21. TETRAPYRROLE PIGMENTS

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BACKGROUND

Both major classes of tetrapyrrole pigments, chlorins and porphyrins, have been found in sediments; chlorins associated with recent sediments and porphyrins with ancient sediments and bitumens (Hodgson *et al.*, 1967; and Baker, 1969). Firm structural identification of the components of these complex mixtures has not been possible because of similarity of spectral and chromamographic properties of homologues.

Mass spectrometry has proved to be the most powerful analytical method in unraveling the structures of porphyrins in bitumens (Baker *et al.*, 1967) and its application to the pigments in recent sediments seems a normal extension. A library of mass spectra must be accumulated so that the spectra may be interpreted, and until the present only a scattered few mass spectra of the types expected in sediments have been reported (Jackson *et al.*, 1965). Neither chlorophyll nor the phytol esters of chlorin derivatives have been reported, probably because of their thermal instability. The author has found that readable mass spectra of such compounds can be obtained, and reports preliminary spectra on model compounds and pigments extracted from deep ocean sediments.

MASS SPECTRA OF PHEOPHYTIN AND ITS ANALOGUES

The partial mass spectrum of pheophytin *a* is shown in Figure 1. The complexity is apparent and a complete analysis of the spectrum is not appropriate here. The significant information has been extracted and is presented in Figure 2 in diagrammatic form along with similar information for pyropheophytin *a* and deoxomesopyropheophrobide *a* dihydrophytol ester (for structures, see Figure 3).

In the high-voltage mass spectrum of pheophytin *a*, two losses dominate the spectrum, 278 and 354. The 592 peak (loss of 278, probably phytadiene) is presumed to arise via the following mechanism (Bedzikievicz *et al.*, 1964):
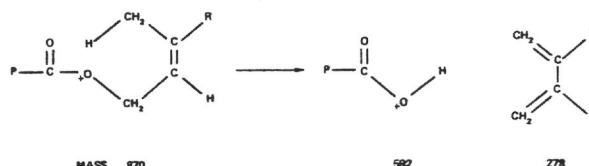
The 278 peak itself is of variable height depending on the source temperature and technique of sample injection. Pheophytin *a* is sufficiently labile that continual exposure to a source temperature of 300° will lead only to pyrolysis (see Experimental section). Interestingly, the loss of the 10-carbomethoxyl group in conjunction with the diene does not lead to the acid (mass 534), but rather to the ketene (mass 516):
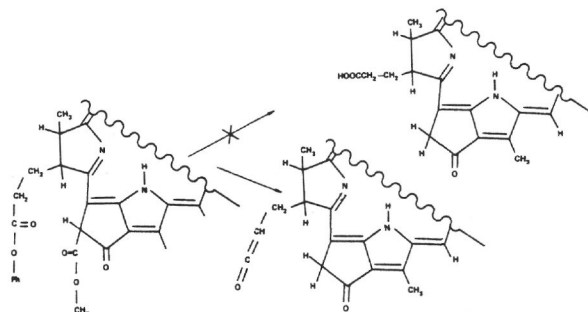
That the 10-carbomethoxyl group does influence and/or control the phytol side-chain fragmentation is shown by the spectrum of pyropheophytin *a*. Here the 534 peak is the major peak in the spectrum while the 516 is all but absent. Compare Figure 2A and 2B. Odd mass peaks corresponding to C-O cleavage ($C_{20}H_{39}O$ loss) are minor in both the pheophytin and pyropheophytin spectra. The mechanism proposed for the formation of pheophorbide (mass 592) from pheophytin requires the intervention of double bond for hydrogen transfer to give even mass fragments. Would fragmentation without hydrogen transfer become dominant if the double bond were absent as is the usual case with long chain fatty acid esters? A dihydrophytol ester was synthesized and its spectrum shown in Figure 2C. The major peak corresponds to a loss of 280 (phytene) presumably via a conventional McLafferty rearrangement.
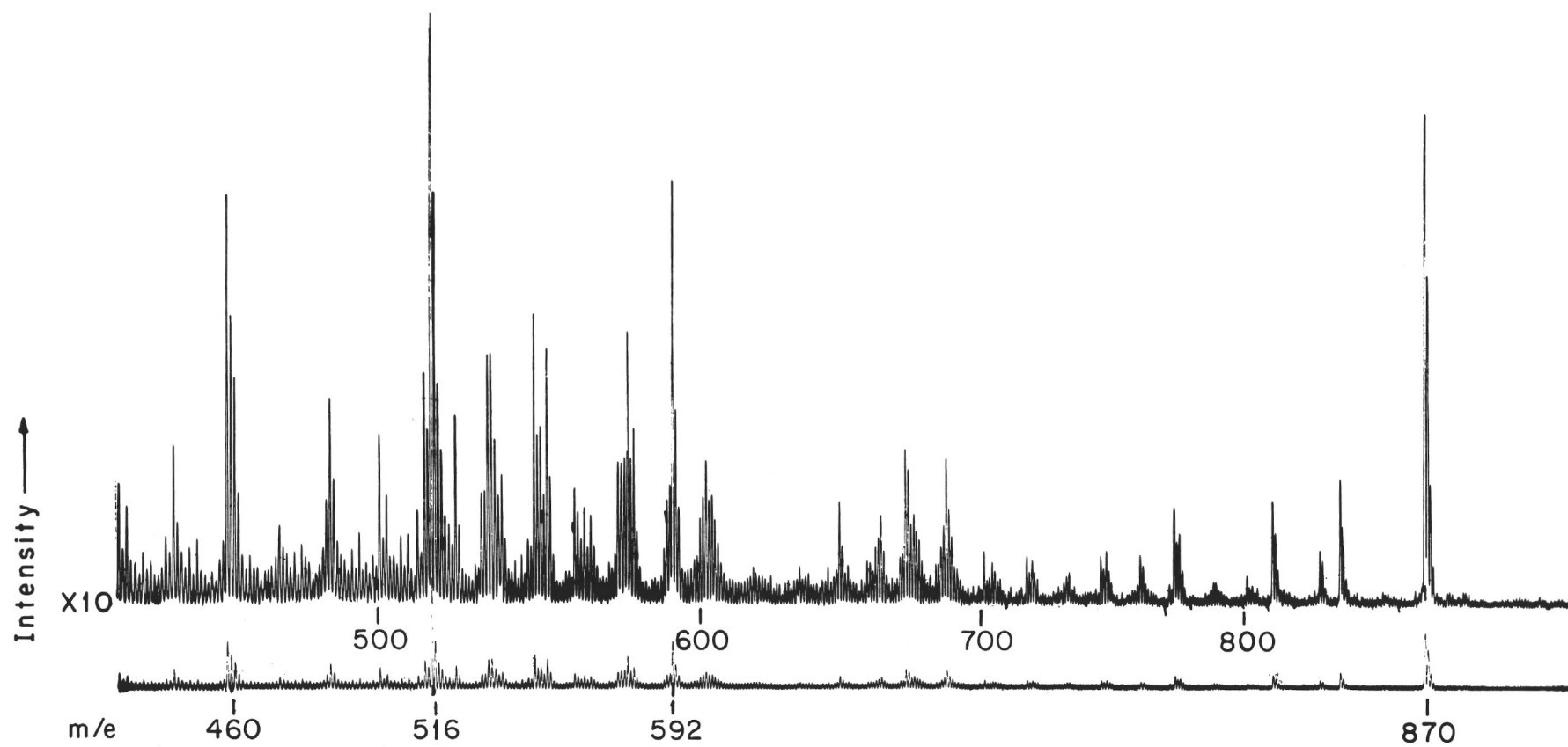
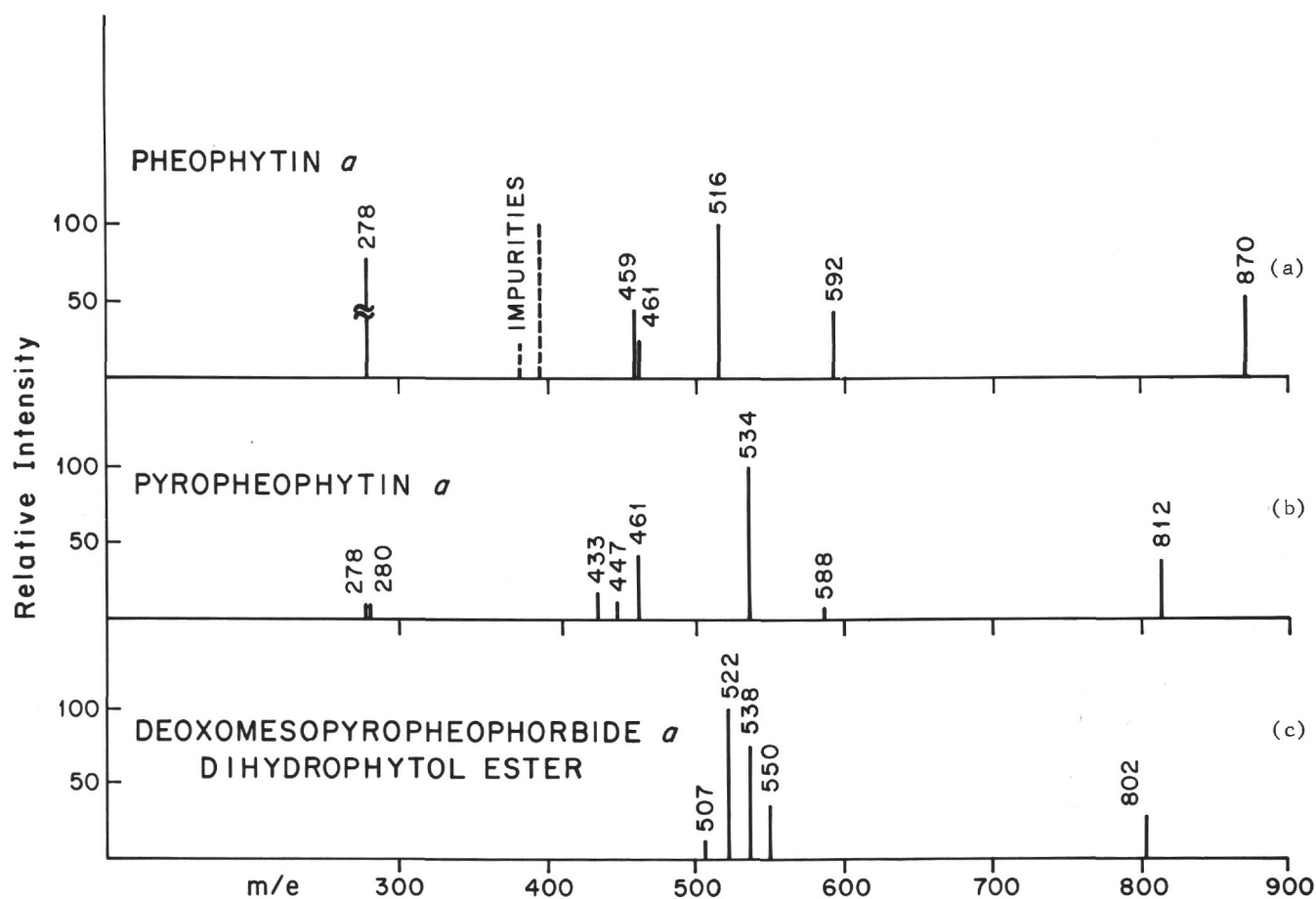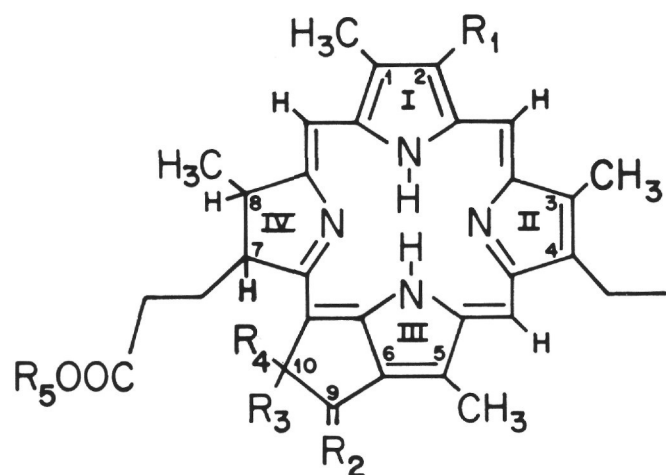Figure 1. *Partial mass spectrum of pheophytin* a.

Figure 2. *Diagramatic mass spectra of pheophytin analogues.*

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Figure 3. *Structures of pheophytin analogues.*

Comp.	Name	R_1	R_2	R_3	R_4	R_5
1	Pheophytin a	$CH=CH_2$	O	$COOCH_3$	H	$CH_2CH=\underset{CH_3}{C}-C_{16}H_{33}$
2	Pyropheophytin a	$CH=CH_2$	O	H	H	$CH_2CH=\underset{CH_3}{C}-C_{16}H_{33}$
3	Deoxomesopyropheophorbide a dihydrophytol ester	CH_3CH_3	H_2	H	H	$CH_2-CH_2-\underset{CH_3}{CH}-C_{16}H_{33}$
4	10-Hydroxy Pheophorbide a	$CH=CH_2$	O	$COOCH_3$	OH	$CH_2CH=\underset{CH_3}{C}-C_{16}H_{33}$

SPECTRA OF FOSSIL PIGMENTS

The pertinent geological data and absorption spectra of the tetrapyrrole pigments extracted from sediments taken at Sites 26, 27 and 30 are given in Table 1. Total pigment was separated into red and green components by chromatography and in line with conventional terminology, the green pigments have been called chlorins and the red ones, porphyrins.

The chlorin from Site 26 has a pheophorbide a type spectrum. The major red absorption at 668 mu suggests that metallochlorins are not present (at least as major components of the mixture) since in that case the band would be shifted to shorter wave lengths (640 mu or lower). Also bacteriochlorins do not seem to be present, since in that case the red band would be at much lower energy (about 750 mu).

The partial 70 ev mass spectrum of the chlorins from sample 4-30-07-01 is shown in Figure 4. The observation of peaks at 877, 858 and 830 was surprising and is most readily explained by assuming the presence of phytol esters of the chlorins similar to their biological procursors, pheophytin a (870) and pheophytin b (884). A nearly identical spectrum was obtained from the chlorins from 4-26-02-02, where peaks at 872, 844 and 830 were observed. In this case sufficient pigment was available for a second injection so that a low voltage spectrum was obtained. The low voltage (14 ev) spectrum showed only two main peaks (872 and 858), and no peaks in the 450 to 600 mass range (as are seen in Figure 2), leading to the impression that the peaks in that region are fragments.

The 872 peak in the mass spectrum indicates a dihydropheophytin structure, and the 668 mu band in the electrons spectrum suggests only an intact chromophore. This tends to rule out reduction of the vinyl group or formation of ring-conjugated carbonyl functions as likely structures.

The mid-range odd mass peaks in the mass spectra (that is, 603, 579, 577, 575 and so forth) could be produced by bond rupture on the phytyl propionate ester (position 7, compound 1, Figure 3) at the ester oxygen, giving a loss of 295. If the alcohol were dihydrophytol, the equivalent loss would be 297 mass units (872-297-575). No model compounds have been prepared yet which produce this type of mass spectrum so that the structures of these geo-chlorins are beyond reasonable speculation. However, the outlook for solution of this problem is encouraging since only a relatively few compounds are present as shown by the rather uncomplicated low voltage mass spectra.

It has generally been assumed that saponification of the phytol ester linkage occurred very early (hours or days after demise of the plant cell) in the diagenetic process.

These analyses, if confirmed, indicate that, at least in deep ocean sediments, ester linkages formed in the Pliocene and early Pleistocene eras are preserved.

EXPERIMENTAL

Treatment of Cores

The cores which are stored in a deep freeze at -20° are broken up; and, a sample (about 60 grams is transferred to a Soxhlet thimble and extracted exhaustively with 90 per cent acetone—10 per cent methanol. The extracting solvent is replaced with fresh solvent every 12 hours, and the extract is reduced to dryness under vacuum and stored under nitrogen at 0°. The pigment fraction is separated by Gel Permeation Chromatography as follows: 45 milliliters (about 25 grams of Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, New Jersey), is slurried with 150 to 200 milliliters of distilled tetrahydrofuran (THF). This slurry is transferred to a 2 × 50 centimeter chromatography column fitted with a teflon cock, and a 1 to 1.5 centimeter layer of coarse red sand is added. A bed height of 25 +3 centimeters gives a suitable flow rate of 1.5 ml/min. The combined extracts are taken up in 3 to 4 milliliters of THF and placed on the sand. A typical column (partially developed) is shown in Figure 5. The fraction containing the pigment which is detected by its green color, if sufficient pigment is present, or a red fluorescence and a recognizable absorption peak at 395-410 mμ, is esterified by treatment with diazomethane. Rechromatography over silica gel (Davison, Grade 923, 100-200 mesh) with cyclohexane, cyclohexane-benzene, benzene and benzene with 0.5 to 4 per cent methanol gives a pigment concentrate suitable for spectrometry.

Mass Spectrometry

The mass spectra were obtained on an AEI MS-9 Spectrometer fitted with a direct probe sample injection system. Pigment samples were transferred to the alumina probe with a drop or two of benzene. The solvent was evaporated by rotating the probe such that the pigment was deposited on the forward face of the cylindrical probe. After removal of most of the absorbed solvent from the probe with the roughing pump the ball valve was opened and the traces of solvent removed. The sample was then injected quickly and the spectrum recorded, because once the pigments were exposed to temperatures sufficiently high (about 320 to 330°) to obtain a readable spectrum, pyrolysis occurred very rapidly.
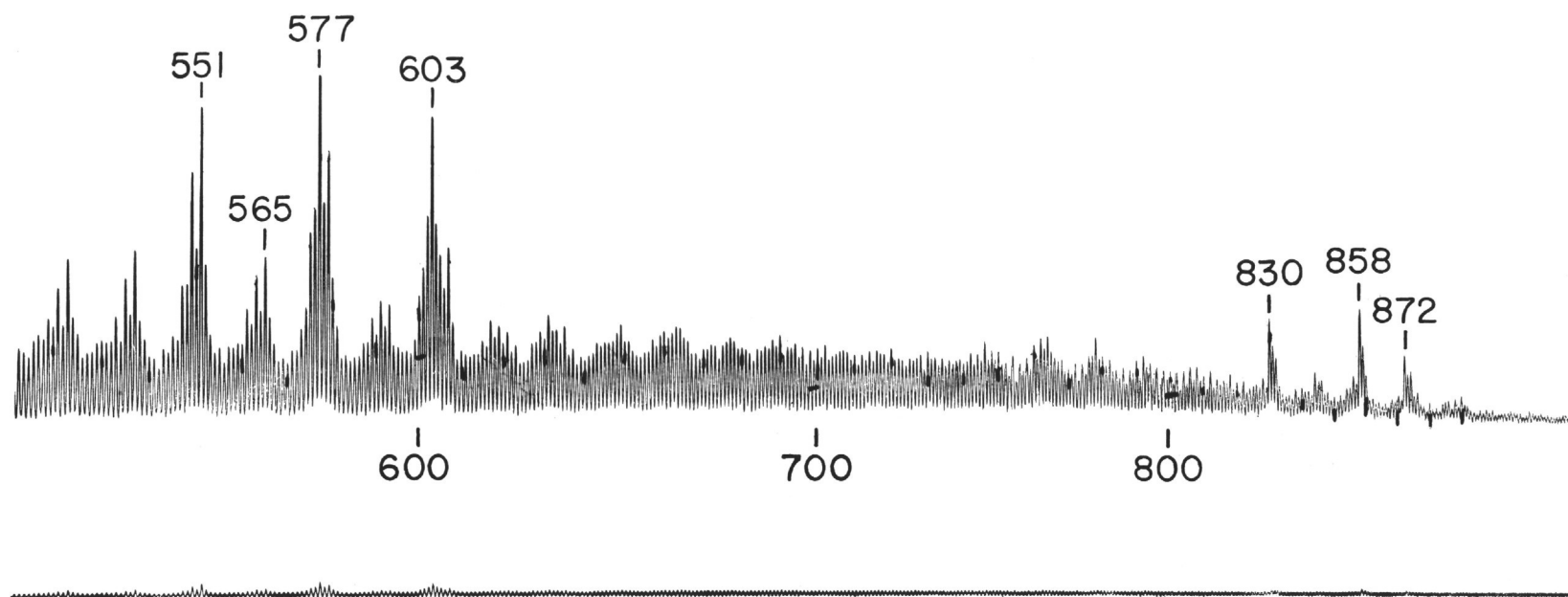
Figure 4. *Partial mass spectrum of chlorins from JOIDES 4-30-07-01.*

IVORY WHITE

YELLOW

GREEN
ORANGE RED
DARK BROWN

OLIVE GREEN

WHITE

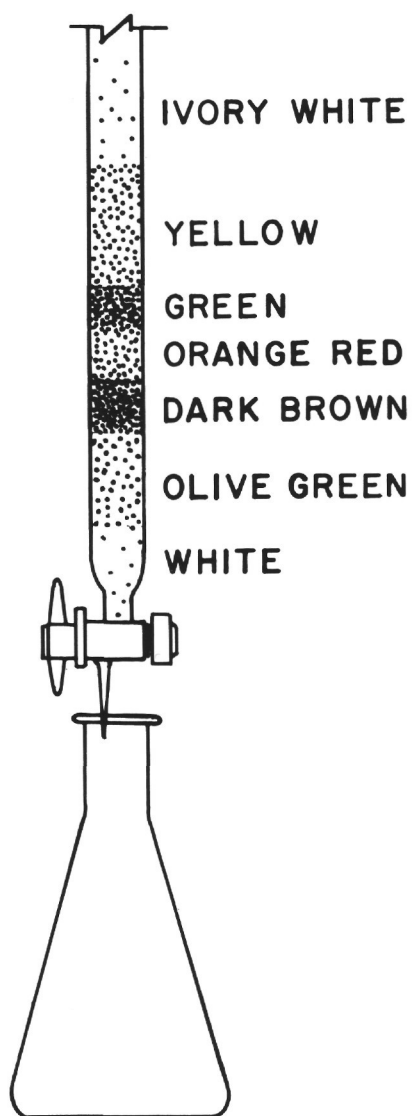Figure 5. *Gel Permeation Chromatography of Core Extracts. See text for details.*

TABLE I
Tetrapyrrole Pigments in Core Samples from Leg 4

Sample	Geologic Age	% Organic Carbon	Tetrapyrrole Pigments			
			Type	Yield μg.	Electronic Spectrum μ (mu)	Mass Spectrum m/e
4-26-02-02[a]	Pleistocene	0.4	Chlorin	180/120 g.	668, 610, 535, 505, 413 Soret	872, 858, 844, 830, 603, 579, 577, 549, 551
			Porphyrin	None detected		
4-27-04-02[b]	Lower Miocene	0.4	Chlorin	None detected		
			Porphyrin Metal?	2/80 g.	Vis. bands not obs. 393 Soret	
4-30-07-01[c]	Pleistocene-Pliocene	0.7	Chlorin	30/160 g.	662, 605, 530, 505, 408 Soret	872, 858, 830, 603, 577, 551
			Porphyrin Metal?	8/160 g.	583, 555, 395 Soret	

[a]Site 26-Off the coast of the Guianas. Sediments continental in origin; depth – 120 meters.

[b]Site 27-North of Barbados in Atlantic basin. Depth – 260 meters.

[c]Site 30-In the Caribbean, west of Grenadines. Depth – 280 meters.

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