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Production of Natural Flavourings, Perfumes, and Other Fine Chemicals by Infected Plants

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Abstract

It was demonstrated that the components of essential oils possess antimicrobial activity. If the formation of secondary metabolites, components of essential oils, is a way to resist the plants against the microorganisms, then the production of essential oils can be stimulated by a "stress" similar to that induced by the microorganisms. However, many volatiles are produced when the plant material is distressed. This is the "incontrollable" secondary metabolite formation by the pathways of degradation of fatty acids, aminoacids, etc. We attempt to demonstrate that many typical plant aromas are the result of plant-microorganism interactions.

Keywords: plant–microorganism interactions, essential oils, Phytoalexins, plants under stress, natural flavourings, secondary metabolites

1. Introduction

A source of natural aroma chemicals is that of essential oils or plant volatiles. Usually the volatiles represent only 0.1–2% of the total plant material, and include some hundred constituents in various concentrations depending upon the plant tissue.

Secondary metabolites can be defined (Bell, 1981) as compounds that have no recognized role in the maintenance of fundamental life processes in the organisms that synthesize them.

Their production can be attributed to four basic biogenetic pathways:

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1. Phenylpropane metabolism
2. Amino acid metabolism
3. Terpene metabolism
4. Lipid degradation.

Many of these compounds appear "after stress" of the plants and can be considered as their response to the aggression. The plant defense system is a complicated one, and it is based on a multistep reaction. Probably many of the essential oils and secondary metabolites, such as linear unsaturated aldehydes are used initially by the plant as a defense response.

For the production of secondary metabolites or constituents of essential oils of interest to the perfume industries, a new procedure can be envisaged: that of "simulated stress" or "simulated infection".

In general the plant is stressed by the presence of microorganisms or other abiotic factors or elicitors. Microorganisms act on the plant cell wall with their extracellular enzymes or elicitors (Albersheim and Darvill, 1985). Mechanical stress is provoked by cutting the plant material. The plant cell after cutting is in a similar situation as after the action of microbial enzymes.

Substitution of microorganisms by their extracellular enzymes or elicitors is the basis for "simulated infection". An unusual way to stress the plant material is the immobilization of plant cells.

2. Phenylpropanoid metabolism and production of secondary metabolites

General phenylpropanoid metabolism is defined as the sequence of reactions involved in the transformation of L-phenylalanine into activated cinnamic acids.

Antimicrobial activity of essential oils is well documented in *in vitro* studies (Bullerman et al., 1977, Conner and Beuchat, 1984, Kurita and Koike, 1982, 1983; Maruzzella et al., 1959, 1960; Dikshit et al., 1983; Reuveni et al., 1984), and it appears that except to their role in plant metabolism the constituents of essential oils, such as eugenol and cinnamic derivatives, play the role of an initial defense system.

There are indications that in plants under stress some of these metabolites appear or disappear. The questions that arise are of which, when, and why, because for the production of these metabolites it is important to know their biosynthetic pathway.

For example, lignification is a common response to infection or wounding in plants (Asada and Matsumoto, 1972). Inoculation of potato with a

sporangial suspension of *Phytophtora infestans* increases the level of phenylalanine ammonia lyase (PAL), chlorogenic acid and lignin (Friend et al., 1973). Similar observations were made for radish roots. Localized lignin for defense was observed in the red Canary grass (Vance and Sherwood, 1976; Vance et al., 1976). These results indicated that lignification is also a form of response to aggression.

However, Tressl and Drawert (1973) demonstrated using labelled (1-^{14}C) caffeic acid, that the distribution of radioactivity in banana tissue slices was located in eugenol, eugenol-methyl-ether and elimicin, and the rest appeared chromatographically after 5-methoxyeugenol. It must be noted here that eugenol, as mentioned above, is known for its antimicrobial activity, and banana tissue slices are a mechanically stressed plant tissue. The results of labeling experiments with banana tissue slices and (2-^{14}C) phenylalanine obtained by Tressl and Drawert (1973), showed that the phenylalanine is converted to 2-phenylethanol under the same conditions. It is interesting to note that the phenylalanine is not transformed into caffeic acid or eugenol but into 2-phenylethanol which is a minor component of banana and which possesses antimicrobial activity. Probably in this case the plant cell — under enzymatic control — "prefers" a short route of defense. This is no firm hypothesis.

All the above observations are summarized in Fig. 1 and indicate that in stressed tissues a particular metabolic pathway takes place, and the tissue accelerates the formation of enzymes involved in the synthesis of compounds destined for plant defense. Therefore this is a promising method for production of the secondary metabolites.

The aromatic phenolics vanillin and eugenol contribute to the flavour of vanilla and banana respectively, each molecule having a characteristic 3-methoxy-4-hydroxy- substitution. The same substitution pattern observed in gingerol in the roots of ginger or in capsaicin in *Capsicum*, is responsible for their pungent taste. This methoxylation and hydroxylation pattern must play an important role in the survival and dispersal of plants as well as in their economic exploitation (Butt and Lamb, 1981).

Immobilization of cells of *Capsicum frutescens* in reticulate polyurethane foams and production of capsaicin was studied by Lindsey and Yeoman (1984, 1984). In the absence of specific precursors to capsaicin, immobilized cells produced 2 and 3 orders of magnitude higher yields than in suspension cells. These results were reflected by an increased rate and extent of incorporation of L-(U-^{14}C) phenylalanine into capsaicin in immobilized, as compared with

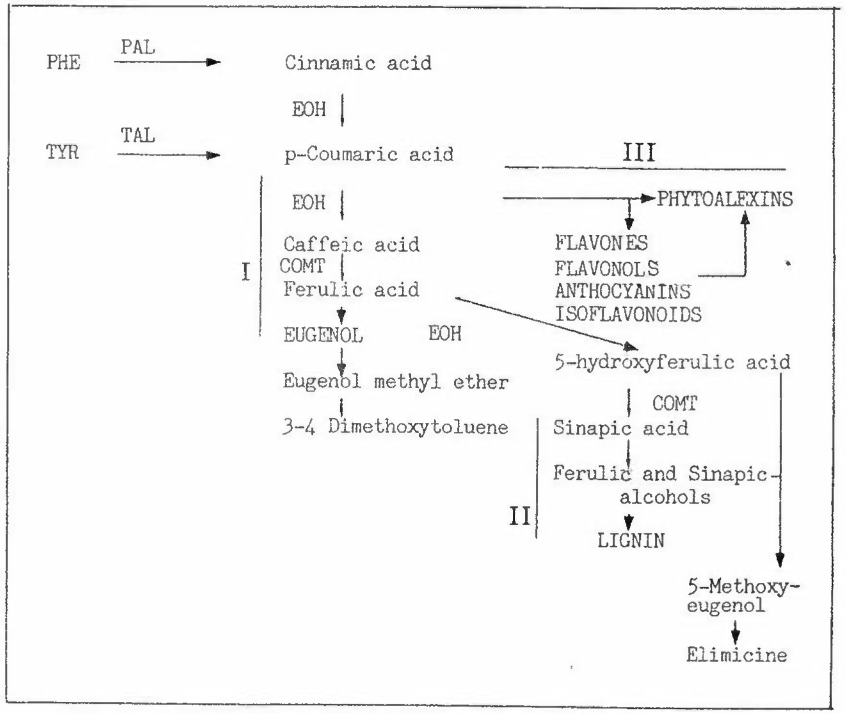


Figure 1. Phenylpropanoid metabolism and defense of plants. PAL: Phenylalanine ammonia-lyase, TAL: Tyrosine ammonia-lyase, COMT: Catechol O-methyltransferase, EOH: Hydroxylase.
I. Plant defense based on volatiles derived from phenyl-propanoid metabolism.
II. Plant defense based on lignin formation: Mechanical defense.
III. Plant defense based on phytoalexin production.

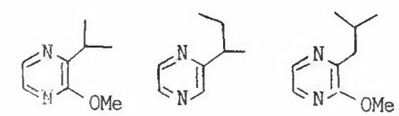
freely-suspended cells and evidence is presented for an inverse relationship between incorporation of labeled phenylalanine into protein and capsaicin.

This experiment indicates that the immobilization is a form of aggression probably caused by the free isocyanate groups and the response of plants to aggression is the production of capsaicin.

In general the term infection is applied to microorganism-plant interactions. Many other factors, such as abscission agents, or other chemicals may play an important role in the production of secondary metabolites as well. Moshonas and Shaw (1980) observed the formation of eugenol, methyl eugenol and elimicin after treatment of orange fruits with abscission agents. None of the phenolic ethers was found in any of the control essential oils from untreated fruits.

3. Amino acid metabolism and production of secondary metabolites

Alkyl methoxy pyrazines are minor odoriferous compounds in vegetables. A hypothetical biosynthetic pathway for the formation of 3-alkyl-2-methoxypyrazines in vegetables was proposed by Murray et al. (1975). This pathway involves amino acid amidation and glyoxal condensation. Such compounds are responsible for the aroma in bell pepper, beetroot and green pea with



0.002	0.001	0.002
ppb	ppb	ppb
green pea	beetroot	bell pepper
galbanum	galbanum	galbanum

very low odoriferous thresholds.

Although, there is no firm evidence, it is possible that these compounds are first produced by *Pseudomonas* bacteria which are known to associate in some ways with the plant. At least two of the above mentioned substances have been formed in relatively large amounts by *Pseudomonas perolens* in various media (Miller et al., 1973).

Regarding the structures of these compounds they are suggested to originate from the amino acids, valine, isoleucine and leucine. This is an unusual pathway for the degradation of amino acids. In general, deamination and α-keto acid formation takes place (Fig. 2).

As opposed to stress metabolites, asparagusic acid and 2-isobutylthiazole are synthesized in intact plant cells of asparagus and tomato.

Sulfur-containing volatiles are normally formed during crushing of the plant material. In *Allium* plants, essential oils possess antimicrobial activity. This anti-yeast activity (Wills, 1956) is probably due to the high allicin content. The effect of allicin on triose-phosphate-dehydrogenase in microbial cell metabolism has been demonstrated by Barone and Tansey (1977). Inhibition of several sulfydryl metabolic enzyme was observed. In onion the principal stress metabolite is the thiopropanal S-oxide. The biogenesis of allicin and thiopropanal S-oxide are summarized in Fig. 2. The precursors and the products possess antifungal activity (Huhtanen and Guy, 1984; Conner and Beuchat, 1984). It is interesting to note that the antifungal properties

Precursor Plant tissue	Products
Phenylalanine banana slices	2-Phenylethanol Antimicrobial activity.
Valine banana slices	2-keto-4-methylpentenoic acid → 2-methylpropanoic ac. Antimicrobial activity
Valine cut onion	2-methylpropanoate → S-(1-propenyl-)-cysteine sulfoxide ↓ thiopropanal S-oxide ← 1-propenylsulfenic acid Antimicrobial activity
Valine cut garlic	2-methylpropanoate → S-(2-propenyl-)-cysteine sulfoxide ↓ 2-propenyl propenethiosulfinate (allicin) Antimicrobial activity
Leucine banana slices	3-methyl-butanal → 3-methyl-1-butanol
Leucine intact tomato	3-methyl-butanal → 2-isobutylthiazole

Figure 2. Secondary metabolites derived from amino acid precursors in cut or intact plant tissues. All the above mentioned metabolites are constituents of the aroma of the vegetables from which they derived.

of the precursors were demonstrated in the gas phase (Huhtanen and Guy, 1984). There probably exists a relationship between microorganisms which act with their extracellular protease on the plant, and biogenesis of secondary metabolites issued from amino acid degradation. This type of interaction involves a protease inhibitor factor (Sequeira, 1985).

4. Terpene metabolism

Sesquiterpenoid phytoalexins with the eudesmane skeleton were isolated from the tissue of sweet potato when infected by *Ceratocystis fimbriata*. Brooks and Watson (1985) recently presented an excellent survey of phytoalexin production by infected plant tissues. The trpenoid phytoalexins,

with eudesmane skeleton, such as beta-selinene, beta-costol, beta-costal, 7-hydroxycostol and 7-hydroxycostal, are described and their action discussed.

We want just to add that the eudesmane skeleton compounds play an important role in fragrance compositions. The above mentioned terpenoids were found to be potential inhibitors of *Ceratocystis fimbriata* spores germination (Schneider et al., 1983).

5. Fatty acid degradation and formation of secondary metabolites with antimicrobial activity

The sensory contribution of such secondary volatiles can be favorable or detrimental depending on their concentrations, composition and type of plant material. In some cases the secondary volatiles formed enzymatically can be accumulated in concentrations sufficient to cause an off-flavor; on the other hand there are examples, such as cucumber melon, where the main characteristic aroma predominates only when the enzymes are allowed to react on decompartmentation of the plant tissue, by cutting, homogenizing, infection, etc. (Schreier, 1984). Green aldehydes and green alcohols are the compounds responsible for the green flavour note. They are produced from linolenic and linoleic acid in wounded plant tissues (Tressl and Drawert, 1973; Galliard, 1978). A large number of enzymes are released after cell membrane damage (Galliard, 1978). Autolysis gives free linolenic and linoleic acid. Lipoxygenase action on the above mentioned acids gives rise to linolenic and linoleic hydroperoxides. The two hydroperoxides have antimicrobial activity (Brooks and Watson, 1985; Shimura et al., 1981, 1983; Sekizawa et al., 1981). Lyase action on the hydroperoxides is responsible for the formation of tr-Hexen(2)-al-1, tr-Nonen-(2)-al-1, Nonadien-(2tr,6c)-al-1. These compounds are the basis of green natural aroma at low thresholds (0.07 ppb).

It has been proved that the above mentioned aldehydes also possess fungistatic action in the gas phase (Gueldner et al., 1985; Huhtanen and Guy, 1984).

An hypothetical defense system of the plant is proposed in Fig. 3, based on the antifungal action of hydroperoxides and green aldehydes. In this defense system the pathway which involves beta-oxidation of linoleic acid is inactivated (Jennings and Tressl, 1984). Inactivation is the result of plant cell disruption.

Enzymatic regulation takes place in wounded tissues. Inhibition of hexenal formation, derived from linoleic acid, was observed in the presence of linolenic acid hydroperoxide (Hatanaka et al., 1983).

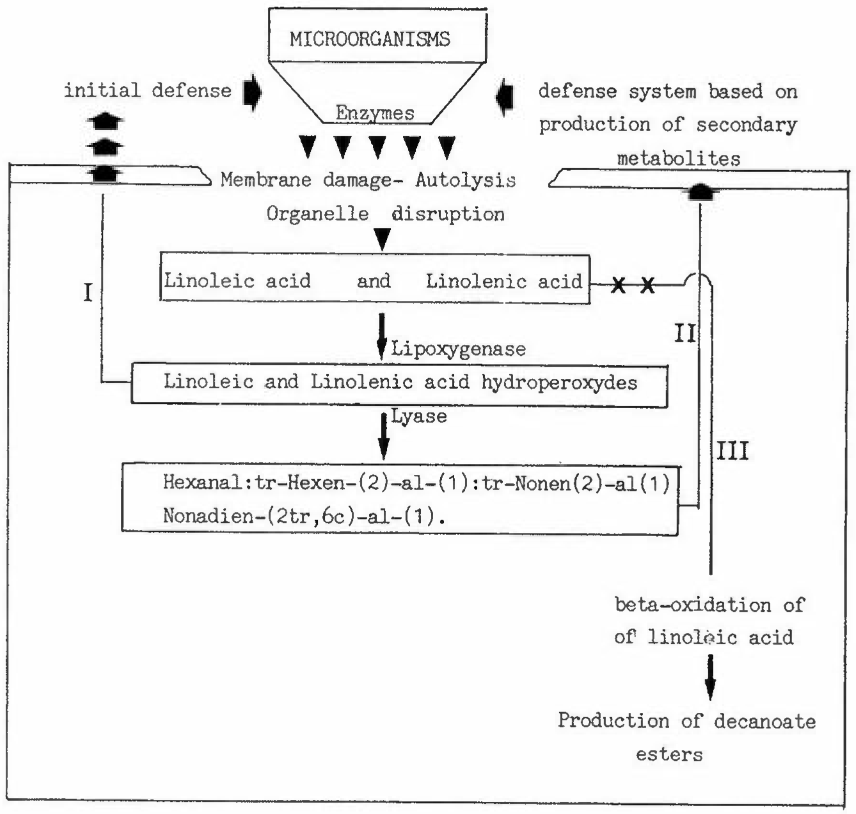


Figure 3. Production of secondary metabolites by fatty acid degradation and defense of plants.
I. Fatty acid hydroperoxides with antimicrobial activity.
II. Green alcohols and green aldehydes with antimicrobial activity.
III. Inactivation of the pathway which involves the beta oxidation of linoileic acid. The enzyme complex catalyzing beta-oxidation is inactivated during disruption of plant material.

In Vioryl SA we have worked for several years on the production of green aldehydes and alcohols (Ragoussis and Yannakopoulou, unpublished results). Studies on the production of nonadienal in cucumber extracts, cucumber plant cells and immobilized plant cells were carried out without success. The presence or absence of the percursor does not affect the yields in nonadienal. Membrane permeabilization, in plant cell cultures, increases in insignificant amounts the yields obtained. Experiments with fruits of different ages affect nonadienal formation, but amounts are insufficient for large scale production.

Based on "simulated stress" with extracellular enzymes of microorganisms we have now obtained total transformation of percursor into nonadienal by cucumber fruit cells. The precursor-linolenic acid was added to suspended cells at the concentration of 3-10 mM. The examination by gas chromatography of the organic phase after extraction shows that only nonadienal was formed in quantitative yield.

6. Conclusion

It appears that many secondary metabolites play the role of an initial defense system in plants. Many others are the precursors in the synthesis of fungitoxic compounds, such as phytoalexins.

For the production of components of essential oils with biotechnological methods it is important to know the plant-microorganism interactions and biogenesis in tissues under stress.

A similation of the stress induced by microorganisms can be envisaged for the acceleration or inhibition of enzymatic reactions in plants in the synthesis of desired compounds. This stress is realized by the factors with which the microorganisms infect the plant.

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