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Extraction of Lipid Biomarkers from Geological Archive Sediments: Sonication

--Manuscript Draft--

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Overview: The material comprising the living "organic" share of any ecosystem (leaves, fungi, bark, tissue; Figure 1) differs fundamentally from the material of the non-living "inorganic" share (rocks and their constituent minerals, oxygen, water, metals). Organic material contains carbon linked to a series of other carbon and hydrogen molecules (Figure 2), which distinguishes it from inorganic material. Carbon's wide valency range (-4 to +4) allows it to form up to four separate covalent bonds with neighboring atoms, usually C, H, O, N, S, and P. It can also share up to three covalent bonds with a single other atom, such as the triple bond in the often poisonous cyanide, or nitrile, group. Over the past 4.6 billion years, this flexibility has led to an amazing array of chemical structures which vary in size, complexity, polarity, shape, and function. The scientific field of organic geochemistry is concerned with the identification and characterization of the whole range of detectable organic compounds, called biomarkers, produced by life on this planet, as well as others, through geologic time.

Principles: Extraction via sonication is the simplest and least expensive method of obtaining a total lipid extract (TLE) from a given sample, and the recovery associated with this method is on par with other more sophisticated techniques. It uses an ultrasonic bath to agitate a sample in a vial in the presence of organic solvent. Biomarkers contained in the sample dissolve into the organic phase based on the rules of solubility, which with organic compounds, are controlled primarily by the polarity of both the biomarker and the solvent. This is summarized by the so-called 'like dissolves like' rule, whereby relatively apolar biomarkers (those containing exclusively C and H; isoprene) dissolve in apolar solvents (such as hexane, polarity = 0.1) and more polar biomarkers (those containing O, N, S, P; GDGTs) dissolve in more polar solvents (such as methanol or dichloromethane, polarity = 5.1 and 3.1). In fact, this is the first step in which the separation of different groups of biomarkers can be achieved via the introduction of a series of solvents, from apolar to polar, each extracting ever more polar compounds from the sample. The solvents from sequential extracts of a target sediment can thus be analyzed separately or combined to form a total lipid extract (TLE) that can be purified later.

Procedure:

1. Collect the necessary materials:

1.1. Samples (leaves, dirt, fungi, bark, tissue), usually frozen, freeze-dried, crushed, and homogenized prior to extraction, extracted in groups to maximize efficiency. Extract three samples.

1.2. Depending on the size of the sample, vials with volumes ranging from 4-60 mL may be used. For this experiment, borosilicate glass vials (40 mL) and solvent safe caps are used. Vials should be combusted at 550C for six hours prior to ensure removal of possible organic contaminants.

1.3. Borosilicate glass pipettes should be combusted at 550C for six hours prior to use.

1.4. Weighing tins should be combusted at 550C for six hours prior to use.

1.5. Pipette bulb(s), lab spatula(s), and a lab scale will be required.

1.6. Dichloromethane and methanol are common in most organic geochemistry laboratories. They are used individually to rinse lab tools and glassware before use. A mixture of dichloromethane (DCM) to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.

1.7. Sonicator. – Here we use a Branson 3510 sonication water bath at room temperature. A range of sonicator sizes, and sonication with or without heat, are available from major scientific equipment retailers.

1.8. Vial racks, which should be waterproof, since they will be placed in sonication bath.

1.9. Solvent approved chemical hood.

2. Preparation of Sample

2.1. Place a combusted weighing tin on the lab scale and then tare.

2.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

2.2.1. The mass of the sample will vary depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) will require much less.

2.3. Quantitatively transfer all of the material in the weighing tin into a combusted, pre-weighed, and labelled vial. Cap the vial, then discard the weighing tin.

2.4. Perform steps 2.1 – 2.3 for each sample that will be extracted.

3. Extraction

3.1. Using a pre-combusted pipette and bulb, transfer ~20mL of the DCM:MeOH (9:1) mixture into each vial (vial should be approximately half full). Recap vials before moving on to next sample, so the volatile solvents do not evaporate.

3.1.1. The volatilities of DCM and MeOH are different. Evaporation of extraction solvent due to uncapped sample vials has the ability to change its polarity, and thus the extraction.

3.2. Place the sample vials into a waterproof vial rack.

3.3. Check that the level of water in the sonication bath is only deep enough to submerge the sample vials up to the top of the extraction solvent. Too much water may cause the vials to float; too little water will stop the samples from being properly agitated.

3.4. Place the vial rack with the samples in it directly into the sonication bath.

3.5. Sonicate for 30 minutes at room temperature.

3.6. Remove sample rack from sonicator. If extracting sediments, let it set for 30 minutes to allow settling to occur. If extracting another set of samples, put in sonicator at this time.

3.7. Remove the DCM:MeOH mixture from the extraction vial, using a pre-combusted pipette and bulb, and insert into another pre-weighed, pre-combusted, and labelled 40mL vial.

3.8. Repeat 3.1 – 3.7 three times for all samples.

3.9. Allow extracted samples to dry in their vials, caps off, and in the hood, covered loosely with a piece of foil. Label as 'extracted residue' and store.

3.10. Label the combined extracts for each sample as 'TLE'.

Results:

At the end of extraction, a total lipid extract (TLE) for each sample is evident. Each vial contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can now be analyzed and their chemical constituents identified and quantified.

Comment [DM1]: Could you add a Results section, discussing the outcome of the experiment? A paragraph or 2 should suffice. Or am I missing it and is the result self-evident in the procedure itself?

Comment [JS2]: Hmmm, yeah, the result of the extraction is that you now have a TLE comprised of a range of different biomarkers reflecting different environmental parameters (temperature, salinity, ecology, etc). This is described briefly in the Application section.

Applications:

Different classes of biomarkers impart information on specific aspects of the Earth system. For example, in its infancy, organic geochemistry was primarily concerned with the formation, migration, and alteration of petroleum, and many of the chemical tools organic geochemists use today are based on those initial investigations. It was through the investigation of a class of compounds called isoprenoids, having a repeating five carbon pattern (Figure 2), that scientists discovered petroleum comprised the chemically altered remains of ancient primary producers, such as plankton in the ocean (converting to oil)(Figure 3) or peat bogs on land (coal) (Figure 4). Chemists at large oil companies used the ratios of a variety of compounds, each with its own known rate of alteration, to estimate how old petroleum was, where it came from, and if it was worth exploiting. Today, new biomarkers are being discovered, identified, and characterized in modern and ancient samples analyzed in organic geochemistry labs around the world. Many of today's applications seek to extract environmental information from biomarkers obtained in modern samples (leaves, soil, microbes, water samples, etc.) in order to extend the biomarker's utility to ancient sediments in an effort to reconstruct the climates, environments, and ecosystems of the past. For example, the distribution of a group of biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs for short), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature. We can therefore use the distribution of these biomarkers in ancient sediments, or

through a series of sediments of known age, to reconstruct air and water temperature back several million years.

Legend:

Figure 1: Organic material, such as trees, leaves, and moss, are chemically and visually distinct from inorganic material, such as pavement.

Figure 2: Isoprene comprises five carbon atoms and two double bonds. When added together in biosynthesis reaction, they can form complex molecules diagnostic for the presence of life. For example, 2, 6,10,15,19-pentamethyleicosane, commonly found in cyanobacterial mats.

Figure 3: Illumination of plankton at Maldives.
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Figure 4: Peat bog at 4500m elevation in the Ecuadorian Andes
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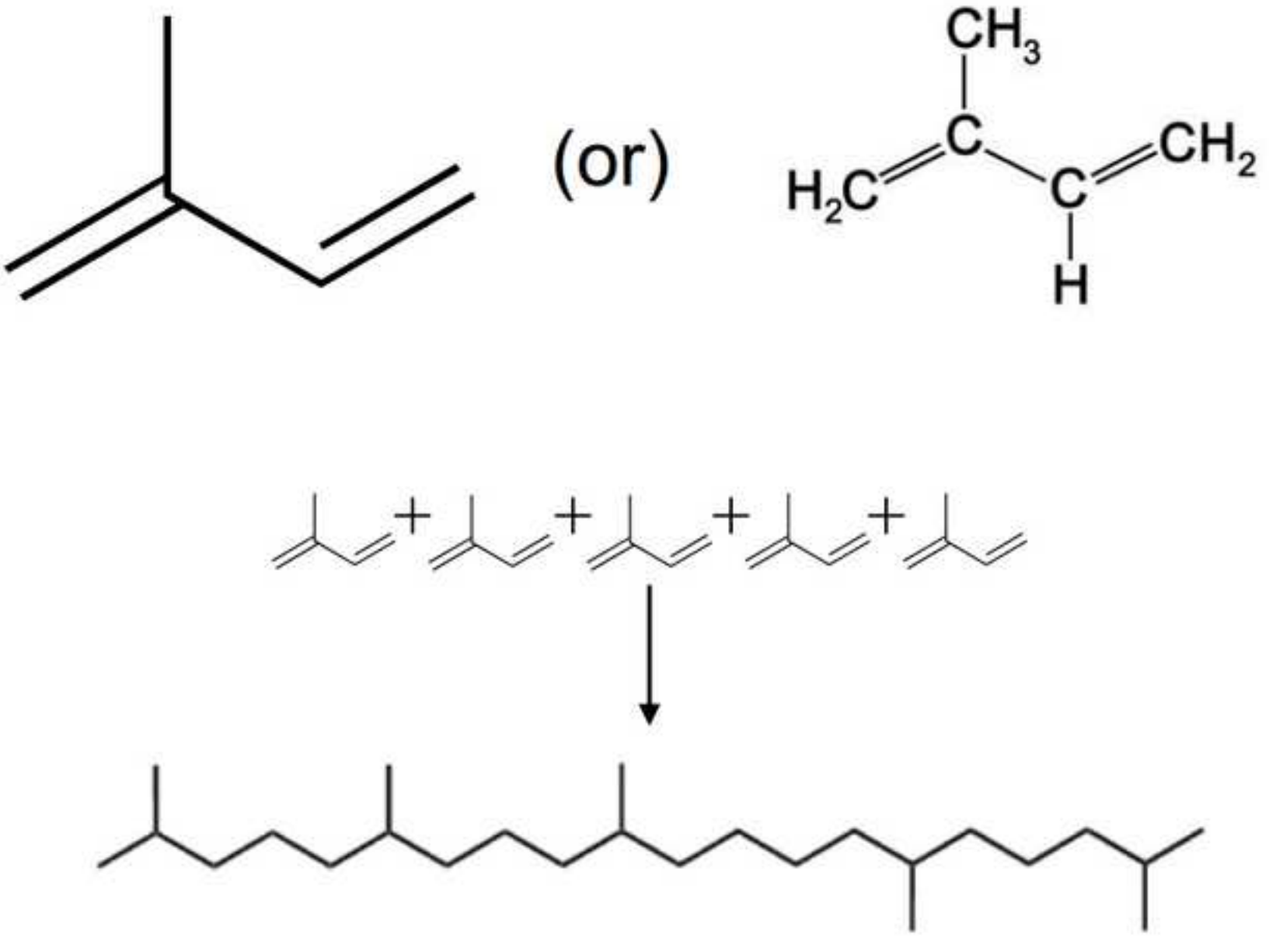

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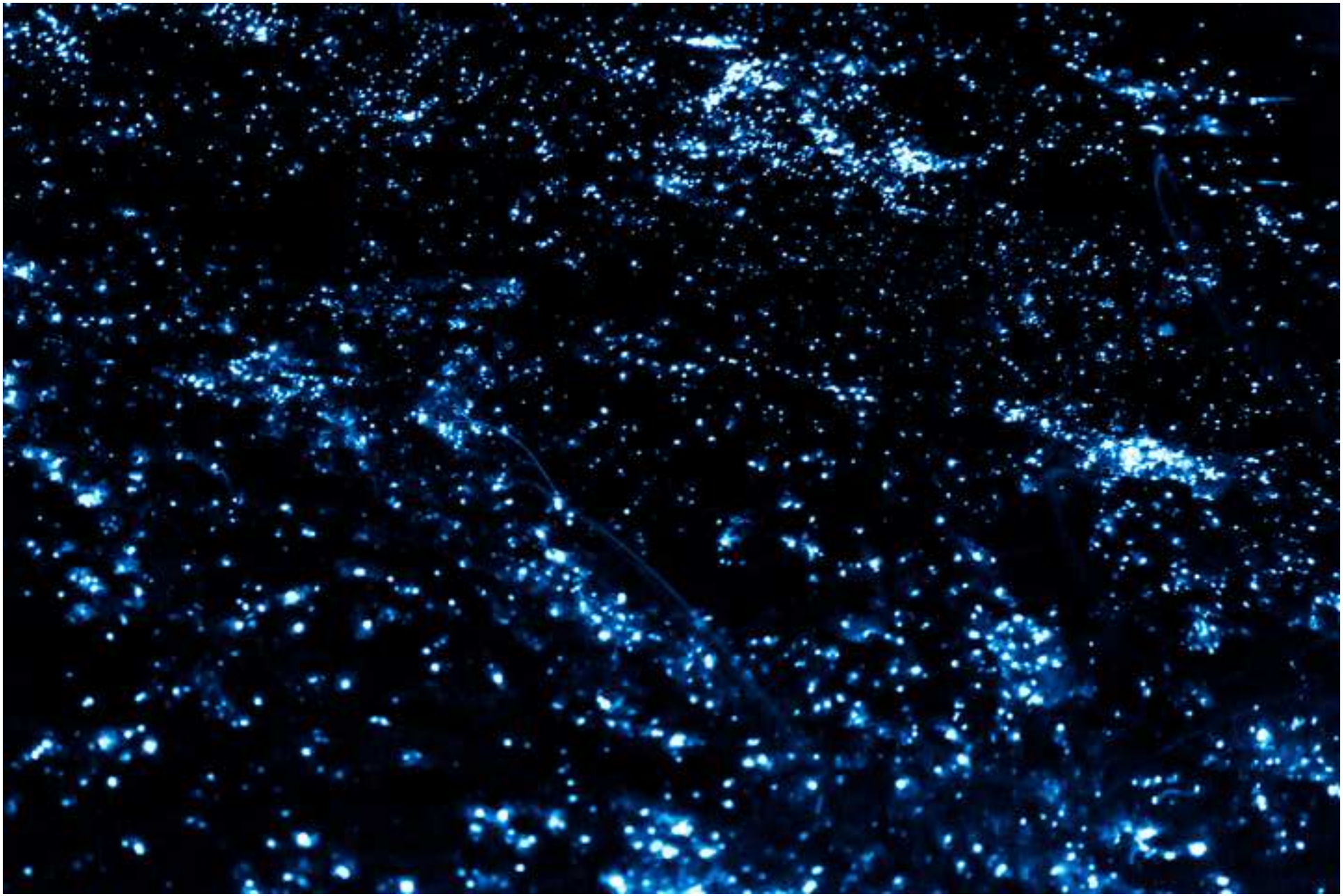

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    ~~1.3.~~

Comment [A1]: glycerol-dialkyl glycerol-tetraethers

Comment [JS2]: Yes.

Comment [A3]: We want to demonstrate some of this process.

Comment [JS4]: Easily done. We can pick 3 very different sample types and show the prep for them.

~~1.4. Borosilicate glass pipettes should be combusted at 550C for six hours prior to use.~~

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Comment [A5]: How do you ensure this?

Comment [JS6]: Buying a good enough solvent like Fisher Optima. Or, you have to distill less pure solvents to remove impurities.

Comment [A7]: How does your lab transfer quantitatively?

Comment [JS8]: All I mean to say is do your best to get all of the sample into the vial.

Comment [A9]: You said multiple extractions can be used to remove biomarkers with different polarities. What other extractions (besides 9:1 DCM:MeOH) can you demonstrate for the samples?

Comment [JS10]: The one described here is the most commonly used. There are others, like extracting in cold acetone to get at temperature sensitive accessory pigments found in some organisms. If it complicates, we can remove that wording.

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Figure 4: Peat bog at 4500m elevation in the Ecuadorian Andes.
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Comment [A13]: Provide more detail about this process. How do temperatures change the distribution? Also, do these break down over time? How far back can they be used to determine environmental conditions?

Comment [JS14]: We're not really all that sure why they are temperature sensitive. We're not sure if a single group of organisms is changing its distribution, or if the make-up of the group is changing. However, for whatever reason, the number of methyl groups in the compounds goes up with decreasing temperature.

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