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Catabolism of Tetrapyrroles

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Abstract. The enzymatic degradation of naturally occurring tetrapyrrolic pigments (heme, chlorophylls, and vitamin B_{12}) is shortly reviewed.

1. Introduction

In contrast to the enormous amount of work accomplished by chemists in the elucidation of biosynthetic pathways of secondary metabolites (terpenes, steroids, alkaloids, among others), only a few attempts have been made until now to understand the mechanisms of their degradation in living organisms. A possible reason for this fact is the irrational association of degradation (catabolism: greek $\kappa\alpha\tau\alpha$= down) with decay and, thus, with unattractive dirty colors and unpleasant odors. The other reason is probably the meaning that degradation processes occur outside the cell rather by uncontrolled chemical reactions which are achieved by environmental factors such as oxygen, water, temperature, *etc.*, than by catabolic (*i.e.*, enzyme-catalyzed) processes. Thus, with exception of proteins, fats, and sugars, which are subjected to a continuous degradation and rebuilding in the living organism, and of nucleic acids, the well-documented catabolism of which is treated in all text books on biochemistry, almost nothing is known concerning the fate of most of the components of living organisms which are liberated from the organelles in the course of morphological differentiation of their cells. Actually, as the main pathways of biogenesis, by which virtually all natural compounds known so far are synthesized, have been already elucidated, it may be anticipated that the study of catabolic processes will attract the interest of more chemists and biochemists in the near future.

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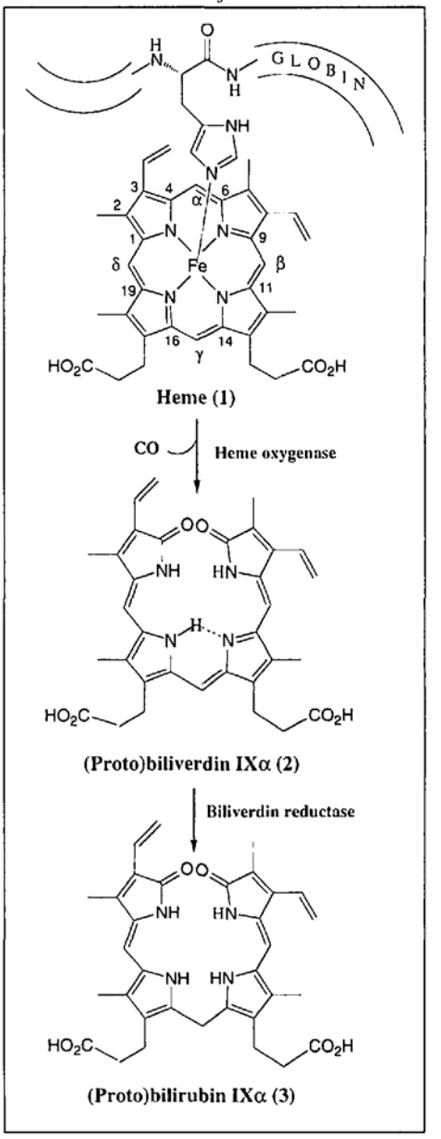
2. Heme Catabolism

It has been known for over half a century that heme, the oxygen-carrier molecule associated with the blood pigment hemoglobin, is converted in animal cells to bile pigments, which are excreted with the faeces and urine [1]. Owing to its significance in human medicine, the catabolism of the heme released upon the degradation of hemoglobin and related chromoproteins, like myoglobin and cytochromes, has been intensively studied during the past decades. Particularly, in recent years the application of modern spectroscopic, chromatographic, and isotopic techniques as well as the development of methodologies which made the most important bile pigments like biliverdin [2], bilirubin [3], phycocyanobilin [4][5], phycoerythrobilin [6][7], and phytochromobilin [8] accessible by chemical synthesis have enabled to elucidate a number of central aspects of the problem but made others, of course, still more puzzling.

Ordinarily, heme degradation occurs in the phagocytic cells of the bone marrow, spleen, and liver but can probably occur in macrophages in other tissues as well. The catabolic process is initiated by the oxidative scission of the protoporphyrin IX macrocycle, the chromophore of heme (**1**), at the α-methene bridge (C(5)) producing CO and an unstable Fe^{III} complex. The latter loses the metal ion to yield the green pigment protobiliverdin IXα (usually abbreviated to biliverdin (**2**)), which is excreted by birds and amphibia, as the final product of heme catabolism (*cf. Scheme 1*). The iron is recovered in the protein called ferritin and can be reutilized for the biosynthesis of new heme molecules. As biliverdin (**2**) has been recognized to be a precursor in the biosynthesis of phycobilins [9], a similar pathway is probably followed for the biosynthesis of this class of light-harvesting chromophores

Scheme 1. *Catabolism of Heme in Mammals*



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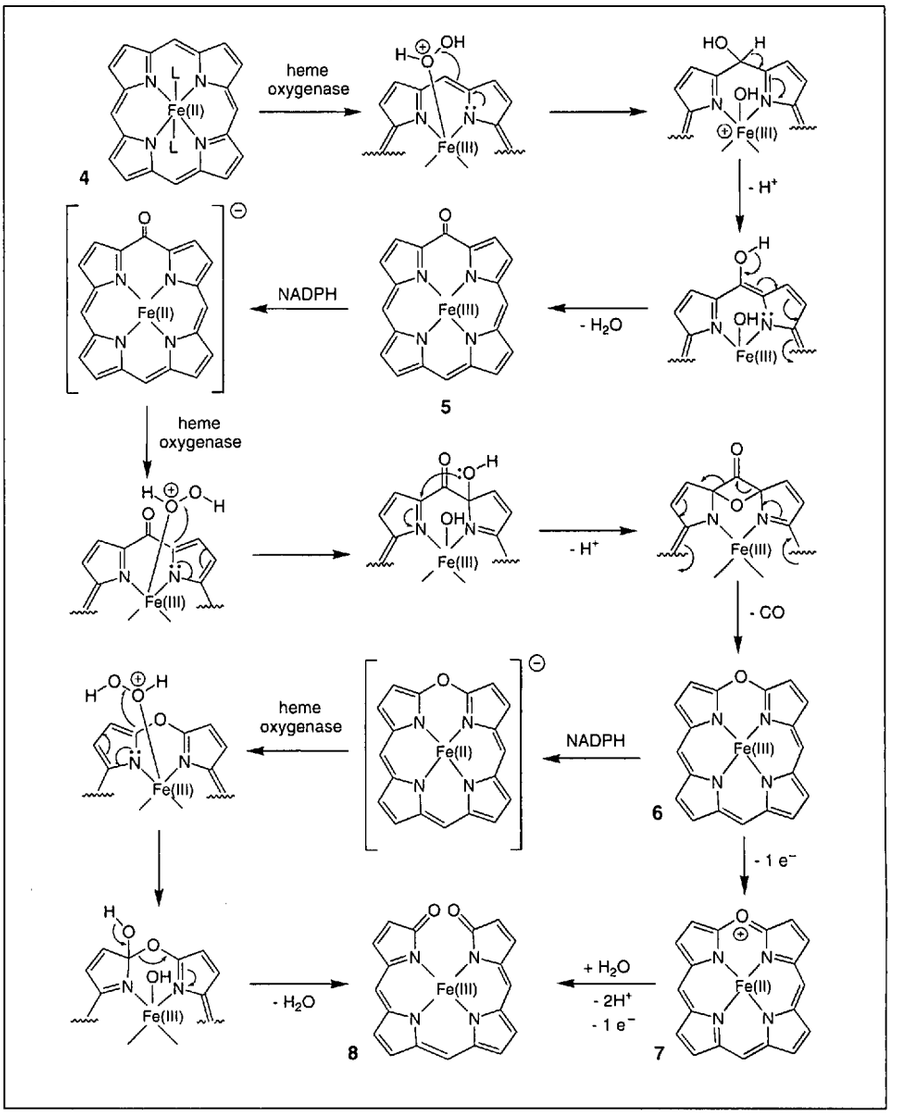
which are associated with photosynthesis in cyanobacteria and in two groups of eukaryotic algae, namely the red algae and the cryptomonads.

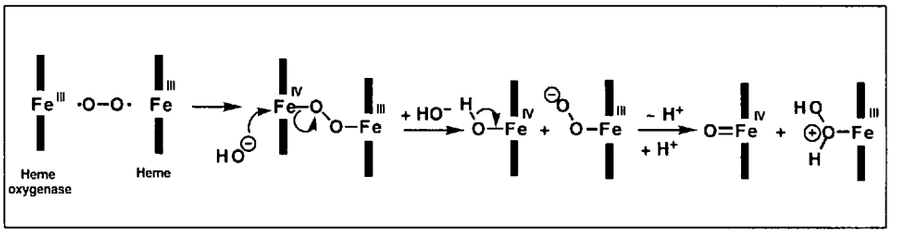
Moreover, the occurrence of bile pigments in arthropoda (insects, crabs, shrimps, and barnacles), mollusca (squids, clams, snails, *etc.*), as well as in egg shells, fins, and scales has been recognized for a long time. Thus, *e.g.*, biliverdin (**2**) is responsible for the green color of the praying mantis (*Mantis religiosa* L.); one of its regioisomers, pterobilin (protobiliverdin IXγ), has been isolated from the wings of certain butterflies, another regioisomer (protobiliverdin IXδ) from the ovaries of the marine snail *Turbo cornutus* [10]. At least for insects of two different taxonomic categories (*Mantis religiosa* L., which feeds on other insects, and *Locusta migratoria* L., a herbivore), *de novo* biosynthesis of endogenous biliverdin has been demonstrated [11]. As insects do not contain heme in their hemolymph, either a biosynthetic pathway peculiar to them or degradation of cytochromes may be taken into consideration to explain the presence of biliverdin (**2**) in their tissues. In humans and mammals, enzymatic reduction of biliverdin, which is catalyzed by the NADPH-dependent enzyme biliverdin reductase, yields yellow bilirubin (strictly speaking protobilirubin IXα (**3**)). Actually, catabolism of heme is the only known source of bilirubin (**3**), of which 300–500 mg are produced daily in adult human beings. Once formed, **3** is esterified with various sugars (mainly glucuronic acid) in the liver, and these water-soluble esters (so-called bilirubin conjugates) are excreted *via* the bile duct into the gut where they undergo further reductive transformations to urobilinoids by the intestinal flora, before their final excretion in the faeces.

On the basis of the experimental evidence available at present, *Scheme 2* is proposed as an outline of the chemical steps involved in the conversion of heme to biliverdin *in vivo* (*cf.* [9]). The mode of action of microsomal heme oxygenase may be analogous to the commonly accepted mechanism of the autoxidation of ferrous porphyrins *in vitro*, as represented in *Scheme 3* (*cf.* [12]). Thus, the suggested pathway explains the requirement for molecular oxygen and provides an explanation for the need for NADPH and a functioning microsomal electron-transport system. Moreover, it is consistent with the finding that the microsomal heme oxygenase is inhibited by CO as well as by azide and cyanide ions, which may interact with the ferrous heme-protein complex. Interestingly, heme oxygenase is inactive against protoporphyrin IX, suggesting that the central Fe-atom of heme is essential for the activity of the enzyme.

Scheme 2. *Hypothetical Mechanism for the Catabolism of Heme to Biliverdin IX*α in vivo. Side chains and apoprotein have been omitted for clarity.



Scheme 3. *Possible Pathway of the Mode of Action of Heme Hydrogenase* (side-view of the porphyrin iron-complex chromophore represented by a bar)



It is known that pyridino-ferrous-porphyrin complexes (so-called pyridine hemochromes (**4**), L = pyridine) are readily oxidized and cleaved to give bile pigment precursors by mild treatment with oxygen in the presence of an excess of a suitable reducing agent (usually ascorbic acid). This reaction, named 'coupled oxidation' because it requires both oxygen and a reductant, was thought in the past to be a suitable model for the enzymatic breakdown of heme, in which basic amino-acid residues of the apoprotein replace pyridine as stabilizing ligands for ferrous iron and NADPH acts as the reducing agent. However, after the origin of the O-atoms

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present in the two lactam groups of the biliverdin molecule has been soundly established by a series of outstanding experiments using oxygen isotopes by *Brown* and coworkers [13–15], the mechanism of the enzymatic reaction appears to be more complex. According to these investigations, the cleavage of the protoporphyrin macrocycle occurs by a 'two-molecule mechanism', *i.e.*, the terminal lactam O-atoms in biliverdin (**2**) are derived from *two* different O_2 molecules. Thus, the formation of **2** by a hydrolytic step involving the insertion of an O-atom from the medium does not occur in the heme-oxygenase system. This reaction pathway (**7** → **8**) has been observed, however, in the coupled oxidation of hemoglobin *in vitro*, in which the formed biliverdin contains one O-atom derived from H_2O and one from molecular O_2 (*cf. Scheme 2*). Nevertheless, in a hydrophobic environment, an intermediate of the oxaporphin type **6** may be reduced by NADPH to the corresponding ferrous complex, which in turn would be able to react with O_2 in the same manner as formulated for the original hemochrome complex, to yield ultimately a ferric complex **8** of biliverdin. On hydrolytic decomposition of the latter, biliverdin (**2**) and a Fe^{III} ion are liberated.

According to the reaction sequence outlined in *Scheme 2*, the transformation of heme into biliverdin (**2**) requires *three* O_2 molecules. The first intermediate formed in the heme-cleavage process is probably the oxyferriheme species (*i.e.*, an iron-oxophlorin chelate **5**). The formation of this intermediate involves a mixed-function oxygenase type of reaction which results in the regioselective hydroxylation of one of the methene bridges of the tetrapyrrole macrocycle. Thus, which isomer of biliverdin (**2**) is produced, is determined at the very first stage of the degradation process. It is interesting that the edge of the heme ring that is preferentially attacked in the coupled oxidation of heme proteins (the α bridge) is the most deeply buried within the hydrophobic core of the protein [16]. The further transformation of oxyheme to biliverdin is a complex one, since the process involves incorporation of two additional O-atoms, loss of the Fe-atom, and loss of the α-methene C-atom as CO.

On the other hand, the finding that biliverdin (**2**) is a biogenetic precursor of phycocyanin [9] (*via* a bilirhodin-like intermediate? [17]) gives rise to the question about the mechanism of the biosynthesis of this photoreceptor molecule in organisms (the cyanobacteria) which populated the earth *before* oxygen was present in the atmosphere in appreciable amounts. Therefore, it is particularly interesting, in this connection that bile pigments (so-called bactobilins) are formed from uroporphyrins I and III by cell extracts of *Clostridium tetanomorphum* under anaerobic conditions [18][19].

In summary, the mechanism of ring cleavage of heme is highly unusual. The coupled oxidation reaction is unique in porphyrin chemistry in that it permits this particularly stable macrocycle to be easily opened under extremely mild conditions. Furthermore, decarbonylation at room temperature in the dark is a rare reaction in organic chemistry, specially in the condensed phase. Because of the lack of appropriate chemical models for these unusual reactions, the pathway suggested on *Scheme 2* remains speculative. None the less, it provides a valuable approach to the better understanding of the mechanisms of degradation of other tetrapyrrolic natural compounds.

As mentioned before, biliverdin (**2**) produced upon cleavage of heme in the mammalian liver is reduced to bilirubin (**3**) by biliverdin reductase, before it is conjugated and excreted in the bile. The small amount of **2** frequently detectable in mammalian bile is probably formed by oxidation of conjugated bilirubin in the bile ducts, in the gallbladder, or during the collection process. In contrast, avian, reptilian, and amphibian bile contains predominantly biliverdin (**2**), but most of this pigment appears to be unconjugated, since biliverdin conjugates have not been identified with certainty. Most recently, bilirubin-10-sulfonic acid has been identified as the end product of heme catabolism in the gallbladder bile from adult bullfrogs (*Rana catesbiania*) [20]. Although biliverdin (**2**) appears to be reduced to bilirubin (**3**) in most fishes, it is interesting that some species have high concentrations of **2** in the serum. It is not clear why **3** rather than **2** is the end product of heme catabolism in mammals. In contrast to biliverdin (**2**), bilirubin (**3**) is cytotoxic and not readily excreted, necessitating the development of a complex series of conjugation and transport functions for its removal and elimination. The biological rationale for the evolution of an apparently ubiquitous, presumably substrate-specific, and regioselective biliverdin reductase remains obscure.

The transport of bilirubin **3** from its tissue sites of formation to its eventual excretion in the intestinal tract can operationally be characterized by five phases: *1*) transport in the plasma, *2*) transfer into the hepatocyte, *3*) hepatic conjugation, *4*) biliary excretion, and *5*) transport and elimination in the intestine. Bilirubin (**3**) released into the plasma is firmly bound to plasma albumin, which serves as its carrier protein. The mechanism by which unconjugated bilirubin is removed from albumin and transmitted into the liver cells is unknown. Indeed, it is not even known with certainty, whether the hepatic uptake of **3** is an active or a passive process. In the liver, **3** undergoes ester formation with a variety of carbohydrate moieties and possibly with other conjugating groups that render the pigment water-soluble. The so-called conjugation of the bile pigment is catalyzed by membrane-bound enzymes localized primarily in the endoplasmic reticulum of the liver cell, the carbohydrate moieties being transferred from their respective uridine diphosphate nucleotides. Conjugation of **3** appears to be essential for biliary excretion, since normally only minute quantities of unconjugated bilirubin occurs in mammalian tissue. The hepatic excretory mechanism for conjugated bilirubin is poorly understood, but it is known that it is shared by diverse groups of endogenous compounds that are secreted into the bile. In normal human bile, bilirubin mono- or di-β-glucuronoside constitute the predominant bile-pigment fraction. Conjugation of bilirubin (**3**) restricts back-diffusion of the excreted pigment across the intestinal mucosa, which is essential for its efficient elimination in the alimentary canal. As a matter of fact, **3** does not appear to be resorbed in significant amounts from the human intestinal tract. In the distal portion of the intestine, the excreted pigment undergoes a series of stepwise reductions to urobilinoid chromogens catalyzed by bacterial enzymes. Removal of the conjugating carbohydrate functions probably also occurs in the intestine, but it is not known whether this takes place before, during, or after reduction. The biological rationale for the fecal excretion of bile pigment in the form of urobilinoids rather than as conjugated bilirubin remains obscure. It is possible that formation of these chromogens is of no consequence to the host and is purely incidental to the presence of microorganisms in the intestine. It is noteworthy, however, that at least one of these intermediates, urobilinogen, is reabsorbed from the gut to the portal circulation and is reexcreted by the liver (so-called enterohepatic circulation). Part of the urobilinogen escapes to the systemic circulation and is excreted by the kidney.

Urobilinoid compounds have also been found in the phycobilisomes, the supramolecular assemblies attached to the outer

surface of the thylakoid membrane which contains the chlorophyll-binding proteins and the reaction centre of the photosynthetic apparatus in cyanobacteria and some microalgae [21]. Their biosynthetic origin is so far unknown.

The bacterial reduction of bilirubin to urobilinoids was studied extensively by *Watson* [22]. The most representative derivatives are urobilinogen (properly: mesourobilinogen (**9**)) and (meso)stercobilinogen (**10**), which upon mild reoxidation by air yield urobilins (*e.g. d*-(meso)urobilin (**11**)) and stercobilins (*e.g. l*-(meso)stercobilin (**12**)), respectively (*cf. Fig. 1*). Although intermediate pigments in the reduction pathway have been found (*e.g.* an optically active *d*-monovinyl-urobilin, the structure and absolute configuration of which was established by synthesis [23]), the mechanism of the bacterial reduction of bilirubin (**3**) is not completely understood; apparently it depends on the strain of bacteria and conditions in the gut.

The microbiological reduction of bilirubin (**3**) is stereoselective. The occurrence of an optically inactive mesourobilin ('*i*-urobilin') in faeces may be explained by racemization of the corresponding optically active compound. In fact, owing to the acidity of their H-atoms attached to the stereogenic centres, urobilins are prone to isomerization both in basic and acidic medium. In the latter, a mixture of diastereoisomeric bilirhodines are formed, the structure and relative configuration of which has been elucidated by synthesis [24][25].

Optical activity of the urobilinoids was first observed by *Fischer et al.* [26] with laevorotatory stercobilin; a dextrorotatory urobilin was discovered later by *Schwartz* and *Watson* in human fistula bile [27]. Uro- and stercobilins are characterized by their extremely high optical activity (*e.g.* $[\alpha]_D = +5000$ for natural *d*-mesourobilin, $[\alpha]_D = -4000$ for *l*-stercobilin). The optical activity of urobilinoids has been explained by the presence of an inherently dissymmetric dipyrrin chromophore, the helicity of which is conditioned by the absolute configuration of the asymmetric C(4)- and C(16)-atoms [28]. Only recently, however, the absolute configurations of two urobilinoid bile pigments could be assigned unequivocally by X-ray diffraction analysis of their synthetic precursors [29][30], so that a relationship between helicity of the chromophore and sign of the *Cotton* effect in this class of compounds can be now established experimentally [31].

As a matter of fact, biliverdin (**2**), which is intrinsically an achiral compound, occurs in solution in helical conformations, which are dissymmetric. However, as the energy barrier for the mutual transformation of enatiomeric biliverdin conformers is rather low (10 ± 0.5 kcal/mol at 200 K) [32], this bile pigment, in contrast to the urobilinoids, is devoid of any optical activity. Bound to proteins, however, biliverdin derivatives may occur either as chiral helical-shaped molecules – *e.g.* in the wing pigment of the cabbage butterfly (*Pieris brassicae* L.) [33] – or as 'stretched' chromophores in the so-called phycobiliproteins. It has been shown experimentally [34][35] that the enhanced extinction of the visible-absorption band of the latter is caused by the presence of 'stretched' conformations of the chromophore, as originally suggested by theoretical calculations. Partially 'stretched' bile pigment chromophores result also from (*Z*) → (*E*) isomerization of the exocyclic C=C bonds [36][37]. This process has been studied intensively with bilirubin as a substrate owing to its relevance in the treatment of human hyperbilirubinemia by phototherapy. In the case of biliverdin and its derivatives, however, this kind of isomers have not yet been found in nature.

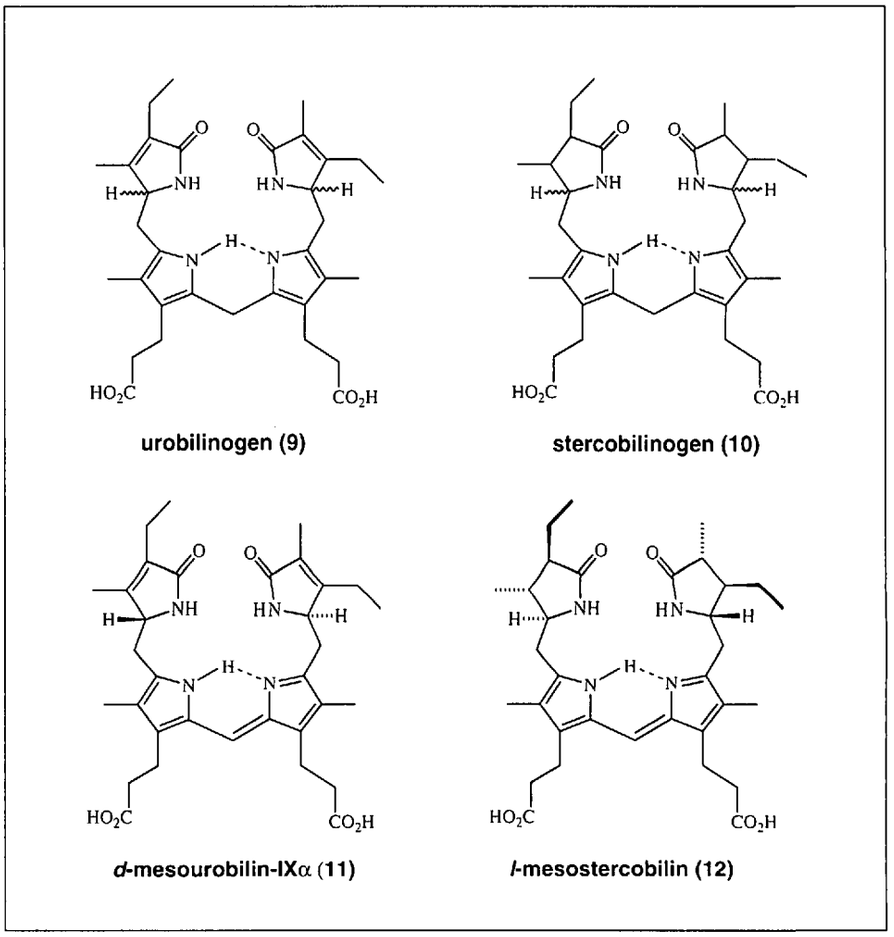


Fig. 1. *Principal bile pigments formed by bacterial reduction of bilirubin in the intestine of mammals*

3. Chlorophyll Catabolism

Besides fucoxanthin, the characteristic pigment of many marine algae and, without question the most abundant natural carotenoid, chlorophyll is the natural pigment that is present in the greatest amounts on earth (at a rough estimate some 10^9 t of chlorophyll are destroyed and re-synthesized each year on land and in the oceans).

In some aspects heme (**1**) and chlorophyll are intimately related natural compounds, in another sense they are antithetic. Thus, although, in contemporary organisms, protoporphyrin IX is a biosynthetic precursor of chlorophyll [38], it is generally agreed that photosynthetic prokaryotes appeared on the earth *before* aerobic live would be able to develop, *i.e.*, the function of chlorophyll *a*, as an ubiquitous photosynthetic pigment in nature, preceded the evolution of hemoglobin, as the most common oxygen-carrier protein in eukaryotic cells. From this point of view, heme symbolizes the (most frequently destructive) heterotrophic metabolism, whereas chlorophyll enables living organisms to build up complex organic mole-

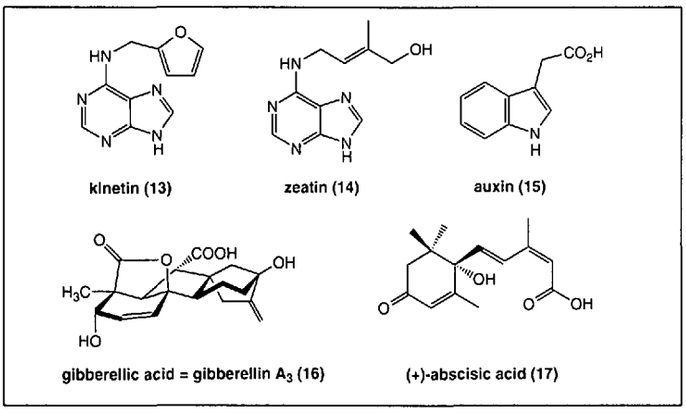


Fig. 2. *Some important plant hormones involved in senescence of plant tissues*

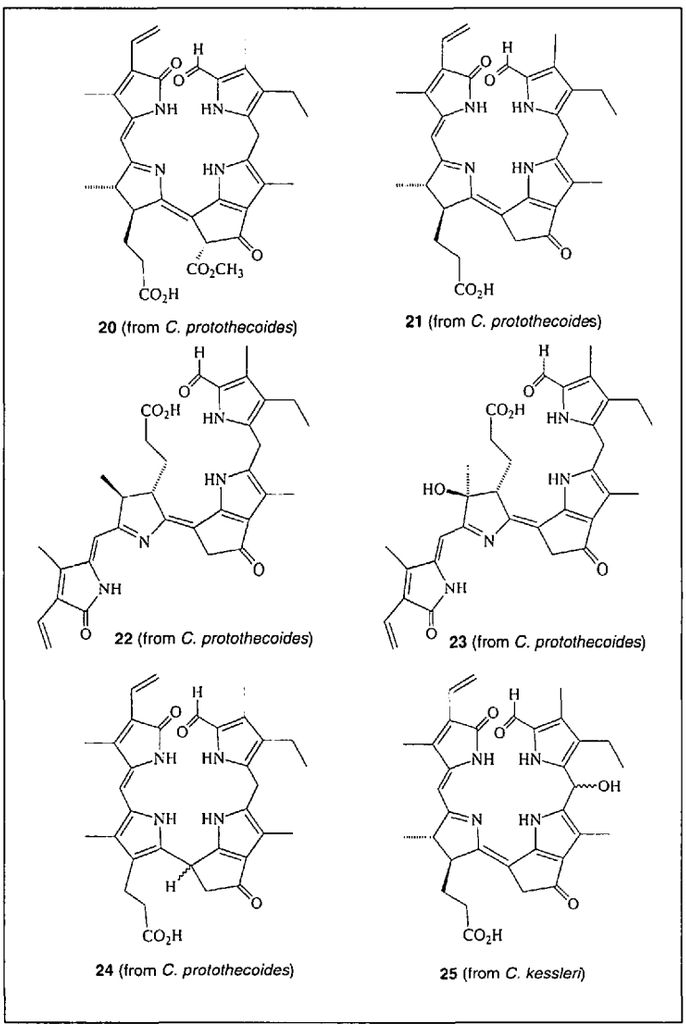


Fig. 3. *Chlorophyll a catabolites from* Chlorella *strains*

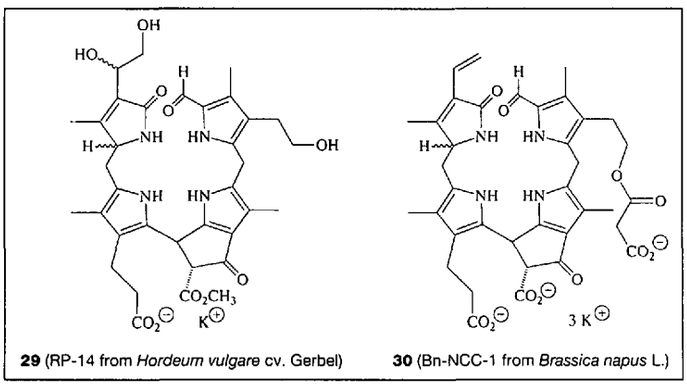
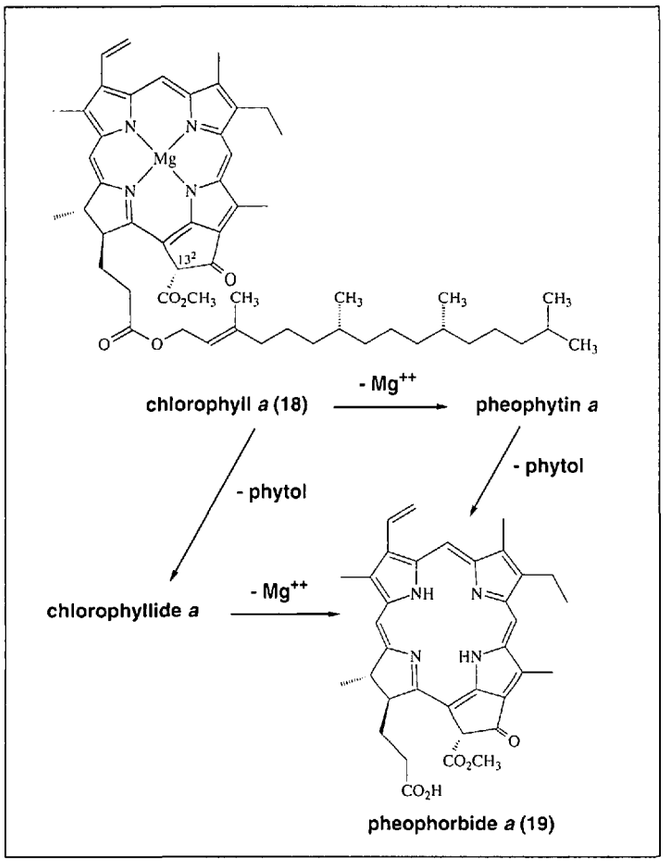


Fig. 4. *Chlorophyll a catabolites isolated from phanerogams*

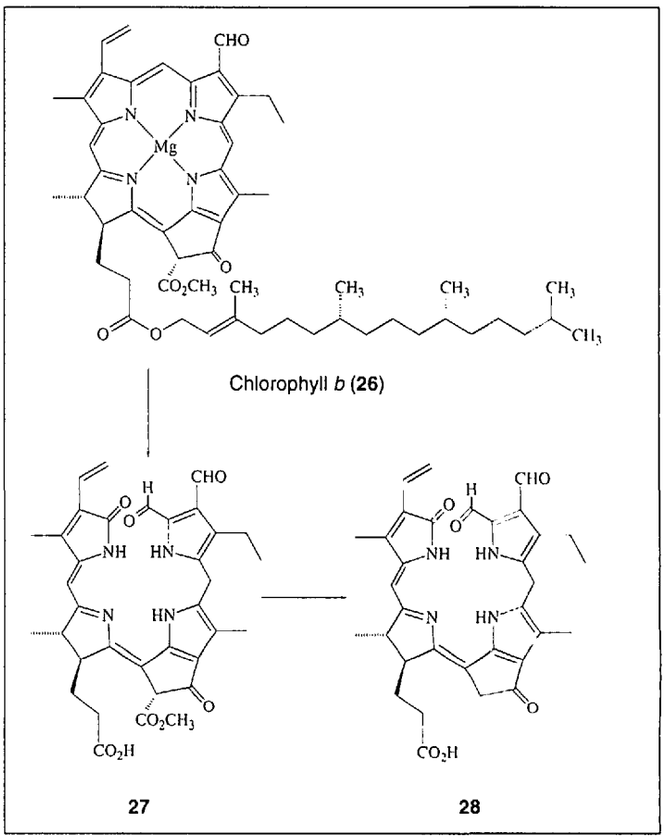
cules from CO_2 and H_2O. Thus, the psychological response (aggressiveness and appeasement) to the colors red and green in higher developed living creatures may be an atavism, which has its origin in the chemical structure of two natural pigments derived from the same biogenetic precursor.

It is all the more surprising that despite the fascination exerted by the yearly play of colors, which takes place in the plant kingdom at the beginning of the autumn in the nontropical countries, the natural fate of chlorophyll in the leaves turning yellow remained an enigma until four years ago [39]. Despite subtle experiments, which were carried out at the end of the last century to get insight in this phenomenon, the only relevant finding from these earlier studies, from the structural point of view, was the recognition that the yellow color of autumn leaves is due to carotinoids (mainly lutein = xanthophyll) which are already present in the plant cells before their senescence, and are not related to the breakdown of chlorophyll [40]. On the other hand, however, the regulation of senescence in plants has been object of a large number of investigations [41]. The prominent role played by some endogenous substances like ethylene, cytokinins (*e.g.* kinetin (**13**), zeatin (**14**), *etc.*), auxin (**15**), gibberellins (*e.g.* **16**), abscisic acid (**17**), and other plant hormones has been recognized, although the regulation mechanisms are until now only rudimentary understood (*cf. Fig. 2*). Moreover, several enzymes involved in the degradation of chlorophyll without affecting the integrity of the macrocycle, have been recognized in the past. Thus, chlorophyllase has long been known to catalyze the hydrolysis of phytol from chlorophyll (**18**) yielding chlorophyllide [42]. Removal of the complexed Mg^{II}-ion by magnesium dechelatase leads to pheophytin. Through the action of both enzymes pheophorbide (**19**) is formed (*cf. Scheme 4*). The corresponding pyropheophytin and pyropheophorbide, which lack the methoxycarbonyl group on C(13^2), have been found in photosynthetic organisms [43]. Both pigments have been characterized as chlorophyll catabolites in the digestive tract and in the faeces of terrestrial herbivores. On the other hand, a few natural pigments which probably proceed from cleavage of the chlorophyll macrocycle have been found in organisms which do not produce endogenous chlorophyll, like krill (*Euphausia pacifica*) [44], dinoflagelates (*Pyrocystis lunula*) [45], and insects (*Anasa tristis* Degger) [46]. Moreover, the presence of a red-fluorescent antiviral glyco-

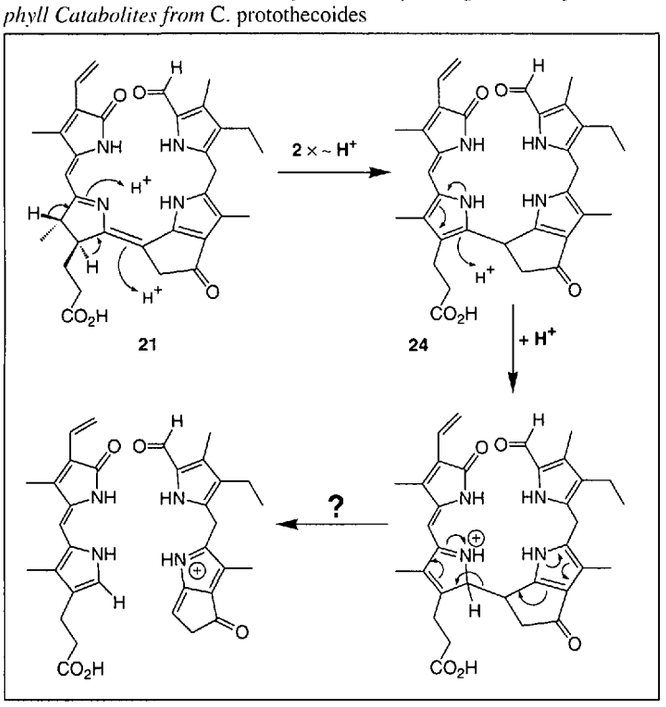
Scheme 4. *Primary Products of Chlorophyll Degradation* in vivo



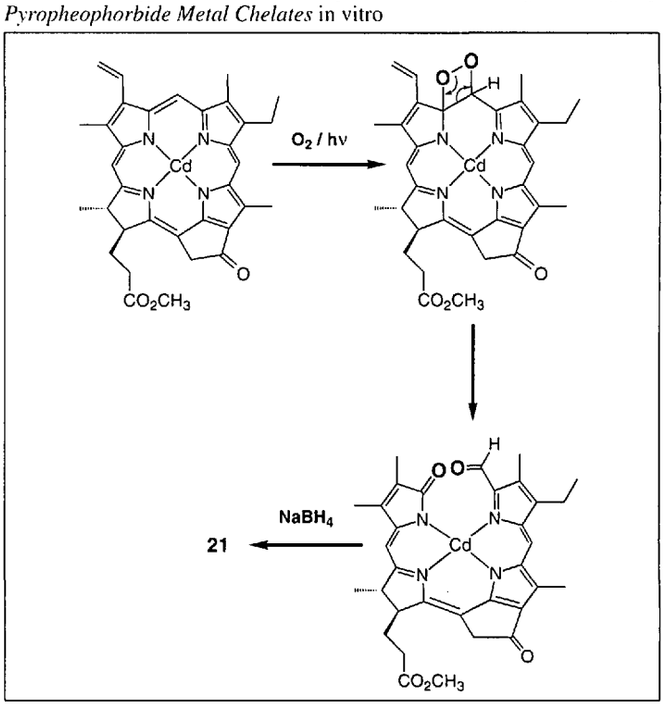
Scheme 5. *Chlorophyll* b *Catabolites Isolated from* Chlorella prototheco-ides



Scheme 6. *Possible Mechanism of the Proteolytic Degradation of Chlorophyll Catabolites from* C. prototheco-ides



Scheme 7. *Suggested Mechanism of the Oxidative Ring Cleavage of Pyropheophorbide Metal Chelates* in vitro



protein [47] in the digestive juice of silkworms (*Bombyx mori* L.) has been reported by Japanese authors [48]. Later work proved that *in vitro* this biliprotein is only formed in the presence of chlorophyll *a* and chlorophyllase, so that a transformation of chlorophyll *a* into the biliprotein may be inferred [49]. Feeding experiments carried out with ^{14}C-labelled chlorophyll *a*, which was prepared from pheophorbide by partial synthesis [50], revealed, however, a very low incorporation of ^{14}C in the biliprotein isolated from the digestive juice of the caterpillars; the main radioactive product being chlorophyllide *a*. Interestingly, up to 9% transformation of chlorophyll *a* into chlorophyll *b* in the intestine of the larvae could be demonstrated [51].

The occurrence of a chlorophyll oxidase which presumably transforms chlorophyll into the corresponding (*R*)- and (*S*)-13^2-hydroxy derivatives (allomerization) has been discussed [52]. As the corresponding nonenzymatic reaction occurs readily in the dark under the influence of air in methanolic solution, it must be born in mind that such compounds may be formed during the procedure of isolation from the organic preparations.

More recent investigations of the chlorophyll catabolism in phanerogams suggested that the degradative processes occurring in senescent leaves serve the reutilization of nutrients in other parts of the plant. This is particularly important regarding the nitrogen which is continuously displaced from senescent into growing organs until it is finally accumulated in the reserve proteins of storage tissues. Whether the four N-atoms of the chlorophyll molecule are reutilized in the same way is still unknown [53].

In 1991, two Swiss research groups succeeded for the first time, independently of each other, in the isolation and characterization of tetrapyrrolic chlorophyll *a* catabolites from dark-bleached excised primary leaves of barley (*Hordeum vulgare* cv. Gerbel) [54] and from the culture medium of the microalgae *Chlorella protothecoides* [55]. Shortly afterwards, the structure of the pigment **21** (*cf. Fig. 3*), isolated from the latter, was confirmed by X-ray diffraction analysis [56], as well as by partial synthesis starting from pyropheophorbide *a* [57]. The common feature of the isolated catabolites is the unexpected regioselectivity of the oxidative cleavage of the macrocycle at the C(4)=C(5) bond instead of the C–C bonds adjacent to the reduced pyrrole ring, as generally prognosticated before. Meanwhile, a related chlorophyll *a* catabolite has been found in the dicotyledon *Brassica napus* L. (rape) [58] (*cf. Fig. 4*), and a series of bilin derivatives, all of them resulting from cleavage of the chlorophyll macrocycle at the α-methene bridge, have been isolated from the culture media of *C. protothecoides* [59] and *C. kessleri* [60] (*cf. Fig. 3*). Moreover, two degradation products, **27** and **28**, of chlorophyll *b* (**26**) have been isolated from the culture medium of the former and characterized for the first time [61] (*cf. Scheme 5*).

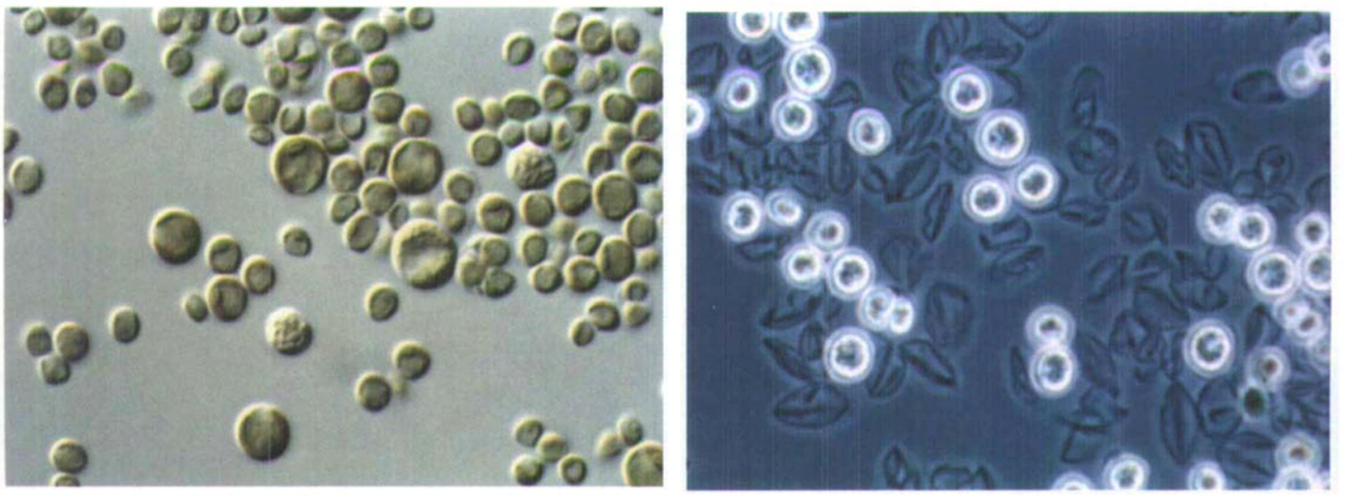


Fig. 5. *Some strains of the microalgae* Chlorella *can be stimulated experimentally to the formation of chlorophyll catabolites which are secreted into the culture medium.* The structures of these catabolites have been elucidated at the Institute for Organic Chemistry of the University of Fribourg for the first time. Growing (*left*) and bleaching cells (*right*) of *Chlorella kessleri*.

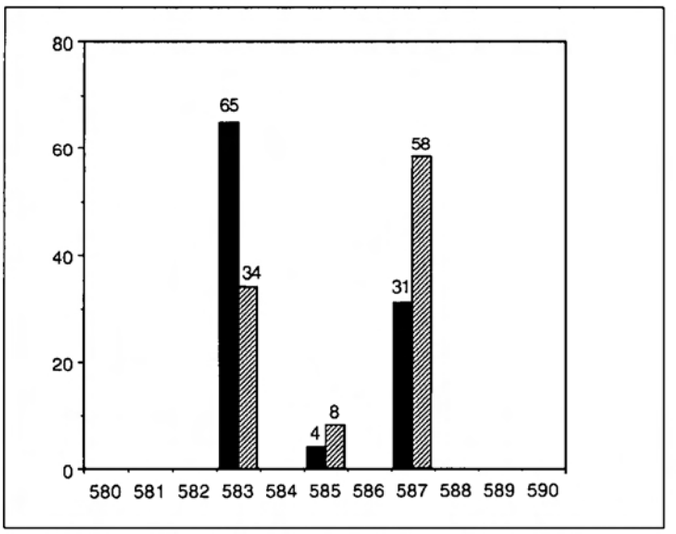


Fig. 6. *Isotopical distribution in the range of the molecular ion peak of* **21**, *obtained by photooxidation of methyl pyropheophorbide-Cd^{II} in an $^{18}O_2$-enriched atmosphere:* ■ *using $^{16}O_2/^{18}O_2$* (62 : 38); ▨ *using $^{16}O_2/^{18}O_2$* (31:69). Percent values have been determined by subtraction of the FAB-mass spectrum of unlabelled **21** from that of the labelled derivative.

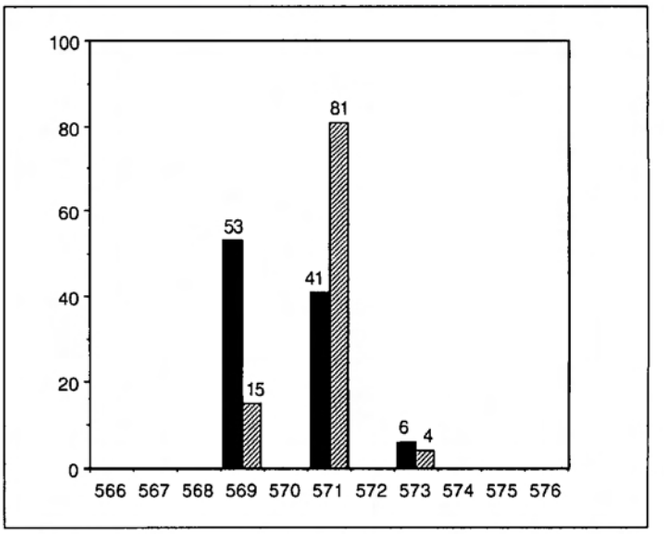


Fig. 7. *Isotopical distribution in the range of the molecular-ion peak of* **21**, *obtained* in vivo *in an $^{18}O_2$-enriched atmosphere:* ■ *using $^{16}O_2/^{18}O_2$* (55 : 45); ▨ *using $^{16}O_2/^{18}O_2$* (11:89). Percent values have been determined by subtraction of the FAB-mass spectrum of unlabelled **21** from that of the labelled derivative.

In contrast to the pigments excreted into the culture medium by *Chlorella* cells, the chlorophyll catabolites **29** and **30** isolated by *Matile et al.* from phanerogams are colorless compounds, which turn a rusty color only on exposure to air (*cf. Fig. 4*). Thus, it becomes clear why the primary degradation products of chlorophyll have been overlooked for such a long time. On the other hand, the present results point out that chlorophyll catabolism may involve several reductive steps which are absent in the degradation of heme. As it is well documented that polypyrrolic compounds in which the pyrrole rings are joined by methylene bridges are readily cleaved by protolysis [62], a further degradation of compounds like **21** should be a rapid process (*cf. Scheme 6*). Until now, however, the fate of the products formed after cleavage of the chlorophyll macrocycle is not known.

Decisive for the results of the Fribourg group was not only the knowledge about the earlier finding that, during the process of bleaching of *C. protothecoides* cells, which takes place when this green algae is grown in a medium rich in glucose but poor in nitrogen (*cf. Fig. 5*), a red pigment is excreted into the culture medium [63][64], but also the discovery that photooxidation of pyropheophorbide metal chelates *in vitro* yields similar products as those isolated from the culture medium of *C. protothecoides* [65], the regioselectivity of the ring cleavage being dependent on the complexed metal ion [66]. However, an investigation of the photooxidative ring cleavage of the Cd^{II}-chelate of pyropheophorbide *a in vitro* and of the enzymatic transformation of chlorophyll *a* into **21**, both in the presence of $^{18}O_2$, revealed that both transformations proceed by different mechanisms. As a matter of fact, the pattern observed in the range of the molecular-ion peaks of the photooxidation product, after reductive demetallation, agrees with the incorporation of two O-atoms proceeding from the *same* molecule (*cf. Fig. 6*). Most likely, therefore, the obtained formylbilinone derivative is formed *via* a dioxetane, resulting from the cycloaddition of a singlet oxygen molecule to the C(4)=C(5) bond of the substrate [67] (*cf. Scheme 7*). On the other hand, after bleaching of *C. protothecoides* cells in the presence of $^{18}O_2$, it could be proved by mass spectrometric analysis of the red bilin derivative **21** excreted into the culture medium that only the O-atom of the formyl group proceeds from molecular O_2, whereas the O-atom of the lactam carbonyl originates from H_2O [68] (*cf. Fig. 7*).

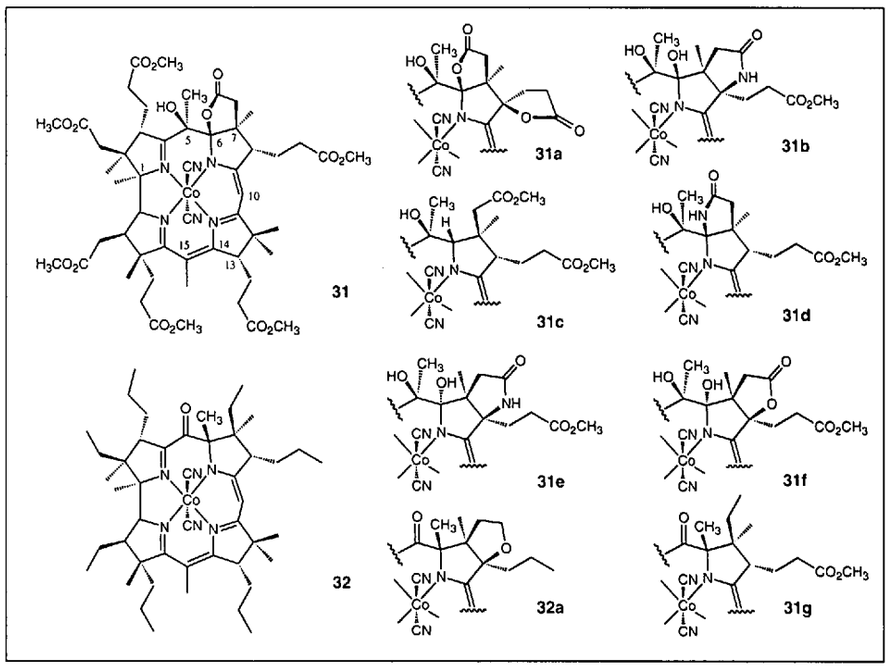


Fig. 8. *Hitherto characterized xanthocorrinoids obtained by 'coupled oxidation'* in vitro *of heptamethyl dicyanocobyrinate and some of its derivatives.* For the sake of clarity, only the part of the molecule, which is modified, is represented in the partial structure, remainder as in the preceding entire formula.

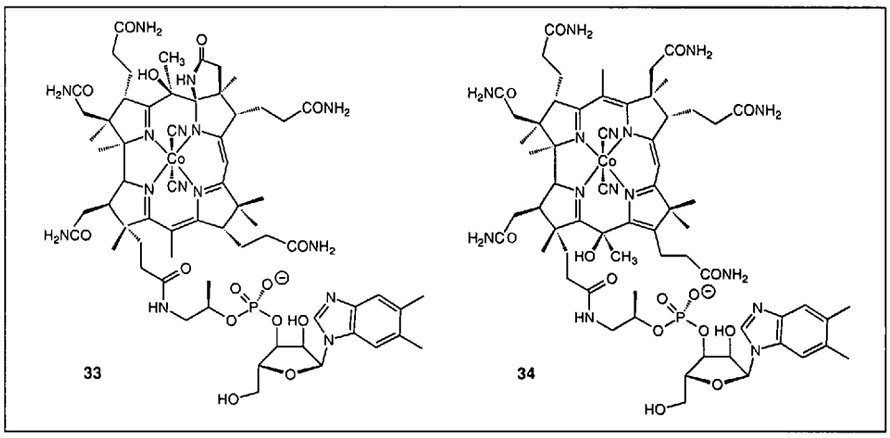


Fig. 9. *Xanthocorrinoids obtained by 'coupled oxidation' of cyanocobalamin (vitamin B_{12})* in vitro

On the basis of the above results, a tentative pathway for the enzymatic degradation of chlorophyll can be suggested, in which the 1,2-diol formed by hydrolysis of a primary pheophorbide-4,5-epoxide is cleaved by a retroaldol-like reaction (*cf. Scheme 8*). As the presence of a Mg^{II}-dechelatase in senescent chloroplasts has been recently demonstrated by the Zurich group [69], it may be anticipated that loss of the Mg-atom precedes the oxidative cleavage of the macrocycle in the enzymatic degradation process. Moreover, as microalgae are considered to be the phylogenetic ancestors of green plants, it may be expected that this pathway holds true for protista and multicellular organisms as well.

4. Vitamin B_{12} Catabolism

Despite the fundamental structural differences between the chromophores of heme and the corrinoids (to which adenosylcobalamin – the vitamin B_{12} coenzyme – belongs), the latter are usually associated to the tetrapyrrolic pigments owing to their manifest global appearance which reflects their common biosynthetic origin.

As the vitamin B_{12} requirement of man is very low (*ca.* 2–5 μg daily), the proof for a metabolism of this compound in the human body is difficult. On an administered oral dose of 0.5–2 μg of pure vitamin B_{12}, 60–80% is absorbed. As the oral dosage increases, the percentage absorbed decreases; at a dose of 5 mg 30% or less is

absorbed. Studies employing radioactive vitamin B_{12} prove that the unabsorbed part of vitamin B_{12} which is taken with the diet, appears in the faeces, and only a negligible amount in the urine. If excessive amounts of vitamin B_{12} are administrated parenterally, however, rapid renal excretion of unbound vitamin is observed.

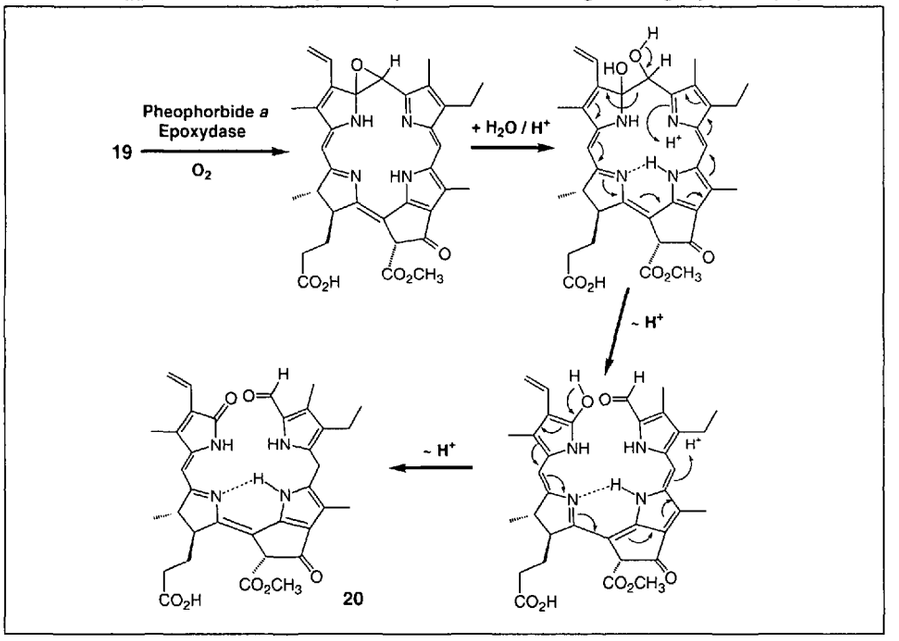
Vitamin B_{12} is probably a primitive coenzyme that was in its prime before O_2 was a significant component of the earth's atmosphere. At present, the biosynthesis of corrinoids is restricted to bacteria. There is no known vitamin B_{12}-dependent reaction in higher plants, and only two vitamin B_{12}-dependent reactions seem to be retained in animals in the course of evolution. Therefore, it may be assumed that corrinoid catabolism should take place, if at all, in microorganisms rather than in more developed living beings.

The microbial degradation of vitamin B_{12} was first studied by *Helgeland, Jonsen* and *Laland* and subsequently by *Pfiffner* and colleagues. *Helgeland et al.* [70] found that cultures of *Aerobacter aerogenes* converted vitamin B_{12} into yellow or brown products, which were separated into two fractions by electrophoresis. On the basis of the UV/VIS spectra of the isolated components, they suggested that their yellow compounds might have a structure analogous to that of the coenzymes, in which the conjugated system had been interrupted. *Pfiffner et al.* [71] obtained yellow compounds from the action of cultures of several different bacteria, of which *Pseudomonas rubescens* attacked vitamin B_{12} the most rapidly. Later on, evolution of CO during aerobic incubation of hydroxycobalamin with either *Bacillus cereus* or α-hemolytic *Streptococcus mitis* was reported by *Engel et al.* [72] who insinuated an analogy between heme and cobalamin catabolism by these bacteria strains. In conformity with this hypothesis, the controlled 'coupled oxidation' of vitamin B_{12} in the presence of ascorbic acid yields a series of products, which showed a greater absorption at 465 nm than cyanocobalamin [73]. At least some of these products appear to be fairly typical 'stable yellow corrinoids' (later called xanthocorrinoids), a class of compounds which are formed as byproducts in most reactions of cobalamin which proceed with alteration of the oxidation state of the complexed cobalt ion. However, the structure of these pigments could not be elucidated at that time.

A systematic study of the 'coupled oxidation' of cobyrinic acid derivatives in the presence of ascorbic acid and Cu^{II} ions (*Udenfriend* reaction) has been carried out in our research group in a series of communications published between 1977 and 1991 [74–80]. The structures of the compounds which have been characterized in the course of this work are represented in *Fig. 8*. Contrarily to the sensitized photooxygenation of heptamethyl dicyanocobyrinate, in which rupture of either the C(5)=C(6) or the C(14)=C(15) bond has been observed by *Kräutler* [81][82], the 'coupled oxidation' of corrin derivatives is regio- and stereoselective. Thus, even though the conversion rate does not account for the totality of the substrate used, in all but one of the investigated reactions (*vide infra*), hydroxylation of the macrocycle takes place on C(5), *cis* to the peripheral acetic-acid chains. In no case, reaction occurred on C(10), which would be the only position of the corrin chromophore capable to be eliminated subsequently as carbon monoxide. A rationale for this behavior has been proposed, which is similar to that of the hydroxylation of the heme chromophore, as given in *Scheme 2* [77]. Usually, the acetic-acid substituent on C(7) is involved in the stabilization of the cationic intermediate formed upon hydroxylation of the C(5) position of the macrocycle (*cf. Fig. 8*). Interestingly enough, an analogous hydroxy-lactone derivative (precorrin-3B) has been identified recently as a biosynthetic intermediate of vitamin B_{12} in the aerobic bacterium *Pseudomonas denitrificans* [83][84].

In the case of substrates in which the acetic acid residue on C(7) (methyl acetate, acetamide or *c*-lactone) had been replaced by a less reactive group (*c*-lactam, *c*-ether or alkyl), either a diol (**31e** and **31f**) was obtained [80] or a pinacol-type rearrangement was observed, in which the Me group on C(5) migrates to the vicinal C(6) position (**31g** and **32a**) [78]. The latter kind of reaction was also observed with a peralkylated corrin derivative as substrate (*cf.* **32**) [75]. The 'coupled oxidation' of vitamin B_{12} itself yields, in addition to xanthocorrinoid **33**, a red-orange product **34**, which is formed by hydroxylation on C(15) and subsequent elimination of the proton bound to C(13) [79] (*cf. Fig. 9*). Although both corrinoids were identified later with pigments present in small quantities in the culture media of different strains of bacteria (*Enterobacter aerogenes, Alteromonas putrefaciens, Pseudomonas putida, Rhizopus arrhizus, Cunninghamella blakesleeana, Beauveria bassiana*, and *Corynebacterium* sp.) [85], the possibility that they are formed as artefacts cannot be ruled out at present. Thus it must be concluded that a convincing evidence for an enzymatic degradation of cobalamin derivatives is still missing.

Scheme 8. *Suggested Mechanism of the Enzymatic Oxidative Ring Cleavage of Chlorophylls*



In the present work, a number of the author's own contributions to the subject treated are reported. All colleagues and coworkers, whose names are mentioned in the references, deserve my sincere thanks for their valuable help, encouraging enthusiasm, and fruitful experimental abilities. Likewise, I wish to thank the *Deutsche Forschungsgemeinschaft* and the *Swiss National Science Foundation* for their generous financial support of our research projects, before and after October 1st, 1982, respectively.

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