Recent Work

Title
A CHEMICAL DEGRADATION STUDY OF SOME BLUE-GREEN ALGAE and BACTERIA and ITS IMPLICATION ON THE ORIGIN OF THE ORGANIC MATTER IN KEROGEN

Permalink
https://escholarship.org/uc/item/4wb8c0bg

Author
Philp, R.P.

Publication Date
1976-03-01
A CHEMICAL DEGRADATION STUDY OF SOME BLUE-GREEN
ALGAE AND BACTERIA AND ITS IMPLICATION ON THE
ORIGIN OF THE ORGANIC MATTER IN KEROGEN

R. P. Philp and M. Calvin

March 30, 1976

Prepared for the U. S. Energy Research and
Development Administration under Contract W-7405-ENG-48

For Reference
Not to be taken from this room
A chemical degradation study on some blue-green algae and bacteria and its implication on the origin of the organic matter in kerogen.

R.P. Philp* and M. Calvin**

ABSTRACT

An alternative method used to try and determine the origin of organic material in kerogen is described. Several species of blue-green algae and bacteria have been degraded, and products obtained include $\alpha,\omega$-dicarboxylic acids, normal carboxylic acids, and a $\alpha,\beta$-isoprenoid ketone. These products are similar to those obtained from ancient kerogens. This would imply that insoluble algal and bacterial cell wall material can be viewed as basic building blocks for the formation of kerogen.
Kerogen is an inert amorphous organic material insoluble in organic solvents, mineral acids, and bases. Its inertness is testified to by the fact that it is isolated by acid treatment of a rock or shale sample to remove minerals such as carbonates and silicates, followed by exhaustive extraction of the remaining residue with organic solvents to remove any soluble lipid material. Any humic-type material can also be removed from this residue by treatment with potassium hydroxide. It is the remaining insoluble organic residue which is known as kerogen. Kerogen is the most abundant reservoir of organic carbon on the Earth, and as an example of its abundance, it has been estimated that there are $3 \times 10^{15}$ tons of kerogen compared to $5 \times 10^{12}$ tons of coal in the Earth's crust.

However, despite its abundance, the precise molecular structure of this insoluble organic material has long remained an enigma. Structural determinations are complicated by the fact that the structure of any kerogen from different samples varies depending on the types of organic material originally responsible for its formation, and variations in the environmental conditions experienced during and since its formation. Tissot and co-workers have characterized kerogens as originating either from (1) algal debris, (2) humic or plant debris, or (3) from a mixture of both types of material. Of the two extreme types, algal-type kerogens are generally considered to have an aliphatic-type structure and humic kerogens an aromatic-type structure. Many chemical and physical degradation studies performed on kerogens from ancient shales and sediments have yielded valuable, though limited structural information. The reagents used, to date in these studies have included oxidative reagents such as potassium permanganate, chromic acid, and ozone, and reductive reagents such as lithium aluminum hydride. Pyrolysis is another technique which has been used to degrade kerogen. The majority of this work has been
performed on kerogens isolated from such ancient shales as the Green River oil shale \(^4\) (Eocene, \(50 \times 10^6\) yrs.) or Tasmanian tasmanite \(^9\) (Permian, 220-275 \(\times 10^6\) yrs.), (see ref. 3 for additional examples of kerogens examined).

We have recently shown that kerogen-like material can also be isolated from recently-deposited algal mats. Upon oxidative degradation this material gives rise to products similar in nature to those obtained by degradation of ancient kerogens known to be of algal origin \(^{10,11}\). These results imply that kerogen-like precursors, or proto-kerogens, are formed very rapidly on a geologic time scale and are found in certain types of organic-rich recent sediments.

To extend this hypothesis further, we felt it necessary to perform similar degradation studies on some pure cultures of blue-green and green algae and bacteria to determine whether the types of degradation products produced would correlate with those obtained from the degradation of ancient kerogens known to be of an algal origin. Microscopic examination of ancient shales such as the Green River oil shale \(^{12}\) or Tasmanian tasmanite \(^9\) clearly shows that the bulk of the organic material in these shales is derived from micro-organisms such as blue-green or green algae, bacteria, fungi and other micro-organisms. It is this material which is also responsible for the origin of the kerogen. Therefore, it seems logical to expect that a study of the insoluble material of contemporary blue-green and green algae, and bacteria should provide valuable information on the origin and method of formation of the algal-type of kerogens.

We have performed degradation studies on the insoluble organic residues obtained from cultures of the algae and bacteria shown in Table 1. The conditions under which these cultures were prepared and the results of the soluble lipid analyses have been described elsewhere \(^{13,14}\). For this study, freeze dried samples of the algae and bacteria (cf Table 1) were treated in a manner similar to that used for the isolation of kerogen from an oil-shale sample. Each sample was exhaustively extracted with a mixture of toluene/methanol (1:1 using ultra-sonication), and
after sixteen successive extractions the organic extracts were colourless, indicating removal of all readily soluble pigments and lipid material. The residues were subsequently hydrolysed with 6N HCl and, exhaustively extracted again with organic solvent, after centrifugation and removal of the aqueous layer. In the case of an oil shale, the kerogen is further concentrated by treatment with 48% HF and HNO₃ to remove silicates and pyrite respectively. However this was deemed to be unnecessary in the case of these pure cultures for obvious reasons.

The resulting residues after drying, were subjected to alkaline permanganate oxidation, using a method previously described by Ogner. The organic oxidation products were extracted from the reaction mixture with heptane and then ethyl acetate and the extracts subsequently combined. Following methylation of the extracts by BF₃/MeOH they were fractionated into branched and unbranched fractions by urea adduction. These fractions were subsequently analyzed by gas chromatography (GC), and computerized-gas chromatography-mass spectrometry (C-GC-MS) and the components identified from their relative retention times, coinjection with standards where possible, interpretation of individual mass spectra, and comparison of mass spectra with authentic spectra wherever possible.

Table 1 shows the elemental analyses of the organic residues from the cultures, the yields and relative amounts of unbranched and branched components after oxidation and fractionation.

Figs. 1 and 2 illustrate the chromatograms obtained from the analysis of the oxidation products of the insoluble residues. The yields of oxidation products are noticeably low. This is not surprising and was indeed anticipated since it is known that the cell-wall material of blue-green algae consists mainly of protein and polysaccharides. Upon oxidation this will give rise to water-soluble amino acids and carbohydrates which are removed in the aqueous layer during the work-up of the oxidation products. These products were not examined.
The unbranched fractions were generally dominated by \( \alpha, \omega \)-dicarboxylic acids in the range \( \text{C}_6-\text{C}_{12} \) with maximum at \( \text{C}_9 \) and n-acids in the range \( \text{C}_{11}-\text{C}_{18} \) with a maxima at either \( \text{C}_{14}, \text{C}_{16}, \) or \( \text{C}_{18} \) (see fig. 1). These acids were not found in the soluble lipid extracts (except for certain of the n-acids \( 13,14 \)) of the algae or bacteria and therefore do indeed represent degradation products of this insoluble cell debris. The branched fractions are less complex than the above mentioned fractions and in the majority of cases consist of benzoic acid, a \( \text{C}_{18} \)-isoprenoidal ketone (6,10,14-trimethylpentadecan-2-one), and in the case of Chlorella pyrenoidosa only, \( \text{C}_{15}, \text{C}_{16}, \text{C}_{17}, \text{C}_{18} \) and \( \text{C}_{19} \) isoprenoidal acids in minor amounts. These degradation products in turn bear a remarkable resemblance to those obtained from the oxidative degradation of ancient kerogens known to be of algal origin, for example kerogen from the Green River oil shale \(^4\) or Tasmanian tasmanite \(^9\). The individual mixtures of products are not anywhere near as complex as those from ancient kerogens, but this is not surprising when one realizes the differences in age, thermal history and environmental conditions that have been experienced by the ancient kerogens at the other end of the geological time scale.

There are several possible origins for these degradation products. Firstly, they could be derived from intracellular chlorophyll, which is not removed during the extraction process. This would tend to support the theory of Oehler et al. that intracellular chlorophyll becomes grafted onto cellular macromolecules, and with increasing maturation finally gives rise to kerogen \(^{17}\). It would also support the theory of Simoneit and Burlingame \(^{18}\), who proposed that the presence of the \( \text{C}_{18} \)-isoprenoid ketone in the degradation products of Green River shale kerogen indicated cross-linking at the allyl rearrangement center of phytol (dervied from the phytol-side chain of chlorophyll) during its incorporation into the kerogen moeity.
Secondly it is possible that the degradation products are derived from cellular macromolecules which on oxidation give rise to the above mentioned products plus other water soluble products not examined in this work. This would support the theory that it is this residual polymeric debris which is the source of the major building blocks of kerogen. With increasing time additional soluble lipid material can become condensed to this basic framework by irreversible chemical reactions, possibly catalyzed by clay minerals in the local sedimentary environment. This mechanism plus condensation between the polymeric debris itself would give rise to the kerogen matrix as found in many sedimentary environments.

In order to extend our theory that the insoluble residues from algae and bacteria are the basic building blocks for kerogen, maturation experiments using \(^{14}\text{C}\)-labelled biolipids are currently under way. It is anticipated that under simulated geological conditions, the degree of crosslinking within the insoluble residues will increase and incorporation of the \(^{14}\text{C}\)-biolipids will also occur.

Acknowledgements

This work was supported by grants from the U.S. National Aeronautics and Space Administration (NGL-05-003-003), the A.L. Day fund, and in part by the U.S. Energy Research and Development Administration. We also wish to thank Mr. S. Brown for assistance in obtaining the C-GC-MS analyses. We also wish to thank Dr. B.R. Simoneit for kindly providing a sample of the oxidative products of the Tasmanian tasmanite kerogen.
References

1) Robinson, W.E., In: Organic Geochemistry. Methods and Results
2) Forsman, J.P., In: Organic Geochemistry (Ed. I.A. Breger), Pergamon,
4) Djuricic, M.V., Vitorovic, D., Andresen, B.D., Hertz, H.S., Murphy, R.C.,
   Preti, G. and Biemann, K., In: Advances in Organic Geochemistry 1971 (Eds.
   in Organic Geochemistry 1973 (Eds. B. Tissot and F. Bienner), Editions Technip,
10) Philp, R.P. and Calvin, M., In: Procs. 2nd International Symposium on
    Environmental Biogeochemistry, (Burlingame, Ontario, March 1975) Ann Arbor, in press.
13) Han, J., McCarthy, E.D., Van Hoeven, W., Calvin, M. and Bradley, W.H.,


Table 1

Elemental analyses, yields and ratios of branched/unbranched products obtained from the insoluble organic residues of the organisms indicated.
<table>
<thead>
<tr>
<th>Organisms</th>
<th>Elemental analyses of residues</th>
<th>A*</th>
<th>B**</th>
<th>Ratio of branched/unbranched products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%C</td>
<td>%H</td>
<td>%N</td>
<td>%O</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>45.4</td>
<td>6.1</td>
<td>14.0</td>
<td>28.6</td>
</tr>
<tr>
<td>(Blue-green)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anacystis nidulans</td>
<td>48.9</td>
<td>7.1</td>
<td>13.2</td>
<td>29.6</td>
</tr>
<tr>
<td>(Blue-green)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phormidium luridium</td>
<td>49.7</td>
<td>6.7</td>
<td>13.0</td>
<td>29.0</td>
</tr>
<tr>
<td>(Blue-green)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>51.4</td>
<td>6.8</td>
<td>13.2</td>
<td>27.7</td>
</tr>
<tr>
<td>(Green)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodopseudomonas spheroides</td>
<td>52.5</td>
<td>6.9</td>
<td>11.3</td>
<td>28.4</td>
</tr>
<tr>
<td>(Photosynthetic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorobium</td>
<td>50.5</td>
<td>6.9</td>
<td>11.0</td>
<td>30.7</td>
</tr>
<tr>
<td>(Sulfur-lacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A* Quantities of insoluble residues used in the oxidation reaction.

B** Yields of products obtained on extraction of the oxidation reaction mixture with heptane and ethyl acetate.
Figure 1. Gas chromatograms of the unbranched oxidation products obtained from the oxidative degradation of the insoluble residues from the algae and bacteria as indicated. The Tasmanian tasmanite kerogen fraction contains the ether soluble oxidation products from a 60 hr. oxidation of the kerogen.

The peaks labelled with arabic numerals, i.e. 9, indicate that these peaks have been identified as \( \alpha, \omega \)-dicarboxylic acids with the corresponding number of carbon atoms. Peaks labelled 14*, indicate that these peaks have been identified as normal acids with the corresponding number of carbon atoms. It should be noticed that in all the chromatograms, except those from the Anacystis and Rhodopseudomonas, the distributions maximize at the \( C_g \alpha, \omega \)-dicarboxylic acid. In the chromatogram from the Tasmanian tasmanite, the products maximize at the \( C_{10} \alpha, \omega \)-dicarboxylic acid. It should be noted that the Tasmanian tasmanite chromatogram represents only the ether soluble degradation products, the normal acids, normally extracted with heptane are not present in this chromatogram. However, it can be said that the major degradation products from these organisms are basically the same as those from certain ancient kerogens although there are minor differences in the distributions.

These chromatograms were obtained using a Varian 2700 GC equipped with a F.I.D. detector. A 15' x 1/16" stainless steel column packed with Dexsil 300 on Gas Chrom Q was used for the analyses under the following conditions: oven temperature 100-280\(^\circ\)C, 6\(^\circ\)/min; injector temperature 280\(^\circ\)C; detector temperature 250\(^\circ\)C, and a helium flow rate of 15ml/min.
Fig. 1

CHLORELLA PYRENOIDOSA

PHORMIDIUM LURIDUM

ANACYSTIS NIDULANS

CHLOROBIUM

RHODOPSEUDOMONAS SPHEROIDES

TASMANIAN TASMANITE KEROGEN
Figure 2. Gas chromatograms obtained of the branched oxidation products from *Phormidium luridium* and *Chlorella pyrenoidosa*. The conditions under which these were obtained are the same as those given in Figure 1. These two chromatograms illustrate the major features seen in the branched component fractions from all the samples. The C\textsubscript{18}-ketone was the dominant product in all cases and the second major product was benzoic acid (as the methyl ester derivative used for GC analysis). The *Chlorella pyrenoidosa* was the exception, in that it was the only sample on degradation to give the isoprenoid acids (the label b-C\textsubscript{16} indicates the peak corresponding to the C\textsubscript{16}-isoprenoid acid) shown in this chromatogram. It is proposed that the C\textsubscript{18}-isoprenoid ketone and the isoprenoid acids, are derived from the phytol side chain of chlorophyll in agreement with the theories put forward for predicting its origin in the degradation products of ancient kerogens.
Fig. 2
LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.