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Preservation of Chlorophyll-Derived Pigments in Sedimentary Organic Matter [and Discussion]

C. B. Eckardt, B. J. Keely, J. R. Waring, M. I. Chicarelli, J. R. Maxwell, J. W. De Leeuw, J. J. Boon, B. Runnegar, S. Macko and J. D. Hudson

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Preservation of chlorophyll-derived pigments in sedimentary organic matter

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SUMMARY

The occurrence in sediments of chlorophyll-derived tetrapyrroles provides evidence for primary photosynthetic communities in palaeo water columns. In ancient sedimentary rocks the components occur mainly as nickel or vanadyl complexes of alkyl porphyrins, and to a lesser extent carboxylic acids. Although extensive loss of functional groups has occurred, the structures of a number of the components reveal that chlorophyll carbon skeletons can survive intact or virtually intact, although in other cases the skeletons have been modified by rearrangement. The structures of a few components indicate an origin from the chlorophylls *c*, and thus an algal input, whereas an origin from photosynthetic bacterial chlorophylls is apparent from the carbon skeletons of other components. Such studies, taken with other distributional features (e.g. ratio of nickel to vanadyl components, extent of tetrapyrrole preservation) can provide information about the productivity and preservation of organic matter and presence of water column anoxia at the time of deposition.

Studies of the tetrapyrrole components in one recent and two highly immature older sediments have provided further evidence for the pathway of defunctionalization, through the identification of the minimum number of components necessary to link a chlorophyll, such as chlorophyll *a*, to the major alkyl porphyrin in sedimentary organic matter. Additionally, evidence for an early and unexpected transformation pathway comes from the recent identification in sediments of chlorin acids esterified with sterols.

1. INTRODUCTION

Chlorophylls comprise one of the most important groups of natural products, being responsible for harvesting solar energy and converting it to chemical energy. Nine basic structure types (**1–9**) have been recognized, and these occur variously in phototrophic eukaryotes and prokaryotes (n.b. in the case of certain bacteriochlorophylls (**7**, **8**) the esterifying alcohol may differ from the major one shown). The principal chlorophylls are (i) chlorophyll *a* (**1**), which occurs in all higher plants, algae and cyanobacteria; (ii) bacteriochlorophyll *a*(**5**), which occurs in all photosynthetic bacteria (although only as the major chlorophyll in the purple photosynthetic bacteria), and (iii) bacteriochlorophylls *c*, *d*, and *e* (**7–9**) which are characteristic of the *Chlorobium* bacteria. The co-occurring secondary pigments, which serve to broaden the spectrum of light absorbed during photosynthesis, are chlorophyll *b* (**2**) in higher plants and green algae, and the chlorophylls *c* (**3**) in several algae including diatoms, dinoflagellates and prymnesiophytes. Among the photosynthetic prokaryotes, bacteriochlorophyll *a* (**5**) functions as the accessory pigment, except in the purple non-sulphur bacteria which contain bacteriochlorophyll *b* (**6**). The homologous nature of the bacteriochlorophylls *c* and *d*, (**7** and **8**) and by inference bacteriochlorophylls *e* (**9**), has been shown to arise from a physiological response to light availability. Hence, increased extent of alkylation alters the aggregation properties of the *in vivo* chlorophyll antenna array, allowing longer wavelengths of light to be used (Smith & Bobe 1987).

It is now widely accepted that chlorophylls and their early stage transformation products, derived from organisms present at the time of deposition, can be preserved in ancient sedimentary organic matter mainly as metal complexes of alkyl porphyrins (M in, for example, **10**). Most of the evidence comes from detailed structural comparisons (through ^1H nuclear magnetic resonance (NMR) spectroscopy) of individual isolated alkyl porphyrins (reviewed by Chicarelli *et al.* (1987)), and to a lesser extent their carboxylic acid counterparts (Ocampo *et al.* 1987), with the carbon skeletons of known chlorophylls. In many cases these comparisons clearly indicate an origin from ancient chlorophylls via loss of the functional groups of the latter. The most abundant tetrapyrrole pigments in sedimentary rocks are various metal complexes (see below) of desoxophylloerythroaetioporphyrin (DPEP, **10**), their abundance outweighing that of any chlorophyll in the biosphere. Although structural comparison of DPEP (**10**) with chlorophylls shows that it could be

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derived from virtually any of them, the presence of the characteristic five-membered exocyclic ring does indicate a chlorophyll origin (figure 1*a*). Other abundant and widely occurring components (**11**, **12**), which similarly cannot be related with certainty to specific precursor chlorophylls, have a greater degree of structural modification arising from condensation (Wolff *et al.* 1983; Fookes 1983; Prowse *et al.* 1987). A few components are, however, markers of specific chlorophylls; for example, the rearranged alkyl components (**13**) and their carboxylic acid counterparts have been related (figure 1*b*) to an origin in the chlorophylls *c* (**3**) through a condensation involving the acrylic acid side chain (Ocampo *et al.* 1984; Callot *et al.* 1990). On the basis of the chlorophylls known at the time of its discovery, the alkyl porphyrin **14** was suggested (Chicarelli & Maxwell, 1986) to have arisen from chlorophyll *b* (**2**), although the recently discovered chlorophyll c_3 (**3c**, Fookes & Jeffrey 1989) is now considered to be a more likely precursor (cf. Verne-Mismer *et al.* 1990). Specific markers (**15**) of certain of the bacteriochlorophylls *d* (**8**) have been identified in the acid porphyrin assemblage of the Messel oil shale of Eocene age (*ca.* 45 Ma; Ocampo *et al.* 1985). Likewise, the recent tentative assignment (e.g. **16**) of an alkyl porphyrin bearing a methine (C-20) methyl substituent, in the Oulad Abdoun oil shale of Cretaceous age (*ca.* 70 Ma), points (Callot *et al.* 1990) to an origin in the bacteriochlorophylls *c* (**7**) or *e* (**9**).

Finally, several unexpected components are found, the structures of which cannot readily be related to known chlorophyll carbon skeletons; these include the benzoporphyrins (e.g. **17**, Kaur *et al.* 1986) and their tetrahydrobenzo counterparts, (e.g. **18**) (Verne-Mismer *et al.* 1987). Clearly, studies of the origins of such components are required, as are those of the unexpected components bearing a methyl substituent at C-13^1, (e.g. **19**) (Chicarelli *et al.* 1987), as this is the position of the ketone functionality in chlorophylls.

Sedimentary cycloalkanoporphyrins appear to be derived predominantly from algal and bacterial chlorophylls rather than the chlorophylls of higher plants. Evidence in support of this conclusion comes from: (i) the occurrence of low abundances of porphyrins relative to the total organic carbon (TOC) content in lignites and humic coals, i.e. where a major higher plant input has occurred (reviewed by Bonnett *et al.* (1987)); (ii) in samples where organic petrography and porphyrin studies have been done, high porphyrin concentrations are generally coincident with the presence of greater amounts of amorphous organic matter (algal or bacterially derived; Mello (1988)) than of figured matter (higher plant derived); (iii) the stable isotope contents ($\delta^{13}C$) of the major porphyrins in the lacustrine Messel shale, which has a significant input of higher plant organic matter, are consistent with an origin from the chlorophylls of algae and bacteria (Hayes *et al.* 1987). Indeed, in vascular plants chlorophylls may be enzymically degraded to colourless products during senescence.

In summary, sedimentary rocks can contain a wide variety of cycloalkanoporphyrins. Many of them can be related to chlorophylls arising predominantly from algal or algal and bacterial sources. The other major alkyl porphyrin structural type that occurs in ancient sediments is the aetio type (e.g. **20**) without an exocyclic ring. Recent stable isotopic evidence suggests that these components arise from both chlorophyll and cytochrome sources (Ocampo *et al.* 1989; Boreham *et al.* 1989).

2. TRANSFORMATIONS OF CHLOROPHYLLS

(*a*) *Main defunctionalization pathway*

Relatively little is known about the nature and timing of the defunctionalization reactions linking chlorophylls to sedimentary alkyl porphyrins with a preserved carbon skeleton such as DPEP (**10**). It is clear from structural elucidations of the major chlorins of a surface sediment (Priest Pot, Cumbria, U.K.; see Keely *et al.* 1990) and from examination of the chlorin distributions of a number of recent sediments (C. B. Eckardt *et al.*, unpublished results), that loss of the magnesium ligand, ester hydrolysis and decarbomethoxylation (to give components (**21–25**) in figure 2), can occur in the water column through biological mechanisms, as suggested by laboratory studies of algal senescence and herbivory (cf. Daley 1973; Daley & Brown 1973; Scoch *et al.* 1981; Owens & Falkowski 1982). It is more difficult to rationalize the causes of reduction of the C-3 vinyl substituent originally present in, e.g. (**1**), dehydration and reduction in the C-3 substituent (in **7–9**), C-13^1 ketone reduction, aromatization, and decarboxylation or reduction in the C-17 substituent in, for example, pyrophaeophorbide *a* (**25**). Components resulting from these reactions have not been identified so far in recent sediments. However, the circumstantial evidence that such reactions must occur comes not only from the structures of the porphyrins from ancient sediments, but also from structural elucidation of the chlorins (**26** and **27**, figure 2) in sediments with a mild thermal history (Keely *et al.* 1990). Despite the fact that the nature and timing of the reactions are uncertain, the presence of several defunctionalized components, including (**25–28**) in figure 2 and DPEP (**10** M = 2H) in a wet, partly consolidated sediment of Pliocene age (*ca.* 1.5 Ma; Willershausen, F.R.G.) and in a highly immature sediment of Miocene age (5.3–23.7 Ma; Maraú shale, Brazil) indicates indirectly that they occur early on through biological or low temperature chemical mechanisms (Keely *et al.* 1990). The components identified in the three sediments (Priest Pot, Maraú and Willershausen) represent the minimum number of 'intermediates' necessary to link a chlorophyll, such as chlorophyll *a* (**1**) to DPEP (**10**, M = 2H), although the order of the reactions is not necessarily that shown for convenience in figure 2. Although an aromatization step is not necessary if the porphyrins are derived from the chlorophylls *c* (**3**), which are themselves porphyrins, indirect evidence that this reaction does occur comes from the identification of the Messel bacterially-derived porphyrin acids (Ocampo *et al.* 1985).

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(*a*)

(*b*)

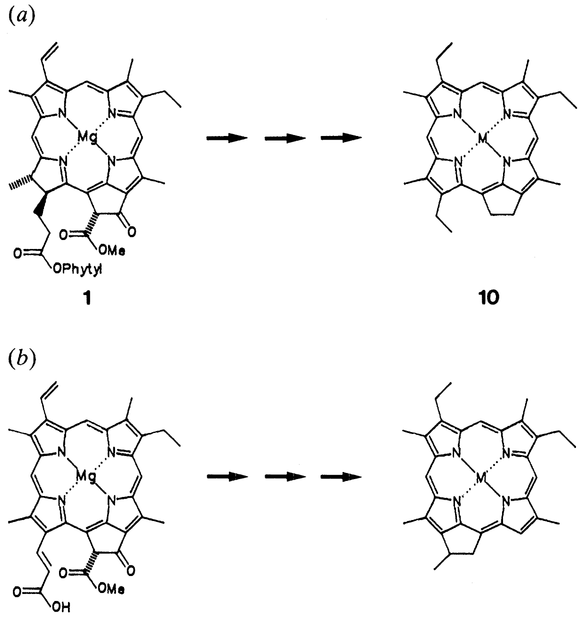

Figure 1. Schematic representations for the proposed origins of sedimentary porphyrins. (*a*) Non-specific origin, e.g. DPEP (**10**) from chlorophyll *a* (**1**), (*b*) specific origin, e.g. **13** from chlorophyll c_1 (**3*a***).

(*b*) *Condensation*

The presence of the bicycloalkano chlorin (**29**) and its porphyrin counterpart (**12*b***, M = 2H) in the partly consolidated Willershausen clay shows that the condensation is also a low temperature reaction. Further circumstantial evidence comes from the recognition of more functionalized bicyclic components (**30**, **31**), bearing the same carbon skeletons, in a sponge (Karuso *et al.* 1986) and a clam (Sakata *et al.* 1990). These components have been suggested as arising from enzymic alteration of ingested chlorophyll, giving precedent to the occurrence of a biologically mediated condensation reaction leading to the carbon skeletons of the sedimentary analogues.

(*c*) *Other transformations*

Another example of the use of tetrapyrrole distributions in immature sediments to infer low temperature transformations comes from the unexpected occurrence of high molecular mass (HMM, i.e. > 900 Da) chlorins. Two of the major tetrapyrrole pigments (**32**) in the lacustrine Maraú shale (see above) have been shown to comprise chlorin nucleii, which appear to be derived from chlorophyll *a* (**1**), esterified to a C_{30} 4-methyl sterol. As sedimentary steroids arise mainly from algae, an algal origin for these pigments was suggested (Prowse & Maxwell 1991). More recently, liquid chromatography-mass spectrometry (LC-MS) studies have shown that complex mixtures of such chlorin esters, with C_{27}–C_{31} sterols and stanols esterified to a (presumably) pyrophaeophorbide *a* nucleus (cf. **32**) occur in several recent lacustrine and marine sediments (Eckardt *et al.* 1991; C. B. Eckardt *et al.*, unpublished results). The detailed structures of the components in these recent samples remain, however, to be proved. As an example, figure 3 shows the chromatogram from LC-MS analysis of the methylated total extract from a Black Sea surface sediment, showing the HMM species as significant tetrapyrrole components. Although they are not considered to be intermediates on the main chlorophyll defunctionalization pathway, they may represent products of a novel transformation pathway. Hence their occurrence, with a sterol as the esterifying alcohol instead of the usual acyclic isoprenoid alcohols (e.g. phytol (**33**) or farnesol (**34**)) typical of chlorophylls, suggests that they arise from a biologically mediated esterification of acidic chlorophyll derivatives, possibly after cellular disruption caused by senescence, decay of herbivory (cf. Prowse & Maxwell 1991). Their derivation from previously unrecognized chlorophyll precursors with sterols replacing, e.g. phytol (**33**), cannot, however, be excluded at present. Clearly, whatever the origin of these components (primary, i.e. biosynthetic, or secondary) further studies are necessary to investigate the processes which control their occurrence and distribution.

(*d*) *Metal incorporation*

In ancient sediments which contain tetrapyrroles, the Ni(II) and VO(II) metallo complexes predominate [e.g. **10**, M = Ni(II) or VO(II)] although the free bases and other metal complexes, such as Cu(II) and Fe(III) have also been reported (see Eckardt *et al.* 1989). Tetrapyrroles complexed to such metal ions are certainly not, however, among the major tetrapyrrole components in surface and shallow sediments (e.g. figure 3) studied to date. The youngest and most immature samples found to contain metalloporphyrins are unconsolidated sediments recovered by the Deep Sea Drilling Project (DSDP). Trace amounts of Ni alkyl porphyrins were reported in Pleistocene (*ca.* 1.5 Ma) sediments from the Black Sea (Site 380A; Baker *et al.* 1978), and both Cu(II) and Ni(II) species were found in Pliocene sediment (*ca.* 3 Ma; DSDP Leg 63 Site 467, offshore California). In the latter case the Ni(II) complex of DPEP [**10**, M = Ni(II)] was shown, after demetallation, to be present by HPLC coinjection with a standard (Bennett 1991). These studies suggest that metallation can occur during the early stages of diagenesis in wet and unconsolidated sediments.

3. PORPHYRIN PRESERVATION AND DEPOSITIONAL CONDITIONS

Although the factors controlling the nature and distribution of porphyrins in sedimentary rocks are still not fully understood, it is becoming increasingly clear that such components can provide information about the types of photosynthetic organisms present at the time of deposition, and the redox conditions in the water column. Three features appear to be important with regard to assessment of the depositional conditions in the palaeoenvironment.

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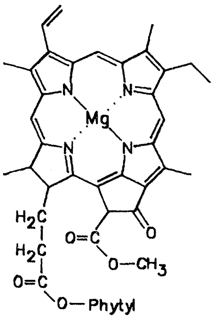

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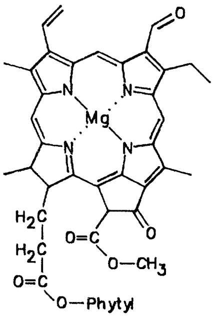

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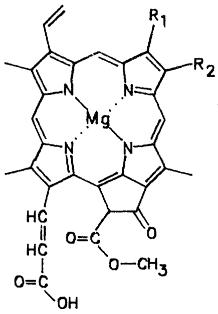
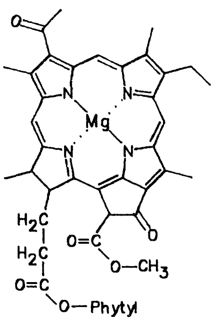


3

3a R_1=Me , R_2=Et
3b R_1=Me , R_2=Vinyl
3c R_1=CO_2Me, R_2=Vinyl



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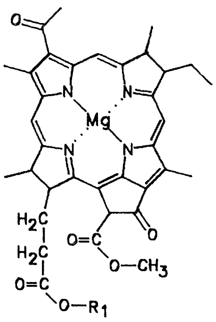

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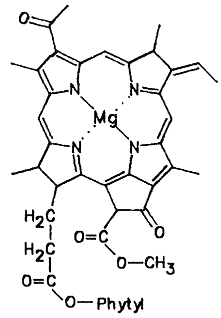
R_1=Phytyl,
Geranyl geranyl



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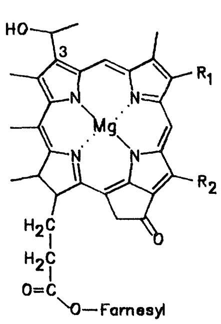

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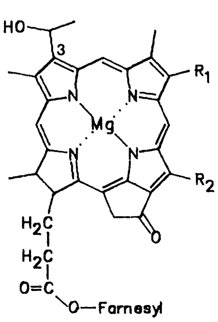
R_1=Et, n–Pr, i–Bu
R_2=Me, Et



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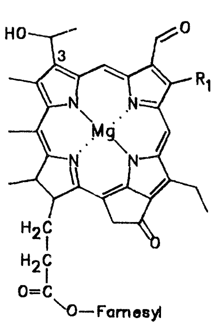
R_1=Et, n–Pr, i–Bu, neo–Pent
R_2=Me, Et



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R_1=Et, n–Pr, i–Bu

Scheme 1.

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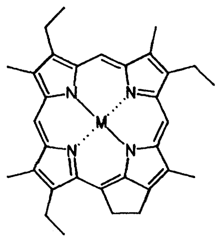

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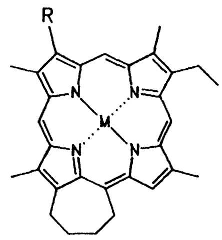
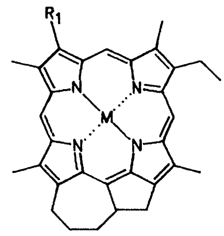
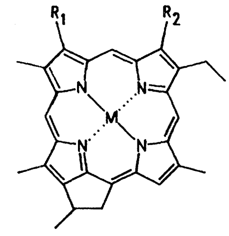


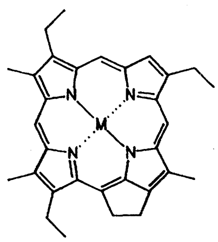
11 R=Me, Et



12a R=Me
12b R=Et



13 R_1=H, Me, Et
R_2=H, Me



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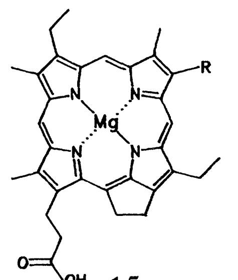
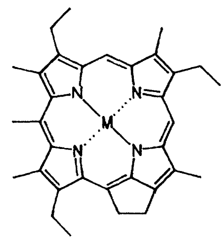


15
R=Et, n–Pr, i–Bu



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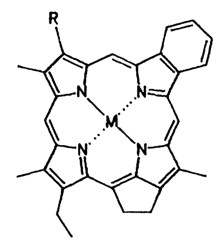
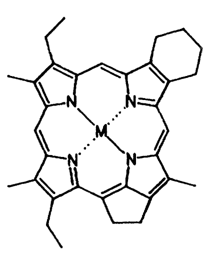


17 R=Me, Et



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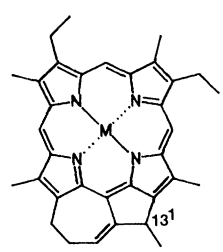

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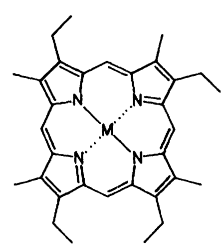

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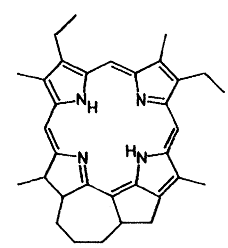

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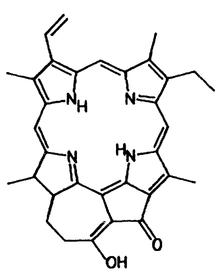

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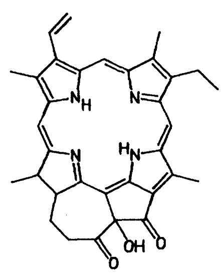

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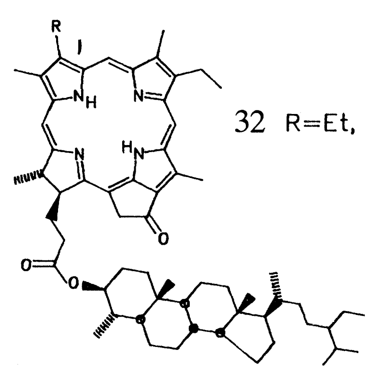
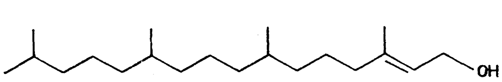


32 R=Et, Vinyl



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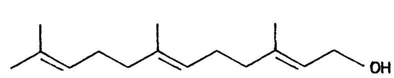

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(Scheme 1. *cont.*)

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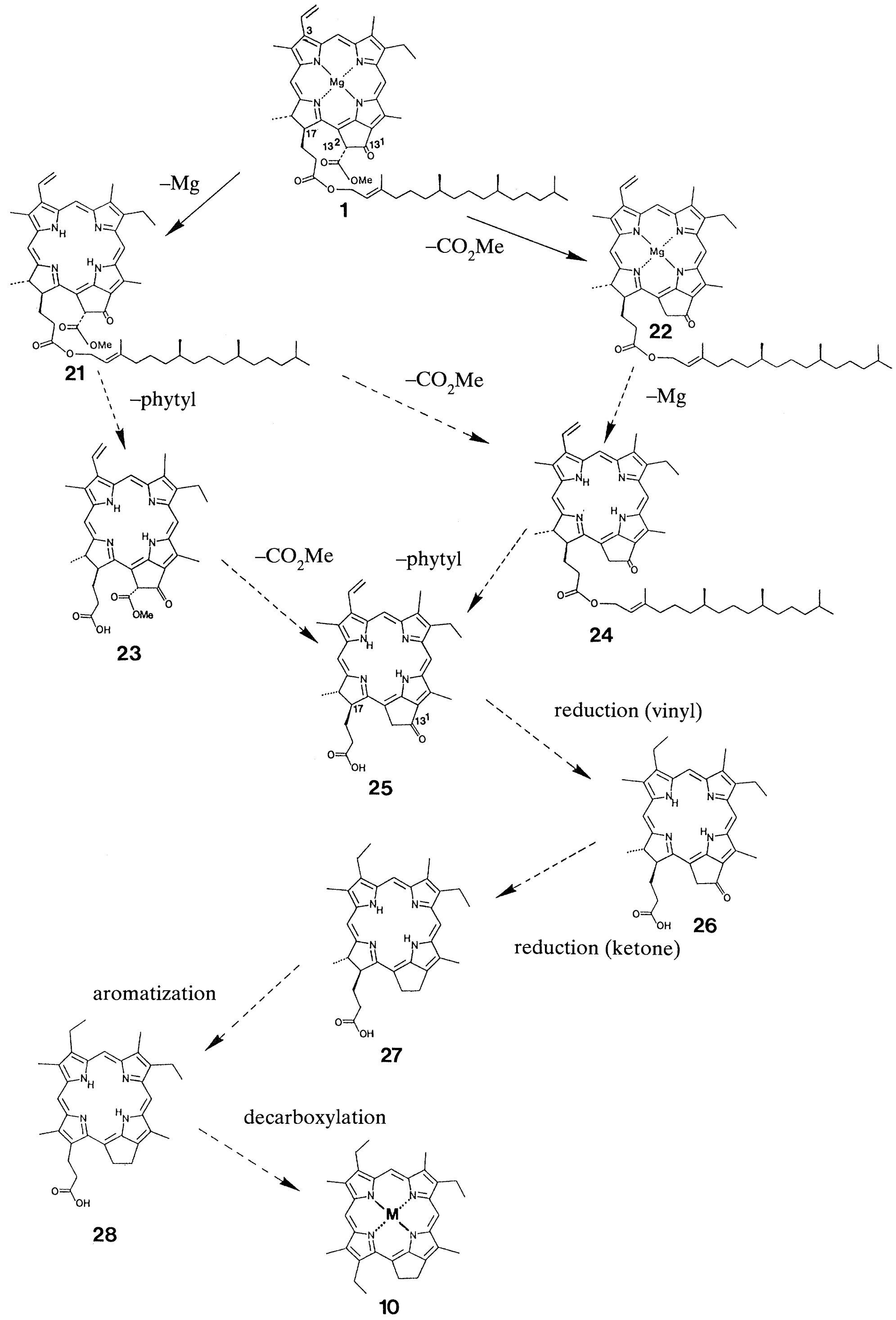

Figure 2. Possible transformation pathways for the conversion of chlorophyll *a* (**1**) to DPEP (**10**; M = 2H) based on characterization of components from Priest Pot (**1**, **21–25**), Maraú (**25**, **26**), and Willershausen (**27**, **28**, **10**) sediments (see text). Solid arrows indicate reactions that must occur. (Modified from Keely *et al.* 1990.)

(*a*) ***Presence of marker components***

The occurrence of components with structures sufficiently specific to act as markers for the former occurrence of particular chlorophylls provides, in turn, some indication of the presence of particular types of photosynthetic organisms. For example, components with a methyl-substituted five-membered ring (e.g. **13**) are thought to arise from chlorophyll *c* (**3**), allowing an input of organic matter to the sediment from a limited number of algal divisions to be inferred (see above and figure 1*b*). Similarly, the identification of highly alkylated (C_{34}–C_{36}) porphyrin acids (**15**) in the Messel shale provides evidence of an origin from bacteriochlorophylls *d* (**8**), in turn indicating the presence of *Chlorobium* bacteria in the palaeo water column (see above). More importantly, however, the presence of such higher molecular mass acids (and related alkyl porphyrins) provides direct molecular evidence for the presence of anoxic conditions extending into the photic

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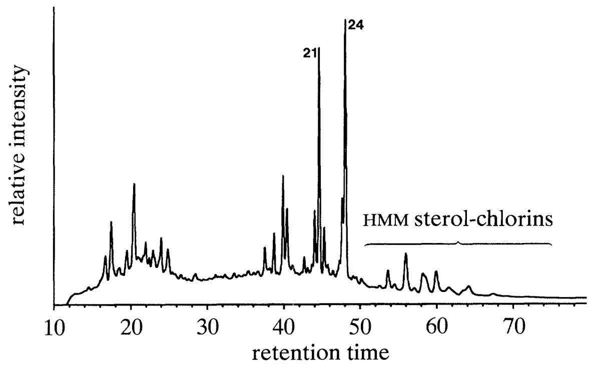

Figure 3. HPLC chromatogram (monitored at 400 nm) of a methylated total extract from a Black Sea surface sediment, showing HMM chlorins with a range of sterols and stanols (C_{27}–C_{31}) esterified to a (presumed) common chlorin nucleus. For identities of **21** and **24** see structures.

zone, all *Chlorobium* bacteria being obligate anaerobes that utilize H_2S instead of H_2O as an electron donor in photosynthesis (see below). We know of no other means, at present, of recognizing photic zone anoxia in palaeo water columns, except the occurrence of certain sedimentary aryl isoprenoid hydrocarbons, thought to be degradation products of *Chlorobium* bacterial carotenoids (Summons & Powell 1987).

(*b*) *Porphyrin preservation*

It is well known that chlorophylls and their early alteration products are sensitive to oxic photodegradation (see Simpson *et al.* 1976). Hence it might be expected that low abundances of porphyrins (relative to, for example, total organic carbon (TOC)) in organic rich sedimentary rocks would reflect the presence of oxygenated conditions in the water column, most likely extending below the photic zone. Conversely, high pigment yields would indicate a high degree of oxygen depletion within the water column and in the sediment, conditions commonly recognized as being associated with excellent preservation of organic matter. An example of this approach comes from a preliminary study of the porphyrins in two immature marine sediments from the Toarcian Posidonia shale sequence (Lias ε; *ca.* 195 Ma) in Germany. Both samples show high TOC and total soluble extract (TSE) yields (table 1), reflecting a high primary productivity in the water column. In both cases the aliphatic hydrocarbon concentrations relative to TOC are similar (table 1) as are the distributions; this similarity is also apparent in the biological markers, which show almost identical distributions of steranes (derived from algal sterols) and hopanes (from bacterial hopanoids), suggesting a similar input of organic matter (Waring 1991). Indeed in relation to other samples we and others have examined, the comparatively high pigment yields relative to TOC suggest the presence of oxygen-depleted water columns in both cases. The conditions during deposition of the two samples appear, however, to have varied (see below).

(*c*) *Relative abundances of Ni and VO porphyrins*

The relative abundance of Ni to VO porphyrins in ancient sediments varies widely between two extremes, where solely Ni or solely VO components occur, although consideration of all the analyses which have been carried out to date seems to indicate that a predominance of Ni species occurs more frequently. Lewan (1984) proposed a model whereby metallation with either Ni(II) or VO(II) is related to water column redox conditions and pH. According to the model a predominance of VO species is thought to indicate an anoxic depositional environment, in which Ni(II) ions become unavailable for tetrapyrrole chelation owing to their removal as nickel sulphides by reaction with H_2S from bacterial sulphate reduction. In contrast, the predominance of Ni porphyrins was thought to reflect lower degrees of oxygen depletion with insufficient reduced sulphur species to remove Ni(II), or the unavailability of VO(II) at higher pH.

The porphyrin distributions of the two Posidonia shale samples suggest that the model may be slightly oversimplified in some cases. The sample from Schömberg (SCH; Lower Lias ε) contains only Ni species (table 1). The relative abundances of the molecular ions corresponding to cycloalkanoporphyrins (the most abundant components) in a probe mass spectrum of the demetallated fraction are shown in figure 4. Although the individual components have not yet been isolated by HPLC for full structural elucidation by NMR spectroscopy, it is clear that components of higher molecular mass (greater than C_{33}) are present, indicating an origin from the chlorophylls of *Chlorobium* bacteria. Hence, there were periods during deposition of the sediment when anaerobic conditions in the water column extended into the photic zone (cf. Repeta *et al.* 1989). The Lewan (1984) model would suggest, however, that sediments deposited under such intense

Table 1. *Selected data for two Posidonia shale samples from Holzmaden* (HOL)[a] *and Schömberg* (SCH)[b]

(TOC, Total organic carbon. TSE, Total soluble extract. HC, Hydrocarbons. TS, Total sulphur. n.d., not detected)

Sample	TOC (%)	TSE (p.p.m. of rock)	aliphatic HC µg per gram TOC	TS (%)	Ni-p.[c] µg per gram TOC	V = O-p.[c] µg per gram TOC	Ni + V = O-p.[c] µg per gram TOC
HOL	11.5	7240	120	1.8	88	15	103
SCH	8.1	7620	130	0.3	314	n.d.	314

[a] Holzmaden (*ca.* 30 km S.E. of Stuttgart, F.R.G.); Middle Lias ε (Unterer Schiefer, horizon II/4).
[b] Schömberg (*ca.* 40 km S.W. Tübingen, F.R.G.); Lower Lias ε (Seegrasschiefer).
[c] Alkyl porphyrins; concentrations calculated from electronic absorption spectra.

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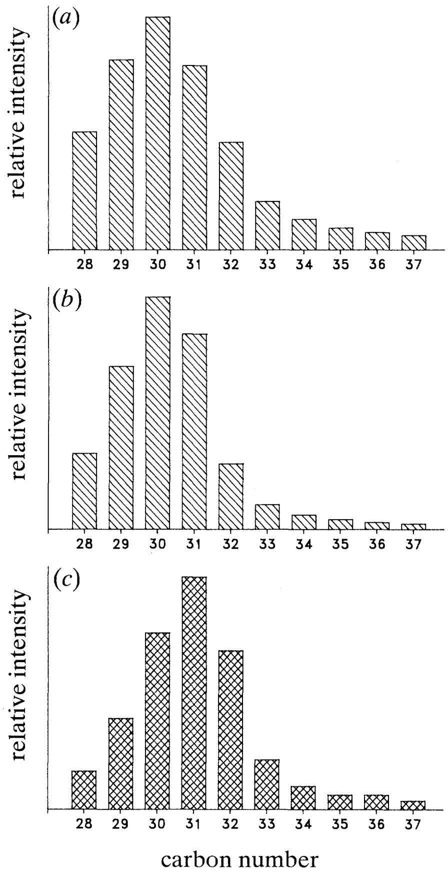

Figure 4. Histograms of cycloalkanoporphyrin distributions obtained from probe mass spectrometry. (*a*) Schömberg demetallated Ni porphyrins; (*b*) Holzmaden demetallated Ni porphyrins; (*c*) Holzmaden demetallated VO porphyrins.

and extensive oxygen depletion should be dominated by VO species. It appears that in this case the photosynthetic bacterial activity, utilizing H_2S, did not allow the effective removal of Ni(II) ions. In the sample from Holzmaden (HOL; Middle Lias ϵ), cycloalkanoporphyrins greater than C_{33} occur in both the demetallated Ni and VO porphyrin fractions (figure 4), again attesting to the occurrence of anoxic conditions extending into the photic zone; yet the porphyrin yields relative to TOC and TSE in this sample are significantly lower (table 1), suggesting an overall lower degree of oxygen depletion in the water column. However, the higher sulphur content in the Holzmaden sample (table 1) presumably indirectly reflects more extensive sulphate reduction. This apparent contradiction would be removed if, during deposition of the Holzmaden sediment, sulphate reduction was stimulated following replenishment during incursions of fresh seawater. During such events, periods of more extensive oxygenation of the water column might be expected before re-establishment of anoxic conditions in the photic zone. It is possible, therefore, that during such periods oxic degradation of the porphyrin precursor pigments occurred, leading to an overall lower preservation of pigments than in the case of the Schömberg sample. In this context it is noteworthy that the Lower Jurassic in Europe is generally characterized by more transgressive periods (Brinkmann 1975), and geological evidence suggests periods of water column oxygen depletion (see Kauffman 1981, and references therein).

4. FUTURE STUDIES

(*a*) *Chlorophyll transformations*

To investigate further the nature and timing of the early stage transformations, additional studies of the tetrapyrrole components in recent and young immature sediments from a variety of depositional environments are required. Such studies may provide information not only about key defunctionalization reactions which must occur, the products of which have not yet been observed in sediments younger than Pliocene (see above), but also on the origin of the unusual components such as those with bicyclic ring systems (e.g. **12** and **29**).

Full structure elucidation of the chlorin steroidal esters in recent sediments is required, because only mass spectrometric and electronic absorption spectral information have been obtained. In particular, algal senescence and decay experiments in the laboratory (cf. Scoch *et al.* 1981; Owens & Falkowski 1982), and experiments simulating zooplankton grazing on algae, may provide information on the origin of such components and of tetrapyrroles with bicyclic ring systems.

(*b*) *High-molecular-mass porphyrins*

To date, the only porphyrins whose structures have been fully characterized, and which can be conclusively related to an origin from photosynthetic sulphur bacteria, are the carboxylic acids from the Messel shale (see above). Clearly, full structural elucidations of individual alkyl porphyrins greater than C_{33}, which are likely to be more widely occurring than the acids, are required to: (i) confirm structural relations with the bacteriochlorophylls of the photosynthetic sulphur bacteria; (ii) provide standards for HPLC coinjection studies incorporating a variety of samples where porphyrins are preserved. This should allow further information about the extent and intensity of oxygen depletion in water columns of samples from different depositional environments to be obtained.

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Discussion

J. W. de Leeuw (*Organic Geochemistry Group, Delft Technical University, The Netherlands*). We are all aware of the fact that during the actual extraction procedure transesterifications and esterfications of components in the extract may occur especially when clays are present. Therefore, did Professor Maxwell do blank experiments to rule out the possibility that the sterol esters of chlorophylls are actually produced during the extraction procedure?

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J. R. Maxwell. The relative abundances of the two esters (*ca.* 4:1) in the Miocene Maraú sediment differ significantly from the relative abundances of the corresponding free acids (*ca.* 1:1), which are also present. Furthermore, in the Black Sea surface sediment the sterol chlorin esters could be obtained in a total extract simply by allowing the sediment to stand for a few minutes in acetone. Finally, we have found sterol chlorin esters in a natural plankton population from the Baltic Sea. I believe therefore, that the components are not artefacts.

J. J. Boon (*Fom-Amolf, Amsterdam, The Netherlands*). Professor Maxwell has shown some wonderful molecular work on what happens to porphyrins when the ring remains intact. Could he give his view on what happens when the ring is opened? Do we currently overlook those molecules because of their polarity? Is it worth looking for them and other metabolites of porphyrins?

J. R. Maxwell. I do not know which products would be formed in the environment if the macrocyclic ring is cleaved, although I could make some guesses. I am fairly sure, however, that this must happen, as I am aware of some work in the U.S.A. aimed at identifying ring-opened products in marine water columns and bottom sediments. In addition, my colleague Dr Chris Eckardt has found high relative abundances of alkyl pyrroles in the Permian Kupferschiefer, which presumably formed through oxidative ring cleavage at the time of deposition. I would say, therefore, that it was very worthwhile indeed to look for ring-opened products in the environment.

B. Runnegar (*Earth and Space Sciences, University of California, U.S.A.*). If I remember correctly, one or more kinds of methanogenic bacteria use a nickel porphyrin. Would Professor Maxwell expect to be able to recognize such Ni-pigments in fossil deposits?

J. R. Maxwell. Professor Runnegar is correct in remembering that methanogenic bacteria contain a nickel pigment, Factor 430. It has a structure similar to porphyrins, although it is not actually a porphyrin, and is a highly functionalized compound with a variety of functional groups round the macrocyclic ring. I would expect it to be present in sediments where there is methanogenic activity, and it would be an excellent molecular marker for such activity. I am not sure, however, how long it could survive in a recognizable form in sediments with increasing burial depth because of the large number of functional groups present.

S. Macko (*Department of Environmental Sciences, University of Virginia, U.S.A.*). My collaborators and I have been approaching characterization of chlorophylls and their breakdown products by using stable isotope ratios of nitrogen and carbon of the tetrapyrrole. The nitrogen is important because it is bound in the pyrrole at synthesis and reflects the source nitrogen. We have also seen fairly consistent relations between chlorophyll *a* and chlorophyll *b* which should eventually help in resolving the products of these two once they are deposited in the sedimentological record and undergo diagenesis. It appears that the signals are fairly well preserved as we have looked at 1600-year-old *Sargassum* preserved in the Orca Basin which has nearly identical isotope signatures to, and the chemistry of, modern *Sargassum*. The major problem with the application of the technique to fossil sediments will be the complexity of the separation you observed and the presence of coelution of minor components.

J. R. Maxwell. I agree entirely with Dr Macko's comments about the $\delta^{15}N$ values reflecting the source nitrogen, whereas changes can be expected in carbon values, depending on the extent of diagenetic alteration in the β-substituents round the macrocycle. Because of this a few $\delta^{13}C$ and $\delta^{15}N$ measurements have already been made, in collaboration with Dr John Hayes, of six of the alkyl porphyrin components isolated from the Triassic Serpiano oil shale. Fortunately, the demetallated vanadyl alkyl porphyrins have a relatively simple distribution so the isolation was not too much of a problem in this case.

J. D. Hudson (*Department of Geology, University of Leicester, U.K.*). The porphyrin results from the Posidonia shale that Professor Maxwell mentioned, indicating that photosynthetic bacteria contributed to the population and hence that anoxic conditions extended into the photic zone, are of great interest. This is particularly because there has been much discussion in the geological and palaeontological literature concerning the depositional environment of the Posidonia shale: anoxic bottom waters with brief oxygenation events according to Seilacher, only a very thin (a few mm or cm) anoxic layer of bottom water according to Kaufmann. There are also variations in oxygenation inferred for different beds within the Posidonia shale sequence. In view of this, it would be important to know from which precise beds the samples are, and eventually to extend the sampling throughout the sequence.

J. R. Maxwell. The HOL sample was collected at Holzmaden (*ca.* 30 km southeast of Stuttgart) at the quarry operated by B. Hauff Jr, and comes from the Middle Lias ε (Unterer Schiefer, horizon II/4). The SCH sample comes from an outcrop at Schömberg (*ca.* 40 km southwest of Tübingen) and is from the Lower Lias ε (Seegrasschiefer). Deposition of the latter, according to Kaufmann (1981), took place mainly under oxygen depleted conditions, as indicated by the presence of *Chondrites*. Our results indicate that, before *Chondrites* activity, i.e. during deposition of the sediment, there were periods of photic zone anoxia. For the Unterer Schiefer Kaufmann suggests an overall greater extent of oxygen depletion than for the Seegrasschiefer; our results again indicate that there were periods of photic zone anoxia and do not contradict the Kaufmann model. I agree that a detailed study of the porphyrins through a sequence from a single core would be fruitful.

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