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INDUCED BIOSYNTHESIS OF TERPENOID INSECT SEMIOCHEMICALS IN PLANTS

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In nature volatiles have a multitude of functions. They may act as antibiotics, fungicides, deterrents, attractants, or, more generally speaking, as INFOchemicals transferring all kind of information into a complex network of mutually interacting organisms. Plants under attack by a herbivore or micro-organism may emit characteristic volatiles that are *inter alia* implicated in the attraction of the natural enemies of the herbivore[1]. To understand the events between primary leaf damage and the emission of volatiles from the damaged plants, our research activities focus on the following topics.

1. Early events involve the interaction of low- and high-molecular elicitors of the attacking organisms with the plant "leaf-biochemistry" and require the identification of high- and low-molecular elicitors from insect herbivores and micro-organisms.

2. The second complex comprises the elements transduction linking the primary events and the known end product (jasmonic acid) of the signalling and addresses the identification of the different pat well as the signalling-quality of individual compounds within the signalling-pathways.

3. Recognition of the signal transducer(s) by cellular receptors and the subsequent events leading to reprogramming the gene expression of the leaf.

4. Enzymatic and regulatory aspects (resource mobilisation, enzyme mechanisms) of volatile biosynthesis.

(1) Cellulysin, a cocktail of endoglucanases and cellulases, produced by the plant parasitic fungus *Trichoderma viride* was identified as a powerful and generally active high-molecular elicitor of volatile biosynthesis in plants (*Phaseolus lunatus*, *Nicotiana plumbaginifolia*, *Zea mays*)[2]. It was found to stimulate the emission of a burst ethylene (3–6 h after stimulation) followed by the release other volatiles (fatty acid metabolites, terpenoids, aromatics) as a later event. In the Lima bean, used as the model system for most of these studies, their emission is not continuous, but follows an endogenous, circadian rhythm. Among the elicitors from oral secretions of insect herbivores (Noctuidae) amino acid conjugates of saturated and unsaturated C_{18} and C_{16} fatty acids were found to be widespread, but the compounds showed limited activity as elicitors for the induction of volatile biosynthesis in test plants.

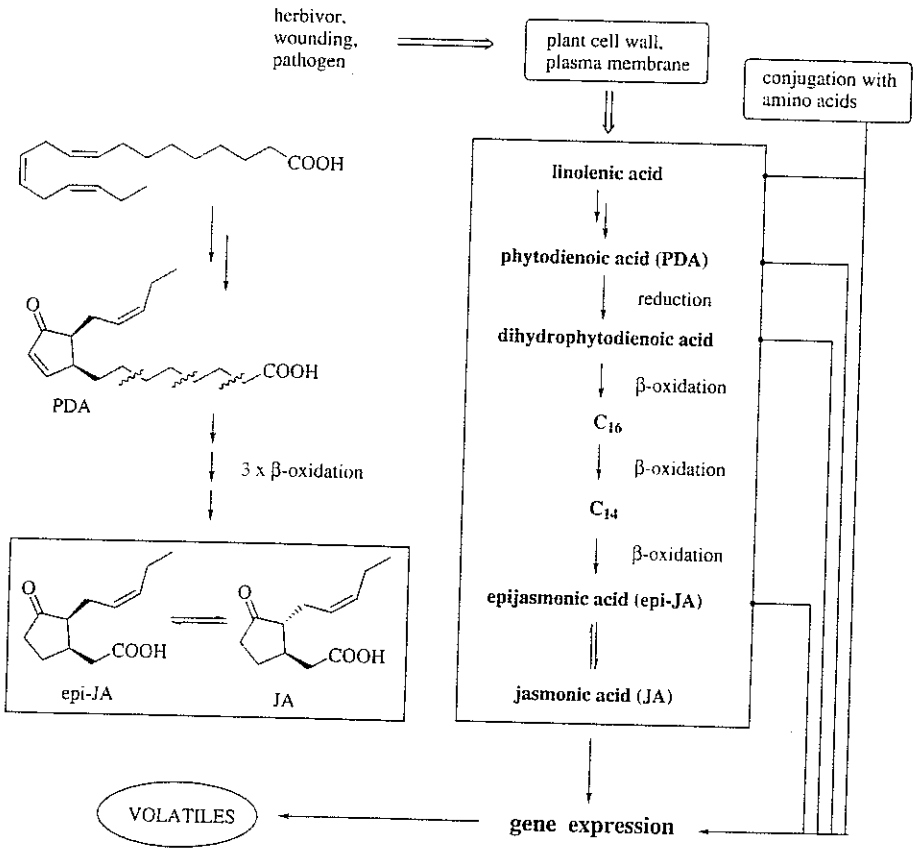

Fig. 1 Octadecanoid signalling pathway in plants

(2) Although the primary events of the leaf-cellulysin-interaction are unknown, it could be shown that the high-molecular elicitor acts via the octadecanoid signalling path-

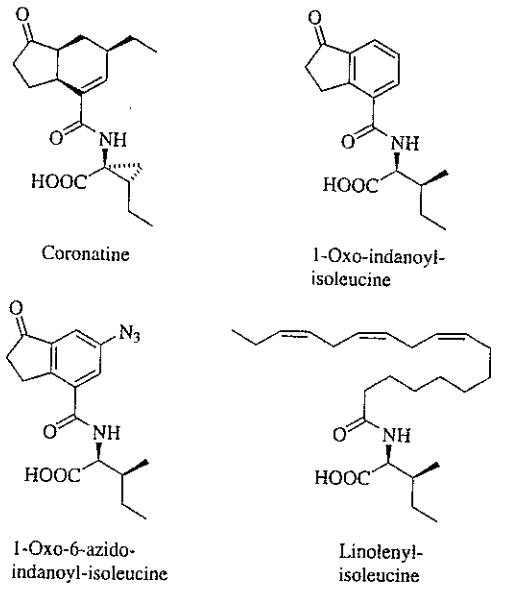

Fig. 2 Low molecular elicitors from microorganisms (coronatine) and insects (LIN-Gln) and some synthetic analogues.

way. Inhibitors preventing lipidperoxidation like phenidone completely blocked the cellulysin-dependent volatile biosynthesis[2]. The upregulation of the JA-pathway could be also directly demonstrated by analysing the time course of the JA-level in cellulysin-treated plants[3]. Analysis of the inducing-power of individual intermediates of the JA-pathway revealed that in the Lima bean exist at least two centres of biologically active compounds. Early intermediates (linolenic acid → 12-oxo-phytodienoic acid (PDA)) induce the biosynthesis of the two homoterpenes 4,11-dimethylnona-1,3,7-triene and 4,8,12-trimethyltrideca-1,3,7,11-tetraene, representing degradation products of sesquiterpenoid and diterpenoid precursors[3]. By using inhibitors, PDA was identified as the active compound in this part of the pathway. (cf. Fig. 1). Subsequent intermediates of the pathway were synthesized (10,11-dihydro-PDA and the $C_{17} \rightarrow C_{13}$ analogues of JA) but failed to induce volatile biosynthesis[4]. JA, the last member of the octadecanoid pathway turned out to be generally active. Modern (dicots, monocts) and evolutionary ancient plants (ferns, Gingko) respond to JA-treatment with the emission of volatiles. Some plants produce novel compounds, others only respond with quantitative shifts within their blends[5].

Besides compounds from the octadecanoid signalling pathway several structurally non-related amino acid conjugates, like the bacterial phytotoxin coronatine[5], the synthetic indanoyl-isoleucine[6], or amino acid conjugates of linolenic acid (Lin-Ile) likewise induce volatile biosynthesis[3]. Minor changes in the amino acid moiety of the indanone conjugates result in different volatile profiles (sesqui- and diterpenoids), attributing to the amino acid substructure a specific role for the recognition and the selective induction[7]. In general, amino acid conjugates with fatty acids like linolenic acid or jasmonic acid need to be hydrolysed by phytogenic enzymes to gain biological activity, but the conjugates coronatine and indanoyl-isoleucine exhibit biological activity only as intact molecules[7].

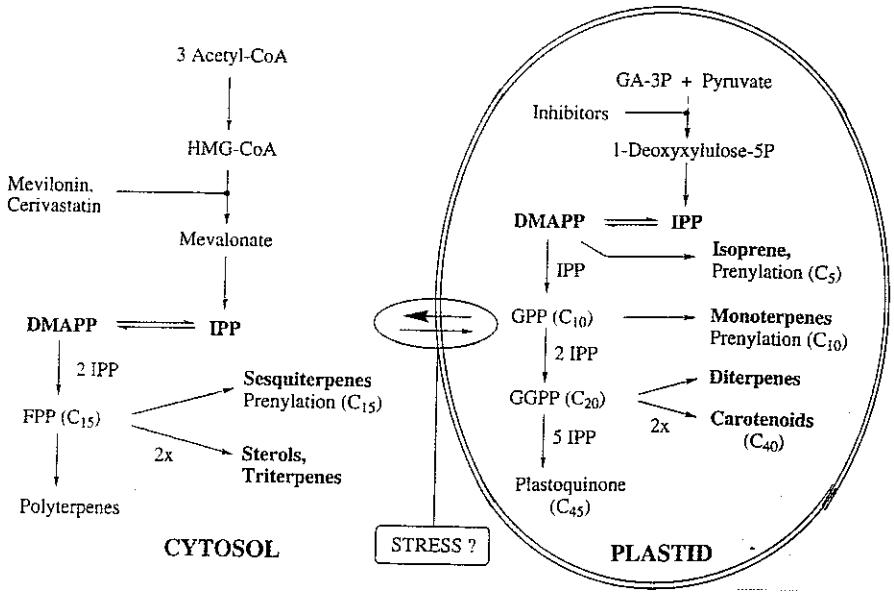

Fig. 3 Localisation of the mevalonate-dependent and the mevalonate-independent biosynthesis of terpenoids in cytosolic and plastidic compartments of the cell

(3) The 6-azido indanone (Fig. 2) was developed as a photolabile "elicitor" principally useful for a photoaffinity approach to study the late events of plant signalling (binding proteins, receptors)[8]. Details of JA/PDA-recognition and transduction of this signal into altered gene expresion are not known.

(4) The origin (mevalonate-dependent and mevalonate-independent biosyntehsis) of the induced terpenoids was studied by feeding labelled mevalonate or labelled deoxy-D-xylulose to herbivore-infested or JA-treated plants. Mass spectroscopic analysis of the emitted terpenoids revealed that mono- and diterpenoids are synthesised *de novo* along the novel desoxy-D-xylulose (DOX) pathway, while the biosynthesis of sesquiterpenes may be fuelled from both, the DOX- and the mevalonate pathway. Since also many sesquiterpenes were found to be highly labelled, these data support that DOX-derived precursors like IPP or GPP are shuttled from the plastid to the cytosol of the cell[9]. The extent of shuttling labelled precursors into the cytosol is especially high, if the cytosolic IPP-synthesis is blocked by inhibitors. This finding may be of importance for the maintaining the success of the plant defense in case of introduction of inhibitors along with the salivary secretion of herbivores. Similar findings concerning the efficiency of incorporation of labelled DOX into mono/di- and sesquiterpenoid volatiles have been made for flower volatiles.

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NEUROSTEROIDS: MEDICINAL CHEMISTRY OF STEROIDS AFFECTING GABA RECEPTOR FUNCTION

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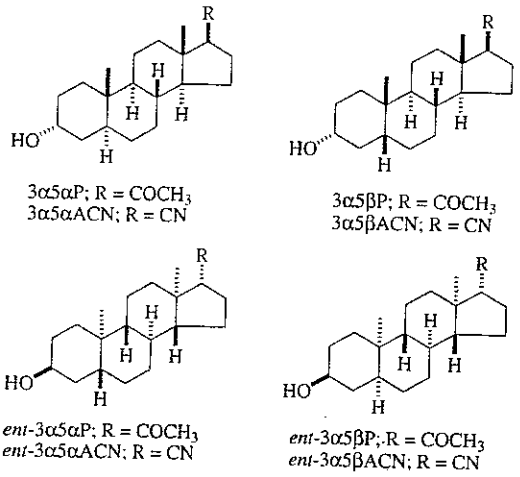
Hans Selye showed in 1941 that pharmacological doses of progesterone caused general anesthesia in rats[1]. His discovery led to the development of intravenous anesthetic steroids by the pharmaceutical industry[2]. It is now established that the anesthetic action of these steroids is correlated with their enhancement of $GABA_A$ receptor-mediated neuronal inhibition[3].

Structure/activity studies have established the structural features that a steroid must contain for it to have anesthetic activity[2]. These structural features are present in the potent steroid anesthetics 3α5αP and 3α5βP (Figure 1). The 3α-hydroxy group, which appears to function as a hydrogen bond donor, is an absolute requirement. For optimal anesthetic activity, a group which can function as a hydrogen bond acceptor (*e.g.*, the acetyl group found in 3α5αP and 3α5βP or the cyano group found in 3α5αACN and 3α5βACN) is also required as a 17β-substituent.

The location of the binding site(s) of anesthetic steroids on $GABA_A$ receptors has (have) not been established. Moreover, there are no radiolabeled ligands for the putative steroid binding sites on $GABA_A$ receptors. Thus, it remains possible that steroid modulation of $GABA_A$ receptor function results from changes in receptor function caused by steroid-induced membrane perturbation rather than by the direct binding of these compounds to $GABA_A$ receptors. Studies of the membrane perturbing effects of anesthetic steroids indicate that the



3α5αP; R = $COCH_3$
3α5αACN; R = CN

3α5βP; R = $COCH_3$
3α5βACN; R = CN

ent-3α5αP; R = $COCH_3$
ent-3α5αACN; R = CN

ent-3α5βP; R = $COCH_3$
ent-3α5βACN; R = CN

Fig. 1