A SEARCH FOR PORPHYRIN BIOMARKERS
IN NONESUCH SHALE AND EXTRATERRESTRIAL SAMPLES*

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Abstract. An organic solvent extract of billion year old Nonesuch Shale was examined for porphyrins by means of fluorometry and high resolution mass spectrometry. It appears to contain at least three or more classes of porphyrins, one similar to tetraphenyl porphin and the others more complex. Many are apparently chelated with copper, nickel, zinc, iron and vanadyl and are highly aromatic. We have also examined the extracts of Apollo 11, 12 and 14 surface fines for porphyrins by spectrophoto-fluorometry but we found none even though our method was capable of detecting $10^{-18}$ moles per gm of sample.

1. Introduction

A wide variety of microfossils and carbonaceous materials of uncertain origin has been found in Precambrian sedimentary rocks. Some of the micro-structures found show 'algae like' and 'filamentous' morphology and occur in carbonaceous argillites, siltstones and cherts of great age (Barghoorn and Tyler, 1965; Schopf et al., 1965; Barghoorn et al., 1965; Cloud et al.; 1965; Barghoorn and Schopf, 1966; Engel et al., 1968; Cloud and Licari, 1968).

Simple morphological evidence for the antiquity of terrestrial life, however, impressive, would be strengthened if supported by other evidence (Nagy and Nagy, 1969; Cloud, 1969) and organic geochemical examination of ancient sediments has therefore been used to supplement these studies (Eglinton et al., 1964; Oró et al., 1965; Meinschein, 1965; Belsky et al., 1965; Prashnowsky and Schidlowski, 1967 and Kvenvolden et al., 1969).

The Precambrian Nonesuch shale of Northern Michigan is about one billion years old. It has been studied extensively by Barghoorn et al. (1965) by both geochemical and micropaleontological means and they have reported the presence of about 50 ppm of porphyrins in siltstones of this formation. This characterization was, however, based only on visible absorption spectra which were those typical of vanadyl porphyrins.

Porphyrin pigments are thermostable 'biological markers' found in many ancient terrestrial rocks (Baker, 1969). This paper presents some data on porphyrins in

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Nonesuch Shale and a discussion of their possible biogenic origin. We also describe the search for porphyrins in the Apollo samples.

2. Experimental

Nonesuch Shale Parting Shale Member, Domino Horizon (45.3 g) was collected by E. S. Barghoorn from the White Pine Copper Mine, White Pine, Mich. (Barghoorn, et al., 1965). It was ground to pass a 200 mesh Tyler standard sieve and was then layered (to ensure drainage) with small increments of borosilicate glass wool in a 'Zitex' microporous Teflon Soxhlet thimble. It was extracted for 24 h sequentially with methanol and benzonitrile and each extract was then concentrated separately in a rotary evaporator. The extracts were chromatographed on a Unisil* silicic acid chromatographic column with discontinuous gradients of hexane, benzene, and methanol. Both extracts were similar on the basis of chromatography but the methanol extract yielded very small amounts of the polar porphyrin class found abundantly in the benzonitrile extract. This latter extract was therefore chosen for mass-spectrometric analysis.

A column 1 cm I.D. x 17.4 cm high was packed with 5 g of activated 100–200 mesh Unisil in hexane. The sample, a dark brown oil (22 mg) which unavoidably contained a small amount of benzonitrile, was applied to the column in 3 x 2 ml of hexane. Subsequent solvent changes were used to rinse the sample vessel contents quantitatively into the column. All operations were carried out in near darkness but without exclusion of air. The column was eluted at 0.5 ml min⁻¹ with hexane, then (volume ratios) with hexane:benzene 9:1, hexane:benzene 2:1, hexane:benzene 1:1, benzene and finally methanol. Ten ml fractions were collected in glass stoppered centrifuge tubes and the bulk of the solvent was removed under a stream of nitrogen to near dryness. The residues were redissolved in benzene to a volume of 1 ml for subsequent examination.

Metalloporphyrins are frequently nonfluorescent so the benzene solution was first treated with methanesulfonic acid (MSA) to demetallate any such present (Erdman, 1965). The aqueous phase was then neutralized with saturated sodium acetate and partitioned with the benzene phase. The aqueous phase was discarded. The benzene phase which would contain the free base porphyrins was washed with a small volume of 6N HCl to concentrate them.

Fluorometric examination of all fractions revealed that a 'nonpolar' porphyrin fraction was eluted with hexane, principally in fraction 5, and a 'slightly polar' porphyrin class was eluted with hexane-benzene mixtures in fractions 15 through 21. Weight quantitation of the porphyrins was not attempted as they were contaminated with hydrocarbons.

Fractions 4 through 6 were pooled separately from fractions 15 through 25. Both samples were analyzed by mass spectrometry. The mass spectra were recorded on a

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