Anthocyanins in the flowers of European orchids

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Summary. The flowers of European orchids contain cyanidin 3-monoglucoside, cyanidin 3-diglucoside and cyanidin 3,5-diglucoside. 2 complex anthocyanins, composed of cyanin and a 2nd organic compound were isolated from the flowers of *Orchis, Dactylorhiza* and *Gymnadenia.*

The anthocyanins of European orchids have so far been determined only in a few cases[1]. For this reason we have studied these pigments from all available species.

Experimental. For the isolation and identification of the anthocyanins, the usual methods of chromatography[2,3], the controlled chemical hydrolysis with acids[4] or bases[5], and the absorption spectroscopy were applied. As far as possible, several plants of the same orchid species, but different geographical and ecological origin were investigated. About 100–200 mg plant tissue were needed for the determination of the anthocyanins. In order to prevent decomposition of the pigments, the flowers were processed without drying.

Results. The investigations showed that orchids of the same species, independent of growth conditions, contain always the same anthocyanins. The results are summarized in table 1. It demonstrates that the European orchids contain almost exclusively cyanidinglucosides. Besides the common anthocyanins cyanidin 3-monoglucoside (chrysanthemin), cyanidin 3-diglucoside (mecocyanin), and cyanidin 3,5-diglucoside (cyanin), 2 unusual anthocyanins of more complex chemical structure occur in the petals. They are characteristic pigments of the subfamily orchidoideae and contain the fundamental structure of cyanin. For this reason, these pigments were named 'orchicyanin'. One of them, the red orchicyanin II, arises already during an early stage of flower development. For this reason, it appears mainly in the buds. A short time before and at the beginning of the anthesis, the biosynthesis of the violet orchicyanin I commences, while the concentration of orchicyanin II is decreasing. The velocity of this metabolism is related to the orchid species.

Both the acid and the alkaline hydrolysis of orchicyanin I and II yield cyanin as the 1st degradation product. This indicates that both orchicyanins contain a 2nd compound, attached to cyanin (organic acid, flavonol). Such a combination may cause the bluer colour of orchicyanin I and its higher stability against chemical and enzymic influences. Similar effects by flavonols[6] and cinnamic acids[7] have been repeatedly reported in the literature, and may be explained

Table 1. Anthocyanins and their relative concentrations (%) in the flowers of the European and related orchid species

	Chrysantemin	Mecocyanin	Epipactin	Cyanin	Orchicyanin II	Orchicyanin I
Cypripedium calceolus	100 P					
C. reginae	100 L					
C. cordigerum	100[a]					
Listera ovata	100 P					
Epipactis atrorubens	40	30	20	10		
E. helleborine	20	40	20	10		
E. palustris	?[d]	40	60			
Calypso borealis	90			10		
Ophrys insectifera	100 L					
O. speculum	80				10	10
O. apifera L	100				–	–
P	–				40	60
Barlia robertiana[b]	10			40	40	10
Serapias lingua[b]	10?	20?		10	30	30
Nigritella nigra	100					
N. miniata[b]				10	40	50
Gymnadenia odorata				10	40	50
G. conopea				10	40	50
Dactylorhiza sambucina				10	30	60
D. majalis				10	30	60
D. maculata				10	20	70
Orchis ustulata	10[c]	90				
O. purpurea	10 P	?[d]		20	50	20
O. militaris				50	30	20
O. simia				10	60	30
O. longicornu				10	20	70
O. morio				10	10	80
O. mascula				5	5	90

The concentration values are round and refer to flowers for 2 days open. Epipactin: Characteristic orange coloured anthocyanidin glycoside of Epipactis species. P: petals and sepals; L: labellum. [a] Dark red spots on the labellum; [b] preliminary results; [c] only in buds and at the beginning of the anthesis; [d] very small quantities.

Table 2. Chromatographic and optical properties of orchicyanin from *Dactylorhiza maculata*

	Rf-value · 100 BAW	BHCl	1% HCl	AHCl	Colour	λmax [nm]
Cyanin	26	07	16	42	red	511, 273
Orchicyanin I	06	02	14	39	red	513, (312), 267
Orchicyanin II	30	20	27	58	violet	526, (322), 350, 267

Rf-values of paper chromatograms on Schleicher u. Schüll, Nr.2043a. BAW n-butanol-concentrated acetic acid-water (4:1:5, top layer); BHCl n-butanol-2N HCl (1:1, top layer); 1% HCl water-concentrated HCl (97:3); AHCl concentrated acetic acid-concentrated HCl-water (15:3:82). λmax absorption maximum in 5% acetic acid; small peaks in parentheses.

by the blocking of the hydroxyl groups in the B-ring of cyanidin. Bayer and Wegmann[8] found that a degradation of anthocyanins by the enzyme cyaninoxidase does not happen unless 2 free hydroxyl groups are present in the B-ring.

The optical and chromatographic properties of the orchicyanins from *Dactylorhiza maculata* are listed in table 2. Particularly the spectral investigations yielded, according to the orchid species, variable values. This finding points to different compounds associated with cyanin in the orchicyanins, and may be of taxonomic importance. Further investigations are being made in order to determine them in the various species.

The relative concentrations in table 1 (pigment contents refered to the sum of all anthocyanins) have, within the same species and at the same development stage of the flowers, almost constant values. Also plants of different origin show this typical relative anthocyanin concentration of the species, even though the variability is a little greater.

The absolute concentrations (anthocyanin contents refered to the fresh plant material) are, of course, much lower. On application of the spectrophotometric method and cyanin for calibration, the following average total anthocyanin concentrations of fresh (about for 2 days opened) flowers (labellum, sepals, petals) were measured: *Dactylorhiza maculata* 0.2%; *Dactylorhiza majalis* 0.7%; *Gymnadenia conopea* 0.15%; *Nigritella nigra* 2.0%. The absolute concentrations are much more variable within the same species, even in plants from the same place of growth.

All concentrations alter during the development of the flowers. Comparing investigations and determinations of variability must be made with this in mind.

The anthers of the European orchids contain either no anthocyanins at all or cyanidinglucosides with a simple structur: cyanidin 3-monoglucoside, cyanidin 3-diglucoside, and cyanidin 3,5-diglucoside. Cyanidin 3-monoglucoside occurs alone in the anthers of *Nigritella nigra* and *Ophrys insectifera.* Both, cyanidin 3-glucosid and the cyanidindiglucosides have been found in following species: *Dactylorhiza majalis, D. maculata, Orchis purpurea, O. simia, O. morio* and *O. mascula.*

These results indicate that the diglucosides occur mainly in the anthers of *Orchis* and *Dactylorhiza* which contain orchicyanin in the petals. In these species, at first the more simple structured chrysanthemin is formed. Therefore it occurs above all in the anthers of the buds. At the beginnung of the anthesis, the biosynthesis of the diglucosides is accelerated, so that their highest concentration is reached in the anthers of flowers several days open.

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Purification de la S-adénosyl-L-homocystéine hydrolase du foie de rat par chromatographie d'affinité[1]

Rat liver S-adenosyl-L-homocysteine hydrolase purification by affinity column chromatography

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Summary. S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) has been purified 240-fold from rat liver by affinity column chromatography on aminohexyl sepharose bound 6-mercaptopurine 9 D-riboside. The purified enzyme was homogeneous by gel electrophoresis.

La S-adénosyl-L-homocysteine hydrolase (EC 3.3.1.1) est très répandue[2-6]. Chez l'animal elle existe dans presque tous les organes, dont le foie et le cerveau[7-10]. Elle catalyse l'hydrolyse en adénosine et en homocystéine de la S-adénosyl-L-homocystéine (SAH) elle-même formée à partir de la S-adénosyl-L-méthionine (SAM) dans les réactions de transméthylation, mais la synthèse de la SAH est thermodynamiquement favorisée[2-11]. Comme la SAH est un inhibiteur puissant des méthyltransférases[11] et présente des propriétés neurotropes récemment mises en évidence[12], il apparait que la SAH-hydrolase doit jouer un rôle régulateur. Afin de préciser ce rôle, nous avons entrepris