



Free lipids of hot spring microbial mats of possible evolutionary significance
by Jentaie Shiea

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Chemistry

Montana State University

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Abstract:

The free lipid compositions of several hot spring microbial mats and bacteria were examined. These included a eucaryotic algal mat, two cyanobacterial mats, and three photosynthetic bacterial mats and the photosynthetic bacteria which build them. Generally speaking, the major free lipids of the mats reflect well the inputs of predominant mat-building photosynthetic microorganisms. Comparative study of these samples permitted evaluation of several unique lipids thought to serve as "chemical fossils" of certain microbial groups representing important steps in microbial evolution during the Precambrian period. Mid-chain branched monomethylalkanes were found only in cyanobacterial mats. The presence of whole suites of structural isomers of these compounds in these modern mats suggests that cyanobacteria may have been direct contributors of the monomethylalkane suites found in Precambrian sediments. Dimethylalkanes found in one of the cyanobacterial mats also resemble similar compounds previously extracted from Precambrian samples. Hopanoid compounds were found in anoxic mats and bacteria as well as in oxic mats, raising doubt that such compounds are produced solely by aerobic and facultative bacteria.

Sterols were abundant and varied only in the eucaryotic algal mat and appear to reflect the eucaryotic nature of this mat. Low levels of common sterols found in cyanobacterial mats are likely to be contaminants, as similar sterols were found in anoxic mats and bacteria which should be unable to synthesize them. Two classes of compounds appeared to be unique to the green bacteria studied. Long chain alkenes found in the green nonsulfur bacterium *Chloroflexus aurantiacus* appear to mark its presence in both hot spring and marine lagoonal mats. Wax esters were detected in both *C. aurantiacus* and the green sulfur bacterium *Chlorobium* sp., and mats which contain them. In most cases, free alcohols and fatty acids were less abundant than hydrocarbons and wax esters. Alcohol distributions seem to reflect the distributions of alcohols esterified in major chlorophylls and wax esters. Fatty acid distributions appear to reflect the complex lipid fatty acids of predominating photosynthetic mat-building microorganisms.

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Chemistry

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 1988

APPROVAL

of a thesis submitted by

Jentaie Shiea

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

16 May 1988
Date

Dis M. Wood
Chairperson,
Graduate Committee

16 May 1988
Date

Eric Drummond
Co-Chairperson,
Graduate Committee

Approved for the Major Department

16 May 1988
Date

Edwin H. Abbott
Head, Major Department

Approved for the College of Graduate Studies

25 May 1988
Date

Henry F. Parsons
Graduate Dean

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ACKNOWLEDGMENTS

I would like to express my special thanks to Dr. David Ward for his guidance in this project. I also appreciate his great patience and help in the writing of my thesis.

I would like to thank Dr. Simon Brassell for his great help in interpreting the mass spectra. I also would like to thank Joe Sears, Dr. James Guckert, Dr. Geoffrey Eglinton, Scott Williams, Pam Refling, and Dave Johnson for their advice and assistance with this project and Dr. Michael Madigan for providing pure cultures. I am indebted to the National Geographic Society for funding Precambrian Paleobiology Research Group field work in New Zealand (UCLA grant no. 3293-86) and to Yellowstone National Park for their support and cooperation in sample collection.

In particular, I would like to thank my wife Shwuneu for all her assistance and loving support during this period.

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ABSTRACT

The free lipid compositions of several hot spring microbial mats and bacteria were examined. These included a eucaryotic algal mat, two cyanobacterial mats, and three photosynthetic bacterial mats and the photosynthetic bacteria which build them. Generally speaking, the major free lipids of the mats reflect well the inputs of predominant mat-building photosynthetic microorganisms. Comparative study of these samples permitted evaluation of several unique lipids thought to serve as "chemical fossils" of certain microbial groups representing important steps in microbial evolution during the Precambrian period. Mid-chain branched monomethylalkanes were found only in cyanobacterial mats. The presence of whole suites of structural isomers of these compounds in these modern mats suggests that cyanobacteria may have been direct contributors of the monomethylalkane suites found in Precambrian sediments. Dimethylalkanes found in one of the cyanobacterial mats also resemble similar compounds previously extracted from Precambrian samples. Hopanoid compounds were found in anoxic mats and bacteria as well as in oxic mats, raising doubt that such compounds are produced solely by aerobic and facultative bacteria. Sterols were abundant and varied only in the eucaryotic algal mat and appear to reflect the eucaryotic nature of this mat. Low levels of common sterols found in cyanobacterial mats are likely to be contaminants, as similar sterols were found in anoxic mats and bacteria which should be unable to synthesize them. Two classes of compounds appeared to be unique to the green bacteria studied. Long chain alkenes found in the green nonsulfur bacterium Chloroflexus aurantiacus appear to mark its presence in both hot spring and marine lagoonal mats. Wax esters were detected in both C. aurantiacus and the green sulfur bacterium Chlorobium sp., and mats which contain them. In most cases, free alcohols and fatty acids were less abundant than hydrocarbons and wax esters. Alcohol distributions seem to reflect the distributions of alcohols esterified in major chlorophylls and wax esters. Fatty acid distributions appear to reflect the complex lipid fatty acids of predominating photosynthetic mat-building microorganisms.

INTRODUCTION

The question of the origin of life is one of the most important problems of natural history. Scientists have studied this question for many centuries. Of course, it is possible that life did not arise on the Earth but no one can prove or disprove this possibility. Much evidence has been found which strongly suggests that life might have originated on the Earth and the scheme by which life evolved can be speculated upon (Woese, 1987).

There is a rich fossil record of plants and animals which occurred in the last 600 million years (Phanaerozoic period 600 million years ago to the present). A less well preserved record of microbial evolution extends throughout the Precambrian period (prior to 600 million years ago) to at least 3.5 billion years (3.5 Ga) ago, only 1 billion years after the Earth was thought to have formed (Schopf and Walter, 1983). In 1983, Schopf and coworkers submitted a possible scheme for the evolutionary events in the Precambrian (Schopf, et al, 1983) as outlined in Figure 1. They suggested that the earliest living cells were presumably simple, aquatic, and small spheroidal anaerobic microorganisms which could have evolved very early (before 3.5 Ga). Only a trace of oxygen from decomposition of

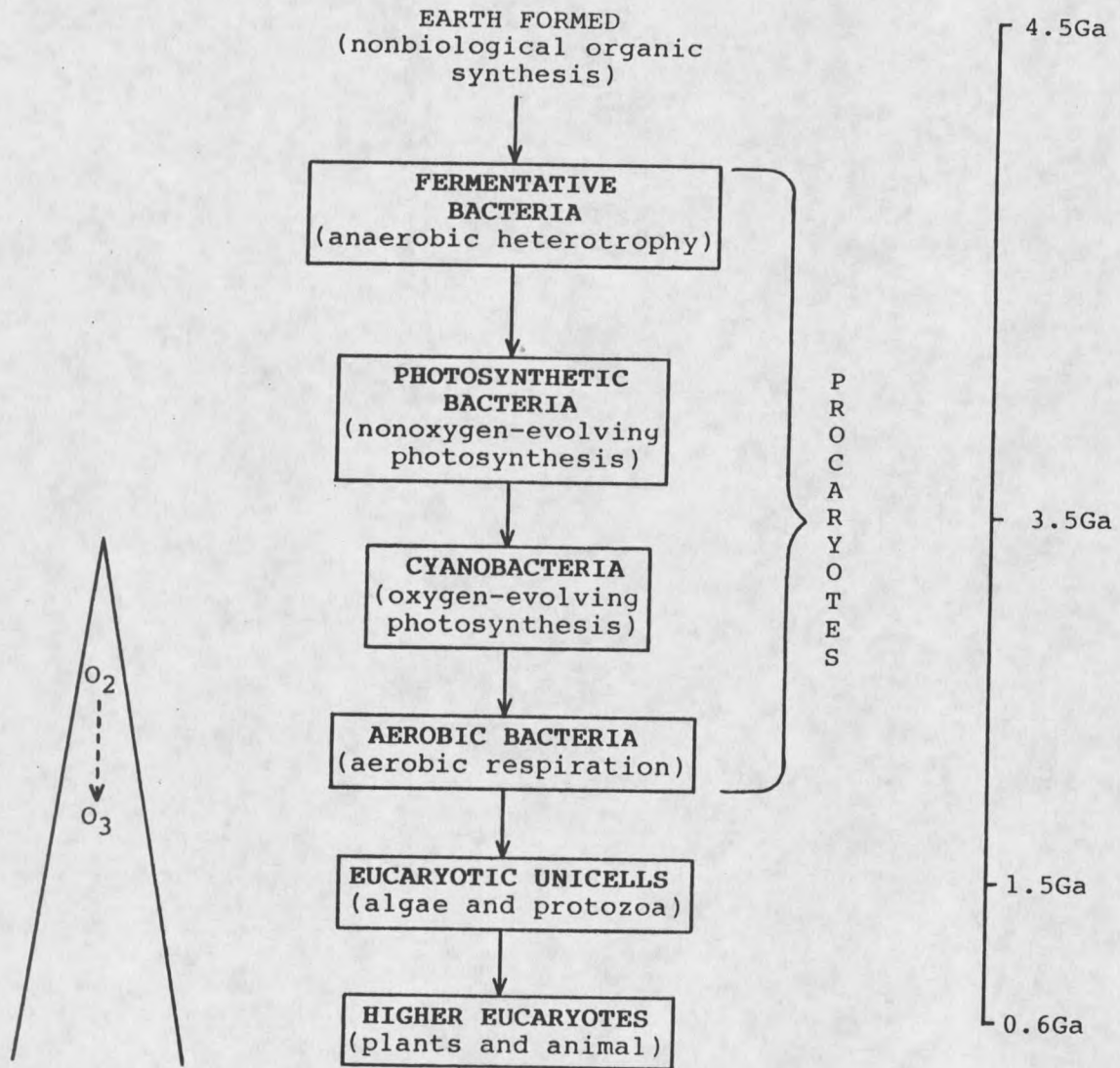


Figure 1. A suggested scheme for evolution during the Precambrian (modified from Schopf and Walter, 1983).

water by ultraviolet (UV) light probably was present in the atmosphere at that time. This earliest microbial life (heterotrophs) could have survived by fermenting organic molecules formed nonbiologically in the anoxic environment. The first photosynthetic organisms may have evolved when nonbiologically derived nutrients were diminished by primitive heterotrophs. Early phototrophs could have derived energy from photosynthesis by using a single photosystem which was entirely anaerobic and anoxygenic. Later, oxygenic photosynthesis could have followed, carried out by the ancestors of modern cyanobacteria. These microorganisms would have possessed photosystem II and thus generated oxygen. As the concentration of free oxygen in the atmosphere began to rise toward the modern level, anaerobic microorganisms were forced to retreat to anoxic habitats leaving the best space for photosynthesis to the cyanobacteria. The ozone (O_3) layer probably formed after oxygenation of the atmosphere. This protecting layer could filter out most ultraviolet (UV) radiation and eventually made it possible for organisms to live in shallow water or on the land with less dependence on the UV-absorbing protection of water. The first eucaryotic cells might have emerged around one and a half billion years ago (Schopf and Oehler, 1976). Eucaryotic cells contain more complex metabolic systems and organelles. When multicellularity and advanced sexual reproduction were evolved later, rapid

diversification of eucaryotic life forms (e.g. plants and animals) could have happened.

Although much evidence has been found to support parts of this suggestion, the major clues to connect all of the events are still missing. Indeed, more evidence is needed to completely understand life history in the Precambrian. This thesis deals with improving our interpretation of some of the evidence which suggests major events in microbial evolution during the Precambrian.

Approaches to Studying Microbial Evolution

Three approaches have been used in exploring the evolution of life: 1) study of the possible genetic and energetic relations among living organisms, 2) observation of ancient microfossils to understand morphological evolution, and 3) study of the inorganic and organic "chemical fossils" in ancient sediments in comparison to the chemistry of relevant modern environments and organisms. In order to understand the whole sequence of the evolution of the earliest life forms all three approaches must be taken.

The Phylogentic Record

Darwin, the father of evolution, pointed out that the various life forms can be produced through the interaction of a changing environment and a changeable organism, but

some clues from the antecedents will be retained and can be found in the descendents (Clark, 1976). From studying the fossil record, such changes have been observed to have occurred over long periods of geological time (Clark, 1976). However, even though no living organism is biochemically identical with its Precambrian antecedents, vestiges of earlier biochemistries have been retained. In the last fifty years, many living organisms have been examined for their genetic and biochemical characteristics, and the mechanism of change is partly understood.

The traditional approach of comparative biochemistry reveals a range of complexity in microbial biochemistry. It is logical to speculate that living organisms evolved from a simple metabolic system to more complex systems. Primitive life was presumed to possess the simplest fermentation system and the necessary organic materials for this metabolism were mainly produced nonbiologically (Schopf, 1978). Only little energy can be conserved in this process. When photosynthetic bacteria first evolved, light could be used to drive reactions for assimilation of exogenous nutrients. It was suggested that ancestral photosynthetic organisms contained only a cyclic electron transport system, the simplest known photosynthetic system in modern living organisms, for the efficient assimilation of external nutrients (Olson, 1970). Other photosynthetic systems such as those in cyanobacteria are more complex

(with a second photosystem) and should have evolved later. Recently, some cyanobacteria have been found in sulfide-rich anaerobic environments in which the cyanobacteria performed anoxygenic photosynthesis (Cohen, et al, 1975; Garlick, et al, 1977; Oren and Padan, 1978; Oren and Shilo, 1979; Castenholz and Utkilen, 1984; Cohen, et al, 1986). The ability to carry out both oxygenic and anoxygenic photosynthesis suggests that cyanobacteria may have played a transitional role between anoxygenic and oxygenic organisms. Eucaryotes with their greater cellular complexity are presumed to have evolved even later.

While comparative biochemistry may lead to speculation about the sequence of evolutionary change, more modern phylogenetic approaches provide a record based on actual mutational change in a biochemical mechanism which has been conserved throughout evolution--protein synthesis. Ribosomal RNA nucleotide sequences for living organisms are unique, and the extent of sequence difference depends on the phylogenetic relatedness of the organisms being compared (Woese, 1987). A phylogenetic tree which reveals the evolutionary relationships among living things is shown in Figure 2. Three major kingdoms--eubacteria, archaeobacteria, and eucaryotes--have been suggested. Each of the kingdoms is quite distinct on the molecular level. Some details are of interest to the speculated scheme described in Figure 1. Fermentative eubacteria (e.g. Thermotoga) and

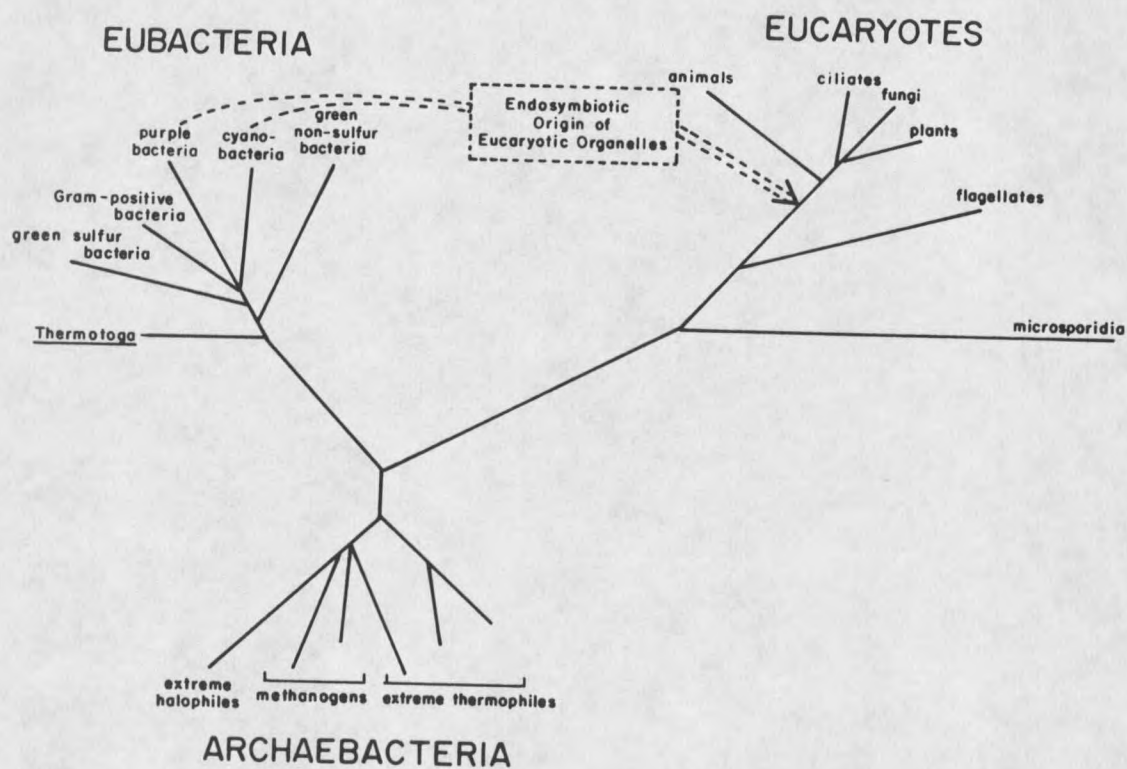


Figure 2. Phylogenetic tree determined from 16S rRNA sequence comparisons (modified from Woese, 1987).

archaebacteria (e.g. extreme thermophiles) may have predated the earliest photosynthetic microorganisms. Compared to some photosynthetic bacteria (e.g. green nonsulfur and green sulfur bacteria), cyanobacteria were relatively late to evolve. Unicellular eucaryotes (e.g. some flagellate and microsporidian protozoa) preceeded multicellular eucaryotes (e.g. animals and plants). Finally higher eucaryotes do seem to have derived their organelles (e.g. mitochondria and chloroplasts) from procaryotes (e.g. "purple bacteria" and cyanobacteria) via intracellular symbiosis.

The Fossil Record

Although the study of modern organisms may show the sequence of evolution, this approach cannot tell us the timing of events. To relate evolutionary events to time, the records (e.g. fossils) which were preserved in the Precambrian need to be examined. Fossils are commonly preserved as body fossils and trace fossils (e.g. tracks). There is a rich fossil record of Phanerozoic life, however large body and trace fossils are rarely found in only the latest part of the Precambrian record. The two best records of Precambrian organisms are microfossils and stromatolites. The oldest known and simplest life forms (procaryote-like) are preserved as microfossils in stromatolites about 3.5 billion years old.

The term "stromatolite", first used by Kalkowsky in 1908, is from the Greek "stroma", meaning bed or coverlet, and "lithos", meaning stone (Schopf, 1978). At first, stromatolites were thought to have had a nonbiological origin, but after a number of fossil microscopic organisms were found in them, stromatolites were thought to be one of the best remains by which to interpret the early evolutionary history of life. According to Walter (1977), a stromatolite is "an organosedimentary structure produced by the sediment trapping, binding and/or precipitation activity of microorganisms, primarily cyanobacteria". Krumbein (1983) restricted the term "stromatolite" to refer only to laminated geological structures, and defined unconsolidated laminated systems which are clearly related to the activity of modern microbial communities as "potential stromatolites". Although the definition of stromatolite is still in debate, this type of geological structure undoubtedly provides valuable clues for exploring early life. The oldest known stromatolites were found in the Warrawoona Group, Western Australia, of the 3.5-Ga-old Towers Formation (Schopf and Walter, 1983). Many other stromatolites have been found in Precambrian rocks (Walter, 1983). Stromatolites increased in abundance throughout the Precambrian, then decreased abruptly at the Precambrian-Phanerozoic boundary (Awramik, 1984).

Living microbial mat communities, with a laminated structure similar to stromatolites, have been found in hot spring and marine lagoon environments (Awramik, 1984). Because of the structural similarity between modern mats and ancient stromatolites, these modern microbial communities are considered as the best models by which to interpret the nature of stromatolite communities (Krumbein, et al, 1977). Most microbial mats are built by cyanobacteria living together with other bacteria (Castenholz, 1984a). However, many mats which are built by photosynthetic bacteria and which lack cyanobacteria have recently been found in hot springs (Ward, et al, in press b). The existence of photosynthetic bacterial mats implies that stromatolites could have been constructed by either anoxygenic or oxygenic photosynthetic microorganisms.

By using transmitted light microscopy or electron microscopy, microfossils can be observed in thin sections of silicified stromatolites. Some microbes are three-dimensionally preserved in the rock, hence it is possible to determine the shape and the size of microbes preserved in the stromatolites. Over one hundred types of stromatolitic microbes have been identified in such micropaleontology studies (Schopf and Walter, 1983). From these studies, the morphologic evolution of stromatolitic microbes has also been observed. It seems that the microbes found in the stromatolites exhibit increasing size

and structural complexity through the Precambrian (Hofmann and Schopf, 1983). This suggests that life might have originated from a simple and small style (procaryotes) and evolved to a more complex and large style (eucaryotes).

Although many stromatolitic microbes have been recognized from microscopic observation, the interpretation of the type of microorganism preserved is simply based on the shape of the microfossils. For example, many microfossils, including the oldest known, have been regarded by some micropaleontologists to represent the cells of cyanobacteria (Schopf, 1978; Schopf and Walter, 1983; Schopf and Packer, 1987). Microbiologists are well aware of the limited value of morphology in identification. Even if shape were meaningful the high pressure and temperature conditions of rock formation might distort the original structures and characteristic components of cells. So far, scientists still can't make a definitive conclusion about the types of these microbes based solely on shape. Other lines of evidence to better understand the sequence and timing of evolutionary events are needed.

The Geochemical Record

The value of geochemistry to the study of evolution is obvious. For example, the decay of radioisotopes is the basis for age dating of rocks (White and Wood, 1986). Many other inorganic geochemical transitions are thought to

record changes in environmental conditions during the Precambrian which were possibly caused by biological evolutionary events. For example, the depositional histories of three minerals-uraninite (UO_2), red beds, and banded iron formations are thought to record a transition of Earth's atmosphere from anoxic to oxic. In the presence of certain amounts of oxygen, grains of UO_2 will be oxidized to U_3O_8 and are thereby dissolved in water. In this case streambed deposits of uraninite can't accumulate any more. It is estimated that this will occur when the concentration of atmospheric oxygen is greater than about one percent (Grandstaff, 1976). Uraninite-bearing deposits have been found only in the sediments older than about 2 Ga, but not in younger sediments. This suggested that an increase in concentration of oxygen probably occurred at that time. Red beds (mostly the mineral hematite, Fe_2O_3) represent a kind of mineral deposit which shows just the opposite pattern as uraninite, having been found only in sediments younger than 2 Ga. This is also consistent with an increase in the oxygen concentration at that time. The oxidation required for hematite deposition would have demanded a large supply of oxidants, presumably O_2 . Banded iron formations (BIF) are comprised of mostly ferric ion and can be accumulated under high oxygen concentration. The major BIF deposits were found to be around two billion years old (Awramik, 1984) also indicating the increased

availability of oxidants (e.g. O_2) at that time. Based on the record of these inorganic deposits, scientists suggested that huge amounts of oxygen must have been generated around two billion years ago and before that time the concentration of oxygen was probably very low. Though nonbiological sources of the oxygen can't be ruled out, a biological source--oxygenic photosynthesis performed by cyanobacteria--seems more plausible.

Organic geochemical transitions may also record information pertinent to biological evolutionary events. It is well known that biological preference for the lighter isotopes of the key elements of life (e.g. carbon, hydrogen, sulfur, and nitrogen) will alter stable isotope abundances. For example, when carbon has passed through a chain of metabolic process of bacteria, the ratio of ^{12}C to ^{13}C increases. Thus, the value of $\delta^{13}C$ defined as

$$\left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1 \right] \times 10^3 \text{ (o/oo, PDB)}$$

(where PDB is the Peedee belemnite reference standard which is used to report the isotopic composition of carbon in samples) (Hayes, et al, 1983; Schidlowski, et al, 1983), becomes more negative. For sediments comprised of organic matter from bacteria or plants the $\delta^{13}C$ value is around -30 to -10 (o/oo,PDB). Samples from marine carbonates and atmospheric carbon dioxide have $\delta^{13}C$ value around 0 to -10

(o/oo,PDB). The preferential selection of ^{12}C is mainly due to autotrophic CO_2 fixation (Schidlowski, et al, 1983). Through isotopic geochemistry studies, scientists suggest that autotrophic biological activity was present at least 3.5 billion years ago ($\delta^{13}\text{C}$ is close to -30 (o/oo,PDB) (Hayes, et al, 1983). Other isotopic ratios such as $\delta^{15}\text{N}$, δD , and $\delta^{34}\text{S}$ can also be used as an index of biological activity, but very few data are available for Precambrian samples. To better understand ancient biochemistries, more stable isotope measurements are needed.

The deficiency of stable isotope geochemistry is that the specific types of autotrophs involved in the ancient biosphere can't be figured out. To overcome this problem, the "biochemical marker" approach, another aspect of organic geochemistry, has been developed. Through analysis of specific organic chemical remains of Precambrian life by modern analytical techniques, it is hoped that more specific information about the types of organisms present in the ancient fossils can be learned. In this type of organic geochemistry "biomarkers", compounds which are produced by distinctive groups of organisms, are sought. In a sense these could be considered "chemical fossils". At least two approaches must be taken. The first is to examine ancient and recent sediments in the hope that some of the characteristic organic material produced by the ancient organisms is still there in recognizable form. The

second is to examine living organisms and mats to understand which organic compounds are diagnostic of which organisms. Combining the results from both approaches, one can attempt to infer when different types of living things were present.

Some organic compounds are unique in certain organisms and can be used as very good biomarkers to reflect the presence of these organisms in sediments (Smith, et al, 1983). The survival of some organic materials such as nucleic acids, proteins, and carbohydrates may be very poor, because of rapid microbial degradation and diagenesis which destroy them (Brown, et al, 1972). Lipids, however, seem to have longer survival potential. The surface of both eucaryotic and procaryotic cells is covered with a mixture of lipids (Goldfine, 1984), including linear hydrocarbons and their oxygenated derivatives such as fatty acids, long chain alcohols, wax esters, sterols, triterpenoids, and other isoprenoids. Some of these compounds are weakly bonded and easy to extract and analyze in organic solvents, and thus are called "free lipids". In many cases, these substances combine with more polar compounds to form complex lipids such as phospholipids, sulfolipids and steroidal glycosides, which are harder to extract and analyze.

Most organic matter in sediments is in the form of kerogen, a macromolecular material with no regular

structure, and which can't be extracted by organic solvents (Farrington, et al, 1977). Analytical methods for analyzing kerogen are still under development (Nagy, 1982). However, many fossil free lipids have been found in ancient (including Precambrian) and recent sediments (Nes and Nes, 1980; ten Haven, et al, 1985). A major problem in such work is the possible contamination of ancient sediments from lipids of more recent origin which have migrated into the rock, or which are introduced during analysis. Careful analysis of well-chosen samples, in which lipids are likely to be derived from organic matter deposited when the sediment formed, still reveals evidence of a surviving biomarker record for at least part of the Precambrian period. Most of these free lipids are hydrocarbons, some formed by defunctionalization of cellular components (e.g. steranes from sterols, hopanes from hopanols and hopenes) which occurs through microbial alteration or during preservation over geological time (Barghoorn, et al, 1965). In comparison to other chemical bonds, the C-C bond in hydrocarbons is quite strong (the bond energy for C-C is 347 KJ/mole) so the carbon skeleton is often preserved when hydrocarbons pass through different organisms or become incorporated into sediments. The approximate lifetime of alkanes subjected to 150°C in the absence of catalysts can be calculated to be about 10^9 years (Nes and Nes, 1980). Hence, biological information represented by a particular

hydrocarbon skeleton should be preserved over very long periods of time. Many steranes, hopanes, monomethyl alkanes, acyclic isoprenoids and other hydrocarbons have been found in Precambrian samples (see, for example, Eglinton, et al, 1964; Barghoorn, et al, 1965; Hoering, 1978; Arefev, et al, 1980; Nes and Nes, 1980; Jackson, et al, 1986; Klomp, 1986; Fowler and Douglas, 1987; Summons, 1987). The major objective for organic geochemists is to correctly assign the origins of sediment free lipids to the organisms which contributed them.

Many living organisms have been surveyed to attempt to understand their free lipid composition. As a result, a number of organic compounds (or their hydrocarbon derivatives) have been suggested as good biomarkers for certain types of organisms. Some hydrocarbon biomarkers which are known to survive in Precambrian rocks, and their suggested biological sources are shown in Table 1. A few of these may be particularly useful in establishing the timing of major evolutionary events, such as the emergence of cyanobacteria and eucaryotes. For example mid-chain branched alkanes, such as 7- and 8-methylheptadecanes may be unique to cyanobacteria, hopanoid hydrocarbons may be unique to aerobic bacteria, and steranes may be unique to eucaryotes (and possibly cyanobacteria). The validity of the suggested assignments is only as good as the choice of organisms studied in such surveys, however. Thus, organic

Table 1. Hydrocarbons with potential as Precambrian biological markers (modified from McKirdy and Hahn, 1982).

Hydrocarbon type	Characteristic feature	Possible sources
normal alkanes	C ₁₂ to C ₂₁ with odd carbon-number preference, C ₁₅ or C ₁₇ dominant	algae, bacteria, cyanobacteria
branched alkanes	7- and 8-methylheptadecane	cyanobacteria
	C ₁₆ to C ₃₀ iso and anteiso alkanes	bacteria
	C ₁₃ to C ₂₀ regular isoprenoids	photosynthetic algae, certain bacteria
	C ₂₁ to C ₄₀ regular and irregular isoprenoids (incl. squalene)	archaebacteria
cyclic alkanes	C ₂₇ to C ₃₅ pentacyclic triterpanes of hopane series	aerobic prokaryotes ^a
	steranes	algae, cyanobacteria

^a restriction to aerobes suggested by Taylor (1934) and Ourisson (1987).

geochemical studies have also been performed on modern mats, as communities of organisms analogous to stromatolite communities. The complexity of marine lagoonal mat communities, which contain cyanobacteria, anoxygenic photosynthetic bacteria, and eucaryotic microorganisms, obscures the assignment of the sources of lipids found there. In comparison to lagoonal mats, hot spring microbial mats are built by much simpler collections of microorganisms, making the assignment of sources of lipids easier. In addition, hot spring microbial mats are built by different types of photosynthetic microorganisms (e.g. photosynthetic bacteria, cyanobacteria, and eucaryotic algae). They are thus especially valuable in verifying biomarker assignment for these evolutionary important microbial groups. Comparing the free lipid distributions of different hot spring microbial mats has been the main point of my research.

Hot Spring Microbial Mats and Microorganisms Studied

For this project, six mats were examined (see Table 2). All of the mats were collected from hot springs located in Yellowstone National Park and New Zealand. In hot spring mats, the environmental conditions are usually too extreme for eucaryotic organisms. In general, eucaryotes can't tolerate temperatures above 40-50°C. An exception occurs in mats of acidic hot springs such as

Table 2. Chemical and microbiological features of microbial mats studied.

Mats	Chemical features			Photosynthetic microorganisms			
	°C	pH	H ₂ S (uM)	Eucaryotic algae	Procaryotic		
					Cyanobacteria	Purple Bacteria	Green Bacteria
Nymph Creek	45	3		<u>Cyanidium</u> <u>caldarium</u>	-	-	-
"Weigert's Channel"	45	6		-	<u>Phormidium</u> sp. <u>Synechococcus</u> <u>lividus</u>	-	<u>Chloroflexus</u> <u>aurantiacus</u>
Orakei Korako	48	7.3			<u>Chlorogloeopsis</u> sp.	-	<u>Chloroflexus</u> <u>aurantiacus</u>
"New Pit" Spring	58	6.3	34	-	-	-	<u>Chloroflexus</u> sp.
"Roland's Well"	55	6.3	41	-	-	<u>Chromatium</u> <u>tepidum</u>	<u>Chloroflexus</u> sp.
"Travel Lodge" Stream	45	5.9	>1000	-	-	-	<u>Chlorobium</u> sp.

Nymph Creek (Yellowstone National Park). Here, mats are formed by the eucaryotic alga Cyanidium caldarium in the absence of cyanobacteria and photosynthetic bacteria which do not tolerate low pH. Comparision of the Nymph Creek mat to mats in near neutral to alkaline springs should then reveal lipid inputs characteristic of eucaryotes.

Other chemical conditions also affect the types of photosynthetic procaryotes which build mats in hot springs. Mats in near neutral or alkaline hot springs can be divided into two groups--cyanobacterial mats and cyanobacteria-free mats formed by photosynthetic bacteria. Cyanobacterial mats appear in hot springs with temperatures below 70°C (Castenholz, 1984a) and very low sulfide concentration. Cyanobacterial mats from "Wiegert's Channel", Yellowstone National Park and a hot spring in the New Zealand Orakei Korako thermal area were studied. The cyanobacteria in these mats included Synechococcus lividus and Phormidium sp. ("Wiegert's Channel"), and Chlorogloeopsis sp. (Orakei Korako). All these are oxygenic photosynthetic procaryotes which develop at the top of the mats. Water is used as electron donor and oxygen is produced through the photosynthetic process. The oxygenic photosynthesis can be measured by oxygen microelectrodes (Jørgensen, et al, 1983; Revsbech and Ward, 1984; Revsbech and Jørgensen, 1986). A filamentous photosynthetic bacterium Chloroflexus aurantiacus, is also found in these mats. C. aurantiacus

is a photoheterotrophic green nonsulfur bacterium. Light is used as the energy source and organic materials are used as carbon sources. The organic materials used by this bacterium can be produced by cyanobacteria or by other nonphotosynthetic bacteria in the mats. Hydrogen sulfide may be used by C. aurantiacus as an electron donor in a true photosynthetic process, but no oxygen can be produced. Many other nonphotosynthetic bacteria also live in the mats and these are presumably mainly decomposers (Ward, et al, 1987).

Cyanobacteria-free mats occur in hot springs which contain sulfide in high enough concentration to inhibit the growth of the cyanobacteria (Castenholz, 1984b). Many photosynthetic bacteria such as Chromatium tepidum, Chloroflexus sp., and Chlorobium sp. are known to build this kind of mat. Sulfide is used as electron donor for the photosynthetic processes of these bacteria, and no oxygen can be produced. Therefore, such mats provide two conditions relevant to evolutionary studies of stromatolite-like communities 1) the absence of cyanobacteria and 2) the absence of oxygen. Chloroflexus sp., a green nonsulfur bacterium, physiologically distinct from the C. aurantiacus found in cyanobacterial mats, is the principal phototroph which builds such mats in Yellowstone (e.g. "New Pit" Spring), Iceland, and New Zealand hot springs (Giovannoni, et al, 1987; Castenholz,

1973 and 1976). C. tepidum, a purple sulfur bacterium, is a major component in some hot spring mats in Yellowstone National Park (e.g. "Roland's Well"). Chlorobium sp., a green sulfur bacterium, is not reported to occur in the mats from Yellowstone National Park. Some mats from New Zealand hot springs (e.g. "Travel Lodge" Stream) are built principally by Chlorobium sp. (Castenholz, in press). Since the free lipid composition of anoxygenic photosynthetic bacteria has not been well studied, these three microorganisms were also examined in this project.

Objectives

The free lipids of hot spring mats, including one eucaryotic algal mat, two "cyanobacterial" mats, and three photosynthetic bacterial mats were analyzed. The main objective was, through comparative analysis, to investigate whether the distribution of specific biomarkers correlates with the microbial distributions in these mats. This should increase or decrease confidence in the interpretation of specific biomarkers which have been found in Precambrian sediments as indicators of 1) the presence of eucaryotes, 2) the presence of cyanobacteria, and 3) the presence of oxygen. I also analyzed the three photosynthetic bacteria which produce the anoxygenic mats studied. This aided identification of sources for lipids found in such mats and revealed biomarkers which might be

used to distinguish such microorganisms.

MATERIALS AND METHODS

Chemicals and Standards

Special precautions were taken to eliminate or identify contaminants. High purity (Omni-grade) dichloromethane, hexane, diethylether, methanol, ethylacetate, acetone, and toluene were purchased from EM Scientific Co.. Each solvent was condensed from ca. 200 ml to 0.5 ml by rotary evaporation (Buchler 78424) and checked for organic contaminants by gas chromatography-mass spectrometry (GC-MS). Alumina (Fisher Scientific Co.) was extracted with dichloromethane before using. After packing prerinsed alumina into columns, 100 ml of hexane was used to preelute nonpolar impurities. Potassium hydroxide was baked at 220°C for 4 hours to remove organic impurities. Hydrochloric acid and distilled water were extracted with dichloromethane to remove organic impurities before using.

Standard reagents included saturated straight-chain alkanes and alcohols, and alkenes (all from Supelco Inc.), and fatty acid methyl esters, 5 α -cholestan-3-one, 24-ethyl-cholesta-5,22-dien-3 β -ol and normal alcohols (all from Sigma Chemical Co.).

Samples

All of the materials used in collecting samples were rinsed with pure dichloromethane or methanol prior to use. Sample was placed in rinsed aluminum foil, frozen in dry ice immediately after collection, and transferred to a -70°C freezer upon returning to the laboratory. Except for a small portion of the sample retained for microscopic observation, the bulk of the sample was lyophilized (Vir Tis, freeze mobile 6). Temperature and pH values were measured in situ with a thermometer and pH meter. The concentration of sulfide in the hot spring water was determined by using the colorimetric method of Cline (1969).

Eucaryotic Algal Mat

A mat built by the eucaryotic alga Cyanidium caldarium was collected at Nymph Creek, a small stream flowing into the east end of Nymph Lake, Yellowstone National Park. At the collection site (45°C, pH 3), the mat was 1-2 cm thick. As this mat received significant inputs of pine needles, care was taken to collect mat in high-flow areas where contamination was slight.

Cyanobacterial Mats

Mats built by cyanobacteria were collected in Yellowstone National Park and New Zealand hot springs. One

was collected from a spring (unofficially called "Wiegert's Channel") in Seredipity Meadow, Yellowstone National Park, formerly used by Wiegert and Fraleigh (1972) to supply an artificial channel. The sample was collected at a site (45°C , pH 6) where the natural effluent channel flowed through a meadow. Grasses which might be a source of contamination were carefully excluded. The other cyanobacterial mat was collected in the Orakei Korako thermal area, located between Lake Taupo and Lake Rotorua, New Zealand. The sample was collected from an effluent channel (48°C , pH 7.3) along the west edge of the thermal area between the lower and upper terraces.

Photosynthetic Bacterial Mats

Mats built mainly by green and purple photosynthetic bacteria were collected from two springs in the Mammoth Hot Spring group of Yellowstone National Park--"New Pit" Spring and "Roland's Well" (unofficial names). The general locations of these springs were between Elephant Back Spring and Orange Mound Spring on the upper terrace loop road (see map in Castenholz, 1977). "New Pit" Spring is ca. 500 m north of Elephant Back Spring. The dark green mat in "New Pit" Spring (ca. 0.5-1.0 cm thick by ca. 0.7 m x 0.4 m) grew in a source pool ca. 2 m beneath a travertine ledge (see Ward, et al, in press b). The mat was collected as free from sediment as possible. "Roland's Well" was

located in a small depression ca. 200 m north of "New Pit" Spring (see Ward, et al, in press b). The purple mat in "Roland's Well" (ca. 0.2-0.5 cm thick by ca. 0.4 m x 0.25 m) grew on the wall and the rocks inside the source pool.

Another green photosynthetic bacterial mat was collected from a New Zealand hot spring unofficially named "Travel Lodge" Stream as it flowed toward Lake Rotorua along the east edge of the parking lot of the Rotorua Travel Lodge Motel. The green mat (ca. 1-3 mm thick) overlying a muddy sediment was collected by scraping with a solvent-rinsed spatula over the surface above the mud in the effluent channel.

Pure Cultures

Pure cultures of three photosynthetic bacteria were provided by Dr. Michael T. Madigan (Dept. of Microbiology, Southern Illinois Univ.). Chloroflexus aurantiacus (strain Y-400-f1) was isolated from the Octopus Spring cyanobacterial mat in Yellowstone National Park.

Chromatium tepidum was isolated from a purple mat in the Mammoth Hot Springs group (Madigan, 1984 and 1986).

Chlorobium sp. was isolated from the green mat of the New Zealand "Travel Lodge" Stream.

Microscopic Observation

Before lipid extraction, a thawed portion of the sample was observed by fluorescence microscopy. A Leitz Ortholux II microscope equipped with an HBO-100 W mercury lamp that provided vertical ultraviolet (UV) light illumination through a Leitz B-cube excitation-emission filter was used to observe red autofluorescence characteristic of chlorophyll a. Interpretation of microscopic observation was under the guidance of Dr. David M. Ward (Dept. of Microbiology, Montana State Univ.).

Pigment Analysis

Lyophilized samples were homogenized in 60% sucrose (w/v) with a glass-tissue homogenizer (Wheaton Scientific, No. 357546), using pestle "A" with clearance of 0.25-0.75 mm), then centrifuged at 3000 g for ca. 3 min. to remove large debris. The absorption spectrum of the whole cell homogenate was measured by a Cary model 14 spectrophotometer scanning continuously from 900 nm to 340 nm.

Total Organic Carbon Measurements

The total organic carbon (TOC) in the samples was determined using an Oceanography Total Organic Carbon Analyzer, (model 0524B). The sample was acidified with concentrated hydrochloric acid and purged with oxygen gas

to remove inorganic carbon, then oxidized with potassium persulfate in an autoclave (121°C, 4 hours). The resultant CO₂ was measured by nondispersive infrared spectrometry.

Lipid Analysis

The overall scheme for extraction and separation of free lipids was described by Dobson, et al, (in press) and is outlined in Figure 3. Obvious impurities (e.g. pine needles) were carefully removed from lyophilized samples which were then ground to powder using a dichloromethane-rinsed mortar and pestle. All glassware used in lipid analysis was cleaned with detergent, rinsed with distilled water, then baked at 180°C for several hours, and finally rinsed with hexane followed by dichloromethane to remove impurities. A blank test was done to check any possibility of contamination in lipid analysis.

Extraction

Free lipids were obtained by repeated extraction of the dry powdered sample with dichloromethane until the extracts were nearly colorless by alternate sonication (Branson D-50 sonicator) and centrifugation (Sorvall RD-5B refrigerated super-speed centrifuge). Extracts were pooled and solvent was removed by rotary evaporation (Buchler 78424).

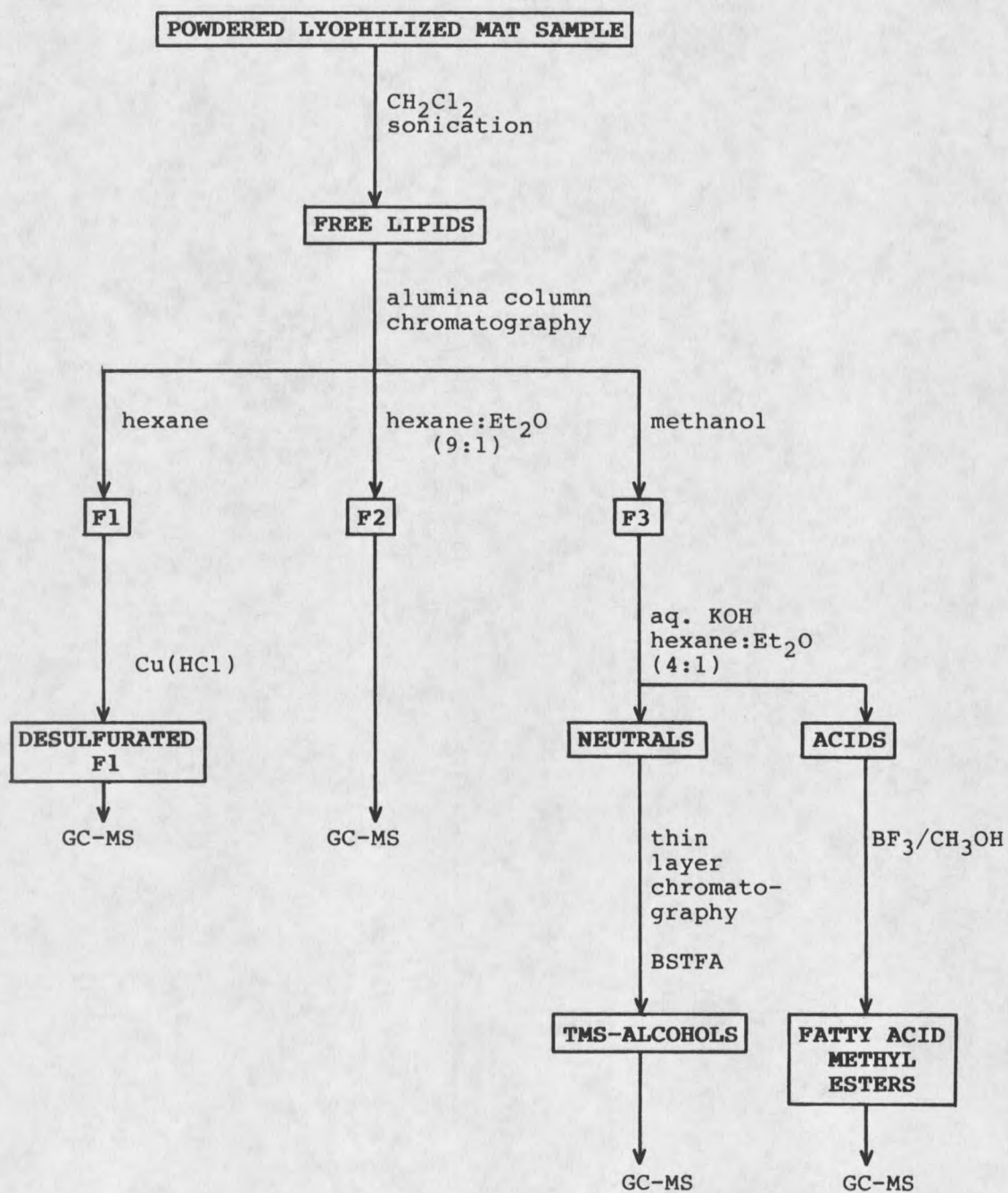


Figure 3. Extraction, fractionation, and gas chromatography-mass spectrometry (GC-MS) analysis of free lipids.

Column Chromatography

The extracted lipids (in dichloromethane phase) were separated on a glass column (3 x 31 cm) provided with a sintered-glass filter disc and filled to a depth of 20 cm with a slurry of alumina (80-200 Mesh, preactivated for 12 hours at 220°C) in hexane. The extract was redissolved in 2 ml of dichloromethane and mixed with ca. 15 g of alumina. After drying in the air, the extract-coated alumina was added to the column and then eluted into three fractions. The first fraction (F-1) containing the aliphatic hydrocarbons was eluted with 160 ml hexane (ca. 2 times dead volume of the alumina packed column). The second fraction (F-2) containing the wax esters was obtained by elution with 160 ml hexane-diethylether (9:1 v/v). The third fraction (F-3) containing alcohols, fatty acids and pigments was obtained by elution with 200 ml methanol. The solvents were removed by rotary evaporation and the residues were blown to dryness under a stream of nitrogen gas.

Desulfuration

Desulfuration of the F-1 fraction of some samples was necessary before gas chromatography-mass spectrometry (GC-MS) analysis. An elution tube (1.25 x 25 cm) packed with copper filings was prerinsed with concentrated hydrochloric acid (HCl) to activate the copper. The column was then

sequentially rinsed with distilled H_2O , acetone and CH_2Cl_2 to remove HCl , H_2O and organic impurities. The F-1 fraction was dissolved in dichloromethane and passed through the acid-activated copper-filing column. Sulfur reacted with copper to form CuS (black color) and this was retained in the elution tube.

Acid-Neutral Separation

The components of the F-3 fraction were separated into acidic and neutral compounds by differential partition between KOH (aq) and hexane-diethylether. The sample was dissolved in 10 ml hexane:diethylether (4:1 v/v) and transferred into a separatory funnel which contained 30 ml KOH (2% w/v). After extraction, the aqueous layer was removed. The organic layer was then extracted two times with 30 ml KOH (10% w/v). The aqueous layers were pooled and reextracted with 20 ml hexane-diethylether (4:1 v/v). The pooled organic layers containing neutral compounds were concentrated under a stream of nitrogen gas. In some cases, alcohols were further purified by thin layer chromatography (see Dobson, et al, in press). The aqueous layers were acidified with concentrated HCl to pH less than one, then extracted three times with dichloromethane. The organic layers, here containing fatty acids, were concentrated by evaporation under nitrogen gas.

Derivatization of Acids and Alcohols

N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (Supelco Inc.) was used to derive trimethylsilylethers of alcohols before GC-MS analysis. About 200 μ l of the reagent was added to the sample in a 5 ml vial, and the vial was put in a heating block (ca. 38°C) for one hour. After cooling, the solvent was dried by a stream of nitrogen gas.

Boron trifluoride in methanol (12-14% w/v, Supelco Inc.) was used as a transesterification catalyst to prepare methylesters of fatty acids before GC-MS analysis. About 3 ml of the reagent was added to the sample and the mixture was refluxed for 20 min. Most of the methanol was then removed by rotary evaporation. A small amount of distilled water was added to dissolve the remaining methanol, and this solution was extracted with CH_2Cl_2 . The organic layer containing methylated fatty acids was then dried under nitrogen gas.

Detailed Analysis of Long-Chain Alkenes

Long chain alkenes found among F-1 compounds in the mat from "New Pit" Spring and in the bacterium C. aurantiacus were analyzed in greater detail as shown in Figure 4. Accurate mass measurement by GC-MS is described in the following section.

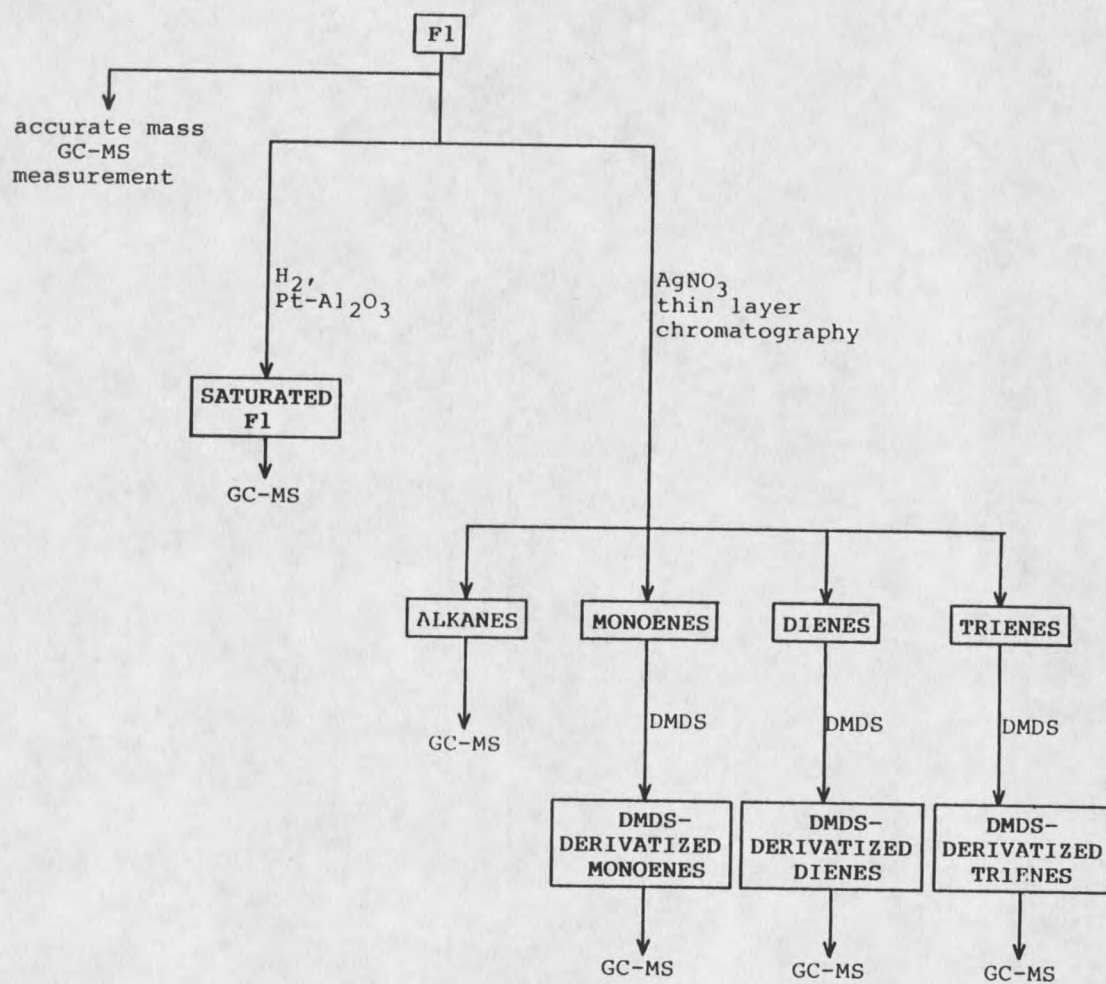


Figure 4. Separation and identification of alkenes (DMDS= dimethyl disulfide).

Hydrogenation

Hydrogenation was done to both samples to determine the number of double bonds in alkenes. The F-1 fraction was dissolved in 2 ml of methanol and put into a 5 ml flask connected to a condenser, then about 0.5mg of Adams' catalyst ($\text{Pt-Al}_2\text{O}_3$) was added. A balloon which was filled with hydrogen gas was set at the top of the condenser. During the reaction, the solution was kept stirring by a magnetic stirrer at room temperature. After twelve hours, the liquid layer was poured into a small vial and dried under nitrogen gas. The sample was then analyzed by GC-MS. An internal standard (tricosane) was injected together with the sample.

Silver Nitrate Chromatography

Silver nitrate chromatography was used to separate alkanes and alkenes with a different number of double bonds (Christie, 1982). A 0.5 mm pre-coated silica gel thin-layer chromatography (TLC) plate (20 x 20 cm)(Fisher Scientific Co.) was prerun with ethyl acetate for four hours. After drying, the top 2 cm of silica gel was scraped off to remove contaminants. The plate was eluted again in an aluminum foil-wrapped tank with a solution which contained 10 g silver nitrate in 60 ml of ethanol together with 30 ml of water for at least twelve hours. It was dried in the air for 15 min., then in an oven for 15

min. at 100°C. To prevent the reaction of silver nitrate with light, all of these steps were carried out in a darkened room.

F-1 fractions were dissolved in dichloromethane and applied as a narrow streak 3 cm from the bottom of the plate, using a syringe. The plate was then placed in a tank containing the eluting solvent--hexane:diethylether (9:1 v/v). Normal alkane, mono-, di-, and triunsaturated alkene standards were applied at the side of plate, separated from the sample by a groove. When the solvent had nearly reached the top of the plate, the plate was removed from the tank, dried in a hood and the side which contained the standards was sprayed with rhodamine solution (Alltech Associates Inc.) to render the lipids visible under UV light. According to the corresponding position of standards, the side which contained the samples was divided into sections containing alkanes, mono-, di-, or trienes which were scraped into small chromatographic columns (plugged with glass fibers). Ethylacetate was then used to elute the samples. The samples were dried under a stream of nitrogen gas.

Dimethyl Disulfide (DMDS) Derivatization

The position of the double bonds in alkenes were determined by DMDS derivatization (Buser, et al, 1983; Nichols, et al, 1986). The dried mono-, di- or

triunsaturated alkenes contained in AgNO_3 -TLC fractions were dissolved in hexane (50 μl) in 2 ml vials with teflon-lined screw-cap lids; 100 μl DMDS (Supelco Inc.) and 1-2 drops of iodine solution (6% w/v in diethylether) were then added. The reaction was carried out at 50°C for 48 hours. After that, 500 μl sodium thiosulfate (5% w/v in water) was added to remove the iodine. The organic layer was removed, and the aqueous layer was extracted with hexane:chloroform (4:1 v/v). The combined organic layers were dried under a stream of nitrogen gas.

Gas Chromatography-Mass Spectrometry

Capillary gas chromatography-mass spectrometry (GC-MS) and fragmentography were performed with a Varian 3700 gas chromatograph connected to a VG MM16 mass spectrometer or a VG 70E-HF mass spectrometer. A fused silica column (30 m length, 0.25 mm internal diameter) coated with 95%-dimethyl-5%-diphenylpoly-siloxane (DB-5) at a thickness of 0.25 μm was used. The GC oven temperature was held initially at 35°C for 4 min., then increased to 80°C at $20^\circ\text{C}/\text{min}$, and to 300°C at $4^\circ\text{C}/\text{min}$; a 20 min. isothermal period followed. For DMDS-derivitized alkenes, the final temperature was set at 320°C and a 50 min. isothermal period followed. The helium carrier gas flow rate was 1 ml/min. The injection port was heated to 300°C for DMDS-

derivitized alkenes, to 280°C for F-2 fractions and to 260°C for other fractions. The electron energy used for ionization in the ion source of the mass spectrometer was 70 eV, except in analysis of long chain hydrocarbons (F-1 fraction) when it was reduced to 40 eV. The electron beam current and the accelerating potential were set at 200 uA and 4 KV in the VG MM16, and at 100uA and 5 KV in the VG 70E-HF. The electron multiplier voltage was set at 2 KV for both mass spectrometers. Each peak was scanned within the range of m/z 33 to m/z 550 (or m/z 650) atomic mass units with a scan rate of 0.7 sec. per mass decade and 0.3 sec. interscan delay for the VG MM16, and 0.3 sec. per mass decade and 0.2 sec. delay for the VG 70E-HF. The mass spectrometer was interfaced to a VG-2035 data system with a PDP 8-A (64 or 16 K memory) computer. The accurate mass measurements for the long chain alkenes were done using the VG 70E-HF mass spectrometer with perfluorkerosene-H as a reference standard.

The identity of compounds was determined by relative retention time, mass spectra interpretation, (confirmed by Dr. Simon C. Brassell, Stanford University, Geology Department), and in one case by coinjection with standard. For each analysis the reconstructed ion chromatograms (RIC) of ions which have been found by experience to be diagnostic of particular groups of compounds (Table 3) were printed out along with the total ion chromatogram (TIC).

Table 3. Mass fragments used in detecting specific groups of compounds (modified from Asselineau and Asselineau, 1984 and Silverstein, et al, 1981).

m/z	Compound Types
57, 85 ^a	alkanes and alkyl groups
55, 69 ^a	alkenes and cycloalkanes
64, 96 ^b	sulfur
68	isoprene
69	squalene
73, 147 ^c	gas chromatograph septum bleed
74	methylester
75	trimethylsilyl ether
78	benzene
91	toluene, alkylbenzenes, phenyl alkyl group
123	phytene
135, 207 ^c	gas chromatograph column bleed
143	trimethylsilyl phytol
149	phthalic acid
189	hopenes, hopenols
191	hopanes, hopanols
217	steranes
^a series of higher peaks with m/z 14 difference	
^b series of higher peaks with m/z 32 difference	
^c series of higher peaks with m/z 74 difference	

This simplified assignment of most compound types. The detailed interpretation of mass spectra will be discussed in the Results section.

The concentration of each component was determined by comparing its peak area in a GC spectrum to that of internal standards. The internal standards were normal tetracosane (for hydrocarbons, wax esters, and fatty acid methyl esters), TMS-derivatized nonanol (for nonsteroidal alcohols) and 24-ethyl-cholesta-5,22-dien-3 β -ol (for sterols).

Since the GC-MS analysis was able to detect trace amounts of compounds, the finding of trace contaminants from this analysis was inevitable. Phthalic acid esters, a common plastic material, was the most frequently encountered contaminant in the samples and could be easily recognized by its mass spectrum (m/z 149 as the base peak) (McLafferty, 1980). Naphthlene (mol. weight 128) and some undefined aromatic compounds (m/z 77, 91, 105, (+14n)) were found in blanks as well as samples. Some other unknown contaminants (e.g. highly volatile compounds with very short retention times on the GC column) and indene (mol. weight 220)) were also found in blanks as well as samples. Column and septum bleed caused serious contamination problems when the concentration of the sample was very low but fortunately, these could be easily recognized through their mass spectra (see Table 3). All of these

contaminants were ignored when drawing the diagrams of free lipid distributions in the Results section.

RESULTS

Microscopic and Spectrophotometric Observations

Mat samples were examined microscopically (Table 4) to confirm that the predominant phototrophic microorganisms were those expected (see Table 2). Fluorescence microscopy confirmed the predominance of oxygenic phototrophs (containing chlorophyll a) resembling C. caldarium in the Nymph Creek mat, S. lividus and Phormidium sp. in the "Wiegert's Channel" cyanobacterial mat and Chlorogloeopsis sp. in the Orakei Korako cyanobacterial mat. Red autofluorescent cells were absent or very rare in photosynthetic bacterial mats (only occasionally seen in the "Roland's Well" mat), confirming the absence of cyanobacteria in these samples. Filamentous bacteria resembling Chloroflexus sp. were predominant in the "New Pit" Spring mat and abundant also in the "Roland's Well" mat where S^0 -containing unicells resembling Chromatium tepidum predominated. The "Travel Lodge" Stream mat contained principally green unicellular bacteria resembling Chlorobium sp..

Spectral analysis of aqueous cell suspensions also confirmed the presence of the expected chlorophylls

Table 4. Microscopic and spectral observations of the mats and bacteria.

Samples	Major Pigments (Wavelength)	Microscopic Observation
Nymph Creek	Ca (680 nm) PC (620 nm)	large sphaeroidal red-fluorescing cells.
"Wiegert's Channel"	Ca (680 nm) PC (620 nm)	large slightly curved unicellular green red-fluorescing cells, wide filamentous green red-fluorescent cells, nonseptate filamentous green cells which do not fluoresce red.
Orakei Korako	Ca (680 nm) PC (620 nm) Ba (750 nm)	cylindrial green red-fluorescing cells in chains, nonseptate filamentous green cells which do not fluoresce red.
"New Pit" Spring	Bc (735 nm) Ba (805 nm)	nonseptate filamentous green cells which do not fluoresce red.
"Roland's Well"	Bc (735 nm) Ba (805 nm and 855 nm)	nonseptate filamentous green cells which do not fluoresce red, small purple rod-shaped cells with internal S ⁰ granules which do not fluoresce red.
"Travel Lodge" Stream	Bc (750 nm)	tiny rod-shaped green cells which do not fluoresce red.
<u>Chloroflexus aurantiacus</u>	Bc (753 nm) Ba (805 nm)	-
<u>Chromatium tepidum</u>	Ba (805 nm and 855 nm)	-
<u>Chlorobium</u> sp.	Bc (750 nm)	-

Ca=chlorophyll a; Ba=bacteriochlorophyll a;
Bc=bacteriochlorophyll c; PC=phycocyanin

characteristic of photosynthetic community members (see Figures 5-7 and Table 4). Absorption maxima at 680 nm and 620 nm, characteristic of chlorophyll a and phycocyanin, respectively, were only found in the cyanobacterial mats and the C. caldarium algal mat. Maxima at 735-750 nm, characteristic of bacteriochlorophyll c were present in green bacteria (Chloroflexus aurantiacus and Chlorobium sp.) and mats containing these species (e.g. all photosynthetic bacterial mats and the Orakei Korako cyanobacterial mat). While bacteriochlorophyll c was not detected in the "Wiegert's Channel" mat, nonfluorescent filamentous bacteria indicated the likely presence of C. aurantiacus which is usually found in cyanobacterial mats of Yellowstone National Park. Maxima at 805 and 855 nm, characteristic of bacteriochlorophyll a of the purple bacterium C. tepidum were only found in abundance in the "Roland's Well" mat.

Gravimetric Analysis and TOC Content

Gravimetric analysis of free lipid fractions (before and after correcting to per gram of total organic carbon), sulfur content, and total organic carbon (TOC) content for each sample are summarized in Table 5. A high concentration of elemental sulfur was detected in each photosynthetic bacterial mat presumably related to the importance of H_2S oxidation in photosynthesis in these

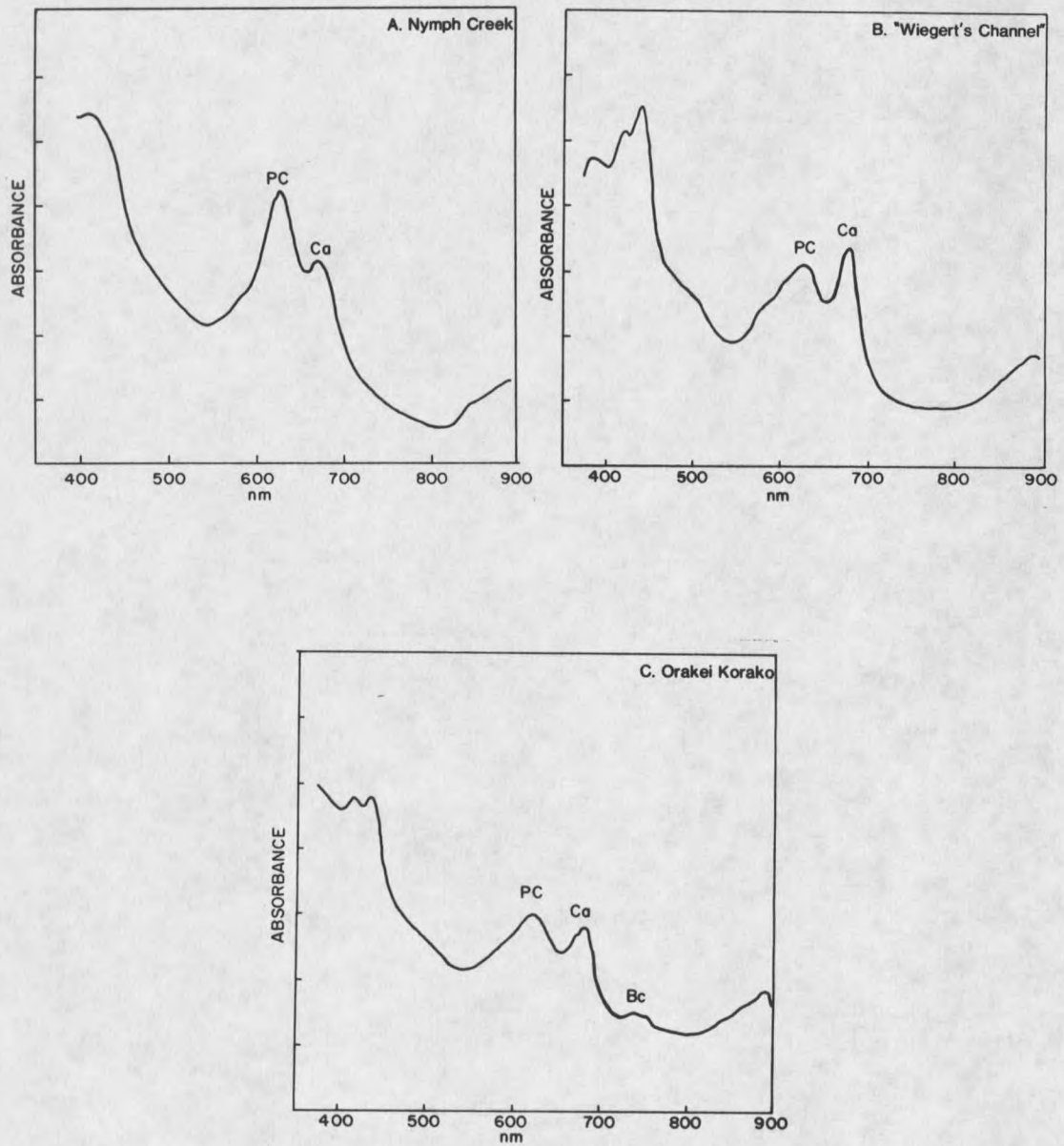


Figure 5. In vivo absorption spectra of oxygenic mats
(see Table 4 for the definition of the symbols).

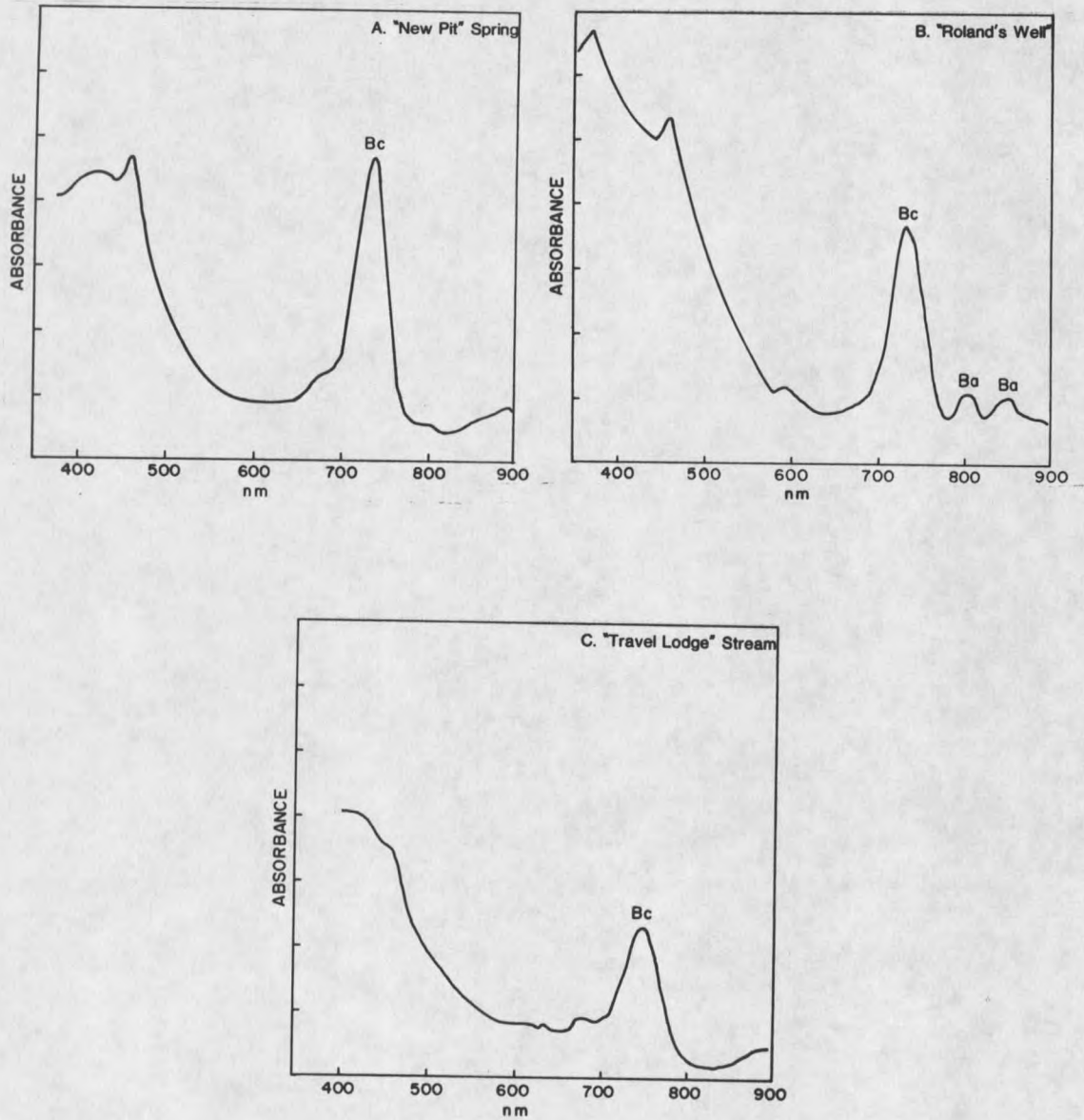


Figure 6. In vivo absorption spectra of photosynthetic bacterial mats (see Table 4 for the definition of the symbols).

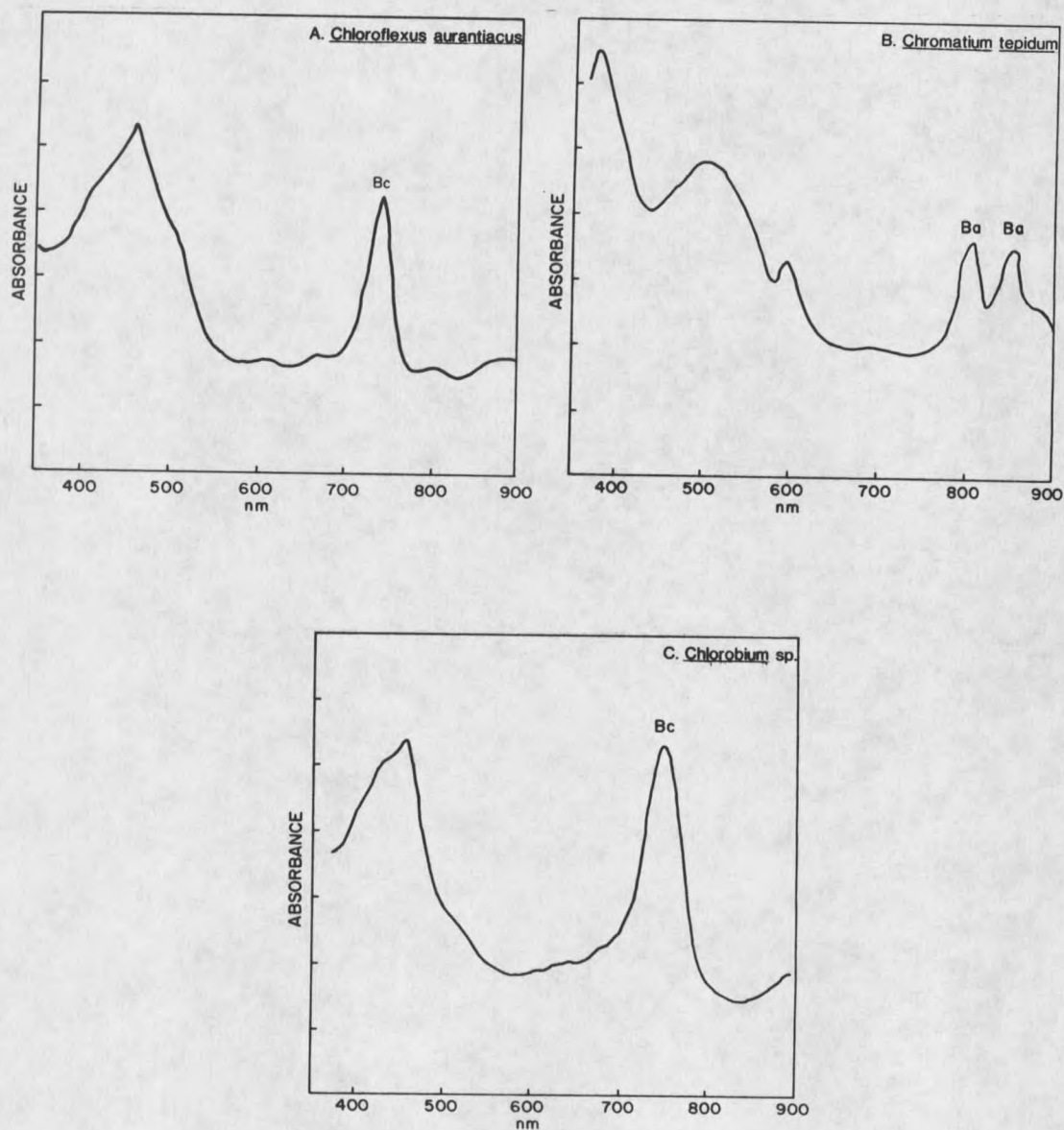


Figure 7. In vivo absorption spectra of photosynthetic bacteria (see Table 4 for the definition of the symbols).

Table 5. Gravimetric weights of lipid classes, sulfur content, and total organic carbon (TOC) content of the mats and bacteria.

Fractions	ug/g dry weight, (ug/g organic carbon)								
	Mats ^a						Bacteria ^b		
	NC	WC	OK	NP	RW	TL	<u>C. aur.</u>	<u>Chr. tep.</u>	<u>Chl. sp.</u>
Sulfur	40	nd	69	4400	3830	1290	nd	nd	7x10 ⁵
Hydrocarbons	50 (2.4)	1046 (37.4)	690 (49.3)	235 (33.6)	24 (8)	65 (32.5)	260 (16.3)	5 (0.1)	27 (9)
Wax esters	nd	489 (17.5)	80 (5.7)	1050 (150)	1520 (507)	14 (7)	1657 (103)	nd	183 (61)
Alcohols	165 (7.9)	217 (8)	57 (4.1)	58 (8.3)	33 (11)	10 (10)	105 (6.6)	145 (3.1)	10 (3.3)
Fatty acids	35 (1.7)	88 (3.1)	52 (3.7)	12 (1.7)	8 (2.6)	10 (5)	66 (4.1)	30 (0.6)	18 (6)
TOC ^c (% dry weight)	21	28	14	7	3	2	16	47	3

^a NC(Nymph Creek), WC("Weigert's Channel"), OK(Orakei Korako), NP("New Pit" Spring), RW("Roland's Well"), TL("Travel Lodge" Stream).

^b C. aur.(Chloroflexus aurantiacus), Chr. tep.(Chromatium tepidum)
Chl. sp.(Chlorobium sp.).

^c TOC analysis was done by Pam Refling (Dept. of Chemical Engineering, Montana State Univ.).

mats. Sulfur was also detected in high abundance in the Chlorobium sp. culture, this almost certainly produced during growth of the isolate on H_2S . Small amounts of sulfur were detected in the Nymph Creek algal mat and the Orakei Korako cyanobacterial mat.

Since it was impossible to collect samples without taking sediments, normalizing the content of free lipids to a per gram TOC basis permits a better comparison of the lipid content among the samples. Hydrocarbons were detected in all of the samples; the two cyanobacterial mats contained higher amounts of hydrocarbons than any other samples. Wax esters were detected in all of the samples except C. tepidum and the Nymph Creek mat. The mats from "New Pit" Spring and "Roland's Well", and C. aurantiacus contained the highest amount of wax esters. Only a trace of wax esters was found in the "Travel Lodge" Stream mat. Relatively low concentrations of alcohols were found in all of the samples. Fatty acids were detected in all of the samples, and were usually the free lipids found in the lowest concentration.

Free Lipid Distributions

The major free lipids of each fraction of all samples are shown in Figures 8 to 16. Due to the problem of scale, these figures do not indicate minor compounds which will be mentioned in the text, or detailed in other illustrations.

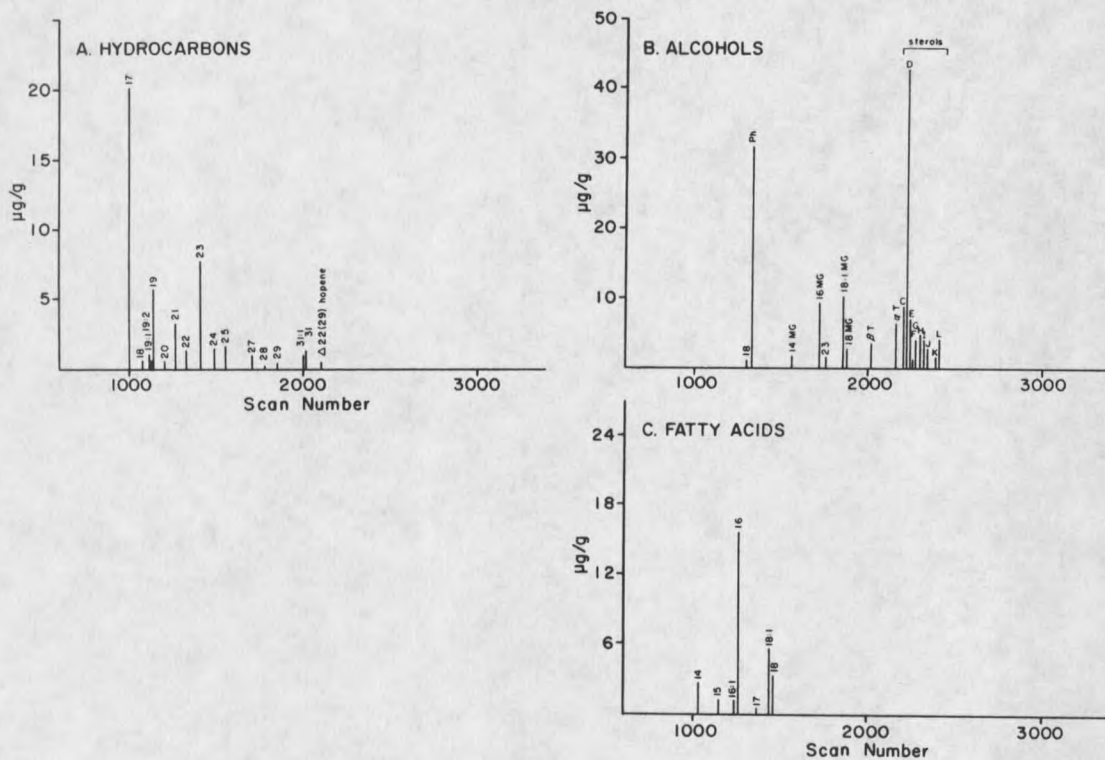


Figure 8. Major free lipids of the Nymph Creek Cyanidium caldarium mat (numbers refer to the carbon number of a compound; the number after colon indicates the number of double bonds; MG= monoglyceride; Ph=phytol; letters designate sterols described in Table 11).

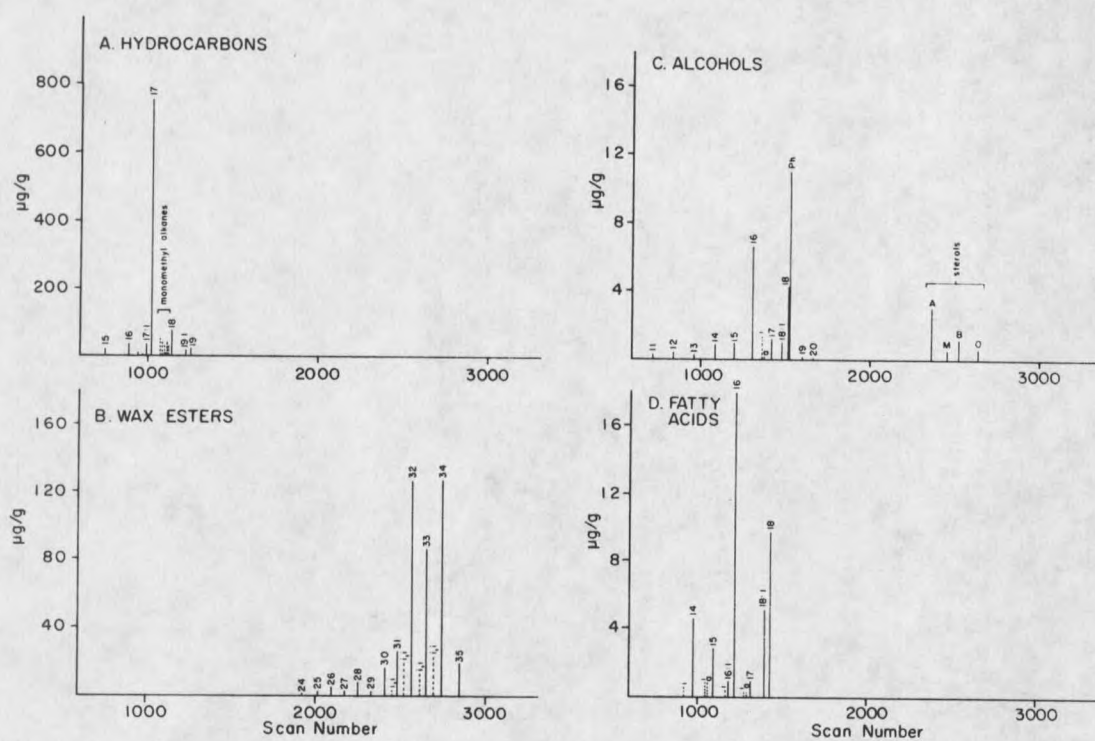


Figure 9. Major free lipids of the "Wiegert's Channel" cyanobacterial mat (dashed lines represent branched compounds; i=iso; a=anteiso; see legend to Figure 8 for definition of the other symbols).

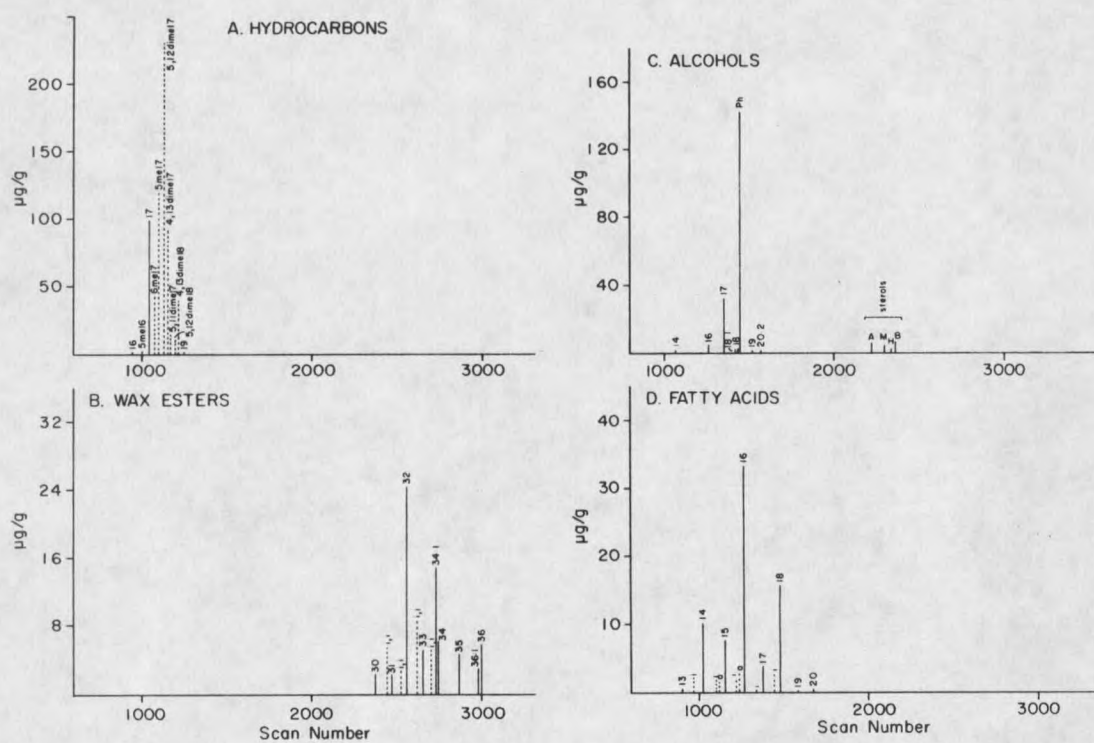


Figure 10. Major free lipids of the Orakei Korako cyanobacterial mat (see legends to Figures 8 and 9 for definition of symbols).

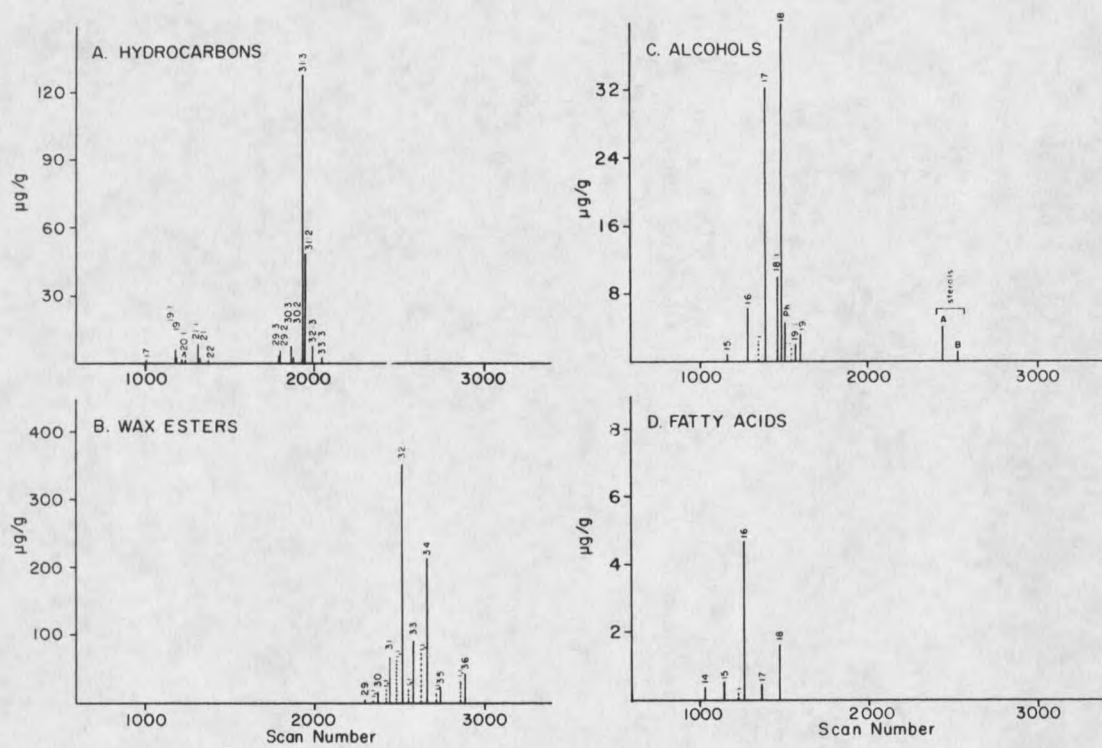


Figure 11. Major free lipids of the "New Pit" Spring photosynthetic bacterial mat (see legends to Figures 8 and 9 for definition of symbols).

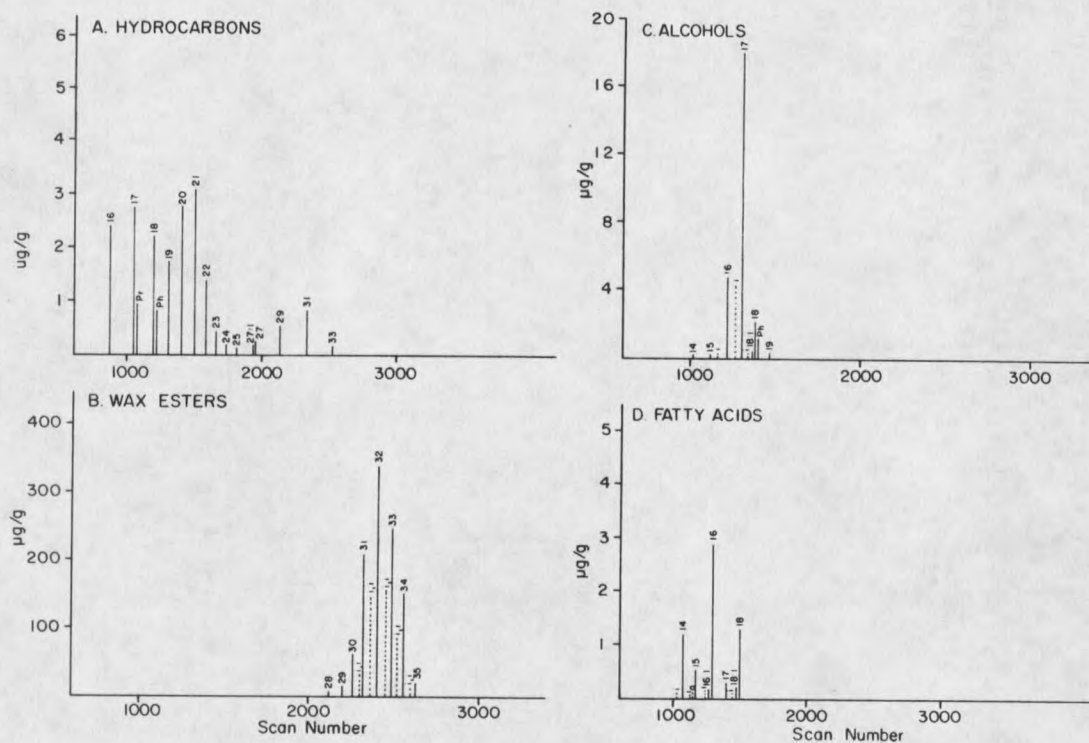


Figure 12. Major free lipids of the "Roland's Well" photosynthetic bacterial mat (see legends to Figures 8 and 9 for definition of symbols).

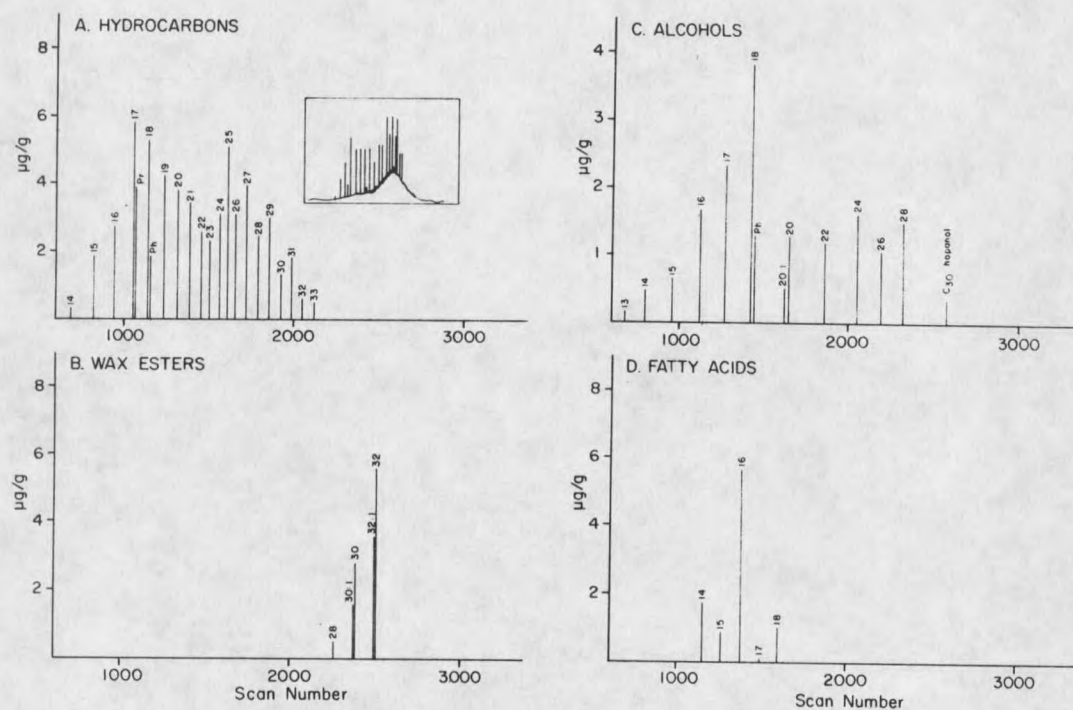


Figure 13. Major free lipids of the "Travel Lodge" Stream photosynthetic bacterial mat (insert in A shows actual total ion current of F-1 fraction; see legends to Figures 8 and 9 for definition of symbols).

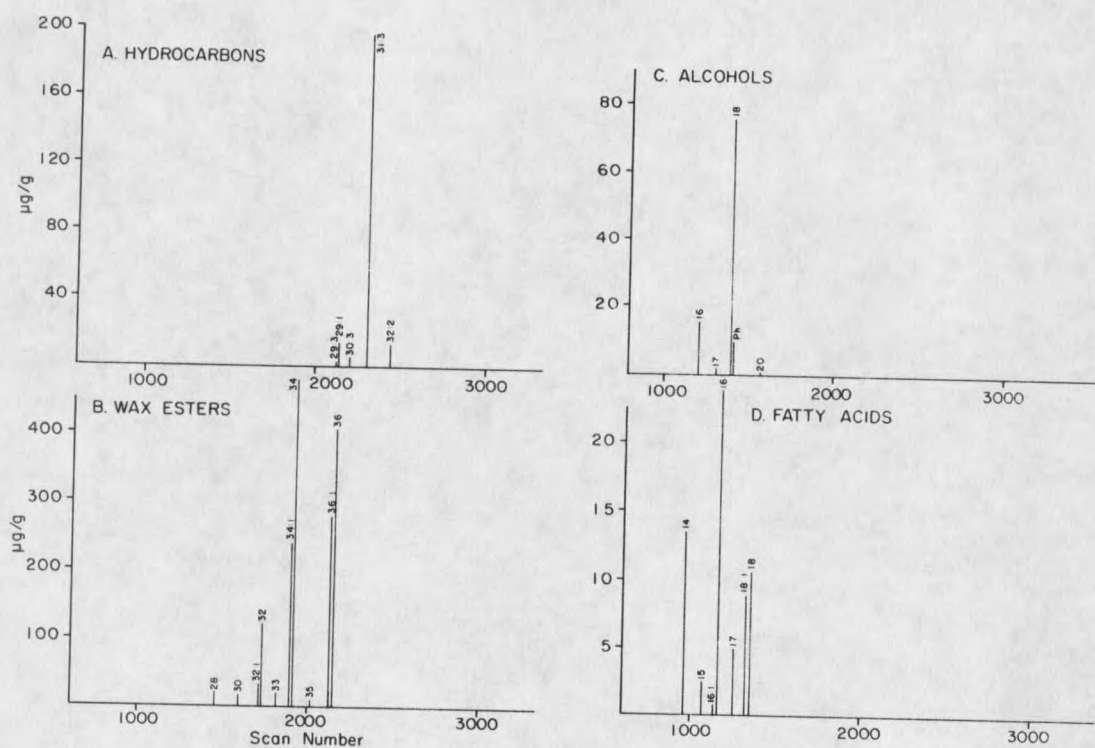


Figure 14. Major free lipids of the green nonsulfur bacterium Chloroflexus aurantiacus (see legend to Figure 8 for definition of symbols).

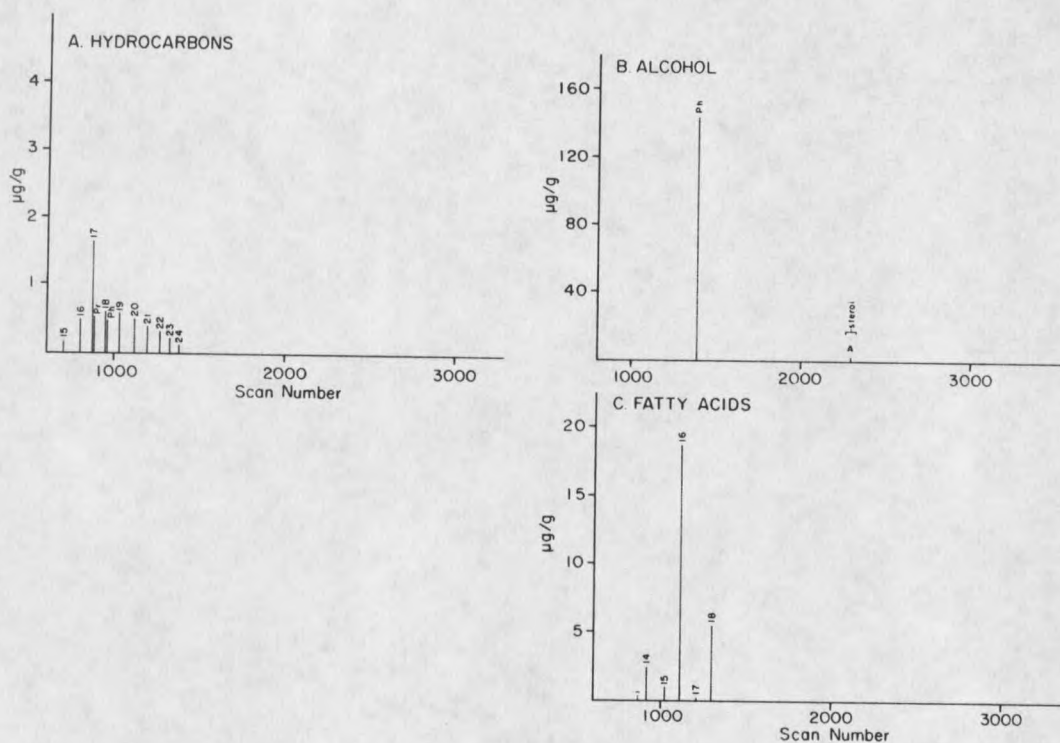


Figure 15. Major free lipids of the purple sulfur bacterium *Chromatium tepidum* (see legends to Figures 8 and 9 for definition of symbols).

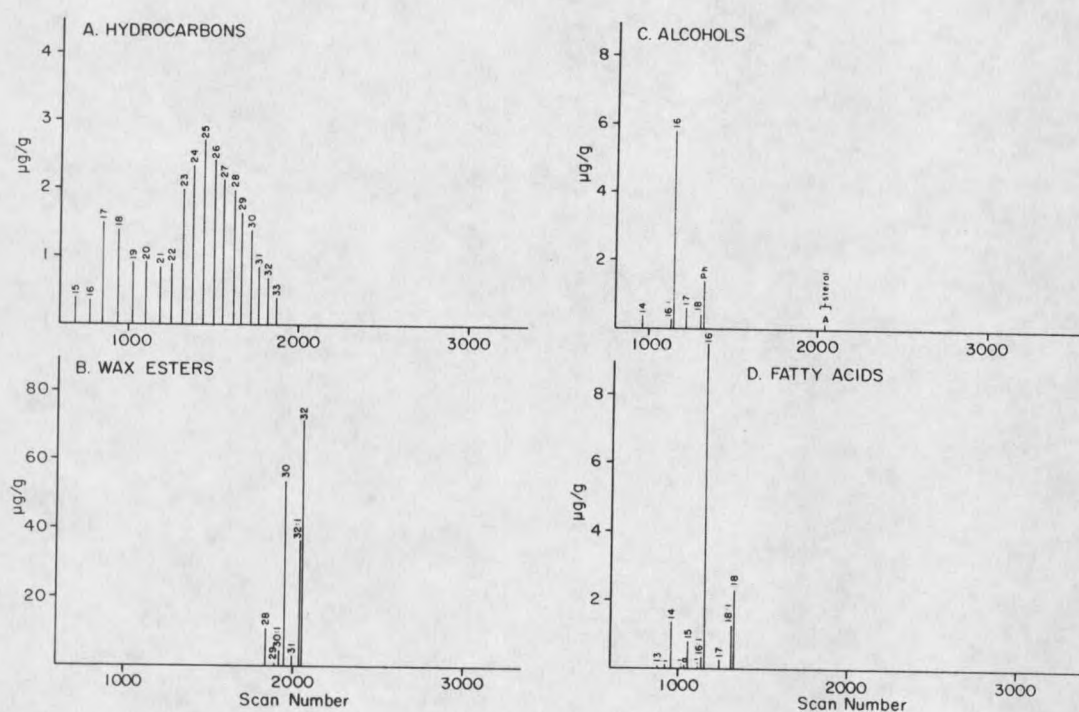


Figure 16. Major free lipids of the green sulfur bacterium *Chlorobium* sp. (see legends to Figures 8 and 9 for definition of symbols).

Representative structures of various free lipids found in this study are shown in Figure 17.

Hydrocarbons

The hydrocarbons found in the samples included normal alkanes, mono- and dimethylalkanes, alkenes, pristane, phytane, hopanes, and hopenes. The major components and the presence or absence of the above hydrocarbons in each sample are summarized in Table 6. The identity of each hydrocarbon found in samples was determined by its GC retention time and mass spectrum.

Saturated or unsaturated long chain acyclic hydrocarbons were the most dominant compounds in the F-1 fraction of all samples. Major hydrocarbons in the bacteria were also found among major components of the hydrocarbons in mats predominated by these bacteria (compare C. aurantiacus and "New Pit" Spring mat, C. tepidum and "Roland's Well" mat and Chlorobium sp. and "Travel Lodge" Stream mat in Table 6). Normal alkanes, often the predominant hydrocarbons, were easily recognized by a series of $(C_nH_{2n+1})^+$ fragments and weak molecular ions $(M)^+$. The most abundant fragments were at C_3 (m/z 43) or C_4 (m/z 57) and the least abundant fragment was at $(M-15)^+$. The chain length was determined by the molecular ion.

Mid-chain branched monomethylalkanes were only detected in mats containing cyanobacteria. The mass

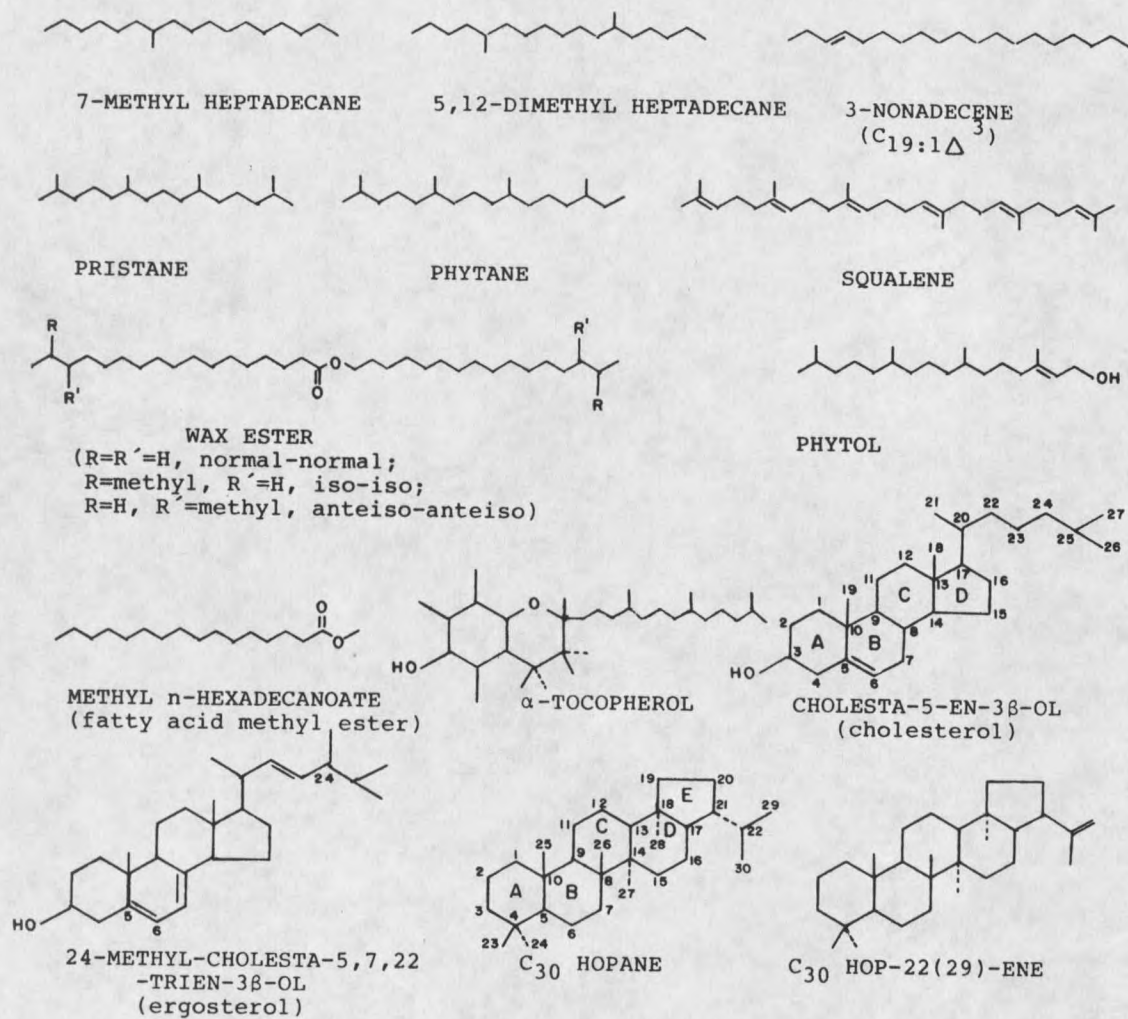


Figure 17. Structures of free lipids found in mats and bacteria.

Table 6. Major classes of hydrocarbons found in mats and bacteria.

Hydrocarbons	Mats ^a						Bacteria ^a		
	NC	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chr.</u> <u>tep.</u>	<u>Chl.</u> <u>sp.</u>
Major Compound	nC ₁₇	nC ₁₇	5,12-dimeC ₁₇	C _{31:3}	nC ₁₆ -nC ₂₂	nC ₁₇ -nC ₂₅	C _{31:3}	nC ₁₆ -nC ₂₃	nC ₁₇ -nC ₃₀
Mono-Methyl Alkanes	-	C ₁₆ -C ₁₉	C ₁₆ -C ₁₉	-	-	-	-	-	-
Dimethyl Alkanes	-	-	C ₁₉ -C ₂₀	-	-	-	-	-	-
Monoun-saturated alkenes	+	+	+	+	+	-	+	-	-
Diun-saturated alkenes	+	-	-	+	-	-	+	-	-
Triun-saturated alkenes	-	+	+	+	+	-	+	-	-
Pristane	-	-	-	-	+	+	-	-	+
Phytane	-	-	-	-	+	+	-	-	+
Hopenes (number)	-	(7)	(4)	(1)	(3)	-	-	-	-
Hopanes (number)	-	-	-	-	-	(13)	-	-	-

^a see Table 5 for definition of the symbols; number under C refer to the carbon number; number after colon refer to the number of double bonds; n=normal alkane; + and - refer to the presence or absence of the compounds; dime=dimethyl.

spectra of monomethyl alkanes were similar to those of normal alkanes, but the smooth curve of decreasing intensities was broken by preferred fragmentation at each branch. Thus, the intensity of the fragment $(C_nH_{2n})^+$ generated by cleavage and charge retention at the branched carbon, increased with the loss of the largest alkyl group favored. For example, the mass spectrum of 7-methyl heptadecane contained enhanced peaks at m/z 112 and 168 because of fragmentation around the site of the substituted methyl group. The molecular ion was usually of low intensity or absent in the mass spectrum. In the nonpolar GC column used (DB-5), the relative retention times of mid-chain branched monomethylalkanes were about 0.6 to 0.7 carbon units less than the normal alkane of the same carbon number (Jackson and Blomquist, 1976). The GC resolution of some structural isomers of monomethylalkanes is poor (especially for the isomers with the methyl group located at central positions). However, mass chromatography of key ions expected due to ionization around the methylation site was used to resolve the mixtures (Klomp, 1986; Fowler and Douglas, 1987) and provided evidence of the presence of suites of such compounds (see Figure 18 and Table 7) in the "Wiegert's Channel" and Orakei Korako cyanobacterial mats.

In the "Orakei Korako" mat, several C_{19} to C_{20} compounds were found with greater volatilities than those of monomethyl alkanes. On the basis of their rapid

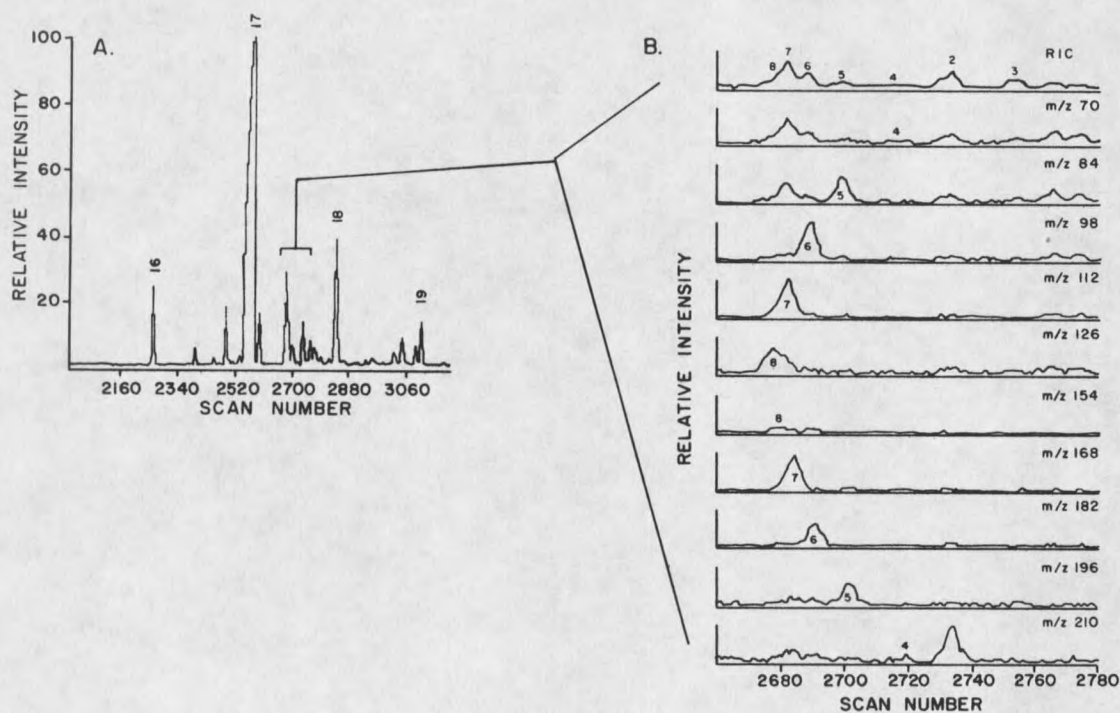


Figure 18. Partial total ion current of the "Wiegert's Channel" mat F-1 fraction (A) and fragmentograms (B) of ions diagnostic of methylation site, showing suites of mid-chain branched monomethylalkanes (numbers in (A) refer to the carbon number of a compound; numbers in peaks in (B) indicate site of methylation).

Table 7. Mid-chain branched monomethylalkanes and dimethylalkanes found in the "Wiegert's Channel" and Orakei Korako cyanobacterial mats.

Mid-Chain Branched Mono- and Dimethylalkanes	"Wiegert's Channel"	Orakei Korako
I. Monomethyl alkanes		
8-methylpentadecane	-	+
7-methylpentadecane	-	+
6-methylpentadecane	-	+
5-methylpentadecane	+	+
4-methylpentadecane	+	+
3-methylpentadecane	-	+
2-methylpentadecane	+	+
8-methylhexadecane	+	-
7-methylhexadecane	+	-
6-methylhexadecane	+	-
5-methylhexadecane	+	+
4-methylhexadecane	+	-
3-methylhexadecane	-	-
2-methylhexadecane	+	-
8-methylheptadecane	+	+
7-methylheptadecane	+	+
6-methylheptadecane	+	+
5-methylheptadecane	+	+
4-methylheptadecane	+	+
3-methylheptadecane	+	+
2-methylheptadecane	+	+
9-methyloctadecane	+	-
8-methyloctadecane	-	+
7-methyloctadecane	+	+
6-methyloctadecane	+	-
5-methyloctadecane	-	+
II. Dimethyl alkanes		
5,12-dimethylheptadecane	-	+
5,11-dimethylheptadecane	-	+
4,13-dimethylheptadecane	-	+
4,12-dimethylheptadecane	-	+
3,12-dimethylheptadecane	-	+
5,12-dimethyloctadecane	-	+
4,13-dimethyloctadecane	-	+
4,14-dimethyloctadecane	-	+

retention time and mass spectral information, they are tentatively interpreted to be dimethylalkanes (see Figure 19 and Table 7). Two of the most predominant hydrocarbons in the sample (5,12-dimethyl heptadecane and 4,13-dimethyl heptadecane; see Figure 17), were far more volatile than C_{18} monomethylalkanes and they in fact obscured the normal octadecane peak. C_{20} dimethylalkanes were even more volatile than normal nonadecane, running ca. 1.3 carbon units less than the corresponding normal C_{20} alkane. The relative retention times of dimethylalkanes from insects (the carbon number is usually bigger than C_{25}) were reported to be about 1.3 to 1.4 carbon units less than the normal alkane of the same carbon number in the nonpolar GC column used (Jackson and Blomquist, 1976). The mass spectra of two predominant dimethylalkanes are also shown in Figures 19B and 19C. Two pairs of fragments, presumably generated from the bond cleavage at each branch, were found in higher intensity compared to normal alkane spectra. For example, the mass spectrum of 5,12-dimethyl heptadecane contained enhanced peaks at m/z 85, 99, 197, and 211 presumably due to the fragmentation shown in Figure 19B.

A series of unusual long chain diunsaturated and triunsaturated alkenes (C_{29} to C_{33}) were found as the predominant hydrocarbon in C. aurantiacus and the mat from "New Pit" Spring. Alkenes were recognized by increases in the abundance of the $(C_nH_{2n-1})^+$ and $(C_nH_{2n})^+$ ion series

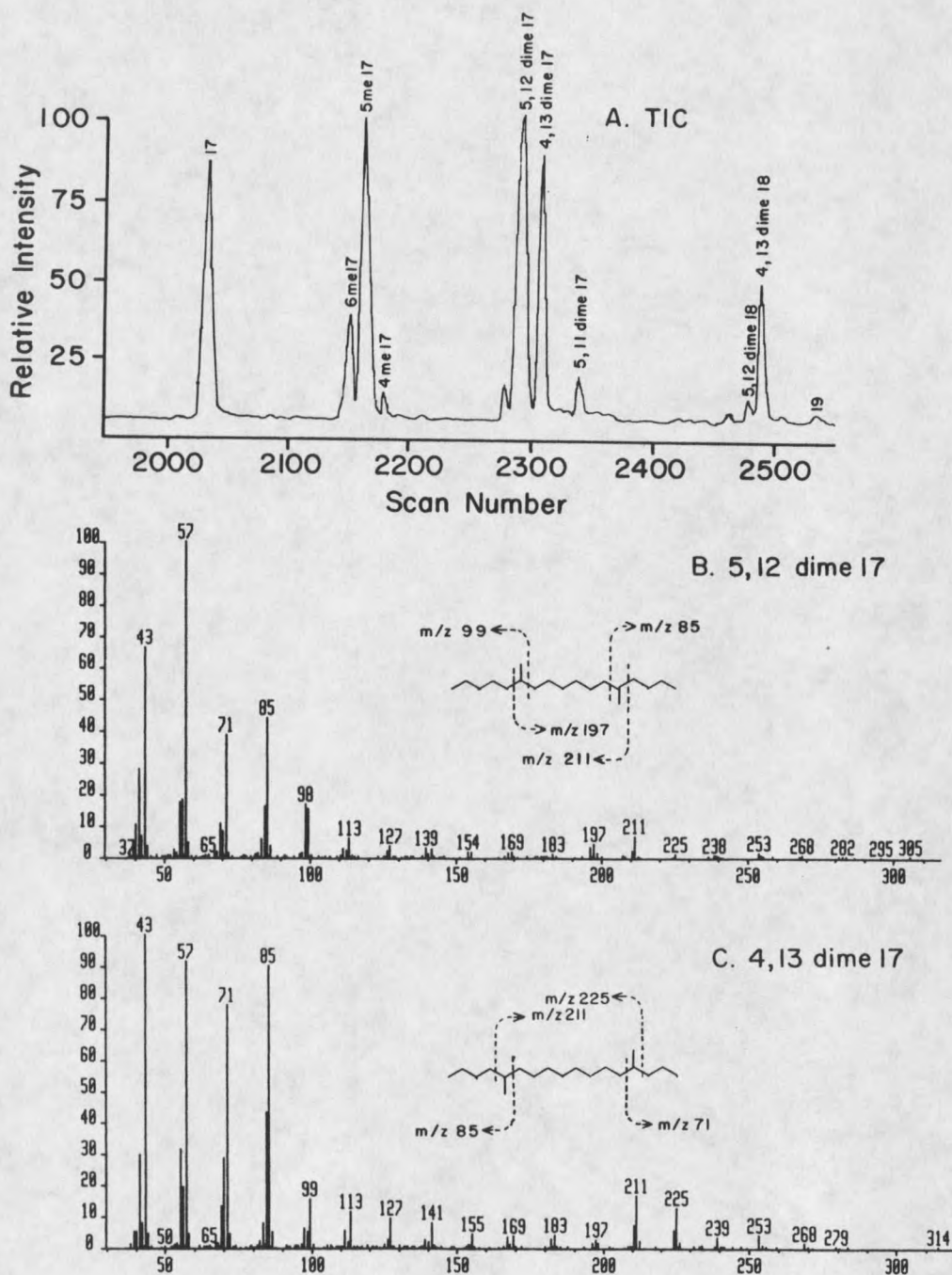


Figure 19. Partial total ion current of the Orakei Korako mat F-1 fraction (A) and mass spectra of two predominant dimethylalkanes (B and C).

compared to the $(C_nH_{2n+1})^+$ ion series found in normal alkanes. After hydrogenation, the straight chain diunsaturated and triunsaturated alkenes (e.g. hentriacontadiene and hentriacontatriene) became saturated and only normal alkanes (e.g. n-hentriacontane) of comparable carbon number were detected (Figure 20). The presence of the normal alkanes was proved by coinjection with n-hentriacontane. Accurate mass measurement confirmed that these compounds contained only carbon and hydrogen, and excluded the possibility that they contained heteroatoms (e.g. nitrogen and oxygen). The dominant alkene in both samples was hentriacontatriene ($C_{31:3}$, mol. weight 430, with two characteristic mass fragments m/z 194 and 208 in the mass spectrum which are of unknown origin). The concentration of this compound was quite high (125 ug/g in the "New Pit" Spring mat and 200 ug/g in C. aurantiacus). Mats from "Wiegert's Channel", Orakei Korako, and "Roland's Well" also contained traces of hentriacontatriene, though it was not shown in Figures 9A, 10A, and 12A. Traces of short-chain C_{17} to C_{21} monounsaturated alkenes were also detected in most of the mats. Due to very low concentrations in some of the samples, these alkenes were not shown in some of the figures.

Location of double bonds in acyclic alkenes from their mass spectra is impossible because of the facile migration of double bonds in the fragments produced in mass

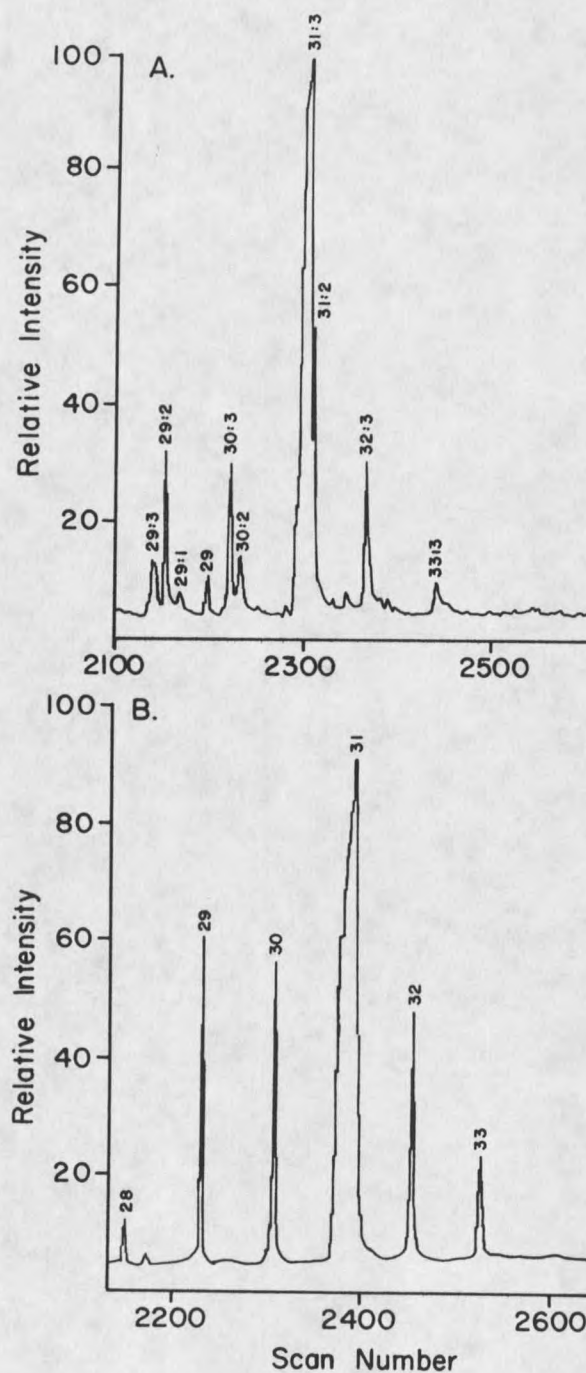
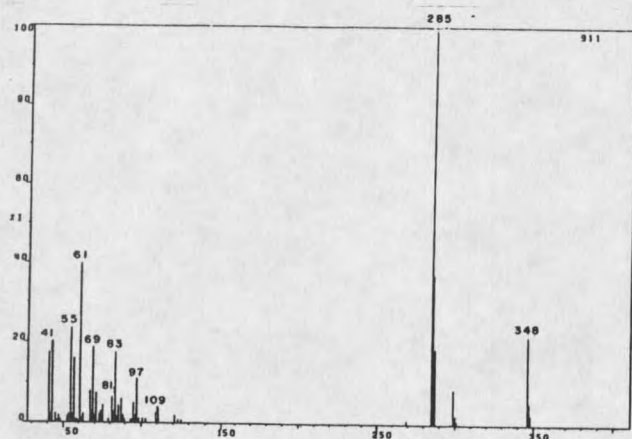


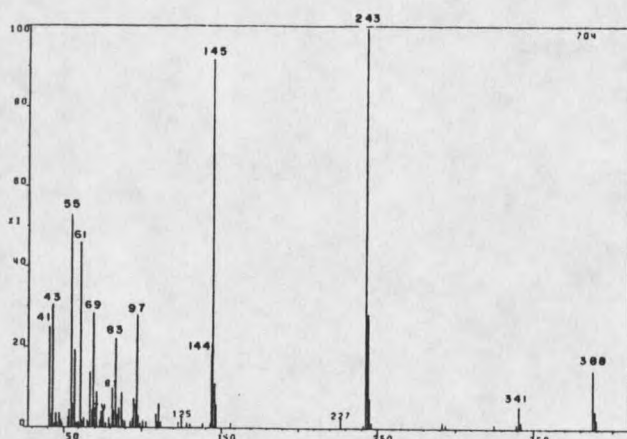
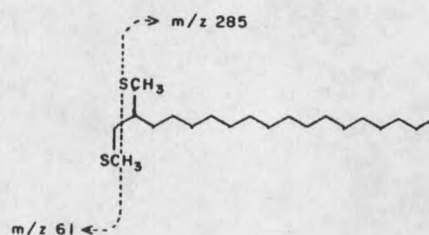
Figure 20. Partial total ion current of the "New Pit" Spring mat F-1 fraction before (A) and after (B) hydrogenation.

spectrometry. Therefore, derivatization of alkenes, using reagents (e.g. DMDS) which hold the double bond in its original position was needed before injecting the samples into the GC-MS. After DMDS derivatization, monounsaturated acyclic alkenes showed a particularly high intensity fragment as $(C_nH_{2n}(SCH_3))^+$ (usually it was the base peak) with the largest alkyl group as the dominant peak in the mass spectrum. The other alkyl group with shorter carbon number and a methylsulfide group on it was also seen in lower intensity. Examples of the mass spectra of two standards of DMDS-derivatized monounsaturated alkenes are shown in Figures 21A and 21B together with diagrams of the expected fragmentation. It was possible to determine that the double bonds in short chain monounsaturated alkenes (C_{19} to C_{21}) of the "New Pit" Spring mat were at carbon 3. The mass spectrum of the derivatized C_{20} monoene is shown in Figure 21C together with explanation of the sources of major fragments. Attempts to determine location of double bonds in di- and triunsaturated alkenes by DMDS derivatization failed. Since dienes and trienes will accept four and six methylsulfide groups, respectively, in the derivatizing process, and thus increase the polarity and boiling point, they become impossible to elute through the nonpolar GC column.

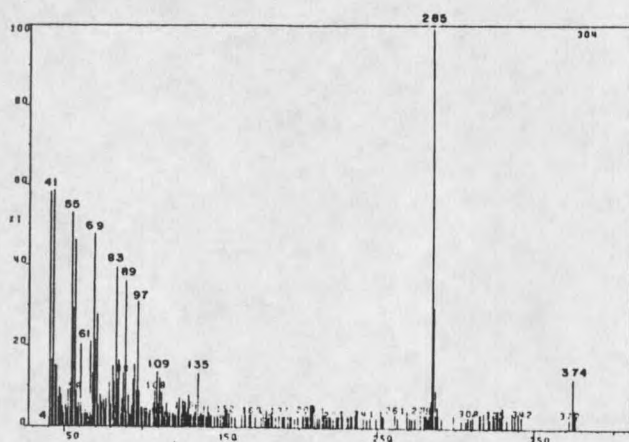
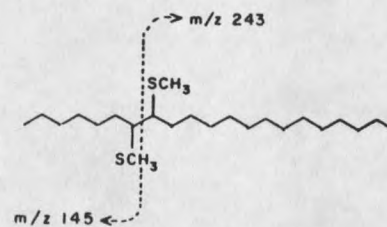
Two acyclic isoprenoids, pristane and phytane, were detected in C. tepidum and in the mats from "Roland's Well"



C18:1 Δ¹ (Std.)



C21:1 Δ⁷ (Std.)



C20:1 Δ³

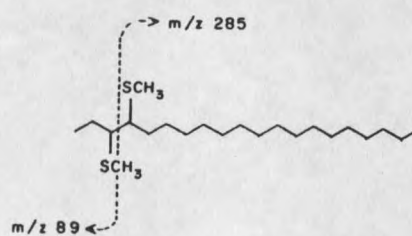


Figure 21. Mass spectra of dimethyl disulfide-derivatized monounsaturated alkenes from standards (A and B) and the C_{20:1} monounsaturated alkene from the "New Pit" Spring mat (C).

and "Travel Lodge" Stream. They were recognized by their retention time in the nonpolar GC column (pristane elutes soon after heptadecane and phytane elutes soon after octadecane), their molecular ions and characteristic fragments (e.g. m/z 183) derived from fragmentation at the branched position with the largest alkyl group.

Hopanoid hydrocarbon distributions are detailed in Table 8. Hopanes were only found in the "Travel Lodge" Stream mat. Hopenes were found in the Nymph Creek mat, both cyanobacterial mats, and two of the photosynthetic bacterial mats ("New Pit" Spring and "Roland's Well"). The identities of hopanes and hopenes were determined by their retention time on the GC column and by mass spectral features. In general, two key fragments were found in the mass spectra. The first, rings A+B (see Figure 17), is derived from bond cleavage between carbons 9 and 11 and 8 and 14, and shows m/z 191 for a saturated fragment (hopane) or m/z 189 for a monounsaturated fragment in rings A or B (hopene). The second, rings D+E, is derived from bond cleavage between carbons 8 and 14 and 12 and 13. The m/z value of this part depends on the alkyl group attached at carbon 21 (e.g. m/z 149, 163, or 177 for C_{27} , C_{28} , and C_{29} hopanes, respectively). The molecular ion, $(M-15)^+$ due to loss of methyl group, and $(M-29)^+$ due to loss of ethyl group were also usually seen. Minor differences in retention time and mass spectra were used to discern

Table 8. Distribution of hopanes, hopenes, and hopanols in mats.

Hopanoids	Mats ^a					
	NC	WC	OK	NP	RW	TL
I. Hopanes						
C ₂₇ hopane(17 α)	-	-	-	-	-	+
C ₂₉ hopane(α , β)	-	-	-	-	-	+
C ₃₁ 22S and 22R homohopanes(α , β)	-	-	-	-	-	+
C ₃₂ 22S and 22R bishomohopanes(α , β)	-	-	-	-	-	+
C ₃₃ 22S and 22R trishomohopanes(α , β)	-	-	-	-	-	+
C ₃₄ 22S and 22R tetrakishomohopanes(α , β)	-	-	-	-	-	+
C ₃₅ 22S and 22R pentakishomohopanes(α , β)	-	-	-	-	-	+
II. Hopenes						
C ₃₀ hop-17(21)-ene	-	+	-	-	+	-
C ₃₀ neohop-13(18)-ene	-	+	-	-	+	-
C ₃₀ hop-22(29)-ene	+	+	+	+	+	-
C ₃₀ neohop-12-ene	-	+	-	-	-	-
C ₃₀ unknown hopenes or triterpenes (kinds)	-	(4)	(3)	-	-	-
III. Hopanols and hopenols						
C ₃₀ hop-12-ol	-	-	-	-	+	-
C ₃₀ neohop-12-ol	-	-	-	-	+	-
C ₃₀ hop-22(29)-enol	-	-	-	-	-	+

^a see Table 5 for the definition of the symbols.

stereoisomers. Three common hopane epimers (17α , 21β (H)- , 17β , 21α (H)-, and 17β , 21β (H)-) have been found in nature and all of them show a different ratio of the intensity of two major fragments described above. The retention times of these epimers are also different ($\beta\beta < \beta\alpha < \alpha\beta$). Two kinds of steric isomers, $22(R)$ - and $22(S)$ -hopanes, also possess different retention times on a nonpolar GC column ($22(S) < 22(R)$). The difference in location of double bond in hopenes causes different types of mass fragments and also different retention times (e.g. $\Delta^{17(21)} < \Delta^{13(18)} < \Delta^{12} < \Delta^{22(29)}$).

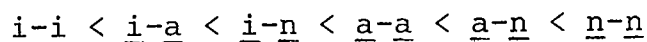
A hump, referred to as an unresolved complex mixture (UCM), was found only in the F-1 fraction of the "Travel Lodge" Stream mat (see insert in Figure 13A).

Wax Esters

Wax esters were found in seven out of the nine samples and included normal, iso-branched, anteiso-branched and monounsaturated isomers. Only the mat from the Nymph Creek and the purple bacterium C. tepidum did not contain wax esters.

The mass spectra of wax esters were characterized by the component fatty acids and alcohols. The most characteristic fragment was due to McLafferty rearrangement and cleavage one bond removed from the carbonyl group (Ryhage and Stenhagen, 1960). For example, the mass

spectrum of the $C_{16}H_{33}COOC_{16}H_{33}$ wax ester showed a characteristic fragment $((C_{16}H_{33}COOH_2)^+)$ with m/z 271 from the C_{17} fatty acid moiety of the compound. The structural isomers of wax esters were separated by GC with a nonpolar column and were recognized by their relative retention times:



(where i represents iso-branched, a represents anteiso-branched, and n represents normal alcohol or fatty acid moieties).

The GC peak for each structural isomer of a given total carbon number, contained compounds with a different pairing of fatty acid and alcohol moieties as detailed in Table 9. For example, in C. aurantiacus, the major wax ester peak (C_{34} with normal acid and alcohol pairing) (see Figure 14B) was actually comprised of a mixture of structural isomers with different normal fatty acid and alcohol pairs. The relative abundance of each isomer in a mixed GC peak was derived by comparing the relative abundances of the fragments diagnostic of the various fatty acid moieties. Thus, the $n,n-C_{34}$ wax ester in C. aurantiacus (bold highlighted in Table 9, see page 78) was a mixture containing 26% $C_{17}H_{33}COOC_{16}H_{33}$, 9% $C_{16}H_{29}COOC_{17}H_{37}$, and 62% $C_{15}H_{37}COOC_{18}H_{29}$, based on the relative abundances of the

Table 9. The relative abundance of wax esters in mats and bacteria.

Carbon Number	Type ^c	Acid-Alcohol ^c	Percent of total in a peak ^a							
			Mats ^b						Bacteria ^b	
			OS	WC	OK	NP	RW	TL	C. aur.	Chl. sp.
24	n-n	14-10	-	6	-	-	-	-	-	-
		13-11	-	9	-	-	-	-	-	-
		12-12	-	30	-	-	-	-	-	-
		11-13	-	33	-	-	-	-	-	-
		10-14	-	22	-	-	-	-	-	-
25	n-n	14-11	-	10	-	-	-	-	-	-
		13-12	-	25	-	-	-	-	-	-
		12-13	-	18	-	-	-	-	-	-
		11-14	-	26	-	-	-	-	-	-
		10-15	-	6	-	-	-	-	-	-
		9-16	-	9	-	-	-	-	-	-
		8-17	-	7	-	-	-	-	-	-
26	n-n	15-11	-	1	-	-	-	-	-	-
		14-12	-	23	-	-	-	-	-	-
		13-13	-	33	-	-	-	-	-	-
		12-14	-	39	-	-	-	-	-	-
		11-15	-	4	-	-	-	-	-	-
27	n-n	16-11	-	4	-	-	-	-	-	-
		15-12	-	13	-	-	-	-	-	-
		14-13	-	32	-	-	-	-	-	-
		13-14	-	18	-	-	-	-	-	-
		12-15	-	12	-	14	-	-	-	-
		11-16	-	10	-	46	-	-	-	-
		10-17	-	11	-	15	-	-	-	-
		9-18	-	-	-	24	-	-	-	-
	i-n	13-14	-	-	-	26	-	-	-	-
		11-16	-	-	-	11	-	-	-	-
		10-17	-	-	-	47	-	-	-	-
		9-18	-	-	-	16	-	-	-	-
28	n-n	18-10	-	-	-	3	-	-	1	-
		17-11	-	-	-	3	-	-	-	-
		16-12	-	6	-	6	-	14	82	6
		15-13	-	14	-	3	-	1	-	-
		14-14	-	40	-	3	2	74	6	77
		13-15	-	14	1	19	12	15	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chl.</u> <u>sp.</u>
28	n-n	12-16	-	13	85	13	38	2	10	17
		11-17	-	13	14	30	44	-	-	-
		10-16	-	2	-	13	4	-	2	-
		9-17	-	-	-	6	-	-	-	-
		8-18	-	-	-	1	-	-	-	-
	i-n	16-12	-	-	-	4	-	-	-	-
		15-13	-	7	-	2	-	-	-	-
		14-14	-	-	-	8	-	-	-	-
		13-15	-	25	-	43	-	-	-	-
		12-16	-	14	-	17	24	-	-	-
		11-17	-	45	-	19	75	-	-	-
		10-18	-	10	-	8	1	-	-	-
	n-n	15:1-13	-	-	-	-	-	-	-	6
		14:1-14	-	-	-	-	-	-	-	4
		13:1-15	-	-	-	-	-	-	-	30
		12:1-16	-	-	-	-	-	-	-	7
		11:1-17	-	-	-	-	-	-	-	29
		10:1-18	-	-	-	-	-	-	-	16
		9:1-19	-	-	-	-	-	-	-	9
29	n-n	17-12	-	3	-	1	-	-	-	-
		16-13	-	10	-	1	1	35	46	1
		15-14	-	23	14	6	5	31	3	27
		14-15	12	23	25	15	12	29	7	59
		13-16	53	25	38	34	33	5	-	10
		12-17	35	13	18	18	41	-	25	1
		11-18	-	3	5	18	7	-	11	-
		10-19	-	-	-	2	-	-	7	-
		9-20	-	-	-	2	-	-	-	-
		8-21	-	-	-	3	-	-	-	-
		7-22	-	-	-	1	-	-	-	-
	a-n	13-16	-	-	57	8	-	-	-	-
		12-17	-	-	43	2	-	-	-	-
		11-18	-	-	-	90	-	-	-	-
	i-n	16-13	-	-	-	1	-	-	-	-
		15-14	-	-	-	18	-	-	-	-
		14-15	-	-	-	23	-	-	-	-
		13-16	-	-	-	9	-	-	-	-
		12-17	-	-	34	34	-	-	-	-
		11-18	-	-	56	15	-	-	-	-
		10-19	-	-	10	-	-	-	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	C. aur.	Chl. sp.
30	n-n	20-10	-	-	-	-	-	1	-	-
		19-11	-	-	-	-	-	1	-	-
		18-12	-	-	-	-	-	4	35	-
		17-13	-	2	-	-	-	1	-	-
		16-14	-	18	15	1	-	20	11	-
		15-15	23	28	5	27	32	5	-	-
		14-16	26	21	66	17	25	66	51	88
		13-17	48	30	15	47	40	1	3	-
		12-18	3	1	-	9	2	1	-	12
		11-19	-	-	-	-	-	2	-	-
	a-n	17-13	-	-	2	-	-	-	-	-
		16-14	-	-	23	-	-	-	-	-
		15-15	-	-	28	-	-	-	-	-
		14-16	-	-	9	-	-	-	-	-
		13-17	-	-	38	-	-	-	-	-
	i-n	18-12	-	2	-	-	-	-	-	-
		17-13	-	4	8	-	-	-	-	-
		16-14	-	5	-	-	1	-	-	-
		15-15	27	29	55	23	33	-	-	-
		14-16	27	10	-	24	14	-	-	-
		13-17	47	32	37	52	51	-	-	-
		12-18	-	8	-	-	2	-	-	-
		11-19	-	10	-	-	-	-	-	-
	n-n	17:1-13	-	-	-	-	-	-	69	-
		16:1-14	-	-	-	-	-	-	-	-
		15:1-15	-	-	-	-	-	44	8	25
		14:1-16	-	-	-	-	-	21	15	24
		13:1-17	-	-	-	-	-	16	4	37
		12:1-18	-	-	-	-	-	12	4	10
		11:1-19	-	-	-	-	-	6	-	4
31	n-n	19-12	-	1	-	1	-	-	-	-
		18-13	-	2	-	2	-	2	1	1
		17-14	-	3	-	3	-	5	1	1
		16-15	7	11	12	7	7	27	13	23
		15-16	47	44	83	39	57	55	36	69
		14-17	43	26	4	39	34	10	24	6
		13-18	3	6	1	9	2	-	20	-
		12-19	-	1	-	-	-	-	5	-
		11-20	-	1	-	-	-	-	-	-
	a-n	17-14	-	-	-	1	-	-	-	-
		16-15	-	-	-	2	-	-	-	-
		15-16	-	-	-	57	-	-	-	-
		14-17	-	-	-	24	-	-	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chl.</u> <u>sp.</u>
31	a-n	13-18	-	-	-	12	-	-	-	-
		12-19	-	-	-	4	-	-	-	-
	i-n	17-14	-	8	-	-	-	-	-	-
		16-15	9	4	-	6	11	-	-	-
		15-16	42	66	-	59	60	-	-	-
		14-17	45	4	-	32	30	-	-	-
		13-18	3	17	-	3	-	-	-	-
32	n-n	19-13	-	2	-	-	-	-	-	-
		18-14	-	-	-	-	-	3	2	2
		17-15	3	6	2	15	1	-	-	-
		16-16	14	17	4	40	24	88	94	98
		15-17	79	72	81	27	73	1	-	-
		14-18	4	2	8	15	2	6	4	-
		13-19	-	-	5	1	-	2	-	-
		12-20	-	-	-	1	-	-	-	-
	a-n	19-13	-	3	-	-	-	-	-	-
		18-14	-	2	-	-	-	-	-	-
		17-15	-	6	-	-	-	-	-	-
		16-16	-	18	72	-	-	-	-	-
		15-17	-	68	10	-	-	-	-	-
		14-18	-	1	18	-	-	-	-	-
	i-n	19-13	-	2	-	-	-	-	-	-
		18-14	-	-	-	-	-	-	-	-
		17-15	3	5	16	4	1	-	-	-
		16-16	22	6	19	10	18	-	-	-
		15-17	70	82	53	81	79	-	-	-
		14-18	5	3	11	5	1	-	-	-
	i-i	17-15	-	-	22	-	-	-	-	-
		16-16	15	-	-	-	-	-	-	-
		15-17	76	-	78	-	100	-	-	-
		14-18	9	-	-	-	-	-	-	-
	n-n	17:1-15	-	-	-	-	-	-	5	-
		16:1-16	-	-	-	-	-	1	7	-
		15:1-17	-	-	-	-	-	55	53	63
		14:1-18	-	-	-	-	-	18	9	14
		13:1-19	-	-	-	-	-	9	3	2
		12:1-20	-	-	-	-	-	11	18	18
		11:1-21	-	-	-	-	-	4	6	3
		10:1-22	-	-	-	-	-	3	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chl.</u> <u>sp.</u>
33	n-n	19-14	-	2	-	1	-	-	-	-
		18-15	-	12	4	3	-	-	4	-
		17-16	20	19	42	25	17	-	41	19
		16-17	61	65	40	48	69	46	37	81
		15-18	20	12	14	22	14	20	14	-
		14-19	-	-	-	2	-	17	4	-
		13-20	-	-	-	-	-	16	-	-
	a-n	20-13	-	1	-	-	-	-	-	-
		19-14	-	2	-	-	-	-	-	-
		18-15	-	1	-	-	-	-	-	-
		17-16	-	26	-	-	-	-	-	-
		16-17	-	56	-	100	-	-	-	-
		15-18	-	10	-	-	-	-	-	-
		14-19	-	2	-	-	-	-	-	-
		13-20	-	2	-	-	-	-	-	-
	i-n	18-15	-	1	3	1	-	-	-	-
		17-16	13	15	49	4	15	-	-	-
		16-17	70	55	23	34	80	-	-	-
		15-18	18	26	25	56	7	-	-	-
		14-19	-	15	-	4	-	-	-	-
34	n-n	20-14	-	-	-	-	-	-	1	1
		19-15	1	4	1	3	1	-	-	-
		18-16	2	5	45	6	2	-	26	17
		17-17	81	75	26	77	82	-	9	6
		16-18	13	10	27	9	11	-	62	57
		15-19	3	5	-	4	4	-	-	-
		14-20	-	-	-	1	-	-	1	14
		13-21	-	-	-	-	-	-	-	1
	a-n	21-13	-	1	-	-	-	-	-	-
		20-14	-	1	-	-	-	-	-	-
		19-15	-	2	-	-	-	-	-	-
		18-16	-	2	-	-	-	-	-	-
		17-17	-	75	-	-	-	-	-	-
		16-18	-	16	-	-	-	-	-	-
		15-19	-	2	-	-	-	-	-	-
		14-20	-	1	-	-	-	-	-	-
		13-21	-	1	-	-	-	-	-	-
	i-n	21-13	-	1	-	-	-	-	-	-
		20-14	-	-	-	-	-	-	-	-
		19-15	-	1	-	-	-	-	-	-
		18-16	3	1	-	2	-	-	-	-
		17-17	69	80	81	75	83	-	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chl.</u> <u>sp.</u>
34	i-n	16-18	26	12	14	16	17	-	-	-
		15-19	2	2	5	7	1	-	-	-
		14-20	-	2	-	-	-	-	-	-
		13-21	-	1	-	-	-	-	-	-
	n-n	17:1-17	-	-	-	-	-	-	25	-
		16:1-18	-	-	-	-	-	-	10	-
		15:1-19	-	-	-	-	-	-	32	-
		14:1-20	-	-	-	-	-	-	11	-
		13:1-21	-	-	-	-	-	-	3	-
		12:1-22	-	-	-	-	-	-	7	-
		11:1-23	-	-	-	-	-	-	8	-
		10:1-24	-	-	-	-	-	-	4	-
35	n-n	21-14	-	4	-	-	-	-	-	-
		20-15	1	2	-	-	-	-	-	-
		19-16	1	3	-	5	9	-	-	-
		18-17	27	28	55	29	29	-	30	-
		17-18	56	52	11	44	47	-	70	-
		16-19	9	7	-	20	15	-	-	-
		15-20	6	5	35	-	-	-	-	-
		14-21	-	1	-	-	-	-	-	-
	a-n	22-13	-	2	-	-	-	-	-	-
		21-14	-	8	-	-	-	-	-	-
		20-15	-	2	-	-	-	-	-	-
		19-16	2	8	-	-	-	-	-	-
		18-17	9	26	-	-	-	-	-	-
		17-18	72	19	-	-	-	-	-	-
		16-19	13	28	-	-	-	-	-	-
		15-20	4	8	-	-	-	-	-	-
	i-n	22-13	-	3	-	-	-	-	-	-
		21-14	-	3	-	-	-	-	-	-
		20-15	1	3	-	-	-	-	-	-
		19-16	2	11	-	-	-	-	-	-
		18-17	32	16	-	-	-	-	-	-
		17-18	54	24	-	-	-	-	-	-
		16-19	6	14	-	-	-	-	-	-
		15-20	5	18	-	-	-	-	-	-
36	n-n	14-21	-	7	-	-	-	-	-	-
		20-16	2	-	-	-	-	-	1	-
		19-17	13	21	-	-	-	-	-	-
		18-18	25	38	-	-	-	-	95	-
		17-19	46	24	-	-	-	-	-	-
		16-20	11	13	-	-	-	-	4	-
		15-21	3	4	-	-	-	-	-	-

Table 9. Continued

Carbon Number	Type	Acid-Alcohol	OS	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chl.</u> <u>sp.</u>
36	n-n	17:1-19	-	-	-	-	-	-	43	-
		16:1-20	-	-	-	-	-	-	18	-
		15:1-21	-	-	-	-	-	-	-	-
		14:1-22	-	-	-	-	-	-	8	-
		13:1-23	-	-	-	-	-	-	16	-
		12:1-24	-	-	-	-	-	-	-	-
		11:1-25	-	-	-	-	-	-	4	-
		10:1-26	-	-	-	-	-	-	2	-
		9:1-27	-	-	-	-	-	-	9	-

^a the percentage of each structural isomer in a GC peak.

^b see Table 5 for the definition of the symbols; OS=Octopus Spring (data from Dobson, et al, in press).

^c n=normal alkyl group; i=iso alkyl group; a=anteiso alkyl group; number represents the chain length of fatty acid and alcohol moieties; the number after a colon represent the number of double bonds.

characteristic fragments--m/z 285 (C₁₈ acid), 271 (C₁₇ acid), and 257 (C₁₆ acid), respectively. Two other isomers represented ca. 1% of the n,n-C₃₄ wax ester peak. In order to have better comparison of the molecular composition of the wax esters in hot spring mats, the data from the Octopus Spring cyanobacterial mat (from Dobson, et al, in press) are also included in Table 9.

Most of the mats contained wax esters ranging from C₂₈ to C₃₆ with unbranched compounds dominating (Figures 9B to 13B). This was similar to the wax esters found in C. aurantiacus (Figure 14B), except that this bacterium produced normal and monounsaturated wax esters, but no branched isomers. A second series of wax esters ranging from C₂₄ to C₂₇ was detected in the "Wiegert's Channel" cyanobacterial mat (Figure 9B). The wax ester distribution in the "Travel Lodge" Stream mat (Figure 13B) and the green sulfur bacterium Chlorobium sp. (Figure 16B) were nearly identical (C₂₈ to C₃₃) with both monounsaturated and saturated wax esters in the mat and the isolate, but were distinctly different from those found in other samples.

Alcohols

The alcohols found in samples included acyclic normal, branched, and unsaturated alcohols, phytol, sterols, tocopherols, monoglycerides, and hopanols. The distribution of these alcohols is summarized in Table 10.

Table 10. Major classes of alcohols found in mats and bacteria.

Alcohols	Mats ^a						Bacteria ^a		
	NC	WC	OK	NP	RW	TL	<u>C.</u> <u>aur.</u>	<u>Chr.</u> <u>tep.</u>	<u>Chl.</u> <u>sp.</u>
Major Compounds	Ph ^b Er ^c	Ph Er	Ph nC ₁₇	nC ₁₈	nC ₁₇	nC ₁₈	nC ₁₈	Ph	nC ₁₆
Phytol	+	+	+	+	+	+	+	+	+
α,β Tocopherols	+	+	-	-	-	-	-	-	-
Monoglycerides	+	+	-	-	-	-	-	-	-
Sterols (kinds)	(14)	(5)	(5)	(6)	(5)	(2)	-	(1)	(1)
Hopanols (kinds)	-	-	-	-	(2)	(1)	-	-	-

^a see Table 5 for the definition of the symbols.^b phytol.^c ergosterol.

Normal alcohols (e.g. n -C₁₆, C₁₇, or C₁₈) were found in all samples except the purple bacterium C. tepidum and were the major free alcohols of the other two photosynthetic bacteria and all three photosynthetic bacterial mats. The mass spectrum of TMS-derivatized normal alcohols showed (M-15)⁺ as base peak and a characteristic fragment m/z 75 from the protonated trimethylsilate. The molecular ion and the other fragments showed low intensity. Branched or monounsaturated alcohols, minor compounds in some samples, were recognized by their shorter retention time. Also, for these alcohols, the fragment (M-15)⁺ was less dominant and m/z 75 became the base peak.

Phytol, characterized by m/z 143 (from the bond cleavage between the γ and the δ carbons), was found in all of the samples and was predominant in the algal and cyanobacterial mats and in the purple photosynthetic bacterium C. tepidum.

Tocopherols (see Figure 17) were detected in the mats from the Nymph Creek and "Wiegert's Channel" (only trace amounts were found in this mat and they were not shown in Figure 9C). The mass spectrum of derivatized tocopherol showed M⁺ as the base peak and a fragment (M-265)⁺ which is from loss of the pristyl group from the molecular ion.

Monoglycerides were also present in the alcohol fraction of these two mats (again, not shown in Figure 9C). The mass spectra of the derivatized monoglycerides were

characterized by the fragment $(M-103)^+$ which is from loss of $CH_3O(TMS)$ from the molecular ion. A fragment from α cleavage of the bond next to the α -carbon was also seen and was used in judging the chain length and unsaturation of the esterified alkyl group.

Sterols were detected in all of the samples except the bacterium C. aurantiacus. The identities of TMS-derivatized sterols were determined from relative retention time and mass spectra. Major fragments were derived through bond cleavage between carbons 13 and 17, 14 and 15 (in ring D) and 17 and 20. The molecular ion and the fragments $(M-15)^+$, $(M-90)^+$, and $(M-105)^+$, derived from loss of a methyl group, loss of HO-TMS group, and loss of both groups from the molecular ion were also seen; the intensity of these fragments changed dependent upon the location of the double bond in the ring. The distribution and abundances of sterols are shown in Table 11. Sterols were greater in number and concentration in the Nymph Creek mat than in any other samples (see also Figure 8C).

Traces of hopanols were detected only in the mats from "Roland's Well" and "Travel Lodge" Stream (see Table 10). The mass spectrum of the TMS-derivatized hopanols was similar to that of hopanes except that the fragments $(M-15)^+$, $(M-90)^+$, and $(M-105)^+$ were seen, as described above for sterols.

Table 11. Distribution and concentration range of sterols in mats and bacteria.

Peak ^a	Sterols	Abundance ^b						
		Mats ^c						Bacteria ^c
		NC	WC	OK	NP	RW	TL	Chr.Chl. tep.sp.
I. C ₂₇ stenol								
A	cholest-5-en-3 β -ol	-	+	+	+	tr	+	+
II. C ₂₈ stenols								
C	24-methyl-cholesta-5,7,9(11),22-tetraen-3 β -ol	++	-	-	-	-	-	-
D	24-methyl-cholesta-5,7,22-trien-3 β -ol	+++	-	-	-	-	-	-
E	24-methyl-cholesta-7,22-dien-3 β -ol	++	-	-	-	-	-	-
F	24-methyl-cholesta-5,7-dien-3 β -ol	+	-	-	-	-	-	-
	24-methyl-cholesta-5,22-dien-3 β -ol	-	+	-	-	-	-	-
G	24-methyl-cholest-7-en-3 β -ol	+	-	-	-	-	-	-
	24-methyl-cholest-5-en-3 β -ol	-	+	+	-	tr	-	-
III. C ₂₉ stenols								
	4 α ,24-dimethyl-cholesta-5,22-dien-3 β -ol	tr	-	-	-	-	-	-
	24-ethyl-cholesta-5,7,22-trien-3 β -ol	tr	-	-	-	-	-	-
	24-ethyl-cholesta-5,7-dien-3 β -ol	-	-	-	-	tr	-	-
H	24-ethyl-cholesta-5,22-dien-3 β -ol	+	+	+	-	-	-	-
J	24-ethyl-cholest-7-en-3 β -ol	+	-	-	-	-	-	-
B	24-ethyl-cholest-5-en-3 β -ol	-	+	+	-	+	-	-
I	4 α ,24-dimethyl-cholest-8(14)-en-3 β -ol	+	-	-	-	-	-	-
L	4 α ,24-dimethyl-cholesta-5,7-dien-3 β -ol	+	-	-	-	-	-	-
	C ₂₉ unknown stenol	tr	-	-	-	-	-	-
IV. C ₃₀ stenols								
C ₃₀	unknown stenol	-	-	-	tr	-	-	-
C ₃₀	unknown stenol	tr	-	-	-	-	-	-
C ₃₀	unknown 4methyl stenol	+	-	-	-	-	-	-
C ₃₀	of two unknown stenols	-	-	-	-	tr	-	-
C ₃₀	unknown stenol	-	-	+	tr	-	-	-
C ₃₀	unknown stenol	-	-	-	-	tr	-	-

^a see Figures 8-11, and 15-16.^b tr= < 1 ug/g; += 1-5 ug/g; += 5-10 ug/g; +++= >10 ug/g.^c see Table 5 for the definition of the symbols.

Fatty Acids

Free fatty acids were minor components in the free lipids of all of the samples and the distribution of these compounds was very similar among the samples (Figures 8D to 16D). The mass spectrum of the normal fatty acid methylester showed a characteristic fragment m/z 74 which is from α -cleavage between α and β carbons with γ -hydrogen rearrangement. The other fragments such as m/z 87 $((C_2H_4COOHCH_3)^+)$ and $(M-31)^+ ((C_nH_{2n+1}COOH)^+)$ were also quite characteristic in the mass spectrum of the normal fatty acid methylesters. A methyl group substituted at the β -carbon (iso-) gives rise to McLafferty ion at m/z 88. 3-methyl alkanolic acid methyl ester (anteiso-) was identified by absence of the ion at m/z 87 and presence of an intense ion at m/z 101 (Jacob, 1976).

Hexadecanoic acid was the dominant fatty acid in all of the samples. Octadecanoic, octadecenoic, or tetradecanoic acid were the next most abundant fatty acids. A small amount of C_{15} and C_{17} fatty acids were detected in all of the samples. Trace of iso- and anteiso-fatty acids were also detected in most of the samples.

DISCUSSION

Microbiology of the Mats Studied

It was important to ensure that the microbial distribution was as expected in the samples analyzed for lipids. This was done by both pigment and microscopic analysis. The whole cell absorption spectra for C. aurantiacus, Chlorobium sp., and C. tepidum agree well with the published results for these bacteria (see Figures 5 to 7, Table 4, and Pierson and Castenholz, 1974; Schmidt, 1980; Schmidt, et al, 1980; Blankenship, et al, 1983; Madigan, 1984). C. aurantiacus and Chlorobium sp. are green photosynthetic bacteria. Both possess a small amount of bacteriochlorophyll a in their photosynthetic reaction centers and bacteriochlorophyll c, d, and e as antenna pigments used in light harvesting (Stanier, et al, 1986). The predominant pigment is bacteriochlorophyll c. C. tepidum is a purple sulfur bacterium, with bacteriochlorophyll a and b as reaction center and antenna pigments.

Cyanobacteria and green algae contain chlorophyll a in the photosynthetic reaction center and as an antenna pigment. Phycocyanin is also a major accessory photo-

pigment. The absorption spectra of these pigments differ from those of the bacteriochlorophylls and thus distinguish the presence of these oxygenic types of microorganisms. Chlorophyll a can also be detected by its red autofluorescence when excited by ultraviolet (UV) light.

Combining the absorption spectra (Figures 5 to 7 and Table 4), fluorescence and other microscopic observations (Table 4), knowledge of the chemical conditions of the springs in situ (Table 2), and culture work done by other microbiologists (Brock, 1978; Castenholz, 1984a; Ward, et al, in press b) the major microbial components of the mats can be established. The "New Pit" Spring mat showed a single absorption maximum characteristic of bacteriochlorophyll c (Figure 6A). No autofluorescent cells were seen (Table 4). The predominant organisms were greenish long filamentous bacteria resembling Chloroflexus sp., which has been previously isolated from high sulfide thermal springs of this type (Giovannoni, et al, 1987). Similar spectral and fluorescence measurements suggested that the "Travel Lodge" Stream mat is built by green bacteria (Figure 6C and Table 4). The morphology of the major organism suggests Chlorobium sp. as the predominant photosynthetic organism, consistent with the isolation of a thermophilic Chlorobium sp. from the mat (Castenholz, in press). The absorption spectrum from the "Roland's Well" mat showed peaks characteristic of bacteriochlorophylls a,

b, and c (Figure 6B), suggesting that both green and purple bacteria live in this mat. Very few cells with red fluorescence were observed in the mat, implying that very few cyanobacteria were present. Long filamentous bacteria resembling Chloroflexus sp. suggested this as the predominant green bacterium (Table 4). Small cylindrical rod-shaped purple cells with interior sulfur granules confirmed that purple bacteria resembling Chromatium tepidum are also abundant in this mat (Table 4). The high temperature and elevated sulfide content excluded eucaryotes which were never seen in samples from high sulfide springs (Table 2).

Chlorophyll a and phycocyanin were the major photopigments in cyanobacterial mats (Figures 5B and 5C). The "Wiegert's Channel" mat contained two types of red autofluorescent cyanobacterial cells (Table 4); rod-shaped cells were characteristic of Synechococcus lividus, and thin filamentous organisms were likely to be thermophilic Phormidium sp. (see Brock, 1978). Nonfluorescent filamentous bacteria also suggested the presence of C. aurantiacus, a common member of hot spring cyanobacterial mats (Ward, et al, in press b) though bacteriochlorophyll c was not abundant (see Figure 5B). Similar spectral data for the Orakei Korako mat suggested the coexistence of cyanobacteria and green bacteria. The predominant red fluorescent cyanobacterium resembled Chlorogloeopsis sp.

(formerly recognized in New Zealand hot springs as Mastigocladus sp. "HTF" by Castenholz (in press)). The presence of nonfluorescent filamentous bacteria and a small bacteriochlorophyll c peak (see Figure 5C) suggested the presence of C. aurantiacus. No eucaryotic organisms were seen in cyanobacterial mat samples.

The Nymph Creek mat contained phycocyanin and chlorophyll a (Figure 5A) and was, as expected, predominated by red-autofluorescent cells characteristic of the eucaryotic alga Cyanidium caldarium (Brock, 1978).

These analysis provided confidence as to the predominant photosynthetic microorganisms of the mats. Other work (Dobson, et al, in press; Ward, et al, in press b) suggests that the predominant lipids of mats appear to reflect the dominant photosynthetic microorganisms. However, it must be pointed out that as they are natural communities the mats also contain many other microorganisms which are involved in decomposition of the mat (see Dobson, et al, in press). The mats are also subject to lipid contamination from nonmicrobial sources; this will be discussed below.

Free Lipid Distribution in Mats and Bacteria

Hydrocarbons (including normal and branched alkanes, alkenes, acyclic and cyclic isoprenoids), wax esters, alcohols (including saturated and unsaturated long chain

alcohols, phytol, sterols, and hopanols), and fatty acids were the major free lipids found in samples investigated in this study. The possible sources of these compounds in the mats and their geochemical significance will be discussed, as some of the free lipids appear to reflect the microbial components of mats quite well and may be good biomarkers for particular microorganisms in modern and ancient sediments.

Hydrocarbon Contaminants

It is important to distinguish between hydrocarbons which are obviously contaminants in samples and those which are far more likely contributed by the microorganisms themselves. If contamination was low, there was good correspondence between the hydrocarbons of a microorganism and the mat in which it was predominant (e.g. Chloroflexus and "New Pit" mat) (Figures 11A and 14A). Two kinds of contaminants were evident in the hydrocarbon fraction of samples studied. The most obvious was the presence of oil in the "Travel Lodge" Stream mat, clearly suggested by the presence of UCM (see insert in Figure 13A) which is usually used as an indicator for the presence of petroleum hydrocarbons (Zafirliou, 1973; Keizer, et al, 1978; Ward, et al, 1980; Gundlach, et al, 1983). Other hydrocarbons such as pristane, phytane, and hopanes which are likely of geologic, rather than biologic origin (Ourisson, et al,

1984) (see Figure 13A and Table 8) were also found in this mat. This is consistent with the location of "Travel Lodge" Stream adjacent to a parking lot and the likelihood that rain water might transport oil from the parking lot to the stream. A second type of contamination was the input of plant hydrocarbons in the "Roland's Well" and Nymph Creek mats, indicated by long chain normal alkanes predominantly of odd carbon number especially C₂₉, C₃₁, and C₃₃ (see Figures 8A and 12A and Table 8) (Sever, et al, 1972). Pine trees which grow around the hot spring and creek are possible sources of plant input. If these contaminating compounds are subtracted from the analysis, there is quite good agreement between the hydrocarbons detected in the microorganisms and the mats which contain them (e.g. Chlorobium sp. and the "Travel Lodge" Stream mat and C. tepidum and the "Roland's Well" mat) (compare Figures 12A and 15A, 13A and 16A). This is also true for the Nymph Creek mat (Figure 8A) and C. caldarium, which is known to contain heptadecane and alkenes (C_{19:1} or C_{19:2}) as predominant hydrocarbons (Nagashima, et al, 1986).

Normal Hydrocarbons

Heptadecane was found as a major hydrocarbon in most of the mat samples (Table 6 and Figures 8A to 13A). It was the most dominant hydrocarbon in "Wiegert's Channel" and Nymph Creek mats, the second most dominant hydrocarbon in

the oxygenic mat from Orakei Korako Spring, and a major hydrocarbon in mats from the "Travel Lodge" Stream and "Roland's Well". It was also a major hydrocarbon detected in C. tepidum and Chlorobium sp.. Many algae (including C. caldarium) and cyanobacteria are known to produce heptadecane in significant amounts (Han, et al, 1968; Han and Calvin, 1969a; Rezanka, et al, 1982; Nagashima, et al, 1986). It seems that heptadecane is a qualitatively significant hydrocarbon in certain types of photosynthetic microorganisms, no matter whether they are procaryotes or eucaryotes, oxygenic or anoxygenic. Heptadecane is not abundant and is generally absent in higher plants (Han and Calvin, 1969a).

Although the function of heptadecane in photosynthetic microorganisms is still unknown, the unusually high content of heptadecane in these microorganisms makes it a good biomarker for judging recent photosynthetic microbial inputs to sediments (Johnson and Calder, 1973; Philp, 1980; Hamilton, et al, 1984). It has been suggested that photosynthetic microorganisms were one of the earliest types of life on the Earth (Schopf, 1978). Theoretically this suggestion should correlate with a high content of heptadecane in the hydrocarbon fractions in ancient (e.g. Precambrian) sediments. Unfortunately, most of the hydrocarbons found in ancient sediments were normal alkanes with a nearly equal abundance of odd and even carbon number

(Oro', et al, 1965; Han and Calvin, 1969b). These compounds are believed to be the products of diagenesis, the original hydrocarbon composition having been altered through long periods of geological time.

However, some ancient sediments were preserved under special geological conditions (e.g. low temperature and pressure) and may have retained their original hydrocarbons. For example, organic compounds are well preserved in many ancient sediments, including those with heptadecane as the dominant hydrocarbon, implying the presence of phototrophic microorganisms which contributed a high content of heptadecane to the sediment. Heptadecane was also the dominant hydrocarbon reported to occur in Precambrian stromatolites (ca. 1.5 to 0.9 Ga) from the Aravalli Group, India (Banerjee and Klemm, 1985).

Mid-chain Branched Mono- and Dimethylalkanes

Mid-chain branched monomethyl alkanes (C_{17} to C_{20}) have been found in many cyanobacteria (Han, et al, 1968; Han and Calvin, 1969a; Winters, et al, 1969; Fehler and Light, 1970; Gelpi, et al, 1970; Blumer, et al, 1971; Oehler, 1976) and in cyanobacterial mats in marine lagoons and hot springs (see Dobson, et al, in press; Ward, et al, in press a). Since there is no evidence that other microorganisms produce this kind of alkane, they have been suggested as good biomarkers for cyanobacteria (Table 1).

Cyanobacteria are thought to have played a central role in oxygenating Earth's atmosphere. Perhaps the record of mid-chain branched monomethyl alkanes in Precambrian sediments can help us better understand the history of cyanobacteria. Actually, mid-chain branched monomethyl alkanes have been detected in Precambrian sediments dating back to ca. 1.7 Ga (Brooks, et al, 1977; Klomp, 1986; Jackson, et al, 1986; Summons, 1987). In these sediments, suites of all possible monomethyl-substituted structural isomers ranging from C₁₇ to C₂₈ were found, unlike the reported occurrence of one or a few such compounds (e.g. 7- and 8-methyl heptadecane) in cyanobacteria and cyanobacterial mats. The lack of whole suites of structural isomers in modern cyanobacteria and cyanobacterial mats lead some geochemists (e.g. Hoering, 1978; Summons, 1987) to suggest that the suites originated through diagenesis of some other organic compounds in the sediments. Some bacteria, for example, are known to make mid-chain branched fatty acids which might be converted to alkanes via diagenesis (see Summons, 1987). Alternately, they may have been contributed by extinct microorganisms or by organisms which haven't yet been studied.

In this study, mid-chain branched monomethyl alkanes were not detected in the eucaryotic algal mat or in any of the anoxygenic mats and bacteria, but were found in the two cyanobacterial mats. Unlike other cyanobacterial mats

which have been studied, suites of mid-chain branched monomethyl alkanes ranging from C_{17} to C_{19} were detected in both samples (Figure 18 and Table 7). This is the first discovery of suites of these alkanes in living cyanobacterial mats, and suggests that cyanobacteria might be direct contributors of the compounds found in Precambrian sediments. However, the chain lengths of mid-chain branched monomethyl alkanes found in hot spring cyanobacterial mats do not quite fit with those reported for all ancient sediments (Brooks, et al, 1977; Klomp, 1986; Jackson, et al, 1986; Summons, 1987).

Many animals and plants have been reported for their ability to synthesize long chain monomethyl alkanes (Mold, et al, 1966; Jackson, 1969; Tartivita and Jackson, 1969; Nelson and Sukkestad, 1970; Solidary, et al, 1974) but these are mainly of higher molecular weight (C_{24} to C_{40}) and none of these organisms are known to contain suites of monomethyl alkanes. Plants and animals would also not have been present in the Precambrian, excluding the possibility of their having contributed these compounds to sediments of that age.

The detection of suites of mid-chain branched monomethyl alkanes in the cyanobacterial mats studied does not prove that the cyanobacteria of these mats synthesize all these compounds. The communities in both mats are complex and may include other microorganisms which cause

the formation of the structural isomers through biodegradation processes. Analysis of the hydrocarbon composition of cyanobacteria isolated from both mats should be done in the near future.

Several hydrocarbons likely to be dimethyl alkanes (C_{19} to C_{20}) were detected in the mat from the Orakei Korako Spring (Figure 19 and Table 7). The dominant dimethyl alkanes in this mat are tentatively identified as 5,12- and 4,13-dimethyl heptadecanes and octadecanes. Alternatively, the symmetric mixtures (e.g. 5,13-dimethyl heptadecane and 6,12-dimethyl heptadecane for 5,12-dimethyl heptadecane) cannot be excluded. Together with monomethyl alkanes, these are the major compounds in the hydrocarbon fraction of this mat. Because of the similar substitution positions of methyl groups in dimethyl and monomethyl alkanes, these compounds might be metabolically related. This means that monomethyl alkanes might be biosynthetic precursors (or alternatively degradation products) of the corresponding dimethyl alkanes. Dimethylalkanes (tentatively assigned as 6,10-dimethyl heptadecane and 6,10- and 7,10-dimethyloctadecane) have also been found in one lagoonal cyanobacterial mat (de Leeuw, et al, 1985).

Dimethyl alkanes have been detected in Precambrian sediments (Klomp, 1986). The chain lengths (C_{22} to C_{25}) and the positions of substituted methyl groups (11 or 12 for the first methyl group and 2 to 10 for the second

methyl group) do not fit exactly with those found in the Orakei Korako mat. A dimethylalkane (13,16-dimethyl octacosane) was produced through degradation of Messel oil shale kerogen (Chappe, et al, 1979), suggesting that this type of hydrocarbon may have more oxidized precursors. Understanding the sources of these compounds is of obvious evolutionary importance. Some dimethyl alkanes and fatty acids were reported in insects, but usually with carbon numbers ranging from 25 to 55 (Jackson and Blomquist, 1976), significantly different from those found in the mat. Although dimethyl alcohols and acids were found in several marine bird waxes (Jacob, 1976), it seems very unlikely that the mat from Orakei Korako Spring is contaminated by marine birds. Since the chain length of dimethyl alkanes is similar to that of other hydrocarbons found in the mat, the most likely source of these compounds seems to be the microorganisms in the mat itself. The dimethyl fatty acid (2,4-dimethyl tetradecanoic acid) has been reported in Mycobacterium gordonae (Julák, et al, 1980) but the positions of the substituted methyl groups (2 and 4) are different from those found in the Orakei Korako mat. So far, there is no reported occurrence of dimethyl alkanes in photosynthetic procaryotes.

Alkenes

C. aurantiacus, and all of the mats studied which contained Chloroflexus sp., were found to contain long chain diunsaturated or triunsaturated alkenes. The most dominant alkene was hentriacontatriene ($C_{31}H_{58}$). In addition, hentriacontatriene was also found in the Octopus Spring cyanobacterial mat, also known to contain Chloroflexus (Dobson, et al, in press). Recently, long chain alkenes were reported to occur in a marine cyanobacterial mat from Hao Atoll, French Polynesia (Boudou, et al, 1986a), where hentriacontatriene was the most dominant hydrocarbon (ca. 200 ug/g) found in the 1.0-1.2 cm vertical interval. This may indicate the presence of a low temperature strain of Chloroflexus in this mat, previously suggested on morphological evidence for lagoonal cyanobacterial mats (see Castenholz, 1984b).

Long chain diunsaturated and triunsaturated alkenes have also been found in other modern sediments and organisms. C_{39} and C_{40} di- and triunsaturated alkenes were detected in a mat from an Antarctic Oasis Lake (Orcutt, et al, 1986). The major components of the mat are thought to be cyanobacteria, diatoms, and some other algae. Different types of alkenes which contain 20, 25, and 30 carbon atoms and usually possess rings have been found in some marine sediments (Barrick and Hedges, 1980; Requejo

and Quinn, 1983). The sources of these ring-containing alkenes are still uncertain. Microbial input or diagenesis of acyclic isoprenoids leading to cyclization were suggested as ways that these alkenes might have been produced.

So far, very few photosynthetic microorganisms such as Botryococcus braunii (a golden-brown alga), Anacystis montana (a cyanobacterium) and Emiliana huxleyi (a marine alga) have been reported to contain long chain alkenes (Gelpi, et al, 1968; Gelpi, et al, 1970; Weete, 1976; Volkman, et al, 1980; Wolf, et al, 1985). The dominant alkenes in these microorganisms were different from those found in C. aurantiacus ($C_{29:2}$ in B. braunii, $C_{27:1}$ in A. montana, and $C_{37:3}$ in E. huxleyi). Hentriacontatriene was absent in these microorganisms.

The reason why C. aurantiacus synthesizes long chain multiple unsaturated alkenes is not understood. Like wax esters which C. aurantiacus synthesizes in abundance, these compounds may serve as storage materials (see below). However, it seems odd that Chloroflexus would not store both compounds if it were growing under conditions of carbon or energy excess. Note that wax esters were abundant in the "Roland's Well", Orakei Korako and "Wiegert's Channel" mats, whereas long chain alkenes were not. Thus, the two types of compounds might have different functions. Phylogenetic evidence suggests that C.

aurantiacus may play a very important role in microbial evolution. It is necessary for geochemists to understand the fate of these long chain alkenes which might serve as biochemical markers.

In addition to the long chain multiple unsaturated alkenes, three short chain monounsaturated alkenes ($C_{19:1}$, $C_{20:1}$, and $C_{21:1}$) with the double bond located at carbon 3 were detected in the "New Pit" Spring mat. Some monounsaturated alkenes with the same carbon range were also found in C. aurantiacus, and the mats from "Wiegert's Channel", Orakei Korako Spring, and "Roland's Well". The presence of Chloroflexus and monounsaturated alkenes in the samples suggests that these short chain alkenes may be synthesized in each case by Chloroflexus. Three alkenes with the same carbon number were also detected in the bottom layer (1.2 cm-1.5 cm) of the Octopus Spring cyanobacterial mat (Dobson, et al, in press) which also contains Chloroflexus in upper mat layers.

Many short or long chain monounsaturated alkenes have been found in other bacteria and sediments (Albro, 1969 and 1976; Keizer, et al, 1978; Barrick, et al, 1980; Řezanka, et al, 1982). In most cases, Δ^1 or branched alkenes were reported. A series of monounsaturated alkenes-- Δ^1 , Δ^2 , and Δ^3 --were detected in Shale Oil from Rundle, Queensland (Regtop, et al, 1982). Since the distribution of monounsaturated alkenes is continuous from C_{10} to C_{32} , they

are quite different from those found in the hot spring mats.

C. aurantiacus is certainly not an exclusive source of C₁₉ to C₂₁ alkenes as two alkenes (C_{19:1} and C_{19:2}) were also detected in the mat from Nymph Creek. Strains of Cyanidium caldarium, the major microbial inhabitant of this mat were found to contain these monounsaturated alkenes (Nagashima, et al, 1986). Since the position of the double bond of monounsaturated alkenes in the Nymph Creek mat and C. caldarium was not determined, comparison of these compounds to those found in C. aurantiacus cannot be made.

Acyclic Isoprenoid Hydrocarbons

Pristane and phytane are the two most common acyclic isoprenoids produced from biological activities in nature. So far, acyclic isoprenoids have been widely found in both procaryotes and eucaryotes. Thus, it is not amazing to find these compounds in most sediments (Blumer, et al, 1963; Eglinton, et al, 1964; Oro', et al, 1965; McCarthy and Calvin, 1967; Han and Calvin, 1969a; Blumer, et al, 1971; Moldowan and Seifert, 1979; Barrick, et al, 1980; Nes and Nes, 1980; ten Haven, et al, 1985). The oldest ancient sediment found to contain pristane and phytane is from the Soudan iron formation, Minnesota (ca. 2.7 Ga) (Johns, et al, 1966; Calvin, 1969). Although these compounds are not specific biomarkers for any microbial group, the presence

of them in sediments is symbolic only of biological activities.

In my study, the purple bacterium C. tepidum and the "Roland's Well" mat (which contains C. tepidum) showed significant content of pristane and phytane (Figures 15A and 12A). The origin of one or both isoprenoids in C. tepidum may relate to degradation of bacteriochlorophyll a and b which possess a phytyl group esterified to the porphyrin ring (Stanier, et al, 1986). Since chlorophyll a also has a phytyl group attached to the porphyrin ring, it seems surprising that no acyclic isoprenoid was detected in the two cyanobacterial mats or the eucaryotic algal mat (Figures 9A to 11A). Except in a few cases (Oro', et al, 1965; Blumer, et al, 1971), pristane and phytane were not reported in cyanobacteria (Han, et al, 1968; Han and Calvin, 1969a; Rezanka, et al, 1982). Acyclic isoprenoids (e.g. phytene, phytadiene, phytane, or pristane) were detected in some cyanobacterial mats in which biodegradation of the pigments could have been performed by other bacteria (Cardoso, et al, 1976; Cardoso, et al, 1978; Philp, 1980; Boon, et al, 1983; Boudou, et al, 1986b; Dobson, et al, in press). Though the abundance of phytol among free alcohols in the cyanobacterial and algal mats studied suggests degradation of chlorophyll a (see below), it is possible that conversion of phytol to more reduced isoprenoid hydrocarbons in these mats was not as extensive.

Phytane and pristane were also found in the "Travel Lodge" Stream mat, but not in its major component Chlorobium sp.. As described before, this mat was probably contaminated by petroleum, the more likely source for both compounds.

Methanogenic archaeobacteria have been found in the Octopus Spring and "Wiegert's Channel" cyanobacterial mats (Ward, et al, 1978; Sandbeck, 1980) and it is also possible that these or other archaeobacteria might be present in other hot spring microbial mats studied. Phytyl or biphytyl groups are very common in diethers and tetraethers of glycerol in archaeobacteria (Brassell, et al, 1981; Chappe, et al, 1983; Risatti, et al, 1984; Mancuso, et al, 1985) and many acyclic isoprenoids have been found in these bacteria (Holzer, et al, 1979). Phytanyl and biphytanyl ether lipids have been found in the Octopus Spring and Nymph Creek mats (see Ward, et al, 1985). These might represent other sources of acyclic isoprenoids found in the mats.

Cyclic Triterpenoid Hydrocarbons

Recently, because of improvements in analytic methods, equipment, and synthesis of standard compounds, the study of complex compounds such as cyclic triterpenoids has become possible (Langworthy and Mayberry, 1976; Seifert, et al, 1978; Rullkotter and Wendisch, 1982; Volkman, et al, 1983; Moldowan, et al, 1984). Cyclic isoprenoids such as

steroids are very common in living organisms; mostly they are found in eucaryotes. The only cyclic triterpenoids commonly found in bacteria are the hopanoids (including the hopenes and hopanols) (Das and Mahato, 1982; Rohmer, et al, 1984; Taylor, 1984, Ourisson, 1987). Although a few hopanoids have been found in lower plants (e.g. fungi and ferns) (Das and Mahato, 1982; Ourisson, et al, 1987), none of these are extended hopanoids (e.g. $>C_{31}$). Thus, bacteria seem to be the major source of these compounds. So far, the distribution of hopenes found in bacteria is very wide. In general, they have been found in most but not all cyanobacteria, purple nonsulfur bacteria and some chemoheterotrophic bacteria (Rohmer, et al, 1984). Diploptene (hop-22(29)-ene) is the most common hopanoid found in bacteria. Purple and green sulfur bacteria and archaebacteria have not yet been proven to synthesize hopanoids (Rohmer, et al, 1984).

It has been noted that so far, anaerobic bacteria have not been reported to contain hopenes or other hopanoid compounds (Taylor, 1984; Ourisson, 1987), a particularly interesting idea as hopanoids might then serve as biomarkers for the presence of aerobic conditions. In my study, no hopenes were detected in any of the photosynthetic bacteria. However, except for the "Travel Lodge" Stream mat, hopenes were detected in low concentration in all of the mats (Table 8). Thus, the

presence of these compounds seems to be unrelated to the presence or absence of oxygen.

Hopanes, either directly contributed by microorganisms or diagenetic products of hopenes and hopanols have been found in many ancient sediments (especially in petroleum) (Kimble, et al, 1974; Rubinstein, et al, 1977; Simoneit, 1977; Curiale, et al, 1985; Tannenbaum, et al, 1986; Ourisson, 1987). As a result, it is believed that bacteria may play a very important role in the formation of petroleum. Thus, understanding the distribution and fate of hopanoids in bacteria and sediments becomes very important for exploring the source and the formation of petroleum (Tannenbaum, et al, 1986). As mentioned before, the mat from "Travel Lodge" Stream was contaminated by petroleum. The series of hopanes accompanied by their steric isomers in this mat, also confirmed the presence of oil contamination. Almost all of the hopanoid producing bacteria synthesize 22(R) rather than 22(S) hopanoids (Rohmer, et al, 1984). During diagenesis, some of 22(R) hopanoids will isomerize to form 22(S) hopanoids and the ratio of 22(S) to 22(R) increases to ca. 1.5 as the diagenetic process progresses to equilibrium (Philp, 1986). Both isomers are usually present in ancient sediments but only 22(R) hopanoids can be found in living microorganisms.

Wax Esters

Although wax esters (i.e. simple esters of fatty alcohols and fatty acids) have been found in many eucaryotes (Nevenzel, 1969; Kolattukudy, 1976) they have rarely been reported in bacteria (Kolattukudy, et al, 1976; Bryn, et al, 1977; Nes and Nes, 1980). In general, palmitic or stearic acids (C_{16} or C_{18}) and hexadecanol are the most common fatty acid and alcohol moieties in bacterial wax esters. Except for Serratia marcescens (ca. 2% of the cell lipids), the amount of wax esters in bacteria is usually very small (Kolattukudy, et al, 1976).

C. aurantiacus is the first phototrophic bacterium which has been reported to contain abundant (ca. 3% of dry weight) wax esters (Knudsen, et al, 1982). The function of wax esters in C. aurantiacus is uncertain, though recently, intracellular organelles, termed "wax oleosomes", which contain ca. 60% wax esters were found, and they were suggested to be storage materials (Beyer, et al, 1983). In my study, the wax esters which were detected in C. aurantiacus ranged from C_{28} to C_{36} and monounsaturated wax esters were found only in even carbon number wax esters (i.e. C_{30} , C_{32} , C_{34} , and C_{36}) (Figure 14B and Table 9). There was only a small difference in concentration from those reported previously (Knudsen, et al, 1982).

There was excellent correspondence between wax esters

and predominant photosynthetic organisms in the mats studied. For example, saturated normal, iso- and anteiso-wax esters with chain lengths ranging from C₂₉ to C₃₆ were found in the mats from "New Pit" Spring, "Roland's Well", "Wiegert's Channel", and the Orakei Korako Spring (Figures 9B to 12B, and Table 9), all of which are believed to contain Chloroflexus. Similar wax esters were found in the Octopus Spring cyanobacterial mat which is also known to contain Chloroflexus (Dobson, et al, in press). There also appeared to be a reasonable correspondence between wax ester concentrations (Table 5) and the abundance of Chloroflexus, as indicated by bacteriochlorophyll c which was higher in "New Pit" Spring and "Roland's Well" mats than in cyanobacterial mats (Figures 5B and 5C and 6A and 6B). The similarity in the carbon number of branched and unbranched wax esters, suggests that they may also be produced by Chloroflexus, though an alternate source cannot be excluded.

A small amount of saturated and unsaturated wax esters (C₂₈ to C₃₂) were found in Chlorobium sp. (Figure 16B and Table 9), suggesting that green sulfur bacteria are also able to synthesize wax esters. However, another strain of Chlorobium was found to lack wax esters (Knudsen, et al, 1982). The same type of wax esters were also detected in the "Travel Lodge" Stream mat (Figure 13 and Table 9). The concentration of wax esters found in both the mat and

Chlorobium sp. increased with increasing carbon number and reached a maximum at C₃₂. The pattern of wax esters found in these two samples was quite unlike that in Chloroflexus or mats containing it.

A unique group of shorter chain wax esters (C₂₄ to C₂₇) was detected in the mat from "Wiegert's Channel" (Table 9). These wax esters probably come from unrecognized organisms in the mat.

Detailed analysis of fatty acid and alcohol moieties of the wax esters (Table 9) suggested some dissimilarity between Chloroflexus and mats containing it. For example, in this bacterium the dominant wax esters C₃₂, C₃₄, and C₃₆ contained almost exclusively C₁₆ or C₁₈ acids and alcohols, whereas in mats which contain Chloroflexus, the predominanting acids and alcohols were often C₁₅, C₁₇, or C₁₉ compounds. This presumably relates to nutritional differences between culture conditions and the natural environment. Such differences were not apparent in the Chlorobium and "Travel Lodge" Stream mat, where C₁₄ and C₁₆ acids and alcohols were predominant in both the bacterium and the mat.

Wax esters or their diagenetic products might be good biomarkers for some anoxygenic photosynthetic bacteria (e.g. Chloroflexus and Chlorobium sp.) in modern mats. Since simple wax esters have never been reported in ancient sediments, the survival of these compounds could be very

poor. Thus, using these compounds as biomarkers for ancient bacterial communities requires an understanding of the fate of wax esters in sediments (see below).

Nonsteroidal Alcohols

Phytol is the most dominant nonsteroidal alcohol in all of the algal and cyanobacterial mats and in the purple bacterium C. tepidum. As described before, chlorophyll a and bacteriochlorophyll a and b all possess a phytol alcohol side chain attached to the tetrapyrrole ring. This side chain may be the most likely source of phytol found in the samples.

C. aurantiacus and the "New Pit" Spring mat (which was built mainly by Chloroflexus sp.) contained octadecanol as the major alcohol. Bacteriochlorophyll C_s, the major photopigment found in C. aurantiacus, has an octadecanol side chain instead of phytol (Gloe and Risch, 1978). This is a likely source of octadecanol found in both samples. Since a trace of bacteriochlorophyll a is also present in Chloroflexus (Stanier, et al, 1986), it was not surprising to also find a small amount of phytol in both samples (Table 10).

Aside from phytol and octadecanol, the mats from "Wiegert's Channel", "New Pit" Spring, "Roland's Well", and Octopus Spring (from Dobson, et, al, in press) all showed the same type of normal alcohol distribution with

heptadecanol predominating (Figures 9C, 11C, and 12C). The dominant alcohol moiety of wax esters in these mats was also heptadecanol (Table 9), suggesting a close structural relationship between the free alcohols and the alcohol moiety of wax esters. The same parallel between the alcohol moieties of wax esters and free alcohols was found in Chlorobium sp. and the mat from "Travel Lodge" Stream. Here, hexadecanol was the dominant component among both free alcohols and alcohol moieties of wax esters in both samples. Only in the Orakei Korako mat is there a lack of correlation between the alcohol moiety of wax esters and free alcohols. No wax esters were detected in C. tepidum and the mat from Nymph Creek and the free normal alcohols were also very low in both samples.

There is correlative evidence that the sources of free alcohols in the samples can include chlorophylls, bacteriochlorophylls, and wax esters. The distribution of phytol and octadecanol as dominant alcohols appears to reflect the distribution of pigments in the various mats and organisms studied. With a single exception, other normal alcohols reflect the composition of wax esters and may come from the chemical or biochemical degradation of these lipids.

No data are available on the alcohols in Precambrian sediments. Recent experiments showing the release of ether-linked alcohols from kerogens from ancient sediments

may provide new insight into studying alcohols which are truly native to the rocks in which they are found (Chappe, et al, 1977).

Two types of vitamin E, α - and β - tocopherol, were detected in the mats from "Wiegert's Channel" and Nymph Creek. Although vitamins are important growth factors for bacteria, tocopherol seems not to be an important one. Since plants (e.g. grasses) are the major sources of the tocopherol in nature (Marks, 1975), the finding of these compounds in both mats may reflect contamination from extraneous plant input.

Monoglycerides were detected in the mats from Nymph Creek and "Wiegert's Channel". The same type of fatty acids (e.g. C_{16} , C_{18} , and $C_{18:1}$) were found in both free fatty acids and fatty acids moieties of monoglycerides (see Figures 8C, 8D and Table 10). Since this type of fatty acids were also found in the polar lipids of C. caldarium (Ikan and Seckbach, 1972), monoglycerides and free fatty acids may come from degradation of polar lipids.

At least three C_{30} hopanols were found at low concentration in the photosynthetic bacterial mat from "Roland's Well" and "Travel Lodge" Stream (Table 8). As with the hopenes, so far, it is still impossible to be sure about the exact source of these hopanols among bacteria. Two hopanols were reported in the cyanobacterial mat of Octopus Spring (Dobson, et al, in press). The presence of

both hopenes (see above) and hopanols in anaerobic mats suggest further that hopanoids might be synthesized in both oxic and anoxic environments. Thus, the presence of hopanoids in ancient sediments may not necessarily indicate the oxic or anoxic nature of the environment in which the microorganisms lived (see above). More studies on hopanol distribution and synthesis under both aerobic and anaerobic conditions are needed.

Sterols

Sterols are among the most common biological products on Earth. They are also ubiquitous in nature. Even in the air, small amount of sterols can be detected (Christie, 1982). Eucaryotes are the most suggested sources for these compounds. Even though, trace amounts of sterols have been reported in aerobic bacteria and cyanobacteria (Souza and Nes, 1968; Bird, et al, 1971), the ability of procaryotes (including cyanobacteria) to synthesize sterols is in debate (see Ward, et al, in press a; Ourisson, et al, 1987). Since oxygen is involved in the biosynthesis of sterols (Schopf, 1978; Stryer, 1981), it is believed that photosynthetic bacteria are unable to synthesize these compounds.

Except for C. aurantiacus all of the samples examined in this project contained one or more sterols (Table 11). Cholesterol was found in seven out of nine samples

including Chlorobium sp. and C. tepidum, which should, as anaerobes, be unable to synthesize sterols. This suggests that cholesterol is a common contaminant from the culture medium, analytical procedures, operator, or air.

Sterols found in small amounts in the two cyanobacterial mats showed a similar distribution. In addition to cholesterol, three sterols--24-methyl-cholest-5-en-3 β -ol, 24-ethyl-cholesta-5,22-dien-3 β -ol, and 24-ethyl-cholest-5-en-3 β -ol--were detected in both mats. Except for 24-methyl-cholest-5-en-3 β -ol, the same types of sterols were also reported in small amounts in the cyanobacterial mat from Octopus Spring (Dobson, et al, in press). These sterols were also reported in some other cyanobacteria and cyanobacterial mats (Boudou, et al, 1986b; Orcutt, et al, 1986). Because of the similarity of sterols found in cyanobacterial mats and cyanobacteria, it is possible that the cyanobacteria which live in the mats are the major producers of these compounds. However, since the same types of sterols have been reported in many eucaryotes (e.g. animals, plants, and algae) (Kolattukudy, et al, 1976; Volkman, 1986), the possibility for extraneous input from eucaryotes can't be excluded (Ward, et al, in press a).

Trace amounts of a few sterols (C₂₈ to C₃₀) were also found in the three photosynthetic bacterial mats (Table 10). Since the bacteria that live in photosynthetic

bacterial mats would be unable to synthesize sterols in the anoxic mat environment, the most possible source of these compounds is extraneous contamination. The fact that many of the same sterols were found in anoxic and cyanobacterial mats suggests that contamination may be the more likely source of the sterols found in cyanobacterial mats as well.

Compared to the procaryotic mats and bacteria studied, the eucaryotic algal mat from the Nymph Creek showed a significantly greater abundance and variety of sterols. Fourteen sterols (C_{28} to C_{30}) were found in this mat, thirteen of which were unique to this mat. Ergosterol was the most dominant component in the alcohol fraction, with a concentration even higher than that of phytol. Cyanidium caldarium, the major microorganism living in this mat, is known to synthesize ergosterol, sitosterol, and stigmasterol (Seckbach and Ikan, 1972). The first two of these sterols were detected in the Nymph Creek mat. Other eucaryotic microorganisms, such as the fungus Dactylaria gallapava, which inhabit this mat (see Brock, 1978) may contribute the other sterols.

Many lagoonal "cyanobacterial" mats have been shown to contain a variety of sterols in relatively high amounts (Cardoso, et al, 1976; Cardoso, et al, 1978; Huang and Meinschein, 1978; Boon, et al, 1984; Edmunds and Eglinton, 1984; de Leeuw, et al, 1985; Boudou, et al, 1986 b; Ward, et al, in press a). Eucaryotes (e.g. diatoms and

meiofauna) which inhabit these mats (Krumbein, et al, 1977; Javor and Castenholz, 1981; Gerdes and Krumbein, 1984; Ehrlich and Dor, 1985; Gerdes, et al, 1985 a; Gerdes, et al, 1985b), have been suggested as the most probable sources for the sterols (Ward, et al, in press a). In sum, mats known to contain eucaryotes also contain an abundance and variety of sterols, while mats known to lack eucaryotes contain traces of a limited range of sterols. Similar sterol distributions in anoxic photosynthetic bacterial mats as well as oxic cyanobacterial mats suggest the likelihood that sterols in these mats are contaminants. Thus, diagenetic products of sterols are likely to serve as biomarkers of eucaryotic organisms in the geochemical record.

The fate of sterols in sediments has been studied for quite a long time. Generally speaking, sterols are very common compounds in recent sediments (Volkman, 1986) and steranes are usually found in petroleum and ancient sediments (Barrick and Hedges, 1981; MacKenzie, et al, 1982; Moldowan, 1984). Through diagenesis, the double bonds and the functional groups of sterols can be easily saturated and removed leaving the basic carbon skeleton in sediments (Gagosian and Farrington, 1978; Nishimura, 1978; Mackenzie, et al, 1982). Biodegradation may also change the structure of steroids (Eyssen and Parmentier, 1974; Seifert and Moldowan, 1978; Brassel and Eglinton, 1981;

Owen, et al, 1983, Wolff, et al, 1986). Steroid hydrocarbons are the terminal products of diagenesis which survive in ancient sediments (Seifert and Moldowan, 1979; Philp, 1986). By understanding the concentration and chemical properties of the diagenetic products of sterols in ancient sediments (especially in petroleum), the maturation, migration and the sources of crude oils can be explored (Rubinstein, et al, 1977; Seifert and Moldowan, 1979; Tannenbaum, et al, 1986). As described before, sterols may be good biomarkers of eucaryotic organisms, thus, through the finding of their diagenetic products (steranes and sterenes) in ancient sediments the evolutionary scheme for the eucaryotes can be better understood. Steranes reported to occur in late Precambrian sediments as old as ca. 1.7 Ga (Arefev, et al, 1980; Grantham, 1986; Fowler and Douglas, 1987; Summons, et al, unpublished) may imply the existence of eucaryotic microorganisms at that time.

Fatty Acids

Free fatty acids were in relatively low abundance in all of the samples examined and the distribution of these compounds in all of the samples was quite similar. Hexadecanoic acid was the dominant fatty acid and octadecanoic, octadecenoic, or tetradecanoic acids were the next most abundant. Two major sources of free fatty acids

seem likely--wax esters and complex lipids. As described before, the types of free alcohols in the mats and bacteria were similar to those found in the alcohol moiety of wax esters, suggesting that degradation of wax esters may occur in these mats and bacteria. If the degradation of wax esters does occur, both alcohols and acids should accumulate. Because 1) only low concentration of fatty acids were detected in the samples, 2) the same type of fatty acids were found in samples in which wax esters were absent, and 3) the chain lengths of the free fatty acids were different from those found in the acid moieties of wax esters (see Table 9), the free fatty acids found in the samples are likely not to arise from decomposition of wax esters. Presumably, other bacteria in the mats (e.g. consumers) recycle fatty acids for their nutrition and leave the alcohols in the environment.

The more likely source of free fatty acids may be from other components in the cells (e.g. phospholipids or glycolipids)(Zepke, et al, 1978). It was shown that in the Octopus Spring cyanobacterial mat the type of fatty acids in polar and free lipids are similar, with lipids characteristic of thermophilic phototrophs (cyanobacteria and photosynthetic bacteria) predominating over the lipids characteristic of thermophilic heterotrophs (Ward, et al, in press a). Perhaps this means that the free fatty acids of the mats studied here also reflect the polar lipid fatty

acids of phototrophs. This correlation holds for the "New Pit" Spring (Chloroflexus dominant) and the Nymph Creek mats (Cyanidium caldarium dominant) as mat free fatty acids resemble polar lipid fatty acids of the predominating phototrophic microorganisms (Ikan and Seckbach, 1972; Knudsen, et al, 1982). In other mats, the lack of knowledge of complex lipids of major phototrophs prevents such a comparison.

Saturated, unsaturated, and branched chain fatty acids have been reported to occur in many recent and ancient sediments (Leo and Parker, 1966; Cranwell, 1973; Farrington, et al, 1977; Matsuda and Koyama, 1977; Philp, et al, 1978; Perry, et al, 1979; Nes and Nes, 1980). For example, unsaturated fatty acids were reported to occur in McMinn Shales, Australia (ca. 1.6 Ga)(see Nes and Nes, 1980) and saturated C₁₄ to C₁₈ fatty acids (C₁₆ dominant) were reported to occur in the sediment in Onverwacht, South Africa (ca. 3.4 to 3.7 Ga) (Han and Calvin, 1969b). It is inconceivable that unsaturated fatty acids would survive in such ancient sediments (McKirdy, 1974). In general, these compounds should be regarded as contaminants from extraneous or recent organic inputs. It may be that as with alcohols, fatty acids which are more definitely native to ancient sediments, are bonded in kerogen and can be released through degradative reactions to yield information about the nature of microbial life in the Precambrian.

CONCLUSIONS

- 1) Except for extraneous inputs, the major free lipids and photopigments of mats reflect well the predominant mat-building photosynthetic microorganisms.
- 2) Suites of mid-chain branched monomethyl alkanes were only found in cyanobacterial mats. This is consistent with the suggestion that these compounds are cyanobacterial biomarkers. Similar compounds found in Precambrian sediments may have been directly produced by cyanobacteria rather than diagenetically produced from noncyanobacterial lipids. The source of monomethyl alkanes suites with carbon number greater than 20 found in ancient sediment is still unknown.
- 3) Several dimethyl alkanes, accompanied by structurally related monomethyl alkanes, were found in high abundance in one cyanobacterial mat. This implies that both types of compounds may be metabolically related. The source of these dimethyl alkanes is of particular interest since similar compounds have been reported to occur in Precambrian sediments.

- 4) Unusual long chain alkenes were found as major hydrocarbons in the green nonsulfur bacterium C. aurantiacus. These compounds reflect the distribution of Chloroflexus in hot springs mats and may provide evidence for Chloroflexus in marine-type cyanobacterial mats.
- 5) Hopanoid compounds (hopenes, hopanols) were detected in both cyanobacterial and photosynthetic bacterial mats. This does not fit with the suggestion that they are synthesized only by aerobes and facultative anaerobes. Further study is needed to more vigorously test the hypothesis that hopanoid compounds might serve as biomarker for such organisms.
- 6) Wax esters may be good biomarkers (especially in modern mats) of the green nonsulfur bacterium Chloroflexus and the green sulfur bacterium Chlorobium sp..
- 7) The major free nonsteroidal alcohols in hot spring microbial mats reflect the distribution of chlorophylls, bacteriochlorophylls, and the alcohol moieties of wax esters. Thus, pigments and wax esters may be sources of nonsteroidal free alcohols.
- 8) Compared to one eucaryotic mat, only a simple pattern and low abundance of sterols were found in cyanobacterial mats. Low levels of a limited range of sterols

in photosynthetic bacteria and the anoxic mats they produce suggest that sterol contamination is a serious problem in studying modern mats and organisms. Sterols are more likely indicators of eucaryotic organisms.

- 9) Similar types of free fatty acids were detected in cyanobacterial mats and photosynthetic bacteria mats, so that free fatty acids cannot be used to distinguish the types of phototroph predominating in a mat.

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