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Chlorophyll breakdown and chlorophyll catabolites in leaves and fruit†

Bernhard Kräutler

Institute of Organic Chemistry & Centre for Molecular Biosciences, University of Innsbruck, A-6020, Innsbruck, Austria

Abstract

Chlorophyll metabolism probably is the most visible manifestation of life. Total annual turnover of chlorophyll has been estimated to involve more than 1000 million tons. Surprisingly, chlorophyll catabolism has remained an enigma until less than twenty years ago, when a colorless chlorophyll catabolite from senescent plant leaves was identified and its structure was elucidated. In the meantime, chlorophyll breakdown products have been identified in a variety of plant leaves and their structural features have been elucidated. Most recently, chlorophyll breakdown products have also been identified in some ripening fruit. Chlorophyll breakdown in vascular plants only fleetingly involves enzyme-bound colored intermediates. The stage of fluorescent catabolites is also passed rapidly, as these isomerize further to colorless nonfluorescent tetrapyrrolic catabolites. The latter accumulate in the vacuoles of de-greened leaves and are considered the final products of controlled chlorophyll breakdown. The same tetrapyrroles are also found in ripening fruit and are effective antioxidants. Chlorophyll breakdown leads to tetrapyrroles that appear to have physiologically beneficial chemical properties, and it may thus not merely be a detoxification process.

1. Introduction

Due to their unique roles in the capture and transformation of sun light, chlorophylls occupy a very special position among the natural pigments.¹ Indeed, their seasonal metabolism is probably the most visual sign of life on earth, observable even from outer space (see Fig. 1).² The appearance of the green plant pigments in spring and their disappearance in the autumnal foliage of deciduous trees and in ripening fruit belong to the most colorful and fascinating natural phenomena. It is estimated that more than 10⁹ tons of chlorophyll (Chl) are biosynthesized and degraded every year on the earth.³ However, only within the last two decades has Chl breakdown in plants begun to yield some of its mysteries.^{2,4-6} Matile and coworkers provided the first evidence for the presence of colorless Chl catabolites in senescent leaves (*Festuca pratensis*^{7,8} and barley^{9,10}), which easily decomposed into rust-colored compounds.¹¹ Surprisingly, these catabolites were found in the vacuoles, rather than in the chloroplasts.⁹ The main catabolite from barley, now named *Hv-NCC-1* (a 3¹,3²,8²-trihydroxy-1,4,5,10,15,20-(22*H*,24*H*)-octahydro-13²-[methoxycarbonyl]-4,5-dioxo-4,5-seco-phytylporphyrinate), was the first non-green Chl catabolite from a higher plant to be identified.^{4,13} Its structure revealed it to be derived from Chl *a* and gave first-hand clues as to the major structural changes occurring in the degradation of Chl during plant senescence.^{2,4,14}

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2. Chlorophyll breakdown and catabolites in senescent leaves of vascular plants

The initial identification of the colorless, nonfluorescent Chl catabolite *H_v*-NCC-1 was followed by an intensive search in senescent leaves from a variety of vascular plants.^{2,5,12,15} The structures of all these Chl catabolites (except for one, see below¹⁶) pointed to a direct lineage with Chl *a*, and not with Chl *b*, the minor chlorophyll component in plants.⁵ The puzzling fate of the *b*-type Chls in the course of Chl breakdown was clarified by the discovery of a biochemical pathway from Chl *b* to Chl *a*¹⁷ as part of a “Chl *a*/Chl *b* cycle”.¹⁸ This reductive conversion and dephytylation are now indicated to precede the loss of the magnesium ion, an important first step dedicated to actual chlorophyll breakdown.^{14,19} Activity of a magnesium dechelating enzyme depends on the assistance of a heat stable “metal dechelating substance”.²⁰

Tiny amounts of nearly colorless but fluorescent compounds (provisionally named “fluorescing” Chl catabolites or FCCs) could be observed in senescent cotyledons of oil seed rape (*Brassica napus*).^{21,22} None of these fluorescent compounds accumulated *in vivo*. The structure of an FCC from *Brassica napus* (as a 3¹,3²-didehydro-1,4,5,10,17,18,20,(22*H*)-octahydro-13²-(methoxy-carbonyl)-4,5-dioxo-4,5-seco-phytytoporphyrin, see Scheme 1) was elucidated and it was, indeed, identified as a “primary” FCC (pFCC).²² Its structure was consistent with oxygenolysis of the porphinoic macroring of pheophorbide *a* (Pheo *a*) by an oxygenase,²³ followed by a reduction and saturation of the “western” 6-*meso* position, thus generating the pFCC.²² The putative oxygenase was remarkably specific for Pheo *a*.²³ It was identified as a non-heme iron-dependent a mono-oxygenase^{24,25} and was named pheophorbide *a* oxygenase (PaO).²⁶ Accordingly, a red tetrapyrrole was suggested as an elusive intermediate in Chl breakdown as the product of PaO and as the direct precursor of the FCCs.^{22,27} Such a “red” Chl catabolite (RCC) would be similar to some red bilinones, which were found to be excreted as final degradation products of the Chls in the green alga *Chlorella protothecoides*.^{28,30} Authentic RCC (3¹,3²-didehydro-4,5,10,17,18-(22*H*)-hexahydro-13²-(methoxycarbonyl)-4,5-dioxo-4,5-seco-phytytoporphyrin) was prepared by chemical degradation of Pheo *a*²⁷ and could be identified in tracing experiments in senescent plant material.^{31,32} PaO is located in the chloroplast envelope and its activity depends upon the presence of reduced ferredoxin.²⁶ As PaO opens the porphyrinoid macrocycle to give a non-green product, and its activity is senescence dependent, it was suggested to catalyze a key step of chlorophyll breakdown.^{5,6,24}

As we know now, red chlorophyll catabolite RCC is bound strongly to PaO, and inhibits it. Therefore, RCC could not be observed in the course of natural senescence in higher plants.^{14,31} A cytosolic reductase, now named red Chl catabolite reductase (RCC-reductase),^{33,34} directly reduces the bound RCC and sets free the “primary” FCC (pFCC),^{22,35,36} The reductase depends on reduced ferredoxin as electron donor, while (other) cofactors appear not to be involved in its task.³³ The central steps of Chl breakdown in the leaves of higher plants thus depend on the intimate cooperation of the membrane bound PaO and RCC-reductase, which is located in the stroma of the plastids and whose action is associated with the release of the primary FCC as the reduction product of RCC.²⁶ A second FCC (*Ca*-FCC-2) was isolated from sweet pepper (*Capsicum annuum*) and was shown to be a stereoisomer (the C(1)-epimer) of the pFCC from oil seed rape.³⁵ The absolute configuration at C(1) of the pFCCs may be functionally irrelevant.⁵ The stereo-dichotomy at this chiral center of the pFCCs, the configuration of which is defined by (in *Arabidopsis thaliana* a small protein domain of) RCC-reductase,³⁷ points to a remarkable, species dependent divergence of the RCC-reductases.³⁶

The apparent independence of RCC-reductase of cofactors for the ferredoxin-driven reduction of RCC at first appears puzzling. However, electrochemical reduction of RCC (and of its methyl ester (Me-RCC)),²⁷ provided direct (but stereo-unselective) access to the two strongly luminescent epimeric pFCCs (see Scheme 2), or their methyl esters.^{38,39} These experiments suggest that (i) RCC might be inherently sufficiently redox-active to undergo ferredoxin-driven and enzyme mediated reduction to the pFCCs and (ii) the reduction of RCC by RCC-reductase may come about from (enzyme controlled) protonation and single electron reduction steps,^{15,38} as also proposed for the homologous bilin reductases.⁴⁰

FCCs, such as the pFCCs, do not to accumulate during Chl breakdown in vascular plants.²⁶ They are transformed further to the colorless and nonfluorescent chlorophyll catabolites (the NCCs), such as *Hv*-NCC-1.^{4,5,12,14,41} Complete de-conjugation of the four pyrrolic units is characteristic of the chromophore of the tetrapyrrolic NCCs.⁴ This de-conjugation is indicated to directly result from a thermodynamically favorable acid-catalyzed (chemical) isomerization of FCCs to NCCs.⁴¹ The isomerization of the pFCCs (*epi*-pFCC (= *Ca*-FCC-2) or pFCC) to the “primary” NCCs (*epi*-pNCC (= *Cj*-NCC-2) or pNCC, respectively) occurs readily and with high stereo selectivity in a slightly acidic medium (as suggested to exist in the vacuoles, see Scheme 3).^{39,41}

So far, over a dozen colorless and nonfluorescent chlorophyll catabolites (NCCs) from de-greened leaves of a variety of higher plants have been characterized, which all have the same basic structure as the one of *Hv*-NCC-1 from barley (see Scheme 4).^{4,13,41,51} In senescent cotyledons of oil seed rape (*Brassica napus*), *e.g.* four NCCs were found, termed *Bn*-NCC-1 to *Bn*-NCC-4.^{45,46,49,51} In a variety of other senescent higher plants, NCCs were identified, such as the *So*-NCCs in naturally de-greened leaves of spinach.^{42,48} The autumn leaves of the tree *Cercidiphyllum japonicum* turned out to be a particularly efficient source of one NCC (*Cj*-NCC-1).^{41,47}

The constitution of *Hv*-NCC-1^{4,13} indicated peripheral modification reactions, which were tentatively assumed to occur in the later stages of the breakdown pathway.^{2,5,6,14} Indeed, a hydroxyl group at the terminal position of the ethyl side chain at ring B is particularly remarkable among the indicated peripheral functional groups.^{4,5,13,51} This particular hydroxyl group appears to serve both the purpose of increasing the polarity of the catabolites as well as of providing an anchor point for further, secondary refunctionalization with hydrophilic groups.

In this context, the recent observation of a less polar NCC (*Cj*-NCC-2) in extracts of senescent leaves of *C. japonicum* is noteworthy,⁴¹ which is an isomer of the pFCCs⁴¹ and lacks the hydroxyl group at ring B. Another unusual observation concerned the identification and structural characterization of an NCC from *A. thaliana*, called *At*-NCC-3, that proved to be carry a hydroxymethyl group at C(7) (see Scheme 4),¹⁶ whereas a methyl group is the characteristic C(7)-substituent of all other known NCCs.⁵ The identification of this NCC (besides its isomer with a hydroxyl group at the ethyl side chain at C(8), as generally observed) can be rationalized by a possible unselective hydroxylation reaction.^{16,51} Else, it could indicate PaO not to be strictly selective for oxygenation of Pheo *a*, but to also accept 7-hydroxymethyl-Pheo *a* as a second acceptable substrate in course of the degradation of Chls in this plant.¹⁶

The spatial localization in the senescent leaf cell, as well as the timing of the isomerization of FCCs into the corresponding NCCs during Chl breakdown, is another matter of high interest.^{2,5,26} The vacuoles are not only the final storage vessel for the NCCs, they also appear to be likely sites for the final isomerization of FCCs to NCCs, considering the low pH values typical of these storage organs of the plant cell.⁵ Indeed, experiments with

authentic pFCCs showed a considerable readiness of the natural FCCs to undergo acid induced, stereo-selective tautomerization to the corresponding NCCs in the absence of enzymes (see Scheme 3).^{39, 41}

Breakdown of Chl in senescent leaves beyond the stage of the NCCs has not been well established and may not follow a specific pathway. Indeed, the NCCs appear to accumulate in the vacuoles of senescent leaves of some higher plants.^{9, 52} The amount of *Bn*-NCCs present in de-greened cotyledons from oil seed rape, roughly corresponded to the calculated amount of Chls present initially in the green leaf.⁴⁶ Likewise, in de-greened leaves of barley¹¹ and of French beans⁵³ the total content of NCCs appeared not to decrease strongly over a time of several days. Accordingly, it has been suggested that NCCs may represent the final products of controlled chlorophyll breakdown in senescent vascular plants.^{2, 6, 26}

However, the original characterization for *Hv*-NCC-1 as a “rusty” pigment pointed to the readiness of these reduced linear tetrapyrroles to undergo spontaneous reactions, which become manifest by the appearance of the rust color.^{11, 13} Evidence for further degradation of NCCs was provided by the identification of colorless uro-bilinogenoidic linear tetrapyrroles⁵⁴ in extracts of de-greened primary leaves of barley. These tetrapyrroles were associated with specific further degradation of *Hv*-NCC-1, from which their constitution differs on account of the absence of the formyl group. These tetrapyrroles possibly arose from further (yet non-enzymatic) transformation of the NCCs in the tissue of the senescent barley leaves. Oxidative loss of the formyl group from related linear tetrapyrroles has been noted.⁵⁴

Degradation of Chl clearly plays a very prominent role in the recycling of nutrients (from senescent leaves to other parts of the plant), such as of reductively fixed nitrogen and magnesium ions.⁵⁵ As shown, there is evidence that rapid breakdown of Chl beyond the stage of tetrapyrroles does not occur in senescent leaves. Obviously, Chl breakdown is not aimed at reusing the four nitrogen atoms of the chlorin macrocycle (which represents only a few percent of total leaf nitrogen).^{4, 14, 26} It rather results in the dismantling of chlorophyll protein complexes, which is a prerequisite for rapid proteolytic degradation.⁵⁶ When bound to the proteins (in light harvesting complexes and reaction centers) Chl, in turn, is inhibited from causing photo-oxidative damage. Accordingly, when set free from the protein complexes, Chl becomes a phototoxic agent, and the sophisticated machinery of Chl catabolism must be interpreted as serving a vitally important detoxification process.^{6, 57} Indeed, it is important for cells to remain viable since the breakdown of proteins, and the recycling of nutrients depend on an organized metabolism to the very end of the senescence period.⁵⁸

3. Colorless chlorophyll catabolites from ripening fruit

De-greening and the typical appearance of appealing colors are frequent signs of fruit ripening. However, whereas in the last two decades considerable attention has been given to Chl breakdown in leaf senescence in higher plants, a form of “programmed cell death”,^{58, 60} Chl breakdown and the structure of the catabolites in ripening fruit have largely remained to be clarified. We have recently begun to study the occurrence of Chl catabolites in ripening fruit, starting with commercially available apples and pears.

In freshly cut and extracted peelings of a yellow pear (of the “Williamine” brand) the presence of two fractions with spectral characteristics typical of an NCC were revealed. By spectral and chromatographic analysis *Pc*-NCC-2, the less polar NCC from the pear (*Pyrus communis*), was indicated to be a 3¹,3²-didehydro-8²-hydroxy-1,4,5,10,15,20,22,24-(21*H*, 23*H*)-octahydro-13²-(methoxycarbonyl)-4,5-dioxo-4,5-seco-phytoporphyrinate,⁵⁹ and identical with *Cj*-NCC-1 from *Cercidiphyllum japonicum*.⁴⁷ By the same means, *Pc*-NCC-1,

the more polar NCC from the pear was revealed to be a $3^1,3^2$ -didehydro- 8^2 -(1- β -glucopyranosyl)-oxy-1,4,5,10,15,20,22,24-(21*H*,23*H*)-octahydro-13²-(methoxycarbonyl)-4,5-dioxo-4,5-seco-phytoporphyrinate.⁵⁹ *Pc*-NCC-1 could be identified with *Nr*-NCC-2, an NCC recently characterized in extracts of senescent leaves of tobacco (*Nicotiana rustica*).⁴³

Likewise, freshly cut and extracted yellow peelings of a ripe apple (of the “Golden Delicious” brand, see Fig. 2) were similarly indicated to contain two fractions with the characteristics of the NCCs that were identified in the pear already.⁵⁹ Both fruit thus harbor the same two NCCs. The amount of the two “fruit” NCCs in the fruit peelings was found to correlate roughly with the apparent fruit ripening and with the disappearance of chlorophyll. However, it corresponded to only about 10% of the estimated original chlorophyll in the fruit skin.⁵⁹ In each of the two fruit, as well as in the pear leaves, the more polar NCC (e.g. *Pc*-NCC-1) is derived (in a formal sense) from the less polar one (e.g. *Pc*-NCC-2) by glucosylation of the hydroxyl-ethyl side chain. As mentioned, the two “fruit” NCCs were also identified with “leaf” NCCs. This structural identity of NCCs from ripe fruit and from de-greened leaves clearly strengthens the long-held view, that degreening in senescent leaves and in ripening fruit show similar traits of Chl breakdown (see e.g. ref. ⁶⁰). The color changes in ripening fruit and in leaf senescence in higher plants are thus accompanied by a remarkably common biochemical path of chlorophyll breakdown.⁵⁹

In the peelings of the yellow pear, the two NCCs *Pc*-NCC-1 and *Pc*-NCC-2 were present in a total amount of about $6.7 \mu\text{g g}^{-1}$, corresponding to about 10% of the chlorophylls extracted from the peel of a green unripe pear. However, also the peeled main body of a ripe pear was indicated to contain the two *Pc*-NCCs (see Fig. 3). The two NCCs, *Pc*-NCC-1 and *Pc*-NCC-2, were considerably more abundant in the fruit flesh near the skin (about $1.2 \mu\text{g g}^{-1}$) than in a sample from an inner layer ($<0.2 \mu\text{g g}^{-1}$). The whole pear (of about 290 cm^3 volume) was indicated to contain about 300 mg of NCCs, coming up for about 7% of the estimated amount of Chls in a green unripe pear of the same size.⁵⁹ Senescent leaves of the pear tree also contained *Pc*-NCC-1 and *Pc*-NCC-2 (i.e. the two NCCs also available in the fruit). Their estimated amounts of about 29 mg cm^{-2} (*Pc*-NCC-2) and $4.9 \mu\text{g cm}^{-2}$ (*Pc*-NCC-1), respectively, in the de-greened leaves came up for over 70% of the Chls analyzed in fresh green leaves.⁵⁹

4. Colorless chlorophyll catabolites as antioxidants

The notorious tendency of NCCs to degrade to “rust” colored compounds in the presence of oxidants, induced us to test their activity as antioxidants. Specifically, *Pc*-NCC-2, the less polar of the two NCCs from fruit,⁵⁹ was tested in a standard auto-oxidation experiment.⁶¹ The presence of *Pc*-NCC-2 inhibited the formation of hydro-peroxides of linoleic acid strongly, as monitored as a function of time and of the concentration.⁵⁹ The effect of added *Pc*-NCC-2 was only slightly inferior to that of bilirubin.⁶¹ The NCCs have thus been shown to be remarkable antioxidants. Chl breakdown thus provides tetra-pyrrolic compounds naturally, that may play a more active part during fruit ripening and plant senescence. In this respect it may be important that NCCs are first formed in the vacuoles, which appear to only slowly develop leakiness during senescence.⁵⁸ Indeed, the tetra-pyrrolic NCCs are structurally related to bilirubin, a tetra-pyrrolic product from heme breakdown.⁶² Bilirubin has been shown not only to be a remarkable antioxidant, but also to be a cytoprotective component, relevant in the reduction of coronary heart diseases, of retinal damage and of cancer mortality in mammals.⁶³

5. Conclusions and outlook

In the last twenty years, the elucidation of Chl breakdown in higher plants has made significant progress and, gradually, Chl catabolism has lost a major portion of its “enigmatic” nature.^{4,5,26} Indeed, subsequent to the loss of the Mg-ion from Chlide *a*, the early dedicated step in the Chl degradative path, an oxygenolytic cleavage of the porphyrinoid macrocycle of Pheo *a* is the step that leads to loss of the green color. In higher plants, the oxygenolytic cleavage occurs with Pheo *a* as a specific substrate for a membrane-bound and largely senescence-induced mono-oxygenase. Clearly, the result of this primary ring cleavage reaction and of the subsequent steps can be envisaged as the rapid conversion of the chromophore of a photoactive and intensely colored chlorin into that of a colorless tetrapyrrole with de-conjugated hetero-cyclic rings. A well-controlled and sequentially operating enzymatic machinery appears as being characteristic of the degradation of the Chls and Chl breakdown in senescent leaves has been considered a detoxification process.⁶ In support of this view, the absence of activities of the oxygenase PaO²⁵ or of the presumed reductase of RCC (RCCR),³⁷ was found to correlate with the observation of necrotic lesions in *A. thaliana*, when grown in the presence of light, and the corresponding enzymes have been linked with the “accelerated cell death” genes, *acd-1* and *acd-2*.^{64,65} The intermediary catabolites, Pheo *a* and (enzyme bound) RCC, were indeed indicated to be photo-sensitizers: by binding (and transforming) these phototoxic tetrapyrroles as their substrates, the two enzymes were suggested to help protect the senescent plant.³⁷

Important parallel work has also revealed the structures of Chl catabolites from a green alga and from marine organisms. The red pigments from the green alga (*Chlorella protothecoides*) were determined to be linear tetrapyrroles with the same basic skeleton as found in the colorless chlorophyll catabolites from plants.³⁰ These red tetrapyrroles are thus also correlated to the Chls by an oxygenolytic cleavage of the macroring at the northern *meso*-position.²⁹ However, the red catabolites from the alga were found to be derived from Chl *a* as well as from Chl *b*.²⁹ In contrast, linear tetrapyrroles from marine organisms (from krill and the dinoflagellate *Pyrocystis lunula*) are of a differing structural type and relate to the Chls by an oxidative opening at the western *meso*-position of the porphyrinoid macroring.^{66,67}

In various leaves, such as those of *Brassica napus*, NCCs were found to account largely for the Chl broken down, so that in senescent leaves of the higher plants NCCs appear to constitute the “final” chlorophyll breakdown products.⁴⁹ While NCCs have been considered as detoxification products⁶ it is unclear, as yet, whether these colorless tetrapyrrolic remnants of the Chls would not have a further function and Chl breakdown could play a more active role in the plants. Indeed, NCCs are amphiphilic, low molecular weight compounds and effective antioxidants, which are found not only in the senescent leaves, but also in fruits (peelings and other parts of the ripe fruit). These findings may induce new directions in plant biological research dealing with Chl breakdown, and the possible later fate of the Chl catabolites, beyond the stage of leaf senescence, deserve more attention. Indeed, evidence has been provided for the formation of substituted imides in senescent leaves (which are considered to be mono-pyrrolic cleavage products of Pheo *a*).⁶⁸

On the other hand, the identification of “fruit” NCCs and their availability in ripe fruit also calls for attention as to their possible physiological relevance in humans and higher animals. Plant-derived components are generally recognized as beneficial constituents of human nutrition.⁶⁹ Perhaps, the identification of de-greened catabolites of chlorophyll as so far overlooked components of fruit is of particular relevance. Whereas Chls are considered to be phototoxic and are assumed not to be absorbed *via* the intestinal tract,⁷⁰ NCCs, natural products of chlorophyll breakdown, are tetrapyrrolic antioxidants and candidates for having

important physiological properties. The occurrence of NCCs in fruit might thus even give a new turn⁷¹ to the meaning of the saying: “An apple a day keeps the doctor away”.

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Biography

Bernhard Kräutler, Professor of Organic Chemistry and Head of the Centre of Molecular Biosciences at the University of Innsbruck. Interests in molecular life sciences and molecular engineering; current research topics: chlorophyll breakdown in leaves and fruit; chemical biology of vitamin B12 and B12-binding macromolecules; functionalized fullerenes and porphyrins.

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Fig. 1. Satellite images of Europe, color coded according to the Vegetation Index and recorded in March, June and October 2000 (made available by Deutsches Fernkundungs-Datenzentrum (DFDZ), Oberpfaffenhofen, Germany).

Scheme 1.

Overview of chlorophyll breakdown in senescent higher plants.^{5, 12} The Chls (Chl *a* or Chl *b*) are degraded *via* pheophorbide *a* (Pheo *a*), “red” chlorophyll catabolite (RCC), the primary “fluorescent” chlorophyll catabolites (pFCCs) to “nonfluorescent” chlorophyll catabolites (NCCs, such as *Hv*-NCC-1, for structural details see Scheme 4).

Scheme 2.

Electrochemical reduction of the red chlorophyll catabolite (RCC) leads to the “primary” fluorescent catabolites, pFCC and *epi*-pFCC, which also result from enzymatic reduction of the elusive RCC by RCC-reductase. Yellow products are also formed as isomers of the pFCCs.^{38, 39}

Scheme 3.

Non-enzymatic isomerization of pFCC to the “primary” NCC (pNCC) and of *epi*-pFCC (*Ca*-FCC-2) to the *epi*-pNCC (*Cj*-NCC-2); the stereochemical assignment in natural NCCs is derived from the suggested isomerization mechanism.^{39, 41}

Compound	R ¹	R ²	R ³	Ref.
<i>Hv</i> -NCC-1	OH	CH ₃	CH(OH)CH ₂ OH	[4, 13]
<i>Cj</i> -NCC-1	OH	CH ₃	CH=CH ₂	[41, 47]
<i>Cj</i> -NCC-2	H	CH ₃	CH=CH ₂	[41]
<i>Bn</i> -NCC-1	O-Mal	H	CH=CH ₂	[45, 46]
<i>Bn</i> -NCC-2	O-β-Glc	H	CH=CH ₂	[46]
<i>Bn</i> -NCC-3	OH	H	CH=CH ₂	[46]
<i>Bn</i> -NCC-4	H	H	CH=CH ₂	[51]
<i>So</i> -NCC-1	OH	H	CH(OH)CH ₂ OH	[42, 48]
<i>So</i> -NCC-2	OH	CH ₃	CH(OH)CH ₂ OH	[42]
<i>So</i> -NCC-3	OH	H	CH=CH ₂	[42]
<i>So</i> -NCC-4	OH	CH ₃	CH=CH ₂	[42]
<i>So</i> -NCC-5	H	CH ₃	CH=CH ₂	[42]
<i>Nr</i> -NCC-1	O-β-(6'-O-Mal)Glc	CH ₃	CH=CH ₂	[43]
<i>Nr</i> -NCC-2	O-β-Glc	CH ₃	CH=CH ₂	[43]
<i>Zm</i> -NCC-1	O-β-Glc	CH ₃	CH(OH)CH ₂ OH	[44]
<i>Zm</i> -NCC-2	O-β-Glc	CH ₃	CH=CH ₂	[44]
<i>At</i> -NCC-1	O-β-Glc	H	CH=CH ₂	[51]
<i>At</i> -NCC-2	OH	H	CH=CH ₂	[51]
<i>At</i> -NCC-4	O-β-Glc	CH ₃	CH=CH ₂	[51]
<i>At</i> -NCC-5	H	H	CH=CH ₂	[51]
<i>Pc</i> -NCC-1	O-β-Glc	CH ₃	CH=CH ₂	[59]
<i>Pc</i> -NCC-2	OH	CH ₃	CH=CH ₂	[59]
<i>Ms</i> -NCC-2	OH	CH ₃	CH=CH ₂	[59]

Abbreviations: Mal = malonyl; Glc = glucopyranosyl

Scheme 4.

Constitution of nonfluorescent Chl catabolites (NCCs) from higher plants and separate constitutional formula of the exceptional *At*-NCC-3 from *A. thaliana*.¹⁶

Fig. 2.
Left: fall colors developing on a tree in Kyoto, pictured on December 9, 2007. Right: yellow (ripe) “Golden Delicious” apples, as obtained from the farmers market in Innsbruck.⁵⁹

Fig. 3. Distribution of NCCs in ripe pear. Analysis of total amounts of NCCs (*Pc*-NCC-1 and *Pc*-NCC-2) in pear peelings, in the outer, central and inner part of the flesh of the pear (data given as µg g⁻¹ wet weight).⁵⁹