

III. CHLOROPHYLL DERIVATIVES IN DSDP LEG 14, 20, 26, 27, AND 29 SEDIMENTS

Earl W. Baker and G. Dale Smith, Department of Chemistry,
Northeast Louisiana University, Monroe, Louisiana

INTRODUCTION

Background

As part of the continuing participation of organic geochemists in the Deep Sea Drilling Program, we report herein the findings of investigations of tetrapyrrole pigment isolated from selected sediment core samples from DSDP Legs 14, 20, 26, 27, and 29.

Experimental

Electronic absorption spectra were obtained using a Beckman ACTA CIII ultraviolet-visible linear wavelength spectrophotometer and a Beckman DK-2 spectrophotometer. An Aminco-Bowman 8202 spectrofluorometer was employed for fluorescence measurements, and mass spectra were recorded using an AEI MS9 mass spectrometer equipped with a solid sample probe inlet system.

The general procedure described for treatment of Leg 15 (Baker and Smith, 1973) and Leg 22 (Smith and Baker, 1974) cores was used in the analysis of Leg 14, 20, 26, 27, and 29 sediment samples (Table 1). Core extracts yielding insufficient tetrapyrrole pigment for UV-visible analysis were treated with methane sulfonic acid (MSA) following the method of Baker (1969). The extract was treated with MSA (5-ml MSA for each gram of extract concentrate) in a ball mill containing 16 glass marbles, rolling at low speed for 4 hours at $105 \pm 5^\circ\text{C}$. After 4 hours, a mixture of 5 g of ice and 5 ml water per gram of extract was added with stirring. The solution (ca 50°C) was filtered and extracted with methylene chloride in a liquid-liquid extractor. The porphyrin was converted from the dication to the free base by treatment with

sodium acetate (100 ml, 10% wt/v). These solutions were analyzed for free-base porphyrins by both emission and excitation spectrofluorometry. The porphyrins were then partitioned between the organic solvent and 6*N* hydrochloric acid and the acid solution analyzed for dication fluorescence. Sample size of the standard compounds was approximately 10 mg, and the instrument parameters were set at a moderate sensitivity level.

SPECTRA OF FOSSIL PIGMENTS

Each of the sediment samples investigated in this series of cores was low in tetrapyrrole pigment concentration (Table 2). Samples 27-263-6-5 and 29-281-14-0 yielded approximately 115 ppb pigment, whereas most of the other cores analyzed gave tetrapyrrole yields of 5 ppb or lower.

The youngest sample of the suite of cores from Leg 29 (281-14-0, Oligocene age) contained both major classes of tetrapyrrole: chlorin and porphyrin. The electronic spectrum of the core extract consisted of a band in the red region at 660 nm, and two major bands in the Soret region (ca 400 nm). A band at 408 nm is taken to indicate the presence of chlorin pigment, while the band at 395 nm may be ascribed to metalloporphyrin. Leg 29 Eocene samples gave electronic spectra characteristic of metalloporphyrin, with the Soret appearing at 394 nm.

In addition to Leg 29 cores, samples from Legs 14, 20, 26, and 27 were also investigated (Table 2). The absorption spectra obtained followed the pattern observed in previous DSDP core analyses (Baker, 1970; Baker and Smith, 1973; Smith and Baker, 1974). The Oligocene to late Eocene samples exhibited both chlorin and metalloporphyrin peaks in the visible spectra. The older

TABLE 1
Experimental Procedure Flow Sheet

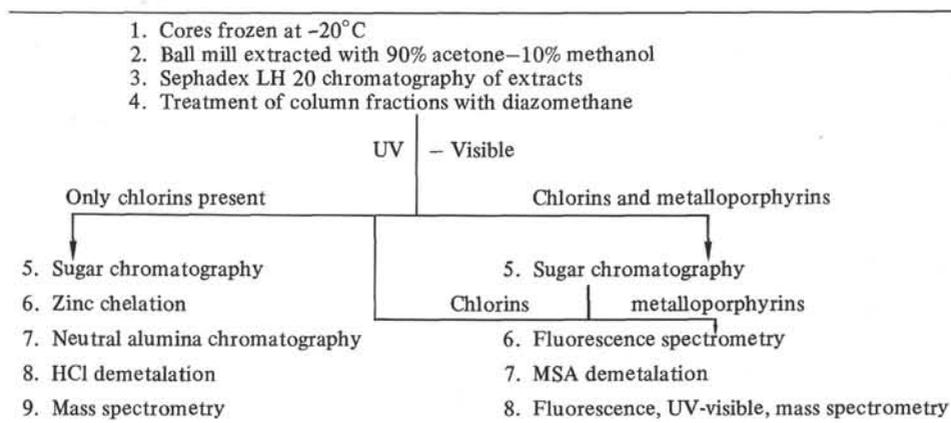


TABLE 2
Core Description and Analytical Results

Sample	Geologic ^a Age	Sediment ^a Depth (m)	Pigment yield ppb	Electronic Spectrum (nm)		Tetrapyrrole Type	Fluorescence Check
14-144A-2-6	Middle Eocene	45	<5	408	660	Chlorin Nickel porphyrin	Positive
14-144B-3-6	Early Oligocene	35	30	408	660	Chlorin Nickel porphyrin	Positive
20-198A-4-3	Early Campanian	120					Negative
26-250A-10-6		100	<5	392		Nickel porphyrin	Positive
27-259-9-5	Upper Cretaceous	100	<5			?	Positive
27-263-6-5	Upper Cretaceous	150	120	389	513 551	Nickel porphyrin	
29-280A-10-4	Late Eocene	165	<5	394		Nickel porphyrin	Positive
29-280A-15-2	Late Eocene	280	<5	393		Nickel porphyrin	Positive
29-280A-19-0	Late Eocene	450					Negative
29-281-11-0	Middle Miocene	90					Negative
29-281-14-0	Oligocene	130	110	408	660	Chlorin Nickel porphyrin	Positive

^aGeologic age and sediment depth from shipboard hole summaries.

sediments (Upper Cretaceous) contained no detectable free-base chlorin, with only tetrapyrrole belonging to the metalloporphyrin class.

Samples containing less than 5 ppb pigment (Table 2) were treated with MSA to remove chelating metals and to enhance the pigment fluorescence, thus lowering the detection limit. The fluorescence spectra of all porphyrin extracts and reference compounds (mesoporphyrin IX dimethyl ester and etioporphyrin I) were obtained by exciting at 390-405 nm and scanning the 550-700 nm emission range for the two principal dication fluorescence peaks. As indicated in Table 2, the presence of tetrapyrrole was detected in all but three of the sediment core samples investigated by fluorometry.

DISCUSSION

Previous Results

The data obtained by electronic absorption in this investigation are in good agreement with previously reported studies of DSDP cores.

In investigations of deep-ocean sediments spanning the geologic age range from Recent to Upper Cretaceous, a spectrum of structures from barely altered chlorophyll to nickel porphyrins has been found. In the Leg 15 analysis (Baker and Smith, 1973), we reported the identification of a series of dihydrophytol pheophorbides isolated from Pleistocene sediments by the use of electronic and mass spectroscopy. The electronic absorption spectrum was identical to that of pheophytin a. In cores of increasing age and depth of burial (Miocene to Eocene) the visible bands in the spectra of extracted pigments undergo blue shifts, suggesting reduction of ring-conjugating groups.

Analytical results for the Eocene and Oligocene sediments reported here (Table 2) agree well with the

predicted chlorophyll diagenetic pathways outlined for Leg 22 (Smith and Baker, 1974). In addition to the observation of green (unchelated chlorin) pigments, characterized as pheophorbides and chlorin *p*₆ compounds, porphyrins chelated with nickel were detected. In the older sediments (late Eocene to Upper Cretaceous) only nickel porphyrin was observed. This absence of metallochlorins and free-base porphyrins suggests that introduction of the metal ion, and the dehydrogenation of porphyrin are possibly concerted reactions.

Special Study—Leg 27, Site 263

The analytical results of Sample 27-263-6-5 are of special interest due to the relatively high yield of pigment. One half of the core sample made available for investigation in our laboratory (360 g) was exhaustively extracted with 90% acetone-10% methanol. The visible spectrum indicated the presence of ca 43 μ g of nickel porphyrin.

After treatment with diazomethane, the pigment was chromatographed on neutral alumina to yield two fractions. The fraction eluted with benzene-methanol (ca 35 μ g) had UV-visible peaks at 551, 513, and 389 nm. The more polar fraction eluted with tetrahydrofuran absorbed at 548, 510, and 391 nm. Since the first fraction contained most of the pigment, it was selected for treatment with methanesulfonic acid (MSA) and the tetrapyrrole was demetaled to determine its porphyrin structure using characteristic absorption patterns.

Because of a high level background, interpretation of the UV-visible spectrum of the extract was not possible. After further treatment an extract was obtained which after neutralization under ether with sodium bicarbonate gave a visible spectrum with peaks at 648.5, 595, 530, 505, and 395 nm, very unlike a typical free-base porphyrin (Figure 1a).

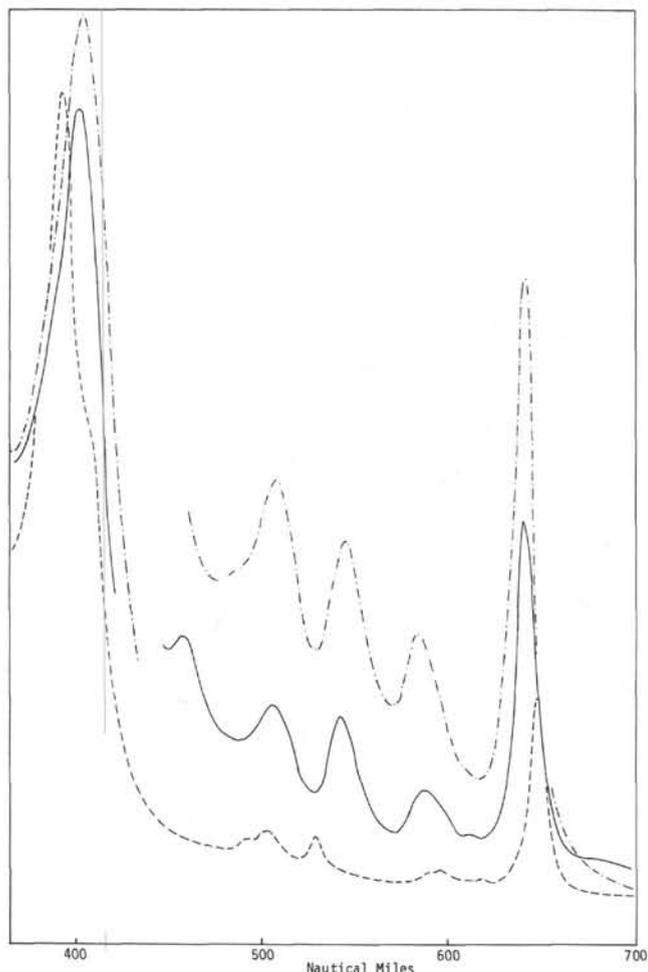


Figure 1a. Visible spectrum peaks. — dioxy-mesoporphyrin IX; - · - · - dioxy-deoxophylloerythrin; - - - - first half of 27-263-6-5 core extract.

In addition to the tetrapyrrole recovered, a small amount of pigment was obtained by treatment with sodium carbonate under ether. The electronic spectrum of the concentrated ether indicated the presence of peaks at 620 and 565 nm, in addition to the 648, 595, 530, 505, and 395 nm peaks. Shaking the ether solution with 3% hydrochloric acid removed the compound(s) absorbing at 620 and 565 nm, leaving a solution with an identical spectrum to that obtained on the dichloromethane extracts.

The pigment recovered from the 6*N* hydrochloric acid extract has a chlorin-type absorption spectrum with peak 1 at 649 nm. The overall spectrum appearance is very similar to that obtained for a compound isolated from a petroleum porphyrin aggregate by Fisher and Dunning (1961). They reported the pigment to be a non-carboxylated material which resists conversion from chlorin to porphyrin upon treatment with a quinone by Eisner's method (Eisner, 1955; Eisner and Linstead, 1956), thereby indicating that the material is probably not a chlorin.

Suspecting that the compound(s) was an artifact, the dioxy derivative of mesoporphyrin IX dimethyl ester

(dioxy-meso IX DME) and the dioxy derivative of deoxophylloerythrin methyl ester (dioxy-DPE ME) were prepared by the method of Fisher and Orth (1937). As shown in Figure 1a, the spectra of dioxy-meso IX, dioxy-DPE and the extracted compound(s) are very similar.

In addition, the three compounds exhibited similar spectral changes, noticeably the appearance of a peak at 615-630 nm, when treated with diazomethane to convert free-acid functions to methyl esters (Figure 1b).

The unknown sediment pigment and the two dioxy-model compounds were treated with nickel acetate in acetic acid-acetone to produce the metal chelates. Again the results reveal a close similarity in spectra, especially between the unknown compound and the dioxy derivative of deoxophylloerythrin (Figure 1c).

Lembert et al. (1938) and Lembert and Legge (1949) reported that the hydroxyporphyrins or oxyporphyrins have a chlorin-like spectrum, and this material may very well be an oxidation product, as it forms during prolonged standing of benzene solutions of porphyrins.

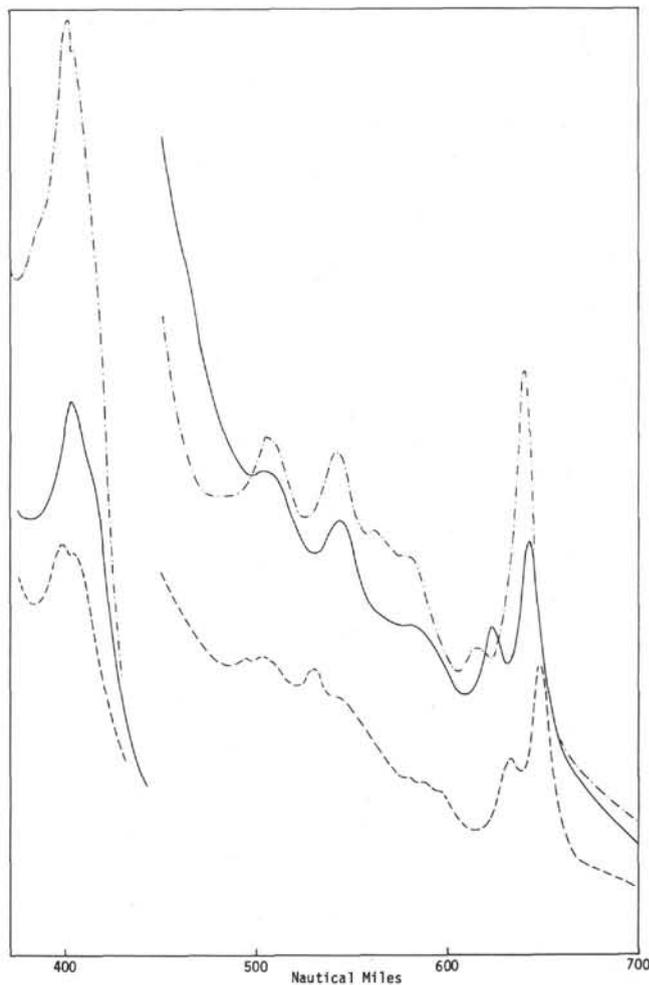


Figure 1b. Visible spectrum peaks. — dioxy-mesoporphyrin IX DME; - · - · - dioxy-deoxophylloerythrin ME; - - - - 27-263-6-5 methyl ester.

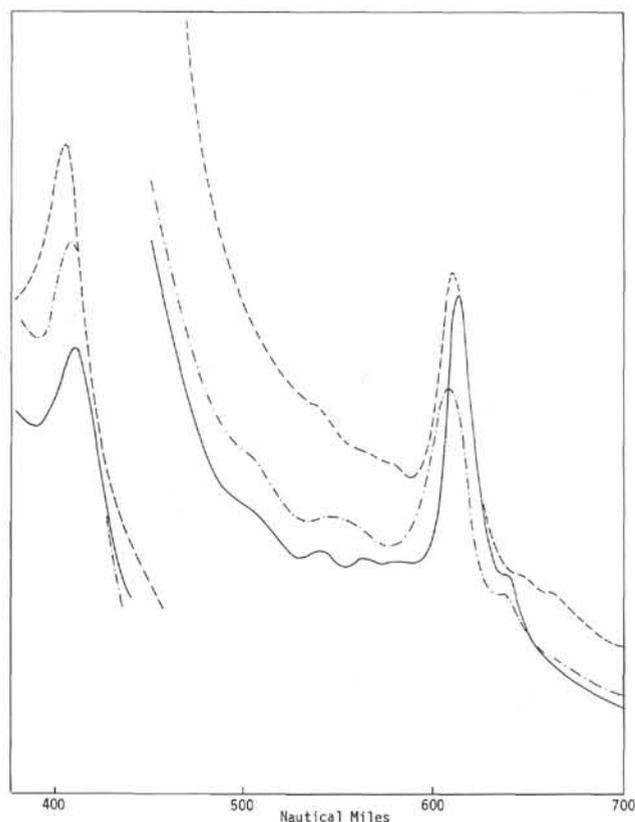


Figure 1c. Visible spectrum peaks. — nickel dioxy-mesoporphyrin IX DME; - - - nickel dioxy-deoxyphylloerythrin ME; - · - · - nickel 27-263-6-5 methyl ester (first half).

Caution must be exercised in the selection of acidic reagents for demetalation or for extraction of porphyrins from bitumens. Treatment of deuterio- or protoporphyrin in HBr-AcOH leads to a compound of the chlorin class when bromine was present (Chang et al., 1967).

Baker et al. (1967) reported satisfactory yields in the extraction of asphaltenes with methanesulfonic acid, but destruction of the porphyrin when attempts were made to demetalate either purified vanadyl petroporphyrin fractions or vanadyl porphyrins with specially purified MSA (Baker, 1969). However, the addition of hydrazine sulfate to the reaction mixture produced essentially quantitative yields of free-base porphyrin in each case.

Comparative results with the model dioxy compounds indicated that during MSA treatment of the first half of Sample 27-263-6-5 extract oxidation to the dioxy derivative occurred. The experimental procedure was then repeated using the second half of the core with precautions taken to prevent oxidation.

The remaining core (360 g of 27-263-6-5) was extracted and the isolated pigment was treated with technical MSA and about 50 mg of phenylhydrazine. After heating for 2.5 hr at 105°C, the solution was worked up as before. The recovered free-base tetrapyrrole had an acid number below 4, unlike the dioxy-porphyrin which had an acid number of around 15. Comparison of the electronic spectrum with standards suggests a mix-

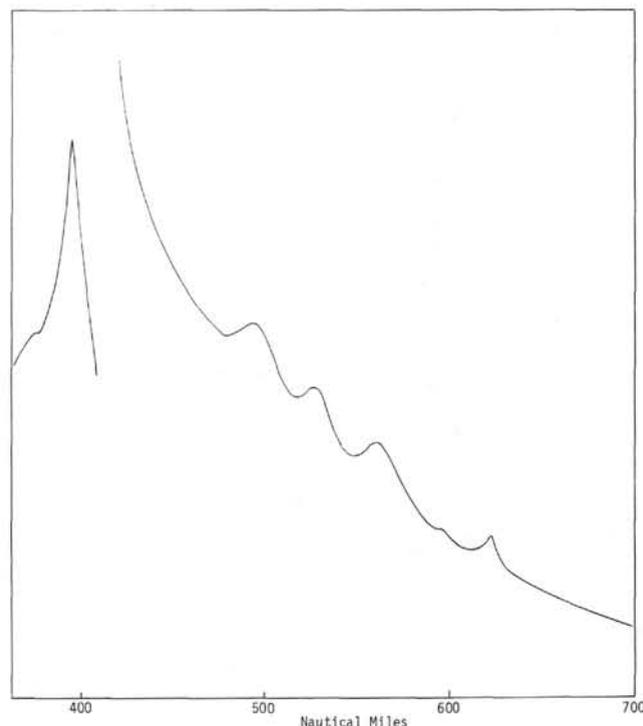


Figure 2a. Electronic spectrums. — electronic spectrum of porphyrin extracted from second half of 27-263-6-5.

ture of DPEP-type and Etio-type free-base porphyrins (Figure 2a).

Reaction of the porphyrin mixture with nickel acetate gave a product having an electron spectrum very similar to that of the nickel chelates of various synthetic porphyrins and identical with that of the pigment isolated from the original core acetone extract (Figure 2b). Again, the spectrum of the nickel derivative of the sediment porphyrin indicated a mixture of DPEP and Etio-type tetrapyrroles. A comparison of the relative intensities of the α and β bands points to such a mixture (Table 3).

Inspection of the total collected data leads to the speculative identification of the 27-263-6-5 Upper Cretaceous core pigments as predominantly nickel deoxyphylloerythrin, and closely related nickel phylloporphyrins, with a lesser amount of other carboxylated nickel porphyrins. The existence of such a mixture is consistent with the report by Treibs (1934) of the identification of carboxylated compounds in petroleum porphyrin aggregates.

Observation of the intact carboxylic acid is significant, since the presence of such a functional group indicates a low-temperature history. Treibs (1934) has shown that porphyrin carboxylic acids lose carbon dioxide when subjected to temperatures above 200°C in the laboratory for extended periods of time.

The tentative identification of the chlorin-like compound as a dioxy by-product of the separation scheme is of substantial importance. These findings could possibly explain the origin of the secondary-chlorins reported by many workers (Treibs, 1936; Blumer, 1950; Blumer and Omenn, 1961).

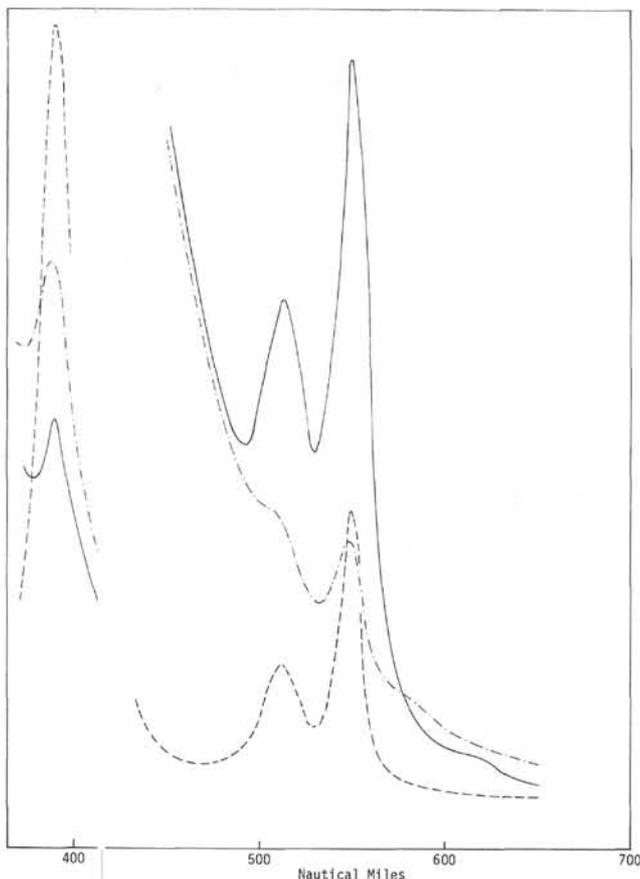


Figure 2b. Electronic spectrums. — 27-263-5 acetone-methanol sediment core extract; - - - nickel chelate of 27-263-6-5 (second half) demataled petroporphyrin; - · - · - nickel deoxyphylloerythrin.

ACKNOWLEDGMENTS

This research was supported by the Oceanography Section of the National Science Foundation, NSF Grant GA-37962. The mass spectra were obtained through the cooperation of the associated personnel of the Jet Propulsion Laboratory (JPL) of the California Institute of Technology under Contract Number NAS7-100.

Appreciation is expressed to Dr. Heinz G. Boettger of JPL for assistance in operation of the AEI-MS9 HRMS Data System. Our sincere thanks are extended also to Dr. Phillip Jobe for his generous assistance in obtaining the fluorescence spectra.

REFERENCES

- Baker, E. W., 1969. *In* Eglinton, G. and Murphy, M. T. J. (Eds.), *Organic geochemistry*: New York (Springer-Verlag), p. 479.
- , 1970. Tetrapyrrole pigments. *In* Bader, R. G., et al., *Initial Reports of the Deep Sea Drilling Project, Volume 4*: Washington (U.S. Government Printing Office), p. 431.
- Baker, E. W. and Smith, G. D., 1973. Chlorophyll derivatives in sediments, Site 147. *In* Heezen, C., MacGregor, I. D., et al., *Initial Reports of the Deep Sea Drilling Project, Volume 20*: Washington (U.S. Government Printing Office), p. 943-947.
- Baker, E. W., Yen, T. F., Dickie, J. P., Rhodes, R. E., and Clark, L. R., 1967. Mass spectrometry of porphyrins II. Characterization of petroporphyrins: *J. Am. Chem. Soc.*, v. 89, p. 3631.
- Blumer, M., 1950. Porphyrinfarbstoffe und porphyrin-metallkomplexe in schweizerischen Bitumina: *Helv. Chim. Acta*, v. 33, p. 1627.
- Blumer, M. and Omenn, G., 1961. Fossil porphyrins: Uncomplexed chlorins in a Triassic sediment: *Geochim. Cosmochim. Acta*, v. 25, p. 81.
- Chang, Y., Clezy, P., and Morell, D., 1967. The chemistry of pyrrolic compounds. VI. Chlorins and related compounds: *Australian J. Chem.*, v. 20, p. 959.
- Eisner, U., 1955. Chlorophyll and related substances: Part II. The dehydrogenation of chlorin to porphin and the number of extra hydrogen atoms in the chlorins: *J. Chem. Soc.*, p. 3749.
- Eisner, U. and Linstead, R., 1956. Chlorophyll and related substances: Part III. The synthesis of octamethylchlorin: *J. Chem. Soc.*, p. 1655.
- Fisher, L. and Dunning, H., 1961. Chromatographic resolution of petroleum porphyrin aggregates: *Bur. Mines Rep. Investig.* 5844.
- Fisher, H. and Orth, H., 1937. *Die chemie des pyrrols*: Part II: *Akad. Verlag., Leipzig*, p. 273.
- Lembert, R. and Legge, J., 1949. *Hematin compounds and bile pigments*: New York (Interscience), p. 91.
- Lembert, R., Cortis-Jones, B., and Norrie, M., 1938. Chemical mechanism of the oxidation of protohematin to verdohematin: *Biochem. J.*, v. 32, p. 171.
- Smith, G. D. and Baker, E. W., 1974. Chlorophyll derivatives in DSDP Leg 22 sediments. *In* von der Borch, C. C., Slater, G., et al., *Initial Reports of the Deep Sea Drilling Project, Volume 22*: Washington (U.S. Government Printing Office), p. 677-679.
- Treibs, A., 1934. Occurrence of chlorophyll derivatives in an oil shale of the Upper Triassic: *Ann.*, v. 509, p. 103.
- , 1936. Chlorophyll and hemin derivatives in organic mineral substances: *Agnew. Chem.*, v. 49, p. 682.

TABLE 3
Spectrum of Nickel Derivatives

Compound	α -band (nm)	β -band (nm)	α/β Ratio
Nickel DPEP ^a	555	522	2.09
Nickel deoxyphylloerythrin	551	512	2.55
Nickel etioporphyrin ^a	555	526	3.01
Nickel mesoporphyrin DME	553	517	3.36
27-263-6-5 ^b	551	513	2.78
27-263-6-5 ^c	551	513	2.65

^aIsolated from a sample of gilsonite.

^bAcetone-methanol extract of sediment core.

^cNickel chelate of free base porphyrin isolated from MSA extracts.