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Occurrence of the Plant Growth Regulator Jasmonic Acid in Plants

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Abstract. The natural occurrence of jasmonic acid and its methyl ester in plants has been studied using different methods such as GC, GC-MS, HPLC, radioimmunoassay, and bioassay. Jasmonic acid was detected in several Leguminosae plants and a number of species belonging to nine other Angiospermae families. Highest amounts occurred in fruit parts, especially the immature pericarp, but it was found also in flowers and vegetative plant parts, e.g. leaves, stems, and germs. Young apple fruits contain both jasmonic acid and methyl jasmonate, and in Douglas fir, the only Gymnospermae species studied, only the methyl ester could be detected. Jasmonic acid is discussed as an endogenous plant growth regulator widely distributed in higher plants.

Methyl jasmonate (JA-Me) (Fig. 1) is known as an odoriferous compound in the essential oil of *Jasminum grandiforum* L. and *Rosmarinus officinalis* L. (Demole et al. 1962, Crabalona 1967). The free acid jasmonic acid (JA) (Fig. 1) was first isolated as a plant growth inhibitor from the culture filtrate of the fungus *Lasiodiplodia theobromae* (Aldridge et al. 1971).

Yamane et al. (1980) described the identification of JA as a plant growth inhibitor and compared its activity with other structurally related compounds. JA-Me could be identified in leaves and stems of *Artemisia absinthium* L. as a senescence-promoting substance (Ueda and Kato 1980). Furthermore, the free acid has been found in immature seeds of *Phaseolus vulgaris* L. and *Dolichos lablab* L., as well as in leaves and insect galls of *Castanea crenata*

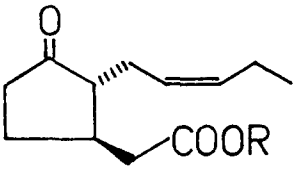

Fig. 1. Formula of jasmonic acid (R = H) and its methyl ester (R = CH_3)

Sieb et Zucc. as a growth inhibitor of rice seedlings (Yamane et al. 1981). Studying the endogenous plant hormones of *Vicia faba* L., Dathe et al. (1981) identified (-)-JA in the pericarp of immature broad bean fruits as a growth inhibitor of wheat seedlings. Recently, JA was shown to occur as a senescence-promoting substance in mature leaves of *Cleyera ochnacea* DC (Ueda and Kato 1982a) and furthermore, both JA and JA-Me have been detected in pollens and anthers of three *Camellia* species with JA being considered as a possible endogenous regulator of pollen germination (Yamane et al. 1982). Cucurbic acid, a plant growth inhibitor structurally related to JA, has been isolated from seeds of *Cucurbita pepo* L. (Koshimizu et al. 1974, Fukui et al. 1977a, b). We now report further studies on the occurrence of JA as a plant growth regulator possibly widely distributed in higher plants.

Materials and Methods

Plant Material

From a number of plant species (Table 1), different parts were harvested and stored at −20°C until extraction. Plants of *Vicia faba* L. var. *minor* cv. 'Fribo,' *Vicia narbonensis* L., *Pisum sativum* L. var. *cimitari* cv. 'Meteor,' *Dolichos lablab* L., and *Solanum tuberosum* L. cv. 'Adretta' were cultivated in a greenhouse in 1981/1982. The following species were grown in the Institute's field in 1981 and 1982: *Phaseolus vulgaris* L. var. *vulgaris* cv. 'Helidor'; *Phaseolus vulgaris* L. var. *nanus* cv. 'Harzgold'; *Phaseolus coccineus* L. var. *coccineus* cv. 'Preisgewinner'; *Lupinus albus* L.; *Cucurbita maxima* L.; *Brassica oleracea* L. var. *capitata* cv. 'Dithmarscher Früher' (dwarf); *Sinapis alba* L; *Foeniculum vulgare* Mill.; *Petroselinum crispum* (Mill.) A. W. Hill; *Helianthus annuus* L. (dwarf); *Taraxacum officinale* Wiggers; *Tussilago farfara* L.; and *Hordeum vulgare* L. var. *nutans* cv. 'Trumpf.'

Malus sylvestris Mill. cv. 'Golden Delicious' was received from a plantation; *Pseudotsuga menziesii* Carr. was collected in a forest near Berlin, and *Quercus robur* L. and *Fagus sylvatica* L. near the Institute. *Glycine max* L., *Citrus aurantifolia* Swingle cv. 'Persian,' *Citrus sinensis* Osbeck cv. 'Valencia late,' and *Calliandra haematocephala* Hassk. were cultivated and extracted in Cuba in 1979 and 1982.

Extraction and Purification

The frozen plant material was homogenized in methanol, the extract filtered, and the material extracted twice, using 80% aqueous methanol. After evapo-

Table 1. Occurrence of JA in various plants.

Plants	Organs	Methods			
Leguminosae:		GC	HPLC	RIA	GC-MS
Vicia faba L.	Pericarp	+++	−	+++	x
	Leaves	−	++	++	x
Vicia narbonensis L.	Immature pericarp	+	−	−	x
	Immature seeds	+	−	−	−
	Young fruits	++	−	−	x
Pisum sativum L.	Immature pericarp	+	−	−	x
	Immature seeds	+	−	−	−
	Young fruits	+	−	+	x
Phaseolus vulgaris L. var. *vulgaris*	Fruits	+++	−	++	x
Phaseolus vulgaris L. var. *nanus*	Fruits	+	−	+	−
	Immature pericarp	+	−	(+)	x
	Seeds	+	−	(+)	−
Phaseolus coccineus L.	Fruits	+	−	(+)	−
	Immature pericarp	+	−	−	x
Glycine max L.	Immature pericarp	+++	−	++	x
	Ripe seeds	−	−	+	−
Calliandra haematocephala Hassk.	Immature pericarp	++	−	++	x
Dolichos lablab L.	Mature seeds	−	−	+	−
	Immature pericarp	+	−	−	x
Lupinus albus L.	Immature pericarp	+	−	−	x
	Immature seeds	+	−	−	−
	Young fruits	++	−	−	x
Other families:					
Pseudotsuga menziesii Carr.	Stems (March)	++[a]	−	+[a]	−
	Needles (March)	+[a]	−	+[a]	−
	Needles (September)	(+)[a]	−	(+)[a]	−
Quercus robur L.	Young leaves	(+)	−	−	x
Fagus sylvatica L.	Young leaves	(+)	−	−	x
Cucurbita maxima L.	Young fruits	++	−	−	x
Brassica oleracea L.	Leaves (rosettes)	+	−	(+)	−
Sinapis alba L.	Young fruits	+	−	−	−
Malus sylvestris Mill.	Young fruits	+	x	−	x
		++[a]	x[a]	−	x[a]
Citrus aurantifolia Swingle	Young fruits	++	x	−	x
Citrus sinensis Osbeck	Fruits:albedo	−	x	+++	x
Foeniculum vulgare Mill.	Leaves	+	−	−	−
Petroselinum crispum (Mill.) A.W. Hill	Leaves	+	−	−	−
Solanum tuberosum L.	Dark germs	−	++	−	x
	Light germs	−	(+)	−	−
Helianthus annuus L.	Immature seeds	+	+	−	x
Taraxacum officinale Wiggers	Buds	+	−	−	−
	Flowers	+	−	−	−
Tussilago farfara L.	Buds	+	−	−	−
	Flowers	+	−	−	−

Table 1. (cont'd)

Plants	Organs	Methods			
		GC	HPLC	RIA	GC-MS
Hordeum vulgare L.	Vegetative shoots	+	–	(+)	–
	Ears	+	–	(+)	–

x = Identification without quantification
– = Not analyzed
(+) = Traces
+ ≧ 10 < 100 ng/g FW
++ ≧ 100 < 500 ng/g FW
+++ ≧ 500 ng/g FW

[a] Ja-Me occurring

ration of the methanol, the aqueous concentrate was frozen, thawed, filtered, and subsequently partitioned (at pH 2.5) with ether (3 × 1/3 volume). The ether extract was dried with anhydrous Na_2SO_4, evaporated, and chromatographed on DEAE-Sephadex A-25 according to Graebner et al. (1976), using a discontinuous gradient of acetic acid in 80% methanol.

Aliquots of each fraction were bioassayed for inhibitory activity, and the active fractions eluted with 80% methanol (JA-Me) and 1.5% acetic acid (JA) were collected. Both the JA-Me- and the JA-containing fractions (after methylation with diazomethane) were purified by preparative thin-layer chromatography (TLC) on activated silica gel GF_{254} (Merck) using the solvent system n-hexane –ethyl acetate (70:30 by vol.; JA-Me: R_f = 0.70–0.75; ABA-Me: R_f = 0.30–0.35). TLC of the JA-Me gave a much better separation from the impurities than TLC of the JA. JA-Me was detected by vanillin reagent (5 – 10 min, 120°C) (Randerath 1965). For further identification and quantification, JA-Me samples eluted from TLC plates with ethyl acetate were analyzed by gas chromatography (GC), combined gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), radioimmunoassay (RIA), and the rice seedling bioassay, respectively.

Gas-Liquid Chromatography and Combined Gas Chromatography-Mass Spectrometry

GC was carried out on a Chromatron GCHF 18.3 under the following conditions: steel column (3 m × 4 mm); 10% EG SS-X on Gas Chrom P (125 – 150 μm); carrier gas N_2, 46 ml min^{-1}; isothermal column temperature 180°C; hydrogen flame ionizing detector. Retention time of JA-Me is 12 min.

Combined GC-MS was performed on a VARIAN MAT 111 with 80 eV-mass spectrometer and a 1.80 m × 2 mm glass column containing 10% EG SS-X on Gas Chrom P (100–120 μm). Fragmentation pattern of the plant substances has been compared with that of authentic (±)-JA-Me, which corresponds to the data given in literature (Ueda and Kato 1980).

High-Performance Liquid Chromatography

For reversed phase analysis, a Pye UNICAM PU 4020 was used. A stainless steel column (250 mm × 4.6 mm) packed with Polyol RP 18 on Si 100 was eluted with 70% methanol containing 0.1% phosphoric acid at a flow rate of 1 ml/min^{-1} monitored at 228 nm. Retention time of JA-Me is 5.7 min.

Radioimmunoassay

A radioimmunoassay was developed by Knöfel et al. (1984) and applied as described there. The RIA is based on an antiserum raised in rabbits against (±)-JA linked to bovine serum albumin via its carboxyl group. The antibodies respond to the (+)− and (−)-enantiomers of JA-Me, and the assay has been standardized with (−)-JA-Me.

Bioassay

Inhibitory activity of crude column fractions was checked using the wheat seedling bioassay. (*Triticum aestivum* L. cv. 'Hatri') (Dathe et al. 1978). The biological activity of the samples purified by TLC was determined by means of the more sensitive rice seedling bioassay (*Oryza sativa* L. cv. 'Tan-ginbozu') in the presence of 10^{-6} M GA_3 (Sembdner et al. 1976).

Results and Discussion

The natural occurrence of JA and JA-Me has been studied after extraction of freshly frozen plant material and purification of the extracts by GC, GC-MS, HPLC, RIA, and bioassay. After separation of the neutral JA-Me and the acidic JA containing fraction by chromatography on DEAE-Sephadex A-25, JA was methylated, and all further determinations have been made with the purified methyl ester. For identification and quantification, at least two independent methods, e.g. GC and RIA, and sometimes HPLC, have been used; for selected samples GC-MS was applied. Additionally, the biological "anti-GA" activity was determined in dwarf rice. The applied analytical methods differ with respect to their sensitivity. The following detection limits (μg JA-Me) were observed: RIA-0.002; rice bioassay-0.2; TLC-0.2; GC-0.2; HPLC-1.0. The RIA can be applied as is to the crude ether extract without any further purification. Up to this step, losses of only 10% occurred. The physical methods and the rice bioassay require further purification steps including TLC, which altogether give losses up to 70%, as determined by adding radioactive-labeled JA during the extraction procedure. In general, the JA values determined by GC, HPLC, and bioassay are in good agreement. Also the RIA normally gave comparable values; however, in some cases lower amounts were measured by RIA than by GC. This may be due to losses of volatile JA-Me during a prolonged storage of samples before RIA.

The plant species analyzed and the results obtained are summarized in Table 1. In all members of the Leguminosae studied, JA was found to occur naturally.

The highest amounts were determined in fruit parts. Thus, the pericarp of developing broad bean fruits contained 3,100 ng JA/g FW, the immature pericarp of *Glycine max* 1,260 ng/g FW, and that of *Calliandra haematocephala* about 430 ng/g FW (GC data), whereas in ripe soybean seeds only 38 ng JA/g FW was determined by RIA. In young fruits of *Phaseolus vulgaris,* also, a high amount of JA (1,140 ng/g FW) was found, using GC.

In *Vicia faba,* JA is present not only in the different fruit parts, e.g. pericarp and seeds (cf. Dathe et al. 1981, Büttner 1982), but also in vegetative plant parts such as leaves. Thus, young broad bean leaves showed a content of 280 ng JA/g FW. This amount is much lower than that of immature broad bean pericarp (3,100 ng), but in the same order of magnitude as the JA content of young chestnut leaves (Yamane et al. 1981), whereas mature leaves of *Cleyera ochnacea* contained only 6 ng JA/g FW (Ueda and Kato 1982a).

Besides Leguminosae, plant species belonging to nine other Angiospermae families have been analyzed, and JA has been detected in all species studied (Table 1). JA was found to be present in a variety of plant organs—flowers, young fruits, seeds, buds, germs, shoots, and leaves. It is notable that in pumpkin fruits known to contain cucurbic acid (Koshimizu et al. 1974, Fukui et al. 1977a, b) JA could also be found (GC-MS identification) in rather high amounts. Young apple fruits contain both JA (80 ng/g FW) and an even higher amount of JA-Me (390 ng/g FW). The identity of the JA ester was proved by TLC, HPLC, GC, and GC-MS. The occurrence of JA-Me in applies may explain data published by Baker et al. (1981) and Nishida et al. (1982). They observed that hairpencils of the male oriental fruit moth, *Grapholitha molesta* (Busk.), contain JA-Me as a component of sex pheromone complex, only, when the insects are reared on young apple fruits. *Pseudotsuga menziesii,* the only member of Gymnospermae studied, also was shown to contain JA-Me, but no JA could be detected. The amount of JA-Me was higher in young stem parts than in the needles, the content of which apparently changes during the season.

The results presented and data already published (Ueda and Kato 1980, 1982a, Dathe et al. 1981, Yamane et al. 1981, 1982) indicate that JA is widely distributed in Angiospermae. In some plant species, JA is accompanied by its methyl ester, e.g. in apple (Table 1) and *Camellia* sp. (Yamane et al. 1982). In wormwood leaves and stems only the JA-Me was detected (Ueda and Kato 1980), and the same is true with needles and stems of Douglas fir, a coniferous plant (Table 1). Pteridophyta, mosses, and algae have not been studied at all, and only one species of fungi is known to produce JA (Aldridge et al. 1971). Thus, in order to complete our knowledge of the natural occurrence of JA, many more examples have to be studied. Such a screening program analyzing a large number of selected species of higher and lower plants by means of a RIA will be the subject of a separate report (Müller, Brückner, Knöfel, Kramell, and Sembdner, in preparation).

Concerning the distribution of JA within the plant, the results (Table 1) demonstrate the occurrence of the plant growth regulator in both generative and vegetative organs. Furthermore, some indications about changes in the JA content in relation to fruit development and other physiological processes are given. However, these questions have to be studied in much more detail. First results on the distribution of JA in organs and tissues of the broad bean plant

and changes during development have been obtained using RIA (Knöfel et al. 1984).

JA and JA-Me have been shown to possess different physiological activities when applied exogenously to plants. Thus, JA and JA-Me are known to exhibit a senescence-promoting activity in oat leaves (Ueda and Kato 1980, 1981; Ueda et al. 1981), to induce stomata closure (Satler and Thimann 1981), to inhibit cytokinin-induced soybean callus growth (Ueda and Kato 1982b), and to reduce seedling growth in rice and other cereals (Yamane et al. 1980, 1981, Dathe et al. 1981, Sembdner et al. 1983). Some of these activities are similar to ABA effects; however, with respect to the kinetics of seedling growth inhibition, JA and ABA differ. Unlike ABA, JA was shown not to delay the development of wheat seedlings (Dathe et al. 1981). Therefore, JA may be considered to be an endogenous plant growth regulator widely distributed in the plants. The question of whether it has a general physiological significance of a hormonelike nature has to be studied further.

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