physiol 75:458–461

Wettstein D (1957) Chlorophyll lethals and the submicroscopic changes in plastid structure. Exp Cell Res 12:427–506

Yamane H, Abe H, Takahashi N (1982) Jasmonic acid and methyl jasmonate in pollens and anthers of three *Camellia* species. Plant Cell Physiol 23:1125–1127

Yamane H, Takagi H, Abe H, Yokota T, Takahashi N (1981a) Identification of jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol 22:689–697

Yamane H, Takahashi N, Ueda J, Kato J (1981b) Resolution of (±)-methyl jasmonate by high performance liquid chromatography and the inhibitory effect of (+)-enantiomer on the growth of rice seedlings. Agric Biol Chem 45:1709–1711