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IDENTIFICATION OF 180 MILLION YEARS OLD, PROBABLY UNCHANGED, MELANINE

K. Beyermann and D. Hasenmaier

(NASA-TM-75132) IDENTIFICATION OF 180 MILLION YEARS OLD, PROBABLY UNCHANGED MELANINE (Kanner (Leo) Associates) 10 p HC A02/MF A01

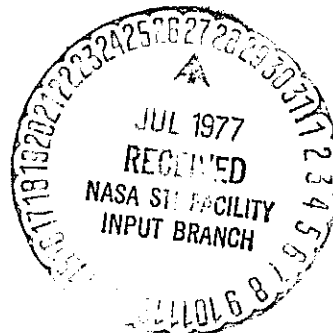
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| 16. Abstract The comparison of the infrared spectra of recent sepia melanine and of the content of the ink sac of fossilized cuttlefish indicates that the 180 million years old substance is unchanged melanine. Both substances behave identically on heating. Other procedures for identification of melanine are surveyed critically. They are not suited for a specific detection of melanine. | | | |
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IDENTIFICATION OF 180 MILLION YEARS OLD, PROBABLY
UNCHANGED, MELANINE

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As long as 100 years ago black-colored substances were /202*
observed in cuttlefish fossils in places corresponding to those
in which the ink sacs are located in recent cuttlefish. For a
long time the question has remained unanswered of whether this
fossil substance is unchanged melanine or decomposition products.

In what follows we will discuss the analytical problems
connected with the identification of recent and fossil melanine.
Infrared spectrography proves to be a suitable detection method,
even for the fossilized ink sacs. The similar behavior of
recent and fossilized sepia on heating can be referred to in
support of the findings obtained spectroscopically.

Experimental Procedure

Test Material

Fossilized sepia: ink sacs of three specimens of "cuttlefish"
Geothautis bollensis from the posideonian schist of the Lias
epsilon. Comparison material for determining the inorganic and
bituminous components of the Lias schist was taken at a small
distance from the fossil in the fossilized layer.

Recent sepia: a) dried sepia (made by Schwabe of Karlsruhe
and Schminke of Düsseldorf) and ink sacs of *sepia officinalis*,
approximately 4 days old, refrigerated and brought from Sappin.

1. A discussion of the paleontological aspects will follow in an
article in the *N. Jb. für Geol. etc.*, Monthly journal, Stuttgart,
(1973).

* Numbers in the margin indicate pagination in the foreign text.

Comparison substance from the muscle arms of the same sepias.

Pretreatment

After drying at 100° C the material (both fossilized and recent) was very finely pulverized in an agate mortar and then boiled with concentrated HCl with a reflux condenser for 48 hours. The undissolved portion was filtered off and evaporated with concentrated hydrofluoric acid in a platinum crucible. The black evaporation residue was rinsed with distilled water, extracted for 24 hours with acetone and then dried in a drying chamber at 100°C. (All the steps in this preparation process were checked by infrared spectrography. There were no changes in the bands typical for melanine. In this treatment, however, silicate and chalk impurities as well as bituminous residues were largely removed from the fossilized sepia.)

Physical Examination Methods

Infrared spectra were taken of the dried material (KBr micro-briquets). In addition, the samples were heated in each case for 2 hours at 200°, 300°, 400°, 500° and 600° C. Infrared spectra were again taken from the heated substances after they had cooled. In addition, the weight loss on heating was determined gravimetrically.

Reflection spectra were taken in the wavelength range between 400 and 800 nm. Moreover, NMR spectra were taken of the sepia /203 samples in a large variety of solvents. Finally, mass spectrography examinations were made.

Dissolution Tests

Sepia samples were treated with the following solutions or

solvents: saturated aqueous KOH, concentrated sulfuric acid, acetonitrile, ethylene glycol monomethylether [13], ethylene glycol monobutylether, ethyl acetate and benzyl alcohol [13].

Chemical Identification Method

Oxidation and reduction experiments were done according to references [6,7,19] with solutions of KMnO_4 , H_2O_2 , sodium dithionite and diamino silver chloride. Following different instructions [3-5, 14, 20-22] sepia or sepia-free muscle substance with the addition of NaOH or K_2CO_3 was oxidized in solution by heating in the air at 300°C or by adding KMnO_4 . The oxidation products were acidified and extracted with ether or ethylacetate. The extracts were released by evaporating from the solvent. The thus obtained evaporation residue was separated chromatographically. The separated substances were made visible by spraying with diazotized sulfanilic acid or FeCl_3 solution.

Results

Both recent and fossilized melanine are soluble only in concentrated sulfuric acid or in a solution of caustic potash. When diluted with water the dissolved components -- at any rate only an insignificant amount -- again precipitate. Purification cannot be determined by means of IR spectroscopy. All other solvents -- also those recommended in the literature -- do not dissolve sepia melanine.

In the case of recent sepia the oxidation methods coupled with chromatography resulted in spots whose position and appearance were in agreement with data given in the literature. Fossilized sepia produced no dyeable substances. Since the completely sepia-free muscle substance of recent cuttlefishes

also produced the same chromatography spots like sepia, it can certainly be assumed that the oxidation and chromatography methods detect protein components which are not identical with the sepia.

The oxidation methods with aqueous solutions of oxidants as well as the reduction method with sodiumdithionite did not produce any utilizable findings either for recent or fossilized sepia.

NMR spectroscopy in the physical examination methods did not produce any usable evidence because of the insolubility of the dye in the solvents. Whereas the recent sepia sample produced a mass spectrum with few lines, with the fossilized sample an abundance of additional lines could be seen which must be attributed to the bituminous impurities.

The reflection spectra of the fossilized and recent sepia are in complete agreement in the range from 400 to 800 nm. Unfortunately they show only an uncharacteristic maximum at 560 nm.

Fig. 1 shows the infrared spectra. Fig. 2 shows the intensities of the band both for recent and fossilized sepia at 2.9 μ and 6.1 μ as a function of the temperature to which the materials were heated. Fig. 3. shows the ratio of the extinction of the bands at 2.9 μ to the extinction at 6.1 μ as a function of the heating temperature.

Discussion

In spite of the relative frequent occurrence of melanines as dark dye pigments, the exact structure and molecular weight of the substances is not yet exactly known [16]. Analysis is made more difficult by the fact that all natural melanines are practically insoluble in all solvents because the dark pigments

always occur tightly bound with granular protein [19]. Synthetically produced melanines which are protein-less have quite different solubilities.

Because of this lack of solubility methods for separating and purifying the melanine (from protein in the case of recent sepia and inorganic rock impurities and bitumens in the case of fossilized sepia) cannot be used.

Since the oxidative treatment methods for recent sepia and sepia-free musculature also produce similar results, the value of these methods for characterizing fossilized sepia is not very large.

By contrast, infrared spectrography proves to be well suited for identification of melanine. This method has already been suggested several times for identifying recent melanines [9,16, 23], although on the other hand it has been rejected as being unsuitable [17]. The method was only used once for the identification of fossilized sepia [14]. But in this case only "aliphatic compounds" were detected and "indication of the presence of amino compounds" were given. Spectra of fossilized melanine have not been previously published.

Comparison of IR spectra of recent and fossilized melanine (Fig. 1) shows good agreement, also with spectra of recent melanine described in the literature [16]. Just the spectrum of fossilized sepia additionally show the CH bands of bituminous impurities at about 3.4 which also similarly appear in the schist impurities.

The proof of identity on the basis of not very characteristic 205 spectra -- which, however, are only to be expected because of the structure of melanine -- can be confirmed by behavior on

heating. Here the fossilized and recent sepia show the same curves (Figs. 2 and 3). Striking here is the relatively large stability for an organic substance which first begins to show decomposition phenomena on heating at temperatures above 400-500°C. Here too both recent and fossilized sepia behave similarly.

By means of the suggested identification method it is also possible to examine undissolved samples. Because of the similar behavior on heating and the agreement of the infrared spectra the conclusion suggests itself that recent and fossilized sepia are practically the same. Favored by ideal isolation conditions (isolated from the air in the oil shale) and the high degree of stability of the melanine, the fossilized material seems to have remained largely unchanged over a period of 180 million years.

The presence of organic substances in specimens as old as this is not completely unusual. Porphyrine-like substances have been found in petroleums [1,2,11, 12]. Residues of waxes, scleroproteins, chitin and cellulose have been found in deposits which formed under strongly reducing conditions [11]. Amino acid residues have been reported in the skin substance of Holzmaden sauria [10]. However more or less largely converted products were always involved.

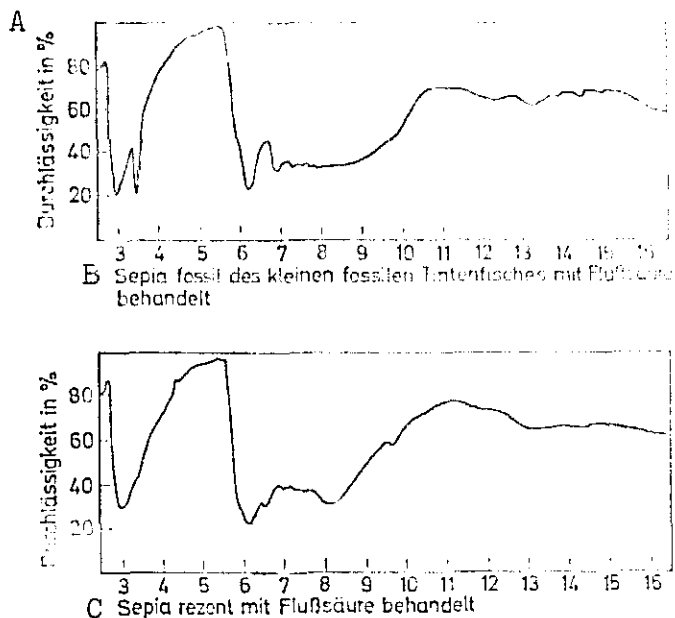


Fig. 1. Infrared spectra of a fossilized (above) and recent (below) sepia specimen. Equal weights of material treated identically with concentrated HCl, concentrated HF and acetone. (Details in test.) KBr briquets.

Key: A) Transperancy in %
 B) Fossilized sepia of a small fossilized cuttlefish treated with hydrofluoric acid
 C) Recent sepia treated with hydrofluoric acid

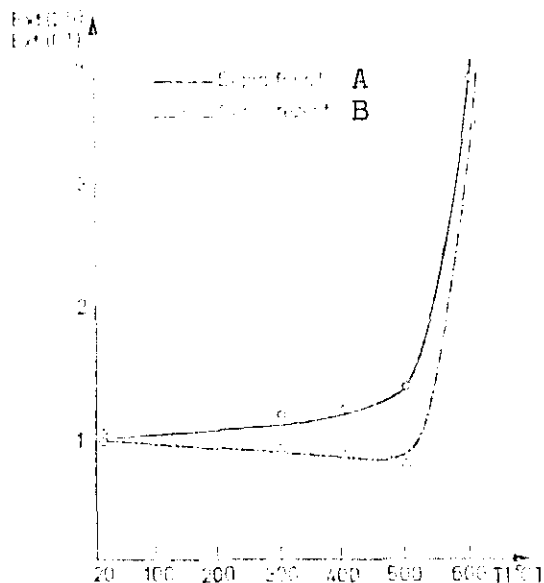


Fig. 3. Ratio of the intensity of extinction of the bands at 2.9 divided by the extinction at 6.1 for specimens which were heated to different temperatures.

Key: A) Fossilized sepia
 B) Recent sepia

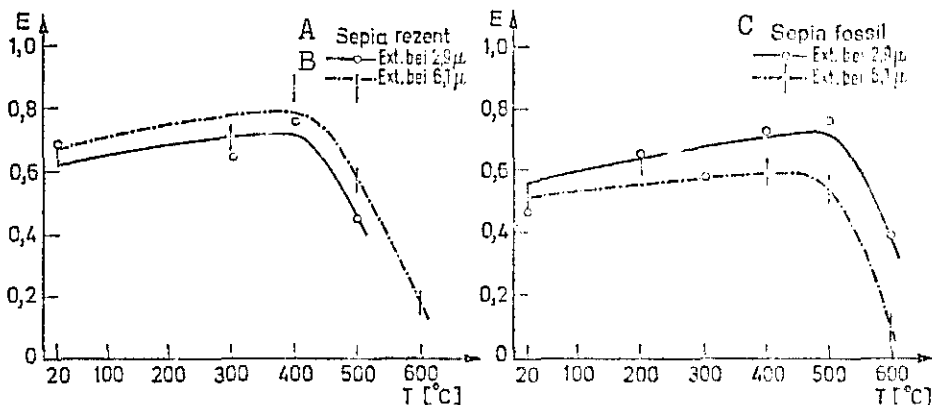


Fig. 2. Intensity of the bands at 2.9 and 6.1 μ after heating recent and fossilized melanine for 2 hours at different temperatures.

Key: A) Recent Sepia
 B) Extinction at
 C) Fossilized sepia

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