14 01

Enzymes involved in acridone alkaloid biosynthesis

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Acridone alkaloids which are yellow in colour are found in about 20 genera of the *Rutaceae*. The acridone skeleton is biosynthetically derived from anthranilic acid and acetate via a polyketo acid. Enzymes catalyzing the first steps in acridone alkaloid formation have been studied using cell suspension cultures of *Ruta graveolens* L. The first pathway-specific reaction is the N-methylation of anthranilic acid. The next step involved the activation of N-methylanthranilic acid which is catalyzed by a specific CoA ligase. Acridone synthase catalyzes the formation of 1,3-dihydroxy-N-methylacridone by condensing N-methylanthraniloyl-CoA and malonyl-CoA. The properties of these particular enzymes will be discussed and compared with related plant-specific enzymes.

14 02

Distribution of mucilage cells and mucilage production of some *Alcea* species (*Malvaceae*) in ontogeny

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Distribution and function of mucilage cells in stems and roots of 4 species of *Alcea* have been studied. The mucilage cells occurred especially in roots to the end of the first year. The quantity of mucilage cells in roots increased to the phase of budding in next year. Accumulation mucilage cells in stems was maximum to the same phase also. Production of mucilage in stems and roots were decreasing gradually during flowering and fruiting. It may be suggested the mucilage containing polysaccharides mainly is a storage substance. There are two processes accumulation and expence of polysaccharides in plants. But did not understand: whether transport of polysaccharides take place in organ to organ, whether the mucilage is useful in growing processes. On the other hand appearance of mucilage cells in young parts of plants may be an independed process. It will be very interesting to know about utilization of mucilage from cells without cytoplasm completely.

14 03

The flavonoids in some *Scutellaria* species

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The aglycone flavonoid compositions have been investigated in *S.adenostegia, S.adsurgens, S.phyllostachya, S.oxystegia, S.schugnanica, S.physocalyx, S.baldshuanica* and *S.strigillosa*. 9 flavonoid aglycones have been isolated. There are two groups of flavonoids in investigated *Scutellaria* species. The first group (apigenin and luteolin) is with substitution at 4' position in B ring. The second group (chrysin, baicalein, oroxylin A, 2'-methoxychrysin, 5,2'6'-trihydroxy-6,7,8-trimethoxyflavon, 5,2'6'-trihydroxy-7,8-dimethoxyflavon and (-)-5,2'-dihydroxy-6,7,8,6'-trimethoxyflavanon) is without these substitution. The first group flavonoids have been found in *S.adsurgens* and *S.adenostegia*; the second group ones - in all 8 species.The active biosynthesis of the second group flavonoids in *Scutellaria* is one of the peculiarities of its.

14 04

Partial purification and several characteristics of shikimate dehydrogenase in *Capsicum annuum* L.

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Shikimate dehydrogenase catalyzes the fourth step in the biosynthetic pathway for aromatic amino acids, which are the precursors of the alkaloid called capsaicin, the pungent principle of hot pepper. The enzyme was purified 79-fold using two chromatographic steps: Reactive Red 120-agarose and Q-sepharose, with a recovery of 13 % of the total activity. Shikimate dehydrogenase was unstable during the entire purification procedure, even with the addition of several protective agents (cysteine, 2-mercaptoethanol, dithiothreitol, glycerol) to the buffer. Various isozymes, three or more depending on the age of seedlings, were resolved by PAGE. Molecular mass and catalytic characteristics were determined for the total purified shikimate dehydrogenase and for the major isozymes separated by preparative PAGE.

14 05

On the activity of secretory leaf glands in *Ocimum* L.

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Biochemical processes, leading to the formation and accumulation of the lower terpenes, together with characteristics of the corresponding secretory structures are aggregately regarded as a special system - terpenogenous complex. Various forms of such complexes for several *Ocimum* L. species and forms, which are of interest for breeding of essential oil producing cultivars, have been investigated. A number of plant groups, distinguished by chemical composition and productivity of essential oil were identified. It is shown that in addition to the accumulation of cinnamic acid derivatives common for *Ocimum*, in some cases the synthesis of the acyclic oxygenated terpens takes place. It is shown in the development of so called "citral forms". A structure of peltate secretory glands and their distribution on leaf surface have been studied as well. Data received reflect particularities of the consisting and capacity of terpenogenous complexes, and, in the content of the available data on structure of analogous systems, make it possible to achieve a more concrete interpretation of the processes of peltate glands secretory activity in higher plants.

14 06

Oxidase and peroxidase activities of cell wall acidic peroxidase isoenzymes are correlated with cell wall lignification during xylogenesis

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The oxidase (H_2O_2-generating) and peroxidase (H_2O_2-consuming) activities of cell wall acidic peroxidase isoenzymes were determined along the xylogenesis gradient in etiolated lupin (*Lupinus albus* cv. Multolupa) hypocotyls. The results showed that both activities were correlated with the lignification of the secondary thickening of xylem vessels, suggesting that acidic peroxidase isoenzymes themselves may generate the H_2O_2 that it later uses for the oxidative polymerization of cinnamyl alcohols to lignins. These results were confirmed by histochemical methods that showed that peroxidatic activities are located in the secondary thickening of xylem vessels during lignification and xylogenesis.

14 07

Plants, alkaloids and insects: a chapter of chemical ecology

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Secondary plant constituents may have important functions in the interaction of the producing plants and their living and physical surroundings, i.e. chemical ecology. The pyrrolizidine alkaloids (PAs) have been selected as a typical example to document and verify these functions. The biosynthesis of PAs in roots of *Senecio* species (Asteraceae), their specific long-distance translocation into shoots via the phloem path, their channelling to the preferred sides of storage (i.e. inflorescences), and safe storage as *N*-oxides in the vacuoles is discussed. A great number of adapted insects belonging to different orders such as Lepidoptera, Coleoptera, Orthoptera and Homoptera are known to sequester PAs from plants. These insects use the plant acquired compounds as protective chemicals for their own benefit. The biochemical quality of PAs as plant defense compounds shaped by evolution and their successful acquisition by various insects will be discussed.

14 08

Primary metabolism and secondary metabolites

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The metabolic and evolutionary relationships between primary and secondary metabolism is demonstrated using two examples of the shikimate pathway. In the first example the isochorismate hydroxymutase is decribed [1], an enzyme equilibrating chorismic and isochorismic acid. The latter compound is a precursor of an array of primary and secondary metabolites.
In the second example experiments are decribed on the biosynthesis of aminohydroxybenzoic acid, a compound formed by a new variant of the shikimate pathway [2]. Aminohydroxybenzoic acid is a prescursor of maytansinoids, compounds occurring in a bacterium (Actinosynnema pretiosum) and some higher plant families (Euphorbiaceae, Rhamnaceae, Celastraceae).

[1] P.M.M. Schaaf, L. Heide, Y. Tani, M. Karas R. Deutzmann and E. Leistner, J. Nat. Products 56 , 1294 (1993)
[2] C.G. Kim, A. Kirschning, P. Bergon, Y. Ahn, J.J. Wang, M. Shibuya and H.G. Floss, J. Am. Chem. Soc., 114, 4941 (1992).

14 09

Nicotine content in *Nicotiana rustica*: Effect of application times of molybdenum

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The effect of different molybdenum application times on nicotine content in leaves, stem and root of *N. rustica* was studied. The plants were grown in hydroponic cultures (vermiculite). The experiment was carried out throughout development cycle of *N. rustica*. Three lots of plants were used: Control lot, receiving 0.01 ppm of Mo; (1-Mo) lot, receiving 1 ppm of Mo throughout their development and (1-Mo + C) lot which received 1 ppm of Mo during the vegetative stage and 0.01 ppm of Mo from the flowering.
The results suggest that the Mo supply does not increase the nicotine content in *N. rustica*. However, during the vegetative growth, the highest nicotine content was observed in stem of the (1-Mo) lot; the difference between (1-Mo) lot and Control lot was significant (90%). But, from the flowering a gradual decrease in the alkaloid content in leaves was observed, above all in the (1-Mo) lot; the difference between these lots was significant (92%).

14 10

Cell suspension culture of spruce (*Picea abies*): Induction of a transient release of hydrogen peroxide (oxidative burst) by fungal elicitor

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Plants can mobilize a large variety of different mechanisms in response to a pathogenic attack. These are designed to either strengthen the barriers against invasion (*e.g.* lignin) or to inhibit the invading pathogen (antibiotic compounds). H_2O_2 is suggested to participate in all of these mechanisms.
Elicitation of spruce cells with a wall fraction of the fungus *Rhizosphaera kalkhoffii* causes an immediate release of H_2O_2 from the cells. Release was estimated using 2 methods: ABTS (for kinetic measurements), phenol red (total amount of H_2O_2 released). The release is a biphasic process with an initial small maximum at 1 h and a second large maximum at 5-6 h. The amount released is significantly higher and the maximum release is later than reported for other plant systems. A relation of the oxidative burst to an influx of Ca^+ and an efflux of K^+ is postulated. The ionophores A 23187 (Ca^{2+}) and cycloheximide (K^+) induce a release of H_2O_2 and, when together with elicitor, induction is synergistically increased. Protoplasts which are required for studying the fluxes of Ca^{2+} and K^+ have been prepared and found to retain the elicitor-induced oxidative burst.

14 11

Infraspecific variation of essential oil composition of *Mentha longifolia* L. (Huds.)

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Genus *Mentha* is characterized with the great inter- and intra-specific variability of essential oil composition. Biosynthesis of mono- and sesquiterpenes of essential oil are influenced both by genetic and environmental factors. In the present study the composition of essential oil of *Mentha longifolia*, collected from six different area of Serbia, Monte Negro and Macedonia have been investigated, by the means of GC-MS method. In essential oil 60 compounds were detected and 58 identified. The great differences in qualitative and quantitative essential oil composition have been found. Examined samples have been classified into four chemotypes, contained as dominants compounds: carvone, menthofuren, piperitoneoxide and aromatic monoterpenes thymol and *p*-cymene.

14 12

Alcohol dehydrogenase from *Vanilla planifolia* green embryo tissue culture

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Properties of cytoplasmic alcohol dehydrogenase from green embryo cultured in light in liquid Gamborg B-5 medium were investigated. The optimum pH of extraction and reaction, substrate specificity and electrophoretic (PAGE) mobility were determined. The enzyme appeared to be very specific towards C_6-C_1 aldehydes and carried exclusively reduction of aldehydes to corresponding alcoholes. It is postulated that the enzyme plays important role in biosynthesis of p-hydroxybenzyl alcohol in *Vanilla planifolia* tissues.

14 13

Enzymology of betalain synthesis in *A. muscaria*: Characterisation of a tyrosinase and a dioxygenase

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The betalains are water soluble pigments occurring in plants of the order *Caryophyllales* and in certain fungi, such as *Amanita muscaria*. The first step in betalain biosynthesis is the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA). DOPA is then transformed to betalamic acid, the betalain chromophore, by the action of DOPA-4,5-dioxygenase. Conjugation of betalamic acid with amino acids or amines gives rise to yellow betaxanthins; reaction with (glycosylated) cyclo-DOPA gives violet betacyanins.
DOPA-4,5-dioxygenase was the first enzyme of the pathway to be purified. Now we report the purification of a tyrosinase from the colored parts of the hat of *A. muscaria*. Both enzymes were present only in betalain producing tissues. Cell fractionation showed that they were localized in the cytoplasm.
The tyrosinase was characterized with respect to its molecular weight and subunit structure, enzyme activity, specificity and co-factor requirements. Its immunological relationship with other tyrosinases will be discussed.

14 14

Cyanogenic glucosides in flax (*Linum usitatissimum*)

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Identification and quantification of cyanogenic glucosides in different organs of flax (*Linum usitatissimum* L. cv LCSD 200) plants at different stages of development was performed using TLC and specific colour reactions. Monoglucosides (linamarin and lotaustralin) and diglucosides (linustatin and neolinustatin) appeared in developing embryos soon after anthesis, but in mature seeds accumulated only diglucosides. Monoglucosides appeared again in germinating seeds and in young seedlings they presented the only class of cyanogens. The high level of linamarin and lotaustralin was found in green parts of plants during all vegetation period, being the highest in flowers. In contrast these glucosides were present in relatively small amounts in roots and in lignified parts of stem.

14 15

Identification of three flavonoid O-glycosyltransferases in alfalfa

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O-glycosyltransferases (OGTs) conjugate a range of flavonoids involved in the defence response and nodulation. Our investigations suggest there are at least three closely related flavonoid OGTs in alfalfa; flavonol-(eg. quercetin)-3-OGT (I), isoflavone-(eg. formononetin)-7-OGT (II) and coumestan\pterocarpan-(eg. coumestrol\medicarpin)-3-OGT (III). These OGTs are all cytosolic, possess similar MWs and isoelectric points and are of low abundance. Unlike OGT-II and OGT-III, OGT-I was unaffected by low molecular weight inhibitors in crude extracts, had higher specific activities in light-grown than in etiolated leaves and was partially resolved by hydrophobic interaction chromatography from the other OGTs. Levels of conjugated formononetin and medicarpin, together with OGT-II activity, increased in roots immersed in water. The PAL inhibitor AOPP abolished both the accumulation of isoflavonoid conjugates and the induction of OGT-II. Experiments using cycloheximide revealed that OGT-II and OGT-III have much shorter half-lives than OGT-I. OGT-II activity was also selectively increased in nodulated roots of mature field-grown plants. These results are further evidence for the differential regulation of alfalfa OGTs.

14 16

Ontogenetic aspects of 7-methyljuglone accumulation in some *Drosera* L. species

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The naphthoquinones with important biological activity are synthetized in species of the genus *Drosera*. Accumulation of 7-methyljuglone was studied in *Drosera rotundifolia* and *D. anglica*.

Plants were collected on seven localities in Finland. Content of 7-methyljuglone was estimated by HPLC. Their identity was confirmed by GC-MS.

High content of 7-methyljuglone was found in young leaves up to 2 cm long (2.7 % in *D. rotundifolia* dry weight; 2.1 in *D. anglica*). Mature leaves of *D. rotundifolia* contained from 0.8 to 1.2 % of 7-methyljuglone and *D. anglica* from 0.6 to 1.6, respectively. High content of 7-methyljuglone was found in flowers (*D. rotundifolia* 2.7 %). Accumulation of this secondary metabolite in unmature leaves and flowers can be explained by their defence role.

Two red pigments were recorded in older reddish leaves. Their presence was related to dramatic decrease of 7-methyljuglone content in leaves (0.04 to 0.1 % of 7-methyljuglone in both species).

14 17

Diosgenin content in *Dioscorea balcanica* tissue culture

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Some *Dioscorea* species, including the relict and endemic *D. balcanica*, contain diosgenin and other steroidal sapogenins, which are precursors for synthesis of steroidal hormones. As the propagation from seed or by the conventional vegetative methods is too slow to provide sufficient plant material, the aim of our work was to obtain a callus culture and to optimize conditions for growth and diosgenin yield in callus of *D. balcanica*.The callus culture was obtained from immature embryos and grown on MS medium with 0.66 mg/l 2,4-D and 0.5 mg/l BAP. For inducing organogenesis, 0.178 mg/l IAA was substituted for 2,4-D. The regenerated plants were transfered to soil and grown in the glasshouse. Diosgenin was quantified using gas chomatography, in callus. roots and leaves from *in vitro* cultures, as well as in roots, leaves and rhizomes from glasshouse grown regenerates. The influence of various concentrations of sucrose, growth regulators (2,4-D ; BAP) and light/dark conditions on growth and diosgenin yield in callus were also examined.

14 18

Toxicity of arsenate and phytochelatin production in *Silene vulgaris*

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The toxicity of arsenate in *Silene vulgaris* was studied in a nutrient solution in short- and long term experiments, using growth of the longest root or total plant dry weight as an indication of arsenate toxicity.
Arsenate is competitively taken up by the phosphate uptake system. It appeared that low doses of arsenate (2.5 microM) inhibited root growth to a high extent (EC_{80}), especially in plants grown at a low phosphate-concentration (10 microM), wheras at a higher phosphate-concentration (100 microM) the same amount of inhibition of root length growth occurred at 50 microM arsenate. Long-term experiments were carried out with plants grown at a phosphate-concentration of 100 microM. After 18 days of exposure to 5 microM of arsenate, the total dry weight of the plants had been reduced with 80 %, as compared to the unexposed controls.
Internal arsenic concentrations in the roots reached levelled off at 250 micromol per g dry weight after 18 days of exposure to 5 microM of AsO_4, whereas shoot arsenic concentrations did not exceed 5 micromol per g dry weight. Exposure to arsenate did not affect internal phosphate-concentrations.
Administering arsenate strongly induced phytochelatin-production in roots and to a lesser extent in shoots. Roots exposed to 25 microM of arsenate for 28 days contained 17 micromol per g dry weight, shoots only 0.3 micromol per g dry weight. The induction of PC-synthase by arsenate *in vitro* is currently under investigation.

14 19

Coupling of catharanthine and vindoline to form anhydrovinblastine by FMN in the presence of light

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Catharanthine and vindoline are the monomeric precursors of vincristine and vinblastine, which are potent anticancer drugs produced by *Catharanthus roseus*. These compounds exist in very low yield in the intact plant and were never detected in cell suspension cultures. The system responsible by the coupling of catharanthine and vindoline *in vivo* has not been characterized and seems to be a key regulatory step. As an effort to elucidate the mechanisms of the coupling reaction in the intact plant, we screened leaf extracts for coupling activity in the presence of several cofactors and we discovered that FMN alone in the presence of light was able to mediate the coupling of catharanthine and vindoline to form 3',4'-anhydrovinblastine. This reaction was characterized and optimized and a maximum yield of 34% was obtained. The hypothesis that the coupling might be a non-enzymatic step, mediated by FMN, should now be considered. Previous reports on the coupling activity of cell free extracts from cell suspension cultures in the presence of FMN should be regarded with caution.

14 20

The possible detoxification mechanism of lead excess by means of synthesis of phytochelatins in lupin roots

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The main site of lead uptake from the soil into the plants are roots. The production of lead binding peptides in roots of lupin in response to Pb supply was recorded. Plants were cultivated hydroponically for 4 d and then exposed to the 1 mM $Pb(NO_3)_2$ concentration for three days. Pb phytochelatins were analysed using fast performance liquid chromatography on a FPLC-Q Sepharose anion exchange column and eluted by a linear 0 - 1 M NaCl gradient. Simultaneously absorption at 245 nm and Pb amount were monitored. Chromatograms showed two lead-binding peptides peaks. The production of phytochelatins was estimated by HPLC method. The production was attended by a decrease of glutathione content in lupin root cytosol.

14 21

Biosynthesis of glucosinolates in oilseed rape leaves

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Glucosinolates are synthesised from amino acids, and in rape leaves the most abundant glucosinolates are derived from chain-extended methionine and phenylalanine homologues. Microsomal flavin mono-oxygenase enzymes have been identified which are specific for these precursors, and catalyse the synthesis of aldoximes which are subsequently converted to thiohydroximates, desulphoglucosinolates and glucosinolates. The characteristics of these enzyme systems will be described, and contrasted with the quite different aldoxime-forming enzymes of cyanogenic glucoside and indole glucosinolate biosynthesis. Current progress in elucidating the biochemistry of thiohydroximate synthesis will also be described. A variety of developmental and environmental factors influence glucosinolate synthesis and accumulation, and the roles of light, nutrition, and pest and pathogen attack will be discussed in relation to the biochemistry. The effects of putative signal molecules on glucosinolate accumulation will also be presented.

14 22

Betalain biosynthesis as a model for the regulation of secondary metabolites synthesis in tissue cultures

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Betalains are vacuolar pigments occurring in plants of the order *Caryophyllales* and in some fungi (*Amanita muscaria* for ex.). The biosynthetic pathway of these chromo-alkaloids is fairly short involving a maximum of three to four enzymatic steps from tyrosine to betaxanthins or betacyanins. We have been studying the behaviour of red beet (*Beta vulgaris*) cell cultures in relation to their ability to synthesise different types of pigments. In liquid batch cultures the different betaxanthins are synthesised for some of them during the log-phase and for others during the stationary-phase indicating a very subtle regulatory mechanism. The relations between morphological differentiation and pigment biosynthesis under various conditions of stable or induced specific pigmentation, have been studied by a combination of computer assisted image analysis and HPLC analysis. Automatic Image analysis (including mathematical morphology and monochromic colour analysis) and fuzzy logic analysis of the results lead to a better understanding of the relations between morphology and pigment biosynthesis