

RECENT ADVANCES IN THE STUDY OF POTENTIAL KEROGEN  
PRECURSORS FROM RECENTLY-DEPOSITED ALGAL-MATS

R. P. PHILIP

Dept. of Chemistry and Laboratory of Chemical Biodynamics  
University of California, Berkeley, Cal. 94720

INTRODUCTION

The most abundant form of organic carbon on Earth is kerogen. It is commonly defined as the residual organic material remaining after minerals have been removed from a sample of shale or sediment with hydrochloric and hydrofluoric acids, after humic material has been removed with base, and after the soluble organic material has been removed by exhaustive extraction with organic solvents. It has been estimated that there are  $1.3 \times 10^{18}$  tons of kerogen on the Earth compared with  $1.5 \times 10^{15}$  tons of coal (1). Kerogen is generally found in sedimentary rocks in the form of oil shales. The best known, and most widely studied shale, the Green River oil shale, has been estimated to contain 8,000 billion barrels of oil currently in the form of kerogen (2). But in spite of its vast abundance, relatively little is known about the origin, structure, or method of formation of any type of kerogen. Tissot has classified kerogens into three general categories, i.e., a) humic-type kerogens, derived from higher plant material and generally aromatic in structure; b) algal-type kerogens derived from algae and generally aliphatic in structure; and c) kerogens which are intermediate between these two and are a mixture of algal- and humic-type materials (3).

Many attempts have been made to obtain structural information on kerogens using various chemical degradation techniques. These have included chromic acid and alkaline potassium permanganate oxidation, saponification with methanolic potassium hydroxide, reduction with lithium aluminum hydride, hydrogen bromide treatment and ozonolysis (4). Another widely-used technique in this type of study is pyrolysis. However, the major problems associated with all of these studies include: a) the lack of reactivity of kerogen with most reagents due to the fact that the degradation reactions are generally heterogeneous; b) the random nature of the degradation by some of these reagents; and c) the fact that some products are secondary degradation products. As a result of these problems any information obtained is usually not very structurally specific and only gives general information on whether a kerogen is aliphatic or aromatic in nature.

To say that there is one common solution to all of these problems is very misleading. However, valuable structural information could be obtained if: a) specific reagents for cleaving only one type of bond were used; or b) information was first obtained on the structure of potential kerogen precursors and the ways in which the precursors are involved in the formation of kerogen.

In an attempt to examine this latter line of approach and to get more structural information on algal-type kerogens, an investigation has been made into the structure of the insoluble organic residues isolable from recently-deposited algal ooze and from some pure cultures of green and blue-green algae. The major aim of the study was to determine whether or not there was any kerogen-like material in the sample of algal ooze; if so, what was its structure, and can it react to form the more complex kerogen moiety found in ancient shales and sediments; and finally if there was kerogen-like material in the algal ooze, can it be said that the basic "building blocks" of kerogens are actually in the organisms themselves prior to being deposited in the sedimentary environment?

To try and provide answers to some of these questions, a series of degradation

experiments, previously performed on ancient kerogens, were repeated on the insoluble organic residues from an algal ooze and some pure algal cultures. The sample of algal ooze for this study was obtained from Laguna Mormona, Baja California, in an environment where the sources of organic matter are well documented. The environment at Laguna Mormona is characterized by extreme local variations in sedimentological, geochemical, and biological properties (5). Semi-arid climatic conditions and restricted water movement from the ocean have combined to produce an evaporite flat and hyper-saline marsh environment. Large quantities of organic matter are slowly accumulating because of the high salinity which inhibits organisms that normally consume and degrade the organic material. The intertidal algal mats examined at Laguna Mormona are characterized by the blue-green algae *Microcoleus chthonoplastes* and *Lyngbya aestuarii*. Pure cultures of the blue-green algae *Phormidium luridum* and *Anacystis nidulans* were obtained from an earlier study by Han and McCarthy on the soluble lipid components of the same organisms (6).

This paper will describe results obtained from the degradation of the insoluble organic residue from the algal ooze using saponification, oxidation and pyrolysis. It will also summarize the results obtained from alkaline potassium permanganate oxidation of the pure cultures of the blue-green algae. As described above, these degradations were performed in an attempt to determine whether there were any apparent structural similarities between the algal ooze, the organisms and algal kerogens. The paper will also attempt to illustrate some of the major potential problems involved in trying to make molecular structure determinations of kerogens using these types of chemical degradations.

#### EXPERIMENTAL

The kerogen fraction was isolated from the sample of algal ooze in the same way as kerogen would be isolated from an ancient shale or sediment. This involved removal of carbonates and silicates by treatment with 6N HCl and 48% HF respectively, followed by exhaustive extraction of the remaining residue with toluene/methanol (1:1). No attempt was made to remove any pyrite from the residue with concentrated HNO<sub>3</sub> since this generally leads to the alteration of certain structural features of the kerogens. The residues from the pure cultures of algae were obtained by acid treatment of the cultures with 6N HCl followed by removal of the soluble organic material by extraction with toluene/methanol (1:1).

##### (i) Saponification.

The algal ooze residue was refluxed for three days with 0.5% KOH/MeOH. After termination of the reaction, the methanolic solution was decanted, acidified, and extracted with heptane and ethyl acetate. The extracts were combined, evaporated and subsequently methylated with BF<sub>3</sub>/MeOH (13% w/v). The methylated extracts were fractionated into normal and branched/cyclic fractions by urea adduction prior to analysis by gas chromatography (GC) and computerized-gas chromatography-mass spectrometry (C-GC-MS).

##### (ii) Oxidation.

a) Oxidative degradation of the algal ooze residue was performed using alkaline potassium permanganate and a method similar to that previously described by Ogner (7). The residue was subjected to three four-hour periods of oxidation, a total of twelve hours oxidation. After each oxidation, the reaction mixture was acidified and excess MnO<sub>2</sub> solubilized by addition of sodium bisulphite. The clear reaction mixture was extracted with heptane and ethyl acetate, the extracts combined and methylated using BF<sub>3</sub>/MeOH (13% w/v). The methylated extracts were fractionated into normal and branched and cyclic fractions by urea adduction prior to analysis by GC and C-GC-MS.

b) The residues from the algal cultures were oxidized in the same manner as described in (a) except that only one four-hour oxidation period was required to

totally degrade the residue.

(iii) Pyrolysis.

The pyrolysis experiment was performed by initially heating the algal ooze residue in a quartz tube for 5 min. at 200°C to remove any unbound soluble lipid material, and then increasing the temperature to 600°C for 30 mins. The pyrolysate was collected in a U-tube using a liquid nitrogen trap. When the reaction had been terminated, the products were dissolved in ethyl acetate and transferred from the U-tube to a small vial, the solvent removed and the crude pyrolysate weighed. The pyrolysate was subsequently filtered through an alumina column and fractionated into a non-polar (heptane eluate) and a polar fraction (ethyl acetate eluate). The heptane fraction was further purified by thin-layer chromatography and urea adduction prior to analysis by GC and C-GC-MS.

(iv) Analytical Procedures.

GC analyses of the various degradation products were performed on a Varian 2700 GC equipped with a flame ionization detector and a 15' x 0.03" i.d. glass column packed with Dexsil 300 coated on Gas-Chrom Q. Helium was used as carrier gas with a flow rate of 12 ml/min and temperature conditions were: oven temperature 70 to 280°C, programmed at 6°/min; injector temperature 280°C and detector temperature 250°C.

C-GC-MS analyses were performed using a DuPont 491-2 double-focussing mass spectrometer coupled directly to a Varian Aerograph 204 gas chromatograph. A glass capillary column, 22' x 0.03" i.d., packed with Dexsil 300 coated on Gas-Chrom Q was used for the analyses; oven temperature: 90 to 280°C, programmed at 6°/min. Data were continually acquired during the C-GC-MS analyses using a DuPont 21-094 data system.

#### RESULTS AND DISCUSSION

Saponification of the algal ooze residue provided some specific information on the types of compounds attached to the residue as esters. The normal fraction was dominated by the n-C<sub>16</sub>, n-C<sub>18</sub> and C<sub>18</sub>:1 carboxylic acids with the latter two in a 1:1 ratio. Other normal acids were present in the C<sub>12</sub>-C<sub>32</sub> range in relatively minor proportions. The branched and cyclic fraction was dominated by a cyclopropyl-C<sub>19</sub> acid, and phytanic and pristanic acids. The distribution of these fatty acids is similar to that observed in the soluble lipid fractions from other samples of these algal-mats and oozes which have been examined and also other Recent sediments (8,9, 10). However, there is not a great deal of similarity between these products and those obtainable from saponification of ancient kerogens. One plausible explanation for this is that, with increasing time, additional lipid material becomes complexed to the insoluble residue. Alternatively, the material already linked to the residue can undergo structural alteration by either bacterial or geochemical effects during maturation.

Oxidation of the algal ooze residue, when performed in a stepwise fashion, produced mainly normal carboxylic acids and isoprenoid acids in the first step, but with increasing time gave rise to increasing amounts of  $\alpha,\omega$ -dicarboxylic acids and only small amounts of isoprenoid components as summarized in Table I. These results suggest that the normal and isoprenoid components are attached to the outer part of the kerogen nucleus and the  $\alpha,\omega$ -dicarboxylic acids result from degradation of a highly cross-linked nucleus in a similar manner to the degradation of ancient kerogens (11). Alternatively these degradation products may not be truly representative of the original kerogen structure at all but are formed by secondary degradation of the initial oxidation products. However, until more specific and less severe oxidizing reagents are found for use in this type of study, the above problem will never be completely eliminated. It is also noteworthy that the time taken to completely degrade the kerogen-like material from the algal ooze was only twelve hours compared to the ninety

Table I  
 DISTRIBUTION OF OXIDATION PRODUCTS OBTAINED FROM DEGRADATION OF RESIDUE A

Oxidation Step	ADDUCTED PRODUCTS						NON-ADDUCTED PRODUCTS
	$\alpha, \omega$ -Dicarboxylics		n-Carboxylics		Monomethyl-Carboxylics		
	Range <sup>1</sup>	Max.	Range	Max.	Range	Max.	
Step 1	9-16	11	11-31	16	13-19	15	Isoprenoid Acids <sup>2</sup>  C <sub>20</sub> , C <sub>16</sub> , C <sub>19</sub> , C <sub>17</sub> , C <sub>15</sub> , C <sub>13</sub>
Step 2	8-14	11	10-28	16, 18	12-18	15	
Step 3	6-15	8	14-20	Minor amounts only	-	-	

<sup>1</sup>Carbon number range

<sup>2</sup>Arranged in decreasing order of abundance

hours required to completely degrade the kerogen from the Green River oil shale (11). This again illustrates the less complex nature of this immature kerogen-like material compared to that from ancient kerogens.

Pyrolytic degradation of the insoluble organic residues from the algal oozes and ancient kerogens is of little use in obtaining specific structural information on the residues. It is of use in classifying kerogens as algal or humic depending on whether aliphatic or aromatic compounds respectively are the dominant products of pyrolysis. The hydrocarbons obtained from the pyrolysis of the algal ooze residue used in this work were predominantly aliphatic, confirming that algal kerogen-precursors have an aliphatic type of structure. The major products were homologous series of n-alkanes and n-alkenes in the range C<sub>15</sub>-C<sub>34</sub> with a C.P.I. =1, and one isomer of pristene (C<sub>19</sub>H<sub>38</sub>) plus three phytene isomers (C<sub>20</sub>H<sub>40</sub>) and minor amounts of phytadiene (C<sub>20</sub>H<sub>38</sub>). The distributions of the products were similar to those obtainable from the pyrolysis of mature kerogens which again supports the theory that there are structural similarities between these kerogen-precursors and ancient algal kerogens.

In work published elsewhere, results from the comparative pyrolytic degradations of several different algal residues has been reported (12). In that study significant differences between the distributions of the cyclic hydrocarbons from the various residues were observed. Pyrolysis coupled with microscopic examination of the residues, should be a valuable technique for correlating source material with structural information.

It is clear from these three degradation studies that there are structural similarities between the algal ooze residue and ancient algal kerogens. The oxidation studies on the insoluble organic residues from the blue-green algae produced results which enable this statement to be extended to say that there are structural similarities between the organisms, the algal ooze residue and the ancient algal kerogens. This is based on the fact that the major oxidation products from the Phormidium luri-dium residue were  $\alpha,\omega$ -dicarboxylic acids (range C<sub>7</sub>-C<sub>10</sub>; max. C<sub>9</sub>) with minor amounts of n-carboxylic acids (range C<sub>11</sub>-C<sub>16</sub>; max. C<sub>12</sub>), and from the Anacystis nidulans residue,  $\alpha,\omega$ -dicarboxylic acids (range C<sub>7</sub>-C<sub>12</sub>; max. C<sub>9</sub>) and equal amounts of n-carboxylic acids (range C<sub>11</sub>-C<sub>18</sub>; max. C<sub>14</sub>).

#### CONCLUSIONS

The work described above has shown by various chemical degradation techniques that there are structural similarities between the insoluble organic residues from blue-green algae, algal oozes, and ancient algal-type kerogens. The conclusion is based on similarities between the degradation products obtained from the insoluble organic residues. Further work is now required using the insoluble organic residues from the algal oozes to explore new and specific types of degradation reagents. More emphasis needs to be placed on using reagents which are known to cleave specific linkages, such as ether linkages, to obtain larger molecular fragments from the degradation. These larger fragments, although less volatile, could be examined initially using high pressure liquid chromatography, gel permeation chromatography, ultra-violet, or infra-red spectroscopy, which should give valuable information as to their structure. After this initial examination the fragments could be mildly and specifically degraded, again, to give compounds more readily identifiable by GC and GC-MS.

It is clear that although these studies have demonstrated the presence of kerogen precursors in algae and algal-oozes, a great deal of work remains to be done in order to determine the exact origin of kerogen and the way in which it attains the degree of molecular complexity found in ancient sediments.

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