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N69-35755

NASA CR-105396

# ORGANIC GEOCHEMISTRY

William Van Hoeven
(Ph.D. Thesis)

January 1969

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UCRL-18690

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory

Berkeley, California

AEC Contract No. W-7405-eng-48

& NASA - NGL-05-003-003

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## ABSTRACT

Organic geochemistry has, in the past decade or two, been increasingly concerned with the problems of the time, manner and place of the origin of life, both terrestrial and extraterrestrial. This concern is shared and actively investigated by the complementary fields of chemical evolution and micropaleontology. The first portion of this thesis attempts to place organic geochemical research on the origin of life into its proper context, both historically and scientifically. The basic assumptions on which rests this research are critically examined; the concept of biological markers and chemical fossils are discussed in detail.

Each of the four major classes of biochemicals, carbohydrates, proteins and peptides, nucleic acids, and lipids are analyzed as to the suitability, either of individual compounds or of conglomerations within a compound type, for use as chemical fossils. Structural specificity, biosynthetic relatability, possible abiotic synthesis and thermal stability are the main points discussed.

The second portion of this thesis describes experimental results relevant to the origin of life problem. The alkane constituents of nine geological samples ranging in age from 2000 years to $2.7 \times 10^9$ years, have been examined. Some pigments have been identified in the two youngest sediments. Fatty acids from the six oldest samples have also been examined.

The alkane distributions from the youngest sediments are readily relatable to the presumed algal and plant contributors, consisting mostly of normal alkanes of 25 to 33 carbon atoms. The alkanes of the ancient oils and sediments are more complex and apparently the product of considerable diagenetic transformation; however, normal alkanes (usually $C_{15}$-$C_{25}$) and polyisoprenoid alkanes are generally the major components. Alkane distributions, such as steranes and high molecular weight n-alkanes can provide more specific information as to the organisms contributing uniquely to a particular sediment.

All of the saturated fatty acid distributions are indicative of biological activity, being dominated by the n-$C_{16}$ and n-$C_{18}$ components. In two cases, the occurrence of significant amounts of polyisoprenoid fatty acids is noted. The validity of the results is strengthened by separate analyses of the free (solvent extractable) and bound (released by HF/HCl digestion) acids, as well as by control experiments. Attempts have been made to relate both alkanes and fatty acids to the identical or similar components in biological systems. It is obvious from distributional patterns that the alkanes of a sediment do not arise from fatty acids solely by decarboxylation.

The validity of the various compounds and/or distributions as biological markers has been thoroughly discussed, particularly in respect to recent reports of "abiotic" syntheses. The results of subjecting methane to a high energy polymer-producing process are described and discussed.

The possibility of utilizing the unbranched, 4-carbon fragment of such biological polyisoprenoids as squalene and carotenoids is

investigated. Synthetic operations necessary for examining this problem are described as is the result of the preliminary search for such a fragment.

At the conclusion, several suggestions are offered as to future experiments within the field of organic geochemistry. The final analysis is that organic geochemists do have the ability to examine the alkanes (and perhaps fatty acids) of ancient terrestrial and extraterrestrial geological samples and can make a valid decision as to whether or not the distributions of these compounds are indicative of a presence, in time, of biological activity.

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# CHAPTER I

## INTRODUCTION

Any suggestion as to when man first became interested in the origin of living systems can be nothing but speculation; certainly interest in the origin of life predates written history. Until recently, the approaches toward shedding light on this problem have been dominated by the philosophers and the theologians.[1] In the past one hundred years science has directed more and more of its energies and techniques to examining the many aspects of this question, until today it can be said that the study of the time and manner of the origination of life is predominantly the domain of the scientist. Certainly the origin of the entire universe presents itself as a quite different problem, and theologians, philosophers and scientists, among others, have given this question much consideration as well. It is not the intention of this writer to examine this latter question, but to accept as fact that it occurred and to consider only subsequent events which presumably had a bearing on the origin of life.

Most of the scientific research on the origin of life has been directed toward the origin of life on the Earth. The reason is the obvious one, for to gather the necessary data from extraterrestrial locations has been, until a few years ago, an impossibility. This is not to say that the really basic answers do not lie somewhere other than the Earth; indeed it is very possible that they do.[2,3]

Throughout this thesis, when information relevant to extraterrestrial life is available it will be considered.

Current research on the origin of life is based upon the hypothesis that life evolved from inanimate matter.[1] Certainly an impressive array of philosophers and scientists have considered this notion throughout the years. Charles Darwin, prior to 1871, suggested to a friend that his findings on biological evolution could reasonably be extrapolated to include the formation of the first living organism when he stated in a letter:[4]

> "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present, that a proteine compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed."

Certainly the vast amount of information collected by paleontologists since Darwin's time has not contradicted his extrapolation. This concept, that life evolved from inanimate matter, has been amplified and altered during the years and in 1935 Oparin laid the foundations for most modern research when he published a book entitled: Origin of Life.[1] The importance of his book lies in the fact that he critically reviewed the numerous theories which have been proposed to explain the occurrence of life, and he subjected these ideas and past experiments to rigorous scientific criticism. Having thus ruled out various possibilities, he suggested an approach which could be tested by proper experimentation. The scientific area with which Oparin dealt has become known as the field of Chemical Evolution.

In terms of a scientific approach to obtaining information about the origin of life on Earth, the emergence of the field of chemical evolution and its early successes served as a stimulant to other scientific disciplines, whose findings were also relevant to this question.

Before discussing these "other scientific disciplines" it is useful to understand a bit more about chemical evolution. A comprehensive survey of the basic results in this area can be found in the book by M. Calvin.[5]

Chemical evolutionists examine astronomical data and extract from it information as to the conditions which existed on and around Earth during and since its formation. In general, the starting point is taken when the atmosphere was a reducing one, consisting primarily of $CH_4$, $NH_3$, $H_2O$ and $H_2$.[6] The theory is that proper energy inputs (from radioactivity, heat, ultraviolet rays, etc.) have produced simple organic molecules, which in turn have combined by one or more possible mechanisms into polymeric substances. Indeed, virtually all types of monomers important in biological systems of today have been produced from such "primitive-Earth atmosphere" experiments.[7] For example, in 1951 Calvin identified HCOOH and HCHO from $\alpha$-irradiation of $CO_2$ and $H_2O$.[8] In 1953, Stanley Miller reported the formation of amino acids using an electrical descharge in a $CH_4$, $NH_3$, $H_2O$ atmosphere.[9] Since that time a considerable number of experiments of the primitive Earth type have been performed, yielding sugars, amino acids, nucleotides, purines and pyrimidines, porphyrins, etc.[7,10]

Once these monomers were formed on the primitive Earth, they supposedly combined to form polymers, which, because of the nature of the monomers and their polymeric forms, gradually evolved to higher orders of specific complexity. The research to establish the validity of this notion is quite abundant and convincing, and is presented in Calvin's book.[5]

It will be shown later that this concept of abiogenic compound synthesis is of a direct concern to specific aspects of organic geochemistry. This brief survey of the basic principles of chemical evolution has been included here to help define the scientific climate which prevailed in the 1950's and 1960's in regard to the "origin of life" experiments.[1]

Chemical evolution assumes the posture of looking from the past to the present. The complementary point of view is to position ourselves in the present and look into the past. This latter point of view has also provided clues to the origin of life, mainly from two fields, paleontology and geochemistry.

Paleontology, the study of fossils, is another branch of science which is directly concerned with the origin of various species, and indeed with the origin of the first living organisms. Many volumes have been written describing the findings of researchers in this field, and it is beyond the scope of this author and this thesis to discuss all of these findings.[11]

It has long been realized that from the geological era known as the Precambrian (earlier than 600 x $10^6$ years ago) well recognized macroscopic morphological remains are very scarce, in fact almost

non-existent.[11] The abundance of species at the start of Cambrian times (500-600 x $10^6$ years) suggested that life did exist earlier; to find the evidence for this life required the development of micropaleontology, which utilized the enhanced visibility afforded by the microscope.

In 1954, S. Tyler and E. S. Barghoorn announced the presence of microfossils in the Gunflint Chert,[12] a sedimentary rock from Minnesota which was dated by radioactive dating techniques as approximately 1.9 x $10^9$ years old.[13] The significance of this finding was large and twofold. In the first place it provided evidence for the occurrence of life hundreds of millions of years earlier than had previously been demonstrated. Secondly, it established a scientific approach and technique which has continued to provide exciting information on the occurrence of microorganisms at various stages in the Earth's history. Barghoorn,[14] P. Cloud,[15] B. Nagy[16] and others have applied the powerful electron microscope to their search for microfossils in terrestrial sediments and also meteorites. The most impressive results, in terms of numbers and quality, which have appeared in the past several years have been the work of J. W. Schopf and Barghoorn. An excellent example of this work appeared in 1968,[17] in which is discussed the identification of well recognized microscopic morphological remains in the Bitter Springs Formation from Australia (1-1.5 x $10^9$ years). Recently Engels and Nagy _et al._[18] have described microfossils present in the Onverwacht Series of South Africa (3.2 x $10^9$ years), the oldest known sedimentary rocks on Earth.[18]

Certainly this sort of identification is not without its perils,[19] but there can be little doubt as to the authenticity of most, if not all, of the results recently described for sedimentary rocks.

Micropaleontology seems capable of providing the most straightforward and visible proof of the existence of various organisms during the early period of biological evolution, which may also be the latter portion of the period of chemical evolution. However, it is limited in that it cannot provide information for those periods of time before which definite and preservable forms for microorganisms were existent. To approach this period of time, it was necessary to hypothesize another type of fossil. It was, again, during the 1950's, that this question arose. The question which arose was whether or not it was possible to demonstrate the occurrence of biological indicator(s) on a molecular level. If such biological markers could be found, might it not be reasonable to expect certain of these compounds to be capable of survival over the entire period of the Earth's history, now estimated to be approximately 4.5-5 x $10^9$ years old?[20] Any biological marker possessing this degree of stability is a chemical fossil.* Indeed it was reasonable, and in the 1950's organic geochemistry (which can be defined as the chemistry of organic compounds within a geological environment) in collaboration with organic chemistry, biochemistry, and geology set forth the criteria for such chemical fossils and suggested specific possibilities.

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*It is important to distinguish between these two definitions. Biological markers are molecules which verify the existence of biological activity. Chemical fossils (or molecular fossils) are biological markers which have the additional characteristic of being stable, within a geological environment (sedimentary), for periods of time approaching the age of the Earth.

The remainder of this thesis is concerned with the critical examination of the criteria for chemical fossils and the utilization of the chemical fossil concept to providing clues to the origin of life on Earth. Integration of organic geochemistry with chemical evolution, micropaleontology, comparative biochemistry, etc. will be made, when warranted, for elucidation and completeness.

Ab initio determination of the criteria for chemical fossils did not appear to be a major problem. The first step is to set forth the criteria which set a molecule apart from other molecules as a biological marker. The criteria for a biological marker are three: 1) The molecule must have a specific and characteristic structure; 2) the structure of the molecule must be relatable to known biosynthetic sequences; and 3) this compound should not be produced in significant amounts, relative to other compounds, in any reasonable abiogenic synthesis of the compound type. When such a biological marker also has chemical stability for the billions of years of geological time, it fulfills the four criteria demanded of a chemical fossil. What follows is a critical examination of these criteria, in which the necessity of each and the sufficiency of the four is considered.

1) The molecule must have a specific and characteristic structure. The architectural skeleton of the organic molecules to be considered as chemical fossils must possess some unique arrangement of at least some of its atoms, an arrangement which can be unambiguously identified. There must be something unusual about the structure of such molecules. Whether this combination of atoms is a strange pattern

of branching, a specific stereochemical arrangement, an unusual arrangement of ring structures, etc. is of small consequence--there must be something extraordinary. This is certainly a necessary criterion, for one would expect, and in fact it is true, that within the geological environment there exists the potential to synthesize a wide variety of organic molecules (Chemical Evolution), and this extraordinary structure should set the biological markers apart from these general organic molecules.

2) The second criterion, closely related to the first and the third, is that the structure of the biological marker must be relatable to a biosynthetic sequence. This criterion often seems to be unnecessary, and indeed this may be so. What it says is that there must exist, within living organisms, the biosynthetic mechanism to form this type of compound, and to form it from structurally nonspecific starting materials. The importance here lies in the fact that if a molecule is relatable to a known biosynthetic sequence there is a rationalization for its occurrence, a rationalization which is dependent upon the living system. It is important to realize that "relatable" does not demand that the exact compound considered be produced biosynthetically. There may have been distinguishable and/or understandable minor changes in the biosynthetic sequence with time, or the originally biosynthetically produced compound may have undergone transformations, which, though major, would not detract from its utility as a biological marker. The only viable criticism of this criterion is that there may exist a situation in which the biological marker decided upon may not be a product of

biosynthesis, but may be a product of biological alteration of a compound, perhaps even of a compound not formed biologically. Such a product would still be indicative of the presence of life. However, it would probably be easier to establish the biogenicity of biosynthesized compounds than of those produced by biological alterations.

3) The third criterion for a biological marker is also intimately related to the first and second criteria, and is concerned with the question of abiogenesis or biogenesis. The more involved discussion of this matter occurs later in this thesis (Chapter IV); at this time the basic criterion will be explicated. What this criterion says is, that if a group of compounds is abiotically synthesized, or produced by abiotic alteration of previously synthesized compounds, there should not result, as a prominent constituent of the mixture, the very compounds which are taken as biological markers. The necessity of this criterion is obvious and generally accepted. The debates which have arisen in recent years,[21] and which will be discussed later, arise from acceptance or nonacceptance of proposed abiogenic processes as reasonable, and from the limits at which a compound is "prominent" or "significant".

4) The fourth criterion, which determines the use of biological markers as chemical fossils, is that of stability. The matter for concern here is straightforward survival of the compound. If a given compound is accepted as a biological marker, but by virtue of its chemical instability cannot generally be expected to survive for several billion years, its usefulness is obviously limited. For with this particular compound one could not hope to trace life back

in time beyond the lifetime of the compound itself. Such instability may arise from the tendency of the compound to undergo chemical reactions under the influence of the temperatures, pressures, pH, etc., found in the geological environment. If such chemical reactions result in a loss of the structural specificity previously noted as necessary, the usefulness of the compound as a chemical fossil is terminated. Certain reactions which alter the biological marker may, indeed, not prevent it from being used, and in some cases in which a series of compounds might be considered as valid indicators of biological activity, even complete destruction of some of this series does not prevent the remainder from being used. A hypothetical case is the total destruction of some of the biological amino acids, while the remaining amino acids are examined for optical activity.

Of course, the author realizes that it is possible to gain much organic geochemical information from short- or medium-lived compounds, but this information covers only short or medium periods of the Earth's history, and the organic geochemist must consider the entire history of the Earth (and perhaps longer) if he wishes to find the complete answer to the origin of life problem.

Having considered the four basic criteria used by organic geochemists to characterize chemical fossils, the conclusion is that certainly the need for structural significance, lack of abiogenic synthesis and stability is real and that relatability to biosynthesis, though desirable, is not absolutely necessary. In defense of the wide use of this last criterion, and indeed its use in this thesis, is the fact that a suitable biochemical alteration product, such as

mentioned previously, which fulfills the other criteria, has not been established.

The question then arises as to whether or not the four above mentioned criteria are sufficient. This perhaps is best answered by considering other indicators, if any, of biological activity which would manifest themselves in the individual compounds. Probably the one best single indicator (though certainly not a proof) of the presence of biological activity is the occurrence of optical activity. The specificity of organisms for only one of the two possible optically active configurations of such compounds as amino acids is certainly a valuable source of information. But to impose this requirement on a chemical fossil is to eliminate many compounds which, though certainly biological, either do not possess asymmetric carbon atoms (e.g., β-carotene) or possess asymmetric carbon atoms which are quite readily racemized (amino acids).[22] The possibility, however, of optical activity being the only biologically significant structural feature can be accomodated by the first criterion stated earlier.

The above says that the absence of optical activity does not prove or disprove the absence of biological activity. It must also be quickly and emphatically stated that the presence of optical activity does not prove the presence of biological activity. Chemical reactions and physico-chemical phenomena may result in the selective formation of one of the two possible optical isomers. There are a number of reports in the literature which purport to do precisely this, although such reports must be viewed with considerable caution.

Havinga[23] has reported the selective crystallization of one of two optical isomers (with no significant preference for one over the other) in a system in which that portion of the substance remaining in solution is capable of rapid racemization. More recently, Garay[24] has reported a selective destruction of one of two isomers of tyrosine under the influence of radioactive strontium. This experiment lacks statistical verification and must be performed with a mixture of the two isomers. The selectivity exhibited by various metal complexes, such as the cobalt complexes of Allen and Gillard,[25] as discussed and extrapolated by Calvin,[5] provides a means for the exclusive formation of one optical isomer of peptides.

Autocatalysis is the mechanism by which these single events could be propagated. This stereospecific autocatalysis, as defined and explained by Calvin,[5] is capable of forming, abiotically, optically active compounds such as amino acids. The existence of this capability precludes the conclusion that optical activity in ancient chemicals is proof of the existence of biological activity at that time.

There is one more indicator of biological activity which can be of use to the organic geochemist, namely the biological fractionation of isotopes. This phenomenon, which is documented for C, O, and S, has been investigated to a considerable extent,[26] and has been frequently accepted as proof of biological activity in the case of studies done on entire fractions or extracts.[27] It must, however, be realized that the isotopic fractionation (particularly that of carbon) in biological systems is simply the result of physical and

chemical processes.[28] Abiotic chemical reactions and physical processes, similar to those responsible for biological fractionation, will also result in an isotopic discrimination; the only question is one of the relative magnitude.[29] Most primitive Earth syntheses are high-energy processes which would be expected to have miniscule isotope effects compared with the low energy processes within biological systems in which kinetics play a large role. A mild thermal treatment of $CH_4$, a relatively low-energy process, perhaps also a catalytic process, would preferentially form $^{12}CH_3\cdot$ versus $^{13}CH_3\cdot$ and the higher molecular weight compounds formed would be enriched in $^{12}C$. Admittedly any equilibration of the heavier hydrocarbons with $CH_4$ would reverse this fractionation. The magnitude of these abiotic fractionations is difficult to predict. Melander discusses some C-C bond breakage experiments in which the $\frac{K_{^{12}C-^{13}C}}{K_{^{12}C-^{12}C}}$ is 0.981.[30] In other such experiments involving most likely a C-O bond cleavage, $\frac{K_{^{14}C-O}}{K_{^{12}C-O}}$ is $0.88^9$ at 0°C, and $0.91^4$ at 24.75°C.[31] These data, which verify the theory as detailed by Melander, show the possible importance of abiotic chemical reactions as well as temperature factors in isotopic fractionation. To insist that a mixture of compounds or that a single compound is biological because its isotopic distribution is the same as known biological compounds, is to draw invalid conclusions. To conclude a mixture or compound is abiotic because of its $^{13}C/^{12}C$ ratio is just as invalid, for the reverse of the above mentioned situation is also possible.

Outside of such indirect evidence as cited above, there are no data which support or refute the suggestion proposed here. Until

such data are available it is necessary to consider as a real possibility that the phenomenon of isotopic discrimination may be due to abiotic processes, and to excercise restraint in attaching significance to isotopic distributions.

In spite of the above possibility, much use has been made of isotope ratios in sediments.[27] Such measurements have been used to study the origin of the insoluble kerogen in sedimentary rocks, and to support the claim of indigenosity or migration of extractable carbon compounds.[27,32] Such studies are certainly not to be discounted, and will become more useful as this approach is extended to more samples as well as single components of complex mixtures.

Again, the criticisms and ambiguities resulting from such possibilities as the above abiotic fractionation render isotopic distribution invalid as a necessary criterion for a biological marker. In passing, it should also be pointed out that this same conclusion, of non-validity, would be reached if one considered only the fact that the isotopic fractionations of carbon are due to the photosynthetic process, which certainly did not exist in the most primitive organisms. Such a limitation would also result in rejecting isotopic fractionation as a criterion.

The above discussion gives the organic geochemist a starting point for his experimental work--it lays down the rules to be followed. The next step is to choose, from the vast repertoire of biological compounds, those which are useful as biological markers, and then to test their stability to decide their suitability as chemical fossils. This portion of this thesis proposes to review

the information and decisions which have led to the selection of specific compounds or groups of compounds as chemical fossils; all of the major classes of biological compounds found in present-day living systems will be mentioned. Reports of the findings of the compounds will be mentioned and discussed.

There are four major classes, or types, of compounds (mostly biopolymers) dominant in living organisms today. These classes, in which we shall include also the monomeric species, are: carbohydrates, nucleic acids, proteins and peptides, and lipids.

Carbohydrates

The first class of biological compounds to be considered here is the carbohydrates. Probably the only definition which can define all members of this class is that a carbohydrate is a polyhydroxyaldehyde or a polyhydroxyketone, or a substance which yields such aldehydes and/or ketones upon hydrolysis. Chemically these substances are generally divided into three classes on the basis of the number of monomeric units. Thus there are monosaccharides (e.g. glucose and ribose), oligosaccharides with 2 to ∿10 monosaccharides (e.g. sucrose), and polysaccharides (e.g. cellulose, starch and glycogens).[33] Geochemists tend to base some of their definitions on solubility characteristics and on the manner of isolation, a disadvantage which frequently leads to ambiguities and confusion. Thus in the case of carbohydrates, there are two classes, the "free" and the "combined". According to Degens,[34] "free sugars are those that can be extracted from sediments with $H_2O$ or 80% EtOH without a preceding acid hydrolysis....combined carbohydrates require an acid treatment for their final release".

To consider the usefulness of carbohydrates as biological markers, it is necessary to apply the previously discussed criteria. The question of structural specificity becomes a matter of the determination of a very subtle arrangement of atoms. The occurrence of a polyhydroxy ketone or aldehyde, with no designated arrangement of the hydroxy groups, is not to be considered a specific structure but a random structure. The fact that 5 and 6 carbon sugars predominate can be interpreted as a chemical, nonbiological phenomenon.

The only significant structural specificity found in carbohydrates is concerned with the stereoisomerism. Natural carbohydrates generally have the D- configuration at $C_2$, although some L- sugars do exist in nature.[35] Also, of the 8 D-aldohexoses possible, only five have been found in nature.[36] Such subtle anomalies could permit the monosaccharides to be used as biological markers, in spite of their seemingly general structure.

The fact that polysaccharides are generally polymers of a single monosaccharide linked in a regular way (as the alpha glucoside linkage to $C_4$ of successive D-(+)-glucose units in amylose) is not to be considered a structural specificity, since such linkages could easily be favored for abiotic, chemical reasons. The singularity of the monosaccharides in a polymer may be due to an abiotic availability of this particular monomer or to some abiotic interaction, perhaps autocatalysis, which promotes dehydration between these identical units.

The same can be said about many of the oligosaccharides. However, certain of these compounds are composed of more than one monosaccharide. Again, these are not necessarily specific structures and only if natural oligosaccharides are shown to be selective and not general, in the same manner as discussed for monosaccharide distributions, can these sugars be considered biologically formed.

The need for a biological marker to be related to a known biosynthetic sequence is easily fulfilled in the case of carbohydrates. The path of carbon in photosynthesis, as described by Bassham and Calvin[37] has been elucidated to show the origin of various carbohydrates. The route to the polysaccharides has also been investigated and demonstrated.[38]

Difficulties as to the suitability of carbohydrates as biological markers arise when their ease of abiogenic formation is considered. Numerous recordings of sugar formation under non-biological conditions have been made, some of which fit into the "primitive-Earth atmosphere" class. In 1861, Butlerow[39] obtained a complex mixture of sugars from formaldehyde in dilute aqueous alkali. More recently, and more germain to chemical evolution, ribose and deoxyribose have been reported by a number of workers under presumed primitive-Earth conditions.[40,41,42] As is pointed out in Lemmon's[7] and Ponnamperuma's[10] summaries of abiogenic syntheses, although no specific sugar has been established as a product of a $CH_4$-$NH_3$-$H_2O$ primitive atmosphere, such a synthesis is not difficult to visualize and indeed almost certainly occurred.

The conclusion which seems inevitable, from the above brief discussion, is that in general carbohydrates are not usable as biological markers. Only if a geochemical occurrence of saccharides stereochemically identical and distributionally similar to the naturally occurring saccharides can be found, do the carbohydrates seem a likely biological marker.

If carbohydrates can be used as biological markers, it is necessary to evaluate their utility as chemical fossils by determining their geochemical stability. It is necessary to consider the major factors which might reduce the amounts of these compounds within the geological environment, and this includes such phenomena as microbial action and thermal stability. Results reported by Whittaker and Vallentyne reinforce the concept of the microbial destruction of

large fractions of sedimentary sugars.[43] Microbial destruction would probably be selective and upset the needed distributional pattern of the sugars. Certainly other factors contribute to the observed rapid initial decrease in sugar concentration with depth of sediment, and these are discussed by Vallentyne[44] and in the work of Rittenberg *et al.*[45]

Thermal stability also suggests a limit to the utility of carbohydrates as chemical fossils. Unfortunately, no thermal stability studies have been reported which permit an accurate estimation of the half lives of either monosaccharides or polysaccharides. Vallentyne[44] presents the data of Staudinger and Jurisch;[46] however, the only conclusion that can be reached from these data is that the decomposition of cellulose is a complex process dependent on numerous factors, none of which were examined in sufficient detail. Puddington[47] reported on the thermal degradation of various mono-, di- and polysaccharides. From a chemical standpoint, the data and details are insufficient to permit a calculation of half-lives of the various carbohydrates. Only for starch at 200°C and 210°C can a half-life be calculated. At 200°C, the calculated half-life is between 216-495 hours, while at 210°C, the value is 61-150 hours. Again, it must be emphasized that this data is insufficient to attach large significance to it. Its possible usefulness is the qualitative conclusion that sugars can be expected to decompose within a relatively short period of geological time.

The only feasible means by which carbohydrates might survive for long periods of time is if they are stabilized by complex

formation. Vallentyne discusses this matter,[48] and reports that indeed the interaction of saccharides with clay minerals does slow down the decomposition rates. The effectiveness of these interactions is still uncertain.

The polyfunctional sugars have been suggested as a significant progenitor of kerogen, the insoluble organic matter in sediments.[49] This hypothesis has not yet been unequivocally demonstrated; however, chemical transformation of saccharides is almost certainly a major cause for their disappearance in sediments.

An additional influence within sedimentary environments is the redox potential at the time of and subsequent to deposition. Sugars are very susceptible to chemical alterations, and as is demonstrated by Prashnowsky et al.,[50] their abundance in recent sediments is a reflection of the redox potential at the time of deposition. In sediments obtained from the Mohole project, Rittenberg et al.[45] found that "carbohydrates are more easily eliminated than other components of the organic matter in the first stages of diagenesis" in an oxidizing environment.

Having considered the potentiality of carbohydrates for survival over long periods of geological time, it is useful to turn to the literature reports of the occurrence of saccharides in various geological environments. Vallentyne has reviewed the findings up to 1963.[44] Much of this work has been concerned with sugars in soils, lake waters, recent sediments, etc., and it is this work which demonstrates the selective and rapid alteration of sugar content in sediments. F. M. Swain and co-workers have probably done the most

extensive analyses of carbohydrates in ancient sediments,[51] and report the presence of sugars as far back as the Early Precambrian, including several sediments in excess of 2 x $10^9$ years.[52] (See Tables 1 and 2.) His data suggest that after the initial rapid decline in carbohydrate concentration, the concentration of total sugars remains roughly the same for billions of years, again suggesting a protective mechanism such as binding. Recently, Oberlies and Prashnowsky[53] have reported rather high concentrations (10-25 mg/kg) of seven individual sugars in the 2.1 x $10^9$ year old Witwatersrand System.

In considering the possibility of using carbohydrates as chemical fossils, one must consider all of the above information. The most important factor is whether or not it can be stated, with a reasonable certainty, that a given mixture of saccharides has a biological origin. The mixtures found within a geological environment will reflect the contributing species of plant and animal life. In addition, the amounts of individual monosaccharides will depend not only on the monosaccharides present in the contributing organism, but also on the past-depositional situation, including formation of monosaccharides by depolymerization. The geochemical importance, relative and absolute, of any of the many factors operating within the sediment environment has not been sufficiently determined to permit one to draw final conclusions.

Certainly one of the most powerful arguments against placing the highest priority on this class of compounds is their facile synthesis by abiotic means. This certainly is not to say that past

Table 1

Monosaccharides in Precambrian Samples (ppm)[52]

| Geological formation | Sugars Gal | Glu | Ara | Unkn | Total |
|---|---|---|---|---|---|
| Coutchiching | -- | -- | -- | a) | -- |
| Thomson | 0.19[a)] | 0.19[a)] | 0.09[a)] | -- | 0.47[a)] |
| | -- | 0.41[b)] | | | |
| Rove, Gunflint | -- | 0.29[b)] | -- | -- | 0.29[b)] |
| Rove (Evenkite) | -- | 0.87[a)] | -- | -- | 0.87 |
| | | 0.30[b)] | -- | -- | |

[a)] Paper chromatographic analysis.

[b)] Enzymatic analysis.

Table 2

Total Carbohydrate Contents of Precambrian Rocks[52]

| Formation | Locality | Type of Rock | Tot. Carboh. (ppm) | Abs. max. (nm) |
|---|---|---|---|---|
| Coutchiching | Ont. | Chlor. schist | 6-14 | 488 |
| Soudan | Minn. | Carb. schist | 7-8 | 488 |
| Thomson | Minn. | Argillite | 25* | 486 |
| Cuyuna | Minn. | Argillite | 16 | 486 |
| Biwabik | Minn. | Argillite | 0-tr | 488 |
| Biwabik | Minn. | Algal chert | tr | 490 |
| Rove | Minn. | Argillite | 0-tr* | 490 |
| Rove | Ont. | Carb. argill. | 15* | 484 |
| Rove | Minn. | Evenkite | 0-tr | 490 |
| Wynniatt | Victoria Is. | Argillite | 9 | 488 |
| Killiam | Victoria Is. | Argillite | 0 | - |

*Presence of D-glucose verified by glucose-oxidase test.

studies or future studies are invalid; it does say that additional groundwork is needed and that caution must be exercised, especially when one is concerned with samples from the "Very-Early" Precambrian.

Nucleic Acids

The importance of nucleic acids to living substances has recently received much attention and research effort. Though certainly not the most abundant of the biopolymers, the significance of nucleic acids within living systems demands their consideration by the organic geochemist. The nucleic acids differ from carbohydrates and peptides by being constituted of monomeric units, nucleotides, which are a composite of three dissimilar entities, a sugar (ribose or deoxyribose), phosphate, and a purine or pyrimidine base.

The structure of the biopolymers has been extensively studied and it is known that generally only five bases are found in these nucleic acids. Adenine and guanine are purines; and thymine,

cytosine and in RNA, uracil (in place of thymine) are the pyrimidines. The specific arrangement of these bases on the ribose-phosphate chain has also been studied. The ratios of the various bases to each other have been determined.[54] In a very few cases, the exact sequence of these bases in several low molecular weight RNA's has been determined.[55]

The geochemical importance of the above mentioned studies is that they provide the geochemist with a basis for comparison when the polymers or monomers are isolated from a geological environment.

It is useful then to again consider the criteria for chemical fossils, to try to decide if this class of compounds has potential for the organic geochemist. Some mention has already been made concerning the specificity of the nucleic acid structure. Certainly the combination of base sequence and base ratios constitutes a degree of structural specificity sufficient to meet the geochemical criterion. The biosynthetic pathways to the ribose and bases has been studied,[56] as well as the route to the polymer.[57] Although this study is not complete, the available information is sufficient to meet the second geochemical criterion. At this point it can also be stated with certainty that no abiotic primitive-Earth synthesis of such a complex molecule as RNA or DNA has been accomplished.[7,10] In fact, from "primitive-Earth-atmosphere" experiments only adenine of the five bases has been demonstrated; from primitive-Earth experiments, other bases (not including thymine) have been produced. By combination with various compounds also produced in primitive-Earth experiments, nucleosides, nucleotides, and polymeric nucleotides (of only a single base) have been synthesized. Again, no combination of bases has been

polymerized to give a nucleic acid remotely similar in chemical composition to the natural substances, and it must presently be concluded that the third criterion of non-abiotic synthesis has been fulfilled. However, further progress in chemical evolution may necessitate a disposal of this class of compounds for this reason.

To transform a biological marker into a chemical fossil, it must be a stable species. Certainly the sugar-phosphate and base-sugar bonds are subject to hydrolysis in the aqueous environment of sedimentation.[58] This means that the geochemist should be concerned with a search not only for large segments of fossil nucleic acids, but for small segments of perhaps only a few monomeric units or, more likely, for the individual bases separated from the sugars. Possibly in protected environments such as within fossil shells, etc., preservation of large nucleic acid fragments is possible. These have not been found, though they would be destroyed by the rather vigorous processes used to isolate the individual bases.[59]

Before considering the geochemical search for this class of compounds, one must question their thermal stability. One study, of direct geochemical significance, on the thermal stability of the individual bases, has been carried out by Minton and Rosenberg;[60] no comparable study has been done on the nucleosides, nucleotides, or nucleic acids. The results of these workers is that at 25°C adenine and cytosine have half-lives of $\sim 10^6$ years, guanine and uracil have half-lives of $\sim 10^5$ years, and thymine has a half-life of $<10^3$ years.

Although such half-lives are based on aerobic decomposition in the solid phase, and result from extrapolation of values determined

at higher temperatures, they do suggest that any study concerning these compounds in Precambrian environments would be difficult to perform and positive identifications subject to question. Since from the previous discussion it would seem that a comparison of the amounts of the individual bases is of absolute necessity, the extremely short half-life of thymine as well as guanine and uracil would preclude such a study.

Attempts to isolate purines and pyrimidines from geological environments have been rather limited. In 1964 Rosenberg[59] reported a very useful study on sediments from the experimental Mohole, the ages of which vary between 0 - $25 \times 10^6$ years. Until Rosenberg's report, and since this report, virtually no additional statements on the occurrence of these substances have been made. Rosenberg's work is valuable because it incorporates the variations with increasing age, permitting some correlation with his stability studies.[60] Although he states that his findings are compatible with the order of stability, the absence of uracil in all samples would seem to either contradict this or require another explanation.

The conclusion must be that if a polymer of polymer segment of nucleotides with a geometrical configuration or sequential constitution resembling that of today's nucleic acids could be found, this would indeed constitute strong evidence for biological activity. However, the scant data available on geochemical study of nucleic acids and their composite bases leads one to the conclusion that, although by proper knowledge of sequences and relative base or base/sugar concentrations, nucleic acids are useful biological markers,

for the organic geochemist interested in extending his studies into the Early Precambrian, thermal stability precludes the use of nucleic acids and nucleic acid residues as chemical fossils.

Proteins, Peptides, and Amino Acids

The importance of the amino acids (and their polymeric forms, the proteins and peptides) to the living systems has been known for many years. This class of compounds has received an enormous amount of attention from the chemical evolutionists and from the organic geochemists. To chronicle the entire effort in this area would demand a separate treatise, beyond the scope of this thesis. However, an attempt is made to note those findings which contribute directly to the goals of this thesis.

In a situation quite analogous to the nucleic acids, the discussion of the proteins and peptides is largely, though not exclusively, a discussion of the monomers, the amino acids. Hydrolysis of the polymers within the aqueous sedimentation environment, is to be expected[61] and the search for individual amino acids is more

likely to bear fruit. At the start it must be realized that geochemists have been concerned with free amino acids (those not formally bound to the rock matrix or to other amino acids) and combined amino acids (liberated by acid hydrolysis and presumably either from polymers or bound to the rock matrix).

Living systems produce and utilize about 20 α-amino carboxylic acids, and evolution has resulted in a situation where, almost exclusively, only one of the two possible stereoisomers is used in these systems.[62] The detailed structure, including the stereochemical configuration of all these common amino acids, has been known for a long time. Much is also known concerning the amino acid sequences in proteins and peptides.[63] Thus, when the organic geochemist confronts the question of choosing amino acids as chemical fossils, he finds that nature seems to have provided a structurally specific narrow group of compounds--20 L-amino acids. However, there is nothing very unique about the structure of these 20 amino acids, aside from their stereoisomeric configuration. In fact, the only other structural feature common to all is the $-NH_2$ group alpha to the -COOH; the residues are quite varied. Whether or not the stereoisomeric selectivity is a result of biological action or the result of non-biological phenomena has been discussed earlier. The conclusion from that discussion, when applied to amino acids, is that this stereoisomeric selectivity is not a proof of biogenicity.

Certainly the sequence of amino acids in geochemical polymers, if correlatable to sequences in biological systems, eminently fulfills the criterion of structural specificity. Considering the

narrow range of amino acids found in biological systems, with a knowledge of the distribution of these acids and a possible correlation with optical activity, it must be admitted that the structural requirement of biological markers is met, in both the polymeric and monomeric portions of this class of compounds. But again the geochemist may have to find a biological distribution within a compound class.

As in the case of the carbohydrates and the nucleic acids, a great deal of work has been done to elucidate the steps in the biosynthesis of amino acids.[64] Also, the biosynthesis of the proteins has been extensively studied,[65] and it is accurate to say that the criterion for biosynthetic information is fulfilled for this class of compounds.

The question of the abiotic synthesis of amino acids has received a great amount of attention since Miller's report in 1959.[9] An extremely large range of alpha amino acids has been produced in primitive Earth experiments. Lemmon[7] reports these findings in tabular form in his review, and Ponnamperuma[10] also reviews the many reports of such syntheses. A few of the amino acids common in biological systems today have not been reported--e.g., histidine and methionine. In addition to the individual acids, peptides can be formed quite readily under primitive Earth conditions.[66] Fox has done a considerable amount of work on heating amino acids to give "proteinoids"[67] and he also finds a certain selectivity--or "non-randomness" of the amino acids incorporated.

The facile synthesis of amino acids under many abiotic conditions raises considerable doubt as to their merit as biological merkers, a doubt increased by the fact that often the same acids prominent in biological systems are also prominent in abiotic syntheses.[7,10] Again, to satisfy the criterion of non-abiotic synthesis one must broaden his scope and discuss the distribution of the amino acids and their optical activity as well.

The geochemical stability of the amino acids has been studied and discussed by Abelson[68] and by Vallentyne.[69] Both of these workers subjected various amino acids, in dilute aqueous solution, to elevated temperatures (approx. 200°C - 300°C) and measured the disappearance of the acids with time. Abelson combines his data with that of Conway and Libby[70] and finds a half-life for alanine of billions of years at room temperature. He also classifies a number of the other naturally occurring α-amino acids as stable, moderately stable, and relatively unstable.

Vallentyne[69] provides an extensive discussion of his experiments and shows the percentage of a given amino acid which would be expected to survive if held at a given temperature for certain time periods. Again, the conclusions suggest that some of the more stable amino acids would survive for billions of years. Vallentyne does warn that some of his results can be deceiving, since they do not include various geochemical factors, such as protection by fossils, adsorption, etc.

Degens[71] provides some discussion concerning the question of proteins and/or peptides being hydrolyzed to simpler compounds,

and notes that work on shell and bone amino acids[68] indicates that rapid depolymerization is to be expected.

Any attempt to evaluate the utility of amino acids as chemical fossils must take into account all of the fact and factors discussed above. From the discussion of the use of amino acids as biological markers, one must conclude that it is the distribution of the amino acids and not the presence or absence of single compounds which permits their use as biological markers. It must also be realized that unless some general preserving influence has been exerted, it is not possible to directly compare amino acid distributions from Early Precambrian samples with more recent or modern distributions. (Admittedly, such a comparison may not be permissible anyway without a more detailed knowledge of the amino acid distributions in the primitive contributing organisms.) In order to utilize distributions as a chemical fossil, the stability data must be accomodated. Unfortunately this stability information is far from complete. A more detailed knowledge of the geochemical stability is needed. It would also be valuable if the stability data could be used to provide information on the initial concentrations and distributions of amino acids in the sediments. It should also be restated that ancient amino acids need not be optically active since racemization without destruction is a relatively facile process.[22]

The conclusion of the above most be that without an operating preservation phenomenon, the stability information available presently is insufficient to permit amino acids to be used as chemical fossils.

Although amino acids, free and combined, have long been reported as present in various geological environments, it is only with great caution that workers have recently attempted to isolate, identify, and draw conclusions from amino acids in ancient sediments. Concerning the proteins and peptides, considerable effort has been directed toward these substances in the protected environments of shells and other calcerous structures. Abelson reports the presence of proteins or peptides in fossil specimens as old as $1 \times 10^6$ years, but finds no such substances, only free amino acids, in $25 \times 10^6$ year old shells.[72] More recently, Florkin has published an extensive manuscript[73] describing the occurrence of fossil proteins in a number of calcerous bodies, some ranging in age up to $400\text{-}500 \times 10^6$ years. He has also studied the amino acid constituents of these fossils.

As has been mentioned, there are numerous reports of amino acids within various environments. For a detailed review of the early reports, one should consult the report of Abelson.[74] Turning to the more recent reports of free amino acids in ancient sediments, one finds results in direct contradiction to the experiments of Vallentyne[69] and Abelson.[68] Degens[75] discusses the amino acid profile in an experimental Mohole core, and points out the unusual differences between the amino acids of the sea water and the sediments, and discusses the origin of this and other phenomena.

The most direct contradictions are the recent reports of amino acids in Early Precambrian sediments. Within the past few months, two reports have stated the occurrence in these old sediments. The

first, by Oberlies and Prashnowsky,[53] lists 11 amino acids from the Bulawaya Formation of Rhodesia (2.6 - 2.7 x $10^9$ years). They stress the effect on amino acid preservation not only of the processes to which the rock has been subjected, but also the nature of the initial substances. They attempt to demonstrate the preservative significance of additional functionality such as the presence of sulfur, or an additional $-NH_2$ or -COOH group.

Certainly the most dramatic recent report of amino acids from Early Precambrian rocks is that of Schopf et al.[76] These workers have used extreme caution in an attempt to rule out laboratory contamination. They examined the Bitter Springs Formation ($\sim$1 x $10^9$ years), the Gunflint Iron Formation (1.9 x $10^9$ years), and the Fig Tree Series (3.2 x $10^9$ years). They examine "free" (extractable by ammonium acetate leaching) and "combined" amino acid (obtained by HCl hydrolysis). Only glycine and $\alpha$-alanine were found in the leachates. Twenty one amino acids were detected in the hydrolysate. Of particular interest is the fact that the quantity of amino acids detected is inversely related to the ages of the cherts, possibly due to gradual chemical degradation.

More recently, the optical activity of the amino acids isolated from the Fig Tree Series has been determined.[77] Previous work, as discussed above,[22] suggests that these amino acids should be completely racemized. This is not the case; the amino acids possess almost exclusively the L-configuration! This surprising result suggests either an excellent preservation of the amino acids for 3 x $10^9$ years, or recent contamination, perhaps through micro-

cracks, etc., in the rocks.

Abelson and Hare[78] have recently provided evidence which suggests that the latter explanation is correct. In addition to supporting the previous work on the Gunflint Chert, finding essentially the same distributions of amino acids, predominantly L, these workers gently heated the Chert and found that the amino acids were racemized and partially destroyed. Combined with a permeability study, this stability study implies that the amino acids in the Gunflint Chert are epigenetic and not syngenetic with the inorganic rock matrix.

Certainly Abelson and Hare's report does not settle the debate as to the suitability of amino acids as chemical fossils. Each geological sample must be critically examined, individually, to determine the answer as to the origin of these amino acids. However, caution is a necessity before attaching significance to any result.

## Lipids

The fourth major class of compounds found in biological systems today is the lipid fraction. By definition, the lipids are those compounds extractable with common organic solvents,[79] and such a broad definition results in a broad spectrum of compounds being included in this class. For the purposes of this discussion it is best to subdivide this class and consider various compound types by themselves. The subdivision used here is one of geochemical convenience more than of biochemical tradition, for it tends to group compounds together on the basis of method of analysis and geochemical significance. Such grouping provides one with four "sub-classes" of compounds:

A) Pigments
B) Polycyclics
C) Hydrocarbons
D) Esters, alcohols and acids

This is not meant to be comprehensive listing into which all the biological molecules not already discussed can be fit, but it does include the vast majority, and experience suggests no major compound, or type of compound of general geochemical interest, is excluded.

### A. Pigments

Pigments are generally limited, geochemically, to mean the carotenoids and the porphyrins and chlorins. Other pigments, such as quinones, polynuclear phenols, authocyanins, etc., are generally discounted because of their more limited distribution in nature or their tendency toward rapid alteration in the geological environment.[80]

Turning then to the carotenoids, one again must apply the four criteria for a chemical fossil. The first of these, a unique structural characteristic, seems to be eminently satisfied. The polyisoprenoid structure based on the polymerization of $C_5$ units results in a unique carbon chain possessing a methyl group on every fourth carbon. In the case of the carotenoids, two such chains are joined to produce a mid-chain 4-carbon unbranched fragment.* Such a structure appears to be non-random and specific enough to fulfill the first criterion for a biological marker.

The biosynthetic route to the carotenoids has been well studied.[81] The formation of the polyisoprenoid chain is a classic in the study of biosynthetic mechanisms, and much effort has also been directed toward discovering the mechanism of joining two such isoprenoid chains[82] as well as the mechanism of formation of the terminal rings found in certain carotenoids.[83]

There has been absolutely no report of the synthesis of a carotenoid in any sort of promitive Earth experiment. Although synthetic polyisoprenoids are known,[84] no synthesis has been demonstrated which incorporates the unbranched four-carbon piece symmetrically into the middle of the chain. More attention will be given to the polyisoprenoid synthesis later, but the conclusion regarding these compounds, the carotenoids, is that no abiotic synthesis has produced them.

It is, in part, thermal stability which precludes the use of carotenoids as chemical fossils. Mulik and Erdman have subjected

*For a further discussion of the significance of this fragment see Chapter V of this thesis.

β-carotene to thermal treatment in a sedimentary environment.[85] Rapid decomposition of the carotenoid to smaller aromatic molecules such as toluene, xylenes, naphthalenes, and ionene occurred.[86] Although in nature the xanthophylls (O-containing carotenoids) are 3-10 times the abundance of the hydrocarbon carotenoids, in sediments this ratio is much lower--suggesting an even greater destruction of xanthophylls than hydrocarbon carotenoids.[87] Certainly thermal degradation is not the only destructive force operating on carotenoids; light, oxidation, enzymic conversions, etc., all cause rapid destruction of these pigments.[88] Nevertheless, over geological time, the thermal process probably will be the most significant factor.

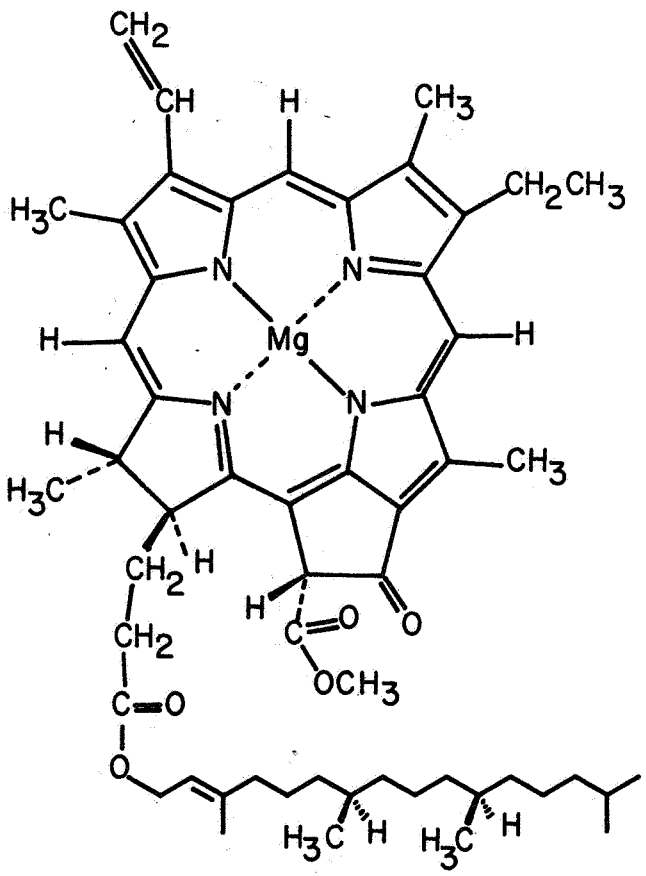
A consideration of the reports of the occurrence of carotenoids in sediments demonstrates that indeed they are very unstable. There have been numerous reports of their occurrences in recent sediments and these are reviewed by H. N. Dunning.[80] In this laboratory a recent sediment has been analyzed, and the occurrence of carotenoids in it will be discussed in Chapter III. The oldest sediment in which carotenoids have been demonstrated to occur is a 100,000 year old gytta.[89] It is conceivable that the unsaturated carotenoid could become fully reduced in the appropriate sedimentation environment, and such a reduction should preserve the basic structure as a carotane. Recently Eglinton,[90,91] and Robinson[92] have separately reported the occurrence of a $C_{40}$ carotane in the $60 \times 10^6$ year old Green River Shale of Colorado. This is, however, the only report of a saturated carotenoid.

Both the consideration of the criteria and the lack of reports of their presence in sediments seem to suggest that the carotenoid skeleton does not provide the geochemist, interested in ancient sediments, with a useful chemical fossil.

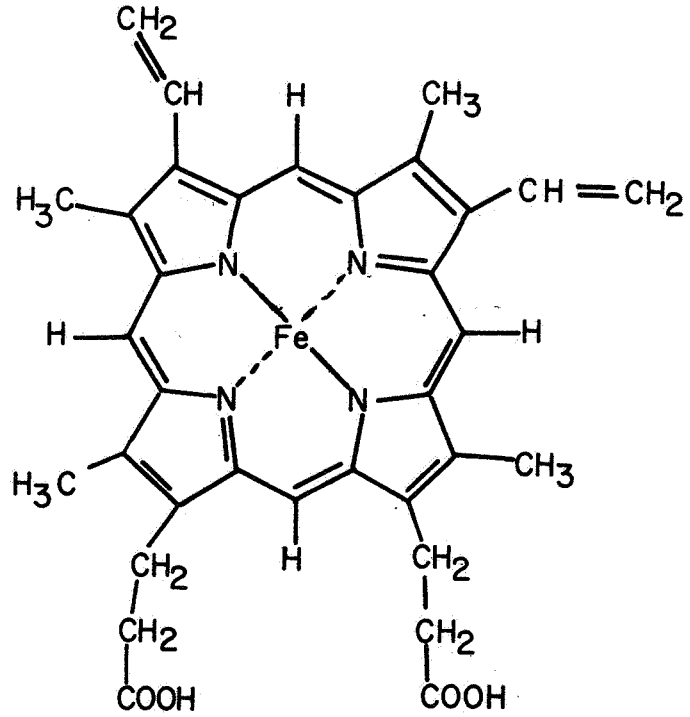
The tetrapyrrole pigments, the porphyrins and chlorins, constitute an extremely important group of biochemicals, because of their participation in photosynthesis as the primary energy conversion components, and in mammals as $O_2$ transporters.[93] Since the evolution of the photosynthetic mechanism was of great importance in the evolution of living systems, this type of compound becomes extremely important to the organic geochemists.

In fact, Treibs'[94] isolation, in 1934, of porphyrins from bituminous rocks is considered one of the initial milestones of organic geochemistry. Ever since that time considerable attention has been given to the search for these compounds in various geological environments. It is, however, still necessary to apply the four criteria to this group of pigments.

The structures of heme and chlorophyll-a are shown in Figure 1. Both of these structures consist of four pyrrole nuclei attached by one-carbon bridges and specifically substituted at the peripheral position by 1,2 and 3 carbon chains. In the case of chlorophyll, there is a long-chain alcohol bound to one of these sidechains via an ester linkage. The geochemical search for these pigments has been concerned primarily with the basic tetrapyrrole nucleus since the long-chain alcohol would be quickly removed in the environment of sedimentation.[95] (This side chain will be discussed later.)




CHLOROPHYLL – A




HEME

XBL6811-6693

Figure 1. Structures of chlorophyll-a and heme.

The tetrapyrrole nucleus is a relatively complex structure, so complex that it has been felt to be proof of biological activity. However, if one considered this nucleus as a cyclic polymer of a simple five-carbon heterocycle, joined by one-carbon bridges, the structure does not seem particularly unique. Therefore, one must take into account the distribution of the various side chains around the nucleus. The distributions found in such molecules as heme and chlorophyll-a suggest a selective positioning of these substituents, which, if also found to be present in the other biological pigments of this type, would seem to be structurally indicative of biological activity, thus fulfilling the first criterion for a biological marker. Even if this exact pattern of substituents is not found in other pigments, a limited number of substitution patterns would still adequately fulfill this criterion.

Once again, it is possible to state that the biosynthetic route to these compounds is well elucidated,[96] fulfilling the second criteria.

The abiotic synthesis of such a large molecule would seem to be an unlikely possibility. But again, considering it as a polymer, the "polypyrrole" nucleus might be synthesized in a primitive Earth experiment. Szutka[97] has reported several experiments which produce such a nucleus, but his starting materials are either too ideally chosen or, as in the case of δ-amino levulinic acid, not known from primitive Earth experiments. Recently Hodgson[98] has demonstrated the presence, in very small yields, of this tetrapyrrole nucleus from primitive Earth atmosphere experiments. That

porphyrins have been isolated seems of little doubt; whether or not the side-chain distribution, if present, is even remotely similar to that of biological systems is not known. These side chains may well be the only factor which permits the porphyrins to be used as biological markers.

The porphyrins are very stable compounds due to the aromatic ring structure of the tetrapyrrole nucleus. Dunning[80] has discussed possible reactions which might alter the pigment during sedimentation, but such diagenetic changes as hydrolysis (as mentioned above) demetallation, complexing with other metals, etc., do not alter the basic nucleuc-side chain structure. Blumer[99] has also discussed the long-term fate of fossil porphyrins and discusses the occurrence of homologous porphyrins resulting from decarboxylation and reduction of the side chains. Reactions which alter these side chains are extremely important since they could eliminate the very structural features necessary to utilize the compounds as biological markers. An Arrhenius activation energy of 53.5 kcal/mole has been given for the porphyrins,[68] which would suggest a half-life of $10^{18}$ years at room temperature. The conclusion is that while the original pigment will almost certainly be subject to considerable diagenetic alteration, the basic nucleus and non-functional side chains should be sufficiently stable to permit the use of these pigments as chemical fossils.

As mentioned earlier, a great deal of geochemical effort has been directed toward porphyrin and chlorin analysis. Dunning[80] provides a review of work in this field and from his discussion it

is evident that these pigments do exist in sediments ranging from the very young to the Cambrian. More recently this search has been extended into the Precambrian with some success. Probably the greatest effort has been that of Hodgson and coworkers, who recently reported on the presence of porphyrins, chlorins and polycyclic aromatics in nearly 250 soils, sediments and rocks, ranging in age from Recent to the Early Precambrian.[100] Of greatest interest here is the report of porphyrins in the $2.6 \times 10^9$ year old Witwatersrand System, and the $2.7 \times 10^9$ year old Soudan Formation.

Concerning the question of homologous series and side-chain occurrences, Hodgson has no comment, for the techniques used by him permit no such differentiation. The work of Blumer mentioned previously,[99] as well as that of Baker and coworkers,[101] does give some attention to this matter, but does not provide precise information as to the exact locations and length of these side chains. The difficulty in separating pure components differing only in the presence or absence of one carbon atom, or in the position of one $-CH_3$ or $-CH_2$ group is a formidable one. Encouraging signs that this may become feasible is seen in the work of Boylan et al.[102,103]

Success in the derivitization and volatilization of porphyrins should permit the organic geochemist to isolate and more accurately define the exact structure of these pigments as they occur in sediments. Correlation of this information with the biological structures, as explained earlier, should provide very strong evidence for biological activity. The only limitation remaining is that ancient non-photosynthetic organisms and the most primitive

life forms may not have contained these macromolecules. In spite of this limitation the porphyrins and chlorins must be considered one of the most valuable chemical fossils.

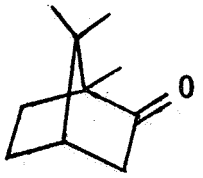
B. Polycyclics

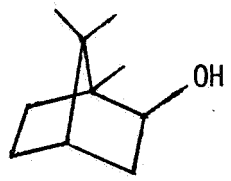
The geochemical subdivision of the lipids includes a group of compounds broadly classified as polycyclics. Although many types of biological compounds contribute to this group, attention here is directed to those which are the largest or most important geochemical contributors. This includes polynuclear aromatics, tetra- and pentacyclic terpenoids, and some heterocyclic compounds.

Many of the biological polycyclics are apparently too unstable to be preserved, in any easily recognizable form, within the geological environment. Included in this group are compounds containing strained rings, heterocyclics (especially those with several heteroatoms), and stable rings with highly reactive side chains. Many of these compounds by decomposition, combination, polymerization and reaction with other molecules such as sugars, nucleic acid residues, etc., are probably incorporated into kerogen. Although much work has been done attempting to elucidate the structure of kerogen, relatively little can be said about it; there does seem to be a close relationship between kerogen and humic acid. This relationship, as well as the importance of lignin to the kerogen and the chemical and biochemical transformations which are important in this chain of transformations, is discussed by Degens[104] and Swain.[105] Whatever its exact nature and origin, kerogen seems to be the repository for sedimentary organic matter which is not

sufficiently stable or non-functional to survive in isolation. Its utility in organic geochemistry depends on whether or not analytical methods can effectively isolate from it unaltered molecules which can be related to biological precursors. At the moment this cannot be done.

It is important here to note the recent report of the presence of camphor and borneol




<u>camphor</u> and <u>borneol</u>

in the 2 x $10^9$ year old Ketilidian sedimentary rocks of Greenland.[106] In most organic geochemical work done so far, such compounds would not have been detected due to the analytical processes used. This rather surprising occurrence must be substantiated because the significance of such presumably unstable compounds of such unique structure could be very important.

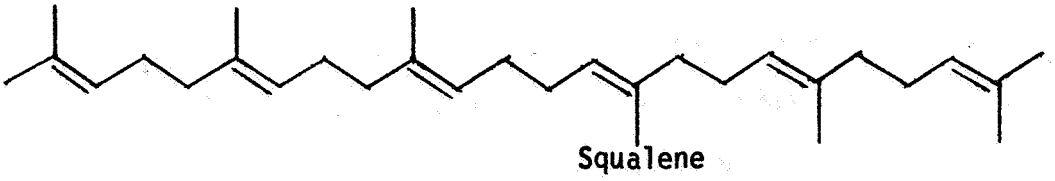
Heterocyclic compounds in coals and petroleum have been studied quite extensively because of their commercial significance. A large number of sulfur and nitrogen heterocyclic compounds have been identified in petroleums.[107] Also, flavinoids and similar compounds have been identified in some recent sediments.[108] Such studies may become very important in the future, but at the moment their scope is too limited, especially in regard to ancient occurrences of these compounds, to permit any definite conclusions

concerning the origin of life or presence of biological activity. It is reasonable to expect, in an abiotic process, that nitrogen and sulfur would be incorporated into aromatic heterocycles. Without much more information, these compounds cannot be used as biological markers or chemical fossils.

Polynuclear aromatic hydrocarbons can be easily formed by dehydrogenation of such cyclic compounds as steroids and triterpenes, or by some of the processes responsible for the formation of humic acids and kerogen.[105] However, they can as easily be formed by the abiotic reaction of carbon and hydrogen.[109] Even the presence of substituent methyl groups, such as would be expected if formation is from terpenoid compounds, is not very significant since these may also be easily formed in abiotic reactions. Such ease of formation, without a biological agent, demands that these compounds be rejected as potential chemical fossils.

The rejection of the polycyclic aromatics does not extend to the polycyclic aliphatics, especially the steranes and pentacyclic triterpanes. While the aromatic counterparts may be easily formed abiotically, and the aliphatics by a subsequent process, the latter have much greater potential for structural specificity, if the positional and stereochemical arrangement of substituents is considered. The structural specificity of the polyisoprenoid compounds containing a central four-carbon unbranched fragment has been discussed in connection with the carotenoids, and the comments made at that time are equally relevant here, since the steroidal and triterpenoid compounds in biological systems are formed by

cyclization of squalene, the $C_{30}$ analog of the carotenoid skeleton.[110]




As has already been suggested in the case of other compound types, in considering a class of compounds for organic geochemical purposes, it is best to concentrate on the basic carbon skeleton, and accept the fact that functionality such as hydroxyl, amino, vinyl and carbonyl groups will tend to disappear with time. The question then is one of whether the steroidal skeleton (or pentacyclic triterpenoid skeleton), is a useful chemical fossil.

The structural specificity of the steranes and triterpanes lies in their stereochemical preferences and in the positions of the substituents (mostly $-CH_3$ groups). The basic question here is whether or not these preferences as observed in biological systems are the result of the operation of a biogenetic mechanism or are due to the inherent geometrical preferences of the acyclic isoprenoid from which they are formed. Recently a great deal of attention has been given to the non-enzymatic (non-biological) cyclization of squalene and similar compounds.[111] Although some success has been achieved, it is only partial, for no one has yet reported the cyclization of squalene to the precise A-B-C-D ring structure of the steranes. However, should this be accomplished, the utility

of the cyclic terpenoids as biological markers is not necessarily invalidated since the starting material, squalene, has not been abiotically produced.

If squalene can be formed in a reasonable abiotic process, or if steranes can arise from other compounds (such as "regular" polyisoprenoids), the usefulness of the steranes and triterpanes as biological markers is reduced or non-existent. If not, a caution that should still be exercised would be against interpreting cyclic terpanes in geological situations as necessarily having been derived only from cyclic terpenoids in biological systems.

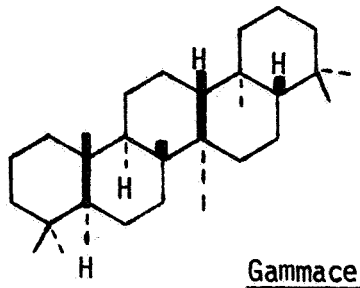
Following the discussions in the previous paragraphs, the conclusion is that with the information currently available, the criteria for a biological marker are fulfilled. However, this is a situation where such conclusions must be made with reservations and where future findings may necessitate a change in the significance attached to the compounds.

The question of geochemical instability of these compounds is probably predominantly a question of the alkyl substituents, and perhaps only in extreme cases a question of nuclear rearrangement or alteration. The stability of saturated C-C bonds has been estimated from pyrolysis data. From the data it has been calculated that there is an Arrhenius activation energy of $\sim$58 kcal/mole,[68] which amounts to a room temperature half-life of $\sim 10^{21}$ years. For hydrocarbons constantly maintained at 400°K the estimated half-life is $>10^{10}$ years.

Certainly the above values are not directly extrapolatable to geological situations;[112] however, they do suggest that hydrocarbons are sufficiently stable to be used as chemical fossils as old as the Earth itself.

There is a paucity of reports dealing with the geological occurrences of steranes and triterpanes. Most of the early reports, as noted by Bergmann,[113] have dealt with occurrences in petroleum. Until recently, techniques have not been developed sufficiently to permit separation of one compound from its isomers. Since this fraction of petroleum is considered to be important in the occurrence of optical activity in oil,[114] much attention has recently been given to this separation problem. Thus a number of reports have been made of particular components being isolated from petroleum, and their relationship to biological precursors has been discussed.[115]

In several sediments, particularly those with a large non-marine (terrestrial land-plant) contribution, the occurrence of steranes and triperpanes has been noted; most prominent among these sediments has been the Green River Shale.[116] Hills and Whitehead were the first to identify a pure sedimentary triterpane isolated by Cummins and Robinson; they showed it to be gammacerane:[117]




Gammacerane

More recently, Henderson et al.[118] have determined the structure of a large number of triterpanes and steranes from this Green River Shale. Another occurrence of steroidal compounds is in the 130 x $10^6$ year old Pierre Shale. Since this work was done in the author's laboratory, further data will be presented later.

Individual steranes and triterpanes have not been isolated from Precambrian sediments, although they have been reported in at least one instance. This report, of much interest, was that of Burlingame et al.,[116] who reported the presence of steranes in the 2.7 x $10^9$ year old Soudan Shale; however, there is some question about the age of the extractable hydrocarbons of this sample.* Other hydrocarbon analyses of old shales has not been sufficient to refute or support the presence of steranes in this era. This group of compounds does seem to be able to provide valuable information concerning the time and manner of evolution of various living systems. But again it must be stated that extra caution is needed in attaching significance to the steranes and triterpanes.

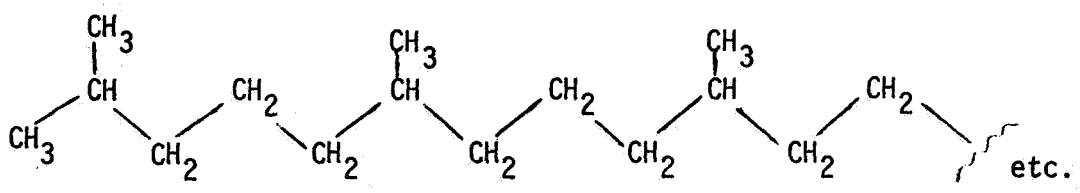
C. Hydrocarbons

Although widespread in the plant and animal world, hydrocarbons do not normally constitute a very large percentage of the total mass of such systems.[119] Nevertheless, as discussed in the previous section, these compounds are stable enough to survive for billions of years, may eventually accumulate, and should be

*For a discussion of this matter, see Johns et al.[32]

considered for use as chemical fossils. Eglinton and Hamilton[120] have reviewed the occurrences of alkanes in biological systems, and although most work on natural alkanes has been concerned with leaf waxes, wool wax, etc., these compounds are known to occur in many other biological situations. The majority of biological alkanes reported are the straight-chain or normal alkanes, the distribution of which, in nature, has been reviewed by Clark.[121]

The iso- (2-methyl) and anteiso- (3-methyl) alkanes seem to be the most widespread and abundant non-normal alkanes, particularly in plants, but also in bacteria.[122,123] The "regular" polyisoprenoid alkanes, as depicted below,




with a methyl branch on every fourth carbon, have also been found in various plants and animals.[124,125] These three types of alkanes, normals, mono-methyl branched and polyisoprenoid, have received the greatest attention and have been most thoroughly documented. Since reports of other alkanes do not permit generalizations, due to limited distributions, etc., only the above three types will be considered as potential biological markers.

Aside from the length of the chain, there is no structural specificity in normal, iso- or anteiso- alkanes. Only the anteiso-alkanes have an optically active center, but no stereoselectivity

has been reported to occur in these compounds. The examination of chain length distribution reveals that most biological systems form the odd-carbon-number normal alkanes.[121] In the case of the iso- and anteiso- alkanes, although some reports suggest a predominance of odd-carbon-number compounds in the iso- series and even-carbon-number compounds in the anteiso- series,[122] other work, with alkenes and alkanes suggests that such preferences are not universal.[123] In view of the relatively few reports on these compounds, no conclusion can be drawn as to the overall odd/even ratio.

The polyisoprenoid alkanes present the greatest structural specificity among the saturated hydrocarbons. The presence of a methyl group on every fourth carbon is certainly indicative of a biological specificity, the same specificity seen in the carotenoids and triterpenoid compounds. The added possibility of stereoselectivity at all or some of the asymmetric carbons increases the likelihood of these acyclic polyisoprenoid alkanes being structurally specific molecules; however, no one has yet reported on the configurations at these asymmetric centers in the alkanes.

The conclusion from the foregoing discussion is that the acyclic polyisoprenoid alkanes fulfill the criterion of being structurally specific. The iso- and anteiso- structures do not seem to be specific, although the presence of these methyl alkanes and the absence of the other methyl alkanes (i.e., 4-methyl-, 5-methyl-, etc.,) might be considered an indicator of biological

activity. This rests upon the assumption that the other methyl alkanes are generally absent from living systems, an assumption not necessarily valid.[126,127] The normal hydrocarbons can fulfill the specificity criterion only if their distribution is considered. The predominance of odd/even chain lengths, especially in the higher carbon numbers (i.e., $C_{25}$-$C_{35}$), would seem to provide an indicator of biological activity.

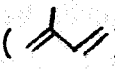
The biosynthesis of hydrocarbons has been studied to only a limited degree.[128] It has generally been assumed that these compounds arise either directly from, or in a manner analogous to, the long chain functional compounds such as fatty acids and alcohols.[120] In many cases, especially among the animals, these hydrocarbons may result from biogenetic processes acting upon ingested fatty acids and other lipids. Although the route to the exact compounds discussed here has not been determined, sufficient work has been done on related compounds to permit the statement that the criterion for biosynthetic knowledge is fulfilled.

The major objection to the use of both normal and monomethyl alkanes arises from the ease of abiotic syntheses of these compounds. Anxieties in this matter are well founded for such compounds have been formed by very simple processes. Perhaps the most widely known synthesis of these compounds uses the Fischer-Tropsch process, the equation for which is:

$$nCO + (2n + 1)H_2 \xrightarrow[200°C]{(Fe,Co\ Ni)} nH_2O + C_nH_{2n+2}$$

This process or a minor modification of it, is not unreasonable as a primitive-Earth situation. The catalysts used are not unlike many natural substances and the presence on the primitive Earth of suitable starting materials is likely. A survey of the Fischer-Tropsch literature[129] and results from this laboratory[130] show that normal, iso-, and anteiso- compounds can be produced abiotically in good yields.

Even the odd/even predominance of the n-alkanes can be produced in the laboratory by simple processes. Telomerization of ethylene (or ethane or acetylene) with any one-carbon species, such as CO or $CO_2$, and subsequent reduction to the hydrocarbon would give an odd/even predominance. Although the reasonableness of such a process, within the context of organic geochemistry, can be questioned and debated, the knowledge that this type of chain formation has been demonstrated[131] causes skepticism about the significance which can be attached to the distributions of normal hydrocarbons.

The abiotic synthesis of a molecule as complex as pristane or phytane has long been thought to be a very remote possibility. However, the fact that such molecules are simply polymers or modified polymers of such simple molecules as isoprene (  ) or 2-methyl-butane has recently led to speculation and doubts as to the suitability of these compounds as biological markers. The logic behind such questioning has been discussed by McCarthy and Calvin.[21] The fact that Studier et al.[109] have reported the synthesis of lower members of this series has lent credence to the doubts

surrounding these compounds. More will be mentioned later concerning the logic and validity of these abiogenic syntheses, but this author feels that the acyclic polyisoprenoid alkanes are still valuable as indicators of biological activity. The willingness to accept (with reservations) these compounds is due to the inability of any abiotic experiment yet reported to synthesize the $C_{15}$-$C_{20}$ (minus the $C_{17}$) acyclic polyisoprenoid alkanes in large amounts relative to other alkanes. Traditionally organic geochemistry has rested on the assumption that these compounds are proof of biological activity. The change from proof to suggestive evidence fortunately does not negate work already reported; it suggests the need for substantiation, and this is now being realized and carried out.

Accepting then, the acyclic polyisoprenoid alkanes (and less so the other alkanes discussed here) as possible biological markers, one need only question their stability. This matter has been discussed in connection with the triterpanes, and that discussion suffices for these acyclic compounds. With the fourth criterion fulfilled, one can conclude that these compounds deserve attention as chemical fossils.

Any attempt to discuss in detail the hydrocarbons found in oils and sediments would constitute many more volumes than this thesis is designed to be. Whitehead and Breger[132] present a table of over 200 hydrocarbons (some of which are aromatic) which have been isolated from oils. They note that this is <50% of the constituents of crude petroleum; it is to be expected that, in

addition, quite different hydrocarbons may be found in sediments, coals, etc. Since many of the results and discussion of this thesis are concerned with fossil hydrocarbons, the remaining discussion of occurrences and significances will be presented later in the thesis.

D. Esters, alcohols, and acids

The final group of compounds to be considered for use as chemical fossils consists of those long-chain alcohols, esters, and acids found predominantly in waxes, glycerides, phosphatides, etc. Although such fragments are often only a portion of very interesting and certainly biological compounds, the bonds joining these fragments to the remainder of the molecule are apparently too unstable to survive within the environment of sedimentation. Hydrolysis of ester bonds, dehydration of alcohols, reduction of double bonds, cyclizations, etc., are reasonable processes; even carboxyl groups may be reduced as demonstrated by Blumer.[99] The two most stable end products of such diagenetic transformations would be the saturated hydrocarbons and the saturated fatty acids, and it is these two groups of compounds which are potential biological markers and chemical fossils.

Turning then to the basic carbon skeletons found in this group of compounds, one finds that the majority of these compounds consist of skeletal structures already discussed--unbranched chains, iso- and anteiso- chains and acyclic polyisoprenoid chains. It seems unnecessary to repeat all the discussions relating to the suitability of these carbon skeletons for use as biological

markers. Perhaps the most valuable approach is to consider the distributions of these compounds and the contributions they will make to the hydrocarbon distributions already discussed.

The redox potential of a sediment is one of the most important factors determining the diagenetic processes which occur. It is closely associated with the presence or absence of various bacteria, which as mentioned in connection with carbohydrates, play a large role in transformations of organic (and inorganic) matter within sediments. The precise magnitude of all of the many factors operating during diagenesis is difficult, if not impossible, to measure, and certainly is unique for each sediment.[133]

Most marine sediments rich in organic matter are highly reducing.[134] It is reasonable, therefore, to assume that many long-chain functional molecules will ultimately be transformed into alkanes. Only those compounds originally present as acids or those oxidized to acids during the early stages of deposition would be expected to appear as acids in older sediments. Unfortunately no data are available to permit a prediction of the percentage of the sedimentary acids which would be reduced, in time, to alkanes. It is not unreasonable to anticipate some acids surviving for very long times. Similar to the case of carbohydrates,[48] the acids may become chemically bound, via the carboxylate, to the inorganic rock matrix, thus permitting preservation of at least a small fraction of the original acids.

Much attention has been given to the lipids present in plants,[135] bacteria,[136] etc. The great majority of these compounds have carbon

chains with an even number of carbon atoms. In the case of fatty acids, n-$C_{14}$, n-$C_{16}$ and n-$C_{18}$ are the predominant members. Any contemplation of the use of fatty acids for geochemical studies must accept the fact that a great deal of emphasis will have to be placed on the distributions among the normal acids. The branched acids present, as such, or derived from other long chain moieties, can also be used for geochemical studies. Unfortunately, the unusual mono-methyl- or cyclopropyl-acids found in such organisms as Lactobacilli and Micobacteria[135] may not be widespread enough to permit their use as general chemical fossils. On the other hand, the acyclic polyisoprenoid structures may be common to most sediments. Though the acids themselves apparently have a limited biological distribution,[137] the possibility of their formation by oxidation from phytol, the side chain of chlorophyll, may give them a wide distribution in sediments.

This discussion of the feasibility of using fatty acids as biological markers is based in large part on suppositions and probabilities which are either impossible to accurately state a priori or for which insufficient data are available to draw more tenable conclusions. Nevertheless, these compounds cannot be ruled out as possible biological markers.

The hydrocarbons which result from complete reduction of lipids are not likely to be a precise duplication of the carbon chains of the original molecules. Certainly other reactions, some due to bacteria in sediments, will alter some lipids in preference to others. One of the great lacks in organic geochemistry is a

knowledge of the diagenetic transformations which occur during sedimentation. One really cannot state what hydrocarbons will be produced in this way. Most of the suggestions for processes which occur are made after examining sediments, a very rough, if not invalid approach. Assuming a direct reduction, with no change in chain length, one can expect that even-carbon-number alkanes will be produced in large amounts, in accordance with the predominance of even-carbon-number fatty acids. On the other hand, if fatty acids undergo simple decarboxylation, the odd-carbon-number hydrocarbons would be expected to be dominant. In attempting to explain some results from recent sediments, Cooper and Bray[138] have proposed the opeartion of this second mechanism. Bendoritis[139] also used this idea in connection with the isoprenoid alkanes. Jurg and Eisma[140] have recently demonstrated that while such a process does occur, it is complicated by a number of other reactions, ultimately resulting in a great number of products. Meinschein,[141] on the contrary, has suggested that reduction is a major cause, not only of even-carbon alkanes, but also of the odd-carbon alkanes. Although this possibility cannot be ruled out, it is important to point out that the work on which this suggestion is based[142] is not definitive according to modern chemical standards. This hydrogenolysis reaction should be repeated to certify the loss of methane in the process.

The conclusion of this discussion of processes and compounds leading to alkanes is that the matter is too complicated to permit accurate statements. Since hydrocarbons have already been considered

as potential chemical fossils, the important question here is whether or not contributions from functional lipids demand any change in this consideration. The answer is that only if a combination of the two contributing elements cannot be distinguished from abiotic hydrocarbons, do these compounds lose their potentiality as biological markers and chemical fossils.

Most of the above discussion of both fatty acids and hydrocarbons is concerned with structural specificity attending the expected hydrocarbons and fatty acids, attempting to establish some basis for the expectations. The criteria of biosynthetic information, abiogenic formation and stability must also be attended to. Concerning the alkanes, all of these matters have previously been discussed. The only addition to that discussion is the fact that now there is perhaps a different distribution to consider in connection with abiogenic mixtures. One cannot say the criterion is <u>not</u> met without a knowledge of the geochemical distribution which results from all biological contributions.

The biosynthetic route to the lipids has been studied quite thoroughly, especially the biosynthesis of fatty acids.[143] However, formation of fatty acids by abiotic means has also been studied and shown to be feasible.[144] Johnson and Wilson have devised a scheme by which predominantly unbranched fatty acids would be abiotically synthesized.[145] In a manner quite analogous to the selective formation of odd or even alkanes, it has been shown that it is possible to telomerically form the fatty acids.[146] It is conceivable that mineral structures could limit such

processes so that only a very few members of the series might be formed. As in the case of the acyclic polyisoprenoids, no abiogenic mixture of fatty acids has yet been demonstrated which produces a biological distribution, and one must conclude that this criterion is fulfilled.

The stability of fatty acids has been mentioned, circuitously, in the preceeding discussions. Jurg and Eisma's[140] experiments demonstrate that these compounds are thermally labile. To invoke a protective mechanism by the minerals is a tenuous matter in view of their result that no hydrocarbons were formed in the absence of the clay mineral. (However, this does not mean some acid is not bound to the rock and stabilized.) Unfortunately, no geochemical stability studies have been reported which permit an estimation of fatty acid half-lives. Normally such compounds are considered to be very stable and one can conclude that fatty acids may be potential chemical fossils.

As has already been stated, the reports concerning the occurrences of alkanes in geological environments will be discussed later; the same is true concerning the fatty acids. Only a few comments will be made here concerning the reports on acids.

n-Fatty acids have been studied to a great extent, and at least one author has concluded that the biological predominance of the even-carbon-numbered acids disappears with time.[147] Certain exceptions would suggest a need for more careful analysis of older, Precambrian samples.[148] Only recently have the isoprenoid acids been reported to be present in oils and sediments,[149-154]

although in quantities considerably less than the normal acids. If acids are preserved by some interaction with the matrix, perhaps even the ancient sediments should contain these branched acids. Of great interest and importance is the report on stereochemical studies of the isoprenoid acids, isolated from the Green River Shale. By comparison with the acids made from natural sources it was possible to show that the geological fatty acids had a stereoisomeric distribution compatible with derivation from chlorophyll.[155] Results of this nature are quite definitive and indicative of the level of scientific sophistication organic geochemistry has attained in its brief history.

## Conclusion

The facts described in the preceeding pages provide the organic geochemist with a context within which he can easily operate. The variety of experiments which need to be done is multitudinous and the implications of his results far-reaching.

The decision as to which of the potential chemical fossils should be chosen for detailed study is a difficult one. Historically, the choice was simplified by a lack of knowledge, by expected situations and by available techniques.

Techniques for elaborate and complete separations of carbohydrates, amino acids, porphyrins and fatty acids were in initial stages of development. The various chromatographies--paper, thin-layer, electrophoretic, etc.,--were relatively new and unsophisticated. Derivitizations were also not well developed, limiting the

scope of most isolation techniques. One powerful tool did show promise, namely gas-liquid (or vapor phase) chromatography. This method had been used with considerable success in the petroleum industry, for separation of complex mixtures of hydrocarbons. Also, the mass spectrometer was in the initial stages of development and could be used for structure determination. Both gas chromatography and mass spectrometry had the additional capability of needing only small amounts (1-100 μg) for structure determination.

Since by the 1950's most of the stability studies mentioned earlier had not been carried out, hydrocarbons seemed to be the best choice; no one could be sure amino acids, fatty acids, etc., would survive for the billions of years perhaps necessary.

In addition to these very practical motivations, persons within the oil industry, such as Bendoritis,[139] had noted the occurrence of large amounts (relative to other branched and cyclic alkanes) of the isoprenoid hydrocarbons.

In any event, the ground work for a search for biogenic hydrocarbons, especially the acyclic polyisoprenoids, had been laid, and numerous investigators began to pursue the goal of tracing, via chemical fossils, the origin of biological activity. The expectations of these workers were fulfilled seemingly, in numerous situations, as reports of the presence of isoprenoid hydrocarbons in progressively more ancient sediments[32,156-161] and some extraterrestrial samples[162] began to be published. Attending these results was the additional information on other

organic geochemicals which prompted some of the further investigations mentioned earlier.

The extension of the organic geochemists' search to other classes of compounds could have been predicted at the very start of the program; the advances in instrumentation, separation science, and chemistry made this extension inevitable. As stability information became available it became reasonable to add new compound types to the list of potential chemical fossils. But certainly one of the major causes for such an extension came from that group of persons who questioned the fact that pristane and phytane were biological markers.

Whether it was the extraterrestrial findings, the regularity of the methyl branching, the fact that isoprenoid hydrocarbons could be found in nearly everything, or any combination of these facts or others, doubt as to the importance of these findings was expressed. Although more will be mentioned later about the reasonableness of attempted abiotic formations of these biological markers, such criticisms could not be set aside; they had to be considered. As already discussed, McCarthy and Calvin[21] have given attention to this matter, and their information suggests that although additional experiments (e.g., stereochemical determinations) may remove any doubts, at the present time, some doubt must exist concerning the biogenicity of all isoprenoid hydrocarbons. By the time of this conclusion, research with other biological markers had progressed sufficiently that it seemed reasonable to seek corroborative data from other sources. A more

scientifically based choice among potential chemical fossils could now be made, with the advanced techniques and knowledge available. The choices seemed to be between porphyrins, amino acids, and fatty acids. Fortunately for organic geochemistry, all three have been sought. Hodgson et al.[100] have done preliminary work on the porphyrins, Schopf and Kvenvolden and Barghoorn[76] have dealt with amino acids, and this laboratory has concentrated on fatty acids.

Ideally, all four compound types should be analyzed concurrently, within a single laboratory, on a single sample, and such results should be verified by reproducibility from laboratory to laboratory. Certainly such verifications (or refutations) will eventually be done. Presently the various reports stand isolated and this should be considered during interpretation. Only in the case of the fatty acids has a thorough concurrent analysis of the hydrocarbons been performed. A partial hydrocarbon analysis accompanies the report on porphyrins.[101]

In addition to investigations on other types of chemical fossils, it is certainly advisable to expand knowledge concerning the hydrocarbons to include other branched and cyclic compounds. In addition, extension of the study to different types of sediments--that is, sediments with widely varying geological histories, would seem informative. Any additional sediment, when analyzed for hydrocarbons, adds a piece to the puzzle being worked by the organic geochemists.

The remainder of this thesis is concerned with reporting the analyses of the hydrocarbons found in various geological samples and also the fatty acids and hydrocarbons of a number of these samples. The full implications of all the findings will be discussed.

# CHAPTER II

## EXPERIMENTAL

The procedures and experimental techniques described in this section of the thesis are the general processes used in this laboratory for the analysis of hydrocarbons and fatty acids in sediments. Because portions of these procedures have been published elsewhere,[156,130,163] emphasis will be placed on modifications and innovations designed to meet the demands of ultra-micro analysis and to avoid contamination. The quantitative results for the individual samples will be given when the results from that sample are discussed.

Portions of the work reported in this thesis do not involve, directly, the analysis of sediments, and the experimental details of these portions will be attended to when they are discussed. In the latter part of this section, the problem of laboratory and handling contamination is considered.

The Moonie Oil from Queensland, Australia was the first sample examined and was analyzed according to the basic procedure reported by Eglinton _et al._[156] The second sample analyzed was the Florida Mud Lake and was also analyzed, with some alterations, by the scheme indicated above. Although several other samples have been examined by the above procedure, these and the reamining samples were analyzed concurrently for hydrocarbons and fatty acids in a procedure similar to that of Eglinton _et al._[151]

The exact scheme used for the analysis of the Moonie Oil is given in Figure 2. The gas chromatography used for this sample is described by Van Hoeven et al.[164] The collected samples obtained from the sequential preparative gas chromatographies were analyzed by mass spectrometry using a modified CEC-103 (low resolution) mass spectrometer.

The Florida Mud Lake samples were analyzed according to the scheme in Figure 3. Thin layer chromatography was done using either Silica Gel G or $Ca(OH)_2$ as adsorbent. The ultraviolet-visible spectra were recorded on either a Cary 11 or a Cary 14 u.v.-visible recording spectrophotometer.

The remaining geological samples were treated according to the following schemes (Figures 4 and 5). Since portions of this procedure involve new techniques or modifications of old techniques, this procedure shall be described in some detail at this time.

The rock specimen used in the analysis has certainly been handled, wrapped, stored and shipped in such a manner that contamination is inevitable. For this reason, the outer surface is removed by means of a water-cooled diamond saw. This saw is thoroughly cleaned just prior to use, and washed with benzene and methanol. The amount of material removed in this way is occasionally limited by the sample shape and size. When possible, at least 1/4" was removed. Only in the case of the Gunflint Chert was this step omitted, and this will be discussed later.

ANALYTICAL SCHEME: MOONIE OIL

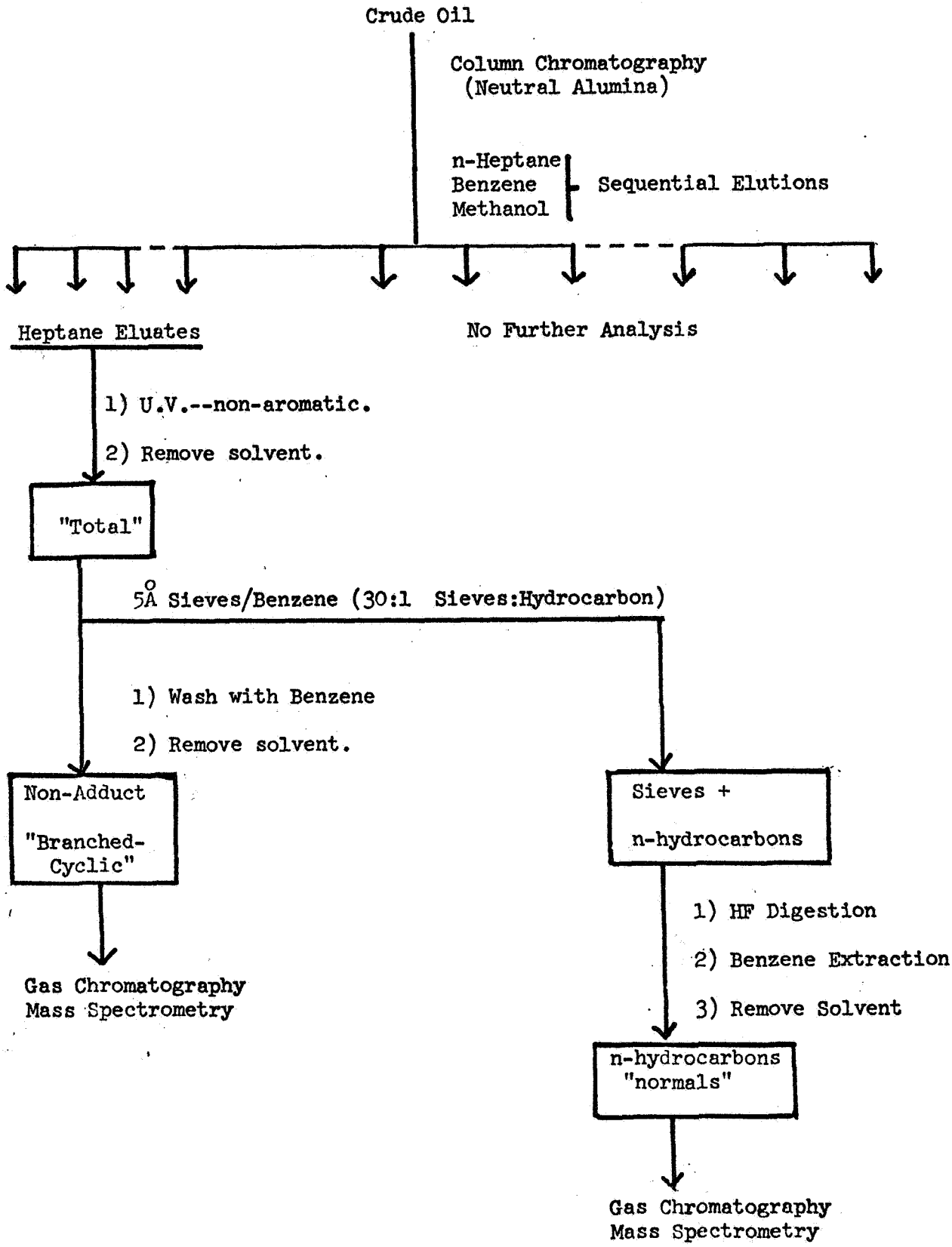



Figure 2. Analytical procedure for the analysis of the Moonie Oil.

ANALYTICAL SCHEME: FLORIDA MUD

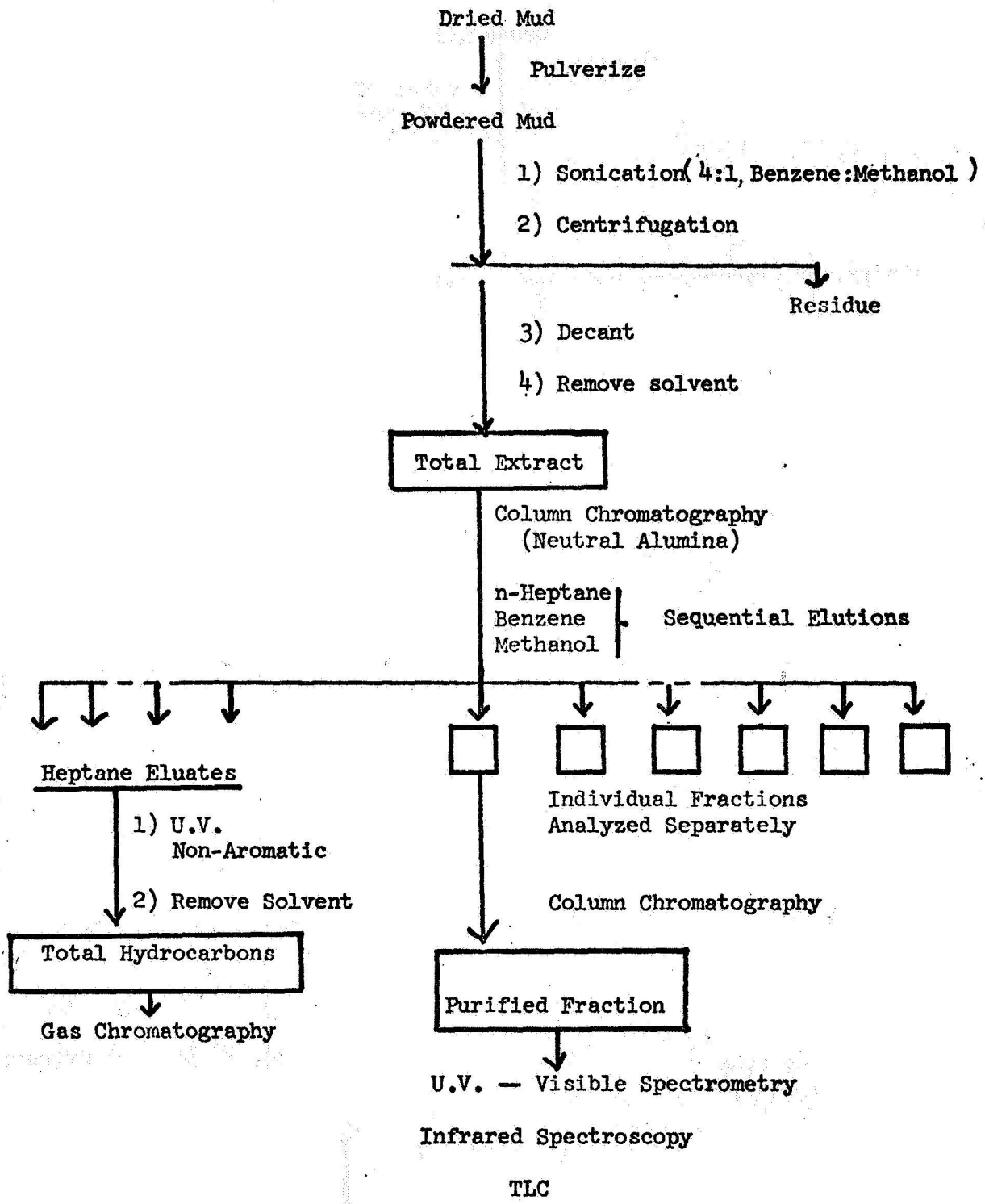
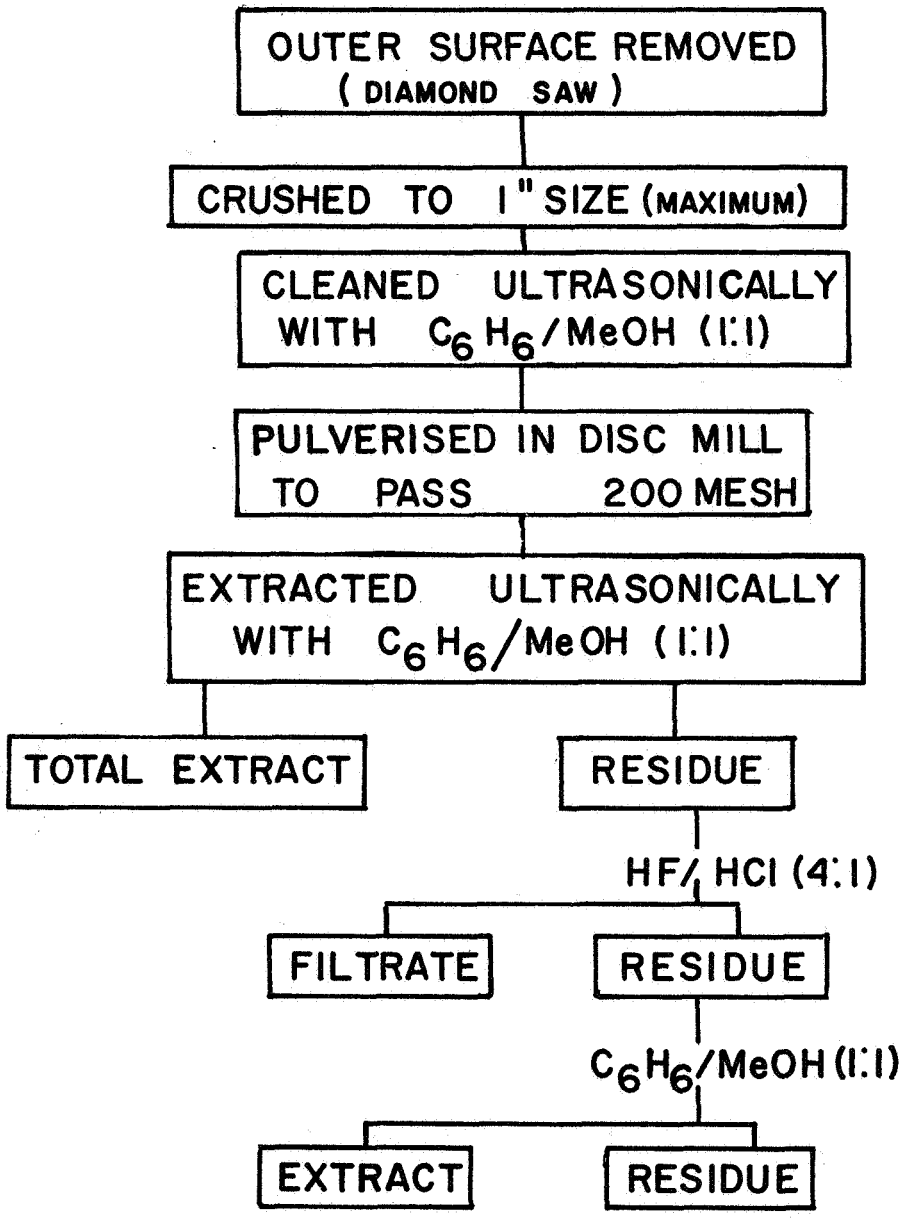


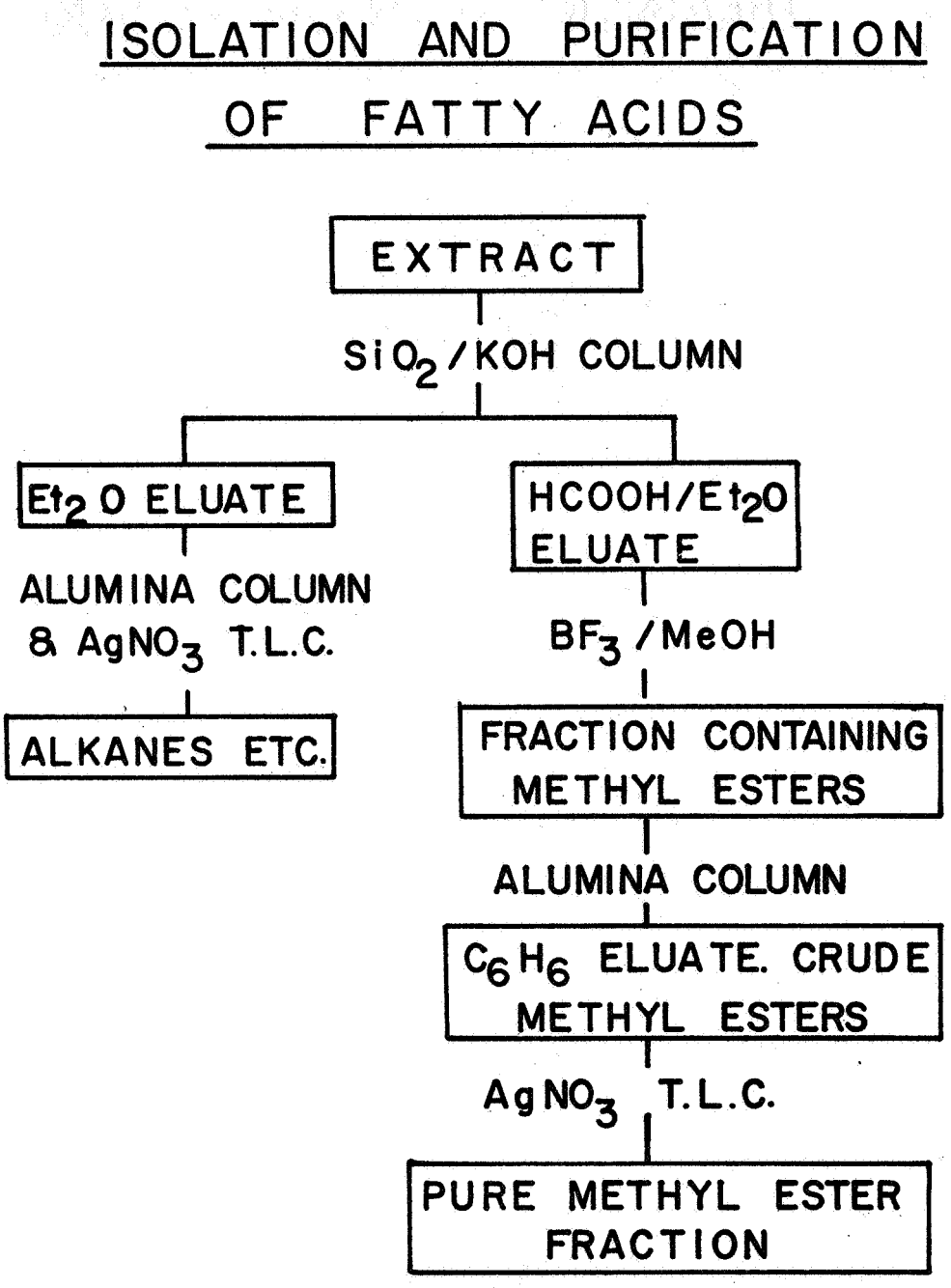

Figure 3. Analytical procedure for the analysis of the Florida Mud Lake samples.

# TREATMENT OF SEDIMENT SAMPLES




XBL 684-4143

Figure 4. Analytical procedure for obtaining sediment extracts.




XBL 684-4142

Figure 5. Analytical procedure for isolation of alkanes and fatty acids (as methyl esters) from sediment extracts.

The large rock segment(s) was crushed into small pieces by hand, by wrapping the sample in aluminum foil and striking with a hammer. These small pieces were placed in a beaker, covered with benzene:methanol (1:1) and sonicated for 30 minutes with a Mettler Electronics ultrasonic cleaner (M 1.5 -- over 1000 peak watts at 28 kc). This washing process was done at least twice. After the crushed sediment dried it was pulverised, by means of a disc mill, to pass through a 200 mesh screen. The disc mill used for this purpose is a Model 8701, Type T250 Laboratory Disc Mill produced by Angstrom, Inc., Chicago, Illinois. The mill is fitted with a Teflon gasket. The rock does not come in contact with any moving or lubricated parts of the machine.

After the rock has been pulverized, it is extracted ultrasonically with 1:1 benzene:methanol in order to obtain the extractable organic compounds. This extraction is carried out by placing no more than 100 g of pulverised sediment into a 250 ml centrifuge bottle, and adding at least 100 ml of the solvent. These bottles are kept covered during the sonication. The sonication process is sufficiently rigorous to prevent the sediment from settling. The sonicator used in this extraction is a Sonogen Automatic Cleaner, Model A-300 with a power output of 300 W and an operating frequency of 25 kc (available from Branson Instruments, Inc., Stamford, Conn.).

After centrifugation at 1000 rpm for at least 20 min, the solvent is pipetted off and evaporated with a Buchi evaporator, yielding the "total extract". This extraction procedure is

repeated a minimum of two times and may be repeated four times for organic-rich samples. This extract is then examined for saturated hydrocarbons and for fatty acids. The sediment remaining in the bottles is dried in a vacuum oven (∿20 mm pressure) at no greater than 65°C.

In order to obtain additional organic material from the sediment, it is necessary to dissolve the inorganic rock matrix. This dissolution, or digestion, is accomplished by using concentrated hydrofluoric acid (40-50% by weight) and concentrated hydrochloric acid (20 or 37% by weight) in the approximate ratio of 4:1. Since reagent grade chemicals are known to contain high molecular weight organic compounds, the possibility of the HF and HCl being contaminated with such compounds had to be considered.

Extraction of either acid with pure benzene, washing the benzene with water, and gas chromatographic analysis of the residue obtained from evaporation of the benzene showed the presence of a very large number of high molecular weight compounds ($C_{10}$-$C_{30}$) in a distribution not unlike that found in sediments. The HCl could be purified by continuous extraction with benzene for 25 hours, followed by six extractions in a separatory funnel, or it can be diluted to 21% and distilled, as are other solvents.

Hydrofluoric acid is normally shipped and stored in polyethylene containers and after extraction several times with benzene, and storage in polyethylene, still contains detectable amounts of organics (e.g., up to 1 mg/liter). In order to purify HF for use in this work, it was necessary to use a modification of the method

of Kwestroo and Visser.[165] In the process used in this laboratory, 500 ml of technical grade 70% HF (obtained from the Industrial Chemicals Division of Allied Chemical) is placed in a 1 gallon polyethylene bottle, the top of which has been cut off. Directly into this crude HF was placed a 400 ml Teflon beaker containing ∿300 ml of pure water. The polyethylene bottle was then covered by placing a 1/8" Teflon-covered wooden block on top and securing it by the use of a lead brick. After four or five days, the crude acid was replaced by fresh crude acid. After another 4-5 days, the HF in the Teflon beaker (now ∿400 ml) was poured into a pre-washed Teflon bottle and stored until use. In most cases this acid was used within one week. Titration showed the pure acid to be 40± 5% HF by weight.

Dissolution of the sediment residue was accomplished by slowly adding the sediment (800-1000 g) to a 4:1 mixture of HF:HCl (∿1500 ml total, contained in Teflon beakers) and stirring occasionally with a Teflon stirring rod. After 10-12 days the digestion seems to be complete and is unaffected by the addition of fresh acid. Magnetic stirring of the sediment-acid mixture is not advised in this situation, since the abrasive properties of the finely divided sediment tends to destroy the base of the beaker.

After digestion, the acid-residue mixture is diluted with water (1:1) and filtered through a sintered glass filter funnel using a water aspirator. The solid is washed several times with water to remove the HF/HCl. This filtration may take several hours or up to five days, depending upon the nature of the residue. The water

used throughout has been deionized and distilled by departmental apparatus and then is distilled in the laboratory from a basic $KMnO_4$ solution. It has been shown to be contaminant-free after this treatment.

Both the HF/HCl solid residue and the HF/HCl filtrate have been analyzed for hydrocarbons and fatty acids. If the HCl has been purified by benzene extraction, it is absolutely necessary to analyze the filtrate, since the benzene dissolved in the HCl seems to extract some of the organic matter during digestion. The filtrate is best examined by extracting several times (at least three) with benzene, washing the benzene with water to remove traces of acid, evaporating the benzene and treating this residue as in the case of the other extracts.

In order to efficiently extract the HF-HCl solid residue, it is necessary to dry it throroughly. This is best accomplished by heating in a vacuum oven at 80°C (>25 mm) overnight. The dried residue is generally very hard, and must be repulverized in the disc mill before extraction. The extraction procedure is the same as before, and centrifugation and evaporation of the 1:1 benzene:methanol gives an extract which is analyzed according to the following procedure.

The procedure used in this laboratory is ideally suited to the simultaneous analysis of the alkanes and fatty acids from a given sediment. All three extracts, the direct solvent extraction of the sediment, the benzene extraction of the HF/HCl, and the extraction of the HF/HCl residue are treated in the same manner.

The extracts are sonicated for a few seconds with diethyl ether to dissolve most of the organic compounds, including the acids and alkanes. This diethyl ether solution is then put onto a silicic acid/KOH/isopropanol column according to the description of McCarthy and Duthie.[166] The successive elutions provide an ether eluate, which contains, among other types of compounds, the alkanes, and an ether/formic acid eluate, which contains the fatty acids.

The isolation of the alkanes proceeds in a straightforward manner. The complex mixture from diethyl ether elution is column chromatographed on neutral alumina, the alkanes being eluted with n-$C_7$. The n-$C_7$ solvent is almost completely removed and the remaining n-$C_7$ solution is applied to a $AgNO_3$ impregnated Silica Gel G thin layer chromatoplate (10% $AgNO_3$), which is pre-washed with ethyl acetate. The sample is applied with an Applied Sciences Streaker (Cat. No. 17700) available from Applied Sciences Laboratories, Inc., State College, Pa. The final development of the plate is with n-$C_7$ in the case of the alkanes. Standard compounds (usually a normal alkane and alkene) are simultaneously chromatographed to permit determination of Rf values. Visualization is accomplished by spraying with a 0.2%, in ethanol, 2,6-dichloroflourescein solution and observing the plate under 254 nm u.v. light. The saturated alkane band is scraped off the plate and the alkanes are isolated by three extractions of the silica gel with diethyl ether. Evaporation of the diethyl ether gives the saturated alkanes which are then analyzed by gas chromatography and mass spectrometry.

The analysis of the fatty acid fraction also proceeds in a straightforward manner. After removal of the $HCOOH/Et_2O$ with a Buchi evaporator, a small amount of recently opened $BF_3/MeOH$ (Applied Sciences Laboratory) is added, and the solution is warmed for 5-10 minutes. After the methylation and destruction of excess $BF_3$ with water, the methyl esters are extracted from the $BF_3/MeOH$ + water by means of n-$C_6$ or n-$C_7$. If evaporation yields a substantial residue with large amounts of colored impurities, the sample is column chromatographed on neutral $Al_2O_3$ and the benzene eluate is then purified by TLC. In cases where the residue is small, it is purified by TLC directly. The TLC is done as before, except that the developing solvent is a 1:1 n-$C_6$:$Et_2O$ solution. The purified methyl esters are obtained in the same manner as the alkanes. It should be mentioned that some sediments have large percentages of sulfur which must be removed. In all cases discussed here, the sulfur present in the sample was removed by the $AgNO_3$-TLC, thereby making unnecessary the common removal of sulfur by a colloidal Cu column.[167]

The gas chromatography of the alkanes and the fatty acids was accomplished with a 100 ft x 0.01 in I.D. stainless steel capillary column coated with Apiezon L. Low boiling components in the Apiezon L had been removed by sublimating it at reduced pressure (<5 mm) for 24 hrs at 250°C. Using the residue to coat the columns resulted in g.c. columns capable of being used at up to 300°C, with little or no "column bleed". The actual coating of the columns was accomplished by Jerry Han.

The mass spectra of all but the Moonie Oil hydrocarbons were run on an A.E.I. M.S.-12 (low resolution) mass spectrometer. Some of the results were obtained by means of combined gas chromatography-mass spectrometry. Although this is not a new development, the mechanics of the interfacing, as worked out by D. Boylan and F. Walls and subsequently modified by J. Maxwell, P. Harsanyi, J. Han and W. Van Hoeven, demand some comment. The system used in this laboratory is presented in Figure 6.

The g.c. effluent, consisting of 1.5 - 3 ml/min He plus the individual components separated in the column, is split into two fractions by a simple T-connector. Approximately 20-40% (depending on the flow before the "T") of the effluent goes directly into the mass spectrometer without any enrichment. The pressure drop (as well as the determination of the fraction going into the M.S.) is effected by the 0.002" I.D. capillary. Two records of the g.c. column effluent are made: 1) the recorder tracing due to the signal from the flame ionization detector of the gas chromatograph, and 2) the pattern due to the ionization of the organic molecules in the ion chamber of the mass spectrometer. In theory these two tracings should be essentially identical, and in practice this has been the case. It is certainly desirable to have this double record to permit correlation of mass spectra with retention times, and to be certain mixing and inversion of retention times is not occurring. When no mass spectra are being recorded, the ionization beam is at 20 eV, insufficient to ionize the vast amount of He present in the chamber but sufficient to ionize the organic

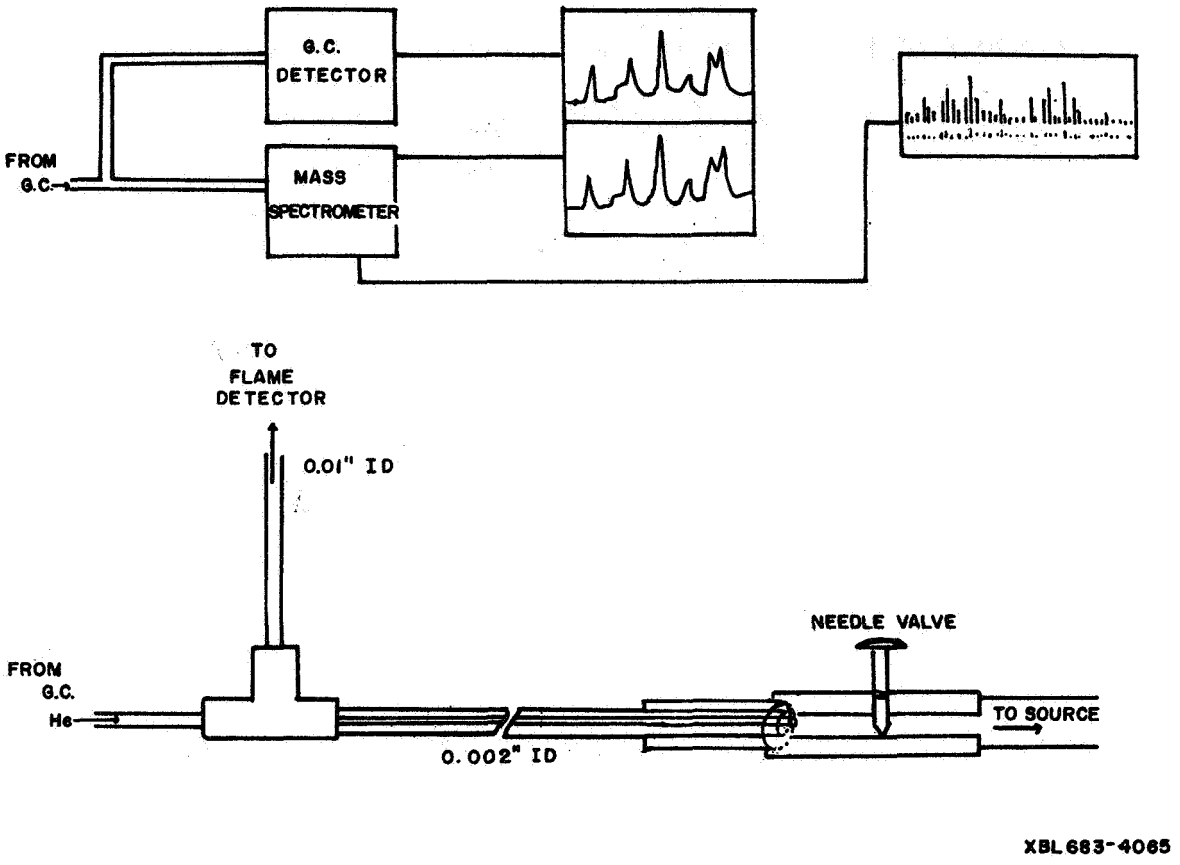



Figure 6. Schematic of gas chromatograph-mass spectrometer interface.

molecules therein. When the mass spectrum is taken, the beam energy jumps instantaneously to 70 eV to give the high energy spectra normally used to determine structure.

Proper placement of valves, the multiple-port source on the mass spectrometer, and the use of a dual column gas-chromatograph permit facile and prompt conversion to or from the combined gc-ms. The major difficulty with the system as described above is the decomposition and apparent adsorption of compounds on the hot stainless steel. Adsorption seems to occur in the case of the methyl esters and decomposition (via dehydrogenation) has been observed in the case of saturated hydrocarbons. Silanation of this stainless steel according to a modification of the method of McLeod et al.[168] has proved effective in reducing or eliminating this problem.

Certainly gas chromatography-mass spectrometry is the most powerful tool available today for ultramicro organic analysis. As more geochemical laboratories acquire the facilities, and as the facilities are modified and developed, more and more results will be made available to help solve some of the problems now extant.

As geochemists have decreased the amount of a given compound necessary for structure determination, they have increased the possibility of laboratory contamination. Contamination has been a problem for many years, and the passage of time has not decreased the problem. This discussion shall be concerned only with contamination due to treatment of the sample in the laboratory (or in the field) and not with a geological contamination such as the migration of young organic compounds into an ancient sediment.

Laboratory contamination can be identified and controlled. This positive statement is justified by the experiences of geochemistry in this lab and in other laboratories. Reagents used in the analyses can all be checked and purified. The equipment can also be checked for residual contaminants or artifacts. In all of the work reported here, each step in each process has been tested for contamination; in addition, the analysis for the fatty acids (and simultaneously the hydrocarbons) was begun only after the entire process had been carried out on 800 g of Sierra granite and showed no significant contamination.

It is not the intention of this author to provide a detailed account of each contamination check. Each solvent, each adsorbent, each organic chemical and each inorganic chemical were thoroughly checked, normally by using an amount in excess of that used in any analysis. Each mechanical operation, such as transfer, evaporation, etc., has been shown to be contaminant free. The most likely sources of contamination used in the analytical schemes previously outlined are as follows: 1) Benzene. This is the most difficult to purify of the solvents used in our procedures. Sufficient purification can be achieved by distillation of the reagent grade benzene through a 30 plate Oldershaw column with a variable reflux take-off, operating at a ratio of between 1:6 and 1:8, take-off:reflux. 2) The mineral acids HF and HCl. These have been discussed previously and purification methods presented. 3) The KOH. This reagent contains fatty acids, presumably as the potassium salts.[169] Pure KOH can be obtained by heating reagent grade

pellets (85% KOH, 15% water) to 500°C for 1-2 hours in a Pt or Ni crucible. In the case of the remaining reagents and equipment purification by distillation, washing with copious amounts of solvent, etc., are sufficient to insure against contamination. Some of the latter work reported here was performed in a laminer flow clean air cabinet (Agnew-Higgens, Model No. 43, or Model No. 168, available from Agnew-Higgens, Inc., Garden Grove, Calif.) to provide an additional precaution.

All of the above should not lead one to think that contamination is no longer a concern. Minor and perhaps imperceptible events may affect the purity of even a single reagent, once purified. A person tarring the roof of an adjacent building or smoking a cigarette outside of the laboratory (or inside) may pollute the air and this may contaminate the sample being examined. For reasons such as these, frequent, though not necessarily regular, checks of all reagents and processes should be carried out. Using such care and with a knowledge of the organic chemistry potentially or actually attending each manipulation, it is possible to restate that "laboratory contamination can be identified and controlled".

# CHAPTER III

# RESULTS

## THE FLORIDA MUD LAKE

The method of presentation of organic geochemical results depends on the aim of the research. In most cases in which an attempt is made to relate the results to evolutionary events, the chronological approach has been used, and it is this approach which will be used in this thesis. The chronological sequence, beginning with the most recent sediment and progressing to the most ancient, has the advantage of permitting one to view general changes which have occurred with time. The difficulty of this approach is that the nature and history of individual sediments may vary so much that direct comparison with other sediments, varying in age by perhaps billions of years, may be a tenuous comparison. The most informative study would be one in which the sediments examined had similar origins--that is, were formed in a similar manner geologically, and which had similar post-depositional histories, so that variation with time would have more specific evolutionary significance. Unfortunately, the sediments available, particularly those from the Early Precambrian, are too limited in number and type to permit much choice for such studies. Nonetheless, accepting the limitations and remembering that interpretations and extrapolations must

take these limitations into account, the chronological approach has been chosen for use here.

It has already been mentioned that it is desirable to concurrently analyze as many possible types of compounds as possible. Although the alkanes of many of the samples discussed here have previously been examined, the alkane distributions obtained in the concurrent analyses are presented, in part because of slightly different techniques having been used, and in part because some samples, though geologically related, were not from the same piece of rock as previous analyses.

The sediments examined and reported on here are:

| | | |
|---|---|---|
| 1) | Florida Mud Lake MW-0 | 2000 years |
| 2) | Florida Mud Lake MW-6 | 5200 $\pm$ 250 years |
| 3) | Pierre Shale | 75-80 x $10^6$ years |
| 4) | Moonie Oil | 160-200 x $10^6$ years |
| 5) | Antrim Shale | 350 x $10^6$ years |
| 6) | Nonesuch Seep Oil | 1 x $10^9$ years |
| 7) | Nonesuch Shale | 1 x $10^9$ years |
| 8) | Gunflint Chert | 1.7-1.9 x $10^9$ years |
| 9) | Soudan Shale | 2.7 x $10^9$ years |

The Florida Mud Lake represents, according to W. H. Bradley,[170] a situation quite analogous to that which must have given rise to the Green River Shale. This apparent analogy is based upon the fact that the Mud Lake constitutes an algal ooze and that it is a

fresh water sediment, and that geological and fossil studies of the Green River Shale[171] show a large contribution from similar algae to its formation. The theory that such an algal ooze results in an oil shale has been postulated for many years.[172] The examination of this recent sediment was undertaken for two basic reasons: 1) to obtain an alkane distribution for comparison with the Green River Shale, and 2) to correlate the hydrocarbon distribution of the sediment with the hydrocarbons found in various algae. The environment and nature of the Florida Mud Lake has been previously described by McCarthy.[163] The individual samples are numbered according to the distance from the mud-water interface--e.g., MW-0 represents the mud from the interface to a depth of 1 ft, MW-1 represents the mud between 1-2 ft, etc. The youngest of the mud samples is MW-0, with a $^{14}C$ date of ∿2000 years.[173]

The elemental composition of the dried mud is:

| | |
|---|---|
| C | 40.06% |
| H | 6.39% |
| N | 4.16% |
| S | 1.13% |

This sample (3.3183 g) was examined by pulverizing to a fine powder, (pulverization was accomplished with a Pica Blender-Mill, Model 3800, Pitchford Mfg. Corp., Pittsburg, Pa.), and then extracting ultrasonically in 200 ml of 4:1 benzene:methanol. Sonication was carried out with a Branson Sonifier, Model S-75, with a "Step-

Horn" Mechanical Transformer (Power output RF 75 w. ave. 150 w peak. Frequency, 20 kc/s). The solvent was decanted off and evaporated with a water aspirator to give the total extract. The heptane soluble fraction of this extract was column chromatographed on ∿4 g of neutral alumina (TLC grade) and eluted with progressively more polar combinations of n-heptane, benzene and methanol. In the first (of two) analysis of the mud, only the first 10 ml fraction, eluted with n-$C_7$, was colorless and, having been shown to be essentially free of aromatics by u.v. (240-260 nm) (Perkin-Elmer 202 u.v. visible recording spectrophotometer), this was used as the total alkanes (0.2 mg).

A second analysis was carried out more recently, to permit re-examination of the alkanes. The two alkane distributions were qualitatively similar, although the first analysis indicated that the lower alkanes, ∿$C_{17}$-$C_{18}$, were present in greater amounts than was evident in the second analysis; this is almost certainly due to small variations in technique.

The gas chromatogram of the total alkanes (second analysis) is shown in Figure 7 (Aerograph 204, 100' x 0.01" Apiezon L, program rate 2°/min, He flow 3 ml/min). The assignment of the normal alkanes is based on retention times, coinjection of some components and gc-ms of several peaks (e.g., n-$C_{17}$ and n-$C_{25}$). The relative areas of the n-$C_{27}$ - n-$C_{31}$ and n-$C_{33}$ compounds are approximately as follows: n-$C_{33}$; n-$C_{31}$; n-$C_{30}$; n-$C_{29}$; n-$C_{28}$; n-$C_{27}$ = 1.0; 6.3; 0.7; 7.4; 0.8; 3.2. Peak A appears to be composed mainly of two components, 6-methyl heptadecane and 7-methyl heptadecane, with a slight

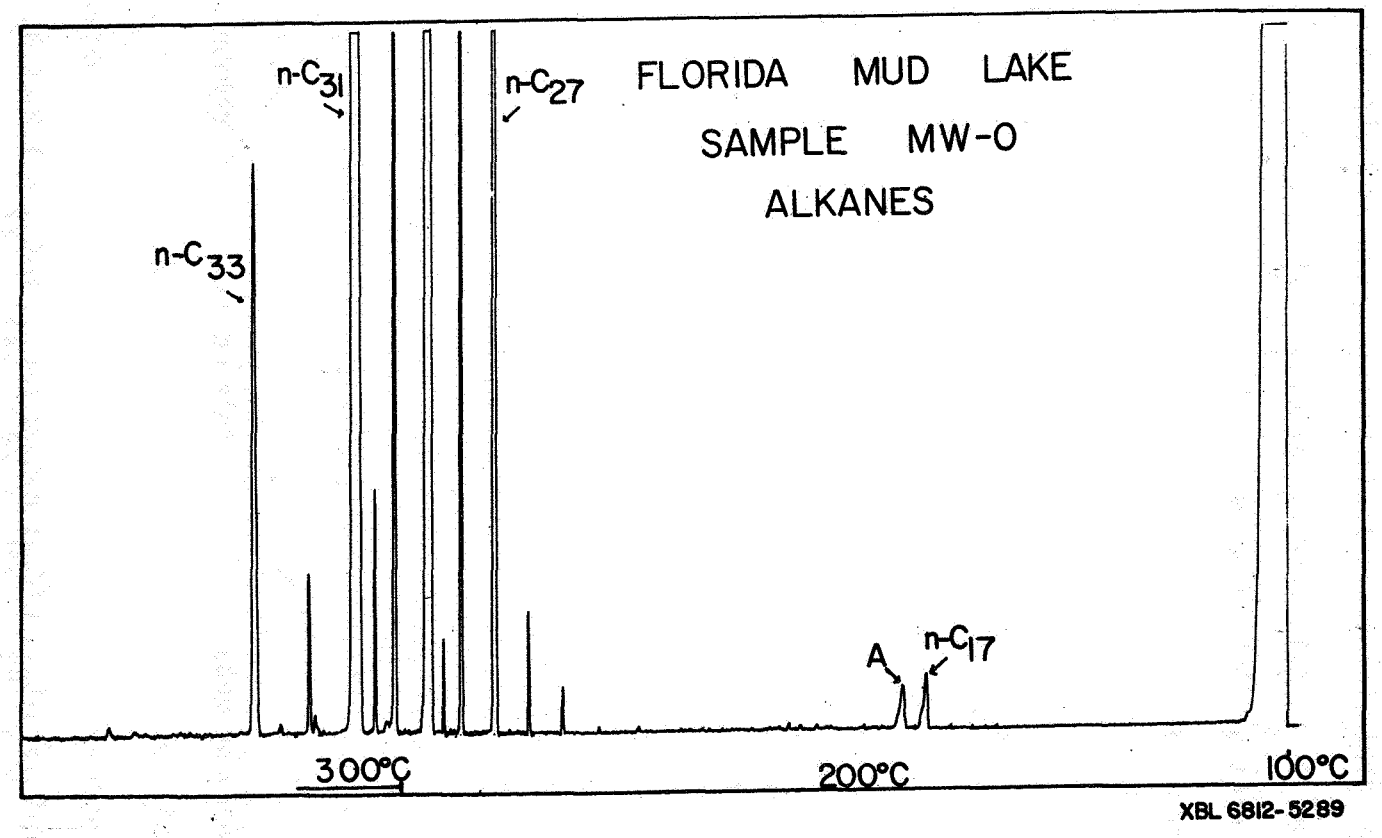



Figure 7. Gas chromatogram of alkanes from Florida Mud Lake sediment, sample MW-O (mud-water interface to one foot depth).

indication of the presence of 5-methyl heptadecane. This assignment is based on the coinjection of a mixture of 7-methyl heptadecane and 8-methyl heptadecane, known to be inseparable under the gas-chromatographic conditions used, and on mass spectrometry. Figure 8 shows the mass spectrum of this peak and of the 7- and 8-methyl standard (from J. Han). Of particular attention is the dominance of the even mass peaks at 98, 112, 168 and 182 over the odd numbered peaks of one higher mass unit, a situation totally analogous to that found for the 7- and 8-methyl heptadecanes.[174] Also, from the series of mass spectra taken across the gc peak, it is evident that the peak is not of a single compound (7-methyl has a slightly shorter retention time).

The second 10 ml fraction off the $Al_2O_3$ column (first analysis) contained a yellow pigment, and this fraction was investigated further. A second chromatography on neutral alumina was performed in an attempt to purify this pigment. Visible spectra of the pigment were suggestive of this compound being β-carotene. These spectra, along with those for standard β-carotene, are given in Figure 9. Absorption maxima are listed in tabular form in Table 3 (spectra recorded on a Cary 14 u.v.-visible recording spectrometer). Thin-layer chromatography on $Ca(OH)_2$ using 2% $CH_2Cl_2$ in n-$C_7$ as solvent showed that the mud pigment and β-carotene had identical retention times. The chromatographic data and the spectral data combine to prove the existence of β-carotene in MW-0. No other pigments were characterized.

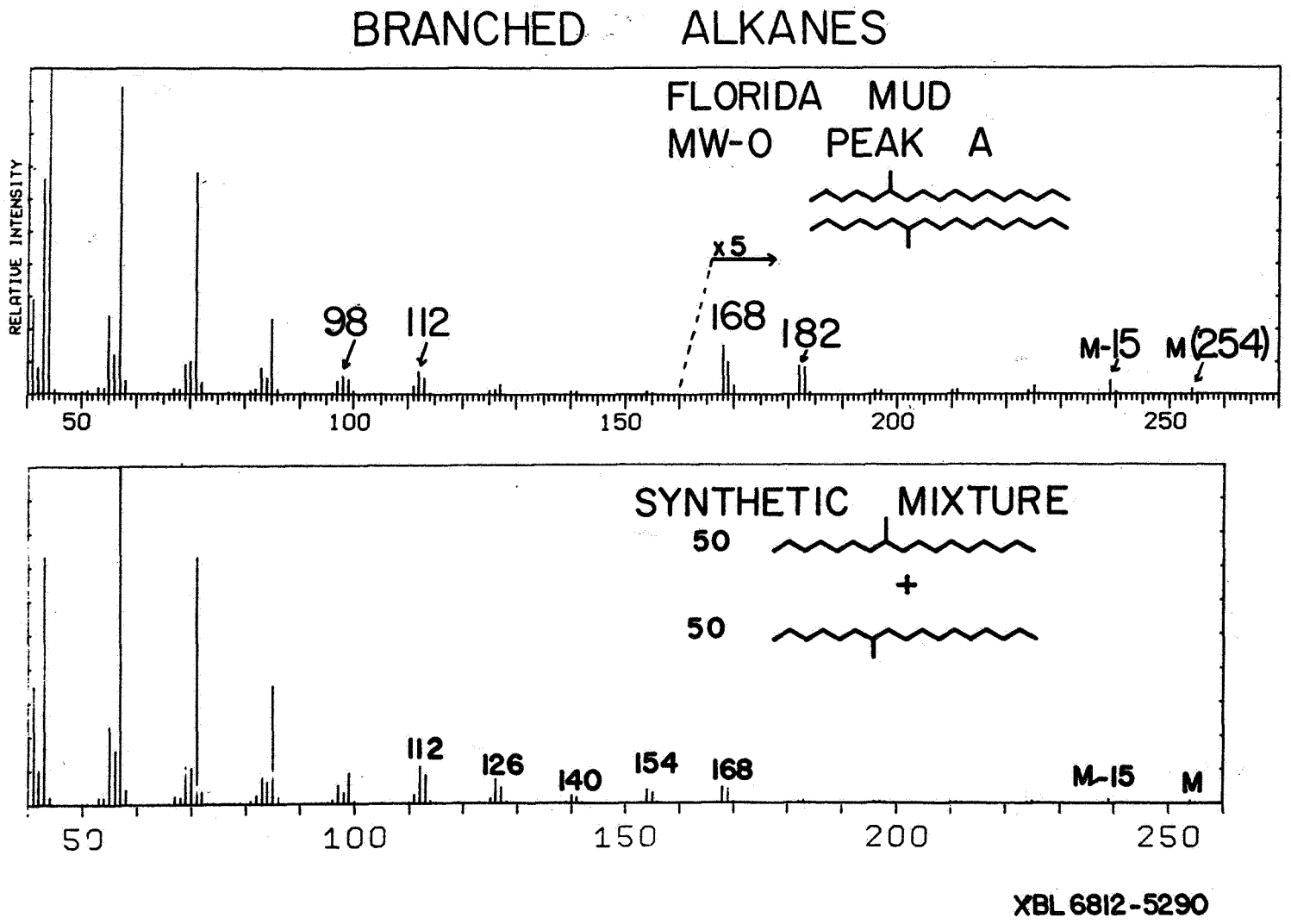
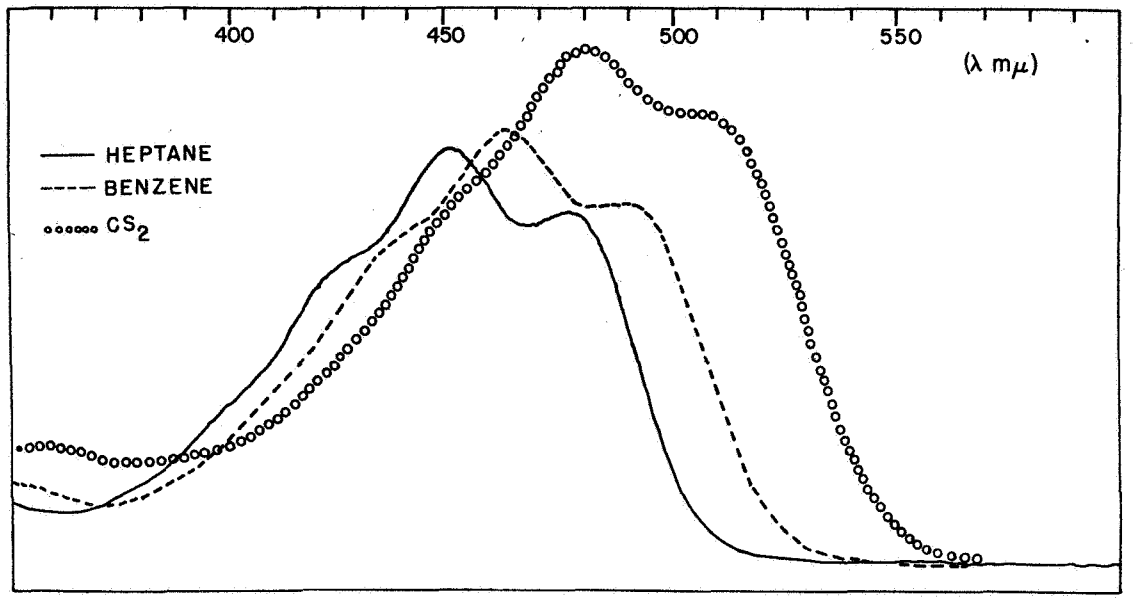
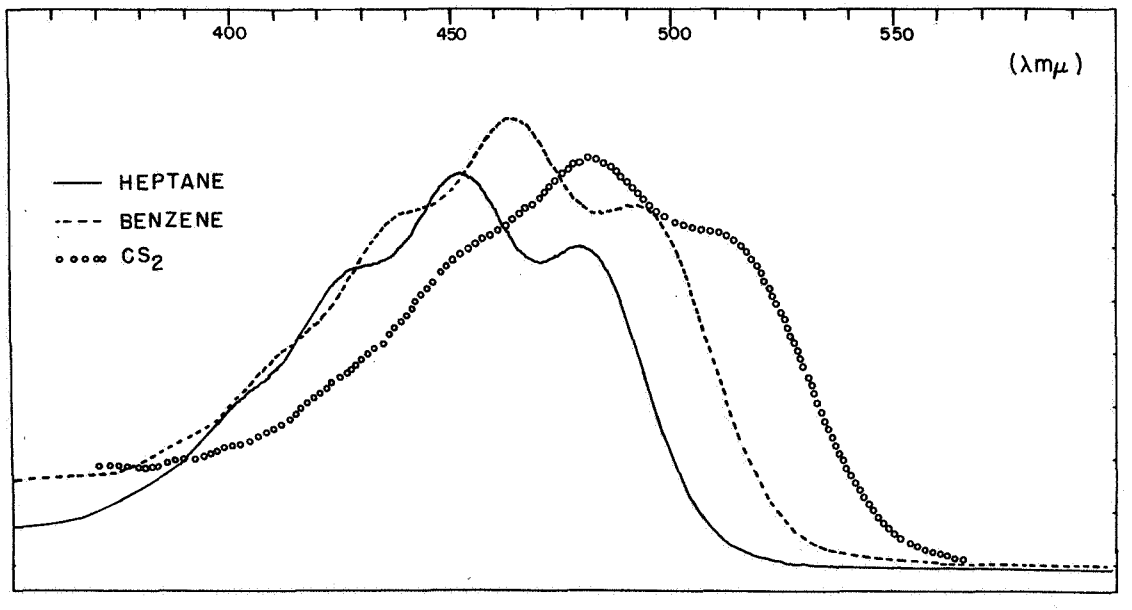



Figure 8. Mass spectra of branched alkanes from Florida Mud Lake, MW-O (Peak A) and synthetic methyl heptadecanes.




VISIBLE SPECTRA OF β-CAROTENE
FROM FLORIDA MUD LAKE (LEVEL MW-O)




VISIBLE SPECTRUM OF STANDARD β-CAROTENE

XBL6812-5265

Figure 9. (top) Visible spectra of β-carotene from Florida Mud Lake (MW-O), and (bottom) of standard β-carotene.

Table 3

Florida Mud Lake-MW-0

β-Carotene Spectral Data (maxima)

| SOLVENT | MAXIMA | | |
|---|---|---|---|
| **Mud Lake Pigment** | | | |
| n-heptane | 430 | 451 | 476 |
| benzene | 440 | 462 | 490 |
| carbon disulfide | 454 | 480 | 507 |
| **Standard β-Carotene** | | | |
| n-heptane | 429 | 452 | 478 |
| benzene | 440 | 463 | 491 |
| carbon disulfide | 455 | 481 | 508 |
| **Literature Values**[175] | | | |
| n-hexane | 430 | 451 | 479 |
| benzene | 441 | 463 | 494 |

The second sample examined was MW-6, consisting of the 6-7 ft layer of mud, with an approximate age of 5000 years and an elemental composition of C, 52.20%; H, 5.32%; N, 1.39%; S, 2.59%; P, 0.0%; residue, 11.1%. The dry mud was crushed by hand in an agate mortar and then extracted for 30 min, by sonication, as for MW-0. The weight of mud used was 8.2844 g and 200 ml of benzene:methanol (4:1) was used as solvent. Centrifugation, followed by decantation and solvent evaporation yielded a total extract of 98 mg.

The total extract was placed on an alumina column (~5 g TLC grade neutral $Al_2O_3$) and eluted with the sequence of solvent mixtures mentioned earlier to isolate the non-aromatic hydrocarbons. Elution with n-heptane and fractionation into 10 ml aliquots gave only two fractions prior to the elution of colored (orange) material. Examination of these two fractions by u.v. (Perkin-Elmer, Model 202) indicated low quantities of aromatic compounds to be present (240-260 nm), and these two fractions were combined and treated as the "total alkanes" from MW-6 (2.6 mg). The GLC of this sample is shown in Figure 10 (Aerograph 665, 10' x 1/16" O.D. column, 3% SE-30 on 100/120 mesh Chrom Z, $N_2$ carrier gas at 30 ml/min). The assignment of structure and carbon number to the large peaks is again based on retention time of coinjected standards. The relative amounts of the $C_{25}$, $C_{27}$, $C_{29}$ and $C_{31}$, calculated from peak areas, is approximately 1:1.9:5.3:6.1. No attempt was made to sieve this mixture.

In view of the fact that a number of the fractions eluted from the $Al_2O_3$ column were distinctly colored, as in the case of

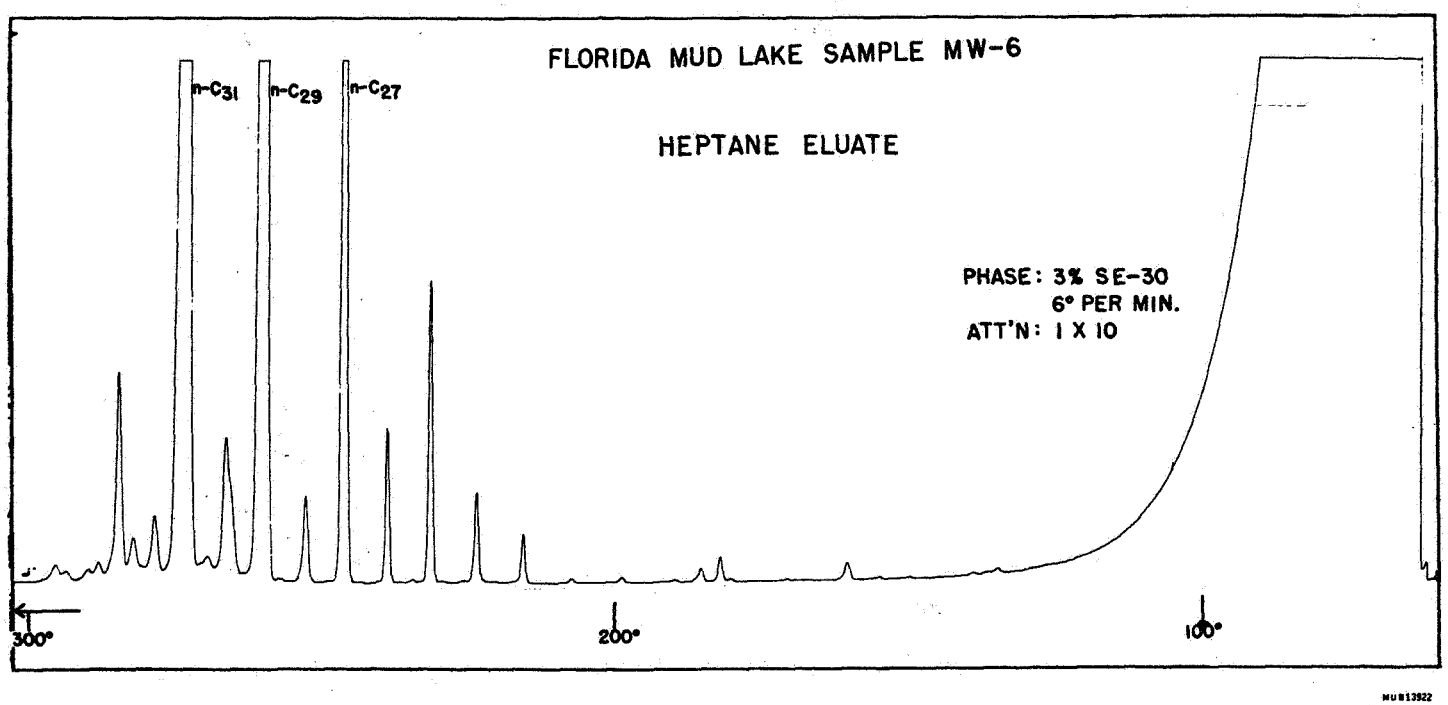



Figure 10. Gas chromatogram of alkanes from Florida Mud Lake sediment sample MW-6 (6-7 foot depth).

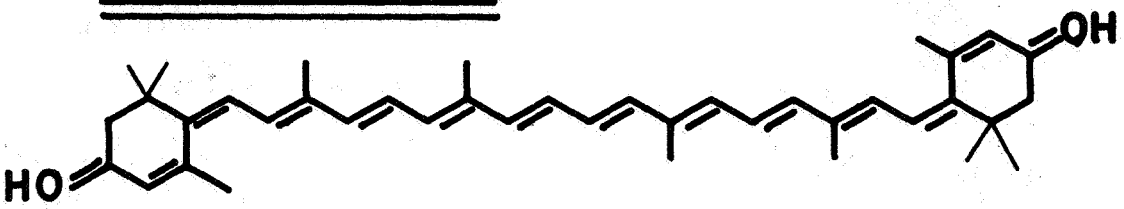
MW-0, the u.v.-visible absorption spectrum of each fraction was recorded in a variety of solvents. Of these spectra, those from fraction 9 (0.3 mg) eluted with 100% benzene, were most distinct and most informative. The spectra were characteristic of a carotenoid and could readily be compared with literature values. This comparison suggests that this Mud Lake carotenoid is rhodoxanthin (Figure 11). The spectral data of the sediment pigment and the literature data[176] data for rhodoxanthin are given in Figure 11. Also presented is spectral data for crude rhodoxanthin isolated from jew berries. (All spectra were recorded on either a Cary 14 or a Cary 11 u.v.-visible recording spectrophotometer.) In addition to the u.v.-visible data, an attempt was made to record the infrared absorption spectrum; however, too little sample was available and no meaningful data were obtained.

The confirmation of the geochemical pigment as rhodoxanthin is certainly not final. Spectral evidence is only suggestive in this case, and is not substantiated sufficiently to state that rhodoxanthin has been isolated from MW-6.

An attempt was made to isolate more of the alkanes and the pigments by pulverizing the extracted mud to a fine powder and re-extracting as before. No quantities sufficient for additional characterizations were obtained, although the alkane distribution from the first extraction was substantiated.

The results from these two samples of the Florida Mud are to be compared, not only with other Florida mud analyses,[163,177] but also with the components of algae, such as the type supposedly

## RHODOXANTHIN




### SPECTRAL DATA

| MUD LAKE PIGMENT | | | |
|---|---|---|---|
| SOLVENT | MAXIMA | | |
| n-HEXANE | 461 | 484 | 517 |
| CYCLOHEXANE | 464 | 490 | 521 |
| BENZENE | 478 | 500 | 531 |
| METHANOL | 456 | 481 | 512 |
| STANDARD PIGMENT | | | |
| HEXANE | 460 | 483 | 510 |
| BENZENE | 480 | 500 | 530 |
| LITERATURE VALUES (KARRER)[176] | | | |
| PETROLEUM ETHER | 456 | 487 | 521 |
| BENZENE | 474 | 503 | 542 |

XBL 6812-5262

Figure 11. Rhodoxanthin structure and spectral data (geological pigment, standard pigment, literature[176]).

contributing to this sediment, and with other sediments and known contributors to sediments. In all of the Florida Mud Lake analyses, the higher alkanes--i.e., $C_{20}$-$C_{33}$, are prominent constituents; indeed they are often by far the major components (e.g., MW-6). This is consistent with a plant wax contribution to the sedimentary hydrocarbons. The presence of these higher alkanes is not surprising in view of the dense vegetation surrounding and covering the lake. Although the first six inches of the ooze consist wholly of minute fecal pellets of blue-green algae,[173] with no mention of higher plants, some contribution from these plants is to be expected. The presence of the n-$C_{25}$, n-$C_{27}$, n-$C_{29}$ and n-$C_{31}$ hydrocarbons is analogous to the situation in the Green River Shale,[156] known to have a plant contribution.[171]

Of considerable importance is the absence, in all the Mud Lake samples, of significant amounts of pristane and phytane. In view of other indications of plant contribution to these sediments (e.g., pigments and the normal alkanes mentioned above) it must be concluded that the series of geochemical reactions from phytol to the alkanes has not yet been effected. Perhaps such compounds as the phytadienes identified by Blumer[178] are present in these young sediments; however, no attempt was made to locate and identify these intermediates.

The presence of the n-$C_{17}$ hydrocarbon and the methyl heptadecanes in MW-0 is not really surprising, since such compounds are present in blue-green algae. In fact, the alkanes from the Nostoc blue-green algae consists almost entirely of n-$C_{17}$ and a mixture

of the 7-methyl and 8-methyl heptadecanes.[174] Also, as mentioned before, there is an abundance of blue-green algal fecal pellets in the MW-0 level of this sediment. The origin of these branched alkanes within organisms has been studied by Han,[179] and his conclusion is that they are derived from octadecenoic acids via the cyclopropane acids. The acid responsible for the 7- and 8-methyl heptadecanes is cis-vaccenic acid ($\Delta^{11}$-octadecenoic acid). An analogous sequence of reactions could be responsible for the 6- and 7-methyl heptadecanes reported here, with either $\Delta^{7}$- or $\Delta^{12}$-octadecenoic acid being the initial acid. Neither of these two acids seems to be abundant in organisms, although the former has been found in a biotin deficient mutant of *E. coli*.[180] A more thorough analysis of the algae and bacteria contributing to the Florida Mud Lake sediment would help to answer the questions posed by the appearance of this distribution.

The alga *Spirogyra* is supposedly one contributor to this ooze.[173] J. Han has analyzed a sample of these alga taken from the Mud Lake.[181] No evidence for these branched ($C_{18}$) alkanes was found, but the presence of some higher alkanes (*i.e.*, $C_{25}$-$C_{31}$) was noted. This sample is almost certainly contaminated by the compounds from other organisms in and near the lake. On the other hand, laboratory cultures of such organisms may not be wholly indicative of the chemical composition of "wild" algae since the carbon sources and compounds ingested vary from one condition and environment to another.

One other comment must be made concerning the alkane distribution from the MW-0 level of the Mud Lake. Kvenvolden has previously reported the normal hydrocarbon distribution of this same sample,[182] and finds that n-$C_{17}$ is the largest component and that the higher n-alkanes are also present in approximately the relative amounts shown here. There is some question in his analysis about whether or not some of the lower alkanes ($C_{15}$-$C_{19}$) are in fact normal alkanes.[183] Since his method of separation was by urea adduction, it is possible that they are not, since mono-methyl alkanes are adducted by urea. Finally, it is interesting to note that the n-$C_{17}$ hydrocarbon is by far the largest normal alkane in the $C_{15}$-$C_{20}$ region of the Green River Shale.[156]

The absence of the n-$C_{17}$ and methyl heptadecanes in the older (i.e., deeper) Mud Lake samples is somewhat puzzling. The most obvious explanation is a selective destruction, with time, of these alkanes. Such selectivity could be exhibited by bacteria, etc., operating within the first several feet of the sediment. It is possible that these alkanes are converted into the higher alkanes in a manner analogous to the biosynthetic processes of Koluttkudy.[128]

The fatty acids from this lake sediment have been examined by Kvenvolden,[182] who finds that the n-$C_{16}$ acid, palmitic acid, constitutes 40% of the total acids. Although the acids range from $C_{12}$-$C_{34}$, the lower acids are by far the most abundant. In view of the low molecular weight distribution of these acids,

it does not seem reasonable to postulate a direct conversion to alkanes of similar molecular weight. Conversion, as suggested for the alkanes, to compounds of higher molecular weight is not to be ruled out.

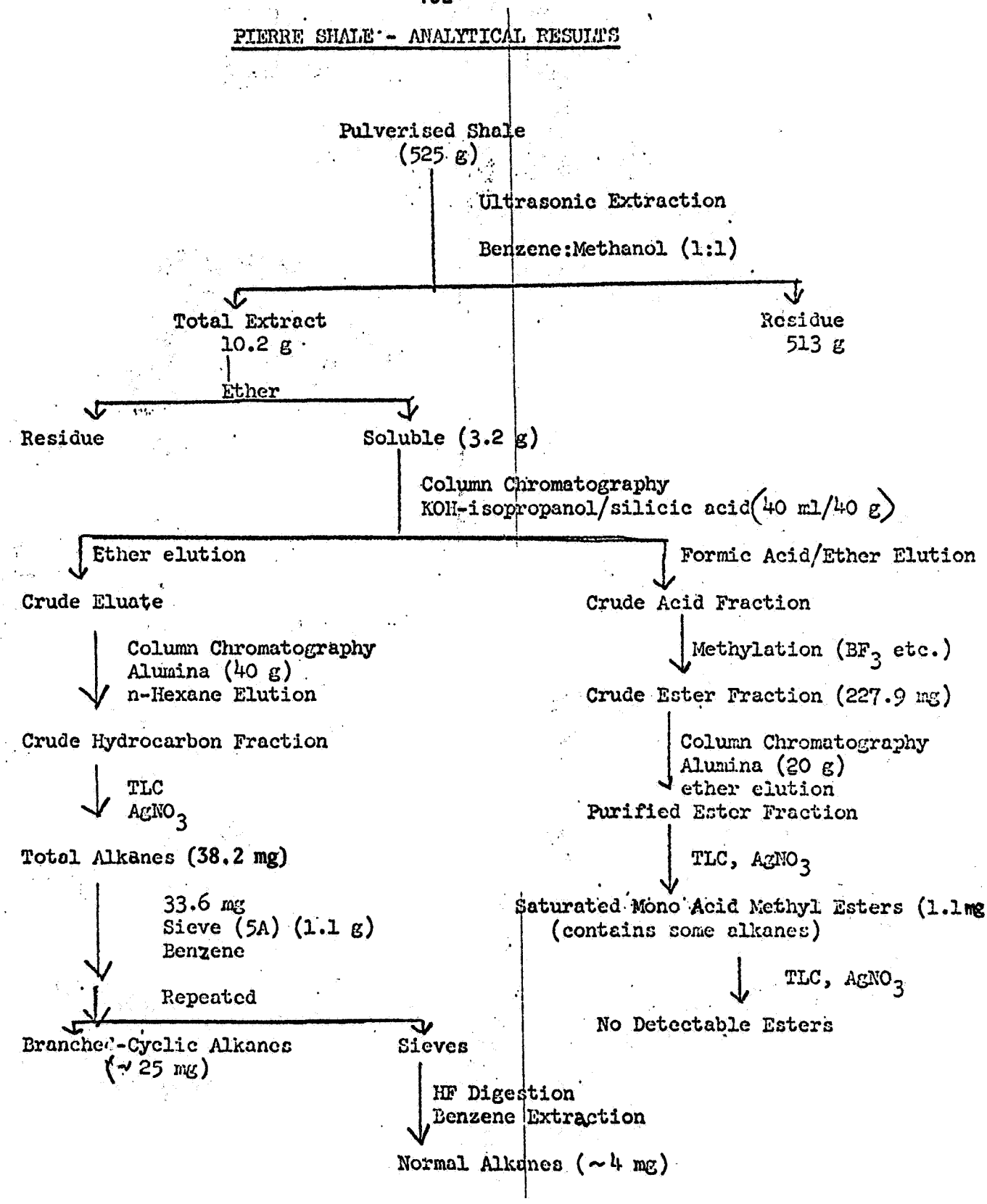
The conclusion from the Mud Lake study must be that the sediment alkanes do not bear a direct relationship to the alkanes of the presumed algal contributors. Other contributing organisms, such as higher plants, may contribute directly to the higher n-alkanes. The significance of algal contribution cannot be denied, however, and the absence of the alkanes from these algae must depend on diagenetic transformations.

THE PIERRE SHALE

The Pierre Shale (75-80 x $10^6$ years), from the central United States, constitutes an interesting situation because it presumably arises from both marine and terrestrial sources.[184] Preliminary analyses of a sample of the Sharon Springs member of this shale, supplied by I. A. Breger, suggested further investigation might be warranted. Accordingly, additional samples were obtained and a more extensive analysis performed. The particular sample analyzed here was also from the Sharon Springs Member of the Pierre Shale. It was collected by H. Tourtelot, A. L. Burlingame and E. D. McCarthy from the S.W. 1/4 N.E. 1/4 of sec. 23, T.38 N R.62 W Niobrara County, Wyoming. The sample contains 5-10% organic carbon. According to Tourtelot, this particular sample represents an accumulation of organic matter far from shore (at least 100 miles), accumulated under completely marine conditions. The contributing organisms are apparently both marine organisms and land plants, with the land derived material seemingly the most abundant.[185]

The sediment is very brittle and was not obtained in large pieces. Also, it appears to be a rather porous sediment, and this must be borne in mind in considering the results. The sediment was analyzed according to the previously defined process, and the amounts obtained are indicated in the following diagram.

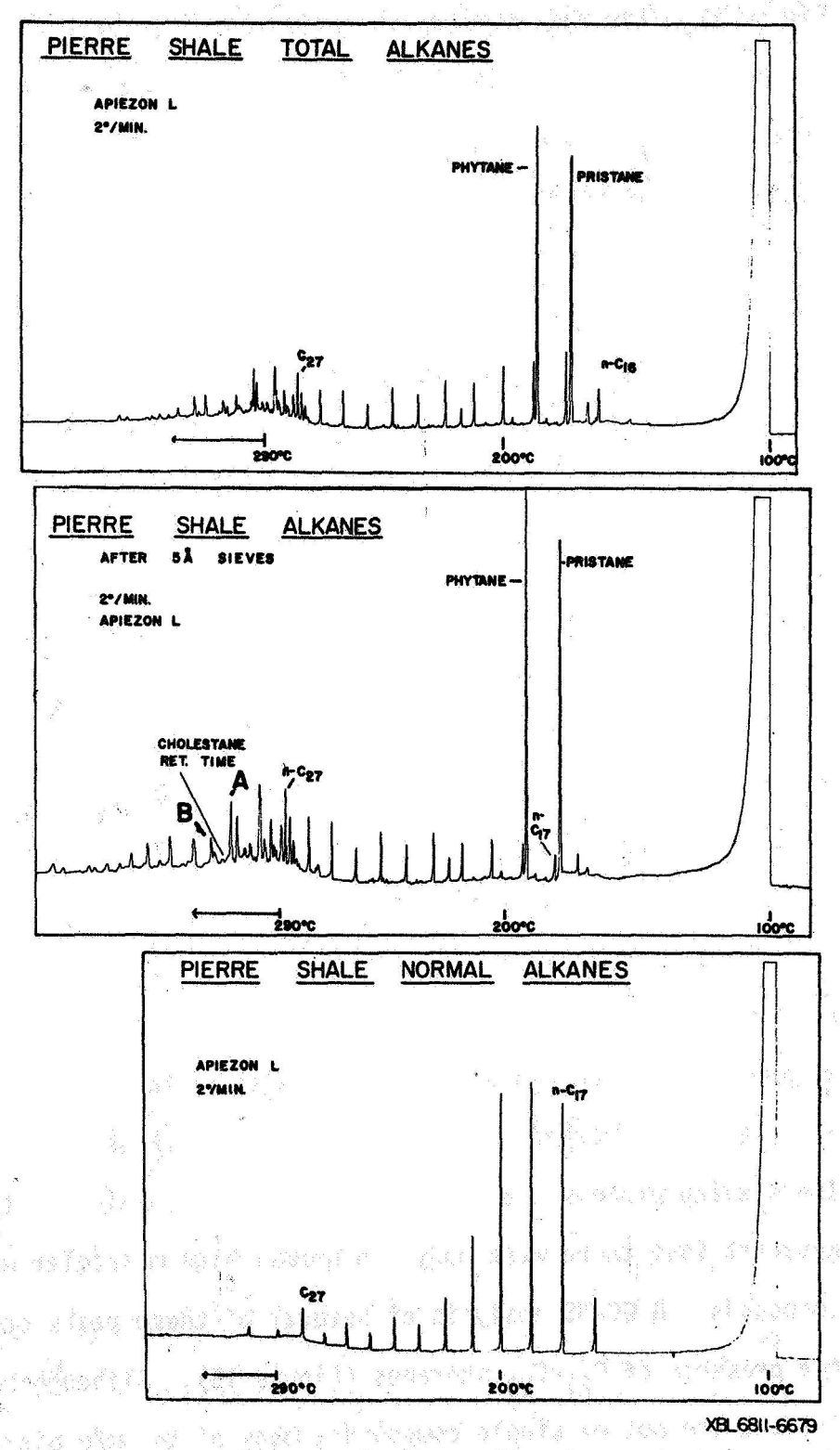
PIERRE SHALE - ANALYTICAL RESULTS



The capillary gas chromatogram of the alkanes is shown in Figure 12. The most obvious characteristic is the presence of the bimodal distribution, in which there is a maximum centering around $C_{17}$-$C_{18}$ and another centering around $C_{25}$-$C_{29}$. The carbon numbers of the normal hydrocarbons have been assigned by means of coinjections and retention times as before, as well as by combined gas chromatography-mass spectrometry; the mass spectra of the n-$C_{16}$ and n-$C_{21}$ are given in Figure 13. The alkanes are dominated by pristane and phytane, whose identity has been confirmed by coinjection of these standards with the alkane mixture, and by GC-MS as shown in Figure 14.

The Pierre Shale has been examined several times by this author. In all cases the bimodal distribution has been apparent. The earlier examination, in which the hydrocarbons were obtained in the same manner as the Florida Mud alkanes, revealed the presence of steroidal type components in the high molecular weight region of the chromatogram.[186]

The high molecular weight compounds of the latter sample have also received some additional attention. The higher normal alkanes exhibit an odd/even carbon number ratio of >1, although this is not as marked as in the Florida Mud alkanes. Although the sieving process, repeated twice, was not complete, it was apparent that there were many non-normal high molecular weight compounds. A GC-MS analysis of several of these peaks confirmed the presence of $C_{27}$-$C_{29}$ steranes (Figure 15). Although such spectra are not of single compounds, many of the m/e peaks are




XBL6811-6679

Figure 12. Gas chromatogram of alkane fractions of Pierre Shale.

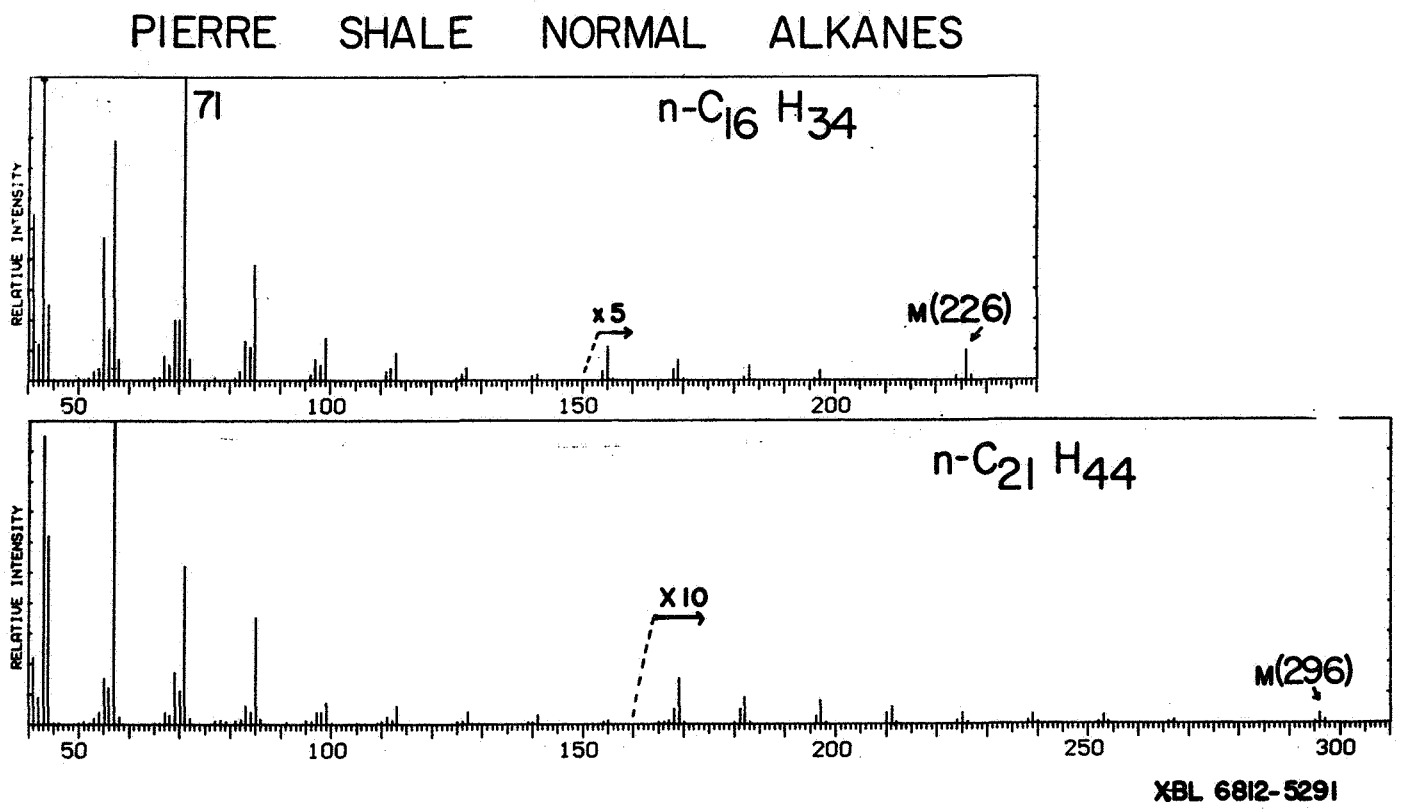



Figure 13. Mass spectra of n-alkanes from the Pierre Shale.

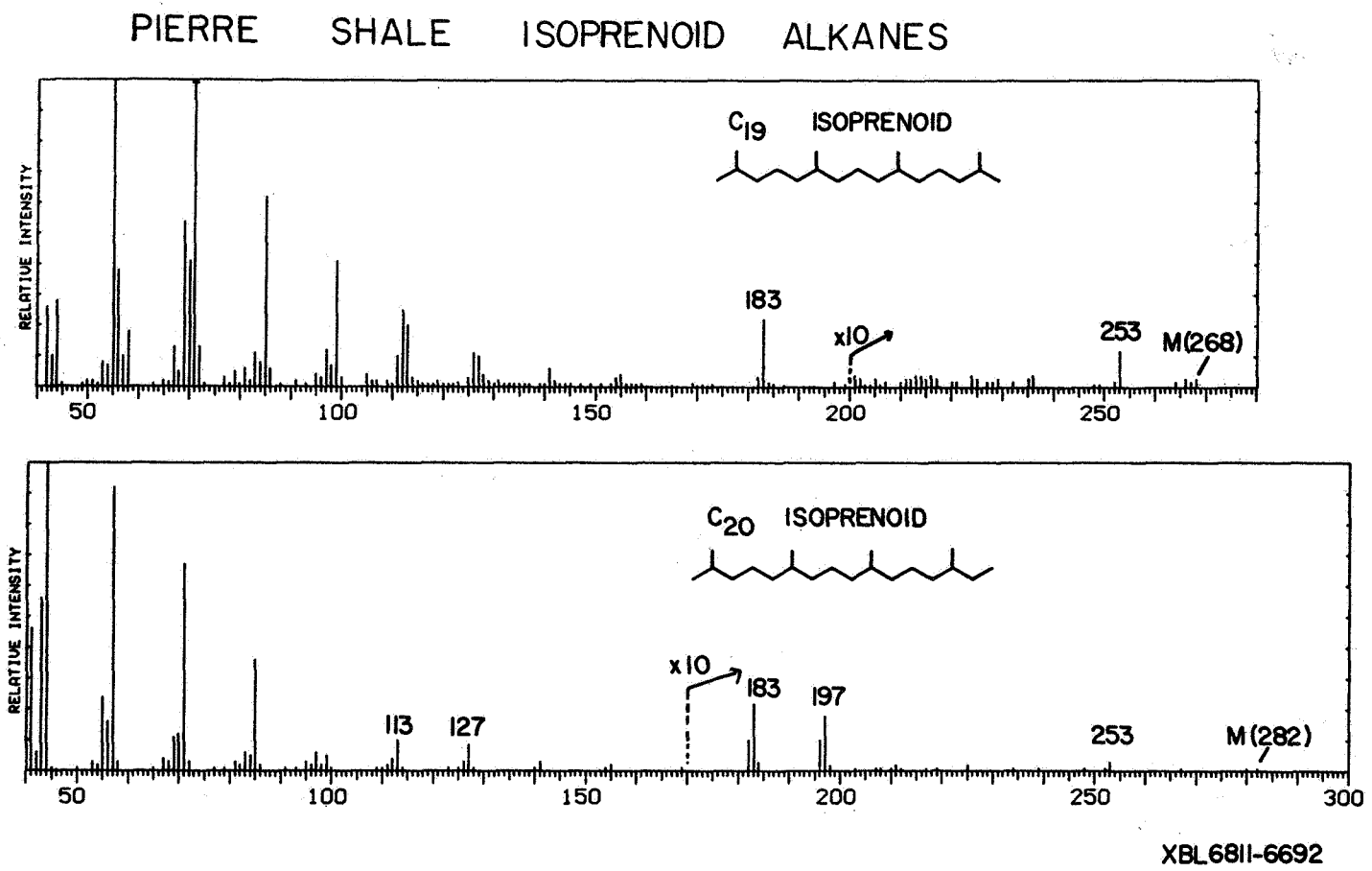



Figure 14. Mass spectra of isoprenoid alkanes from the Pierre Shale.

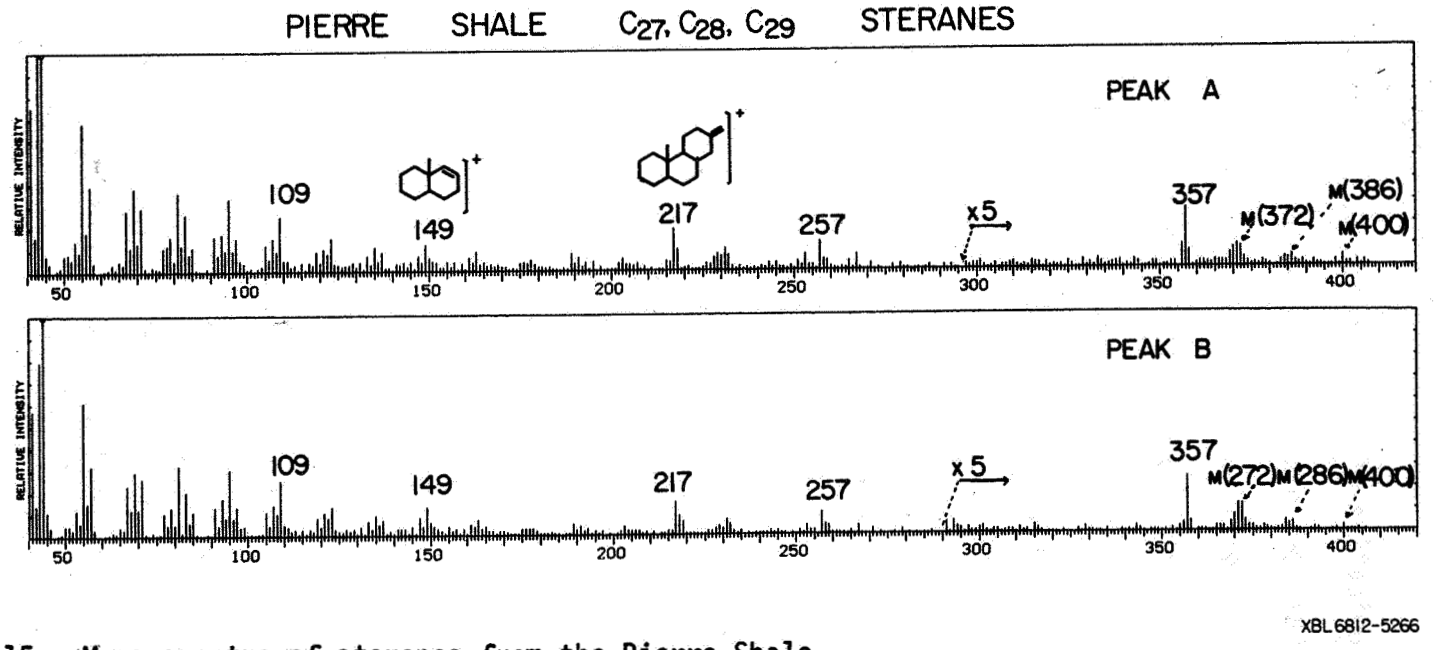



Figure 15. Mass spectra of steranes from the Pierre Shale.

indicative of known fragmentations of steranes.[186] The fact that some peaks, including molecular ions, are lower by two mass units than would be expected for steranes, can be explained by dehydrogenation of the alkanes in the GC-MS system.

The biogenetic character of the Pierre Shale, specifically the Sharon Springs member, can hardly be doubted, since both the geological[184,187] and geochemical data are in accord with this statement. Of greater interest here is the marine/terrestrial origins of some of the hydrocarbons. Pristane and phytane, being the most abundant compounds, suggest a large contribution from plants, presumably originally in the form of phytol from chlorophyll. The predominance of the odd-carbon number alkanes over the even numbered also suggests a contribution from higher plants.[120] Of considerable interest to the question of marine/terrestrial origin is the presence of steranes. These compounds are, very likely, products of diagenesis of sterols and other triterpenoid compounds which are often products of biosynthetic mechanisms of higher plants. These three factors: 1) the pronounced abundance of triterpenoid compounds, 2) the odd carbon alkanes, and 3) extremely large amounts of pristane and phytane, seem to be indicators of plant (i.e., terrestrial) origin of sedimentary material. The analogy with the Green River Shale is notable in this respect.[116,156]

That a marine environment was partially responsible for the organic matter in the Pierre Shale is suggested by the presence

of numerous compounds in the n-$C_{15}$ to n-$C_{20}$ region, the maximum area for many marine oils and sediments.[130,163] In situations where the sediment has a non-marine origin (e.g., the Green River Shale[156] and the Florida Muds), there are very few compounds of this low molecular weight.

The previously mentioned concern about the condition of the sediment does not seem to be of great importance, as the results are in accord with those from other ancient sediments, and not in accord with the alkanes in recent sediments or microorganisms.

THE MOONIE OIL

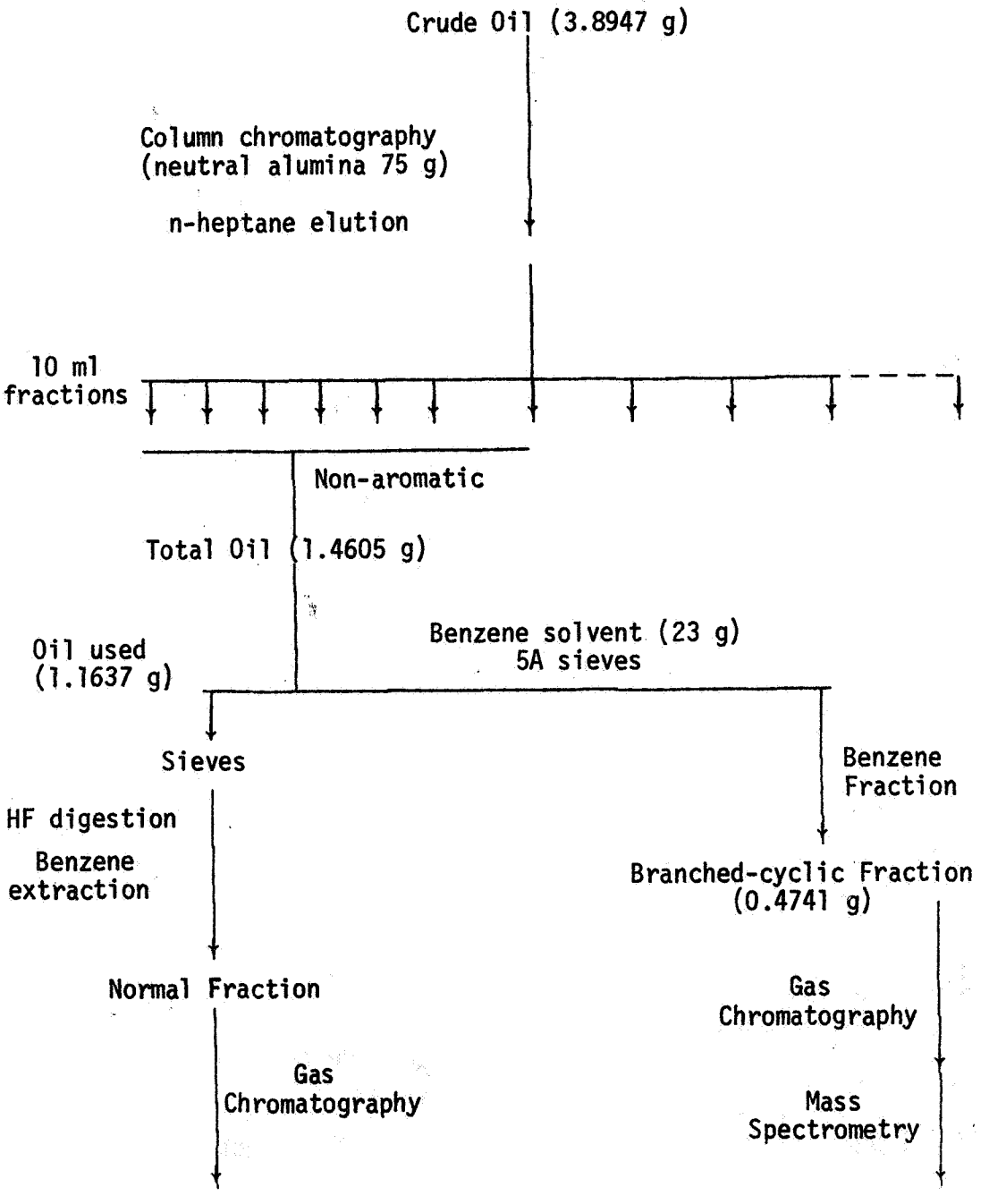
The Moonie Oil of Queensland, Australia (200 x $10^6$ years) is thought to have, at least in part, a non-marine origin. The situation is apparently unsettled, being complicated by the presence of two different oils located in two different basins of the same oil field; apparently the oil examined in this laboratory is a composite of the two different oils.[188] An attempt has been made to relate the oil to the Evergreen Shale.[189]

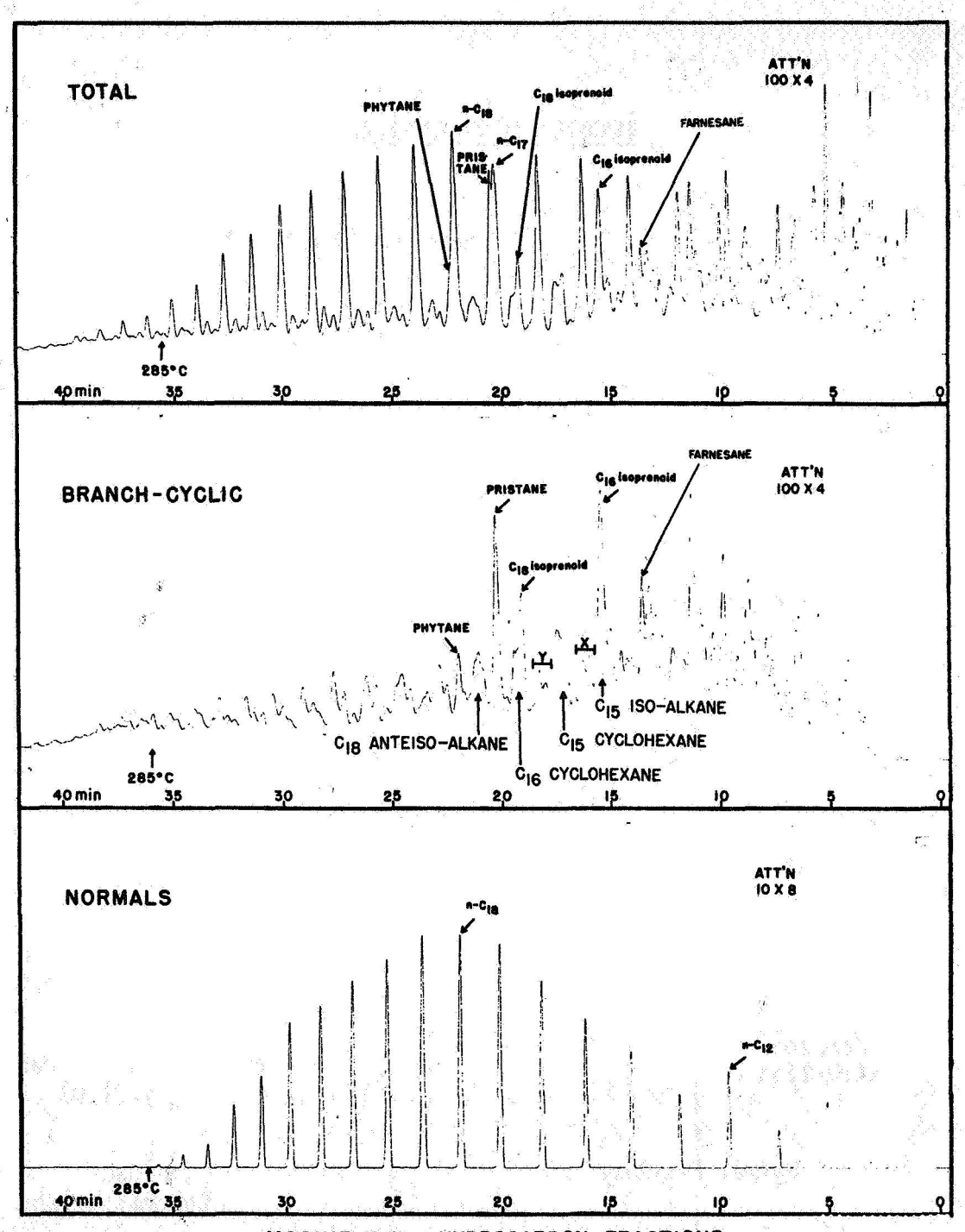
The analytical scheme used was detailed in the experimental section; the quantities obtained are outlined in the scheme on the following page.

The gas chromatograms of the various fractions are shown in Figure 16. These chromatograms are obtained by means of a packed 10' x 1/16" O.D. column of 3% SE-30 (Aerograph 665, 6°/min, 70°-300°C). Those peaks which are labeled in the branched-cyclic chromatogram were identified by successive preparative GLC and mass spectrometry, as described elsewhere.[169]

The effectiveness of this method of isolation can be seen in Figure 17, which is a photograph of the actual mass spectrometer trace, for the $C_{16}$, $C_{18}$ and $C_{19}$ polyisoprenoid alkanes. The plotted mass spectra for the $C_{15}$, $C_{16}$, $C_{18}$, $C_{19}$ and $C_{20}$ polyisoprenoid alkanes are given in Figure 18. There is also mass spectrometric evidence and gas chromatographic data suggesting the presence of the regular $C_{21}$ polyisoprenoid alkane (Figure 19); however, the isolated compound was not sufficiently pure to provide an

## MOONIE OIL ANALYTICAL RESULTS






MOONIE OIL. HYDROCARBON FRACTIONS

~200 X $10^6$ YEARS

Figure 16. Gas chromatogram of hydrocarbon fractions of Moonie Oil.

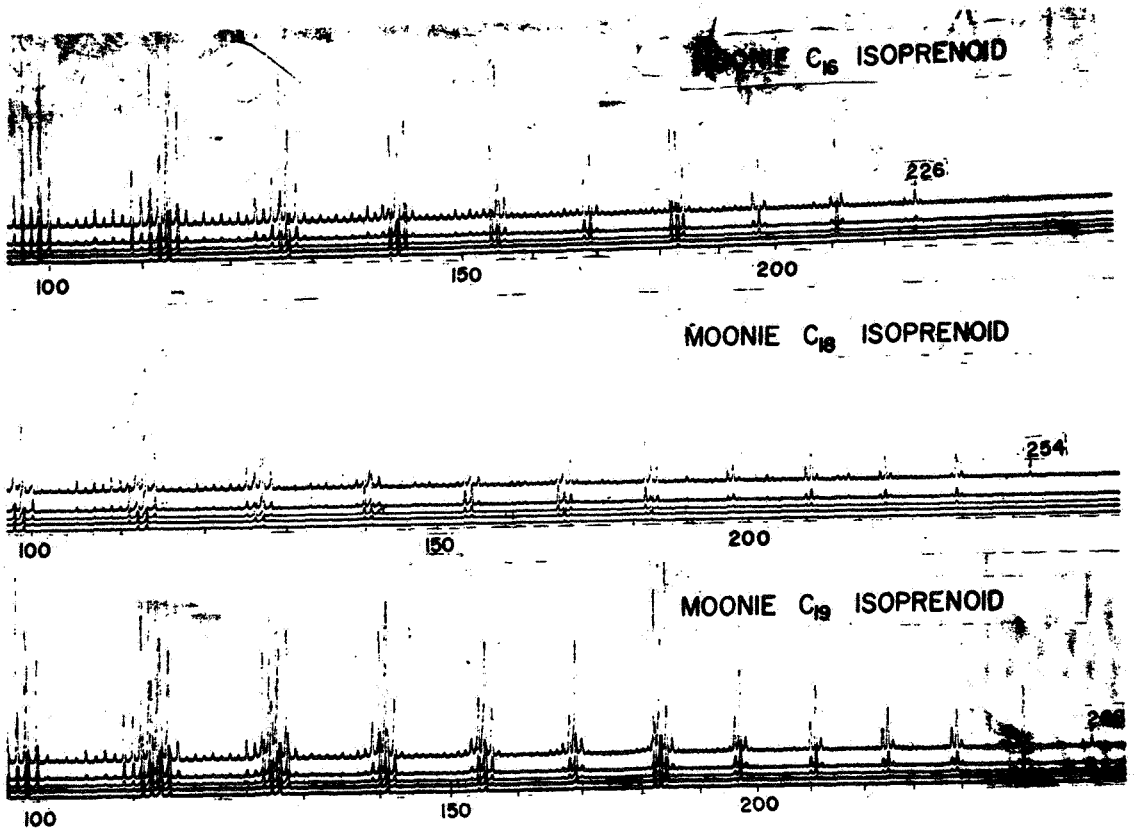



Figure 17. Mass spectrometer oscillograph tracings of polyisoprenoid alkanes isolated from the Moonie Oil.

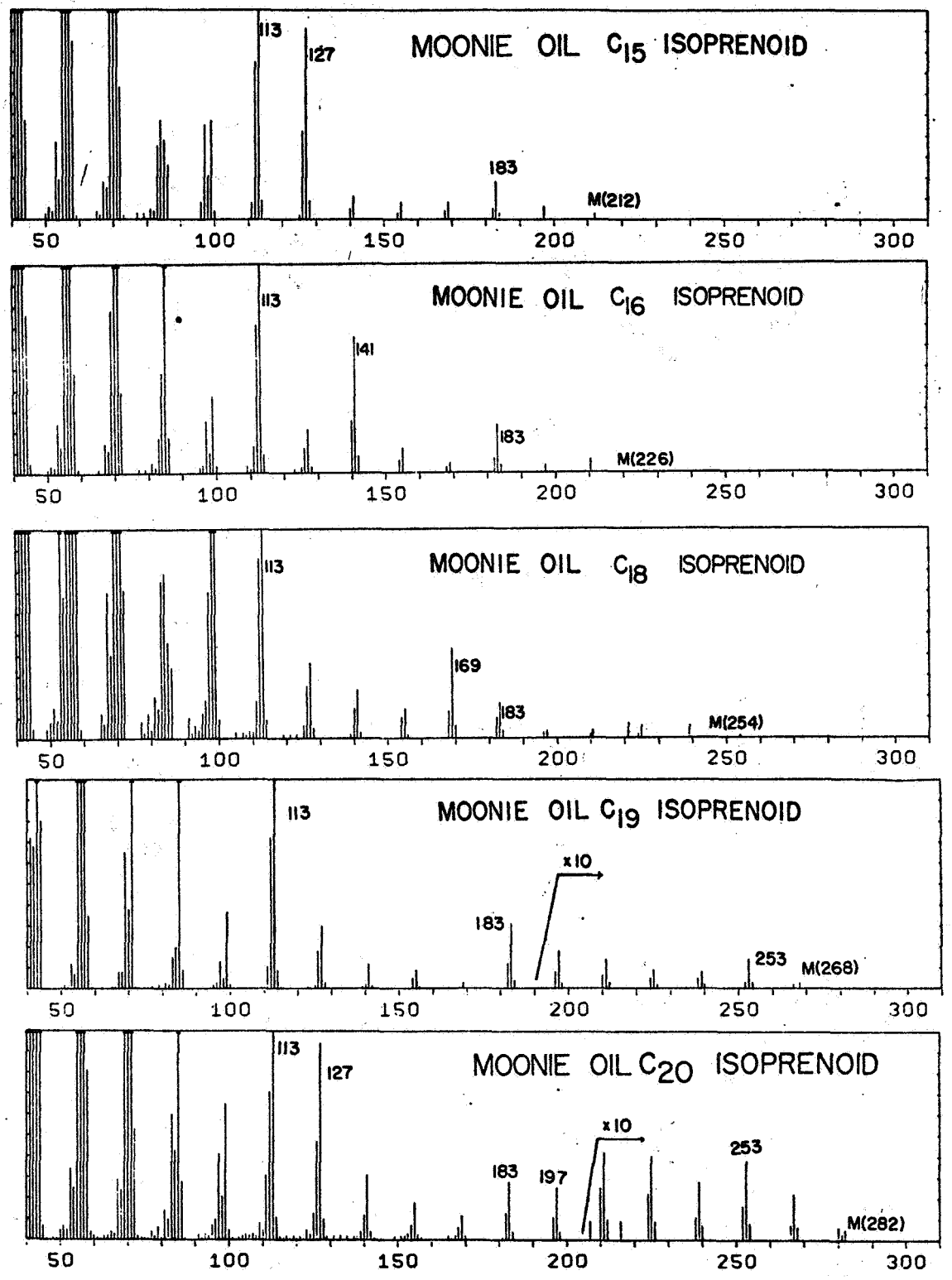
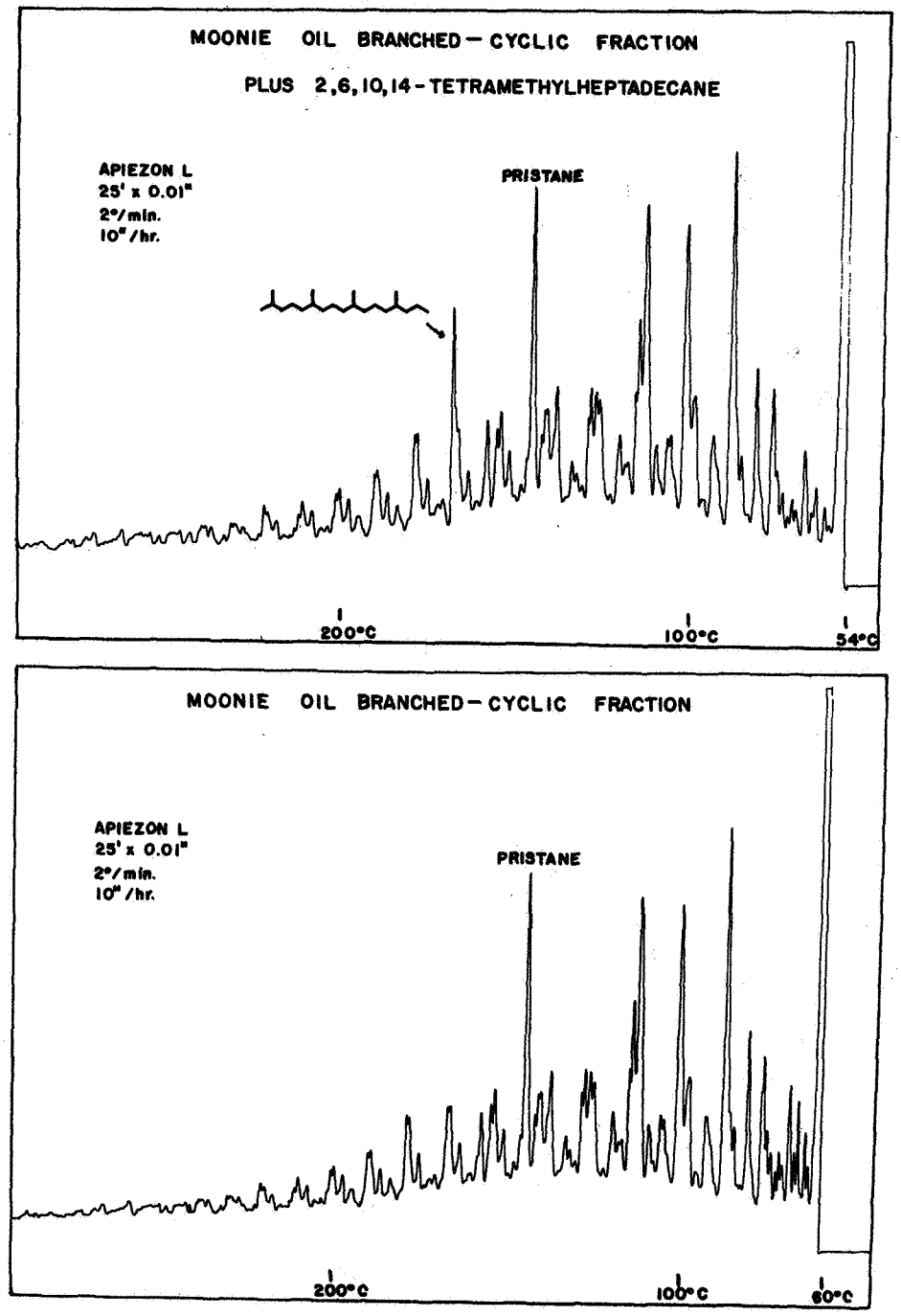



Figure 18. Mass spectra of polyisoprenoid alkanes from the Moonie Oil.

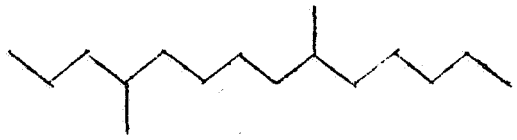



XBL 683-4071

Figure 19. Gas chromatograms of Moonie Oil alkanes, with and without added 2,6,10,14-tetramethyl heptadecane.

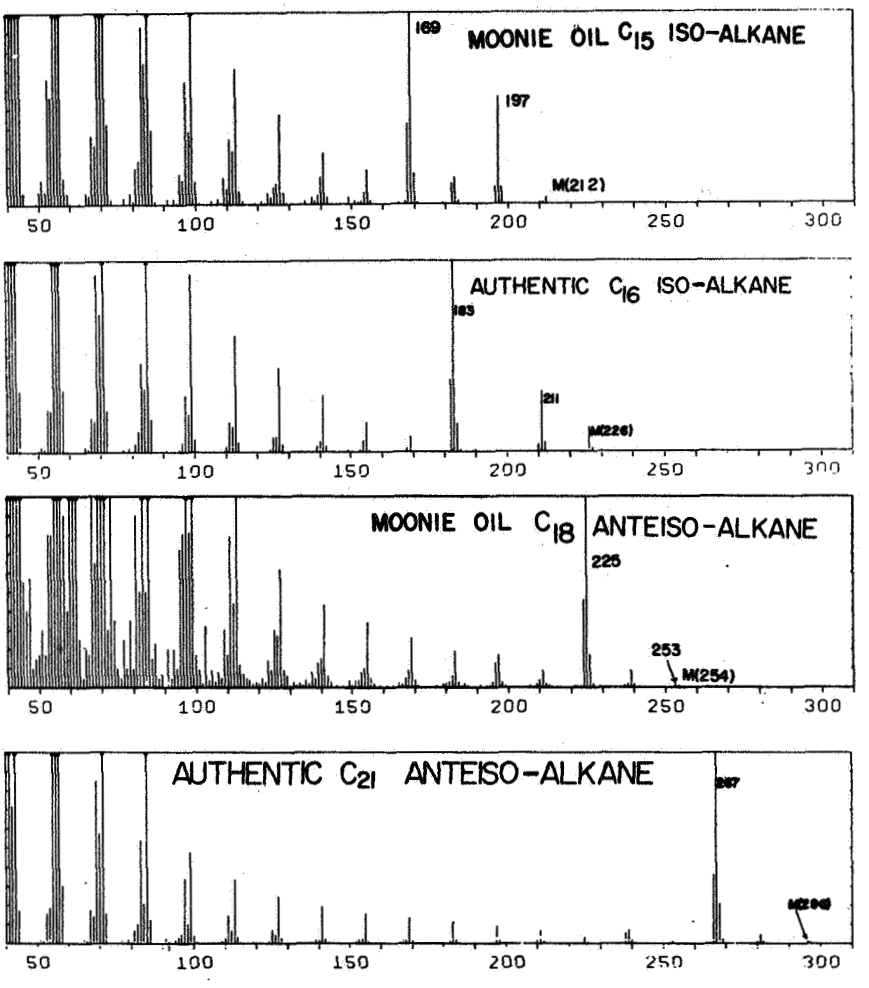
unequivocal mass spectrum. One iso-alkane and one anteiso-alkane were also proved to be present in the Moonie Oil. The mass spectra for the $C_{15}$ iso- and $C_{18}$ anteiso-alkanes are given in Figure 20 along with that of standard iso- and anteiso-alkanes. Two members of the alkyl cyclohexane series have also been identified, and the mass spectra of these two compounds are shown in Figure 21. Four compounds were isolated from the Moonie Oil whose structure could not be readily deduced by mass spectrometry. The mass spectra of these are shown in Figure 22. All off-scale mass spectral intensities are given in Table 4. In a previous publication, the structure of $X_1$ was suggested to be 5,9-dimethyltetradecane, and that of $X_2$ as 4,9-dimethyltetradecane.

$X_1$



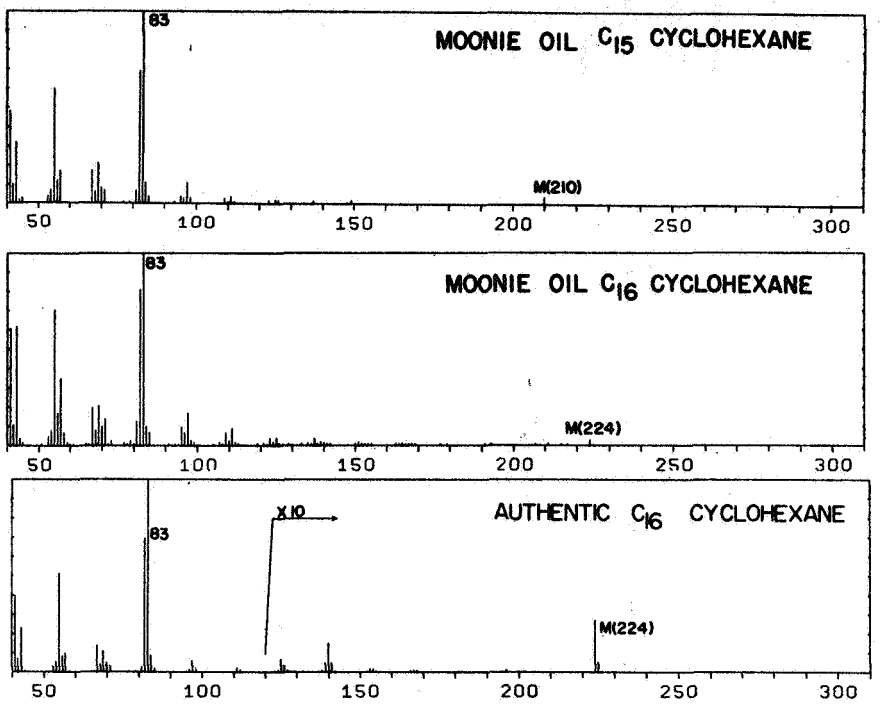
$X_2$

No tentative structure was proposed for $Y_1$ and $Y_2$. No further work has been performed in an attempt to validate these structural assignments. However, in view of recent advances in the interpretation of mass spectra,[163] the previous assignments must be considered rather tenuous. Figure 23 shows a capillary gas chromatogram of the branched-cyclic fraction of the Moonie Oil (150' x 0.01", SE-30, program rate 1°/min). Of special note is the way in which single or double peaks become complex multiplets.




XBL 6812-5260

Figure 20. Mass spectra of iso- (2-methyl-) and anteiso- (3-methyl-) alkanes, authentic and from Moonie Oil.




XBL 6812-5261

Figure 21. Mass spectra of n-alkyl-cyclohexanes, authentic and from Moonie Oil.

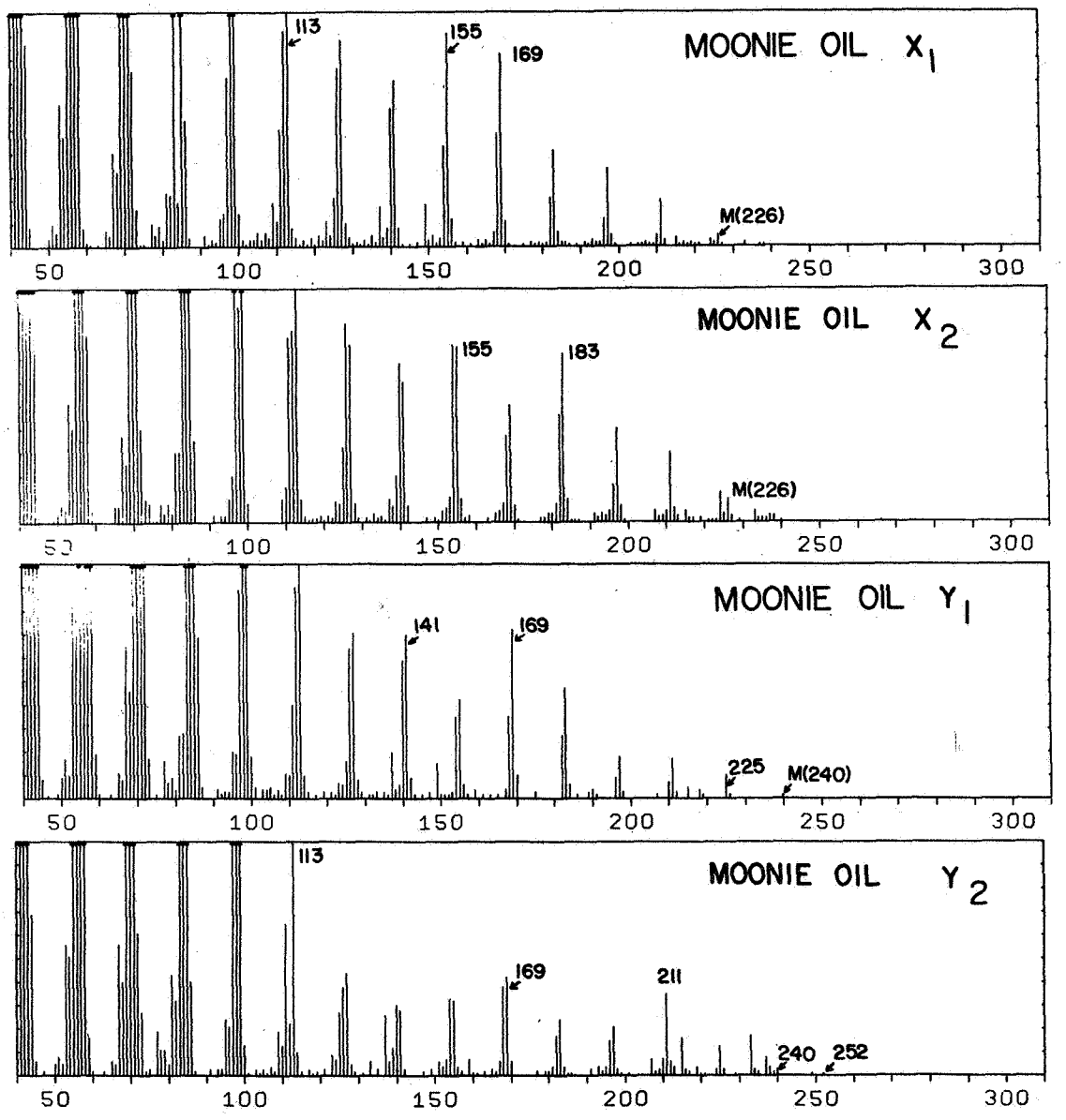



Figure 22. Mass spectra of branched alkanes from the Moonie Oil.

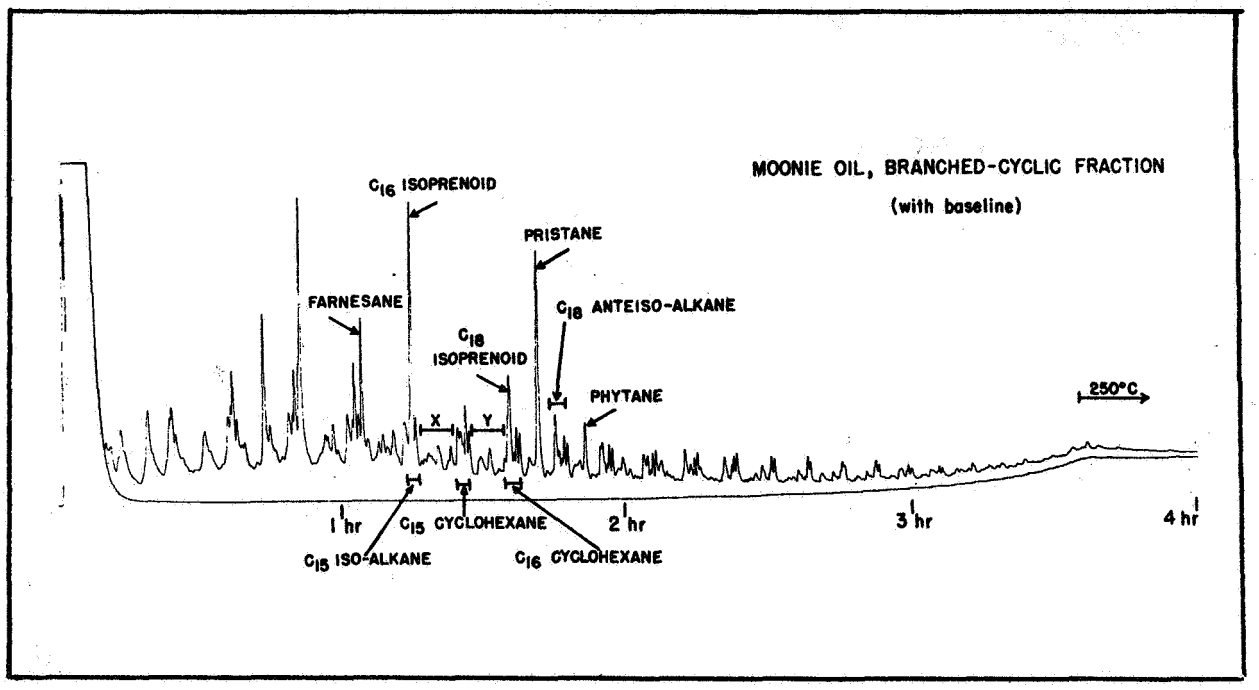
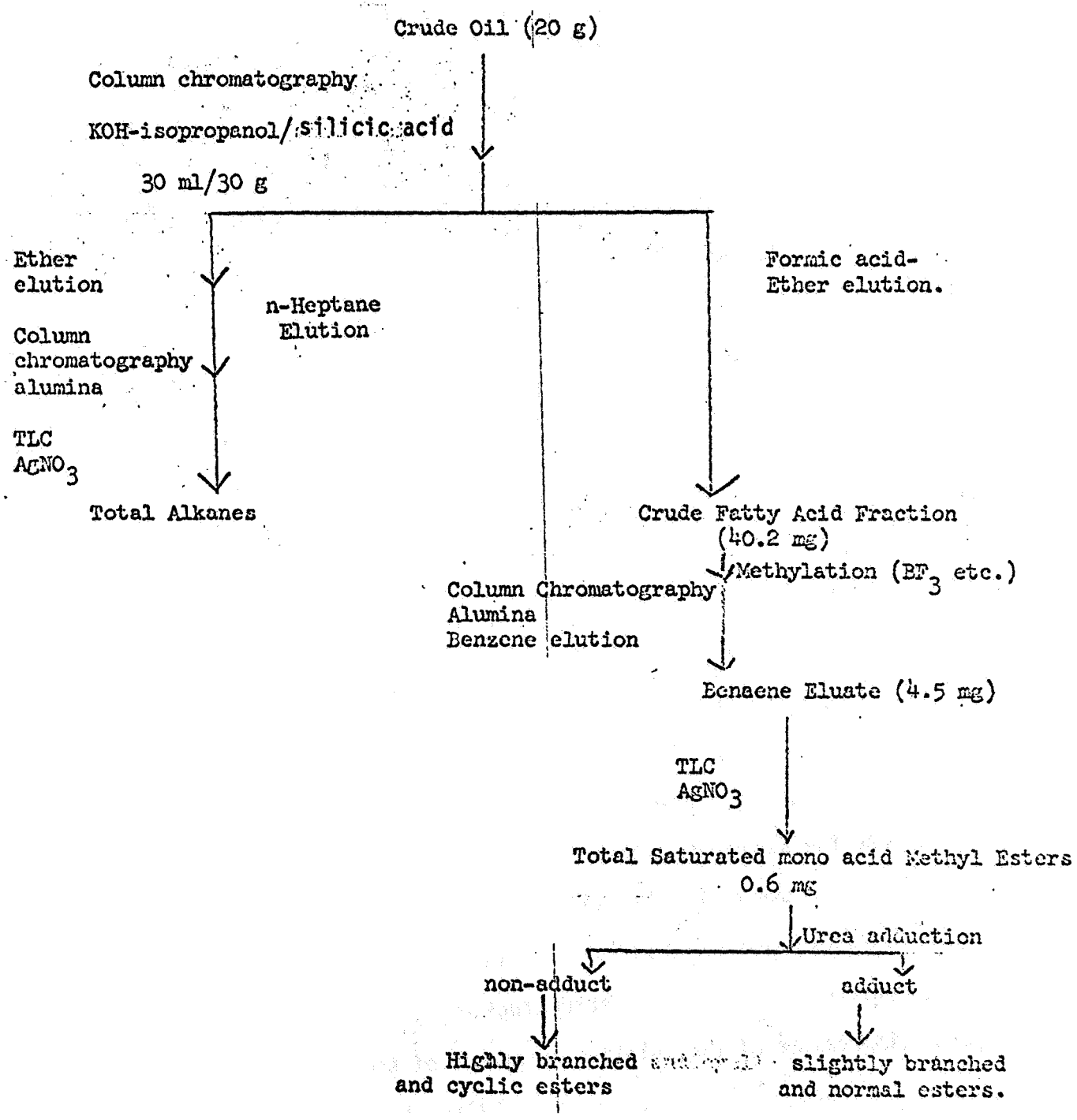



Figure 23. Capillary gas chromatogram of Moonie Oil, branched-cyclic fraction.

More recently, as part of this laboratory's program for the analysis of fatty acids, the Moonie Oil has been reexamined, primarily by J. R. Maxwell. The analytical scheme was as previously discussed, treating the oil as a "total extract." The quantities are depicted on the following page. The gas chromatogram of this acid fraction is shown in Figure 24. The labeled peaks and positions are determined by coinjection of the standard esters, plus urea adduction substantiation. In some cases, mass spectral evidence has been obtained as well, particularly in the case of methyl phytanate and several of the unbranched acids; however, the spectra are not of single compounds. The distribution is dominated by methyl phytanate. Of the normal acids, $C_{16}$ and $C_{18}$ are prominent, but not excessively so. The urea adduction is obviously not as selective as 5A sieves are with hydrocarbons, but some separation is effected. The large number of peaks in the adduct fraction may be due to such acids as <u>iso</u>- and <u>anteiso</u>- acids, which are known to be adducted. The presence of the methyl phytanate and other bulky acids can be attributed to the tendency of acids to adsorb on the urea.

Figure 25 shows a comparison between the saturated mono acids and the saturated alkanes. There is no apparent direct relationship between these two distributions. Also, it is difficult to compare this hydrocarbon distribution with that of the previous investigation. Capillary gas chromatography often gives a false first impression of the relative abundance of the major (in this case the normal alkanes) components. Also, the methods of sample

## MOONIE OIL ANALYTICAL RESULTS



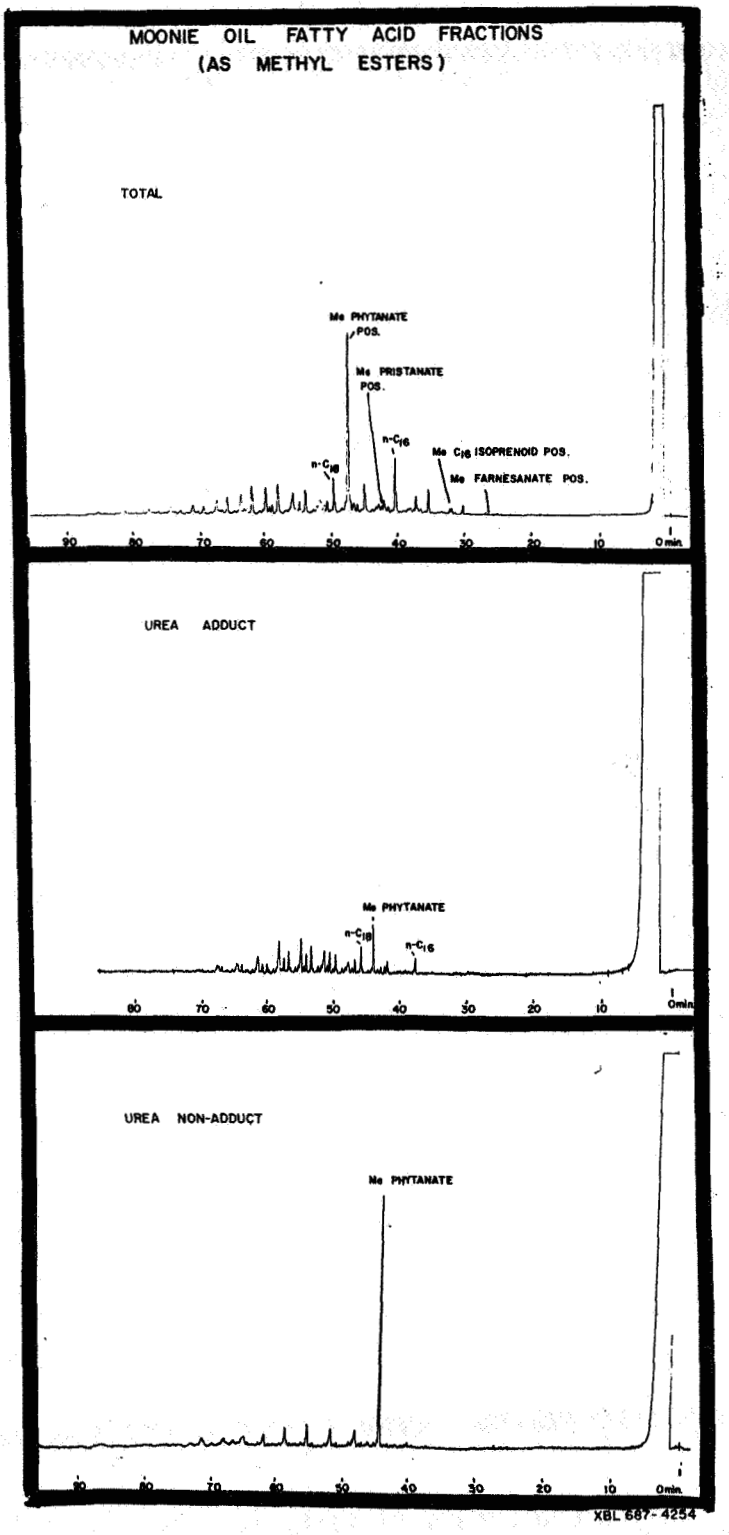



Figure 24. Gas chromatograms of fatty acid fractions (as methyl esters) from Moonie Oil.

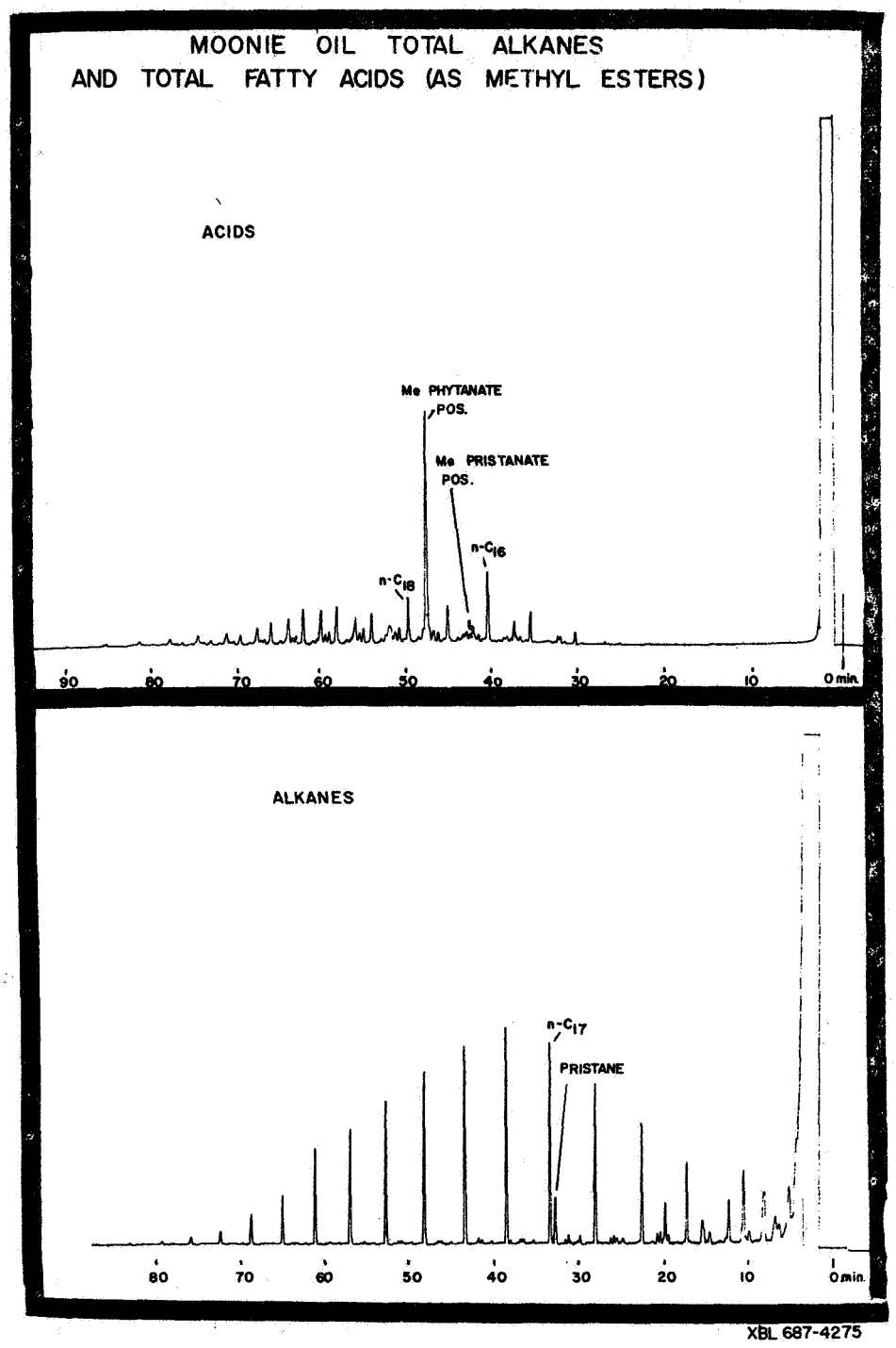



Figure 25. Gas chromatograms of fatty acids (as methyl esters) and alkanes isolated from the Moonie Oil.

preparation were slightly different, with no specific removal of alkenes from the earlier sample.

The question of a non-marine origin for the Moonie Oil (or a portion of it) receives no direct answer from the alkane distribution. The preponderance of odd-numbered alkanes, n-$C_{23}$ through n-$C_{31}$, often associated with non-marine sediments, is not evident. Also, there is no indication of steranes as in the Green River and Pierre Shales. The n-alkane maximum at $C_{18}$ is not unusual for oils examined in this manner.

The branched alkanes provide greater information than do the normals. The presence of the polyisoprenoid alkanes supports the contention of a large biological contribution to this oil. By coinjection of the $C_{17}$ regular polyisoprenoid, and subsequent mass spectrometric examination, it is obvious that the ubiquitous $C_{17}$ polyisoprenoid is not present in large amounts relative to the other polyisoprenoid alkanes, providing further support for the formation of this alkane series from one of the higher members--*e.g.*, phytol.

The presence of *iso*- and *anteiso*-alkanes has been observed previously. Although these compounds may derive directly from *iso*- and *anteiso*-alkanes or from *iso*- or *anteiso*-fatty acids in nature, their formation by thermal processes is not unreasonable, and little significance can be attached to the identification of single members of each series. Reports of alkyl cyclohexyl compounds in natural systems are relatively scarce.[190,191] For the most part these are thought to arise by cyclization due to non-

biological reactions, with unsaturated fatty acids being often mentioned as a feasible starting product. Again, no great biological significance can be placed on their occurrence in oils, unless it can be demonstrated that these arise by cyclization of biological acids or are naturally occurring, in large quantities, as the alkanes.

It is again the question of distributions and relative amounts which permits one to conclude the nature and presence of biological contributions to the sedimentary deposit. The distribution of minor components, perhaps such compounds as the $X_1$ and $X_2$ previously described, can provide more information as to the mode of formation or origin of some of the branched compounds. The unbranched 4-carbon chain of $X_2$, as mentioned in the introduction, may have great significance if the presence of this could be confirmed (see Chapter VI).

The fatty acids, considered by themselves, present a most interesting picture. Why phytanic acid should be so dominant is not understood. One can conjecture that phytol was oxidized and that subsequent decomposition of the acids has been limited, but there is no evidence from other compounds which supports this view. Simple decarboxylation to pristane would suggest a larger amount of this alkane should be present, although it may have gone on to the other polyisoprenoid alkanes. The ratio of normal acids to polyisoprenoid acids is lower than the ratio of normal alkanes to polyisoprenoid alkanes, suggesting again that decarboxylation is not the sole process operating.

A more reasonable explanation for the large quantity of this acid is its presence, in extraordinary amounts, in the contributing organisms. This may be a situation quite analogous to the phytanyl moiety in Kates' halophilic bacteria.[192] It is known which organisms may have contributed to this oil,[189] but no study has been done to support or refute the suggestion of phytanic acid or a readily oxidizable phytyl group being a major constituent of such organisms. It is even possible that this acid may be representative of the ecology unique to this continent at the time of formation of the oil.

THE ANTRIM SHALE

The Antrim Shale constitutes the first and youngest of the ancient sediments examined in this laboratory for fatty acids. It and the remaining samples were examined primarily for this purpose, and so greater attention is given to the fatty acid results obtained. In all the remaining samples the alkanes have previously been reported, and the distributions obtained in the analyses reported here are compared with these published reports. Several things should be kept in mind relative to the results reported here. The free acids and extractable alkanes were obtained by Dr. J. R. Maxwell, and the remaining samples by the author; some minor variations may result from this division of labor. Also, the weights reported for the various small samples are certainly not completely accurate, and may vary by $\pm 0.5$ mg; the values given in the various schemes should be interpreted in this light. Also, most of the discussion concerning the acids from the various sediments will be given after all the results have been stated. Only minor discussion is given following the results for individual samples.

The alkanes from the Devonian Antrim Shale ($350 \times 10^9$ years) have already been examined in considerable detail.[163] This analysis by McCarthy was done on a core sample from a depth of 2608 ft. In the work reported here, the 2608 ft sample was again examined, as well as a sample from 2614 ft, and a partial analysis

of a 2611 ft sample. According to R. D. Matthews,[193] all three of these samples are, geologically, essentially identical.

The 2608 ft core was examined by the hydrocarbon fatty acid scheme previously outlined. However, drying the HF-HCl residue prior to extraction was apparently incomplete, a fact which becomes evident only after extraction has begun. Accordingly, the sample was later extracted by means of a Soxhlet extractor, first with methanol and then with benzene, for about 12 hours each.

The samples available and analyzed from the above procedures are as follows:

Initial benzene: methanol extract

Saturated hydrocarbons

Monomethyl saturated acid esters

Ultrasonic extract of HF/HCl digestion residue

Fatty acid esters

Soxhlet extract of HF/HCl digestion residue (after sonication)

Saturated hydrocarbons

Fatty acid esters

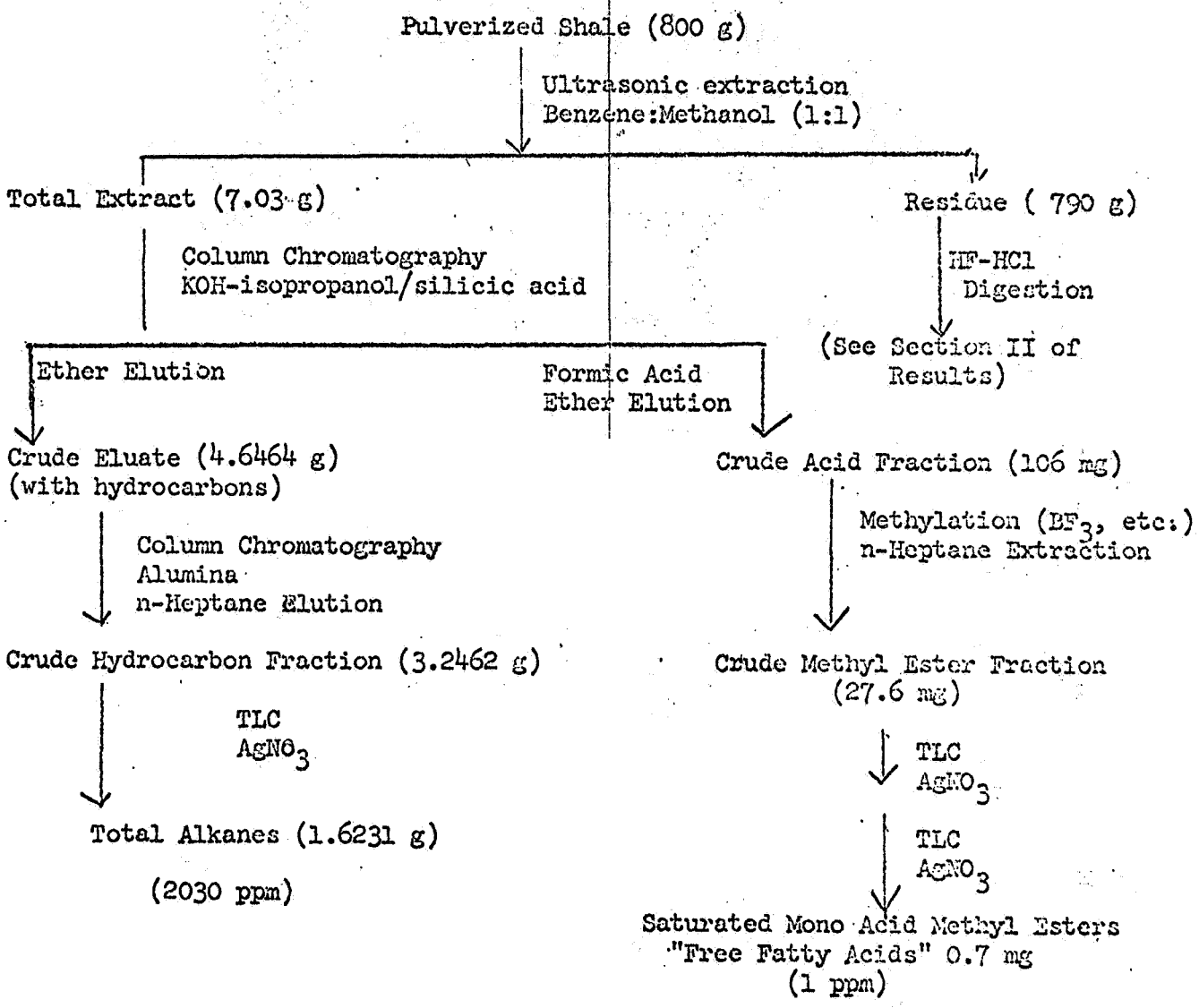
HF/HCl (filtrate)

Not analyzed

The quantities obtained in this complex analytical sequence are given in the following schemes.

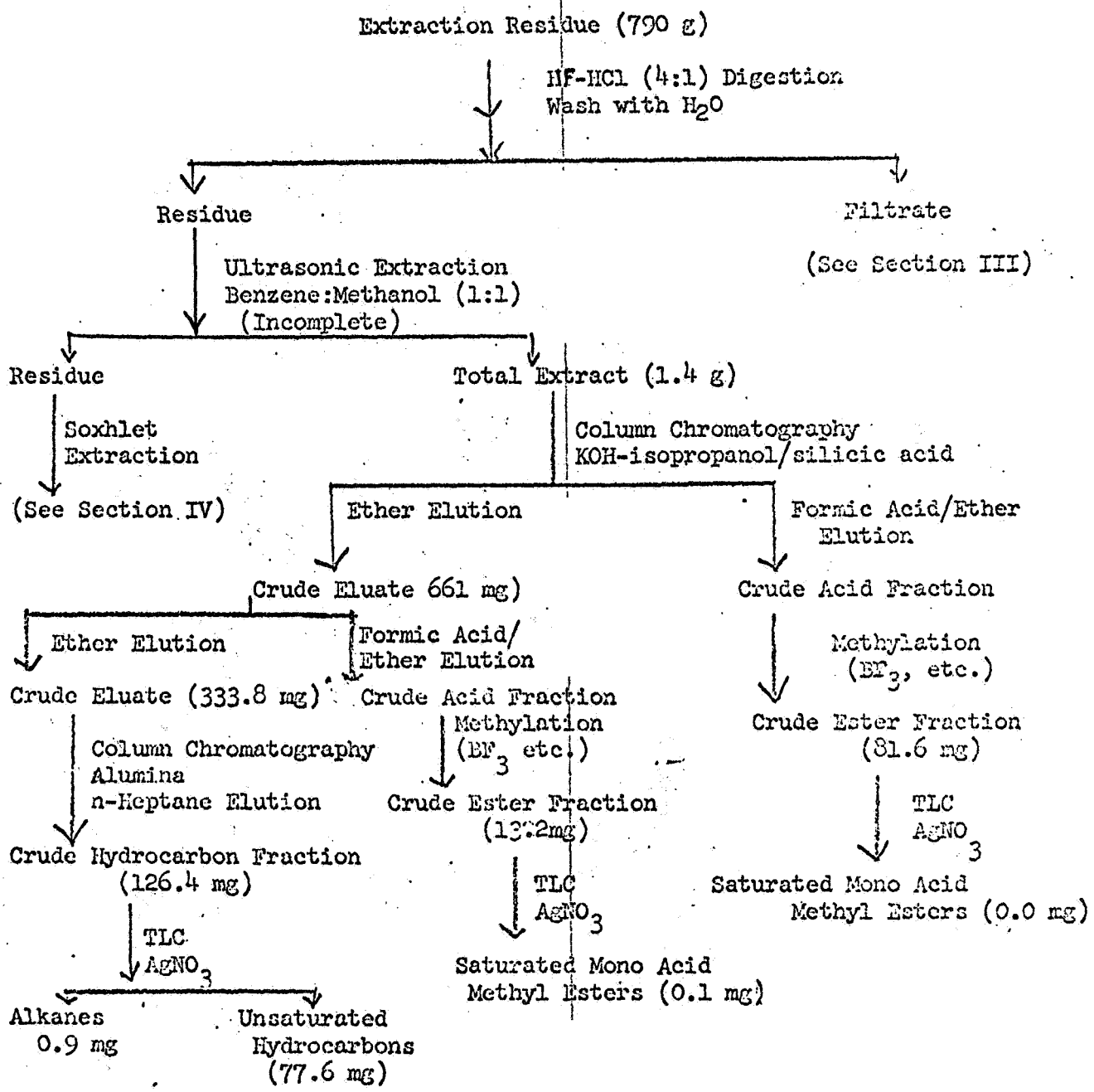
SECTION I

ANTRIM SHALE ANALYTICAL RESULTS



## SECTION II

## ANTRIM SHALE ANALYTICAL RESULTS



## Section III

## ANTRIM SHALE ANALYTICAL RESULTS

HF-HCl Used in Digestion
($+H_2O$ from washing)

↓ Benzene Extraction

Total Extract

Column Chromatography
KOH-Isopropanol/Silicic Acid

↓ Ether Elution

Crude Hydrocarbon Fraction
(2.7 mg)

↓ Formic Acid/
Ether Elution

Crude Acid Fraction

↓ Methylation
($BF_3$ etc.)

Crude Ester Fraction (12.9 mg)

↓ TLC
$AgNO_3$

Saturated Mono Acid Methyl Esters
(0.6 mg)

## SECTION IV

## ANTRIM SHALE ANALYTICAL RESULTS

Digestion Residue

↓ Soxhlet Extraction
1. Benzene 12 hrs.
2. Methanol 12 hrs.

Total Extract (8 g)

↓ Column Chromatography
KOH-Isopropanol/Silicic Acid

**Left branch:**

↓ Ether Elution

Crude Eluate

↓ Column Chromatography
Alumina (50 g)
n-Heptane Elution

Crude Hydrocarbon Fraction (45.7 mg)

↓ Colloidal Copper Column
n-Heptane Elution

Crude Hydrocarbon Fraction (32.9 mg)
(minus S)

↓ TLC
$AgNO_3$

Total Alkanes (26.0 mg)

**Right branch:**

↓ Formic Acid/
Ether Elution

Crude Acid Fraction

↓ Methylation
($BF_3$ etc.)

Crude Ester Fraction (11.9 mg)

↓ TLC
$AgNO_3$

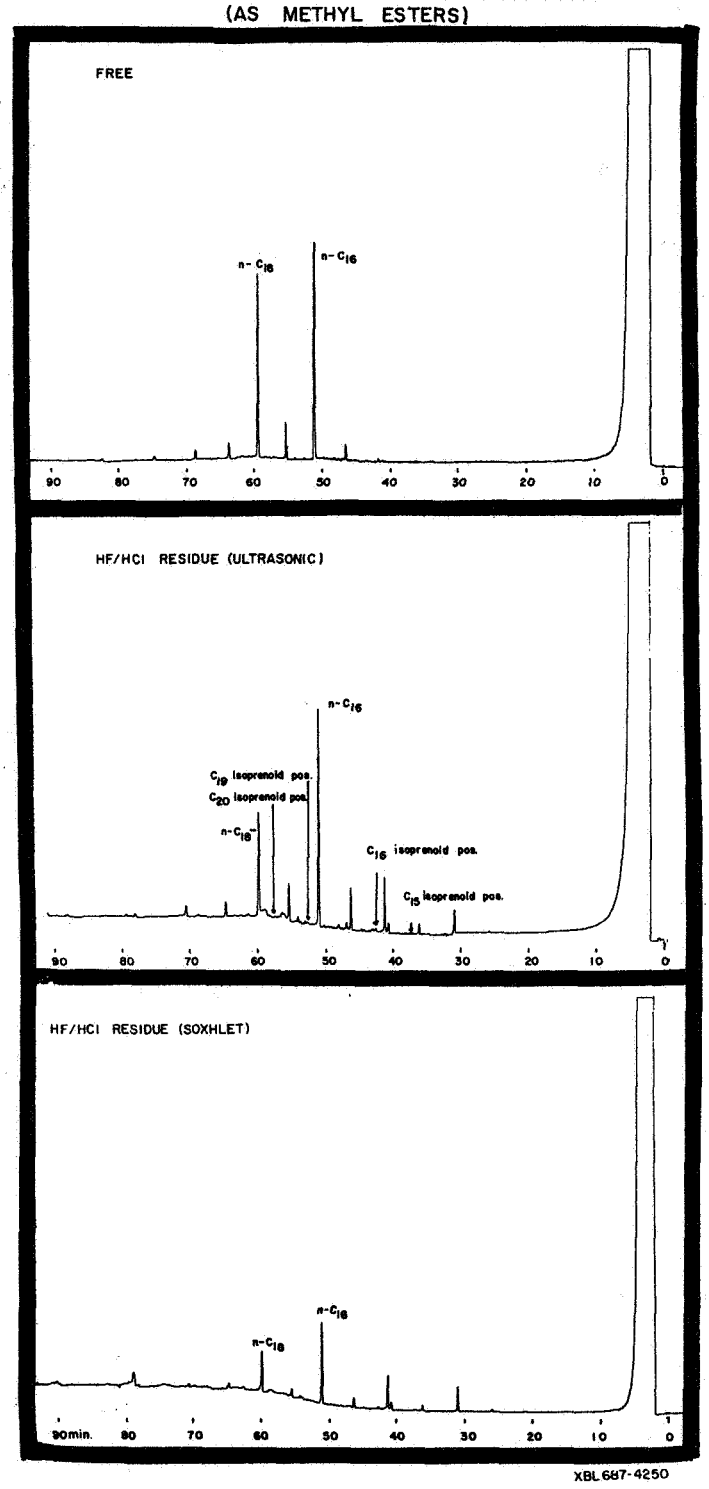
Saturated Mono Acid Methyl Esters
(0.0 ± 1 mg)

Figure 26 shows a comparison of the fatty acids from the initial extraction, the ultrasonic extraction of the HF/HCl residue, and the Soxhlet extraction of the HF/HCl residue. Qualitatively the three patterns are similar, with the normal acids, particularly $C_{16}$ and $C_{18}$ (identified by coinjections) being the dominant components. It is important here to note that the latter two patterns are essentially identical, suggesting that the two methods of extraction are quite equivalent in their behavior toward the fatty acids. The bound acids exhibit more of the low molecular weight components; perhaps this difference is due to minor fluctuations in work-up--i.e., evaporation of the $C_{12}$, $C_{14}$ (and $C_{16}$?), etc., compounds, or to minor differences in the free and bound acid distributions.

Figure 27 shows the comparison between the bound fatty acids and the alkanes from the initial extraction. In general, this is the best way to compare alkanes and fatty acids from a given sample, since the bound acids are generally 2-10 times more abundant than the "bound" alkanes. Of note here is the lack of correlation between the two patterns.

Figure 28 shows the difference in the alkanes obtained by initial ultrasonic extraction (∿2000 ppm) and those obtained via Soxhlet extraction (∿30 ppm). Obviously the former process tends to extract a greater proportion of the lower molecular weight alkanes than of the higher alkanes. The peak labeled "pristane" is determined by coinjection; the distribution of the alkanes

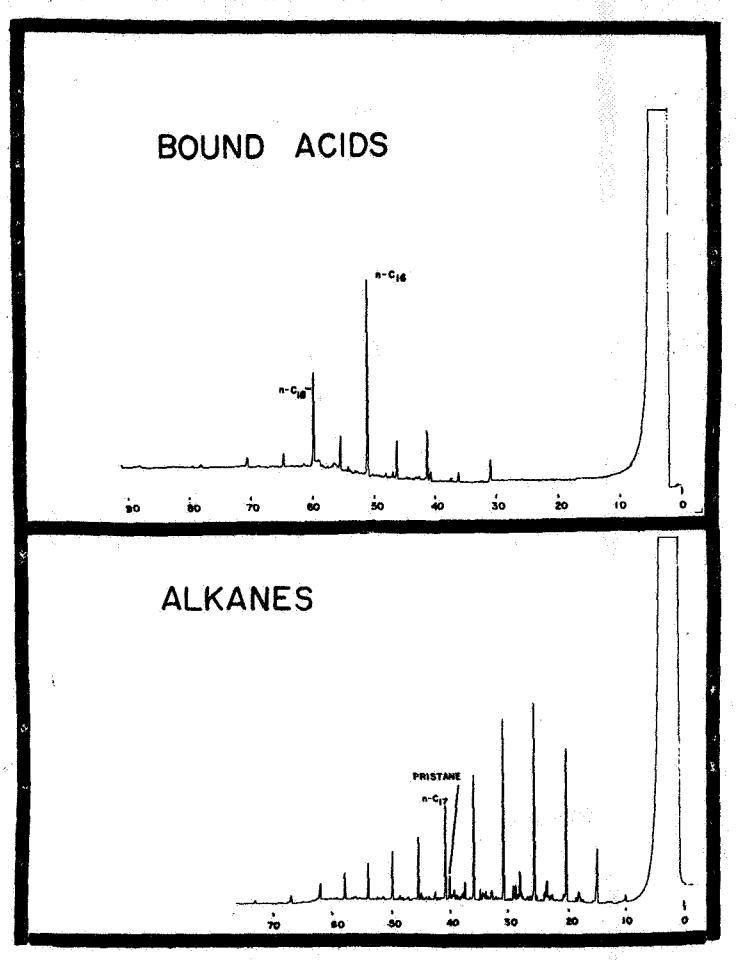
ANTRIM SHALE FATTY ACID FRACTIONS
(AS METHYL ESTERS)




XBL 687-4250

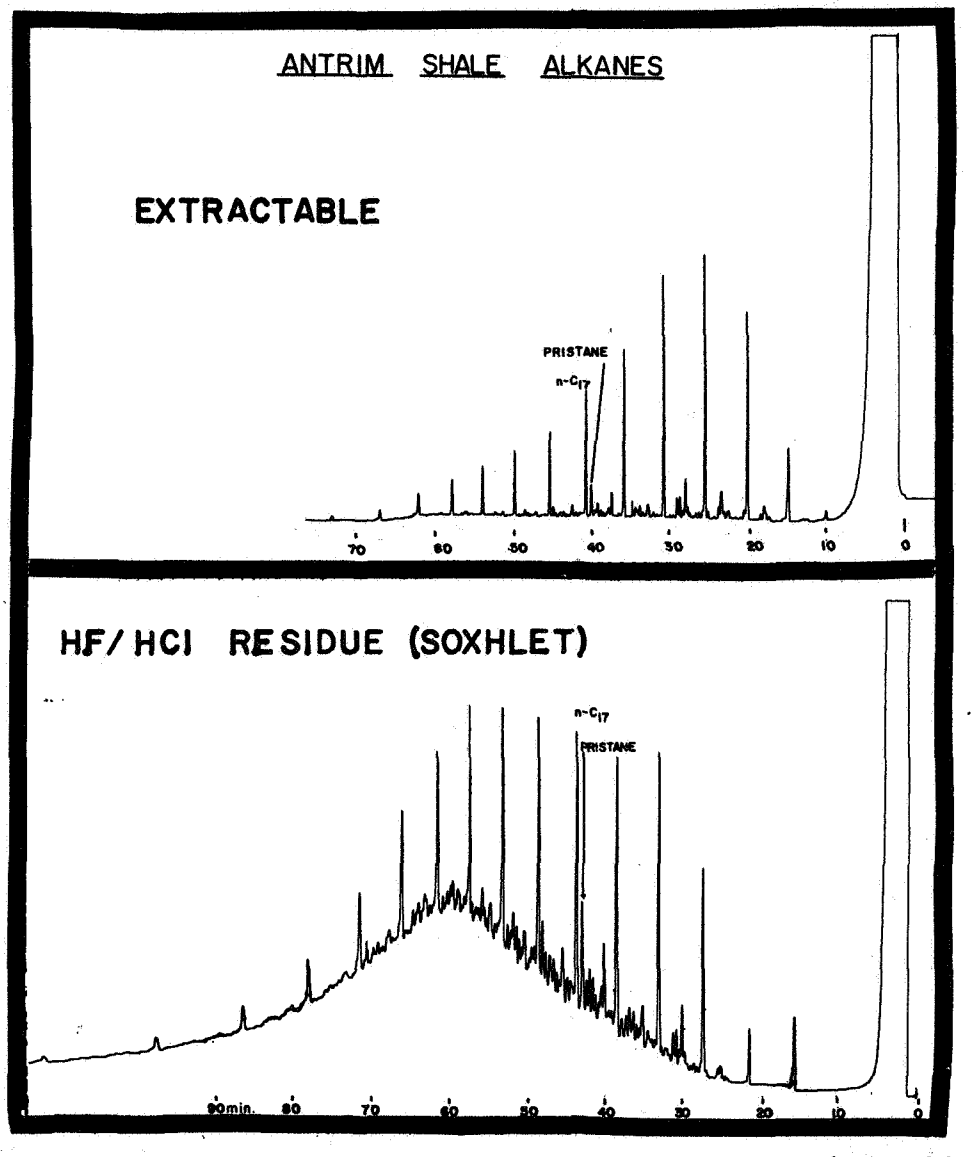
Figure 26. Gas chromatograms of fatty acids (as methyl esters) from the Antrim Shale initial extract (free) and HF/HCl residue.

ANTRIM SHALE ALKANES & BOUND FATTY ACIDS (as methyl esters)




XBL 688-4389

Figure 27. Gas chromatograms of the Antrim Shale bound fatty acids (as methyl esters) and the extractable alkanes.




XBL 6811-6694

Figure 28. Gas chromatograms of alkanes from the Antrim Shale, by initial extraction and extraction of the HF/HCl residue.

from the initial extract is essentially that of McCarthy,[163] and no additional characterizations were carried out.

Because of the difficulties involved in extracting the bound acids of this 2608 ft sample, another analysis for free and bound acids was carried out, using the 2614 ft sample. The quantities of the various fractions obtained in this analysis were roughly equivalent to those from the previous sample. The distributions of the free and bound fatty acids are essentially identical to those from the 2608 ft sample. Although the extractable alkanes were not examined, the "bound" alkanes were given a cursory glance and this revealed a distribution in which the maximum lay somewhere between the initially extracted alkanes and the Soxhlet extracted alkanes of the earlier analysis.

The HF/HCl used for digesting the 2614 ft sample was examined for alkanes and fatty acids. The alkane distribution ($\sim$0.1 ppm original sediment) parallels that of the alkanes from the digestion residue. The acid distribution (<0.1 ppm), parallels that of the bound acids. Again, it is noted that the HCl used in this particular digestion had been purified by benzene extraction.

Finally, the 2610 ft sample was examined for free fatty acids, and the distribution pattern was identical to that of earlier samples. No further analysis of this sample was performed.

As is true of the hydrocarbons, the fatty acids of the Antrim Shale have a distribution which is consistent with a biological origin; the predominance of $C_{14}$, $C_{16}$ and $C_{18}$ acids correlates

well with the predominance of these acids in modern biological systems.[135,136] The failure of the acids to obtain an odd/even carbon ratio equal to one is different than what has been suggested would be the case.[147] Cooper[194] and Kvenvolden[182] have reported on the distribution of fatty acids in the geologically related Chattanooga Shale. In their reports, the n-acids exhibit an odd/even ratio nearly equal to unity. There are several factors which prevent extrapolating the results from the Chattanooga Shale to the Antrim. In the first place, the two shales are related but not identical. According to Matthews,[193] the Antrim Shale, though lithologically similar to and occupying the same stratigraphic position as black shales in the East-Central United States, is not continuous from Michigan southward. There is apparently some margin for discussion as to whether or not the two shales have exactly the same date of origin, since the Chattanooga contains both Devonian and Mississippian fossils,[193] and no paleontological evidence has been found which definitely establishes that the ages of the Michigan unit and those from further south (i.e., Ohio) are the same.[195]

In addition to this argument about initial deposition, there is always a question about differences in diagenetic condition. As Kvenvolden explicitly states in his report,[147] the post-depositional situation may be the most influential factor in determining the final (i.e., present) fatty acid distribution. Such factors as

thermal histories and inorganic elemental abundances may greatly affect the preservation of the initial distribution of such compounds.

Finally, no one can doubt that ecological differences exist now and probably also existed 350 x $10^6$ years ago between Michigan and Oklahoma (from where Kvenvolden's sample came). Perhaps such differences in contributing organisms are responsible for the observed differences.

The fact that the bound acids are equal or greater in quantity than the free acids supports the notion that some of the original acids become bound to the inorganic matrix. The fact that the free and bound acids have similar distributions suggests that this binding is not absolutely necessary for preservation of the acids for 350 x $10^6$ years (but is helpful), although it is possible that the acids described here as free were liberated from the matrix by the extraction.

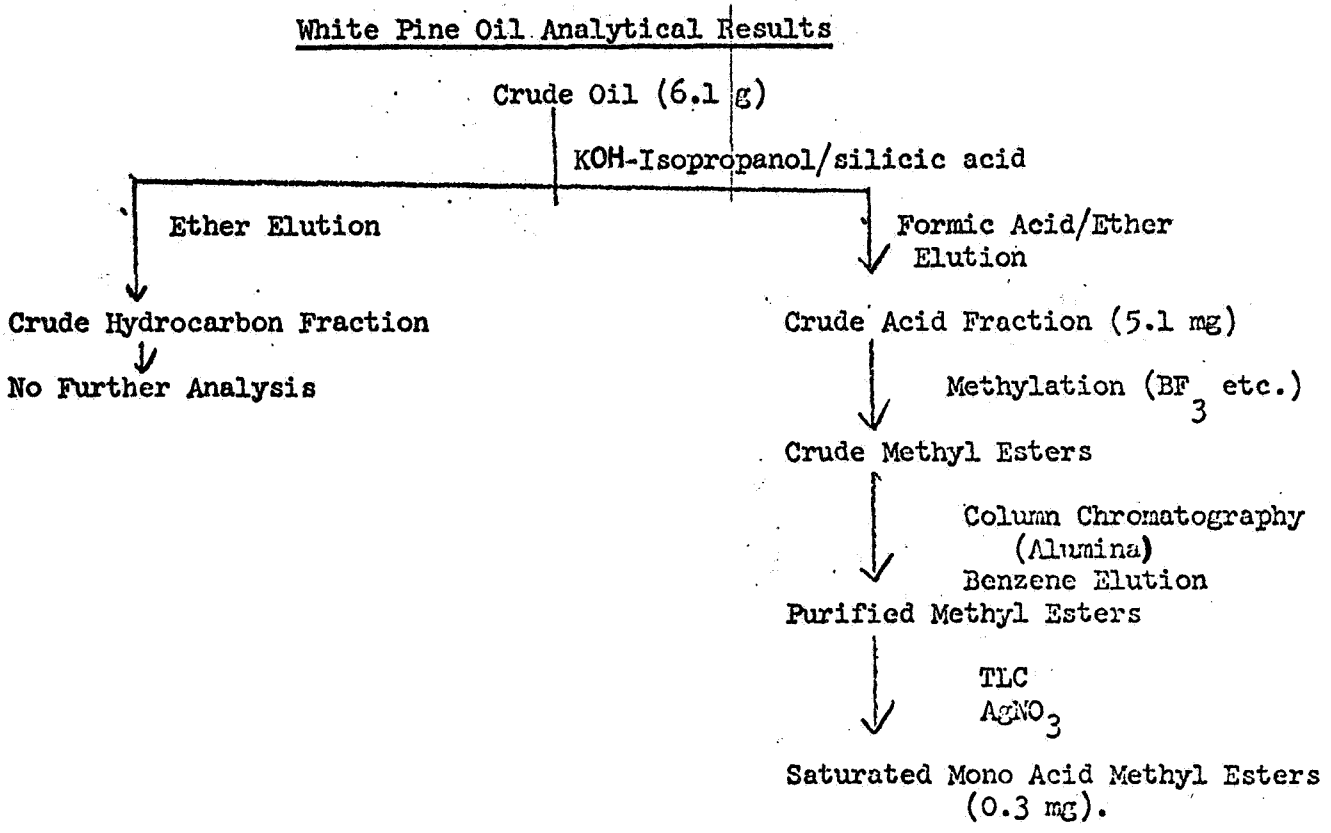
As was the case in the Moonie Oil, there is no evidence to support the hypothesis of acids going directly to hydrocarbons solely by decarboxylation.

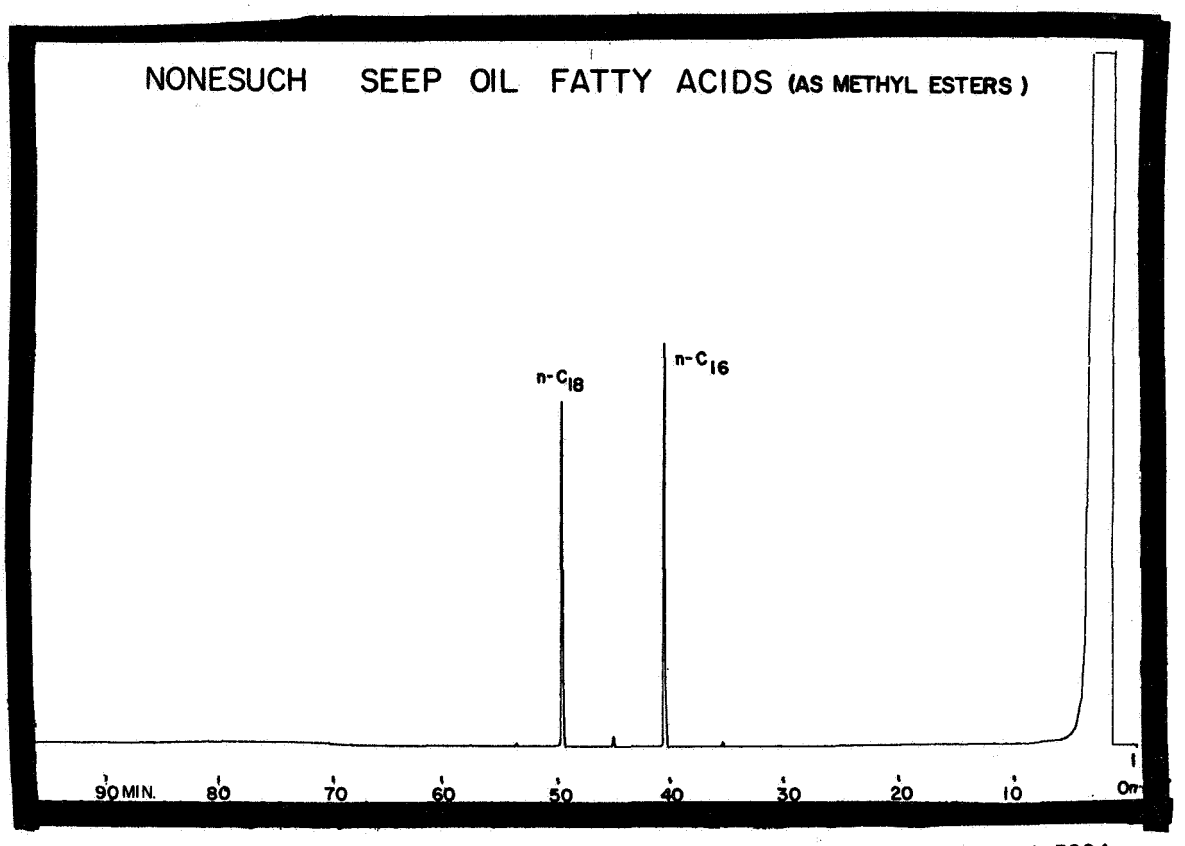
THE NONESUCH SHALE

Of the Precambrian samples analyzed for fatty acids, the Nonesuch Shale and associated seep oil are the youngest, with an age of $1 \times 10^9$ years. The geological factors attending this deposit have previously been discussed in detail.[196] Associated with the shale and oozing from faults, is a seep oil, which is reportedly the same age as the shale.[196] In the case of old oil seeps, some bacteria seem to feed on this petroleum. In the collection of this oil, a concerted effort was made to avoid areas of obvious bacterial growth. Recent collections of both the oil and the subsurface shale were made by W. Van Hoeven with the assistance of Dick Thompson, Resident Geologist, White Pine Copper Co. As in the case of the Moonie Oil, the seep oil was examined for fatty acids and hydrocarbons. The results are shown on the following page.

Figure 29 is the gas chromatogram of the fatty acid fraction (as methyl esters). It is dominated by methyl palmitate ($n\text{-}C_{16}$) and methyl stearate ($n\text{-}C_{18}$) with very few other components being evident.

The apparently biological nature of the fatty acids distribution might be interpreted as biological contamination from the bacterial sources mentioned earlier. Obviously the pattern of fatty acids from the shale is relevant to this question.

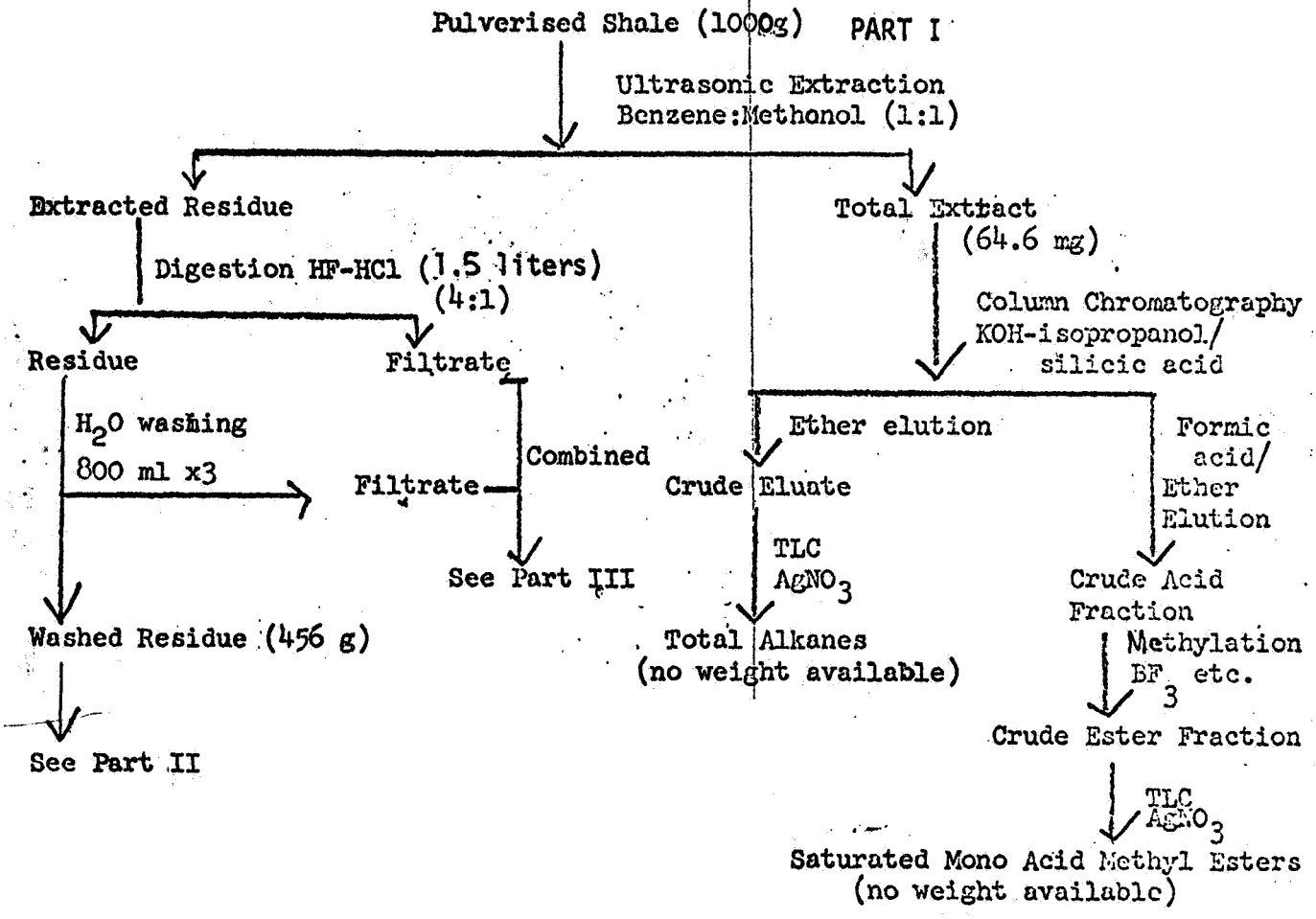

White Pine Oil Analytical Results
Crude Oil (6.1 g)
KOH-Isopropanol/silicic acid
Ether Elution
Formic Acid/Ether Elution
Crude Hydrocarbon Fraction
No Further Analysis
Crude Acid Fraction (5.1 mg)
Methylation ($BF_3$ etc.)
Crude Methyl Esters
Column Chromatography (Alumina) Benzene Elution
Purified Methyl Esters
TLC $AgNO_3$
Saturated Mono Acid Methyl Esters (0.3 mg).




XBL6812-5264

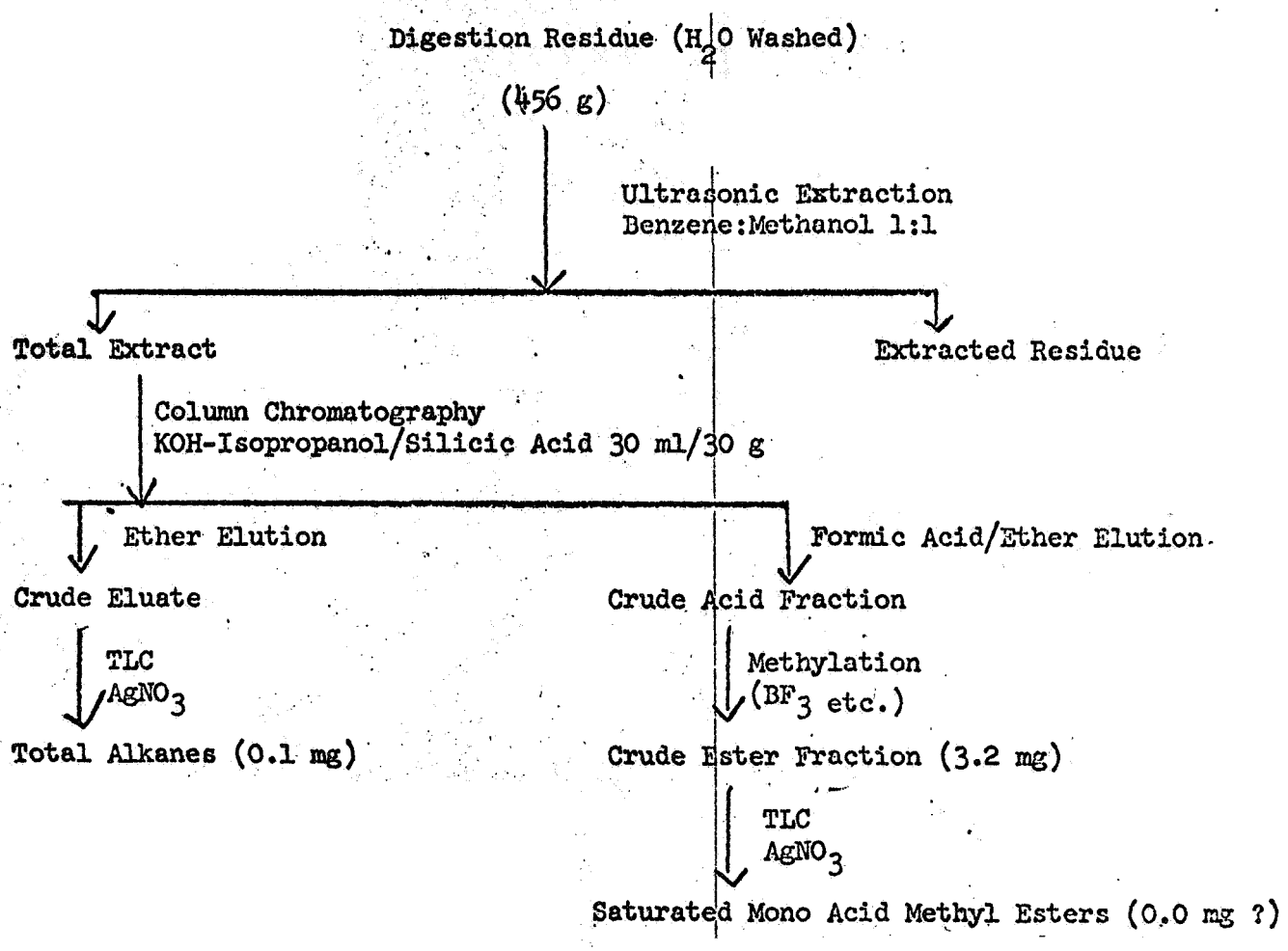
Figure 29. Gas chromatogram of fatty acids (as methyl esters) from the Nonesuch Seep Oil.

NONESUCH SHALE ANALYTICAL RESULTS



NONESUCH SHALE ANALYTICAL RESULTS

PART II



NONESUCH SHALE ANALYTICAL RESULTS

Part III

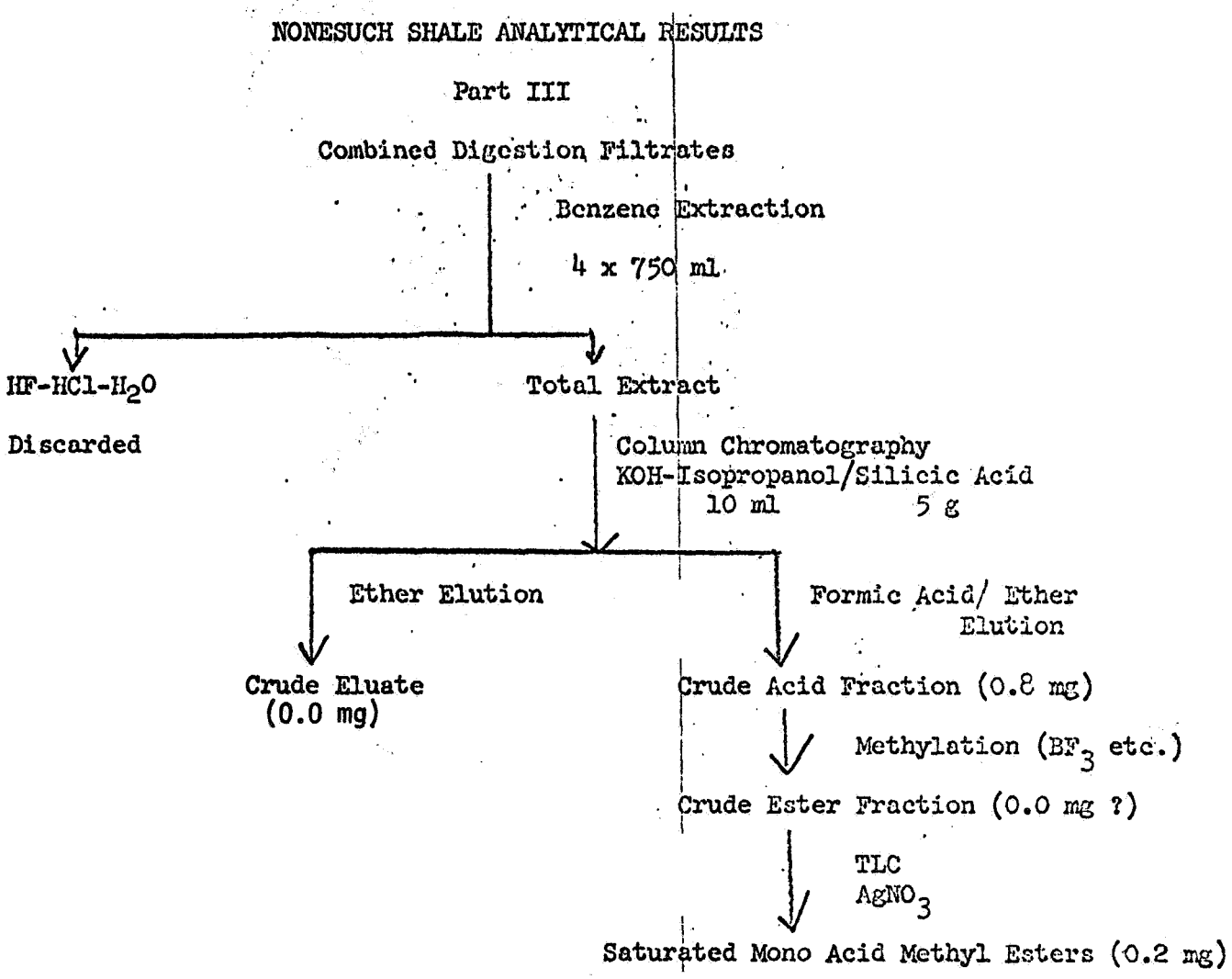


Figure 30 presents the fatty acid distributions from the initial extraction, the HF/HCl digestion residue, and the HF/HCl used in digestion. In all three cases, the distribution is essentially composed of only two components, the n-$C_{16}$ and n-$C_{18}$ acids (determined by coinjection). The bound acids pattern results from inadvertant injection of too large a sample (essentially the total available) and shows that some small amounts of other compounds are present. The insert represents the record of the relative abundances of the two major compounds. The HF/HCl-filtrate pattern was run at high sensitivity, and the appearance of the shoulder at $C_{16}$ and other miscellaneous small peaks is deceiving, since only the two major peaks appeared on other chromatograms of this fraction used for coinjection. The comparison between the acid distribution and the extractable alkanes is shown in Figure 31. This alkane distribution is essentially that previously reported,[156] and the assignment of structures is based on coinjection and analogy.

The dominance of n-$C_{16}$ and n-$C_{18}$ is not unexpected for this sample since, qualitatively, such a distribution was suggested by other workers.[148] The similarity between the seep oil distribution and the shale distribution, in view of the steps taken to prevent bacterial contamination of the shale, combine to suggest that the seep oil fatty acid distribution is characteristic of the ancient oil and is not due to recent bacterial activity. The simplicity of the acid pattern, compared with the younger samples, may be explained in several ways. Perhaps only the

NONESUCH SHALE FATTY ACID (METHYL ESTERS).

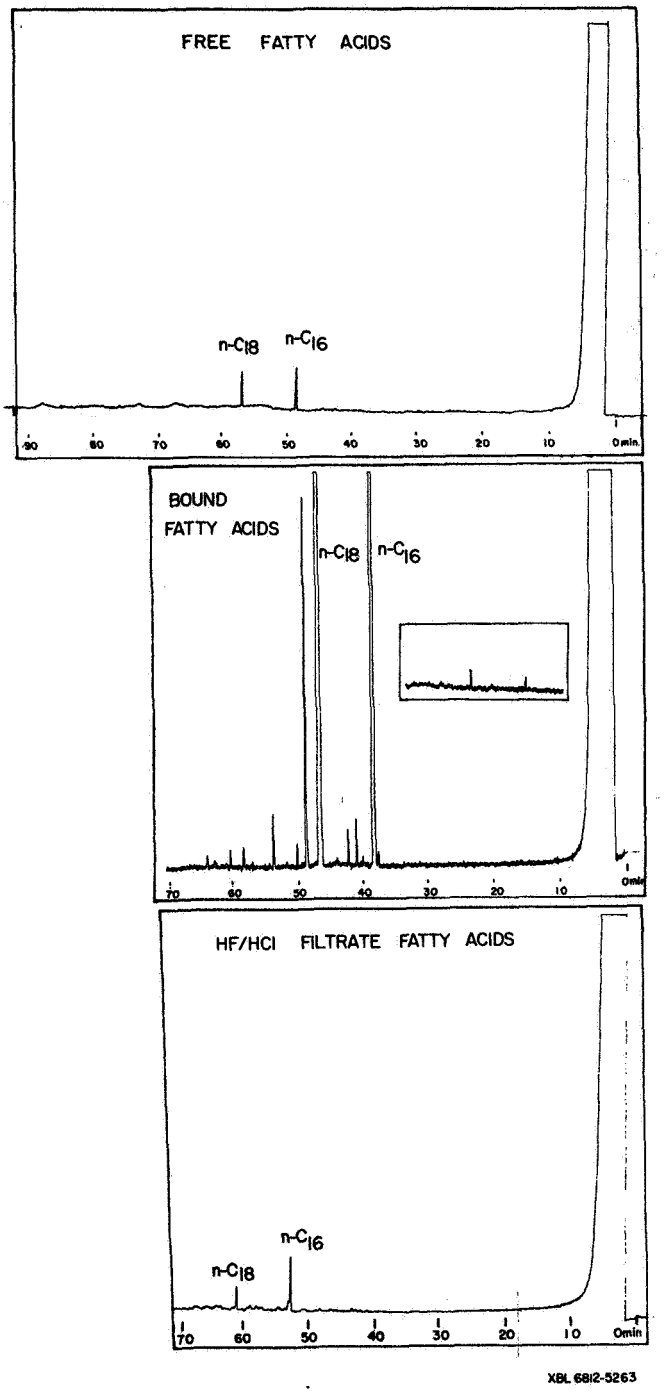
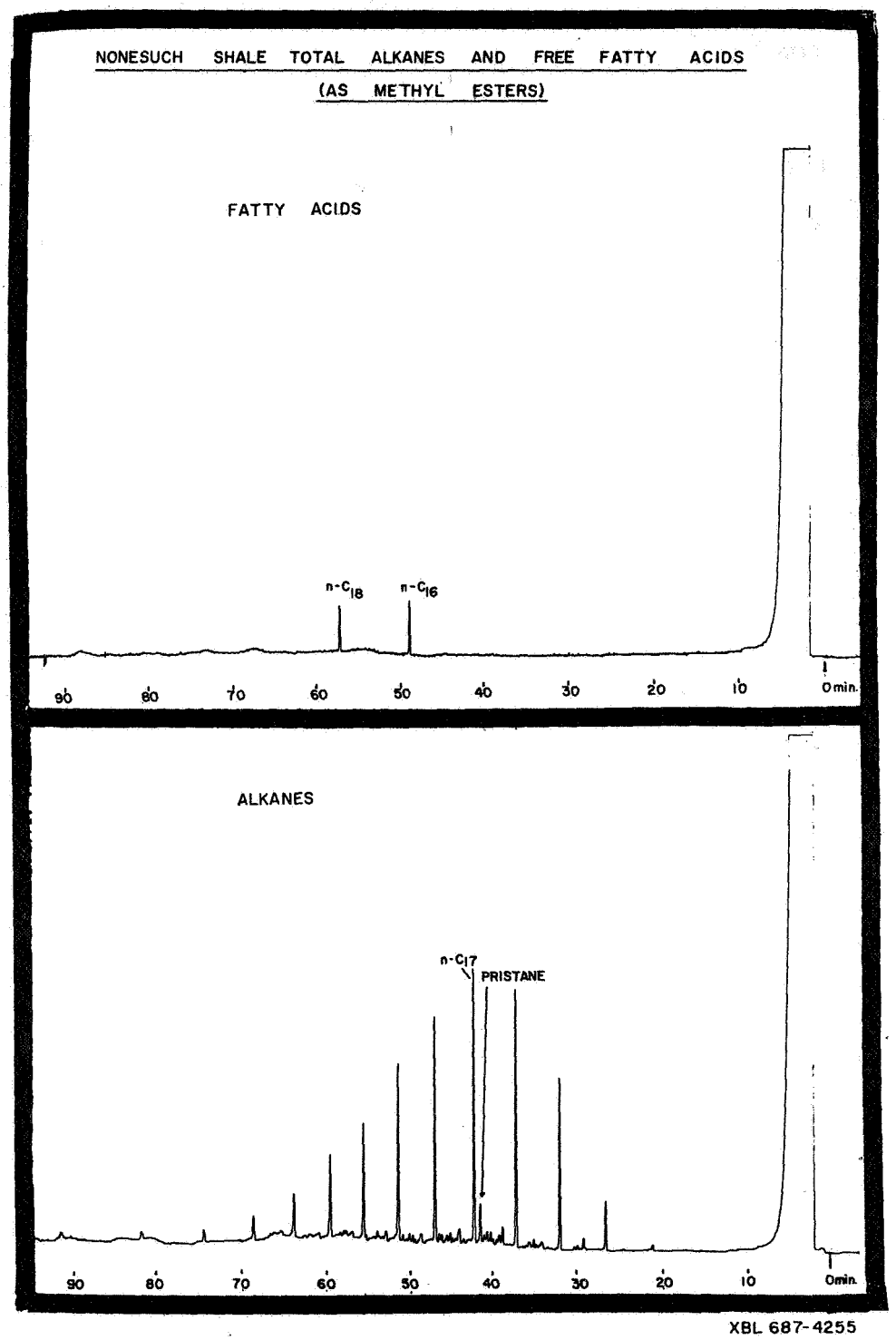



Figure 30. Gas chromatograms of Nonesuch Shale fatty acids (as methyl esters) from initial extraction (free), HF/HCl residue (bound), and HF/HCl filtrate.




XBL 687-4255

Figure 31. Gas chromatograms of extractable fatty acids (as methyl esters) and alkanes from the Nonesuch Shale.

normal acids were initially deposited, a not unlikely possibility if the contributing organisms were relatively primitive or limited in type. Modern day primitive algae and bacteria do contain a rather limited range of acids in terms of carbon numbers, with n-$C_{16}$ and n-$C_{18}$ (saturated and unsaturated) being by far the most abundant.[181] Another possible explanation is that only the normal acids have survived for a billion years, and this may also be the case. Unfortunately, there is not sufficient data on geochemical stabilities of fatty acids to provide substantiation or refutation of this latter concept.

Whatever the case, initial selective deposition, or subsequent selective preservation, the fatty acids which have survived for one billion years exhibit a most interesting and certainly biological distribution. Their presence at this early time supports the hypothesis that fatty acid distributions can provide information on the time and nature of the origin of life.

THE GUNFLINT CHERT

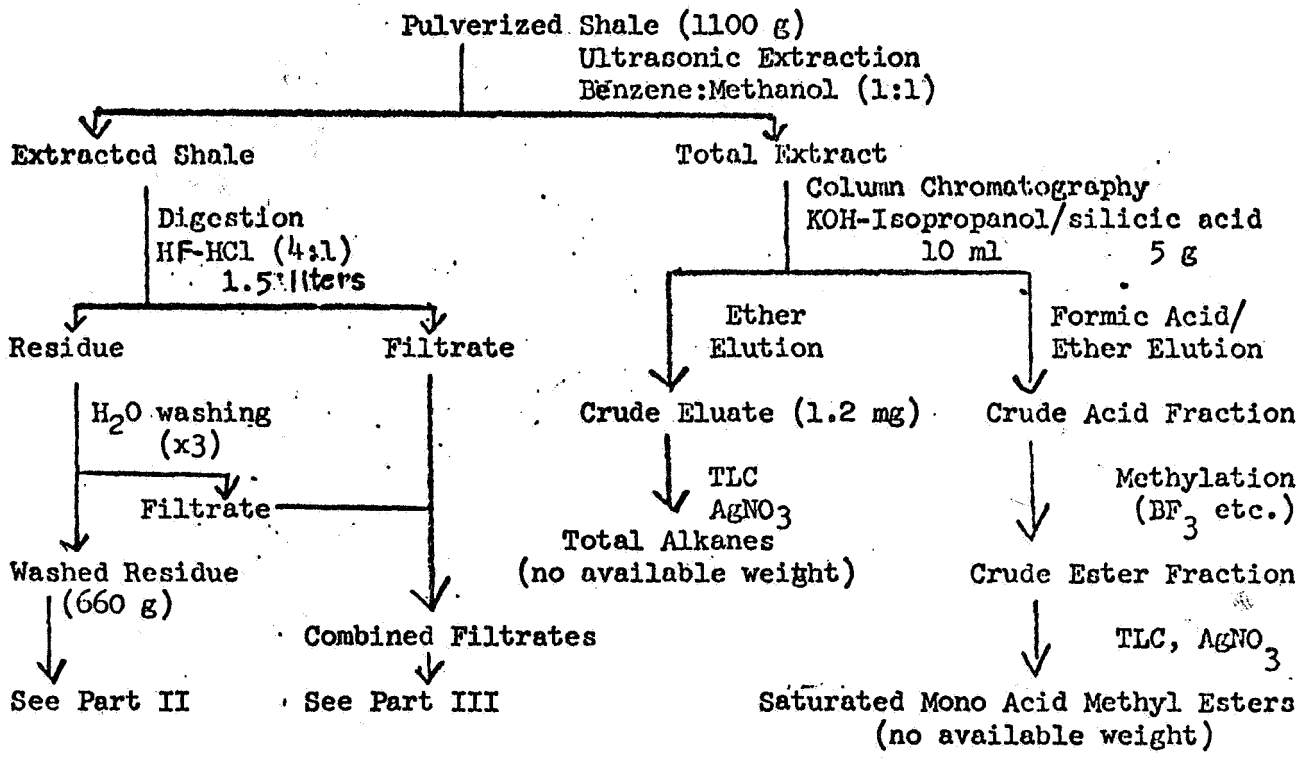
The Gunflint Chert represents a well-defined[197] Precambrian sediment (1.9 x $10^9$ years) from which alkanes[158] and amino acids[76] have previously been reported. The sample reported here was obtained from P. Cloud; unfortunately it consisted of such small pieces (∿25-100 g) that removal of the outer surface was not possible. Accordingly these pieces were ultrasonically washed several times to remove surface contamination. The sample was then pulverized and treated according to the previously outlined procedure. The quantities obtained are outlined on the following page. Another procedural departure from the previous samples was that the HCl used was not the 37% solution purified by benzene extraction, but a 21% azeotrope purified by distillation.

The results obtained from solvent extraction are presented in Figure 32. This single fatty acid has a retention time equal to the n-$C_{18}$ acid. The extractable alkanes have a maximum at n-$C_{22}$, and no indication of pristane and/or phytane. For the acids as well as the hydrocarbons, the quantities obtained were diminutive; in fact, the total fatty acids were injected for this one chromatogram.

The distributions of fatty acids and hydrocarbons obtained by extraction of the HF-HCl digestion residue are shown in Figure 33. By far the most dominant acid is the n-$C_{16}$, although n-$C_{14}$, n-$C_{15}$ and n-$C_{18}$ are also apparently present. Confirmation that the major acid is indeed the n-$C_{16}$ comes from coinjection

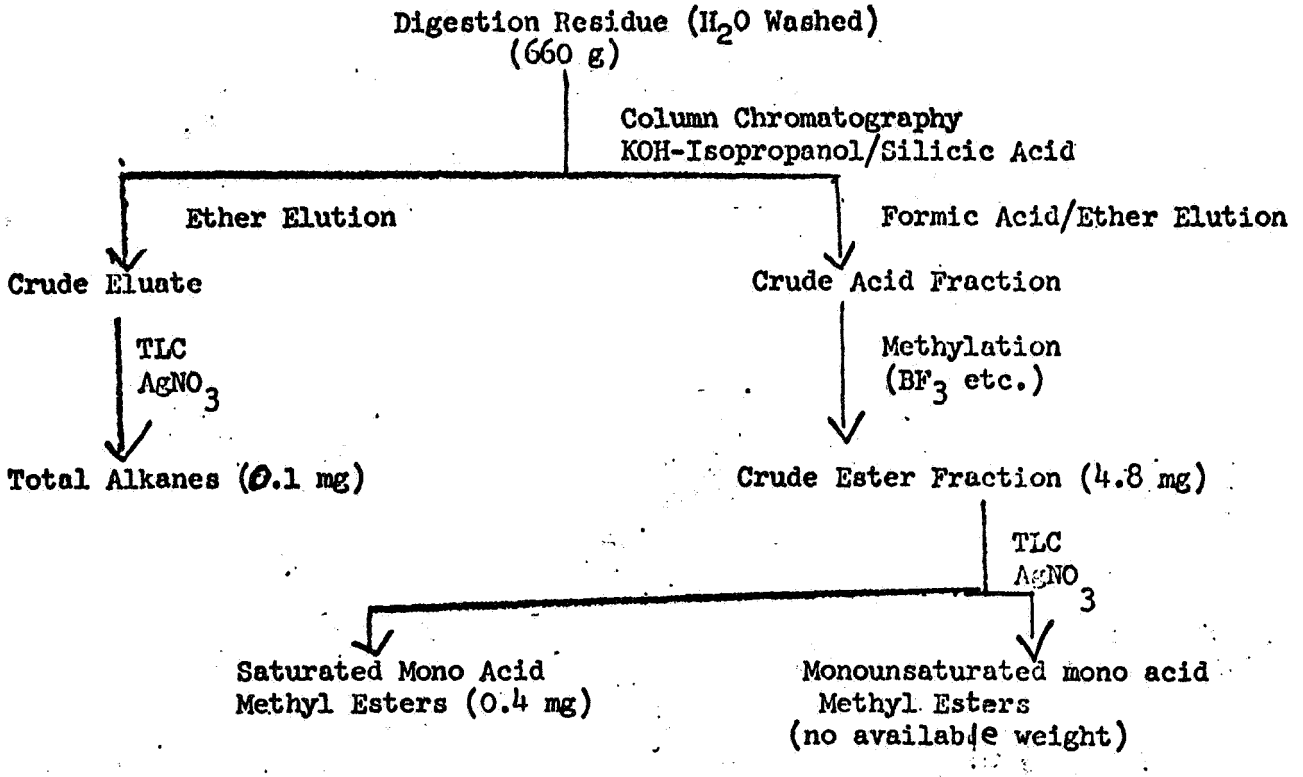
Part I

Gunflint Chert Analytical Results

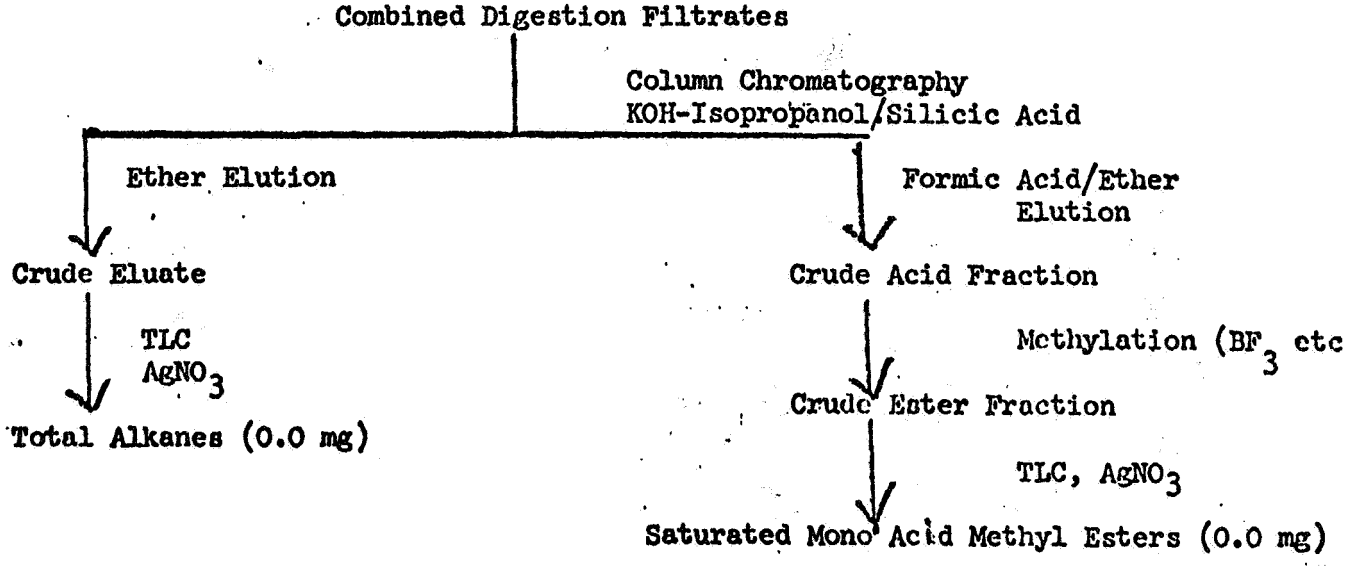


GUNFLINT CHERT ANALYTICAL RESULTS

Part II




Part III



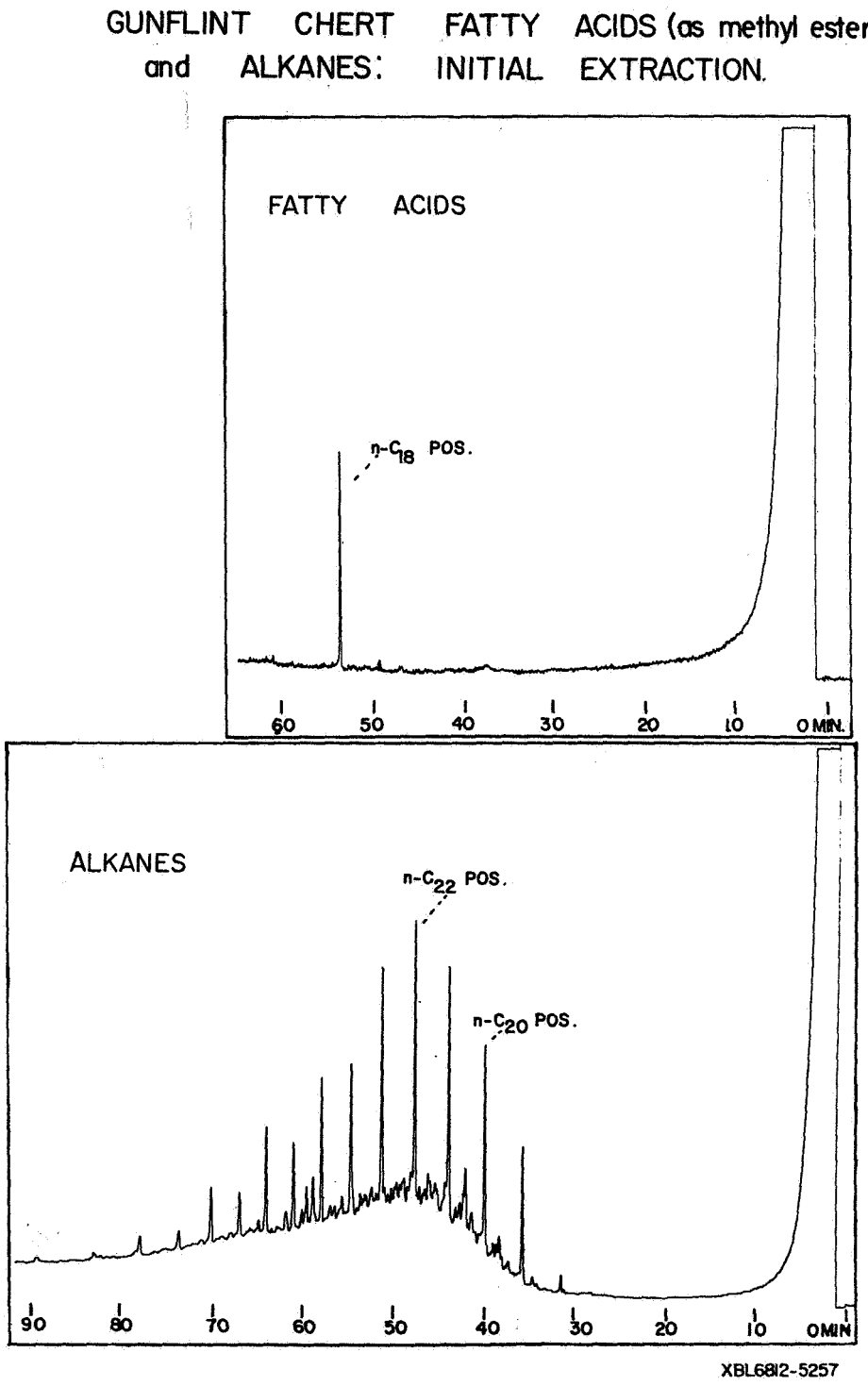
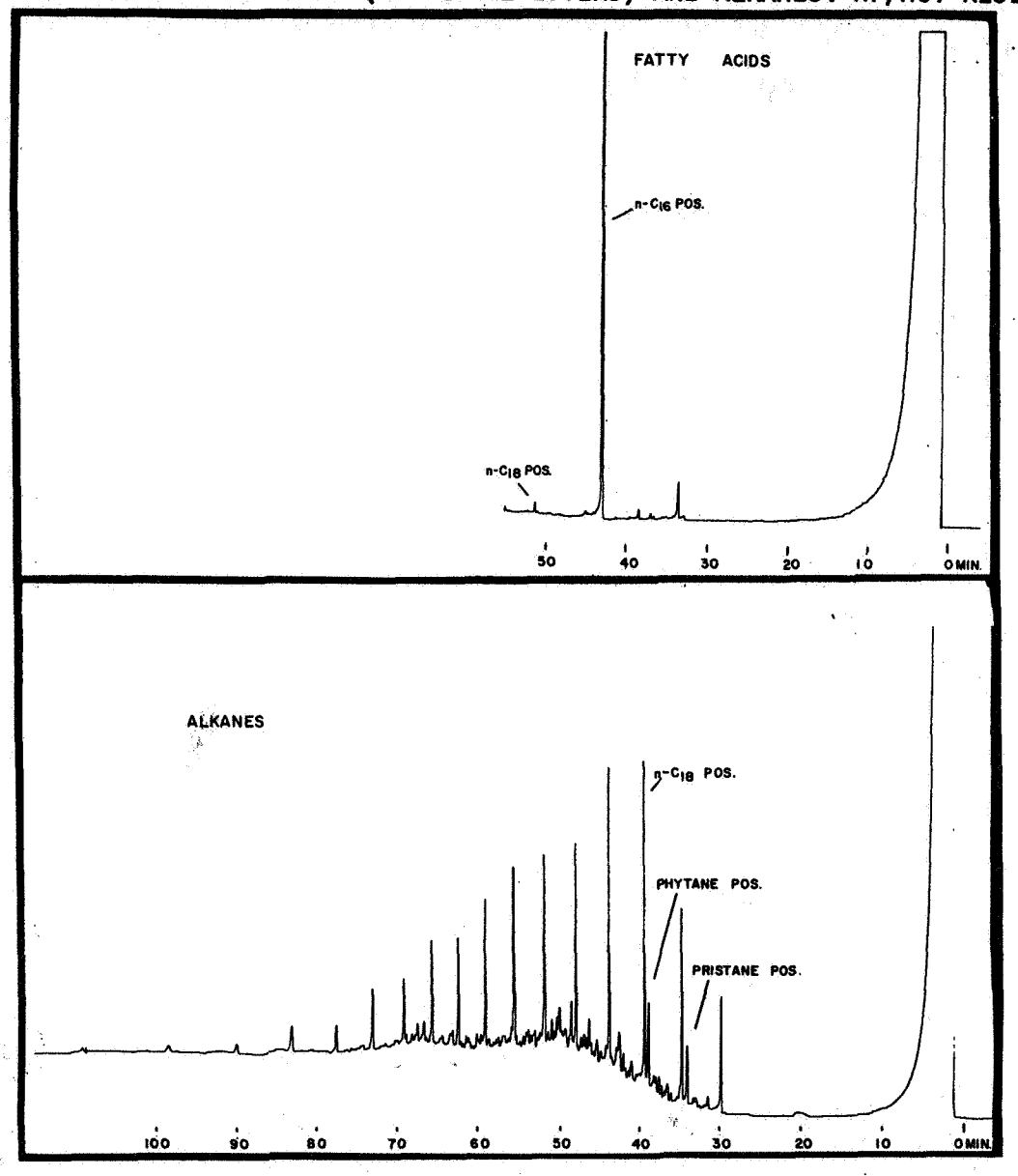



Figure 32. Gas chromatograms of extractable fatty acids (as methyl esters) and alkanes from the Gunflint Chert.

GUNFLINT CHERT FATTY ACIDS (AS METHYL ESTERS) AND ALKANES: HF/HCl RESIDUE




XBL 688-4379

Figure 33. Gas chromatogram of fatty acids (as methyl esters) and alkanes obtained by extraction of the HF/HCl residue of the Gunflint Chert.

and from gas chromatography-mass spectrometry. The mass spectra of standard methyl palmitate and the Gunflint major acid component are given in Figure 34.

The alkane distribution is in accord with the published results of other workers.[158] Of particular note is the presence of pristane and phytane (based on coinjection and analogy) and the very slight odd/even dominance of the $C_{23}$ to $C_{29}$ n-alkanes, a factor which is indicative of biological activity. Whereas in the other samples reported here, the bound acids were compared with "free" alkanes, since the two quantities of alkanes were nearly identical and since there was some question about the effects of not removing the outer surface, the comparison here must be between the bound acids and the "bound" alkanes.

An encouraging result was obtained by analyzing for fatty acids in the HF-HCl used in digestion (plus the water used in washing). The amount of fatty acids, if any, obtained by the analysis of this filtrate, is at least 100 times less than that of the residue extract and also 100 times less than that obtained from earlier (benzene purified) HF-HCl filtrates (proportions are based on gas chromatography). This serves to illustrate the advantage of not using organic solvents to purify the inorganic reagents.

Since there existed a difference between the free and bound fatty acid distributions, and since the bound acids resembled the saturated acids from modern bacteria, to eliminate the

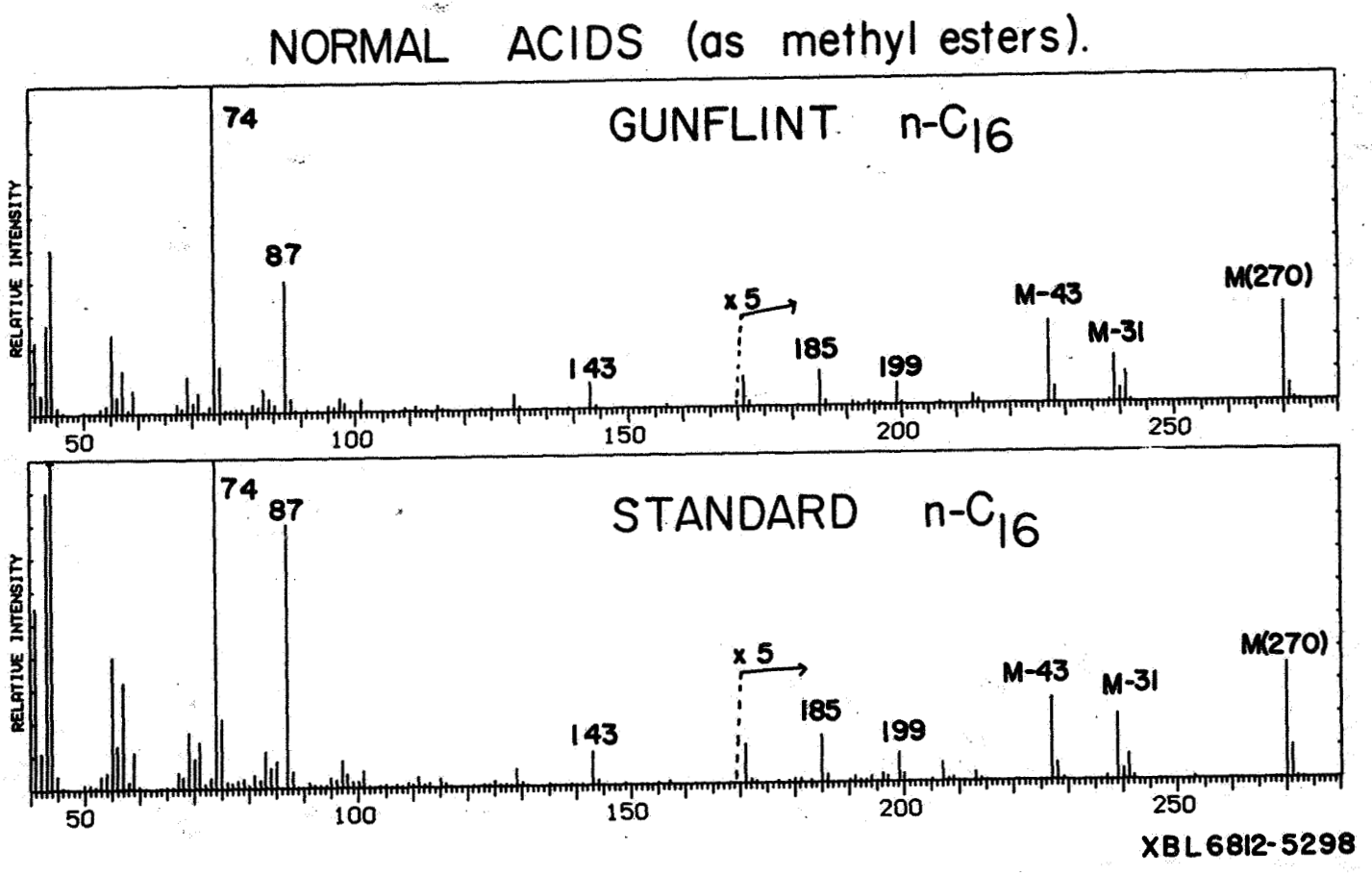



Figure 34. Mass spectra of authentic methyl palmitate and methyl ester from the Gunflint Chert HF/HCl residue.

problem of recent bacterial contamination, the unsaturated acids from the HF-HCl digestion residue were examined. After determining precisely the Rf values for stearic and oleic acids, and after showing that the oleic acid was stable in the $AgNO_3$-Silica Gel TLC system, the TLC band corresponding to the Gunflint Chert bound unsaturated acids was examined. This investigation showed that there were no detectable biological unsaturated acids in this sample. This conclusion is based on the fact that there are no cis- alkenoic acids in the extract. Since cis- acids (and not trans-) are by far the dominant biological isomers of the biological monoalkenoic acids,[198] the conclusion is straightforward and definitive. Also, if bacterial contamination gave rise to the bound saturated acids, certainly the unsaturated acids from these bacteria should also be "bound".*

Again, as in the case of the Nonesuch, there seems to be no direct relationship between the acid and the hydrocarbon distributions. Both groups of compounds are indicative of biological activity. The dominance of a single fatty acid is suggestive of a limited contributory process. The fact that this acid is $C_{16}$ and not $C_{18}$ is compatible with the notion that the contributing organism(s) were relatively simple, since it is in the higher organisms that n-$C_{18}$ becomes more significant.[135]

---

*An anomolous narrow yellow band with a low Rf value was apparent, in the TLC of the bound acids. However, since the responsible component(s) was not elutable from alumina with heptane, benzene or methanol, and since the alkanes seem to be free of contamination, nor further attention was given to this matter.

The case for the fatty acids being ancient and not due to recent contamination is fairly well documented because of the absence of any biological unsaturated fatty acid. Thus there is additional evidence that fatty acids can survive several billion years, and also that the acids extracted from the $1.0 \times 10^9$ year old Gunflint Chert are predominantly biological in origin.

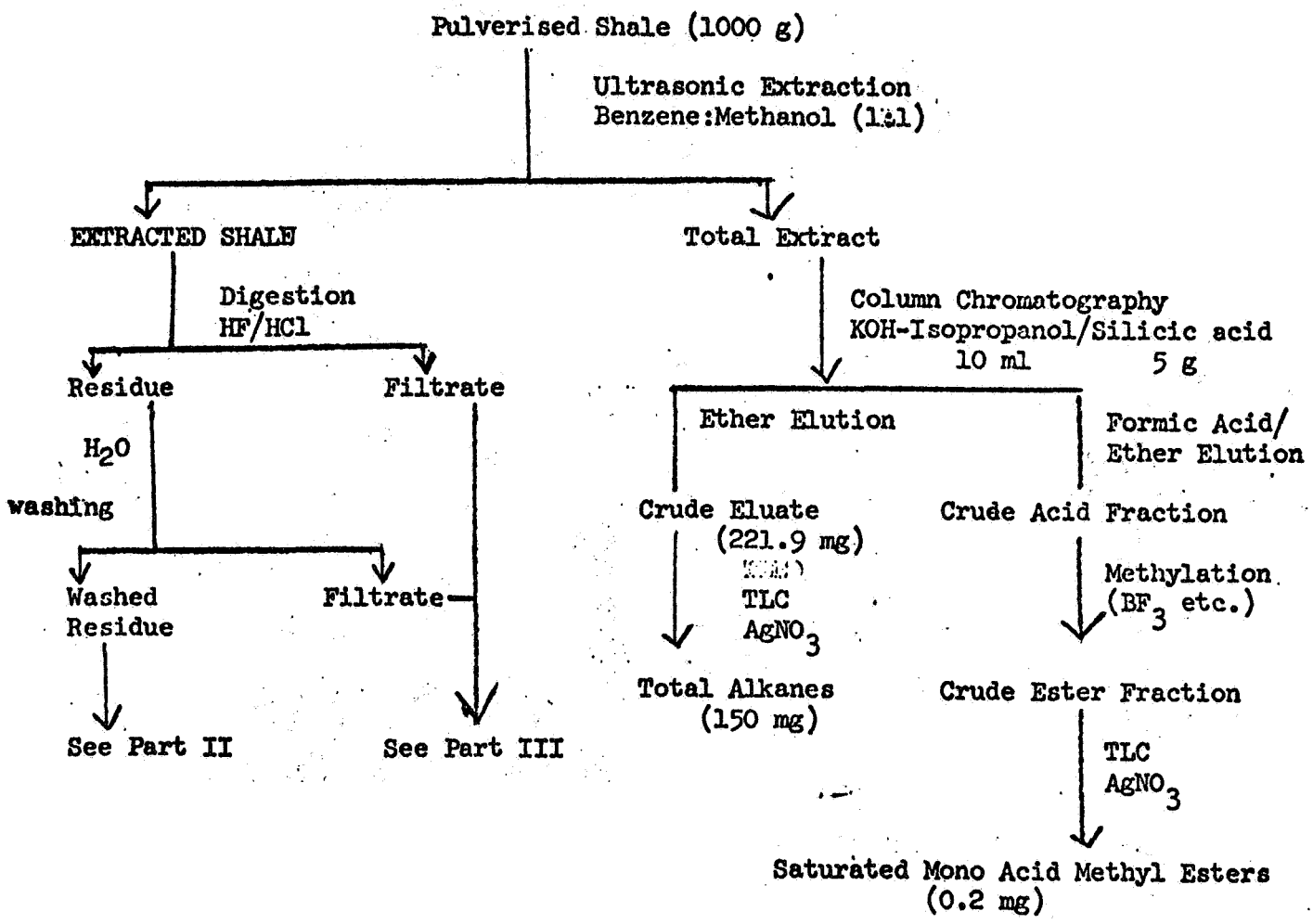
THE SOUDAN SHALE

The Soudan Shale, from Minnesota, is the oldest Precambrian sediment[199] ($2.7 \times 10^9$ years) analyzed for fatty acids. An extraordinary amount of attention has been given to this shale because of the question of the origin of the extractable organic matter. The carbon isotope studies of Hoering[200] have been interpreted as evidence that the extractable organic matter has been introduced into the sediment considerably after the original sedimentary matter (that which produced the kerogen) was deposited. A discussion of the information relevant to this matter can be found in the papers by Johns *et al.*[32] and Meinschein.[201] Their conclusion was that the matter could not be resolved, and little information has been forthcoming in the past few years which would provide a solution to the problem. Because of its considerable age and because a knowledge of the fatty acid distributions might speak to this migration question, this laboratory undertook the investigation reported here. The procedure was as previously described, and the analytical results (a surface outcrop--Sample I of Johns *et al.*[32]) are shown in the following diagram.

Figure 35 shows the fatty acids and alkanes obtained from the pulverized shale by ultrasonic extraction. The hydrocarbon distribution is very similar to that of the previous work,[32,201] and the labeling of specific peaks is based on coinjection and comparison with the earlier results. The fatty acid distribution

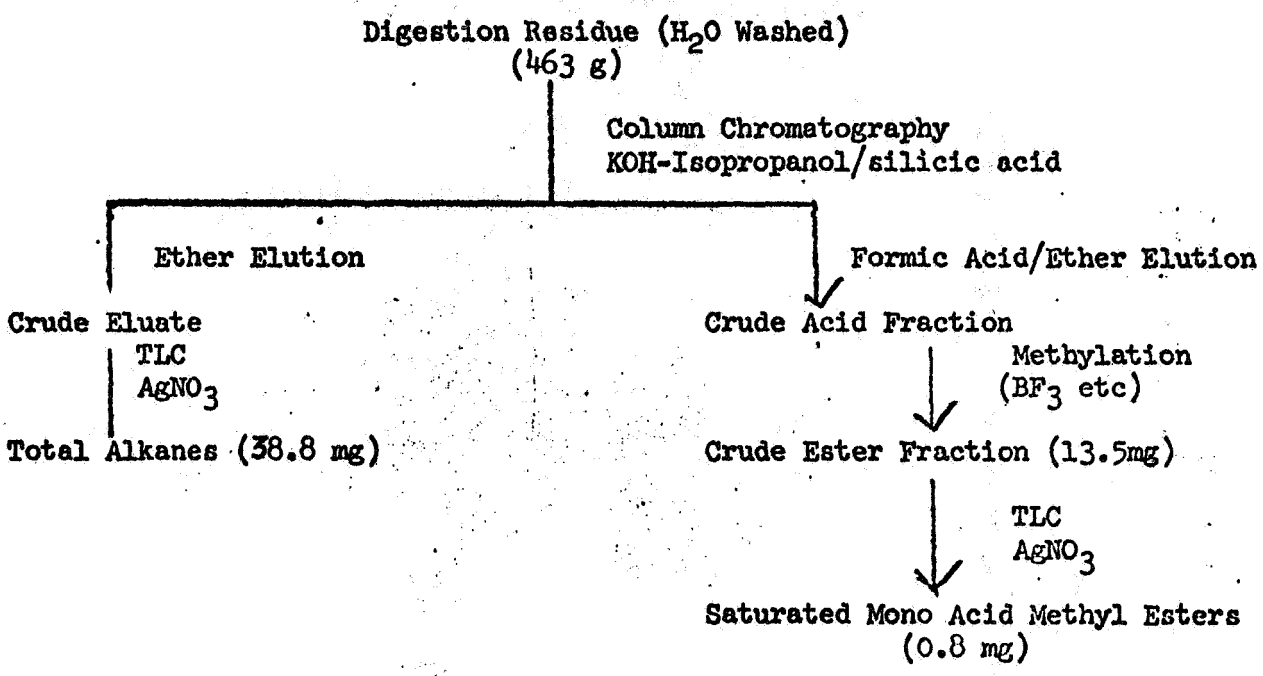
# SOUDAN SHALE ANALYTICAL RESULTS

## PART I

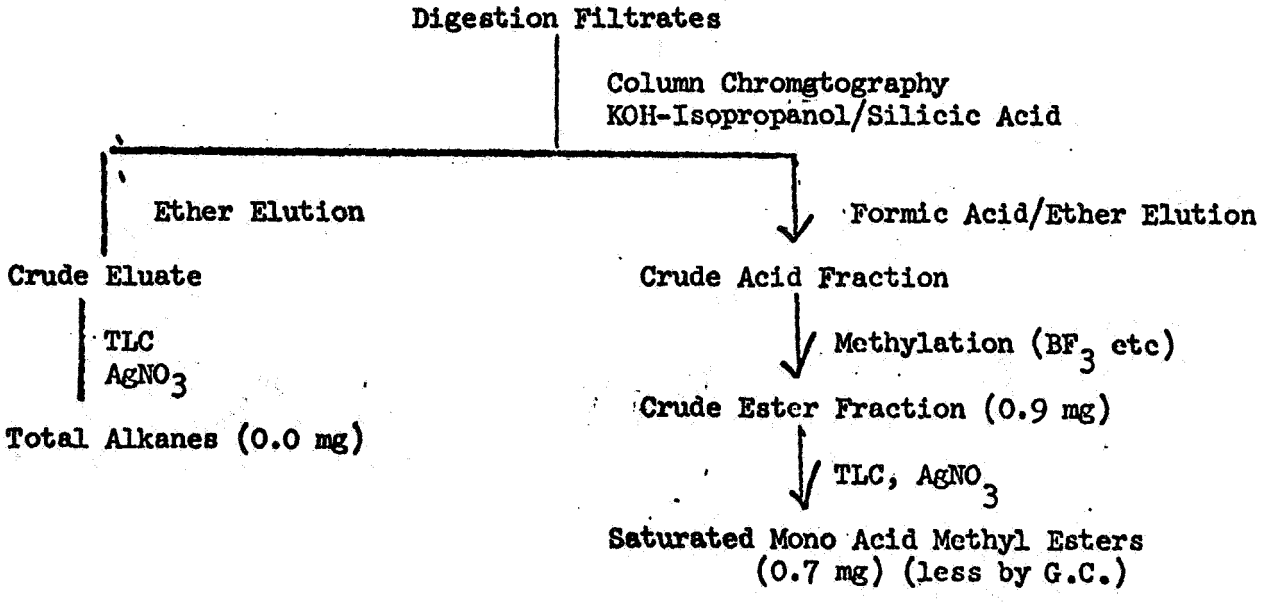


SOUDAN SHALE ANALYTICAL RESULTS

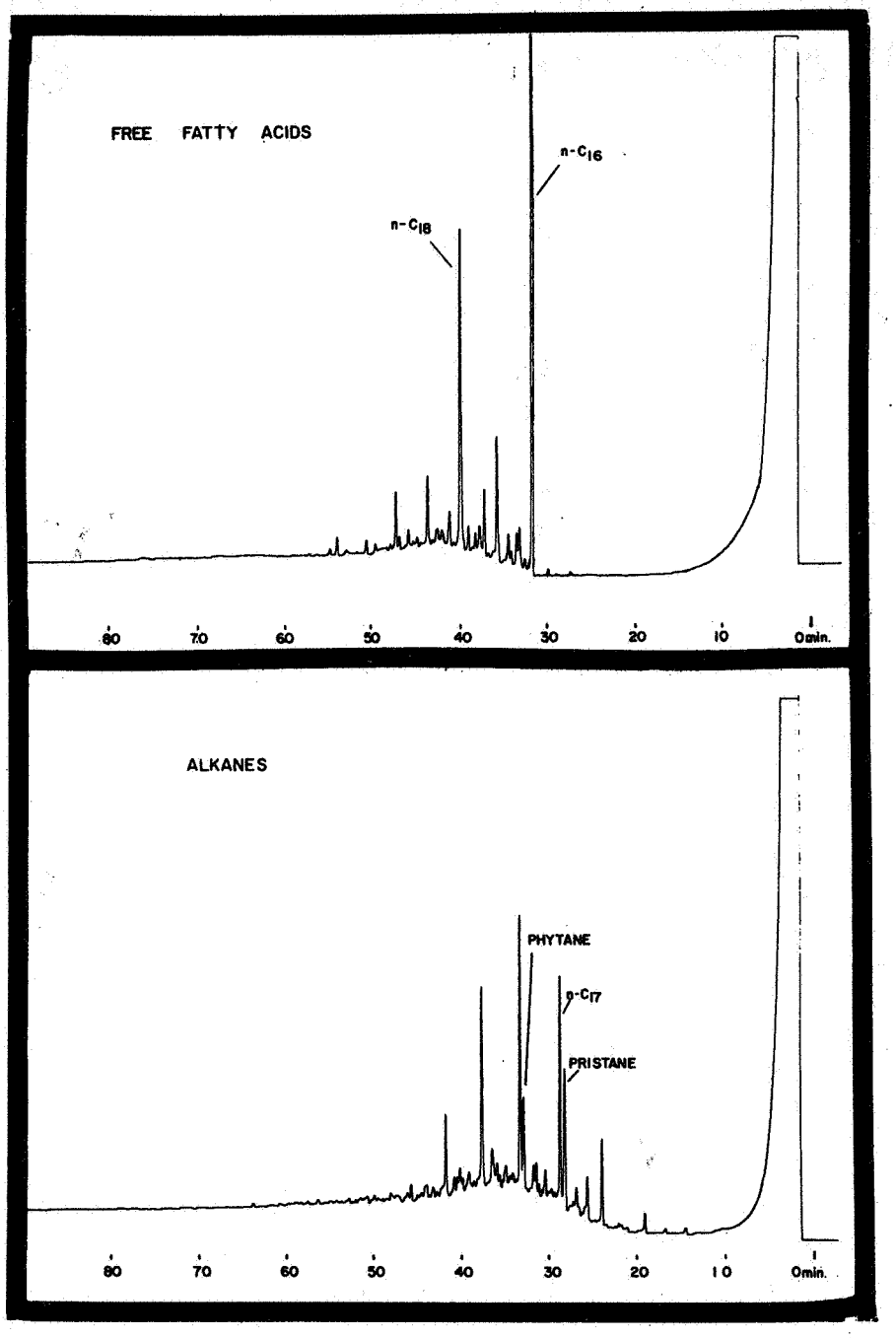
Part II




Part III



SOUDAN SHALE ALKANES and FREE FATTY ACIDS (AS METHYL ESTERS)



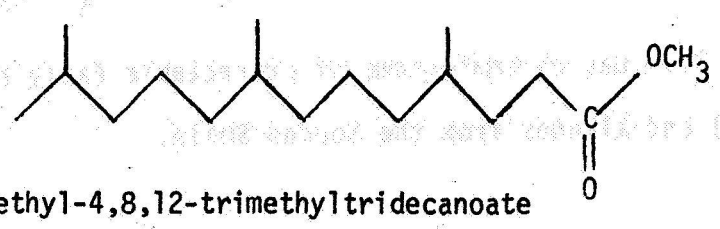

XBL 687-4272-A

Figure 35. Gas chromatograms of extractable fatty acids (as methyl esters) and alkanes from the Soudan Shale.

is certainly more complex than that of the other Precambrian samples, although n-$C_{16}$ and n-$C_{18}$ are still the dominant acids. In this case, there seems to be a greater amount of branched and/or cyclic fatty acids.

Figure 36 represents the distribution of the bound fatty acids, again compared with the extractable alkanes. The alkanes obtained by HF-HCl digestion and subsequent extraction have essentially the same distribution as those from the initial extraction, shown in Figure 37. Again the HF-HCl showed no significant amounts of fatty acids, compatible with results from the Gunflint Chert.

The bound fatty acids, especially those from n-$C_{16}$ to greater retention times, are essentially identical to the free fatty acids. The absence of the lower molecular weight free acids, especially n-$C_{14}$, n-$C_{15}$ and the $C_{15}$ and $C_{16}$ isoprenoid acids are almost certainly due to laboratory techniques since, in the case of the bound acids, greater care was exercised in evaporation of solvents. Again the even carbon-numbered acids dominate the distribution. Of greatest interest here is the implication that there are relatively large amounts of the polyisoprenoid acids in the Soudan Shale. The labeled positions are based on coinjection of the standard polyisoprenoid acid methyl esters.




Methyl-4,8,12-trimethyltridecanoate

SOUDAN SHALE ALKANES & BOUND FATTY ACIDS (AS METHYL ESTERS)

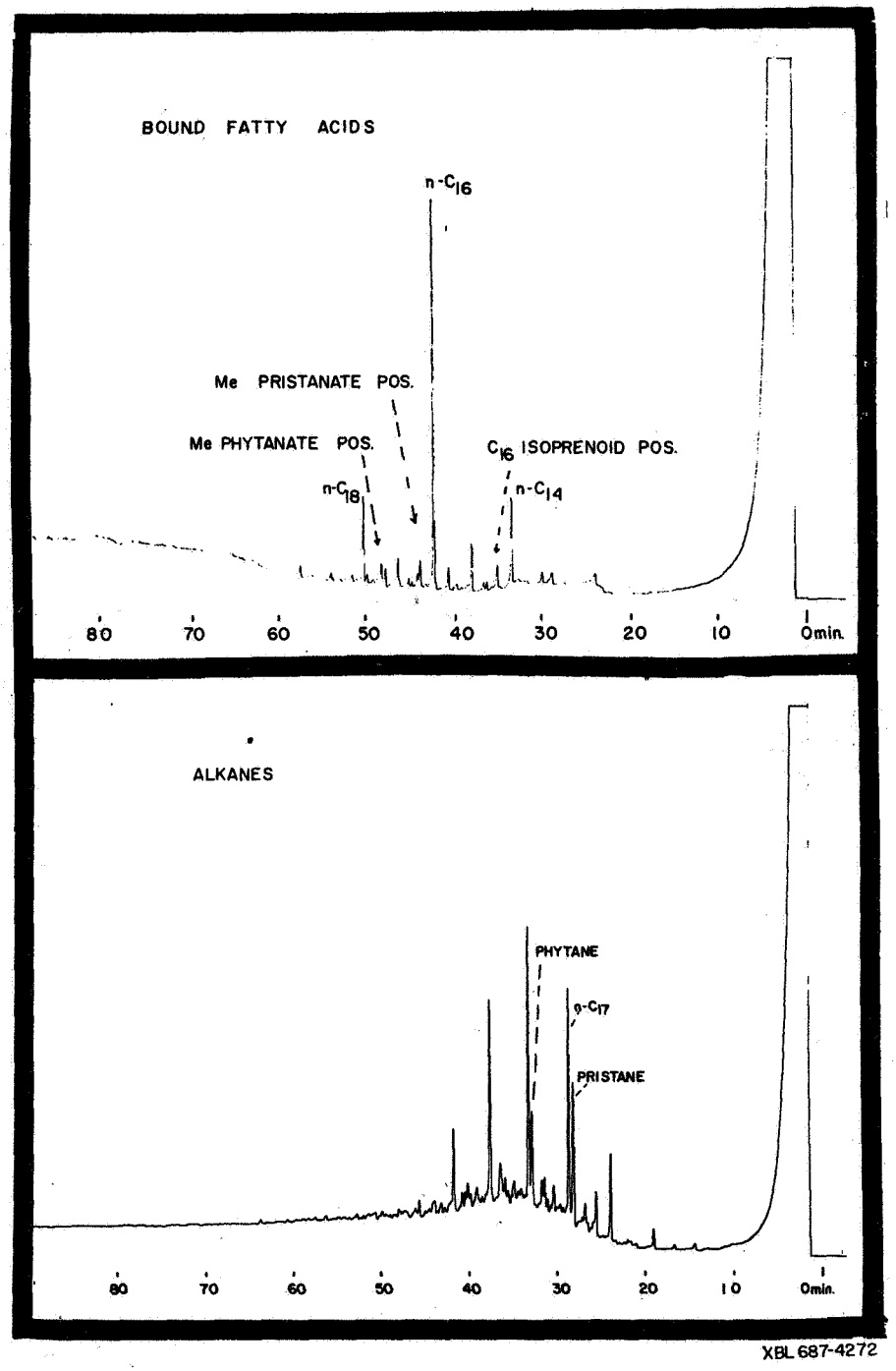



Figure 36. Gas chromatograms of the bound fatty acids (as methyl esters) and the extractable alkanes from the Soudan Shale.

SOUDAN SHALE ALKANES

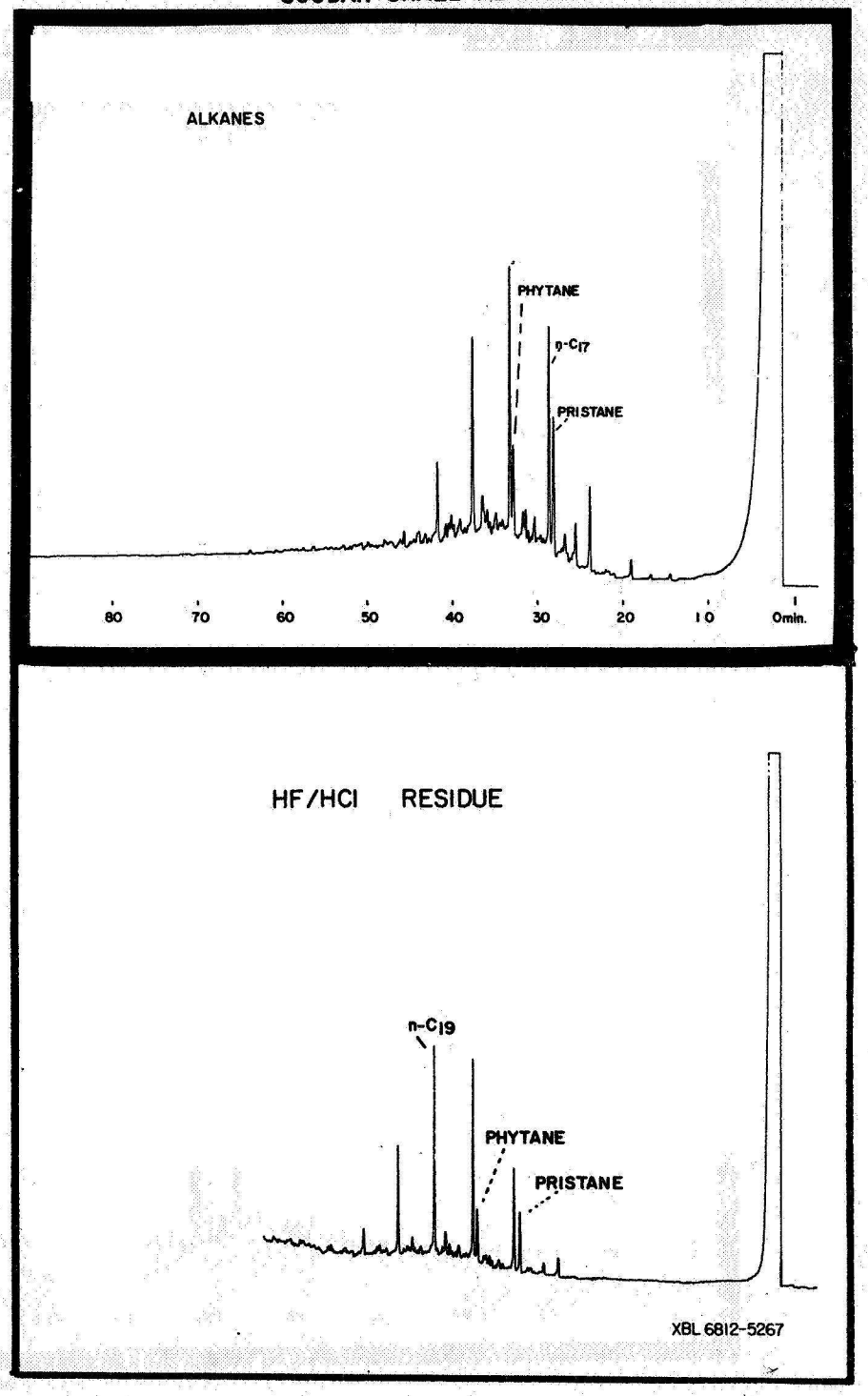
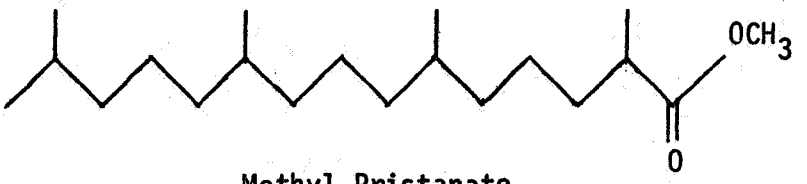
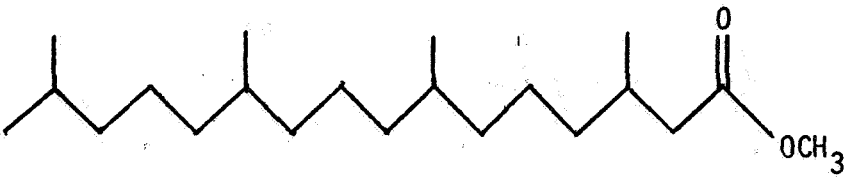



Figure 37. Gas chromatograms of the Soudan Shale alkanes obtained by initial extraction and extraction of the HF/HCl residue.

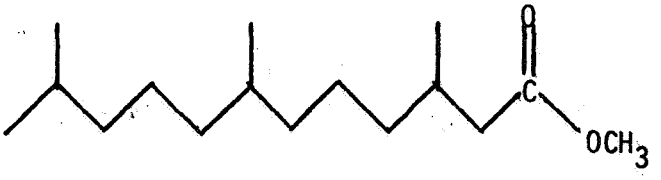



Methyl Pristanate




Methyl Phytanate

The third significant peak in the chromatogram, with a retention time of ∿29 min (last significant peak prior to elution of n-$C_{14}$) corresponds in retention time to methyl farnesanoate, the saturated $C_{15}$ polyisoprenoid acid methyl ester.




Methyl Farnesanoate

Confirmation of the presence of at least the first two members of the series, the $C_{15}$ and $C_{16}$ polyisoprenoid esters, was obtained by means of GC-MS analysis. Figure 38 shows the mass spectra obtained for these compounds after GC injection of the complex mixture. Included in Figure 38 are the mass spectra of the standard esters, also obtained by GC-MS under conditions identical to those of the geological sample. Unfortunately, since GC-MS resolution is lower than that for GC alone, since the compounds

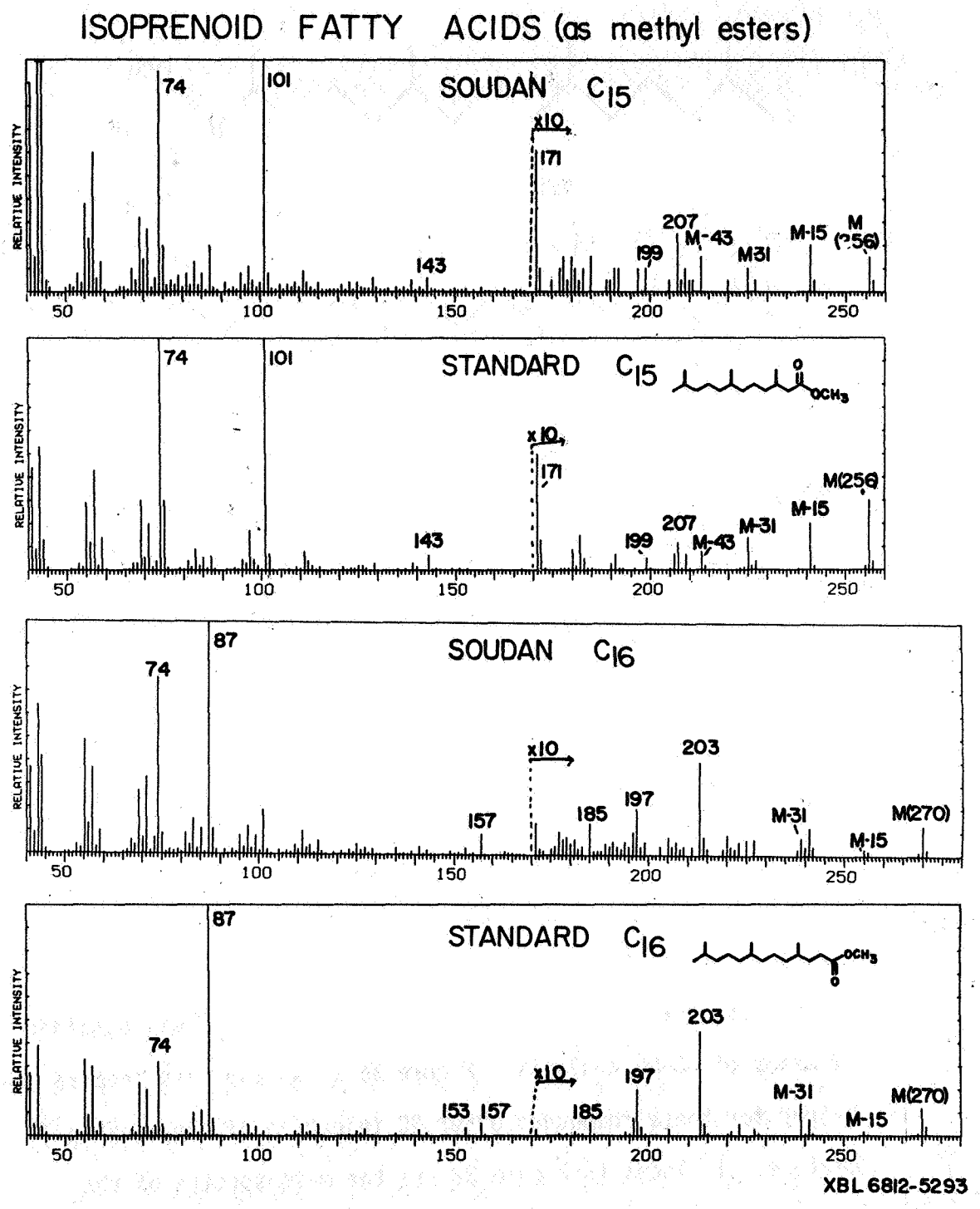
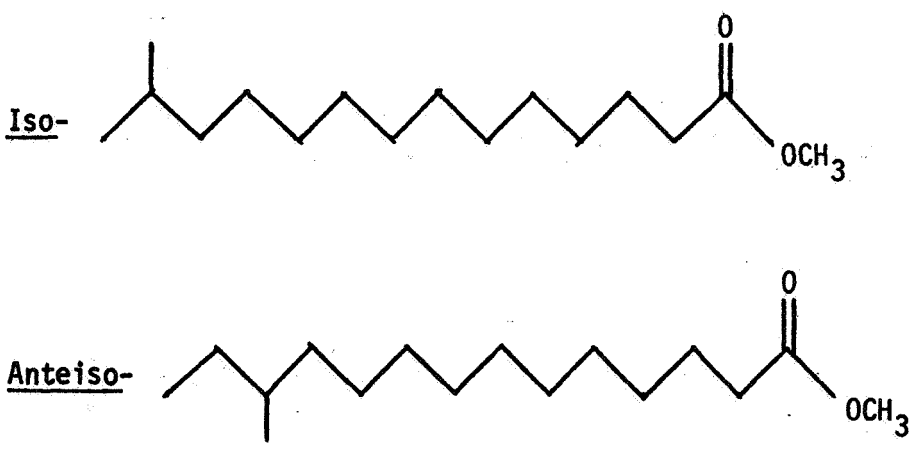



Figure 38. Mass spectra of authentic polyisoprenoid acid methyl esters and bound fatty acids (as methyl esters) from the Soudan Shale.

are more numerous in the region of methyl phytanate and methyl pristanate, definitive spectra of these compounds were not obtained.

The GC-MS spectra for the n-$C_{15}$, n-$C_{16}$ and n-$C_{18}$ acid methyl esters are shown in Figure 39, along with standard spectra. The structure of some of the other acids is uncertain, although GC-MS suggests they are acyclic. Two of these compounds, seen on the gas chromatogram as a doublet immediately following the $C_{16}$ isoprenoid, have been identified by mass spectrometry as the methyl esters of the iso-$C_{15}$ acid (methyl-13-methyl tetradecanoate) and the anteiso-$C_{15}$ acid (methyl-12-methyl tetradecanoate). (See Figure 40.)




All of the comments relevant to the occurrences of the n-acids in the other sediments examined are relevant to their occurrence and predominance in the Soudan Shale. The distribution is indicative of a biological origin for these acids and suggestive (though less so) of a limited contributory element--i.e., relatively

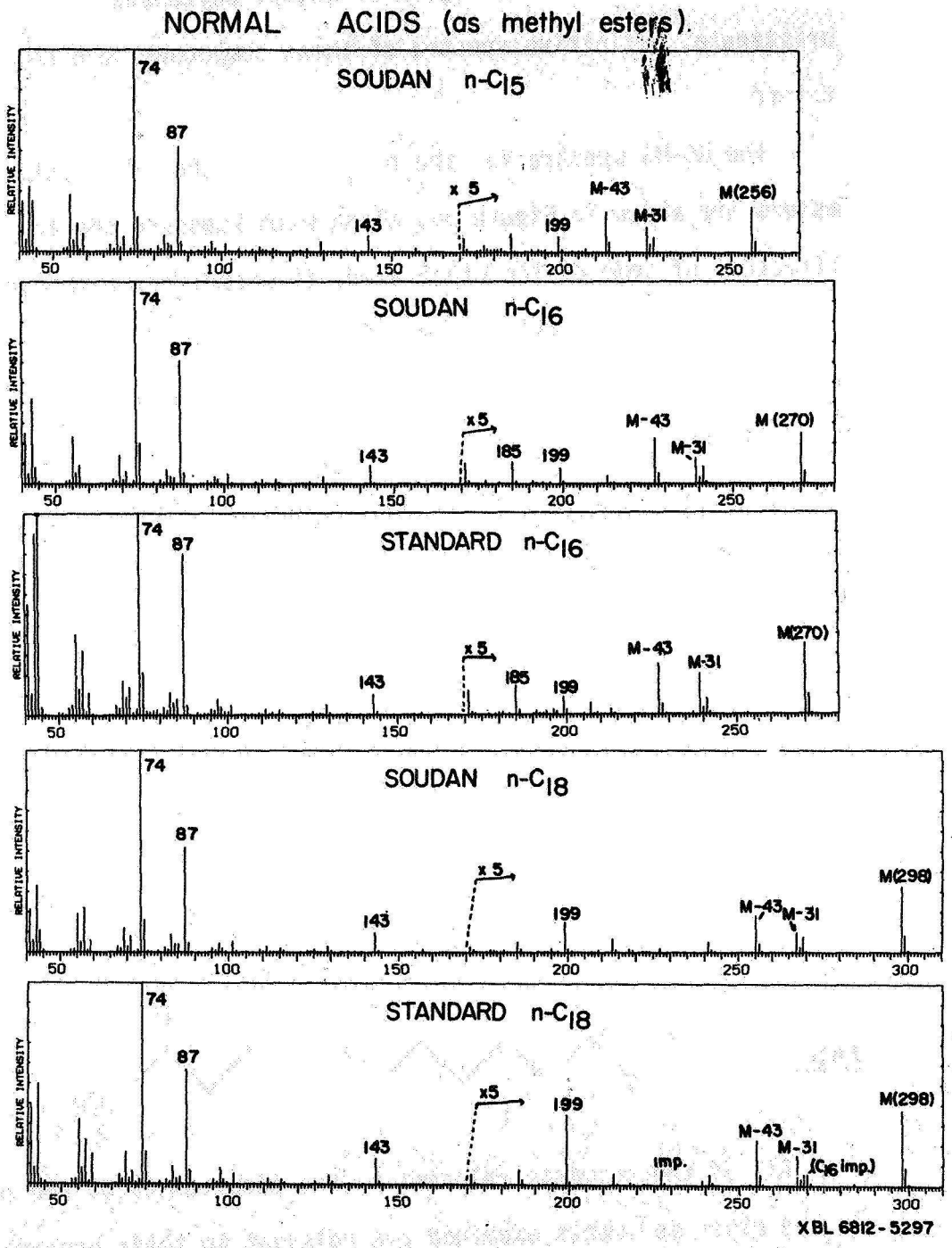



Figure 39. Mass spectra of authentic methyl palmitate and methyl stearate and bound fatty acids (as methyl esters) from the Soudan Shale.

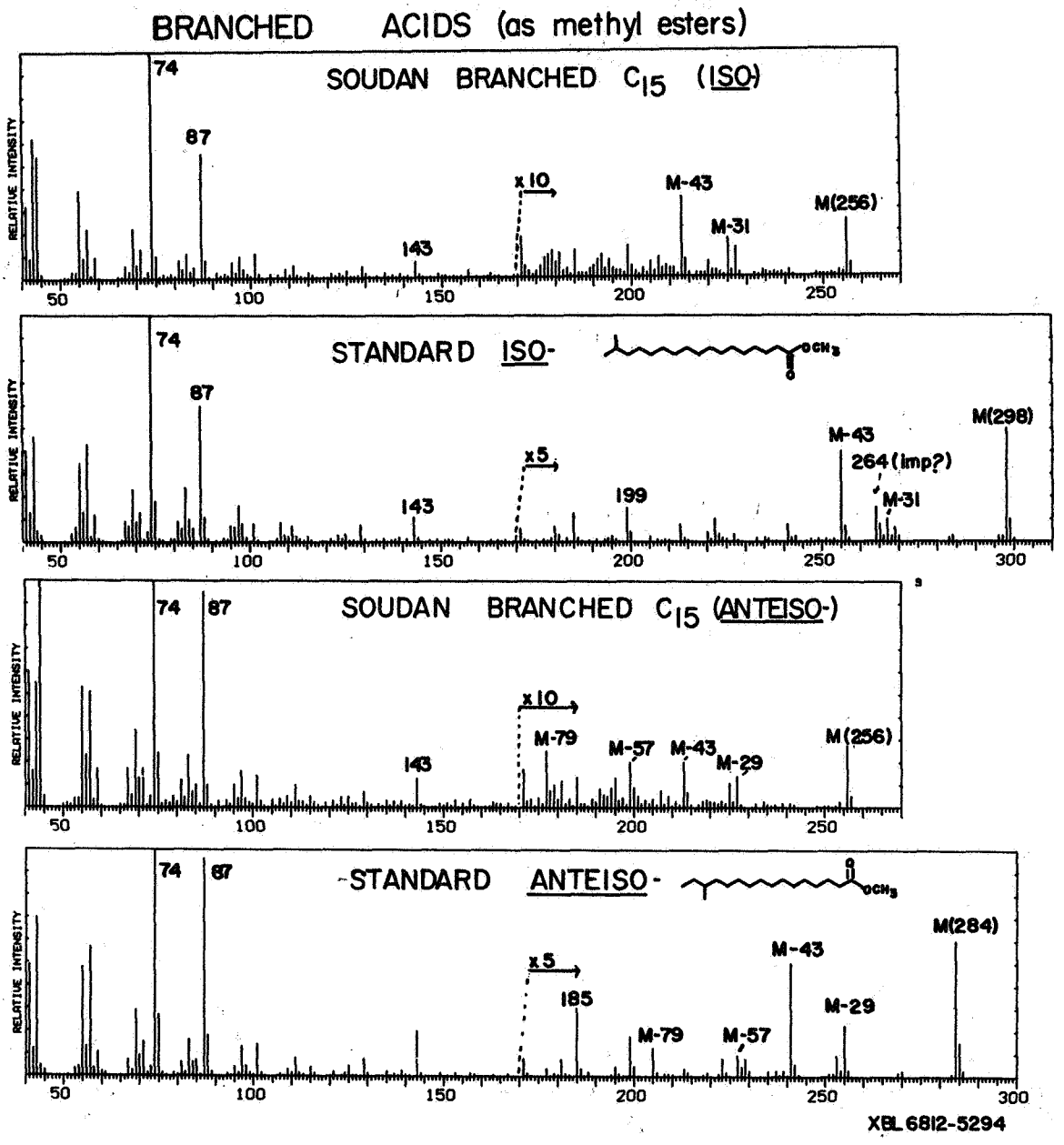



Figure 40. Mass spectra of authentic iso- and anteiso-acid methyl esters and bound acids (as methyl esters) from the Soudan Shale.

undeveloped organisms.[135] It is the polyisoprenoid acids which are unique to this sample, and which arouse interest and demand comment.

There are several possible explanations for the occurrence of these compounds. Certainly it is possible that these were originally deposited by some organism not responsible for the fatty acids of the Nonesuch Shale or the Gunflint Chert. However, such polyisoprenoid acid-containing organisms would be rather unique; their modern day analogs have not been reported.

The most likely explanation for the presence of these compounds is that other polyisoprenoid compounds, such as phytol (and perhaps even phytane, pristane, etc.) more abundant in organisms, were converted to the acids by diagenetic factors unique to this sediment. The suggestion of high temperatures, and the knowledge that oxygen (as oxides) is abundant in this sediment,[199] lends credence to this hypothesis. The simultaneous occurrence of polyisoprenoid fatty acids and steranes may be significant, although the other sediment containing both these types of compounds, the Green River Shale, has a considerably different geological history.

Whether or not the acid distribution, and specifically the polyisoprenoid acids, provides information about the contemporaneity of the extractable organic matter and the kerogen is a matter of interpretation. There is no information about the fatty acids present as part of the kerogen; surely knowledge of the presence or absence of the polyisoprenoid acids in the

kerogen would be valuable. The fact that the bound acids and the free acids have similar distributions is a factor which points toward a contemporaneous origin for the readily extractable matter and the difficultly extractable matter, a situation totally analogous with Meinschein's results.[201]

The conclusion from this particular sample must be that the migration-origin problem is still unsolved. Certainly the extractable hydrocarbons and fatty acids are derived from biological sources. The quantity and type of non-normal fatty acids indicates differences between this shale and other sedimentary rocks examined here.

## DISCUSSION

Having considered these geological samples individually, it is desirable to examine them collectively and to identify any generalities or trends which are present. Once such phenomena are recognized it is essential that one tries to place these into the overall context of organic geochemistry and tries to understand the significance of variations due to time, original situation, etc. As has been suggested throughout this thesis, the hydrocarbons have been considered to be the most valid and valuable source of information on the existence of life. Primarily the polyisoprenoid alkanes have been sought, with the implication, if not direct statement, that these compounds, by themselves, are proof of biological activity. However, the increased knowledge about abiotic syntheses, combined with the vastly greater information about organic geochemical processes and products, has led to some doubt as to the sufficiency of the polyisoprenoid alkanes as such proof. Accordingly, as mentioned earlier, other alkanes and other compound types have recently received considerable attention. But this is not to say that polyisoprenoid alkanes are no longer useful, for these compounds have a sufficiently high degree of structural specificity and biological association and a sufficiently low degree of probable abiotic synthesis that they may still be used as chemical fossils. The recognition of the <u>potential</u> failure to meet the abiotic synthesis criterion has necessitated a degree of caution.

Of the samples reported here, only the Florida Mud Lake samples (with the exception of the n-alkanes), the Pierre Shale and the Moonie Oil were analyzed first or exclusively by this author. The alkane distributions from all the remaining samples have previously been examined and discussed, and those discussions will not be reiterated here.

Placed within the context of organic geochemistry, the Mud Lake samples constitute a unique and informative situation, for it appears that a portion of the alkanes initially deposited are nearly totally transformed into other components. It is suggested here that perhaps these low molecular weight alkanes, $C_{17}$ and $C_{18}$, specifically, are a substrate for various organisms within the top few inches or feet of the sediment. They are thus removed from the sediment and converted into other compounds via the metabolisms of the organisms which ingest them. The higher alkanes (e.g., $C_{25}$-$C_{31}$) are not consumed in this way and remain (at least partially) as distinct entities, not only for the thousands of years encompassing the Mud Lake samples, but for millions of years as seen in the higher alkane distributions of the Pierre Shale and even the Gunflint Chert. It is then imperative to suggest an origin for the many lower alkanes (e.g., $C_{15}$-$C_{20}$) found in all the ancient oils and sediments so far examined. Certainly the most obvious and reasonable explanation is that they are diagenetic products of either higher alkanes or of other geochemicals. Relevant to this suggestion is the absence of phytane and pristane from the

young sediments and its presence, and often dominance, in the older sediments. As suggested by the stability studies mentioned in Chapter I, most compound types do exhibit some geochemical stability, so the diagenetic transformations responsible for their disappearance (and the appearance of less functional, more stable compounds) cannot be expected to be spontaneous. The tendency for ancient oils and sediments to have a maximum of $C_{15}$ to $C_{20}$ alkanes is undoubtedly due to a complex interplay of biogenetic, diagenetic, geologic and thermodynamic factors about which volumes have been written and no more will be said here.

Of the ancient sediments reported here, one--the Pierre Shale--is known to have a large contribution from terrestrial plants. Interestingly, it is in this sample that the high molecular weight normal alkanes have the highest odd-carbon-number/even-carbon-number ratio. Also, this sample is the only one which contains large amounts of steranes. The Florida Mud Lake does also have a high ratio of odd/even n-alkanes, and this is also interpreted as due to the higher land plants associated with the lake. Comparison of the Pierre Shale with the Green River Shale, known to be non-marine and also with a plant contribution, shows these two to be similar in the high molecular weight region. The conclusion to be drawn from the sediments discussed here is that the alkane distribution can reflect terrestrial plant contribution to the sediments. This may not be obvious in the Precambrian sediments but is obvious for recent sediments and those up to 75 x $10^6$ years of age.

If such a conclusion can be demonstrated to be valid for a large number of such sediments, this means of identification could become useful for descriptions of geological specimens.

The main interest of the majority of the sediments analyzed here is with the fatty acids, both their variations due to differing conditions and their relationship to the alkanes. Concerning the latter point, the evidence presented here says that there is no direct correlation between the carbon number of the normal acids and the carbon number of the normal alkanes. Cooper's[194] suggestion that n-alkanes may arise from n-acids solely by decarboxylation is not supported, and is in fact refuted.

It has been pointed out that often the odd-carbon fatty acids increase in abundance with time, relative to the even-carbon fatty acids.[182] This does not seem to be the case for the sediments examined here. The billions of years which are encompassed by these sediments should provide an excellent opportunity to view such a phenomenon, if in fact it is general. However, Kvenvolden's results[182] demonstrate the lack of generality of this process. Unfortunately there are not sufficient experimental data available to provide any useful information as to the products of fatty acid decomposition in sediments. That it is a complicated process is shown by the work of Jurg and Eisma.[140] All that can be stated concerning this matter is that while it may be true that in some sediments the even/odd

ratio of fatty acid chain lengths decreases with time and approaches unity, it is not the situation in the sediments examined here. Which of the two situations is the "normal" case is not known.

The biggest concern in reporting these results, especially those from the Early Precambrian, is that the fatty acids may not be of the same age as the sediment from which they were obtained. To answer this question it is useful to examine the possible sources of contamination and to pass judgement on whether or not they may be operating in these cases. The most obvious source of contamination is that which results from laboratory handling and techniques. As was discussed and emphasized in Chapter II, this contamination source, though potentially operative, is controllable. In the samples reported here, certainly the variations in the fatty acid distributions, combined with blank experiments, suggest that laboratory contamination is <u>not</u> producing the results. Support for this comes from a knowledge that the Antrim Shale, analyzed three times over a period of several months, showed the same fatty acid distribution in each analysis.

A second obvious potential source of contamination is from recent bacterial action on or in the sediment. Such contamination might be expected in view of the fact that the samples have been handled, exposed, stored, etc. There are two pieces of information which refute this concept. The first of these is that no bound biological unsaturated fatty acids were found in that Precambrian sample which had the greatest surface area

prior to treatment and of which the surface was not removed. This is the strongest refutation of recent bacterial contamination now available. The only possible factor not experimentally checked is that by some mechanism the unsaturated acids are quickly and completely destroyed in the sediment. This seems unlikely in view of the chemistry necessary for such transformations and the limits to which the unsaturated acids are detectable.

The second piece of information arguing against this type of contamination is that in the most porous and most exposed sediment examined, the Pierre Shale, no significant amounts of free fatty acids were isolated. If recent microbial contamination had occurred, it should be evident in the free fatty acid fraction, for the other samples provide ample evidence that binding to the rock matrix does not go to completion.

These two arguments convincingly demonstrate the lack of recent bacterial contamination of the sediments. Nevertheless, it is the suggestion of this author that in the future all analyses include a search for biological and non-biological unsaturated fatty acids.

The last obvious source of contamination is a geological one, in which the fatty acids have migrated into the sediment considerably after the sediment was formed. Unfortunately, this type of contamination is both difficult to identify and difficult to prove or disprove. In regards to the alkanes, the arguments involving porosity, difficulty of migration from such distant reservoirs, etc., have been invoked, and these are

certainly applicable here. It is important also to note that in all but one case (Gunflint Chert) the free fatty acids and the bound fatty acids have the same origin. It also says that the dissolution of the rock matrix, which makes more of the inner portions of the rock available, does not release significant amounts of new compounds. Unfortunately, with a knowledge of the binding equilibrium and the kinetics of the process, it does not solve the migration problem.

In the final analysis, there is currently no way to prove that migration has or has not occurred. Carbon isotope ratios, particularly on individual compounds and compound types would be a valuable indicator. The analysis of the fatty acids of the kerogen, making sure that no alteration of structure has occurred, would also be useful, though not necessarily indicative since the kerogen acids may be derived from precursors different from those of the non-kerogen fatty acids.

In considering the value of fatty acids as chemical fossils, it is necessary to consider the possibility of their abiogenic formation. This possibility was discussed in Chapter I, and it was pointed out at that time that the abiotic formation of a limited range of fatty acid type compounds has been accomplished.[146] Certainly the selective formation of a limited number of chain lengths is also feasible by means of the surface characteristics and dimensions of catalyzing surfaces. However, such a distribution has not been realized in any abiotic experiment, and it

must be concluded that the distributions from the sediments examined here are still in total accord with a biological origin.

In conclusion, with regard to fatty acids, it is obvious that the distributions of fatty acids demand serious consideration as chemical fossils, since they have been unequivocally identified in very ancient sediments. The acid distributions reported for three Precambrian sediments are compatible with a biological origin, since it appears that the fatty acids reflect the acid distributions of the contributing organisms or they reflect other compounds of these organisms which can be converted to fatty acids (e.g., phytol). Taken with other organic geochemical results, fatty acids can be used to provide information on the time, place and manner of the origin of life.

# CHAPTER IV

# ABIOTIC VERSUS BIOTIC

If there is one basic assumption upon which rests all of the organic geochemistry related to the origin-of-life problem, it is the assumption that it is possible to distinguish an abiotic mixture of chemicals from a biotic mixture of chemicals. It is hypothesized that any group of atoms or molecules which has been through biological processes, whether those processes are degradative, synthetic, or otherwise transformative, will reflect the selectivity and non-randomness of biological systems, and that it is possible to identify these deviations from randomness, thus identifying the existence of biological activity. Although this hypothesis has been generally accepted, recently there have been some questioning and doubts concerning its validity. It is the intention here to more closely define the bases of the various assumptions, relevant to this matter, which have been made and to attempt to draw some conclusion about the validity of the basic assumption and hypothesis just defined.

There has never been a serious doubt that biological systems are selective and non-random and that biogenetic processes produce a limited, non-random group of compounds. The preference for L-amino acids, for limited distributions of fatty acids and nucleic acid constituents, to mention only a few of the most obvious examples, is the basis upon which part of organic geochemistry's

basic assumption has been made. It is not this part which has recently been challenged, doubted, and supposedly tested. A major concern of some scientists in the past several years has been to suggest and demonstrate that supposedly abiotic systems can also exhibit selectivity and non-randomness. The way in which this has most commonly been approached has been to demonstrate the possibility of "abiotically" synthesizing those very compounds used as biological markers, and it is the general philosophy of this type of research which is discussed here.

There is no doubt that it is possible to "abiotically" synthesize any of the biological markers used in organic geochemistry today, if "abiotic" does not include the decisions and actions of the researcher, but refers only to the nature of the starting materials and the catalysts used in the conversions. But the omission of the role played by the scientist is an intolerable disregard of a biological selective agent. Any non-biological selectivity, which is used as an argument against the acceptance of a compound as a biological marker, must result from use of reaction conditions and reagents which are not so limited in their characteristics that selectivity is inevitable.

An example of such a possible misinterpretation could result from the report of the possible occurrence of farnesane and phytane as the predominant $C_{15}$ and $C_{20}$ compounds produced by $^{60}Co$ irradiation of isoprene in the presence of vermiculite and subsequent hydrogenation.[202] It must be pointed out that the authors

do not conclude that these two compounds have been synthesized and/or rigorously identified and that emphasis is placed by them on the differences which occur between those irradiations performed with and without vermiculite. However, it is the opinion of the author of this thesis that very little significance can be attached to such a result, for the selectivity in using a pure monomer and subjecting it to these well defined conditions would inevitably produce farnesane and phytane. The gas chromatographic and mass spectrometric behavior of the various isomers possible are not sufficiently determined to state that these compounds are the prominent constituents of the reaction mixture. The only information which can be used by geochemists from this report is that pure isoprene polymerizes and that its polymeric products differ when polymerized in the presence of the clay mineral vermiculite. One must be certain, in experiments of this type, that he realizes that the degree of selectivity exhibited by the end products of the experiment may be a direct reflection of the selectivity put into the experiment by himself.

Another approach to abiotic synthesis of the complex biological markers, for example--porphyrins and polyisoprenoid alkanes, has been to identify, within a very complex mixture, very small amounts of these compound types. One of the most important reports of this type has been that of Studier, Hayatsu and Anders,[109] who describe the presence of a series of 2,6-dimethyl alkanes in the reaction product of a Fischer-Tropsch process, in which

powdered meteorite is used as a catalyst. One look at the gas chromatographic data contained in this report obviates the fact that the 2,6-dimethyl series is not present in quantities greater than other dimethyl series (e.g., 4,5; 2,5; 2,3; etc.). The question is simple--Why so much emphasis on a minor component?

Another case in point is that of "prebiotic porphyrin genesis", in which great significance is attached to the crude structures of a product, (not a pure compound) with a $10^{-5}$% yield.[98] Can one justifiably ignore 99.99999% of his reaction product?

Only one phenomenon permits such emphasis on a minor constituent, namely the process of autocatalysis. This process, discussed in Chapter I and demonstrated in the references mentioned at that time, may result in the eventual accumulation of an originally minor component. However, the burden of proof must rest on the person who relies on this process, for although such phenomena can and do occur, their scope so far seems limited and one cannot assume that autocatalysis will occur for any compound or compound type.

Certainly conclusions based on the selection of components as minor as those mentioned above are not acceptable, by themselves, as an outcome of these abiotic processes. Although the starting materials are not biological, in terms of their complexity, and apparently the total products are essentially non-selective, the emphasis and identification of such a small percent seems contrary to the assumptions underlying abiotic syntheses of biological markers.

The past several paragraphs have brought up the question of distributional significance. It is the conclusion of this author that current abilities to determine the presence of biological activity when based on distributions and not on individual compounds, though certainly less than ideal, are sufficient for the purposes for which they are now used. And this distributional concern encompasses not only the distributions of single compounds within a compound type, but also the distributions of groups and types of compounds within the total range of possible chemical compounds.

One question which inevitably arises is, what does an abiotic distribution of compounds look like? There have been several attempts to answer this question, most of which are concerned with abiotic hydrocarbon distributions. In 1964, Davis and Libby[203] reported that polymeric hydrocarbons were formed by irradiation of solid methane with $^{60}Co$ gamma rays. However, no details were provided as to the molecular weights, chemical characteristics or isomeric distributions of the products obtained. Since Libby's experiment basically consisted of the input of large amounts of energy into solid methane, it was decided to duplicate this type of process and to elaborate on the product composition. To avoid the hazards and inconveniences of $^{60}Co$, the high energy source chosen was a linear accelerator capable of providing an electron beam composed of approximately 7.5 mev electrons; it was also decided to irradiate solid methane, since

of the three phases of methane, structural specificity would be most expected in the most structured phase.

The experimental conditions and apparatus ultimately chosen are briefly described here. Each piece of apparatus was thoroughly cleaned and washed prior to use. After connecting the various components, the system was evacuated then flushed with methane, evacuated, etc., until the flushing process had been repeated a minimum of five times. A U-tube was placed between the methane supply and the irradiation tube during flushing, and was immersed in liquid nitrogen. After evaporation of most of the methane (in this U-tube), mass spectrometry showed that some ethane and propane was trapped, but no quantitative determinations were made. The methane was solidified by gradually lowering a 4 mm I.D. glass tube into liquid nitrogen, until approximately 400-500 mg of methane were frozen (∿3-5 in.). Several irradiations were carried out; however, experimental difficulties and a high probability of contamination have limited the validity of some runs. In the most certain and informative run, the methane was irradiated during several hours with a total dose of 100 Mrads, using 7.5 mev electrons. (Dose is based on calibration with Co glass and is uncorrected for the glass and quartz tube thicknesses.) That the methane remained a solid was determined by direct observation. After irradiation the sample was gradually warmed to room temperature, the excess methane as well as other volatile gases escaping through a check valve. The tube was broken and the liquid

products were dissolved in n-$C_5$ and $CH_2Cl_2$; the solvent was removed and the product (0.8 mg) analyzed by gas chromatography. Figure 41 is a photograph of the Dewar and sample during irradiation. The beam enters from the brass object on the far left. Coloration is due to the effect of the beam on quartz; the sample tube is contained inside the whitish inner tube of the Dewar.

The g.c. analysis of this irradiation was carried out on a 150' x 0.01" I.D. Apiezon L column (Perkin-Elmer 226, conditions as shown), and is shown in Figure 42. The results are too incomplete to permit many conclusions, but it is obvious that at low molecular weights, where the number of possible isomers is limited, the peaks are more distinct than in the high molecular weight region, where the potential isomers are very numerous. No homologous series is evident and no distinct peaks are apparent which have the retention times of the common polyisoprenoid alkanes.

The results which have been described here are of a preliminary nature only. However, additional experiments have not been performed. After this background work was done, Meinschein reported his thorough analysis of the products formed by Davis and Libby's experiments and also found no structural specificity in the products.[204] Electric discharge experiments with methane give qualitatively the same results.[130]

Before considering other types of abiotic experiments, it is necessary to point out that these three experiments are obviously concerned only with hydrocarbons. Whether or not the same

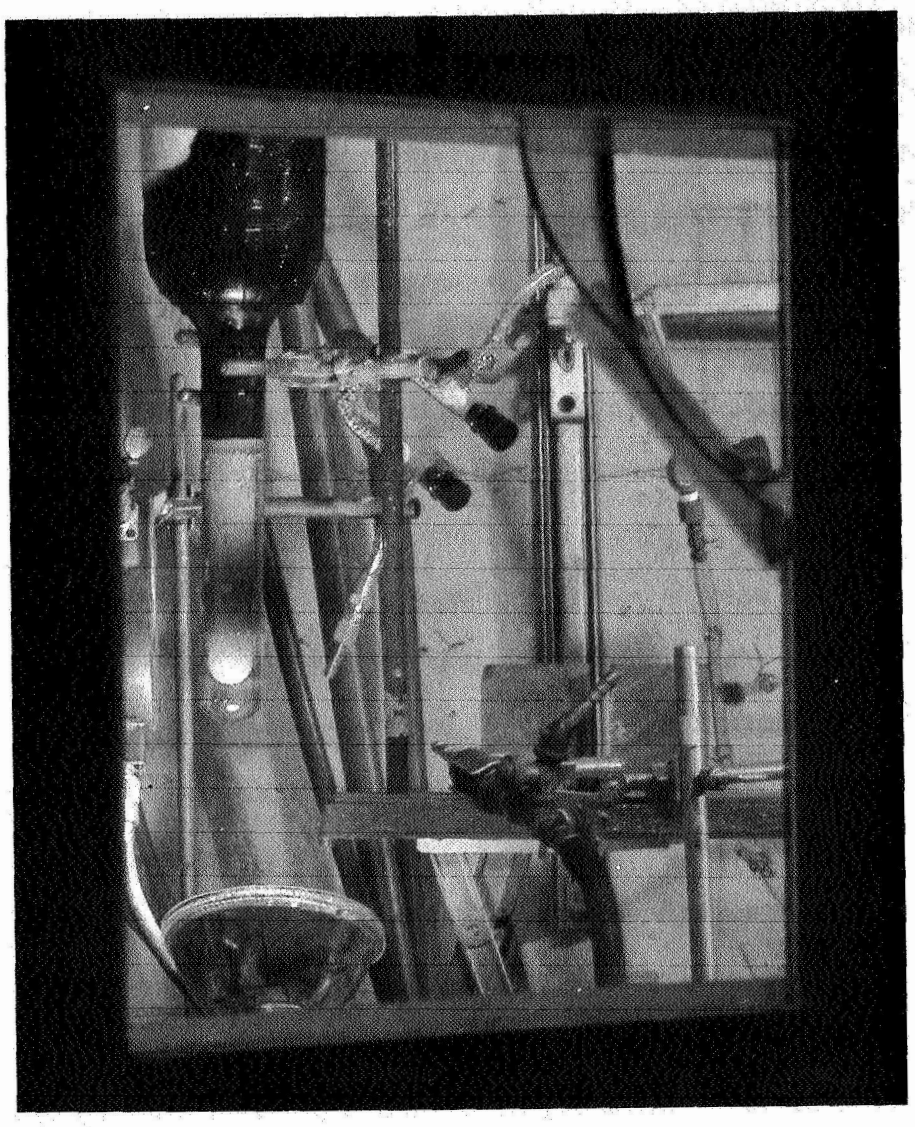


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Figure 41. Photograph of the irradiation (in progress) of solid methane by an electron beam.

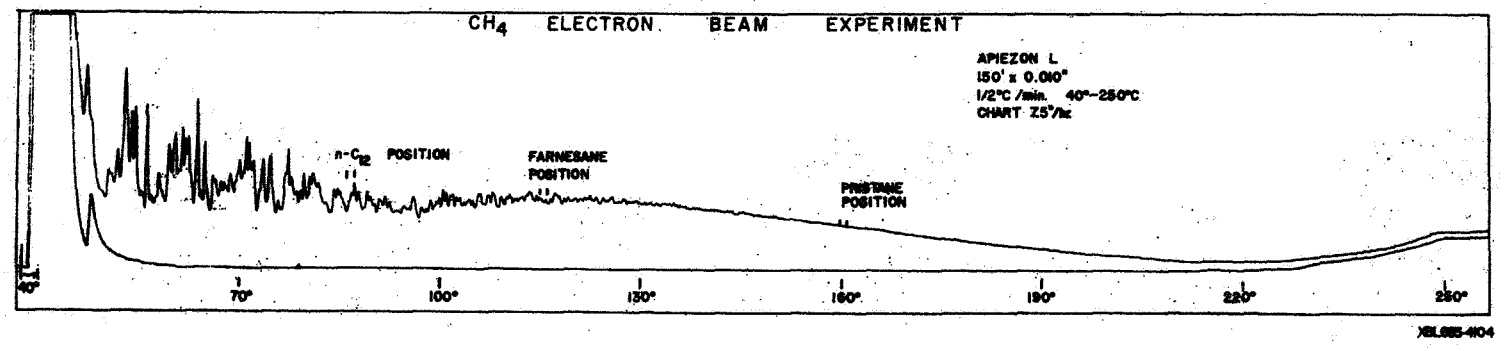



Figure 42. Gas chromatogram of the products obtained by electron beam irradiation of solid methane.

conclusions can be drawn concerning other classes of compounds is uncertain. Several other experiments which attempt to represent abiotic compound formation were mentioned in Chapter I, but in general the total mixtures have not been examined. Until it is shown to be incorrect, it is valid to assume that all reported abiotic experiments producing any or all compound type(s) have produced essentially non-specific mixtures.

The experiments just described are all of the same type. Whether effected by $^{60}Co$, electron beam, or electric discharge, these processes can almost certainly be considered to be high energy processes in which the product distributions are determined more by the thermodynamic stabilities of the compounds produced than by kinetic factors. The experimental distributions are certainly in accord with this suggestion. One of the factors which might alter the distribution is the presence of a catalyst. The Fischer-Tropsch processes are of this type and the products can show some specificity. The final outcome is very dependent on the exact conditions, but in Fischer-Tropsch products examined so far, structural specificity has been limited to a predominance of the unbranched structures.[109,130]

There has as yet been no abiotic synthesis which produces a distribution and specificity similar to that of biological systems. Perhaps what is needed is an experimental system which differs markedly from those previously tried. Such a system comes out of the interesting calculations of A. Hochstim,[205] who has considered

the synthetic potential of a meteorite's impact, especially one which impacts in the water. The excited species in the attendent shock wave would be rapidly quenched, and in such a situation it is likely that the kinetic relationships would determine the products to a far greater extent than in other abiotic syntheses yet reported. This hypothesis is certainly subject to experimental verification. As long as one is careful to not restrict his experimental conditions too severely, considerable information can be obtained on kinetic vs. thermodynamic control of product distribution.

There are several other questions which are relevant to the abiotic vs. biotic question. The most important of these, suggested by the results and discussions in the past several pages, is--what are the characteristics of a truly abiotic mixture of compounds? Of the many mixtures of carbon compounds postulated to be of non-biological origin, how many have and have not been through the biosphere--i.e., have or have not been affected either directly or indirectly by the presence of biological activity? Such compounds as inorganic carbonates, graphites, carbides, etc., may have originated as biochemicals or may be the residue of biological depletion of an earlier carbon source, so that carbon isotope ratios, elemental compositions, etc., might be misleading. At the moment it is probably impossible to definitely state whether or not any terrestrial (or extraterrestrial) carbon compound has never passed through the

biosphere. One can only estimate the probabilities based on current geochemical and geological information.

The basic assumption of organic geochemical searches for information on the origin of life has been tested and considered in the preceding pages. Realizing that, once again, information is incomplete, at this point in organic geochemical research, it must be concluded that it is possible to state whether a distribution of compounds, particularly of hydrocarbons, represents a biological distribution. It is not possible with present information, to state that a certain mixture is abiotic as opposed to a transformed biotic (or partially biotic) mixture.

Of course the test of such a conclusion is its application to an actual situation. This has been tried in studies of carbonaceous chondrites, but the conclusions are uncertain.[206,207] The uncertainties are complicated by the question of terrestrial vs. extraterrestrial origin for these compounds. In most cases, however, the reported hydrocarbon distributions do appear to have a biological origin.

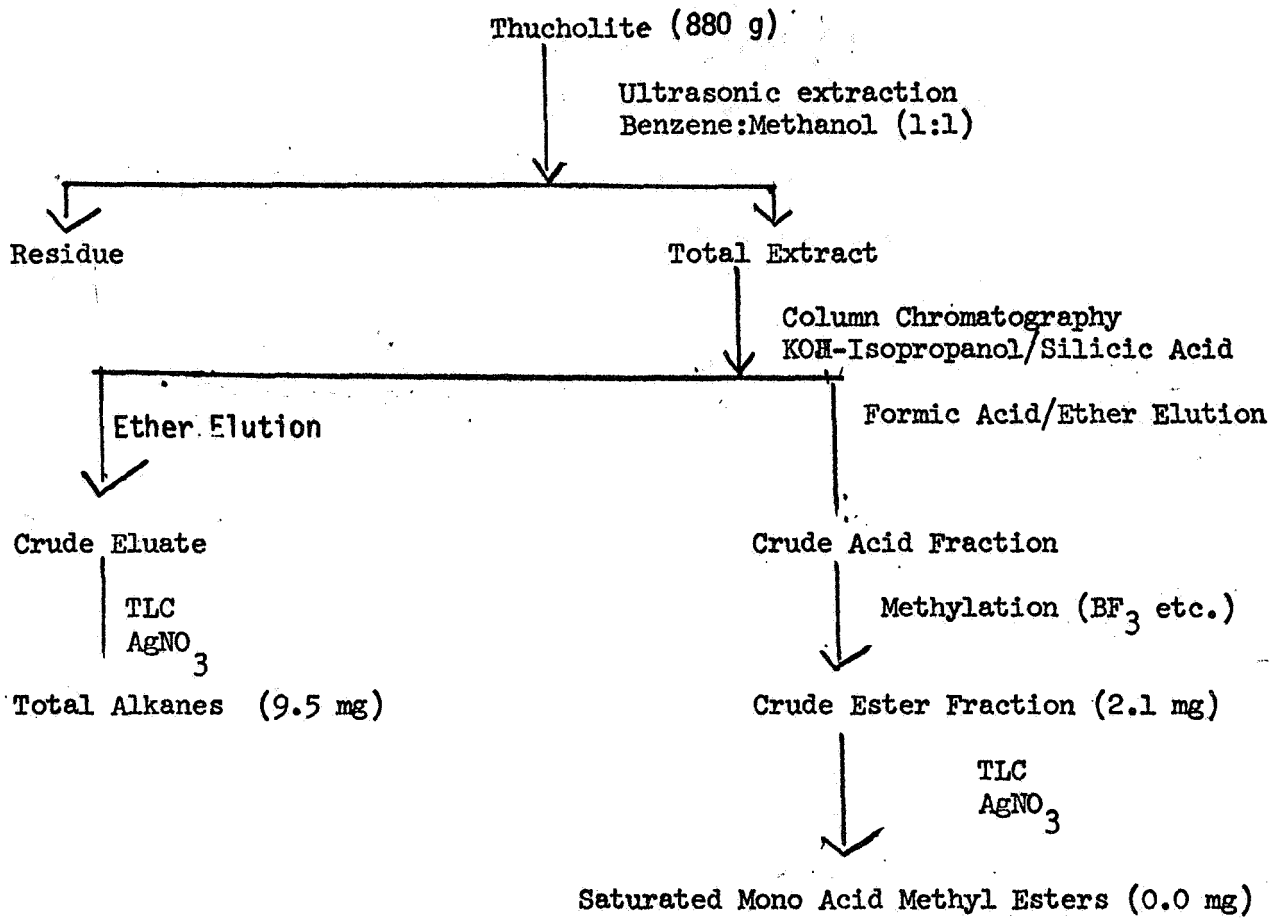
Another test of the use of alkane and fatty acid distributions is that of the analysis of a Precambrian thucholite. Historically, because of the presence of the carbonaceous veins within radioactive minerals, it has been felt that the thucholite present may be the product of radioactivity interacting with some carbon source.[208] One of the more significant occurrences of a thucholite is from the Besner Mine in Conger Township, Parry Sound District, Ontario, Canada. A sample of this thucholite

was provided by C. Frondel, and is that described in 1930 by Spence.[208] This sample was analyzed according to the fatty acid-hydrocarbon scheme previously outlined. Only the free fatty acids and extractable hydrocarbons were examined; no HF-HCl digestion was carried out. The carbon containing fractions were imbedded within the mineral matrix. These were removed by hand, resulting in the carbon-containing pieces being broken into small (∿1" diameter maximum) pieces. These pieces were thoroughly cleaned ultrasonically before pulverization. The quantities obtained are shown in the following scheme.

Figure 43 represents the extractable alkanes and saturated mono acids present in the sample. The fatty acids are dominated by the normal acids n-$C_{16}$, n-$C_{18}$ and n-$C_{14}$, representative of biological activity as discussed in Chapter III. The peaks are determined by coinjection and retention times.

The alkane distribution is typical of that found in ancient sediments. Pristane and the $C_{18}$ polyisoprenoid (2,6,10-trimethylpentadecane) have been determined not only by retention times, but by g.c.-m.s. as well, as shown in Figure 44. The g.c.-m.s. also provided evidence for the presence of phytane. The conclusion from both the fatty acid distribution and the alkane distribution is that this mixture of carbon compounds has a biological origin. One can say that it is impossible to state absolutely that the distribution is due to biological activity and that the original suggestion of an abiotic formation affected

THUCHOLITE - ANALYTICAL RESULTS



THUCHOLITE ALKANES and FREE FATTY ACIDS (AS METHYL ESTERS)

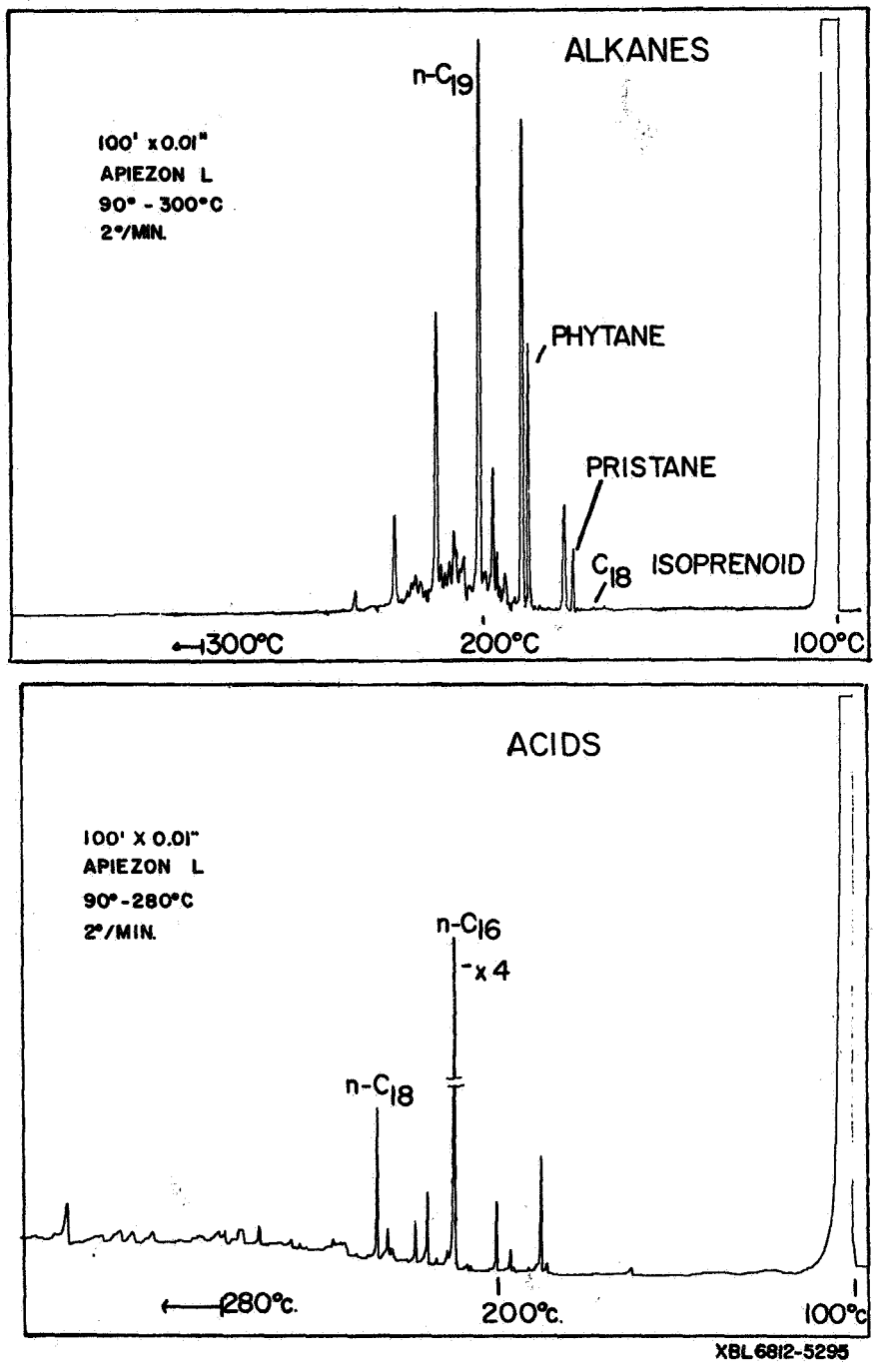



Figure 43. Gas chromatograms of extractable alkanes and fatty acids (as methyl esters) from the Thucholite.

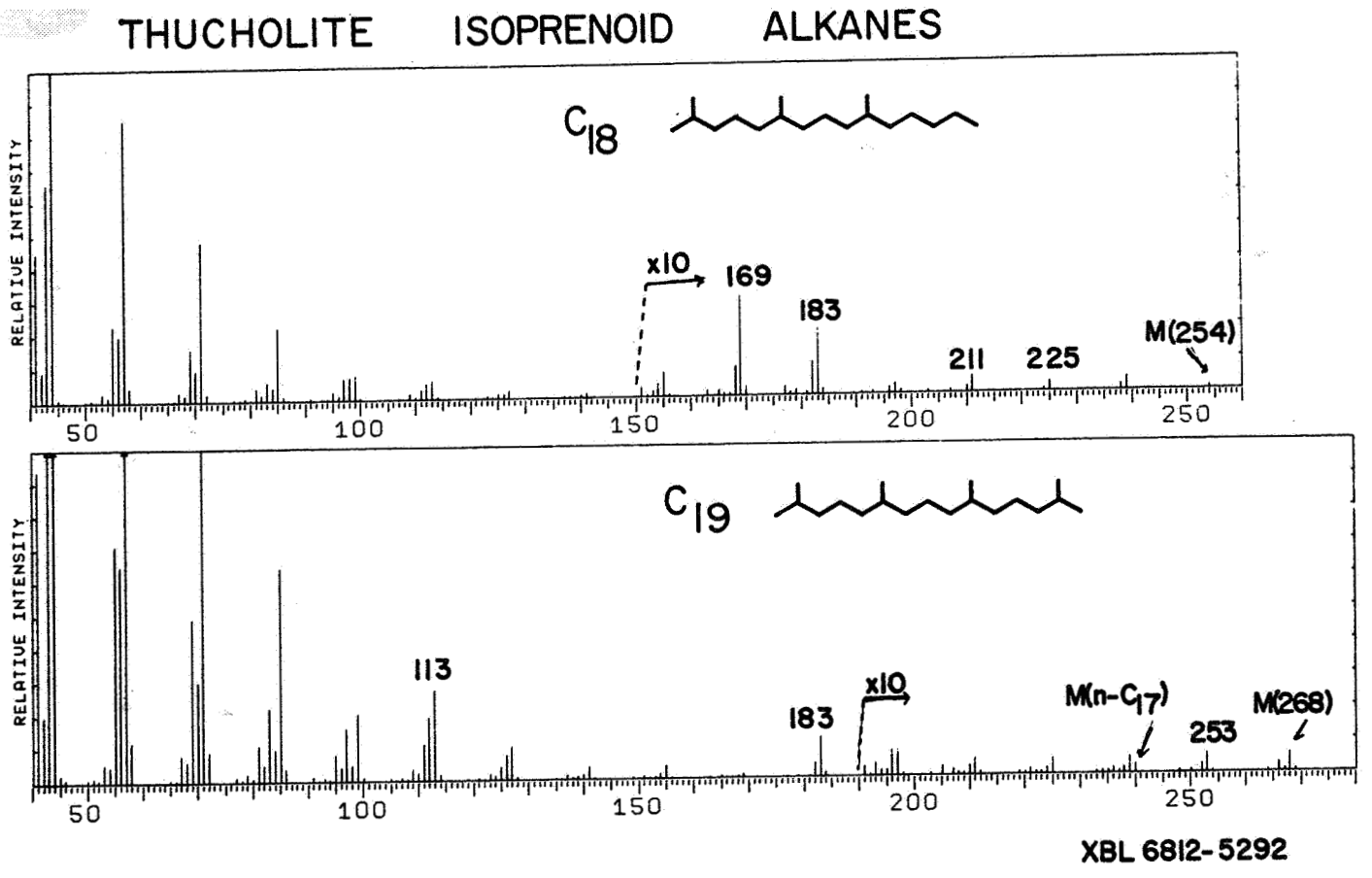



Figure 44. Mass spectra of polyisoprenoid alkanes from the Thucholite.

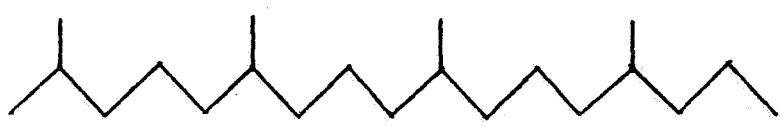
by mineral structures, etc., may still be accurate. This is true, but in view of the abiotic experiments described earlier and the experience and evidence obtained by analyses of both types of distributions, the balance of evidence still lies heavily in favor of the conclusion reached here.

# CHAPTER V

## THE TAIL-TO-TAIL LINKGAGE IN BIOLOGICAL POLYISOPRENOIDS

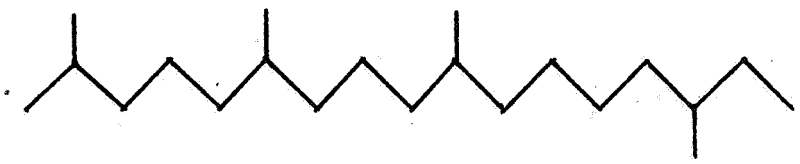
## -A NEW BIOLOGICAL MARKER?-

The previous chapter has attempted to deal with the differences between biotic and abiotic distributions of compounds. In the course of the concern about the validity of the polyisoprenoid alkanes as biological markers, attention in this laboratory was focused on the $C_{21}$ polyisoprenoid alkane, which had been shown to be present in a number of sediments.[164,209] A consideration of the origin of this alkane suggested that two different structures could reasonably be postulated for this $C_{21}$ compound. If this alkane was a homolog of the regular polyisoprenoid series, it would have the 2,6,10,14-tetramethylheptadecane structure:



This structure would be expected if the $C_{21}$ alkane were derived from any of the regular polyisoprenoid structures ($>C_{20}$) known in nature (e.g.--betulaprenols,[210] ubiquinones, bombiprenone,[211] geranylnerolidol,[212] etc.), or if it were derived from the acyclic $C_{40}$ carotenoids, such as lycopene.

Biogenetic considerations, however, suggest that this $C_{21}$ alkane could also be derived from compounds such as squalene, in which case it would have the 2,6,10,15-tetramethylheptadecane structure:



Since thermal cracking processes have apparently played a large role in the formation of petroleum,[213] the direct formation of these $C_{21}$ compounds from higher alkanes is not unreasonable. Logical diagenetic pathways to these alkanes are shown in Figure 45.

The difference between these two compounds is a very important one. Whereas abiotic polymerizations of isoprene produce either regular head-to-tail compounds or a random mixture of head-to-tail, tail-to-tail, and head-to-head linkages of the isoprene units, no abiotic polymerization has been devised which is entirely head-to-tail except for one tail-to-tail linkage exactly in the middle of the chain. In nature, the head-to-tail linkage is dominant, as evidenced by the many polyisoprenoid precursors discussed throughout this thesis. Squalene and the carotenoids are the most obvious exceptions (Figure 46). Of special note is the unbranched 4-carbon fragment. If, in fact, the regular polyisoprenoid alkanes (e.g., pristane and phytane) are not sufficiently structurally specific for use as biological

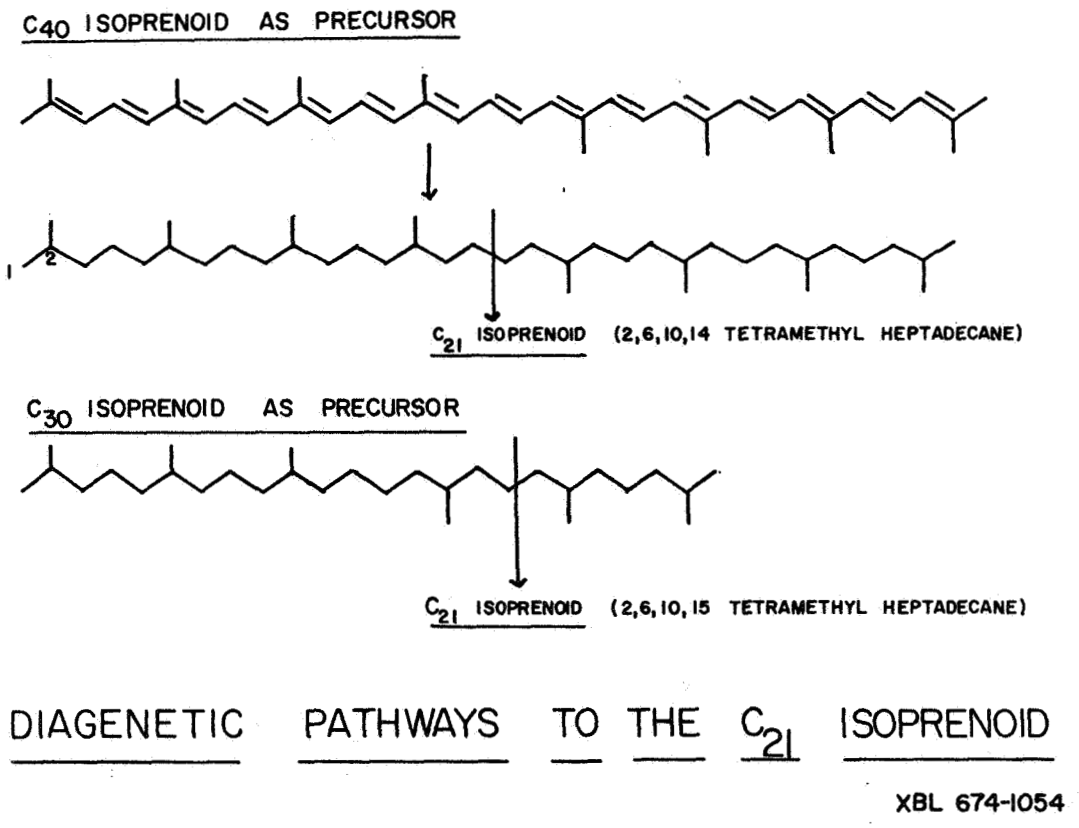



Figure 45. Possible diagenetic pathways to $C_{21}$ polyisoprenoid alkanes.

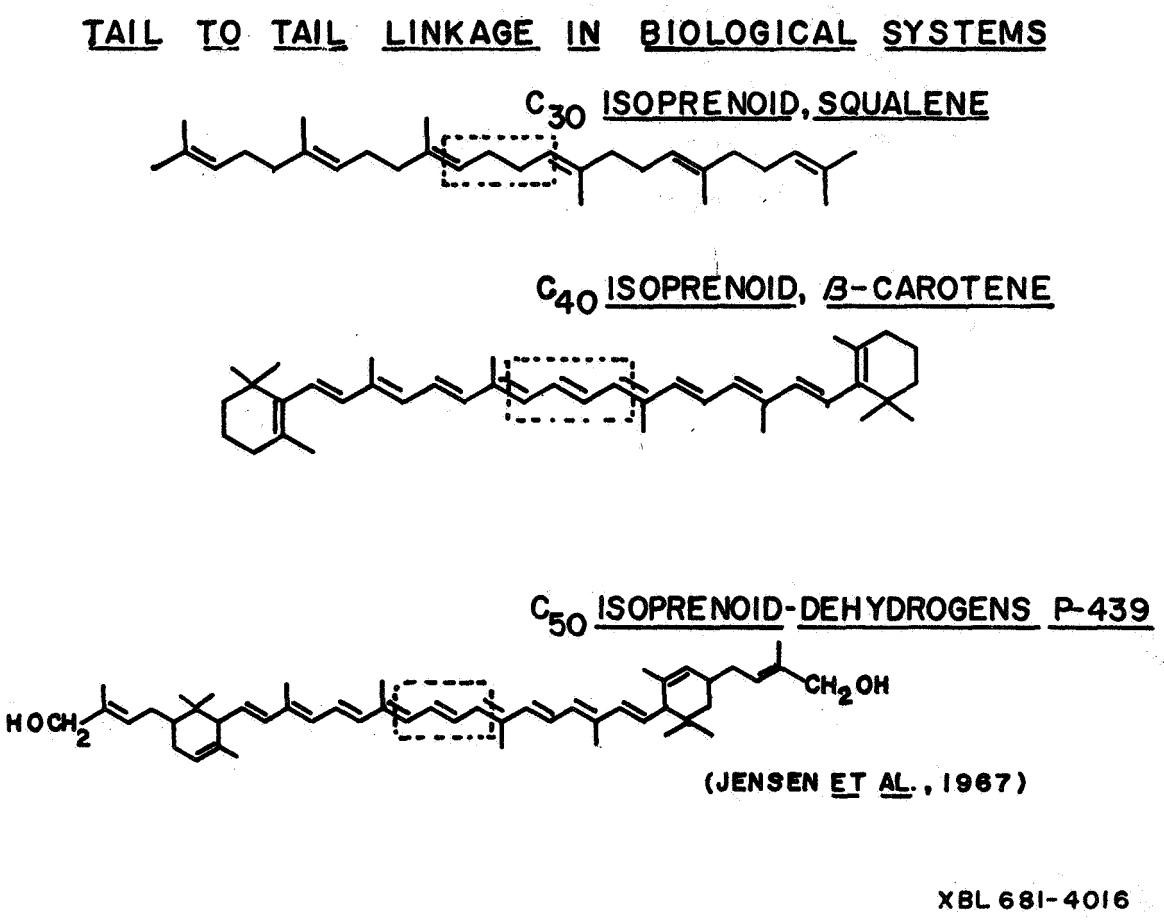



Figure 46. Tail-to-tail isoprenoid linkages found in biological systems.

markers, the polyisoprenoid alkanes containing this 4-carbon unbranched fragment could be used.

Since it had been well established that small differences (e.g., the displacement of a methyl group one carbon along the chain) between molecules such as pristane and related $C_{19}$ alkanes could not be detected by mass spectrometry, if the isolated compounds were slightly impure,[163] it became imperative to prove the structure of the isolated $C_{21}$ polyisoprenoid alkane. The only way in which the choice between the above two compounds could be made was by their synthesis and a precise study of their gas chromatographic and mass spectrometric behavior.

The two compounds were synthesized according to the scheme in Figure 47. The 2,6,10,15- compound was synthesized by E. D. McCarthy, and the details of that synthesis are recorded elsewhere.[163] The following is a detailed account of the synthesis of the 2,6,10,14-tetramethylheptadecane from phytol.

Infrared spectra were recorded on either a Perkin-Elmer Model 257 or a Perkin-Elmer Model 137 infrared spectrophotometer. Mass spectra were taken on either a modified C.E.C. 103 or an A.E.I. MS-12 low resolution mass spectrometer. Which of the instruments was used is indicated for the individual analyses. Mass spectral data is to be interpreted as follows: Mass number (origin of peak), (percent of base peak). The starting material for the synthesis was crude phytol (General Biochem.) $C_{20}H_{40}O$. I.R. (257) 3300 (O-H), 2920 (C-H), 1660 (C=C), 1455 (C-H), 1370

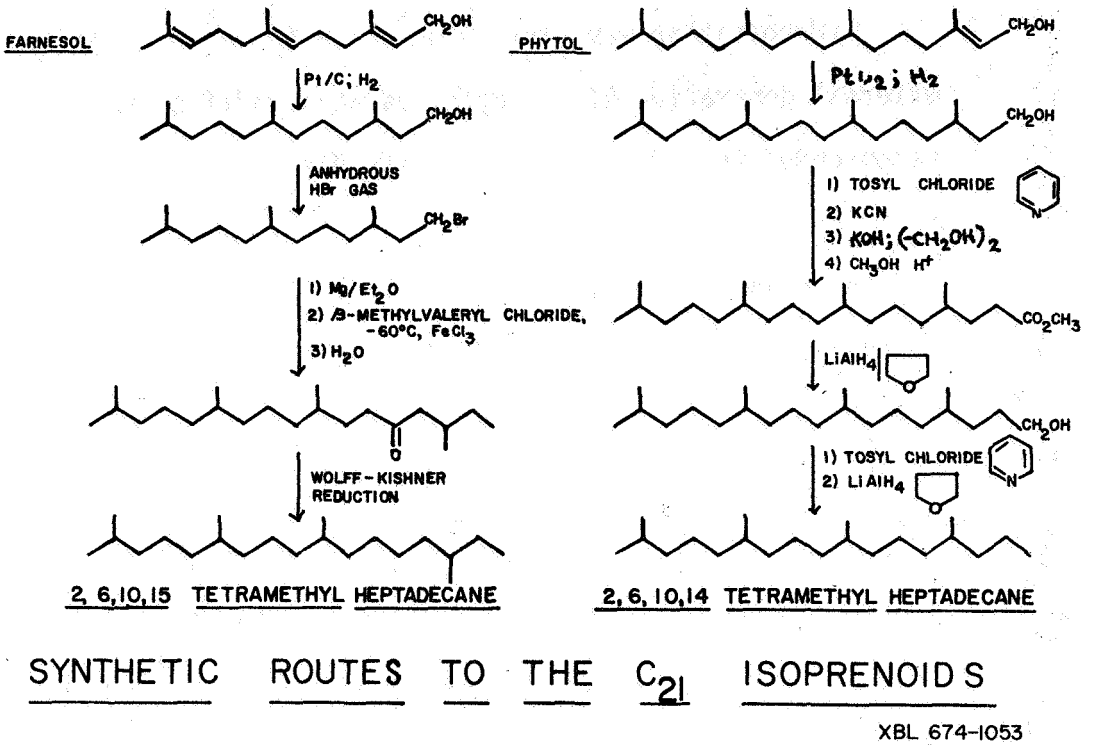



Figure 47. Synthetic routes to the $C_{21}$ polyisoprenoids.

(C-H), 1000 $cm^{-1}$ (allylic O-H). Mass spectrum (MS-12) (70 eV) m/e 296 ($M^+$) (0.5), 278 (M-18) (1.2), 123 (17), 71 (100), 43 (70).

Hydrogenation was carried out in a Brown[2] Hydrogenator with external generation of hydrogen, using a modification of the recommended procedure. The reaction flask initially contained 50 ml of absolute ethanol, to which was added five drops of 10% HCl in ethanol and 0.3 g of fresh $PtO_2$ (84%). The hydrogen generator contained 40 ml of 8.7 M acetic acid, into which was injected 15 ml of 1.0 M $NaBH_4$ in 80% aqueous ethanol. Ten minutes were allowed for this flushing operation, after which 25 g (84.5 mmoles) of phytol was added. Experimental difficulties prevented an accurate measure of hydrogen uptake; however, after 24 hours the system was stable and the reaction was discontinued. Centrifugation and removal of the ethanol gave 25 g (84 mmoles-99% yield) of a yellow solution which was not purified.

Crude dihydrophytol. ($C_{20}H_{42}O$) IR (257) 3315 (O-H), 2920 (C-H), 1460 (C-H), 1370 (C-H), 1050 $cm^{-1}$ (saturated primary O-H). Mass spectrum (MS-12) 280 (M-18) (1.2), 252 (M-46) (2.3), 196 (3), 183 (2), 182 (2), 71 (70), 57 (100), 43 (80). Gas chromatography (Aerograph 665, 25' x 0.01 " Apiezon L) showed three peaks in a 1:1:8 ratio, none of which corresponded to phytol.

Tosylation of the crude dihydrophytol (25 g - 84 mmoles) was according to the procedure described elsewhere,[214] except

in this case the solution was allowed to reach room temperature and was stirred for 72 hours. Isolation of the product, after acidification, was accomplished by extraction with ethyl ether, drying ($MgSO_4$) and solvent evaporation to give 24 g (55 mmoles - 65% yield) of crude tosylation product.

Crude dihydrophytol-tosylate. IR (137) 2900 (C-H), 1460 (C-H), 1360 (C-H), 1175 $cm^{-1}$ (sulfonic ester).

The alkyl cyanide was made according to the procedure of Cava.[215] The source of cyanide ion was KCN, and the reaction temperature was maintained at 130°C for 19 hours; ethyl ether was used to extract the product. From 23 g (51 mmoles) of the crude tosylation product, 18.5 g of crude cyanolation product was obtained. Gas chromatography (same conditions as with dihydrophytol) indicated a three component product in a 1:3:6 ratio. Preparative gas chromatography (10' x 1/4", 3% SE-30, Aerograph A-90-P) followed by mass spectrometry confirmed that the largest component was the desired alkyl cyanide (11.1 g - 36 mmoles - 71% yield).

Crude alkyl cyanide product. IR (257) 3420 (-O-H), 2920 (C-H), 2244 (-C≡N), 1460 (C-H), 1375 (C-H), 1150 (-$\overset{|}{\underset{|}{C}}$-OH), 1120 (i-Pr), 1060 $cm^{-1}$ (-O-H).

Purified alkyl cyanide. $C_{21}H_{41}N$. Mass spectrum (MS-12) 307 (M) (2.5), 292 (M-15) (5), 222 (22), 152 (30), 96 (27), 71 (60), 57 (100), 43 (70), 41 (40). On the basis of mass spectrometry, the 10% component could be identified as a phytene (MW = 280) and

the 30% component as the product formed by allylic rearrangement of phytol and subsequent hydrogenation.

Hydrolysis of the alkyl cyanide was accomplished by the method of Cason and Rapaport.[216] Fifteen grams of the crude cyanide (60% alkyl cyanide - 29 mmoles) was used. The acidification with 12 N HCl produced a gummy mass, which when dissolved in ethyl ether, washed ($H_2O$), dried ($MgSO_4$), and recovered by solvent removal gave 13 g of a viscous brown liquid. Spectral analysis suggested this to be largely the acid salt. Infrared (257) 3320 (-O-H), 2920 (C-H), 1560 ($-CO_2^-$), 1460 (C-H), 1405 ($CO_2^-$), 1375 (gem-di-$CH_3$), 1090 (C-O), 1050 $cm^{-1}$ (O-H). Less than 1 g of acid was extracted from the acidified hydrolysis mixture. A small portion of this acid salt was esterified by reaction with $BF_3$-MeOH (Applied Science) for five minutes, followed by extraction with n-$C_7$. Gas chromatography showed the product to be ∿70% methyl ester (therefore ∿9.1 g of acid - 28 mmoles - 97% yield).

A portion of the $C_{21}$ acid was methylated by refluxing in 50 ml MeOH (plus 1 ml conc. $H_2SO_4$) for 65 hours. The mixture was poured into 150 ml of $H_2O$ which was extracted with n-$C_6$. The extract was dried ($MgSO_4$) and the solvent removed to give the $C_{21}$ ester (322 mg).

<u>Methyl-3,7,11,15-tetramethylheptadecanoate</u>. $C_{22}H_{44}O_2$. Mass spectrum (C.E.C. 103) 340 (M) (3), 311 (M-29) (1), 309 (M-31) (1), 283 (M-57) (6), 157 (12), 87 (100), 74 (32), 71 (32), 57 (52).

The $C_{21}$ methyl ester was reduced to the alcohol by lithium aluminum hydride ($LiAlH_4$) reduction.[217] The ester (322 mg) - 0.95 mmoles) was dissolved in tetrahydrofuran (THF) and 0.95 g $LiAlH_4$ was added. The mixture was heated to reflux for 43 hours, after which time 10 ml of methanol was cautiously added to destroy the excess $LiAlH_4$. To saponify the unreacted ester, 5 ml of 10% KOH was added and the thick mixture was refluxed for 1 hour. Acidification with 10% $H_2SO_4$ and extraction with $Et_2O$ (4 x 75 ml) gave an extract which when dried ($MgSO_4$) and evaporated gave 222 mg of crude alcohol (222 mg - 0.69 mmoles - 72.5% yield). To 200 mg (61 mmoles) of the crude alcohol in 15 ml pyridine (0°C) was added 356 mg tosyl chloride. The reaction mixture was allowed to rise to room temperature and was stirred for 24 hours. The solution was acidified with 75 ml of 25% HCl and extracted with ether (3 x 20 ml). The extract was washed ($H_2O$), dried ($MgSO_4$), and the ether evaporated to give 114 mg of crude tosylation product (0.24 mmoles - 40% yield). The $C_{21}$ tosylate was dissolved in 15 ml THF; $LiAlH_4$ (512 mg) was added and the mixture heated to reflux for 19 hours. Saturated $Na_2SO_4$ was slowly added until a precipitate formed. Filtration, followed by evaporation of the THF gave 81 mg of crude product. The product was dissolved in ether, filtered through sintered glass to remove traces of $Al(OH)_3$, and the ether evaporated to give the $C_{21}$ alkane crude product, 56 mg (-0.19 mmoles - 79% yield). This mixture was analyzed

by analytical gas chromatography and shown to be ∿90% the desired compound. Preparative gas chromatography followed by mass spectrometry gave the mass spectrum shown in Figure 48.

With the two $C_{21}$ isomers, it is possible to directly compare their mass spectra with each other and those of the $C_{21}$ polyisoprenoids isolated from the oils and sediments. Comparison of the mass spectrum of one standard with the other does show some real differences which permit one to distinguish between the two. However, as mentioned earlier, compounds isolated from complex mixtures are seldom pure, and the small differences between the mass spectra of individual isomers are obscured by impurities. Thus in the case presented in Figure 48, it is impossible to determine which of the two isomers has been isolated from the Soudan Shale (isolation by E. D. McCarthy). (It must be mentioned that there are many tetramethylheptadecanes, and that the isolated compound from the Soudan may not be one of the two considered here. However, these two are the most likely from biogenetic and diagenetic considerations.)

Capillary gas chromatography, using conditions such as those shown in Figures 49 and 50, is capable of separating the two isomers (from E. D. McCarthy). Coinjection of the standards with the alkanes from various sediments has shown that in all cases examined, the isolated $C_{21}$ polyisoprenoid corresponds to the 2,6,10,14-tetramethylheptadecane (Figures 51 and 52--52 from E. D. McCarthy).

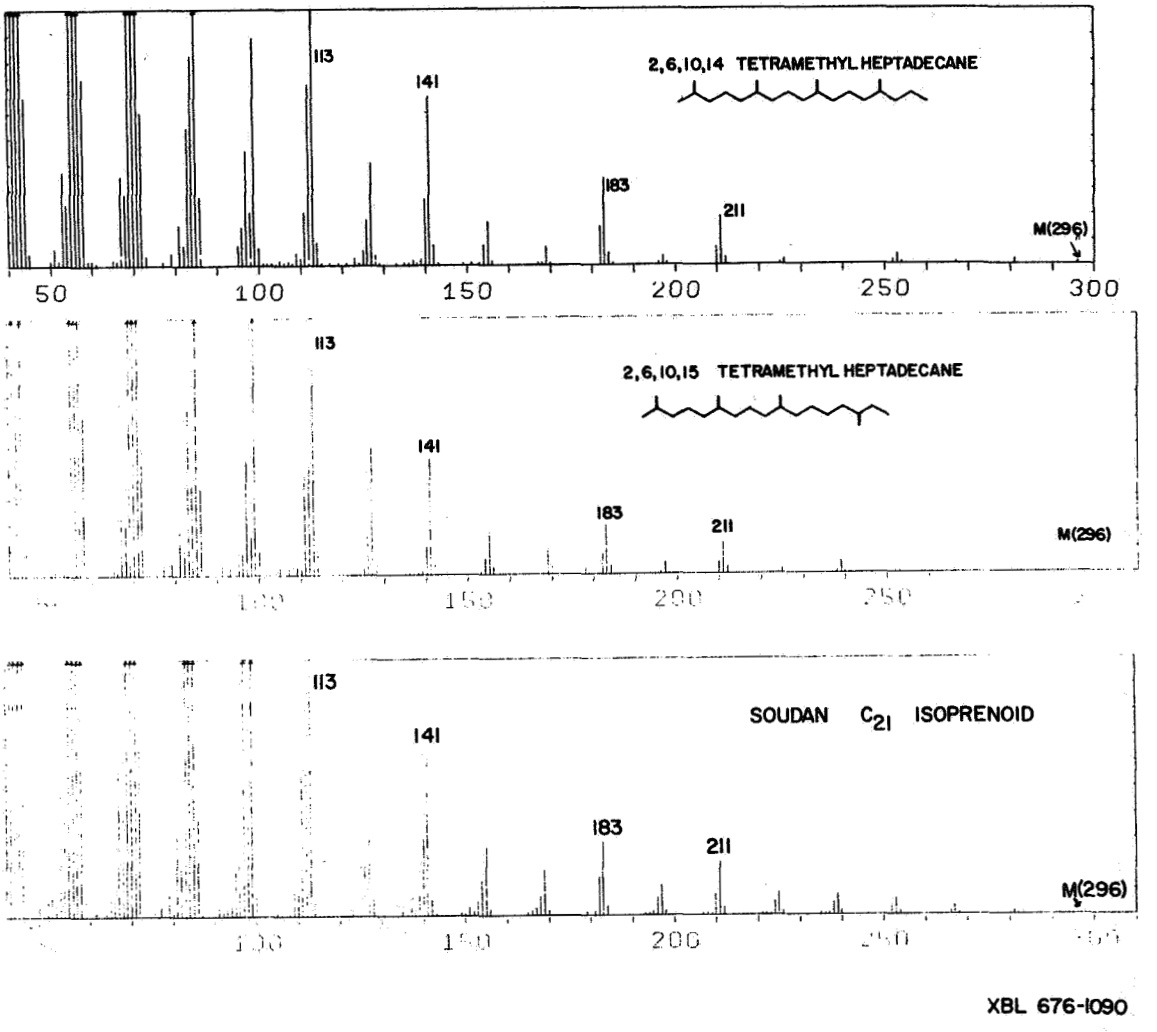



Figure 48. Mass spectra of authentic $C_{21}$ polyisoprenoid alkanes and Soudan Shale alkane.

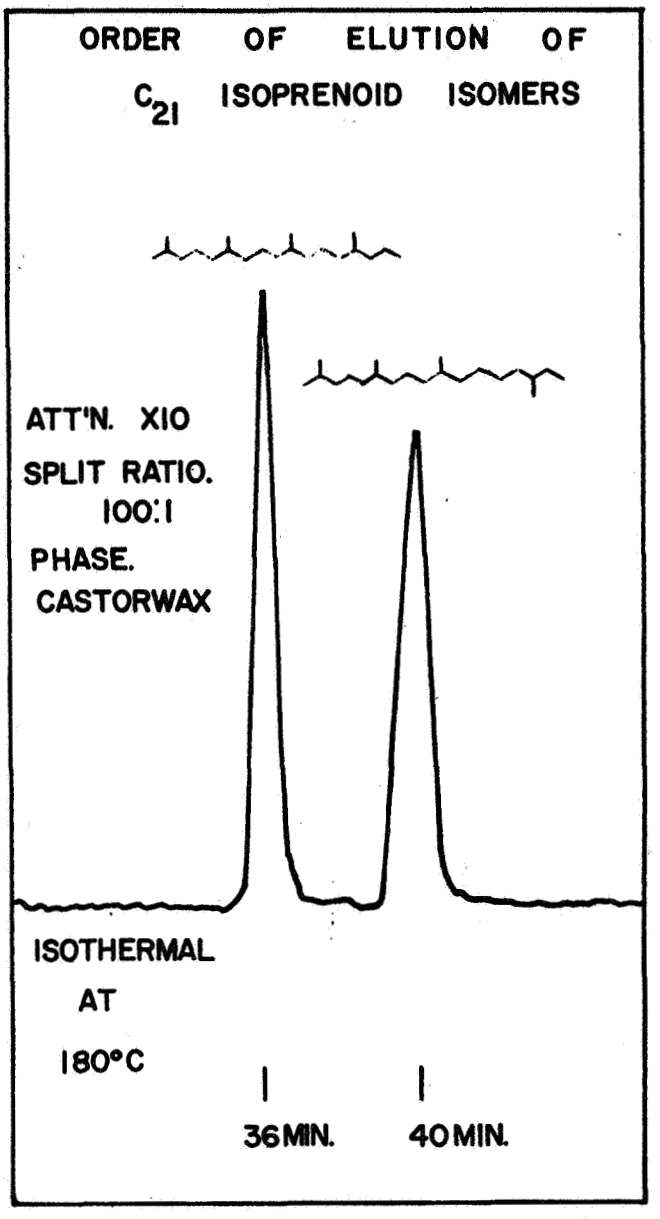



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Figure 49. Gas chromatogram of $C_{21}$ polyisoprenoid alkanes (on castor-wax).

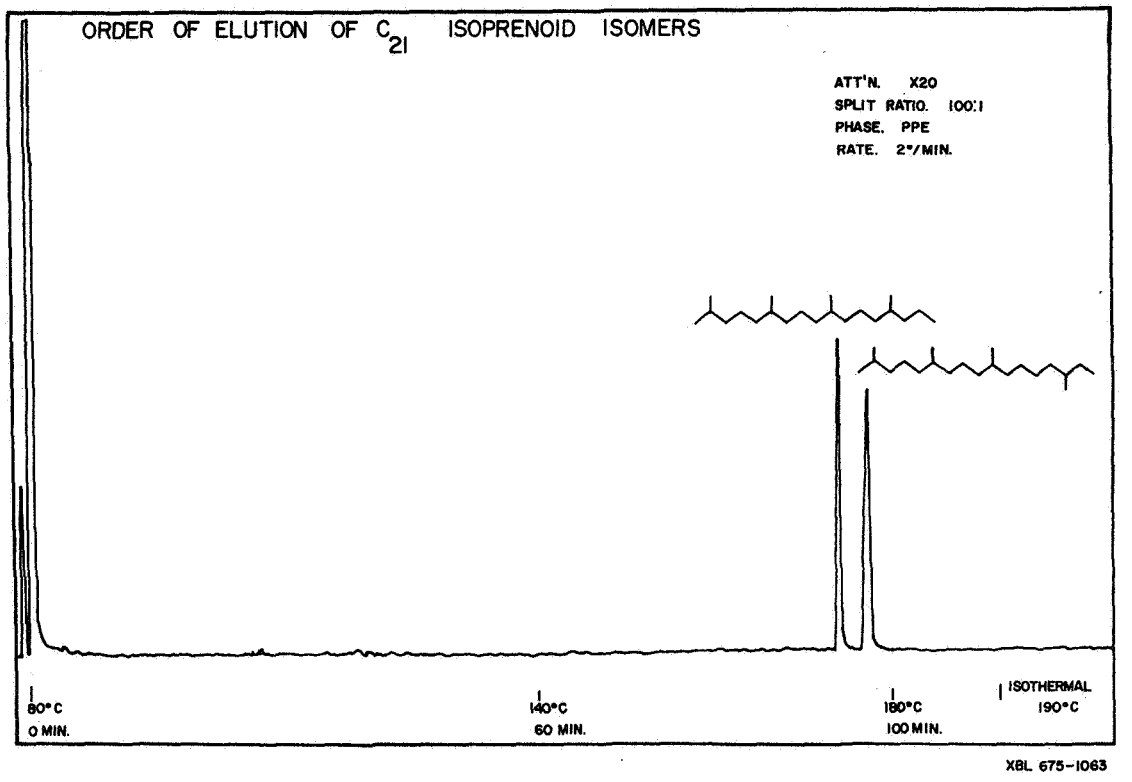



Figure 50. Gas chromatogram of $C_{21}$ polyisoprenoid alkanes (on polyphenylether).

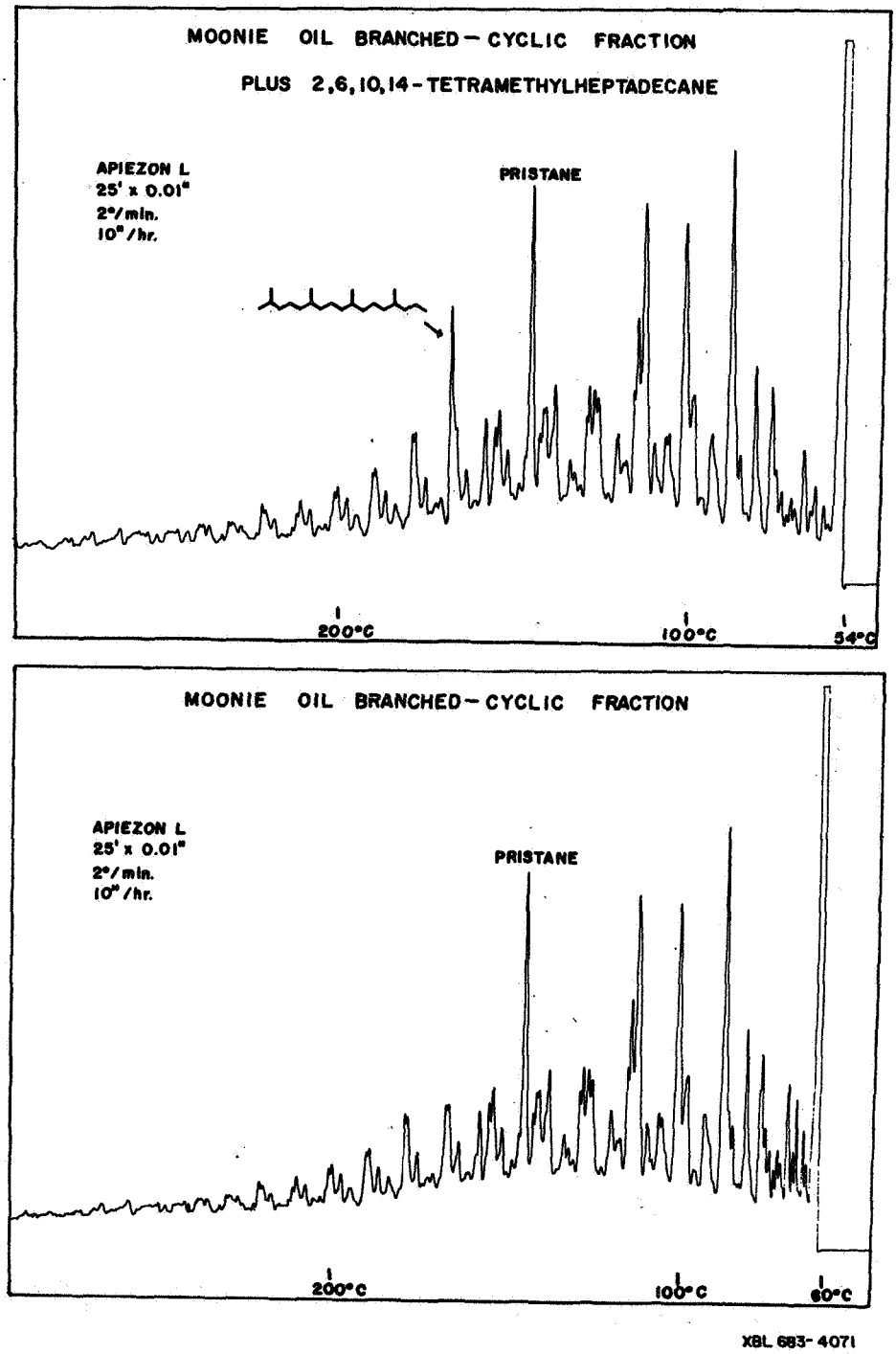



Figure 51. Gas chromatograms of Moonie Oil alkanes with and without added 2,6,10,14-tetramethylheptadecane.

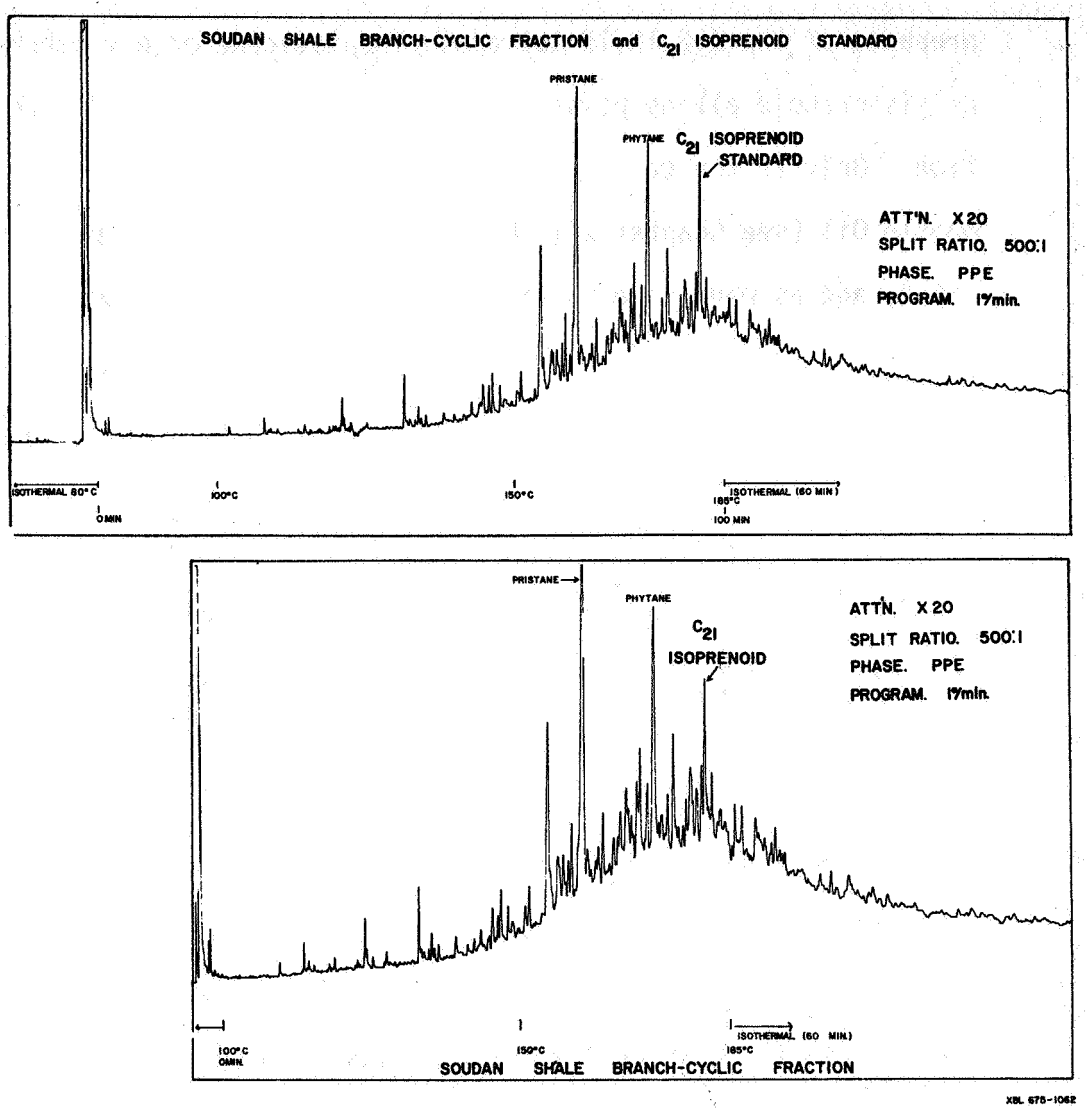
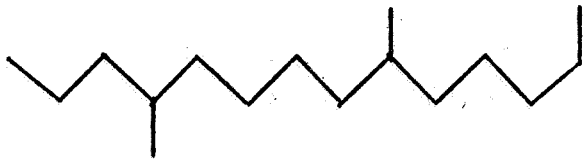



Figure 52. Gas chromatograms of Soudan Shale alkanes with and without added 2,6,10,14-tetramethylheptadecane.

In fact, there is not yet any evidence which confirms the presence of the 2,6,10,15-tetramethylheptadecane or any other polyisoprenoid alkane possessing the 4-carbon unbranched portion. Only in the case of the compound labeled $X_2$ from the Moonie Oil (see Chapter III) has such a structure been postulated, and as mentioned earlier, the evidence is not complete.



($X_2$)

From the absence of the 2,6,11,15-tetramethylheptadecane as a major component in the ancient sediments thus far examined, it can be concluded that neither squalene nor the carotenoids contribute greatly, in a direct manner, (i.e., single bond cleavages) to the acyclic polyisoprenoid alkanes in these sediments. The concepts governing the research reported here are, however, no less valid and should be continued to be considered as of significant value in organic geochemistry.

# CHAPTER VI

# CONCLUSION

The preceeding five chapters have attempted to place organic geochemistry within the proper context, to consider the choices for biological markers and chemical fossils which are available for the organic geochemist, to report on recent findings within the field, particularly concerning hydrocarbons and fatty acids, to relate these findings not only to the general field but also to the problem of the origin of life, and to discuss individually some specific points of concern. Throughout all of this discussion, conclusions have been drawn and suggestions made on the basis of the results reported. This final chapter contains the overall conclusions as well as suggestions for future research.

It is the firm belief of this author that the organic geochemical approach to origin-of-life problems, extraterrestrial life problems, and geological and petrological problems is a valid one. Even at present its foundation is solid enough to justify research such as reported here and to accommodate the results of that research.

The research on hydrocarbon constituents of sediments and oils has attained a sufficient level of sophistication that, from the distributions of alkanes, it is possible to state whether or not those compounds are (at least partially) the

result of biological activity. The evidence for the existence of biological activity extends back in time to the oldest sedimentary rocks yet discovered, approximately 3.5 x $10^9$ years of age. The results of the research reported here have permitted some greater degree of understanding about some of the phenomenon which do and do not affect the alkane distributions, by examining in some detail those distributions and their variation with source material and post-depositional conditions.

In spite of honest and necessary questioning of the validity of the approach, experiments have not destroyed this validity in any way. These abiotic syntheses have, however, forced the organic geochemists to consider other possible chemical fossils and to extend the foundation upon which their research rests.

One of the chemical fossils which has been studied for many years, though limited almost exclusively to sediments less than 500 x $10^6$ years, is the fatty acid distribution. The work presented here extends these studies, for the first time, into the Early Precambrian. In a rather comprehensive analysis of each of several sediments and oils, the presence of a biological distribution of fatty acids has been recognized in sediments as old as 2.7 x $10^9$ years. Contamination from laboratory handling and recent bacterial action has been appropriately considered and eliminated as the cause of these distributions. The only question not sufficiently answered, and this is true of virtually <u>all</u> organic geochemical results, is whether or not these

compounds are syngenetic or epigenetic (perhaps by billions of years) with the inorganic matrix of the sediment. It is encouraging to the author to note that the research reported, while it has answered some very basic questions, has stimulated additional questions and research within the field.

It was pointed out at the start of this thesis that the origin-of-life problem can be approached in several ways. Within the approach of organic geochemistry, it is useful and necessary to recognize the relevancy of results from geology, paleontology, inorganic, organic and physical chemistry, biochemistry, etc. The general trend of organic geochemical research today seems to be in the direction of a closer look at more well-defined systems, which are part of the overall picture. Comparative biochemical results, recent sediment analyses, diagenetic factors, to mention but a few, have recently received considerable attention.

There are several important aspects of organic geochemistry which, though often invoked, are not well documented or understood, not necessarily because of neglect but because of insufficient techniques or because these aspects have, until recently, not been recognized. One of the most important factors governing the use of a compound or compound type is its geochemical stability. Only for amino acids is there considerable stability data. The modes of decomposition and the thermodynamics and kinetics involved should be given greater attention. And

work should be extended from the pure compound to its decomposition in a sedimentary environment, with as much duplication of heat, pressure, and chemical environment as possible. The "protective binding mechanism" so often invoked should be critically examined.

Isotope effects, particularly those of carbon, are often invoked as substantiative evidence for biological activity. However, these effects are due to physico-chemical and physical processes which have not been studied for all those molecules of interest to the organic geochemist. One of the most necessary studies in this field is the study of the magnitude of isotope effects for various individual compounds (such as phytol) within living systems, and the magnitude of the effects in the geochemical analogs (e.g., phytane). With information as to the isotope ratios of individual atoms within complex molecules, their decompositions to various geochemicals might be better understood (i.e., why some bonds seem to preferentially break).

Optical activity is one of the best single indicators of biological activity, and yet relatively few advances have been made which permit the measurement of very small effects. The recent advances involving resolution of optical isomers by gas chromatography should be extended to as many classes of compounds as possible. One of the difficulties here is the formation of suitable derivatives; and this is one area which organic chemists have neglected. Needless to say, adequate

derivitization would be useful for the analyses of several classes of compounds (e.g., porphyrins and nucleic acids) which are currently very difficult to handle.

The question of additional biological markers opens up an area of considerable potential. The 4-carbon unbranched fragment which was discussed in Chapter V is but one of several possibilities. Other individual compounds which may be of use are the mono-methyl compounds such as those from the blue-green algae and the Florida Mud Lake sample. This latter possibility will almost certainly become a distributional problem; other unusual distributions may become more apparent as the exact compounds present in living organisms are discovered.

The question of abiotic vs. biotic distributions can be studied in greater detail. The concept of a meteoritic impact can be examined by means of shock tube experiments, rapid hot tube experiments, etc.

As initially expressed, a major concern of the work and results included in this thesis was to provide information on the time, place, and manner of the origin of life on Earth. That problem has not been solved by the work reported here. However, new information relevant to that question has been provided. Additionally, results of importance, hopefully of considerable importance, to the field and future of organic geochemistry have been provided. It is also toward the arrival on Earth of lunar samples that this work has been directed. The scientific and non-scientific future in all these areas in indeed bright and exciting.

## ACKNOWLEDGEMENTS

To adequately acknowledge, in the space available here, the many contributions and much assistance given during the years which have culminated in this dissertation is certainly impossible, but a sincere effort must be made.

The tasks associated with such doctoral studies are, of course, many and varied, but, of them all, developing the ability to properly approach scientific questions and answers is undoubtedly the most important. If I have attained any measure of competence in this area, it is largely through the guidance of Professor Melvin Calvin. His example, advice, and personal encouragement have been most helpful and I appreciatively acknowledge his contribution.

From many people in the Department of Chemistry, the Space Sciences Laboratory, and the Lawrence Radiation Laboratory I have received a great deal of assistance and many helpful suggestions. Drs. Richard Lemmon and Douglas Pounds made possible the methane polymerization experiment. Mr. William Neuman also deserves a special thank you for his generous and excellent technical assistance. Mr. Howard Ono has given me numerous valuable suggestions in our many discussions.

Throughout the years, a number of professors, post-doctoral personnel, and fellow students have been associated with this research effort, and their contributions, mentioned throughout the thesis, are more personally acknowledged here. Particularly I thank Drs. G. Eglinton, A. L. Burlingame, and R. B. Johns for

helpful guidance and discussions. I sincerely hope that the reader of this thesis recognizes the valuable contribution of Dr. James R. Maxwell, without whose efforts much of the story presented here would be incomplete.

A number of people have been most generous in making available various geological samples and standard isoprenoid acids. The former were provided by the persons mentioned in the text of the thesis. The acids were provided by Professor James Cason, and Drs. R. P. Hansen and G. J. Popjak.

The preparation of this manuscript has been made possible by the patience and effort of several people. Mrs. Sandra Norton admirably translated my original heiroglyphics and prepared most of the figures. Mrs. Marilyn Taylor provided many suggestions and much assistance, and Mrs. Evie Litton typed the final copy and also assisted in the preparation of the photographs.

Finally, I want to express my deep appreciation to the one individual without whose personal encouragement and expressed faith and confidence this degree would not have been obtained.

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